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PROTEIN, DNA, RNA AND AMINO ACIDS CONTENTS FROM STOMACH OF MICE INFECTED WITH *ANCYLOSTOMA CANINUM* LARVAE

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ABSTRACT

The present study was conducted to know the protein, DNA. RNA and amino acids level in the stomach of male swiss albino mice infected orally each with 500 (group A), 1000 (group B) and 2000 (group C) larvae of *Ancylostoma caninum*. All the mice of infected groups showed increased level of protein, RNA and amino acids and decreased level of DNA from day 1 to 30 of infection period compared to uninfected controls. It was understood that the infection induced significant alteration in the synthesis and/or release of those biochemical constituents.

Key words: *Ancylostoma caninum* larvae, mice, stomach, protein, DNA, RNA, amino acids.

INTRODUCTION

Hookworms (nematodes) are parasites of the intestinal tract in their normal hosts. *Ancylostoma caninum* (the dog hookworm) can cause Cutaneous Larva Migrans (CLM) in humans. Some zoonotic hookworms can also reach the intestine and mature in humans resulting in classic hookworm disease (ancylostomiasis) characterized by bleeding and anemia. The human beings become an accidental host in the developing countries because of contamination of domestic environment by canine faces containing eggs of *A. caninum* (Croese, 1995; Barcat, 2000). Adults of *A. caninum* occur commonly in the small intestine of dogs (Urquhart *et al*., 2000). The larval stages of *Ancylostoma* are associated with creeping eruption in man which is generally referred as cutaneous larva migrans (Prociv and Croese, 1996). *A. caninum* is responsible for the induction of eosinophilic enteritis and unexplained abdominal pain with peripheral eosinophilia in man (Bahgat *et al*., 1999; Sabray and Lofty, 2009). Potential reasons for the emergence of these infections include changes in social, dietary and environmental changes (Mc Carthy and Moore, 2000). Hookworms are blood sucking nematodes and in fact 740 million people in developing countries have been suffering from this disease (de Silva *et al*., 2003). Hookworms that survive and attempt to colonize the intestinal tract face immune cells such as eosinophils (Landmann and Prociv, 2003; Meeusen *et al*., 2005 and Achaiah, 2013). Globally, dogs remain as important source of emerging diseases in humans (e.g. eosinophilic enteritis by *A. caninum*), a bridge for re-emerging infections (*Echinococus granulosus*) and a source of parasites in immunocompromised persons (Eguta-Aguilar *et al.*, 2005; Sabray and Lofty, 2009). Zoonotic parasites are animal parasites that can infect humans. One of the major zoonotic nematode parasites in Korea is *A. caninum* (Youn, 2009).

Due to the high prevalence and its zoonotic importance, *A. caninum* has gained much

attention in the field of veterinary as well as public health research. In recent years, it is realized that *A. caninum* can cause human gut disease and this has sparked to conduct research on ancylostomiasis. The present investigations are designed to study the level of protein, DNA, RNA and amino acids from the stomach of male mice infected with various single doses of *A. caninum* infective larvae and controls.

MATERIALS AND METHODS

Four groups of healthy male Swiss albino mice (*Mus musculus albinus*) (6-8 weeks of age, Av. wt. 25-31 g) were maintained under suitable conditions and fed with standard balanced diet and water *ad libitum*. The third stage, infective, filariform larvae of *A. caninum* were cultured from faecal samples of the infected pup following the petridish method of Sen *et al.*, (1965) and doses prepared according to the dilution method of Scott (1928). Three groups of mice (10 in each group) were orally infected each with a single dose of 500 (group A), 1000 (group B) and 2000 (group C) larvae. Another group (D) of mice (10) was kept as uninfected control for comparison. All the experiments were performed according to the rules laid down by CPCSEA. Two mice from each of groups A, B and C were sacrificed on day 1, 4, 9, 16 and 30 after infection, 2 mice from controls (group D) were also sacrificed, on the same designated days. The total protein, DNA, RNA and amino acids were estimated from the stomach of experimental and control male Swiss albino mice following methods Lowry et al., (1951), Burton (1971) and More and Stein (1948) respectively and the results were analyzed using students 't' test.

