EVALUATION OF SURFACE REACTIVITY PATTERNS BETWEEN OVINE NEMATODES BY IMMUNOPEROXIDASE ASSAY

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Abstract: Surface-reactivity amongst ovine nematodes viz., *Haemonchus contortus,Oesophagostomum columbianum and Bunostomum trigonocephalum* was evaluated by immunoperoxidase test. In this assay, third stage larvae of *H. contortus* was used as antigen source in the interface of heterologous sera of *O.columbianum* and *B.trigonocephalum*. A strong and intense surface reactivity was observed in homologous sera whereas mild and less intense surface reactivity was observed with heterologous sera.

Keywords: Immunoperoxidase assay, nematodes ,surface reactivity.

Introduction: Infections with gastro- intestinal nematodes are prevalent world wide including India and pose a major constraint on sleep and goat health and production [6]. Though the evidence of cross-reactivity among the helminths has been reported, the literature on cross reactivity amongst strongylid nematodes is however scanty. Hence, the present study was undertaken to elucidate the surface reactivity amongst strongylid nematodes viz., *H.contortus*, *B. trigonocephalum* and *O. columbianum* using immunoperoxidase assay.

Materials and methods: Three species of gastro-intestinal nematodes viz., *H. contortus, B. trigonocephalum* and *O. columbianum* were collected from a local abattoir of sheep and goats. The parasites were recovered from their respective sites of predilection at necropsy following standard technique [5]. The collected worms were washed repeatedly with distilled water followed by physiological saline and phosphate buffered saline (pH 7.4). The worms were identified uptospecies level using standard keys [7].

Soluble extract antigen (SEA) for each species of the referral nematodes was obtained by processing adult parasites of *H. contortus, O.columbianum* and *B. trigonocephalum* separately using standard technique [1],[3]. The protein concentrationof the referral antigens (SEA) was estimated using bovine serum albumin fraction V as the standard [4]. Rabbit hyper immune sera (RHIS) were raised against SEA of *Haemonchus contortus,Oesophagostomum columbianum* and *Bunostomum trigonocephalum* using standard immunisation protocol to serve as reference sera.

Larval culture: Immediately after collection of adult worms, they were used for preparation of copro-cultures using the standard technique [7]. Adult female worms of *H. contortus* were teased out for isolation of eggs and were inoculated into sterile faeces-charcoal mixture, contained in glass petridish. The cultures were incubated for 5-7days at 27-30°C with 70-75% relative humidity. Several such cultures were prepared to obtain sufficient quantity of larvae. The third stage sheathed larvae (L_3) were obtained in 4-5 days and all infective larvae were collected in outside water jacket form culture. The larvae were washed several times in distilled water with low speed centrifugation at 1000 rpm for 5-10 minutes after 30 minutes storage at 4 ° C and finally suspended in known volume of distilled water. These larvae were stored alive into sterilized vial in a refrigerator with adequate arrangement for aeration until use for the study.

Immunoperoxidase assay: An immunoperxiase assay was employed to examine the surface reactivity patterns of larvae in fixed preparation as described by Farr and Nakane(1981) with slight modifications.

The chilled acetone fixed larva of *H.contortus* (L_3) on microscopic glass slides were air dried at room temperature for 15 minutes and the reaction zones marked out. Three glass slides (larvae fixed) were used for this test. The slides were treated with 1% gelatine and washed in PBS (pH 7.4) gently. One slide was used for treating with homologous sera (*H. contortus* hyperimmune sera) and other two slides were used for treating with heterologous sera (hyper immune sera of *O. columbianum* and *B. trigonocephalum*). The diluted primary antibody (1:100) of 25µl was added exactly on the larval spot and allowed to react for 45 minutes. Then, the slides were washed with PBS carefully. The enzyme labeled secondary antibody of 25µl (goat-anti rabbit IgG-HRPO, dilution 1:1000) was added on the larval spot and allowed to react for 45 minutes. Then, the slides were washed with PBS gently. The substrate solution, Diaminobenzidine (DAB) of 50 µl was added and allowed to react for 20 minutes.Then,the slides were washed and mounted with 50 % glycerol in PBS for microscopy.

