Biolife 2(2):543-549
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

# HISTOLOGICAL ALTERATIONS IN THE STOMACH OF MICE AGAINST ANCYLOSTOMIASIS

**Tarakalakshmi, Y[1] and Viveka Vardhani, V[2]**

[1,2]Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar, 522510 (AP)

.

**E-mail:** vadlamudi_vv@yahoo.co.in

## ABSTRACT

Three different doses of *Ancylostoma caninum* larvae were given per OS to 3 varied groups (group A, 500 larvae; group B, 1000 larvae; group C, 2000 larvae) of male swiss albino mice. Histological changes in stomach of 3 groups of experimental mice were studied on day 1, 4, 9, 16 and 30 of infection comparing with uninfected controls (group D). *A. caninum* infection induced marked changes in the histology of stomach like destruction of gastric folds and glands, infiltration of cells and vacuolization from day 1 to 30 of infection.

**Key words:** Histology, stomach, mice, *A caninum* infection.

## INTRODUCTION

Infection of intestinal nematodes is a global health problem and more than 25% of world's population is suffering due to hookworm anemia (McCarthy and Moore, 2000; Maizels and Yazdanbakhsh, 2003; Elliot et al., 2007; Hewitsoh et al., 2011). Rural children infected with hookworm showed reduced level of RBC, Hb, MCH, MCHC and albumin (Avhad et al., 2013). Hookworms cause chronic pathogenesis in their host in relation to their invasion, colonization and toxicity (Periago and Bethony, 2012). The L3 larvae of *A. caninum* are highly infectious both to man and dog (Brooker et al., 2004). It is found that *A. caninum* larvae induce mastocytosis, eosinophilia, neutrophilia anaphylaxis in the small intestine of mice (Vardhani and Johri, 1979; Vardhani and Gowri., 1996; Nirmala Devi and Vardhani., 2007; Vardhani, 2002, 2005). However, no information is available on the histopathology of stomach during canine hookworm infection in mice. The present investigations are designed to understand the histologic reactions of stomach in mice infected with various single doses of infective *A. caninum* larvae.

