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Haplorchis taichui: Worm recovery rate and immune responses in infected rats (*Rattus norvegicus*)

Supap Saenphet [a,*], Chalobol Wongsawad [a], Kanokporn Saenphet [a], Amnat Rojanapaibul [a], Pramote Vanittanakom [b], Jong-Yil Chai [c]

[a] *Department of Biology, Faculty of Science, Chiang Mai University, Chiangmai, Chiang Mai 50200, Thailand*
[b] *Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*
[c] *Department of Parasitology, College of Medicine and Institute of Endemic Diseases, Seoul National University Medical Research Center, Seoul 110-799, Republic of Korea*

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ABSTRACT

Worm recovery rate, mucosal mast cells (MMCs), eosinophils and serum IgE concentration in rats were investigated after orally feeding 300 *Haplorchis taichui* metacercariae to male rats. The duodenal, jejunal and ileal tissue sections were stained with 1% alcian blue and 0.5% safranin-O for MMC count. Eosinophil count and the serum IgE concentration assay were measured from cardiac puncture blood. The average worm recovery rates were 20.00%, 13.00%, 0.67%, 1.67% and 0.00% on day 3, 7, 14, 21 and 28 post-infection (PI), respectively. The number of MMCs in the infected rats were significantly higher than in the controls ($P<0.01$), reaching a peak on day 21 PI. They decreased thereafter, with the decline in worm recovery. Eosinophil count and Serum IgE concentration were also increased but not significantly higher than the controls. However, they showed a positive relationship to worm recovery. It could be concluded from the results that MMCs, eosinophils and IgE may play an important role in the expulsion of *H. taichui* from rat intestine. However, the mechanism by which the MMC result in the helminth expulsion still need to be understood, and it is recommended that other cells such as goblet cells be studied further.

1. Introduction

Heterophyid fluke infections are an important public health problem in Southeast and Far-East Asia. They inhabit the intestinal mucosa, where they cause a mild inflammatory reaction with some necrosis (Chi et al., 1988), acute abdominal pain or appendicitis (Tantachumrun and Kliks, 1978). The eggs may also be deposited in the heart, brain, spinal cord and other organs. The lesions found in the myocardium led to heart failure in some patients (Africa and Garcia, 1935; Africa et al., 1936a,b). Among heterophyid infections in man, *Haplorchis taichui* was first documented from a human autopsy in Udonthani Province, Thailand (Manning et al., 1971). Later, various *Haplorchis* species have been reported in Taiwan, Laos PDR, Thailand and the Philippines (Ditrich et al., 1990; Giboda et al., 1991; Waikagul, 1991; Radomyos et al., 1994). A survey of metacercariae in fish showed that the *H. taichui* was the dominant species of *Heterophyidae* family (Waikagul, 1998). It is predictable that *H. taichui* infection will also become the most common species of intestinal fluke in Thailand in the near future.

In laboratory infections it was shown that the recovery of *H. taichui* from the gut of infected mice and chicks gradually decreased with the increasing time (Sukontason et al., 2001; Kumchoo et al., 2003). Mechanisms such as inappropriate ecological niche and the host immune response have been considered to describe this phenomenon. Helminth infections in mice and humans usually induce Th_2 cell responses (Else et al., 1993; Pearlman et al., 1993; Jabara et al., 1988; Mossman, 1991). Activated Th_2 cells secrete cytokines; Interleukin (IL) 5, IL-4, IL-9 and IL-10, resulting in the induction of intestinal mastocytosis, blood and tissue eosinophilia, and immunoglobulin (Ig) secretion by B cells, particularly IgE and IgG_1 (Mossman and Coffman, 1989; Mossman, 1991; Finkelman et al., 1991; Thompson-Snipes et al., 1991). In *Nippostrongylus brasilensis* infection, the characteristic immune responses of the host are eosinophilia, elevated serum IgE, mucosal mastocytosis and goblet cell hyperplasia (Rennick et al., 1990; Abe et al., 1993; Uchikawa et al., 1994; Chen et al., 1995). However, no information is available regarding of the host immune response to *H. taichui* infection.

