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SDS PAGE Protein profile in small intestine of mice during ancylostomiasis

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ABSTRACT

Qualitative protein profile in the small intestine of singly and multiply infected mice were studied during ancylostomiasis. In singly infected animals, polypeptide changes occurred on day 4 and 9 of infection. In multiply infected animals also, the polypeptide pattern was markedly altered during day 4 and 9 of experiment. These studies demonstated that both low molecular and high molecular weight protein bands are formed due to antigen – antibody reactions in the small intestine of the host.

Key words : SDS PAGE, Protein profile, Small Intestine, Mice

INTRODUCTION

Hookworm infection, one of the major cosmopolitan diseases of mankind causes iron deficiency anemia effecting nearly one billion people worldwide. Hookworms feed on blood from capillaries in the lamina propria and submucosa of the small intestine (Cappello *et al*., 1995). The larval nematodes of hookworms like *Ancylostoma braziliense, Ancylostoma caninum* or *Uncinaria stenocephala* penetrate the corneal layer of epidermis and/or visceral organs of humans causing CLM and VLM respectively (Black Well and Vega-Lopez, 2001). Zoonotic infection with infective larvae of *A. caninum* can also occur, where in certain individuals can induce eosinophilic enteritis (Prociv and Croese, 1996).

During single infections (oral) in mice, most of the larvae were found within the gastrointestinal tract during day 1 to 4 of infection and expel from the gastrointestinal tract by day 9 of infection. However, a threshold larval burden remain in the muscles causing pathogenic reactions in the abnormal host. In immunized groups the larval migration and distribution was comparatively less and most of the larvae were expelled from the gastrointestinal tract by day 1 of infection (after the lost dose of the infection) (Vardhani, 2006). The pathogenic larvae may cause disturbance in the host's metabolism, physiology and pathology. Therefore, the present study was undertaken to assurs the protein profile (Qualitatively) in the small intestine of mice infected with single and multiple doses of *A. caninum* larvae.

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MATERIALS AND METHODS

Infective (L3) larvae of *A. caninum* were cultured and various infective doses were prepared following the method of Sen *et al*., (1965) and Scott (1928). Infective larvae were given orally to 6 groups of male swiss albino mice (6 to 8 wks old; 25-31 g); 3 groups (A, 500 dose; B, 1000 dose; C, 2000 dose) (4 in each group) served as single doses and another 3 groups (D, 125 + 125 + 250 dose; E, 250 + 250 + 500 dose; F, 500 + 500 + 1000 dose at weekly interval) (4 in each group) as multiple doses. One group (c) of 12 mice was kept as uninfected controls for comparison. Two mice from each group were necropsied on day 4 and 9 of infection (after the last dose of infection in groups D, E and F). Two mice

from controls were also sacrificed on day 4 and 9 of experiment. Tissues of small intestine were separated and analysed for protein profile using SDS-PAGE apparatus.

