International Journal of Science, Environment and Technology, Vol. 13, No 2, 2024, 116 - 123
ISSN 2278-3687 (O)
2277-663X (P)

# IMMUNOLOGICAL STUDIES OF TICK SALIVARY GLAND ANTIGEN OF *Rhipicephalus haemaphysaloides* IN RABBITS

**Dr. P.J. Gawande[1], Dr. B.S. Baviskar[2], Dr. A.K. Jayraw[3], Dr. Alka Sawarkar[4] and Dr. Kranti Kharkar[5]**

[1]Assistant Professor, Department of Veterinary Parasitology, Nagpur Veterinary College, Nagpur

[2]Director Wild-CER, Nagpur.

[3]Professor and Head, Department of Veterinary Parasitology, College of Veterinary Science & A. H. Mhow

[4]Assistant Professor, Department of Veterinary Pharmacology & Toxicology, Nagpur Veterinary College, Nagpur

[5]Assistant Professor, Department of Animal Genetics and Breeding, Nagpur Veterinary College, Nagpur

E-mails: [1]priyagawande@mafsu.in, drpriyagawande@gmail.com (*Corresponding author*)
[2]baharbaviskar@gmail.com

**Abstract:** The immunological studies of salivary gland antigen of *Rhipicephalus haemaphysaloides* in rabbits were carried out for one academic calendar year. The protein concentration from the salivary gland was estimated to be 2.1 mg/ml of antigen. It was characterized by using SDS- PAGE technique which 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. The SGA of *R. haemaphysaloides* was partially purified by Ion Exchange chromatography using DEAE-Sepharose beads. It is followed by alkaline, acidic `and sodium phosphate buffer treatment. Hyperimmune sera were raised in two groups of four New Zealand white rabbits. Animals in immunization groups were injected at multiple sites on days 0, 14 and 28, followed by blood collection. The second booster dose was given at two weeks intervals, and final blood collection was performed two weeks post the second booster. The control rabbits received Freund's complete and incomplete adjuvant (FIA) alone on days 0, 14 and 28. Experimental rabbits were aseptically bled before immunization on days 0, 14, 28, 42 post-infection. Humoral immune response was assessed by using the Agar gel immunodiffusion test (AGID).

**Keywords: S**alivary glands, *Rhipicephalus haemaphysaloides*, rabbits.

## INTRODUCTION

Among ectoparasites, the ticks are second to mosquitoes as vectors of infectious pathogens to humans and from one animal species to another. Ticks and tick-borne diseases affect 80% of the cattle population and are widely distributed throughout the world, which causes significant production losses (de Castro, 1997) in cattle. In India, several reports are available on the use of different tick crude extracts for immunization of rabbits, viz., the antigen extract

*Received March 14, 2024 * Published April 2, 2024 * www.ijset.net*

from whole tick and salivary glands of *Hyalomma anatolicum anatolicum* and immunized the rabbits (Manohar and Banerjee, 1992a), with the use of an extract of the midgut of *H. a. anatolicum* (Kumar *et al*., 2003). Similarly, an immunological reactivity study was conducted using larval, nymphal and adult antigens of *Boophilus microplus, H. a. anatolicum* and *Rhipicephalus sanguineus* (Ghosh and Khan, 1998), besides midgut antigen of different ticks infesting small ruminants.

**MATERIALS AND METHODS**

New Zealand white rabbits of 3-4 months age, either sex weighing 1-1.5 kg with no tick exposure, were used in the present experimental trials. They were maintained in heat sterilized cages and fed on Bengal gram, Lucern, Green vegetables and adlibitum water during the study. An engorged female *Rhipicephalus haemaphysaloides* ticks were collected from severely infested pathogen-free donor cattle (Fig 1). The ticks were picked in glass vials and incubated at a temperature ranging from 20.00 C to 30.00 C and relative humidity 48.0 to 85.0 per cent for depositing the eggs. After 6 to 7 days, the eggs deposited were separated in glass tubes.

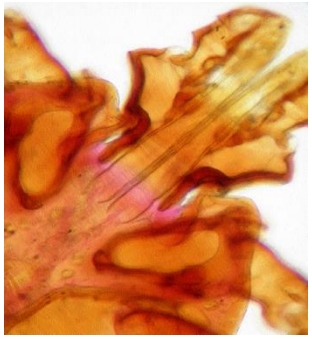

**Fig. 1 Anterior end of *Rhipicephalus haemaphysaloides* tick**

The tubes containing eggs were hatched at 280 C temperature and 85 per cent relative humidity. The larvae hatched after 21 days were allowed to feed on ears of pathogen-free experimental calves using thick cotton ear bags. The nymphal and adult stages were also fed on experimental calves. The partially fed female ticks were maintained in the laboratory. Ticks were dissected according to the standard procedure described by Till (1961) and Blewett and Branagan (1973) (Fig. 2). The isolated salivary glands were homogenized, and the sonicated homogenate was centrifuged.

