

## International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 7, Issue 5, May 2018

# Immune Response of New Zealand White Rabbits Immunized with Midgut Antigen of *Rhipicephalus haemaphysaloides*

Dr.A.Aruljothi<sup>1</sup>, Dr.Bhaskaran Ravi Latha<sup>2</sup> and Dr.K.Sivakumar<sup>3</sup>

Assistant Professor, Veterinary University Training and Research Centre, Coimbatore, Tamil Nadu, India<sup>1</sup>,

Professor and Head, Dept. Veterinary Parasitology, Madras Veterinary College, Chennai, Tamil Nadu, India<sup>2</sup>,

Associate Professor and Head, Veterinary University Training and Research Centre, Coimbatore, Tamil Nadu, India<sup>3</sup>.

**ABSTRACT:** New Zealand white rabbits were experimentally immunized with crude and partially purified midgut antigen of *Rhipicephalus haemaphysaloides*. The crude and partially purified midgut antigen of *Rhipicephalus haemaphysaloides* characterized by SDS- Page revealed 11 protein bands with Coomassie blue staining ranging from 20.1 to 80.4 kDa and 3 protein bands at 35.9, 43.0, 53.2 kDa respectively while with silver staining 8 protein bands were seen ranging from 20.1 to 58.3 kDa. Western blot studies of crude and partially purified midgut antigen of *R. haemaphysaloides* revealed 5 immunogenic bands at 25.3, 29.0, 35.4, 66.0 kDa and 4 immunogenic bands at 25.4, 35.9, 43.0 and 58.3 kDa respectively. The midgut antigen was found to be immunoprotective as the weight of engorged females, egg masses and egg rate conversion efficiency reduced by 40 and 47.4, 70 and 75, 49.31 and 56.27 percent respectively in the case of crude midgut antigen 54.56 and 40.1, 76 and 78, 46.62 and 65 percent respectively in the case of partially purified midgut antigen on both the 28th and 63rd day post immunization (DPI) challenge. Agar gel immunodiffusion test revealed antibody response from day 21 to 56 DPI in case of crude midgut antigen and from day 21 to 63 DPI against partially purified midgut antigen. ELISA revealed an increase in antibody titre from 7 to 63 DPI in immunized animals when compared to control animals. The lymphocyte transformation test revealed the maximum stimulation was observed on 42 DPI in group I and in group II.

**KEYWORDS:** midgut antigen, *Rhipicephalus haemaphysaloides*, antibody response, ELISA

### I. INTRODUCTION

Globally ticks and tick-borne diseases are of veterinary and public health concerns. Economic impact due to direct effect of infestation and disease transmission are significant. The global economic losses from ticks and tick-borne diseases are estimated to be in billions of dollars annually (Sonenshine, 1993). The major tick control method is the application of acaricide. This however has a number of demerits such as chemical pollution of the food chain and the environment as well as quick development of resistance against acaricides by ticks. A series of elegant studies led to the production of commercial vaccine Tick GARD™, Tick Guard Plus (Willadsen *et al.*, 1995) and Gavac™ (Rodriguez *et al.*, 1995) against the one-host tick, *Rhipicephalus (Boophilus) microplus* in Australia and Cuba respectively. In Tamil Nadu immunization of cattle against *Hyalomma marginatum isaaci* using crude midgut antigen (Latha, 1998) and a similar work against *Rhipicephalus (Boophilus) microplus* (Raman 2000) had been conducted with encouraging results.

### II. RELATED WORK

Essumen *et al.*, (1992) reported that the solubilised midgut membrane fraction represented a promising source of immunogen for the development of an anti-tick vaccine for *Rhipicephalus appendiculatus*. Antigen from extracts of

# International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 7, Issue 5, May 2018

eggs and gut membrane of *Rhipicephalus (Boophilus) microplus* were known to provide significant protection in cattle (Kimaro and Opdebeeck, 1994). The tick extract supernatant antigen of *Rhipicephalus (Boophilus) microplus* was purified by gel filtration chromatography and SDS-PAGE analysis of the first peak detected 4 bands of 105.4, 92.2, 67.0 and 62.0 KDa (Ghosh and Khan, 1997). Rabbits immunized with *H. dromedarii* tick derived midgut antigens with FCA subcutaneously, provided partial protection against *H. a. anatolicum* (Kumar and Kumar, 1996b). Prominent precipitation bands were seen by agar gel immuno diffusion test, only from day twenty one post immunization with midgut antigen of *H. m. isaaci* which persisted till 63 DPI. No such reaction was observed on 0, 7, and 14 days (Latha, 1998).

