MODELLING MULTI-DRUG RESISTANCE DURING CHEMOTHERAPY OF ANIMAL AFRICAN TRYPANOSOMIASIS IN KWALE, KENYA.

WANGWE IBRAHIM INERTIA, BSc., MSc.

I56/75532/2014

A THESIS SUBMITTED TO THE UNIVERSITY OF NAIROBI

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN BIOINFORMATICS

OCTOBER 2018

Declaration

I declare that this research thesis is entirely my work and has not been submitted for a degree in any other University

Wangwe Ibrahim Inertia,

I56/75532/2014

Signature:_____ Date:_____

Approval

This research thesis has been submitted with our approval as supervisors:

Dr. Benard Kulohoma,

Center for Biotechnology and Bioinformatics (CEBIB), University of Nairobi

Signature: _____ Date: _____

Dr. Lillian Wambua,

School of Biological Sciences, University of Nairobi

Signature: _____ Date: _____

Dedication

To my parents, Mr. Peter Wangwe Wamukoya and Mrs. Anne Masitsa Wangwe for their encouragements as well as Dr. George Obiero, Dr. Benard Kulohoma and Dr. Lillian Wambua for anchoring my career in Bioinformatics.

Acknowledgments

First and foremost, I want to thank the Almighty God for His mercy and grace that has enabled me to successfully finish this work. My sincere appreciation goes to Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for their support to my graduate studies by RUFORUM grant award RU 2014 GRG-086 to Dr. Benard Kulohoma. I would like to thank CEBIB, the University of Nairobi for preparing me for my graduate career. In particular, I would like to thank my supervisor Dr. Kulohoma for supporting me and believing in me and in my prior mathematical modeling knowledge. His willingness to meet on Monday, Wednesday and Friday mornings to help me decipher my preliminary outputs of the models will never be forgotten. His mentorship without any promise of reward is a selfless act, which proved to be invaluable during my final semesters. I would also like to thank my supervisor Dr. Wambua for her candor, wisdom, endless support and guiding me through the laboratory work. Many thanks to Sarah Akinyi for her review comments.

I wish to thank Dr. Isabella Oyier at CEBIB for her willingness to advise me during progress report presentations. I am sincerely grateful to Dr. Obiero and Dr. Kulohoma for reigniting my love for biological sciences and medicine through their effortless and commonsense approach. I wish to thank Dr. Mark Kimathi for his relentless support, his encouragement and peer review. I wish to thank Abraham Parwos for his gracious administrative assistance in all things. I wish to thank my family for their endless encouragement and prayers. Mum, dad, brothers and sisters: I could not do anything without your support and expression of confidence in my abilities.

Many thanks to *icipe* for providing resources for building my capacity as an early career scientist. In particular, I acknowledge the Molecular Biology and Bioinformatics Unit (MBBU) for hosting me. The suggestions and comments during journal club really helped to make this work a success.

Finally, I would like to thank Dr. Asborn Misiko, my best friend and one true love. Thank you for listening, loving and encouraging. Our relationship is a foundation upon which dreams can be realized.

Abstract

Multi-drug resistance (MDR) has complicated treatment and control of numerous infectious diseases. The extent and rate of increase of MDR varies widely with different drug-pathogen combinations. Each year, animal African trypanosomiasis (AAT), contributes to high cattle mortality rates throughout sub-Saharan Africa (SSA). Treatment of AAT in SSA is complicated by increasing MDR in trypanosomes. Over reliance on trypanocides and poor treatment practices has ultimately led to the serious negative effects of non-susceptibility to multiple trypanocides. Mathematical models can effectively predict the most judicious trypanocide use, and thereby help to restrict widespread MDR. This study aimed to predict the most effective use of trypanocides in Kwale County, Kenya. This was performed among farming communities of Shimba Hills, an area with high AAT incidence and in close proximity to the Shimba Hills National Reserve that has high vector densities. This involved designing, validating and implementing a compartmental susceptible, exposed, infectious and recovered (SEIR) epidemiological model for simulating trypanosomes transmission in tsetse flies and cattle. A smart mobile phone Health App was developed to collect field data on trypanocide non-susceptibility. Data was collated on a central database and analyzed to determine the mathematical model parameters. This information was coupled with that from laboratory analyses of trypanocide induced gene mutations, and used to develop mathematical models that predict increasing MDR. It demonstrates how trypanocide treatment in cattle influences the tsetse infectivity and transmissibility of drug non-susceptible trypanosome strains. The results show that treatment of all cattle with a combination of two trypanocides was the most optimal treatment strategy to restrict development of MDR to AAT. Furthermore, starting the treatment with the least effective trypanocide and ending with the most effective trypanocide, was an ideal trypanocide prescribing practice. The optimal threshold for mass treatment of infected cattle populations was 80%. This study highlights best practices for communal use of multiple drugs to restrict widespread trypanocide non-susceptibility.

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List of abbreviation

AAT	Animal African Trypanosomiasis
AUC	Area under the curve
CDC	Centres for Disease Control and Prevention
et al.	and colleagues
ha	hectares
HAT	Human African Trypanosomiasis
kb	Kilo bases
Km^2	Kilometers squared
MDR	Multi-Drug Resistance
NTD	Neglected Tropical Disease
nm	nanometer
R_{h1}	Non-susceptibility to drug 1
R_{h2}	Non-susceptibility to drug 2
R_{h3}	Non-susceptibility to drug 3
R_{h12}	Non-susceptibility to drugs 1 and 2
R_{h13}	Non-susceptibility to drugs 1 and 3
R_{h23}	Non-susceptibility to drugs 2 and 3
SEIR	Susceptible, Exposed, Infected, Recovered
SIR	Susceptible, Infected, Recovered
Spp.	Species
T. brucei	*Trypanosoma brucei*
T. congolense	Trypanosoma congolense
T. gambiense	Trypanosoma *gambiense*
T. rhodesiense	Trypanosoma *rhodesiense*
TMDR	Trypanosome Multi-Drug Resistance

CHAPTER 1

Introduction

1.1 Trypanosomes

Trypanosomes are protozoan parasites that cause life-threatening human African trypanosomiasis (sleeping sickness) in humans (1–5) and animal African trypanosomiasis (AAT) (or nagana) in livestock (6,7). AAT is a tropical disease caused by multiple diagnostically distinguishable trypanosome species that include: *Trypanosoma congolense*, *Trypanosoma brucei brucei* and *Trypanosoma vivax* (8,9). Tsetse flies (*Glossina* species) are the vectors of trypanosomes, and can be categorised into three major groups (8):

- *Morsitans* group which includes: *Glossina longipalpis, Glossina pallidipes*, *Glossina morsitans, Glossina swynnertoni* and *Glossina austeni*, largely found in the savanna and woodland habitats.
- *Palpalis* group which includes: *Glossina palpalis palpalis*, *Glossina fuscipes fuscipes* and *Glossina palpalis gambiensis,* all found in riverine habitats.
- *Fusca* group which includes: *Glossina fusca fusca*, *Glossina schweitz, Glossina brevipalpis* and *Glossina longipennis* that mostly inhabit the rain forests of West Africa.

T. vivax, T. congolense and T. b. brucei are transmitted majorly by the *morsitans* group kind of vectors. *G. fuscipes* subspecies are most important vectors in the *palpalis* group, whereas all members of the *fusca* group apart from *Glossina brevipalpis*, have no medical or veterinary importance (8). *T. evansi* could be transmitted through infected blood (10) whereas tsetse fly bites transmit *T. brucei rhodesiense, T. congolense*, *T. brucei gambiense* and *T. brucei brucei* (10). Genetically is similar to *T. brucei rhodesiense* and *T. brucei gambiense* (11). Cyclical transformation does not occur for

T. evansi within the tsetse flies. In the course of its life cycle *T. evansi* remains monomorphic unlike *T. brucei* sub-species, which are pleomorphic (11).

1.1.1 Geographic distribution of trypanosomiasis in Kenya

Smallholder cattle farmers in Shimba Hills (coastal region), Teso, Busia, Suba, Lambwe Valley (western region), Mtito Andei, Makueni and Maasai Mara (south Rift region), are some of the areas affected by AAT in Kenya (12–14) (Figure 1). Farmers in these areas receive trypanocide prescriptions from extension workers and veterinary officers. These areas have the highest AAT incidence rate in Kenya (15,16). Cases of multi-drug non-susceptible trypanosomes have previously been reported in Kwale (17,18), where this study was performed. Most people in Kwale, living near the neighboring Shimba Hills National Reserve, a tsetse fly reservoir, practice small-scale livestock farming. Wildlife therefore provides a constant reservoir of trypanosomiasis infection transmitted to livestock.

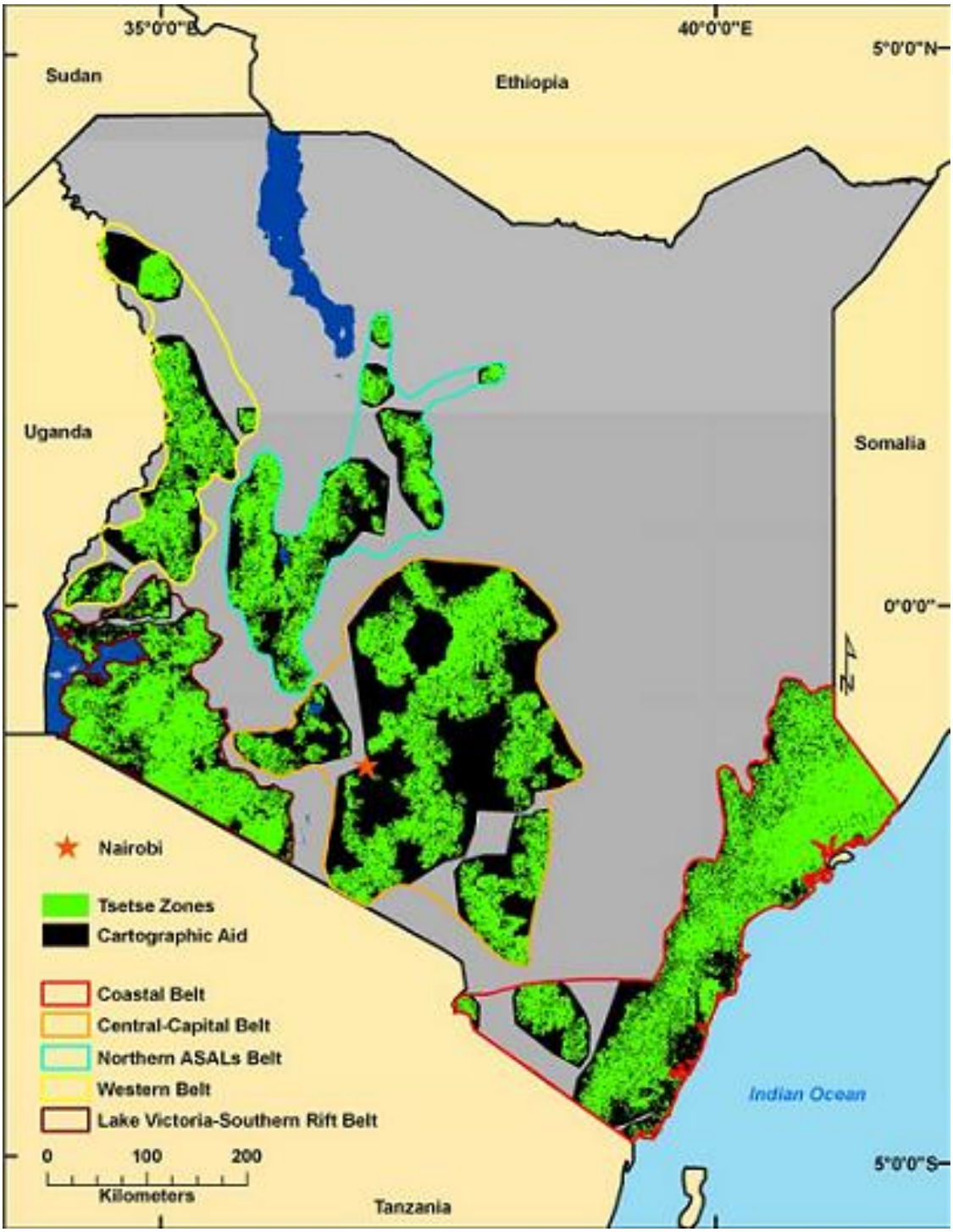


Figure 1: A of Kenya showing the prevalence of tsetse flies. Adapted from (19)

1.1.2 Tsetse fly infection by trypanosomes

Trypanosomes undergo cyclic development in the tsetse fly before infecting vertebrate hosts. Unfed, teneral and starved (young) tsetse flies are more susceptible to trypanosome infections than older flies (20).Trypanosomes develop from procyclic blood stream forms acquired from the mammalian host to infective metacyclic trypanosomes (21). *T. vivax* has the shortest development cycle lasting 5 to10 days, and the whole development occurs in the proboscis. The procyclic forms multiply in the tsetse midgut before entering the proboscis in *T. congolense*, taking approximately 15 days. *T. brucei* undergo a more complicated development cycle involving stages in the salivary glands with a mean duration of 20 days (8). Some trypanosomes do not develop to the infectious stages, an example is *Trypanosoma brucei* where only 3% of them mature and are capable of causing infectious disease (22,23). This is referred to as refractoriness, and results in relatively low infection rates in tsetse flies (vector) compared to cattle (host) (11).

1.2 Trypanosomiasis challenges and control

1.2.1 AAT challenges

AAT reduces animal work rate, meat and milk yield, and increases expenses incurred on trypanocidal drugs. Economic losses due to AAT can best be approximated through pecuniary losses due to paucity of large-scale veterinary public health records. AAT has been estimated to result in losses of up to US $ 4.5 billion annually. It affects 37 resource poor African countries (24–27). Approximately 60 million cattle are at risk of infection (24), which can lead to anemic condition, mortality and muscle loss (28). Techniques for AAT management in cattle such as: use of trypanocides; vector control using screens and flytraps; and breeding of trypano-tolerant cattle breeds have been used (29). Trypanocides are widely used by farmers to control trypanosomiasis since they are affordable (30). Poor or lack of information about disease management among

smallholder farmers is a significant setback to disease control as a result of non-susceptibility to trypanocides.

1.2.2 Drug control

Current tools for trypanosomiasis management are limited to trypanocidal drugs. In Africa, approximately 35 million doses of trypanocidal drugs are prescribed by veterinary officers to farmers each year (34). The three classes of trypanocidal drugs administered in Africa are Isometamedium (ISM), 40%, homidium salts (such as ethidium bromide (EtBr)), 26%, and diminazene aceturate (DA), 33% (31). There are emerging reports of drug non-susceptibility, and it is difficult to develop vaccines due to parasitic antigenic variation (32). Consequently, vector control is the preferred alternative (33). The main trypanocide used in Kwale, Kenya is veriben, a diminazene group trypanocide. This is complemented by antibiotic use.

1.2.3 Biological control

Biological control involves the use of a living antagonist to lower pest (vector or parasite) population to un-harmful acceptable levels. Biological control methods have no environmental side effects. However, there are some side effects associated with chemical control methods.

1.2.3.1 Biological control of vector of protozoan parasite

Biological control of intermediate carriers in form of hosts and vectors can be used to control protozoans, such as *Plasmodium, Babesia, Theileria, Leshmania* and *Trypanosoma*. This can be achieved either by direct predatory enemies or by other mechanisms, such as infection or altering their biology such as mode of reproduction (34,35).

1.2.3.2 Trypano-tolerant breeds

Trypano-tolerant livestock breeds that require little or no chemotherapy include: N'Dama, Baoule, Muturu, Laguna, Somba and Dahomey (West African), zebu cattle breeds such as Maasai zebu (East African) (36). Other trypano-tolerant breeds include indigenous small ruminant breeds like dwarf sheep from West African and goats from both West and East African regions (36). They are more non-susceptible to trypanosome infection than imported breeds (classically temperate 'European' taurine breeds but also including Asian-derived Bos indicus breeds, relatively new to trypanosome endemic areas, such as Boran). Since an animal's ability to control parasitaemia and anaemia has a major effect on production, weight gain when infected, and decreased mortality rates (36), monitoring infection rates and haematocrits of individuals in a herd is recognized as a useful tool for the selection of trypanotolerant breeding stock such as Trail, d'Ieteren, Colardelle, Maille, Ordener, Sauveroche and Yangari (37). Crosses of non-susceptible breeds have better weight, faster growth rate, packed cell volume (PCV) checks, anemia control, and provide great motivation of the farmer due to satisfaction as a result of economic efficiency (38,39).

1.2.4 Surveillance and management of trypanosomiasis

Trypanosomes are progressively becoming non-susceptible to multiple drugs (40,41). Nevertheless, Trypanosomiasis surveillance has been inadequate. Data collection tools like paper questionnaires that require the physical presence of investigators have for long been the main methods exploited to collect health demographic surveillance information of AAT and other infectious diseases. This has led to bottlenecks in collecting relevant information, and significantly slowed down the process of formulating policies after data analysis; and providing feedback to the affected communities (42). New software applications like Open data Kit (ODK) have been developed to replace prior practices but they remain untested in most endemic areas (42).

The lack of consistent surveillance efforts of AAT coupled with the misuse of trypanocides potentially poses serious concerns. This is because the accumulation of populations of non-susceptible trypanosomes would complicate the management of the disease. There is also a great need for new effective and safe trypanocides, since long-term exposure to the existing drugs increases the risk of trypanosome acquiring non-susceptibility (43–45). The limited resources and fewer veterinary personnel in developing countries where trypanosomiasis is endemic further complicate the situation (46). Poor compliance to prescribed drug regimens (misuse of drugs and prolonged use), unsuitable drug choice, the porous African borders that allow nomadic migrations to infected areas, and unfavorable dosing have significantly increased annual treatment failure rates; and promote the emergence of non-susceptibility. Trypanocide vendors are not monitored and the illiteracy of the farmers leads to poor storage and administration of trypanocides. These limitations alongside varied farming practices and absence of new trypanocidal drugs favor the development of trypanocide non-susceptibility. Overreliance on trypanocides in the control of trypanosomiasis ultimately leads to the rise of multi-drug resistant (MDR) trypanosome populations rendering the use of trypanocides infeasible. There have been several studies on detection and prevalence of MDR trypanosomes (47,48), but little has been done to determine prevalence and control of MDR trypanosomes in Kenya. More effective approaches that provide accurate information on the level of drug non-susceptibility in the livestock population are required in resource poor settings (24).

1.3 Mathematical models

Dynamic mathematical models can be exploited in epidemiology to describe how diseases are transmitted. Methods for minimizing trypanocide non-susceptibility are becoming more important due to the increasing rate of trypanocide non-susceptibility (49).

Mathematical models have for a long time been used to develop a better understanding of systems in order to control or optimize results (17,50–68). Thus mathematical models of infectious disease are now commonplace, and have helped predict development of antibiotic non-susceptibility and the temporal spread of diseases (51,62–71). Models are used to understand treatment and infection rates in order to optimize ability to predict, quarantine and control disease.

1.3.1 The susceptible, infected, recovered (SIR) model

The susceptible, infectious, recovered (SIR) is a nonlinear, compartmental model that allows studies on movement of populations between the susceptible compartment, the infected compartment, and the recovered (removed) compartment, hence the name, SIR model, (Figure 3) (72,73).

Figure 2: Susceptible-Infected-Recovered Flowchart.

Differential equations that represent the status of infection within the compartments are first described. The rate of change of susceptible cattle is proportional to the product of the number of cattle in the susceptible, $S(t)$ and infected $I(t)$ compartments at a given time t. This rate is negative since the number of cattle in this compartment is decreasing.

Beta, β, represents the contact rate, or the likelihood that susceptible cattle, S would become infected through interaction with infectious cattle, I.

$$\frac{dS}{dt} = -\beta SI \qquad 1.1$$

The rate of change of the infected cattle population grows at the same rate the susceptible compartment decreases. The recovery rate, υ, is the likelihood that infected cattle would recover (the removal rate), with $\frac{1}{\upsilon}$ being the time it takes to recover (the period of infectivity). The number of new infected number of cattle is represented by βSI.

$$\frac{dI}{dt} = \beta SI - \upsilon I \qquad 1.2$$

The recovered cattle, R population is growing proportional to the product of the infected compartment and the recovery rate, υ.

$$\frac{dR}{dt} = \upsilon I \qquad 1.3$$

Equations 1.1, 1.2, and 1.3 are defined such that

$$\frac{dS}{dt} + \frac{dI}{dt} + \frac{dR}{dt} = 0 \qquad 1.4$$

And

$$S + I + R = N \qquad 1.5$$

Where N represents the total population, assuming the total population is constant. The parameter, β, is called the infectious disease rate of transmission.

1.3.2 An SIR model with vital dynamics

Many other variables and parameters can be considered when creating an SIR model. These include: exposure, temporary immunity, drug non-susceptibility, multiple infections, and vaccination. The tradeoff remains a balance between creating a more accurate model that affords the ability to study details or a model that is easier to work with functionally omitting some of the facts (72,73). The following model accounts for vital dynamics, or changes within the cattle population size due to birth and death rates. This model is a revised form of the previously mentioned SIR model to account for birth and immigration, represented by, λ, as well as death, represented by, μ, (72,73). Equations 1.1, 1.2, and 1.3 can be modified.

$$\frac{dS}{dt} = -\beta SI - \mu S + \lambda N \qquad 1.6$$

$$\frac{dI}{dt} = \beta SI - \upsilon I - \mu I \qquad 1.7$$

$$\frac{dR}{dt} = \upsilon I - \mu R \qquad 1.8$$

Such that

$$\mu = \lambda \qquad 1.9$$

to keep a constant total population.

The susceptible population grows in accordance with the birth rate, λ, which is proportional to the total population, N. The susceptible population decreases as a result of infection, $-\beta SI$, or death, $-\mu S$.

The infected number of cattle grows in relation (what is called the infection rate as the constant of proportionality) to the product of the number infected, the number susceptible, βIS. The infected compartment decreases due to recovery, $-\upsilon I$, and death, $-\mu I$.

The recovered population grows in accordance with the recovery rate, which is proportional to the size of the infected population, υI , and decreases due to the death rate which is proportional to the recovered population, $-\mu R$. This is in line with the fact that natural death rate should be proportional to total population and the recovered population is part of the total population.

1.3.3 Ross-MacDonald model

Ronald Ross was one of the first to conduct breakthrough research on malaria due to his discovery that malaria is transmitted to human hosts via bites from mosquitoes in 1902 (74). The model was first published initially by Ross in 1908 and further explorations and refinements were mentioned subsequently through 1916. The model did not experience significant updates until 1952 when Dr. George MacDonald discovered some interesting results as he studied the endemic equilibria of the model. MacDonald also made advances in his studies of the reproductive number, a ratio that determines whether or not the epidemic will sustain or fail (74). Ross and MacDonald's work continue to be the foundation for epidemic models today.

This study develops models for infection with multiple strains to model the spread of MDR in trypanosomes which heavily borrows from Ross and MacDonald’s work and that from other scholars (51,70,71).

The three factors that influence the epidemiology of trypanosomiasis considered are: the tsetse flies’ (vectors) geographic distribution, the trypanosomes’ (parasite) virulence and the cattle’ (host) response. Therefore, each of the factors between the host and the vector was considered as separate entities in the sub-model development.

1.3.4 The vector compartments

The vector model comprised of two sub-models:

(i) Tsetse fly population dynamics sub-model, which estimates densities of adult tsetse flies at each time step.

(ii) Infection dynamics sub-model which simulates the rate of infection, and development of infection in the vector population through susceptible and infectious categories.

Vector population dynamics is driven by environmental and climatic data including presence of sandy soil (for breeding), rainfall, humidity, temperature and vegetation cover. Changes in these climatic variables affect tsetse fly breeding and survival. There is an optimal temperature range of 24^0C to 26^0C for survival, development and breeding of tsetse flies. Conditions related to climate affect tsetse population through some or all of birth, mortality, immigration and emigration rates (75,76).

