DFG German-African Cooperation Projects in Infectiology

Vaccine and Diagnostics improvement: Genotyping and Application of genomics to *Theileria parva* pathogens in Uganda

Ann Nanteza[1], Richard Bishop[2] and George William Lubega[1]
[1]Faculty of Veterinary Medicine, Makerere University, Uganda; [2]ILRI, P.O. Box 30709, Nairobi, Kenya
nantezaa@vetmed.mak.ac.ug

Introduction:

The major tick-borne diseases of livestock in sub-Saharan Africa are East Coast fever (ECF), anaplasmosis, babesiosis and cowdriosis. The principal method employed to control tick-borne diseases is intensive acaricide treatment which is no longer sustainable due to increasing resistance to acaricides and concerns relating to environmental contamination and food safety. A sustainable approach would be a recombinant vaccine against *T. parva* (Morzaria *et al.,* Ann N Y Acad Sci 916:464 (2000)) to circumvent the logistical difficulties in delivering the existing live 'Infection and Treatment" (IT) vaccine (Radley, *et al*., Vet Parasitol 1:35 (1975)). As efforts towards a recombinant vaccine continue, the IT vaccine remains the foreseeable interim measure. The pre-requisite for effective deployment of any anti-*Theileria parva* vaccine is an understanding of the level of polymorphism in *T. parva* populations and development of proper tools for diagnosis and surveillance. The reverse line blot (RLB) is a promising practical tool since a single PCR reaction is sufficient to discriminate between *Theileria species* (*T. parva*, *T. velifera*, *T. mutans* and *T. taurotragi*) and other tick-borne diseases. The p67 gene is a conserved and antigenic molecule which makes it suitable for inclusion in a subunit vaccine and as a diagnostic tool. This study aims at assessing the *Theileria parva* strain diversity in Uganda in order to generate information for selection of appropriate candidate strains to include in a cocktail IT or subunit vaccine. With the recent information that the genome sequencing of a *T. parva* isolate from Northwest Uganda is complete, comparative genomics of various *T. parva* isolates will be done to show how the various isolates from this study resemble or differ from this reference strain and other isolates documented within the region.

Objective:

