USE AND MISUSE OF TRYPANOCIDES AND COMPARISON OF PUTATIVELY DRUG SENSITIVE AND RESISTANT STRAINS OF *TRYPANOSOMA CONGOLENSE* ISOLATED FROM TANZANIA

ANNA FLOWINO NGUMBI

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA.

2020

EXTENDED ABSTRACT

This thesis was prepared based on the “publishable manuscripts” format of the Sokoine University of Agriculture. The thesis discusses the findings on two *Trypanosoma congolense* parasites causing the African Animal trypanosomosis. Two laboratory maintained stocks, putatively drug-sensitive and drug-resistant strains of *T. congolense* were compared in this study. The objectives were to: assess the use and misuse of commonly used trypanocides (isometamedium chloride and diminazene aceturate) and their contribution towards the development of resistant trypanosomes; determine whether or not the two *T. congolense* strains vary in terms of their morphometry, transmissibility, pathogenicity and genotypes.The qualitative study was conducted using semi-structured questionnaire to assess the level of knowledge and practice of the farmers that may contribute directly or indirectly to the development of drug resistance in pastoral and agropastoral communities. Furthermore, experimental studies were conducted on the *T. congolense* strains to assess their relative transmissibility, pathogenicity and morphometry using Swiss albino mice and Zebu steers. Molecular identification and phylogenetic analysis were used to genetically characterize the resistant and sensitive strains of the *T. congolense* stocks. This study revealed a high level of trypanocides misuse which poses a high risk of trypanosome drug resistance development. However, drug resistant trypanosomes in this study have shown high transmission potential with short prepatent period following syringe passage transmission between hosts (mice and bovine). Despite the exhibition of high infectivity to its hosts, bloodstream drug-resistant trypanosomes demonstrated significantly short morphological length with less pathogenic effects in a normal bovine host as compared to drug sensitive bloodstream trypanosomes. These findings must be taken into consideration when devising control and/ or surveillance strategies against this disease.

DECLARATION

I, Anna Flowino Ngumbi, do hereby declare, to the Senate of the Sokoine University of Agriculture, that this thesis is my own original work and that it has neither been previously submitted nor is it being concurrently submitted for any kind of award by any other institution.

…… ……

Anna Flowino Ngumbi **Date**

(Candidate)

This declaration has been approved by:

…… …….

Dr. Ladslaus L Mnyone **Date**

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

Firstly, I thank the almighty God for giving me the strength and enthusiasm to accomplish my PhD studies and research successfully. Secondly, my heartfelt gratitude goes to my supervisors, Prof. R.S. Silayo and Dr. L.L. Mnyone, for their tireless supervision, guidance and other forms of support provided throughout my studies. Prof. Silayo, your words of encouragement during tough moments of my PhD renewed my energy and enthusiasm; I will remain grateful forever. Thirdly, I extend my heartfelt appreciation to the Government of the United Republic of Tanzania for the financial support awarded to me through the Commission for Science and Technology (COSTECH). Fourthly, I extend my thanks to the staff of Sokoine University of Agriculture who in one way or the other contributed to this research work. The list includes staff from the Department of Veterinary Microbiology, Parasitology and Biotechnology, Department of Animal, Aquaculture and Range Sciences as well as the Animal Breeding Unit. Equally, I am indebted to my fellow PhD students, with whom we strived to attain this goal. Finally, but equally important, I extend special thanks to my family, my beloved husband Dr. Andrew Chota and our children Janeth, Joseph and Jacob for patience, moral support, encouragement and prayers. All of you were very instrumental to this endeavor and you will remain part and parcel of this victory. Furthermore, I thank my beloved father, Mr. Flowino Ngumbi, the late mother, Mrs. Yvonne Ngumbi, and my siblings for their sacrifice and encouragement throughout my education.

DEDICATION

To my parents, Mr. Flowino Ngumbi and the late Mrs. Yvonne Ngumbi (May, her soul rest in eternal peace), for their tireless love, care and sacrifices towards my education and what I am today. Similarly to my beloved husband and children for their encouragement and evalasting support.

TABLE OF CONTENTS

EXTENDED ABSTRACT.....ii

DECLARATION.....iii

COPYRIGHT.....iv

ACKNOWLEDGEMENTS.....v

DEDICATION.....vi

TABLE OF CONTENTS.....vii

LIST OF TABLES.....xi

LIST OF FIGURES.....xiii

LIST OF ABBREVIATIONS.....xv

CHAPTER ONE……...1

1.0 GENERAL INTRODUCTION …….1

1.1 Tsetse-transmitted Animal trypanosomosis and its epidemiology ……..1

1.2 Trypanosomes ……...2

1.2.1 Trypanosome development in the mammalian hosts ……..3

1.2.2 Trypanosome development in the tsetse vector ……..4

1.3 Impact of Tsetse-transmitted Trypanosomosis ……5

1.4 Control of tsetse transmitted trypanosomosis ……...6

REFERENCES ……8

CHAPTER TWO.....17

2.0 A cross-sectional study on use and misuse of trypanocides in selected pastoral and agropastoral areas of Eastern and North-Eastern Tanzania. ……17

Abstract ……..17

2.1 INTRODUCTION ……..19

2.2 MATERIALS AND METHODOLOGY ……21

2.2.1 Aim, design and study area ……21

2.2.2 Questionnaire administration ……21

2.2.3 Data analysis ……..22

2.3 RESULTS ……..22

2.3.1 Respondents' demographic characteristics ……22

2.3.2 Respondents' knowledge on tsetse, tsetse control methods and trypanosomosis ……..24

2.3.3 Availability of drugs and knowledge on trypanocide drugs' indications and adherence to the indications ……26

2.4 DISCUSSION ……30

2.5 CONCLUSIONS AND RECOMMENDATIONS ……34

REFERENCES ……..36

CHAPTER THREE.....40

3.0 Morphological appearance of trypanosomes in relation to drug sensitivity: comparative studies between drug-sensitive and drug-resistant *Trypanosoma congolense* strain in murine and bovine model ……40

Abstract ……..40

3.1 INTRODUCTION ……..41

3.2 MATERIALS AND METHODS ……43

3.2.1 Trypanosomes ……43

3.2.2 Maintenance of Experimental animals ……..43

3.2.3 Study design ……
44

3.2.4 Morphology measurement
……..45

3.2.5 Statistical analysis
……..46

3.3 RESULTS ……...47

3.3.1 Mean body lengths of Trypanosomes ……..47

3.3.2 The relative abundance of different morphological forms
…….47

3.4 DISCUSSION ……52

3.5 CONCLUSION ……..55

REFERENCES ……..56

CHAPTER FOUR.....60

4.0 Pathogenicity and transmissibility of trypanosomes in relation to drug sensitivity: comparative studies on two *Trypanosoma congolense* strains in bovine and murine models ……...60

Abstract ……...60

4.1 INTRODUCTION ……..62

4.2 MATERIALS AND METHODS ……64

4.2.1 Experimental animals
…….64

4.2.2 Trypanosomes ……65

4.2.3 Trypanocidal drugs
…….66

4.2.4 Inoculation of experimental mice with trypanosomes ……
66
4.2.5 Drug sensitivity of *T. congolense* stocks ……
67
4.2.6 Pathogenicity testing in mice
…….68
4.2.7 Pathogenicity testing in cattle ……
68
4.2.8 Data analysis
……..69
4.3 RESULTS ……70
4.3.1 Drug sensitivity in mice
……70
4.3.2 Pathogenicity of trypanosomes ……
72
4.4 DISCUSSION ……76
4.5 CONCLUSIONS AND RECOMMENDATIONS ……81
3.3 REFERENCES ……82

CHAPTER FIVE.....90
5.0 Molecular characterization of drug-sensitive and drug-resistant strains of *Trypanosoma congolense* isolated from Tanzania ……..90
Abstract ……...90
5.1 INTRODUCTION ……..91
5.2 MATERIALS AND METHODS ……..93

5.2.1 Blood sample collection ……93

5.2.2 Oligonucleotides ……93

5.2.3 DNA extraction ……..94

5.2.4 DNA amplification ……94

5.2.5 Gel electrophoresis ……95

5.2.6 Sequence and phylogenetic analysis ……..96

5.3 RESULTS ……...96

5.3.1 Identification of the Nannomonas group …… 96

5.3.2 Identification of *T. congolense* subgroups ……97

5.3.3 Phylogenetic relatedness of the *T. congolense* strains …… 98

5.4 DISCUSSION ……..99

5.5 CONCLUSION……101

REFERENCES ……102

CHAPTER SIX.....108

6.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS ……..108

6.1 Conclusions……...108

6.2 Recommendations ……..109

LIST OF TABLES

Table 2 1: Responses on trypanosomosis and tsetse control among respondents in the three study districts.....24

Table 2 2: Trypanosomosis control approaches used by respondents in the study districts.....25

Table 2 3: Sources of drugs and knowledge on indications and adherence in the three study districts.....27

Table 2 4: General comments given by farmers on the use of trypanocides for controlling animal trypanosomosis.....30

Table 3.1: Range of trypanosome lengths used to characterize the three morphological forms of *Trypanosoma congolense* in the present study. .46

Table 3. 2: Mean lengths of trypanosomes in mice infected with drug-sensitive and drug-resistant *T. congolense* strains.....49

Table 3. 3: Mean (Mean ± stdv) and range of lengths of drug-sensitive and drug-resistant strains of *T. congolense* over time post infection in cohorts of mice.....49

Table 3. 4: Mean (Mean ± stdv) and range of lengths of drug-sensitive and drug-resistant strains of *T. congolense* over time post infection in cattle.....50

Table 3. 5: Abundance of different morphological forms, congolense type, intermediate type and dimorphon type of trypanosomes measured in cohorts of mice infected with drug-sensitive and drug-resistant *T congolense* strains.....51

Table 3. 6: Abundance of different morphological forms, congolense type, intermediate type and dimorphon type, of trypanosomes measured over time post infection of mice with drug sensitive and resistant *T congolense* strains. Congolense type (≤ 12µm long); Intermediate type (>12 - <14 µm long); Dimorphon type (≥ 14µm long).....51

Table 3. 7: Abundance of different morphological forms, congolense type, intermediate type and dimorphon type, of trypanosomes in cattle infected with drug sensitive and drug resistant *T congolense* strains.....52

Table 3. 8: Percentage of different morphological forms, congolense type, intermediate type and dimorphon type, of trypanosomes measured over time post infection of cattle with drug sensitive and resistant *T congolense* strains.....52

Table 4. 1: Proportions of mice that were parasitaemic for *T. congolense* Mikese

(drug-sensitive stock/strain) over time post treatment (carried out at day-4 post infection) with different doses of diminazene aceturate.....70

Table 4. 2: Proportion of mice that were parasitaemic for *T. congolense* SIO-201 Mbagala (drug resistant stock/ strain) over time post treatment (carried out at day-4 post infection) with different doses of diminazene aceturate 71

Table 4. 3: Proportions of mice that were parasitaemic for *T. congolense* Mikese (drug-sensitive stock/strain) over time post treatment (carried out at day 4 post infection) with different doses of isometamedium chloride…72

Table 4. 4: Proportion of mice that were parasitaemic for *T. congolense* SIO-201 Mbagala (drug resistant stock/ strain) over time post treatment (carried out at day 4 post infection) with different doses of isometamidium chloride.....72

Table 5. 1: Oligonucleotide primer sequences and their expected product size.....94

LIST OF FIGURES

Figure 2. 1: Age distribution of respondents in the three study districts.....23

Figure 2. 2: The level of education of respondents in the three study districts.....23

Figure 2. 3: The responses of respondents from three study districts on intervals of chemoprophylaxis administration.....26

Figure 2. 4: Respondents responses on who administered drugs to the animals.....28

Figure 2. 5: Respondent responses on the route of drug administration.....29

Figure 3. 1: Giemsa stained thin blood smear showing a portion of 100µm scale bar with 1µm inter-dividers space illustrating measurement of trypanosomes in this study. Trypanosomes are indicated by arrows....46

