CHRONOLOGICAL OBSERVATIONS OF INTESTINAL HISTOPATHOLOGY IN RATS (*RATTUS NORVEGICUS*) INFECTED WITH *CENTROCESTUS CANINUS*

Supap Saenphet[1], Chalobol Wongsawad[1], Kanokporn Saenphet[1], Amnat Rojanapaibul[1], Pramote Vanittanakom[2] and Jong-Yil Chai[3]

[1]Department of Biology, Faculty of Science; [2]Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; [3]Department of Parasitology and Tropical Medicine, Seoul National University, College of Medicine, and Institute of Endemic Diseases, Seoul National University Medical Research Center, Seoul, Korea

Abstract. Intestinal pathological enzyme activity changes were studied chronologically in rats after *Centrocestus caninus* infection. A single inoculation of 300 metacercariae isolated from the gills of goldfish (*Carassius auratus*), was orally administered to male rats (n = 15). Uninfected animals were used as controls (n = 5). At days 3, 7, 14, 21, and 28 post-infection (PI), three infected rats, and one from each control group, were sacrificed. The duodenum, jejunum, and ileum were removed separately and fixed in 10% formalin and 10% cold formal calcium solution for histopathological and alkaline phosphatase activity investigations, respectively. The worms were found intruded into the intervillous space of the mucosa and the mucosa showed villous atrophy, crypt hyperplasia and stromal inflammation with inflammatory cell accumulations. Alkaline phosphatase (ALP) activity also showed retardation. However, it seemed that these phenomena would return to normal at the end of the experiment. It can be concluded, from our data, that *C. caninus* could cause mild histopathological alterations and reduce ALP activity in the small intestines.

INTRODUCTION

Centrocestus caninus (Trematoda: Heterophyidae) is a minute food-borne parasite inhabiting the small intestines of birds and mammals (Yamaguti, 1958). Natural human infection by *C. caninus* in Thailand was first reported from Chiang Mai and Chiang Rai provinces (Waikagul *et al*, 1997). Moreover, a synonym species of *C. caninus, C. formosanus,* was reported in humans from Taiwan who had eaten partially cooked freshwater fish, frogs, and a toad (Chen, 1942), and from India (Nath and Pande, 1970). Natural infection with *C. armatus* has also been reported in humans in Korea (Hong *et al*, 1988).

It has long been known that the epithelial cells lining the villi of the small intestine contain many enzymes for final digestion, such as disaccharidase, peptidase, and alkaline phosphatase (Dawson and Pryse-Davies, 1963; Forstner *et al*, 1968; Fujita *et al*, 1972). These enzymes activities are reduced if the villous epithelial cells are destroyed by diseases such as coeliac disease. The pathology of this heterophyid infection has not yet been reported in Thailand and because of the zoonotic significance of this trematode, the chronologically histopathological and alkaline phosphatase activity changes in the intestines of rats experimentally infected with *C. caninus* was first described in Thailand.

Correspondence: Supap Saenphet, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50202, Thailand.
Tel: 66 (0) 53 943 346-61
E-mail: saenphet@chiangmai.ac.th; supap_saenphet@yahoo.co.th

MATERIALS AND METHODS

Collection of metacercariae

Metacercariae of *C. caninus* were collected from the gills of goldfish, *Carassius auratus*, by digestion technique. Briefly, after being sacrificed, the fish gills were removed and mixed with 1% pepsin solution, then minced with a blender and incubated at 37 ºC for 1.5 hours in a shaking water bath. The digested material was filtered through a sieve (size 60 μm) and the filtrate rinsed several times with normal saline. Then, the metacercariae were collected and counted under a stereomicroscope.

Infection of rats

Twenty male rats, aged 4-6 weeks, were purchased from Sala Ya, Mahidol University, Nakhon Pathom Province. After acclimatization for 1 week in the animal house, Department of Biology, Faculty of Science, Chiang Mai University, Thailand, a single inoculation of 300 of *C. caninus* metacercariae was administered orally to each rat (n = 15); the rest served as uninfected controls (n = 5). The rats were fed with food and water *ad libitum*.

Histopathological observation

Three rats from the infected group were sacrificed on each of days 3, 7, 14, 21, and 28 post-infection (PI) for pathological investigation. Five to ten millimeters of duodenum, jejunum, and ileum were removed and fixed with 10% formalin solution for 24 hours. They were then prepared following routine histopathological processes and stained with hematoxylin and eosin. For alkaline phosphatase activity, 5-10 mm of each small-intestine segment was immersed in 10% cold formal calcium solution for 24 hours. After tissue processing, alkaline phosphatase activity assay was performed on 5-μm paraffin sections using biotinylated mouse anti-human alkaline phosphatase antibody (R and D Systems, South Korea) (modified from Hsu *et al*, 1981).