1.3.5 The parasite

Animals with parasitaemia frequently survive for longer periods. This accelerates disease transmission by tsetse flies. The antigenic coat change by the trypanosomes helps them to overcome the immune response of the host. This is an important factor of AAT, hence parasitaemic condition continues even after treatment (77,78).

The trypanosome glycoprotein coat that is targeted by the host's immune responses repeatedly switches to evade parasite immune elimination (79). It is known that trypanosomes have multiple antigenic types each expressed from a different genetic repertoire (79,80). Thus, successfully treated domestic animals are likely to have recrudescent infection.

1.3.6 The host

The host compartments were composed of the cattle population density and infection dynamics sub-model whereby they can be treated and become free of trypanosomes or

carry non-susceptible trypanosome strains. The population density was determined by the birth, migration from the area, and natural and trypanosomiasis related death rates. The infection dynamics in cattle was determined by factors such as; immunity status, level of exposure to infected tsetse flies, duration of exposure, location and disease management interventions. These determine the number of animals and transmission rates in susceptible, exposed, infected, and recovered compartments.

1.4 African animal trypanosomiasis and trypanocide non-susceptibility

1.4.1 Definition and etiology

Trypanocide non-susceptibility is the reduction or absence of trypanosome strains sensitive to standard quality trypanocides as prescribed by the pharmaceutical companies and administered according to the best veterinary practice (81). Currently, it is possible to understand underlying mechanisms of drug resistance by inducing trypanocide non-susceptibility *in vitro* (82). Detection of drug resistant trypanosomes from wildlife that were never in contact with the drug may be indicative of circulation of non-susceptible trypanosomes in the area, or that there was existence of trypanocide non-susceptibility without any drug medication (82,83). These factors are useful in determining the origin of trypanocide non-susceptibility. Multidrug resistance (MDR) is non-susceptibility to more than one trypanocide. Cross resistance is non-susceptibility to a specific trypanocide that often results in non-susceptibility to another trypanocide, to which the trypanosome may not have been exposed, usually of a similar chemical class. Most trypanocides target drug transportation or detoxification pathways in trypanosomes, and a single targeted mechanism could result in multi-drug resistance. An example is quinapyramine that causes non-susceptibility to diminazene aceturate (DA), and isometamidium chloride (ISM) (81,84). Although quinapyramine was banned in the 1970s, it was re-introduced in Africa due its acceptability in Asia. Quinapyramine should only be allowed for the treatment of *T. evansi* in camels and horses (81).

1.4.2 Mechanisms of trypanocide non-susceptibility

1.4.2.1 Isometamidium chloride (ISM)

Isometamidium chloride (ISM) is commercially available as Veridium® and Samorin®, and has been in use for nearly 7 decades. ISM's specific mode of action in trypanosomes remains unclear, but it interacts closely with the kinetoplastid (84). Diffusion translocates ISM in to the trypanosome with no expenditure of metabolic energy, and then it is actively transported in to the kinetoplast (84). Fewer intakes and more efflux of the trypanocide in the mitochondrion have been associated with ISM non-susceptibility, and include:

(i) Mitochondrial membrane reduced transport (electrical potential of the mitochondrion is low).
(ii) Inner mitochondrion membrane with a transporter modification.
(iii) More cytoplasmic compartment efflux.
(iv) Multiple of these strategies (84,85).

An insertion mutation of a conserved *GAA* codon in a transporter gene has been associated with the ISM non-susceptible clone (86). These ABC transporters require ATP energy to transport substrate across biomembranes, irrespective of the concentration gradient (87). Some trypanosome strains classified as non-susceptible in mouse models of non-susceptibility (88) did not show the *GAA* insertion codon (86). Altered trypanocide target sites are also thought to result in ISM non-susceptibility at the kinetoplast DNA-topoisomerase complexes (81,89). Reduction in size and depletion of the kinetoplast DNA network is induced by the silencing mitochondrial topoisomerase (90). Modification of topoisomerase II in *T. congolense* has been associated with ISM non-susceptibility (85). Hence there could be more than one non-susceptibility strategy involved.

1.4.2.2. Homidium salts (ethidium and novidium)

Homidium also translocates in to trypanosomes by diffusion (91), and are widely used in East Africa despite their harmful side effects (92,93). Mechanisms of resistance to homidoums salts still remain unclear although some findings point towards similarity to those in ISM (94).

1.4.2.3. Diminazene aceturate (DA)

Diminazene requires unique transporters for movement across biomembranes due to its highly charged nature, and modifications of transporters reduce drug sensitivity and may result in non-susceptibility (95,96). Trypanosomes depend on hosts purines that are transported through a variety of mechanisms; an example is the adenosine carriers P1 and P2. P2 is a high affinity transporter of amino purine, adenine and adenosine, while P1 is a broad-specificity purine transporter (95,96). Modification of *TbAT1* genes encoding the P2 amino purine carrier has been linked to DA non-susceptibility in *T brucei brucei* (85). The mutation *Val 306-Ile 306* in *Tb*AT1 gene of *T. brucei* correlates with the DA non-susceptibility phenotype (84,95). Other modifications associated with DA non-susceptibility exist. An example is the modification of the gene *TeDR40* that encodes a protein with ubiquitous cellular localization, which is associated with high DA non-susceptibility in *T. evansi* (97). High levels of DA non-susceptibility may be a consequence of the cumulative effect of these two none identical resistance mechanisms.

1.4.3. Strategies for combating trypanocide non-susceptibility

Efficacious use of the available trypanocides by smallholder farmers is essential to restrict widespread multi-drug resistance. Therefore, guidelines and policies recommending rational trypanocide use, that delay the development of trypanocide

non-susceptibility are of crucial importance (98,99). These strategies include the use alternative trypanocides when non-susceptibility to previously used trypanocides has developed. The use of DA/ISM trypanocide and DA-homidium sanative pairs by small holder farmers has been encouraged. These approaches may restrict the emergence and widespread development of MDR, and include (44,45):

a) Providing farmers with information and education on rational use of trypanocides.
b) Training veterinary health care practitioners and farmers on integrated methods of trypanosomiasis control and prevention.
c) Avoidance of wrong treatment by improving on disease diagnosis and trypanocide prescription.

Continuous surveillance of trypanocide non-susceptibility, particularly in endemic areas is of vital importance.

1.5 Hypothesis

Multiple trypanocide use in Kwale County, Kenya, has an impact on the transmission dynamics of drug non-susceptible trypanosomes.

1.6 Objectives

1.6.1 General objective

Non-susceptibility is the inevitable outcome of persistent use of the same drugs for long durations. Community-led data collection using a mobile app and data from previous studies were used as input data in mathematical models of MDR trypanosome strains to predict optimal treatment strategies for infectious diseases (63,100).

This study aimed to restrict widespread development of MDR due to poor treatment practices and policies during chemotherapy of AAT. This was done by establishing practices of trypanocide use among smallholder cattle farmers in Kwale County, Kenya. Surveillance data was collected using a mobile application (App) as primary input for susceptible, exposed, infected, recovered (SEIR) mathematical models that predict the most optimal treatment regimens that curb widespread multi-drug non-susceptibility.

1.6.3 Specific objectives

i. To develop a mobile application (App) to collect community-led baseline data, on the use of multiple trypanocides and awareness on non-susceptibility, from small-holder farmers. The developed app was used to collect data on the significance of AAT on the trypanosomiasis disease management of farming communities in Kwale, Kenya.
ii. To design, validate and implement a compartmental susceptible, exposed, infected and recovered (SEIR) epidemiological mathematical model for simulating ordinal and combination of trypanocide treatment regimens that reduce widespread transmission of drug non-susceptible trypanosome strains by tsetse flies to cattle.
iii. To establish, using the developed SEIR model, the communal minimum threshold for infectious population-wide treatment using multiple drugs, which reduces wide-spread trypanocide non-susceptibility.

1.7 Justification

The trypanosome is one of the most economically destructive livestock parasites unique to Africa. Trypanosomes have a devastating effect on livestock, causing AAT, a fatal disease, reducing animal work rate, and meat and milk yield (5,11). Current remedy for trypanosomiasis heavily relies on trypanocidal drugs such as: homidium salts, isometamedium chloride and diminazene aceturate, which were developed over 60

years ago. Treatment of AAT in SSA is becoming more complicated due to increasing MDR in trypanosomes (44,45).

Widespread non-susceptibility in endemic areas such as Shimba Hills can be restricted by judicious use of available trypanocides. Poor farming practices drive trypanosome non-susceptibility. Most smallholder farmers lack knowledge on best practices of trypanocide use, resulting in widespread trypanocide non-susceptibility (44,45).

Mathematical models have the potential to uncover the mechanisms that underpin development of MDR, and their deterrents (51,60,62–65,68,69,71). This study aimed to develop and test a mobile application as a tool for community-led surveillance of trypanosomiasis epidemiology, and therapy in areas with high trypanosomiasis burden and drug usage. These data were examined using mathematical modelling to highlight factors that influence the development and transmissibility of MDR trypanosome strains.

Information on the MDR trypanosomes prevalence and the existing non-susceptibility profiles of their locales will be of great benefit to farmers and veterinary health workers in the endemic regions. This can be used to inform appropriate practices and policies for veterinary drug prescription, thereby greatly improving drug prescription practices to the farmers. These well-informed farmers and veterinary health workers will use knowledge acquired to exercise more precautionary use of trypanocides.

CHAPTER 2

Materials and methods

2.1 Study site

The study site was Shimba Hills located at latitude -4.174 (4.1744° S) and longitude 39.4603 (39.4603° E), in Kwale County in the coastal region in Kenya, a trypanosomiasis endemic region (Figure 4). Kwale covers an area of more than 823,000 hactares. Kwale County has low population density, and is adjacent to a national wildlife park, the Shimba Hills National Reserve.

Two villages: Kizibe and Mbegani at different locations within Shimba Hills were identified for sample collection. Kwale County veterinary health care officials helped identify the locations with reference to information from previous prevalence studies to assess livestock numbers, trypanosomiasis risk and tsetse prevalence.

2.2 Biological data collection and processing

2.2.1 Data collection using a smart phone application (App)

Closed ended questionnaire was developed where sixteen farmers were interviewed. Hence, demographic, social and economic status data on the small holder farmers was collected to understand trypanosome and trypanocide non-susceptibility management (Appendix B).

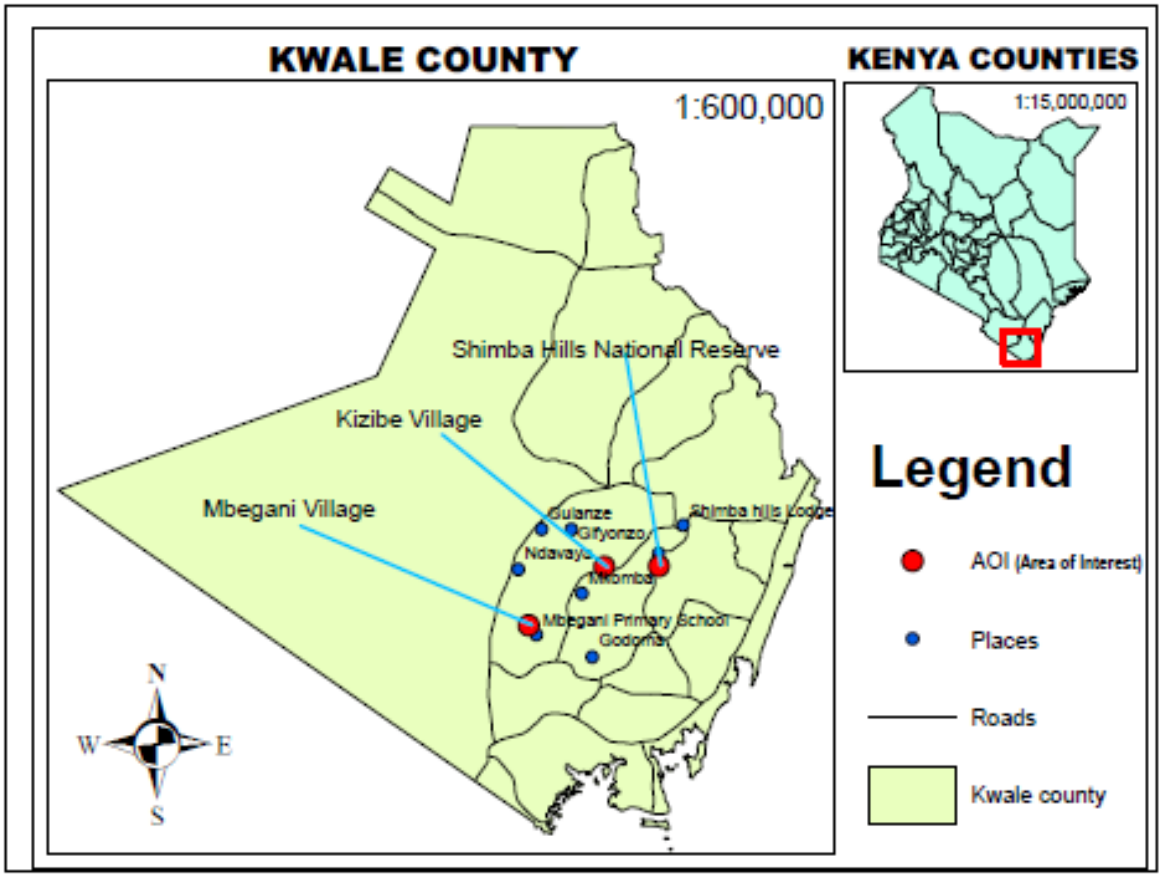


Figure 3: A map of Kwale County showing the study site locations. This research was performed using samples from Mbegani and Kizibe villages where there is a high prevalence of MDR trypanosomes (18).

The side effects of AAT on animal productivity, which include: milk off-take, animal traction losses, growth rates, cost of trypanocides and mortalities inclusive of abortions were also collected from the farmers. Swahili language was used to interview the farmers, which is well understood in the area. This study also evaluated how farmers make decisions based on their understanding of disease management. The closed ended questionnaires used in the study were designed on the mobile application, Open Data Kit (ODK). A smart mobile phone application for data collection was developed to enable monitoring livestock trypanocide non-susceptibility and understand smallholder farmer perceptions on trypanosomiasis in Shimba Hills, Kenya. Open Data Kit (ODK) technology, an open-source smart phone application (App) development software that is publicly available, was used to create a data collection App (101). It had a user-friendly

web interface for designing forms and programming simple logic. The App was developed offline by initially generating the appropriate form on an Excel spread sheet. The forms generated could be viewed by ODK collect App that was downloaded by a smartphone. The questionnaire was developed and uploaded in the mobile application before a pilot study was run to confirm that the application was functioning as expected. The application was loaded onto several android phones to ensure that any malfunction on a single device could not slow down the progress of the field work. Pre-evaluation was important to detect whether the questionnaire was simple, understood, precise, and user-friendly. Improvements after pre-evaluation included: changing multiple answers questions to forced-choice, dichotomous questions with 'yes' and 'no' options; changing to multiple choice responses; adjusting the numbering of the questions starting with the most important ones; ordering words to improve understandability; and covering wider range of possible responses by addition of multiple choice answers. Adaptive questioning or skip logic was employed to direct interviewees through different paths as a result of their choices. During data collection filled forms were initially stored on the phone's memory, prior to storage in a remotely located centralized database at the University of Nairobi's Centre for Biotechnology and Bioinformatics for further analysis. Results from the data collection App can be converted to Excel spreadsheet format for analysis (102,103). This App incorporated global positioning system (GPS) functionality allowing automatic storage of related geographic location, as well as photographs and videos (104).

2.2.2 Sample collection

Experienced smallholder farmers with multiple cattle (n>3) in the area were selected to participate in the study. The Kwale County government Chief Veterinary Officer gave consent for the study to be done in the area. Employees of the Kwale County veterinary department accompanied all the researchers during the field work and ensured that all farmers, male and female, from the two villages were invited to participate. The village elders were informed concerning the study, before the field work began and were duly notified on the objectives of the research to be carried out. They invited all the farmers

in their respective villages without any bias. The study was approved by the University of Nairobi.

The blood samples were collected from 80 cattle: 40 from Mbegani and 40 from Kizibe. This was a good representative sample given that the 50 famers that were interviewed had a combined cattle population of 230. This was around 35% of the total population. The identified cattle were safely restrained and blood was drawn from a major vein in the hind leg of the animal using a standard bovine hypodermic needle and syringe (5ml). The blood extraction was done with care to ensure that the animal was not injured by the needle. Only cattle with visible symptoms of nagana infection were selected. The blood was then placed in uniquely lablled heparin lined tubes to prevent clotting. The sample was temporarily stored in dry ice in the field. At the end of the day, the samples were divided into cryo-tubes, which were marked with the corresponding sample number and placed in liquid nitrogen for shipment to the laboratory and stored at -80^0 C.

Tsetse flies were captured from the environment by use of biconical and Ngu traps along periphery of the forests and game reserve, and near animal sheds. Male and female tsetse flies were identified.

2.2.3 Assays and biological sample processing methodology

Polymerase chain reaction (PCR) experiments were used to differentiate if the tsetse flies were infected by various trypanosome species (9).

Polymerase Chain Reaction (PCR) analysis of tsetse and blood was carried out to detect trypanosomes strains in tsetse and blood samples collected from randomly selected cattle (n = 5) from each homestead in the villages, as described by (105). PCR amplification and sequencing of genetic markers for drug non-susceptibility was then performed to determine the prevalence and genetic diversity of non-susceptible strains. The prevalence of non-susceptible strains from both cattle and the tsetse flies was then

determined. Variation of drug non-susceptibility at village level was also assessed, after certain periods of drug use. This was in addition to previously collected data on drug non-susceptibility prevalence using the mobile App. This allowed the calculation of new infection incidence and the prediction of the prevalence of drug non-susceptible parasites. The incidence level was determined by use of the blood sample specimens that had trypanosome infection divided by the total number of cattle sampled while prevalence was determined from the field data.

2.3 Mathematical models

In this case mathematical models were used to explore fitness costs associated with non-susceptibility to multiple trypanocides (drugs) and its influence on non-susceptible strain prevalence. Susceptible-exposed-infected-recovered (SEIR) models were used to predict MDR development. Mathematical models have been used to develop a better understanding of dynamical systems in order to control the dynamics or optimize results. Additionally, mathematical models are used to study treatment and infection rates of diseases in order to optimize ability to predict effective quarantine and control disease strategies.

2.4 Model development

The three factors that influence the epidemiology of trypanosomiasis were considered i.e., the tsetse fly distribution (vectors), the trypanosome virulence (parasite), and the cattle response (host). Therefore, each of the factors was considered as separate compartments in the model development.

The differences between single-drug and combination therapy was evaluated, and the differences compared in non-susceptibility magnitudes by comparing effect sizes. Numerical simulations were conducted using a simple forward Euler scheme and varied both the percentage of cattle that were treated as well as the non-susceptibility rates for a period of three years. An approximation that the cattle population size was 5,000 and

the tsetse fly population size was 40,000 was made. This was based on the population density of the area whereby the 50 farmers that were interviewed were a representative of about 0.5% of the total population in the two villages sampled. A total of 546 flies were trapped and based on the land area and proximity to Shimba Hills national reserve we approximated the tsetse density to be 40 000. It was also assumed that initially there was no treatment occurring with approximately 5% of the population being exposed and approximately 29% of the population being infected. Overall from the analysis of the collected data it was observed that 8% of the cattle were colonized by non-susceptible trypanosome strains. Further 2% of the total cattle population was colonized by trypanosome strains non-susceptible to multiple trypanocides.

Non-susceptibility rates varied from village to village. One of the “hot spots” for non-susceptibility was Mbegani village, where 11% of its cattle population was colonized by non-susceptible strains whereas 5% were colonized by strains non-susceptible to multi-trypanocide. In Kizibe village, 4% of the cattle were colonized by non-susceptible trypanosomes while 1% of the cattle population was colonized by trypanosome strains non-susceptible to multiple trypanocides. Since the interest of the study was drug non-susceptibility, it was decided to vary parameters that would affect the level of non-susceptibility experienced by the cattle population. It evaluated trypanosome drug non-susceptibility using three different transmission rates (low 4%, medium 7.5%, and high 11%) that reflect differences in disease prevalence rates at two different endemic sampling locales (Kizibe 4%, and Mbegani 11%), which are distant and proximal to the Shimba Hill National Reserve respectively. Hence forth, low drug non-susceptibility is referred to as 4%, medium non-susceptibility as 7.5%, and high non-susceptibility as 11%. Here these percentages controlled the likelihood that cattle would become non-susceptible to one drug or MDR.

Exploration of treatment and non-susceptibility was partitioned into two categories. First, it focused on how increasing the percentage of infected cattle that receive treatment affects the number of cattle in each compartment. The changes to the infectious group as a whole was investigated, which is the sum of infectious, I_h, treated, T_h, non-

susceptibility to drug 1, R_{h1}, non-susceptibility to drug 2, R_{h2}, non-susceptibility to drug 3, R_{h3}, non-susceptibility to combinations of drugs 1 and 2, R_{h12}, non-susceptibility to combinations of drugs 1 and 3, R_{h13} and non-susceptibility to combinations of drugs 2 and 3, R_{h23} compartments. Then investigation of the effects of drug non-susceptibility and MDR by comparing low, medium, and high levels of non-susceptibility was performed.

Two treatment protocols were compared:

(i) Drugs were cycled periodically (cycling or ordinal treatment).

(ii) Drugs were given simultaneously to each infected host (combination treatment).

The model accounts for low, medium and high transmission rates to evaluate fluctuating non-susceptibility. The model highlighted key non-susceptibility drivers, which can be incorporated in the early warning systems for MDR surveillance, namely:

- Timing for drug regimens.
- Changes in population densities of cattle and tsetse flies.
- The number infected by non-susceptible strains.

2.4.1 Model for drug non-susceptible trypanosomes

2.4.1.1 Drug non-susceptibility

An important component of the MDR model developed was drug non-susceptibility to three commonly used trypanocides classes (drugs), namely: isometamedium chloride, diminazene aceturate and homidium salts. This model was compartmentalized into three compartments. These three compartments were further extended into six compartments by taking into consideration susceptibility or non-susceptibility to the drug combinations. The model was developed to evaluate the effects of treatment of the

cattle with drug 1, drug 2 and drug 3. This means that drug 1, drug 2 and drug 3 correspond separately to the three different classes of trypanocides.

This study considered non-susceptibility to single trypanocides as well as non-susceptibility to a combination of trypanocides:

- Non-susceptibility to trypanocide 1
- Non-susceptibility to trypanocide 2
- Non-susceptibility to trypanocide 3
- Non-susceptibility to a combination of trypanocide 1 and trypanocide 2
- Non-susceptibility to a combination of trypanocide 1 and trypanocide 3
- Non-susceptibility to a combination of trypanocide 2 and trypanocide 3

2.4.1.2 Assumptions and deterministic model compartments

A deterministic susceptible-exposed-infected-recovered (SEIR) mathematical model that also included parameters from publicly available data (17,106) was developed (Table 1). Tsetse flies do not recover after trypanosome infection throughout their lifespan (107). Currently available trypanocides were introduced at different times (homidium - 1952, diminazene - 1955 and isometamidium - 1960) (49). However, information of when and in what order they were introduced in disease endemic locales is unavailable. The effects of three different trypanocides both individually and when used in combination were examined by making the following assumptions:
:

a) Trypanosomiasis incidence is unaffected by environmental factors such as rainfall and temperature, and the infection rate is uniform throughout the year (108).

b) Drugs are used to prevent or treat a wide range of perceived conditions; and it was assumed that treated cattle were infected by the trypanosomes.
c) Transmission was only between tsetse flies (vector) and cattle (host) only.
d) The model only considered population density independent death rates.
e) Immigration and birth rate were combined to a single value for both host and vector.
f) The time spent to treat an infection is 7 days.
g) There is a constant tsetse fly bite rate.
h) Trypanosomes are most non-susceptible to drug 1, least non-susceptible to drug 3, and that drug 2 is intermediate between them.

