RESEARCH

Evaluation of the recombinant Bm86 antigen (Gavac™) against Hyalomma dromedarii *and* H a anatolicum *(*Acari: Ixodidae*) in Sudan*

✉ Hassan IA[1], Hassan SM[2], El Hussein AM[3], Rodríguez M[4]

[1]Department of Microbiology and Parasitology, University of Nyala
PO Box 155, Sudan
E-mail: ibrahimsulieman@msn.com
[2]Department of Parasitology, University of Khartoum, Sudan
[3]Central Laboratory, Ministry of Science and Technology, Sudan
[4]Center for Genetic Engineering and Biotechnology

ABSTRACT

This study evaluated the Bm86 glycoprotein that was originally isolated and characterized from *Boophilus microplus* and used as a commercial recombinant anti-tick vaccine against this species. Current research revealed the ability of this antigen to induce an effective immune response against another tick genera, namely *Hyalomma* spp. Immunization experiments were carried out on rabbits and camels. Immunity developed by Bm86 was assessed by monitoring the performance of ticks fed on immunized hosts compared with those fed on the control groups. The acquired resistance was expressed as a significant lengthening of feeding period, preoviposition period, oviposition period and a reduction in mean engorgement weight, mean egg mass weight, and hatchability percentage of larvae compared with ticks fed on the non-immunized group. An ELISA was carried out on samples collected before, during and after vaccination sessions to determine levels of antibody response and dynamics throughout vaccination experiments. Optical densities of immunized rabbits showed a mean of 0.22 at 405 nm against 0.06 for the control group, while immunized camels showed 0.46 at 405 nm compared to 0.095 for the control group. On measuring the peak point of antibodies against Bm86, rabbits showed the highest response on week 13 (OD 0.79 at 405 nm), while camels highly respond on week 9 with an OD of 0.93 at 405 nm.

Keywords: Hyalomma anatolicum anatolicum, *Hyalomma dromedarii*, Gavac, recombinant vaccines, Bm86

Biotecnología Aplicada 2007;24:122-125

RESUMEN

Evaluación del antígeno recombinante Bm86 (Gavac ™) contra *Hyalomma dromedarii* y *H. a. Anatolicum* (*Acari: Ixodidae*) en Sudán. En este estudio se evaluó la glicoproteína Bm86 originalmente aislada y caracterizada de *Boophilus microplus*, y utilizada como vacuna recombinante comercial contra esta especie de garrapata. En este trabajo se mostró la capacidad de este antígeno para inducir una respuesta inmune efectiva contra otro género de garrapata, *Hyalomma* spp. Los experimentos de inmunización fueron realizados en conejos y camellos. La inmunidad desarrollada contra Bm86 se evaluó mediante el monitoreo del desempeño de garrapatas alimentadas en los animales inmunizados, en comparación con garrapatas alimentadas en animales de los grupos controles. La resistencia adquirida se expresó como el alargamiento significativo de los períodos de alimentación, períodos de pre-ovoposición y de ovoposición, y la reducción del peso medio por ingestión de sangre, peso medio de la masa de huevos y el porciento de nacimiento de las larvas correspondientes, comparadas con garrapatas alimentadas en los animales no inmunizados. Se evaluó los niveles y la dinámica de la respuesta de anticuerpos mediante ELISA de muestras colectadas antes, durante y después de los experimentos de vacunación. La densidad óptica media, medida a 405 nm, fue de 0.22 en conejos inmunizados contra 0.06 en el grupo control, y de 0.46 contra 0.095 en camellos inmunizados y el correspondiente grupo control, respectivamente. En conejos, el pico de respuesta máxima de anticuerpos se obtuvo en la semana 13 (0.79 D.O. a 405 nm), mientras que en camellos se alcanzó en la semana 9 (0.93D.O. a 405 nm).

