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(MS Received: 10.07.2023; MS Revised: 21.08.2023; MS Accepted: 22.08.2023)



MS 3083

(RESEARCH PAPER IN VETERINARY PARASITOLOGY)

**Abstract**

Ticks are very common ectoparasites of bovine and other domestic ruminants across the world. The bovine tick *Boophilus annulatus* and *B. microplus* have been estimated to cause an annual weight loss of 0.7 kg/tick. They have also been found to spread serious protozoal diseases like Babesiosis, Anaplasmosis among cattle and buffaloes in India. In the present study, twelve healthy cattle divided into two groups (A and B) of six animals in both the groups. Engorged adult *Boophilus microplus* female ticks were reared for hatching and larval emergence. Bm95 recombinant tick antigen was obtained from Indian Immunological Ltd., Hyderabad, India. Group A was inoculated with 1 ml of antigen on zero day and second dose in the same amount on 15<sup>th</sup> day after primary dose and group B kept as unvaccinated control. All animals were challenged with larval ticks on 30<sup>th</sup> day, 70<sup>th</sup> day and 120<sup>th</sup> day post vaccination. Blood samples were collected on zero day and weekly interval till one month and then fortnightly up to 5 months. Globulin, Albumin and Total serum protein (TSP) values were significant ( $P < 0.01$ ) increased whereas the values of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were significant ( $P < 0.01$ ) decreased in group A (Bm95) animals as compared to control animals.

**Key words:** Bm95 recombinant tick antigen, *Boophilus microplus*, unfed larvae of *Boophilus microplus*, Engorged adult *Boophilus microplus* female ticks.

**Introduction**

The bovine tick *Boophilus annulatus* and *Boophilus microplus* have been estimated to cause an annual weight loss of 0.7 kg/tick (Soulsby, 1982) and also been found to spread serious protozoan diseases like Babesiosis, Anaplasmosis among cattle and buffaloes in India. In the early 1990s, two such vaccines using recombinant Bm86 were subsequently registered in Latin American countries (Gavac, Heber Biotech S.A., Havana, Cuba) and Australia (TickGARD, Hoechst Animal Health, Australia) during 1993–1997. The present investigation was planned to assess the biochemical changes during the course of administration of Bm95 antigen.

**Materials and Methods**

Twelve healthy cross bred cattle of about 2 years age having no previous exposure to ticks. They were selected from Instructional Bovine Farm, RVC, Kanke, Ranchi. Engorged adult *Boophilus microplus* female ticks were collected from naturally infested cattle herds in and around Ranchi. They were washed and placed in B.O.D. incubator at temperature  $28 \pm 1^{\circ}\text{C}$  and relative humidity (R.H.)  $85 \pm 5\%$ . After oviposition, larval emergence was used for challenged on experimental animals (Fig-1). Bm95 recombinant tick antigen was obtained from Indian Immunological Ltd., Hyderabad. (Genebank accession no. AF150891.2).



**Fig-1: Engorged adult female ticks (*B. microplus*) harvested and preserved in B.O.D. incubator.**

Experimental cattle were divided into two groups (A and B) of six animals in both the groups. Animals of group A were inoculated with the first dose of 1 ml of Bm95 (200 $\mu\text{g}$  protein/ml) intramuscular on zero day and the second dose in the same amount on 15<sup>th</sup> day after primary immunization whereas group B was kept as unvaccinated control. Biochemical parameters such as Globulin (g/dL), Albumin (g/dL), Total serum protein (TSP g/dl), Alanine aminotransferase (ALT-IU/L) and Aspartate aminotransferase (AST-IU/L) were

conducted by standard methods on 0-day, weekly interval up to 1-month and then fortnightly up to 5 months. For knowing the biochemical response, all animals were challenged with larval ticks on 30<sup>th</sup> day, 70<sup>th</sup> day and 120<sup>th</sup> day post vaccination with unfed larvae (Fig-2).