2. Materials and methods

2.1. Collection of H. taichui metacercariae and infection to rats

The metacercariae of *H. taichui* were procured from *Henicorhyncus siamens* is, collected from Mae-ngud Reservoir, by pepsin digestion method as previously described (Srisawangwong et al., 1997).

* Corresponding author. Fax: +66 53 892259.
E-mail addresses: papom2345@yahoo.com, saenphet@chiangmai.ac.th (S. Saenphet).

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The fish homogenate was filtered through a sieve mesh series, and the filtrate was rinsed repeatedly with normal saline. Finally, the metacercariae were isolated and counted under a dissecting stereomicroscope. The morphology of the *H. taichui* metacercariae was characterized according to the description of Yamaguti (1958) and Pearson and Ow-Yang (1982).

Thirty male rats, aged between 4 and 6 weeks old, were purchased from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom Province, Thailand. After acclimatizing for a week in the Animal House, Department of Biology, Faculty of Science, Chiang Mai University; a single inoculation of 300 active metacercariae of *H. taichui* was orally introduced into the rats ($n=25$) and the rest of the rats served as uninfected controls ($n=5$). The rats were fed a standard diet (C.P. 082) and water *ad libitum*. All experimental procedures were in accordance with Institutional regulations for Animal Care and Use (No. Re 001/07), Department of Biology, Faculty of Science, Chiang Mai University.

2.2. Worm recovery rate and mucosal mast cell (MMC) counts

Five rats from each infected group were sacrificed on day 3, 7, 14, 21 and 28 post-infection (PI), respectively. To observe the worm recovery rate, the duodenum, jejunum and ileum, from three infected rats, were removed and opened longitudinally. Each segment was placed the Baermann's apparatus for 1.5 h at 37 °C with periodically shaking (Chai et al., 1998). The free adult worms were collected and counted under a dissecting stereomicroscope. To evaluate the MMC count, 5–10 mm of duodenum, jejunum and ileum from both infected ($n=5$, in each group) and control rats ($n=1$, in each group) were removed and fixed in Carnoy's solution; dehydrated and embedded in paraffin. The sections, cut at 5 μ thickness, were stained with 1% alcian blue (pH 0.3) and counterstained with 0.5% safranin-O (pH 1.0) (Strobel et al., 1981). The numbers of MMC were counted in well-oriented sections using an eyepiece equipped with a graticule (10/100) at 10× and objective lens at 40×. At least five fields of each tissue section were counted. The mean numbers of MMC/0.0625 mm^2 was expressed as mean and standard deviation and analysed using the Student *t*-test.

2.3. Eosinophil counts

At least 2.0 ml of blood from both infected and control rats were collected from the left ventricle for eosinophil count and serum IgE concentration. For eosinophil count, 20 μl of EDTA-mixed blood was combined with Hinkelman's solution and gently agitated for 3 min. A direct eosinophil count was measured using an improved Neubauer hemocytometer.

2.4. Serum immunoglobulin E (IgE) concentration assays

The remaining blood from the cardiac puncture was centrifuged to obtain serum. IgE concentration measurements were performed using a rat IgE ELISA quantitation kit (Bethyl Laboratories, Inc.). Briefly, 96-well plates (NUNC) were coated with 10 μg/ml of captured antibody (sheep anti-rat IgE, sigma) in carbonate buffer and then incubated at 4 °C overnight. Plates were washed with PBS/Tween 20 (sigma) for 1–2 times. Standards and sera (1:50) were serially diluted with sample diluents (1% BSA/PBS, 0.05% Tween 20, pH 8.0) and the total 100 μl were added to microwells. The plates were incubated at room temperature for 2 h, washed 4–5 times with PBS/Tween 20, pH 8.0. The detection antibodies (sheep anti-rat IgE labeled with horse radish peroxidase, Sigma; 1:2500) were diluted with 1% BSA/PBS, 0.05% Tween 20, pH 8.0 and 100 μl was added and incubated at room temperature for 1 h. The plates were washed 4–5 times with PBS/Tween 20, pH 8.0. The enzyme reaction was started by adding a substrate (TMB, Sigma), incubated in the dark at room temperature for 30 min and stopped by adding 100 μl of 1 M H_2SO_4. The absorbance at 450/630 was measured by Anthos 2010 (Austria) microplate reader. The results were compared to the control groups and the values were expressed as mean ± SD.

