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PATHOLOGICAL CHANGES INDUCED IN MICE DUE TO EXPERIMENTAL INFECTION OF CANINE HOOKWORM LARVAE

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ABSTRACT
Four groups of each mice were infected orally with a single dose of 500 (group A), 1000 (group B), 2000 (group C) and 4000 (group D) third stage, infective larvae of *Ancylostoma caninum*. A single group (E) of mice was kept as uninfected control for comparison. The survival pattern of larvae and/or the pathogenic reaction in small intestine of different experimental groups of mice occurred in a dose dependent manner. The rapid termination/ expulsion of *A. caninum* larvae from the experimental mouse of gastrointestinal tract is associated with a marked accumulation of various types of white cells in the mucosa of bowel. The persistence of *A. caninum* larvae in abdominal muscles of mice (in groups A, B, C and D) triggered the immune response thereby causing diffuse infiltration of inflammatory cells. Muscle fibers showed increase in the number of sarcolemmal nuclei on day 9,16 and 30 of infection in groups A and B and degeneration in groups C and D (on day 4) of infection.

INTRODUCTION

Hookworms are commonly found in the warm, moist climates but may reach abundant proportions in cold areas. *Ancylostoma caninum* is a parasite of dogs, foxes, and cats; it inhabits the small intestine, attaching with bucal capsule to the mucosal lining. Ancylostomiasis occurs in warm and temperate climates, especially where there is adequate moisture. A. *caninum* and *Uncinaria stenocephalia* infections are relatively common in pups, although the former is much more frequent. *A. caninum* is the most pathogenic species of all hookworms in pet animals. The primary sign of hookworm infection and disease is anemia in dogs (Miller, 1973). This anemia in dogs in almost invariable of the acute hemorrhagic type and is distinctly different from the chronic iron deficiency anemia of hookworm disease in man (Cooper *et al.*, 2003; Loukas *et al.,* 2005). The mucosal response was assessed in hamsters immunized by *A. ceylanicum*; mast cells, goblet cells and eosinophils showed a marked increase in infected animals (Alkazmi and Behnke, 2010).

The work of Bhopale and Johri (1975) and Vardhani and Johri (1981) have demonstrated that mice can be immunized against subsequent severe challenge of *A. caninum* by the oral administration and/or the subcutaneous infection of normal infective larvae. This resistance to challenge was manifested by reduced number of larvae and/or expelled larvae from the challenge of normal larvae. The mechanism of worm expulsion from mice is thought to involve several aspects of immune response. Release of pharmacological mediators like histamine, may cause expulsion of larvae from bowel (Vardhani and Johri, 1979a;1979b). Sensitized cells may affect the actual expulsion of larvae once they have been affected by antibodies (Vardhani and Johri, 1987) .Observations on previous reports (Nirmala and Vardhani 2007, and Vardhani, 2002, 2003a;2003b) indicated that the larvae of *A. caninum* are much pathogenic in the abnormal host, Swiss albino mouse. This paper describes the effect of infection of the host and an attempt to describe the cellular reactions in the intestine and abdominal muscles of normal and infected mice during the oral administration of infective larvae of *A. caninum*.

MATERIALS AND METHODS

Culture of *A. caninum* larvae

A pure strain of *A. caninum* is maintained in an experimentally injected pup where the infection had been maintained in dogs for several years. Faeces from infected pup collected from the floor of the kennel, was cultured in the dark at 26^{c} for 8 days using the petridish method of Sen *et al.,* (1965).

Acquisition and preparation of experimental animals

Male Swiss albino mice (6-8 weeks old; 26-28 g wt.) were purchased from dealers and were divided into 5 groups. Four experimental groups A, B, C and D with 10 in each were infected with a single dose of 500, 1000, 2000 and 4000 larvae of *A. caninum*. Another group E, with 10 mice, was kept as control for comparison. The retention and expulsion of larvae were measured by the recovery of larvae from different organs and muscles of mice through Baermann's process on day 1, 4, 9, 16 and 30 after infection. Pieces of intestinal and abdominal muscle tissues from both experimental and control mice were fixed, sectioned, stained and studied for histopathological changes.

RESULTS AND DISCUSSION

The results of larval recoveries from experimental animals are shown in table-1. The total recovery was 61.4% in group A, 69.2% in B, 55.0% in C and 75.0% in group D on day 1. Greater number of larvae was expelled in group C (45.0%) and D (55.0%) which received 2000 and 4000 doses respectively in comparison to groups A (38.6%) and B (30.8%) that received 500 and 1000 doses respectively. Thereafter, the larval recoveries decreased gradually from day 1 to 30 of infection. Rapid expulsion of larvae from gastrointestinal tract in groups C and D confirms that it is due to the action of sensitized cells in this mouse model. Ogilvie *et al.,* (1977) explained that expulsion of immunologically damaged worms is due to inflammatory reactions during nippostrongyliasis in rats.