RESULTS

Histopathological observations: a summary of the pathological changes in rat small intestines infected with 300 *C. caninus* metacercariae is shown in Table 1. Pathological changes were mainly seen in the duodenum; pathologic findings were identical for days 3 to 14 PI. Some worms were located between the intervillous spaces of the small intestines and some were observed deeper against the outlet of the crypts (Fig 1a). Interestingly, some worms were found to invade closely to the muscularis mucosae layer of the duodenum (Fig 1b). However, the worms were confined to the mucosal layer of the small intestines. Pathologically, epithelial compression was occasionally seen around the site of worm attachment (Fig 1a). The tip of villous erosion and adhesion of villi were clearly observed in the duodenum (Fig 1c). The villous/crypt ratios were decreased. Crypt hyperplasia was also observed. Stromal inflammation was apparently infiltrated with inflammatory cells, such as eosinophils and lymphocytes (Fig 1d). On days 21 to 28 PI, however, pathologic findings were closely restored to the controls. The villous and crypt ratio returned to a normal range. However, the stroma were still infiltrated with inflammatory cells but of a smaller degree.

Alkaline phosphatase activity: degrees of ALP activity in the small intestines of infected rats were much lower than the control. Particularly, in days 3-21 PI, duodenum ALP activities were lower (Fig 2b, 2c) than the controls (Fig 2a). At the end of the experiment, ALP activity tended to return to levels close to the control (Fig 2d). ALP in the jejunum and ileum seemed to be unaffected by this infection, and demonstrated similar degrees of ALP activity throughout the experiment.

Table 1
Histopathological alterations in small intestines of *C. caninus*-infected rats.

Microscopic findings	Degree of pathologic change														
	3 days			7 days			14 days			21 days			28 days		
	D	J	I	D	J	I	D	J	I	D	J	I	D	J	I
Changes of villi															
Blunting	≠	-	+	≠	+	+	+	+	+	+	+	-	+	-	-
Thickening	+	-	-	+	+	-	+	-	-	+	-	-	+	-	-
Adhesion	+	+	-	+	+	-	+	+	+	+	+	-	+	-	-
Tip erosion	+	+	-	-	+	-	+	-	-	+	-	-	+	-	-
Loss of villi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stromal changes															
Edema	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
Congestion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cell infiltration	≠	≠	+	+	≠	≠	≠	≠	+	≠	≠	+	≠	+	+
Fibrosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Crypt changes															
Epithelial hyperplasia	+	+	+	≠	≠	+	≠	≠	+	≠	+	+	+	+	+
Villus/crypt ratio	3/1	3/1	3/1	3/1	3/1	3/1	3/1	4/1	4/1	4/1	3/1	3/1	4/1	4/1	4/1
Sectioned worms	4	2	0	0	1	0	0	0	0	0	1	0	0	0	0

- : no changes, + : slight, ≠ : moderate
D : Duodenum, J : Jejunum, I : Ileum

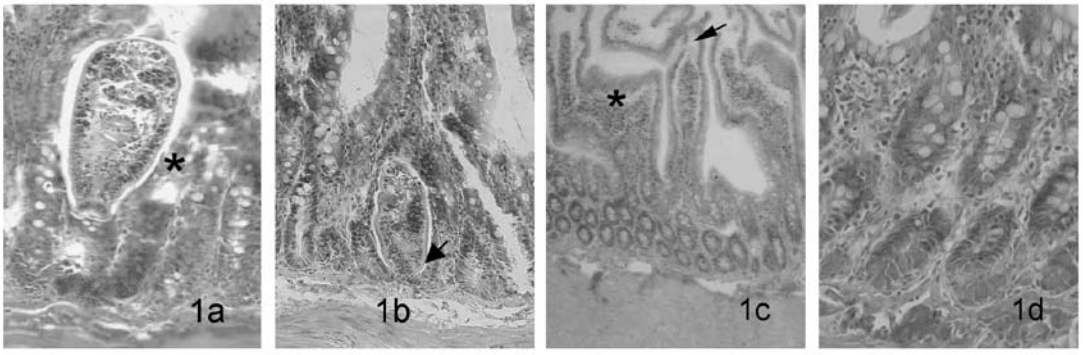

Fig 1- 1a Duodenum; day 3 PI, worm was facing against the opening of the crypt and epithelial compression (*) were also observed nearby the site of attachment. H&E. 20X; 1b duodenum; day 3 PI, some of the worms (arrow) were found invaded deeply into the muscularis mucosae. H&E. 10X; 1c duodenum; day 14 PI, tip if villous erosion (arrow) and villous fusion (*) were observed. H&E. 20X; 1d Duodenum; dy 14 PI, crypt hyperplasia and accumulation of eosinophils and lymphocytes were observed in lamina propria. H&E. 40X.

