GENETIC CHARACTERIZATION OF MULTI-DRUG RESISTANT TRYPANOSOME VARIANTS IN ENDEMIC REGIONS OF KENYA.

ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

Ms. Sarah Akinyi Opondo Wamwenje
I56/75314/2014

INTRODUCTION

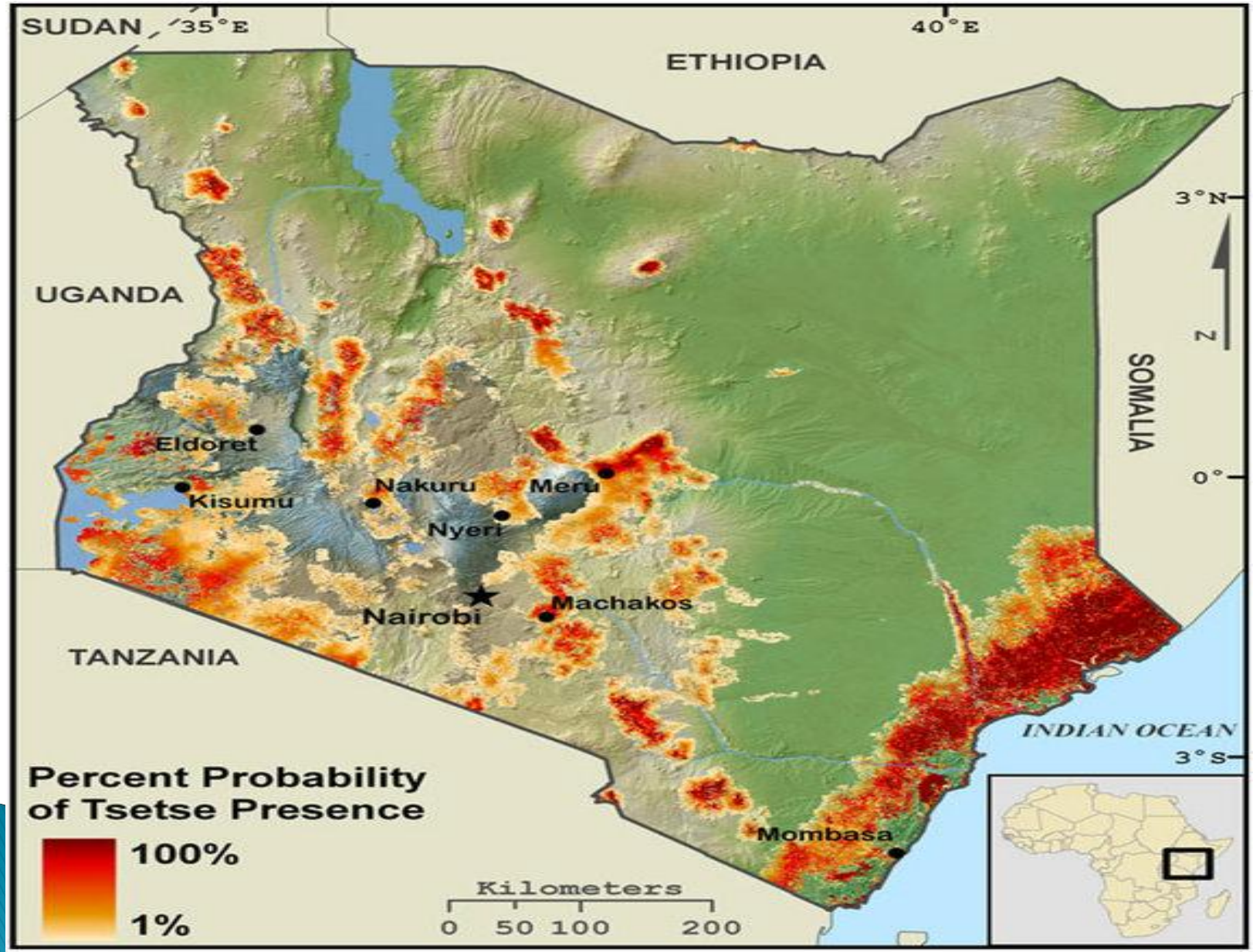
•Trypanosomes cause nagana in bovid animals living in sub-Saharan Africa.

•They are transmitted by tsetse flies (*Glossina fuscipes, G. Palpalis* and *G. morsitans*).

•Vaccine and new drug development initiatives are not feasible possibilities in the near future.

