THE OCCURRENCE OF HETEROPHYID METACERCARIAE IN CYPRINOID FISH IN CHIANG MAI PROVINCE

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Abstract. The occurrence of the heterophyid metacercariae in cyprinoid fish from 10 districts of Chiang Mai Province, Thailand, was investigated from November 2001 to October 2002. Seven hundred sixty-seven out of 849 (90.34%) cyprinoid fish were infected with heterophyid metacercariae, namely: *Stellantchasmus falcatus, Haplorchis taichui, Centrocestus caninus* and *Haplorchoides* sp. The highest prevalence of heterophyid metacercariae (95%) was observed during the winter November - January (95%) and the lowest (90%) was observed during the summer months February - April (90%). *S. falcatus* metacercariae were found in *Dermogenus pusillus* only. The highest numbers of *H. taichui, C. caninus* and *Haplorchoides* sp metacercariae were collected from *Henicorrhyncus siamensis, Rasbora perviei* and *Mystacoleucus marginatus*, respectively. The distributions in the fish hosts also varied. The most common site for *S. falcatus* and *H. taichui* metacercarial infection was the muscle, whereas the greatest numbers of *C. caninus* and *Haplorchoides* sp metacercariae were found in the gills and scales, respectively. The high prevalence of heterophyid flukes observed in this survey suggests that people who live in this area are at high risk for heterophyid fluke infection.

INTRODUCTION

More than 21 species of heterophyid flukes have been reported in humans (Waikagul, 1991). Numerous species of Heterophyidae, such as *Haplorchis taichui, H. pumilio, H. yokogawai, Stellantchasmus falcatus* and *Centrocestus caninus*, have been documented to cause infection in humans residing in northeastern and northern Thailand (Manning *et al*, 1971; Kliks and Tantachamrun, 1974; Tantachamrun and Kliks, 1978; Radomyos *et al*, 1990, 1998; Tesana *et al*, 1991; Waikagul, 1991, 1998). The major cause of fish-borne trematodiasis in northern Thailand is the consumption of local Thai fish dishes, such as *Lab-pla* (Radomyos *et al*, 1998; Sukontason *et al*, 1999). Heterophyid metacercariae have been reported in freshwater fish collected from Mae Sa stream and in some areas of Chiang Mai Province (Wongsawad *et al*, 2000; Sripalwit *et al*, 2003) and in northeastern Thailand (Srisawangwong *et al*, 1997). A survey of metacercarial infections in their second intermediate hosts, freshwater or brackish fish, showed that the dominant species are the members of Heterophyidae family, particularly *H. taichui* (Waikagul, 1998). It is predictable that heterophyid flukes will become the most dominant species of intestinal flukes in Thailand in the near future. It is necessary to know where it is endemic, therefore a year long survey of metacercarial infection in fish from 10 districts of Chiang Mai Province, Thailand, was performed.

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MATERIALS AND METHODS

A survey of the seasonal prevalence of heterophyid metacercariae in fish caught from 10 districts of Chiang Mai, Thailand (Mae Rim, Mae Taeng, San Sai, Doi Saket, San Kamphaeng, Saraphi, Mueang, Hang Dong, San Pa Tong and Chom Thong), was

conducted from November 2001 to October 2002. Various species of freshwater fish were captured from and/or bought from the fishermen living near natural reservoirs in these districts and then examined in the laboratory. Taxonomic identification of the fish was based on the guidelines and atlas of freshwater fish in Thailand by Smith (1945), Department of Fisheries, Ministry of Agriculture and co-operative, and Wittayanon (2003). To determine the distribution of the metacercariae within the fish body, various organs of the fish were examined for heterophyid metacercarie, including the gills, scales, fins, muscles and body cavity, using digestion techniques as previously described by Srisawangwong *et al* (1997). The heterophyid metacercarial species were identified following the morphological descriptions given by Yamaguti (1958), Pearson and Ow-Yang (1982), and Wongsawad *et al* (2000).

RESULTS

A total of 849 freshwater fish, consisting of 18 fish species belonging to the family Cyprinidae, were collected during a year-round survey from November 2001 to October 2002.