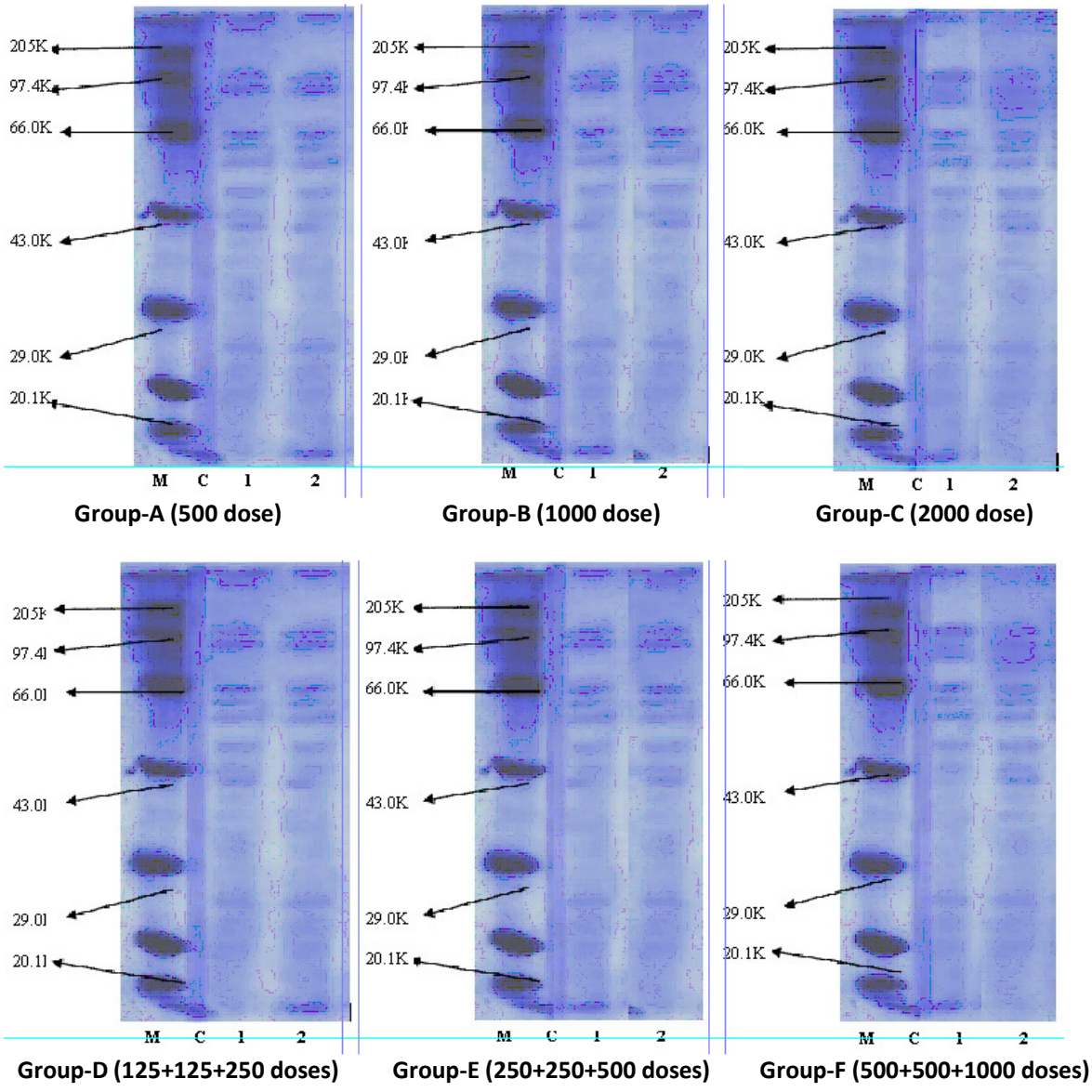
RESULTS AND DISCUSSION

The protein bands pattern in small intestine of singly and repeatedly infected mice (on day 4 and 9 of infection) is shown in Figure-1.

The small intestine samples showed a series of several protein bands ranging between 265 to 20.1 kDa of the marker. Difference was observed in the pattern of protein profiles on day 4 and 9 of infection in both singly (groups A, B and C) and repeatedly (group D, E and F) infected mice in comparison with marker and control value.

500 dose: Eight polypeptides (~ 25, ~ 42, ~ 46, ~ 58, ~ 62, ~ 66, ~ 96 and ~ 97.4 kDa) were found common on day 4 and 9 of infection. Low molecular weight polypeptides (~ 20.1 kDa) were absent in controls and on day 4 of infection. However, one low molecular weight polypeptide was found on day 4 of infection. The expression of polypeptide was not clear in intestinal region other than the above specified polypeptides.

Figure-1. Polypeptides pattern in small intestine of mice on day 4 and 9 of infection in groups A-F.
(M-Marker; C-Control; 1-Day 4 of infection; 2-Day 9 of infection)



1000 dose: Eight polypeptides (~ 27, ~ 43, ~ 46, ~ 54, ~ 60, ~ 66, ~ 94 and ~ 97.4 kDa) were found common on day 4 and 9 of infection.

2000 dose: Six polypeptides (~ 28, ~ 46, ~ 56, ~ 62, ~ 66 and ~ 97.4 kDa) were present common on day 4 and 9 of infection. The polypeptide bands were not clearly expressed nearing 97.4 kDa band on day 4 and 9 of infection. One low molecular weight polypeptide below ~ 20.1 kDa was recorded on day 1 of infection. Two polypeptides (~40 and ~ 43 kDa) were present only on day 9 of infection.

125+125+250 dose: Eight polypeptides (~ 27, ~ 43, ~ 50, ~ 58, ~ 62, ~ 66, ~ 97.4 and ~100 kDa) were found common on day 4 and 9 of infection. However, one polypeptide (below ~ 20.1 kDa) was present on day 9 of infection.

250+250+500 dose: Eight polypeptides (~ 26, ~ 43, ~ 50, ~ 57, ~ 62, ~ 66, ~ 95 and ~ 99 kDa) were found common on day 4 and 9 infection. One low molecular weight polypeptide (~ below 20.1 kDa) was present on day 4 of infection.

500+500+1000 dose: Six polypeptides (~ 26, ~ 50, ~ 58, ~ 62, ~ 66 and ~97.4 kDA) were found common on day 4 and 9 of infection. However, two additional polypeptides (~ 40 and ~ 43 kDa) appeared on day 9 of infection. The expression of polypeptides in between ~ 66.0 and ~ 97.4 kDa (as observed in the marker) is not clear on day 4 and 9 of infection.

The present investigation clearly demonstrated qualitative alterations in protein fraction and their intensity profiles in small intestine of both singly and repeatedly infected animals on day 4 and 9 of infection. The occurence of some common protein bands, and the difference in the pattern of protein bands in both singly and repeatedly infected mice (on day 4 and 9 of infection) would clearly suggest the effect of larva migrans at molecular level. Infectious organisms may produce ill-effects in fish/rats/mice etc., at physiological, tissue, cellular and molecular level as suggested by Das and Mukherjee (2003) and Begum (2004). When mice are subjected to stress due to invasive larvae of *A. caninum*, there may be synthesis of stress proteins which was evident in electropherogram.

The protein profile of small intestine in singly and repeatedly infected mice showed much variation in the molecular weight of protein bands when compared with marker and control values. Low molecular weight and high molecular weight polypeptide bands were evident in mice received infection. These results compare well with that of Tarakalakshmi and Viveka Vardhani (2014) who reported significant changes in the quantitative estimation of protein at day 1, 4, 9, 16 and 30 of infection in mice during ancylostomiasis.

Variation in resistance to infection with gastro-intestinal nematodes is influenced by complex factors which involve environmental, physiological and behavioural as well as immunological mechanisms. The inflammatory reaction provoked by the immunological response against the parasites/larvae spreads down the gut. This phenomenon is clearly seen in the present studies of ancylostomiasis in mice. Zoonotic infection with infective larvae of *A. caninum* can also occur, where in certain individuals can induce eosinophilic enteritis (Prociv and Croese, 1996). The adverse environment in the gastrointestinal tract might have brought significant changes in the quantitative as well as qualitative protein content in experimental mice as suggested by Vardhani (1979, 2002) during ancylostomiasis in mice.

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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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