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Haplorchis infections in intermediate hosts from a clonorchiasis endemic area in Meinung, Taiwan, Republic of China

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Abstract

Snails and freshwater fish were examined from four ponds in the Meinung township in which *Clonorchis sinensis* was known to be endemic 18 years ago. No metacercariae were found in 478 *Tilapia nilotica*, whereas of 451 *Ctenopharyngodon idellus* examined, 16.2%, 3.3% and 0.9% were found to be infected with *Haplorchis pumilio*, *H. taichui* and *Clonorchis sinensis*, respectively. In addition, there were some unidentified metacercariae in 12.0% of *Ctenopharyngodon idellus* examined. Overall, no positive correlation between infection rates and sizes of infected fish was shown. Six species of snails were collected in this survey and two frequently-occurring snails, *Melanoides tuberculata* and *Thiara granifera* were commonly infected with *H. pumilio*. Reasons for the prevalence of *Haplorchis* species and the absence of *Clonorchis sinensis* in fish and snail hosts in a previously reported endemic area for human clonorchiasis are discussed.

Introduction

Clonorchis sinensis is a common parasite of fish-eating mammals and humans in the Far East. Adult flukes inhabit the bile ducts and induce irritation by mechanical means and toxic secretions. Human infections in Taiwan were first reported by Ohoi (1915), who found eggs of the parasite in the stools of hospitalized patients. After this first report, the Meinung township in southern Taiwan was the first endemic area of clonorchiasis to be found (Chow, 1960; Kuntz *et al.*, 1961). Therefore, *C. sinensis* is considered a major zoonotic parasite in Taiwan (Hsieh, 1959). Various studies on the infection rate and distribution of human, reservoir host, the first and second intermediate hosts of the parasite have been carried out throughout the island. Thus, *C. sinensis* has long been known to be endemic in several areas of Taiwan, especially in the Meinung township (Chow, 1960; Kim & Kuntz, 1964; Huang *et al.*, 1965; Kuntz, 1966; Clarke *et al.*, 1971; Ong & Lu, 1979; Yen *et al.*, 1988).

The most frequently occurring metacercariae in freshwater fish from endemic areas examined 18 years ago were those of *C. sinensis* (Muto, 1938; Huang & Khaw, 1964; Wang *et al.*, 1980). However, more recently, Ooi & Chen (1997) examined the freshwater fish *Hemiculter leucisculus* from the Sun Moon Lake in central Taiwan, and found the infection rate of *C. sinensis* to be only 0.05%, in contrast to an increase in infections by *Haplorchis* spp., especially a 96.2% prevalence of *H. taichui* in the fish of that lake. Following the report by Ooi & Chen (1997), the aim of the present investigation was to establish whether or not a similar phenomenon occurred in the Meinung township.

To date, inhabitants with clonorchiasis in the Meinung township are diagnosed by examining stools for eggs of *C. sinensis*, but, as it is difficult to distinguish eggs of *Haplorchis* spp. from those of *C. sinensis* in stool specimens, it is possible that the infection in the inhabitants could be *Haplorchis* spp. rather than *C. sinensis*. In the present study, we examined freshwater snails and fish from aquatic habitats in the Meinung

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Table 1. Prevalence (%) of metacercarial infections in *Ctenopharyngodon idellus* from pond A.

Fish size (cm)	No. examined	Number (%) of metacercariae found			
		H. t.	*H. p.*	*C. s.*	Unidentified
40–49	43	6 (14.0)	29 (67.4)	3 (7.0)	12 (27.9)
50–59	13	1 (7.7)	6 (46.2)	0	4 (30.8)
Total	56	7 (14.3)	35 (62.5)	3 (5.4)	16 (28.6)

H. t., Haplorchis taichui; H. p., Haplorchis pumilio; C. s., Clonorchis sinensis.

township for trematode infections to update the epidemiological data. In addition, we postulate the decreased likelihood of *C. sinensis* infection occurring in the inhabitants of Meinung if the infection rate of this parasite in snails and fish is low.