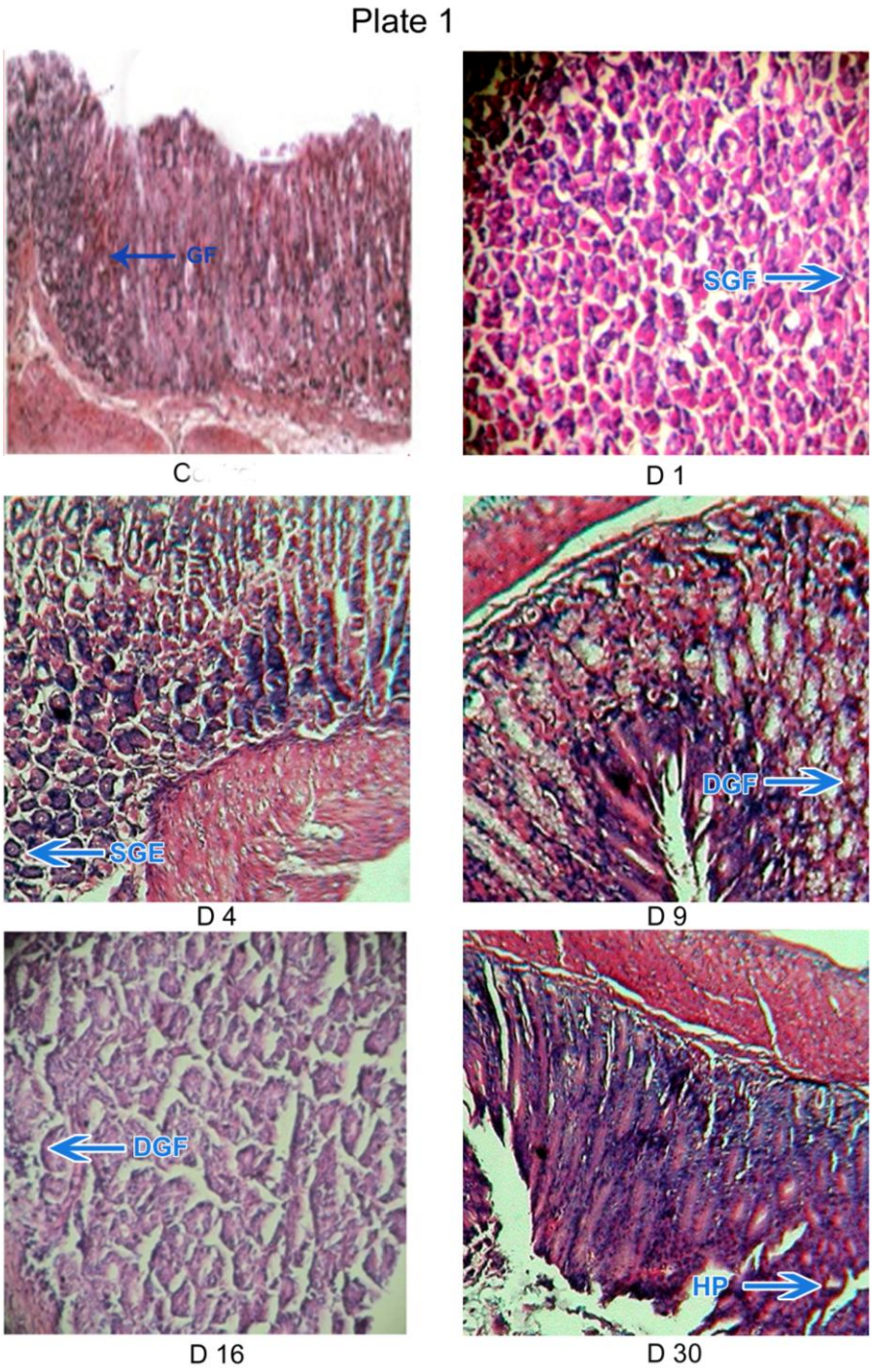
## MATERIALS AND METHODS

Infective larvae of *A. caninum* were obtained according to the method of Sen et al., (1965). Thirty male swiss albino mice (6 – 8 wks; 23 – 25 g) were divided into 3 groups and inoculated orally each with a single dose of 500 (group A), 1000 (group B) and 2000 (group C) larvae. Another group (D) of ten mice was kept as controls