Palabras clave: Hyalomma anatolicum anatolicum, *Hyalomma dromedarii*, Gavac, vacunas recombinants, Bm86

*I*ntroduction

The application of an anti-tick vaccine has been shown to be the most promising alternative tick control strategy compared to acaricides that have a number of drawbacks. The success of this strategy depends on the cloning, and characterization of tick molecules that are involved in their basic physiological roles [1]. Thus far, research has shown the presence of a small number of well characterized molecules with the ability to stimulate an immune response against ticks, among which Bm86 is the most effective one. Recombinant Bm86 is a commercially available molecule under two names Gavac™ and TickGARD™.

It is a tick gut cell membrane-related molecule of approximately 86 kDa [2]. It has been applied successfully in countries of South America and Australia in *B. microplus* controlling programmes.

Hyalomma ticks are a major concern for livestock development in Sudan. While *H. a. anatolicum* is the main vector of tropical theileriosis (*Theileria annulata* infection). *H. dromedarii* is a very harmful to camels since it ingests large amounts of blood resulting in large liveweight losses.

The purpose of this study was to evaluate recombinant Bm86 of *Boophilus microplus* against *H.*

1. Imamura S, da Silva Vaz Junior I, Sugino M, Ohashi K, Onuma M. A serine protease inhibitor (serpin) from *Haemaphysalis longicornis* as an anti-tick vaccine. Vaccine 2005; 23(10):1301-11.

2. Rand N, Moore T, Sriskantha A, Spring K, Tellam R, Willadsen P, Cobon G. Cloning and expression of a protective antigen from the cattle tick *Boophilus microplus*. Proc Natl Acad Sci 1989; 86:9657-61.

✉ Corresponding author

dromedarii and *H. a. anatolicum* fed on camels and rabbits in Sudan.

Materials and methods

Tick species

Unfed adult stages of ticks were kept in the laboratory. *Hyalomma dromedarii*, were collected as engorged females at the central livestock market, Khartoum State, and were kept on goat kids and reared to the adult stage. *H. a. anatolicum* dropped nymphs were collected at the Khartoum University Farm and kept until reaching the adult stage.

Experimental animals

Eight male camels (2-2.5 years old) were used in the study. They were divided into two groups of 4 animals each; group-1 (vaccinated) and group-2 (control). The rabbits were also divided into a vaccinated and a control group of 8 animals each .

Gavac™ application

Vaccination was applied on weeks 0, 4 and 7 following the manufacturer's instructions. Camels were injected intramuscularly, with 2 ml of Gavac™ containing 100 µg of recombinant Bm86 in montanide 888 (Heber Biotec SA., Havana, Cuba), while rabbits received 0.5 mL (25 µg) also intramuscularly. Serum samples were collected prior to each dose administration. This was followed by a tick challenge 21 days after the last booster dose.

Tick challenge

Laboratory reared unfed adults of *H. dromedarii* and *H. a. anatolicum* were used in this study. Forty unfed adults of only *H. dromedarii* (20 females +20 males) were applied on each camel using a cotton bag attached to each ear of the camels, while on rabbits, 20 unfed adults (10 females + 10 males) of both *H. dromedarii* and *H. a. anatolicum* were applied simultaneously on the ears according to the method described by Bailey [3]. During feeding, the ticks were monitored and the individually dropped females were collected from inside the bags and immediately weighed. They were incubated at 28 ± 1 ºC and 75.5% relative humidity. The weights of the egg batches of each female were recorded.