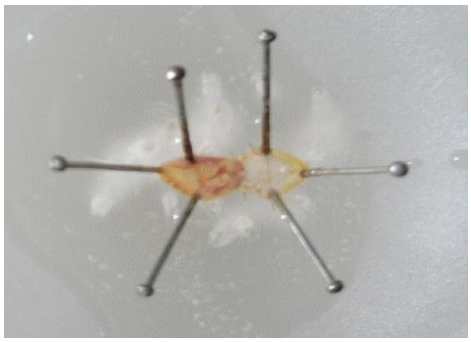

**Fig. 2 Dissection of *R. haemaphysaloides* tick**

**Characterization of salivary gland antigen (SGA):**

The crude salivary gland antigen of *R. haemaphysaloides* (Fig 3) was characterized by 10.0% Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS- PAGE) on a 1 mm mini gel (10 X 10.5 cm) using a discontinuous system (Laemmli 1970) in a vertical slab gel electrophoresis system. A molecular weight protein marker in the range of 97.4- 14.3 kDa was used. A power supply of 50 V was applied for the migration of protein through the stacking gel and 100 V through the separating gel. After an electrophoretic run, the gel was removed carefully and immersed in 0.25% Coomassie Brilliant Blue R- 250 staining solution for one hour and then followed by destaining until the bands of polypeptide became unclear. The molecular weights of the unknown polypeptides were determined using the Syngene programme. The SGA of *R. haemaphysaloides* was purified by Ion Exchange chromatography [Ganapamo *et al.* (1997), Gordon and Allen (1987), Brown and Askenase (1986)] followed by Alkaline treatment, Acidic treatment, Sodium Chloride Treatment.

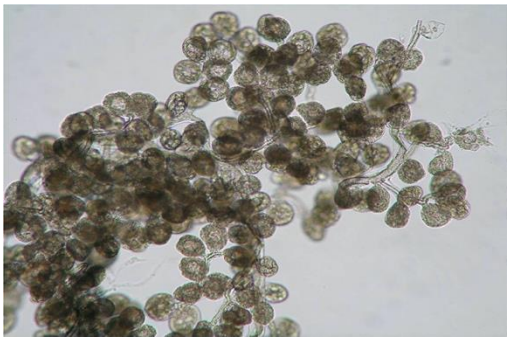

**Fig. 3 Salivary glands of *R. haemaphysaloides* tick**

**Raising of hyperimmune sera**:

Animals in immunization groups received 500 mg of antigen per rabbit (Fig 4). Salivary gland antigen was mixed with an equal volume of Freund's Complete Adjuvant (FCA) and was injected at multiple sites in rabbits in divided doses on day 0, 14 and 28, followed by blood collection. The second booster dose was given at 2 weeks interval, and final blood

collection was performed 2 weeks post second booster. The control rabbits received Freund's complete and incomplete adjuvant (FIA), alone on day 0, 14 and 28 All experimental rabbits were aseptically bled from the heart before immunization on day 0, 14, 28, 42 post-infection and subsequently at weekly interval up to 10 weeks post-immunization)**.** 5 ml of blood was collected from each animal and allowed to clot at room temperature for 2 hrs. followed by 4 hrs incubation at 40 c and finally serum was separated and centrifuged at 40 c for 30 minutes at 2500 pm. Serum was aliquated and labeled properly and stored at -200 c after adding 0.01% Merthiolate (thiomersal). Blood from all experimental animals were collected from 0 day at weekly intervals to day 63 post-immunization. The sera were separated and preserved with 1:1, 00,000 thiomersal and kept at –200C.

**Fig. 4 Inoculation of experimental rabbit with SGA of *R. haemaphysaloides***

**Assessment of humoral immune response:**

Agar gel immunodiffusion test (AGID) was conducted to assess humoral immune response in the experimental rabbits. It was based on the precipitation reaction between antigen and antibody. The antigen and antibody migrate in the gel and form a precipitation line (Fig 5).