Figure 3.2: Giemsa stained thin blood smear from mice showing different morphological forms of *T. congolense*: a = short form, b = intermediate form and c = long form.....48

Figure 4. 1: Parasitaemia level (10^xparasites/ml) in mice post infection with *T. congolense* drug-sensitive and drug-resistant stocks/ strains…74

Figure 4. 2: Body temperature (^oC) of mice post infection with *T. congolense* drug-sensitive and drug-resistant stocks/ strains.....74

Figure 4. 3: Packed cell volume (%) of steers following infection with *T. congolense* stocks/ strains.....75

Figure 5. 1: Gel electrophoresis profile of drug-resistant stock/ strains lanes 2 and 4 and drug-sensitive stock/ strains lanes 3 and 5 and a negative control lane 6; lane 1 and 7 is a 100bp DNA ladder.....97

Figure 5. 2: Gel electrophoresis profile showing amplification of a positive control for *T. congolense* savannah subgroup in lane 1, drug-resistant stock/ strains S1-3 in lanes 2,3 and 4 and positive control for *T. congolense* Kilifi subgroup in lane 7 and drug-sensitive stock/ strains K1-3 in lanes 8,9 and 10; lanes 6 was a 100bp ladder and lane 5 and 11 were negative controls......98

Figure 5. 3: Phylogenetic tree showing relationships of sequences generated from this study shown with black dots and sequences retrieved from database shown with their accession numbers......99

LIST OF ABBREVIATIONS

µm	Micrometer
DAARS	Department of Animal Aquaculture and Range Sciences
AAT	African Animal Trypanosomosis
Amsl	Above mean sea level
COSTECH	Commission for Science and Technology
DA	Diminazene aceturate
DPI	Days Post Infection
DVO	District Veterinary Officer
IM	Intramuscular
ISMM	Isometamidium chloride
IV	Intravenous
PCV	Packed Cell Volume
PP	Prepatent Period
SUA	Sokoine University of Agriculture
VAT	Variable Antigenic Type
VSG	Variable Surface Glycoprotein

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Tsetse-transmitted Animal trypanosomosis and its epidemiology

Tsetse-transmitted animal trypanosomosis continue to constitute a major constraint on agricultural development in many areas of the African continent. Its epidemiology is complex and involves three elements; the *Trypanosoma* parasite, the tsetse fly (*Glossina)* vector, and the mammalian vertebrate host (Firesbhat and Desalegn, 2015). The distribution and abundance of tsetse vector is an important variable in the epidemiology of trypanosomosis (Malele *et al.,* 2011), although the disease can be transmitted mechanically. Tsetse distributions positively impact the disease distribution since tsetse provides an ambient environment for the trypanosomes to complete their unique life cycle. Two third of 945,090 km^2 of the land resource in Tanzania is infested by ten (10) species and subspecies of tsetse flies belonging to three subgenera: Fusca , Palpalis and Morsitans. However, recent studies have reported shrinkage of tsetse belt due to changes in land use associated with increase in human population in many areas of the country (Malele, 2011).

In Africa, the disease affects 37 sub-Saharan countries extending to about 9.8 million km^2 of land; an area representing approximately one-third of the Africa's total land area (Mattioli *et al.,* 2004; Meyer *et al.,* 2016). Tsetse-borne animal trypanosomosis is a widely spread protozoan disease affecting various species of livestock in sub-Saharan Africa, however, cattle are the most frequently diagnosed and treated species. The disease affects all age groups though high prevalence has been shown in older animals of above two (2) years of age compared to young animals (Simwango *et al.,* 2017), this may be ascribed by variation in grazing areas between the two age group.

In southern Africa, tsetse-transmitted animal trypanosomosis is referred to as nagana from the Zulu word N'gana which means powerless/useless (Steverding, 2008). The disease is caused by several species of protozoa of the genus *Trypanosoma*, transmitted cyclically by vector tsetse flies under the genus *Glossina*.

1.2 Trypanosomes

The tsetse-transmitted trypanosomes are flagellate protozoans that inhabit the blood and tissues of their hosts. They are elongate and usually slightly curved with a single nucleus. There are many species of mammalian *Trypanosomes*, the main causative agents of the disease are *T. congolense* and *T. vivax* in cattle and to a lesser extent *T. brucei* (Auty *et al.,* 2015). However, in tropical Africa, the most widely spread of these species is *T. congolense* (Peacock *et al.,* 2012; Auty *et al.,* 2015). Trypanosomes can readily be distinguished from one another on morphological grounds; the size and shape of the body, the position of the nucleus and kinetoplast and the length and form of the undulating membrane and flagellum, as observed in stained slide preparations of the parasites (Godfrey, 1960; Nantulya *et al.,* 1978). All those *Trypanosoma* species which undergo a cycle of development in the tsetse fly and transmitted via tsetse's mouthparts are regarded as members of the Section Salivaria (Stevens and Gibson, 1999). They belong to three different subgenera which can be identified based on their sites of development within the fly as; trypanosomes that develop in the proboscis are in the subgenus Duttonella (Osorio *et al.,* 2008), trypanosomes that develop in the midgut and infective metacyclic stages found in proboscis are in subgenus Nannomonas (Peacock *et al.,* 2012), and those which develop in the midgut but then migrates to salivary glands are in subgenus Trypanozoon (Rico *et al.,* 2013; Ooi and Bastin, 2013).

Difference in developmental sites in tsetse vector alone and distinguishing them on morphological basis were found insufficient for species identification because of similarity in sites of development in tsetse vector and morphology in some species of trypanosoma, such as Subgenus Nannomonas contains the species *T. congolense*, *T. simiae* and *T. godfrey* which are morphologically indistinguishable (Gibson, 2003), but they are known to infect different host species. Thus, their pathogenicity in different hosts has been used to differentiate between *T. congolense* which grows well in mice, and *T. simiae* which is infectious only to pigs, similarly between *T. simiae* which cause acute infection in domestic pigs and termed as lightning destroyer of the pig and *T. godfrey* which cause subacute infection in pigs (Sturn *et al.*, 1998).

Development of molecular techniques, Polymerase Chain Reaction (PCR) in particular has overcome this shortcoming and has been used successfully for identification and characterization of African trypanosomes to species and sub-species level (Gibson, 2009). Within *T. congolense* for instance, at least four (4) different genotypes or sub-species have been identified which include Savannah, Kilifi West African forest/ Riverine and Tsavo (currently named *T. simiae* Tsavo) genotypes as reviewed by Gibson, (2003).

1.2.1 Trypanosome development in the mammalian hosts

The life cycle of trypanosomes has two developmental phases, one occurring in the mammalian host and the other one occurring in the insect vector. In the mammalian host, as the infected tsetse fly feeds, the infective metatrypanosomes are inoculated intradermal, they undergo development and multiplication at the site of infection where a major route of dissemination to the general circulation is via the draining lymphatics. Thus, finally the mature blood trypanosomes (or trypomastigotes) are released into the blood circulation via

lymph vessels. Reproduction in mammalian host occurs through binary fission (FAO, 1998).

The trypanosome stage in the bloodstream possesses an electron-dense surface coat that covers the membrane and is present in all mammalian stages of the parasite but absent during cyclical development in the tsetse fly until the infective metacyclic stage is reached in the tsetse's mouthparts (Dubois *et al.,* 2005). It consists of tightly packed antigenic molecules known as the Variable Surface Glycoprotein (VSG) or Variable Antigenic Type (VAT) (Turner, 1997). The VSG produces a protective cell coat that provides robust protection to trypanosomes against host immunological response throughout the mammalian cycle. However, this protection extends only to trypanosomes that consecutively change their surface antigen that cannot be destroyed by the antibodies of the host's immunological response (Frank and Barbour, 2006; Horn, 2014).

1.2.2 Trypanosome development in the tsetse vector

Trypanosomes are transmitted between mammalian hosts by tsetse species. When a tsetse fly ingests trypanosomes (trypomastigotes) via blood-meal of an infected mammal, the trypanosomes undergo development and multiplication in the digestive tract of the fly (Van de Abbeele *et al.,* 2013; Matthews, 2015). In the insect gut, the VSG coat of bloodstream trypanosomes replaced with a less-dense surface coat composed of procyclins. This transformation is accompanied by metabolic change from the mammalian host's bloodstream to the tsetse fly (Matthews, 2015). Only trypomastigotes forms occur in tsetse gut at this initial stage and transform into epimastigote forms prior to their further transformation into metacyclic forms in the mouthparts or salivary glands, the only forms which are infective to the mammalian host. At this stage the metacyclics have re-acquired

a VSG coat in preparation for transmission into a new mammalian host (Turner, 1997; Morrison *et al.,* 2009).

The developmental period in tsetse flies varies according to the species of trypanosome; 29-30 days for *brucei*-group (Van Den Abbeele *et al.,* 1999), about 18 days on average but with broad variations from 7-53 days for *T. congolense* (Dale *et al.,* 1995), and about 5-14 days for *Trypanosoma vivax* (Ooi *et al.,* 2016). Transmission of trypanosomes after they have undergone morphological and metabolic changes within the vector ending in the production of infective metacyclic trypanosomes is referred to as cyclical transmission. However, trypanosomes also may persist on the mouthparts of various species of biting flies including *Glossina species* and transmitted without undergoing any developmental changes and as such referred to as mechanical transmission for example the transmission of *T. vivax* by *Stomoxys* spp (Osorio *et al.,* 2008).

1.3 Impact of Tsetse-transmitted Trypanosomosis

Trypanosomosis is probably the most important single factor which profoundly affects human settlement and agricultural development in most of Africa. The disease affects not only the health and productivity of livestock, but also it affects socio-economic development (Charnie *et al.,* 2013). It is considered that if trypanosomosis did not exist in some regions of Africa, those areas would otherwise be three to five times more suitable for livestock and crop-livestock production (Mortelmans, 1984).

Trypanosome infection may be acute and rapidly fatal, subacute, but mild chronic infections are more common (Sturm *et al.,* 1998). In susceptible breeds of cattle, the disease causes severe anemia that occurs largely due to phagocytic removal of

erythrocytes damaged by lashing action of trypanosome flagella, undulating pyrexia, toxins and metabolites from trypanosomes. (Naessens, 2006; Mbaya *et al.,* 2012).

Clinically affected animals lose condition, become weak and unproductive (Shaw, 2009). Trypanosomosis causes widespread endocrine malfunction in cattle leading to abnormalities of the thyroids, ovaries, testes, adrenals and pituitary, thus rapidly impairs their functions in susceptible cattle breeds At herd level, the impact of trypanosomosis is reflected in various ways including impaired fertility due to ovarian anomalies in female animal such as cysts, fibrosis, reduced number of follicles and persistence of corpus luteum (Silva *et al.,* 2013). As a result, trypanosome-infected cows often have an irregular oestrus cycle and may be infertile or completely sterile. In pregnant animals, trypanosomosis may lead to endometritis, foetal death, abortion, still birth and neonatal death. Furthermore, milk and meat production are reduced. Furthermore, milk and meat production are reduced (Oluwafemi *et al.,* 2007). In male animals degenerative changes of the reproductive organs (testes and epididymis) have been observed (Raheem, 2014).

The indirect impact of the disease are the effects upon human welfare and crop production system whereby, presence of tsetse flies and animal trypanosomosis in large parts of Africa result to restricted access to fertile and cultivable land area, imbalance of land use, decrease in animal draft power which limits cultivation and local transport (Connor, 1994; D'leteren *et al.,* 1998; Swallow, 2000).

1.4 Control of tsetse transmitted trypanosomosis

Over many decades, considerable efforts have been invested in the control of trypanosomosis, mainly targeted towards prevention of tsetse bites through vector control and control of the causal agent. Vector control is based on directly or indirectly

elimination of tsetse flies and various methods have been employed to achieve this goal (Bourn *et al.,* 2005; Abd-Alla *et al.,* 2014; Kaba *et al.,* 2014; Meyer *et al.,* 2016).

