

HIGH PREVALENCE OF *HAPLORCHIS TAICHUI* METACERCARIAE IN CYPRINOID FISH FROM CHIANG MAI PROVINCE, THAILAND

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Abstract. This study aimed to investigate *Haplorchis taichui* metacercarial infection in fish collected from the Chom Thong and Mae Taeng districts, Chiang Mai Province during November 2001 to October 2002. A total 617 cyprinoid fish of 15 species were randomly collected and examined for *H. taichui* metacercariae. All the species of fish were found to be infected with *H. taichui*. The infection rates were 91.4% (266/290) and 83.8% (274/327), with mean intensities of 242.9 and 107.4 in the Chom Thong and Mae Taeng districts, respectively. The portion of the fish body with the highest metacercarial density was the muscles, and second, the head, in both districts. In addition, the fish had mixed-infection with other species of trematodes, namely: *Centrocestus caninus*, *Haplorchoides* sp., and *Haplorchis pumilio*.

INTRODUCTION

In Thailand, fish-borne trematode infections have been commonly found in the northeastern and northern regions, including Chiang Mai Province (Maning *et al*, 1971; Kliks and Tanta-chamrun, 1974; Pungpak *et al*, 1998; Radomyos *et al*, 1998). *Haplorchis taichui* is one of the intestinal trematodes of the family Heterophyidae. The adult fluke is able to develop in the small intestine of birds and mammals, including humans (Faust and Nishigori, 1926; Yamaguti, 1958; Cheng, 1974). This worm has been reported as the predominant species of intestinal flukes in the northern region of Thailand (Pungpak *et al*, 1998; Radomyos *et al*, 1998). Humans acquire the infections from eating raw or undercooked freshwater fish containing the metacercaria of *H. taichui*. Cyprinoid fish have been reported as an important intermediate host of this fluke (Srisawangwong *et al*, 1997; Wai-kagul, 1998; Sukontason *et al*, 1999; Wongsawad *et al*, 2000).

In *H. taichui*, there is no documented re-

port of severe pathogenicity, as is seen in the liver or lung flukes. It is known that heterophyid flukes irritate the intestinal mucosa and cause colicky pain and mucousy diarrhea, with the production of excess amounts of mucus and superficial necrosis of the mucus coat (Beaver *et al*, 1984; Chai and Lee, 2002). Moreover, the eggs of *Haplorchis* species can be deposited in the spinal cord, brain, and cardiac valves of man (Africa *et al*, 1935, 1936, 1937a, b).

The present study aims to demonstrate the prevalence and intensity of *H. taichui* metacercarial infection in several species of cyprinoid fish from Chom Thong and Mae Taeng districts, Chiang Mai Province. These areas have many kinds of cyprinoid fish that are an important source of food and commerce for the local people.

MATERIALS AND METHODS

Cyprinoid fish, the common intermediate host for *Haplorchis taichui*, were collected from Wangpan Dam, Chom Thong district and Mae Ngad Reservoir, Mae Taeng district in Chiang Mai Province. A year-round examination for metacercaria-parasitizing fish was performed monthly, from November 2001 to October 2002. Fish were obtained from the local fishermen, placed in ice

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boxes, and then transported to the laboratory. Each fish was separated into 5 parts: head, gill, scales, fin rays (all the fins: dorsal, pectoral, pelvic, anal and caudal) and muscles of the body. Metacercarial cysts were isolated from individual fish by 1% acid pepsin solution for 2 hours at 37°C. The digested material was passed through graded sieves and rinsed with 0.85% NaCl, then examined for the metacercariae under a stereomicroscope. The number of metacercariae were recorded. The data were analyzed by ANOVA. Some specimens of encysted metacercariae were excysted by 100% pineapple juice for 3 hours at 37°C. Encysted and excysted flukes were fixed in 5% formalin under a cover glass, then stained with borax carmine or hematoxylin, counterstained with eosine and fast-green, dehydrated in an alcohol series, cleared in xylol, and mounted in permount. The encysted and excysted metacercariae from living specimens and permanent slides were morphologically identified based on Yamaguti (1958), Pearson (1964) and Pearson and Ow-Yang (1982).