The sets of parameter values that take into account non-susceptibility transmission dynamics within Kwale County were considered (Table 1).

Table 1: The host and vector parameter description, measurements and initial conditions for various transmission cases.

Host (Cattle)				
Parameter	**Description**	**Transmission initial conditions**	**Measurement**	**References**
λ_h	Rate at which new recruits (birth rate and immigration) enter the susceptible cattle population	6	Cattle $\times$ Day^{-1}	Obtained from field data
ρ	Rate at which cattle lose partial immunity	0.045	Day^{-1}	Obtained from field data
μ_h	Natural death rate for cattle	0.0005	Day^{-1}	(109)

ω_h	Rate at which cattle become infectious after they have been exposed	0.1	Day $^{-1}$	Obtained from field data
δ_h	Trypanosomiasis-induced death rate for cattle	0.002	Day $^{-1}$	(109)
β_h	Rate at which cattle receive a successful bite from an infected tsetse fly	0.46	Day $^{-1}$	(30)
c	Tsetse fly bite rate	0.75		Obtained from field data
θ_h	Treatment rate for cattle	0.1, 0.2, 0.4, 0.8		Obtained from field data
η_I	Rate that cattle acquire partial immunity prior to treatment	0.01		(52)
η_T	Rate that treated cattle acquire partial immunity	0.14		Obtained from field data
η_1	Rate that cattle in the first non-susceptibility compartment acquire partial immunity	0.04		Obtained from field data
η_2	Rate that cattle in the second non-susceptibility compartment acquire partial immunity	0.03		Obtained from field data
η_3	Rate that cattle in the third non-susceptibility compartment acquire partial immunity	0.02		Obtained from field data
λ_1	Rate that cattle become non-susceptible to drug 1 after receiving treatment	0.11		Obtained from field data

λ_2	Rate that cattle become non-susceptible to drug 2 after receiving treatment	0.075		Obtained from field data
λ_3	Rate that cattle become non-susceptible to drug 3 after receiving treatment	0.04		Obtained from field data
a_1, a_2, a_3	Coefficients for trypanosomiasis-induced death rate due to drug 1, 2 and 3 non-susceptibility respectively	0.3, 0.2, 0.1		Obtained from field data
Vector (Tsetse fly)				
Parameter	**Description**	**Transmission initial conditions**	**Measurement**	**References**
λ_v	Rate at which new recruits enter the susceptible tsetse fly population	10 000	Tsetse flies $\times$ Day^{-1}	Obtained from field data
μ_v	Natural death rate for tsetse flies	0.03	Day^{-1}	(109)
ω_v	Rate that tsetse flies become infectious after they have been exposed	0.091	Day^{-1}	Obtained from field data
β_v	Rate at which a susceptible tsetse flies successfully bites infected cattle	0.025	Day^{-1}	(30)

The model was composed of two components that took into account transitions in cattle (host) and tsetse flies (vector) population, and has a time-step of one day (Figure 4).

The cattle population comprises of the following health states: susceptible (S_h), exposed (E_h), infectious (I_h), treated (T_h), withdrawn (recovered) (W_h), and non-susceptible (R_h). The host non-susceptible compartment was further sub-divided into three, for drugs 1, 2 and 3 respectively: R_{h1}, R_{h2} and R_{h3} (Figures 4 and 5). The vector (tsetse fly) population comprises of three compartments: susceptible: S_v, exposed: E_v, infectious: I_v populations (Figure 4).

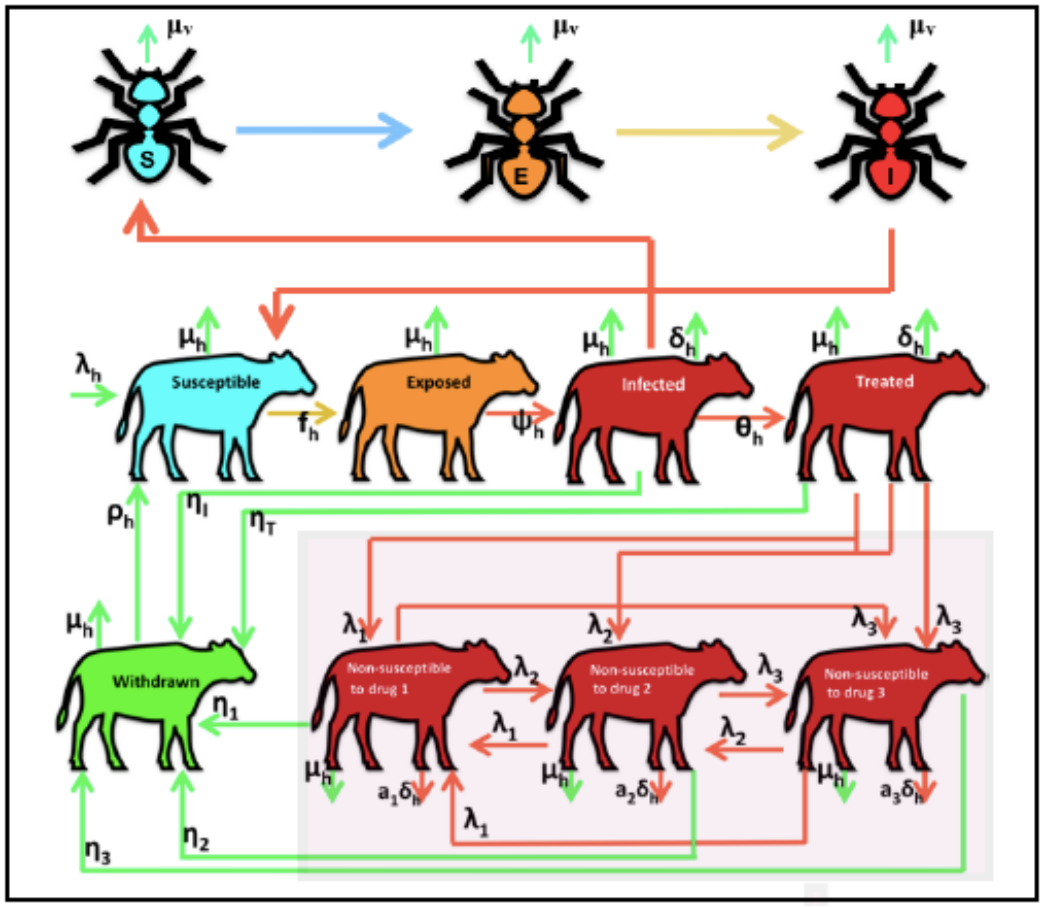


Figure 4: Compartments in the deterministic mathematical model. The vector (tsetse fly), host (cattle) components of the model. This shows the host-vector interactions flowchart, showing transmission cycle between cattle and tsetse flies.

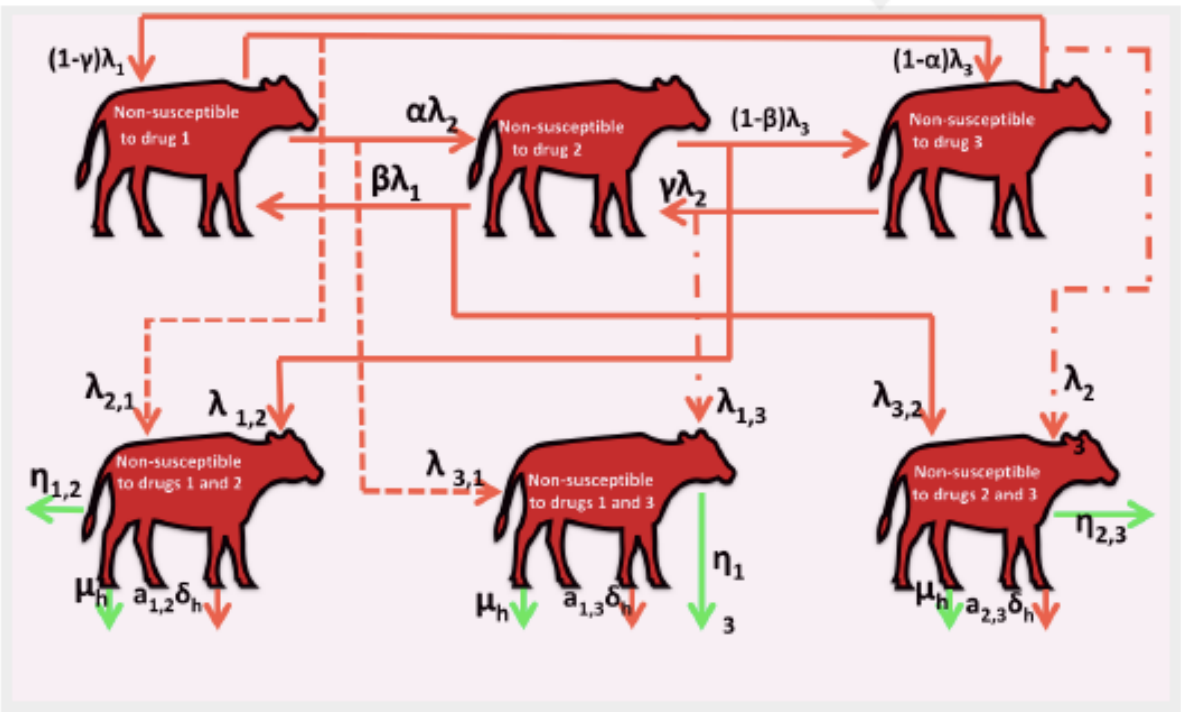


Figure 5: Compartments in the deterministic mathematical model. The non-susceptible compartment interactions flowchart of the host, showing transmission cycle between single drugs and combinations.

Simulations with discrete time-steps of one day, highlighted differences in a range of drug regimens, and changes in compartment size for host and vector population infected with non-susceptible trypanosomes.

2.4.1.3 The deterministic model equations and parameters

Trypanosomes are transmitted at a constant rate, β, and λ_1, λ_2 and λ_3 denote the fitness cost of non-susceptibility acquisition for drugs 1, 2 and 3 respectively. The Ordinary Differential Equations for the host (cattle) population based on the model given in Figure 5 are given by:

The host infection rate is given by:

$$f_h(t) = \beta_h C I_v(t)/N_h(t) \quad 2.1$$

Where β_h is the host transmission rate; C is a coefficient of infection that satisfies $0<C<1$; $I_v(t)$ is the infectious vector population at a given time, t; and $N_h(t)$ is the total host population at a given time, t.

The susceptible host sub-population is given by:

$$S_h'(t) = \lambda_h - f_h(t)S_h(t) + \rho W_h(t) - \mu_h S_h(t) \quad 2.2$$

Where $S_h'(t)$ is the derivative of the susceptible cattle population, $S_h(t)$ with respect to time; λ_h is the combined birth rate and immigration of the host population; $W_h(t)$ is the recovered or withdrawn cattle population at a given time, t; ρ is the rate at which withdrawn population become susceptible; and μ_h is the natural death rate of the susceptible host population.

The exposed host sub-population is given by:

$$E_h'(t) = f_h(t)S_h(t) - (\psi_h + \mu_h)E_h(t) \quad 2.3$$

Where $E_h'(t)$ is the derivative of the exposed host population, $E_h(t)$ with respect to time; and ψ_h is the rate at which the exposed cattle population become infectious.

The infectious host sub-population is given by:

$$I_h'(t) = \psi_h E_h(t) - \theta I_h(t) - \eta_I I_h(t) - \delta_h I_h(t) - \mu_h I_h(t) \quad 2.4$$

Where $I_h'(t)$ is the derivative of the infectious host population, $I_h(t)$ with respect to time; θ is the proportion of the infectious population subjected to treatment; η_I is the proportion

of the infectious cattle that naturally recovers from AAT; and δ_h is the AAT induced death rate, the deaths that occur as a result of trypanosomiasis infection.

The treated host sub-population is given by:

$$T_h'(t) = \theta I_h(t) - \lambda_1 T_h(t) - \lambda_2 T_h(t) - \lambda_3 T_h(t) - \lambda_{1,2} T_h(t) - \lambda_{2,1} T_h(t) - \lambda_{1,3} T_h(t) - \lambda_{3,1} T_h(t) - \lambda_{2,3} T_h(t) - \lambda_{3,2} T_h(t) - \eta_T T_h(t) - \delta_h T_h(t) - \mu_h T_h(t) \quad 2.5$$

Where $T_h'(t)$ is the derivative of the treated cattle population, $T_h(t)$ with respect to time; λ_1, λ_2, λ_3 is the rate of resistance acquired as a result of treatment by drugs 1, 2, and 3 respectively; and η_T is the proportion of the treated cattle population that recovers as a result of treatment.

The host sub-population resistant to drug 1 is given by:

$$R_{h1}'(t) = \lambda_1 T_h(t) - \lambda_2 R_{h1}(t) - \lambda_3 R_{h1}(t) - \eta_1 R_{h1}(t) - a_1\delta_h R_{h1}(t) - \mu_h R_{h1}(t) + \lambda_1 R_{h2}(t) + \lambda_1 R_{h3}(t) - \lambda_{2,1} R_{h1}(t) - \lambda_{3,1} R_{h1}(t) \quad 2.6$$

Where $R_{h1}'(t)$ is the derivative of the cattle population that become resistant to drug 1, $R_{h1}(t)$ with respect to time; $\lambda_2 R_{h1}(t)$, and $\lambda_3 R_{h1}(t)$ is the proportion cattle resistant to drug 1 that develop resistance after treatment with drugs 2, and 3 respectively; $\lambda_1 R_{h2}(t)$, and $\lambda_1 R_{h3}(t)$ is the proportion of cattle resistant to drugs 2, and 3 respectively that develop resistance after treatment with drug 1; η_1 is the proportion of cattle population resistant to drug 1 that recovers naturally; and a_1 is the proportion of AAT induced cattle that die due to resistance to drug 1.

The host sub-population resistant to drug 2 is given by:

$$R_{h2}'(t) = \lambda_2 T_h(t) - \lambda_1 R_{h2}(t) - \lambda_3 R_{h2}(t) - \eta_2 R_{h2}(t) - a_2\delta_h R_{h2}(t) - \mu_h R_{h2}(t) + \lambda_2 R_{h1}(t) + \lambda_2 R_{h3}(t) - \lambda_{1,2} R_{h2}(t) - \lambda_{3,2} R_{h2}(t) \quad 2.7$$

Where R_{h2}‘(t) is the derivative of the cattle population that become resistant to drug 2, R_{h2}(t) with respect to time; η_2 is the proportion of cattle population resistant to drug 1 that recovers naturally; and a_2 is the proportion of AAT induced cattle that die due to resistance to drug 2.

The host sub-population resistant to drug 3 is given by:

$$R_{h3}‘(t) = \lambda_3 T_h(t) - \lambda_1 R_{h3}(t) - \lambda_2 R_{h3}(t) - \eta_3 R_{h3}(t) - a_3\delta_h R_{h3}(t) - \mu_h R_{h3}(t) + \lambda_3 R_{h1}(t) + \lambda_3 R_{h2}(t)) - \lambda_{1,3} R_{h3}(t) - \lambda_{2,3} R_{h3}(t) \quad 2.8$$

Where R_{h3}‘(t) is the derivative of the cattle population that become resistant to drug 3, R_{h3}(t) with respect to time; η_3 is the proportion of cattle population resistant to drug 1 that recovers naturally; and a_3 is the proportion of AAT induced cattle that die due to resistance to drug 3.

The host sub-population resistant to both drugs 1 and 2 is given by:

$$R_{h1,2}‘(t) = (\lambda_{1,2} + \lambda_{2,1})T_h(t) + \lambda_{1,2} R_{h2}(t) + \lambda_{2,1} R_{h1}(t) - (\eta_{1,2} + a_{1,2}\delta h + \mu_h)R_{h1,2}(t) \quad 2.9$$

Where $R_{h1,2}$‘(t) is the derivative of the cattle population that become resistant to both drugs 1 and 2, $R_{h1,2}$(t) with respect to time; $\lambda_{1,2}$ is the proportion of cattle resistant after successive treatment with drug 1 followed by drug 2, and eventually non-susceptible to both ($\lambda_{1,2=}\ \lambda_1 \times \lambda_2$); $\lambda_{2,1}$ is the proportion of cattle resistant after successive treatment with drug 2 followed by drug 1, and eventually non-susceptible to both ($\lambda_{2,1=}\ \lambda_2 \times \lambda_1$). Similar definitions follow for $\lambda_{1,3}$, $\lambda_{3,1}$, $\lambda_{2,3}$, and $\lambda_{3,2}$. $\eta_{1,2}$ is the proportion of cattle population resistant to treatment with both drugs 1 and 2 that recovers naturally; and $a_{1,2}$ is the proportion of AAT induced cattle that die due to resistance to both drugs 1 and 2.

The host sub-population resistant to both drugs 1 and 3 is given by:

$$R_{h1,3}'(t) = (\lambda_{1,3} + \lambda_{3,1})T_h(t) + \lambda_{1,3}R_{h3}(t) + \lambda_{3,1} R_{h1}(t) - (\eta_{1,3} + a_{1,3}\delta h + \mu_h)R_{h1,3}(t) \quad 2.10$$

Where $R_{h1,3}'(t)$ is the derivative of the cattle population that become resistant to both drugs 1 and 3, $R_{h1,3}(t)$ with respect to time; $\eta_{1,3}$ is the proportion of cattle population resistant to treatment with both drugs 1 and 3 that recovers naturally; and $a_{1,3}$ is the proportion of AAT induced cattle that die due to resistance to both drugs 1 and 3.

The host sub-population resistant to both drugs 2 and 3 is given by:

$$R_{h2,3}'(t) = (\lambda_{2,3} + \lambda_{3,2})T_h(t) + \lambda_{2,3}R_{h3}(t) + \lambda_{3,2} R_{h2}(t) - (\eta_{2,3} + a_{2,3}\delta h + \mu_h)R_{h2,3}(t) \quad 2.11$$

Where $R_{h2,3}'(t)$ is the derivative of the cattle population that become resistant to both drugs 2 and 3, $R_{h2,3}(t)$ with respect to time; $\eta_{2,3}$ is the proportion of cattle population resistant to treatment with both drugs 2 and 3 that recovers naturally; and $a_{2,3}$ is the proportion of AAT induced cattle that die due to resistance to combination of drugs 2 and 3.

The withdrawn host sub-population is given by:

$$W_h'(t) = \eta_I I_h(t) + \eta_T T_h(t) + \eta_1 R_{h1}(t) + \eta_2 R_{h2}(t) + \eta_3 R_{h3}(t) + \eta_{1,2}R_{h1,2}(t) + \eta_{1,3} R_{h1,3}(t) + \eta_{2,3} R_{h2,3}(t) - ((\rho + \mu_h) W_h(t)) \quad 2.12$$

Where $W_h'(t)$ is the derivative of the cattle population that recover naturally or after treatment by trypanocides as already described with respect to time.

The ordinary differential equations for the vector (tsetse fly) population are described below.

The susceptible vector sub-population is given by:

$$S_v'(t) = \lambda_v - f_v(t)\ S_v(t) - \mu_v S_v(t) \quad 2.13$$

The other parameters have similar definitions to those of the host differential equations. $S_v'(t)$ is the derivative of the susceptible tsetse population, $S_v(t)$ with respect to time; λ_v Is the combined birth rate and immigration of the vector population; and μ_v is the natural death rate of the susceptible tsetse fly population.

The exposed vector sub-population is given by:

$$E_v'(t) = f_v(t)\ S_v(t) - \psi_v E_v(t) - \mu_v E_v(t) \quad 2.14$$

Where $E_v'(t)$ is the derivative of the exposed vector population, $E_v(t)$ with respect to time; and ψ_v is the rate at which the exposed tsetse fly population become infectious.

The infectious vector sub-population is given by:

$$I_v'(t) = \psi_v E_v(t) - \mu_v I_v(t) \quad 2.15$$

Where $I_v'(t)$ is the derivative of the infectious vector population, $I_v(t)$ with respect to time.

The vector infection rate is given by:

$$f_v(t) = \beta_v c(\eta_I I_h(t) + \eta_T T_h(t) + \eta_1 R_{h1}(t) + \eta_2 R_{h2}(t) + \eta_3 R_{h3}(t) + \eta_{12} R_{h12}(t) + \eta_{13} R_{h13}(t) + \eta_{23} R_{h23}(t))/N_h(t) \quad 2.16$$

Where β_v is the vector transmission rate while C is a coefficient of infection that satisfies $0<C<1$; and $I_h(t)$ infectious host population at a given time, t.

A list of host and vector model component parameters is shown in Table 1.

Infection was passed from tsetse flies to cattle in proportion to the contact rate, β_h. The magnitude of the contact rate, β_h, defined in $f_h(t)$ could be affected by several factors, including the proportion of infectious vectors relative to the total host population, , $I_v(t)/N_h(t)$ as well as the size of the susceptible compartment, $S_h(t)$.

Infection could also be passed from cattle to tsetse flies via tsetse fly contact rate, β_v, defined in the function $f_v(t)$. A tsetse fly could be infected by biting cattle in any of the infectious compartments, that is, infectious , $I_h(t)$, treated, $T_h(t)$, non-susceptibility to drug 1, $R_{h1}(t)$, non-susceptibility to drug 2, $R_{h2}(t)$, and non-susceptibility to drug 3, $R_{h3}(t)$, non-susceptibility to a combination of drugs 1 and 2, $R_{h1,2}(t)$, non-susceptibility to a combination of drugs 1 and 3, $R_{h1,3}(t)$, and, non-susceptibility to a combination of drugs 2 and 3, $R_{h2,3}(t)$. Thus, the proportion of the populations within these compartments relative to the total cattle population was taken into account, along with the size of the susceptible tsetse fly subpopulation, $S_v(t)$.

The susceptible compartment increased in size in accordance with the birth rate, λ_h, and the recovery rate proportional to the withdrawn subpopulation, $\rho W_h(t)$. The susceptible compartment population decreased due to the contact rate proportional to the susceptible population, $- f_h(t) S_h(t)$, or due to the natural death rate proportional to susceptible population at time t , $- \mu_h S_h(t)$.

The exposed compartment became larger by introducing susceptible cattle that had been bitten by an infectious tsetse fly, $f_h(t) S_h(t)$. The exposed compartment was reduced by the proportion of cattle experiencing symptoms of trypanosomiasis (becoming infectious), $-\psi_h E_h$, or by the natural death rate, $- \mu_h E_h(t)$.

The infectious compartment increased proportionally to the decrease experienced within the exposed population, $\psi_h E_h$. There were several decreasing rates of change within the infectious compartment: the treatment rate, $-\theta I_h(t)$, the recovery rate, $- \eta_I I_h(t)$, the

natural death rate, - $\mu_h I_h(t)$., and the infection-induced death rate, - $\delta_h I_h(t)$ with all rates used in proportion to the infected compartment.

The treated compartment was increasing due to the treatment rate proportional to the infected population, $\theta I_h(t)$. Treated cattle experienced a reduction due to; the rate of change due to recovery, - $\eta_T T_h(t)$, the appearance of drug 1 non-susceptibility, - $\lambda_1 T_h(t)$, the appearance of drug 2 non-susceptibility, - $\lambda_2 T_h(t)$, the appearance of drug 3 non-susceptibility, - $\lambda_3 T_h(t)$, the AAT-induced death rate, - $\delta_h T_h(t)$ or due to the natural death rate, - $\mu_h T_h(t)$. The outcome of treatment determined whether or not the infection was sensitive or non-susceptible to drugs.