There are several measures that are recommended and/or have been used for control of tsetseflies depending on the severity of the problem. Such measures include clearance of trees and shrubs to devoid breeding and resting habitats, exclusion of game animals through fences and selective hunting, use of sterile male technique and application of chemical insecticides on animals and other targets such as traps, vegetation, cattle and other tsetse hosts. Use of trypanotolerant animals is another alternative in areas affected by the disease (Naessens, 2006).

Beside these, trypanocidal drugs represents the most frequently used and widely adopted control approach in many livestock keeping communities (Delespaux and de Koning, 2007). Currently, only three compounds, isometamidium chloride, diminazene aceturate and homidium, are available on the African markets (Holmes *et al.,* 2004). Of those drugs, diminazene aceturate and isometamidium chloride have been in use to achieve the respective goals for more than 55 years. Isometamidium chloride is used for cure and prophylaxis with variable protection period of 2-4 months, while diminazene and homidium are primarily used for curative purpose with short prophylactic effect of 2-3 weeks for diminazene (Achenef and Bekele, 2013). Following privatization of veterinary services, many of these drugs are available and are now purchased directly by farmers through local Agroveterinary suppliers and the informal sector hence they are frequently used without accurate diagnosis. Estimates revealed that about 35 million doses of such drugs are dispensed every year for approximately 45-60 million cattle exposed to trypanosomosis (D'leteren *et al.,* 1998; Kristjanson *et al.,* 1999; Sones, 2001). Despite the wide and successful application of trypanocides as a major means of controlling

trypanosomosis, the development of resistance in trypanosomes against such drugs is increasingly reported in most of the sub-Saharan Africa (Pinder and Authie, 1984; Dolan *et al.,* 1992; Chitanga *et al.,* 2011; Kulohoma *et al.,* 2020).

The occurrence of drug resistance was found to be greater in those regions, where drug use has been more intensive (Van den Bossche *et al.,* 2000; Ngumbi and Silayo, 2017). This study assessed the use and misuse of trypanocidal drugs with focus on revealing various characteristics of trypanosomes resistant to diminazene (DA) and/ or Isometamidium (ISMM).

REFERENCES

Abd-Alla, A. M. M., Bergoinb, M., Parkera, A. G., Manianiac, N. K., Vlakd, J. M., Bourtzise, K., Bouciasg, D. G. and Aksoy, S. (2013). Improving Sterile Insect Technique (SIT) for tsetse flies through research on their symbionts and pathogens. *Journal of Invertebrate Pathology* 112: S2-10 doi:10.1016/j.jip.2012.07.009.

Achenef, M. and Bekele, B. (2013). Drugs and Drug Resistance in African Animal Trypanosomosis: A review. *European Journal of Applied Science* 5 (3): 84 – 91.

Auty, H., Torr, S.J., Michoel, T., Jayaraman, S and Morrison, L. J. (2015). Cattle trypanosomosis: the diversity of trypanosomes and implications for disease epidemiology and control. *Revue scientifique et technique (International Office of Epizootics)* 34(2): 587-598.

Bourn, D., Grant, I., Shaw, A. and Torr, S. (2005). Cheap and safe tsetse control for livestock production and mixed farming in Africa. *Aspects of Applied Biology* 75:

12pp.
http://www.fao.org/docs/eims/upload/agrotech/1937/CheapSafeTsetseControl.pdf

Chitanga, S., Marcotty, T., Namangala, B., Van den Bossche, P., Van Den Abbeele, J. and Delespaux, V. (2011). High Prevalence of Drug Resistance in Animal Trypanosomes without a History of Drug Exposure. *PLoS Neglected Tropical Diseases* 5(12): 5 pp. doi:10.1371/journal.pntd.0001454.

Connor, R. J. (1994). The impact of nagana. *Onderstepoort Journal of Veterinary Research* 61: 379-383.

D'Ieteren, G., Authie, E., Wissocq, N. and Murray, M. (1998). Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomiasis. *Revue Scientifique et Technique*, (*Office International des Epizooties*) 17:154 -175.

Dale, C., Welburn, S. C., Maudlin, I. and Milligan, P. J. M. (1995). The kinetics of maturation of trypanosome infections in tsetse. *Parasitology* 111: 187-191.

Delespaux, V. and de Koning, H. P. (2007). Drugs and drug resistance in African trypanosomiasis. *Drug resistance updates* 10: 30-50.

Dolan, R. B., Stevenson, P. G. W., Alushula, H. and Okech, G. (1992). Failure of chemoprophylaxis against bovine trypanosomiasis on Galana Ranch in Kenya. *Acta Tropica* 51: 113-121.

Dubois, M. E., Demick, K. P. and Mansfield, J. M. (2005). Trypanosomes expressing a Mosaic variant surface glycoprotein coat escape early detection by the immune system. *Infection and Immunity* 73(5): 2690-2697.

FAO. (1998). Drug management and parasite resistance in Bovine trypanosomiasis; Pathogenicity of drug-resistant parasites and the impact on livestock productivity. Viale delle Terme di Caracalla, 00100 Rome, Italy. http://www.fao.org/docrep/003/W9791E/w9791e04.htm.

Firesbhat, A. and Desalegn, C. (2015). Epidemiology and impacts of Trypanosomiasis in cattle. *European journal of applied science* 7(5): 220-225.

Frank, A. S. and Barbour, G. A. (2006). Within-host dynamics of antigenic variation. *Infection, Genetics and Evolution* 6: 141-146.

Gibson, W. (2003). Species concepts for trypanosomes: from morphological to molecular definitions? *Kinetoplastid Biology and Disease* 2(10): 6pp. https://doi.org/10.1186/1475-9292-2-10.

Gibson, W. (2009). Species-specific probes for the identification of the African tsetse-transmitted trypanosomes. *Parasitology* 136(12): 1501-1507.

Godfrey, D. G. (1960). Types of *Trypanosoma congolense* I. Morphological differences. *Annals of Tropical Medicine & Parasitology* 54(4): 428-438.

Holmes, P. H., Eisler, M. C. and Geerts, S. (2004). Current chemotherapy of animal trypanosomiasis. In the trypanosomiases. Maudlin I, Holmes P H and Miles M A (Eds). CABI Publishing. London, UK, pp.431 – 444.

Horn, D. (2014). Antigenic variation in African trypanosomes. *Molecular and biochemical parasitology* 195: 123-129.

Kaba, D., Zacarie, T., M'Pondi, A.M., Njiokou, F., Bosson-Vanga, H., Krober, T, McMullin, A., Mihok, S. and Guerin, P. M. (2014). Standardising Visual Control Devices for Tsetse Flies: Central and West African Species *Glossina palpalis* palpalis. *PLoS Neglected Tropical Diseases* 8(1): 11pp. doi:10.1371/journal.pntd.0002601

Kinabo, L. D. B. (1993). Pharmacology of existing drugs for animal trypanosomiasis. *Acta Tropica* 54: 169-183.

Kristjanson, P. M., Swallow, B. M., Rowlands, G. J, Kruska, R. L. and De Leeuw, P. N. (1999). Measuring the costs of African Animal trypanosomosis, the potential benefits of control and returns to research. *Agriculture systems* 59: 79-98.

Kulohoma, B. W., Wamwenje, S. A. O., Wangwe, I. I., Masila, N., Mirieri, C. K. and Wambua, L. (2020). Prevalence of trypanosomes associated with drug resistance in Shimba Hills, Kwale County, Kenya. *BMC Research Notes* 13(234): 6pp. https://doi.org/10.1186/s13104-020-05077-3

Malele, I. (2011). Fifty year of tsetse control in Tanzania: challenges and prospects for the future. *Tanzania Journal of Health Research* 13 (5 suppl. 1): 10 pp. DOI: http://dx.doi.org/10.4314/thrb.v13i5.9.

Matthews, K. R., McCulloch, R. and Morrison, L. J. (2015). The within-host dynamics of African trypanosome infections. *Philosophical Transactions of the Royal Society B* 370: 10 pp. http://dx.doi.org/10.1098/rstb.2014.0288.

Mattioli, R. C., Feldmann, U., Hendrick, G., Wint, W., Jannin, J. and Slingenbergh, J. (2004). Tsetse and trypanosomiasis intervention policies supporting sustainable animal-agricultural development. *Food, Agricultural and Environment* 2(2): 310-314.

Mbaya, A., Kumshe, H. and Nwosu, C. (2012). The Mechanisms of Anemia in Trypanosomosis: A Review. In: Silverberg, D. (Ed.): Anemia. Shanghai: InTech. pp. 269-282.

Meyer, A., Holt, H. R., Selby, R. and Guitian, J. (2016). Past and ongoing tsetse and animal trypanosomiasis control operations in five African countries: A

systematic review. *PLoS Neglected Tropical Disease*, 10 (12): 29pp: doi:10.1371/journal.pntd.0005247.

Morrison, L. J., Marcello, L. and McCulloh, R. (2009). Antigenic variation in the African trypanosome molecular and phenotypic complexity. *Cellular Microbiology* 11(12): 1724-1734.

Naessens, J. (2006). Bovine trypanotolerance: A natural ability to prevent severe anaemia and haemophagocytic syndrome? *International Journal for Parasitology* 36: 521–528.

Nantulya, V. M., Doyle, J. J. and Jenni, L. (1978). Studies on *Trypanosoma (Nannomonas) congolense* I. On morphological appearance of the parasite in the mouse. *Acta Tropica* 35: 329-337.

Ngumbi, F. and Silayo, R. S. (2017). A cross-sectional study on the use and misuse of trypanocides in selected pastoral and agropastoral areas of eastern and northeastern Tanzania. *Parasites and Vectors* 10(607): 9 pp. DOI 10.1186/s1307-017-2544-3

Oluwafemi, R. A., Ileomobade, A. A. and Laseinde, E. A. O. (2007). The impact of African animal trypanosomosis and tsetse on the livelihood and well-being of cattle and their owners in the BICOT study area of Nigeria. *Scientific Research and Essay* 2(9): 380-383.

Ooi, C. P. and Bastin, P. (2013). More than meets the eye: understanding *Trypanosoma brucei* morphology in the tsetse. *Frontiers in Cellular and Infection Microbiology* 3 (71): 12 pp. Doi.10.3389/fcimb.2013.00071.

Ooi, C. P., Schuster, S., Cren-Travaille, C., Bertiaux, E., Cosson, A., Goyard, S., Perrot, S. and Rotureau, B. (2016). The cyclical development of *Trypanosoma vivax* in

the tsetse fly involves an asymmetric division. *Frontiers in Cellular and Infection Microbiology* 6(115): 16pp. Doi:10.3389/fcimb.2016.00115.

Osorio, A. L. A. R., Madruga, C. R., Desquesnes, M., Soares, C. O., Ribeiro, L. R. R and Goncalves da Costa, S. C. (2008). Trypanosoma (Duttonella) vivax: its biology, epidemiology, pathogenesis, and introduction in the New World – A review. *Memórias do Instituto Oswaldo Cruz* 103 (1): 1-13.

Peacock, L., Cook, S., Ferris, V., Bailey, M. and Gibson, W. (2012). The life cycle of *Trypanosoma (Nannomonas) congolense* in the tsetse fly. *Parasites and Vectors* 5(109): 13pp. http://www.parasitesandvectors.com/content/5/1/109.

Peregrine, A. S. and Mamman, M. (1993). Pharmacology of diminazene-A review. *Acta Tropica* 54: 185-203.

Pinder, M. and Authie, E. (1984). The appearance of isometamidium resistant *Trypanosoma congolense* in West Africa. *Acta Tropica* 41: 247-252.

Raheem, K. (2014). A review of trypanosomosis-induced reproductive dysfunctions in male animals. *Agrosearch* 14(1): 30 -38.

Rico, E., Rojar, F., Mony, M. B., Szoor, B., MacGregor, P, and Matthews, R. K. (2013). Bloodstream form pre-adaptation to the tsetse fly in *Trypanosoma brucei*. *Frontiers in Cellular and Infection Microbiology* 3(78): 15pp. Doi:10.3389/fcimb.2013.00078.

