ANALYSIS OF POLYPEPTIDE PROFILE OF EXCRETORY/SECRETORY ANTIGENS OF *FISCHOEDERIUS ELONGATUS* FROM CATTLE ORIGIN USING SILVER STAINING

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Abstract: In the present study, *Fischoederius elongatus* adult flukes were collected from cattle slaughtered at the abattoir in Thanjavur , Tamil nadu. The flukes collected were confirmed to be *Fischoederius elongatus* 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 1.20 mg/mL. On Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining were carried out to analyse the polypeptide profile. Six protein bands were observed in E/S antigen under study. Out of which, three prominent bands at 66 kDa, 20 kDa and 14 kDa and three minor bands at 95kDa, 40 kDa and 35 kDa were observed in the E/S antigen of *Fischoederius elongatus*. Further studies are warranted to identify the immunogenic proteins, which will be useful for serodiagnosis of *Fischoederius elongatus* infection in cattle.

Keywords: Antigenic profile, Cattle, *Fischoederius elongatus*, Silver staining.

Introduction: Amphistome parasites of domestic ruminants are abundant in the tropical and subtropical countries. The disease, amphistomosis, causes high morbidity and mortality resulting in great economic losses through reduced productivity (Manna, 1994 and Hassan *et* al., 2005). The adult worms inhabiting the rumen have low pathogenicity, while the migrating immature stages cause severe pathological disturbances (Yadav *et al*.,2005; Raina *et al*.,2006). A striking feature of the rumen dwelling parasites particularly *Fischoederius elongatus* is marked seasonality in egg production (Hanna *et al*., 1988). As a result eggs cannot be detected in faeces by routine parasitological investigation during the non-reproductivephase. Therefore, immunological diagnosis would be of considerable significance during the non-egg producing period. Hence the aim of the present study was to identify the potential antigenic polypeptides of *F.elongatus* that may be useful for the immunological diagnosis of amphistome infection particularly during the non-reproductive phase of the parasite.

Materials and Methods: Live, mature *F.elongatus* flukes were collected from the rumen of cattle slaughtered at the local abattoirs of Pattukkottai and Thanjavur areas. 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 etal.,(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 reconstituted in DPBS and stored at -20°C till further use. The total protein content of the samples was estimated (Lowry etal,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 1.20 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 were carried out to analyse the polypeptide profile. Six protein bands were observed in E/S antigen under study. Out of which, three prominent bands at 66 kDa , 20 kDa and 14 kDa and three minor bands at 95 kDa, 40 kDa and 35 kDa were observed in the E/S antigen of *Fischoederius elongates.*

No information is available in literature on protein profiles of *Fischoederius elongatus.*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 *Gastrothylaxcrumenifer.*

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 95kDa. Three prominent bands having molecular weight of 66, 20 and 14kDa and three minor polypeptide bands in the range of 95, 40 and 35 kDa, which corroborated with the results of Ahmad *et al.*, (2004). However, the slight variations in the relative molecular weight of 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 *Fischoederius elongatus* which could help to develop specific serodiagnostic test for earlier detection of Paramphistomosis in cattle.

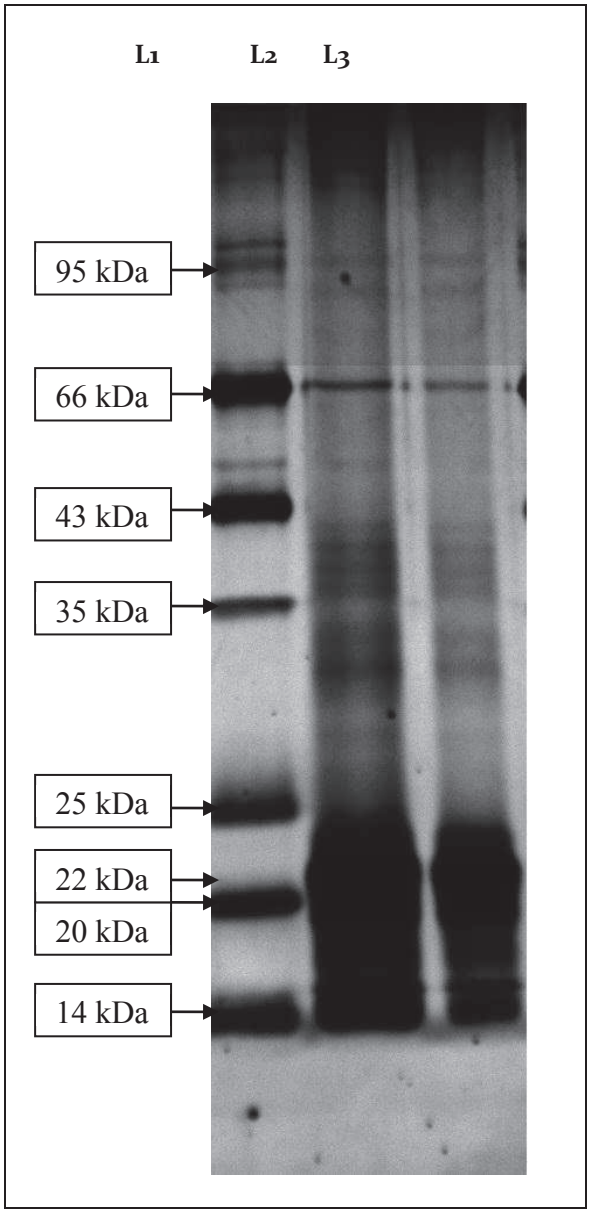

Fig: SDS-PAGE analysis of E/S antigens of *Fischoederius elongates* (Silver staining)

Lane 1. Mid rangeprotein marker

Lane 2. E/S antigens of *F.elongatus* (10 microliters)

Lane 3. E/S antigens of *F.elongatus* (5 microliters)

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