

**Research Article**

## **POST MORTEM WORM COUNTS IN SHEEP AFTER IMMUNIZATION WITH PURIFIED METABOLIC PRODUCTS FROM BLOOD FEEDING OVINE GASTROINTESTINAL NEMATODE**

**\*S. Arunkumar**

Department of Veterinary Parasitology, Veterinary College and Research Institute, Orathanadu,  
Thanjavur -614625, Tamil Nadu

\*Author for Correspondence

### **ABSTRACT**

*In-vitro* culture was made for isolating the metabolic products from ovine gastro intestinal nematode, *Haemonchus contortus* and the culture supernatant was used as antigen. On immuno blot studies, it was demonstrated that the purified metabolic products showed single reactive band at 66.0 kDa. In immunization trial, sheep were immunized with 500 µg of purified antigen along with montanide as adjuvant on day 0, 30 and 60 intramuscularly. On ELISA, it was observed that the mean absorbance values were significantly ( $P \leq 0.01$ ) higher up to 20 weeks post immunization in Group-I compared to Group-II (unimmunized control). Further, the mean faecal egg count values was lower in Group-I ( $200 \pm 32.17$  to  $700 \pm 48.14$ ) than Group-II ( $2300 \pm 105.01$  to  $5300 \pm 123.65$ ) and the percentage reduction in mean faecal egg counts was 84.5 per cent. Similarly, the mean post-mortem worm counts was lower in Group-I ( $845.13 \pm 24.15$ ) than Group-II ( $3465 \pm 51.43$ ) and the percentage reduction in mean abomasal worm count was 68.5 per cent.

**Keywords:** *Haemonchus Contortus*, *Metabolic Products*, *Post-Mortem Worm Counts*, *Sheep*

### **INTRODUCTION**

Parasitic nematode infections of livestock are responsible for significant economic losses and welfare concerns worldwide. The appearance of anthelmintic resistant strains of *Haemonchus contortus* has necessitated the development of alternative strategies for the effective control of this highly pathogenic, blood feeding nematode of small ruminants. The protective immunity against *H. contortus* infection can develop in sheep, suggests that vaccination has potential as a feasible method of control and several highly protective native antigens have been isolated from extracts of *H. contortus*. Recently, attempts have been made to characterize the excretory / secretory products of *H. contortus* as these substances could be potential target for immunological control. Experimental studies suggested that these E/S products have greatly enriched for cysteine proteases and conferred high levels of protection against homologous challenge in sheep. The present work was carried out to ascertain the protective effects in sheep immunized with purified metabolic products of *H. contortus*.

### **MATERIALS AND METHODS**

Fresh *H. contortus* adults worms were collected from abomasum of sheep slaughtered at local abattoir, Chennai. Following washing in normal saline and phosphate buffered saline (PBS, pH 7.4) the fresh, live worms were transferred to RPMI 1640 medium containing penicillin (500 IU/ml) and streptomycin (5 mg/ml) and cultured at a concentration of approximately 50 worms per ml in a culture flask and maintained at 37°C for 24 hours. The medium was changed every 6 hours of incubation. After incubation, the culture medium was collected and centrifuged at 10,000 rpm for 30 minutes at 4°C and the supernatant was used as metabolic antigen or excretory / secretory (E/S) antigen. This antigen was purified by affinity chromatography method (Knox *et al.*, 1999). Further, the antigen was characterized using sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE) and the immunogenic fraction was identified using western blotting (Towbin *et al.*, 1979). The protein content was determined using bicinchoninic acid method using kit (Smith *et al.*, 1985).

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Immunization trial was conducted in sheep to evaluate the protective effects of purified E/S antigen of *H. contortus*. Twelve Madras red breeds of male sheep aged around 6 months old were maintained indoor as per the guide lines of institutional animal ethical committee. All the animals were dewormed with fenbendazole at the dose rate of 5 mg /kg body weight 21 days prior to start of the trial. They were then divided into two groups of 6 sheep each. In group-I, sheep were immunized with 500 µg of purified E/S antigen adjuvanted with montanide ISA 206 on day 0,30 and 60 through deep intramuscular injection. In group-II, sheep were used as unimmunized control.

