

DIFFERENTIAL DIAGNOSIS OF OPISTHORCHIID AND HETEROPHYID METACERCARIAE (TREMATODA) INFECTING FLESH OF CYPRINID FISH FROM NAM NGUM DAM LAKE IN LAOS

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Abstract. The examination of flesh of 313 cyprinid fish from the Nam Ngum water reservoir in Vientiane Province, Laos, revealed the presence of four morphologically similar opisthorchiid and heterophyid metacercariae of medical importance. The following morphological characters, visible under dissecting microscope with low magnification, were typical of trematodes recorded in fish: (1) *Opisthorchis viverrini*, the liver fluke, has oval cysts containing quickly moving C-shaped metacercariae provided with a rather large excretory bladder; (2) metacercariae of *Haplorchis pumilio* and *H. taichui* are surrounded by a thin-walled, oval cyst; they are actively motile, C- or S-shaped, and possess a relatively small excretory bladder; (3) cysts of *Haplorchoides mehrai* are considerably variable in shape, size, and thickness of cyst wall; larvae are slowly moving, C- or S-shaped, or often not folded, and provided with a rather small excretory bladder. Some cysts with dead or degenerated *H. mehrai* larvae are filled with brownish or yellowish pigment. Morphological variability of some larvae, however, excluded correct identification of all metacercariae during routine examination of fish. Therefore, we consider isolation of several larvae from each fish host as well as all "atypical" metacercariae from fish flesh and their examination under the light microscope to be necessary. Even though this method of examination is rather time consuming, it decreases the possibility of false identification of trematode larvae encysted in flesh of cyprinid fish during field studies on opisthorchosis and heterophyiosis.

INTRODUCTION

In Laos, only one species of small fluke, the liver fluke, *Opisthorchis viverrini* (Poirier, 1886), has hitherto been reported to occur in humans (Segal *et al*, 1968; Sicard *et al*, 1977). Commonly consumed cyprinid fish of the genera *Hampala*, *Puntius*, *Cyclocheilichthys*, and *Barbodes*, whose flesh is infected with *O. viverrini* metacercariae, represent a main source of human infection in some countries of Southeast Asia. The examination of cyprinids from Nam Ngum Dam Lake in Laos revealed the presence of three additional trematode species of the family Heterophyidae with morphologically closely similar metacercariae (Ditrich *et al*, 1990). Considering the different medical importance of individual parasites, their correct identification is important. This paper presents data about the routine identification and differential diagnosis of metacercariae encysted in flesh of cyprinid fish that might be misdiagnosed as *O. viverrini*.

MATERIAL AND METHODS

The study was carried out at the Nam Ngum water reservoir situated north of the Lao capital, Vientiane (Ditrich *et al*, 1990). This reservoir represents the most important source of fish for inhabitants of the whole Vientiane Province. Flesh of 313 fish of 18 species were examined under a dissection microscope (magnification 30 ×) using the compression method.

RESULTS

Metacercariae of the following four species of small flukes were recorded: *O. viverrini* (family Opisthorchiidae), *Haplorchis pumilio* (Looss, 1896), *H. taichui* (Nishigori, 1924), and *Haplorchoides mehrai* Pande et Shukla, 1976 (family Heterophyidae).

Simultaneous infections of one fish with two species were recorded in 11 cases: *O. viverrini* and *H. mehrai* metacercariae were recorded six

times (twice in *Hampala dispar*, twice in *H. macrolepidota*, once in *Barbodes gonionotus*, and once in *Cyclocheilichthys repasson*); *H. taichui* and *H. mehrai* larvae were found five times (twice in *H. dispar*, twice in *H. macrolepidota*, and once in *B. gonionotus*).

The following morphological features were used for identification of most larvae during routine fish examination (Fig. 1): *O. viverrini* has mostly oval cysts containing quickly moving C-shaped metacercariae provided with a large excretory bladder. *Haplorchis pumilio* and *H. taichui* metacercariae are surrounded by a thin-walled, oval cyst; they are actively motile, C- or S-shaped, and their excretory bladder is rather small. Cysts of *H. mehrai* are variable in shape, size, and thickness of cyst wall; metacercariae are slowly or very slowly moving, C-, S- or often I-shaped (not folded). They are also provided with a relatively small excretory bladder. Some *H. mehrai* metacercariae were dead or degenerated; their cysts were filled with brownish or yellowish pigment.

