DEVELOPMENT OF *HAPLORCHIS TAICHUI* (TREMATODA: HETEROPHYIDAE) IN *MUS MUSCULUS* MICE

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Abstract. The development of *Haplorchis taichui* was studied in sixteen mice, *Mus musculus*. Metacercarial cysts of *H. taichui* were obtained from the freshwater fish, *Thynnichthys thynnoides*, collected in Chiang Mai Province, north Thailand. Approximately 200 active metacercariae were orally introduced into each mouse. Two mice were randomly sacrificed and necropsied daily from day 2-9 postinfection (pi). Two peaks of increment in the length and width of worms were found at day 3 and 7 pi. *H. taichui* was rapid in maturation, similar to other minute intestinal flukes. Rudimentary sex organs were found at day 2 pi. Spermatozoa in a seminal receptacle, vitellaria and eggs were seen as early as 3 days pi. The number of eggs increased daily to ≈50-60 eggs thereafter. Mice can serve as a suitable experimental definitive host for harvesting adult *H. taichui*, especially in 1-week pi.

INTRODUCTION

Haplorchis taichui is a minute intestinal fluke (MIF) of the Heterophyidae family that parasitizes the small intestine of several kinds of birds and mammals including humans (Faust and Nishigori, 1926). In Thailand, it has been reported as the largest degree of trematode recovered in the gastrointestinal tract of humans living in the northern region (Pungpak *et al*, 1998; Radomyos *et al*, 1998) and the most metacercarial species found in cyprinoid fish in northeastern and northern regions (Srisawangwong *et al*, 1997b; Sukontason *et al*, 1999). Although there is no report of clinical importance caused by *H. taichui*, other MIF can elicit a mild inflammatory reaction at the site of contact with the intestine or burrow into the mucosa. The worms produce mild irritation, accompanied by colicky pain and mucous diarrhea with production of excess mucous and a superficial necrosis of the mucous coat (Beaver *et al*, 1984).

Human infection with *H. taichui* is caused by consumption of raw and/or undercooked fresh water fish harboring the viable infective stage or metacercariae, in which they grow to be adults in the small intestine. Some of the Thai traditional food prepared from raw fresh water fish can serve as the source of infection (Sukontason *et al*, 1998). Although information is available on the development of MIF in experimental animals (Yasuraoka and Kojima, 1970; Lee *et al*, 1997; Srisawangwong *et al*, 1997a; Wongsawad *et al*, 1998; Chai *et al*, 1999), the *H. taichui* *per se* strain studied was from Formosa (Taiwan) (Faust and Nishigori, 1926). No detailed information regarding the infectivity and development of this parasite was found. Since parasitic stains may differ biologically and/or physiologically, the purpose of this study was to determine development of the parasite in mice with the Thai strain of *H. taichui*, with emphasis placed on the degree of infectivity, increment of body size and differentiation in the morphology of its genital organs.

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MATERIALS AND METHODS

Metacercarial cysts of *H. taichui* were obtained from the natural cyprinoid fish, *Thynnichthys thynnoides*, caught from Mae Ngud man-made reservoir of Chiang Mai Province, north Thailand. Metacercarial cysts were immediately collected using acid pepsin solution (conc hydrochloric acid 1 ml: pepsin (Sigma®, Germany) 1 g: 0.85% sodium chloride solution 99 ml) in mixer/blender at a ratio of 1 g of fish: 10 ml acid pepsin solution. The digested material was transferred into a water bath shaker for 1 1/2 h at 37 ˚C and subsequently passed through 2 layers of wet gauze. The digested material was then rinsed with normal physiological saline and examined for metacercariae with a stereo-microscope. The identification of metacercariae was carried out by morphological examination based on Pearson and Ow-Yang (1982) and Scholz *et al* (1991).

