COMPARISON OF PREDICTED BINDERS IN *Rhipicephalus (Boophilus) microplus* INTESTINE PROTEIN VARIANTS BM86 CAMPO GRANDE STRAIN, BM86 AND BM95*

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ABSTRACT:- ANDREOTTI, R.; PEDROSO, M.S.; CAETANO, A.R.; MARTINS, N.F. **Comparison of predicted binders in *Rhipicephalus (Boophilus) microplus* intestine protein variants Bm86 Campo Grande strain, Bm86 and Bm95**. [Comparação da previsão de ligação das variantes de proteína de intestino de *Rhipicephalus (Boophilus) microplus* Bm86 cepa Campo Grande, Bm86 e Bm95]. *Revista Brasileira de Parasitologia Veterinária*, v. 17, n. 2, p. 93-98, 2008. Department of Animal Health, Embrapa Beef Cattle, C.P. 154, Campo Grande, MS 79002-970, Brazil. E-mail andreott@cnpgc.embrapa.br

This paper reports the sequence analysis of Bm86 Campo Grande strain comparing it with Bm86 and Bm95 antigens from the preparations TickGard^{PLUS} and Gavac^{TM}, respectively. The PCR product was cloned into pMOSBlue and sequenced. The secondary structure prediction tool PSIPRED was used to calculate alpha helices and beta strand contents of the predicted polypeptide. The hydrophobicity profile was calculated using the algorithms from the Hopp and Woods method, in addition to identification of potential MHC class-I binding regions in the antigens. Pair-wise alignment revealed that the similarity between Bm86 Campo Grande strain and Bm86 is 0.2% higher than that between Bm86 Campo Grande strain and Bm95 antigens. The identities were 96.5% and 96.3% respectively. Major suggestive differences in hydrophobicity were predicted among the sequences in two specific regions.

KEY WORDS: *Rhipicephalus (Boophilus) microplus*, tick, Bm86, Bm95, antigen.

RESUMO

O objetivo deste estudo foi analisar a seqüência de Bm86 cepa Campo Grande comparando-a com os antígenos Bm86 e Bm95 das preparações TickGard^{PLUS} e Gavac^{TM}, respectivamente. O produto de PCR foi clonado em PMOSBlue e seqüenciado. Para calcular os conteúdos de alfa-hélice e fita beta do polipeptídio previsto, foi utilizada a ferramenta de prognóstico de estrutura secundária PSIPRED. O perfil de hidrofobicidade foi calculado usando os algoritmos de Hopp e Woods, além da identificação das possíveis regiões de ligação com MHC classe I nos antígenos. O alinhamento "pair-wise" revelou que a similaridade entre Bm86 cepa Campo Grande e Bm86 é 0,2% maior que aquela entre Bm86 cepa Campo Grande e Bm95. As identidades foram de 96,5% e 96,3%, respectivamente. Com relação à hidrofobicidade, os resultados sugerem que a maior diferença entre as seqüências está localizada em duas regiões específicas.

PALAVRAS-CHAVE: *Rhipicephalus (Boophilus) microplus*, carrapato, Bm86, Bm95, antígeno.

INTRODUCTION

Tick control remains a serious problem for cattle farms in Brazil. Limited success is achieved when acaricides are used as the main control method, and major drawbacks are associated with development of resistance by ticks and chemical residue toxicity in the environment and animal products (GRISI et al., 2002). The use of vaccines for tick control in association with chemicals and pasture rotation may open possibilities for integrated control, reducing the problems caused by development of resistance by the parasites and environmental and food product contamination (GARCIA-GARCIA et al., 2000).

An effective vaccine was developed based on the *Rhipicephalus (Boophilus) microplus* gut Bm86 antigen (KEMP et al., 1989; DE LA FUENTE et al., 1995). The

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recombinant Bm86-containing vaccines Gavac™ (BOUÉ et al., 1999; Heber Biotec S.A., P.O. Box 6162, Havana, Cuba) and TickGard^{PLUS} (WILLADSEN, 1997; Intervet Australia Pty. Ltd., 91-105 Harpin Street Bendigo, East Vic.) have been shown to be effective in providing immune protection against this cattle tick (DE LA FUENTE et al., 1998; DE LA FUENTE et al., 1999; WILLADSEN; KEMP, 1988), especially when combined with acaricides (REDONDO et al., 1999).

However, regional *R. (B.) microplus* strains, such as the Argentinian *R. (B.) microplus* strain A, have shown varied sensitivity to antibodies produced by Gavac™ and TickGard^{PLUS}. Further research is therefore necessary to identify and develop additional antigens that might prove effective against a broader spectrum of tick strains (GARCIA-GARCIA et al., 1999).