RESULTS

Six hundred and seventeen fish of 15 species were investigated for *Haplorchis taichui* metacercariae. The results show that 540 of 617

fish were infected with *H. taichui* and other heterophyid metacercariae. Among the fish collected, *Henicorhynchus siamensis* was the most predominant (24.0%; 148/617) while 2 species of fish were found in the smallest numbers, namely, *Amblyrhynchichthys truncatus* and *Cyclocheilichthys armatus* (0.3%; 2/617) (Tables 1, 2).

Four species of heterophyid metacercariae were found in cyprinoid fishes from both locations; *Centrocestus caninus*, *Haplorchoides* sp, *H. pumilio* and *H. taichui*. The most abundant species was *H. taichui*, which was found in all the species of fish examined. The results show that *Haplorchoides* sp infected large numbers of fish, and was predominant in some species of fish, while *C. caninus* and *H. pumilio* were presented in small numbers (Tables 1, 2).

The infection rates of *H. taichui* in Chom Thong and Mae Taeng districts for the year-round survey are shown in Fig 1. In Chom Thong district, 266 of 290 fish in 14 species harbored metacercariae of *H. taichui* (Table 1). The infection rate for *H. taichui* was 91.7% (266/290), and the monthly prevalence of *H. taichui* in all fish was 56-100% with a mean intensity of 242.9 metacercariae per fish. The highest prevalences were found in January, February, May, July, and

Table 1
The distribution of *Haplorchis taichui* metacercaria and other heterophyid metacercariae found in 14 species of fish from Chom Thong district.

Fish species	Number of fish Infected/ examined	Number of metacercariae, mean (min-max/fish)			
		<i>Haplorchis taichui</i>	<i>Haplorchis pumilio</i>	<i>Haplorchoides sp</i>	<i>Centrocestus caninus</i>
<i>Amblyrhynchichthys truncatus</i>	2/2	29.50 (23-36)	0	197.50 (140-255)	0
<i>Barbodes gonionotus</i>	25/25	192.72 (0-814)	0.16 (0-4)	184.56 (0-1,629)	2.44 (0-17)
<i>Barbodes schwanenfeldi</i>	13/13	109.0 (0-613)	0	28.38 (0-122)	0
<i>Cyclocheilichthys armatus</i>	2/2	76.50 (34-119)	0	352.50 (261-444)	0
<i>Cyclocheilichthys repasson</i>	13/13	11.54 (0-26)	0	617.54 (70-2,054)	0
<i>Henicorhynchus siamensis</i>	91/93	391.57 (0-2,248)	0.12 (0-4)	12.19 (0-102)	0.72 (0-15)
<i>Labiobarbus siamensis</i>	33/34	68.97 (0-327)	0	41.03 (0-184)	0.18 (0-3)
<i>Mystacoleucus marginatus</i>	37/37	532.78 (8-3,165)	0	1,055.68 (52-2,872)	0.24 (0-5)
<i>Osteochilus hasselti</i>	3/5	2.60 (0-9)	0	0	0
<i>Paralaubuca barroni</i>	8/8	16.75 (2-44)	0	0	0
<i>Puntioplites proctozysron</i>	37/42	27.0 (0-180)	0	97.12 (0-644)	0.67 (0-8)
<i>Raiamas guttatus</i>	3/3	990.0 (648-1,356)	0	0	0
<i>Rasbora tornieri</i>	2/3	0.67 (0-2)	0	13.67 (0-23)	0
<i>Systemus orphoides</i>	10/10	112.70 (0-264)	0	227.60 (42-639)	1.60 (0-7)
Total	266/290	242.93 (0-3,165)	0.05 (0-4)	214.12 (0-2,872)	0.64 (0-17)

Table 2

The distribution of *Haplorchis taichui* metacercaria and other heterophyid metacercariae found in 7 species of fish from Mae Taeng district.