Infectivity and development of *H. taichui* was studied in 4-week-old mice (*Mus musculus*), weighing approximately 30 g each. Two hundred metacercarial cysts were washed in normal physiological saline before being introduced orally into each mouse using

a drawn out polyethylene tube (feeding tube). The mice were kept at room temperature and fed on commercial mouse food. Mice were sacrified at 2-9 days pi. The intestine was dissected, scraped and rinsed with normal physiological saline in a glass petri dish. The material inside the petri dish was then examined for adult *H. taichui* under the stereo-microscope. When found, all worms were rinsed briefly in normal physiological saline, fixed in hot alcohol-formalin-acetic acid, dehydrated in ethanol, stained in Mayer's carmine and mounted on a glass slide in Permount® (Fisher, Fairlawn, NJ). The internal organ development of worms was examined under light microscope at a magnification of 400 x and 1,000 x. The length and maximum width measurements of all worms recovered were made with the aid of a calibrated ocular micrometer.

RESULTS

All mice infected with *H. taichui* had mature worms in their intestine. When microscopically viewed, the worms were ovated to elongated in shape, and their usual posture resulted in them being convex dorsally and concave ventrally. The worms showed a pronounced oral sucker, while 16 sclerites on the ventrogenital sac were apparent. The alimentary canal seemed to be well developed. The esophagus was relatively long and bifurcated into the intestinal ceca extending exceed the testis. The excretory bladder was constricted in the extremity of the posterior, due to the growth of the testis.

The degree of infectivity in mice with *H. taichui* varied, as shown in Table 1. Average worm recovery rate was found mostly at 2 days pi (n = 21) followed at 4 days (n = 27) and decreased progressively at 6-9 days (n = 6-15).

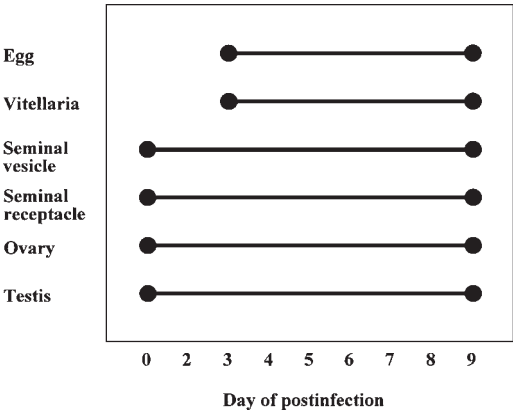


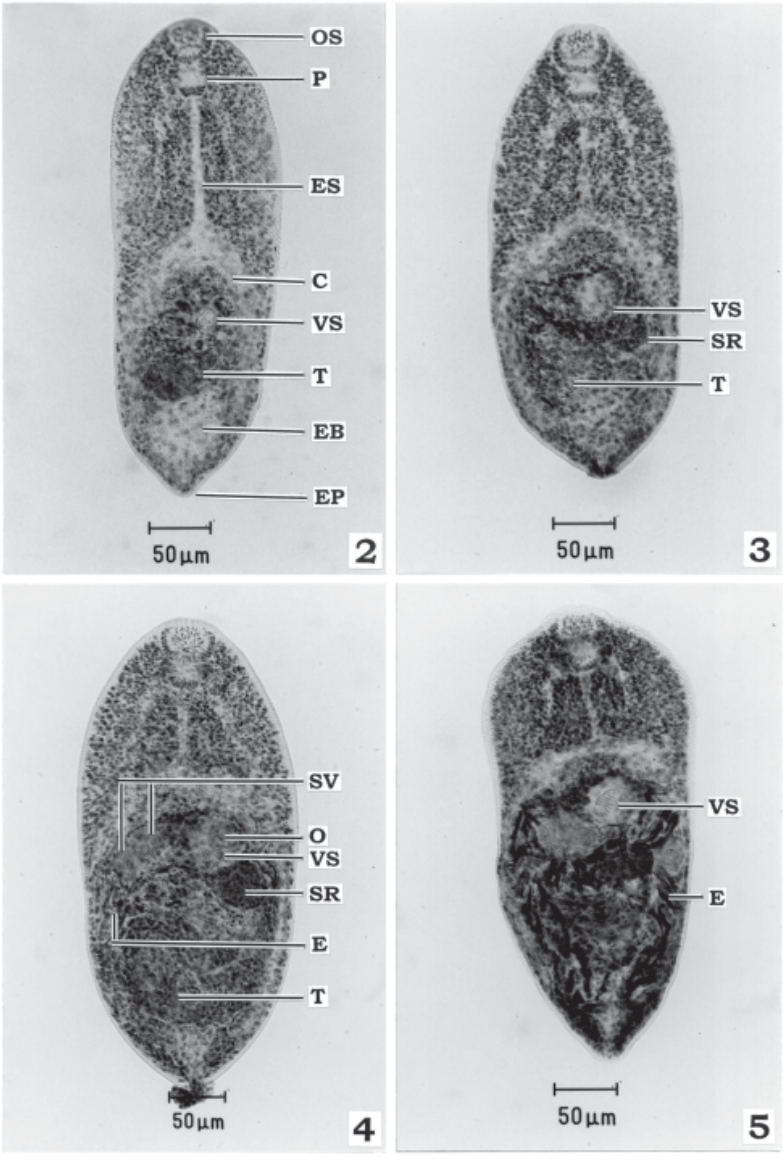
Fig 1- Appearance of the genital organs of *Haplorchis taichui* during the development in mice 2-9 days pi. These remarks are applied to specimens that developed at best each day. Note: Data of 0-day-old worms based on Sukontason *et al* (2000).