Kimaro and Opdebeeck (1994) evaluated the effect of exposure of cattle to ticks in the field during vaccination by using ELISA to assess the immune response to gut antigen of *Rhipicephalus (Boophilus) microplus*.

The humoral antibody response of calves to tick extract supernatant of *Rhipicephalus (Boophilus) microplus* when assessed by ELISA showed significant antibody level as early as 7 DPI which continued up to day 91 with a peak on 63 (Ghosh and Khan, 1996). The lymphocyte transformation test by colorimetric method using MTT is based on the metabolism of the yellow tetrasolium salt (3-(4, 5 - dimethyl thiazol - 2-yl) - 2,5 - diphenyl tetrasolium bromide) by mitochondrial dehydrogenases of active cells into blue formazan crystals.

## III. MATERIALS AND METHODS

### Experimental Animals

The experimental trials were conducted using New Zealand white rabbits of 3 to 4 months of age with no previous tick exposure. They were fed with bengal gram, lucerne and adlibitum water during the study.

### Rearing of ticks

Pathogen free *Rhipicephalus haemaphysaloides* colonies were maintained using experimental rabbits. The larval, nymphal and adult stages were allowed on ears of these rabbits using thick cotton ear bags. The unfed stages were maintained in the laboratory in BOD incubator (Forma Scientific Inc., USA) at  $20 \pm 2^\circ\text{C}$  using Pottassium hydroxide solution for maintenance of 85 per cent relative humidity.

### Preparation of midgut antigen

The midgut antigen was prepared as per the methods of Johnston *et al.*, (1986) with some modifications. The protein concentration of the antigen was determined (Lowry *et al.*, 1951).

### Purification of midgut antigen

The midgut antigen of *R. haemaphysaloides* was purified by gel filtration chromatography using sephadex G-200 column (Ghosh and Khan, 1997).

### Preparation of Gel bands

It was done according to Amero *et al.*, (1994) with minor modification. It was used in the immunization trial as partially purified midgut antigen.

### Immunization

Immunization trials were conducted involving eighteen New Zealand white rabbits comprising of 3 groups of 6 rabbits each for crude (Group I), partially purified (Group II) and control (Group III).

### Assessment of immune response

#### Humoral Immune response

#### Agar gel Immune diffusion test

The test was done according to the method of Ouchterlony (1958) with minor modifications. Agarose 1% (Bangalore Genei) was used and the gel slides were stained with Coomassie Brilliant blue R-250 for 30 minutes and destained using destaining solution.

#### Enzyme linked immunosorbent assay

The ELISA plate was coated with 1 in 100 dilution of crude and partially purified midgut antigen. On hundred  $\mu\text{l}$  of test sera, diluted 1 in 100 in PBS, and 100  $\mu\text{l}$  of 1 in 5000 dilution goat anti-rabbit IgG conjugated to horse radish peroxidase were added to each well. The substrate was ABTS 2, 2<sup>1</sup> Azino-bis (3-Ethylbenz - Tiazoline-6-Sulfonic acid) and the optical densities of unimmunized and test sera were determined with an ELISA reader at 405 nm.

# International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 7, Issue 5, May 2018

## Cell mediated immune response

### Lymphocyte transformation test (LTT)

The LTT was carried out as per the method described by Wagner *et al.*, (1999) with minor modifications.

