

Commercial vaccines based on the Bm86 tick gut antigen are used to control *Boophilus microplus*, *B. decoloratus* and *B. annulatus* ticks in Australia, South America and Asia. The vaccine also exhibits potential for control of *Hyalomma anatolicum* and *H. dromedarii* but do not induce significant protection against cattle infestation by *Rhipicephalus appendiculatus*. This therefore indicates the need to develop a vaccine that would protect cattle against *R. appendiculatus*. In this study, homologues of Bm86 were cloned from *R. appendiculatus* to determine if they could confer protective immunity in cattle against this tick species. Polymorphism in the *R. appendiculatus* antigen was assessed in a laboratory stock (Muguga laboratory stock, reared in the laboratory for over 40 years) and four Kenyan field populations from Kiambu, Kakamega, Makuyu and Uasin Gishu. A preliminary experiment was conducted to evaluate possible cross-protection against *R. appendiculatus* by the Bm86 based TickGARD™ Plus vaccine using five vaccinated and three unvaccinated *Bos indicus* calves. Full-length cDNA encoding the homologues of Bm86 from *R. appendiculatus* (designated Ra86) were isolated using Polymerase Chain Reaction (PCR) and by screening a cDNA library made using RNA extracted from the mid-gut of engorged female ticks. After cloning the cDNA in plasmid vectors and sequencing, the predicted amino acid sequences were analysed for polymorphism and used to draw phylogenetic tree using OMIGA® and Phylip® computer programs respectively and also for positive selection using synonymous-nonsynonymous mutation analysis. An *E. coli* derived recombinant Ra86 antigen was evaluated for protection against homologous tick challenge in rabbits. The results of the tick challenge experiment revealed that TickGARD™ Plus vaccine induced non-significant protection in cattle against *R. appendiculatus*. However the mean engorged weight of *B. decoloratus* and the egg weight per surviving adult female tick was significantly reduced by vaccination (ANOVA, d.f=6,  $\alpha=0.05$ ,  $P<0.001$ ). Analysis of 19 Ra86 sequences from the laboratory stock and 20 sequences from each of the four field sites revealed polymorphism in the Ra86 antigen both within the laboratory stock and the field sites. Polymorphism involved deletion/insertion of continuous amino acid domains as well as single nucleotide polymorphisms resulting in amino acid changes. Structural analysis revealed that Ra86/Bm86 homologues are made of apparent epidermal growth factor repeat (EGF-like) modules, which are cysteine rich, and probably involved in maintenance of the tertiary structure of the protein. The ratio of nonsynonymous to synonymous mutations, according to both selective neutrality and neutral evolution hypotheses, revealed positive selection in regions of the protein, perhaps driven by selection for improved metabolic function. Phylogenetic analysis of sequences from Ra86, Bm86 from different regions, *B. decoloratus* (Bd86) and the *H. anatolicum* homologue (Ha86), revealed inter-specific variation. *H. anatolicum* (sub-family Hyalominae) clustered separately from *Rhipicephalus* and *Boophilus* are closely related. High levels of Ra86 cross-reactive antibodies in sera from Bm86-vaccinated cattle were detected using enzyme linked immunosorbent assay (ELISA). Bacterially expressed recombinant Ra86 induced insignificant protective effect against adult female *R. appendiculatus* in rabbits. The findings of this study present a potential vaccine that can be developed to control *R. appendiculatus* or be combined with other antigens in a polyvalent vaccine.