Table 1
Worm recovery rate and body measurement of *Haplorchis taichui* recovered from mice.

Day pi	Ave worms recovered	Median (range) of flukes in μm	
		Body length	Body width
0[a]	11	286 (220-346)	134 (116-147)
2	21	243 (223-375)	132 (101-162)
3	15	406 (203-497)[b]	152 (117-193)[b]
4	27	376 (304-507)[c]	183 (132-203)
5	19	304 (223-426)[c]	142 (112-183)[c]
6	9	406 (345-507)[b]	183 (152-213)[b]
7	6	548 (466-649)[b]	233 (203-254)[b]
8	9	314 (209-375)[c]	193 (132-213)[c]
9	15	304 (234-477)[c]	162 (142-193)

Total 2 mice were necropsied in each day of pi.
[a] Data from previous study by Sukontason *et al*, (2000).
[b] Significant increasing in size compared to that of the previous day at $p<0.05$.
[c] Significant decreasing in size compared to that of the previous day at $p<0.05$.

Body size measurement of *H. taichui* found at 2-9 days pi is also presented in Table 1. The length and maximum width of 0-day-old worms (newly excysted metacercariae) averaged 286 μm (range = 220-346) and 134 μm (range = 116-147), respectively (n = 11) (Sukontason *et al*, 2000). The increase in body length and width of the worms was found in two peaks. They were significantly increased from day 2 to day 3 and between day 5 to 7, while they were significantly decreased from day 3 to 5 and day 7 to 9 (Table 1).



Figs 2-5- Light micrographs of *Haplorchis taichui* grown in experimentally infected mice. (2) Two-day-old worm showing pronounced oral sucker, ventrogenital sac, pharynx, esophagus, branced intestinal ceca, enlarged excretory bladder. Testis, ovary, seminal vesicles and seminal receptacle are in the form of a condensation of cells. (3) Three-day-old worm showing enlarged genital organs. Some worms became ovigerous. (4) Four-day-old worm showing enlarged genital organs and diminished excretory bladder. Several eggs are seen. (5) Five-day-old worm showing attenuated posterior end and numerous eggs in the posterior half of body. Abbreviation: intestinal ceca (C), egg (E), excretory bladder (EB), excretory pore (EP), esophagus (ES), Ovary (O), oral sucker (OS), pharynx (P), seminal receptacle (SR), seminal vesicle (SV), testis (T), ventrogenital sac (VS).

Fig 1 shows the order and day of appearance for genital organs during the development of the parasite 2-9 days pi. The development at 0-day-old worms was also included.

Flukes recovered on day 2: This stage showed little difference from that in 0-day-old worms. The position of the testis could be detected by the presence of a single density of dark stained cells with a smooth outline anterior to the excretory bladder (Fig 2). The rudiment of ovary, seminal vesicles and seminal receptacle were formed as a condensation of cells in the proximity of the ventrogenital sac.