for comparison. Two mice from each infected and control groups were sacrificed on day 1, 4, 9, 16 and 30 of infection period and tissues of stomach were fixed, sectioned (5 um) and stained by H & E method for histopathological studies.

## RESULTS AND DISCUSSION

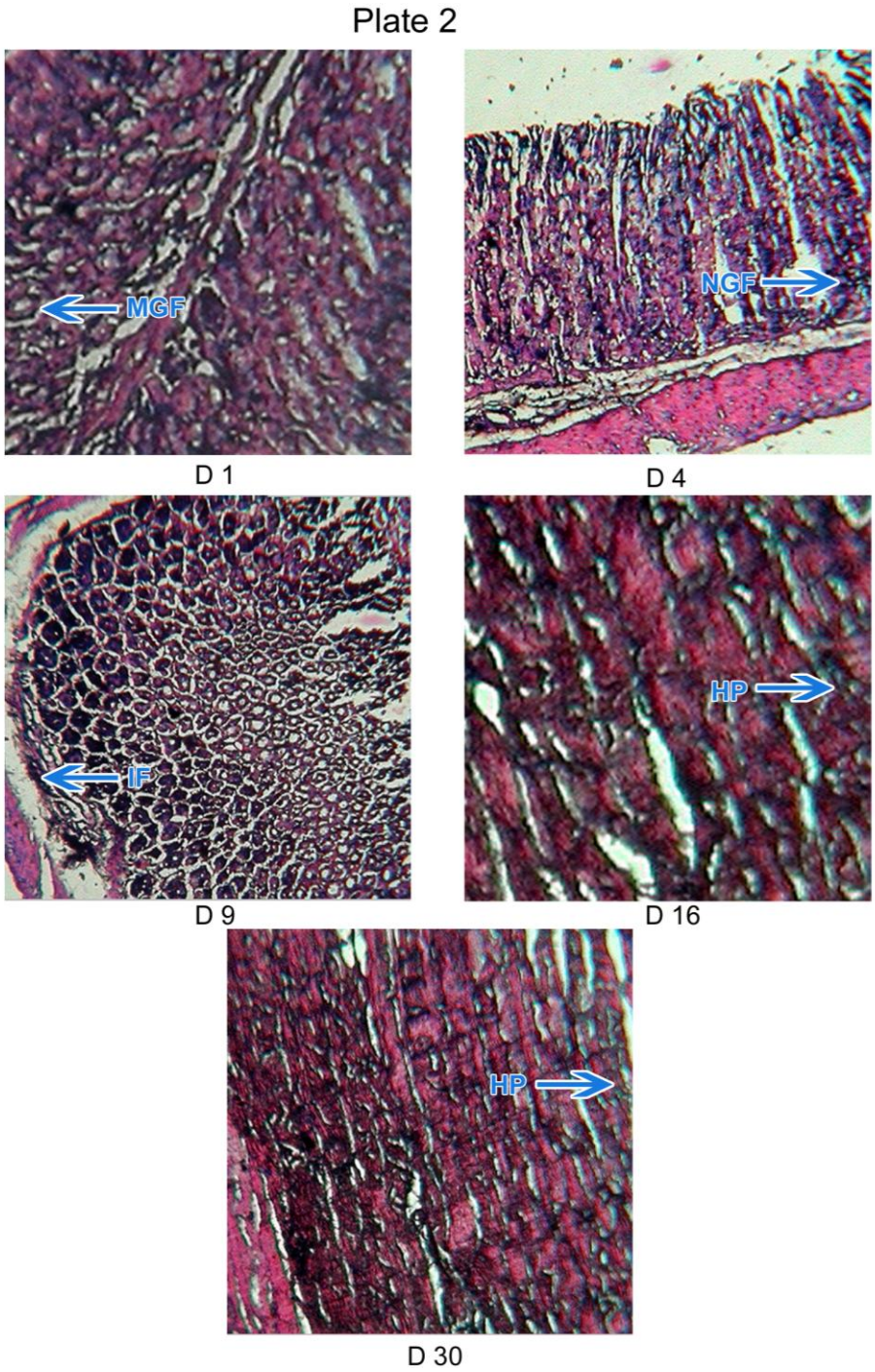
The histological changes recorded in the stomach of mice exposed to various single doses

