

PERFORMANCE OF TWO BM86 ANTIGEN VACCIN FORMULATION AGAINST TICK USING CROSSBREED BOVINES IN STALL TEST¹

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ABSTRACT: - ANDREOTTI, R. **Performance of two Bm86 antigen vaccin formulation against tick using crossbreed bovines in stall test.** [Performance de duas formulações vacinais do antígeno Bm86 contra carrapato usando bovinos cruzados em teste de baias]. *Revista Brasileira de Parasitologia Veterinária*, v. 15, n. 3, p. 97-100, 2006. Embrapa Gado de Corte, BR 262, Km 04, Caixa postal 154; Campo Grande, MS 79002-970. Brazil. E-mail andreott@cnpqc.embrapa.br

Cattle tick control remains a serious problem for cattle farms in Brazil due to the limited success achieved with chemicals. In Brazil, the use of vaccines for tick control associated with the use of chemicals and pasture rotation may open possibilities for integrated control. However, it is important to know whether regional *Boophilus microplus* strains are sensitive to antibodies produced by the available antigens: antigen preparations GavacTM and TickGard^{PLUS}. The aim of this research was to evaluate the performance of two Bm86 antigen vaccin formulation against tick using crossbreed bovines in stall test antigen against a regional *B. microplus* strain. The experiment was carried out in central Brazil (20°27'S, 54°37'W). A trial was conducted in stall conditions on crossbred cattle under controlled infestation. Two groups of 16 animals each, homogeneous in weight and sex, were vaccinated with GavacTM or TickGard^{PLUS}, two groups of eight animals as control. Challenge was performed on three alternate days, with 5,000 larvae each time, beginning 21 days after the second injection. The antibody response was measured by ELISA and vaccinated animals presented immune response considering IgG levels. The results showed 49.2% and 46.4% protection efficacy for GavacTM and TickGard^{PLUS}, respectively.

KEY WORDS: *Boophilus microplus*, tick, Bm86, Tickgard, Gavac.

RESUMO

O controle do carrapato dos bovinos, *Boophilus microplus*, ainda é considerado um sério problema para os produtores de bovinos no Brasil, devido ao limitado sucesso do controle alcançado pelo uso de produtos químicos. No Brasil, o uso de vacinas para o controle do carrapato associado ao uso de produtos químicos e rotação de pastagens pode abrir possibilidades para um controle integrado. Entretanto, é importante saber se isolados regionais de *B. microplus* são sensíveis aos anticorpos produzidos pelos antígenos disponíveis: GavacTM e TickGard^{PLUS}. O objetivo deste trabalho foi avaliar a performance de duas formulações vacinais do antígeno Bm86 contra carrapato usando bovinos cruzados em teste de baias. O experimento foi desenvolvido no Brasil central (20°27'S, 54°37'W), utilizando bovinos cruzados com infestações con-

troladas. Dois grupos de 16 animais cada, homogêneos em peso e sexo, foram vacinados com GavacTM ou TickGard^{PLUS}, dois grupos de oito animais serviram como controle. O desafio foi desenvolvido em três dias alternados com a inoculação de 5,000 larvas por vez, iniciando 21 dias após a segunda inoculação. A resposta de anticorpos foi avaliada por ELISA. Os animais vacinados apresentaram resposta imune considerando os níveis de IgG, e com eficácia de proteção de 49,2% e 46,4% para GavacTM e TickGard^{PLUS}, respectivamente.

PALAVRAS-CHAVE: *Boophilus microplus*, carrapato, Bm86, Tickgard, Gavac.

INTRODUCTION

Cattle tick control remains a serious problem for cattle farms in Brazil, not only due to the limited success achieved when chemicals are used as the main control method, but also because of major drawbacks associated with resistance, toxicity and residues (GRISI et al., 2002).

In recent years an effective vaccine was developed based

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on *Boophilus microplus* gut Bm86 antigen (KEMP et al., 1989; DE LA FUENTE et al., 1995). Experiments have demonstrated its effectiveness against this tick species when appropriately combined with chemical treatment (REDONDO et al., 1999).

The recombinant Bm86-containing vaccines GavacTM and TickGard^{PLUS} against the cattle tick, *B. microplus*, has proved its efficacy in a number of experiments (DE LA FUENTE et al., 1998; WILLADSEN; KEMP, 1988), especially when combined with acaricides.

