DFG German-African Cooperation Projects in Infectiology

Genotyping of *Theileria parva* isolates from cattle and buffalo in Southern Sudan and Kenya

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Summary

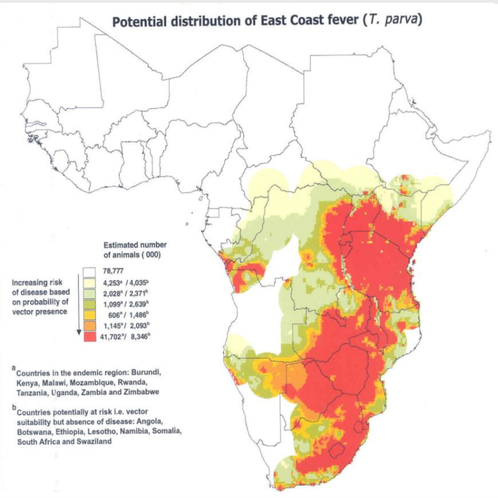
Interaction between the bovine host, tick vector and *Theileria parva* results in co-evolution between the parasite's genotype which shows a high level of genetic diversity. Molecular characterization of field isolates using variable number tandem repeat (VNTR) markers and genes encoding several parasite antigens provides information on the level of genetic diversity in field isolates of the parasite and allows estimation on the frequencies of crossover in recombining populations.

Output

Determine the levels of genetic diversity among field populations of *T. parva*, as an indication of the extent to which *T. parva* is subject to immune selection by the bovine host. This will have relevance in indicating the likely success in the field of improved vaccines against *T. parva* based on a combination approach targeting both sporozoite and schizont stages.

East Coast fever

East Coast fever (ECF) is a lympho-proliferative disease of cattle caused by *T. parva* and is endemic in 11 countries in Eastern, Central and Southern Africa. ECF has recently become recognized as an important disease in Southern Sudan that is rapidly increasing it's geographic range. The disease is coincident with the distribution of the tick vector *Rhipicephalus appendiculatus*.



Combating East Coast fever

Acaricides are currently the main control method, but this is not a sustainable approach. A live vaccine has recently been used in pastoral systems in Tanzania, but this vaccine is difficult to produce and deliver.

Current research is thus focused on development of improved vaccines based on recombinant schizont antigens of *T. parva* that induce T cell responses in the bovine host.

There is considerable genetic diversity among field populations of *T. parva*. The bovine host's T cell immune response may be influenced by the parasite diversity and the host MHC haplotype.

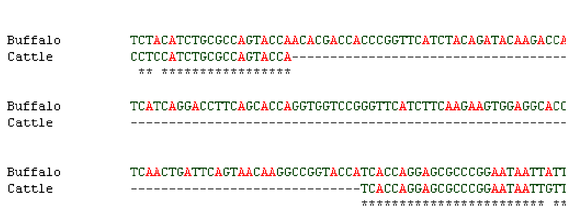
Objectives

- To investigate the role of buffalo derived genotypes of *T. parva* in the epidemiology of ECF using VNTR analysis and antigen gene sequencing.
- To obtain data on the genotypic diversity of *T. parva* isolates that are in circulation in Southern Sudan as a baseline prior to deployment of the live vaccine.
- To determine the extent of antigenic diversity between different stocks of *T. parva*.

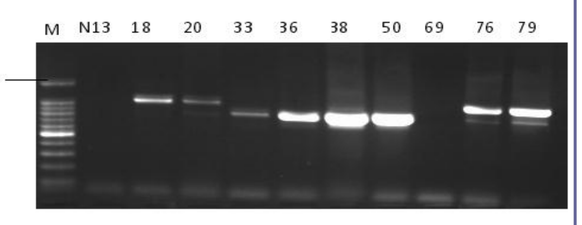
Genotyping strategies

1. Examining cattle -buffalo genetic exchange of *T. parva*

- Molecular characterization of isolates from cattle grazing in close proximity with buffalo.
- Conserved p67 gene sequence from cattle-derived *T. parva* isolates can be differentiated from buffalo-derived isolates based on amplification and sequence analysis of the p67 gene.



129 bp deletion in the central region of the p67 gene from cattle derived isolates.



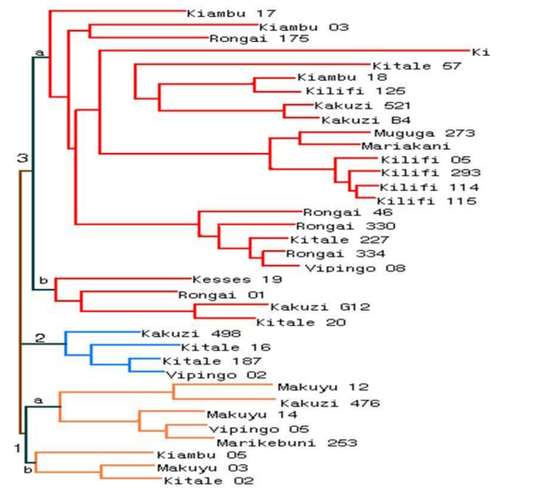
PCR amplification of the central region of the p67 gene. Cattle-derived isolates have smaller sized amplicons.

2. Molecular characterization to determine *T. parva* population genetic structure

- PCR amplification at multiple VNTR loci followed by fragment sizing discriminates parasite stocks at high resolution.

Genetic relationships of Kenyan *T. parva* stocks

- Diversity does not correlate to geographical origin of isolates
- Cattle movements may be a likely factor in population structure



3. Determining antigenic diversity between *T. parva* stocks by sequencing of candidate antigen genes

- Polymorphic, immunodominant molecule (PIM): is the predominant antigen recognized by sera from infected cattle.
- p67 sporozoite antigen: antibodies to p67, can neutralize sporozoites derived from heterologous parasite stocks, suggesting a conservation antigenic determinants.
- TP 2: contains MHC class I motifs and has been demonstrated as a potential candidate for inclusion within a sub-unit vaccine.