OCCURRENCE OF TWO HETEROPHYID METACERCARIAE *HAPLORCHIS* AND *HAPLORCHOIDES* IN CYPRINOID FISH OF SOME DISTRICTS IN CHIANG MAI AND LUMPHUN PROVINCE

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Abstract. According to currently available information, the metacercaria of one heterophyid trematode, *Haplorchis*, is commonly found in freshwater fishes, especially the cyprinoid group. Thus, in an investigation into the level of *Haplorchis* metacercaria infection in cyprinoid fishes, comprising ten species, 811 cyprinoid fishes were captured from canals, reservoirs and rivers in six districts of Chiang Mai Province and 4 districts of Lumphun Province, Thailand, 43.03 % of the fishes comprising seven species viz *Puntius leiacanthus, P. gonionotus, P. orphoides, P. stolickkae, Cirrhina* spp, *Rasbora* spp and *R. lateristriata,* were found infected, with the prevalence of infection at 77.29, 70.31, 56.52, 47.14, 18.48, 7.94 and 5.18 % respectively. Infected fishes were collected from Mae Rim and Sansai districts of Chiang Mai, and Pasang, Ban Hong and Li districts of Lumphun. It should be noted that the *Haplorchis* metacercariae are commonly found along with *Haplorchoides* metacercariae. Both minute heterophyids metacercariae were mainly localized in scales, a few were found in fins and gills of fishes.

INTRODUCTION

Trematodes in the genus *Haplorchis* of the family Heterophyidae are found in the small intestines of various definitive hosts such as birds, cats, dogs and rats (Yamaguti, 1958; Pearson, 1964; Cheng, 1974). These small flukes are able to develop to maturity in man. Human or another definitive hosts are infected by eating raw freshwater fishes containing encysted metacercariae. In Thailand, adult *Haplorchis yokogawai, H. pumillio* and *H. taichui* have been reported to occur in human population from the northeast (Manning *et al,* 1971; Radomyos *et al,* 1983). Due to the similarity in both morphology and size of the eggs of the liver fluke, opisthorchid and heterophyid flukes, most minute trematode eggs appearing in stool have been diagnosed as liver flukes (Manning *et al,* 1971; Tesana *et al,* 1991). From the results of stool examinations, Chiang Mai and Lumphun Provinces of northern Thailand have been reported as endemic areas of the liver fluke, *Opisthorchis viverrini* (Thitasut *et al,* 1973; Keawvichit *et al,* 1993; Pornpibool *et al,* 1993). However, investigations of *O. viverrini* metacercariae in the second intermediate host, freshwater fish, found low rates of infection. (Ratanasri- tong and Kliks, 1974; Eusang, 1979; Poolphol, 1995). To confirm helminth species, surveys for larval stages in the intermediate hosts or adults in some definitive hosts should be done (Ditrich *et al,* 1990). Thus, our present study was to determine the prevalence of *Haplorchis* metacercariae in the second intermediate hosts.

MATERIALS AND METHODS

The investigation was carried out from October 1995 to January 1997. Cyprinoid fishes were caught by seine from natural habitats (canal, river and reservoir) in six districts of Chiang Mai Province: Doi Saket, Hang Dong, San Khamphaeng, Mae Rim, San Pa Tong, and San Sai, and four districts of Lumphun Province: Pa Sang, Ban Hong, Li and Muang Lamphun. Some specimens were obtained from local fishermen. The fish were individually examined: their scales, fins, gills and visceral organs were observed under stereo microscope for *Haplorchis* metacercariae. The encysted metacercariae found were studied and identified alive together with excysted metacercariae under light microscopy. The morphological description for identification was based on the descriptions given by Pearson (1964), Kliks and Tantachamrun (1974), and Pande and Shukla (1976). Some encysted and excysted metacercariae specimens were fixed in 4% formalin, stained with borax carmine, dehydrated and mounted in permount. Some were preserved in 70% alcohol.

