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Research Article

AN ANALYSIS OF ANTIGENIC PROFILE OF EXCRETORY/ SECRETORY ANTIGENS OF *OESTRUS OVIS* LARVAE FROM SHEEP USING SILVER STAINING

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ABSTRACT
The present investigation was aimed to ascertain the polypeptide profile of excretory/secretory (E/S) antigens of larvae of *Oestrus ovis* by silver staining procedure. Mature larvae of *Oestrus ovis* were collected from sheep slaughtered at local abattoir in Orathanadu and Thanjavur. Live, intact mature larvae were washed thoroughly with Phosphate buffered saline (PBS, pH 7.4) and suspended in PBS 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 E/S antigen. On SDS-PAGE and silver staining analysis, eight protein bands were observed in E/S antigen under study. Out of which, six prominent bands at 66 kDa, 55kDa, 40 kDa,30 kDa, 23 kDa and 16 kDa and two minor bands at 85 kDa, and 74 kDa were observed in the E/S antigen of *Oestrus ovis*. Further studies are warranted to identify the immunogenic proteins, which will be useful for developing immunodiagnostic, prophylactic and targeting drug molecule against *Oestrus ovis* infection in sheep.

***Keywords*:** *Oestrus Ovis, Excretory/Secretory Antigen, Protein Profile, SDS-PAGE, Sheep*

INTRODUCTION
Nasal myiasis is the infestation of living animals with the larvae of dipteran flies in nasal passages, which at least for a certain period feed on the host's dead or living tissues, body substances or ingested foods. The larvae of the sheep nasal bot fly, *Oestrus ovis* are commonly found in the nasal cavities and frontal sinuses, sometimes also in the maxillary sinuses of domestic sheep. The viviparous females swarm around the heads of the animals and deposit the larvae from a distance of several centimeters into the nostrils, and sometimes also into the eye orbits, in batches of one to several dozen. The larvae then migrate into the nasal cavities and paranasal sinuses where they envelop. Infested animals excrete a purulent discharge from the nostrils, shake their heads, grate their teeth, sneeze, have difficulty breathing, and rub their noses on the ground or against their forelegs.
Larval feeding activity involves secretion of enzymes into the upper respiratory mucosal substrate and such enzymes degrade the substrate into smaller units that are then swallowed to support larval growth and development (Tabouret *et al.*, 2003). These parasitic larvae use proteolytic enzymes for larval migration, establishment, feeding, growth, and development (Muharsini *et al.*, 2000). Hence, the present study was undertaken to identify the polypeptide profiles in excretory/secretory (E/S) antigens of larvae of *Oestrus ovis* by SDS-PAGE analysis.

MATERIALS AND METHODS
The *Oestrus ovis* larvaewere collected from sheep slaughtered at local abattoirs in Orathanadu, Pattukkottai and Thanjavur areas. The larvae were washed thoroughly with PBS (pH 7.4) and were identified on the basis of morphological keys (Zumpt, 1965). Twenty five live, intact larvae were washed thoroughly with Phosphate buffered saline (PBS, pH 7.4) and suspended in 10 ml of PBS 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 E/S antigen. The supernatants thus collected were stored at -20˚C

till further use. The protein content was estimated by Lowry's method (1951). SDS-PAGE was carried out, as described by the method of Laemmli, (1970). Each gel well was loaded with 80 µL of cystic fluid sample in 10 % SDS-PAGE under non - reducing conditions at 100 V for 8 hours. The gel was silver stained by the method of Merril *et al.,* 1981. The electro- phoretogram was studied using the protein marker (low molecular weight Genei, Bangalore) in the range of 3.5 to 205 kDa.

RESULTS AND DISCUSSION

The present investigation was aimed to ascertain the polypeptide profile of excretory/secretory (E/S) antigens of larvae of *Oestrus ovis* by silver staining procedure. Mature larvae of *Oestrus ovis* were collected, washed thoroughly with Phosphate buffered saline (PBS, pH 7.4) and suspended in PBS 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 E/S antigen. The protein content of excretory/secretory (E/S) antigens of larvae of *Oestrus ovis* was found to be 1.648mg / ml. The presence of polypeptide bands in excretory/secretory (E/S) antigens of larvae of *Oestrus ovis* was assessed by SDS-PAGE as shown in figure-1.