The drug 1 non-susceptibility compartment of cattle experiencing the first level of drug non-susceptibility grew after treatment failure, $\lambda_1 T_h(t)$. Cattle could either recover, $-\eta_1 R_{h1}(t)$, progress to drug 2 non-susceptibility compartment, - $\lambda_2 R_{h1}(t)$, progress to drug 3 non-susceptibility compartment, - $\lambda_2 R_{h1}(t)$ or they died, -($a_1\delta_h+\mu_h)R_{h1}(t)$.

The drug 2 non-susceptibility compartment increased in size after treatment failure, $\lambda_2 T_h(t)$. It also increased due to cattle movement from drug 1 non-susceptibility compartment, $\lambda_2 R_{h1}(t)$ and drug 3 non-susceptibility compartment, $\lambda_2 R_{h3}(t)$. Cattle could then either recover, $-\eta_2 R_{h2}(t)$, progress to drug 3 non-susceptibility compartment, - $\lambda_3 R_{h2}(t)$, progress to drug 1 non-susceptibility compartment, $-\lambda_1 R_{h2}(t)$, or they died, - $(a_2\delta_h+\mu_h)R_{h2}(t)$.

Additions to drug 3 non-susceptibility compartment were as a result of cattle experiencing non-susceptibility to drug 3, $\lambda_3 T_h(t)$, failing to recover or respond to treatment, $\lambda_3 R_{h1}(t)$, $\lambda_3 R_{h2}(t)$. At this stage, cattle can recover, $-\eta_3 R_{h3}(t)$, progress to drug 2 non-susceptibility compartment, $-\lambda_2 R_{h3}(t)$, progress to drug 1 non-susceptibility compartment, $-\lambda_1 R_{h3}(t)$, or they died, - $(a_3\delta_h+\mu_h)R_{h3}(t)$.

.

The combination of drugs 1 and 2 non-susceptibility compartment of cattle experiencing the second level of drug non-susceptibility grew after combination treatment failure,

$\lambda_{1,2}R_{h2}(t)$ and $\lambda_{2,1}\ R_{h1}(t)$. Cattle either recovered, $-\eta_{1,2}R_{h1.2}(t)$, or they died, $-(a_{1,2}\delta_h+\mu_h)R_{h1,2}(t)$.

The combination of drugs 1 and 3 non-susceptibility compartment increased as cattle moved from the drugs 1 and 3 combination treatment non-susceptibility compartment, $\lambda_{1,3}R_{h3}(t)$ and $\lambda_{3,1}\ R_{h1}(t)$. Cattle either recovered, $-\eta_{1,3}R_{h1.3}(t)$, or they died, $-(a_{1,3}\delta_h+\mu_h)R_{h1,3}(t)$.

Increase in combination of drugs 2 and 3 non-susceptibility compartment resulted from the drugs 2 and 3 combination treatment failure, $\lambda_{2,3}R_{h3}(t)$ and $\lambda_{3,2}\ R_{h2}(t)$. Cattle either recovered, , $-\eta_{2,3}R_{h2,3}(t)$, or they died, $-(a_{2,3}\delta_h+\mu_h)R_{h2,3}(t)$.

The withdrawn population, $W_h(t)$, included all cattle that have recovered and were temporarily immune regardless of which compartment they came from. Thus $W_h(t)$ was increasing due to the following rates of change:

$\eta_I I_h(t)+ \eta_T T_h(t)+ \eta_1 R_{h1}(t))+ \eta_2 R_{h2}(t))+ \eta_3 R_{h3}(t)+ \eta_{1,2} R_{h1.2}(t)+ \eta_{1,3} R_{h1.3}(t)+ \eta_{2,3} R_{h2.3}(t)$.

Cattle left the withdrawn compartment after losing immunity, and thus , $W_h(t)$ also had a decreasing rate of change to account for losing immunity, $-\rho W_h(t)$, or dying, $-\mu_h W_h(t)$.
After a period of time, $1/\rho$ cattle would lose temporary immunity to re-enter the susceptible compartment. These cattle are fully susceptible to re-infection.

Similarly, the tsetse fly population was adjusted based on their infection status. The susceptible tsetse fly population increased due to their birth rate, λ_v. Susceptible tsetse flies were decreasing via the contact rate, β_v contained in the function $f_v(t)$ or by the natural death rate, $-\mu_v S_v(t)$, both of which were proportional to the susceptible tsetse fly compartment. The exposed tsetse fly population experiences an increasing rate of change proportional to the susceptible compartment, $f_v(t)S_v(t)$. Exposed tsetse flies could then either obtain the infection, $-\psi_v E_v(t)$, or die, $-\mu_v E_v(t)$. While the infectious tsetse fly compartment would become larger due to the rate at which exposed tsetse flies become

infectious, $\psi_v E_v(t)$, the infectious tsetse flies would eventually die without recovering from infection, $\mu_v I_v(t)$.

2.4.1.4 Effect size and ODE solver

The differences in effect size (ES) was computed using the following formulae, (110–116):

ES=(Mean 2- Mean 1)/(Standard deviation 1) 2.17

Mean 1 was always chosen to be the smallest mean of the two sets of data from the Matlab output. Then the standard deviation of mean 1 is computed and used to determine the effect size between the two sets.

We used ode45 as our solver. This versatile ODE solver is used to solve systems of differential equations representing different problems that are non stiff. This solver implements one of the Runge-Kutta method of solving numerical peoblems.

The model was implemented in MATLAB version R2017a.

CHAPTER 3

Results

3.1 Data collection application

Shimba Hills region is one of the most resource poor settings in Kenya. This study was focused at two villages of smallholder farmers: Kizibe and Mbegani, with varying economic capacities, and different farming practices that had made understanding temporal trends in trypanocide non-susceptibility challenging. The efficacy of a community-led data collection approach to acquire accurate information on trypanocide non-susceptibility and perceptions of smallholder farmers on trypanosomiasis was evaluated. A great disparity between gender and cattle rearing was observed (Figure 6). The proportion of female farmers was smaller (6%, n=3), and they also had a lower proportion of the overall livestock ownership (4%, n=8). These female farmers were all from a single community, Mbegani, and their cattle were not infected with non-susceptible parasites. This study compared farming practices between the communities (Figure 7). Smallholder farmers in Mbegani lost more cattle due to trypanosomiasis, spent less on trypanocides, regained milk production earlier after treating livestock, keenly followed drug prescription instructions, and noticed that recurrent infections are non-susceptible to treatment.

A large proportion of smallholder farmers and veterinary health care givers interviewed (94%, n=47) welcomed the App developed on the ODK platform as a method of trypanosomiasis data collection. This suggests that information technology is continuously becoming an important tool to smallholder farmers in resource poor settings to access and organize agricultural information.

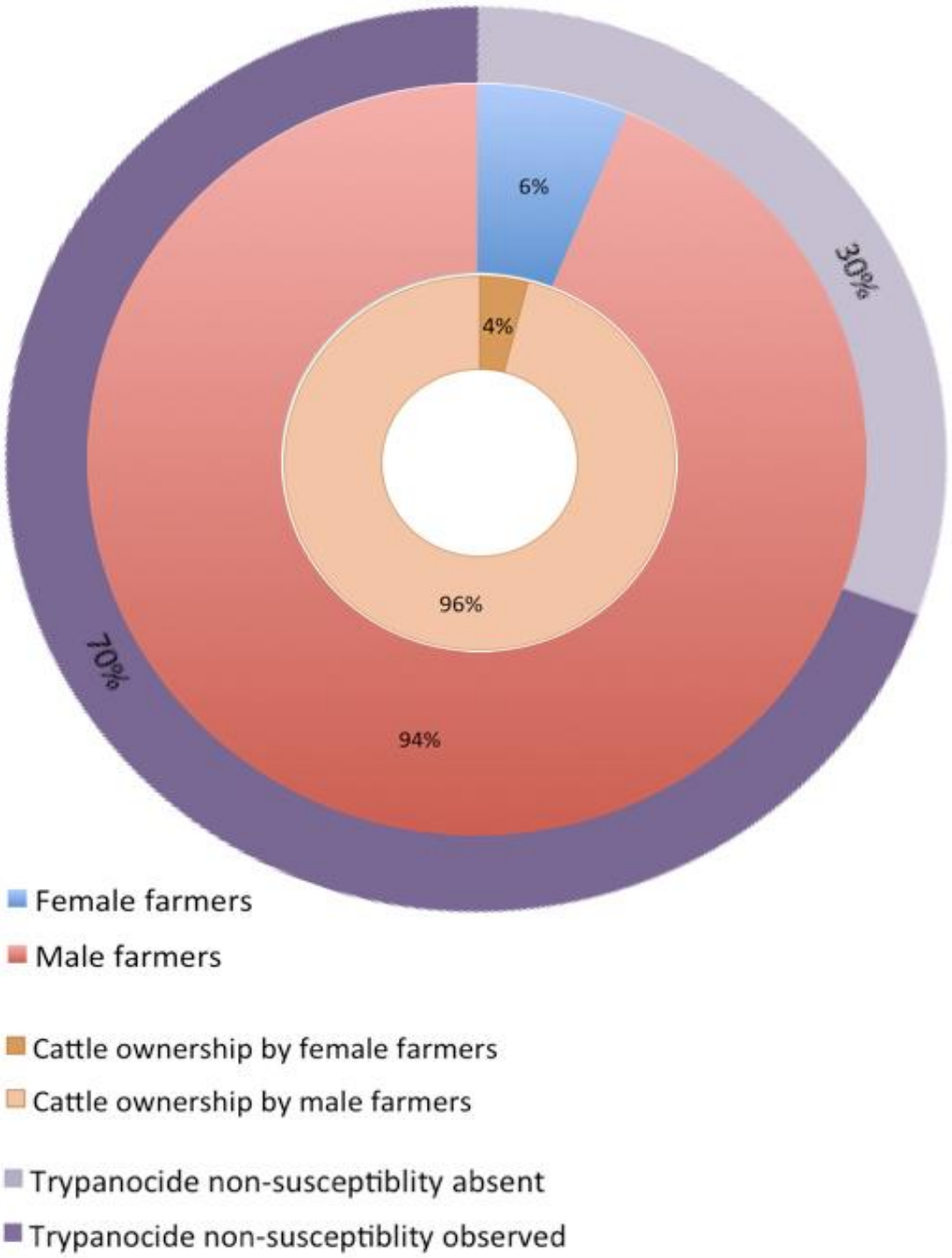


Figure 6: Gender representation in animal husbandry. The proportion of female farmers was smaller (6%, n=3 of 50 (n for the number of farmers)), and they also had a lower proportion of the overall livestock ownership (4%, n=8 of 211 (n for the number of cattle)).

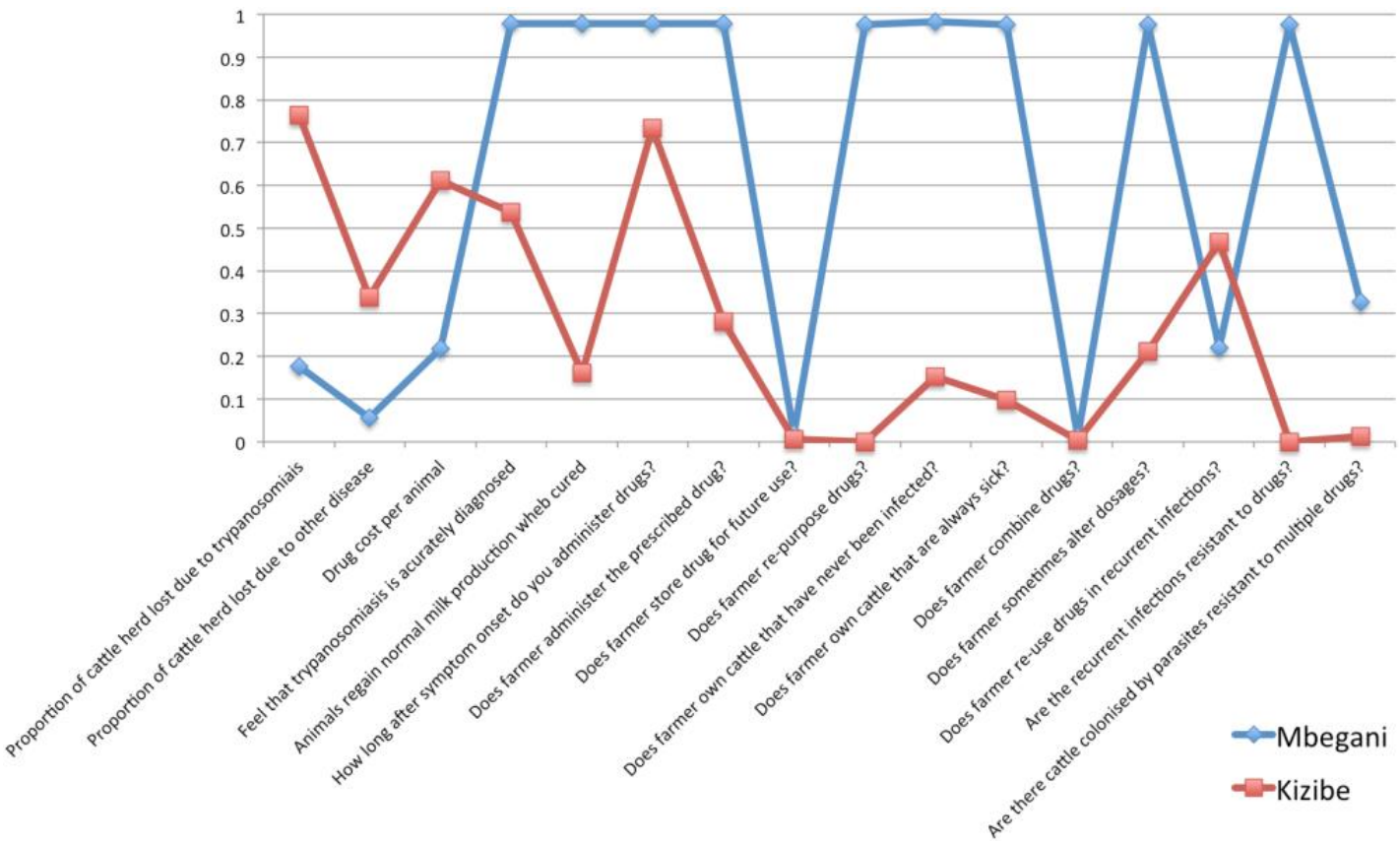


Figure 7*:* Farmers perceptions on livestock trypanosomiasis. Shows the perception of farmers based on results from multiple correspondence analysis (MCA) conducted separately for Mbegani and Kizibe.

3.2 Appropriate trypanocide use results

3.2.1 Single use and combination of trypanocides

The appropriate proportion of cattle to treat when there is a perceived trypanosome infection was determined. The common practice was to treat as many cattle as possible, if not all. It was unclear if this severely affects trypanocide non-susceptibility, or whether

there were benefits that accrue from increasing the extent of population treated. The difference in non-susceptibility effect size was examined by evaluating cattle treatment with a single drug or a combination of two drugs. In this study's model, trypanosomes were most non-susceptible to drug 1 by virtue of having more extended use compared to drug 2, and drug 3 that showed the least non-susceptibility. Interestingly, although there was undetectable change in non-susceptibility when increasing the extent of population treated (from 10% to 100%) for each of the three drugs. There was a decrease in non-susceptibility when a combination of two drugs is used for treatment (Table 2) (Figure 8, Figure 9). This suggests that using combination therapy to treat a larger extent of the infected cattle population results in lowest levels of trypanocide non-susceptibility. The combination of drug 2 and 3 was the most optimal for reduced population-wide non-susceptibility.

Table 2: The difference in effect size of treated population for trypanocide non-susceptibility. Differences in effect size of treated population for trypanocide measured when the proportion cattle treated in the infected population is increased from 10% through 100%. These differences are measured for low (4%), medium (7.5%) and high (11%) transmission rates, which represent different endemic regions within Kwale County, Kenya. Values across the table show differences in effect size for trypanocide non-susceptibility between two different treatments. A large value indicates a large difference in non-susceptibility elicited when the treatments are compared. The strategies examined are both treatment with a single drug, for example drug 1 (Rh1), and treatment with a combination of drugs, for example drug 1 and 2 (R_{h12}). Treating 80% of the cattle with drug 2 and 3 (R_{h23}) gives the best result.

Percentage of population treated	10%			20%			40%			80%			100%		
Prevalence of trypanocide non-susceptibility (%)	4	7.5	11	4	7.5	11	4	7.5	11	4	7.5	11	4	7.5	11
R_{h2} vs. R_{h3}	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01
R_{h1} vs R_{h2}	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01
R_{h1} vs R_{h3}	0.05	0.03	0.02	0.05	0.03	0.02	0.05	0.03	0.02	0.05	0.03	0.02	0.05	0.03	0.02
R_{h13} vs.R_{h23}	0.15	0.15	0.16	0.15	0.16	0.16	0.15	0.16	0.16	0.15	0.16	0.17	0.15	0.16	0.17
R_{h12} vs. R_{h13}	0.17	0.18	0.18	0.17	0.18	0.18	0.17	0.18	0.19	0.17	0.19	0.19	0.17	0.18	0.19
R_{h12} vs. R_{h23}	0.33	0.35	0.37	0.33	0.36	0.37	0.33	0.36	0.37	0.35	0.37	0.38	0.34	0.16	0.38
R_{h3} vs. R_{h12}	0.39	0.57	0.67	0.39	0.58	0.68	0.39	0.59	0.69	0.41	0.62	0.72	0.40	0.60	0.70
R_{h2} vs. R_{h12}	0.42	0.59	0.68	0.42	0.60	0.69	0.43	0.61	0.71	0.44	0.64	0.73	0.43	0.62	0.72
R_{h1} vs. R_{h12}	0.45	0.62	0.70	0.46	0.62	0.71	0.46	0.63	0.72	0.48	0.66	0.75	0.47	0.64	0.73
R_{h3} vs. R_{h13}	0.61	0.83	0.95	0.61	0.84	0.96	0.62	0.85	0.97	0.64	0.89	1.01	0.63	0.87	0.99
R_{h2} vs. R_{h13}	0.65	0.86	0.96	0.65	0.87	0.98	0.66	0.87	0.99	0.68	0.92	1.03	0.66	0.89	1.01
R_{h1} vs. R_{h13}	0.68	0.88	0.98	0.69	0.89	1.00	0.69	0.90	1.01	0.72	0.94	1.05	0.70	0.92	1.03
R_{h3} vs. R_{h23}	0.83	1.09	1.22	0.84	1.10	1.24	0.84	1.11	1.26	0.88	1.16	1.30	0.85	1.13	1.28
R_{h2} vs. R_{h23}	0.87	1.12	1.24	0.88	1.13	1.26	0.88	1.14	1.28	0.92	1.19	1.33	0.89	1.16	1.30
R_{h1} vs. R_{h23}	0.92	1.14	1.26	0.92	1.16	1.28	0.93	1.17	1.30	0.97	1.22	1.35	0.94	1.19	1.32

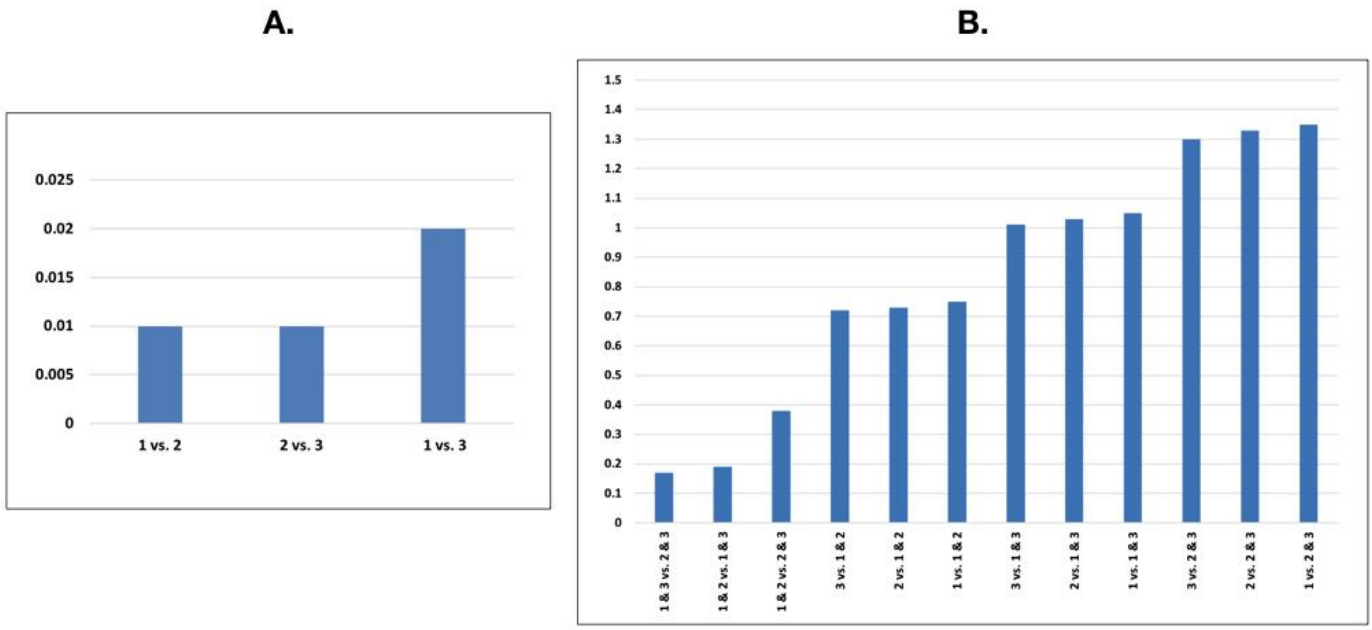
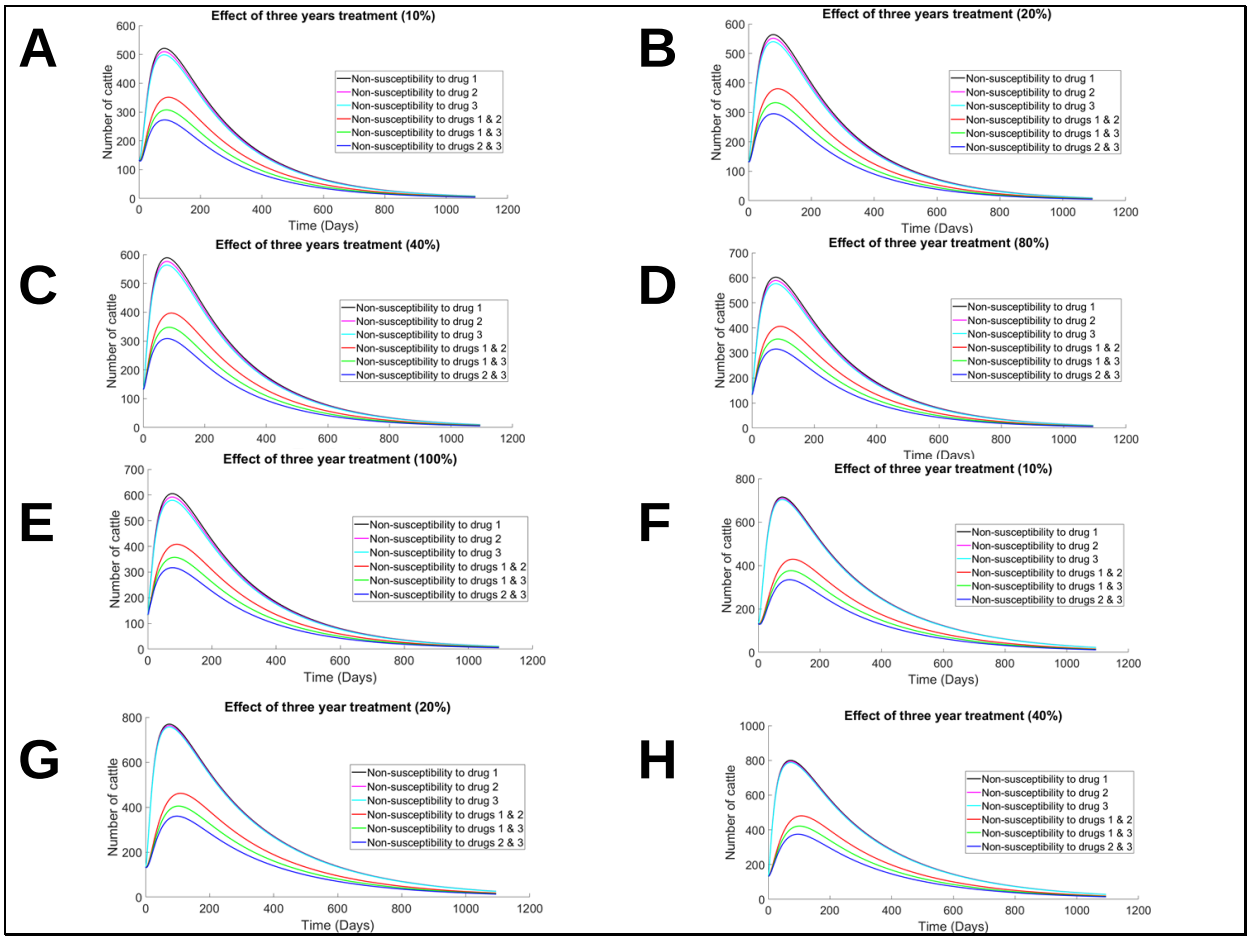


Figure 8: The difference in effect size for trypanocide non-susceptibility. Effect size for trypanocide measured when 80% proportion of infectious cattle is treated in the infectious population. These differences are measured for 11% non-susceptibility rate, which represent one of the different endemic regions within Kwale County, Kenya.