Shaw, A. P. M. (2009). Assessing the economics of animal trypanosomosis in Africa – history and current perspectives. *Onderstepoort Journal of Veterinary Research* 76: 27-32.

Silva, T. M. F., Olinda, R. G., Rodrigues, C. M. F., Camara, A. C. L., Lopes, F. C., Coelho, W. A. C., Ribeiro, M. F. B., Freitas, C. I. A., Teixeira, M. M. G. and Batista, J. S. (2013). Pathogenesis of reproductive failure induced by *Trypanosoma vivax*

in experimentally infected pregnant ewes. *Veterinary research*, 44(1): 9pp. http://www.veterinaryresearch.org/content/44/1/1.

Stevens, J. and Gibson, W. (1999). The evolution of salivarian trypanosomes. *Memórias do Instituto Oswaldo Cruz* 94 (2): 225-228.

Steverding, D. (2008). The history of African trypanosomiasis. *Parasites and Vectors* 1(3): 8pp. Doi:10.1186/1756-3305-1-3.

Sturm, R. N., Murthy, V. K., Garside, L. and Campbell, D. A. (1998). The mini-exon gene of *Trypanosoma (Nannomonas) simiae*: sequence variation between isolates and a distinguishing molecular marker. *Acta Tropica* 71: 199 -206.

Swallow, B. M. (2000). Impacts of trypanosomiasis on African agriculture. *PAAT Scientific and Technical Series* 2: 52 pp. http://www.fao.org /ag/againfo/ programmes/en /paat /documents /papers/Paper_1999.pdf.

Turner, M. C. R. (1997). The rate of antigenic variation in fly-transmitted and syringe-passaged infections of *Trypanosoma brucei*. *FEMS Microbiology Letters* 153: 227-231.

Van den Abbeele, J., Claes, Y., Van Bockstaele, D. and Le Ray, D. (1999). *Trypanosoma brucei* spp. Development in the tsetse fly: characterization of the post-mesocyclic stages in the foregut and proboscis. *Parasitology* 118: 469-478.

Van den Bossche, P., Doran, M and Connor, R. J. (2000). An analysis of trypanocidal drug use in the Eastern Province of Zambia. *Acta Tropica* 75: 247-258.

CHAPTER TWO

2.0 A cross-sectional study on use and misuse of trypanocides in selected pastoral and agropastoral areas of Eastern and North-Eastern Tanzania.

A.F. Ngumbi[1, 2*] and R.S. Silayo[1]

[1]Department of Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, P. O. Box 3019, Chuo Kikuu, Morogoro, Tanzania.

[2]Livestock Training Agency, P. O. Box 603, Morogoro, Tanzania.

E-mail: ngumbianna0@gmail.com

Published: Journal of Parasites & Vectors (2017) 10:607

Available online at DOI: https://doi.org/10.1186/s1307-017-2544-3

Abstract

Background: Tsetse-borne African Animal Trypanosomosis (AAT) greatly influences livestock distribution and significantly slows livestock productivity in sub-Saharan Africa. While a number of control methods targeting the vector tsetse are in field application, treatment with the few available trypanocides continues to be the most widely applied control method. Unfortunately, improper and frequent use of these few available drugs, accelerated by poor veterinary service delivery, promote trypanosome drug resistance, the magnitude of which has not been delineated. In the present study, current practices on trypanocides application for control of bovine trypanosomosis in the field in Tanzania were studied with a view to policy advice on safe and sustainable use of trypanocides.

Methods: A cross-sectional study was conducted using a semi-structured questionnaire administered to a total of 200 randomly selected livestock keepers in selected pastoral and agropastoral areas within three districts namely Korogwe, Pangani and Mvomero in eastern and north-eastern Tanzania. The data were handled using excel spreadsheet and later exported to Epi-Info™ software program version 7 for descriptive analysis.

Results: In total, 50% of respondents in all three study districts had primary level of education; over 40% had informal education and 5% with university education (all from Pangani district). Most of the respondents aged 30-59 years with exception of Korogwe district where 35% aged 20 -29 years. Over 95% of the respondents had knowledge on tsetse as a vector of trypanosomosis and correctly identified tsetse in provided pictures. Furthermore, 98.7% of the respondents applied pyrethroids for tsetse vector control. Regarding parasite control practices, this study revealed significant variation in the usage and application intervals of trypanocides. Whereas only 20% of the respondents used chemoprophylaxis for trypanosomosis control, 69-95% wrongly used diminazene aceturate thinking it is prophylactic while it is not. About 5-30% of the respondents used the prophylactic drug isometamidium chloride. Most of the respondents (95% in Korogwe, 60% in Pangani and 93.1% in Mvomero) administered the drugs on their own. Improper administration of trypanocides was significantly high in all study districts. The respondents in Korogwe (75%) and Mvomero (72%) administered the drugs intravenously with a view to achieve faster drug effect contrary to manufacturers' recommendations. The respondents (40%) in Pangani district used both intravenous and intramuscular routes. Additionally, all respondents did not observe the recommended withdrawal periods for the drugs.

Conclusion: This study revealed high level of trypanocides misuse that poses a high risk of trypanosome drug resistance development as well as risks to human health from drug residues in consumed animal products. This calls for improvement of veterinary service delivery in pastoral and agropastoral areas of Tanzania to forestall misuse of chemotherapeutics.

Keywords: Trypanocides, insecticides, trypanosomosis control, tsetse, veterinary service delivery, drug misuse.

2.1 INTRODUCTION

Tsetse-borne African Animal Trypanosomosis (AAT) causes significant economic losses as it lowers livestock productivity [1, 2] and highly influences livestock distribution in Africa [3, 4]. In endemic areas of Tanzania, control of the disease relies on vector (tsetse) and parasite (trypanosome) control [5, 6].

Parasite control by chemotherapy and chemoprophylaxis predominates as a method of choice in controlling AAT [7] as it is easier than controlling the vector especially in communities where livestock production involves transhumance in search of water and pasture [8]. Trypanocides are probably the most commonly used drugs in Sub Saharan Africa [9]. It is estimated that 35 million doses of trypanocides are used annually in Africa where about 50-70 million animals are at risk of contracting trypanosomosis [10, 9, 2]. However the use of drugs to treat and control trypanososmosis is fraught with a number of problems including paucity of available drugs, trypanosome drug resistance development and lack of industry interest in discovery and development of new trypanocidal drugs for field use [11, 12].

Despite animal trypanosomosis being controlled principally by trypanocides, farmers depend mainly on three compounds: homidium chloride/bromide, diminazene aceturate and isometamidium chloride. The latter drug was first released for field use more than 55years ago [13, 7]. Prospects for new drugs being availed for field application against trypanosomoses are few on account of the African market for the drugs being less attractive to the pharmaceutical industry [14].

As a measure to ensure the available drugs remain useful for a long time, there is need for judicious use of the currently available drugs to slow down the speed of trypanosome drug

resistance development against them. Similarly, there is need for close monitoring of trypanocides usage under real life situations [9].

The way trypanocides are being used in Tanzania can be related to circumstances which began in 1984 when the government devolved veterinary service delivery into private hands. This has led to near-zero availability of veterinary service delivery in pastoral communities particularly in areas where transhumance for pasture and water prevails as a consequence of them being commercially and logistically unattractive. As a result, untrained pastoralists are delivering the veterinary service themselves adding unnecessary cost to livestock production from overuse/misuse, risk of drug resistance development and danger to health of those handling the drugs as well as those consuming the animal products with drug residuals.

In Tanzania, veterinary drugs including trypanocides were strictly controlled by the veterinary department [15]. However, following the privatization of veterinary services, veterinary drugs including trypanocides are freely available. These drugs can currently be purchased directly by farmers from local veterinary shops and even worse, from livestock markets where they are often displayed under direct sun light and heat.

This study was conducted to establish the level of use and misuse of trypanocides in selected pastoral communities of Tanzania with a view to policy advice to mitigate the problem of trypanosome drug resistance development.

2.2 MATERIALS AND METHODOLOGY

2.2.1 Aim, design and study area

The aim of the study was to establish the level of use and misuse of trypanocides in selected pastoral and agropastoral areas of Tanzania. A multistage randomized survey was employed and the districts of Korogwe and Pangani in Tanga region (North-Eastern Tanzania) and Mvomero in Morogoro region (Eastern Tanzania) were selected. Village information was obtained from the District Veterinary Officers (DVOs). The selection of villages for survey was purposive based on information on presence of pastoral communities, reported presence of tsetse/trypanosomosis and use of trypanocides. In Korogwe district (5^{o} 0’ S and 38^{o}25’E; altitude 1,093 meters above mean sea level [amsl]), surveys were carried out in three villages namely Mkalamo, Mgambo and Changalikwa. In Pangani district (5^{o}30’ S and 38^{o} 49’ E; altitude 69m amsl), surveys were carried out in two villages namely Masaika and Mivumoni. These two villages were different in terms of average herd size whereby there were 20-100 and 300 cattle for Masaika and Mivumoni respectively. In Mvomero district (6^{o} 21’ S and 38^{o} 27’ E; altitude about 160 meters amsl), surveys were carried out in five villages namely Msongozi, Mangae, Mela, Wamiluhindo and Kambala.

2.2.2 Questionnaire administration

The study involved administration of semi-structured questionnaire to heads (or representatives) of households randomly selected from a list of those owning cattle as provided by ten-cell leaders. A total of 200 cattle owners/ trypanocides users participated in the study. The questionnaire was contained questions to assess knowledge as well as practice towards the use of trypanocides. Specifically, the questions posed sought to assess knowledge on clinical signs of trypanosomosis, identification of tsetse flies from presented pictures, presence of flies of that kind in their areas and determine types of control

measures applied, how controls were carried out, knowledge on the use of trypanocidal drugs as control measures for trypanosomosis, preference of trypanocidal drugs, routes and frequency of drug administration, drug dilution, and observation of withdrawal period. Focus was on isometamidium use as a prophylactic agent, though questions were also directed to a lesser extent on the use of the curative drug diminazene aceturate. Furthermore, questions were posed to explore people's knowledge and attitudes on the application of pyrethroids insecticides.

2.2.3 Data analysis

Data obtained were entered into excel data sheets and descriptive statistics obtained using Epi-Info™ software program version 7.

2.3 RESULTS

2.3.1 Respondents' demographic characteristics

Of 200 cattle owners that were interviewed, 131 (65.5%) and 69 (34.5) were males and females respectively. Most of the respondents were in the age of 20 - 59 years in all three study districts, majority being 40 - 49 years old in Mvomero and Pangani with an exceptionally high number of respondents in the age group 20 - 29 years in Korogwe (Figure 2.1).The vast majority (75%, 80%, and 70.62% respectively) of respondents in Korogwe, Pangani, and Mvomero were heads of households.