RESULTS AND DISCUSSION

500 dose (group A) (Table 1):

Protein content:

There was an increase of protein from day 4 to 30 when compared to controls with a brisk increase on day 9 (165.77 μg/mg). On day 1 of infection the content of protein (136.75 μg/mg) is equal to controls (136.53 μg/mg).

DNA content:

The DNA values were found to be below normal level on day 1 and 30. From day 4 to 16, a gradual increase has taken place which is slightly higher than controls on day 4 and 9. The increase of DNA on day 16 (4.59 μg/mg) was significant when compared to other days of infection.

RNA content:

The RNA levels on day 1 and 4 are slightly higher than controls. There was a gradual increase from day 1 to 9 and gradual decrease from day 9 to 30. The increase of RNA was significant on day 9 (4.17 μg/mg) and 16 (4.06 μg/mg).

Amino acids content:

Higher amino acids levels were found from day 1 to 30 of infection period. There was a gradual increase from day 9 to 30 which was at its zenith on day 9 (677.5 μg/g).

1000 dose (group B) (Table 2):

Protein content:

Higher level of protein was found from day 1 to 30 of infection when compared to that of uninfected controls. From day 1 to 9, there is a gradual increase in protein content. Again there was a decrease from day 9 to 30 (still higher than controls). The increase of protein on day 9 (172.71 μg/mg) was significant when compared to other days of infection.

DNA content:

On day 1 of infection, the DNA level in group A (1.67 μg/mg) is somewhat equal to controls (1.60 μg/mg). From day 4 to 30, an increase of DNA level has taken place. The increase of DNA on day 16 (2.91 μg/mg) was significant when compared to other days of infection.

RNA content:

The level of RNA on day 1 (3.7 μg/mg), 4 (3.92 μg/mg) and 30 (3.33 μg/mg) is somewhat equal to controls (3.02 μg/mg). From day 9 (4.29 μg/mg) to 16 (4.03 μg/mg), there was an increase in experimental mice.

Table-1: Protein (μg/mg), DNA (μg/mg), RNA (μg/mg) and amino acids (μg/g) values in the stomach of control (uninfected) (group D) and *Ancylostoma caninum* larvae (500) infected (group A) mice at different periods of infection (Values are expressed in mean derived from 5 observations).

Days of necropsy	Experimental group A				Control group D			
	Protein	DNA	RNA	Amino acids	Protein	DNA	RNA	Amino acids
1	136.75	1.59	3.34	551.00	136.53	1.60	3.02	510.50
4	145.49	1.74	3.80	602.50	136.52	1.61	3.03	510.40
9	165.77	1.85	4.17	677.50	136.51	1.59	3.01	510.50
16	150.20	4.59	4.06	655.00	136.53	1.60	3.03	510.40
30	143.69	1.59	3.79	650.50	136.52	1.61	3.02	510.50

Table-2: Protein (μg/mg), DNA (μg/mg), RNA (μg/mg) and amino acids (μg/g) values in the stomach of *Ancylostoma caninum* larvae (1000 group B) and (2000 group C) infected mice at different periods of infection (values are expressed in mean derived from 5 observations).

Day of Necropsy	Experimental group B				Experimental group C			
	Protein	DNA	RNA	Amino acids	Protein	DNA	RNA	Amino acids
1	149.70	1.67	3.70	605.00	173.22	1.86	3.80	675.50
4	167.64	1.77	3.92	667.00	190.87	1.88	3.93	737.00
9	172.71	1.92	4.29	687.50	211.77	1.91	4.47	779.00
16	161.02	2.91	4.03	683.00	201.11	3.83	5.77	539.00
30	160.25	1.83	3.33	620.00	192.82	1.89	3.80	689.00

Amino acids content:
Mice of group B showed higher amount from day 1 (605 μg/g) to 30 (620 μg/g) and this rise reached its zenith on day 9 (687.5 μg/g). From day 1 to 9, there is a gradual increase and from day 9 to 30, there is a gradual decrease (still higher than controls).