Serum antibody responses in immunized and control sheep was evaluated by ELISA (Knox *et al.*, 2003). Test serum samples were collected at weekly intervals from all the sheep from 0 to 21 weeks of experimental period. The optimum concentration of antigen, serum and conjugate (anti-sheep IgG/HRP, Sigma, USA) were determined by checker board titration method using serial dilution of antigen, serum and conjugate

For challenge studies, the dung sample was cultured for the recovery of third stage infective larvae ( $L_3$ ) of *H. contortus*. A total of 5000 infective larvae ( $L_3$ ) of *H. contortus* were administered orally to each sheep of both the groups on 90<sup>th</sup> day post immunization. The rectal samples were collected from each sheep from 21 days after challenge infection at weekly intervals up to 8 weeks for egg count. The faecal egg count was estimated by modified McMaster method and the percentage reduction in faecal egg counts were calculated using the standard formula. The data were statistically analysed by ANOVA (Snedecor and Cochran, 1968)

All the experimental sheep were slaughtered on day 60 after challenge infection according to Eysker and Kooyman (1993). The abomasum was tied off and removed intact. Then, it was opened along the lesser curvature and flooded with normal saline. The contents were washed again with normal saline and the supernatant fluid was discarded until clear sediment was obtained. Then, the adult worms were collected from the sediment. The adult worms present in the abomasum were counted manually. The level of total worm reduction per cent was calculated using the following formula.

## RESULTS AND DISCUSSION

During incubation in RPMI 1640 medium, the adult worms remained viable as assessed qualitatively by both motility and clumping tendency. The protein content of purified antigen was found to be 2.4 mg/ml. On western blot analysis, the purified E/S antigen probed with serum from sheep infected with *H. contortus* showed single reactive band at 66.0 kDa. These results are in accordance with the reports of Knox *et al.*, (2005), Vervelde *et al.*, (2001) and Rathore *et al.*, (2006). In ELISA, the mean absorbance values in Group-I were gradually increased from second week post immunization and reached a peak value of  $1.2 \pm 0.05$  on 8<sup>th</sup> week post immunization. The serum antibody levels in immunized group were significantly ( $P \leq 0.01$ ) higher and was maintained up to 20 weeks post immunization compared to that of unimmunized controls while, the mean absorbance values in group II were remained low ( $0.09 \pm 0.01$  to  $0.13 \pm 0.01$ ) throughout the observation period. The reason for increase in serum antibody levels in Group-I might be due to the effect of administration of purified E/S antigen which induced strong antibody responses. Similar observations were made by many workers. Redmond and Knox (2004) observed a significant increase in serum antibody levels in purified antigen immunized sheep. Knox *et al.*, (2005) demonstrated a gradual elevation of serum antibody responses following immunization.

In Group-I, the mean faecal egg counts were significantly low ( $300 \pm 40.17$  to  $500 \pm 43.12$ ) up to 42<sup>nd</sup> day after challenge in immunized sheep followed by gradual increase up to 70<sup>th</sup> day post challenge. Whereas in control group, the mean egg counts increased gradually and were maintained high up to 70 day post challenge. The percentage reduction in mean faecal egg counts was found to be 84.5%. Many workers reported different percentage of mean faecal egg counts in immunized animals. Schallig *et al.*, (1997) observed that sheep vaccinated with E/S antigen induced significant reduction in mean faecal egg counts ( $>70\%$ ) compared to that of controls. Bakker *et al.*, (2004) vaccinated sheep with E/S antigen and observed a significant reduction in faecal egg output of 62%. Redmond and Knox (2004) observed a reduction in faecal egg output of more than 50% in immunized sheep. Knox *et al.*, (2005) reported a reduction in mean faecal egg count of 64% in vaccinated sheep.

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The study further revealed that the mean post-mortem worm counts was significantly low in Group I compared to Group II. Whereas, the mean post-mortem worm counts was higher in control groups. The percentage reduction in mean post-mortem worm counts was found to be 68.5 per cent. Several workers reported different percentage of mean worm counts in immunized animals. Bakker *et al.*, (2004) reported a significant reduction in abomasal worm burden of 50 per cent in vaccinated sheep compared to control group. Redmond and Knox(2004) observed a reduction in abomasal worm count of 46 per cent in vaccinated sheep. Knox *et al.*, (2005) reported a mean abomasal worm count of 43 per cent in vaccinated sheep compared to control group. In the present study, no immature worms could be recovered from the abomasal mucosa of immunized animals, This finding is in agreement with the reports with the reports of Smith *et al.*, (1993) who reported no arrested larvae and immature worms in the abomasal mucosa of immunized sheep. The variations observed in reduction percentage of mean faecal egg count and abomasal worm count in the present study might be due to the nature of antigen, dosage of antigen, type of adjuvant used etc. Based on the results of the present study, it was observed that immunization with purified E/S antigen produced immune effect on female worm and it was operating before sexual maturity. Because of the immunizing effect, female worms were stunted in their development which could reduce their egg laying capacity. Hence, protection levels were higher in terms of reduction in faecal egg counts than in abomasal worm counts.

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