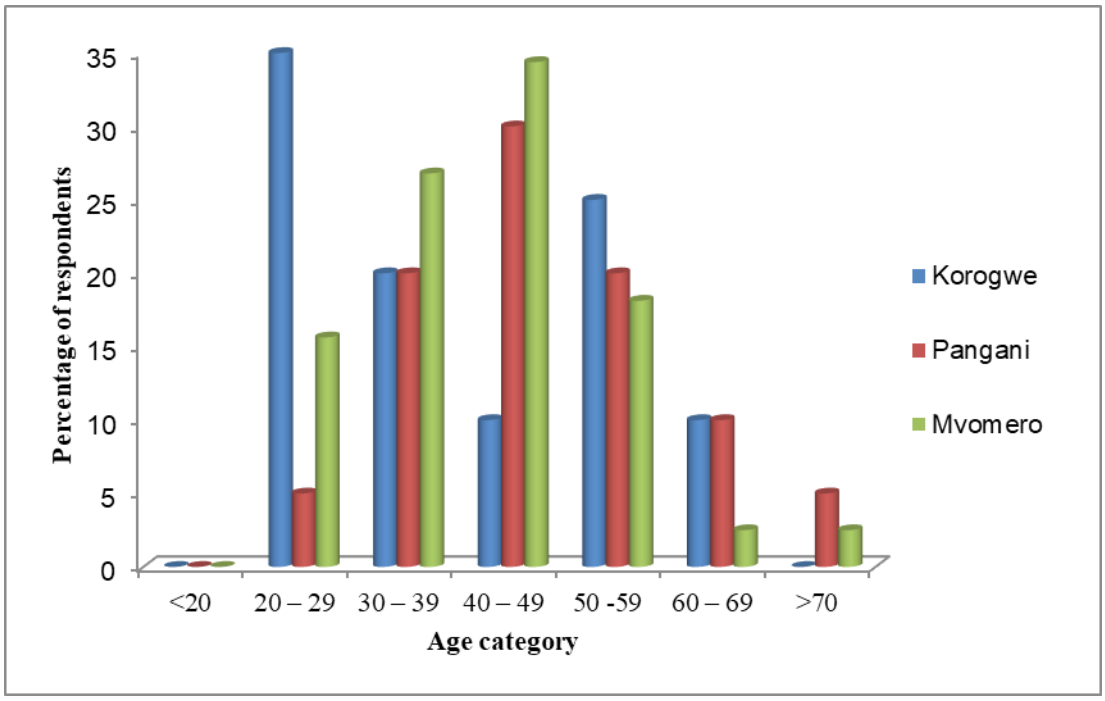

Figure 2. 1: Age distribution of respondents in the three study districts

Generally most of the respondents had informal or primary level education with Korogwe and Mvomero districts having relatively higher percentages of respondents with informal education than Pangani district where more respondents had formal education including 5% advanced level secondary education, 15% college and 5% university education (Figure 2.2).

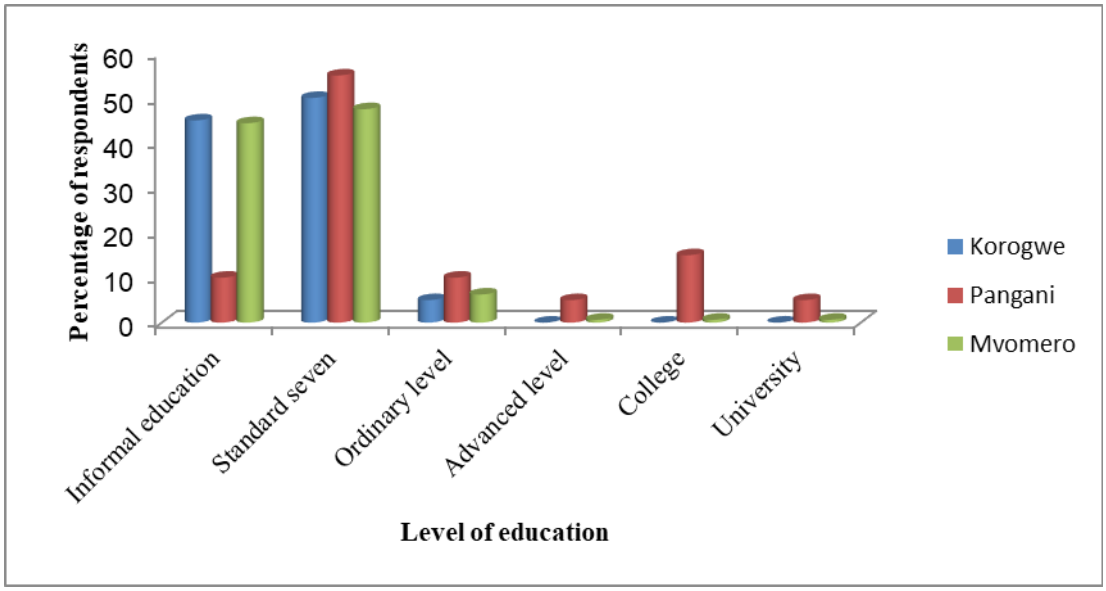

Figure 2. 2: The level of education of respondents in the three study districts

2.3.2 Respondents' knowledge on tsetse, tsetse control methods and trypanosomosis

The awareness of respondents on presence of the disease was significantly high in Pangani and Mvomero districts (75% and 73.8%) respectively relative to Korogwe district (30%) (Table 2.1).Tsetse flies are known and seen in the field by most of the farmers. The study indicated that 95% to100% of the farmers in all three districts knew and do see tsetse flies in the field. The results indicated that 98.7% of respondents in Mvomero and 100% respondents in both Korogwe and Pangani apply pyrethroids on their animals as a control measure against tsetse flies. Ninety-five percent of respondents in both Korogwe and Pangani and 100% in Mvomero use insecticides without additional bush clearing or target panel application (Table 2.1).

Table 2 1: Responses on trypanosomosis and tsetse control among respondents in the three study districts

Item	Response	Korogwe (%)	Pangani (%)	Mvomero (%)
Presence of trypanosomosis	Yes	30	75	73.75
	No	70	25	26.25
Presence of tsetse	Yes	95	100	98.13
	No	5	0	1.87
Tsetse control methods	Insecticides application	95	95	100
	Bush clearing	0	0	0
	Target panel	0	0	0
	Insecticides and bush clearing or target panel	5	5	0
Pyrethroids application	Yes	100	100	98.75
	No	0	0	1.25

Tsetse control as a method for trypanosomosis control was mentioned by 45%, 6.9% and 0% of respondents from Pangani, Mvomero and Korogwe respectively (Table 2.2). Chemoprophylaxis for trypanosomosis control was practiced by 20%, 10% and 11% of the respondents in Korogwe, Pangani and Mvomero respectively. On the other hand, 60% and 43% of the respondents in Korogwe and Mvomero respectively practiced chemotherapy as the control method. In contrary, none of the respondents from Pangani indicated to use

chemotherapy as the control method. The remaining 20%, 45% and 39.4% respondents from Korogwe, Pangani and Mvomero respectively indicated that they combine and or interchange tsetse control with either chemoprophylaxis or chemotherapy. Furthermore, 5%, 45% and 32.5% respondents in Korogwe, Pangani and Mvomero districts respectively use chemoprophylaxis as a routine way of controlling trypanosomosis. Few respondents, 5% and 30%, in Korogwe and Mvomero respectively indicated that they use isometamidium for prophylaxis while the remaining 95% and 69.4% used diminazene aceturate. The situation is different in Pangani where 45% of respondents administered isometamidium for prophylaxis and 30% used diminazene aceturate.

Table 2 2: Trypanosomosis control approaches used by respondents in the study districts

Item	**Criteria**	**Korogwe (%)**	**Pangani (%)**	**Mvomero (%)**
Trypanosomosis control method	Tsetse control	0	45	6.88
	Chemoprophylaxis	20	10	10.63
	Chemotherapy	60	0	42.50
	Slaughter	0	0	0.63
	Either of the two	20	45	39.38
Routine Chemoprophylaxis	Yes	5	45	32.50
	No	95	55	67.50
Drug used for prophylaxis	Isometamidium chloride	5	45	30.63
	Diminazene aceturate	95	30	69.38

Respondent response on intervals of isometamidium administration showed considerable variation. More farmers especially in Mvomero 56% indicated they provide prophylaxis after every three months, but there was a wide range from one week to as much as one year. The extremes were from 15% of respondents from Korogwe applying isometamidium once a week and 15% in Pangani applying isometamidium once a year (Figure 2.3).

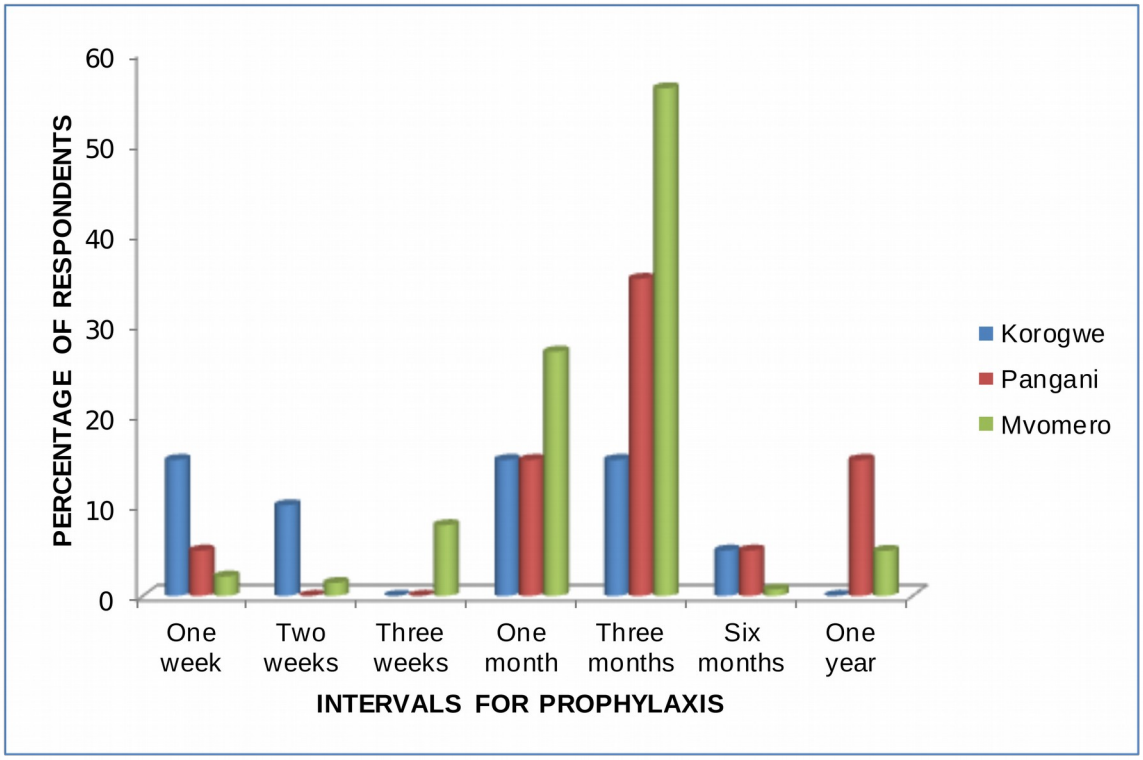

Figure 2. 3: The responses of respondents from three study districts on intervals of chemoprophylaxis administration

2.3.3 Availability of drugs and knowledge on trypanocide drugs' indications and adherence to the indications

All Pangani respondents indicated they obtained drugs from veterinary pharmacies in contrast to those from Korogwe and Mvomero of who 35% and 14.4% respectively indicated they obtain their animal drugs from veterinary pharmacies, while 35% and 81.3% of respondents from Korogwe and Mvomero respectively indicated they obtained the drugs from both veterinary pharmacies and livestock markets. Those indicating they obtained the animal drugs only from livestock auction markets were 30% and 3.1% from Korogwe and Mvomero respectively.

Knowledge on withdrawal period was high (85%) in Pangani followed by Mvomero (70%) and lastly in Korogwe (60%). However, despite of a good knowledge on

withdrawal period 32%, 72% and 80%, of respondents from Pangani, Mvomero and Korogwe respectively indicated they were consuming products from treated animals. Reasons given by those consuming products from treated animals were lack of awareness of the risks associated as mentioned by 100% of respondents from Korogwe, 25% from Pangani and 85% in Mvomero, while 75% and 15% of respondents from Pangani and Mvomero respectively indicated that they consumed as a way of avoiding economic loss (Table 2.3).

Table 2 3: Sources of drugs and knowledge on indications and adherence in the three study districts

Item	**Source**	**Korogwe (%)**	**Pangani (%)**	**Mvomero (%)**
Drug availability	Veterinary pharmacies	35	100	14.4
	Veterinary professional	0	0	0.6
	Livestock auction market	30	0	3.1
	Veterinary pharmacies & livestock markets	35	0	81.3
Withdrawal period if known	Yes	60	85	70
	No	40	15	30
Products from treated animal	Thrown away	15	47	26
	Used for human consumption	80	32	72
	Consumed by other animals	5	21	2
Reason for not observing withdrawal period	Unaware of risks to be encountered	100	25	85
	Avoiding economic loss	0	75	15

In all the three districts, drug administration as indicated by 95%, 60% and 93.1% of respondents in Korogwe, Pangani and Mvomero respectively was carried out by family members (Figure 2.4). Pangani district was the only district where a considerable

proportion (35%) of respondents indicated getting veterinary services from veterinary professionals, paraprofessionals and assistant paraprofessionals (Figure 2.4).