Table 1
Numbers of heterophyid metacercariae collected from 18 fish species in 10 districts of Chiang Mai Provinice, Thailand from November 2001 to October 2002.

Fish species	Numbers of fish infected /examined	Numbers of heterophyid metacercariae isolated				Total
		Sf	*Ht*	*Cc*	*Hsp*	
Dermogynus pusillus	426/442	191,218	0	0	0	191,218
Labiobarbus siamensis	54/60	0	3,609	31	7,327	10,937
Systomus orphoides	45/54	0	1,520	41	10,920	12,481
Tricopsis vittatus	23/38	0	0	11	147	158
Barbonymus gonionotus	30/33	0	2,438	51	3,179	5,668
Henicorhynchus siamensis	55/62	0	10,542	124	7,346	18.012
Mystacoleucus marginatus	36/42	0	1,329	31	9,334	10,694
Tricogaster microlepis	13/15	0	0	64	279	343
Hampala macrolepidota	14/17	0	35	0	243	278
Total	696/763	191,218	19,473	353	38,775	249,789
Puntioplites proctozysron	31/37	0	751	26	2,361	3,138
Rasbora parviei	26/31	0	0	437	29	466
Rasbora tornieri	1/3	0	0	17	1	18
Systomus stoliezkei	1/2	0	0	0	747	747
Amblyrhynchichthys trutcatus	3/3	0	59	0	57	116
Ratamus guttatus	1/1	0	745	0	0	745
Barbonymus schwanenfeldi	5/5	0	301	0	280	581
Paralaubuca harmandi	2/2	0	48	0	0	48
Osteochilus hasselti	1/2	0	0	0	278	278
Total	71/86	0	1,904	480	3,473	6,137

Sf = Stellantchasmus falcatus *Cc = Centrocestus caninus*
Ht = Haplorchis taichui *Hsp = Haplorchoides* sp

The overall infection rates and mean numbers of heterophyid metacercariae recovered from each fish species are shown in Table 1. The overall infection rates varied from 33.3% to 100% (Fig 1). Throughout the course of our survey, 255,926 heterophyid metacercariae were recovered from 18 fish species. Four species of heterophyid metacercariae, namely: *Stellantchasmus falcatus, Centrocestus caninus, Haplorchis taichui* and *Haplorchoides* sp. The highest mean density of *S. falcatus* metacercariae per fish (432.6; range 0 to 2,738) was observed in *D. pusillus*. *D. pusillus* was the only cyprinoid fish from which *S. falcatus* metacercaraie were obtained. The highest mean intensities of *H. taichui, C. caninus* and *Haplorchoides* sp, were observed in *Henicorhyncus siamensis* (175.7; 0-769), *Rasbora parviei* (14.1; 0-113) and *Mystacoleucus marginatus* (222.2; 0-572), respectively. In some fish species such as *Labiobarbus siamensis, Systomus orphoides, Barbonymus gonionotus, Henicorhynchus siamensis, Mystacoleucus marginatus, Puntioplites proctozysron* and *Hampala macrolepidota*. There were mixed infections of heterophyid flukes, for example: *H. taichui* and *Haplorchoides* sp were found as mixed infections in the fins and scales of infected fish (Table 1). During the survey, *S. falcatus* metacercariae had the greatest prevalence followed by *Haplorchoides* sp and *H. taichui*, respectively. *C. caninus* had the lowest infection rate of all seasons (Fig 2).

Heterophyid metacercariae were found in verious parts of the fish body (Fig 3). *S. falcatus* metacercariae were found in the muscles, head and viscera, with the highest numbers recovered from the muscles. *H. taichui* metacercaraie were found in the muscles, head, fins and scales; the greatest number were found in the muscles. *C. caninus* metacercariae were recovered from the gills only. *Haplorchoides* sp metacercariae were recovered from the muscles, fins, scales and gills with the greatest number of metacercariae obtained from the scales.