ELISA assay

With a minor modification, the ELISA test was carried out according to the methods described by Willadsen and McKenna [4] and Triguero *et al.* [5]. The antigen was diluted in carbonate/bicarbonate buffer (pH 9.6) to a final concentration of 1 µg/mL. A 96-well microtitre plate (Maxisorp-Nunc, Denmark) was coated with 100 µL of the antigen preparation. The coated plates were incubated overnight at 4 ºC. The plates were washed with distilled water containing 0.05% (w/v) Tween 20. The same solution was used for other washing steps. A blocking buffer consisting of PBS + 0.05% Tween 20 + 2% skimmed milk powder (pH 5.0) was used after the coated plates were washed. The plates were incubated at 37 ºC for 1 hour. Thereafter, the plates were washed using the washing solution previously prepared, followed by the addition of 100 µL of rabbit test sera diluted at 1:160 in PBST (PBS + 0.1% Tween 20 + 1% skimmed milk powder, pH 5.0) to the plate wells and incubated at 37 ºC for 1 hour. After washing, a peroxidase labelled goat anti-rabbit IgG (H + L) antibody (Sigma, USA) was diluted 1:1000 in PBST and added to the wells (100 µL per well). For camel sera, a known anti-camel antibody was not commercially available, so a bacterial cell wall protein known to be able to combine to the heavy chain moiety of IgG antibody type was used in a recombinant form to detect camel antibodies in this trial. This protein, commercially known as protein G, labeled with peroxidase (1:1000) was also used for the sensitization of camels' antibodies. The plates were then incubated at 37 ºC for 1 hour. After washing, a substrate buffer (pH 5.0) consisting of ABTS (2, 2-Azinodi-Ethylbenzothiazolinesulfonic acid) tablets (0.5 mg/mL, Sigma, US) + Na_2HPO4 (134 mg/mL) + citric acid (52.5 mg/mL) was added. After 20 minutes, the reaction was stopped by adding 50 µL of 2.5% sulphuric acid and optical densities were read at 405 nm using an Immunoskan-BDSL ELISA reader. The reader was connected to a computer with ELISA data analysis (Ascent) software.

Statistical analysis

Data collected from vaccination experiments, were processed using a general linear model (GLM) procedure with a statistical analysis system (SAS version 6.12) package. The SAS system was used to perform analyses of variance (ANOVA) and mean significance tests were performed according to Ryan-Einot-Gabriel-Welsch Multiple Q test (REGWQ) [6].

Results

Immunization of rabbits with Gavac™

Comparisons were made between reproductive parameters of female ticks fed on immunized and non-immunized rabbits to evaluate the immune response stimulated by vaccination with Gavac™.

The feeding periods of both tick species fed on vaccinated rabbits were not significantly prolonged, ($P \geq 0.07$ for *H. a. anatolicum* and $P \geq 0.19$ for *H. dromedarii*), while pre-oviposition periods were significantly lengthened ($P \leq 0.001$ for both ticks). Also female ticks of both species had significantly longer periods of oviposition ($P \leq 0.001$ for *H. a. anatolicum* and $P \leq 0.01$ *H. dromedarii*) (Table 1).

Engorgement weights were significantly reduced for both tick species ($P \leq 0.001$), and consequen-tly there was a significant drop in egg mass weights ($P \leq 0.001$) and a reduced percentage of hatched larvae ($P \leq 0.001$ for *H. a. anatolicum* and $P \leq 0.01$ *H. dromedarii*) (Table 1).

Immunization of camels with Gavac™

Hyalomma dromedarii ticks fed on immunized camels showed significantly prolonged feeding ($P \leq 0.05$), preoviposition ($P \leq 0.001$) and oviposition ($P \leq 0.01$) periods. The mean engorgement weights were notably reduced ($P \leq 0.001$) in these ticks leading to decreased egg batch weights ($P \leq 0.001$). The percentages of

3. Bailey KP. Notes on the rearing of *Rhipicephalus appendiculatus* and their infection with *Theileria parva* for experimental transmission. Bull Epiz Dis Afri 1960; 8:33-43.

4. Willadsen P, McKenna R. Vaccination with "concealed" antigens: Myth or reality? Para Immunol 1991; 13:605-16.

5. Triguero A, Blanco R, Machado H, Rodríguez M, de La Fuente J. Development of enzyme linked immunosorbent assays to measure Bm86 antigen of *Boophilus microplus* (cattle tick) and to detect anti-Bm86 antibodies in serum samples. Biotech Tech 1999; 13:119-25.

