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Protein, DNA, RNA and amino acids content from large intestine 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 large intestine of male swiss albino mice infected orally each with 500 (group A), 1000 (group B) and 2000 (group C) larve of *Ancylostoma caninum*. All the mice of infected groups showed increased level of protein, DNA (except in group A), RNA and amino acids 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 these biochemical constitutents.

Key words : *Ancylostoma caninum* larvae, mice, large intestine, protein, DNA, RNA, amino acids

INTRODUCTION

The soil-transmitted helminths are a group of parasitic nematode worms causing human infection through contact with parasitic eggs or larvae that thrive in the warm and moist soil in tropical and subtropical countries. As adult worms, the soil transmitted helminths live for years in the human gastrointestinal tract. More than a billion people are infected with at least one species (WHO, 2005). Of particular worldwide importance are the round worms (*Ascaris lumbricoides*), whip worms (*Trichuris trichiura*), and hookworms (*Necator americanus* or *Ancylostoma duodenale*). They are considered together because it is common for a single individual, especially a child living in a less developed country, to be chronically infected with all three worms.

Such children may suffer due to malnutrition, growth stunting, intellectual retardation, and cognitive and educational deficits. Hookworms are infecting over one billion people in the developing world (WHO, 1996). Throughout their long history of domestication, dogs have been a source of zoonotic parasites and served as a link for parasite transmission among livestock, wildlife and humans.

Humans can develop patent infection with a wide range of helminth parasites whose natural host is another vertebrate. Examples include intestinal capillariasis, anisakidosis, eosinophilic enteritis, oesophagostomaiasis and gnathostomiasis. Potential reasons for the emergence of these infections include changes in social, dietary and environmental changes (Mc Carthy and Moore, 2000). The most wide spread of all hookworm species is *A. caninum* and it parasitizes dog in many parts of the world. 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. One among the zoonotic parasites that have been widely studied and recognized as a significant public health problem worldwide is *A. caninum* spp. (Dutta, 2002). 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).

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In the nematode research community, *A. caninum* is the best studied of all the hookworms. The main pathology associated with hookworm infection is the loss of blood and anemia, diarrhoea and intestinal pain, which is problematic in immuno-compromised humans and animals, because they are unable to eliminate the hookworms as effectively as normal individuals. Eosinophilic enteritis is a zoonotic intestinal infection characterized by abdominal pain but no blood loss (Kwon *et al*., 2003). The symptoms vary with the number of parasites and the tissue(s) invaded (Acha and Sztyfres, 2003; Douglass and Juzych, 2004).

A study on most recent parasites survey in determining prevalence and epidemiology of canine parasitic zoonoses among tea-growing communities of north east India demonstrated the presence of endemicity parasites (Traub *et al*., 2005). The occurrence of infection in humans and environmental contamination with *A. caninum* eggs was notified in Argentenia (Martin and Demonte, 2008). Stray dogs were more commonly affected and higher prevalence was noticed in puppies than adults (Ashraf *et al*., 2008). The present investigation was undertaken to estimate the level of protein, DNA, RNA and amino acids in the large intestine of mice infected orally with 3 different single doses of *A.caninum* larvae and to assess their significance with regard to ancylostomiasis.

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) larveae. 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 large intestine of experimental and control male Swiss albino mice following methods Lowry et al., (1951), Burton (1971) and Moore and Stein (1948) respectively and the results were analyzed using students 't' test.

RESULTS AND DISCUSSION

500 dose (group A) (Table 1)

Protein content :

Table 1 reveal that mice which received 500 dose of larvae (group A). has a protein level which is higher than control value (98.33 µg/mg) on day 1 (99.3 µg/mg), 4 (119.19 µg/mg), 9 (135.39 µg/mg), 16 (129.1 µg/mg) and 30 (120.62 µg/mg) of infection; there was an abrupt increase on day 9.

DNA content :

On day 1 of infection, the large intestine of infected mice showed below normal level of DNA, which was again increased slightly on day 4 and 9. The DNA level on day 16 is below normal and on day 30 is equal to normal.

RNA content :

The RNA value is lower on day 1 of infection than normal value and the RNA content increased slightly from day 4 to 30 and it is at peak on day 9 (4.21 µg/mg).

Amino acids content :

The result of amino acids are shown in table 1. From day 1 to 30 of infection, there is gradual increase in amino acids content which is higher than normal amino acid levels.

Table-1: Protein (µg/mg), DNA (µg/mg), RNA (µg/mg) and amino acids (µg/g) content in the large intestine 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).

Day of Necropsy	Experimental group A				Control group D			
	Protein	DNA	RNA	Amino acids	Protein	DNA	RNA	Amino acids
1	99.30	1.69	2.82	510.50	98.33	1.78	2.86	476.01
4	119.19	1.83	3.42	575.00	98.32	1.77	2.85	476.03
9	135.39	1.89	4.21	676.50	98.31	1.78	2.87	476.02
16	129.10	1.47	4.01	882.50	98.33	1.76	2.85	476.01
30	120.62	1.78	3.81	580.00	98.32	1.77	2.87	476.03

Table-2: Protein (µg/mg), DNA (µg/mg), RNA (µg/mg) and amino acids (µg/g) content in the large intestine (group B) of *Ancylostoma caninum* larvae (1000) infected (group B) and (2000 group C) 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	105.19	1.81	2.87	550.00	148.62	2.04	3.50	625.50
4	113.76	1.85	3.37	575.00	173.07	2.23	3.73	683.00
9	139.98	2.06	4.40	682.50	190.33	2.38	4.43	718.00
16	170.00	1.81	4.25	972.50	578.10	2.22	8.03	652.50
30	129.27	1.86	3.30	620.00	165.30	2.23	3.64	650.00

The increase of amino acids on day 16 (882.5 µg/g) was significant when compared to other days of infection.

