ASSESSMENT OF ANTIGENIC PROFILE OF EXCRETORY/SECRETORY PRODUCTS OF *COTYLOPHORON COTYLOPHORUM* USING SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS

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Abstract: In the present investigation, *Cotylophoron cotylophorum* adult flukes were collected from sheep slaughtered at the abattoir in pattukottai, Thanjavur district, Tamil nadu. The flukes collected were confirmed to be *Cotylophoron cotylophorum* using their predilection site, size and morphology. Live, intact mature flukes were washed thoroughly with Phosphate buffered saline (PBS, pH 7.4) and suspended in RPMI-1640 medium at 37°C in a incubator for 16 hours. After incubation, the fluid was collected, centrifuged at 10,000 rpm for 15 minutes at 4°C and the supernatant was used as Excretory/Secretory(E/S) antigen. The total protein content of the antigen estimated by Lowry method was 0.876 mg/mL. On Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining, six protein bands were observed. Out of which, three prominent bands at 66 kDa, 40 kDa and 20 kDa and three minor bands at 90 kDa, 32 kDa and 25 kDa were observed in the E/S antigen of *Cotylophoron cotylophorum.* . Further studies are warranted to identify the immunogenic proteins, which will be useful for immunodiagnosis of *Cotylophoron cotylophorum* infection in sheep.

Keywords: Excretory/Secretory antigen, *Cotylophoron cotylophorum,* SDS-PAGE, Sheep.

Introduction: Paramphistomiasis has been a ubiquitous trematode infections in ruminants, but has recently emerged as an importance cause of productivity loss (Anuracpreeda,2008). Adult fluke that lives in rumen and reticulum of ruminants does not cause serious problem, but massive number of immature paramphistomes can migrate through intestinal tract causing acute gastroenteritis in th small intestine with high morbidity and mortality rate especially in young animals (Manna, 1994 and Hassan *etal.,*2005). In general, mixed infections are common in ruminants.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 excretory/ secretory (E/S) antigens of *Cotylophoron cotylophorum* in sheep through SDS-PAGE

Materials and Methods: Live, mature *Cotylophoron cotylophorum* flukes were collected from the rumen of sheep 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 37°C. 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 hrs. 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 re constituted 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).

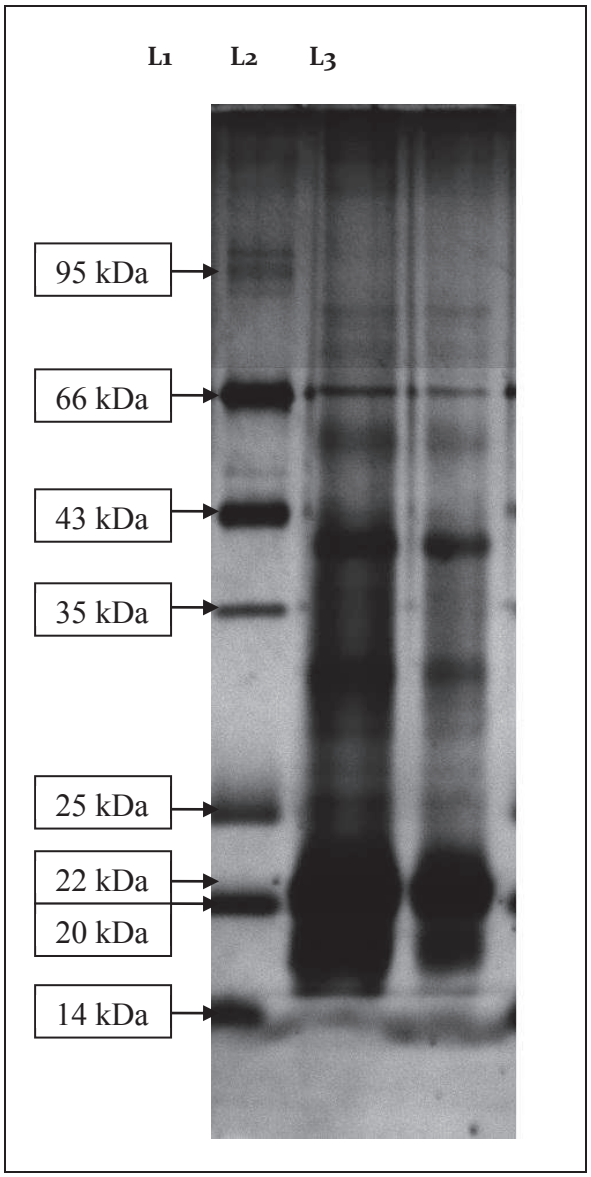
Results and Discussion: In the present study, the total protein concentration was 0.876 mg/mL. Each gel well was loaded with 80 µl of E/S sample. 10 % SDS-PAGE (discontinuous method) under non-reducing conditions was carried out at 100V for 8 hours. Then, the gel was silver stained by adopting the method of Laemmli (1970). The electrophoretogram was studied using the protein marker (medium range molecular weight, Genei, Bangalore).

On Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining, six protein bands were observed. Out of which, three prominent bands at 66 kDa , 40 kDa and 20 kDa and three minor bands at 90 kDa, 32 kDa and 25 kDa were observed in the E/S antigen of *Cotylophoron cotylophorum.*

No information is available in literature on protein profiles of *Cotylophoron cotylophorum.* Hence, the results were compared with other ruminal amphistomes. 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 molecular weight 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 molecular weight in the range of 14 to 90 kDa. Three prominent bands having molecular weight of 66, 40 and 20 kDa and three minor polypeptide bands in the range of 90,32 and 25 kDa, which corroborated with the results of Ahmad *et al.*, (2004). However, the slight variations in the relative molecular weightof the polypeptides may be due to the influence of season on the reproductive cycle of parasites 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 *Cotylophoron cotylophorum* which could help to develop specific serodiagnostic test for earlier detection of paramphistomosis in Sheep.



SDS-PAGE analysis of E/S antigens of *C.cotylophorum* (Silver staining)
Lane 1. Mid-range protein marker
Lane 2. E/S antigens of *C.cotylophorum* (10 microliters)
Lane 3. E/S antigens of *C.cotylophorum* (5 microliters)

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