CHAPTER 1

INTRODUCTION

1.1 General consideration

Opisthorchiasis, a food-borne parasitic disease caused by the liver fluke *Opisthorchis viverrini*, is one of the important communicable diseases in Thailand. Harinasuta (1986) estimated that more than 7 million people in the northeastern region were infected. The majority of individuals with light infections usually have no or few symptoms, and occasionally flatulent dyspepsia. In moderate infections, there may be diarrhea, dyspeptic, flatulence, pain over the liver, jaundice, fever and enlargement of liver (Wykoff *et al.*, 1966; Upatham *et al.*, 1982; Upatham *et al.*, 1984). Chronic and heavy infections have been found to be associated with hepatobilialy disease, cholangitis, cholangiohepatitis, liver abscess and cholangiocarcinoma (Viranuvatti and Stitnimankarn, 1972; Sonakul *et al.*, 1978; Kim, 1984; Bunnag and Fungladda, 1989; Elkins *et al.*, 1990). In recognition of its public health impact, the Ministry of Public Health of Thailand decided to implement an intervention program to control the infection. The opisthorchiasis control program in Thailand has been developed in three phases. Between 1951 and 1968, pilot control programs using chloroquine for treatment of infected people were unsuccessful in reducing the prevalence and maintaining it at a low level. An intensive health education effort began in 1968 as the second phase of control; this lasted until 1987. At the beginning of this phase, health education has been promoted, aimed at persuading villagers to cook their favorite raw fish dishes. Praziquantel became available in 1984 and was incorporated into the control program. By 1987, the prevalence of

opisthorchiasis had declined considerably, but not below the level of public health importance.

The control program is now continuing with the aims of providing diagnosis and treatment for all infected people after individual fecal examination using mainly the Kato's thick smear technique. The strategy includes health education of opisthorchiasis, aimed at changing behavior of people eating raw fish and increasing number of latrines in the villages. The program has been implemented in all provinces in northeast Thailand. Although health education about opisthorchiasis has greatly been promoted, this appears to obtain little success in changing the eating behavior of people. Nonetheless, an interim evaluation showed that the prevalence of opisthorchiasis in the northeast had decreased from more than 70% in 1984 to 24.3% in 1990 (Division of communicable disease control, Ministry of Public Health, 1992), probably mainly due to the campaign of stool examination and treatment. Currently, the control program has been extended to the northern provinces where many endemic areas have also been reported with the prevalence rates varied from 2.4 to 60% (Sadun, 1955; Vajrasthira and Harinasuta, 1957; Yasmuth *et al.*, 1968; Manning *et al.*, 1969; Papasarathorn *et al.*, 1969; Na bangxang *et al.*, 1969; Thitasut *et al.*, 1973; Khamboonruang *et al.*, 1978; Yamaguchi *et al.*, 1982; Keha *et al.*, 1986; Kasuya *et al.*, 1989; Keawvichit *et al.*, 1993; Pornpibool *et al.*, 1993), suggesting that opisthorchiasis in the northern region is as important as in the northeastern region.

The route of getting infection in human is by eating raw fish containing encysted metacercariae of *O. viverrini*. The most important raw-fish food that contribute to opisthorchiasis in the northeastern region is "*koi-pla*" (ก้อยปลา) (Sadun, 1955; Harinasuta, 1969). In northern region, however, "*koi-pla*" seems to be less popular whereas

"*pla-jom*" (ปลาจ่อม) and "*pla-som*" (ปลาส้ม) are more common (Pornpibool *et al.*, 1993). Cyprinoid fish are usually used for preparing these kinds of food.

At present, not only cyprinoid fish caught from their natural habitats, but those from fish farms are increasingly available in the markets. However, it is not clear whether the latter is safe for people to consume without high risk of opisthorchiasis. The infection of *O. viverrini* metacercariae among cyprinoid fish in fish farms needs to be explored. In addition, the role of "*pla-jom*" and "*pla-som*" in transmission of opisthorchiasis remains unclear and also needs to be investigated.