•Drug resistance of the current chemotherapies has become widespread .

TSETSE PRESENCE IN KENYA



INTRODUCTION

In Kenya currently used drugs are in **Diminazene**, **Isometadium** and **Homidium** groups.

Main mechanisms of drug action are:-

•Adenosine Transporters - gene of interest denoted as **AT /P2 (Adenosine Transporter)**

•Aquaglyceroporin Channels – gene of interest denoted as **HAPT 1 (High Affinity Pentamidine Transporter)**

ADENOSINE TRANSPORTERS

•Purine salvage is essential for the survival of trypanosomes, and this is facilitated by uptake using these transporters.

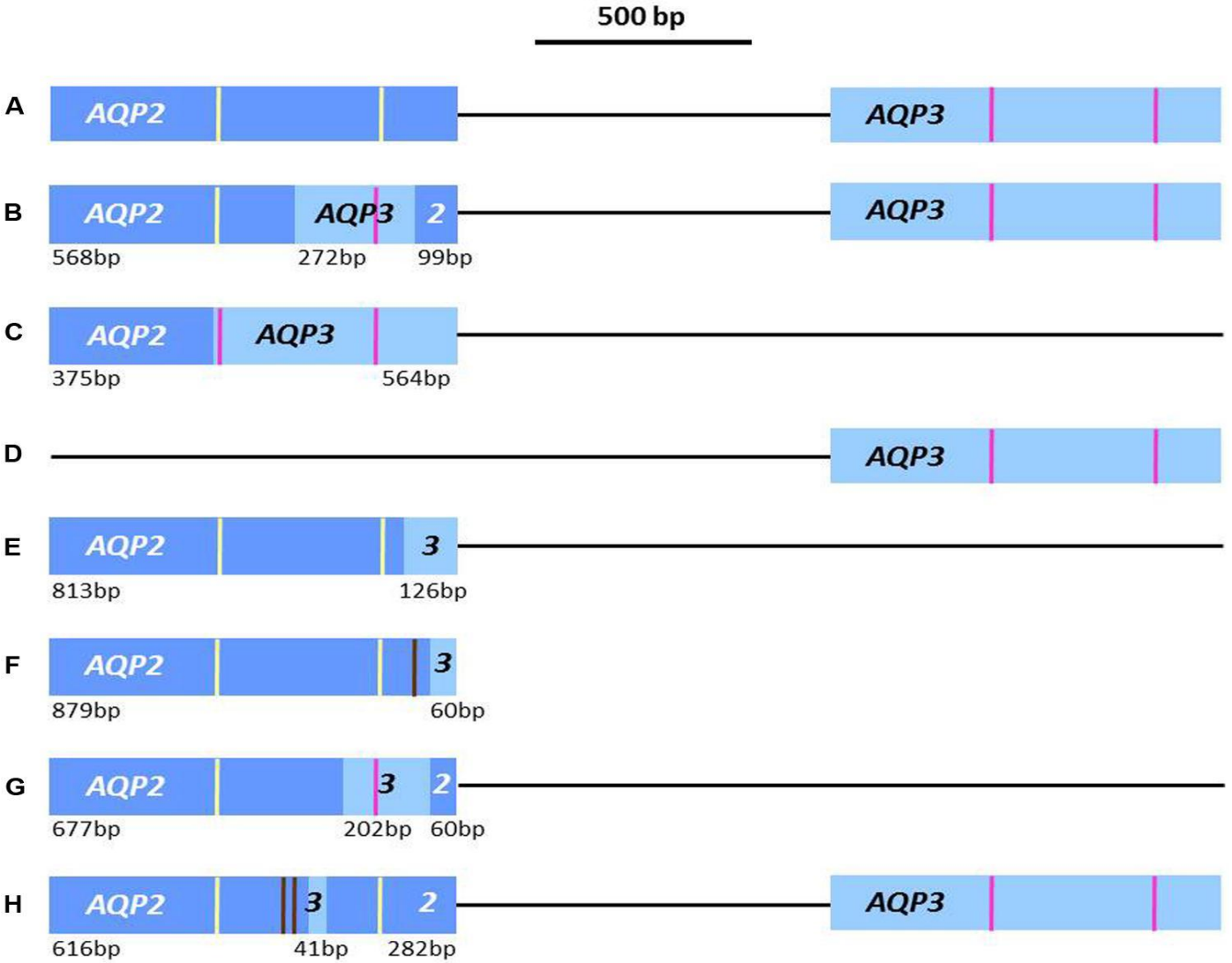
•They are sufficiently different from their mammalian homologs making them ideal drug targets.