Fish species	Number of fish Infected/ examined	Number of metacercariae, mean (min-max/fish)			
		<i>Haplorchis taichui</i>	<i>Haplorchis pumilio</i>	<i>Haplorchoides</i> sp	<i>Centrocestus caninus</i>
<i>Barbodes gonionotus</i>	44/55	30.42 (0-496)	0	8.62 (0-127)	1.13 (0-17)
<i>Hampala macrolepidota</i>	37/60	23.20 (0-447)	0	12.18(0-70)	0.08 (0-3)
<i>Henicorhynchus siamensis</i>	57/60	403.87 (0-1,731)	0.05 (0-2)	9.23 (0-197)	0.38 (0-7)
<i>Labiobarbus siamensis</i>	46/48	29.65 (0-191)	0.02 (0-1)	23.48 (0-135)	0.14 (0-7)
<i>Mystacoleucus marginatus</i>	18/18	289.33 (2-2,131)	0	387.72 (15-1,492)	0.11 (0-2)
<i>Puntioplites proctozysron</i>	55/60	15.48 (0-126)	0	24.80 (0-148)	0.17 (0-6)
<i>Systomus orphoides</i>	17/26	10.58 (0-49)	0	28.80 (0-179)	0
Total	274/327	107.44 (0-2,131)	0.01 (0-2)	37.01 (0-1,492)	0.35 (0-17)

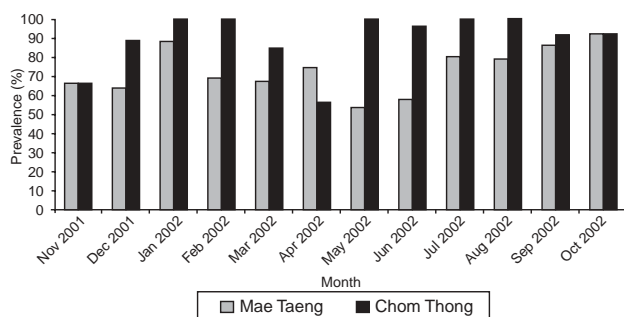


Fig 1—Monthly infection rate of *H. taichui* metacercariae in 15 species of cyprinoid fish in Chom Thong and Mae Taeng districts, Chiang Mai.

August and the lowest in April. The prevalence of infection did not differ significantly between the three seasons (cool=November-February, summer=March-June, rainy=July-October) ($p > 0.05$). In Mae Taeng district, 274 of 327 fish in 7 species harbored *H. taichui* metacercariae. The infection rate for *H. taichui* was 83.8% (274/327), and the monthly prevalence was 54.2-93.1%, with a mean intensity of 107.4 metacercariae per fish. The highest prevalence was found in October and the lowest in May. The prevalences were compared between the three seasons. Significant differences were found during the summer season (March-June) and rainy season (July-October) ($p < 0.05$). Our study showed that, in both locations, the muscles had the highest number of *H. taichui* metacercaria, followed by the head, fins, scales, and finally the gills. The numbers of *H. taichui* metacercariae were significantly differ-

ent in the muscles, head, and fins of parasitised fish ($p < 0.05$), but in the scales and gills, there was no significant difference ($p > 0.05$) (Fig 2A, B).

DISCUSSION

Haplorchis taichui infections are commonly found in the northern and northeastern regions of Thailand. Several species of cyprinoid fish have been reported as the second intermediate hosts (Kliks and Tantachamrun, 1974; Srisawangwong *et al*, 1997; Namue *et al*, 1998; Waikagul, 1998; Sukontason *et al*, 1999; Wongsawad *et al*, 2000). In our study, 15 species of cyprinoid fish were found infected with *H. taichui* and other heterophyid metacercariae, namely *C. caninus*, *H. pumilio* and *Haplorchoides* sp. Among the metacercariae, *H. taichui* had the highest number found throughout the year-round survey. *Haplorchoides* sp was found in mixed-infections and was predominantly found in several species of fish. This species is not a human parasite and adult worms parasitize the intestines of catfish (Waikagul, 1998). From our study, *H. taichui* metacercariae were mainly recovered from the muscles of fish. A previous study had found them mainly in the caudal fin (Sukontason *et al*, 2001). The metacercariae were predominantly localized to the muscles in both districts, while the lowest numbers tended to be the gills. *H. taichui* metacercariae were also commonly found in the head of fish in a large number. The distribution of the infective stage of this fluke was mostly found in the muscles. It is not surprising