1.2 Review of literatures

1.2.1 Life cycle and morphology

O. viverrini, as described by Wykoff *et al.* (1965), requires two intermediate hosts for its larval stages to develop. The life cycle follows man—fresh water snail—fresh water fish—man (Figure 1). Fully embryonated eggs of *O. viverrini* are found in the stools of infected humans, the definitive host, and other mammalian reservoir hosts, especially dogs and cats. Approximately 20% to 60% of cats and 6.3% to 40% of dogs examined were found to be naturally infected with *O. viverrini* (Sadun, 1955; Wykoff *et al.*, 1965). Normally, these eggs do not hatch until they are eaten by the snail, the first intermediate host, which is usually feeding in bodies of water polluted by stools of humans and animals. Within the snail, the miracidia hatch out in the esophagus, intestine or rectum, then penetrate the wall of these organs to become sporocysts and rediae respectively, in the hemolymph spaces of the snail. Finally, rediae migrate to the digestive gland area and form cercariae, which soon leave the snail and swim in the adjacent water. They then penetrate under the skin of the fish

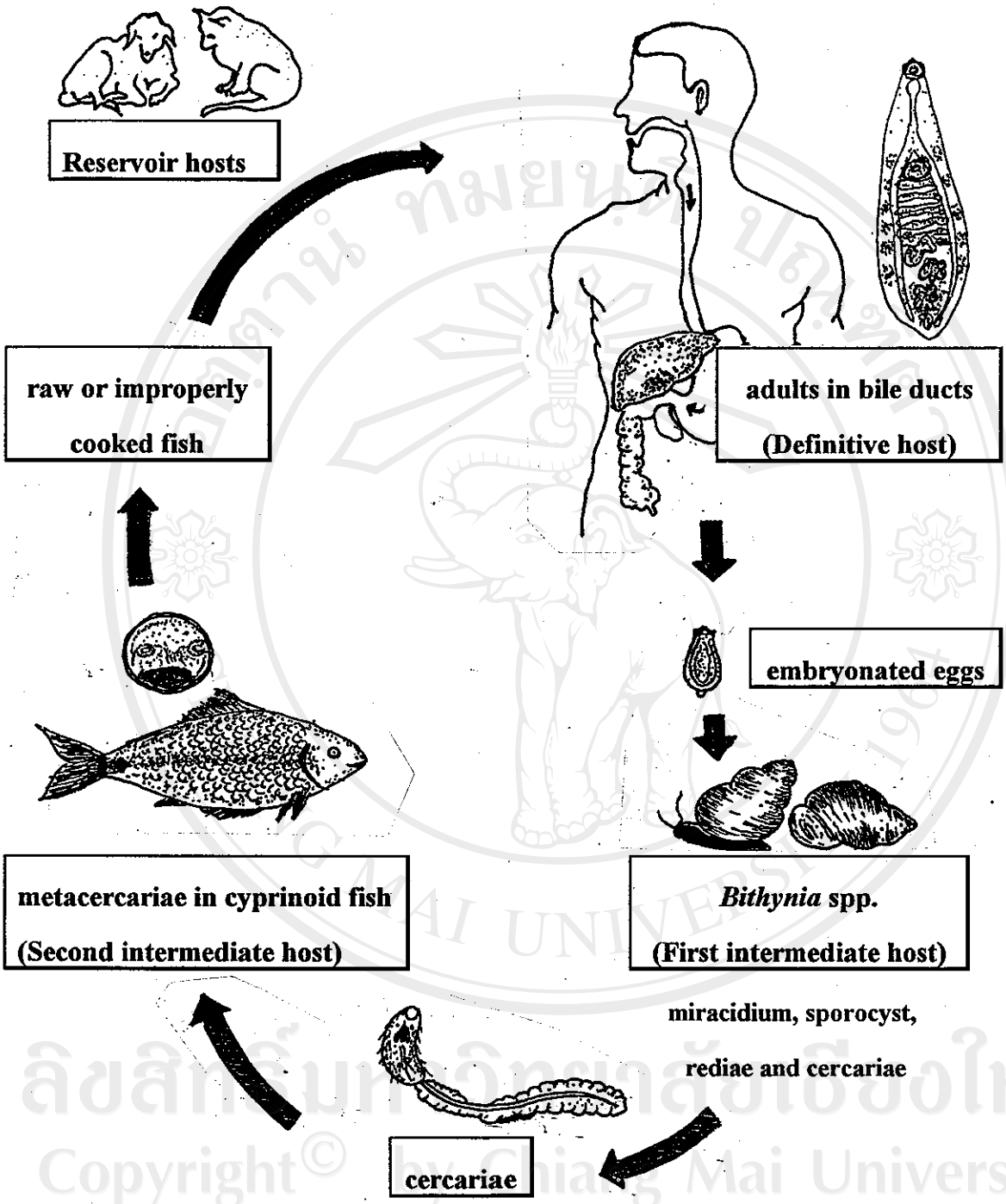

Figure 1. Life cycle of the liver fluke *Opisthorchis viverrini*

second intermediate host and become encysted metacercariae in the muscles. After encystation, the metacercariae become infective within a month. When the infected fish is ingested raw, or slightly cooked or pickled, by man or other mammalian hosts, the metacercariae excyst in the duodenum, move towards the ampulla of Vater into the common bile duct and extrahepatic biliary system, which they reach in about a day or less. After reaching the bile passages, the young worms mature. The first eggs are laid about 4 weeks after infection, but the worm continues to grow for some months after (Wykoff and Ariyaprakai, 1966; Tansurat, 1971; Viranuvatti and Stitnimankarn, 1972; Bhamarapravati *et al.*, 1978). The entire life cycle requires from 4 to 4.5 months.

The adult fluke is thin and transparent, lancet-shaped, rounded posteriorly and attenuated anteriorly. The average size is about 7.4×1.65 mm. The worms are occasionally found in the gall bladder; only in heavy infections have they been encountered in the pancreas. The living worm in the bile tract is reddish-bile colored. The integument is smooth, although in immature worms there is a trace of the spines that are present on the integument of the cercaria. The two suckers are nearly equal in diameter (0.25 mm); the oral sucker is subterminal, while the ventral sucker is situated about one-fifth the body distance from the anterior end. The excretory bladder is a long sac-like tube extending as far forward as the ovary. The two testes are lobed and are situated obliquely to each other in the posterior fourth of the worm (Wykoff *et al.*, 1965). The worms have a life span of 25 to 30 years (Belding, 1965).

