

INTENSITY OF TREMATODE METACERCARIAE IN CYPRINOID FISH IN CHIANG MAL PROVINCE, NORTHERN THAILAND

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Abstract. The intensity of infection of trematode metacercariae was determined in five species of cyprinoid fish collected from Mae Ngud reservoir, Chiang Mai Province. These species were *Thynnichthys thynnoides*, *Puntioplites proctozysron*, *Hampala macrolepidota*, *Puntius leiacanthus* and *Puntius gonionotus*. *T. thynnoides* contained the highest number (83.0%) of metacercariae, whereas *P. gonionotus* had the fewest (0.19%). The caudal fin was the area of highest infection of metacercariae (49.39%), with the anal fin having the fewest (5.93%). Most metacercariae was isolated from *Haplorchis taichui* (63.27%), with an intensity variation of 0.3-165.2 metacercariae/fish.

INTRODUCTION

Trematode infections are the common helminthic infections in humans residing in northern Thailand (Radomyos *et al*, 1994; 1998; Pungpak *et al*, 1998). *Haplorchis taichui* (the heterophyid fluke of the Heterophyidae family) and *Opisthorchis viverrini* (the liver fluke of the Ophisthorchiidae family) were the most common flukes recovered from northern Thai patients treated with praziquantel. The prevalence of the former species found at 6 times more than that of the latter (Radomyos *et al*, 1998). Humans acquire fluke infection by ingesting raw and/or undercooked cyprinoid fish that harbor metacercariae. The most famous local fish dishes for Thai northerners are "Lab Pla" and "Pla Som" (WHO, 1995). The former dish is traditionally prepared by mixing the raw-chopped cyprinoid fish with spices, while the latter is a salted semi-fermented fish dish. The most common cyprinoid fish for both local dishes is *Thynnichthys thynnoides* (Pla Soy). Sukontason *et al* (1998) indicated that *H. taichui* was the only trematode metacercariae found in Lab Pla.

Recent fish surveys revealed that most trematode metacercariae were heterophyid flukes from several areas in northern Thailand and fish farms (Khamboonruang *et al*, 1997; Waikagul, 1998; Sukontason *et al*, 1999). The region where metacercariae infected cyprinoid fish varied according to the study area and species of fish (Komalamisra and Setasuban, 1989; Namue *et al*, 1998). Since there is no report concerning the distribution of metacercariae in northern Thai fish, the present investigation was undertaken to determine the intensity and distribution of trematode metacercariae in natural cyprinoid fish

collected from Mae Ngud reservoir in Chiang Mai. This area represents the most important source of fish for the inhabitants of the province.

MATERIALS AND METHODS

Cyprinoid fish were collected from Mae Ngud reservoir, Mae Tang district, Chiang Mai Province. Five species of fish (10 fish for each species) were investigated for trematode metacercariae: *Thynnichthys thynnoides*, *Puntioplites proctozysron*, *Hampala macrolepidota*, *Puntius leiacanthus* and *Puntius gonionotus*. The fish were rinsed using tap water until clean and the internal organs were removed. Each individual fish was dissected into 5 portions for determining the metacercariae (*ie* caudal fin and muscle, pectoral fin and muscle, ventral fin and muscle, dorsal fin and muscle, and anal fin and muscle). Each portion from the same kind of fish was pooled and then digested using acid pepsin solution [conc hydrochloric acid 1 ml: porcine pepsin (Sigma[®]: Germany) 1 g: 0.85% sodium chloride solution 99 ml] in a mixer/blender at a ratio of 1 g of fish: 10 ml acid pepsin solution. The digested material was transferred into a water bath shaker for 1 1/2 hours at 37°C and subsequently passed through 2 layers of wet gauze. The digested material was then rinsed with 0.85% sodium chloride solution and examined for metacercariae with a stereomicroscope. The identification of trematode metacercariae was carried out by the 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

A total of 3,136 metacercariae were isolated from the 5 species of cyprinoid fish examined (Table 1).

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Among them, *T. thynnoides* harbored the highest number (83.0%: 2,603/3,136), whereas, *P. gonionotus* harbored the lowest (0.19%: 6/3,136). For the overall metacercariae isolated, the caudal fin was the area with the highest infection (49.39%: 1,549/3,136), with the anal fin being the lowest (5.93%: 186/3,136) (Table 1).