In Brazil, the use of vaccines for tick control associated with chemicals and pasture rotation may open possibilities for integrated control, reducing the problems caused by resistance and environmental contamination in an integrated manner.

However, it is important to know whether regional *B. microplus* strains are sensitive to antibodies produced by the available antigens: the recombinant Bm86 (rBm86) antigen preparations GavacTM (Heber Biotec S.A., P.O. Box 6162, Havana, Cuba) (BOUÉ et al., 1998) and TickGard^{PLUS} (Intervet Australia Pty. Ltd., 91-105 Harpin Street Bendigo, East Vic.) (WILLADSEN, 1997).

Boophilus microplus isolates that showed low susceptibility to vaccination with Bm86, prompting research on additional antigens that might prove effective against a broader spectrum of tick strains. *Boophilus microplus* isolates such as the Argentinian strain A show low susceptibility to this vaccine (GARCIA-GARCIA et al., 1999).

Bm95 antigen was effective against different tick strains in a pen trial, including the *B. microplus* strain A, resistant to vaccination with Bm86. A Bm95-based vaccine was used to protect cattle against tick infestations under production conditions, lowering the number of ticks on vaccinated animals and, therefore, reducing the frequency of acaricide treatments. The Bm95 antigen from strain A was able to protect against infestation with Bm86-sensitive and Bm86-resistant tick strains, thus suggesting that Bm95 could be a more universal antigen to protect cattle against infestations by *B. microplus* strains from different geographical areas (GARCIA-GARCIA et al., 2000).

When ticks ingest blood from an animal immunized with Bm86, tick gut cells lyse via antibody-mediated mechanisms of host defense, leading to a reduction in tick number, weight, and reproductive capacity (KEMP et al., 1989).

In this paper we report a trial conducted to evaluate the performance of two Bm86 antigen vaccine formulation against a regional *B. microplus* strain from Campo Grande, Mato Grosso do Sul, Brazil, using crossbred bovines in stall test.

MATERIALS AND METHODS

Controlled pen trial. The experiment was carried out in Campo Grande, Mato Grosso do Sul, central Brazil (20°27'S, 54°37'W). A controlled pen trial was conducted under stall conditions on crossbred cattle under controlled infestation. Eight-month-old crossbred calves, homogeneous in weight (140-170 kg) and sex, were randomly distributed into three groups of 16 animals each. One group was vaccinated with

GavacTM, another with TickGard^{PLUS}, whereas two groups of eight animals remained unvaccinated and served as control.

All the animals in the immunized groups were inoculated intramuscularly with 2 mL of vaccine at weeks 0 and 4. Serum samples were taken from each animal weekly. Beginning 21 days after the last immunization, challenging was performed three times on alternate days with 5,000 larvae of the Campo Grande strain each time.

The larvae were delivered from separate vials on the back of the animals. Three weeks later, engorging adult female ticks were collected, counted, weighed, and their egg-laying capacity and egg fertility were assessed (DE LA FUENTE et al., 1999).

The mean amount of eggs laid was used to express the reproductive capacity of ticks, and fertility was expressed as the percent of viable eggs. With these parameters we calculated the efficacy of each immunogen as a combination of the reduction in the number of engorging ticks, their egg-laying capacity and the fertility of the eggs (DE LA FUENTE et al., 1999):

$$\text{Efficacy (\%)} = 100 \times [1 - (\text{CRT} \times \text{CRO} \times \text{CRF})]$$

where CRT, CRO and CRF are, respectively, the reduction in the number of engorging ticks, the egg-laying capacity, and the fertility, as compared with the control group.

Serum collection and analysis. Serum samples were taken from the animals once a week during the periods of immunization and tick challenge. The sera were stored at -20 °C until assayed.

Antibody levels were determined by enzyme-linked immunosorbent assay (ELISA). Briefly, plates were coated with 100 mg/mL of recombinant Bm86 antigen in phosphate buffered saline solution (PBS) for 3 h at 37 °C. The plates were washed with PBST (0.05% Tween-20 in PBS) and blocked with 5% of fat-free milk overnight at 4 °C. The sera were serially diluted from 1:20 to 1:3200 dilution. After loading the diluted sera, the plates were incubated for 2 h at 37 °C, washed with PBST and then incubated with a goat anti-bovine IgG-horseradish peroxidase conjugate. The reactions were developed with orthophenylene diamine (OPD) and hydrogen peroxide for 15 min in the dark and finally stopped with sulfuric acid. Measurements were performed at 492 nm in an ELISA reader. Titers were expressed as the maximum dilution having an OD 492 higher than two-fold the negative control average OD (TRIGUERO et al., 1999; GARCIA-GARCIA et al., 2000).

