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IMMUNOLOGICAL STUDIES ON SALIVARY GLAND ANTIGEN OF *RHIPICEPHALUS HAEMAPHYSALOIDES* IN CATTLE

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Abstract:

Background - The immunological studies of salivary gland antigen of *Rhipicephalus haemaphysaloides* in calves was carried out for one academic calendar, in the Department of Parasitology, Nagpur Veterinary College, Nagpur. Maharashtra Animal and Fishery Sciences University, Nagpur (M. S.).

Material and Methods - Partially fed *R. haemaphysaloides* ticks were surface sterilized and salivary gland was taken after dissection and washed with phosphate buffer saline (Ph 7.2) and then stored in PBS at 40^0C. Isolated salivary glands were homogenized in PMSF, EDTA, EGTA and merthiolate and sonicated. After centrifugation the salivary gland antigen (SGA) were stored at -20^0 C. The concentration of protein was estimated to be 2.1 mg/ml of antigen.

Result - Crude salivary gland antigen of *R. haemaphysaloides* was characterized by SDS-PAGE and it revealed 17 protein bands. Immunization trials were conducted involving eight cross bred calves comprising of 2 groups. It was observed that the impact of immunization of calves by crude salivary gland antigen of *R. haemaphysaloides* exhibited adverse effect on the engorged tick weight, egg mass weight, preoviposition period and oviposition period.

Keywords: *Rhipicephalus haemaphysaloides,* cattle, salivary gland, antigen.

INTRODUCTION

Amongst parasitic diseases, the tick infestation is well known to affect the growth and productivity of animals adversely. Globally, the economic losses due to tick population are estimated for more than 90 per cent of all reported vector borne diseases (Oliver, 1996). Each

engorged female tick is responsible for the loss of 8.9 ml of daily milk production and 1.0 g of body weight (Johnson *et al*.,1998). A single adult female removes 0.5-2.0 ml of blood and transmit haemoprotistant diseases *viz.* Thieleriosis, Anaplasmosis, Ehrilichiosis etc. Thus tick disease of animals does produce economic losses for hundreds of millions of dollars per year (Soulsby, 1986). This necessitated the researcher to develop novel methods to control tick population. The use of antitick vaccine has an edgeover being economically viable, safe and environmentally sustainable (Banerjee *et al*., 1995). The immunological control of bovine ticks has an advantage of undesirable expedient of indiscriminate application of acaricides and it avoids environmental contamination and prevent selection of drug resistant ticks. These vaccines are potentially important in the control of the disease agents, mainly for not being chemical agents but for being cheaper (Willadsen, 1997). The effect of these vaccines is not a direct killing of ticks, but a successive reduction in numbers, as consequence of the reduction of adult female fertility (Rodriguez *et al*., 1995).

In abroad, several studies conducted for controlling tick infestations through immunization of hosts with selected tick antigens/ development of recombinant Bm 86 gut antigen that reduced *Boophilus* spp. infestation by causing lysis of tick gut cells due to the binding of anti- Bm 86 antibodies with subsequent leakage of material from the gut into the hemocoel. The resulting consequences are mortality of engorging ticks with a substantial effect upon tick reproductive performances (Willadsen, 1997).

MATERIALS AND METHODS

Experimental Animals: The experimental trial was conducted using male cross bred calves of 6- 15 months age with no any previous tick exposure which were procured from Cattle Breeding Farm, Nagpur Veterinary College, Nagpur and housed in tick free pens.

Tick Colony: An engorged female *Rhipicephalus haemaphysaloides* ticks were collected from severely infested pathogen free donor cattle. Blood smear of the donor animals were examined for the presence of any protozoan parasites. The tubes containing eggs were incubated at 28^0 C temperature and 85 per cent relative humidity. The hatched larvae, nymphal and adult stages were allowed to feed on ears of pathogen free experimental calves The partially fed female ticks were used for dissection of salivary glands.

Tick Dissection: Ticks were dissected according to the standard procedure described by Till (1961) and Blewett and Branagan (1973).

Preparation of Salivary Gland Antigen (SGA): The isolated salivary glands were homogenized in presence of 2mM Polymethylene sulphonyl fluroide, 2mM EDTA, 3mM

EGTA and 0.01% merthiolate. Homogenate was subjected to sonication and were centrifuged. The supernatant which designated as Salivary Gland Antigen (SGA) were stored at -20^0 C. The protein concentration was determined by standard method (Lowry *et al.*, 1951).

Characterization Of Salivary Gland Antigen (SGA): The crude salivary gland antigen of *R. haemaphysaloides* was characterized by (SDS- PAGE) and the serum samples from immunized as well as control calves were collected at weekly interval between 0 to 70 days post immunization. These sera were tested by AGID.

Immunization Trials: Immunization trial was carried out using eight crossbred cattle calves. They were divided into 2 groups and were challenged after 35 days after first immunization for study of Rejection %, Weight of engorged tick, Preoviposition period, Oviposition period and Egg mass weight.

