PREVALENCE OF TREMATODE METACERCARIAE IN CYPRINOID FISH OF BAN PAO DISTRICT, CHIANG MAI PROVINCE, NORTHERN THAILAND

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Abstract. The prevalence of trematode metacercariae in natural cyprinoid fish was carried out in Ban Pao district, Chiang Mai Province, northern Thailand. Fish were collected from both man-made water reservoirs and natural sources during July 1996 - May 1997. Nine species of cyprinoid fish harbored the metacercariae of *Opisthorchis viverrini* and small intestinal flukes of the family Heterophyidae, *ie Haplorchis taichui, H. pumilio* and *Centrocestus* spp. Metacercariae of *H. taichui* were found approximately 384 times more than *O. viverrini*. Among the fish collected, *Puntius leiacanthus* contained the highest number of metacercariae of *H. taichui* (182 metacercariae/fish) whereas *P. orphoides* contained the highest number of metacercariae of *O. viverrini* (1.4 metacercariae/fish). Metacercariae of *H. taichui* found in this area were year-round, with the highest and lowest incidence rates being in winter and rainy season, respectively. The difference between *H. taichui* and *O. viverrini* with respect to their prevalences are discussed.

INTRODUCTION

Food-borne trematode infections have been considered as an important public health problem throughout the world (WHO, 1995). In Thailand, the majority of these infections are occurred from consumption of raw and/or undercooked fresh water fish. The most serious food-borne trematode infections is opisthorchiasis caused by the liver fluke, *Opisthorchis viverrini,* in which seven million people in northeast Thailand have been estimated of harboring this parasite (Jongsuksantigul *et al*, 1992). The severity of this disease is ranging from nonmalignant diseases such as cholangitis, choletithiasis and cholecystitis (Pungpak *et al*, 1985) to bile duct cancer (Parkin *et al*, 1991). With the same mode of transmission, the other trematodes particularly heterophyid flukes have also been recorded from Thai people (Radomyos *et al*, 1984, 1994; Waikagul, 1991; Waikagul *et al*, 1998). Although these flukes have not been reported as the etiologic agent of the clinical manifestation in Thailand; however, some of heterophyid flukes were incriminated producing mild mucosal irritation accompanied by colicky pains and mucous diarrhea, with producing of excess mucus and a superficial necrosis (Beaver *et al*, 1984). The cardiac, brain and spinal cord lesions caused by ova of heterophyid flukes were also recorded (Africa *et al*, 1935, 1936, 1937a, 1937b).

Recently, the infective stage or metacercariae of *Haplorchis* spp were found more than those of *O. viverrini* in natural cyprinoid fish of Khon Kaen Province, northeast Thailand (Srisawangwong *et al*, 1997) and in cultured cyprinoid fish of Chiang Mai Province, northern Thailand (Khamboonruang *et al*, 1997). Even though there is no information regarding trematode metacercariae in natural fresh water fish of northern Thailand, however, the prevalence of this minute intestinal fluke from human cases in this area has also been reported much far above that of *O. viverrini* (Radomyos *et al*, 1998). The present investigation was, therefore, undertaken as the pilot study to determine the prevalence of trematode metacercariae in cyprinoid fish of Ban Pao district, Chiang Mai Province. This area represents the most important source of fish for inhabitants of a great part of Chiang Mai Province.

MATERIALS AND METHODS

Fish collection site

The project was carried out in Ban Pao district of Chiang Mai Province, northern Thailand. This district was chosen because of its location proximity to 3 rivers (Ping, Lerm, Mae Ngud), the presence of a large reservoir (Mae Ngud reservoir) and a large man-made dam (Mae Ngud Somboonchol dam or Mae Ngud dam), a small man-made reservoir, the ease of access by the available transport, and comprising of separated small villages (I, II, III, IV) along the banks of Lerm river, the branch of Ping river. Schematic map of the studied areas is shown