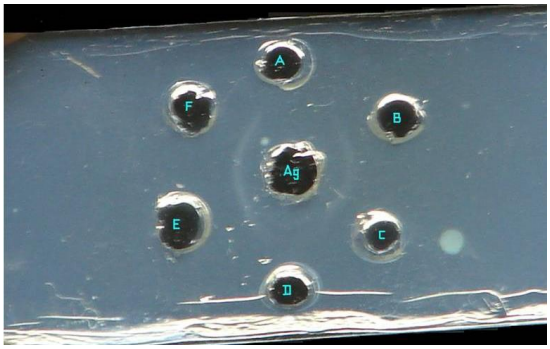


**Fig. 5 Agar gel Immunodiffusion test.**

## RESULTS AND DISCUSSION

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 (Fig 6). Ghosh *et al*.

(1998) prepared homogenized antigens from unfed larvae and nymphs of *H. a. anatolicum* he inoculated rabbits with 8.56 mg Hylomma Larval antigen and 9.34 mg Hylomma Nymphal antigen subcutaneously. Characterization of Salivary Gland Antigen of *R. haemaphysaloides* was carried out by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (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.

Smith (1994) responses sodium dodecyl sulphate gel electrophoresis technique in which proteins were reacted with anionic detergent SDS to form negatively charged complexes. SDS-PAGE of SGE and ME from *A. variegatum* revealed the presence of 48 protein bands in SGE and 29 bands in midgut extract. Although reports on such studies on salivary gland antigen of *R. haemaphysaloides* are absent. However the reports on other ticks include of Lee and Opdebeeck (1991) who 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. Similarly, Ghosh and Khan (1997) 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 infraction of TESA. While Raman (2000) revealed twelve protein bands ranging from 20.6 to 140.0 kDa of partially purified and 62.2 to 92.2 kDa of purified antigenic fraction of *B. microplus* midgut.

The crude salivary gland antigen was purified by ion-exchange chromatography using beads of DEAE- Sepharose. The graph plotted based on the OD value of each of the 10 antigenic fractions revealed a peak (Fig. 11) and the protein concentration of 8 antigenic fractions forming the peak was estimated. Similarly size-exclusion ion-exchange chromatography has been used for purification of *Ixodes ricinus* by Ganapamo *et al.* (1997) and for*Dermacentor andersoni* by Gordon and Allen (1987). Similarly, Brown and Askenase (1986) also purified the salivary gland antigen of *Amblyomma americanum* by ion-exchange chromatography, while the purification of midgut antigen of *B. microplus* by gel filtration chromatography has been reported by Ghosh and Khan (1997) and Raman (2000).

The humoral immune response was assessed by Agar Gel Immuno Diffusion Test (AGID). The agar gel immunodiffusion test was conducted for groups I and II (control group) by using sera collected from animals on 0, 14, 28 and 42 days following immunization. Prominent precipitation line appeared only from 10 days – II booster (Fig. 12) which persisted 42 days post II booster in animals belonging to group I. No such reaction was observed in group II

control rabbits. Ghosh and Khan (1996) immunized calves with tick extract supernatant antigen and assessed humoral immune response by double immunodiffusion test wherein a single band was seen up to 21 DPI and 2 bands were noticed from 28-84 DPI. This is similar to the present study, with the exception that only one band was noticed from 21 to 63 DPI. Earlier reports on double immunodiffusion (DID) are that of Allen and Humphreys (1979), Johnston *et al*. (1986). Similarly, Kumar and Kumar (1996a) showed that DID was positive up to 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. Manohar and Banerjee (1992a) immunized rabbits with *Hylomma anatolicum anatolicum.* The sera of immunized rabbits showed a single precipitation band on 14 and 21 days. On 28, 35, 49th day one or two bands were seen.

Latha (1998) showed prominent precipitation bands by agar gel immunodiffusion test, only from day twenty one post-immunization with midgut antigen of *H. marginatum isaaci* which persisted till 63 DPI. No such reaction was observed on 0, 7 and 14 days. Kumar and Kumar (1995) reported the presence of serum antibody in rabbits after their immunization with midgut antigen from partially fed *Hyalomma dromederii* ticks with double immunodiffusion (DID) and capillary tube agglutination tests by giving three inoculations subcutaneously on days 0, 14 and 21 at a dose rate of 1 mg antigen per animal. Ghosh *et al*. (1998) Larval antigen immunized rabbits showed significant antibody levels from 28-126 days while with Hylomma nymphal antigen (HNAg) elevated antibody levels were recorded up to 112 days. Anti- Hylomma larval antigen (HLA) and anti-HNAg sera recognized common antigenic bands of 97.4, 85, 66, 47.3, 42 and 31 kDa in homogenates of larvae, nymphs and adults.