•They have been characterized as they are key in the uptake of most trypanocides.

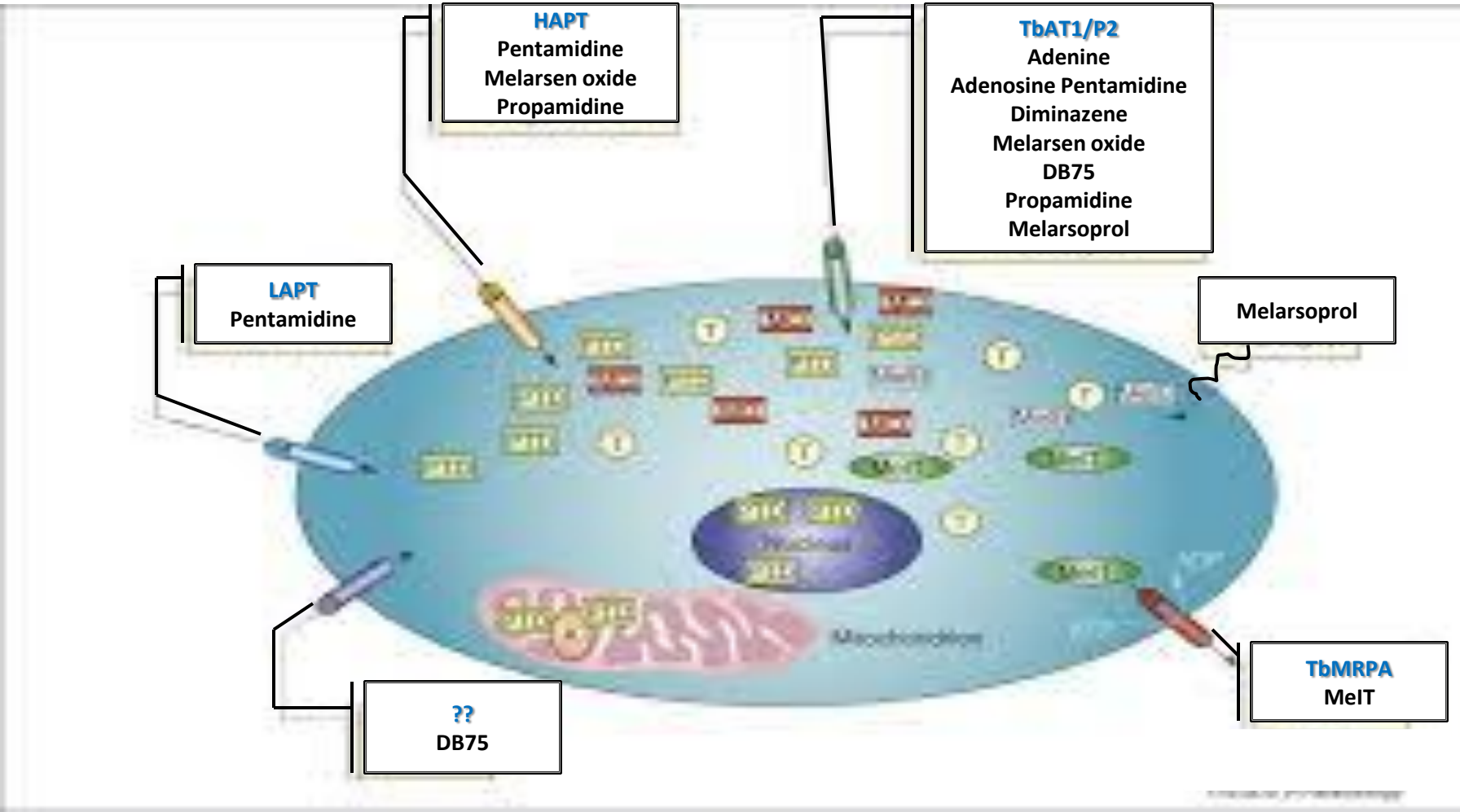
•Secondary uptake systems do exist for all these drugs, unfortunately.

•The loss of this transporter may be a necessary but not sufficient condition for resistance to be expressed.