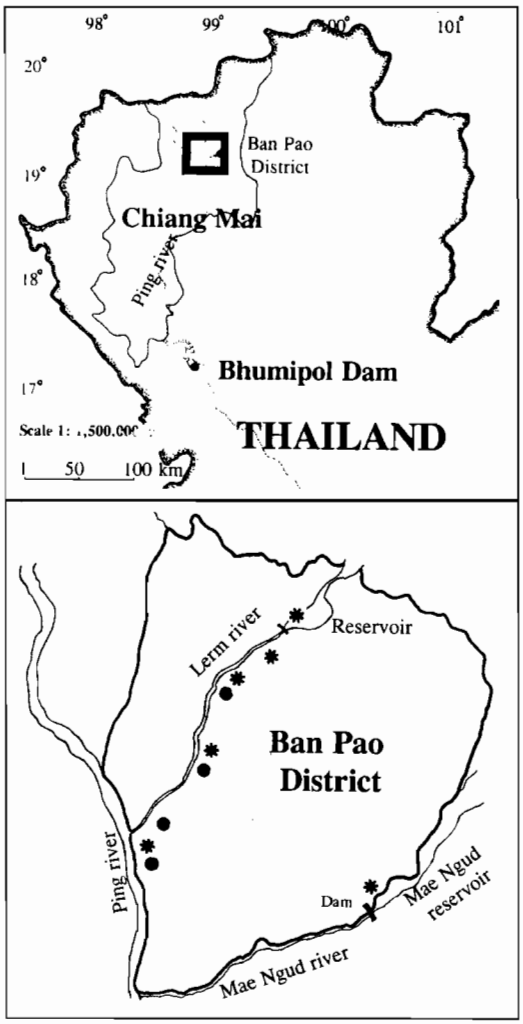

Fig 1–Area in Ban Pao district, Chiang Mai Province, surveyed for prevalence of trematode metacercariae.
● Approximate locations of the villages.
✱ Approximate locations of sites where cyprinoid fish infected with trematode metacercariae were collected.

in Fig 1. The large reservoir and dam serve as a source of water for the hydroelectric power station. Climatically, Chiang Mai Province has rainy season from July till October, winter from November till February, and summer from March till June.

Experiments

Fish were collected using fishing net at the fish collection sites totally 6 times from July 1996 - May 1997. These fish were placed in iced - box at 10°C and transported to the laboratory. All fish were identified morphologically to their being species according to the illustrations distributed by Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand. Trematode metacercariae were immediately collected using acid pepsin solution (100% hydrochloric acid 1 ml : porchine pepsin (Sigma®) 1 g : 0.85% sodium chloride solution 99 ml) in mixer blender at the ratio of 1 g of fish : 10 ml acid pepsin solution. The digested material was transferred into water bath shaker for $1\frac{1}{2}$ hour at 37°C, then it was subsequently passed through 2 layers of wet gauze. The digested material was rinsed with 0.85% sodium chloride solution and examined for metacercariae with the stereo microscope. The identification of metacercariae was carried out by morphological examination based on Scholz *et al* (1991) and the identification of sclerites on the ventrogenital sac of *Haplorchis* species was based on Pearson and Ow-Yang (1982).

RESULTS

Out of 699 cyprinoid fish collected, ten species were obtained, *ie Puntius leiacanthus, P. gonionotus, P. orphoides, Thynnichthys thynnoides, Danio regina, Puntioplites proctozysron, Hampala macrolepidota, Aplocheilus panchax, Larbiobarbus burmanicus* and *Tilapia nilotica* (Table 1). Among them, *T. thynnoides* was the most predominant collected fish (43.2%; 302/699), and nearly all of this species were from Mae Ngud reservoir. *Danio regina* was the second most common fish found, and all of them were from paddy fields in the villages. Fish were consistently collected all year-round from Mae Ngud reservoir. In contrast, there was small amount of fish collected from the villages in summer since these areas were droughty.

Two families of medically important trematode metacercariae were obtained. They consisted of Family Heterophyidae (*Haplorchis taichui, H. pumilio* and *Centrocestus* spp) and Family Opisthorchiidae (*O. viverrini*). Among these species, *H. taichui* was the highest prevalence (87.0%; 34,169/39,267 metacercariae) whereas *O. viverrini* was the lowest (0.2%; 89/39,267 metacercariae) (Fig 2). However, there were some unidentified metacercariae (7.5%; 2,932/39,267 metacercariae) due to the metacercariae being not yet developed and/or during the acid pepsin digestion.

The species of parasites isolated from cyprinoid fish are shown in Table 1, representing the total number of metacercariae and average number of metacercariae per fish. High density of *H. taichui*

was obtained from *P. leiacanthus, P. gonionotus* and *T. thynnoides* (181.7, 80.3 and 59.2 metacercariae per fish, respectively). *Haplorchis pumilio* metacercariae were found only in *D. regina* and *L. burmanicus*. *Centrocestus* spp metacercariae were mostly found in *P. gonionotus*. *Opisthorchis viverrini* metacercariae were found only in *P. orphoides, P. gonionotus, H. macrolepidota, L. burmanicus,* and *T. thynnoides*, with the highest intensity of 1.4 metacercariae per fish in *P. orphoides*. There was no trematode metacercariae found in *T. nilotica.*

Trematode metacercariae were found highest in winter (27,479 metacercariae in 208 fish) and lowest in rainy season (4,860 metacercariae in 389 fish) as shown in Table 2. *Haplorchis taichui* was the most metacercariae found all year-round, with the highest intensity being in winter as well as the occurrence of *O. viverrini* and *Centrocestus* spp. *Haplorchis pumilio* metacercariae were detected only in summer and rainy season but highest in summer.