Values across the table show how the effect sizes compare and differ for trypanocide non-susceptibility between two different treatments. A large value indicates a large difference in non-susceptibility elicited when the two treatments are compared. Therefore there is a cap in effect sizes at 80% treatment. The strategies examined are both treatment with a single drug, for example drug 1, and treatment with a combination of drugs, for example drug 1 and 2 (these two treatment cases provide an effect size of 0.75). This is an excerpt of the 80% infectious population wide treatment at 0.11 non-

susceptibility column from Table 2 above, for clarity. (A) represents the first three rows (where the effect size is almost negligible) while (B) represents the remaining rows.



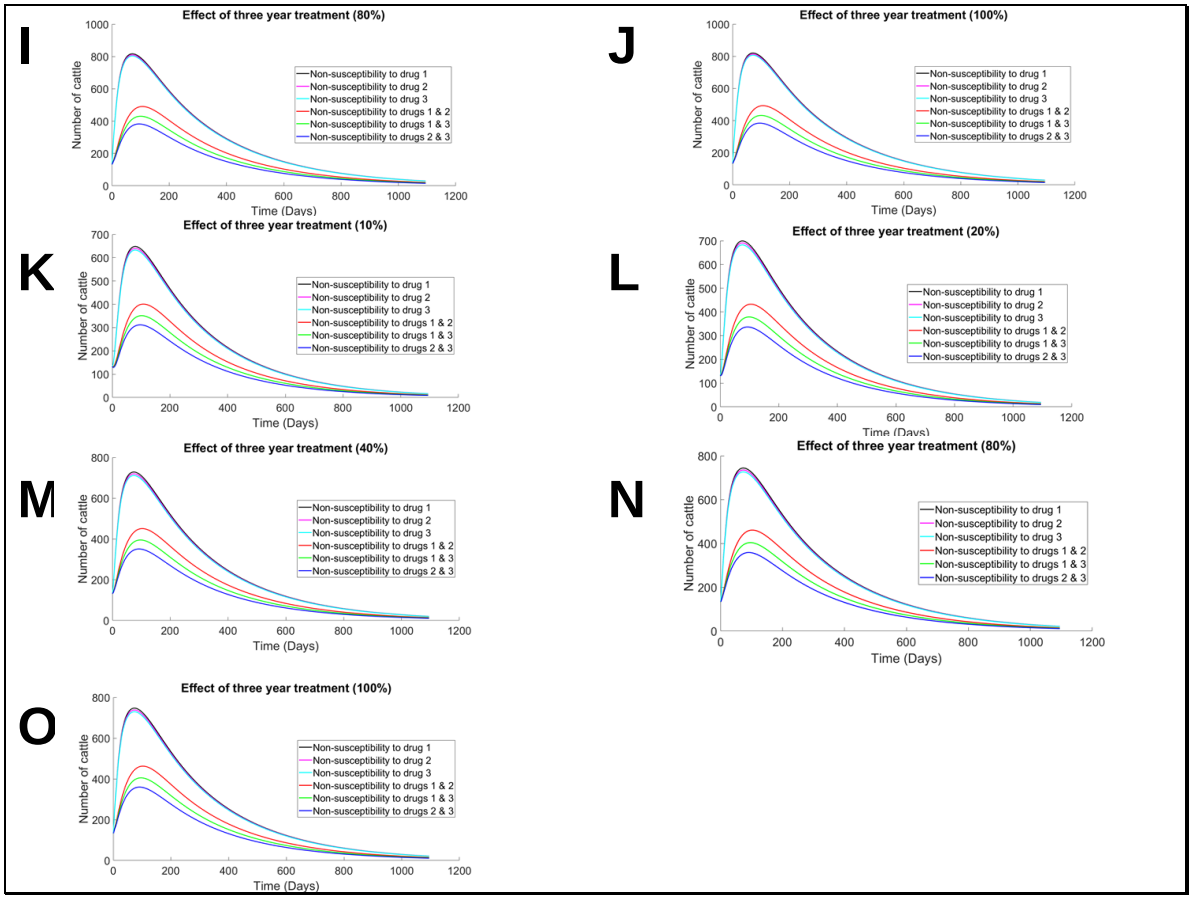


Figure 9: Non-susceptibility in the host population for a period of three years. It highlights non-susceptibility to drug 1, R_{h1}, non-susceptibility to drug 2, R_{h2}, non-susceptibility to drug 3, R_{h3}, non-susceptibility to combination of drugs 1 and 2, R_{h12}, non-susceptibility to combination of drugs 1 and 3, R_{h13} and non-susceptibility to combination of drugs 2 and 3, R_{h23}. (A-E) show non-susceptibility rates at 4% trypanosomiasis prevalence after treating 10%, 20%, 40%, 80% and 100% of the infected cattle population respectively. (F-J) show non-susceptibility rates at 7.5% trypanosomiasis prevalence after treating 10%, 20%, 40%, 80% and 100% of the infected cattle population respectively. (K-O) show non-susceptibility rates at 11% trypanosomiasis prevalence after treating 10%, 20%, 40%, 80% and 100% of the infected cattle population respectively.

3.2.2 Ordinal use of drugs

The ordinal use of these drugs in increasing and decreasing order of non-susceptibility was evaluated. Absence of evidence-based approaches on the appropriate trypanocide made it difficult to select the appropriate drugs given the non-susceptibility prevalence. More specifically, in what order of non-susceptibility should drugs be used in a setting where non-susceptible trypanosomes are prevalent to ensure efficacy? This was established by assessing the absolute number of cattle with non-susceptible infection in each of the host (cattle) non-susceptible compartments. The use of drug 1, 2 and 3 consecutively (decreasing non-susceptibility order) was first examined, and the reverse (increasing non-susceptibility) order in the subsequent analysis. It was demonstrated that using drugs in increasing non-susceptibility order resulted in a negligible increase in number of cattle with non-susceptible infection, in contrast to a more pronounced increase from trypanocide use in decreasing non-susceptibility order (Figures 10 and 11).

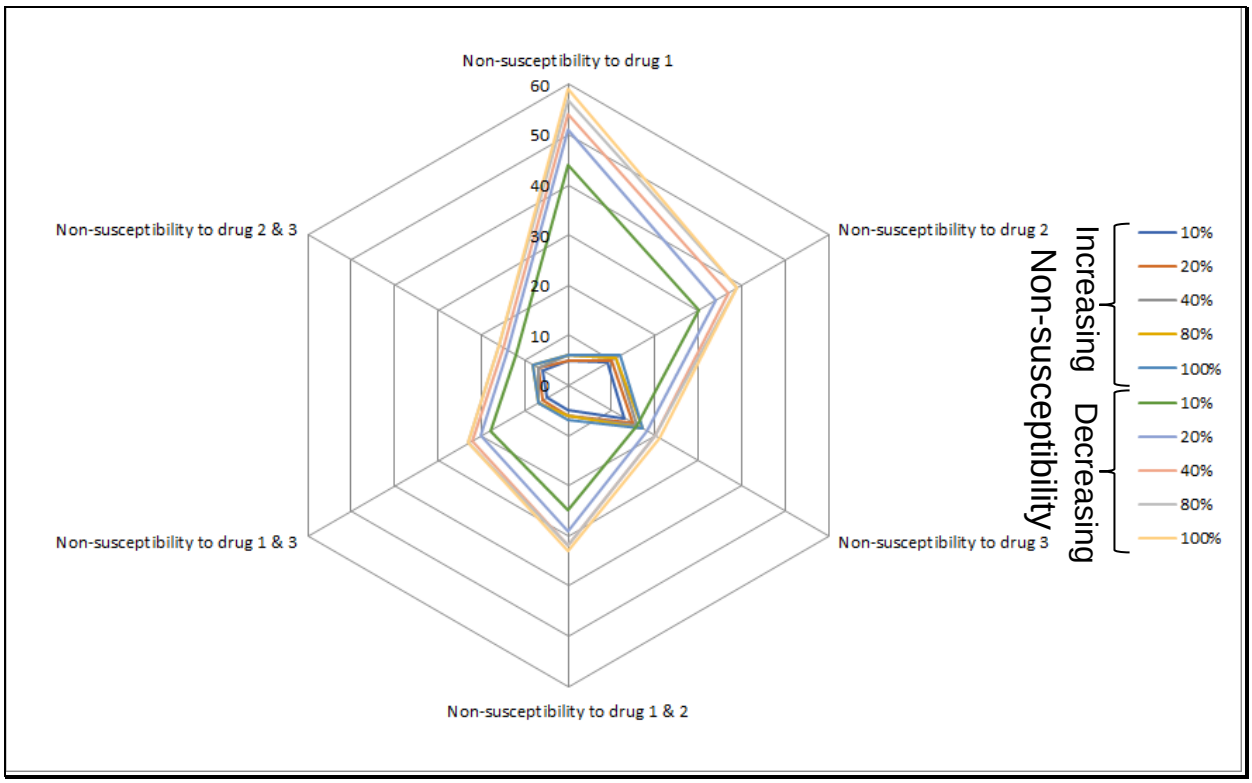
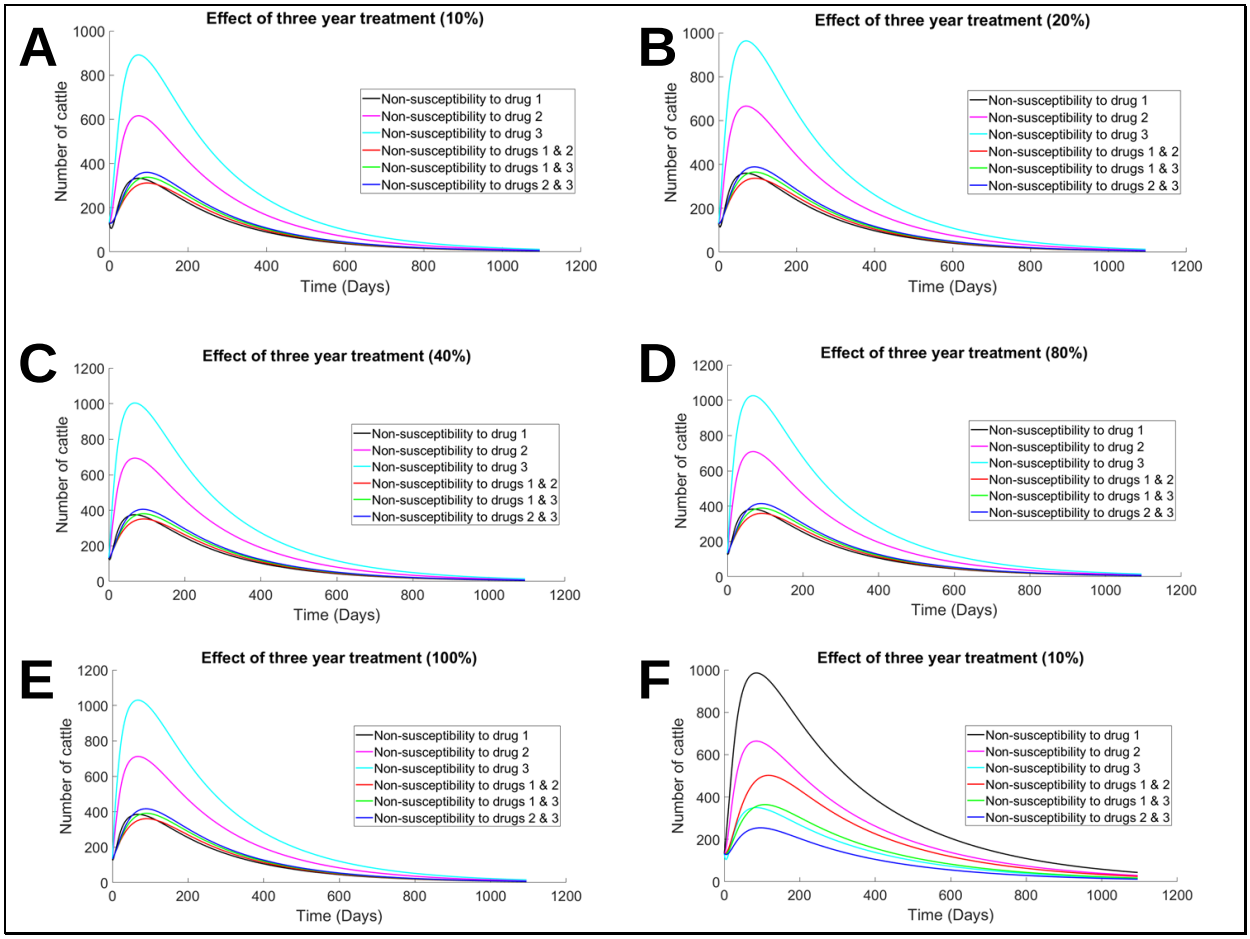


Figure 10: Trypanocide use in increasing and decreasing non-susceptibility order. Trypanocide use in increasing non-susceptibility order of 0.04, 0.075, 0.11 respectively, is represented by the innermost plots, and the reverse (decreasing non-susceptibility) order of 0.11, 0.075, 0.04 respectively, is represented by the outermost plots. The use of drugs in increasing non-susceptibility order results in a negligible increase in the absolute number of cattle with non-susceptible infection in the different non-susceptibility compartments, in contrast to a more pronounced increase from trypanocide use in decreasing non-susceptibility order. The series 0, 10, 20, 30, 40, 50, 60 indicates the number of cattle non-susceptible to the indicated trypanocides, single or their combination.


A
Effect of three year treatment (10%)
B
Effect of three year treatment (20%)
C
Effect of three year treatment (40%)
D
Effect of three year treatment (80%)
E
Effect of three year treatment (100%)
F
Effect of three year treatment (10%)
Number of cattle
Time (Days)
Non-susceptibility to drug 1
Non-susceptibility to drug 2
Non-susceptibility to drug 3
Non-susceptibility to drugs 1 & 2
Non-susceptibility to drugs 1 & 3
Non-susceptibility to drugs 2 & 3

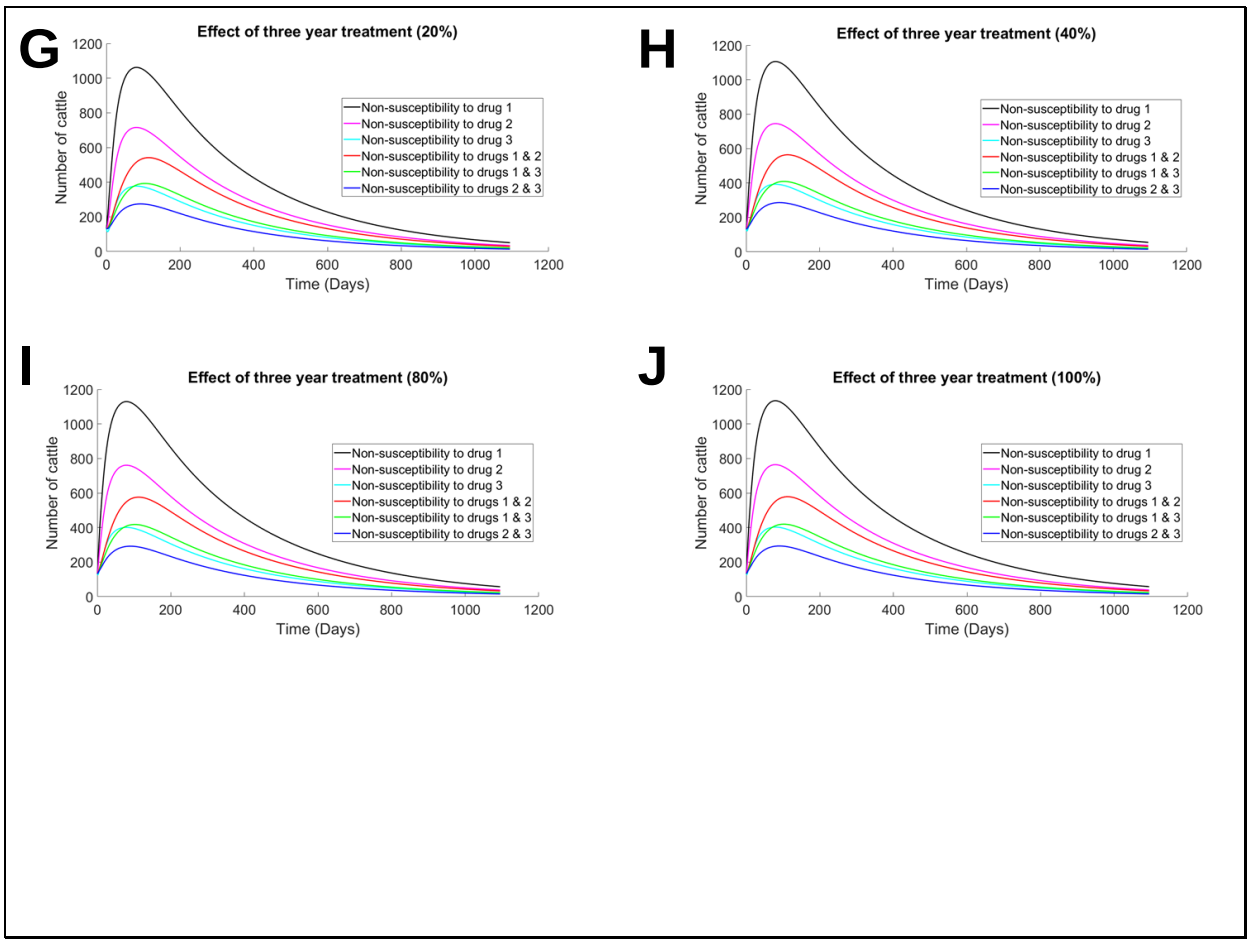


Figure 11: Increasing and decreasing non-susceptibility. (A, B, C, D, E) Trypanocide use in increasing non-susceptibility order of 0.04, 0.075, 0.11 after treating 10%, 20%, 40%, 80% and 100% of the infected cattle population respectively, and (F, G, H, I, J) the reverse (decreasing non-susceptibility) order of 0.11, 0.075, 0.04 after treating 10%, 20%, 40%, 80% and 100% of the infected cattle population respectively.

3.2.3 Optimal proportion of cattle to treat

The threshold of infectious cattle to be treated was examined. Once drug non-susceptibility, and consequently MDR, becomes prevalent, treatment was less likely to be successful. However, drug non-susceptibility could be kept at manageable levels. Increasing treatment led to reduction in the drug non-susceptible cattle population up to 80%. From 80% to 100% there was a reduction in the effect size showing increment of

non-susceptible cattle. Mathematical models suggested thresholds at which treatment would be effective, and when treatment should be used conservatively to avoid creating more problems by aggravating drug non-susceptibility. From the effect size it can be deduced that 80% infectious population wide treatment is the most optimal across board (Table 2, Figure 8). For example, in Table 2 the last row shows that treatment with drug 1 is the least effective of all and treatment by a combination of drugs 2 and 3 is the most effective of all. In particular, the larger the effect sizes the further the plots are from each other, one being more effective while the other less.

CHAPTER 4

4.1 Discussion and conclusions

African animal trypanosomiasis (AAT) is mainly managed by chemotherapy, and up to 70 million doses are sold in Africa annually (49). This presents a trade-off between restoring animal health, and developing rapid widespread non-susceptibility to multiple trypanocides. Efficacious regimens are therefore necessary to achieve the right balance. In a given trypanosomiasis endemic locale, knowing the order in which to use available trypanocides with a range of parasite susceptibilities after recurrent disease episodes could help to achieve this balance. However, absence of evidence-based approaches on the appropriate trypanocide makes it difficult to select the appropriate drugs given the non-susceptibility prevalence rates. More specifically, in what order as well as which kind of combination of non-susceptibility should drugs be used in a setting where non-susceptible trypanosomes are prevalent to ensure efficacy? Despite the importance of diagnostics prior to AAT treatment, smallholder farmers in resource poor settings often administer trypanocides by intuition. Uninfected cattle may benefit from this prophylactic treatment before exposure, especially if entering tsetse-infected common grazing fields. Although effective in reducing parasitaemia levels, trypanocides are toxic and have a prolonged excretion duration (117,118). Treatment without diagnosis exposes animals to unnecessary adverse side-effects, elevates the risk of consuming contaminated animal products, and drives development of trypanosome non-susceptibility *in vivo*. The appropriate number of cattle to treat for optimal disease management in an exposed population, and whether there are benefits accruing from increasing the number treated still remains unclear. Combination therapy may result in beneficial (additive or synergistic effects) or detrimental (antagonistic effects) compared to single-drug treatments (119). Combination therapy has been used to treat human African trypanosomiasis (HAT), and has led to lower doses of trypanocide use, decreasing toxicity and improving effectiveness (120,121). Single-drug use is customarily used for AAT management, and there's renewed interest in development of novel biocompatible formulations that improve therapeutic outcomes (120,121).

Randomized clinical trials are necessary to evaluate the benefits and adverse effects attributable to these novel treatments. This will help in assessing that combination therapy would be a significant improvement of monotherapy for AAT.

The evaluation involved the use of smart phone app for data collection that was used for evaluating farming practices and policies regarding community surveillance on drug use and misuse. Mobile app data was used to estimate some parameters as part of input for mathematical modelling to evaluate and predict the effect of using multiple trypanocides on multi-drug non-susceptibility development in Kwale County, Kenya. Therefore, design, validation and implementation of a compartmental susceptible, exposed, infected and recovered (SEIR) epidemiological model for simulating use of ordinal and combination of trypanocides to reduce trypanosomes transmission involving tsetse flies and cattle was done. The results from the developed model were used to establish, the minimum communal threshold for infectious population wide treatment by use of multiple drugs to reduce and prevent the development and spread of trypanocide non-susceptibility. Animal African trypanosomiasis (AAT) reduces agricultural productivity in arable Africa by up to 10% leading to food shortage, malnutrition, and in extreme cases famine (17,122,123). The most employed strategy for nagana management is the use of trypanocides for disease prevention and management. The lack of consistent surveillance efforts of AAT coupled with the misuse of trypanocides has led to widespread non-susceptibility (124). This raises serious concerns as the accumulation of populations of non-susceptible trypanosomes complicates disease management.

