Assessment of Cross – Antigenicity of Excretory / Secretory Antigens Amongst *Oesophagostomum columbianum* and *Haemonchus contortus* of Goats Using Western Blotting

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Serodiagnosis of helminthic infections continues to be a difficult task on account of cross-reactivity and sharing of antigenic molecules. Despite the evidence of cross-reactivity among helminths, the information on gastro-intestinal nematodes is, however limited (Cuquerella *et al.*, 1994). The present study was carried out to assess the cross-reactivity of excretory/secretory (E/S) antigen of *Oesophagostomum columbianum* with *Haemonchus contortus* of goat origin.

Materials and Methods

Adult live *Oesophagostomum columbianum* worms were collected from the colon of goats slaughtered at corporation slaughter house, Perambur, Chennai. The collected worms were washed five times in normal saline and subsequently washed five times in Phosphate buffered saline (PBS, pH 7.4), containing penicillin (100 IU/100ml), streptomycin (1 mg/100ml) and nystatin (1 mg/100ml). The worms were then identified based on morphological features using standard keys (Soulsby, 1982). The fresh and highly motile worms were transferred to RPMI 1640 medium containing penicillin (100 IU/100 ml) and streptomycin (1 mg/100 ml) and cultured at 37°C for 24 h at a concentration of approximately 300 worms per 20 ml in a culture flask at 5 per cent CO₂ concentration. The medium was changed every 6 h after incubation and fresh medium with 2 per cent glucose was added throughout incubation. Worm viability was monitored throughout this period on the basis of motility and integrity of the worms. After the incubation period, the culture medium was collected by decantation and filtered through a 0.22 mm filter (Millipore). Then, the culture medium was centrifuged at 10,000 rpm for 30 min at 4°C and the supernatant was labelled as excretory/secretory (E/S) antigen. The protein concentration of the E/S antigen was determined using bicinchoninic acid (BCA) method using protein estimation kit (Genei, Bangalore).

Immune goat serum against E/S antigen of *H. contortus* was procured from Division of Parasitology, Indian Veterinary Research Institute (IVRI), Izatnagar (Uttar Pradesh) for this study.

Immune goat serum against E/S antigen of *O.columbianum* was obtained from Department of Parasitology, College of Veterinary Science, Kolkata for this study.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to observe the polypeptide patterns of E/S antigen of *Oesophagostomum columbianum* according to their molecular weights under reducing gel conditions as per method of Laemmli (1970).

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Cross-reactive study on E/S antigen of *O. columbianum* was done by western blotting technique (Towbin *et al*., 1979) using the goat immune serum against E/S antigen of *Haemonchus contortus*.

**Results and Discussion**

In this study, a total of 3000 live adult *O. columbianum* worms were collected from the colon of goats slaughtered at local abattoir. During the incubation period, adult worms remained viable as assessed qualitatively by both motility and clumping tendency. No bacterial contamination was detected during *in-vitro* culture. The protein content of E/S antigen of *O. columbianum* was 1.3 mg/ml. On characterization, the E/S antigen revealed six polypeptide bands at 29.0, 38.0, 49.0, 71.0, 84.0 and 95.0 kDa molecular weights by SDS-PAGE analysis. Jas *et al.* (2010) analysed the antigenic composition of E/S antigen of *O. columbianum* by SDS-PAGE. They observed ten polypeptides with molecular weights ranged between 22.5 to 98.0 kDa. On western blot analysis, the E/S antigen of *O. columbianum* probed with serum from goat immunized with E/S antigen of *O. columbianum* revealed four reactive bands. The E/S antigen of *O. columbianum* was also probed with serum from goat immunized with E/S antigen of *H. contortus* and no reactive bands were detected. Based on this finding it was concluded that there was no cross-reactivity between E/S antigen of *O. columbianum* and *H. contortus*.

In contrast to the present findings, Cuquerella *et al.* (*loc. cit*) reported cross-antigenicity among sheep strongylids viz., *H. contortus*, *Trichostrongylus colubriformis* and *Ostertagia circumcincta* using western blotting. But in this study, they used somatic antigens of *H. contortus* as antigenic source probed with sera from lambs with nonspecific heterologous infection. Molina *et al.* (1999) confirmed the cross-reactivity of somatic antigens of *H. contortus* using serum collected from goat infected with *Teladorsagia circumcincta* by immune blotting. This could be due to the fact that somatic antigens contain complex cross-reactive antigenic determinants whereas, E/S antigen usually display a simple antigenic polypeptide composition. Based on immune assays, it was also observed that the sensitivity and specificity of E/S antigens were high compared to somatic antigens (Mir *et al.*, 2008; Bashir Ahmada *et al.*, 2011).

![Fig 1. SDS–PAGE analysis of E/S antigen of *O. Columbianum*](image)

**Fig 1. SDS–PAGE analysis of E/S antigen of *O. columbianum***

- M – Molecular weight marker
- L1, L2 – E/S antigen

![Fig 2. Western blot analysis of E/S antigen of *O. Columbianum* with homologous sera](image)

**Fig 2. Western blot analysis of E/S antigen of O. Columbianum with homologous sera**

- M – Molecular weight marker
- L1–E/S antigen probed with homologous sera (Positive control)

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Fig 3. Western blot analysis of E/S antigen of *O. Columbianum* with heterologous sera
M - Molecular weight marker
L1, L2-E/S antigen probed with heterologous sera.

Summary

In this study, the E/S antigen of *O. columbianum* was prepared and characterized by SDS-PAGE analysis. The cross reactivity of E/S antigen was also assessed by immune blotting. Based on the results, it was concluded that there was no cross-reactivity between E/S antigens of *O. columbianum* and *H. contortus*.

References


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Post-Synchronization Progesterone Profile of Indigenous Goats During Early Pregnancy*

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For effective reproductive management of goats, early and accurate diagnosis of pregnancy by estimation of serum progesterone during post mating period by radioimmunoassay (RIA) method is important. The present study was conducted to determine serum progesterone concentration during oestrus and thereafter till 40 days post-mating and its effectiveness for early diagnosis of pregnancy following synchronization of oestrus by PGF₂α and buck

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