DISCUSSION

The seasonal dynamics of the prevalence of heterophyid metacercariae in fish were observed for *H. taichui* and *C. caninus* infections. The highest prevalence of *H. taichui* was observed in the summer (February-April) and the lowest was observed during the rainy season (May-October). The highest prevalence of *C. caninus* infection was observed during the rainy season. These patterns are in contrast to those reported

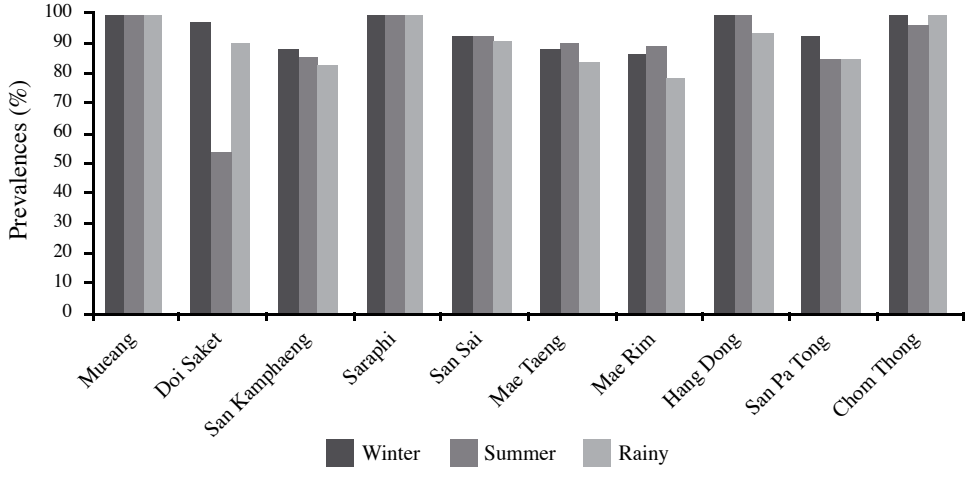

Fig. 1- The seasonal prevalences of heterophyid metacercariae in fish collected from 10 districts in Chiang Mai Province, Thailand.

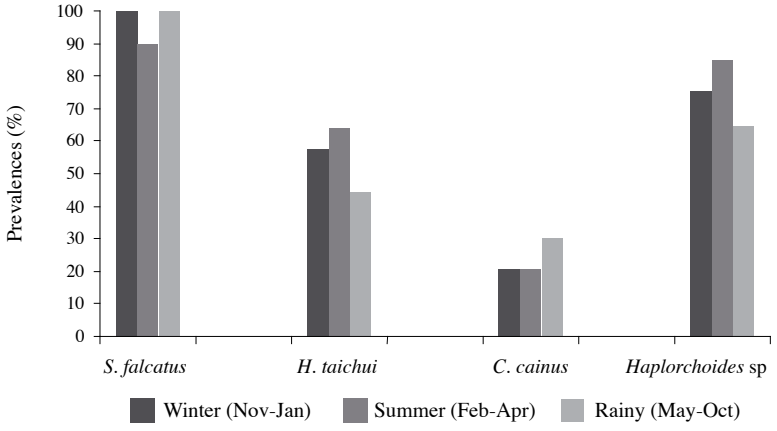

Fig. 2- The seasonal prevalences of 4 heterophyid metacercariae in fish collected from 10 districts in Chiang Mai Province during a year-round survey from November 2001 to October 2002.

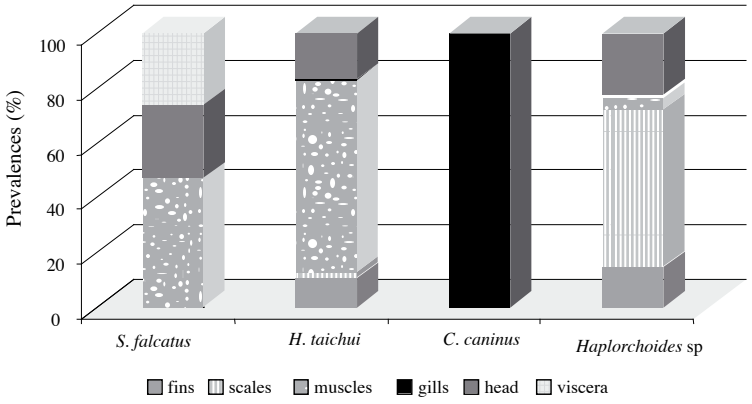

Fig. 3- The distributions of 4 heterophyid metacercariae isolated from various parts of cyprinoid fish caught from 10 districts of Chiang Mai Province, Thailand.

