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THE CONTENT OF PROTEIN, DNA, RNA AND AMINO ACIDS FROM SMALL INTESTINE OF MICE DURING EXPERIMENTAL ANCYLOSTOMIASIS

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ABSTRACT

Protein, DNA, RNA and amino acids levels were estimated in the small intestine of 3 groups (group A, infected orally each @ 500, group B @ 1000 and group C @ 2000 larvae of *Ancylostoma caninum*) of male swiss albino mice. Significant increase of protein, DNA, RNA and amino acids was found in all the infected groups of mice when compared to uninfected controls. The synthesis and/or release of these biochemical constituents was influenced by the host parasite interactions.

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

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INTRODUCTION

Sapro-zoonotic parasites which infect humans are found in soil or water are mainly *Ancylostoma caninum, Ascaris suum, Capillaria hepatica, Strongyloides stercoralis, Trichuris vulpis* and *Hypoderma bovis* (Urquhart *et al*., 2000) Many of carnivorous parasites are zoonotic parasites because dogs and cats have lived with humans for a long period of time (Youn *et al*., 1995, Youn, 2009; Seo *et al*., 2002). Larval infection of humans with *A. caninum* may occasionally give rise to adult worms that inhabit the small intestine and can cause eosinophilic enteritis (Bowman *et al*., 2010). 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). In addition to cutaneous lesions, *A. caninum* has also been reported to cause blood eosinophilia (Prociv and Croese, 1990) and eosinophilic enteritis (Sabrosa and de Souza, 2001; Landmann and Prociv, 2003) in humans. An adult hookworm of species *A. caninum* was recovered at colonoscopy from the terminal ileum of a patient and an epidemiological survey conducted on 33 Australian patients showed abdominal pain associated with eosinophilia. *A. caninum* has also been found in an adult form in the human small intestine and has been implicated in cases of eosinophilic enteritis (Khoshoo *et al*., 1994). Adults of *A. caninum* occur commonly in the small intestine of dogs (Urquhart *et al*., 2000).

Free-ranging animals with sporadic or indirect contact with domestic livestock and humans may serve as reservoirs or sentinels for diseases (Aschfalk and Holler, 2006). Continuous contact between diseased or carrier dogs and

their attendants under improper hygienic measures initiate the development of endemic foci for spreading of different pathogens, specially zoonotic ones with direct life cycle (Pullola *et al*., 2006). The worldwide community has recognized the importance of ascariasis, trichuriasis and ancylostomiasis and reported that their combined disease burden might be as great as those of malaria or tuberculosis (Chan, 1997). Soil transmitted helminthic infections also increase susceptibility to other important infections like malaria, tuberculosis, and HIV (Fincham *et al.,* 2003). In humans and other paratenic hosts, the larvae do not complete their migration and eventually encyst in the tissues (Sakakibara *et al*., 2002; Velho *et al*., 2003; Vardhani, 2006). In experimental mice, infective *A. caninum* larvae which invade the visceral organs, migrate and persist in the muscles causing much histopathological reactions in the small intestine (Vardhani, 2002) and significantly altered the protein, DNA, RNA and amino acids in the stomach (Tarakalakshmi and Viveka Vardhani, 2012). Therefore, a new vista has been opened to estimate the level of protein, DNA, RNA and amino acids in the small intestine of mice infected with *A. caninum* larvae.

MATERIALS AND METHODS

Culture of infective *A. caninum* larvae (from fecal samples of the infected pup) and preparation of doses were made following the petridish method of Sen *et al.,* (1965) and the dilution method of Scott (1928). Experimental male Swiss albino mice (*Mus musculus albinus*) (6-8 weeks of age, Av. wt. 25-31 g) (fed with standard balanced diet and water *ad libitum)* of groups A, B and C (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 ten mice was kept as uninfected control for comparison. All the experiments were performed according to the rules laid down by CPCSEA. Two mice from all the 3 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 for the estimation of total protein, DNA, RNA and amino acids from the small intestine following the methods Lowry et al., (1951), Burton (1956) 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:
The level of protein is somewhat equal to controls on day 1 of infection. From day 4 to 30 there was an abrupt increase and this increase reached its peak on day 9 (149.22 μg/mg)

DNA content:
The level of DNA on day 1 is lower than normal level. A slight increase of DNA has occurred from day 4 to 30 when compared to controls.

RNA content:
Table 1 reveal that the group of mice (A) which received 500 larvae has a RNA level which is lower than control value on day 1 of infection. The RNA value enhanced on day 4 (3.6 μg/mg), 9 (4.29 μg/mg), 16 (4.08 μg/mg) and 30 (3.54 μg/mg); the rise of RNA on day 9 is noticed as peak level of immune response.

Amino acids content:
Mice received small dose (group A, 500) showed higher amino acid levels from day 1 (529.0 μg/g) to 30 (599 μg/g). From day 1 to 9, there is a gradual increase and from day 9 to 30, there is a gradual decrease, but yet higher than control values. The increase of amino acids was at its zenith on day 9 (673.5 μg/g).

1000 dose (group B) (Table 2)
Protein content:
There is a slight increase of protein (97.64 μg/mg) on day 1 compared to controls (96.69 μg/mg). From day 4 to 30, there is a marked increase of protein; this increase is at its peak on day 9 (160.25 μg/mg)

DNA content:
The level of DNA is equal to normal on day 1

(1.71 μg/mg). There is a slight increase of DNA level on day 4 (1.85 μg/mg) and 30 (1.89 μg/mg) and marked increase on day 9 (2.06 μg/mg) and 16 (2.03 μg/mg) when compared to control values.

