

## MUCOSAL MAST CELL RESPONSES IN RATS (*RATTUS NORVEGICUS*) EXPERIMENTALLY INFECTED WITH *CENTROCESTUS CANINUS*

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**Abstract.** Mucosal mast cell (MMC) responses and worm recovery rates in rats experimentally infected with *Centrocestus caninus* were investigated. Metacercariae of *C. caninus*, procured from goldfish, *Carassius auratus*, were orally administered to twenty-five male rats (300 metacercariae each rat). The infected rats were sacrificed on days 3, 7, 14, 21 and 28 post-infection (PI) along with the control rats. Worm recovery was performed from each part of small intestine. To investigate MMC, duodenal, jejunal and ileal paraffinized-tissue sections were processed and stained with 1% alcian blue and 0.5% safranin-O. The average worm recovery rates were 42.8, 37.7, 21.2, 12.5 and 3.7% on days 3, 7, 14, 21 and 28 PI, respectively. The majority of the worms (98.9%) were collected from the duodenum and jejunum. The MMC numbers in the infected rats were significantly higher than those of the controls ( $p < 0.05$ ). A peak level was observed on days 14 PI and the numbers gradually decreased thereafter. The results reveal that MMC plays an important role in the expulsion of *C. caninus* from the host intestine. A more precise description of the role the MMC plays in helminth expulsion is still needed to understand the mechanism of host defense against intestinal helminthic infection, along with other effector cells, such as goblet cells.

### INTRODUCTION

*Centrocestus caninus* (Trematoda: Heterophyidae) is a food-borne parasite, infecting the small intestines of birds and mammals. Its morphological characteristics include 26-32 circumoral spines arranged alternately in two rows around an oral sucker and a distinct 'X'-shaped excretory bladder at the posterior end. The number of the circumoral spines is the most reliable character for species classification (Yamaguti, 1958). Recently, *Centrocestus* metacercariae have been reported in several kinds of freshwater fish from Nakhon Nayok Province (Waikagul *et al*, 1990), Khon Kaen Province (Komalamisra and Setasuban, 1989; Srisawangwong *et al*, 1997) and Chiang Mai Province (Wongsawad *et al*, 2000). A natural infection of *Centrocestus* sp in rats was reported in Chiang Mai Province

(Namue and Wongsawad, 1997). In Thailand, natural human infection with *C. caninus* was first reported in Chiang Mai and Chiang Rai Provinces (Waikagul *et al*, 1997; Radomyos *et al*, 1998). *C. caninus* and *C. formosanus* have been reported in humans in Taiwan (Chen, 1942). *C. armatus* has also been reported in humans in Korea (Hong *et al*, 1988, 1989).

In the laboratory the number of adult worms decreased as the experimental time progressed. Many mechanisms have been suggested for this observation, such as host immune defense, goblet cell hyperplasia and different nutritional status in various hosts (Miller, 1984; Fujino *et al*, 1993; Kim *et al*, 2000). With regard to host defense mechanism, mast cells have been reported to be an important effector cell in expulsion of some intestinal helminths (Woodbury *et al*, 1984; Chai *et al*, 1993; Kim *et al*, 2000). Little is known about the mucosal mast cell response in this trematode infection.

This study set out to observe the dynamics of intestinal mast cells at various sites in the small intestine of rats infected with the heterophyid

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trematode, *C. caninus*.

## MATERIALS AND METHODS

### Collection of metacercariae

The metacercariae of *C. caninus* were collected from the gills of goldfish, *Carassius auratus*. Briefly, after being sacrificed, the gills were removed and then minced in a blender. They were then mixed with 1% pepsin solution, containing 1 g of pepsin (porcine, Sigma) and 1 ml of concentrated hydrochloric acid and then adjusted to 100 ml with distilled water. The specimens were then incubated at 37°C in a shaking water bath for 1.5 hours. The digested material was filtered through a sieve (size 60 µm) and the filtrate was repeatedly rinsed with normal saline. Finally, the metacercariae were collected and counted under a stereo microscope.

### Infection to rats

Thirty male rats, age 4-6 weeks old, were purchased from the National Laboratory Animal Center, Nakhon Pathom Province, Thailand. After acclimatized for 1 week in the animal house, Department of Biology, Faculty of Science, Chiang Mai University, Thailand, a single inoculation of 300 active metacercariae of *C. caninus* was orally introduced into the rats ( $n = 25$ ). The rest of the rats served as uninfected controls ( $n = 5$ ). The rats were fed with a standard diet (C.P. 082) and water *ad libitum*.