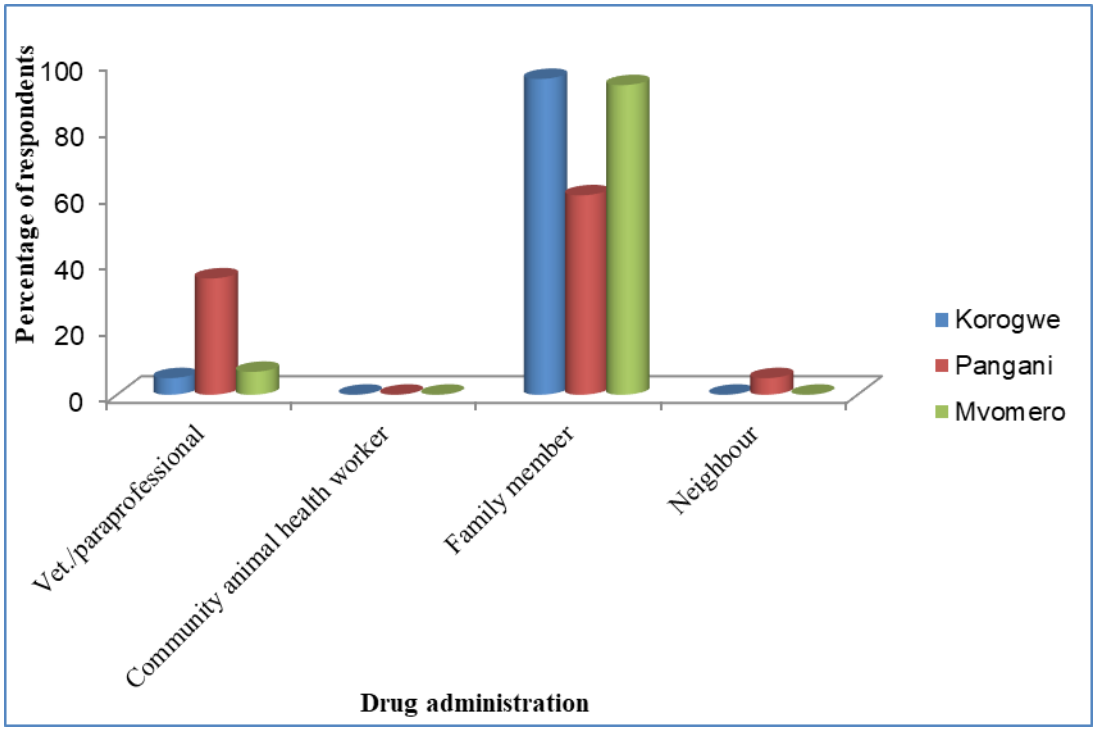

Figure 2. 4: Respondents responses on who administered drugs to the animals

Unprofessional handling of drugs was observed when respondents answered a question on the route of trypanocidal drugs administration with most (75% and 72% respectively) of respondents in Korogwe and Mvomero preferring to use intravenous (IV) route to administer prophylactic drug rather than the recommended deep intramuscular (IM) route. This recommended IM route was used by 40% respondents interviewed in Pangani, while a similar proportion (40%) of respondents from Pangani indicated they administer via both IV and IM route interchangeably (Figure 2.5) regardless of the risk encountered when using intravenous route. Respondents intimated that they use the IV route because it results in faster drug action.

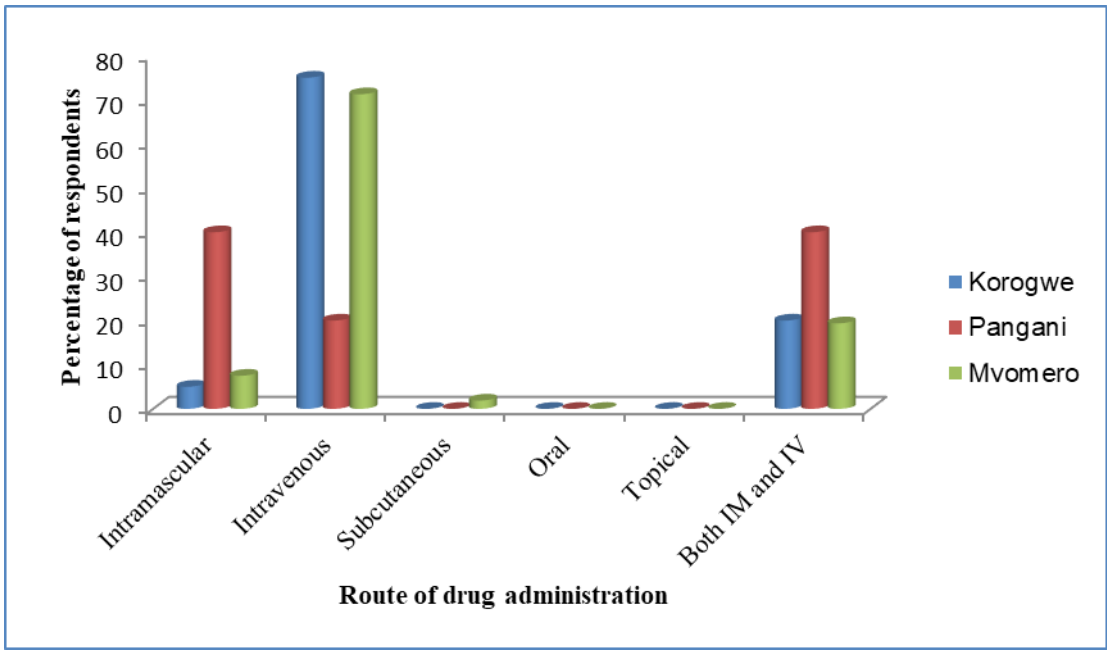

Figure 2. 5: Respondent responses on the route of drug administration

Generally, respondents gave different remarks as to why they did not predominantly practice control of trypanosomosis using trypanocides though most of them except 10.14% from Mvomero seemed willing to control the disease using trypanocides. There were farmers who indicated unavailability of drugs particularly isometamidium (samorin®) and these were 20% in Korogwe, 35% in Pangani and 44.6% in Mvomero. Lack of knowledge was another factor mentioned by 15%, 5% and 20.27% of respondents in Korogwe, Pangani and Mvomero districts respectively. Insufficient Veterinary services was a complaint from 45% respondents in Korogwe, 10% of those from Pangani and 17.57% of those from Mvomero, Financial constraint was not a case in Korogwe while this was mentioned by 7.43% of respondents from Mvomero and a relatively high proportion (35%) of respondents from Pangani (Table 2.4).

Table 2 4: General comments given by farmers on the use of trypanocides for controlling animal trypanosomosis

General observation	Korogwe (%)	Pangani (%)	Mvomero (%)
Unavailability of drugs	20	35	44.59
Unwillingness to control	0	0	10.14
Lack of knowledge	15	5	20.27
Lack of veterinary services	45	10	17.57
Financial constraint	0	35	7.43

2.4 DISCUSSION

This study has indicated that animal trypanosomosis is still a disease of concern in the study areas as many respondents acknowledged presence of trypanosomosis. This concurs with observations by Nonga and Kambarage [5] and presence of tsetse flies in the history of their areas especially in villages of Korogwe and Mvomero, similarly reported by Malele [16]. It is despite the more recent report [17] that the area of Tanzania currently occupied by the tsetse is now only one third, a dramatic decrease from the previous two thirds.

Respondents had varying levels of education with most having primary level of education. Literacy level in Pangani respondents was relatively higher as proportion of those with secondary or tertiary education was higher than in Korogwe and Mvomero. This also corresponded to the ability of the respondents to understand the diseases and the decision on who treats the animals, drug to use and where to purchase the drugs. In Pangani district respondents appeared more knowledgeable on the disease and its control than in other two districts, Korogwe and Mvomero. This knowledge level of Pangani respondents can be attributed to the existence of two missionary centers for sisters and brothers (Capuchin Franciscan sisters and brothers) but largely also due to Mivumoni Tsetse and Trypanosomosis research farm whereby a few respondents were retired workers of this farm, so they have dealt with tsetse and trypanosomiasis before in their livelihood.

This knowledge level was comparable to studies previously reported in Tanzania [18] and Kenya [19].

Despite of low levels of formal education in Korogwe and Mvomero compared to Pangani, respondents were informally knowledgeable of the tsetse and trypanosomosis. High proportion of respondents indicated that they know tsetse flies which they were able to identify. Similar knowledge has been reported in and around Serengeti national park [18, 20] in Serengeti. They are knowledgeable of the control means and a larger proportion indicated use of insecticide as a choice over bush clearing or target panel. In all study areas use of pyrethroids insecticides to simultaneously control tsetse flies and tick borne diseases was well adopted by respondents corresponding to similar findings by Magwisha et al. [21] and Muhanguzi et al. [22]. Additional information from the farmers (not included in questionnaire) indicated that the frequency of insecticides application differed among farmers ranging from daily to two weeks interval, and this was influenced by family manpower whereby a households having more children applied more frequently since they were using hand sprayers.

In some cases, livestock farmers may not be aware that they are controlling tsetse through vector control by insecticide application. In Korogwe for example there was considerable use of pyrethroids on cattle ostensibly for tick and tick-borne disease control but they were not aware that the acaricide was also very effective as insecticide against tsetse. The author, while at Korogwe noted that livestock keepers showed ignorance on how trypanosomosis is transmitted, that some believed cattle only get infection when they lick a site where a tsetse has just bitten. These findings correspond to the observation made in Kenya [23], Southwestern Ethiopia [24] and Tanzania [20].

Chemoprophylaxis and chemotherapy were mentioned as a means of trypanosomosis control, but chemotherapy (whereby farmers administered trypanocides when disease was encountered) was being employed more than chemoprophylaxis. However, where chemoprophylaxis was applied, application regime differed among farmers, with frequency ranging from once per week to once per annum. This study revealed that a frequency treatment at one week intervals was practiced in Korogwe, while a prolonged interval (year) was practiced by some respondents in Pangani while three months interval (as per manufacturers' recommendation) was practised in Mvomero district. This variation in prophylactic regimes, especially the prolonged annual interval may promote emergence of the drug resistant trypanosomes as pointed out by others [6, 25, 26, 27].

The present study has indicated use of the curative diminazene aceturate in preference to the prophylactic isometamidium chloride for trypanosomosis control. Use of curative trypanocidal drugs such as diminazene for prophylactic purposes is regarded as mass treatment in which both infected and non-infected animals are treated. Treatment of uninfected animals' results in unnecessary cost to animal production while treatment of animals which happen to be infected results in bonus immunity against the local stocks of trypanosomes. It can therefore be said that use of diminazene in mass treatment in situations where there is possibility of laboratory confirmation of infection is misuse of the drug. Diminazene aceturate is rapidly cleared from the body reducing its potentiality for use as a prophylactic drug [28]. Misuse of these drugs may be associated with inadequate veterinary service providers in their areas and tendency of farmers to obtain drugs from informal traders who sell these drugs in livestock auction markets without information on correct use of these drugs. A similar trend of farmers getting drugs from unqualified service providers was reported by Machila et al. [29] in Busia and Kwale Districts in Kenya. Veterinary service provision is more in Pangani as compared to Korogwe and

Mvomero, and geographic coverage may be the reason but level of education of livestock keepers may also influence the situation.