Materials and methods

The collection of freshwater fish was conducted randomly during January and June 1999 from four ponds designated as A to D. On average, 15 to 20 freshwater fish, *Ctenopharyngodon idellus* and/or *Tilapia nilotica*, were caught weekly during these months and brought to our laboratory for examination. In June 1999, aquatic snails on the bottom and near the edge of the ponds, as well as in the irrigation canals near the ponds, were randomly collected alive using a stainless steel sieve.

Examination of fish

Each freshwater fish was equally divided into five sections from head to tail. About 20 g of muscle from each section were cut from one side of the fish. A total of 100 g of muscle from each fish was thoroughly homogenized in a blender and digested in artificial gastric juice (1:10,000 pepsin, 2 g; 8 ml concentrated HCl; 1 l distilled water) with magnetic stirring at 25°C for 6 h. The digested material was strained through a fine mesh, and the fluid allowed to stand for 20 min. Two-thirds of the fluid was discarded and the beaker was then filled with tap water again. After several washes, the sediment was transferred into a Petri dish and examined under a dissecting microscope for metacercariae, followed by identification of metacercariae at × 400.

Emergence of cercariae

Aquatic snails from each pond were identified as *Melanoides tuberculata, Austropeplea ollula, Sinotaia quadrata, Cipangopaludina chinensis, Ampullaria canaliculatus* and *Thiara granifera* and each snail species divided into groups of 10. Snails of each group were individually placed in 50-ml beakers containing 20 ml tap water and exposed under an incandescent lamp for 2 h to stimulate cercarial emergence.

Results

Examination of fish

For this study, 111, 286 and 81 *T. nilotica* ranging from 21 cm to 43 cm were examined from ponds A, B and C, respectively, but no metacercariae were found.

A total of 451 *C. idellus* were examined from ponds A and D. Metacercariae of *H. taichui, H. pumilio* and *Clonorchis sinensis* were found in 14.3%, 62.5% and 5.4%, respectively, of 56 *Ctenopharyngodon idellus* from pond A (table 1). Of 395 *C. idellus* examined from pond D, 2.0%, 9.6% and 0.3% were found to be infected with metacercariae of *H. taichui, H. pumilio* and *Clonorchis sinensis*, respectively (table 2). Some specimens of metacercariae in fish from ponds A and D could not be identified, either because they were not fully developed or because they had degenerated. Generally, the infection rate in fish from pond A (table 3), was significantly higher than that in fish from pond D ($X^2 = 52.0$, $P < 0.0001$). There was no positive correlation between metacercarial infection rates and size of infected fish examined from both ponds.

Examination of snails

Six species of snails were identified in pond A (table 4) and only cercariae of *H. pumilio* could be found in

Table 2. Prevalence (%) of metacercarial infections in *Ctenopharyngodon idellus* from pond D.

Fish size (cm)	No. examined	Number (%) of metacercariae found			
		H. t.	*H. p.*	*C. s.*	Unidentified
40–49	44	1 (2.3)	3 (6.8)	0	1 (2.3)
50–59	296	5 (1.7)	27 (9.1)	1 (0.3)	27 (9.1)
60–69	52	2 (3.9)	8 (15.4)	0	10 (19.2)
70–79	3	0	0	0	0
Total	395	8 (2.0)	38 (9.6)	1 (0.3)	38 (9.6)

H. t., Haplorchis taichui; H. p., Haplorchis pumilio; C. s., Clonorchis sinensis.

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Table 3. Prevalence (%) of metacercariae in *Ctenopharyngodon idellus* from ponds A and D, relative to fish size.

	Pond A		Pond D		Both ponds	
Fish size (cm)	No. examined	No. (%) positive	No. examined	No. (%) positive	No. examined	No. (%) positive
40–49	43	23 (53.5)	44	5 (11.4)	87	28 (32.2)
50–59	13	6 (46.2)	296	32 (10.8)	309	38 (12.3)
60–69	0	0	52	13 (25.0)	52	13 (25.0)
70–79	0	0	3	0	3	0
Total	56	29 (51.8)*	395	50 (12.7)*	451	79 (17.5)

* $X^2 = 52.0$, $P < 0.0001$.

Melanoides tuberculata. No cercariae were recovered from five and two species of snails collected from ponds B and C respectively. Among four species of snails collected from pond D, only cercariae of *H. pumilio* were found in *Melanoides tuberculata* and *Thiara granifera*.

