Assessment of Immunoprophylactic Efficacy of Recombinant Midgut Antigen (Bm95) of *Rhipicephalus microplus* in *Bos indicus*

Parthasarathy Sugumara*, Jadhav Vishwanath Tukaram*, Mukhulesh Gatne*, Dev Chandran*, Lakshmi Narasu Mangamoori†, Pundi Narasimhan Rangarajan* and Villuppanoor Alwar Srinivasan*

*Indian Immunologicals Limited, Rakshapuram, Gachibowli, Hyderabad, India.
†Department of Veterinary Parasitology, Bombay Veterinary College, Mumbai, India.
‡School of Biotechnology, Jawaharlal Nehru Technological University, Hyderabad, India.
§Department of Biochemistry, Indian Institute of Sciences, Bangalore, India.
*For Correspondence - srini@indimmune.com

Abstract

Use of recombinant Bm95 isolated from an Argentinean strain of *Rhipicephalus microplus* A as a vaccine candidate to control the tick infestation in India was investigated. Recombinant Bm95 expressed in yeast, *Pichia pastoris*. Purified protein blended into a vaccine using aluminium hydroxide as an adjuvant. An adverse effect on the reproduction of ticks was observed in vaccinated animals indicating reduced the environmental contamination and source of infestation indicating its use of Bm95 in the immunological control of ticks in India.

Key words: Tick, Bm95, *Rhipicephalus*, tick-borne diseases.

Introduction

Ticks are hematophagous ectoparasites belonging to the suborder Ixodida (1). In India, cattle ticks of the genera *Rhipicephalus, Haemaphysalis, Hyalomma*, and *Argas* cause significant economic losses to the tune of US$ 498.7 million per annum (2). The use of chemical acaricides for the control of tick infestation has led to high incidence of acaricide resistance within tick populations and food safety concerns relating to the presence of toxic residues in milk and meat (3). Control of ticks by vaccination has the advantages of being cost effective, reducing environmental contamination and preventing the selection of drug-resistant ticks. In addition, development of vaccines against ticks using multiple antigens may also prevent or reduce transmission of pathogens (1). Several approaches have been used to actively immunize bovines against the cattle tick (4). The first approach was to evaluate the protective efficacy of a vaccine comprising of a large number of antigens derived from progressive fractionation of crude tick extracts against tick challenge (5). Such studies paved way for the isolation and characterization of Bm86, the first protective tick antigen (6). These studies led to the development of commercial vaccines incorporating Bm86 such as Tickgard Plus® and Gavac® which were successful in the control of tick populations, especially when appropriately combined with acaricidal treatments (7). With the advent of more modern technologies such as construction of cDNA libraries and expressed sequence tag (EST) databases from different tick tissues, analysis of developmental stages and from gene expression changes in response to various stimuli (i.e., tick feeding or infection with pathogens), new antigens were identified. Though a large number of potential vaccine candidates for control of tick infestations have been discovered, only a
few such as 64P (8), P29 (9) have been evaluated in animal trials which yielded mixed results. In this study, we have examined the efficacy of Bm95 gut antigen, a Bm86 homologue, isolated from an Argentine Bm86-resistant strain A of *R. microplus* (10) and demonstrate its utility as a potential anti-tick vaccine.

**Materials and Methods**

**Vaccine formulations**: Recombinant vaccine consisting of 200 mgs of recombinant Bm95 and 0.01% thiomersal were adjuvanted with 0.5% aluminium hydroxide into doses of 1 ml each. The vaccine was stored at 4°C till further use.

**Animal studies**

**Determination of the efficacy of recombinant Bm95 in Bos indicus**: A shed containing 48 calves from the “Mumbai Gow-Rakshak Mandal” with moderate tick infestation was selected for the experiment. All the gross ticks were removed from 24 randomly selected healthy calves which were treated with 200 mg/kg body weight of ivermectin in addition to (15 mg/kg body weight). The animals were deticked and the ticks were counted. Immunized group containing 12 calves were vaccinated subcutaneously with 4 doses of vaccine at monthly intervals and control group containing 12 calves was kept as the unimmunized control. These calves were placed in a shed containing other 24 calves with moderate levels of tick infestation. Following live challenge, the efficacy of the tick vaccine was evaluated on the basis of comparison of degree of tick infestation and reproductive potential of female ticks collected on the 120th dpv.