by Sukontason *et al* (1999) where the highest *Haplorchis* sp metacercariae prevalence was observed in the winter (November-January). Some researchers have reported an increase in the emergence of cercariae from the first intermediate host when the water temperature increases (Taskinen *et al*, 1994; Layholt and Buchman, 1996). However, in the present survey no seasonal changes were observed for the prevalence of *S. falcatus*. These results correspond to the prevalence of *C. armatus* metacercariae in some cyprinoid fish caught in the Chikusa River, in which there were no associated seasonal changes (Kimura and Uga, 2005). The changes in the prevalence of metacercariae with seasons may not be very conspicuous because metacercariae flourish for long periods of time inside the fish host and therefore no drastic changes in metacercarial prevalence may be observed at different seasons of the year (Chubb, 1979). *C. caninus* infection was the most prevalent during the rainy season. Cercarial infection may already have occurred during the summer before entering the rainy season.

The present study found that heterophyid metacercariae may be found in different parts of the fish body, such as the gills, fins, scales, muscles and visceral organs. *S. falcatus* and *H. taichui* metacercariae were principally found in the muscles of their fish hosts. Sripalwit *et al* (2003) found a 100% prevalence of *S. falcatus*

metacercariae in *D. pusillus*, with the highest density in the muscles. *H. taichui* metacercariae were commonly found in cyprinoid fish, where the greatest abundance of metacercariae were in the muscles (Kumchoo *et al*, 2005). The above two studies reported no seasonal changes in the prevalence of metacercarial infections. This is in contrast with the findings of Sukontason *et al* (2001), who reported that *H. taichui* metacercariae were found more commonly in the caudal fin. The metacercariae are mostly found in the muscles of the base of the caudal fin. *Haplorchoides* sp was found in mixed infections and was recovered from many kinds of cyprinoid fish, mostly in the scales, however the adults are known to parasitize the intestines of catfish (Waikagul and Radomyos, 2005). *C. caninus* metacercariae were only recovered from the gills in the present study, in contrast to Rim *et al* (1996) who found *C. armatus* metacercariae in different organs of the fish *Zacco temminckii* and *Z. platypus*. The metacercaraie were distributed in the gills, muscles, intestines, scales and fins, where the greatest abundance of metacercariae was observed in the intestines. The observations of Kimura and Uga (2005) are similar to those of Rim *et al* (1996) except for the presence of *C. armatus* metacercariae in the brain. Heterophyid flukes have a low specificity for cyprinoid fish since their metacercariae are commonly isolated from various fish species. The distribution of heterophyid flukes may depend on the biochemical properties of the fish hosts and parasite species themselves. This present study found heterophyid flukes in fish from 10 districts of Chiang Mai Province. People who live in these endemic areas are at high risk for heterophyid fluke infections based on the high infection rates of the heterophyid trematodes in cyprinoid fish. Heterophyid infections found in fish which serve as the protein-food sources or are economically important, such as exported ornamental fish, can result in a reduction in fishery yields and economic losses.

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REFERENCES

Chubb JC. Seasonal occurrence of helminthes in freshwater fishes. Part II. Trematoda. In: Lumsden WHR, Muller R, Baker JR, eds. Advances in parasitology. London: Academic Press, 1979:141-313.

Kimura D, Uga S. Epidemiological studies on *Centrocestus armatus* metacercariae in Chikusa river, Hyogo Perfecture, Japan. *Trop Med Int Health* 2005;33:7-11.

Kliks M, Tantachamrun T. Heterophyid (Trematoda) parasites of cats in north Thailand, with notes on a human case found at necropsy. *Southeast Asian J Trop Med Public Health* 1994;5:547-55.