6. Day RW, Quinn GP. Comparison of treatment after an analysis of variance in ecology. Ecological Monographs 1989; 59:433-63.

Table 1. Means (±SE) of feeding, preoviposition and oviposition periods, engorgement and egg mass weights of *Hyalomma anatolicum anatolicum* and *Hyalomma dromedarii* fed on Bm86 immunized and control rabbits.

Tick spp.	Treatment	N	Feeding (days)	Preoviposition (days)	Oviposition (days)	Engorgement weight (g)	Egg mass weight (g)	Hatchability (%)
H. a. anatolicum	Control	74	7.70 ± 0.22a	4.95 ± 0.19b	14.17 ± 0.28b	0.397 ± 0.013a	0.233 ± 0.0a	69.0 ± 0.02a
	Immunized	58	8.38 ± 0.31a	5.81±0.14a	15.70 ± 0.70a	0.291 ± 0.012b	0.144 ± 0.0b	50.1 ± 0.13b
H. dromedarii	Control	74	7.84 ± 0.19a	4.72 ± 0.146b	15.41 ± 0.30b	0.832 ± 0.026a	0.484 ± 0.02a	71.6 ± 0.33a
	Immunized	67	8.16 ± 0.17a	5.82 ± 0.102a	16.52 ± 0.20a	0.524 ± 0.028b	0.290 ± 0.02b	58.8 ± 0.25b

*Means (± SE) followed by the same letter in each column for each tick species are not significantly different at 5% level based on Ryan's Q test (REGWQ)

*N = number of observations

Table 2. Means (±SE) of feeding, preoviposition and oviposition periods, and engorgement and egg mass weight of *Hyalomma dromedarii* fed on immunized and non-immunized camels.

Treatment	N	Feeding period (days)	Preoviposition period (days)	Oviposition period (days)	Engorgement weight (g)	Egg mass weight (g)	Hatchability (%)
Control	41	8.37 ± 0.30b	3.17 ± 0.09b	16.66 ± 0.33b	0.754 ± 0.028a	0.467 ± 0.02a	63.2 ± 0.23a
Immunized	41	9.29 ± 0.03a	4.37 ± 0.180a	17.63 ± 0.21a	0.568 ± 0.027b	0.341± 0.02b	38.8 ± 0.34b

* Means (± SE) followed by the same letter in each column are not significantly different at 5% level based on Ryan's Q test (REGWQ)

* N = number of observations

hatching larvae were significantly lower than that of the control group ($P \leq 0.001$) (Table 2).

Antibody responses to the recombinant Bm86

Rabbit sera

Levels of antibodies induced by the Bm86 vaccination were measured by ELISA. Significant antibody levels were detected in serum samples obtained in week 7 after the second booster dose. Optical densities (OD) of the immunized rabbits showed different values with a mean of 0.22, while mean OD of the negative sera were 0.06 (Figure 1).

Camel sera

Immunized camels showed a mean OD of 0.46 in week 7, while for the negative group the mean OD was 0.095 (Figure 2).

Dynamics of anti-Bm86 Abs

Sera of Bm86 vaccinated animals were collected until week 13 and analyzed by ELISA to monitor the kinetics of antibodies after the last booster. Differences were observed during the vaccination process , starting from the initial response, during the booster shots and a number of weeks after the last booster. For rabbits the highest OD (0.79) was recorded in samples collected 6 weeks after the last booster (Figure 3), while camels showed an OD of 0.93 on week 9 (Figure 4).

Discussion

Few animals were used in these trials, hence the values obtained are preliminary. In the rabbit trial both tick species were successfully established, while in the camel only *H. dromedarii* were used. Attempts to have *H. a. anatolicum* feed on camels were unsuccessful (data not shown). Previously *H.a. anatolicum* was found to be not well adapted for feeding on camels and it comprised only 3.3% of all ticks collected from this animal [7].