### Worm recovery rate and mucosal mast cell (MMC) counts

Five rats from the infected group were

sacrificed on days 3, 7, 14, 21, and 28 PI, respectively. To observe the worm recovery rate, three segments of the small intestine were removed and opened longitudinally. Each segment was placed on a Baermann's apparatus for 1.5 hours at 37°C (Chai *et al.*, 1998). The free adult worms were collected and counted under a dissecting stereo microscope. To investigate the MMC count, 5-10 mm of duodenum, jejunum and ileum from both infected and control rats were fixed in Carnoy's solution for 4-6 hours, 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 (Strobel *et al.*, 1981). The MMC numbers were counted in well-oriented cross-sections using an eyepiece equipped with a graticule (10/100) at 40x power, at least 5 fields of each tissue section were counted. The number of MMC/0.0625 mm<sup>2</sup> was expressed as a mean and standard deviation. The value was analysed using the Student's *t*-test.

## RESULTS

### Worm recovery rate in rats

The average worm recovery rate was 23.6%, with the highest rate of 42.8% on days 3 PI. The rate was relatively high from days 3 to 21 PI, after which it decrease rapidly from 12.5% on day 21 PI to 3.7% on day 28 PI (Fig 1). Of 353.5 recovered worms, most were collected from the duodenum and jejunum (98.9%). They were few found in the ileum (1.13%) (Table 1).

Table 1  
Mean worm recovery rates in rats infected with *C. caninus*.

Day PI	No. of rats	No. of Mc introduced	Mean numbers of worm recovery			
			Duodenum	Jejunum	Ileum	Total
Day 3	3	300	43.0	81.5	4.0	128.5
Day 7	3	300	40.5	72.5	0.0	113.0
Day 14	3	300	32.5	31.0	0.0	63.5
Day 21	3	300	25.0	12.5	0.0	37.5
Day 28	3	300	10.0	1.0	0.0	11.0
Total	15	1,500	151.0	198.5	4.0	353.5

Mc. : metacercariae

Table 2  
Mean numbers of mucosal mast cell (MMC) in rats infected with *C. caninus*.

Day PI	No. of Rats	No. of Mc Introduced	No. of MMC (mean±SD)		
			Duodenum	Jejunum	Ileum
Control					
Day 3	3	0	17.80±0.57	17.10±0.14	17.65±0.78
Day 7	3	0	14.45±0.21	17.00±0.85	15.05±1.48
Day 14	3	0	17.85±0.78	17.55±0.49	17.10±0.99
Day 21	3	0	17.65±0.64	17.30±0.99	15.30±1.70
Day 28	3	0	16.55±0.07	15.50±0.42	15.35±0.07
Infected					
Day 3	2	300	30.67±2.61	31.50±1.56	27.00±3.39
Day 7	2	300	29.80±1.98	36.10±6.08	22.90±1.56
Day 14	2	300	44.10±4.58	35.60±1.41	26.50±1.84
Day 21	2	300	41.25±1.06	37.30±7.21	29.40±0.57
Day 28	2	300	30.50±3.54	29.60±0.57	23.80±3.11