This study has also shown that farmers are left with no option but to treat their own animals without veterinary supervision due to inadequate private veterinary service delivery in the study area which is the case worldwide where provision of veterinary services is insufficient and many small farmers deliver animal health services [30]. As a result of this practice where treatment is done by farmers themselves and there is limited training of community animal health workers there is a high degree of misuse of drugs as similarly observed by Geerts et al. [9]. This is as a result of wrong choice of drug (considering farmers' ability to make diagnosis is limited since most of them rely on clinical signs), incorrect routes of drug administration, inconsistent administration intervals for prophylaxis and more importantly ignored observation of the withdrawal period as a consequence of disregard (or lack of knowledge) of manufacturers' recommendations. As a result of this haphazard use of drugs, possibilities of creating resistant strains of trypanosomes are high. This calls for emphasis on training of more paraprofessionals and assistant paraprofessionals from amongst transhumant pastoralists

A general observation from the studies is that availability of different approaches for control of trypanosomosis makes livestock keepers give little attention to the disease, as compared to other diseases such as viral diseases (FMD) and tick borne diseases especially heartwater. This could be due to the fact that today trypanocidal drugs are available at affordable price as compared to drugs for tick borne diseases as also observed by Magwisha et al. [21]. Furthermore the use of insecticides particularly pyrethroids permits cattle and tsetse flies to co-exist.

2.5 CONCLUSIONS AND RECOMMENDATIONS

The present studies have corroborated previously reported misuse of trypanocides in Tanzania as a consequence of poor veterinary service delivery in transhumant pastoral areas that are unattractive to private veterinary practice. Experience shows that even private veterinary services delivery faces difficulties in pastoral settings because of livestock movements and lack of permanent settlements, leading to Most of the livestock keepers in such situations are left with no choice but to deliver the veterinary service on their own without proper training. Because diagnosis is largely clinical-sign based, treatment is unspecific and adds to animal production costs. Such haphazard use of trypanocides likely is promoting trypanosome resistance to the few drugs still available for field use and moreover due to lack of observation of drug withdrawal periods results in health risks to consumers of the animal product. To overcome these problems it is being recommended that more studies be carried out on the issue of veterinary service delivery in transhumant pastoral situations especially where vector borne parasitic diseases (in particular trypanosomosis) are concerned. Furthermore, farmers' education on importance of laboratory-based disease diagnosis, indications of trypanocidal drugs and consequences of not adhering to those indications both economically and for public health concerns is important.

Ethical approval and consent to participate

The study obtained ethical clearance from the research and publication committee of the Sokoine University of Agriculture, Morogoro Tanzania and the respondents were educated on the importance of the study and had been given a freedom to accept or decline the request to participate.

Consent for publication

All the authors gave their consent for the manuscript to be published.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no competing interest in this study.

Funding

This study was supported by a PhD scholarship granted to Anna Flowino Ngumbi (AFN) by the Tanzania Commission for Science and Technology (COSTECH).

Authors' contributions

The conceptualization of the research, field work and data analysis and preparation of the manuscript draft was done by AFN and the supervision of the studies, scrutinization and further corrections and advices on the manuscript for publication was done by RSS

Acknowledgements

The authors acknowledge the financial support from The Government of the United Republic of Tanzania through The Commission for Science and Technology (COSTECH). The Sokoine University of Agriculture (SUA) is also acknowledged for granting permission to carry out the research and providing materials and technical support. The support from the District Veterinary Officers (DVO's) and livestock keepers during data collection is highly appreciated.

REFERENCES

1. Mersha C, Dulecha A and Basaznew B. Socio-Economic assessment of the impacts of Trypanosomiasis on cattle in Girja District, Southern Oromia Region, Southern Ethiopia. Acta Parasitol Glob. 2013; 4(3): 80-85.
2. Shaw APM. Assessing the economics of animal trypanosomosis in Africa – history and current perspectives. Onderstepoort J of Vet Res. 2009; 76: 27 – 32.
3. Swallow BM Impacts of trypanosomiasis on African agriculture. PAAT Scientific and Technical Series 2000; No.2, 52 pp http://www.fao.org /ag/againfo/ programmes/en /paat /documents /papers/Paper_1999.pdf.
4. Jahnke HE, Tacher G, Keil P, and Rojat D. Livestock production in tropical Africa, with special reference to the tsetse-afferent zone. In the Livestock production in tsetse affected areas of Africa. The African trypanotolerant livestock network proceedings of a meeting held 23-27 November 1987, Nairobi, Kenya. 1988pp 3-21 http://www.fao.org/wairdocs/ilri/x5443e/x5443e04.htm#1.
5. Nonga HE and Kambarage DM. Prevalence of Bovine trypanosomiasis in Morogoro, Tanzania. Pakistan J of Nutr, 2009; 8(3): 208-213.
6. Silayo RS, Kimbita EN, Mutayoba BM, Maselle RM. Use and abuse of acaricides and trypanocides in the field. Preliminary findings from Morogoro. Tanzania. Vet J. 1996; 2: 123-130.
7. Holmes PH, Eisler MC and Geerts S. Current chemotherapy of animal trypanosomiasis. In the trypanosomiases. 2004; Maudlin I, Holmes P H and Miles M A (Eds).CABI Publishing. London, UK pp.431 – 444.
8. Jordan AM. Arguments for and against considering trypanosomiasis as different from other animal diseases. In a systematic approach to tsetse and trypanosomiasis

control. Proc. of the FAO panel of experts 1-3 December 1993 in Rome, 1994 http://www.fao.org/docrep/004/t4885b/T4885B00.htm#TOC.

9. Geerts S, Holmes PH, Diall O and Eisler M. African bovine trypanosomiasis: the problem of drug resistance. Trends Parasitol.2001; 17: 25-28.

10. Kristjanson PM, Swallow BM, Rowlands GJ, Kruska RL and De Leeuw PN. Measuring the costs of African Animal trypanosomosis, the potential benefits of control and returns to research. Agric. systems. 1999; 59:79-98.

11. Delespaux V, Geysen D, Van De Bossche P and Geerts S. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol. 2008; 24: 236 -241.

12. Geerts S, and Holmes PH. Drug management and parasite resistance in bovine trypanosomiasis in Africa. PAAT Technical and Scientific Series No. 1. 1998. FAO, Rome.

13. Achenef M and Bekele B. Drugs and Drug Resistance in African Animal Trypanosomosis: A review. Europ J of Appl Scien. 2013; 5 (3):84 – 91.

14. Shiferaw S, Muktar Y and Dinaol B. A review on trypanocidal drug resistance in Ethiopia. J of Parasitol and Vect. Biol. 2015; 7(4): 58 – 66.

15. National Livestock Policy. Ministry of Livestock Development. The United Republic of Tanzania. 2006. http://www.mifugouvuvi.go.tz/.

16. Malele I. Fifty year of tsetse control in Tanzania: challenges and prospects for the future. Tanzania J of Healt. Research. 2011; 13 (1), 10 pp.

17. Daffa J, Byamungu M, Nsengwa G, Mwambene E and Mleche M. Tsetse distribution in Tanzania: 2012 status. Tanzania Vet J. 2013; 28: 1-11.

18. Kinung'hi SM, Malele II, Kibona SN, Matemba LE, Sahani JK, Kishamawe C and Mlengeya TDK. Knowledge, attitudes and practices on tsetse and sleeping sickness

among communities living in and around Serengeti National Park, Tanzania. Tanzania Health Res Bull, 2006; 8(3): 168-172.

19. Machila N, Emongor R, Shaw AP, Welburn SC, McDermott J, Maudlin I and Eisler MC. A community education intervention to improve bovine trypanosomiasis knowledge and appropriate use of trypanocidal drugs on smallholder farms in Kenya. Agric Systems. 2007; 94:261-272.

20. Byamungu M, Nkwengulila G and Matembo S. Evaluation of knowledge, attitude and practices of agropastoralists on tsetse fly (Glossina sp.) in western Serengeti Tanzania. J Vet Med Anim Health. 2016; 8(11):169-175.

21. Magwisha HB, Malele II, Nyingilili HS, Mamiro KA, Lyaruu EA, Kapange LA, Kasilagila GK, Joseph JM, Lwitiko NK and Kimbita, E.N. (2013) Knowledge, Attitude and Control Practices of Tsetse Flies and Trypanosomiasis among Agro-Pastoralists in Rufiji Valley, Tanzania. J Commonwealth Vet Assoc. 29 (1):5-11.

22. Muhanguzi D, Okello O W, Kabasa JD, Waiswa C, Welburn SC and Shaw AP M. Cost analysis of options for management of African Animal Trypanosomiasis using interventions targeted at cattle in Tororo District; South-eastern Uganda. Parasit Vectors, 2015; 8: 387.

23. Ohaga SO, Kokwaro ED, Ndiege IO, Hassanali A and Sain RK. Livestock farmers perception and epidemiology of bovine trypanosomosis in Kwale District, Kenya. Prev Vet Med, 2007; 80:24-33.

24. Seyoum Z, Terefe G and Ashenafi H. Farmers' perception of impacts of bovine trypanosomosis and tsetse fly in selected districts in Baro-Akobo and Gojeb river basins, Southwestern Ethiopia. Vet Res, 2013; 9: 214.

25. Roderick S, Stevenson P, Mwendia C and Okech G. The use of trypanocides and antibiotics by Maasai pastoralists. Trop Anim. Heal. and Prod. 2000; 32960: 361-74.

26. Van den Bossche P, Doran M and Connor RJ. An analysis of trypanocidal drug in the eastern province of Zambia. Acta Trop. 2000; 75 (2): 247-58.

27. Magona JW, Walubengo J and Olaho-Mukani W. Knowledge and attitudes of cattle owners regarding trypanosomiasis control in tsetse-infested areas of Uganda. J of South African Vet Ass. 2004; 75(4): 173 -176.

28. Delespaux V and de Koning HP. Drugs and drug resistance in African trypanosomiasis. Drug resistance updates, 2007; 10 (1 -2): 30-50.

29. Machila N, Eisler MC, Wanyangu SW, McDermott JJ, Welburn SC, Maudlin I. Cattle owners perceptions of African Bovine Trypanosomiasis and its control in Busia and Kwale Districts of Kenya. Acta Trop, 2003; 86:25-34.

30. Grace D, Christine J, McGregor-skinner G and Jeffrey CM. Participation of small farmers in Animal Health Programmes. conf. OIE, 2008; 19-34.

CHAPTER THREE

3.0 Morphological appearance of trypanosomes in relation to drug sensitivity: comparative studies between drug-sensitive and drug-resistant *Trypanosoma congolense* strain in murine and bovine model

A.F. Ngumbi[1, 2] and L.L. Mnyone[3]

[1]Livestock Training Agency, P. O. Box 603, Morogoro, Tanzania

[2]Department of Veterinary Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, P. O. Box 3019, Chuo Kikuu, Morogoro, Tanzania

[3]Sokoine University of Agriculture, Pest Management Centre, P.O. Box 3110, Chuo Kikuu, Morogoro, Tanzania

E-mail: ngumbianna0@gmail.com

Published: Journal of Entomology and Zoology Studies 2020;8(4):1860-1866.

Abstract

The pathogenicity and transmissibility of *T. congolense* vary with its morphological appearance in the bloodstream. Similarly, we speculated that the morphological appearance of *T. congolense* varies with therapeutic sensitivity. Therefore, this study aimed to investigate whether the morphological appearance of drug-sensitive *T. congolense*-Mikese and drug-resistant *T. congolense*-Mbagala in host's bloodstream vary from each other. Either *T. congolense* strains were inoculated into mice and cattle and monitored for morphological appearances and abundance in the bloodstream over time post-infection. In mice, the mean body length of drug-sensitive (12.38±1.66µm) and drug-resistant trypanosomes (12.58±2.03µm) was similar, except at day 6-8 post-infection. In

cattle, the mean body length of drug-resistant trypanosomes (10.08±1.32µm) was significantly shorter than that of drug-sensitive trypanosomes (12.38±1.56µm). The categorization of observed trypanosomes based on their length demonstrated short-form intermediate-form and long-form. The abundance of short-form of bloodstream trypanosomes in both mice and cattle was significantly higher in drug-resistant than drug-sensitive *T. congolense*. In conclusion, these findings preliminarily suggest a correlation between the therapeutic sensitivity and morphological appearance of *T. congolense*. However, we recommend additional studies involving fine-scale measurements of other determinant morphological and/or behavioral features (kinetoplast, nucleus, organelle, flagella, pathogenicity and transmissibility).