Some authors observed a discoloration of ticks. This might be attributed to the leakage of blood through the damaged tick guts into the haemolymph of *B. microplus* females fed on Bm86-immunized hosts, while other authors [2, 8, 9] did not observe this discoloration. In this study, this change was not noticed, probably be cause of the immunological cross-reaction between the Bm86 and Bm86-like homologue present in different tick species [10].

The overall impact of vaccination with the recombinant Bm86 antigen on experimental infestations with *H. dromedarii* and *H. a. anatolicum* was observed in the affected biological parameters. The results showed an acceptable level of protection since significant reductions were evident in engorgement weights, egg mass weights and per cent of hatched larvae, besides

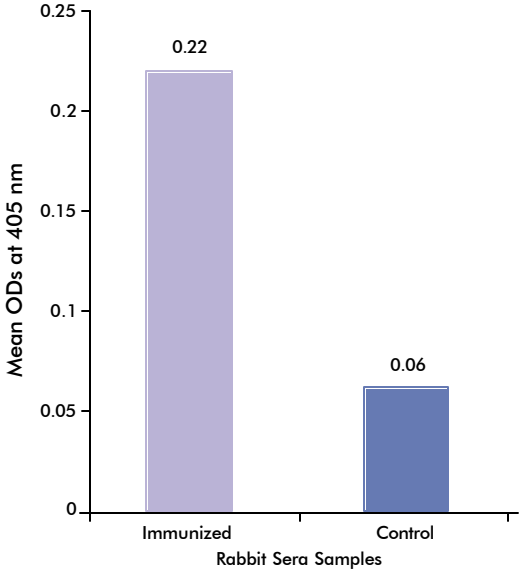

Figure 1. Antibody levels of control and Bm86 immunized rabbits on week 7.

7. Ahmed AE. Studies on ecology and behavior of camel ticks in Nhr Elnil State, Sudan with particular reference to *Hyalomma dromedarii* (*Acari: Ixodidae*). PhD thesis, university of Khartoum. 2005.

8. Valle MR, Rubiera M, Penichet M, Montesinos R, Cremata J, Falcon G, Sanchez R, Brings C, Cordoves M, Valdés R, Lleonart R, Herrera L, de la Fuente J. High level expression of a protective Bm86 antigen from the cattle tick *Boophilus microplus* in the yeast *Pichia pastoris*. Recombinant Vaccines for the Control of Cattle Tick. Editor de La Fuente. 1995. pp. 205-27.

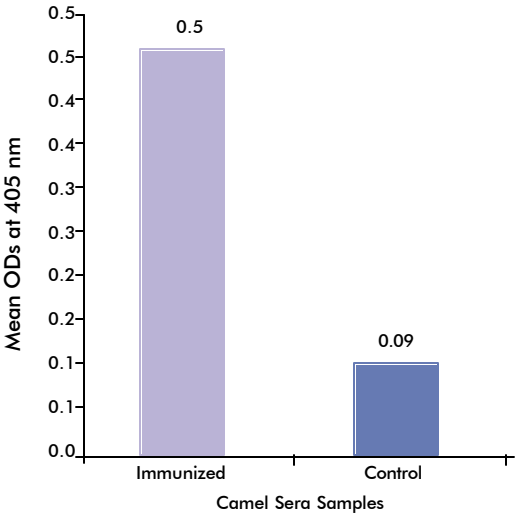

Figure 2. Antibody levels of control and Bm86-immunized camels on week 7.

the significantly long feeding period must be taken into account in interpreting data obtained from these trials. A number of factors are likely to be affecting the potential of the Bm86 antigen in providing cross-protection when used against other tick genera, despite the fact that certain authors found that this antigen

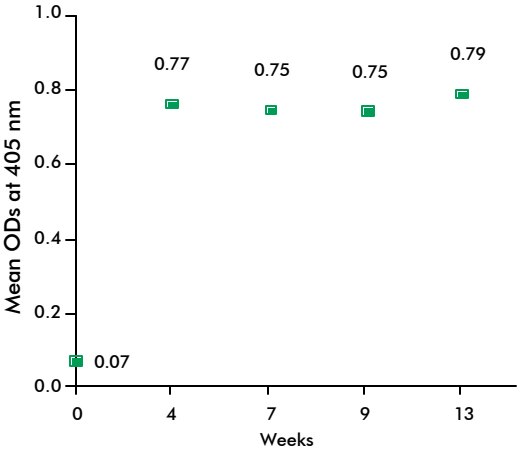

Figure 3. Antibody levels of Bm86-immunized rabbits.