DISCUSSION

The results confirmed the possibility of simultaneous infection of cyprinid fish with opisthorchiid and heterophyid flukes. Of four trematode species found, only *H. mehrai* is of no medical importance. This trematode matures exclusively in predatory fish and cannot infect mammals. The liver fluke *O. viverrini* is a common and medically important human parasite in some countries of Southeast Asia, particularly in Thailand (see, eg, Sadun 1955; Harinasuta 1969). Members of the genus *Haplorchis* Looss, 1899, mainly the two species found in Nam Ngum Dam Lake, occasionally infect humans in the same region (Manning *et al*, 1971; Kliks and Tantachamrun 1974; Radomyos *et al*, 1984). Simultaneous infections of man with *O. viverrini* and heterophyid flukes was confirmed in Laos. Considering their common occurrence and morphological similarity to some *H. mehrai* larvae, the correct identification of *Opisthorchis* and *Haplorchis* metacercariae is necessary. All the above species can be easily distinguished from one another under the light microscope: *O. viverrini* possesses a relatively large, strongly muscular ventral sucker, while heterophyid flukes are characterized by the presence of a ventro-

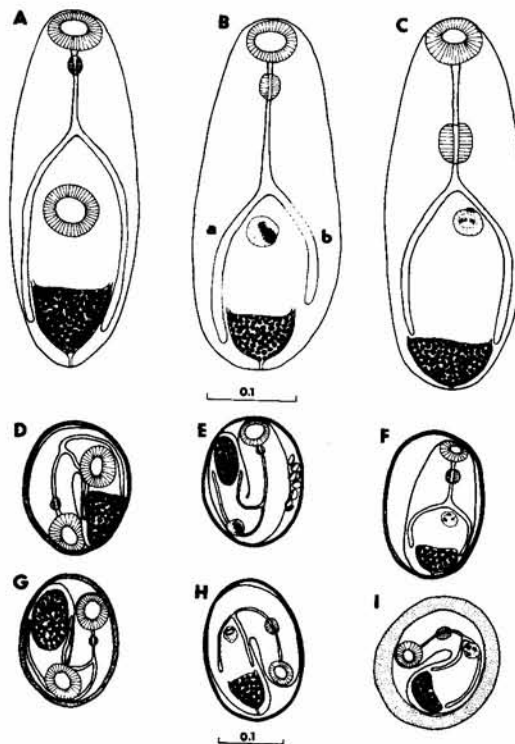


Fig 1 - Free and encysted opisthorchiid and heterophyid metacercariae from cyprinid fish in Nam Ngum Dam Lake, Laos. A, D, G - *Opisthorchis viverrini* (Poirier, 1886); B, E - *Haplorchis taichui* (Nishigori, 1924); B, H - *H. pumilio* (Looss, 1896) {left intestine (a) in Fig. 1 B is of *H. taichui* larva, right intestine (b) of *H. pumilio* larva}; C, F, I - *Haplorchoides mehrai* Pande et Shukla, 1976. A, B, C - free metacercariae, D, E, F, G, H, I - encysted larvae

genital sac, containing a feebly developed ventral sucker, and armed with sclerites and spines of shape, size and arrangement typical for each species (Pearson 1964; Pearson and Ow-Yang 1982). However, the isolation and subsequent examination of all metacercariae under light microscope is practically impossible during field epidemiological studies. Moreover, these features are not recognizable in low magnification. Consequently, morphological features visible under dissecting microscope are necessary for a correct routine identification. We have found such diagnostic criteria. However, morphological variability of some larvae (slightly moving or dead *O. viverrini* larvae, heterophyid metacercariae with a relatively large excretory bladder) made the

correct identification of all larvae under the dissecting microscope not possible. We conclude that a small sample of larvae from each fish host and all variable or dead metacercariae should be identified under the light microscope. Apart from being time-consuming, this method of fish examination, seems to be necessary for the correct identification of metacercariae. It will avoid misinterpretation of results during field studies on food-borne trematodes in Southeast Asia.

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