Discussion

It is of interest to note that no snails were found to be the intermediate host of *C. sinensis*, and only very rarely were freshwater fish infected with *C. sinensis*. In previous epidemiological surveys at the Meinung township, stool examinations revealed that more than 50% of inhabitants were infected with *C. sinensis*, and at the same time, metacercariae of *C. sinensis* were also found in local freshwater fish (Chow, 1960; Wang *et al.*, 1980). Indeed, eggs in the stools of patients from the Meinung township attending the university hospital are still being identified as *C. sinensis*, but based on the present study, it is possible that the eggs are those of *H. pumilio*.

The distribution of metacercariae in Taiwan fish has previously been studied by Hwang & Khaw (1964) and Kawai & Yumoto (1936). It is known that the distribution of different metacercariae may vary in the same species of fish, and that the same species of metacercariae can occur in different locations in different species of fish. Most metacercariae of *C. sinensis* occur in the caudal peduncle of most species of fish (Wang *et al.*, 1980). However, the distribution of metacercariae other than *C. sinensis* is not very well known.

The Meinung township is mostly inhabited by Hakka people, who have preserved customs and special dietary habits such as the consumption of raw fish. Metacercariae of some parasite species have been recovered from many species of freshwater fish, but not all of these fish are considered important in the transmission of the parasites to humans (Kim & Kuntz, 1964). For example, although *Hemiculter kneri*, a species of fish commonly found in southern Taiwan, are infected with large numbers of metacercariae, they are seldom eaten raw (Hwang & Khaw, 1964; Kim & Kuntz, 1964). *Tilapia* spp. and *Ctenopharyngodon idellus* are the most popular species of freshwater fish which are dressed and served raw as 'sashimi'. *Tilapia mossambica* and *C. idellus* were found to have moderate infection rates of *Clonorchis sinensis* in a survey conducted in the Meinung township by Wang *et al.* (1980). In recent years, *T. nilotica* and *Ctenopharyngodon idellus* have commonly been raised in ponds near the Meinung township. In the present study, no metacercariae were found in *T. nilotica*, and most of the metacercariae in *C. idellus* were *Haplorchis* spp. rather than *Clonorchis sinensis*.

Table 4. Examination of snails for cercariae of *Haplorchis pumilio* in four ponds (A to D) in the Meinung township, Taiwan.

	Ponds			
Species of snails	A	B	C	D
Melanoides tuberculata	+	–	N.F.	+
Austropeplea ollula	–	N.F.	N.F.	N.F.
Sinotaia quadrata	–	–	N.F.	–
Cipangopaludina chinensis	–	–	–	–
Ampullaria canaliculatus	–	–	–	N.F.
Thiara granifera	–	–	N.F.	+

N.F., these snail species were not found.
Haplochis pumilio present (+), or absent (–).

Nine species of snails, including *Bithynia* (*Parafossarulus*) *manchouricus*, which are known to be first intermediate hosts of *C. sinensis* were collected from the Meinung township in a previous survey by Chow (1960). Compared with that survey, we have some different findings in our examination of snail hosts. For instance, except for *Thiara granifera*, the composition of snail species is different in the present study, especially the more frequently occurring *Melanoides tuberculata*, which was commonly infected with *H. pumilio*. *Thiara granifera* has been suspected to be the snail host of *C. sinensis* in the Meinung township (Chow, 1960), but the cercariae which emerged from *Thiara granifera* in laboratory examinations were *H. pumilio*. It is known that the activity of snail hosts and the ability of snails to produce cercariae might be related to the seasons of the year (Hwang & Khaw, 1964; Kim & Kuntz, 1964). However, as the snails in the present study were examined in June, a warm month, we believe that environmental changes during the past forty years have led to differences in snail diversity in the township of Meinung.

It is likely that an increase in *H. pumilio* infections among *Ctenopharyngodon idellus* of Meinung is due to the abundance of the snails *Melanoides tuberculata* and *Thiara granifera*. However, some questions still exist. How was the life cycle of *H. pumilio* set up in this area? Are there any definitive hosts or reservoir hosts of *H. pumilio*, such as birds as well as mammals, in this area? How are some freshwater fish infected with metacercariae other than

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H. pumilio? Further studies are therefore needed to answer these questions.

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