### Test procedure

Five ml of heparinised blood sample was gently overlaid on 5 ml of cold biocoll separating solution and centrifuged at 3000 rpm for 30 minutes to separate buffy coat layer. The lymphocytes suspension was distributed in triplicates containing  $5 \times 10^5$  cells in 100  $\mu$ l per well in a flat bottom tissue culture plate. One mg of Con A and 2mg of antigen were added separately in wells maintained for mitogenic stimulation. Control wells were maintained without the addition of stimulants and the plate was incubated at 37°C for 72 hours in a 5 per cent carbon-di-oxide incubator (Forma Scientific Inc, USA). Twenty  $\mu$ l of MTT was added to each of the wells 4 hours before the end of incubation. Then 200  $\mu$ l of lysis buffer was added to each well and the plate was incubated at 37°C over night and the optical density was measured at 570 nm with a reference wave length of 630nm. The stimulation index values were calculated using the following formulae.

$$R = S - B / B$$

S = Absorbance values for antigen stimulated wells

B = absorbance values for control wells

## IV. EXPERIMENTAL RESULTS AND DISCUSSION

The effect of *R. haemaphysaloides* crude and partially purified midgut antigen on the feeding and fertility parameters of ticks fed on immunized and unimmunized control animals were observed on both 28<sup>th</sup> and 63<sup>rd</sup> DPI. No significant variation in the percentage attachment and rejection was observed. The mean feeding period of ticks challenged on 28 and 63 DPI was significantly extended in group I, group II when compared to group III. A highly significant variation ( $P < 0.01$ ) in engorged tick weight was observed in groups I and II compared to group III on both 28 and 63 DPI. The mean feeding efficiency index also decreased significantly in group I and II compared to group III on both 28 and 63 DPI.

The mean pre-oviposition period was significantly extended in group I and group II on 28 DPI, where as it was not significant on 63 DPI. No significant difference in oviposition period was observed in all the three groups on both 28 and 63 DPI. The incubation period was significantly different on 28 DPI in group I and group II compared to group III. The variation was not significant on 63 DPI. A highly significant reduction ( $P < 0.01$ ) in egg mass weight was observed in group I and II compared to group III between 28 and 63 DPI. A reduction percentage of 70, 75 and 76, 78 in the case of group I and group II on both 28 and 63 DPI was observed. The mean incubation period did not significantly vary between group I and II, when compared to group III on 28 DPI. Ticks fed on animals immunized with crude extract of *R. sanguineus* showed insignificant variation in the incubation period compared to ticks fed on unimmunized animals (Bechra *et al.*, 1994). Interestingly the egg mass of ticks belonging to group I and II allowed to feed on 63 DPI failed to hatch the larvae.

A highly significant reduction in fertility efficiency index was observed in group I and group II compared to group III on both 28 and 63 DPI. The reduction was also significant within groups between 28 and 63 DPI. The effect of immunization significantly reduced the egg rate conversion efficiency in group I and II compared to group III on both 28 and 63 DPI. It also differed significantly within group I, II and III on both 28 and 63 DPI.

### Assessment of immune response

#### Assessment of Humoral Immune Response

The humoral immune response was assessed by agar gel immuno diffusion test and enzyme linked immunosorbent assay.

#### Agar gel immuno diffusion test

The agar gel immuno diffusion test was conducted for group I, II and III by using sera collected from animals on 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63 days following immunization. Prominent bands appeared only from day 2 post-immunization which persisted till 56 DPI in group I and 63 DPI in group II. No such reaction was observed in group III control rabbits. 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

## International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 7, Issue 5, May 2018

were noticed from 28-84 DPI. Panda *et al.*, (1992) noticed single precipitation bands on 21 and 28 DPI and double bands on 38 DPI.

### Enzyme linked immunosorbent assay

In ELISA, the antibody response showed a rise from 7 DPI with a peak on 35 DPI. A steady decline in antibody titre was observed in immunized animals till 63 DPI, after that there was a slight rise on 70 DPI. Manohar and Banerjee(1992) observed an increase in anti- tick antibodies upto 28 DPI with a subsequent decrease in antibodies in rabbits immunized with whole tick extract of *H.a.anatolicum*. A significant serum antibody response as early as 7 DPI in calves to tick extract supernatant antigen and crude midgut antigen of *Rhipicephalus (Boophilus)microplus* which continued upto 91 DPI was observed by Ghosh and Khan (1996) and Raman (2000).