**Indirect ELISA using Recombinant Bm95**: Humoral immune response against Bm95 recombinant antigen was measured by ELISA using serum samples collected from animals up to 150 dpv as described elsewhere (11). The absorbance values were recorded at 492 nm in ELISA reader (Microscan MS5605A, ECI Ltd., Bangalore).

**Results**

**Assessment of humoral immune response**: For the assessment of humoral immune response, serum samples (1:640 dilutions) from animals of immunized group and control group were subjected to indirect ELISA. The antibody response following immunization is depicted in Figure 1. Following vaccination, there was an increase in absorbance values till ~0.600 up to 35 dpv, following which the OD values were maintained till the 150 dpv. The humoral response in the unimmunized group showed values of <0.07 and remained unchanged during the course of the experiment.

**Tick counts**: Following live challenge, the animals were examined for tick counts at monthly intervals. Though there was increase in the tick count over the period of study in calves of both the groups, the number of ticks found on the control animals was distinctly higher than on the animals of vaccinated group (Table 1). The percent rejection / reduction of tick count of the body of animals of the vaccinated group was estimated by comparing the average tick counts on the same day on the body of calves from the control group. It is evident from Table 1 that the percent rejection increased from 30.55 on the day 30 to 79.04 on day 120 post immunization. Not only the tick counts but also the average weight of the female tick collected from the animals of vaccinated and control groups showed significant difference. There was steady decrease in average weight of the female tick collected from the animals of vaccinated and control groups was similar throughout the period of study.

Sugumar et al
Reproductive potential of females ticks: The engorged female ticks collected from the calves of vaccinated and control groups on day 120 of study were maintained in the laboratory to note their reproductive performance vis-a-vis adverse effect of vaccination on the parameters. The preoviposition period of the ticks collected from vaccinated animals extended by three days as compared to that of ticks collected from control group. However, oviposition period of the ticks from vaccinated group was reduced to a day or two when compared with ticks collected from control group. The output of eggs expressed as weight of egg mass in milligram from the female ticks of vaccinated group was only 37.5% of the egg mass from the female ticks of control group. This indicates the positive effect of vaccination in the immunized animals wherein the egg mass was reduced by 62.5% when compared to the control group thereby leading to a decrease in the number of ticks. The hatchability of eggs laid by female ticks from vaccinated group was nil indicating 100% reduction. In contrast, female ticks from control animals produced total of 1680 larvae (61.8 larvae/female tick) (Table 2).

Discussion
Cross-bred and exotic breeds of cattle are highly susceptible for tick infestation. Immunological intervention appears to be the best option for reducing tick burden. Hence, immunization of animals with tick antigen is drawing attention of many research groups to protect high productivity cross-bred dairy cattle.

Table 1: Tick counts and average weight of female ticks recovered from vaccinated and unvaccinated calves.

<table>
<thead>
<tr>
<th>Immunized group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPV</td>
<td>Tick count</td>
</tr>
<tr>
<td>Initial screening</td>
<td>398</td>
</tr>
<tr>
<td></td>
<td>10-32 (23.16)</td>
</tr>
<tr>
<td>30th</td>
<td>375</td>
</tr>
<tr>
<td></td>
<td>3-8 (4.25)</td>
</tr>
<tr>
<td>60th</td>
<td>302</td>
</tr>
<tr>
<td></td>
<td>5-12 (7.69)</td>
</tr>
<tr>
<td>90th</td>
<td>281</td>
</tr>
<tr>
<td></td>
<td>6-16 (9.12)</td>
</tr>
<tr>
<td>120th</td>
<td>261</td>
</tr>
<tr>
<td></td>
<td>5-19 (11.8)</td>
</tr>
</tbody>
</table>

DPV: Days post vaccination
Figures in parenthesis indicate averages
NA: Not applicable
Initial screening: indicates counts at before treatment of animals with ivermectin and fenbendazole.