2000 dose (group C) (Table 2):
Protein content:
There is a marked increase from day 1 (173.22 μg/mg)) to 30 (192.82 μg/mg). From day 1 to 9, there was a gradual increase and reached its peak on day 9 (211.77 μg/mg). From day 9, the level of protein decreased to 201.11 μg/mg at day 16 and to 192.82 μg/mg at day 30 (higher than normal value).

DNA content:
Very slight increase of DNA was found from day 1 to 30 of infection period (except on day 16 - 3.83 μg/mg). The content of DNA remained almost constant on day 1 (1.86 μg/mg), 4 (1.88 μg/mg), 9 (1.91 μg/mg) and 30 (1.89 μg/mg).

RNA content:
A higher level of RNA was noted on day 9 (4.47 μg/mg) and 16 (5.77 μg/mg) of infection. From day 1 to 16, there is a gradual increase, the value of RNA on day 30 (3.8 μg/mg) is almost equal to controls (3.02 μg/mg).

Amino acids content:
From day 1 to 30, there is a marked increase of amino acids; the increase of amino acids on day 9 (779 μg/g) was significant when compared to other days of infection. Although the level of amino acids decreased on day 16 (539 μg/g) and 30 (689 μg/g), it is still higher when compared to controls.

Statistical analysis showed that mice received single doses of 500 (group A), 1000 (group B) and 2000 (group C) showed significant increase in protein values when compared with controls

and non-significant values when compared among themselves (Table 3). The increased level of RNA and amino acids was significant in groups A, B and C when compared with controls; but there was no significant difference when compared among themselves (except the RNA level in between groups A and C).

Visceral migration of *A. caninum* larvae in female swiss albino mice has led to marked alterations in the level of cholesterol (Vardhani and Krishna Rao, 1995) and serum and liver enzymes (Vardhani, 1986; 1989). It was found that in case of female swiss albino mice, following the primary infection, larval expulsion

Table 3. 't' values obtained for experimental (infected with 500, A; 1000, B and 2000, C) dose of *Ancylostoma caninum* larvae/mouse) and control (uninfected-D) groups of mice.

Stomach	Experimental groups			Control group
	A	B	C	D
Total Protein: Mean	148.38	162.26	93.95	136.52
't' value	A D 't'=2.73*	B D 't'=7.41*	C D 't'=9.84*	
	A B 't'=2.49*	A C 't'=6.36*	B C 't'=4.75*	
Total DNA: Mean	2.27	2.02	2.27	1.6
't' value	A D 't'=1.28@	B D 't'=2.03@	C D 't'=1.92@	
	A B 't'=0.45@	A C 't'=0.003@	B C 't'=1.12@	
Total RNA: Mean	3.83	3.85	4.35	3.02
't' value	A D 't'=6.31*	B D 't'=5.77*	C D 't'=3.98*	
	A B 't'=0.11@	A C 't'=6.34*	B C 't'=1.35@	
Total amino acids: Mean	627.3	652.5	683.9	510.46
't' value	A D 't'=5.76*	B D 't'=9.42*	C D 't'=4.77*	
	A B 't'=0.99@	A C 't'=0.16@	B C 't'=0.79@	

't' value at 5% level of significance is 2.306

*statistically significant values; @statistically non-significant values

has commenced on day 4 and by day 9 it appeared to be completed (Vardhani and Gowri, 1996).

The present observations on ancylostomiasis in mice reveal that the single doses of the infection was associated with physiological imbalance of the stomach. *A. caninum* infection is known to cause significant changes of weight loss, hematological parameters like WBC count and which may result in anemia in infected mice (Vardhani, 1986). The period of infection in mouse gastrointestinal tract is 1-9 days (Bhopale and Johri, 1975), which may be found in muscles by day 30 as stated by Vardhani and Johri (1981). The significant alteration in the biochemical constituents during the entire study period indicating the host-parasite interactions and thereby producing significant effect on protein, DNA, RNA and amino acid content.

