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Research Article

ELECTROPHORETIC ANALYSIS OF EXCRETORY/SECRETORY ANTIGENS OF *GASTROTHYLAX CRUMENIFER* FROM BUFFALO

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ABSTRACT
Live adult *Gastrothylax crumenifer* worms were collected from buffaloes slaughtered at local abattoirs and washed several times with PBS (pH 7.4). Live intact adult flukes were suspended in DPBS (pH 7.2) and incubated at 37°C in a BOD incubator for 8 hrs. The fluid was centrifuged at 7000 rpm for 30 min at 4°C and the supernatant was designated Excretory and Secretory (E/S) antigens. On Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, seven major bands *viz.*, 240, 220, 180,130, 92, 72 and 30 kDa and three minor bands having molecular weight (MW) lesser than 30 kDa size were observed.

Keywords*: Gastrothylax Crumenifer, E/S Antigens, SDS-PAGE, Buffalo*

INTRODUCTION
Paramphistomosis of livestock is considered as a disease of great economic importance. It is widely prevalent in domestic ruminants in India and several other countries, resulting in heavy losses in terms of morbidity and reduced wool, meat, and milk production (Manna, 1994 and Hassan *et al.*, 2005). A number of investigators have been trying to develop specific serodiagnostic tests for early detection of a related trematode infection in animals (Yadav *et al.*, 2005; Raina *et al.*, 2006). Some of the methods have been tested against experimental and field infections of cattle and buffaloes, but, little effort has been made in immunodiagnosis of ruminal amphistomes. Hence, the study was carried out to analyze the protein profiles of in excretory/ secretory (E/S) antigens of *Gastrothylax crumenifer* through SDS-PAGE.

MATERIALS AND METHODS
Live, mature *Gastrothylax crumenifer* flukes were collected from the rumen of Indian water buffaloes (*Bubalus bubalis)*, slaughtered at the local abattoirs of Pattukkottai and Thanjavur. The flukes were thoroughly washed in Phosphate Buffer Saline without glucose, pH 7.4 and pre maintained at 37ºC. After careful preservation in PBS, the worms were immediately transferred to the laboratory for further processing. The flukes were identified based on specific morphological characters (Soulsby, 1981). Excretory-secretory antigens (ES-Ag) were prepared as per the procedure described by Saifullah *et al.*,(2011) with minor modifications. Live intact adult flukes were weighed and suspended in DPBS (pH 7.2) and were incubated at 37°C in a BOD incubator for 8 hr. Then, the fluid was centrifuged at 7000 rpm for 30 minutes at 4°C and the supernatant collected was designated as E/S antigens. The E/S antigens was further lyophilized in a centrifugal freeze-dryer and then it was reconstituted in DPBS and stored at -20°C till further use. The total protein content of the samples was estimated (Lowry *et al.*, 1951). SDS-PAGE analysis of E/S antigens was carried out as per the method described by Laemmli (1970) and the gels were silver stained by the method of Merril *et al.*, 1981.

RESULTS AND DISCUSSION
In the present study, the total protein concentration was 0.163 mg/mL. Each gel well was loaded with 80 µL of E/S sample. 10 % SDS-PAGE (discontinuous method) under non reducing conditions was carried out at 100V for 8 hours. Then, the gel was silver stained by adopting the method of Laemmli (1970). The

electrophoretogram was studied using the protein marker (low molecular weight Genei, Bangalore) 3.5 to 205 kDa. Seven prominent bands ranging from 240 kDa to 5 kDa were observed as shown in the figure.

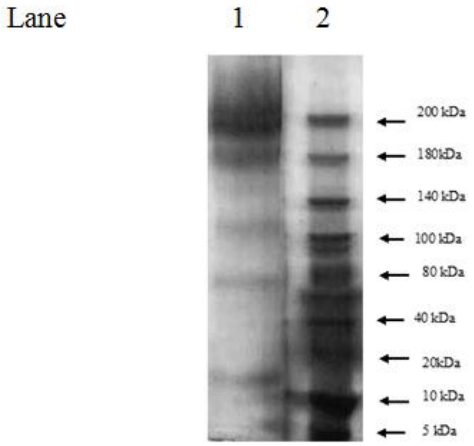

Figure 1: SDS-PAGE analysis of E/S antigens of Gatrothylax crumnifer (Silver staining)
Lane 1- E/S antigens of Gatrothylax crumnifer
Lane 2- Protein markers (Low molecular weight)

On SDS-PAGE analysis, seven major bands *viz.,* 240, 220, 180,130, 92, 72 and 30 kDa and three minor bands having molecular weight (MW) lesser than 30 kDa were observed. Fainter bands around 160 kDa were also observed. Among the prominent bands, the intensity was stronger for higher MW bands (240, 220 and 180 kDa). The intensities of low MW prominent bands were weaker and the separation was not very discrete suggesting the presence of non proteinaceous complex nature of these proteins. Saifullah *et al.,* (2000) reported the presence of heterogeneous population of varying MW ranging from 14 to 205 kDa in eight partially purified fractions of somatic extracts of *Gastrothylax crumenifer*.
Saifullah *et al.,* (2011) reported SDS-PAGE profile of purified fractions of *Gastrothylax crumenifer* containing 8-12 polypeptides having MW. less than 14 to 165 kDa and reported the presence of only three major bands at 105, 141 and 165 kDa. In our study also, we observed bands having MW in the range of < 5kDa. Four prominent bands having MW > 130 kDa and the presence of several polypeptide bands in the range of < 29 to > 205 kDa, which corroborated with the results of Ahmed *et al.,* (2004). However, the slight variations in the relative MW of the polypeptides may be due to the influence of season on the reproductive cycle of parasite as reported earlier by Hanna *et al.,* (1988) and the geographical location of the parasite. Hence, further studies on purification and characterization of E/S antigens of *G. crumnifer*, which could help to develop specific serodiagnostic test for earlier detection of Paramphistomosis in buffaloes.

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