Table 1
Mean worm recovery rate in rats infected with *H. taichui*

	No. of rats	No. of Mc. infected	Mean numbers of worm recovery				
			Duodenum	Jejunum	Ileum	Total	%
Day 3	3	300	0.00	0.00	60.00 ± 9.17	60.00 ± 9.17	20.00
Day 7	3	300	0.00	0.00	39.00 ± 2.00	39.00 ± 2.00	13.00
Day 14	3	300	0.00	0.00	2.00 ± 1.15	2.00 ± 1.15	0.67
Day 21	3	300	0.00	0.00	5.00 ± 2.89	5.00 ± 2.89	1.67
Day 28	3	300	0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Total	15	1500	0.00	0.00	106.00 ± 3.59	106.00 ± 3.59	7.07

Mc, metacercariae.

3. Results

3.1. Worm recovery rate

The average worm recovery rate was 7.07% with the highest rate of 20.00% on day 3 PI. Recoveries remained relatively high until day 7 PI (13.00%) and decreased rapidly to 0.67% on day 14 PI, 1.67% on day 21 PI, disappearing by day 28 PI. Worms were only recovered from the ileum (Table 1). The incidence of *H. taichui* in the small intestines of rats was 60.00% (9/15) and the intensity ranged from 0 to 60.

3.2. MMC counts

The kinetics of MMC numbers is shown in Table 2 and Fig. 1. MMC numbers in the ileum were slightly higher than those in the duodenum and jejunum. The peak level of mastocytosis was observed on day 21 PI. The MMC number in the infected rats was significantly increased in all segments of the small intestine, through the whole period of observation as compared with uninfected controls. They had a tendency to decrease along with the decline in the worm numbers.

Table 2
Mean numbers of mucosal mast cells in small intestines of rats infected with *H. taichui*

Day PI	No. of rats	No. of Mc. infected	Mean no. of MMCs/0.0625 mm^2 (mean ± SD)		
			Duodenum	Jejunum	Ileum
Control					
Day 3	1#	0.00	17.80 ± 0.57	17.10 ± 0.14	17.65 ± 0.78
Day 7	1#	0.00	14.45 ± 0.21	17.00 ± 0.85	15.05 ± 1.48
Day 14	1#	0.00	17.85 ± 0.78	17.55 ± 0.49	17.10 ± 0.99
Day 21	1#	0.00	17.65 ± 0.64	17.30 ± 0.99	15.30 ± 1.70
Day 28	1#	0.00	16.55 ± 0.07	15.50 ± 0.42	15.35 ± 0.07
Infected					
Day 3	5	300	29.40 ± 0.14	30.80 ± 1.13	29.70 ± 1.70
Day 7	5	300	28.55 ± 0.35	27.80 ± 3.39	28.80 ± 0.57
Day 14	5	300	31.85 ± 1.91	32.60 ± 2.26	37.20 ± 1.70
Day 21	5	300	36.80 ± 1.84	37.20 ± 1.70	41.25 ± 1.06
Day 28	5	300	32.50 ± 0.71	22.40 ± 3.11	28.50 ± 0.71

Mc, metacercariae; 1#, the shared data from other two experiments (*Stellantchasmus falcatus* and *Centrocestus caninus* infection groups) were used for increasing the effective number of control animals available for each experimental time to 3. As can be seen from the narrow SDs, there is little variation in uninfected animals.