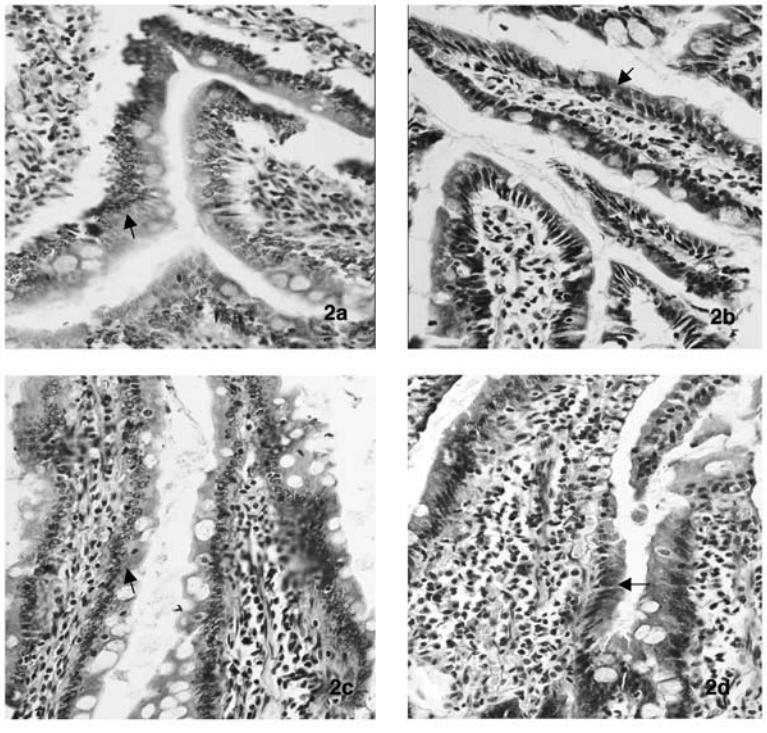

Fig 2- 2a- Duodenum; Control; ALP activity in epithelial cells lining the villi (arrow). 40X; 2b- Duodenum; day 14 PI; marked lower ALP activity in epithelial cells lining the villi (arrow). 40X; 2c- Duodenum; day 21 PI; slightly lower ALP activity in epithelial cells lining the villi (arrow). 40X; 2d- Duodenum; day 28 PI; restoration of ALP activity in epithelial cells lining the villi (arrow). 40X.

DISCUSSION

Observations revealed histopathological changes, such as villous atrophy, adhesion, marked edema, or altered villus/crypt ratio, crypt hyperplasia and stromal inflammation with inflammatory cells, *ie* eosinophils and lymphocytes. Similar findings were observed in other heterophyid fluke infections, such as *C. formosanus* (Nath, 1972), *M. yokogawai* (Chai, 1979; Kang *et al*, 1983) and *C. armatus* (Hong *et al*,

1997), including the intestinal trematode; *Echiostoma hortense* (Lee *et al*, 1990). However, at the end of the experiment, some mucosal lesions were closely restored to their normal condition. A similar finding was observed in metagonimiasis (Lee *et al*, 1981; Kang *et al*, 1983), where recovery of intestinal lesions tended to be spontaneously restored after 4 weeks of infection, although the adult worms were still viable. Nonetheless, heavier infestations possibly cause chronic intermittent diarrhea, abdominal discomfort and nausea (Goldsmith *et al*, 1978). Our results showed the worms were confined only to the mucosal layer of the small intestines, even though some had deeply intruded into the muscularis mucosae. Chai *et al* (1995) found that *M. yokogawai* could invade deeply into the submucosal layer in immunosuppressed mice, even though for a short time. Therefore, immunodeficient persons may be at risk of harmful infections because severe pathologic changes in vital organs have been reported to result from the eggs of the heterophyid flukes, *Haplorchis* sp, *Stellantchasmus* sp and *Procerovum* sp, penetrating the intestinal wall and clustering in the heart and spinal cord (Africa, 1935; Africa *et al*, 1936a,b; 1940).

Mammalian alkaline phosphatase is believed to participate in degrading organic phosphate to free phosphate, and to act in substance transportation across the renal and intestinal membranes (Kaplan, 1972). Our results showed ALP activities were markedly reduced at week 2 of infection and then returned to about the same level as the control. With degenerative changes in the mucosa, it has been proven that brush-border enzyme activities may be reduced in the acute stage of metagonimiasis (Lee *et al*, 1985; Chai *et al*, 2001). The diminished activities of brush-border enzymes may consequently cause absorption disturbances in the secretions from the crypts, suggesting this to be one cause of the osmotic diarrhea found in metagonimiasis (Hong *et al*, 1991).

It can be concluded, from our data, that *C. caninus* infection can cause pathologic changes, in general a mild inflammation, and reduce ALP activity in the small intestines. The pathology of *C. caninus* in rats had not been previously described, and it appears that the present study is the first report in Thailand; it may be useful for future investigations.

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