Since *T. thynnoides* harbored the highest number of metacercariae (83.0%), this species of fish was selected for determining the species of trematode metacercariae. *Haplorchis* spp was the predominant metacercariae found (75.45%: 1,964/2,603), with *H. taichui* being the highest found (63.27%: 1,647/2,603) (Table 2). The highest and lowest number of *H. taichui* metacercariae were localized in the caudal (53.36%: 1,389/2,603) and anal fin (4.69%: 122/2,603), respectively.

The intensity of *H. taichui* metacercariae varied considerably in the cyprinoid fish examined, with those in *T. thynnoides*, *P. proctozystron*, *H. macrolepidota*, *P. leiakanthus* and *P. gonionotus* having 165.2, 21.7, 9.5, 4.5 and 0.3 metacercariae per fish, respectively (Table 3).

DISCUSSION

The intensity of heterophyid metacercariae in cyprinoid fish collected from Mae Ngud reservoir was quite high, particular for *H. taichui*. An unidentified *Haplorchis* species as well as degenerated metacercariae were also found. This is probably due to underdeveloped worm and/or damage to the specimens during the process of metacercariae

preparation. The metacercariae of *H. taichui* were isolated not only primarily from the *T. thynnoides* collected in this study, but also from *P. leiakanthus*, *P. gonionotus*, *P. proctozystron*, *H. macrolepidota*, *Danio regina*, *Aplocheilus panchax* and *Lariobarbus burmanicus* that were collected in other areas of Chiang Mai (Sukontason *et al*, 1999). According to Srisawangwong *et al* (1997), the metacercariae of *H. taichui* was predominant in 6 kinds of cyprinoid fish collected in Khon Kaen Province, northeastern Thailand, especially in *Hampala dispar*, which was similar to the study in Lao PDR (Ditrich *et al*, 1990). The study of Ooi *et al* (1997) in Taiwan also reported that 96.2% of *H. taichui* metacercariae were isolated from the freshwater fish, *Hemiculter leucisculus*. The metacercariae of *H. taichui*, therefore, showed broad ranges in host specificity, since it can live in several species of cyprinoid fish. In addition, the presence of this parasite in the definitive host also being found in several species of mammals (Faust and Nishigori, 1926; Pearson, 1964). For these reasons, this fluke is distributed easily in several geographical regions of Thailand, especially in the northeast and north. Due to the habit of people in the rural community of eating raw and/or improperly cooked freshwater fish, in combination with the broad ranges in host specificity, the occurrence of *H. taichui* is undoubtedly remains present in these regions of Thailand.

The present study showed that the areas most and least infected by metacercariae in fish were the caudal and anal fin, respectively, which resembled report by Tesana *et al* (1985) in that the distribution of *O. viverrini* metacercariae occurred abundantly in the caudal fin, but rarely in the anal fin. Vichasri *et al*

Table 1

Number of trematode metacercariae isolated from each separated organ of cyprinoid fish collected from Mae Ngud reservoir, Chiang Mai Province, northern Thailand.

Species of cyprinoid fish ^a	No. of metacercariae isolated					Total (%)
	Caudal fin	Pectoral fin	Ventral fin	Dorsal fin	Anal fin	
<i>Thynnichthys thynnoides</i>	1,389	521	408	163	122	2,603 (83.0)
<i>Puntioplites proctozystron</i>	34	79	19	65	44	241 (7.69)
<i>Hampala macrolepidota</i>	79	30	40	49	10	208 (6.63)
<i>Puntius leiakanthus</i>	44	4	6	14	10	78 (2.49)
<i>Puntius gonionotus</i>	3	-	1	2	-	6 (0.19)
Total	1,549	634	474	293	186	3,136
(%)	(49.39)	(20.22)	(15.12)	(9.34)	(5.93)	(100.0)

^aTen fish for each species

Table 2
Distribution of trematode metacercariae isolated from each separated organ of *Thynnichthys thynnoides* collected from Mae Ngud reservoir, Chiang Mai Province, northern Thailand.