The eggs of *O. viverrini*, as described by Sadun (1955), are usually elongate ovoid in shape with an operculum resting on its shoulders; they are light yellowish-brown and are about three times as long as broad. The average size is about 26.7×15 μm. At the posterior end of the shell there is usually a minute tubercle-like appendage present in the

middle of the abopercular end. The miracidium, which has an asymmetrical internal organization, is fully developed when the egg is laid. Under light microscope, the eggs of *O. viverrini* are quite similar to those of many small intestinal flukes in both size and morphology. Differentiation can be made under a scanning electron microscope (Tesana *et al.*, 1991), but this method is complicated and not suitable for field or routine work (Kaewkes *et al.*, 1991).

1.2.2 The snail intermediate hosts

Wykoff *et al.* (1965) found that the snails *Bithynia siamensis goniomphalus, B. siamensis funiculata* and *B. siamensis siamensis*, serve as the first intermediate host of *O. viverrini* in northeast, north and central Thailand, respectively. The general habitats of the intermediate snail hosts are stagnant water, e.g. ponds, swamps, and rice fields, with a high content of organic matter rather than flowing water in the rivers (Papasarathorn *et al.*, 1980). The cercarial infection rates of *O. viverrini* in the snails are normally low varying from 0.038-1.6% in Thailand (Wykoff *et al.*, 1965; Wongsaroj, 1988; Thirachantra and Khamboonruang, 1971; Upatham and Sukhapanith, 1980; Brockelman *et al.*, 1986; Impand *et al.*, 1994). Similar situation was also observed in Laos where 0.6% of the snails were infected (Giboda *et al.*, 1991).

1.2.3 The fish intermediate hosts

Hass *et al.* (1990) reported that the cercaria of *O. viverrini* finds and recognizes its fish host by using at least four steps of behavioral patterns: (1) dispersal and selection of plant-free water microhabitat are achieved by intermittent swimming behavior with positive phototactic orientation; (2) attachment to the host is stimulated by water currents

and a hydrophilic component of fish skin surface which has a molecular weight of more than 30 KDa. This component is sensitive to digestion with hyaluronidase and seems to be a glycosaminoglycan other than hyaluronic acid and chondroitin sulfates; (3) remaining on the host's surface is induced by an unknown chemical component of fish skin surface mucus; (4) penetration into the host is triggered by a hydrophilic component of fish skin surface of a molecular weight of more than 30 KDa. On coming into contact with a cyprinoid fish, the cercariae penetrate under the scales or into the flesh, and a period of at least 21 days is required for the full maturation of the metacercaria (Harinasuta and Vajrasthira, 1957; Wykoff *et al.*, 1965).