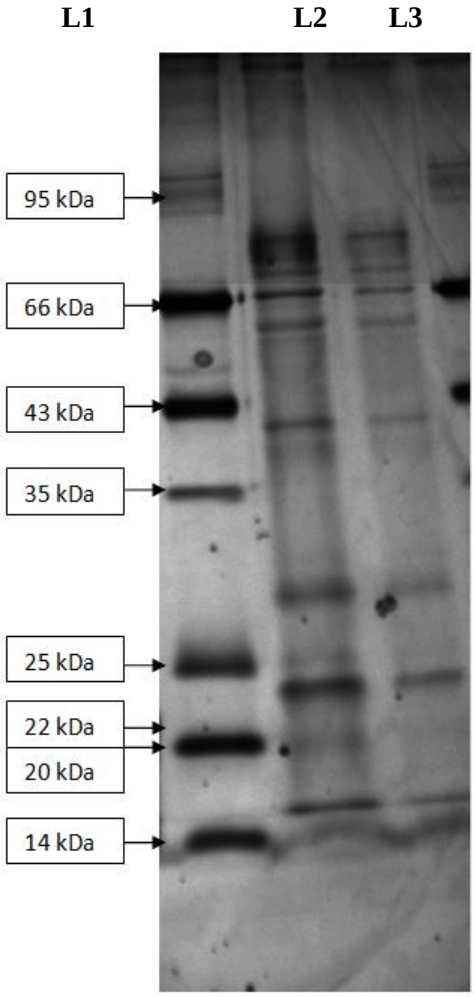

Figure 1: SDS-PAGE Analysis of E/S Antigens of *Oestrus Ovis* (Silver Staining)

Lane 1: Mid range protein marker
Lane 2: E/S antigens of *O.ovis* (10 microliters)
Lane 3: E/S antigens of *O.ovis* (5 microliters)

On SDS-PAGE and silver staining analysis, eight protein bands were observed in E/S antigen under study. Out of which, six prominent bands at 66 kDa, 55kDa, 40 kDa,30 kDa, 23 kDa and 16 kDa and two minor bands at 85 kDa, and 74 kDa were observed in the E/S antigen of *Oestrus ovis*.

Tabouret *et al*., (2001) reported a 28 kDa protein band in excretory / secretory products of *Oestrus ovis* larvae by SDS-PAGE analysis and they assessed humoral immune response to this protein in sheep. Tabouret *et al*., (2003) demonstrated serine proteases activity in excretory / secretory products of *Oestrus ovis* larvae and reported serine proteases from 20 to 100 kDa molecular weights. Further, they observed that these proteases appear to originate mainly from the gut and are exported on nasal or sinusal mucosa.

Chambers *et al*., (2003) reported four proteolytic enzymes, comprising two serine proteases, a metalloproteinase and an aspartyl proteinase with molecular weights ranging from 20 to 40 kDa, with activity across a wide pH range in excretory / secretory products of *Lucilia sericata* larvae. Musleh *et al*., (2015) analyzed the protein profile of excretory/secretory products of camel nasal bot fly, *Cephalopina titillator* by SDS-PAGE and observed a protein band at 32 kDa level. Further, they confirm that the most active areas secreting E/S products are the salivary glands and anterior mid gut regions.

Alborzi *et al*., (2014) observed three polypeptide bands at 58, 42 and 28 kDa level in the E/S antigen of *Oestrus ovis* by SDS-PAGE analysis. Similar results were observed in the present study also. However, the slight variations in the relative molecular weights of the polypeptides may be due to the influx of season on the reproductive cycle of parasite and the geographical location of the parasite.

Further studies are warranted to identify the immunogenic proteins, which will be useful for developing immunodiagnostic, prophylactic and targeting drug molecule against *Oestrus ovis* infection in sheep.

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