The Bm95 antigen was effective in protecting cattle against infestations with different tick strains, including the *R.(B.) microplus* strain A, in a pen trial under production conditions, decreasing ticks numbers on vaccinated animals and, therefore, reducing the frequency of acaricide treatments needed (GARCIA-GARCIA et al., 2000).

The Bm86 protein (RAND et al., 1989) and its variant Bm95 (GARCIA-GARCIA et al., 1999) are the only two vaccine antigens produced commercially. The observed variable efficacy of these antigens on cattle tick strains found in different geographic areas might be due to naturally occurring allelic variations present in the *bm86* gene (DE LA FUENTE; KOCAN 2003).

In this paper we report the results of sequencing *bm86* cDNA (Campo Grande strain – *bm86-CG*) and conducting bioinformatics analysis, comparing previously reported *bm86* and *bm95* sequences, to predict structural and immunogenicity differences in the proteins they codify, aiming at evaluating the reported differences in their antigenic performance.

MATERIAL AND METHODS

Ticks. *R.* (*B.*) *microplus e*ngorged female ticks collected in Campo Grande (20°27'S, 54°37'W), Mato Grosso do Sul, central Brazil, were placed in B.O.D. incubators for oviposition under 28 °C and 80% humidity. Eggs were kept under the same conditions until hatching.

RNA extraction, cDNA synthesis and amplification of the target sequence. The RNA from about 100 mg of larvae was extracted using TRIZOL™ (Invitrogen USA), according to manufacturer's instructions. cDNA was synthesized from total RNA using the TermoScript™ RT PCR System for RT-PCR kit (Invitrogen USA) and used as template in PCR reactions with primers (forward: 5'-ATCATCCATT TGCTCTGACTT-3'; reverse: 5'-AGCACTTGACTTTC CAGGATC-3') based on the previously published bm86 sequence (GenBank acc. # M29321).

PCR reactions were performed as follows: 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Invitrogen USA), 2 mL cDNA, 1.6 U Platinum™ *taq* DNA polymerase (Invitrogen USA) and 0.8 pM of the primers in a final volume of 25 mL. Reactions were carried out at 95 °C for 5 min, followed by 35 cycles of 95 °C for 45 s, 55 °C for 45 s, 72 °C for 2 min and a final step of 72 °C for 10 min.

Cloning and sequencing. PCR products were purified after electrophoresis in 0.8% agarose gels and cloned into pMOSBlue (Amersham Biosciences UK), according to manufacturer's instructions. Colonies were characterized by PCR with primers on the vector. Positive clones were sequenced with an ABI 3100 automated sequencer using BigDye terminator chemistry, according to manufacturer's instructions (Bm86-CG, Acc *# EU352677).*

Sequence analysis. Comparisons of the predicted amino acid sequences from Bm86-CG, Bm86 (Acc. AF150895) and Bm95 (Acc. AF150891) were made using ClustalW from the BIOEDIT suite (HALL, 1999).

Hydrophobicity profile. Hydrophobicity profiles were calculated using the algorithms from Hopp and Woods (1981) and Kyte and Doolittle (1982) with the aforementioned sequences to identify predicted regions of hydrophilicity that are likely to be potential sites of antigenicity.

MHC binding prediction. The ProPred-I on-line service (http://www.imtech.res.in/) was used to identity potential MHC class-I binding regions in the three aforementioned predicted amino acid sequences. Peptides having a score greater than a selected threshold of 4% were considered as predicted binders for selected MHC alleles. Predicted binders are presented on each antigen sequence by a different color or along the primary sequence (SING; RAGHAVA, 2001).

RESULTS AND DISCUSSION

The *bm86* gene from different *R. (B.) microplus* strains derived from various regions of Brazil and from some areas in Argentina, Colombia, Venezuela and Uruguay showed amino acid sequence variations ranging from 3.4% to 6.08% when compared to the recombinant Bm86 protein (acc. # AAA30098, SOSSAI et al., 2005).

Garcia-Garcia et al. (1999) reported that amino acid sequence differences greater than 3.4% between the recombinant antigen and the endogenous Bm86 protein are sufficient to confer vaccination inefficiencies against different tick strains. Pair-wise alignment revealed that the similarity between Bm86-CG strain and Bm86 is 0.2% higher than that between Bm86-CG strain and Bm95 antigens. The identities were 96.5% and 96.3% respectively.

The multiple sequence alignment shown in Figure 1 points out the amino acid identities and discrepancies across the three protein variants (Table 1). For Bm95 and Bm86-CG there are three and four substitutions, respectively; on the other hand, Bm86 has 18 substitutions. Further analyses of the amino acid sequence substitutions observed in Bm86-CG are associated with differences in hydrophobicity predictions (Figure 2).

The predicted surface exposure profiles of the three protein variants show the behavior expected for a globular protein.