An AAT management app was developed. The efficacy of community-led data collection was examined using smart phone mobile health application (MHealth App) for monitoring livestock trypanocide non-susceptibility in Shimba Hills, Kenya. ODK App was successfully used to collect data from the farmers and veterinary officers in the field. It was found that the farmers were highly receptive of the idea of using mobile apps to give detailed and informative information about their problems in regard to farming practices. The study findings agree with the general mobile acceptability as it is known that smart phone telecommunication is the most popular means of data, voice and service transmission and has greatly spread. In Kenya, over 20 million people, of

the approximately 45 million population have access to mobile phones (125,126). The exponential expansion of smart phones use can be attributed to some of its features such as their affordability, ease of use, easy to carry, wide network coverage, voice communication, instant and convenient service delivery. Hence there has been an exponential expansion globally in the number of smart phone applications. This has been facilitated by the availability of wide network coverage, ever increasing mobile function ability and the dwindling handset prices. Several smart phone applications have been developed in Kenya. Examples of Mobile applications are M-Pesa, which is used to transfer money between mobile phone users in Kenya (126,127). Other apps include; M-Farm, ICow and "Kilimo Salama" that are in use in the Kenyan agricultural sector. M-Farm is a mobile application focused on market information, market linkage and network availability over regions, networking of suppliers, farmers and buyers. It also facilitates in enabling the access to agricultural extension services by the farmers (125). iCow is a mobile application that increases productivity and affordability of many of African farmers by the help of relevant information and knowledge as well as networking with other agricultural players. Comprehensibility of iCow is not only a solution for farmers in support of livestock and crop production, but also supports networking of farmers with other important players in their agricultural sector. Among these players are providers of farm input, providers of financial services to farmers, veterinary officers, providers of extension services, government and non-government organizations and many more (128). Kilimo Salama is farming mobile application focused on the insurance of crops where the farmers insure their farm inputs, seeds and the expected harvest against drought and excess rainfall. This application also promotes collective buying and selling and access to information services (125,129). These apps have greatly improved profitability and farming practices among smallholder farming communities. Hence there seems to be a consensus on the general acceptance of the use of mobile phone apps in agriculture.

The mobile App enabled smallholder farmers in Kwale to provide relevant information that helps to understand and improve their farming practices. Despite this study's limited

dataset, a disparity in gender of smallholder livestock farmers in Kwale was highlighted, with a disproportionately smaller number of women included in farming, suggesting that they are less economically empowered compared to men. Although female farmers had smaller cattle herds compared to their male counterparts, trypanocide non-susceptibility was absent in their herds suggesting that they exercise better farming practices, or managing herds correlated challenges with a lower risk of developing drug non-susceptibility since the cattle was better managed. This was a remarkable observation considering all farmers practice free range farming in common community grazing and watered grounds, and hence the animals had similar rates of disease exposure by the carrier. Therefore, female farmers seem to have more information and knowledgeable on AAT management. This is in agreement with the general observation that female farmers are of great importance in farming (130–134). Female farmers do not have control and access to a range of resources such as income, land, and access to farm extension officers. Traditionally, decision-making is reserved for male farmers, and females are generally not involved. Rural female farmers normally perform household chores that are tedious and exhausting as well as pursuing multiple jobs to improve the living standards. Poultry and livestock husbandry within the homestead is a role performed by women and children (130–134). Hence female farmers may acquire resourceful experience that can help in proper management of MDR. Therefore, gender equality for farmers is important for development of agriculture. Agricultural gender equality is beneficial to female farmers, the agriculture and sub-urban areas, and to the whole community.

There are significant differences in the effects of AAT on the productivity of the two villages. The mobile app results showed that farmers in Kizibe village were adversely affected by AAT than the farmers in Mbegani. Farmers in Mbegani lost fewer cattle due to AAT and their cattle regained normal milk production as compared to those from Kizibe. Therefore, Mbegani farmers have good AAT management practices as opposed to Kizibe farmers. The success in Mbegani village can be attributed to good timing of treatment, good interval between chemotherapeutic and chemoprophylactic agents. Although not easy to determine, the next cattle treatment must be concise.

Trypanosome infections have to be economically and practically balanced by ensuring that trypanocides never lose their protective thresholds (37,49,135–137). The transmissibility of trypanosome reservoir and infection can effectively be limited by trypanosome control using trypanocides. Quick recovery of the cattle can be achieved by effective treatment of the acute stage. Clearance of parasitaemia can be attained by the use of trypanocides at the chronic stage. The severity of the infection which includes damage of organs and lose of weight ends may affect the infection recovery clinically, in some cases requiring significantly more treatment time (37,49,135–137). Therapy response may fail due to chronicity of AAT. This is because of irreversibility of the ferrokinetic disturbances and accompanying dyshaemopoeisis. Hence the cattle affected may remain weak, thin with lack of weight and blood even though they have been treated by trypanocides. (37,49,138). Normally AAT that is acute and chronic can become fatal if diagnosis and treatment is delayed. Severity and morbidity of infection is worsened by these delays. Diagnosis must be prompt and it is important that the right prescription and treatment is provided for the cattle quickly. Earlier treatment of AAT is economically empowering as opposed to the later phase just as it is expected in human African trypanosomiasis management (135–137). Normally six chemical trypanocides are used for prophylaxis of cattle infections by trypanosomes as well as treatment. These compounds are; homidium bromide, quinapyramine sulphate, quinapyramine sulphate:chloride and suramin sodium, diminazene aceturate, homidium chloride and isometamidium chloride (49). Most of these trypanocides have been in use for decades while others have been in use in early 20th century (49). The use of these trypanocides is limited by various factors. Index of therapy is small for all the trypanocides and could result irritation at the site of injection. Critically, prolonged use of these trypanocides has resulted in occurrence of non-susceptible trypanosomes. This coupled with the fact that chemically these trypanocides are related has worsened the challenge of MDR resulting into cross-non-susceptibility cases (94,139). Most of the trypanocides in use today appear to be interested in the loss of the kinetoplast (49), but there is no clear knowledge on the biochemical strategies promoting non-susceptibility as well as the way these trypanocides act. Differences in biochemical physiology and distribution of host organ and biochemical physiology have differences resulting into discrimination

against the veterinary trypanosomes. Hence, the trypanocides have different ways of killing relying on precise potency against each trypanosome species and its distribution that is parametrically affected by pharmacokinetic. Phenanthridine isometamidium is normally used for prophylaxis while it is known that most of the other trypanocides are used therapeutically (37,49,138). Factors such as the infection risk, drug availability of trypanocides and infrastructure have to be considered on whether prophylatic or therapeutic trypanocides should be used (49). It can be suggested that in areas of low incidence such as Mbegani village, that mainly treat those cattle with AAT that is clinically attributable to trypanosomes through diagnosis by use of therapeutic trypanocides. Areas of high incidence such as Kizibe village, prophylactic trypanocides should be used for treatment since they are more economically empowering, thus reducing morbidity and mortality and greatly overcoming the AAT's negative productivity effects (49). Chemoprophylaxis mechanisms are determined by the severity of AAT infection locally. For example, cattle entering a tsetse-infested dry season grazing reserve would benefit from prophylactic trypanocide should be used to treat cattle in tsetse fly ravaged grazing reservoir during a dry season to protect them from being exposed to AAT. Before the beginning of high risk cattle in sedentary herds should be treated (37,49,138). Cattle should receive isometamidium chloride every two to three months to reduce mortality rate. When non-susceptibility to isometamidium chloride emerges, the problem can be overcome by the alternating interval of nine month DA treatment, as prophylaxis would be provided by ISM (37,49,138). DA curative treatment is not prophylactic, due to its rapid metabolism and excretion (47,49,85,140).

It was found that the most common cattle breed was the Boran (107). Boran is a taurine breed and therefore highly susceptible to AAT infection. Hence farmers in Kwale could be advised to venture into breeding other cattle breeds that are indigenous. These African breeds include N'Dama, Muturu and Dahomey (Trail, d'Ieteren, Colardelle, Maille, Ordener, Sauveroche and Yangari (37) that have great non-susceptibility to AAT infection as opposed to foreign breeds such as taurine breeds from Europe and Boran. This trypanotolerance phenomena is the ability to continue surviving as well as maintain productivity after infection by trypanosomes (37,49,138). Cattle are able to live with

trypanosome infections majorly because of their ability to limit both parasitaemia (49,141) and anemia (138). Given that controlling parasitaemia and anemia by cattle has a great impact on regaining of weight, being keen on the rates of infection and haematocrits of cattle has been identified as a useful factor for selecting a trypanotolerant cattle breeds (37). The adoption of trypanotolerant breeds will help improve livestock productivity in Kwale County as well several African regions that are endemic. It is a widely accepted AAT infection control mechanism (37,49,135–137).

It was also found that only 4% of the farmers did not appreciate the app. Smallholder farmers with dissenting views (4%, n=2) were illiterate, and had reservations on the use of information technology. This particular group can be taught to use the application during prospective community engagement activities or have reliable literate proxies to assist them with data entry especially if it can be customized in their local dialects. This can be achieved since the app can be customized to local dialects, has user-friendly web-interface, is cost-effective and receives good technical support making it favorable for use in resource poor settings just like iCow app (128). The ease of use will ensure that it is eventually 100% acceptable by local communities. These results emphasized the potential to evaluate pertinent research questions at vast scales, which are unprecedented due to economic restrictions and the laborious nature of field data collection exercises making the app more flexible. The study provided proof of concept for the viability of using mobile Apps to remotely collect reliable large-scale information from smallholder farmers and veterinary healthcare givers in resource poor settings. This information can be collated remotely, and analysis based on the evidence available can be used to inform policies that improve farming practices and economic wellbeing while restricting widespread multi-drug non-susceptibility. Moreover, this approach can be used to monitor and manage other infectious diseases, in multiple different settings.

It has been demonstrated with HAT, that therapeutically combination of nifurtimox and eflornithine is the most preferred second stage treatment (49,121,142). Currently, there are no trypanocides combination used for the AAT treatment. However, there is the use

of trypanocides such as isometamidium chloride (ISM) and diminazene aceturate (DA) alternatingly (sanative), exhibiting lesser cross-non-susceptibility risk. This mechanism can be used for relapse treatment using different tryoanocide class from the previous one. This reinforces trypanocide non-susceptibility selection (49). Drug non-susceptible pathogen infections cause considerable human and animal mortality and morbidity, and widespread drug non-susceptibility is speedily eroding medical progression in trypanocide use in the past seven decades (143). The efficacy of various trypanocide interventions was examined. The information was useful for designing appropriate policy for the control of trypanosomes as well as drug regimens that will be more effective. Efficacy of drugs which plays crucial role in medicine, is at risk by the fact that there is non-susceptibility evolution. Mutations in the trypanosome genome confer non-susceptibility to trypanocides. These mutations are characteristically unlinked and therefore trypanocide non-susceptibility is normally not linked to non-susceptibility to a non-related trypanocide (144–147). Though not conclusive, it has been suggested that there is progression of MDR is due to the genetic predisposition of the host. MDR is also caused by previous treatment failure attributes like treatment compliance that are inadequate and incomplete. Multi-trypanocide non-susceptibility had been reported in several sub-Saharan African countries (3-5). An evidence-based framework was essential to promote appropriate optimal use of the handful of trypanocides to restrict widespread multi-drug non-susceptibility. Knowledge on best practices on rational use of trypanocides by smallholder farmers and veterinary practitioners was at best still incomplete in the endemic resource poor settings of sub-Saharan Africa. These knowledge gaps were addressed by highlighting seemingly easily perceived but previously obscure strategies of restricting widespread multidrug non-susceptibility. The infectious cattle population was responsive to both changes in treatment and increasing the percentage of cattle receiving treatment caused the infectious population to drop. However, this decrease in the infectious group became much less significant as one moved from low to high levels of non-susceptibility. Particularly, the number of cattle population that is at risk can be reduced by effective treatment if the trypanocide non-susceptibility as well as MDR was in the low transmission areas such as Kizibe as compared to Mbegani village.

It was demonstrated that mathematical models such as trypanosome multi-drug resistance (TMDR) could help suggest thresholds at which treatment of a particular trypanocide or a combination would be effective, and when treatment should be used conservatively to avoid creating more problems by aggravating drug non-susceptibility and MDR. Effect-size (ES) measures gives information about the relative size (positive or negative) of the experimental treatment as opposed to statistical significance tests that provides information about how the experiments results likelihood differs from expectations by chance. An ES analysis compares the average mean of the experimental set with the average mean of the control set. Effect size can be defined with respect to an independent or dependent variable or both. Particularly, effect sizes are crucial since they enable people to compare the size (magnitude) of experimental treatment results in several sets (111–115,148). A balance could be found such that treating a certain percentage of cattle would yield optimal effectiveness per dollar spent. ES is a summarization of studies and research that have been undertaken that compares the outcomes over a variety of studies by use of methods that are quantitative. Traditional statistical techniques that include *t, F* and *chi-square* tests are not conclusive for such comparisons since the sample size is a parameter of the function values of such statistics. Therefore, significance testing almost invariably results in confounding by producing research literature deductions that are misleading (74,110–114,159,160,162). Hence hypotheses testing by significance levels are wrong techniques and can mislead. This is because given different sample sizes, research with the same difference between control and treatment sets conditions can have widely varying *t, F* and *chi-square* tests. Since sample sizes have no effect on ES estimation the estimates are used in meta-analyses. Therefore, the model data can be used to provide ES without possibly changing or replacing it (111–115,148). This is because the measures are simply objective standardization of the size (magnitude) of the effect observed. By standardizing it just refers to the fact that ES can be compared against varying researches that have measured differing variables and therefore involving varied scales of measurement (74,110–114,149). Replication of results can be evaluated by these comparisons since replication cannot be resolved by statistical

significance level tests. ES is commonly interpreted as small (ES = 0.2), medium (ES = 0.5), and large (ES= 0.8) as suggested by several researchers (111–115,148). There was need for optimal threshold for the infected population wide treatment. The effect size clearly suggested that treatment of 80% of the infectious population had the most optimal results. In particular 80% of treatment of infectious cattle by a combination of drugs 2 and 3 was the most effective, while treatment by a single drug was the least effective at 80% treatment. The unboundedness of several research processes fail to respect the natural upper bound of host species systems determined by maximal size. The decision problem can have the natural upper bound incorporated endogenously by formulating a stochastic process focused and describing the infected area growth. Thus, ignoring the upper boundary of the system can be nelected thereby overestimating the option to control value. This leads to treatment delay. Actually, if not sure or AAT is spreading at a faster rate, then not considering the upper bound leads to lack of control deployment. Therefore, the results found here have crucial leads for the way in which the real options on control of AAT are to be used to come up with optimal timing of AAT control and being sure about its future progression (160).

It was demonstrated that combination therapy and treating a larger extent of the infected cattle population resulted in lowest levels of trypanocide non-susceptibility as compared to the use of single trypanocides. Moreover, combination perhaps provides the best result of additive or synergistic effects of these regimens. This is economical in resource poor settings where prior disease diagnosis using rapid test-kits is unavailable. Combination therapy is not commonly practiced for animal African trypanosomiasis management (49). Clinical trials of human African trypanosomiasis show that combination therapy averts or delays the emergence of trypanosome non-susceptibility (121). The combination of drugs with the least non-susceptibility imposes a huge fitness cost to trypanosomes and reduces viability (161,162). This lowers parasitemia levels allowing elimination by the host’s immune response. Typically infections consist of multiple parasites with a wide spectrum of drug non-susceptibility. Therefore trypanosomes with a high fitness cost are likely to be overcome by susceptible trypanosomes where there are no imposing trypanocides, and might require

compensating mutations, which improves the fitness cost to the more susceptible trypanosomes level (163,164). The research findings provide an initial evidence-based framework on some essential practices that promote optimal use of the handful of trypanocides. One technique that can help in the prediction and control of MDR infections is by use of combination of two or more trypanocides in the course of treatment. Even though trypanocide-trypanocide interaction (which must be considered during the manufacturing and development of the trypanocide) is an obstacle in this technique, combination regimen is common and important in various medical areas such as cancer, HIV, malaria, mycobacterium tuberculosis and treatment of bacterial infections (143). It has been shown that the selective advantage of non-susceptible pathogens can be reverted in the process overturning the progression of trypanocide non-susceptibility. Given that treatment with a single trypanocide, selective advantage to non-susceptibility is ever present, and can be inhibited by specific trypanocide combination while prohibiting non-susceptibility to the specific compounds. Methods that exploit physiology and evolution of the interactions between trypanocides are advantageous to non-susceptible mutants (165). When one trypanocide partially suppresses the treatment ability of another trypanocide, non-susceptibility to the first trypanocide will destroy its protective nature against the second trypanocide. This destruction of the protective nature makes sure that the non-susceptible mutants are disadvantaged. Second, non-susceptibility to a trypanocide due to mutations can be hindered when synergy is introduced between the trypanocide and another component. There is synergy in trypanocide combination if its killing ability upon the pathogen is better than that of the single trypanocide, and antagonistic if its ability is weak (165). Non-susceptibility to one trypanocide may result in another trypanocides being collaterally sensitive or to a trypanocide whose toxic effect is mediated by the non-susceptibility strategy. The selective advantage of non-susceptible pathogens competing with the susceptible ones can be inverted by these mechanisms resulting into reduction in the rate at which non-susceptibility progresses, or make non-susceptible trypanosomes become trypanocide susceptible. Treatment efficacy and the risk of non-susceptibility can be differentiated by exploring precise interactions between trypanocides, and the means by which non-susceptibility mutations to a specific

trypanocide can regulate interactions or improve the susceptibility of the pathogen to other trypanocides (166). Drug combination can be subdivided into three classes:

i. targets prohibition in different paths,
ii. different target prohibition in the same path, and
iii. same target prohibition in different paths (143).

Combination of the drugs with the least non-susceptibility imposed a huge fitness cost to trypanosomes. *In vitro* studies on loss of fitness in trypanosomes demonstrate reduced viability (167). This reduced viability resulted in decreased parasitemia that allowed the hosts immune response to effectively eliminate infection. Combination treatment has more merits as opposed to use of large doses of a single trypanocide, and in specific cases combinations of fixed-dose have merits against separate use of a single trypanocide. The merits have among others; improved tolerance, efficacy, compliance, better rates of response, favorable pharmacokinetics alterations, economic viability and reduced chances of trypanocide non-susceptibility progression (145–147). The demerits of combination regimens are normally not as common as those of specific single regimens administered. Metabolically disadvantages are less with combined regimens. The action period is frequently longer than if single trypanocides are used. The spectral of response of two trypanocides in combination increases the likelihood of several trypanosomes responding and improves the probability that the initial regimen choice would succeed. Nevertheless, there are demerits to using combination of fixed-dose such as; loss of dose flexibility, lack of the right doses for one or both of the trypanocides required for treatment of certain conditions (168).

However, in the absence of economic capacities of farmers were also tested on the use of a single trypanocide therapy. Clinical and veterinary experience indicates that it is sufficient to adequately treat a disease by a single drug. Hence treatment of AAT by a single trypanocide is generally successful. A trypanocide and the host immune response alleviate disease provided adequate tissue trypanocide supply is attained for a sufficient time with no interruptions. It has been shown that single trypanocide treatment

is only ineffective in two sets of circumstances. These are; trypanosome infections where the normal cattle body defenses are not sufficient, and either by AAT being chronic, or as a result of high trypanosome mutation evading treatment. Essentially combination regimens are useful in both cases (165). However, given the resource poor setting that this research was performed, most farmers were not willing to purchase two trypanocides at once. Therefore, there must be an alternative to trypanocide combination. It also highlighted that using drugs in increasing non-susceptibility order results in a negligible increase in number of cattle with non-susceptible infections. Progressive increase in fitness cost restricts the cattle number with non-susceptible trypanosomes. Clonal expansion of susceptible parasites due to a drug change eventually results in a reversal of non-susceptibility to drugs that were in long use, and that evoked the most non-susceptibility (164). Cycles of trypanocide use in this order markedly decrease spread of non-susceptible parasites. Hence for those farmers who cannot afford trypanocide combinations, they would be advised to use trypanocides sequentially on increasing non-susceptibility basis to treat cattle. This progressive increase in fitness cost greatly reduces parasitemia levels, and subsequently trypanosome transmission (169,170). The indiscriminate use of trypanocides is not good at all. Pharmacological properties of most trypanocides restrict their importance. These properties must be taken into consideration over the possibility of merits that may evolve due to their utilization in combination regimens (165).

In conclusion, sub-Saharan Africa is normally associated with alarmingly rapid increased pathogen non-susceptibility (17,171). Ensuring detailed and frequent information on the contemporary MDR prevalence for a given trypanosome strain in extensive locales as well as judicious use of trypanocides reaches prescribing extension workers and veterinary officers will greatly curb widespread MDR, especially in high disease burden regions such as Shimba Hills in Kwale County. The control of trypanocide non-susceptibility development requires multiple stakeholders that include: farmers, veterinary officers, extension officers, policy makers, researchers, pharmaceutical companies as well as the government. Technological advances provide new avenues for remote data access in most disease endemic areas. This study has

highlighted the importance of a community-led surveillance app on not only what the farmer is using for treatment of AAT but also for the long-term analysis. These methods are easy to adapt in developing countries, and their success, sustainability and scalability relies on community engagement during implementation. Therefore, the farmers and extension officers have to be educated on the most appropriate way of using trypanocides by policy makers as outlined in the research results and discussion sections. The farmers should be advised that 80% is the optimal infected population wide treatment; trypanocide combination should be used for disease management; and trypanocides used sequentially in increasing order of non-susceptibility to the three respective drugs classes give the best result.

4.2 Recommendation for future studies

Future studies using simulations that examine a reduction in the tsetse fly bite rate parameter, C, due to rearing trypano-tolerant breeds could provide more insight on disease management. There is need for more research on adoption of apps in community surveillance of MDR and for the general livestock management. Customization of the apps into local dialects will greatly improve the acceptability of the apps.

References

1. Wenzler T, Schumann Burkard G, S Schmidt R, Mäser P, Bergner A, Roditi I, et al. A new approach to chemotherapy: drug-induced differentiation kills African trypanosomes. Sci Rep. 2016;6(October 2015):22451.
2. Ruiz-Postigo JA, Franco JR, Lado M, Simarro PP. Human African trypanosomiasis in South Sudan: How can we prevent a new epidemic? PLoS Negl Trop Dis. 2012;6(5):1–7.
3. Sow A, Sidibe I, Bengaly Z, Marcotty T, Sere M, Diallo A, et al. Field detection of resistance to isometamidium chloride and diminazene aceturate in Trypanosoma vivax from the region of the Boucle du Mouhoun in Burkina Faso. Vet Parasitol. 2012 Jun;187(1–2):105–11.
4. Mungube EO, Vitouley HS, Allegye-Cudjoe E, Diall O, Boucoum Z, Diarra B, et al. Detection of multiple drug-resistant Trypanosoma congolense populations in village cattle of south-east Mali. Parasit Vectors. 2012;5:155.
5. Brun R, Balmer O. New developments in human African trypanosomiasis. Curr Opin Infect Dis. 2006;19:415–20.
6. Alingu RA, Muhanguzi D, MacLeod E, Waiswa C, Fyfe J. Bovine trypanosome species prevalence and farmers' trypanosomiasis control methods in south-western Uganda. J S Afr Vet Assoc. 2014 Oct;85(1):1094.
7. Muhanguzi D, Picozzi K, Hatendorf J, Thrusfield M, Welburn SC, Kabasa JD, et al. Collateral benefits of restricted insecticide application for control of African trypanosomiasis on Theileria parva in cattle: a randomized controlled trial. Parasit Vectors [Internet]. 2014;7:432. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25199409%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4262232
8. Leak SGA. Tsetse biology and ecology: their role in the epidemiology and control of trypanosomosis. CAB International, in association with the International Livestock Research Institute, Nairobi, Kenya; 1998.
9. Te Pas MFW. in the Diagnosis and Monitoring of Molecular detection of Trypanosoma infection: PCR and PCR-ELISA techniques. Dev Methodol Use

Polym Chain React Diagnosis Monit Trypanos Final Results a Coord Res Proj 2001–2005. 2007;(June):7–10.

