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Short Note

Infectivity of the Cercariae of *Centrocestus formosanus* and *Haplorchis pumilio* (Digenea: Heterophyidae) in *Cyprinus carpio*

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Chin-Tsong Lo and Kin-Mu Lee (1996) Infectivity of the cercariae of *Centrocestus formosanus* and *Haplorchis pumilio* (Digenea: Heterophyidae) in *Cyprinus carpio*. *Zoological Studies* **35**(4): 305-309. When cercariae of *Centrocestus formosanus* were placed together with the carp *Cyprinus carpio*, all of the cercariae disappeared in 30 min, while the cercariae of *Haplorchis pumilio* totally disappeared in 10 min. However, only 45.2% of *C. formosanus* and 42.6% of *H. pumilio* cercariae were later recovered from *C. carpio* as metacercarial cysts at 1 month postinfection. The lower than expected cyst recovery rates could be explained by the trapping of many *C. formosanus* cercariae in the mucus secreted by the gills, and by the difficulty with which *H. pumilio* cercariae could penetrate the scale-covered parts of the fish. Toxic effects of mucus secreted from the body surface of the fish may be partly responsible for the demise of cercariae. The cysts of *C. formosanus* were encapsulated by layers of cells and fibrous material of host origin, indicating that the gills can elicit a strong tissue reaction against these parasites.

Key words: Heterophyidae, Cercarial penetration, Metacercaria.

Heterophyids are small digeneans that inhabit the digestive tracts of birds and mammals. They have a broad range of final hosts and can often develop to maturity in various avian and mammalian hosts (Nishigori 1924, Kuntz and Chandler 1956). Although not highly pathogenic, they can cause intestinal pain, diarrhea, and chronic enterocolitis in man, or death in experimental animals if heavily infected (Nishigori 1924, Bronshtein et al. 1992, Chieffi et al. 1992,). Human infections by heterophyids have been recorded from a number of countries, especially in Asia where there is the custom of eating uncooked fish (Africa and Garcia 1935, Radomyos et al. 1983 1990, Chai and Lee 1991, Giboda et al. 1991, Chai et al. 1993 1994). Ten and 9 species, respectively, are known to infect man in Korea and China (Chai and Lee 1991, Hsu 1994). In Korea, an infection rate of 42.9% for *Heterophyes nocens* and worm loads of 6,015-24,060 for *Metagonimus* spp. were reported (Chai et al. 1993 1994), while a 66.7% positive rate was recorded for *Metagonimus* infection in the Amur River basin of Russia (Bronshtein et al. 1992). The eggs of heterophyids are small, similar to each other among some species, and some also resemble the eggs of liver flukes *Clonorchis sinensis* and *Opisthorchis viverrini*; these factors cause difficulties in differential diagnosis (Lee et al. 1984, Ditrich et al. 1992), and misidentifications have occurred (cited by Kliks and Tantachamrun 1974).

In a review of heterophyid flukes based on the literature between 1912 and 1932 (Ito 1963), 8 species were listed as occurring in Taiwan, namely *Haplorchis pumilio, H. taichui, H. yokogawai, Centrocestus formosanus, Metagonimus yokogawai, Stellantchasmus formosanus, S. pseudocirrata* and *Heterophyes heterophyes*. Pearson (1964) added *Haplorchis wellsi* to the list. Our observations (unpubl.) on cercarial infections in snails show that *C. formosanus* and *H. pumilio* are presently the 2 most commonly encountered species in Taiwan, possibly due to the wide distribution of the snail *Melanoides tuberculata* which serves as the 1st intermediate host for both parasites. The cercariae of *C. formosanus* are highly pathogenic to piscine hosts because they encyst in the gills and cause respiratory problems. Mass mortalities have been reported in elvers (*Anguilla japonica*) and loaches (*Misgurnus anguillicaudatus*) due to infection by *C. formosanus* (Yanohara and Kagei 1983, Tung et al. 1989). The cercariae of *H. pumilio* are less pathogenic because they encyst mostly in the area at the bases of fins but some ecyst in the muscle. Thirteen species of freshwater fishes have been reported to serve as 2nd intermediate hosts in Taiwan (Nishigori 1924, Faust and Nishigori 1926), including the grass carp (*Ctenopharyngodon idellus*) which is often thinly sliced and served raw as sashimi in certain communities. There is a paucity of information regarding human infections by the heterophyids in Taiwan although *M. yokogawai* infections were noticed as early as 1912 (cited by Ito 1963), and experimentally *H. taichui* and *H. pumilio* are capable of infecting human body (Faust and Nishigori 1926). Erroneous assignment of heterophyid infections to *C. sinensis* may have occurred in the past. Although many fish species can act as metacercarial hosts for *C. formosanus* and *H.*