DISCUSSION

In this present study, *H. taichui* is the most common metacercariae found in Ban Pao district of Chiang Mai Province with lesser incidence of *O. viverrini* metacercariae. This result was in accordance with the recent report of Srisawangwong *et al* (1997) in Khon Kaen Province. In addition, Khamboonruang *et al* (1997) has also reported the abundance of metacercariae of *Haplorchis* spp from cultured cyprinoid fish in Chiang Mai Province, unfortunately the species have not been identified. These results were contrary to the previous studies which revealed that most of metacercariae in cyprinoid fish from both Chiang Mai and Khon Kaen Provinces were those of *O. viverrini* (Sujjanun and Thitasut,1971; Komalamisra and Setasuban,1989). In this regard, the changes with respect to prevalence of trematode metacercariae in cyprinoid fish of both regions have already occurred. High prevalence of adult worm *H. taichui* in northern Thai people treated with praziquantel (Radomyos *et al*, 1998) suggested that the changes not only occurred in the fish second intermediate host but also in the definitive host.

Human behavior of eating raw and/or undercooked fresh water fish is existing according to the high prevalence of adult *H. taichui* in northern Thai people (Radomyos *et al,* 1998). In addition, the usage of sanitary latrines of rural people during working in their farms or paddy fields is impractical. Even

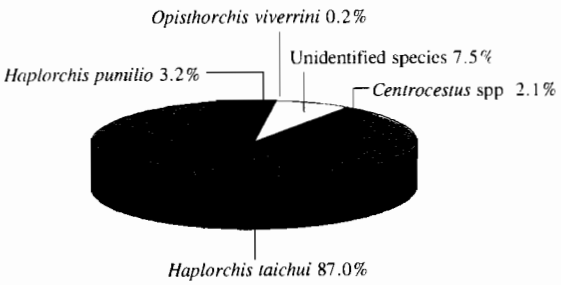

Fig 2–Percentage of trematode metacercariae found in cyprinoid fish collected from Ban Pao district, Chiang Mai Province.

though the people have latrines in their house; however, almost none of them is in paddy fields. In this regard, the disposal of fecal material without latrine usage makes the distribution of fish-borne trematodes more widespread.

The reason for changes of prevalence of both *O. viverrini* and *H. taichui* may be involved in several factors. In Thailand, three taxa of *Bithynia* snails have been reported as the sources of *O. viverrini* infection in different geographical habitats; *B. funiculata* in the north, *B. siamensis goniomphalus* in the northeast and *B. siamensis siamensis* in the central part (Chanawong and Waikagul, 1991). In Chiang Mai, *Thiara tuberculata* serves as the snail first intermediate host of *H. taichui* (Malek, 1980). Even though malacological survey has not been done in this investigation, however the survey in the Bhumipol reservoir, the large storage of water of Bhumipol Dam in Tak Province, has been previously reported (Fig 1). This dam was constructed across Ping River passed from the northern part through the central part of the country. *Bithynia siamensis goniomphalus*, the first intermediate host of *O. viverrini,* was not found in the area of Bhumipol reservoir and the Ping River in front of the Dam (Temcharoen, 1992). In contrast, *T. tuberculata*, the first intermediate host of *H. taichui,* was found in these areas. Since all of the natural water sources in this study are the branches of Ping River (Fig 1), it is possible that the distribution and habitat of snail first intermediate host of *H. taichui* are parallel along with Ping River through in front of the Bhumipol reservoir. Perhaps this might be one of the reasons concerning the high prevalence of *H. taichui* in Ban Pao district of Chiang Mai.

In addition to the snail first intermediate host, the existence of fish second intermediate host is also the important factor involved in prevalence of *O.*

Table 1
Occurrence of trematode metacercariae in cyprinoid fish collected from Ban Pao district, Chiang Mai Province, Thailand.

Fish species	No. of fish examined	Total number of metacercariae (average number of metacercariae per fish)					
		Haplorchis taichui	*Haplorchis pumilio*	*Opisthorchis viverrini*	*Centrocestus* spp	Unidentified	Total
Puntius leiacanthus	49	8,901 (181.65)	-	-	-	345 (7.04)	9,246 (188.69)
Puntius gonionotus	58	4,659 (80.33)	-	55 (0.95)	655 (11.29)	389 (6.71)	5,758 (99.28)
Thynnichthys thynnoides	302	17,864 (59.15)	-	6 (0.02)	69 (0.23)	1,257 (4.16)	19,196 (63.56)
Danio regina	151	2,150 (14.24)	1,264 (8.37)	-	74 (0.49)	777 (5.15)	4,265 (28.25)
Puntioplites proctozysron	34	410 (12.06)	-	-	4 (0.12)	97 (2.85)	511 (15.03)
Hampala macrolepidota	10	95 (9.50)	-	2 (0.20)	8 (0.80)	63 (6.30)	168 (16.80)
Aplocheilus panchax	4	32 (8.00)	-	-	-	2 (0.50)	34 (8.50)
Lariobarbus burmanicus	60	58 (0.97)	2 (0.03)	1 (0.02)	1 (0.02)	2 (0.03)	64 (1.07)
Puntius orphoides	18	-	-	25 (1.39)	-	-	25 (1.39)
Tilapia nilotica	13	-	-	-	-	-	-
Total	699	34,169 (48.88)	1,266 (1.81)	89 (0.13)	811 (1.16)	2,932 (4.19)	39,267 (56.18)