Statistical analysis. Mean antibody levels were determined for each group and compared using ANOVA. The Mann-Whitney nonparametric test was used to compare the results of vaccination efficacy.

RESULTS AND DISCUSSION

The vaccinated animals presented immune response considering IgG levels. The antibody response in vaccinated animals was successful and its kinetics and levels of antibody

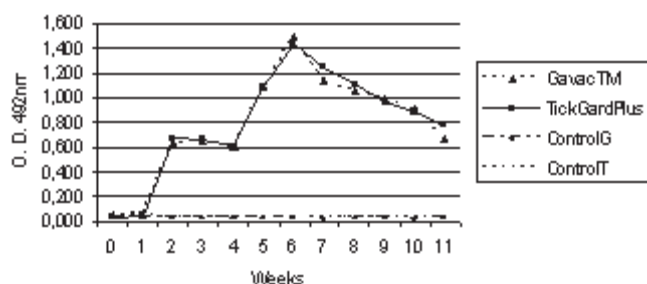


Figure 1. Kinetics of humoral immune response in animals vaccinated with Gavac™ and TickGard^{PLUS}.

response in cattle vaccinated, with either vaccine (Figure 1), showed the same profile by ANOVA, and were similar to those of previous reports using Gavac™ (DE LA FUENTE et al., 1995) and TickGard^{PLUS} (KEMP et al., 1989). Sera from control unvaccinated calves did not react with Bm86 antigen.

Tick development on the animals was supervised during the parasite cycle. The number of engorged ticks was counted during three weeks upon beginning of tick detachment and the profile showed tick production and egg weight peaking on the fourth day in all groups. The final analysis of immunization efficacy showed a reduction in total tick number, total egg weight, and total engorged female weight, as compared with the control group (Table 1).

Table 1. Results of challenge with tick larvae in the pen trial.

Immunogen	Tick number	Tick mean weight (mg)	Egg weight/tick weight	Fertility (%)	Efficacy (%)
TickGard ^{PLUS}	5650	196	0.432	83	46.4
Control	3081	226	0.518	88	-
Gavac™	3224	211	0.508	88	49.2
Control	2721	228	0.512	88	-

As a result of vaccination, ticks engorging on immunized cattle were visibly damaged and had a significantly lower weight and reproductive capacity. Levels of 49.2 and 46.4% of protection efficacy were found for Gavac™ and TickGard^{PLUS}, respectively (Table 1). No significant difference was found when the efficacy of both vaccinated groups was compared.

A preview trial conducted in Brazil on grazing dairy and beef pure and crossbred cattle under natural infestation conditions showed, as in the Cuban study, that the vaccine controlled tick numbers in successive generations in the field (RODRIGUEZ et al., 1995).

In controlled field trials in Cuba, Brazil, Argentina and Mexico, Gavac™ has shown a 55-100% efficacy in the control of *B. microplus* infestations in grazing cattle 12-36 weeks after the first vaccination (DE LA FUENTE et al., 1999).

The parcial effect of Bm86 antigen in Brazil support the idea of vaccin improvement considering differents effect on different strains of *B. microplus*, as showed in Argentinian strain as well(GARCIA-GARCIA et al., 1999).

The results of this study support the idea of poliantigenc vaccin causing differents effects on ticks. Bovine immunization with BmTIs antigen in tick control showed 72.8% of efficacy and could be a good antigen for association with Bm86 because it effect is in larvae attachment (ANDREOTTI et al., 2002).

The antiserum profile of immunized bovines in diferentes tick antigens, BmTI (ANDREOTTI et al., 2002); BYC (DA SILVA VAZ et al., 1998) and Bm86 (WILLADSEN; KEMP, 1988) show that the response decreases some months after vaccination, suggesting the need of a booster when this antigen is used for tick control.

The association of antigens to vaccine production could be advantageous when the antigen targets are different, leading to failure of tick development by distinct ways. BmTIs immunization results from damage in the early stages of tick development and its association with Bm86-like vaccines could increase its degree of protection, acting on the gut antigens of surviving ticks.

The results of this work provide support to develop experiments of integrated control using an association of Bm86 antigen and pasture rotation aimed at reducing the use of chemicals in tick control programs in sensitive environments such as the Amazon.

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