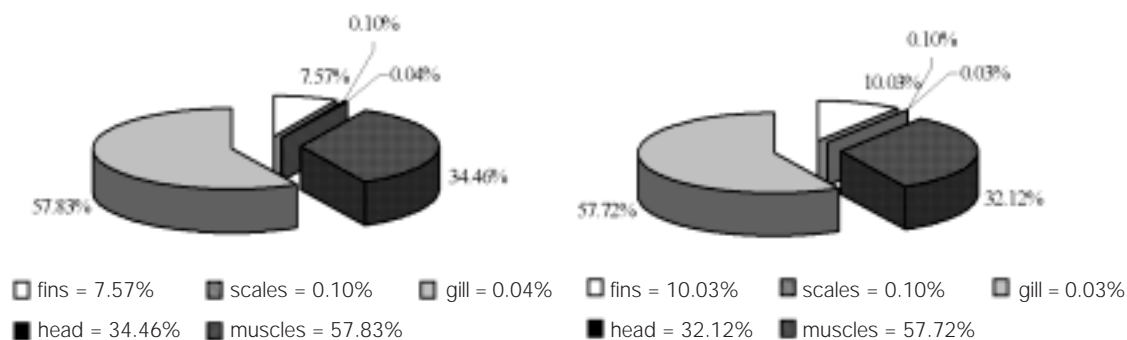


Fig 2—Occurrence of *H. taichui* metacercariae in various parts of the fish body from both locations, A) Chom Thong district and B) Mae Taeng district.

to find them in the muscles at the base of fins, especially the caudal fin, because fish use the caudal fin for swimming more than the other fins, and the water current can enhance the parasite's attachment (Haas *et al*, 1990; Sukontason *et al*, 2001). Infection transmission can be decreased when eating raw fish dishes by avoiding the heavy infected areas, especially the head and the muscles at the base of the fins.

The minute intestinal fluke's egg is similar in shape and size to that of the liver fluke, *Opisthorchis viverrini* (Manning *et al*, 1971; Tesana *et al*, 1991; Radomyos *et al*, 1998). It is difficult to identify species from fecal eggs. Our findings contrast to previous studies, which revealed that most of the eggs found in man were *O. viverrini*, while none were *H. taichui* (Chiang Mai Provincial Public Health Office, 2001). However, investigations of *O. viverrini* metacercariae in the second intermediate host revealed a low infection rate (Ratanasritong and Kliks, 1974; Poolphol, 1995; Sukontason *et al*, 1999). Our results are similar to previous reports in that *H. taichui* had the highest incidence of infection in the northerners (Pungpak *et al*, 1998; Radomyos *et al*, 1998). The distribution of food-borne trematodes depends on many factors, such as the relationship between the host, parasite, environment, and traditional food habits (WHO, 1995). Humans are not the only source of heterophyid fluke egg. Fish-eating animals can also be involved in their life cycle (Faust and Nishigori, 1926; Pearson 1964; Ditrich *et al*, 1990).

In our study, *H. taichui* showed a broad range for host specificity because it can infect several species of cyprinoid fish. Food-borne

trematodes can be assessed by the prevalence of infections. Chom Thong district showed the highest prevalence and intensity of metacercarial infection in fish. It is possible that this area is one of the high-risk areas in Chiang Mai Province. In addition, Thai traditional dishes, such as 'blah-sohm' and 'lahp-blah', are believed to be a sources of infection in people. These raw-fish dishes cannot induce degeneration of the contaminated metacercariae in a short period (Sukontason *et al*, 1998; Wiwanitkit *et al*, 2003), and there is a tendency that the number of worms infecting humans will increase because of acquiring the infection from eating raw-fish dishes.

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