

RECOMBINANT EXPRESSION OF POTENTIALLY  
IMMUNOSUPPRESSIVE PROTEINS OF THE TICK VECTOR

*AMBLYOMMA VARIEGATUM*

NAFTALY WANG'OMBE GITHAKA

A thesis submitted in fulfilment for the Degree of Master of Science in  
Zoology (Animal Parasitology) in the Jomo Kenyatta University of  
Agriculture and Technology.

2007

## ABSTRACT

Development of improved vaccines against tick infestations offers a cost-effective and environmentally sound control method. Although a limited number of protective antigens against tick infestations has been identified and characterized, discovery of novel antigens remains a limiting step for improving the efficacy of tick vaccines. Components of tick saliva/salivary glands, some of which are immunomodulatory proteins, are being considered as candidates for future vaccines. Experiments with a T-cell immunosuppressive protein of *Dermacentor andersoni*, coined p36, elucidated the functional role of one class of these proteins.

The objective of the work reported in this thesis was to produce recombinant proteins from sequences identified in the *Amblyomma variegatum* gene index (AvGI) which have homology to the *D. andersoni* immunosuppressive protein p36. Basic local alignment search tool (BLAST) searches of the AvGI and other tick databases using the p36 sequence identified a total of nine homologues, including four from *A. variegatum*, with both sequence and length polymorphism.

In addition, multiple sequence alignments of these sequences revealed there might be a large family of immunosuppressive proteins (ISPs), perhaps representing a spectrum of immunomodulatory activities.

Two putative p36 homologues of *A. variegatum*, TC183 and TC350, were cloned, sequenced and expressed in recombinant forms. Sequence analysis of TC183 and TC350 predicted the presence of a signal peptide and potential glycosylation sites on both protein sequences. Recombinant proteins were expressed using *E. coli* and purified as insoluble inclusion bodies using immobilized metal affinity chromatography (IMAC). SDS-PAGE and western blot analyses shows that these tick proteins are stable when expressed and purified from *E. coli*, despite their insolubility.

The function and usefulness of these insoluble proteins as protective antigens in cattle remains to be determined. However, the data and reagents described herein will facilitate further characterization of these proteins and evaluation as vaccine candidates.