### Assessment of cellular immune response

#### Lymphocyte transformation test

The blastogenic response of peripheral blood lymphocytes of a normal rabbit to different concentrations of antigen and mitogen (Con A) showed that 2 micro gram of antigen and 1 microgram of Con A gave maximum stimulation index. The results of LTT are presented in Table I and II. However, the antibody levels were significantly higher in immunized animals as compared to the control throughout the period. The lymphocytes derived from control animal cultured in the RPMI 1640 medium with 2 µg of antigen and 1 µg of mitogen were never stimulated and their stimulation index varied between 1.079 to 1.230 throughout the study. In both crude and partially purified midgut antigen immunized rabbits antigen specific responsiveness was highly significant (P<0.01) compared to mitogen. The maximum stimulation was observed on 42 DPI in group I and in group II. Manohar and Banerjee (1992) observed antigen specific responsiveness in immunized rabbits which was significant from 21 to 42 DPI. The maximum stimulation was observed on 35 DPI. Similar to the current study, the blastogenic response of the lymphocytes to purified midgut antigen of *Rhipicephalus (Boophilus)microplus* was evident with significant stimulation index values till 119 DPI with a peak on 42 DPI (Raman,2000).

**TABLE –I**

**Blastogenic response of peripheral blood lymphocytes to immunization with crude and partially purified midgut antigen of *Rhipicephalus haemaphysaloides* in experimental rabbits (Mean S.I. Values ±S.E)**

Days Post Immunization (DPI)	Immunized animals				Control animals	
	Crude Antigen	Mitogen	Partially Purified Antigen	Mitogen	Crude Antigen	Mitogen
0	1.092± 0.0017	1.061± 0.004	1.098± 0.005	1.061± 0.0027	1.078± 0.0025	1.055± 0.0080
14	1.467± 0.004	1.28± 0.004	1.767± 0.0012	1.358± 0.012	1.09± 0.0012	1.075± 0.0080
1	1.601± 0.0017	1.434± 0.0028	1.769± 0.004	1.643± 0.0028	1.184± 0.0023	1.1076± 0.0003
28	1.971± 0.004	1.807± 0.004	1.993± 0.0025	1.876± 0.0016	1.225± 0.008	1.128± 0.0012
35	2.254± 0.004	1.913± 0.0034	2.266± 0.005	1.927± 0.0016	1.278± 0.0084	1.173± 0.004
42	2.298± 0.0028	1.94± 0.0086	2.371± 0.0014	1.946± 0.0052	1.30± 0.004	1.217± 0.0004

## International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 7, Issue 5, May 2018

49	2.290± 0.0023	1.80± 0.0086	2.292± 0.0025	1.82± 0.008	1.212± 0.0025	1.109± 0.0017
56	2.11± 0.041	1.753± 0.0121	2.20± 0.0020	1.782± 0.0028	1.23± 0.0028	1.127± 0.0006
63	2.050± 0.023	1.654± 0.016	2.128± 0.0016	1.67± 0.0048	1.23± 0.0034	1.134± 0.0048

The blastogenic response of peripheral blood lymphocytes of a normal rabbit to different concentrations of antigen and mitogen showed that 2 micro gram of antigen and 1 micro gram of mitogen gave maximum stimulation index.

**TABLE – II**

**Analysis of variance of Blastogenic response of Peripheral blood lymphocytes to immunization with crude and partially purified midgut antigen of *Rhipicephalus haemaphysaloides* in experimental rabbits (Mean S.I. Values ±S.E)**

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Ratio
Replication	8	3.0762	0.3845	12.73**
Treatments	5	5.7274	1.1455	37.94**
Error	40	1.21	0.0302	
Total	53	10.01		

**\*\* Highly Significant**

In both crude and partially purified midgut antigen immunized rabbits antigen specific responsiveness was highly significant compared to mitogen.

### V. CONCLUSION

The humoral immune response was assessed by Agid and Elisa. The Agid test revealed the presence of antibodies against midgut antigen from 21 to 56 DPI and 21 to 63 DPI in group I and group II respectively. Elisa revealed an increase in antibody titre from 7 to 63 DPI in immunized animals when compared to control animals. Significant blastogenic response to crude and partially purified midgut antigen by sensitized lymphocytes was evident from 7 to 63 DPI with a peak in stimulation index values on 42 DPI.