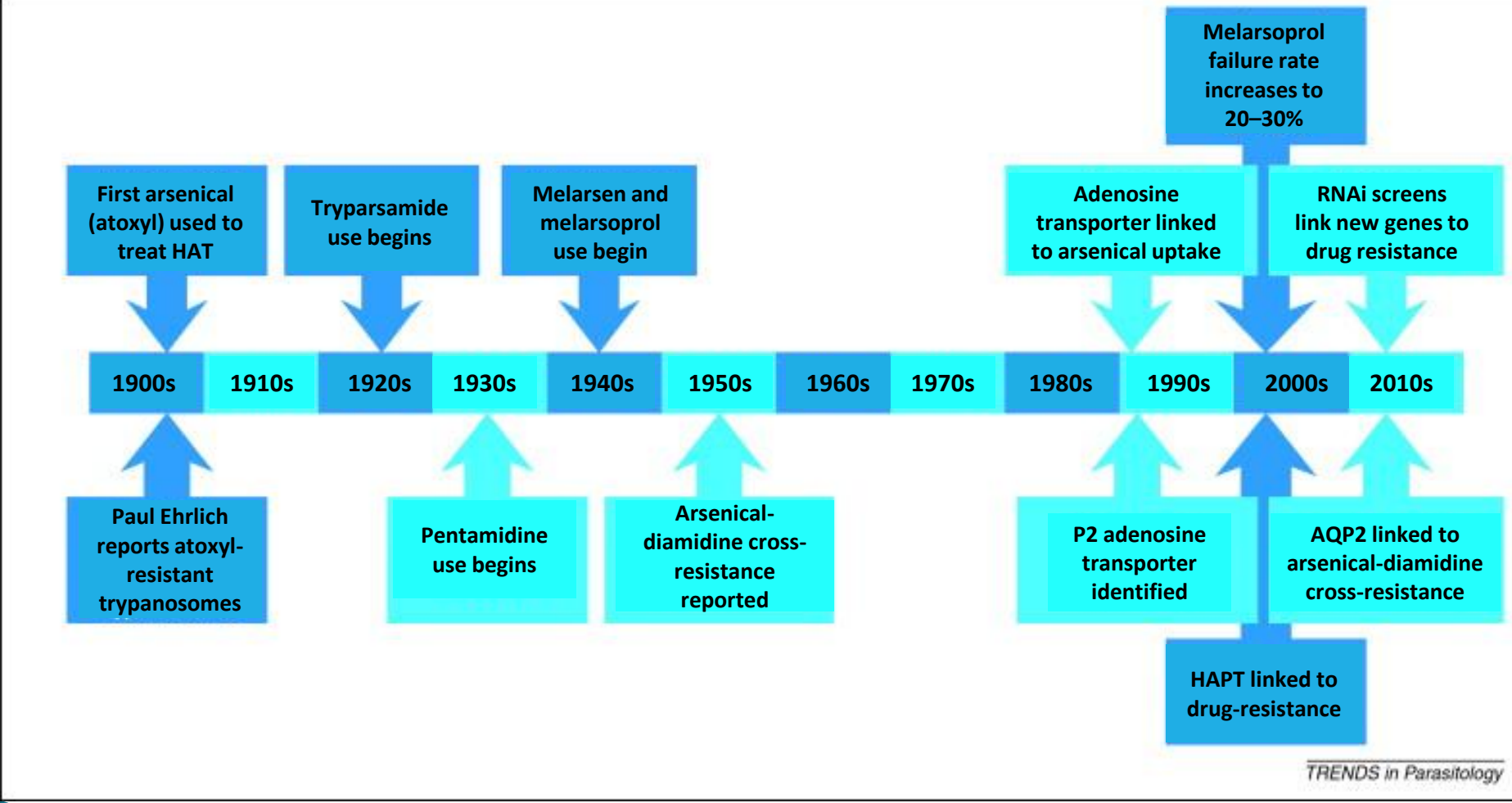
AQUAGLYCEROPORIN ALLELE



DRUGS AND DRUG TARGETS



HISTORY OF TRYPANOCIDAL RESISTANCE



MAJOR AIMS OF THE STUDY

•To characterize previously studied biomarkers that confer multi-drug resistance to trypanosomes that are endemic to Kenya (Shimba Hills) using genome sequencing approaches.

•The molecular tools developed can then be applied to the development of variant specific multiplex diagnostic tools .

•Create a platform from which policies advocating for judicious trypanocide use by farmers can be encouraged.

JUSTIFICATION

•AAT creates huge disease burden.