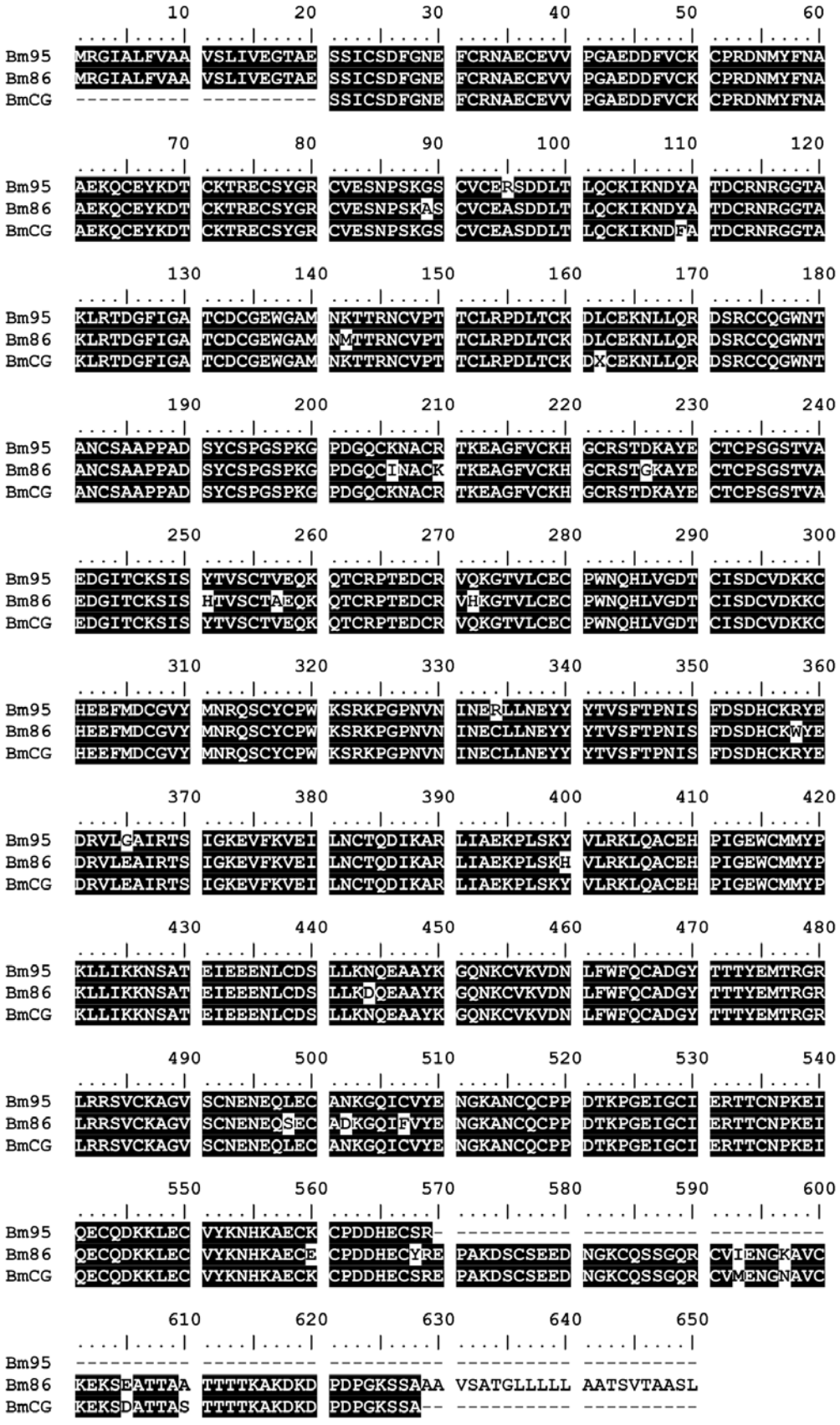

Figure 1. Multiple sequence analysis of Bm86, Bm95 and Bm86 Campo Grande strain performed with ClustalW. Legend: Bm86 (Acc. # AF150895), Bm95 (Acc. # AF 150891), Bm86-CG (Acc. # EU352677)

Table 1. Amino acid divergence between *Boophilus microplus* glycoproteins Bm86, Bm95 and Bm86 Campo Grande strain (Bm86-CG).