**Figure-1. Showing the T.S of stomach from control (group a) and infected (500 dose/mouse) mice. All the panels are at 40 X magnification.**




C : T.S. of stomach of normal mouse (group a); D1: Day 1 of infection; normal mucosa and slightly destructed gastric folds; D 4 : Day 4 of infection; slight destruction in glandular epithelium; D 9 : Day 9 of infection; gastric folds are covered by mucus and slight; destruction in glandular epithelium of gastric folds; D16 : Day 16 of infection showing the slightly destructed gastric folds; D 30 : Day 30 of infection; gastric folds are covered by mucus and showing destruction and hyperplasia.
Abbreviations: GF, gastric folds; SGF, slightly destructed gastric folds; SGE, slightly destructed glandular epithelium; DGF, destructed gastric folds; HP, hyperplasia

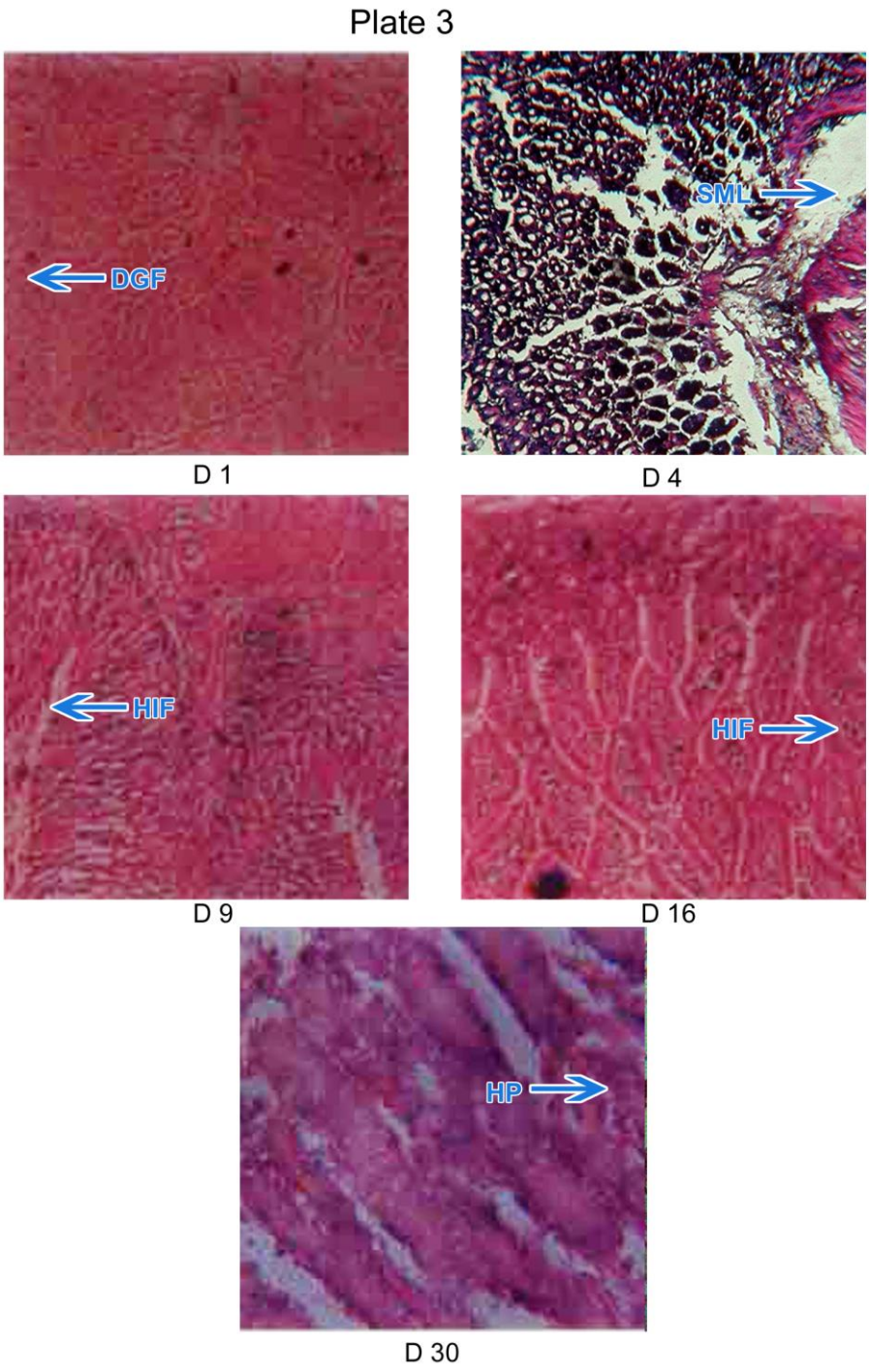
**Figure-2. Showing the T.S of stomach from infected (1000 dose/mouse) mice. All the panels are at 40 X magnification.**




D1 : Day 1 of infection; note the gastric folds covered with excessive mucus; D 4 : Day 4 of infection; slight necrosis in gastric folds; D 9 : Day 9 of infection; slight infiltration of cells ; D 16: Day 16 of infection; hyperplasia in gastric folds; D 30: Day 30 of infection; gastric folds are showing hyperplasia.

Abbreviations: MGF, mucus covered gastric folds; NGF, necrotized gastric folds; IF, infiltration; HP, hyperplasia

**Figure-3. Showing the T.S of stomach from infected (2000 dose/mouse) mice. All the panels are at 40 X magnification.**




D1 : Day 1 of infection -the slight destruction in the gastric folds; D 4 : Day 4 of infection -slight separation of muscle layers; D 9 : Day 9 of infection- heavy infiltration of cells was seen;,D 16: Day 16 of infection- heavy infiltration of cells; D 30 : Day 30 of infection- gastric folds showed hyperplasia and mucus covering.

Abbreviations: DGF, destruction of gastric folds; SML, separation of muscle layers; HIF, Heavy infiltration.

indicate that the infective larvae might have affected the normal histology of stomach. The transverse sections of the stomach (corpus) of control animals (group a) showed clear serosa, longitudinal muscle layer, circular muscle layer, sub mucosa, and gastric folds (glandular epithelium of mucosa) with gastric glands (Plate 1).

**500 dose (group A):**
The T.S. of stomach showed clear serosa, longitudinal muscle (LM) layer, circular muscle (CM) layer and sub mucosa on day 1, 4, 9, 16 and 30 of infection. The layers of stomach and gastric folds were found to be covered with mucus on all days of infection, slight damage was found in glandular epithelium of mucosa of gastric folds and glands on all days of infection.

**1000 dose (group B):**
Marked changes were found from day 1 to 30 of infection. The stomach walls and gastric folds were found to be coated with the mucus covering. Gastric folds were destructed at certain places on day 4 of infection. Heavy infiltration of cells and an increase of spaces in between gastric folds were found on day 9, 16 and 30 of infection.