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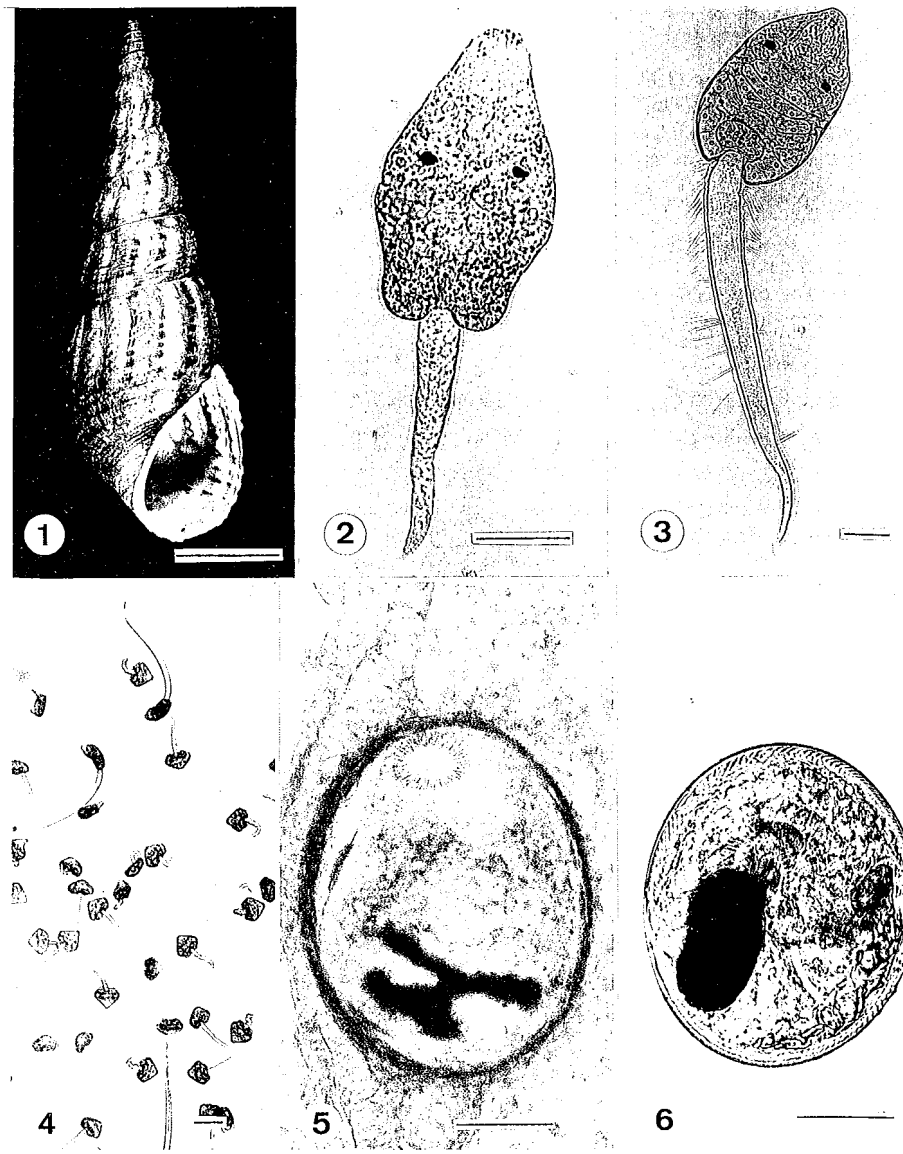
pumilio, the relative infective efficiency of each host species is unknown in quantitative terms. In this study, we examined the infection dynamics of 2 heterophyid cercariae in the carp (*Cyprinus carpio*); this information may prove useful in fish culture and public health. Penetration behaviors of the cercariae were also observed.