RESULTS

811 cyprinoid fishes from ten species were collected and examined. 349 specimens from seven fish species were found to be infected with the *Haplorchis* metacercariae (43.0 %). Infection rates

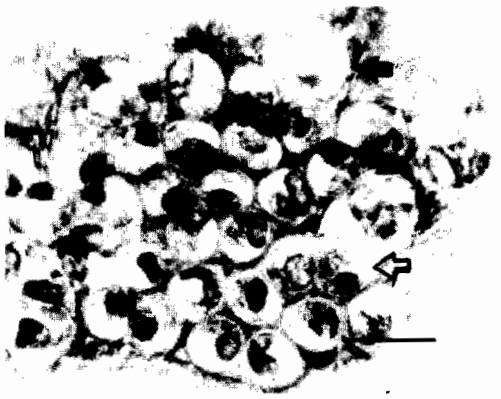

Fig 1–*Haplorchis* (⇨) and Haplorchoides (➡) metacercariae on the fish scale (bar = 0.2 mm).

in 4 cyprinoid fishes belonging to the genus *Puntius: P. leiacanthus, P. gonionotus, P. orphoides* and *P. stotickkae,* were high (47.1%-77. 3 %). Lower infection rates (5.2 - 18.5 %) were found in three

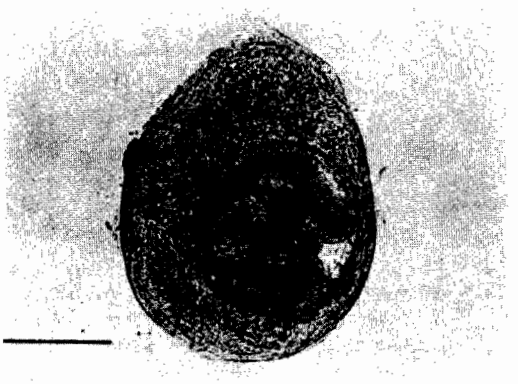

Fig 2–*Haplorchoides* metacercaria recovered from the intestinal wall of fish (bar = 0.2 mm).

species of fishes: *Cirrhinus* spp, *Rasbora* spp and *R. lateristriata.* None of the *Hampala macrolepidota* (n=1), *H. dispur* (n=2) and *Probabus jullieni* (n=1) were infected. The *Haplorchis* metacercariae were frequently found together with *Haplorchoides* metacercariae. Both metacercariae were mainly localized on scales (Fig 1) and few appeared in fins and gills. A number of Haplorchoides metacercariae were found on the intesinal wall of fishes (Fig 2). The total prevalence of *Haplorchis* metacercariae infected in cyprinoid fishes are shown in Table 1.

Some fishes captured from two districts of

Table 1

Total prevalence of *Haplorchis* metacercarial infection in cyprinoid fishes captured from six districts in Chiang Mai and four districts in Lumphun provinces.

Fish species (%)	No. examined	No. infected	Total prevalence
Cirrhinus sp	92	17	18.5
Hampula macrolepidota	2	0	-
H. dispur	2	0	-
Probabus jullieni	1	0	-
Puntius gonionotus	64	45	70.3
P. leiacanthus	273	211	77.3
P. orphoides	46	26	56.5
P. stolickkae	70	33	47.1
Rasbora lateristriata	135	7	5.2
Rasbora spp	126	10	7.9
Total	811	349	43.0

Table 2

Prevalence of *Haplorchis* occurring in each species of cyprinoid fishes from six districts in Chiang Mai.

Districts	Fish species	No. examined	No. infected	Prevalence (%)
Doi Saket	*Cirrhina* spp	4	0	-
	P. gonionotus	9	0	-
	Rasbora spp	9	0	-
Hang Dong	*P. gonionotus*	9	0	-
	Rasbora spp	13	0	-
Mae Rim	*Cirrhina* spp	28	7	25
	H. dispur	2	0	-
	P. gonionotus	6	5	83.3
	P. leiacanthus	130	100	76.9
	P. orphoides	32	19	59.4
	P. stolickkae	70	33	47.1
	R. lateristriata	135	7	5.2
	Rasbora spp	20	8	40
San Khamphang				
	Pr. jullieni	1	0	-
	P. leiacanthus	10	0	-
	Rasbora spp	10	0	-
San Patong	*Rasbora* spp	14	0	-
San Sai	*P. leiacanthus*	36	30	83.3
	Rasbora spp	7	0	-

Table 3

Prevalence of *Haplorchis* occurring in each species of cyprinoid fishes from four districts in Lumphun.