10. Rotureau B, Van Den Abbeele J. Through the dark continent: African trypanosome development in the tsetse fly. Front Cell Infect Microbiol. 2013;3(September):53.
11. Veterinärmedizin F. Tsetse (Diptera : Glossinidae) Bloodmeal Analysis by PCR and Species Differentiation by MALDI TOF MS as Contributions to Rational Vector Control zur Erlangung des Grades eines PhD of Biomedical Sciences. 2012.
12. Thumbi SM, Jung'a JO, Mosi RO, McOdimba FA. Spatial distribution of African Animal Trypanosomiasis in Suba and Teso districts in Western Kenya. BMC Res Notes. 2010;3:6.
13. Nthiwa DM, Odongo DO, Ochanda H, Khamadi S, Gichimu BM. Trypanosoma Infection Rates in Glossina Species in Mtito Andei Division, Makueni County, Kenya. J Parasitol Res. 2015;2015.
14. Turner DA. Tsetse and trypanosomiasis in the Lambwe Valley, Kenya. Trans R Soc Trop Med Hyg. 1986;80(4):592–5.
15. Muraguri GR, McLeod A, McDermott JJ, Taylor N. The incidence of calf morbidity and mortality due to vector-borne infections in smallholder dairy farms in Kwale District, Kenya. Vet Parasitol. 2005 Jun;130(3–4):305–15.
16. Ekuttan CE, Kang'ethe EK, Kimani VN, Randolph TF. Investigation on the prevalence of antimicrobial residues in milk obtained from urban smallholder dairy and non-dairy farming households in Dagoretti Division, Nairobi, Kenya. East Afr Med J. 2007 Nov;84(11 Suppl):S87-91.
17. Liebenehm S, Bett B, Verdugo C, Said M. Optimal Drug Control under Risk of Drug Resistance - The Case of African Animal Trypanosomosis. J Agric Econ [Internet]. 2015;n/a-n/a. Available from: http://hdl.handle.net/10568/69425
18. Mbahin N, Affognon H, Andoke J, Tiberius M, Mbuvi D, Otieno J, et al. Parasitological prevalence of bovine trypanosomosis in Kubo division of Kwale County of coastal: Baseline survey. Am J Anim Vet Sci. 2013;8(1):28–36.
19. McCord PF, Messina JP, Campbell DJ, Grady SC. Tsetse Fly Control in Kenya's

Spatially and Temporally Dynamic Control Reservoirs: A Cost Analysis. Vol. 34, Applied geography (Sevenoaks, England). 2012. p. 189–204.

20. Kubi C, van den Abbeele J, DE Deken R, Marcotty T, Dorny P, van den Bossche P. The effect of starvation on the susceptibility of teneral and non-teneral tsetse flies to trypanosome infection. Med Vet Entomol. 2006 Dec;20(4):388–92.
21. Turner CM, Barry JD, Maudlin I, Vickerman K. An estimate of the size of the metacyclic variable antigen repertoire of Trypanosoma brucei rhodesiense. Parasitology. 1988 Oct;97 (Pt 2):269–76.
22. Weiss BL, Wang J, Maltz MA, Wu Y, Aksoy S. Trypanosome Infection Establishment in the Tsetse Fly Gut Is Influenced by Microbiome-Regulated Host Immune Barriers. PLoS Pathog. 2013;9(4).
23. Franco JR, Simarro PP, Diarra A, Jannin JG. Epidemiology of human African trypanosomiasis. Clin Epidemiol. 2014;6(1):257–75.
24. Brun R, Balmer O. New developments in human African trypanosomiasis. Curr Opin Infect Dis. 2006 Oct;19(5):415–20.
25. Leak SG, Peregrine AS, Mulatu W, Rowlands GJ, D'Ieteren G. Use of insecticide-impregnated targets for the control of tsetse flies (Glossina spp.) and trypanosomiasis occurring in cattle in an area of south-west Ethiopia with a high prevalence of drug-resistant trypanosomes. Trop Med Int Health. 1996 Oct;1(5):599–609.
26. Mulugeta W, Wilkes J, Mulatu W, Majiwa PA, Masake R, Peregrine AS. Long-term occurrence of Trypanosoma congolense resistant to diminazene, isometamidium and homidium in cattle at Ghibe, Ethiopia. Acta Trop. 1997 Apr;64(3–4):205–17.
27. Assefa E, Abebe G. Drug-resistant Trypanosoma congolense in naturally infected donkeys in north Omo Zone, southern Ethiopia. Vet Parasitol. 2001 Aug;99(4):261–71.
28. Geerts S, Holmes PH, Eisler MC, Diall O. African bovine trypanosomiasis: the problem of drug resistance. Trends Parasitol. 2001 Jan;17(1):25–8.
29. Agyemang K. Trypanotolerant livestock in the context of trypanosomiasis intervention strategies. PAAT Tech Sci Ser. 2005;7:1–66.

30. McDermott JJ, Coleman PG. Comparing apples and oranges – model-based assessment of different tsetse-transmitted trypanosomosis control strategies. Int J Parasitol [Internet]. 2001 May 1;31(5–6):603–9. Available from: http://www.sciencedirect.com/science/article/pii/S0020751901001485
31. Rogério dos Santos Alves; Alex Soares de Souza et all. No Title No Title. Igarss 2014. 2014;(1):1–5.
32. Brun R, Blum J, Chappuis F, Burri C. Human African trypanosomiasis. Lancet. 2010 Jan;375(9709):148–59.
33. Aksoy S. Control of tsetse flies and trypanosomes using molecular genetics. Vet Parasitol. 2003 Jul;115(2):125–45.
34. Reichard RE. Area-wide biological control of disease vectors and agents affecting wildlife. Rev Sci Tech [Internet]. 2002;21(1):179–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11974628
35. Ebrahim AY. Biological Control of vector born Protozoan Parasites of Veterinary Importance. Appl Sci Reports [Internet]. 2015;10(3). Available from: http://www.pscipub.com/Journals/Data/JList/Applied Science Reports/2015/Volume 10/Issue 3/1.pdf
36. Mwangi EK, Stevenson P, Gettinby G, Reid SW, Murray M. Susceptibility to trypanosomosis of three Bos indicus cattle breeds in areas of differing tsetse fly challenge. Vet Parasitol. 1998 Sep;79(1):1–17.
37. T0599E01 @ www.fao.org [Internet]. Available from: http://www.fao.org/docrep/004/T0599E/T0599E01.htm
38. Maichomo MW, Ndungu JM, Ngare PM, Ole-Mapenay IM. The performance of Orma Boran and Maasai Zebu crossbreeds in a trypanosomosis endemic area of Nguruman, south western Kenya. Onderstepoort J Vet Res. 2005;72(1):87–93.
39. Rege JEO, Kahi A, Okomo-Adhiambo M, Mwacharo J, Hanotte O. Zebu cattle of Kenya: Uses, performance, farmer preferences and measures of genetic diversity. 2001. 103 p.
40. Unciti-broceta JD, Arias JL, Maceira J, Soriano M. Specific Cell Targeting Therapy Bypasses Drug Resistance Mechanisms in African Trypanosomiasis. 2015;1–20.

41. Genois MM, Paquet ER, Laffitte MC, Maity R, Rodrigue A, Ouellette M, et al. DNA repair pathways in trypanosomatids: from DNA repair to drug resistance. Microbiol Mol Biol Rev [Internet]. 2014;78(1):40–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24600040
42. de la Vega R, Roset R, Castarlenas E, Sanchez-Rodriguez E, Sole E, Miro J. Development and testing of painometer: a smartphone app to assess pain intensity. J Pain. 2014 Oct;15(10):1001–7.
43. Malebo HM, Wenzler T, Cal M, Swaleh SM, Omolo MO, Hassanali A, et al. Anti-protozoal activity of aporphine and protoberberine alkaloids from Annickia kummeriae (Engl. & Diels) Setten & Maas (Annonaceae). BMC Complement Altern Med. 2013;13:48.
44. Grace D, Randolph T, Affognon H, Dramane D, Diall O, Clausen P-H. Characterisation and validation of farmers' knowledge and practice of cattle trypanosomosis management in the cotton zone of West Africa. Acta Trop. 2009 Aug;111(2):137–43.
45. Clausen P-H, Bauer B, Zessin K-H, Diall O, Bocoum Z, Sidibe I, et al. Preventing and containing trypanocide resistance in the cotton zone of West Africa. Transbound Emerg Dis. 2010 Apr;57(1–2):28–32.
46. Holt HR, Selby R, Mumba C, Napier GB, Guitian J. Assessment of animal African trypanosomiasis (AAT) vulnerability in cattle-owning communities of sub-Saharan Africa. Parasit Vectors. 2016 Jan;9:53.
47. Mungube EO, Vitouley HS, Allegye-cudjoe E, Diall O, Boucoum Z, Diarra B, et al. Detection of multiple drug-resistant Trypanosoma congolense populations in village cattle of. 2012;1–9.
48. Mungube EO, Diall O, Baumann MPO, Hoppenheit A, Hinney B, Bauer B, et al. Best-bet integrated strategies for containing drug-resistant trypanosomes in cattle. Parasit Vectors [Internet]. 2012;5(1):1. Available from: Parasites & Vectors
49. GIORDANI F, MORRISON LJ, ROWAN TG, DE KONING HP, BARRETT MP. The animal trypanosomiases and their chemotherapy: a review. Parasitology [Internet]. 2016;1–28. Available from: http://www.journals.cambridge.org/abstract_S0031182016001268

50. Cohen JE, Gürtler RE. Modeling household transmission of American trypanosomiasis. Science [Internet]. 2001;293(5530):694–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11474111
51. Lipsitch M, Bergstrom CT, Levin BR. The epidemiology of antibiotic resistance in hospitals: paradoxes and prescriptions. Proc Natl Acad Sci U S A. 2000;97(4):1938–43.
52. Rogers DJ. A general model for the African trypanosomiases. Parasitology. 1988 Aug;97 (Pt 1):193–212.
53. Ssematimba a., Mugisha JYT, Luboobi LS. Mathematical Models for the Dynamics of Tuberculosis in Density-dependent Populations: The Case of Internally Displaced Peoplesâ€TM Camps (IDPCs) in Uganda. J Math Stat. 2005;1(3):217–24.
54. Grimm V, Berger U, Bastiansen F, Eliassen S, Ginot V, Giske J, et al. A standard protocol for describing individual-based and agent-based models. Ecol Modell. 2006;198(1–2):115–26.
55. Cano DC. Application of Computer Algebra into the Analysis of a Malaria Model using MAPLE TM. Unknown. 2008;
56. Grimm V, Grimm V, Revilla E, Berger U, Jeltsch F, Mooij WM, et al. Pattern-Oriented Modeling of Agent-Based Complex Systems : Lessons from Ecology. 2008;987(2005).
57. Ermert V, Fink AH, Jones AE, Morse AP. Development of a new version of the Liverpool Malaria Model . II . Calibration and validation for West Africa. Malar J [Internet]. 2011;10(1):62. Available from: http://www.malariajournal.com/content/10/1/62
58. Ermert V, Fink AH, Jones AE, Morse AP. Development of a new version of the Liverpool Malaria Model . I . Refining the parameter settings and mathematical formulation of basic processes based on a literature review. Malar J [Internet]. 2011;10(1):35. Available from: http://www.malariajournal.com/content/10/1/35
59. Rogers C, Kribs-zaleta CM. Seasonality in a Two-strain Competition Model for Trypanosoma Cruzi Transmission. U Arlingt Math Prepr Ser. 2013;
60. Prof S, Hargrove J. Modelling the control of tsetse and African trypanosomiasis

through application of insecticides on cattle in Southeastern Uganda by. 2013;(March).

61. Capriles PVSZ, Guimarães ACR, Otto TD, Miranda AB, Dardenne LE, Degrave WM. Structural modelling and comparative analysis of homologous, analogous and specific proteins from Trypanosoma cruzi versus Homo sapiens: putative drug targets for chagas' disease treatment. BMC Genomics. 2010;11:610.
62. Hargrove JW, Ouifki R, Kajunguri D, Vale G a., Torr SJ. Modeling the control of trypanosomiasis using trypanocides or insecticide-treated livestock. PLoS Negl Trop Dis. 2012;6(5).
63. Lipsitch M, Singer RS, Levin BR. Antibiotics in agriculture: when is it time to close the barn door? Proc Natl Acad Sci U S A. 2002;99(9):5752–4.
64. Lipsitch M, Samore MH. Antimicrobial use and antimicrobial resistance: A population perspective. Emerg Infect Dis. 2002;8:347–54.
65. Lipsitch M, Cohen T, Murray M, Levin BR. Antiviral Resistance and the Control of Pandemic Influenza. PLoS Med [Internet]. 2007;4(1):e15. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17253900
66. Lipsitch M, Bacon TH, Leary JJ, Levin BR, Antia R. Effects of Antiviral Usage on Transmission Dynamics of Herpes Simplex Virus Type 1 and on Antiviral Resistance : Predictions of Mathematical Models Effects of Antiviral Usage on Transmission Dynamics of Herpes Simplex Virus Type 1 and on Antiviral Resista. 2000;44(10):2824–35.
67. Lipsitch M, Sousa AO. Historical intensity of natural selection for resistance to tuberculosis. Genetics. 2002;161(August):1599–607.
68. Lipsitch M, Levin BR. The population dynamics of antimicrobial chemotherapy. Antimicrob Agents Chemother. 1997;41(2):363–73.
69. Manuscript A. NIH Public Access. Changes. 2012;29(4):997–1003.
70. Wang YC, Lipsitch M. Upgrading antibiotic use within a class: tradeoff between resistance and treatment success. Proc Natl Acad Sci U S A. 2006;103(25):9655–60.
71. Austin DJ, Anderson RM. Studies of antibiotic resistance within the patient ,

hospitals and the community using simple mathematical models. 1999;
72. Shrestha S, Lloyd-Smith JO. Introduction to mathematical modeling of infectious diseases. Model Paradig Anal Dis Transm Model. 2010;0(75):1–47.
73. Adetunde IA, Yawson N. Modelling the Epidemiology of Malaria : A Case Study of Wassa West District in the Western Region of Ghana. 2010;2(3):105–11.
74. Smith DL, Battle KE, Hay SI, Barker CM, Scott TW, McKenzie FE. Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. PLoS Pathog. 2012;8(4).
75. Fao. Training Manual for TSETSE CONTROL PERSONNEL. Fao. 1982;2:1–105.
76. Covell G. Tsetse Flies. Br Med J. 1955;2:1014.
77. Oie. Trypanosomosis (tsetse-transmitted). www.oie.int/fileadmin/Home/eng/..in./TRYPANO_TSETSE.pdf. 2013;(May):1–5.
78. Baral TN. Immunobiology of African trypanosomes: Need of alternative interventions. J Biomed Biotechnol. 2010;2010.
79. Prowse E. Trypanosomosis , the disease and its control: An analysis of a new tsetse-repellent technology. 2005;32.
80. Jackson AP, Berry A, Aslett M, Allinson HC, Burton P, Vavrova-Anderson J, et al. Antigenic diversity is generated by distinct evolutionary mechanisms in African trypanosome species. Pnas. 2012;109(9):3416–21.
81. Shiferaw S, Muktar Y, Belina D. A review on trypanocidal drug resistance in Ethiopia. 2015;7(May):58–66.
82. Peregrine M. Whether to blow the whistle requires careful consideration. Mod Healthc. 1991 Jul;21(27):26.
83. Chitanga S, Marcotty T, Namangala B, van den Bossche P, van den Abbeele J, Delespaux V. High prevalence of drug resistance in animal trypanosomes without a history of drug exposure. PLoS Negl Trop Dis. 2011;5(12).
84. Delespaux V, Geysen D, Van den Bossche P, Geerts S. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol. 2008 May;24(5):236–42.
85. Delespaux V, de Koning HP. Drugs and drug resistance in African trypanosomiasis. Drug Resist Updat. 2007;10:30–50.

86. Delespaux V, Geysen D, Majiwa PAO, Geerts S. Identification of a genetic marker for isometamidium chloride resistance in Trypanosoma congolense. Int J Parasitol. 2005 Feb;35(2):235–43.
87. Borst P, Elferink RO. Mammalian ABC transporters in health and disease. Annu Rev Biochem. 2002;71:537–92.
88. Eisler MC, Brandt J, Bauer B, Clausen PH, Delespaux V, Holmes PH, et al. Standardised tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle. Vet Parasitol. 2001 Jun;97(3):171–82.
89. Shapiro T a, Englund PT. Selective cleavage of kinetoplast DNA minicircles promoted by antitrypanosomal drugs. Proc Natl Acad Sci U S A. 1990;87(3):950–4.
90. Wang Z, Englund PT. RNA interference of a trypanosome topoisomerase II causes progressive loss of mitochondrial DNA. EMBO J. 2001;20(17):4674–83.
91. Boibessot I, Turner CMR, Watson DG, Goldie E, Connel G, McIntosh A, et al. Metabolism and distribution of phenanthridine trypanocides in Trypanosoma brucei. Acta Trop. 2002 Dec;84(3):219–28.
92. Macgregor JT, Johnson IJ. In vitro metabolic activation of ethidium bromide and other phenanthridinium compounds: mutagenic activity in Salmonella typhimurium. Mutat Res. 1977;48(1):103–7.
93. Delespaux V, Vitouley HS, Marcotty T, Speybroeck N, Berkvens D, Roy K, et al. Chemosensitization of Trypanosoma congolense strains resistant to isometamidium chloride by tetracyclines and enrofloxacin. PLoS Negl Trop Dis. 2010;4(9).
94. Peregrine AS, Gray MA. Cross-Resistance Associated with Development of Resistance to Isometamidium in a Clone of Trypanosoma congolense †. 1997;41(7):1604–6.
95. Delespaux V, Chitanga S, Geysen D, Goethals A, van den Bossche P, Geerts S. SSCP analysis of the P2 purine transporter TcoAT1 gene of Trypanosoma congolense leads to a simple PCR-RFLP test allowing the rapid identification of diminazene resistant stocks. Acta Trop. 2006 Nov;100(1–2):96–102.

96. Carter NS, Fairlamb AH. Arsenical-resistant trypanosomes lack an unusual adenosine transporter. Nature. 1993;361(6408):173–6.
97. Witola WH, Tsuda A, Inoue N, Ohashi K, Onuma M. Acquired resistance to berenil in a cloned isolate of Trypanosoma evansi is associated with upregulation of a novel gene, TeDR40. Parasitology. 2005 Nov;131(Pt 5):635–46.
98. Geerts S, Holmes PH, Eisler MC, Diall O. African bovine trypanosomiasis: the problem of drug resistance. Trends Parasitol [Internet]. 2016 May 3;17(1):25–8. Available from: http://dx.doi.org/10.1016/S1471-4922(00)01827-4
99. Diarra B, Diall O, Geerts S, Kageruka P, Lemmouchi Y, Schacht E, et al. Field evaluation of the prophylactic effect of an isometamidium sustained-release device against trypanosomiasis in cattle. Antimicrob Agents Chemother. 1998;42(5):1012–4.
100. Carrara VI, Zwang J, Ashley EA, Price RN, Stepniewska K, Barends M, et al. Changes in the treatment responses to artesunate-mefloquine on the northwestern border of Thailand during 13 years of continuous deployment. PLoS One. 2009;4(2):1–11.
101. Raja A, Tridane A, Gaffar A, Lindquist T, Pribadi K. Android and ODK based data collection framework to aid in epidemiological analysis. 2014;5(3):1–27.
102. Remote P, Centre S. Mobile Data Collection Using an Android Device. 2013;8491:200–2.
103. Flannery M, Budden DM, Mendes A. FlexDM : Simple , parallel and fault-tolerant data mining using WEKA. Source Code Biol Med. 2015;8–11.
104. Steiner A, Hella J, Gru S, Mhalu G, Mhimbira F, Cercamondi CI, et al. Managing research and surveillance projects in real-time with a novel open-source e Management tool designed for under-resourced countries. 2016;916–23.
105. Thumbi SM, Mcodimba FA, Mosi RO, Jung JO. Parasites & Vectors Comparative evaluation of three PCR base diagnostic assays for the detection of pathogenic trypanosomes in cattle blood. 2008;7:1–7.
106. Chitnis N, Hyman JM, Cushing JM. Determining important parameters in the spread of malaria through the sensitivity analysis of a mathematical model. Bull Math Biol. 2008;70(5):1272–96.

107. Epidemiology C. Epidemiology of human African trypanosomiasis. 2014;257–75.
108. Moore S, Shrestha S, Tomlinson KW, Vuong H. Predicting the effect of climate change on African trypanosomiasis : integrating epidemiology with parasite and vector biology. 2012;(November 2011):817–30.
109. Milligan PJ, Baker RD. A model of tsetse-transmitted animal trypanosomiasis. Parasitology. 1988 Feb;96 (Pt 1):211–39.
110. Merchan-Baeza JA, Gonzalez-Sanchez M, Cuesta-Vargas A. Clinical effect size of an educational intervention in the home and compliance with mobile phone-based reminders for people who suffer from stroke: protocol of a randomized controlled trial. JMIR Res Protoc. 2015;4(1):e33.
111. Vacha-haase T, Thompson B. How to Estimate and Interpret Various Effect Sizes. 2004;51(4):473–81.
112. Kelley K, Preacher KJ. On effect size. Psychol Methods [Internet]. 2012;17(2):137–52. Available from: http://doi.apa.org/getdoi.cfm?doi=10.1037/a0028086
113. Thalheimer W, Cook S, Thalheimer W, Cook S. How to calculate effect sizes from published research : A simplified methodology How to calculate effect sizes from published research articles : 2002;(August).
114. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. Front Psychol. 2013;4(NOV):1–12.
115. Hypothesis N, Testing S, Field A. Effect Sizes. 2005;8057:1–9.
116. Antonio J, Gonzalez-sanchez M, Cuesta-vargas A. Clinical Effect Size of an Educational Intervention in the Home and Compliance With Mobile Phone-Based Reminders for People Who Suffer From Stroke : Protocol of a Randomized Controlled Trial Corresponding Author : 2015;4(1).
117. Mdachi RE, Murilla GA, Omukuba JN, Cagnolati V. Disposition of diminazene aceturate (Berenil) in trypanosome-infected pregnant and lactating cows. Vet Parasitol. 1995 Jun;58(3):215–25.
118. Murilla GA, Mdachi RE, Ismail AA, Karanja WM. Bioavailability, pharmacokinetics, and tissue distribution of 14C homidium after parenteral administration to Boran cattle. J Vet Pharmacol Ther. 1996 Apr;19(2):142–8.