Sequence	Amino acid/position															
Bm95	R95	R334	G365													
Bm86	A89	M142	I206	G226	H251	A257	H272	W358	H400	D444	S498	F557	E560	Y568	I592	K597
	E605	A610														
Bm86-CG	F109	X162	M592	N597												

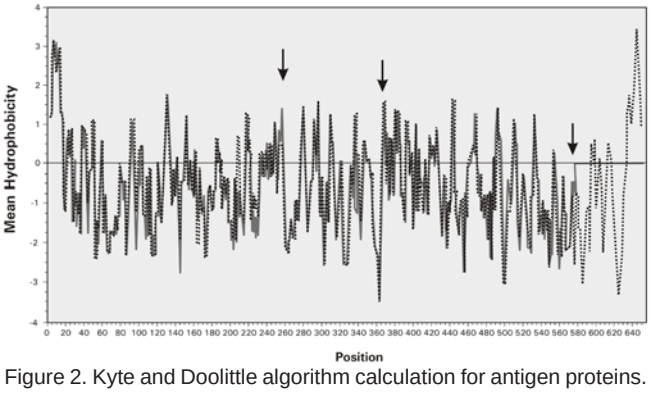

Figure 2. Kyte and Doolittle algorithm calculation for antigen proteins. Dotted gray, CG strain; gray, Bm95; dotted black, Bm86. Legend: Arrows show differences in hydrophobicity predictions.

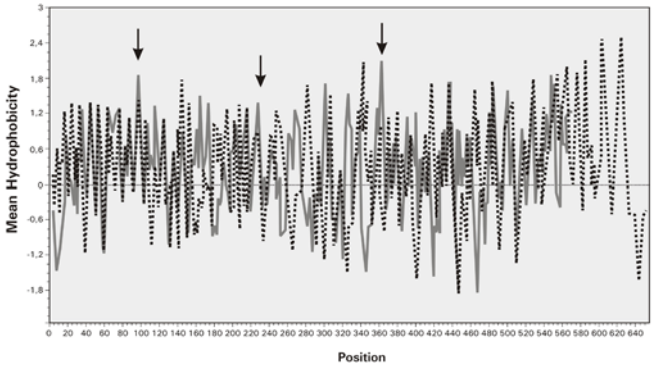

Figure 3. Hopp and Woods algorithm calculation for antigen proteins. Dotted gray, CG strain; gray, Bm95; dotted black, Bm86. Legend: Arrows show differences in hydrophobicity predictions.

No major differences were seen across the three protein variants. On the other hand, the hydrophilicity profile estimated for the three protein variants with the Hopp and Woods (1981) method suggests major differences (Figure 3).

The Bm95 variant shows three regions of high immunogenic potential that were not observed in the other variants. The first region, from R95 to T100 (RSDDLTL), is probably due to the hydophylicity of an Arginine residue. The second region, in position 226, corresponds to an Aspartic acid residue and the third region is at residue number 358, corresponding to an Arginine. For the variant Bm86-CG strain, there were no higher hydrophilic peaks compared with Bm95, but two regions might be of higher immunogenicity: K141, in the sequence DLTCK, and the sequence DRVLEAIR at position 346. This suggests that the respective amino acid changes present in the Bm86-CG variant fall within protein regions normally available to antibody recognition in Bm86 and therefore have a high potential to interfere in the dynamics of antigen recognition. These findings corroborate the observed reduced efficacy of Gavac™ in protecting cattle raised in the Campo Grande region against the local *R. (B.) microplus* strain (ANDREOTTI et al., 2006).

T cells recognize antigens as peptide fragments complexed with MHC molecules, and cattle express high polymorphic classical MHC class I genes (HLA-A, HLA-B and HLA-C; BIRCH et al., 2006). MHC binding site predictions were performed with Bm86, Bm86-CG and Bm95 (results not shown), and with some synthetic peptide sequences derived from Bm86 (PATARROYO et al., 2002, results not shown).

MHC classes I and II have a binding site consisting of a beta-strand flanked by two alpha helices arranged as a pocket. The main difference between the two classes is in the dimensions of the peptide-binding groove, as class I is able to bind to 8-11 amino acid peptides and class II MHC has the capability to bind much larger peptides. The Bm86 protein variants analyzed showed a similar behavior for MHC binding prediction. All proteins and peptides analyzed showed that side chains form favorable hydrophobic environment needed to interact with MHC side chains. The observed differences were not significant with changes in the threshold (results not shown).

On the other hand Garcia-Garcia et al. (1999) suggested that variations greater than 2.8% in the protein amino acid sequence would be enough to confer vaccination inefficiencies when recombinant antigens were used. The results obtained by Sossai et al. (2005) upon analyzing variability of the *bm86* gene in different *R. (B.) microplus* strains from various regions in Brazil and from some areas in Argentina, Colombia, Venezuela and Uruguay revealed variations from 3.4% to 6.08% in the homologous amino acid sequence of the *bm86* gene and, when compared with the *bm95* gene, variations from 1.14% to 4.56%.

In conclusion, all peptides were predicted to probably bind to MHC class I. Further investigation with CD4+ T-cell immunogenic proteins and their epitopes would be necessary to make possible the development of MHC class II to both quantify antigen-specific lymphocytes and discover novel antigenic epitopes properties (BROWN et al., 2006).

The results suggest that the discrepancies in vaccination are not only related to sequence structural features, but might be due to host factor polymorphism. Therefore there is still a need for further studies on Bm86 Campo Grande strain antigen response.

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