Species of metacercariae	No. of metacercariae isolated					Total (%)
	Caudal fin	Pectoral fin	Ventral fin	Dorsal fin	Anal fin	
<i>Haplorchis</i> spp	1,028	396	309	144	87	1,964 (75.45)
- <i>H. taichui</i>	838	350	260	125	74	1,647 (63.27)
- Unidentified <i>Haplorchis</i> spp	190	46	49	19	13	317 (12.18)
Degenerated metacercariae	361	125	99	19	35	639 (24.55)
Total	1,389	521	408	163	122	2,603
(%)	(53.36)	(20.02)	(15.67)	(6.26)	(4.69)	(100.00)

Table 3
Intensity of *Haplorchis taichui* metacercariae in cyprinoid fish collected from Mae Ngud reservoir, Chiang Mai Province, northern Thailand.

Species of cyprinoid fish	No. fish examined	Intensity ^a
<i>Thynnichthys thynnoides</i>	10 (302)	165.2 (59.2)
<i>Puntioplites proctozysron</i>	6 (34)	21.7 (12.1)
<i>Hampala macrolepidota</i>	10 (10)	9.5 (9.5)
<i>Puntius leiacanthus</i>	8 (49)	4.5 (181.7)
<i>Puntius gonionotus</i>	20 (58)	0.3 (80.3)

^aIntensity is given as the mean number of metacercariae per infected fish.

Number in parentheses referred to Sukontason *et al* (1999).

(1982) also reported that the anal fin was the least infected area of *O. viverrini* metacercariae. Moreover, the metacercariae of *H. taichui* was observed mostly in the tail region of fish (Ooi *et al*, 1997). Since fish use the caudal fin in swimming more vigorous than other fins and the attachment of the fish host is stimulated by water current (Haas *et al*, 1990), these may be the reasons why cercariae can recognize and attach to the caudal fin more than others sites. Nevertheless, the site of attachment of *Haplorchis* metacercariae in this study was in contrast to the scales of fish reported by Namue *et al* (1998).

This study showed a higher intensity of *H. taichui* in Chiang Mai Province than that of *O. viverrini* which was in accordance with recent studies in Chiang Mai (Poolpol, 1995; Sukontason *et al*, 1999) and Khon Kaen Province (Srisawangwong *et al*, 1997). However, it was in contrast to those of Sujjanun and Thitasut (1971) in Chiang Mai and Komalamisra and Setasuban (1989) in Khon Kaen. Waikagul (1998) suggested that *O. viverrini* metacercariae was very high in the northeast region of Thailand, particularly in those reports before 1990, which contrasted to *H. taichui*. Up until now, the situation in both regions of Thailand regarding the intensity of *O. viverrini* and *H. taichui* has changed. Several factors may be involved in this change such as the existence of the first intermediate snail host, the second intermediate host of fish as well as the definitive host, usage of sanitary latrines, custom of eating raw and/or undercooked fresh water fish and the presence of infected reservoir hosts. Efforts should be made to determine those factors involving parasitic transmission in order to clarify the cycle of the parasite and eventually prevent humans from acquiring trematode infection.

REFERENCES

- Ditrich O, Scholz T, Giboda M. Occurrence of some medically important flukes (Trematoda: Opisthorchiidae and Heterophyidae) in Nam Ngum water reservoir, Laos. *Southeast Asian J Trop Med Public Health* 1990;21:482-8.
- Faust EC, Nishigori M. The life cycles of two new species of heterophyidae, parasitic in mammals and birds. *J Parasitol* 1926;13:91-132.
- Haas W, Granzer M, Brockelman CR. *Opisthorchis viverrini*: Finding and recognition of the fish host