Materials and Methods—Infected *M. tuberculata* (Fig. 1) were collected from a fish farm in Pingtung County. Mixed infections were rare, and when found they were excluded. About 20 infected snails for each heterophyid species were individually put into 50-ml beakers containing 30 ml conditioned tapwater and placed under an incandescent lamp to stimulate cercarial emergence. The cercariae from these snails were mixed and used within 2 h after emergence. The desired numbers of cercariae were counted by drawing them into a fine pipette under a dissecting microscope. About 100 *C. carpio*, 3-5 cm long, were purchased from a commercial aquarium which obtained the fish from a contracted fish farm. These fish were free of metacercarial infections as revealed by dis-



Figs. 1-6. The snail host, cercariae and metacercariae.
1. Shell of *Melanoides tuberculata*. 2. Live cercaria of *Centrocestus formosanus* under slight coverglass pressure. 3. Live cercaria of *Haplorchis pumilio* under slight coverglass pressure. 4. Cercariae of *C. formosanus* and *H. pumilio* showing their normal shapes and relative sizes. The pentagonal body of *C. formosanus* is indicative of healthy cercariae. 5. Live metacercaria of *C. formosanus* in a fish gill. The presence of 32 circumoral spines and an H-shaped excretory vesicle are distinct. The metacercaria has a thin wall about 2 μm thick, but it is surrounded by thick layers of cells and fibers of host origin. 6. Live metacercaria of *H. pumilio* dissected from fish. Scale bar = 5 mm in Fig. 1; 100 μm in Fig. 4; 50 μm in the others.

section of selected individuals from the batch. The fish were acclimated in the laboratory for 2 wk or more before use. In the cercarial penetration test, each fish was exposed to 50 cercariae in a 100-ml beaker and the rate of disappearance of cercariae was measured by counting the cercariae remaining in the beaker at 1, 2, 3, 4, 5, 10, 20, and 30 min postexposure (PE); the experiment was carried out at 24-26 °C and 4 fish were tested for each exposure time. Another experiment was then carried out to determine the percentage of cyst formation from a given number of cercariae, an ultimate test for the infective success of cercariae. Five fish were individually exposed to 100 cercariae for 1 h, maintained for 1 mo, then dissected. Individual gills, parts of the head, muscles, and fins were pressed between 2 glass plates and examined under a dissecting microscope at 10-40 × magnifications. The tissues were torn further with forceps and allowed to settle in water for the recovery of cysts which might have been missed.

Results—The cercariae of *C. formosanus* are small (Figs. 2, 4). Under test conditions, they swam in more or less straight lines with a speed of about 0.5 mm/sec. Their contact with fish gills was passively achieved by being sucked into the mouth with the respiratory current which passed through the gills. No cecariae were seen penetrating the body surface of the fish. However, when a fish was laid sideways together with several drops of water, and some cercariae were introduced under the tail fin, thus tightly pressing the cercariae against the dish bottom, the cercariae began to penetrate the fin within 10 sec and completed the process in 0.5-3 min with their tails intact.

Compared to *C. formosanus*, the cercariae of *H. pumilio* are twice as long (Figs. 3, 4) and they swam 3-5 times faster; the zigzag path of swimming was due to the presence of bilateral and dorsoventral finfolds on the tail stem. The majority of the cercariae began to penetrate on initial contact with the fish and their tails separated soon afterwards. Those which landed on the fins successfully penetrated in 2-4 min, while those on the scale-covered parts were mostly rejected and died, with some spending up to 40 min trying to penetrate. The penetrated cercariae then migrated towards the trunk and the majority encysted at the basal region of the fins.

The cercariae of *C. formosanus* gradually and steadily disappeared from the exposure vessel as shown in Fig. 7. At 5 min PE 52% remained, at 10 min PE 28%, at 20 min PE 5%, and at 30 min PE there were none. The disappearance of *H. pumilio* cercariae occurred much faster: at 5 min PE only 2% remained and at 10 min PE there were none. For both species about 2% of the cercariae were found dead, weak, or decaudated and crawling at the bottom of the container at the end of the exposure period.

The number of metacercarial cysts of *C. formosanus* (Fig. 5) recovered from the gills of 5 fish ranged from 25 to 73 out of 100 cercariae originally, with an average of 45.2 ± 8.0 SE. They were equally distributed on the right and left sides of the fish, similar to results demonstrated in another cyprinid fish *Aplocheilus panchax* (Madhavi 1986). The degree of cyst development differed among individual fish, ranging from fully mature to retarded. The retarded cysts were smaller, and lighter in color, with some still retaining eyespots or having no circumoral spines. Many of the cysts were surrounded by layers of cells and fibrous material of host origin. The number of *H. pumilio* cysts (Fig. 6) in each fish resulting from 100-cercaria infection ranged from 26 to 63, with an average of 42.6 ± 7.4 SE, which was not significantly different from that of *C. formosanus* (χ^2-test, $p = 0.82$). The cysts of *H. pumilio* were located in spaces between cartilage at the base of various fins and the head, with a few in muscle. Their distribution was as follows: head region (33%); the bases of caudal fin (23%), dorsal fin (19%), pectoral fins (9%), anal fins (9%), and pelvic fins (6%); and the muscle near the fins (1%). The cysts were more uniformly developed compared to those of *C. formosanus* and there were few host cellular reactions.