Mc : metacercariae

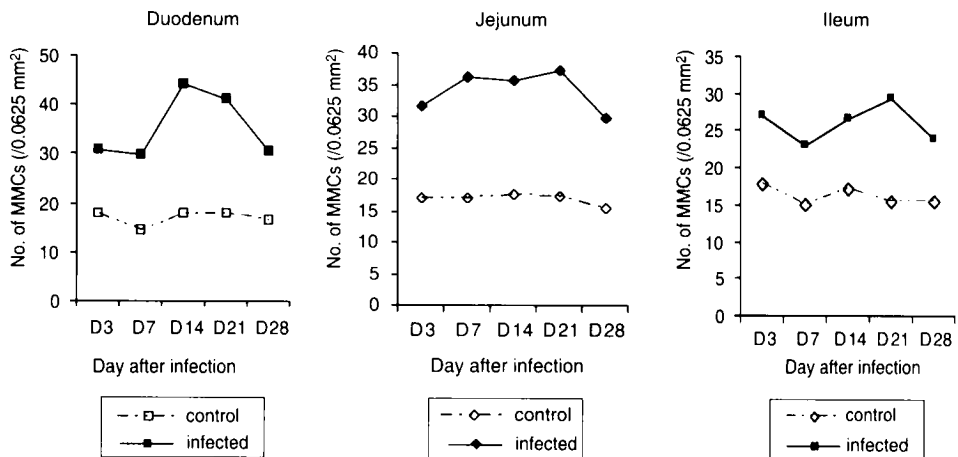


Fig 1—Dynamics of MMC in different sites of the small intestine of rats infected with *C. caninus*, demonstrating that the numbers of MMC (cells/0.0625 mm<sup>2</sup>) were increased throughout the entire length of small intestine, but especially in the duodenum and jejunum, where the worms were located.

### The MMC counted in rats

The dynamics of the MMC counts are demonstrated in Table 2 and Fig 1. The intestinal MMC are shown in Fig 2. The number of MMC increased to nearly twice as much as the controls. The MMC were generally observed throughout the small intestine. However, they were markedly increased in the duodenum on days 14 PI and in the jejunum and ileum on days 21 PI in which the worm recovery rate was low.

The number of MMC in the ileum was lower than the duodenum. They had a tendency to decrease with declining numbers of worms.

### DISCUSSION

Recovery of *C. caninus* was mainly in the duodenum and jejunum. This worm survives in the duodenum where multiple biochemical processes are occurring, indicating this segment of

MMC RESPONSES IN *C. CANINUS* INFECTED RATS

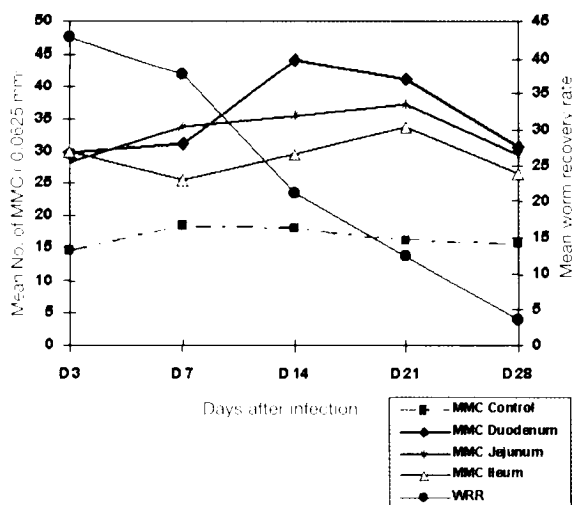


Fig 2—Correlations between the mean worm recovery rate (WRR) and the mean number of mucosal mast cells at different sites in the small intestine of rats infected with *C. caninus*. The highest number of MMC was observed in duodenum, where the worm recovery rate was dramatically lower.

small intestine must form an ecological niche for its development. In contrast to the other two heterophyid flukes, *Stellantchasmus falcatus* and *Haplorchis taichui*, which are located mainly in the ileum (Wongsawad *et al.*, 1998; Saenphet *et al.*, 2002; Kumchoo *et al.*, 2003). Human infection with *C. caninus* had been reported in Thailand and Taiwan (Waikagul *et al.*, 1997). The reservoirs are dogs, cats and rats, but the main sources of infection are fish (*Channa formosana*, *Cyprinus carpio*, and *C. auratus*), frogs and toads (Chen, 1942; Martin, 1958). In the laboratory, however, the number of worms tends to decrease over time. It has been suggested that physicochemical factors and other factors, such as host immunity, are involved in this phenomenon.