- by the cercariae. *Exp Parasitol* 1990;71:422-31.
- Khamboonruang C, Kaewvichit R, Wongworapat K, *et al.* Application of hazard analysis critical control point (HACCP) as a possible control measure for *Opisthorchis viverrini* infection in cultured carp (*Puntius gonionotus*). *Southeast Asian J Trop Med Public Health* 1997;28:65-72.
- Komalamisra C, Setasuban P. Heterophyid flukes and *Opisthorchis viverrini*: intensity and rates of infection in cyprinoid fish from an endemic focus in northeast Thailand. *J Trop Med Parasitol* 1989;12:22-8.
- Namue C, Rojanapaibul A, Wongsawad C. Occurrence of two heterophyid metacercariae *Haplorchis* and *Haplorchoides* in cyprinoid fish of some districts in Chiang Mai and Lumphun province. *Southeast Asian J Trop Med Public Health* 1998;29:401-5.
- Ooi HK, Chen CI, Lin SC, Tung KC, Wang IS, Kamiya M. Metacercariae in fishes of Sun Moon Lake which is an endemic area for *Clonorchis sinensis* in Taiwan. *Southeast Asian J Trop Med Public Health* 1997;28(suppl1):222-3.
- Pearson JC. A revision of the subfamily Haplorchinae Looss, 1899 (Trematoda: Heterophyidae). *Parasitology* 1964;54:601-76.
- Pearson JC, Ow-Yang CK. New species of *Haplorchis* from Southeast Asia, together with keys to the *Haplorchis*-group of heterophyid trematodes of the region. *Southeast Asian J Trop Med Public Health* 1982;13:35-60.
- Poolpol P. A survey of the occurrence of *Opisthorchis viverrini* metacercariae in freshwater fish and raw fish products in Chiang Mai Province. Graduate School, Chiang Mai University, 1995. MSc Thesis.
- Pungpak S, Radomyos P, Radomyos B, Schelp FP, Jongsuksuntikul P, Bunnag D. Treatment of *Opisthorchis viverrini* and intestinal fluke infections with praziquantel. *Southeast Asian J Trop Med Public Health* 1998;29:246-9.
- Radomyos P, Radomyos B, Tungtrongchitr A. Multi-infection with helminths in adults from northeast Thailand as determined by post-treatment fecal examination of adult worms. *Trop Med Parasitol* 1994;45:133-5.
- Radomyos B, Wongsaraj T, Wilairatana P, *et al.* Opisthorchiasis and intestinal fluke infections in northern Thailand. *Southeast Asian J Trop Med Public Health* 1998;29:123-7.
- Scholz T, Ditrich O, Giboda M. Differential diagnosis of opisthorchid and heterophyid metacercariae (Trematoda) infection of fresh cyprinoid fish from Nam Ngum Lake in Laos. *Southeast Asian J Trop Med Public Health* 1991;22:171-3.
- Srisawangwong T, Sithithaworn P, Tesana S. Metacercariae isolated from cyprinoid fishes in Khon Kaen district by digestion technic. *Southeast Asian J Trop Med Public Health* 1997;28(suppl 1):224-6.
- Sujjanun A, Thitasut P. Studies on metacercariae of *Opisthorchis* spp in Chiang Mai, Thailand. *Bull Chiang Mai Med Tech* 1971;4:113-23.
- Sukontason K, Methanitikorn R, Sukontason K, Piangjai S, Choochote W. Viability of metacercariae in northern Thai traditional foods. *Southeast Asian J Trop Med Public Health* 1998;29:714-6.
- Sukontason K, Piangjai S, Muangyimpong Y, Sukontason K, Methanitikorn R, Chaithong U. Prevalence of trematode metacercariae in cyprinoid fish of Ban Pao District, Chiang Mai Province, northern Thailand. *Southeast Asian J Trop Med Public Health* 1999;30:365-70.
- Tesana S, Kaewkes S, Srisawangwong T, Phinlaor S. Distribution and density of *Opisthorchis viverrini* metacercariae in cyprinoid fish from Khon Kaen province. *J Parasit Trop Med Assoc Thai* 1985;8:36-9.
- Vichasri S, Viyanant V, Upatham ES. *Opisthorchis viverrini*: intensity and rates of infection in cyprinoid fish from an endemic focus in northeast Thailand. *Southeast Asian J Trop Med Public Health* 1982;13:138-41.
- Waikagul J. *Opisthorchis viverrini* metacercariae in Thai freshwater fish. *Southeast Asian J Trop Med Public Health* 1998;29:324-6.
- World Health Organization. Control of foodborne trematode infections. Report of a WHO Expert Committee. *WHO Tech Rep Ser* 1995; 849: 157 pp.