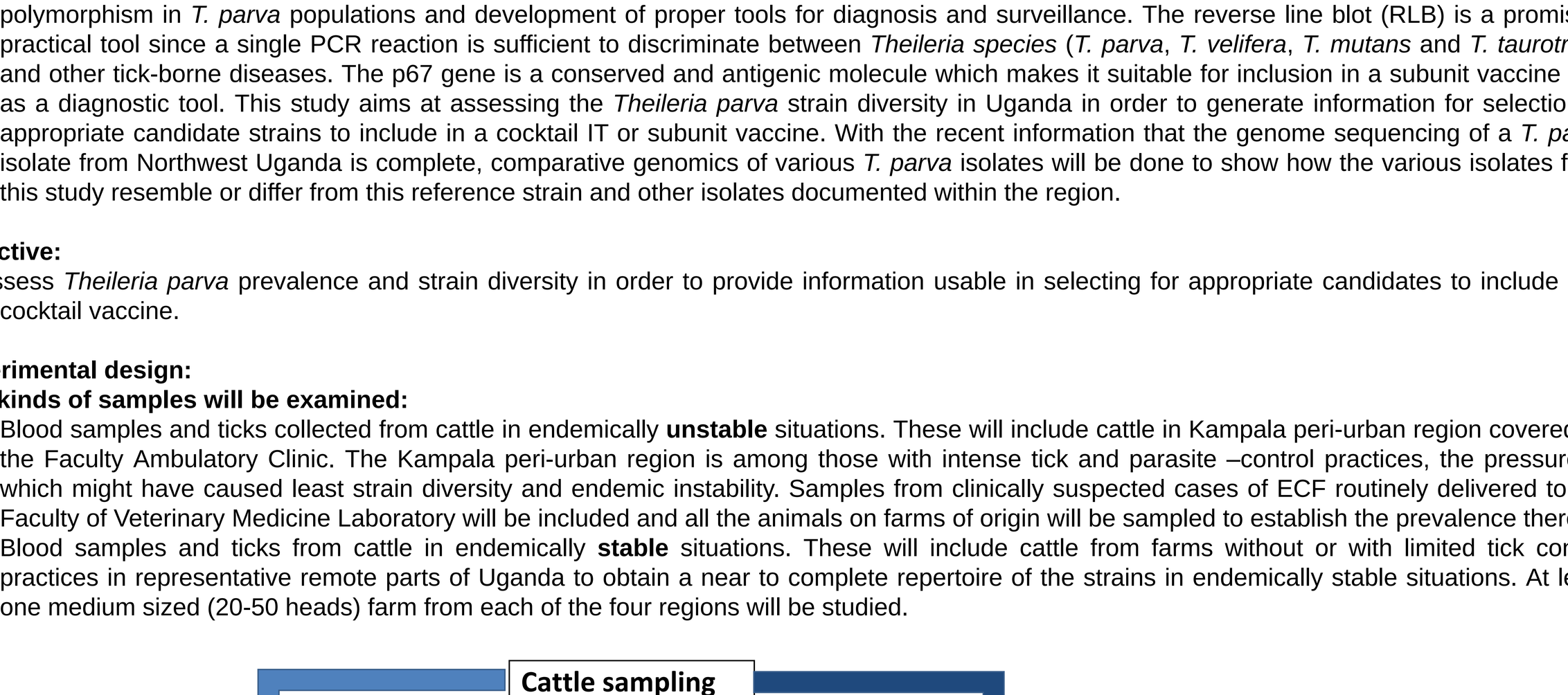
To assess *Theileria parva* prevalence and strain diversity in order to provide information usable in selecting for appropriate candidates to include in a cocktail vaccine.

Experimental design:

Two kinds of samples will be examined:

(i) Blood samples and ticks collected from cattle in endemically **unstable** situations. These will include cattle in Kampala peri-urban region covered by the Faculty Ambulatory Clinic. The Kampala peri-urban region is among those with intense tick and parasite –control practices, the pressure of which might have caused least strain diversity and endemic instability. Samples from clinically suspected cases of ECF routinely delivered to the Faculty of Veterinary Medicine Laboratory will be included and all the animals on farms of origin will be sampled to establish the prevalence thereof.

(ii) Blood samples and ticks from cattle in endemically **stable** situations. These will include cattle from farms without or with limited tick control practices in representative remote parts of Uganda to obtain a near to complete repertoire of the strains in endemically stable situations. At least one medium sized (20-50 heads) farm from each of the four regions will be studied.



Progress:

Some 22 cattle being suspected cases of ECF from four different farms within the ambulatory clinic operation area have been sampled. Out of these, 15/22 samples have been found to be positive microscopically (thin blood smears) for *T. parva* piroplasms. Some blood was spotted on FTA cards and the rest used for preparation of sera or extraction of DNA which have been stored appropriately.

Way forward and expected results :

(i) About 50 samples will be collected from the Kampala peri-urban and from each of the four regions of Uganda amounting to 250 total cattle samples.
(ii) Microscopy and ELISA data will indicate the disease prevalence on individual farms and study areas.
(iii) A number of tick-borne pathogens (protozoan or ehrlichial) are expected to be speciated by RLB. A spectrum differing or similar to that observed by Oura *et al*., 2003 in our laboratory is anticipated.
(iv) PCR amplifications and confirmation by sequencing will generate a repertoire of locus specific (p67, ms 7) genotypes from Uganda to be compared using appropriate phylogenetic analysis tools. A spectrum more or less similar to that observed by Oura *et al*., 2003 in our lab. is anticipated.
(v) Comparative genomics will determine to what extent other *T. parva* genomes differ from the genome of the reference *T. parva* isolate from Northwestern Uganda.