### REFERENCES

- [1] Amero, S., James T.C. and C.R. Elgin, Production of Antibodies using Proteins in Gel Bands. In: Methods in Molecular Biology, Basic protein and peptide protocols, Edited by: J.M. Walker, Humana Press Inc., Totowa, New Jersey. Vol, 32:401 – 406, 1994.
- [2] Bechara G.H., Szabo, M.P.J., Mukai, L.S. and P.C.S. Rosa, Immunization of dogs hamsters and guinea pigs against *Rhipicephalus sanguineus* using crude adult tick extracts. *Vet. Parasitol.*, 52:79-90, 1994.
- [3] Essumen, S., Hassanali, A., Nyindo, M and E.N. Ole-Sitayo, Augmentation of host's naturally acquired immunity by solubilized membrane bound midgut proteins of the tick *Rhipicephalus appendiculatus*. *J. Parasitol.*, 78: 466-470, 1992.
- [4] Ghosh, S and M.H. Khan, Immunological control of ticks. Immunization of cattle against *Rhipicephalus (Boophilus) microplus* using tick extract supernatant antigen. *Ind. J. Vet. Parasitol.*, 10:33-37, 1996.
- [5] Ghosh, S. and M.H. Khan, Identification of tick antigen immunogenic in calves. *Ind. J. Anim. Sci.*, 12:249-252, 1997.
- [6] Johnston, L.A.Y., Kemp, D.H. and R.D., Pearson, 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, 1986.
- [7] Kimaro, E.E and J.P. Opdebeeck, Tick infestation on cattle vaccinated with extracts from the eggs and the gut of *Rhipicephalus (Boophilus) microplus*. *Vet. Parasitol.*, 52:611 – 70, 1994.

## International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 7, Issue 5, May 2018

- [8] Kumar R, and R. Kumar, Cross- resistance to *Hyalommaanaticumanaticum* ticks in rabbits immunized with midgut antigens of *Hyalommadromedarii*. *Ind.J.Anim.Sci.*, 66:657-661, 1996b.
- [9] Manohar, G.S.andD.P.Banerjee, Cross – resistance of heterologous tick species in rabbits immunized with whole antigen extract of *Hyalommaanaticumanaticum*.*Ind.J.Anim.Sci.*,62:513-516, 1992.
- [10] Latha, B.R. Immunological strategies in the control of ixodid ticks. Ph.D., thesis submitted to TANUVAS, 1998.
- [11] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and R.J.Randall, Protein measurement with the folin phenol reagent. *J.Biol.Chem.*,**193**:265-275, 1951.
- [12] Ouchterlony, O. Diffusion in gel methods for immunological analysis.*Progress in Allergy*,**55**:1-78, (1958).
- [13] Panda, D.N., Ansari M.Z., and B.N.Sahai, Immunization of cattle with *Rhipicephalus (Boophylus) microplus* adult female and larval extracts ; Antibody and cutaneous response. *J.Vet. Parasitol.*, 6: 33- 36, 1992.
- [14] Raman, M. Immunoprophylaxis against Boophilid ticks. Ph.D., thesis submitted to TANUVAS, 2000.
- [15] Rodriguez, M., Penichet, M.L., Mouris, A.E., Labarta, V., Lorenzo Luaces, L., Rubiera, R., Cardoves, C., Sanchez, P.A., Ramos, E., Soto, A., Canales, M., Palenzuela, D., Triguero, A., Lleonart, R., Herrera, L. and de la Fuente, Control of *Boophilusmicroplus*populations in grazing cattle vaccinated with a recombinant Bm86 antigen preparation. *Vet. Parasitol.*,**57**:338-349, 1995.
- [16] Sonenshine, D.E. Biology of Ticks, Volume 2.Oxford University Press, New York, pp.3-447, 1993.
- [17] Wagner, U., Burkhardt, E. and K.Failing, Evaluation of canine lymphocyte proliferation: comparison of three different colorimetric methods with the 3 H-thmidine incorporation assays. *Vet.imm.immunopathol.*,**70**: 151-159, 1999.
- [18] Willadsen, P., Bird, P., Cobon, G.S., Hungerford, J., Tomley .F.M and Taylor, D.W. Commercialization of a recombinant vaccine against *Rhipicephalus (Boophylus) microplus*.*Parasitol*, 110: 543-550, 1995.