Also, it is found that anemia and/or the adverse environment in the gastrointestinal tract might have brought significant changes in the biochemical constituents in experimental mice. Although the mechanism responsible for the induction of abnormal environment in GIT and/or disturbances in gut physiology is not fully understood, many points have been elucidated. Experiments performed on female swiss albino mice using sensitized peritoneal exudate cells and mesenteric lymph node cells showed that the expulsion of larvae is promoted by adverse reactions in the gut (Vardhani and Johri, 1980; 1987). The most accepted theory postulates that 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 would induce alterations in the intestinal mucosa which create an unsuitable environment for the stay of the worms (Wakelin, 1993; Bell, 1998; Nirmala Devi and Vardhani, 2007). Larvae specific IgG antibody 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, 2011).The present investigations suggest that in *A. caninum* infection in mice, the adverse local environment of gut and/or serum IgE response (as suggested by Viveka Vardhani and Sakunthala, 2012) reflect true responsiveness of immunological reactions involved in hookworm infection with regard to specific alteration in the biochemical constituents.

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REFERENCES

1. **Achaiah, N. (2013).** Study On Free Amino Acid Levels In *Raillietina Tetragona* (Molin, 1858). Biolife. 1(3), 96-98.
2. **Appleton, J.A., Schain, L.R. and Mc Gregor, D.D. (1988).** Rapid expulsion of *Trichinella spiralis* in sucking rats: mediation by monoclonal antibodies. Immunol. **65:** 487-492.
3. **Bahgat, M.A., El Gindy, A.E., Mahmoud, L.A., Hegab, M.H. and Shahin, A.M. (1999).** Evaluation of the role of *Ancylostoma caninum* in humans as a cause of acute and recurrent abdominal pain. J. Egypt Soc. Parasitol. **29(3):** 873-882.
4. **Barcat, J.A. (2000).** Larva migrans; perros, parasitos., Y. hombres. Medicina (Buens Aries) **60:** 270-272.
5. **Bell, R.G. (1998).** The generation and expression of immunity to *Trichinella spiralis* in laboratory rodents. Advan. Parasitol. **41**: 149-217.
6. **Bhopale, M.K. and Johri, G.N. (1975)**. Experimental infection of *Ancylostoma caninum* in mice. II. Migration and distribution of larvae in tissues after oral infection. J. Helminthol. **49**: 179-185.
7. **Burton, K. (1956).** A study of the conditions and mechanism of the

diphenylamine reaction or the colorimetric estimation of deoxyribonucleic acid. Biochem. J. **62:** 315-323.