Results and Discussion: The surface reactivity patterns among ovine nematodes were analysed using infective larvae (L_3) of *H. contortus* as antigem source in the interface of heterologous sera (*O. columbianum* and *B. trigonocephalum*). A strong and intense surface reactivity (appearance of brown colour) was observed with the homologous sera, in comparsion to

the mild surface reactivity in heterologous sera (**Plate-1 a,b,c**). Demonstration of immunoperoxidase staining of whole, viable *H. contortus* larvae (L_3) was carried out and it was observed that a strong surface staining with serum obtained from vaccinated host compared to no staining with serum from non-vaccinated control sheep[8].

Essentially an immunoperoxidase assay (IPA) is similar to indirect immunofluorescent anti body technique (IFAT) except that anti-immunoglobulin is labeled with an enzyme instead of a fluorochrome. There are some merits for using IPA over that of IFAT in the present study. IPA dose not require an expensive fluorescent microscope and ordinary compound microscope would be sufficient. The stained slides can be visualized and the results can be read at any time after performing the test. Whereas, IFAT results need to be read immediately after performing the assay since the fluorescence of the fluorochrome will quench with time. Above all, in case of immunoperoxidase assay (IPA), it is possible to keep a permanent record of the reaction by preserving the slides, as the colour developed is very stable at room temperature.

Plate 1(a). Third stage larva of *H. contortus* showing strong surface reactivity with homologous sera

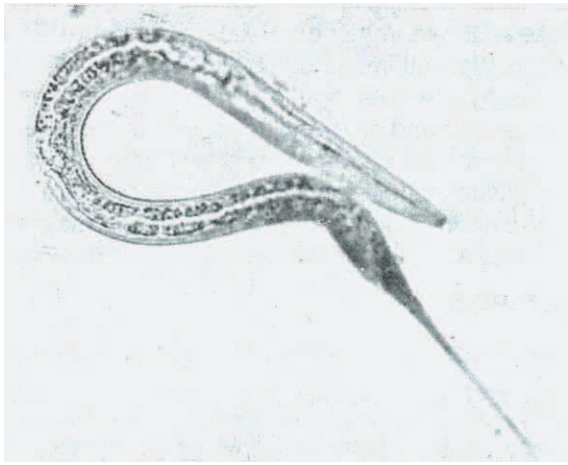

Plate 1(b). Third stage larva of *H. contortus* showing mild surface reactivity with *B. trigonocephalum* hyperimmune sera

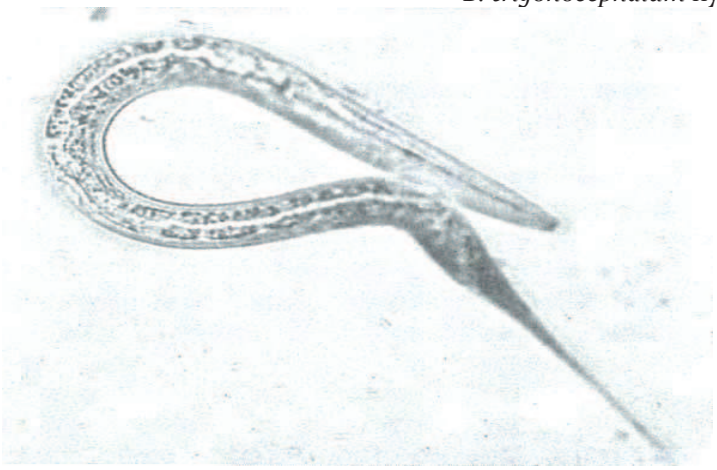

Plate 1(c). Third stage larva of *H. contortus* showing mild surface reactivity with *O. columbianum* hyperimmune sera

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