Received in june, 2007. Accepted for publication in october, 2007.

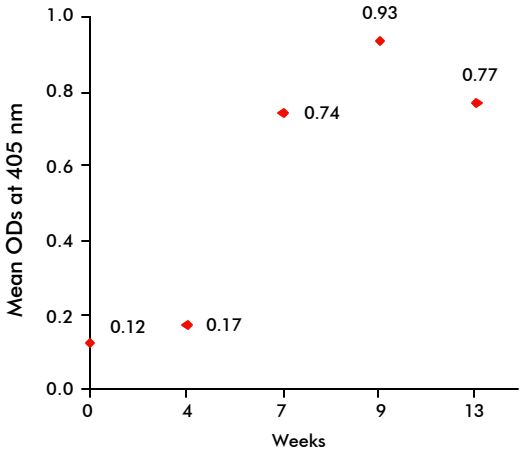

Figure 4. Antibody levels of Bm86-immunized camels.

worked much better against *B. annulatus* [11, 12] and against *H. a. anatolicum* and *H. dromedarii* [10] than *B. microplus*.

These factors are likely to involve the intensity of reaction of Bm86 which is assumed to be homologous in other target tick species. Also, there is a possible variation in the biological nature of the existing homologue and this includes the distribution and concentration of this molecule within the tick gut cells.

Targeted hosts should also be considered, since the response stimulated by vaccination with the Bm86 molecule was found to be affected by many other factors. These considerations were explained on correlating factors such as the physiological state of the animal, age and stress factors. The above factors were found to significantly affect the capacity of the host to respond to vaccination with the Bm86 antigen [13]. Therefore further research on the existence of Bm86 homologues in *Hyalomma* spp. may help improve and better understand the immunological approach in controlling economically important ticks and the disease they transmit.

Acknowledgments

We are grateful to the Integrated Consortium on Ticks and Tick-borne Diseases (ICTTD) for facilitating this study. Thanks are also given to the German Academic Exchange Services (Deutcher Akademischer Austauschdienst-DAAD) for their assitance.

9. Fragoso H, Ortiz R, Rodríguez M, de La Fuente J. Evaluation of the efficacy of the recombinant vaccine Gavac in cattle artificially infested with *Boophilus microplus* (Can). Recombinant Vaccines for the Control of Cattle Tick. (Editor de La Fuente). 1995. pp. 229-37.

10. de Vos S, Zeinstra L, Taoufik O, Willadsen P, Jongejan F. Evidence for the utility of Bm86 antigen from *Boophilus microplus* in vaccination against other tick species. Exp Appl Acarol 2001; 25:245-61.

11. Fragoso H, Rad H, Ortiz R, Rodríguez M, Herrera M, de la Fuente J. Protection against *Boophilus annulatus* infestations in cattle vaccinated with the *B. microplus* Bm86-containing vaccine Gavac. Vacc 1998; 16:1990-2.

12. Willadsen P, Jongejan F. Immunology of the tick-host interaction and the control of ticks and tick-borne diseases. Parasitol 1999; 15:258-62.

13. de la Fuente J, Rodríguez M, Redondo M, Montero C, García-García J, Méndez L, Serrano E, Valdés M, Enriquez A, Canales M, Ramos E, Boue O, Machado H, Lleonart R, de Armas C, Rey S, Rodríguez J, Artiles M, García L. Field studies and cost-effectiveness analysis of vaccination with Gavac™ against the cattle tick *Boophilus microplus*. Vacci 1998; 16:366-73.