Kumchoo K, Wongsawad C, Chai JY, Vanittanakom P, Rojanapaibul A. High prevalence of *Haplorchis taichui* metacercariae in cyprinoid fish from Chiang Mai Province, Thailand. *Southeast Asian J Trop Med Public Health* 2005;36:451-5.

Layholt HCK, Buchmann K. *Diplostomum spathaceum*: effects of temperature and light on cercarial shedding and infection of rainbow trout. *Dis Aquat Organ* 1996; 25:169-73.

Manning GS, Leartprasert P, Watanasirmkit K, Chamroen C. A description of newly-discovered intestinal parasites endemic to northeastern Thailand. *J Med Assoc Thai* 1971;54:464.

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.

Radomyos P, Charoenlarp P, Radomyos B, Tungtrongchitr A. Two human cases of *Stellanthchasmus falcatus* (Trematoda: Heterophyidae) infection in northeastern Thailand. *Jpn J Parasitol* 1990;39:7-11.

Radomyos B, Wongsaroj T, Wilairatana P, *et al*. Opisthorchiasis and intestinal fluke infections in northern Thailand. *Southeast Asian J Trop Med Public Health* 1998;29: 123-7.

Rim HJ, Kim KH, Joo KH, Kim SJ, Eom KS, Chung MS. The infestation states and changing patterns of human infecting metacercariae in freshwater fish in Kyongsang-do and Kyonggi-do, Korea. *Korean J Parasitol* 1996;34:95-105.

Smith HM. The freshwater fishes of Siam or Thailand. Washington: United States Government Office, 1945.

Sripalwit P, Wongsawad C, Chai JY, Anantalabhochai S, Rojanapaibul A. Investigation of *Stellantchasmus falcatus* metacercariae in half-beaked fish, *Dermogenus pusillus* from four districts of Chiang Mai Province, Thailand. *Southeast Asian J Trop Med Public Health* 2003;34:281-5.

Srisawangwong T, Sithithaworn P, Tesana S. Metacercariae isolated from cyprinoid fish in Khon Kaen District by digestion technic. *Southeast Asian J Trop Med Public Health* 1997;28 (suppl):224-6.

Sukontason K, Piangjai S, Muangyimpong Y, Sukontason KL, Methanithikorn 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.

Sukontason K, Sukontason KL, Boonsriwong N, Chaithong U, Piangjai S. Intensity of trematode metacercariae in cyprinoid fish in Chiang Mai Province, northern Thailand. *Southeast Asian J Trop Med Public Health* 2001;32 (suppl 2):214-7.

Tantachumrun T, Kliks M. Heterophyid infection in human ileum: report of three cases. *Southeast Asian J Trop Med Public Health* 1978;9:228-31.

Taskinen J Valtonen ET, Makela T. Quantity of sporocysts and seasonality of two *Rhipidocotyle* species (Digenea: Bucephalidae) in *Anodonta piscinalls* (Mollusca: Bivalvia). *Int J Parasitol* 1994;24:877-86.

Tesana S, Srisawangwaong T, Kaewkes S, Sithithaworn P, Kanla P, Arunyanart C. Egg shell morphology of the small eggs of human trematodes in Thailand. *Southeast Asian J Trop Med Public Health* 1991;22: 631-6.

Waikagul J. Intestinal fluke infections in Southeast Asia. *Southeast Asian J Trop Med Public Health* 1991;22 (suppl):158-62.

Waikagul J. *Opisthorchis viverrini* metacercaria in Thai freshwater fish. *Southeast Asian J Trop Med Public Health* 1998;29:324-6.

Waikagul J, Radomyos P. Intestinal trematode infections in Thailand. *Asian Parasitol Monogr Ser* 2005;1:103-12.

Wittayanon C. Freshwater fishes of Thailand. Bangkok: Nanmeebook, 2003. (In Thai).

Wongsawad C, Rojanapaibul A, Mhad-arehin N, *et al*. Metacercaria from freshwater fishes of Mae Sa stream, Chiang Mai, Thailand. *Southeast Asian J Trop Med Public Health* 2000;31 (suppl):54-7.

Yamaguti S. Systema Helminthum. Vol I. The digenia trematode of vertebrates. Part I & II. New York: Interscience Publication, 1958.