**2000 dose (group C)**:
Stomach exhibited a significant host-parasite infection when compared with controls. There was a slight destruction in gastric folds and gastric glands when comparison was made among the various days (1, 4, 9, 16 and 30) of infection period. The layers of stomach and gastric folds showed a thin layer of mucus covering and the lumen of the stomach is occupied by cell debris. Heavy infiltration of cells was found on day 4, 9 and 16 of infection.

*A. caninum* infection is known to cause weight loss and anemia in infected mice (Vardhani, 1986) and L3 larvae stay in gastrointestinal tract from day 1-9 (Bhopale and Johri, 1975), and in muscles by day 30 (Vardhani and Johri, 1981). Anemia and/or the adverse environment in the gastrointestinal tract might have brought significant changes in the stomach of experimental mice. Immune T cells produce different cytokines that induce many intestinal alterations like eosinophilia and mastocytosis during infections of *Trichinella spiralis* (Ruitenberg *et al*., 1979; Finkelman *et al*., 1997) and *A. caninum* (Vardhani and Johri, 1979; Vardhani, 2002). The allergic inflammation in the gut creates an unsuitable environment for the stay of the worms (Wakelin, 1993; Bell, 1998; Nirmala Devi and Vardhani, 2007) and IgG has been shown to mediate rapid expulsion of *T. spiralis* in rats (Appleton *et al*., 1988) and *A. caninum* in mice (Viveka Vardhani and Sakunthala, 2012). *A. caninum* induced eosinophilic enteritis infection as reported in dogs/humans (Croese *et al*., 1994; Khoshoo *et al*., 1994; Walker *et al*., 1995; McCarthy and Moore, 2000) might have occurred in this host (Vardhani and Gowri, 1996; Madhuri and Viveka Vardhani, 2013)) causing marked histological changes in stomach

Excessive secretion of mucus and destruction of mucosal cells are the marked changes observed in all the 3 singly infected groups (irrespective of the dose given). Similar findings like destruction, vacuolization and infiltration of cells and excessive mucus secretion in stomach were reported in the stomach of mice under the effect of Gene vac B vaccine by Sakunthala et al., (2014). Khogali *et al.,* (2005) found high intensity of lymphocytic infiltration in stomach of mice treated with Dimethoate 40 EC.

## ACKNOWLEDGEENTS

The author (YTL) is thankful to Prof. V. Viveka Vardhani, the then Head of the Department of Zoology & Aquaculture for providing laboratory facilities and to UGC, New Delhi as a partial benefactor in the MRP for conducting this research.

## REFERENCES

1. **Appleton, J.a., Schain, L.r. and MC gregor, D.d. 1988.** Rapid expulsion of *Trichinella spiralis* in sucking rats; medication by monoclonal antibodies. Immunol. 65: 487-492.