**Fig-2: cattle calves challenged with *B. microplus* larvae.**

**Results and Discussion**

The values of globulin and total serum protein in Bm95 vaccinated animals was highly increased from 20<sup>th</sup> day to 100<sup>th</sup> day and then decreased from 120<sup>th</sup> day to 180<sup>th</sup> day post immunization as well as tick challenge infestation as shown in table 1 and 3. The present study revealed that increase in globulin value was probably due to immunological response which increased the immunoglobulin (Ig) or antibody (Kumar *et al.*, 2009). Increased antibody response by Bm86 antigen has been reported by so many workers (Andreotti *et al.*, 2002 and Rodriguez *et al.*, 1995 a/b). The alteration of the concentration of total serum protein might be due to directly depend upon the serum globulin concentration as a result of increase the value of TSP in Bm95 vaccinated animals. In case of unvaccinated animals the value of globulin and TSP were found to be almost unchanged during first and second challenge whereas slightly increased after third challenge infestation. Increase in the serum globulin concentration in tick infested cattle has been reported by El Kammah *et al.* (1982); O'Kielly *et al.* (1971); Springell *et al.* (1971); Williams *et al.* (1977); O'Kielly and Kennedy (1981) and Taylor and Plumb (1981).

The albumin concentration in Bm95 vaccinated animals was initially decreased from day of the vaccination to the last day of the first challenge, after that increased and attained the normal value during rest experimental periods whereas continuously decreased in unvaccinated control groups during different tick challenge infestations as table 2. Lowering of albumin concentration in tick infested animals has been reported by O'Kielly and seifert (1970); O'Kielly *et al.* (1971); Springell *et al.* (1971) and O'Kielly and

Kennedy (1981). Concentration of serum albumin was found to be more decreased in control group as compared to Bm95 vaccinated animals which might be due to liver dysfunction (Albumin is mainly

synthesized in liver, Chatterjea and Shinde, 1999) as a result of toxin secreted by the tick during feeding.

**Table no. 1: Globulin (g/dL) in cattle before and after vaccination and challenge**

Groups (No. of animals)		A (6)	B (6)
Before vaccination	0 DAY	2.72±0.13 <sup>a</sup>	2.71±0.14
Post vaccination	20 <sup>th</sup> day	<sup>B</sup> 4.86±0.23 <sup>b</sup>	<sup>A</sup> 2.64±0.11
First challenge on 35 <sup>th</sup> day	40 <sup>th</sup> day	<sup>B</sup> 5.66±0.18 <sup>bc</sup>	<sup>A</sup> 2.66±0.20
	60 <sup>th</sup> day	<sup>B</sup> 7.54±0.43 <sup>d</sup>	<sup>A</sup> 2.64±0.19
Second challenge on 70 <sup>th</sup> day	80 <sup>th</sup> day	<sup>B</sup> 8.29±0.10 <sup>de</sup>	<sup>A</sup> 2.66±0.22
	100 <sup>th</sup> day	<sup>B</sup> 9.11±0.57 <sup>e</sup>	<sup>A</sup> 2.64±0.12
Third challenge on 120 <sup>th</sup> day	120 <sup>th</sup> day	<sup>B</sup> 8.83±0.28 <sup>e</sup>	<sup>A</sup> 2.65±0.07
	140 <sup>th</sup> day	<sup>B</sup> 8.60±0.31 <sup>e</sup>	<sup>A</sup> 2.53±0.16
	160 <sup>th</sup> day	<sup>B</sup> 8.26±0.26 <sup>de</sup>	<sup>A</sup> 2.73±0.15
	180 <sup>th</sup> day	<sup>B</sup> 6.48±0.15 <sup>c</sup>	<sup>A</sup> 2.91±0.16

Values having the same superscripts in column (small) and row (capital) did not differ significantly

