SURFACE REACTIVITY PATTERNS AMONGST STRONGYLID NEMATODES USING IMMUNOPEROXIDASE ASSAY

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ABSTRACT
Surface-reactivity amongst strongylid 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 with homologous sera whereas mild and less intense surface reactivity was observed with the heterologous sera.

KEYWORDS: Surface reactivity, immunoperoxidase assay, nematodes

INTRODUCTION
Infections with gastro-intestinal nematodes are prevalent world wide including India and pose a major constraint on sheep and goat health and production (Sykes and Coop, 2001; Sood, 1981). Though the evidence of cross-reactivity among the number of nematodes 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 (Sahu and Misra, 1986). The collected worms were washed repeatedly with distilled water followed by physiological saline and phosphate buffered saline (pH 7.4). The worms were identified upto species level using standard keys (Soulsby, 1982).

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 (Klesius et al., 1986) as described by Arunkumar and Sharma (2010). The protein concentration of the referral antigens (SEA) was estimated by the method of Lowry et al. (1951) using bovine serum albumin fraction V as the standard. Rabbit hyper immune sera (RHS) 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 (Soulsby, 1982). 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 5-7 days 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 (L3) were obtained in 4-5 days and all infective larvae were collected in outside water jacket from culture. The larvae were washed several times in distilled water with low speed centrifugation at 1000 rpm for 5-10 min after 30 min 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 their use for the study.