DPV: Days post vaccination
Figures in parenthesis indicate averages
NA: Not applicable
Initial screening: indicates counts at before treatment of animals with ivermectin and fenbendazole.
The suggestion that the blood feeding ectoparasites may be damaged by an immunological reaction of the hosts against their internal organs is not new (12, 13). The first concealed gut antigen which was identified was Bm86 from *Rhipicephalus microplus*. Commercial vaccines (TickGard and Gavac) have been developed by using recombinant Bm86 antigen in Australia (5) and Cuba (14). Other concealed gut antigens from *R. microplus* have been identified viz. Bm91 (15), BmA7 (16) and Bm95 (17), which have been used alone or in combination with Bm86 viz. Bm91 has been combined with Bm86 and has been commercialized as TickGardPlus. Also, Bm95 has shown to induce protection even against Bm86 resistant *R. microplus* strains (17). Later, it was also noticed that combination of Bm86 and Bm95 antigens may provide better protection (5).

The immunoprotective properties of anti-tick antibodies is a well established fact in different tick-host systems (18, 19, 20, 21, 22, 23) In the present study, anti-Bm95 antibody titers peaked on the 35th day and were maintained till the 150th dpv. This rise in antibody titers was statistically significant (P<0.01) as early as day 10 dpv. A direct correlation between the anti-Bm95 antibody titers of vaccinated calves and its effect on feeding and reproductive parameters was noted. Similar results were reported by Ghosh and Khan (24), Rodriguez et al. (14), de la Fuente et al. (4), Andreotti (22), Opdebeeck et al. (23) by using different tick-derived antigens. However, Jackson and Obdebeeck, (19) reported a negative correlation between anti-gut membrane antibodies and its effect on reproductive index.

Cross-protection studies to evaluate the protection potential of Bm95 based vaccine against other tick species prevalent in India viz. *Hyalomma anatolicum anatolicum*, *Rhipicephalus haemaphysaloides* etc. needs to be carried out in India. Another most challenging scientific aspect that needs proper attention from the scientific community is the transmission limiting potential of tick pathogens. In a study, de la Fuente et al. (29) reported that the immunization of animals with Gavac significantly lowered the number of clinical cases of babesiosis. To develop an integrated pest management programme (IPM) for tick and tick-borne diseases suitable to Indian conditions, transmission limiting potentiality of the Bm95 based vaccines needs to be thoroughly studied.

**Conclusions**

The findings on immune response generated by Bm95 antigen in calves during the

<table>
<thead>
<tr>
<th>Reproductive parameters of ticks</th>
<th>Immunized group 120th dpv</th>
<th>Control group 120th dpv</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of engorged females</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>Weight of engorged females</td>
<td>5.49</td>
<td>5.94</td>
</tr>
<tr>
<td>Preoviposition period (days)</td>
<td>6-10</td>
<td>5-7</td>
</tr>
<tr>
<td>Oviposition period (days)</td>
<td>1-2</td>
<td>3-7</td>
</tr>
<tr>
<td>Egg mass (mg)</td>
<td>630</td>
<td>1680</td>
</tr>
<tr>
<td>Hatchability Rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of larvae/female</td>
<td>Nil</td>
<td>927</td>
</tr>
</tbody>
</table>

Table 2: Reproductive performance of female *R. microplus* collected from vaccinated and unvaccinated calves.
present study are of great significance as they support the fact that when ticks feed on immunized animals, a cutaneous inflammatory response is generated at the site of bite followed by an increase in the titer of anti-tick antibodies in the serum. The cutaneous inflammation prevents further feeding and the antibodies that are consumed by ticks along with blood meal damage mid gut producing a deleterious effect, on their feeding and reproductive performances (25, 26, and 27) and eventually causing the death of ticks. Thus, immunization against ticks can have dual action in preventing tick attachment and also tick borne diseases (28).

**Fig. 1**: Humoral response of calves following vaccination using recombinant Bm95 both in the controlled pen and field trial

**References**


its effect as a vaccine against tick infestation in Rabbits. Infection and Immunity 67(4), 1652-1658.


Boophilus microplus following DNA and protein vaccination in sheep. Veterinary Immunology and Immunopathology 71, 151-160.


Immunoprophylactic Efficacy of Recombinant Mid gut Antigen (Bm95)