**Table no. 2: Albumin (g/dL) in cattle before and after vaccination and challenge**

Groups (No. of animals)		A (6)	B (6)
Before vaccination	0 DAY	3.24±0.07	3.32±0.10 <sup>d</sup>
Post vaccination	20 <sup>th</sup> day	3.23±0.04	3.25±0.09 <sup>d</sup>
First challenge on 35 <sup>th</sup> day	40 <sup>th</sup> day	<sup>B</sup> 3.01±0.08	<sup>A</sup> 2.56±0.07 <sup>c</sup>
	60 <sup>th</sup> day	<sup>B</sup> 2.95±0.17	<sup>A</sup> 2.28±0.08 <sup>b</sup>
Second challenge on 70 <sup>th</sup> day	80 <sup>th</sup> day	<sup>B</sup> 2.88±0.11	<sup>A</sup> 2.22±0.06 <sup>ab</sup>
	100 <sup>th</sup> day	<sup>B</sup> 3.08±0.12	<sup>A</sup> 2.13±0.06 <sup>ab</sup>
Third challenge on 120 <sup>th</sup> day	120 <sup>th</sup> day	<sup>B</sup> 3.06±0.04	<sup>A</sup> 2.06±0.08 <sup>ab</sup>
	140 <sup>th</sup> day	<sup>B</sup> 3.17±0.06	<sup>A</sup> 2.08±0.05 <sup>ab</sup>
	160 <sup>th</sup> day	<sup>B</sup> 3.04±0.02	<sup>A</sup> 2.14±0.06 <sup>ab</sup>
	180 <sup>th</sup> day	<sup>B</sup> 3.18±0.04	<sup>A</sup> 2.01±0.10 <sup>a</sup>

Values having the same superscripts in column (small) and row (capital) did not differ significantly

A large number of ticks attached on control animals would have produced more toxins as comparison to a small number of ticks on Bm95 vaccinated animals.

**Table no. 3: Total serum protein (g/dL) in cattle before and after vaccination and challenge**

Groups (No. of animals)		A (6)	B (6)
Before vaccination	0 DAY	5.96±0.13 <sup>a</sup>	6.03±0.16 <sup>c</sup>
Post vaccination	20 <sup>th</sup> day	<sup>B</sup> 8.09±0.27 <sup>b</sup>	<sup>A</sup> 5.98±0.12 <sup>de</sup>
First challenge on 35 <sup>th</sup> day	40 <sup>th</sup> day	<sup>B</sup> 8.67±0.15 <sup>b</sup>	<sup>A</sup> 5.56±0.16 <sup>cd</sup>
	60 <sup>th</sup> day	<sup>B</sup> 10.49±0.36 <sup>cd</sup>	<sup>A</sup> 5.37±0.20 <sup>c</sup>
Second challenge on 70 <sup>th</sup> day	80 <sup>th</sup> day	<sup>B</sup> 11.17±0.17 <sup>de</sup>	<sup>A</sup> 5.18±0.17 <sup>bc</sup>
	100 <sup>th</sup> day	<sup>B</sup> 12.19±0.59 <sup>f</sup>	<sup>A</sup> 4.89±0.08 <sup>ab</sup>
Third challenge on 120 <sup>th</sup> day	120 <sup>th</sup> day	<sup>B</sup> 11.89±0.28 <sup>ef</sup>	<sup>A</sup> 4.76±0.05 <sup>ab</sup>
	140 <sup>th</sup> day	<sup>B</sup> 11.77±0.37 <sup>ef</sup>	<sup>A</sup> 4.61±0.17 <sup>a</sup>
	160 <sup>th</sup> day	<sup>B</sup> 11.3±0.27 <sup>de</sup>	<sup>A</sup> 4.75±0.15 <sup>ab</sup>
	180 <sup>th</sup> day	<sup>B</sup> 9.66±0.14 <sup>c</sup>	<sup>A</sup> 4.92±0.09 <sup>ab</sup>

Values having the same superscripts in column (small) and row (capital) did not differ significantly

