Research paper

Biochemical response in cattle vaccinated with Bm95 recombinant Vaccine

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ABSTRACT

Twelve healthy crossbred cattle divided into two groups (A and B) of six animals in each 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 15th day after primary dose and group B kept as unvaccinated control. All animals were challenged with larval ticks on 30th day, 70th day and 120th 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 where as the values of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were significant (*P<0.01*) decreased in group A animals as compared to control animals.

Keywords: *Boophilus microplus*, Bm95, female ticks, recombinant tick antigen
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Manuscript received: 13.2.2018; accepted: 04.6.2018

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 in crossbred cattle.

MATERIALS AND METHODS

Twelve healthy cross bred cattle of about 2 years age having history of 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 ± 1^{0}C and relative humidity (R.H.) 85 ± 5%. After oviposition, larval emergence was used for challenged on experimental animals. Bm95 recombinant tick antigen was obtained from Indian Immunological Ltd., Hyderabad. (Genebank accession no. AF150891.2).

Experimental cattle were divided into two groups (A and B) of six animals in each group. Animals of group A were inoculated with the first dose of 1 ml of Bm95 (200µg protein/ml) intramuscular on zero day and the second dose in the same amount on 15th day after primary immunization where as 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. To assess the biochemical response, all animals were challenged with larval ticks on 30th day, 70th day and 120th day post vaccination with unfed larvae.

RESULTS AND DISCUSSION

The values of globulin and total serum protein in Bm95 vaccinated animals was highly increased from 20th day to 100th day and then decreased from 120th day to 180th day post immunization as well as tick challenge infestation as shown in Table 1 and 2. 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 Rodriquez *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 3. 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. A large number of ticks attached on control animals

Table 1. Globulin (g/dL) in cattle before and after vaccination and challenge

Groups	Day	A (n=6)	B (n=6)
Before vaccination	0 day	2.72 ± 0.13^{a}	2.71±0.14
Post vaccination	20th day	$B4.86\pm0.23^{b}$	A2.64±0.11
First challenge on 35th day	40th day	$B5.66\pm0.18^{bc}$	A2.66±0.20
	60th day	$B7.54\pm0.43^{d}$	A2.64±0.19
Second challenge on 70th day	80th day	$B8.29\pm0.10^{de}$	A2.66±0.22
	100th day	$B9.11\pm0.57^{e}$	A2.64±0.12
Third challenge on 120th day	120th day	$B8.83\pm0.28^{e}$	A2.65±0.07
	140th day	$B8.60\pm0.31^{e}$	A2.53±0.16
	160th day	$B8.26\pm0.26^{de}$	A2.73±0.15
	180th day	$B6.48\pm0.15^{c}$	A2.91±0.16

Values having the same superscripts in column (small) and row (capital) did not differ significantly n = number of animals

Table 2. Total serum protein (g/dL) in cattle before and after vaccination and challenge

Groups	Day	A (n=6)	B (n=6)
Before vaccination	0 day	5.96 ± 0.13^{a}	6.03 ± 0.16^{e}
Post vaccination	20th day	$B8.09\pm0.27^{b}$	$A5.98\pm0.12^{de}$
First challenge on 35th day	40th day	$B8.67\pm0.15^{b}$	$A5.56\pm0.16^{cd}$
	60th day	$B10.49\pm0.36^{cd}$	$A5.37\pm0.20^{c}$
Second challenge on 70th day	80th day	$B11.17\pm0.17^{de}$	$A5.18\pm0.17^{bc}$
	100th day	$B12.19\pm0.59^{f}$	$A4.89\pm0.08^{ab}$
Third challenge on 120th day	120th day	$B11.89\pm0.28^{ef}$	$A4.76\pm0.05^{ab}$
	140th day	$B11.77\pm0.37^{ef}$	$A4.61\pm0.17^{a}$
	160th day	$B11.3\pm0.27^{de}$	$A4.75\pm0.15^{ab}$
	180th day	$B9.66\pm0.14^{c}$	$A4.92\pm0.09^{ab}$

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

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

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.

The total serum protein and globulin values were found at higher levels whereas ALT and AST values were almost near about normal range in Bm95

Table 3. Albumin (g/dL) in cattle before and after vaccination and challenge

Groups	Day	A (n=6)	B (n=6)
Before vaccination	0 day	3.24±0.07	3.32±0.10^{d}
Post vaccination	20th day	3.23±0.04	3.25±0.09^{d}
First challenge on 35th day	40th day	B3.01±0.08	A2.56±0.07^{c}
	60th day	B2.95±0.17	A2.28±0.08^{b}
Second challenge on 70th day	80th day	B2.88±0.11	A2.22±0.06^{ab}
	100th day	B3.08±0.12	A2.13±0.06^{ab}
Third challenge on 120th day	120th day	B3.06±0.04	A2.06±0.08^{ab}
	140th day	B3.17±0.06	A2.08±0.05^{ab}
	160th day	B3.04±0.02	A2.14±0.06^{ab}
	180th day	B3.18±0.04	A2.01±0.10^{a}

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

Table 4: ALT (g/dL) in cattle before and after vaccination and challenge

Groups	Day	A (n=6)	B (n=6)
Before vaccination	0 day	16.20±2.08^{a}	16.72±2.04^{a}
Post vaccination	20th day	16.73±1.09^{ab}	15.98±1.23^{a}
First challenge on 35th day	40th day	16.60±0.54^{ab}	16.67±0.58^{a}
	60th day	A19.48±0.73^{bc}	B24.07±0.81^{b}
Second challenge on 70th day	80th day	A21.00±1.12^{cd}	B26.98±1.04^{bc}
	100th day	A22.17±0.87^{cde}	B29.88±0.56^{cd}
Third challenge on 120th day	120th day	A22.88±1.01^{de}	B28.60±1.02^{cd}
	140th day	A24.80±1.03^{e}	B31.18±1.33^{d}
	160th day	A23.75±0.83^{de}	B31.65±0.84^{d}
	180th day	A21.08±1.39^{cd}	B28.87±1.04^{cd}

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

Table 5: AST (g/dL) in cattle before and after vaccination and challenge

Groups	Day	A (n=6)	B (n=6)
Before vaccination	0 day	69.17±2.36	68.17±1.70^{a}
Post vaccination	20th day	68.33±1.20	67.83±1.08^{a}
First challenge on 35th day	40th day	68.83±1.17	69.17±1.74^{a}
	60th day	70.67±1.84	76.67±1.87^{b}
Second challenge on 70th day	80th day	A69.00±2.83	B79.50±0.56^{bc}
	100th day	A71.00±1.57	B80.83±0.70^{cd}
Third challenge on 120th day	120th day	A71.67±1.67	B80.50±1.45^{bc}
	140th day	A72.50±2.05	B84.83±0.98^{e}
	160th day	A71.83±1.22	B84.50±1.26^{de}
	180th day	A70.83±1.89	B80.17±1.78^{bc}

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

vaccinated animals as compared to unvaccinated control animals during different tick challenge infestations.

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