8. **Croese, J. (1995).** Seasonal influence on human enteric infection by *Ancylostoma caninum.* Am.J. Med. Hyg. **53:** 158-161.
9. **de Silva, N.R., Brooker, S., Hotez, P.J., Montresor, A., Engels, D. and Savioli, L. (2003).** Soil transmitted helminth infections: updating the global picture. Trends Parasitol. 19**:** 547-551.
10. **Eguta-Aguilar, P., Cruz-Reyes, A. and Martinez-Maya, Z.Z. (2005).** Ecological analysis and description of the intestinal helminthes present in dogs in Mexico City. Vet. Parasitol. **127**: 139-146.
11. **Finkelman, F.D., Shea-Donohue, T.J. and Goldhill, J. (1997).** Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lesions from studies with rodent models. Ann. Rev. Immunol. **15:** 505-533.
12. **Landmann, J.K. and Prociv, P. (2003).** Experimental human infection with the dog hookworm, *Ancylostoma caninum*. Med. J. Aust. **178**: 69-71.
13. **Lowry, H., Rosebrough, N.I., Far, A.L. and Ranall, R.J. (1951).** Protein measurement with Folinphenol reagent. J. Biol. Chem. **193:** 265-275.
14. **Mc Carthy, J. and Moore, T.A. (2000).** Emerging helminth Zoonoses. Internat. J. **Parasitol. 30**: 1351-1360.
15. **Meeusen, E.N., Balic, A. and Bowles, V. (2005).** Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. Vet. Immunol. Immunopathol. **108(1-2**): 121-125.
16. **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.
17. **Moore, S. and Stein, W.H. (1948).** Photometric ninhydrin method for use in chromatography of amino acids. J. Bio. Chem. 176: 367-368.
18. **Ruitenberg, E.J., Elgersma, A. and Kruizinga, W. (1979).** Intestinal mast cell and globule leukocytes: role of the thymus on their presence and proliferation during *Trichinella spiralis* infection in the rat**.** Int. Arch. Allergy, **60**: 302-309.
19. **Sabray, M.A. and Lofty, H.S. (2009).** Captive dogs as reservoirs of some zoonotic parasites. Res. J. Parasitol. **4(4**): 115-122.
20. **Scott, J.A. (1928)**. An experimental study of the development of *Ancylostoma caninum* in normal and abnormal hosts. Amer. J. Hyg. **8**: 158-209.
21. **Prociv, P. and Croese, J. (1996).** Human enteric infection with *Ancylostoma caninum;* hookworms reappraised in the light of "new" zoonosis. Acta. Tropica**. 62**; 23-44.
22. **Sen, H.G., Joshi, U.N. and Seth, D. (1965).** Effect of cortisone upon *Ancylostoma caninum* infection in albino mice. Trans. Roy. Soci. Trop. Med. and Hyg. **59:** 684-689.
23. **Urquhart, G.M., Annor, J.L., Duncan, A.M. and Jennings, F.M. (2000).** In "Veterinary Parasitology". 3rd (ed.). ELBS Longman, UK, pp.50-51.
24. **Vardhani, V.V. (1986).** Serum levels of Aspartate transaminase, alanine transaminase and worm burden in mice infected with *Ancylostoma caninum* larvae. Folia Parasitologica, **33**: 163-167.
25. **Vardhani, V.V. (1989).** Enzyme activity and worm burden in intestine and liver of mice infected with single doses of *Ancylostoma caninum* larvae. J. Hyg. Epid. Microbiol. Immunol. **35**: 149-156.
26. **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.
27. **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.
28. **Vardhani, V.V. and Johri, C.N. (1980).** Delayed (cellular) hypersensitivity in mice during experimental ancylostomiasis. IV. Results in recipients injected with a massive dose of sensitized mesenteric lymph node cell at 7th day before challenging infection. Bioresearch, **4**: 45-47.

29. **Vardhani, V.V. and Johri, G.N. (1979).** Intestinal mast cell counts during experimental ancylostomiasis. J. Helminthol. **53**: 35-39
30. **Vardhani, V.V. and Johri, G.N. (1981).** The migratory behavior and survival pattern of *Ancylostoma caninum* larvae in an adoptively immunized host. Int. J. Parasitiol. **11:** 145-147.
31. **Vardhani, V.V. and Johri, G.N. (1987).** Cell mediated immunity during experimental ancylostomiasis; a review. J. Hyg. Epid. Microbiol. Immunol. **31:** 107-111.
32. **Vardhani, V.V. and Krishna Rao, B. (1995).** The relationship between serum cholesterol and parasitism in mice. Pak. J. Zool. **27**: 373-375.
33. **Viveka Vardhani, V. and Sakunthala (2011).** The specific role of liver in expelling *Ancylostoma caninum* larvae from the host system. The Bioscan. **6(2**): 255-256.
34. **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.
35. **Wakelin, D. (1993).** Allergic inflammation as a hypothesis for the expulsion of worms from tissues. **Parasit. Today, 9**: 115-116.
36. **Youn, H.J. (2009).** Review of zoonotic parasites in medical and veterinary fields in the republic of Korea. Korean J. Parasitol. **47**: 133-141.

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