Table 2
Seasonal variation of trematode metacercariae in fish collected from reservoir and villages of Ban Pao district, Chiang Mai Province, Thailand.

Season	Location	No. of fish examined	Total number of metacercariae (average number of metacercariae per fish)					
			Haplorchis taichui	*Haplorchis pumilio*	*Opisthorchis viverrini*	*Centrocestus* spp	Unidentified	Total
Rainy	Reservoir	224	2,765 (12.34)	-	6 (0.03)	-	338 (1.51)	3,109 (13.88)
	Village	165	1,563 (9.47)	2 (0.01)	26 (0.16)	1 (0.01)	159 (0.96)	1,751 (10.61)
	Total	389	4,328 (11.126)	2 (0.005)	32 (0.082)	1 (0.003)	497 (1.278)	4,860 (12.49)
Winter	Reservoir	158	21,430 (135.63)	-	-	55 (0.35)	971 (6.15)	22,456 (142.13)
	Village	50	3,983 (79.66)	-	55 (1.10)	655 (13.30)	330 (6.60)	5,023 (100.46)
	Total	208	25,413 (122.178)	-	55 (0.264)	710 (3.413)	1,301 (6.255)	27,479 (132.11)
Summer	Reservoir	84	2,758 (32.83)	-	2 (0.02)	26 (0.31)	467 (5.56)	3,253 (38.73)
	Village	18	1,670 (92.78)	1,264 (70.22)	-	74 (4.11)	667 (37.06)	3,675 (204.17)
	Total	102	4,428 (43.412)	1,264 (12.392)	2 (0.019)	100 (0.980)	1,134 (11.118)	6,928 (67.92)
Total		699	34,169 (48.88)	1,266 (1.81)	89 (0.13)	811 (1.16)	2,932 (4.19)	39,267 (56.18)

viverrini and *H. taichui*. Regarding *O. viverrini* in this study, although the prevalence of metacercariae was highest in *P. orphoides*, but the number of metacercariae per fish (1.4) was very low (Table 1). The cyprinoid fish (*ie P. orphoides, P. gonionotus, H. macrolepidota, L. burmanicus*) containing *O. viverrini* were from the paddy fields in the villages, where snail of *Bithynia* spp were greatly found. It is obvious evidence from this study that, the distribution and habitat of both first and second intermediate hosts of parasites, *O. viverrini* in this case, must be related in their ecology in order to complet the parasitic life cycle.

The species of fish as the second intermediate host may be one of the factors involved. Different species harbored the different intensity of metacercariae (Table 1). Sithithaworn *et al* (1997) have also reported the different seasonal variation regarding intensity of *O. viverrini* metacercariae in different type of cyprinoid fish in northeast Thailand. Each species of fish has its own habitat and chance to be infected with cercariae. Fish living nearby the habitat of snail intermediate host may prone to be more infected.

Metacercariae of *H. taichui* found in the collection site of Chiang Mai were year-round, with the highest and lowest incidence rates being in winter and rainy season, respectively. For this reason, people

who still eat raw and/or undercooked fresh water fish have high chance to be infected anytime. The high incidence rate of *H. taichui* in winter may be the effect of the previous rain. In rainy season, water may flood the fecal material including eggs of parasite from the ground to the water resources. This parasite required more than 6 weeks in snail intermediate host to reach the cercarial stage and 2-3 weeks in fish to reach the metacercarial stage, respectively (Faust and Nishigori, 1926). Thus, approximately 2 months of the developmental time in these mentioned hosts correlated to the high incidence of metacercariae in fish collected in the next winter.

It could not be directly concluded that the prevalences of metacercariae of *H. taichui* and *O. viverrini* in the other places of Chiang Mai or elsewhere are the same pattern as in this study since the distribution of parasites varies from time to time and from place to place (Srisawangwong *et al*, 1997). Even within the same region, considerable variation in the prevalence of infection between communities was also recorded (Preuksaraj, 1984). Thus, mass survey of metacercariae in cyprinoid fish as well as malacological survey in total area of Chiang Mai Province is merit to further study.

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