Districts	Fish species	No. examined	No. infected	Prevalence (%)
Pa Sang	*Cirrhina* spp	19	2	10.5
	P. gonionotus	20	20	100
	P. leiacanthus	44	37	84.1
	P. orphoides	6	2	33.3
	Rasbora spp	2	8	16.7
Ban Hong	*Cirrhina* spp	22	8	36.4
	H. macrolepidota	1	0	-
	P. gonionotus	20	20	100
	P. leiacanthus	26	26	100
	P. orphoides	8	5	62.5
	Rasbora spp	10	0	-
Li	*Cirrhina* spp	19	0	-
	H. macrolepidota	1	0	-
	P. leiacanthus	27	18	66.7
	Rasbora spp	2	0	-
Muang Lumphun	*Rasbora* spp	29	0	-

Chiang Mai Province: Mae Rim and Sansai, were infected with metacercariae. In Mae Rim district eight fish species were captured and seven species found infected, and in Sansai district only one species, *P. leiacanthus* was infected with *Haplorchis* metacercaria. In Lumphun Province, the infection rates were high in fishes from Pa Sang, Ban Hong and Li districts. The metacercarial infection rates in *P. leiacanthus* in all three districts were high (66.7-100 %). A summary fish species, the number examined, the number fishes infected and prevalence of infection in each district are shown in Tables 2, 3.

DISCUSSION

As the results show, there is a high prevalence of *Haplorchis* metacercarial infection in cyprinoid fishes. Only three fish species, *H. macrolepidota, H. dispur* and *P. jullieni,* were not infected. This might be due to the small number examined. In Chiang Mai, Kliks and Tantachamrun (1974) have reported the metacercarie of *H. taichui* and *H. yokogawai* from *P. leiacanthus, P. gonionotus* and *P. orphoides,* and adults in domestic cats. Eusang (1979) reported two types of *Haplorchis* metacercariae, which resembles *H. taichui* and *H. yokogawai,* from *P. gonionotus* and *Cycloclilichthys* apogon, respectively. Metacercariae reported were mainly from muscle, oral cavity, fins and scales. Ditrich *et al* (1990) reported infections of *H. pumilio* and *H. taichui* metacercariae in the flesh of *Hampala dispur, H. macrolepidota* and *P. gonionotus* from Nam Ngum water reservoir of Lao PDR. In this investigation, most of the metacercariae were found in the scales.

It should be noted that the *Haplorchis* metacercariae are commonly found with *Haplorchoides* metacercariae. Both minute heterophyid metacercariae inhabitated together on the scales. Both metacercariae are similar in shape and morphology, but *Haplorchoides* is slightly larger. Some *Haplorchoides* metacercariae are attached to the visceral organs, *eg* intestinal wall. In this case, *Haplorchoides* cysts was easily distinguished from *Haplorchis* cysts, Pande and Shukla (1976) reported three species of *Haplorchoides* and recorded the metacercariae from fins, muscle, eye muscle and gills of 12 species of fresh water fish, mainly from the family Cyprinidae. The specimens of *Haplorchoides* were confirmed by Kaewkes, Department of Parasitology, Faculty of Medicine, Khon Kaen University.

Because the egg of the small intestinal flukes, *ie Phaneropsolus bonnei, Prosthodendrium molenkampi, H. pumillio, H. taichui* and *H. yokogawai,* are similar in shape and size to the liver fluke, *O. viverrini* (Manning *et al,* 1971; Tesana *et al,* 1991). Many heterophyid infections have been assigned to the liver flukes (Kliks and Tantachamrun, 1974). Stool examination in Chiang Mai Province have reported a high prevalence of *O. viverini* (Thitasut *et al,* 1973; Keawvichit *et al,* 1993; Pornpibool *et al,* 1993). However, low rates infection of *O. viverrini* metacercariae in fishes were previously reported (Ratanasritong and Kliks, 1972; Eusang, 1979). Recent research also showed the low rate of infection of *O. viverrini* (0.6 - 5 %) in cyprinoid fishes from different habitats and in rawfish products from six districts of Chiang Mai, and the high prevalence of *Haplorchis* metacercariae infection in both a natural habitats (98.1 %) and in cultured fish farms (68.7 %) (Poolphol, 1995).

As with previous studies and the present study found that most cyprinoid fishes were infected with *Haplorchis* metacercariae, implying that the degree of infection of the first intermediate host and definitive hosts would be high in the study area. The ova of the small flukes which appeared in the human stool examination in this area could be due to infection from the *Haplorchis* group instead of the liver flukes, *O. viverrini.*

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