**Table no. 4: ALT (g/dL) in cattle before and after vaccination and challenge**

Groups (No. of animals)		A (6)	B (6)
Before vaccination	0 DAY	16.20±2.08 <sup>a</sup>	16.72±2.04 <sup>a</sup>
Post vaccination	20 <sup>th</sup> day	16.73±1.09 <sup>ab</sup>	15.98±1.23 <sup>a</sup>
First challenge on 35 <sup>th</sup> day	40 <sup>th</sup> day	16.60±0.54 <sup>ab</sup>	16.67±0.58 <sup>a</sup>
	60 <sup>th</sup> day	<sup>A</sup> 19.48±0.73 <sup>bc</sup>	<sup>B</sup> 24.07±0.81 <sup>b</sup>
Second challenge on 70 <sup>th</sup> day	80 <sup>th</sup> day	<sup>A</sup> 21.00±1.12 <sup>cd</sup>	<sup>B</sup> 26.98±1.04 <sup>bc</sup>
	100 <sup>th</sup> day	<sup>A</sup> 22.17±0.87 <sup>cde</sup>	<sup>B</sup> 29.88±0.56 <sup>cd</sup>

Third challenge on 120 <sup>th</sup> day	120 <sup>th</sup> day	<sup>A</sup> 22.88±1.01 <sup>de</sup>	<sup>B</sup> 28.60±1.02 <sup>cd</sup>
	140 <sup>th</sup> day	<sup>A</sup> 24.80±1.03 <sup>e</sup>	<sup>B</sup> 31.18±1.33 <sup>d</sup>
	160 <sup>th</sup> day	<sup>A</sup> 23.75±0.83 <sup>de</sup>	<sup>B</sup> 31.65±0.84 <sup>d</sup>
	180 <sup>th</sup> day	<sup>A</sup> 21.08±1.39 <sup>cd</sup>	<sup>B</sup> 28.87±1.04 <sup>cd</sup>

Values having the same superscripts in column (small) and row (capital) did not differ significantly

The values of ALT and AST were increased more in serum of unvaccinated control animals as compared to Bm95 vaccinated animals as shown in table 4 and 5. It might be due to the involvement of muscles during tick bite which releases some toxins which causing

hypersensitivity reaction (Mustatov, 1969). A large number of ticks feeding on unvaccinated control animals during different infestations might have released large amount of toxins, resulting in significant increase of both enzymes ALT and AST.

Table no. 5: AST (g/dL) in cattle before and after vaccination and challenge

Groups (No. of animals)		A (6)	B (6)
Before vaccination	0 DAY	69.17±2.36	68.17±1.70 <sup>a</sup>
Post vaccination	20 <sup>th</sup> day	68.33±1.20	67.83±1.08 <sup>a</sup>
First challenge on 35 <sup>th</sup> day	40 <sup>th</sup> day	68.83±1.17	69.17±1.74 <sup>a</sup>
	60 <sup>th</sup> day	70.67±1.84	76.67±1.87 <sup>b</sup>
Second challenge on 70 <sup>th</sup> day	80 <sup>th</sup> day	<sup>A</sup> 69.00±2.83	<sup>B</sup> 79.50±0.56 <sup>bc</sup>
	100 <sup>th</sup> day	<sup>A</sup> 71.00±1.57	<sup>B</sup> 80.83±0.70 <sup>cd</sup>
Third challenge on 120 <sup>th</sup> day	120 <sup>th</sup> day	<sup>A</sup> 71.67±1.67	<sup>B</sup> 80.50±1.45 <sup>bc</sup>
	140 <sup>th</sup> day	<sup>A</sup> 72.50±2.05	<sup>B</sup> 84.83±0.98 <sup>e</sup>
	160 <sup>th</sup> day	<sup>A</sup> 71.83±1.22	<sup>B</sup> 84.50±1.26 <sup>de</sup>
	180 <sup>th</sup> day	<sup>A</sup> 70.83±1.89	<sup>B</sup> 80.17±1.78 <sup>bc</sup>

Values having the same superscripts in column (small) and row (capital) did not differ significantly

The total serum protein and globulin values were found at higher levels whereas Alanine aminotransferase and Aspartate aminotransferase values were almost near about normal range in Bm95 vaccinated animals as compared to unvaccinated control animals during different tick challenge infestations.

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