RNA content:
There is a slight increase of RNA on day 1 (3.03 μg/mg), 16 (3.09 μg/mg), 30 (3.35 μg/mg) and a marked increase on day 4 (4.01 μg/mg) and 9 (4.31 μg/mg). The increase of RNA on day 9 was significant.

Amino acids content:
Mice of group B had shown very high level of amino acids from day 1 to 30 of infection. The level of amino acids rose gradually from day 1 to 16 and reached its zenith on day 16 (938.5 μg/g) when compared to other days of infection.

2000 dose (group C) (Table 2)

Protein content:
In group C, there was an increase of protein from day 1 to 30 when compared to controls. There was a gradual increase of protein from day 1 to 9. The increase of protein on day 9 (164.86 μg/mg) was significant when compared to other days of infection. Again there was a gradual decrease of protein from day 9 to 30, but yet higher than control values.

DNA content:
The content of DNA is slightly higher than controls on day 1 (1.87 μg/mg), 4 (1.90 μg/mg), 9 (2.21 μg/mg) and 30 (1.91 μg/mg). On day 16 (1.23 μg/mg) it is slightly lower than normal value (1.69 μg/mg).

RNA content:
Higher RNA levels were found on day 1 (3.39 μg/mg), 4 (3.75 μg/mg), 9 (4.39 μg/mg), 16 (3.29 μg/mg) and 30 (3.48 μg/mg), when compared to that of controls. There is a marked increase of RNA in day 19 when compared to other days of infection.

Amino acids content:

Table-1: Protein (μg/mg), DNA (μg/mg), RNA (μg/mg) and amino acids (μg/g) values in the small 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	96.75	1.68	2.72	529.00	96.68	1.70	2.84	502.49
4	119.82	1.80	3.60	592.00	96.67	1.71	2.85	502.48
9	149.22	1.88	4.29	673.50	96.68	1.70	2.83	502.50
16	132.85	1.81	4.08	611.00	96.67	1.71	2.84	502.48
30	120.62	1.78	3.54	599.00	96.69	1.69	2.85	502.49

Table 2: Protein (μg/mg), DNA (μg/mg), RNA (μg/mg) and amino acids (μg/g) content in the small intestine of experimental groups (B, 1000 larvae; C, 2000 larvae) of 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	97.64	1.71	3.03	574.00	114.27	1.87	3.39	613.50
4	109.88	1.85	4.01	655.00	117.86	1.90	3.75	692.00
9	160.25	2.06	4.31	678.50	164.86	2.21	4.39	703.00
16	134.51	2.03	3.09	938.50	145.20	1.23	3.29	626.50
30	124.81	1.89	3.35	603.00	141.06	1.91	3.48	546.00

Table -3. 't' values obtained for 3 experimental (groups A, B and C) and control (group D) groups of mice.

Small Intestine	Experimental groups			Control group
	A	B	C	D
Total Protein:	125.85	125.41	136.65	96 67
Mean	A D |_____| t=3.52*	B D |_____| t=2.98*	C D |_____| t=4.78*	
't' value	A B |_____| t=0.12@	A C |_____| t=1.12@	B C |_____| t=0.85@	
Total DNA:	1.79	1.90	1.82	1.7
Mean	A D |_____| t=3.03*	B D |_____| t=3.61*	C D |_____| t=0.91@	
't' value	A B |_____| t=1.85@	A C |_____| t=0.23@	B C |_____| t=0.54@	
Total RNA:	3.64	3.55	3.66	2.84
Mean	A D |_____| t=3.31*	B D |_____| t=3.19*	C D |_____| t=4.63*	
't' value	A B |_____| t=0.26@	A C |_____| t=0.04@	B C |_____| t=0.35@	
Total amino acids:	600.9	689.8	636.2	96.67
Mean	A D |_____| t=4.77*	B D |_____| t=3.22*	C D |_____| t=5.23*	
't' value	A B |_____| t=1.44@	A C |_____| t=1.07@	B C |_____| t=0.84@	

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

Higher amino acid levels were recorded from day 1 to 30 when compared to that of controls. From day 1 of infection (613.5 μg/g), the amino acids level increased gradually to day 9 (703.0 μg/g), which was again declined on day 16 (626.5 μg/g) and 30 (546 μg/g) but still it is higher than that of control value. 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 increased levels of protein, DNA, RNA and amino acids of small intestine were significant in groups A, B and C when compared with controls (except the non-significant difference of DNA between groups C and c) (Table 3). No significant difference was found in protein, DNA, RNA and amino acids level of small intestine when compared among the experimental groups A, B and C.

Earlier studies (Vardhani, 2006) showed that the mechanism of immunity against *A. caninum* in mice involves both a specific and non-specific immunological response which may be due to the action of a cellular and/or mediators of the associated response in the gut. In an analysis of the biochemical contents in the stomach of mice to infection with *A. caninum*, Tarakalakshmi and Viveka Vardhani (2014) attributed the responsiveness to functional host parasite interactions.

The statistically significant increased level of protein, DNA, RNA and amino acids in all the three experimental groups of mice indicates the occurrence of potential primary immune response thereby disturbing the metabolism of these four biochemical constituents. It is of interest to note that the increase of protein, DNA, RNA and amino acids was found to be non-significant when comparison was made among the experimental groups; this may be because of the immunological responsiveness/gut anaphylaxis in mice received small dose (500 larvae), medium dose (1000 larvae) and heavy dose (2000 larvae).

Also, it is conceivable that antigenic stimulation may accelerate development and even ontogeny of specific immune responses as suggested by Allen and Maizels (1996) and Alkazmi and Behnke (2010) in various models with reference to gastrointestinal nematodes.

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