•Detection of new reservoirs means increase in cross-resistance patterns.

•Need to understand drug use practices and how they are contributing to development of resistance.

•Our understanding of trypanosome nucleoside transporters and channels has increased in the past decade.

•Much is yet to be done as details are needed from epidemic foci which vary greatly in resistance patterns.

•This knowledge can help in selective design of diagnostic tools and drug targets.

Research Question

Are there multi-drug resistant trypanosome variants in Kenya and could their drug targets be useful as potential drug diagnostic tools?

Hypothesis

There are multi-drug resistant trypanosome variants in Kenya and their drug targets can be useful as potential diagnostic tools.

General Objective

Determine AT/P2 and HAPT1 variants in trypanosomes obtained from cattle living in nagana endemic regions in Kenya.

Specific Objectives

•Identify AT/P2 and HAPT1 gene polymorphisms in trypanosomes living in nagana endemic regions in Kenya.

•Determine variant specific and allelic differences and prevalences of the trypanosomes obtained from cattle living in nagana endemic regions in Kenya.

METHODOLOGY

Study design and sampling

•30 Randomly selected farmers from Kwale county will be interviewed using an APP (**OpenDataKit**).

•Cattle (210) will be sampled, 7 from each farmer.

•Older samples will be obtained from the **ILRI - AZIZI** repository .

PCR Amplification

•To detect trypano-positive samples and distinguish between trypanosome species.

•To amplify target loci (HAPT1 and AT/P2).

•Sequencing outsourced to **Macrogen** (South Korea).