Our study demonstrated that experimentally infected rats had higher numbers

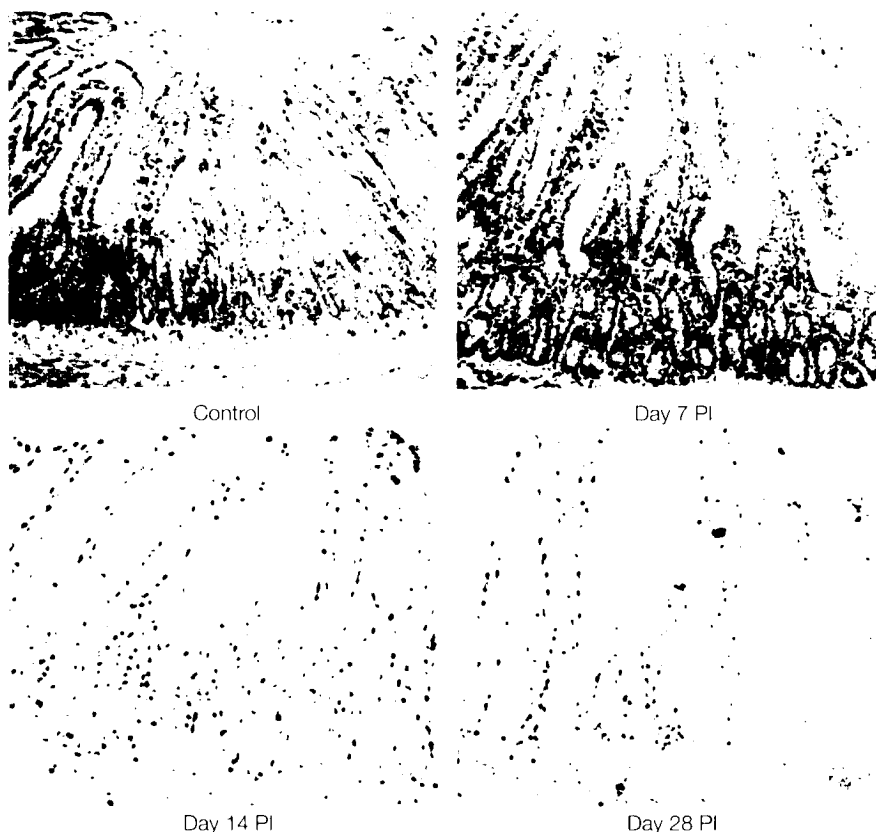


Fig 3—Sections of the duodenum of rats infected with *C. caninus*, compared with controls. Control rats showed only a few mucosal mast cells (MMC, blue spots). The numbers of MMC in infected rats gradually increased after days 3 PI and reached at peak level on days 14 PI and thereafter gradually declined. Alcian blue/safranin-O stain, 100X.

of MMC in all parts of the small intestine than the control rats. Increased numbers of MMC were found in all parts of the small intestine of infected rats, but particularly the duodenum and jejunum where the worms were focally collected. The peak intestinal mastocytosis level was observed on days 14 PI when the worm recovery rate decreased abruptly. This association between the decline in worm recovery and increased numbers of MMC was positively correlated, suggesting this response may be involved in the process of worm expulsion from the host intestine other than the environmental niche. Intestinal mastocytosis has been reported as an important mechanism for expelling other intestinal trematode infections, such as *E. hortense* (Kim *et al.*, 2000) and another heterophyid fluke, *M. yokogawai* (Chai *et al.*, 1993). The number of mucosal mast cells has also shown to be increased during the acute and chronic phases of experimental *Schistosoma mansoni* infection in mice (Kermanizadeh *et al.*, 2001). Lantz *et al.* (1998) demonstrated that expulsion of some intestinal nematodes from the intestines of the mast cell-deficient (W/W<sup>-</sup>) mice and mice lacking the IL-3, the mast cell-stimulating cytokine, was severely impaired. Increased numbers of mast cells had been reported in rats infected with tapeworms, such as *Hymenolepis diminuta* and *H. microstoma* (Andreassen *et al.*, 1978; Novak and Nombrado, 1988) and observed in rats experimentally infected with *Fibricola seoulensis* (Kho *et al.*, 1990). However, increased numbers of mucosal mast cells were seen after *Strongyloides ratti* and *Trichinella spiralis* worms were expelled (Mimori *et al.*, 1982; Woodbury *et al.*, 1984). Miller (1984) stressed that the host response to parasitic helminth infection in the intestine is to increase mast cells numbers in the mucosa. However, there is evidence that the effector cell for the expulsion of *Nippostrongylus brasiliensis* is not the mast cell but a goblet cell (Nawa and Korenaga, 1983). In mice infected with *Echinostoma trivolvis*, the expulsion of the worm was associated with goblet cell hyperplasia (Fujino *et al.*, 1993). From these studies, we may conclude that primary effector cells in immune responses against intestinal parasitic helminth infection may be different depending on the physico-biological properties of both para-

site and host species.

It can be clearly concluded from the results that MMC plays an important role in the expulsion of *C. caninus* from the host intestine. However, the precise role of MMC in helminth expulsion still needs to be understood. There is also the need to study further other effector cells, such as goblet cells.

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