119. Kohanski MA, Dwyer DJ, Collins JJ. How antibiotics kill bacteria: From targets to networks. Nat Rev Microbiol [Internet]. 2010;8(6):423–35. Available from: http://dx.doi.org/10.1038/nrmicro2333
120. Pepin J. Combination Therapy for Sleeping Sickness : A Wake-Up Call. 2007;195:311–3.
121. Priotto G, Kasparian S, Ngouama D, Ghorashian S, Arnold U, Ghabri S, et al. Nifurtimox-eflornithine combination therapy for second-stage Trypanosoma brucei gambiense sleeping sickness: a randomized clinical trial in Congo. Clin Infect Dis. 2007 Dec 1;45(11):1435–42.
122. Okello WO, Muhanguzi D, MacLeod ET, Welburn SC, Waiswa C, Shaw AP. Contribution of draft cattle to rural livelihoods in a district of southeastern Uganda endemic for bovine parasitic diseases: an economic evaluation. Parasit Vectors. 2015 Jan;8:571.
123. Muhanguzi D, Okello WO, Kabasa JD, Waiswa C, Welburn SC, Shaw APM. Cost analysis of options for management of African Animal Trypanosomiasis using interventions targeted at cattle in Tororo District; south-eastern Uganda. Parasit Vectors. 2015 Jan;8:387.
124. Chitanga S, Marcotty T, Namangala B, Van den Bossche P, Van Den Abbeele J, Delespaux V. High prevalence of drug resistance in animal trypanosomes without a history of drug exposure. PLoS Negl Trop Dis. 2011 Dec;5(12):e1454.
125. Masiero S. ECONOMIC AND PRIVATE SECTOR Innovation and Best Practice in Mobile Technologies for Development. 2013;(February). Available from: https://assets.publishing.service.gov.uk/media/57a08a48ed915d622c000661/Innovation_in_mobile_technologies_for_development.pdf
126. Mbiti I, Weil D. Mobile Banking: The Impact of M-Pesa in Kenya. 2011;(May). Available from: http://www.nber.org/papers/w17129.pdf
127. m-pesa-api @ www.safaricom.co.ke [Internet]. Available from: https://www.safaricom.co.ke/about/innovation/technology-innovation/m-pesa-api
128. index @ www.icow.co.ke [Internet]. Available from: http://www.icow.co.ke/
129. index @ kilimosalama.wordpress.com [Internet]. Available from: https://kilimosalama.wordpress.com/

130. Eerdewijk A Van, Danielsen K. Gender Matters in Farm Power. 2015;(February):73. Available from: https://213ou636sh0ptphd141fqei1-wpengine.netdna-ssl.com/gender/wp-content/uploads/publications/56fe4a6ced6cd_Gender Matters in Farm Power.pdf
131. Hoppe R, Korb P. Characteristics of Women Farm Operators and Their Farms. Econ Inf Bull - USDA Econ Res Serv [Internet]. 2013;(111):+44. Available from: http://www.ers.usda.gov/media/1093194/eib111.pdf
132. FAO. State of Food and Agriculture 2010-2011 [Internet]. Vol. 2, Lancet. 2011. 147 p. Available from: http://www.fao.org/docrep/013/i2050e/i2050e.pdf
133. Nisbet JD, Watt JS. Case Study. 1984;102–20.
134. Doss C, Raney T, Anríquez G, Croppenstedt A, Gerosa S, Lowde S, et al. The role of women in agriculture. Food Agric Organ United NAtions [Internet]. 2011;(11):1–48. Available from: http://www.fao.org/docrep/013/am307e/am307e00.pdf
135. Acup C, Bardosh KL, Picozzi K, Waiswa C, Welburn SC. Factors influencing passive surveillance for T. b. rhodesiense human african trypanosomiasis in Uganda. Acta Trop [Internet]. 2017;165:230–9. Available from: http://dx.doi.org/10.1016/j.actatropica.2016.05.009
136. Migchelsen SJ, Büscher P, Hoepelman AIM, Schallig HDFH, Adams ER. Human African trypanosomiasis: A review of non-endemic cases in the past 20 years. Int J Infect Dis. 2011;15(8).
137. Fyfe J, Picozzi K, Waiswa C, Bardosh KL, Welburn SC. Impact of mass chemotherapy in domestic livestock for control of zoonotic T. b. rhodesiense human African trypanosomiasis in Eastern Uganda. Acta Trop [Internet]. 2017;165:216–29. Available from: http://dx.doi.org/10.1016/j.actatropica.2016.08.022
138. ipc-FAO-trypanosomosis-diagnosis-manual-1998.pdf.
139. Byarugaba DK. Antimicrobial Resistance in Developing Countries [Internet]. Antimicrobial Resistance in Developing Countries. 2009. 15-27 p. Available from: http://hdl.handle.net/2299/1942%5Cnhttp://link.springer.com/10.1007/978-0-387-89370-9

140. Chitanga S, Marcotty T, Namangala B, van den Bossche P, van den Abbeele J, Delespaux V. High prevalence of drug resistance in animal trypanosomes without a history of drug exposure. PLoS Negl Trop Dis. 2011;5(12).
141. Singh R, Kone BC, Gounni AS, Uzonna JE. Molecular Regulation of Trypanosoma congolense- Induced Nitric Oxide Production in Macrophages. 2013;8(3).
142. Alirol E, Schrumpf D, Amici Heradi J, Riedel A, de Patoul C, Quere M, et al. Nifurtimox-eflornithine combination therapy for second-stage gambiense human African trypanosomiasis: Médecins Sans Frontières experience in the Democratic Republic of the Congo. Clin Infect Dis. 2013 Jan 16;56(2):195–203.
143. Worthington RJ, Melander C. NIH Public Access. Trends Biotechnol. 2014;31(3):177–84.
144. Pan F, Chernew ME, Fendrick AM. Impact of fixed-dose combination drugs on adherence to prescription medications. J Gen Intern Med. 2008;23(5):611–4.
145. Article R, Science N. International research journal of pharmacy. 2012;3(11):17–21.
146. Wang B, Choudhry NK, Gagne JJ, Landon J, Kesselheim AS. Availability and utilization of cardiovascular fixed-dose combination drugs in the United States. Am Heart J [Internet]. 2015;169(3):379–386.e1. Available from: http://dx.doi.org/10.1016/j.ahj.2014.12.014
147. Sharma SK, Mohan a. Multidrug-resistant tuberculosis. Indian J Med Res. 2004;120(4):354–76.
148. Becker LA. Effect Size (ES). 2000;(1993).
149. Spannagl M, Martis MM, Pfeifer M, Nussbaumer T, Mayer KF. Analysing complex Triticeae genomes - concepts and strategies. Plant Methods [Internet]. 2013;9(1):35. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3847682&tool=pmcentrez&rendertype=abstract
150. The HFOR, Of E, Of O. Statistical Methods for Genome Wide Association Studies. 2012. 1-43 p.
151. Wang WYS, Barratt BJ, Clayton DG, Todd JA. GENOME-WIDE ASSOCIATION

STUDIES : THEORETICAL AND PRACTICAL CONCERNS. 2006;
152. Biology C. Genome-Wide Association Studies. 2012;
153. Paper O, Holmen H, Wahl AK, Jenum AK, Ribu L. A Mobile Health Intervention for Self-Management and Lifestyle Change for Persons With Type 2 Diabetes , Part 2 : One-Year Results From the Norwegian Randomized Controlled Trial Corresponding Author : Related Article : 2014;2.
154. Bolker BM. Ecological Models and Data in R. Ecology [Internet]. 2007;408. Available from: http://books.google.com/books?hl=en&lr=&id=7ZdtfhGHoz8C&oi=fnd&pg=PP2&dq=Ecological+Models+and+Data+in+R&ots=aZwx8lkksC&sig=r7LiufBUdrW_0t06xtTjy2JMeWU
155. Little J, Ioannidis JPA. Genome-wide association studies for complex traits : consensus , uncertainty and challenges. 2008;9(May):356–69.
156. Harris RC, Khan MS, Martin LJ, Allen V, Moore DAJ, Fielding K, et al. The effect of surgery on the outcome of treatment for multidrug-resistant tuberculosis: a systematic review and meta-analysis. BMC Infect Dis [Internet]. 2016;16(1):262. Available from: http://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-016-1585-0
157. Agboola S, Kamdar M, Flanagan C, Searl M, Traeger L, Kvedar J, et al. Pain management in cancer patients using a mobile app: study design of a randomized controlled trial. JMIR Res Protoc. 2014;3(4):e76.
158. Donker T, Petrie K, Proudfoot J, Clarke J, Birch M-R, Christensen H. Smartphones for smarter delivery of mental health programs: a systematic review. J Med Internet Res. 2013;15(11):e247.
159. Stankovich J. Statistical analysis of genome-wide association (GWAS) data.
160. Dangerfield CE, Whalley AE, Hanley N, Gilligan CA. What a Difference a Stochastic Process Makes: Epidemiological-Based Real Options Models of Optimal Treatment of Disease. Environ Resour Econ. 2017;1–21.
161. w9791e00 @ www.fao.org [Internet]. Available from: http://www.fao.org/docrep/003/W9791E/w9791e00.HTM
162. export @ pure.itg.be [Internet]. Available from:

http://pure.itg.be/en/publications/drug-management-and-parasite-resistance-in-bovine-trypanosomiasis-in-africa(00712b8e-41ed-4c36-aeef-cf289f5c8369)/export.html

163. Vaumourin E, Vourc G, Gasqui P, Vayssier-taussat M. The importance of multiparasitism : examining the consequences of co- infections for human and animal health. Parasit Vectors [Internet]. 2015;1–13. Available from: http://dx.doi.org/10.1186/s13071-015-1167-9
164. Laufer MK, Takala-Harrison S, Dzinjalamala FK, Stine OC, Taylor TE, Plowe C V. Return of chloroquine-susceptible falciparum malaria in Malawi was a reexpansion of diverse susceptible parasites. J Infect Dis. 2010 Sep;202(5):801–8.
165. Elek SD. Principles and problems of combined antibiotic therapy. Postgrad Med J. 1956;32:324–7.
166. Baym M, Stone LK, Kishony R. Multidrug evolutionary strategies to reverse antibiotic resistance. Science (80-) [Internet]. 2016;351(6268):aad3292-aad3292. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.aad3292
167. Division I. M-27 isbn 92-5-104. 1998;
168. Neutel JM. The role of combination therapy in the management of hypertension. Nephrol Dial Transplant [Internet]. 2006;21(6):1469–73. Available from: http://ndt.oxfordjournals.org/cgi/doi/10.1093/ndt/gfk064
169. Arya LK, Rathinam SR, Prajna L, Kim U, Tandon V. Presumed trematode induced granulomatousuveitis in south India. Dep Zool [Internet]. 2010; Available from: http://www.klyuniv.ac.in/22ndncp/Proceedings of 22nd NCP_Kalyani.pdf#page=137
170. Orangerie DEL, Al D. 19th International Mouse Genome Conference Final Program & Book of Abstracts Strasbourg Expo-Congrés Centre , Strasbourg , France 19th International Mouse Genome Conference Strasbourg. 2005;
171. Omulo S, Thumbi SM, Njenga MK, Call DR. A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: what can be done better? Antimicrob Resist Infect Control. 2015;4(1):1.

Appendix A: Matlab script (Matlab 2017a)

```
function Ibrahim()
clear all; clc;
global lambdav lambdah miuh miuv omegah omegav rhoh thetah  deltah  deltav ...
lambda1 lambda2 lambda3 nitaI nitaT nita1 nita2 nita3 nita12 nita13 nita23 ...
a1 a2 a3 a12 a13 a23 lambda12 lambda13 lambda23 lambda21 lambda31 lambda32

lambdav=1000;miuv=0.03;omegav=0.091;
lambdah=6;miuh=0.0005;omegah=0.8;
rhoh=0.045; thetah=1.0;  deltah=0.002;  deltav=0.0165;
nitaI=0.01;nitaT=0.14;nita1=0.003;nita2=0.006;nita3=0.009;nita12=0.03;nita13=0.035;ni
ta23=0.04;
lambda1=0.11;lambda2=0.11;lambda3=0.11;
lambda12=0.0095;lambda13=0.0095;lambda23=0.0095;lambda21=0.0095;lambda31=0
.0095;lambda32=0.0095;
betah=0.46; betav=0.025; ch=0.75;cv=0.75;
a1=0.3;a2=0.2;a3=0.1;a12=0.5;a13=0.4;a23=0.3;
options = odeset('RelTol',1e-4,'AbsTol',[1e-4 1e-4 1e-4 1e-4 1e-4 1e-4 1e-4 1e-4 1e-4
1e-4 1e-4 1e-4 1e-4 1e-4]);
[Th,H] = ode45(@host,[0 1095],[3500 250 450 0 133 133 134 133 133 134 0 15000
4000 1000],options);

figure(1)
hold on

plot(Th,H(:,5),'k',          Th,H(:,6),'m',Th,H(:,7),'c',Th,H(:,8),'r',Th,H(:,9),'g',Th,H(:,10),'b',
'linewidth',2)
set(gca,'fontsize',30)

title('Long term population values-High non-susceptibility case (three years)')
xlabel('Time (Days)') % x-axis label
set(gca,'fontsize',30)
ylabel('Number of cattle') % y-axis label
set(gca,'fontsize',25)
legend('Non-susceptibility to drug 1', 'Non-susceptibility to drug 2', 'Non-susceptibility to
drug 3',...
    'Non-susceptibility to drugs 1 & 2', 'Non-susceptibility to drugs 1 & 3', 'Non-
susceptibility to drugs 2 & 3', 'Location', 'East');
hold off
% H(249,1)
H(:,2)
H1T = sum(H(:,1));
H1M = mean(H(:,1));
H1SD = std(H(:,1));
```

```
H2T = sum(H(:,2));
H2M = mean(H(:,2));
H2SD = std(H(:,2));

H3T = sum(H(:,3));
H3M = mean(H(:,3));
H3SD = std(H(:,3));

H4T = sum(H(:,4));
H4M = mean(H(:,4));
H4SD = std(H(:,4));

H5T = sum(H(:,5));
H5M = mean(H(:,5));
H5SD = std(H(:,5));

H6T = sum(H(:,6));
H6M = mean(H(:,6));
H6SD = std(H(:,6));

H7T = sum(H(:,7));
H7M = mean(H(:,7));
H7SD = std(H(:,7));

H8T = sum(H(:,8));
H8M = mean(H(:,8));
H8SD = std(H(:,8));

H9T = sum(H(:,9));
H9M = mean(H(:,9));
H9SD = std(H(:,9));

H10T = sum(H(:,10));
H10M = mean(H(:,10));
H10SD = std(H(:,10));


ES58 =(H5M-H8M)/H8SD
ES59 =(H5M-H9M)/H9SD
ES510 =(H5M-H10M)/H10SD
%
ES68 =(H6M-H8M)/H8SD
ES69 =(H6M-H9M)/H9SD
ES610 =(H6M-H10M)/H10SD
%
ES78 =(H7M-H8M)/H8SD
```

```
ES79 =(H7M-H9M)/H9SD
ES710 =(H7M-H10M)/H10SD

ES56 =(H5M-H6M)/H6SD
ES57 =(H5M-H7M)/H7SD
ES67 =(H6M-H7M)/H7SD

ES89 =(H8M-H9M)/H9SD
ES810 =(H8M-H10M)/H10SD
ES910 =(H9M-H10M)/H10SD

% H(end,2)
% H(end,3)
% H(end,4)
% H(end,5)
% H(end,6)
% H(end,7)
% H(end,8)
% H(end,9)
% H(end,10)

   function dH=host(t,H)
      %H(1)=Sh;H(2)=Eh;H(3)=Ih;H(4)=Th;H(5)=Rh1;H(6)=Rh2;.....
      dH=zeros(14,1);
Nv= H(12)+ H(13)+H(14);
Nh= H(1)+H(2)+ H(3)+H(4)+H(5)+H(6)+H(7)+H(8)+H(9)+H(10)+H(11);
fh=betah*ch*H(14)./Nv;
fv=betav*cv*(nitaI*H(3)+nitaT*H(4)+nita1*H(5)+nita2*H(6)+nita3*H(7)+nita12*H(8)+nita1
3*H(9)+nita23*H(10))./Nh;
     dH(1)=lambdah-fh*H(1)+rhoh*H(11)-miuh*H(1);
     dH(2)=fh*H(1)-(omegah+miuh)*H(2);
     dH(3)=omegah*H(2)-(thetah+nitaI+deltah+miuh)*H(3);
     dH(4)=thetah*H(3)-(lambda1+lambda2+lambda3+nitaT+deltah+miuh)*H(4);
     dH(5)=lambda1*H(4)-
(lambda2+lambda3+nita1+a1*deltah+miuh)*H(5)+lambda1*(H(6)+H(7));
     dH(6)=lambda2*H(4)-
(lambda1+lambda3+nita2+a2*deltah+miuh)*H(6)+lambda2*(H(5)+H(7));
     dH(7)=lambda3*H(4)-
(lambda1+lambda2+nita3+a3*deltah+miuh)*H(7)+lambda3*(H(5)+H(6));
     dH(8)=(lambda12+lambda21)*H(4)+lambda12*H(6)+lambda21*H(5)-
(nita12+a12*deltah+miuh)*H(8);
     dH(9)=(lambda13+lambda31)*H(4)+lambda13*H(7)+lambda31*H(5)-
(nita13+a13*deltah+miuh)*H(9);
     dH(10)=(lambda23+lambda32)*H(4)+lambda23*H(7)+lambda32*H(6)-
(nita23+a23*deltah+miuh)*H(10);
     dH(11)=nitaI*H(3)+nitaT*H(4)+nita1*H(5)+nita2*H(6)+nita3*H(7)...
```

```
        +nita12*H(8)+nita13*H(9)+nita23*H(10)-(rhoh+miuh)*H(11);

      dH(12)=lambdav-fv.*H(12)-miuv*H(12);
      dH(13)=fv.*H(12)-(omegav+miuv)*H(13);
      dH(14)=omegav*H(13)-(miuv)*H(14);
    end

end
```

Appendix B: Closed ended questionnaire

Evaluation of drug use by farmers in Shimba Hills in the fight against nagana

Section to be filled by veterinary officer

Name of area (locale) where attached –

Which villages in your locale are worst hit by the plague?

Name them.....

Which areas report the least infection rates?

Name them.....

What is the cost of a single drug regimen per animal? .....

Disease and drug awareness data

1. How often are you contacted by the farmers over trypanosiamiasis?
   A. Once a week
   B. Twice a week
   C. Once a month
   D. Other (*Please specify*).....

2. At what point do the farmers contact you?
   A. Immediately they notice illness
   B. After self-medicating
   C. As a last resort
   D. Other (*please specify*).....

3. Are farmers generally open and ready to provide information?
   A. yes
   B. no

4. If NO, why?
   A. Myths and suspicions
   B. Ignorance
   C. By choice with no particular reason
   D. Other (*please specify*).....

Diagnosis and prescription patterns

5. How many animals in a week do you think you treat with nagana ?
   A. 1
   B. 2
   C. 3
   D. Other (*please specify*).....

6. How do you go about diagnosing an animal?
   A. I know it is nagana from experience
   B. I collect samples for lab analysis and confirmation
   C. I rely on my colleagues
   D. I rely on the farmers knowledge

7. Are you always confident in your final prognosis*?*
   A. YES
   B. NO

8. Do you experience any difficulties during diagnosis? *If YES, please give details.*
   A. YES
   B. NO

9. If an APP was developed to aid your process, would you use it?
   A. YES
   B. NO

10. Which drug(s) are you most likely to prescribe?
    A. Homidium
    B. Isometamidium
    C. Diminazene
    D. Other (*please specify*).....

11. At what stage of the disease do you prescribe the named drug (s)?
    A. Before onset (prophylaxis)
    B. Early onset
    C. Late disease stage
    D. Other (*please specify*).....

12. What is your reason for choosing the above named drug (s)?
    A. Most available
    B. Most affordable
    C. Most effective

D. Other (*please specify*).....

Drug use and efficacy and toxicity

13. Do the farmers stick to the regimen given?
    A. Yes
    B. no
14. Have you noticed any seasonal variation in drug sensitivity?
    A. Yes
    B. no
15. Have you ever prescribed any of the drugs used to treat nagana for another condition other that nagana in cattle?
    A. Yes
    B. no
16. Do you know of any local remedies used by local farmers as an alternative to chemical therapies?
    A. Yes
    B. no
17. What do you think has caused the multidrug resistance?
    A. over-prescription
    B. self-prescription by farmers
    C. farmers are not completing regimens
    D. Other (*please specify*).....

18. What are some of the toxic effects evidently caused by the drugs?
    A. photosensitivity
    B. skin necrosis
    C. hair loss
    D. Other (*please specify*).....

19. Are they also seen during combinations?
    A. Yes
    B. No

20. Any other livestock other than cattle visibly resistant to the trypanocidal drugs?
    A. Goats
    B. Sheep
    C. donkeys
    D. Other (*please specify*).....

Section to be filled by farmer

Name of village –

Number of cattle owned –

What is the cost of a single drug regimen per animal?

The burden

1. Is there any noticeable difference in productivity between infected and healthy animals?
   A. No difference
   B. Sick animals are obviously less productive
   C. Sick animals are more productive
   D. Both groups are equally productive

2. Out of the animals that fall sick, how many have still died even after receiving medication?
   A. All
   B. None
   C. Half
   D. Other (*please specify*).....

3. What are the other causes of cattle fatalities in your herd aside from nagana?
   A. East Coast Fever
   B. Contagious Bovine Pleuro-pneumonia (CBPP)
   C. I don't know
   D. Other (*please specify*).....

4. What are your options eventually when an animal is not responding to medication?
   A. Slaughter
   B. Sell
   C. Await death
   D. Other (*please specify*).....

5. How do you determine that your animal is infected with Nagana?
   A. I know it is nagana from experience
   B. I wait for the vet to confirm
   C. I rely on fellow farmers knowledge
   D. Other (*please specify*).....

6. After recovery, do the animals regain full productivity?
   A. Yes

B. No

Drug prescription, use and toxic effects

7. How long after symptom onset do you wait before administering medication?

A. immediately

B. I wait for a few days

Other (if B, how many days).....

A. I give immediately
B. It takes a few days as I have to raise funds to buy drugs
C. I pre-medicate to prevent infection
D. Other (*please specify*).....

8. Who recommends the drugs you use?
   A. Myself
   B. Vet
   C. Fellow farmers
   D. Other (*please specify*).....

9. Which of the three drugs do you prefer using (*please confirm brand names*)?
   A. Homidium
   B. Isometamidium
   C. Diminazene
   D. No preference

10. Do you stick to the prescribed drug regimen?
    A. Yes
    B. No
    C. If no, please explain why.....

11. Do you prolong the use of the drug? *(Use for longer than prescribed).*
    A. Yes
    B. No
    C. If yes, please explain why.....

12. When the animal's health is restored, do you store the medication for use later?
    A. Yes
    B. No

13. Have you ever received different dosage levels for the drugs from different vets?

A. Yes
B. No

14. Have you ever used the drugs as a combination?
    A. Yes
    B. No
    C. If yes, please give details.....

15. How many times in a year does the infection recur thus necessitating the need to buy the drug (s)?
    A. Once
    B. Twice
    C. Thrice
    D. Other (*please give details*).....

16. What are the toxic /negative effects of the drug on the cattle?
    A. photosensitivity
    B. skin necrosis
    C. hair loss
    D. Other (*please specify*).....

17. Of your drugs of choice (and combinations) in which doses do you commonly prefer to administer?

    A. As prescribed by vet
    B. As prescribed by pharmacist
    C. As I have always done
    D. As advised by fellow farmers

18. Over time, has the dosing changed?
    A. Yes
    B. No

19. Are you using the medication to treat anything else other than nagana?
    A. Yes
    B. No
    C. If yes (*please give details*).....

Groups of livestock

20. Do you have any cattle in your herd that have never needed medication (***non-colonised***)?
    A. Yes
    B. No

21. (***colonised by susceptible strains***) Are there any cattle in your herd recurrently infected and a particular drug (or particular drugs) seems to be working?
    A. Yes
    B. No
    C. If yes, give details on number of cattle, drug (s), order of drugs, (any combination) and dosage.....

22. Do the doses change when in combination if administered separately?
    A. Yes
    B. No

23. Do you have any cattle in your herd that are recurrently infected and all particular drugs seem to be working?
    A. Yes
    B. No
    C. If yes (*please give details*).....

24. How are they administered?
    A. Combination
    B. Separately
    E. Other (*please specify*).....

25. (***colonised by resistant strains***) Do you have cattle that are not responding to all the medication?
    A. Yes
    B. No

26. (***colonised by strains resistant to two drugs***) Do you have cattle that have fallen ill then recovered after you used two drugs (which failed) and then you administered the third and it worked?
    A. Yes
    B. No
    C. If yes (*please give details*).....

27. Do you use drug combinations often?
    A. Yes
    B. No
    C. If yes (*please give details*).....