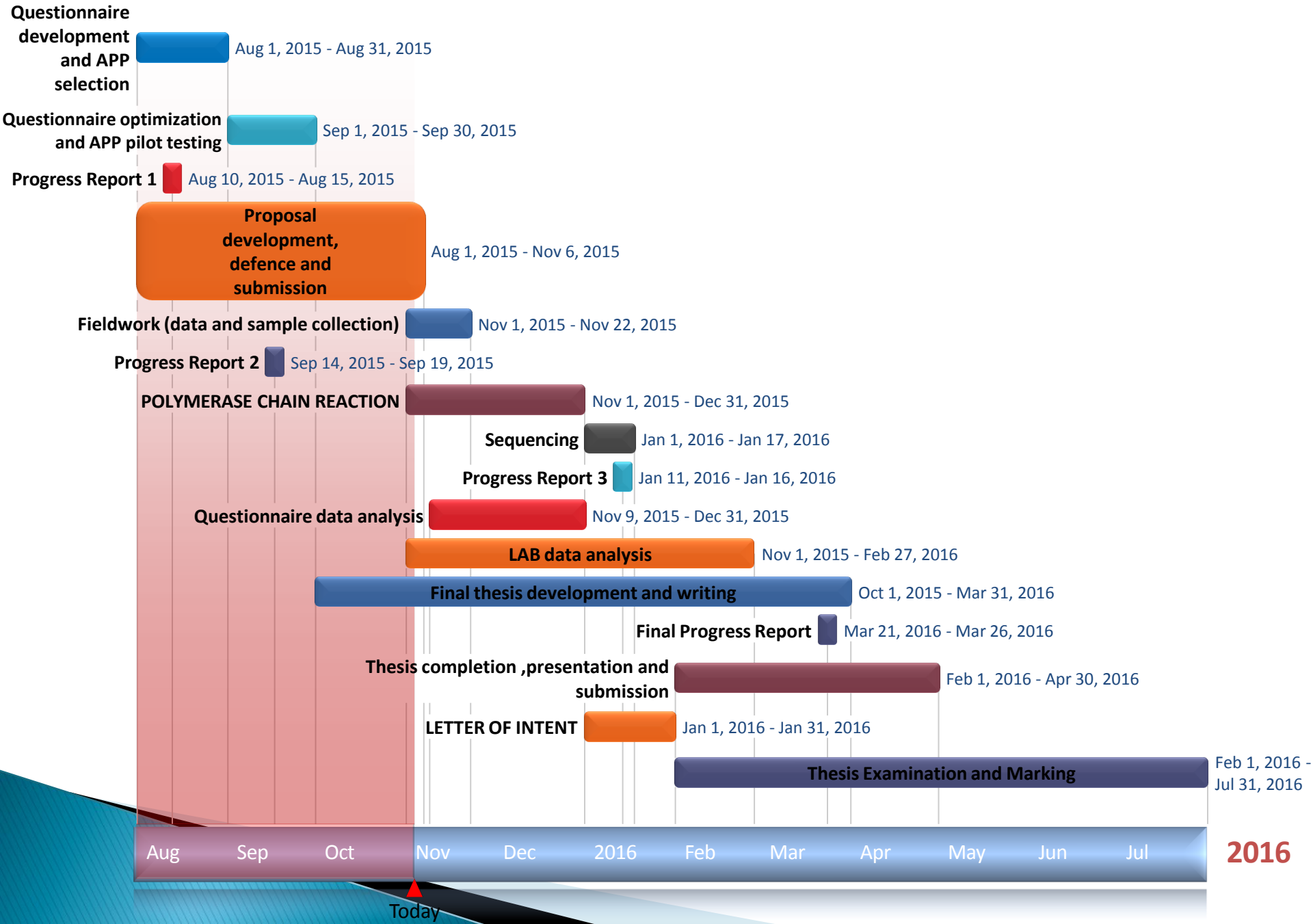
METHODOLOGY

Data analysis

- **Bio Edit** – For analysis of nucleotide polymorphisms from aligned DNA sequence data

- **Artemis Comparison Tool** - Sequence comparisons to determine species variations

- Database to be analysed using **WEKA**.


Questionnaire development and APP selection
Aug 1, 2015 - Aug 31, 2015
Questionnaire optimization and APP pilot testing
Sep 1, 2015 - Sep 30, 2015
Progress Report 1
Aug 10, 2015 - Aug 15, 2015
Proposal development, defence and submission
Aug 1, 2015 - Nov 6, 2015
Fieldwork (data and sample collection)
Nov 1, 2015 - Nov 22, 2015
Progress Report 2
Sep 14, 2015 - Sep 19, 2015
POLYMERASE CHAIN REACTION
Nov 1, 2015 - Dec 31, 2015
Sequencing
Jan 1, 2016 - Jan 17, 2016
Progress Report 3
Jan 11, 2016 - Jan 16, 2016
Questionnaire data analysis
Nov 9, 2015 - Dec 31, 2015
LAB data analysis
Nov 1, 2015 - Feb 27, 2016
Final thesis development and writing
Oct 1, 2015 - Mar 31, 2016
Final Progress Report
Mar 21, 2016 - Mar 26, 2016
Thesis completion ,presentation and submission
Feb 1, 2016 - Apr 30, 2016
LETTER OF INTENT
Jan 1, 2016 - Jan 31, 2016
Thesis Examination and Marking
Feb 1, 2016 - Jul 31, 2016
Aug
Sep
Oct
Nov
Dec
2016
Feb
Mar
Apr
May
Jun
Jul
2016
Today

BUDGET

Category	Supplier	Description	Units	Total Cost
PCR Reagents	Qiagen	500 run kit	1	103,950 kshs
Gel electrophoresis	Bioline	1-10 kb (200 lanes)	1	45,528 kshs
Sequencing	Macrogen	Outsourced	N/A	700,000 kshs
Lab Consumables	Bioline			384,522 kshs
Field consumables	Bioline			200,000 kshs
Stationery costs	UNES	Printing, Airtime, Data		50,000 kshs
Total				**1,484,000 kshs**

ACKNOWLEDGEMENTS

CEBIB Faculty
Cattle farmers from Mbegani and Kizibe areas of Kwale County
Kwale County Director and Deputy of Veterinary Services

Supervisors
Dr. Lillian Wambua
Dr. Benard Kulohoma

Colleagues
Inertia Ibrahim Wangwe
Solomon Kihara Wangóru

Thank
You
Merci
Graciasias
Arigato
Dankscheen
Biyan
Grazie
Juspaxar
Shukuria
Tashakkur
bolzïn
Mehrbani
Komapsumnida
Shukria
suksama
Yaqhanyelay
Efcharisto
Ekhmet
atu
gozaimashita
Maake
Tingki
Mersi unalchéesh
Medawagse
Wabeeja
Paldies
Hatur
aniha
Denkauja
Aguyje
Spassibo
Fakaaue
Spasibo
Nenachalhya
Maketai
hui
Sanco
Maiteka
ekoju
Tavtapuch
Baiika Yuspagaràtam
Minmonchar
Atto
Gaejtho
Sikomo
Gui
Merastawhy
Iah
nuhun
Snachalhuya
Dhanyabaad
Chaltu