2. **Avhad, S.B., Hiware, C.J. and Bhattacharya, M. 2013.** The study correlation of hook worm infection and mean corpuscular hemoglobin concentration in rural pre-school children population of Aurangabad (M.S), India. Internat. J. Res. Biosciences, 2:66-72.
3. **Bell, R.G. 1998.** The generation and expression of immunity to *Trichinella spiralis* in laboratory rodents. Adv. Parasitol. 41: 149-217.
4. **Bhopale, M.K. and Johri, G.N. 1975.** Experimental infection of *A. caninum* in **Mice.** II. Migration and distribution of larvae in tissues after oral infection. J. Helminthol. 49: 179-185.
5. **Brooker, S., Bethony, J. and Hotez, P.J. 2004.** Human hookworm infection in the 21st century. Adv. Parasitol. 58:197-288.
6. **Croese, J., Loukas, A., Opdebeeck, J. and Stephen. F. 1994.** Human enteric infection with canine hookworm. Ann. Int. Med. 120(5): 369-374.
7. **Elliott, D.**E., **Summers, R.W. and Weinstock, J.V. 2007.** Helminthes as governors of immune mediated inflammation. Internat. J. Parasitol.37: 457-464.
8. **Finkelman, F.**D**., Shea-donohue, T. and Goldhill, J. 1997.** Cytosine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. Ann. Rev. Immuno. 15: 505-533.
9. **Hewitson, J.**P**., Harcus, Y., van Agtmaal, M., Filbe, K.J., Grainger, J.R., Bridgett, S., Blaxter, M.L., Ashton, R.M., Curwen, R.S., Wilson, R.A., Dowle, A.A. and Maizels, R.M. 2011.** Proteomic analysis of secretory products from the model gastrointestinal nematode *Heligmosomoides polygyrus* reveals dominance of Venom Allergen-Like (VAL) proteins. J. Prot. 00572:1-22.
10. **Khogali, F.**A**., Jameela, B., Rahman, S.S.A., Rahim, A. and Daghestani, M.H. 2005.** Histopathological and haematological effects of Dimethoate 40 EC on some organs of albino mice. J. King. Saud. Univ. 18(20: 73-87.
11. **Khoshoo, V., Schantz, P. and Craver, R. 1994.** Dog hookworm: a cause of eosinophilic enterocolitis in humans. J. Pediator Gastroenterol. Nutr. 19: 448-452.
12. **Madhuri, D and Viveka Vardhani, Y. 2013.** Influence of immunostimmulant on abdominal muscle aspartate transaminase and alanine transaminase in mice against hepatitis B. Biolife. 2(1), 400-406**.**
13. **Maizels, R.M. and Yazdanbakhsh. 2003.** Regulation of the immune response by helminthic parasites: cellular and molecular mechanisms. Nat. Rev. Immunol.3:733-743.
14. **McCarthy, J. and Moore, T.A. 2000.** Emerging helminth Zoonoses. Internat. J. Parasitol. 30: 1351-1360.
15. **Nirmala Devi, M. and Vardhani, V.V. 2007.** Neutrophilia in immune response to *Ancylostoma caninum* larvae in mice. Eco. Env. & Con. 13(2): 215-219.
16. **Periago, M.V. and Bethony, J.M. 2012.** Hookworm virulence factors: making the most of the host. Microbes. Infect. 14 (15):1451-1464.
17. **Ruitenberg, E.J., Elgersma, A. and Kruizinga, W. 1979.** Intestinal mast cells and globule leucocytes: role of thymus on their presence and proliferation during a *Trichinella spiralis* infection in the rat. Inter. Arch. Allergy, 60:302-309.
18. **Sen, H.G., Joshi, U.N. and Seth, D. 1965.** Effect of cortisone upon *Ancylostoma caninum infection* in albino mice. Trans. Roy. Soc. Trop. Med. Hyg. 59: 684-689.
19. **Sakunthala, G., Nathanael, P.J.R. and Vardhani, V.V. 2014.** Impact of immunostimulant on stomach protein and DNA activity during hepatitis B infection in mice. Ind. J. Sci. Res. Tech. 2(2): 13-17.
20. **Vardhani, V.V. and Johri, G.N. 1979.** Intestinal mast cell counts during experimental ancylostomiasis. J. Helminthol. 53: 35-39.
21. **Vardhani, V.**V**. 1986.** Serum levels of aspartate transaminase, Alanine transaminase and worm burden in mice infected with *Ancylostoma caninum* larvae. Folia Parasitol. 33: 163-167.
22. **Vardhani, V.V. 2002.** The role of intestinal mast cells and eosinophils in the rejection of

the parasite in mice infected with *Ancylostoma caninum*: A review. **J. Ecophysiol. Occup. Hlth. 2:** 117-125.

23. **Vardhani, V.V. and Johri, G.N. 1981.** The migratory behavior and survival pattern of *Ancylostoma caninum* larvae in an adaptively immunized host. Internat. J. Parasitol. 11(2): 145-147.
24. **Vardhani, V.V. and Gowri, P. 1996.** Intestinal eosinophils and worm burden in mice infected with single doses of *Ancylostoma caninum* larvae. Pak. J. Zool. 28: 267-270.
25. **Viveka Vardhani, V. and Sakunthala, G. 2012.** Serum level of IgG and worm load in male swiss albino mice inoculated with L3 larvae of *Ancylostoma caninum*. The Bioscan. 7(1): 65-67.
26. **Wakelin, D. 1993.** Allergic inflammation as a hypothesis for the expulsion of worms from tissues. Parasit. Today. 9: 115-116.
27. **Walker, N.I., Croese, J. and Clauston, A.d. 1995.** Eosinophilic enteritis in Northeastern Australia. Pathology association with *Ancylostoma caninum* and implication. Am. J. Surg. Pathol. 19: 328-337.

DOI:
https://dx.doi.org/10.5281/zenodo.7208632
Received: 7 April 2014;
Accepted; 22 May 2014;
Available online : 14 June 2014