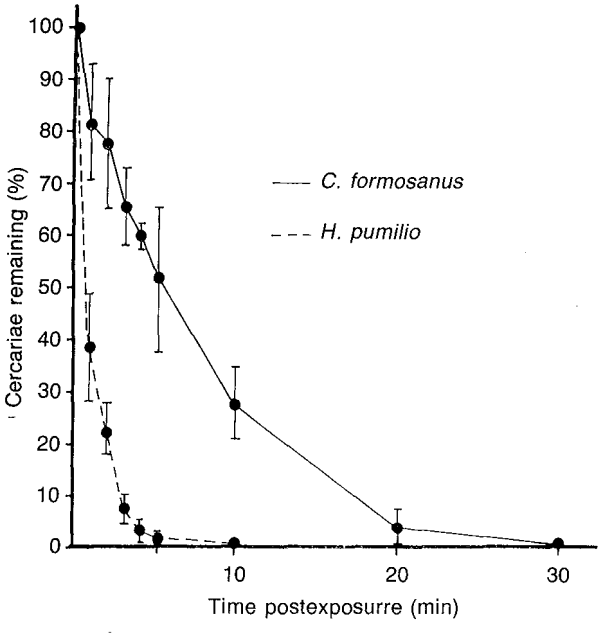


Fig. 7. Disappearance of *Centrocestus formosanus* and *Haplorchis pumilio* cercariae at time intervals postexposure to *Cyprinus carpio* at 25 °C. The mean and range from 4 fish are shown. Total number of cercariae for each exposure time is 200.

Discussion—Only about 2% of the cercariae exposed to the fish remained free in the exposure vessel at the end of observation period, implying that the rest must have successfully penetrated. Yet, cyst recovery rates were less than 50% for both species. The copious mucus secretion by the gills may have acted as a trap and hindered the penetration of *C. formosanus* cercariae; dissection on separate occasions of several fish at 1-2 h PE revealed this to be the case, since many cercariae were found to have been retained by the mucus and were dead. The smaller than expected recovery of *H. pumilio* cysts could be explained by the difficulty in penetrating the scale-covered parts of the fish. Chun (1964) showed that the surface mucus of *C. carpio* and *Carassius carassius* was lethal to the cercariae of *Clonorchis sinensis* which has a life history pattern similar to those of heterophyids. The toxic substances were identified as linoleic acid and ethyl linoleate which were also lethal to the excysted metacercariae of *C. sinensis* (Rhee et al. 1984 1988). Therefore, the smaller than expected numbers of metacercariae could be partly due to the toxic effects of these substances. The uneven development of *C. formosanus* cysts and capsule formation around them indicates that a strong host defense mechanism operates in the gills. Although these 2 species are placed in the same family, they differ considerably in the morphology of larval and adult stages. This study additionally demonstrates that their cercariae also differ in the modes of swimming and penetration behavior.

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臺灣異形吸蟲及普密略異形吸蟲對鯉魚之感染力

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當把臺灣異形吸蟲 (*Centrocestus formosanus*) 之尾動幼蟲 (尾幼) 與鯉魚放在一起時，所有的尾動幼蟲會在 30 分鐘內消失，顯示可能已穿入魚體內。如用普密略異形吸蟲 (*Haplorchis pumilio*) 之尾幼，則消失時間爲 10 分鐘。雖然幾乎所有的尾幼已消失，把魚隻在 1 個月後檢查時，囊狀幼蟲 (囊幼) 之回收率只達 45.2% (臺灣異形吸蟲) 及 42.6% (普密略異形吸蟲)。觀察尾幼之穿入情形時，發現有些臺灣異形吸蟲被困在魚鰓外圍之黏液中而死亡；普密略異形吸蟲之尾幼，如降落在有鱗片覆蓋之部位，則大部分不能順利穿入體內而死亡。魚體表面所分泌的黏液具有毒性，此可能是尾幼死亡之部分原因。臺灣異形吸蟲之囊幼多被細胞包圍，表示魚鰓對侵入的寄生蟲可引起強烈的細胞防禦反應。

關鍵詞：異形吸蟲，尾動幼蟲，囊狀幼蟲，感染力。

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