RESULTS AND DISCUSSION

Preparation of Salivary Gland Antigen: The Salivary gland antigen was prepared as per the method of Johnston *et al*. (1986) and the concentration of protein was estimated to be 2.1 mg/ml of antigen. Characterization of was carried out by SDS-PAGE. The SDS-PAGE profile of the crude salivary gland antigen revealed 17 protein bands with Coomassie blue staining at 25.8, 30.1, 37.5, 40.1, 44.0, 59.2, 64.3, 68.0, 76.2, 81.2, 87.4, 92.8, 104.0, 117.2, 130.4, 140.3, 146.9 kDa. Although reports on such studies in *R. haemaphysaloides* are absent reports on other ticks include that of Ghosh and Khan (1997) who characterized midgut antigen of *B. microplus* and identified 16 protein bands ranging from 34.2 to 105.4 kDa in the crude antigen and 4 protein bands in fraction of TESA. Lee and Opdebeeck (1991) fractionated the purified fraction of the soluble midgut antigen of *B. microplus* Qu 13 by SDS-PAGE and observed protein bands from 30 to 200 kDa.

Assessment of humoral immune response: The humoral immune response was assessed by Agar Gel Immuno Diffusion Test (AGID). It was conducted for group I and II (control group). Prominent precipitation line appeared on 42 DPI in group I. No such reaction was observed in group II control calves. Ghosh and Khan (1996) immunized calves with tick extract supernatant antigen and assessed humoral immune response by double immuno diffusion test wherein a single band was seen upto 21 DPI and 2 bands were noticed from 28-84 DPI. Earlier reports on double immuno diffusion (DID) are that of Allen and Humphreys (1979), Johnston *et al*. (1986). Similarly, Kumar and Kumar (1996a) showed that DID was positive upto 84 DPI in response to supernatant midgut antigen of *H. dromedarii*. With *B.*

microplus Panda *et al*. (1992) noticed single precipitation bands on 21 and 28 DPI and double bands on 38 DPI.

Effect of Immunization: The effect of *R. haemaphysaloides* crude salivary gland antigen on the feeding and fertility parameters of ticks fed on immunized and unimmunized control animals were observed.

Effect of immunization on feeding parameter of ticks: Higher tick rejection (16.03 ± 0.62) was evidenced in immunized animals (Fig: 10) that control group (10 ± 0.2) in the present experimental study. However the role of self grooming in rejection of ticks in these experimental animals would have been prevented since the ticks were confined to thick cotton ear bags. Similarly percentage of attachment was non significant in immunization trials carried out using *H. a. anatolicum* by Manohar and Banerjee (1992).

Engorged tick weight: Significant variation ($P< 0.05$) was observed in engorged tick weight, reduction in weight of engorged tick (198.7 ± 4.59) from immunized group was recorded than ticks from control animals. Essuman *et al.* (1992) reported significantly large reduction of about 43 per cent of engorgement weight in rabbits immunized with *R. appendiculatus* midgut membrane protein. Similarly 30 per cent reduction in tick weight was seen by Banerjee and Manohar (1992) in ticks recovered from *H. a. anatolicum* whole tick extract immunized calves. However, Das *et al*. (2000) reported insignificant changes in the engorgement weight of ticks fed on animals immunized with larval antigen of *H. a. anatolicum.*

Effect of immunization on reproductive parameters of ticks: The effect of immunization on reproductive ability of ticks was assessed by observing the fertility parameters of ticks fed on animals immunized with crude salivary gland antigen and on control animals.

Preoviposition period: Preoviposition period was found 3.5 ± 0.35 in immunized animals while that found in immunized animals was 3.11 ± 0.06. It indicates that there was decrease in the preoviposition period in the immunized animals in contrast, there were increases of 0.4 days, 0.6 days and 0.2 days of ticks obtained from vaccinated hamsters, dogs and guinea pigs respectively when compared with ticks fed on controls (Bechara *et al*., 1994). Banerjee and Manohar (1992) recorded a significantly longer preoviposition period of 8 to 11 days with *H. a. anatolicum* while Sahibi *et al*. (1997) reported a preoviposition period of 18 and 24 days following immunization with *H. m. marginatum* salivary and intestinal extracts respectively. Similarly Kimaro and Opdebeeck (1994) who reported prolonged preoviposition period was recorded in group II. This is in accordance with the findings of Ghosh and Khan (1996) who

observed no significant change in the pre oviposition and oviposition periods in ticks fed on calves immunized with TESA.

Oviposition period: Oviposition period in immunized animals was found higher (24.5 ± 0.99) than that of control animals (17 ± 0.70). Significant difference was recorded in oviposition period of ticks in immunized groups compared to control group. Lee and Opdebeeck (1991) have recorded 62 percent reduction in oviposition period of ticks fed on cattle vaccinated with highly purified antigen of *B. microplus*.

Egg mass weight: A highly significant reduction in egg mass weight was observed in immunized group when compared to control on challenge.

CONCLUSIONS

Salivary Gland Antigen of *Rhipicephalus haemaphysaloides* tick provoked immune response in calves. On characterization by SDS-PAGE, the SGA revealed 17 protein bands ranging from 25.8 to 146.9 kDa were detected. AGID test detected prominent precipitation line on 42 DPI of salivary gland antigen. Thus, the salivary gland antigen of *Rhipicephalus haemaphysaloides* can constitute major immunological molecule as a vaccine candidate for control of tick parasitism of bovines.

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