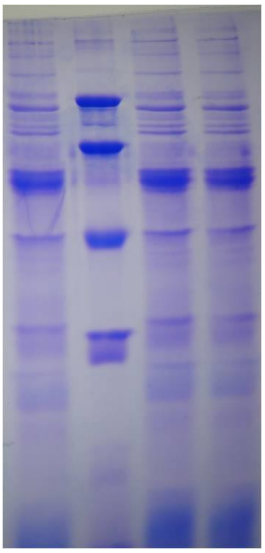

**Fig. 6 Characterization of salivary gland antigen by SDS-PAGE**

## CONCLUSIONS

Salivary Gland Antigen of *Rhipicephalus haemaphysaloides* tick provoked immune response in rabbits. On characterization by SDS-PAGE, the SGA 17 protein bands ranging from 25.8 to 146.9 kDa were detected. On Partial purification SGA revealed protein concentration of 8 antigenic fractions ranging from 0.003 to 0.646 kDa. AGID test detected prominent precipitation line from 38 days DPI of salivary gland antigen. Thus, the salivary gland antigen of *Rhipicephalus haemaphysaloides* can constitute major immunological molecule as a vaccine candidate against tick parasitism of bovines.

## References

[1] Allen, J.R. and S.J. Humphreys (1979). Immunization of guinea pigs and cattle against ticks. *Nature,* 280: 491-493.

[2] Blewett D.A. and D. Branagan (1973). The demonstration of *Theileria Parva* infection in intact *Rhipicephalus appendiculatus* salivary glands. *Trop. Anim. Health. Prod.* 2: 27-34.

[3] Brown S. J. and P.W. Askenase (1986). Amblyomma americanum: physiochemical isolation of a protein derived from the tick salivary gland that is capable of inducing immune resistance in guinea pigs. *Exp Parasitol.* 62(1): 40-50.

[4] De Castro and J. J. (1997). Sustainable tick and tick-born disease control in livestock improvement in developing countries. *Veterinary Parasitology*. 7: 77-97.

[5] Ganapamo F., B. Rutii and M. Brossard (1997). Identification of an Ixodes ricinus salivary gland fraction through its ability to stimulate CD4 T cells present in BALB/c mice lymph nodes draining the tick fixation site. *Parasitology.* 115 (1):91-6.

[6] Ghosh S., M.H. Khan and S.C. Gupta (1998). Immunization of rabbits against *Hyalomma anatolicum anatolicum* using homogenates from unfed immature ticks. *Indian Journal of Experimental Biology*. 36(2): 167-70.

[7] Ghosh, S. and M.H. Khan (1997). Identification of tick antigen immunogenic in calves. *Ind. J. Anim. Sci*. 12: 249-252.

[8] Gordon J.R and J.R. Allen (1987). Isolation and characterization of salivary antigens from the female tick, Dermacentor andersoni. *Parasite Immunol.* 9(3):337-52.

[9] Johnston L.A.Y., D.H. Kemp and R.D. Pearson (1986). Immunization of cattle against *Boophilus microplus* using extracts derived from adult female ticks: Effects of induced immunity on tick populations. *Int. J. Parasitol.* 16: 27-34.

[10] Kumar R and R. Kumar (1995). Artificial immunization of rabbits with *Hyalomma dromedarii* tick-derived midgut antigen. *Exp Appl Acarol.*19(6): 319-24.

[11] Kumar R., D.P. Banerjee and A.K. Gupta (2003). *J. Parasitic Diseases.* 27: 53-63.

[12] Laemmli, U.K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature*. 227:630-684.

[13] Latha, B.R. (1998). Immunological strategies in the control of ixodid ticks. Ph. D., thesis submitted to TANUVAS.

[14] Manohar G.S. and D.P. Banerjee(1992a). Effects of immunization of rabbits on establishment, survival, and reproductive biology of the tick *Hyalomma anatolicum anatolicum*. *Journal of Parasitology.* 78(1): 77-81.

[15] Panda D.N., M. Z. Ansari and B.N. Sahai (1992). Immunization of cattle with *Boophilus microplus* adult female and larval extracts; I. Antibody and cutaneous responses. *Journal of Veterinary Parasitology.* 6(1): 33-36.

[16] Raman, M. (2000). Immunoprophylaxis against Boophilid ticks. Ph. D. thesis submitted to TANUVAS.

[17] Smith, H. (1994). SDS Polyacrylamide Gel Electrophoresis of Proteins. In: Methods in molecular biology, Basic protein and peptide protocols Edited by: J. M. Walker, Humana Press Inc., Totowa, New Jersey. 32: 23-34.

[18] Till, W.M. (1961). The contribution to the anatomy and histology of Brwon Ear Tick *Rhipicephalus appendiculatus* Neumann. Memories of Entomological Society of Southern Africa, 6: 124.