Fifteen species of cyprinoid fish from 7 genera have been reported to be the second intermediate hosts of *O. viverrini* in Thailand with the infection rates varying from 3 to 94.4% and with the average numbers of metacercariae per fish from 0.2 to 98.6 (Table 1). Among them, high infection rates (over 60%) were found in *Cyclocheilichthys apagon*, *C. armatus*, *C. repasson*, *Puntius leiacanthus*, *P. orphoides* and *Hampala dispa.*

At present, fish cultivation (aquaculture) or fish farms continues to grow in economic importance of Thailand. Cyprinoid fish are one of the commonly cultured fish. In Chiang Mai province, for instance, production of cultured cyprinoid fish in 1991 to 1992 increased from 1,857 to 2,853 tons (Saengthong, 1993). However, little is known about the *O. viverrini* infection rate in fish from fish farms.

1.2.4 The metacercaria of *Opisthorchis viverrini*

The *O. viverrini* metacercaria, as described by Vajrasthira *et al.* (1961), is contained in a double-walled cyst surrounded by a thick layer of tissue. Most cysts are oval in shape; a few are rounded. The walls of the cyst are thin, the outer wall being about

Table 1. Cyprinoid fish that have been found to be infected with metacercariae of *O. viverrini* in Thailand.

Fish	Local names	References*
Cyclocheilichthys apagon	ปลาตะโกก, ปลาหนามหลัง	(3), (4)
C. armatus	ปลาขาว	(2)
C. siaja	ปลาแม่สะแด้ง	(6)
C. repasson	ปลาขาวน้อย, ปลาขาวอีไทย	(2)
Esomus metallicus	ปลาซิว	(1), (6)
Puntius gonionotus	ปลาตะเพียนขาว, ปลาปาก	(6), (8)
P. leiacanthus	ปลาตะเพียนทราย	(1), (3), (4), (5)
P. orphoides	ปลาแก้มช้ำ, ปลาขาวมน, ปลาปก	(1), (2), (6)
P. partipentazona	ปลาเสือสุมาตรา	(4)
P. protozysron	ปลากระมัง, ปลาสะกาง	(6)
P. richoever	ปลาขาว	(6)
Hampala dispa	ปลากระสูบจุด, ปลาสูต	(2), (3), (6)
Osteochilus spp.	ปลาสร้อย, ปลาหน้าหมอง	(6)
Larbiobarbus linneatus	ปลาคุยลาม	(7)
Puntioplites sp.	ปลากระมัง	(7)

*(1) Sujjanun and Thitasut (1971); (2) Tesana *et al.* (1985); (3) Impand *et al.* (1994); (4) Vichasri *et al.* (1982); (5) Department of Parasitology, Faculty of Medicine, Chiang Mai University (unpublished); (6) Wykoff *et al.* (1965); (7) WHO (1995); (8) Ratanasritong and Kliks (1972)

3-8 μm thick and the inner wall so thin that it can be recognized only after the worm has escaped. The average size of the cyst is about 201×167 μm. The body of the metacercaria is folded within the cyst and frequently appears to be C-shaped. The mature larva moves vigorously at room temperature. When the metacercaria is at rest, the characteristic excretory corpuscles in the bladder and the brownish-yellow pigment scattered throughout the body are clearly visible. The excretory bladder appears as an oval area composed of masses of dark granules. The oral and ventral suckers are usually clearly seen.

The free metacercaria is oblong in shape, tapering slightly towards the anterior end. The average measurements of living specimens were are about 558×1,454 μm. All the cuticular surface, except the areas of the oral and ventral suckers, is covered with small spines, which become finer and fewer towards the posterior part of the body. Both suckers are roughly rounded. The oral is 60.0×67.9 μm and the ventral 72.7×76.5 μm in average. The oval excretory bladder is filled with numerous calcareous corpuscles. Brownish-yellow pigmented granules are scattered throughout the body. This pigmentation is one of the characteristic features of metacercariae of the family Opisthorchiidae.