Normal small intestine (group E)

Sections of the small intestine at the level of the duodenum, jejunum and ileum from uninfected mice were uniformly free of damage or inflammatory change. The lamina propria and the Bruner's gland regions contained small numbers of mast cells, eosinophils and neutrophils. Furthermore, the muclei of the eosinophils and neutrophils in the tips of the villi were abnormally small, compact and irregularly oval.

Small intestine of mice during single doses of infection (groups A, B, C and D)

The cellular reactions in the small intestine, in which the larvae cause pathogenecity, may be roughly divided into the following four stages: (i) day 1 of infection, very less number of larval migration and there was little inflammation (ii) day 4 of infection, large number of larval migration and there was significant inflammation (iii) day 9 of infection, large number of larval expulsion and with the inflammation (iv) from day 9 to 16 when the larvae passed/expelled out of the intestine and inflammation progressively increased (v) day 30 of infection, no larval retention and mild inflammatory reaction.

Normal abdominal muscles (Group E)

Sections of abdominal muscles showed clear muscle fibers with normal infiltration of cells.

Abdominal muscles during single doses of infection (groups A, B, C and D)

The histologic sections of abdominal muscles of mice showed mild inflammatory reactions and muscle destructions on day 1, 4, 9, 16 of infection in all the singly infected groups. At day 30 of infection there was some alteration in muscle fibers and mild infiltration of mast cells, basophils and eosinophils in groups A (500 dose) and B (1000 dose). At day 1, 4, 9, 16 and 30 of infection, larvae invaded the underlying muscles (migrating through lungs). Larvae were found in a typical coiled position in the muscle fibers. An increase in the number of sarcolemmal nuclei was observed on day 9, 16 and 30 of infection. The enlarged nuclei were found in the center and periphery of muscle fiber. Neutrophils and lymphocytes appeared at day 1 and 4, eosinophils at day 9 and 16 and abundant mast cells at day 30. It is of interest to note that in mice of groups C and D (which received 2000 and 4000 dose of larvae) muscle fibers showed degeneration on day 30 and 4 respectively; there was long chains of sarcolemmal nuclei and heavy infiltration of inflammatory cells (mast cells and eosinophils)

It is evident from the migration and distribution of *A. caninum* larvae in mice that the larvae migrate directly through the tissues. These results indicate that the survival pattern and migratory behavior of *A. caninum* larvae is depended on the dose of inoculum but it was similar mode of migration in all the three singly infected groups during oral infection. Viveka Vardhani and Sakunthala (2011) also suggested that the yield of larvae from the liver was dose dependent in singly infected groups of mice during ancylostomiasis. It is clear from the results that the heightened cellular reactions in the lamina propria and / or Bruner's gland region of the gut about a week after initial infection are associated with developing immunity

Table 1: Larval recovery (%) during various doses of *Ancylostoma caninum* infection in mice

Days of infection	Doses of infection	Larval recovery (%)				Total yield
		GIT	Liver	Lungs	Muscles	
1	500	215(43.0)	60(12.0)	29(5.1)	-	307(61.4)
	1000	438(43.8)	172(17.2)	78 (7.8)	-	692 (69.2)
	2000	800(40.0)	200(10.0)	100 (5.0)	-	1100 (55.0)
	4000	800(20.0)	800(20.0)	200 (5.0)	-	1800 (45.0)
4	500	12(2.4)	02 (0.4)	60 (12.0)	10 (2.0)	273 (56.6)
	1000	46(4.6)	07 (0.7)	198(19.8)	40 (4.0)	705 (70.5)
	2000	180(9.0)	260(13.0)	200(10.0)	40 (2.0)	680 (34.0)
	4000	200(5.0)	600(15.0)	800(20.0)	80(2.0)	1680(42.0)
9	500	-	-	-	229(45.8)	229(40.5)
	1000	-	-	01(0.!)	651(65.1)	652(65.2)
	2000	-	120(5.0)	40(2.0)	600(15.0)	760(19.0)
	4000*					
16	500	-	-	-	221(44.2)	221(44.2)
	1000	-	-	05(0.5)	610(61.0)	615(61.5)
	2000	-	-	20(1.0)	500(25.5)	520(26.0)
30	500	-	-	-	110(22.0)	110(22.0)
	1000	-	-	-	556(55.6)	552(55.2)
	2000	-	-	-	460(23.0)	460(23.0)

- No larval recovery, * died after day 4 of infection

which subsequently terminates / expels the infection. *A. caninum* induced eosinophilic enteritis infection as reported in dogs/humans might have occurred in this host system bringing out cellular changes in the intestine (Croese *et al.,*1994a;1994b; Khoshoo *et al.,*1994;Walker *et.al.*,1995; Mc Carthy and Moore, 2000). From the immunological standpoint the expulsion of larvae from the intestine is similar to the crisis which terminates the acute rise and initiates the developed infection in certain helminthic infection. Earlier migration of larvae into muscles in infected groups reveals that because of the adverse environment in the gastrointestinal tract, larvae were unable to stay in the gut and migrated quickly in to the muscles. These results are similar to that of Nirmala and Vardhani (2007) who explained the occurrence of inflammatory reactions in the gut during helminthic infections.

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