Flukes recovered on day 3: Considerable enlargement of the gonads was observed by this day. In contrast, the size of the excretory bladder became smaller. The worms showed the presence of clear testis, ovary, seminal vesicles and seminal receptacle (Fig 3). Vitellaria appeared along both posterior sides of the body. Spermatozoa were observed in the seminal receptacle of some worms examined. Some worms became ovigerous with a few eggs presented.

Flukes recovered on day 4: Four-day-old worms showed a presence of large testis, dark stained ovary and seminal receptacle, and light stained seminal vesicles. A lot of eggs were clearly seen in almost of worms examined (Fig 4).

Flukes recovered on day 5 to 9: Approximately 50-60 eggs appeared in each worm (Fig 5). Eggs are oval to elongated in shape, with an indistinct operculum seen.

DISCUSSION

Infections with digenetic trematodes in definitive hosts are acquired by the ingestion of encysted metacercariae. The initial stage of an infection involves excystment of the metacercariae followed by its attachment to the wall of the gut (Howell, 1970). The metacercarial excystment usually requires a number of extrinsic factors that are derived from the host (Fried, 1994). These factors within the gut of mice, *eg* trypsin, pH, temperature, bile extract etc, may be suitable to instigate the onset of activation within the cyst and eventually excystment to enable them to become established in the intestine.

Attempts to infect mice with metacercarial cysts of the Thai strain *H. taichui* proved successful. This reflected appropriate physio-chemical conditions for the excystment of *H. taichui* in the gut of mice. As far as the degree of infectivity is concerned, the worm recovery rate of *H. taichui* was, however, highly variable between mice necropsied 2-9 days pi. Dawes (1962) also found this phenomena in his *in vivo* studies of *Fasciola hepatica* in mice. It has been found in previous reports on other trematodes that the number of worms recovered is much smaller than the number of cysts administered (Schumacher, 1938; Dawes, 1962; Yasuraoka and Kojima, 1970; Chai *et al*, 1995; Humphries *et al*, 1997; Lee *et al*, 1997). The highest number of *H. taichui* was found in the intestine of mice during the first 5 days pi, which resembled *Stellantchasmus falcatus*, the other MIF, recovered mostly within the first 7 days pi in mice and chicks (Wongsawad *et al*, 1998). This was probably due to the micro-environment of mice being suitable for adult worms for the first few days (Wongsawad *et al*, 1998). On the other hand, the data of Chai *et al* (1995, 1999) suggested that the immune response of the host resulted in the difference of *M. yokogawai* worm recovery rate between immunocompetent and immunosuppressed mice. Regarding this, the host immune defense mechanism might be involved in the worm's infectivity.

Adults *H. taichui* from the infected mice grew increasingly and became ovigerous as early as 3 days pi, thus, reflecting the rapid maturation of *H. taichui* in experimental animals. This result was similar to several species of MIF [*eg M. yokogawai* (Yasuraoka and Kojima, 1970; Chai *et al*, 1995), *Centrocestus formosanus* (Srisawangwong *et al*, 1997a), *Plagiorchis muris* (Hong *et al*, 1998), *S. falcatus* (Wongsawad *et al*, 1998)] that developed speedily to adulthood and became ovigerous within a few days. The rapid maturation might be attributable to the genital organs already possessed in the metacercarial stage (Faust and Nishigori, 1926).

In digenetic trematodes, it has been assumed that cross-fertilization is mandatory for worms to become ovigerous. According to Yasuraoka and Kojima (1970), the production of normally viable eggs requires cross-fertilization in some species, but not in others. Mono-metacercarial infection with *M. yokogawai* resulted in adult worms capable of producing eggs (Yasuraoka and Kojima, 1970). This indicated that cross-fertilization might not be prerequisite. Whether *H. taichui* adult worms require cross-fertilization to produce eggs, and whether these eggs are viable, have been questioned, and this merits further studies.

The rapid maturation of *H. taichui* in mice agreed with Faust and Nishigori (1926) that mice can serve as the appropriate experimental definitive host for this parasite; but a good result yielded for only the first 5 days. This information may be applied for harvesting mature worms from mice, from which they can be utilized further in a topographic study and/or studies of molecular biology and immunology.

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