1000 dose (group B) (Table 2)

Protein content :

In case of mice which received 1000 dose, the large intestine showed a definite prospect of protein content. There is a marked and gradual increase of protein from day 1 to 16 leading its peak on day 16 (170 µg/mg). Though there is a decrease from day 16 to 30 (129 µg/mg), it is still higher than normal value (98.32 µg/mg).

DNA content :

In group B, there is a slight increase of DNA form day 1 to 30 infection. A marked increase of DNA was found on day 9 (2.06 µg/mg).

RNA content :

In case of mice received 1000 dose (group B), the level of RNA in large intestine is equal to normal on day 1 (2.87 µg/mg). There was a slight increase from day 4 to 30 with a peak value on day 9 (4.4 µg/mg).

Amino acids content :

Mice received 1000 dose showed marked increase of amino acids throughout the experimental period. There was a gradual increase from day 1 to 16 (972.5 µg/g – peak value) and decrease on day 30 (620.0 µg/g) (which is still higher than control value).

2000 dose (group C) (Table 2)

Protein content :

The mice of group C which received 2000 dose of larvae showed a protein level which is higher than control value from day 1 to 30 of infection. There was an abrupt increase of protein on day 16 (578.10 µg/mg) of infection. There was a sudden decrease on day 30 (165.30 µg/mg); however, it is higher than control value (98.35 µg/mg).

DNA content :

The level of DNA enhanced slightly on day 1 (2.04 µg/mg), 4 (2.23 µg/mg), 9 (2.38 µg/mg), 16 (2.22 µg/mg) and 30 (2.23 µg/mg) when compared with normal value (1.81 µg/mg).

RNA content :

In case of group C, the level of RNA content was higher than normal throughout the period of experimentation. On day 16 of infection, the RNA level is at peak (8.03 µg/mg). On day 30, there is some markable decrease (3.64 µg/mg) but still this decreased DNA level is higher than control value.

Amino acids content:

Mice of group C showed marked increase of amino acids from day 1 to 30 of infection when compared that of uninfected controls. From day 1 (625.5 µg/g) to 9 (718.0 µg/g), there is a gradual increase of amino acids. From day 9 to 30, a gradual decrease of amino acids has taken place. However, this decrease is comparatively higher than that of controls.

The results of experiments were analysed by applying Students 't' test. The mean values of protein, DNA, RNA and amino acids with 't' values for 1-30 days of infection period are shown in table 3. The level of protein was statistically significant in large intestine of groups A and B when compared with controls; but there was no significant difference when a comparison was between groups C and D and among the experimental groups (Table 3). Groups B and C showed significantly increased DNA values in large intestine in comparison with controls and in between groups A and C and B and C; there was no significant difference in the level of DNA in group A when compared with controls (group D) and group B.

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.

Large Intestine	Experimental groups			Control groups	
	A	B	C	D	
Total Protein:					
Mean	120.72	134.61	251.08	98.32	
	A D	B D	C D		
	|_____|	|_____|	|_____|		
't' value	t=4.09*	t=3.28*	t=2.08@		
	A B	A C	B C		
	|_____|	|_____|	|_____|		
	t=0.94@	t=1.77@	t=1.61@		
Total DNA:					
Mean	1.73	1.87	2.22	1.77	
	A D	B D	C D		
	|_____|	|_____|	|_____|		
't' value	t=0.66@	t=2.43*	t=8.59*		
	A B	A C	B C		
	|_____|	|_____|	|_____|		
	t= 1.88@	t= 5.99*	t=5.36*		
Total RNA:					
Mean	3.65	3.63	4.66	2.86	
	A D	B D	C D		
	|_____|	|_____|	|_____|		
't' value	t=3.60*	t=2.19@	t=2.34*		
	A B	A C	B C		
	|_____|	|_____|	|_____|		
	t=0.04@	t=1.24@	t=1.25@		
Total amino acids:					
Mean	644.9	680.0	665.8	476.02	
	A D	B D	C D		
	|_____|	|_____|	|_____|		
't' value	t=2.9*	t=2.98*	t=13.32*		
	A B	A C	B C		
	|_____|	|_____|	|_____|		
	t=0.39@	t=0.34@	t=0.2@		

*'t' value at 5% level of significance is 2.306; *statistically significant values; @statistically non-significant values*

Groups A, B and C showed significantly increased values of RNA and amino acids when compared with controls (except the level of RNA in group B) and non-significant difference when compared among themselves (Table 3).

The findings of the present study revealed that there was a significant increase in protein, DNA, RNA and amino acids in large intestine of all the singly infected groups with resultant adverse reactions and/or host-larval interactions in the gastrointestinal tract (GIT) of mice infected with *A. caninum* larvae. Anaemia 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 sensitised 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).

A. caninum infection in mice, which produce adverse local environment of gut and/or serum IgE response (as suggested by Viveka Vardhani and Sakunthala, 2012) reflect abnormal immunological reactions (mechanisms) involved in hookworm infection with regard to specific alteration in the biochemical constituents. The present findings reveal that the protein, DNA, RNA and amino acid alterations were not in accordance with the increasing single dose levels. Tarakalakshmi and Viveka Vardhani (2014 a, b) also reported that the visceral migration of *A.caninum* larve may led to marked disturbance in the gut resulting in abnormal physiological changes.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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