The metacercariae can be found in all parts of the body of fish (Harinasuta and Vajrasthira, 1961; Vichasri *et al.*, 1982; Tesana *et al.*, 1985). Once they have entered the fish they remain infective for about 2 years (Wykoff *et al.*, 1965). Waikagul *et al.* (1977) reported that the *O. viverrini* metacercariae in the fresh fish kept at 4-8°C remained viable and infective for 7 days after the death of the fish host, but the metacercariae which were freed from the fish host and kept at room temperature remained viable and infective for 14 days.

1.3 Raw-fish food

The custom of eating raw-fish food has been considered one of the most important factors in the epidemiology of opisthorchiasis (Sadun, 1955; Harinasuta and Vajrasthira, 1957; Harinasuta and Vajrasthira, 1960; Wykoff *et al.*, 1965). Fresh water fish including cyprinoid fish are present in several Thai dishes, such as "*koi-pla*" (ก้อยปลา), "*pla-som*" (ปลาส้ม), "*pla-lap*" (ปลาลาบ), "*som-fak*" (ส้มฟัก) "*pla-jom*" (ปลาจ่อม), "*pla-ra*" (ปลาร้า) etc. Some of the common species of fish used in the preparation of these dishes are listed in Table 2 (Sadun, 1955). In northeastern Thailand, Kurathong *et al.* (1987) reported that about 70% of people had a history of eating "*koi-pla*". In northern region, by contrast, a recent study by The Public Health Office of Lampang province carried out in Amphoe Mae Prig in 1995 found that only about 15% of people had an experience in consuming this kind of food; other kinds of food containing raw or inadequately cooked fish are more common, i.e. "*pla-jom*" (64.8%), "*pla-ra*" (88.0%), "*pla-som*" (51.3%) and "*pla-lap*" (58.2%). These results are roughly similar to the study of Pornpibool *et al.* (1993) carried out in Lamphun province.

Sadun (1955) demonstrated the presence of viable metacercariae in raw-fish food by experimentally infecting cats with several samples of "*koi-pla*", "*pla-ra*" or "*pla-som*". At necropsy, adults of *O. viverrini* were recovered from the cat which received "*koi-pla*" only, but with "*pla-ra*" or "*pla-som*" no worms were found. In addition, Harinasuta *et al.* (1961) failed to detect *O. viverrini* metacercariae in "*pla-ra*" and "*pla-som*" collected from the markets in northeast region.

Table 2. Some of the common species of fish eaten raw in northeast Thailand.

Family	Species
Siluridae	*Pseudeutropius acutirostris*
	Ompok bimaculatus
Cyprinidae	*Cyclocheilichthys apagon*
	Puntioplites protozysron
	Osteochilus hasseltii
	Osteochilus lini
	Osteochilus melanopleura
	Cirrhinus jullieni
	Paralanbuca viveroi
	Puntius gonionotus
	Puntius leiacanthus
	Hampara dispa
Osphoronemidae	*Tricogaster lerii*
	Tricogaster pectoralis
Notopteridae	*Notopterus notopterus*
Bagridae	*Mystus wolffii*
Naudidae	*Pristolepis fasciatus*
Clariidae	*Clarias batrachus*
Eleotridae	*Oxyeleotris marmoratus*
Chaudidae	*Chauda siamensis*
Chandidae	*Opicephalus striatus*
Engraulidae	*Lycothrissa crocodilus*
Anabantidae	*Anabas testudineus*

1.4 Aims of investigation

1. To compare the prevalence, intensity and density of *O. viverrini* metacercariae in fresh-water fish in the Suborder Cyprinoidei collected from natural habitats and from fish farms in Chiang Mai province.

2. To survey of the occurrence of *O. viverrini* metacercariae in raw-fish products, "*pla-jom*" and "*pla-som*" sold in the markets around Chiang Mai province.

1.5 Significance of the research

The study provides the current status of *O. viverrini* metacercaria infection in fresh-water fish collected from different types of ecology, i.e. natural habitats and fish farms, in Chiang Mai province, as well as the occurence in raw-fish products. The information obtained is important for understanding more the epidemiology of opisthorchiasis in northern Thailand.