Keywords: drug-resistant, drug-sensitive, morphological, *Trypansoma congolense*

3.1 INTRODUCTION

Tsetse-borne African Animal trypanosomosis (AAT) is greatly hindering livestock production and socioeconomic development in many parts of Africa. Different pathogenic *Trypanosoma* species are responsible for the disease in cattle. The major species include *Trypanosoma vivax, T. congolense, and T. brucei.* Of these, *T. congolense* is probably the most pathogenic and causes most of the infections [1]. *T. congolense* belongs to the subgenus Nannomonas, which includes two other species namely *T. simiae* and *T. godfreyi*.

Trypanosoma congolense is distinguishable from the rest of livestock pathogenic trypanosomes based on their developmental cycle in the vector tsetse fly and mammalian host as well as the morphology of bloodstream forms [2-4]. In its bloodstream forms, *T. congolense* is distinguishable from other species based on host specificity and morphological variation [5]. This trypanosome is further divided into three morphologically

indistinguishable but genetically distinct types that are yet to be recognized as subspecies. The naming of these types is based on their ecological and/or geographical origins [6]. They include *T congolense* savannah, *T. congolense* Kilifi and *T. congolense* Forest. Morphometrically, *T. con*golense in the vertebrate bloodstream measures 9-22µm in length compared to 12-24µm for *T. simiae*, 18-26µm for *T. vivax* and 17-30µm for *T. brucei* [7]. The most recently discovered member established as a species due to its level of genetic divergence from the other members of the Subgenus Nannomonas is *T. godfreyi* whose bloodstream forms measures 9.1-21.8µm [5, 8].

Although bloodstream forms of *T. congolense* in the mammalian host have generally been described as being monomorphic, several studies have reported individuals with varying morphological lengths (pleomorphic). Godfrey [9] and Nantulya et al. [10] reported three morphological forms: 1) short-form/congolense type, 2) long-form/dimorphon type and 3) transitional form. The transitional form is the intermediate type which bridges the gap between the short and long types. The two studies described and considered intermediate type as a transitional form towards a long-form, contrary to observations made by Hoare [11], who indicated neither intermediate form nor association between the short forms measuring 10–15 µm and long forms measuring 22–25 µm in length.

Of the different morphological forms of *T. congolense* described so far, two forms (short form/congolense and long-form/dimorphon type) stand out. However, whether *T. congolense* deserves to be termed monomorphic or pleomorphic is still undecided. Godfrey [12] indicated that the pathogenicity of *T. congolense* varies with their morphological appearance in the bloodstream, with the long-form being more pathogenic than the short form. It is speculated that morphological appearance (morphometry) of particular species of trypanosomes may equally vary with drug sensitivities. However, no study has been done so far to confirm that assertion in *T. congolense*.

It was the aim of this study, therefore, to investigate whether the morphological appearance of the drug-sensitive and drug-resistant *T. congolense* in the host's bloodstream vary. Findings of this study will enhance knowledge of *T. congolense* concerning their therapeutic responses. Such knowledge will eventually be exploited to improve disease diagnosis and management.

3.2 MATERIALS AND METHODS

3.2.1 Trypanosomes

Experiments were conducted using two laboratory maintained trypanosomes: *T. congolense*-Mikese (drug-sensitive strain) and *T. congolense*-Mbagala (drug-resistant strain). These *T. congolense* strains were originally isolated from naturally infected cattle at Mikese, Morogoro in 2005 (Mkumbukwa 2005; Unpublished data) and Mbagala Dar es Salaam in 2001 (Mushule, 2007) respectively. These strains are maintained through serial passages in mice at the Small Animal Breeding Unit (SABU) of the College of Veterinary Medicine and Biomedical Sciences (CVMBS), Sokoine University of Agriculture (SUA), Morogoro, Tanzania. Previous studies indicated *Trypanosoma congolense*-Mikese to be highly sensitive to diminazene aceturate in mice at the very low dosage of 2mg/kg body weight (Mkumbukwa 2005; Unpublished data) while *T. congolense* Mbagala was shown to be resistant to treatment with isometamidium up to the dose of 20mg/kg and diminazine aceturate up to the dose of 84 mg/kg (Mushule 2007; Unpublished data). The drug sensitivity status of either strain was confirmed before their use in this study.

3.2.2 Maintenance of Experimental animals

Two types of experimental animals were used in this study: Swiss albino mice and *Bos indicus* cattle. Two groups of experimental mice, five in each, 10–12 weeks of age, were used. The mice were maintained in clean cages with wood shavings as bedding materials, fed on commercial broiler mash and supplied with water *ad libitum*. Two cattle, 8–10

months of age, used in this study were purchased from a Maasai herd in peri-urban Morogoro Municipality. The steers were confined in a fly-proof pen at Department of Animal, Aquaculture and Range Sciences (DAARS) farm at SUA and maintained on cut grass, supplemented with hay, concentrates, minerals and availed water *ad libitum*. The steers were examined for trypanosomes, other haemoparasites and gastrointestinal parasites before they were used. These steers were also dewormed with 10mg/kg Levamisole (KELA N.V Hoogstraten–Belgium) administered subcutaneously and injected intramuscularly with 6.6mg/kg Imidocarb (Essex Animal Health Friesoy the, Germany) to clear any *Babesia* and *Anaplasma* parasites.

3.2.3 Study design

This was an experimental study, which involved infecting mice and cattle, with two strains of *T. congolense* and comparing their morphological appearance over time post-infection. Before infecting experimental animals, the test trypanosomes (drug-sensitive and resistant *T. congolense*) were inoculated intraperitoneally, each into two donor mice, for expansion of the trypanosomes. These mice were monitored for parasitemia by microscopic examination of wet films of tail blood at 400× magnification, every other day over seven days post-inoculation. Subsequently, experimental mice and cattle were infected with trypanosomes from the donor mice. Two groups of 5 mice each were injected with 0.2ml of PBSG diluted donor-mice blood containing $1.0\times10^{6.6}$parasites/mil of drug-sensitive or drug-resistant trypanosomes. All experimental mice were identified using picric acid marker [13]. These mice were monitored for parasitemia, initially three times a week until trypanosomes were detected. Subsequently, they were similarly monitored for parasitemia daily to detect any variations in body lengths and abundance of different morphological forms between drug-sensitive and drug-resistant trypanosomes.

For cattle, two steers were injected with 5ml of PBSG diluted donor-mice blood containing $10^{7.8}$–$10^{8.4}$ parasites/ml of drug-sensitive or drug-resistant trypanosomes intravenously via the jugular vein. The steers were monitored for parasitemia through microscopic examination of wet blood films and buffy coat, initially three times a week until trypanosomes were detected. Subsequently, they were similarly monitored for parasitemia daily to detect any variations in body lengths and abundance of different morphological forms between drug-sensitive and drug-resistant trypanosomes.

3.2.4 Morphology measurement

Thin blood smears were prepared in grease-free microscope glass slides. The blood samples were collected daily from infected mice and cattle. In mice, tail blood samples were dropped directly onto glass slides. In cattle, blood from the jugular vein was collected into vacutainer tubes with EDTA. The air-dried thin blood films were fixed in absolute methanol for 2 min and then stained using 10% Giemsa stain for 30 min. The stains were examined under oil immersion at ×400 magnification for measuring body lengths of the detected trypanosomes. The body lengths of trypanosomes in mice and cattle were measured per procedures and criteria described by Nantulya [10], using 100µm scale bar with calibrations set as one space between dividers equivalent to 1µm (Figure 3.1). Measurable trypanosomes were obtained through observations on multiple microscopic fields from an area with low to moderate concentration of trypanosomes; preferentially towards the tail of thin blood film. The body length of individual trypanosomes was measured by counting inter-divider spaces of scale bar positioned from one end of the body to the other through the middle. The number of trypanosomes measured per slide depended on parasite density. But, at least five trypanosomes were measured per slide. Based on such measurements, the test trypanosomes (drug-sensitive and drug-resistant *T. congolense* strain) from mice and cattle were categorized into

different morphological forms (short/congolense, long-form/dimorphon type and transition/intermediate type) per criteria described by Godfrey [9] (Table 3.1).

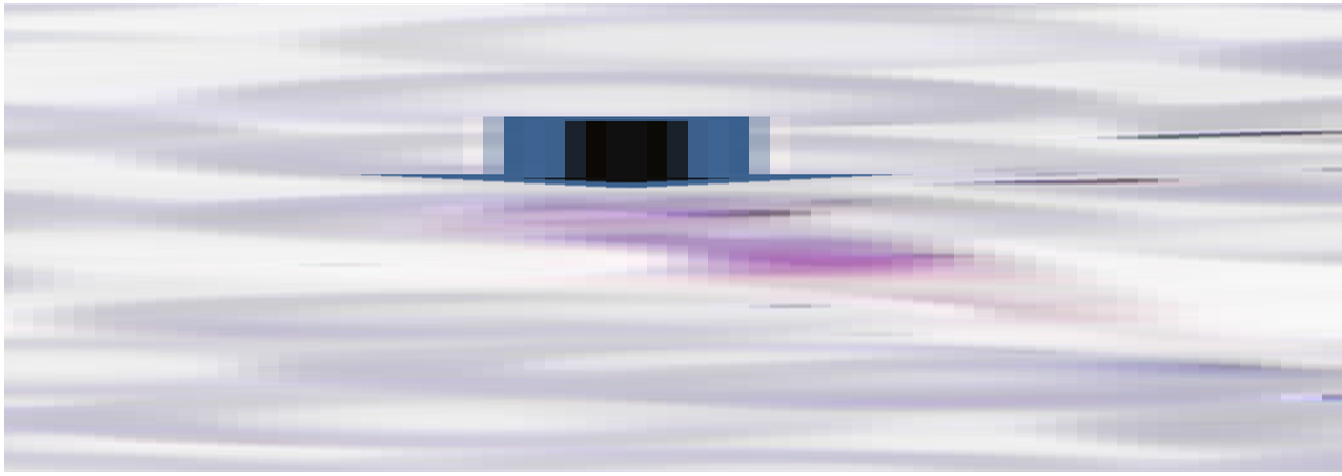

Figure 3. 1: Giemsa stained thin blood smear showing a portion of 100µm scale bar with 1µm inter-dividers space illustrating measurement of trypanosomes in this study. Trypanosomes are indicated by arrows

Trypanosome stocks were categorized in different morphological forms based on the lengths measured, as per criteria described by Godfrey. (1960) but with some modifications (Table 3.1).

Table 3.1: Range of trypanosome lengths used to characterize the three morphological forms of *Trypanosoma congolense* **in the present study**

	Trypanosome length (µm)		
	Short form (congolense type)	Intermediate form	Long form (dimorphon type)
Godfrey, 1960	11.20 – 12.92	12.98 – 13.85	13.75 – 15.10
This study criteria	≤12.92	12.98 – 13.98	14.00 – 18.10

3.2.5 Statistical analysis

All data were entered into an excel spreadsheet and analyzed by student t-test to test for significance between the mean lengths and proportions of morphological forms of the

drug-sensitive and drug-resistant trypanosomes. This analysis was done using SPSS version 16.

3.3 RESULTS

3.3.1 Mean body lengths of Trypanosomes

Drug-sensitive and drug-resistant *T. congolense* trypanosomes with various body lengths were observed in mice and cattle. In mice, the mean body length of drug-sensitive (12.38±1.66µm) and drug-resistant trypanosomes (12.58±2.03µm) was similar (p=0.185, 95% CI = -0.492-0.095; Table 3.2), except at day 6-8 post-infection (Table 3.3). Nevertheless, mean body lengths of either strain of trypanosomes in individual mice ranged from 12.11 µm to 12.70µm. In cattle, they trypanosomes were morphologically distinct, with body lengths ranging from 9–15µm for sensitive- and 9–14 µm for resistant-strain. Furthermore, the mean body length of drug-resistant trypanosomes (10.08±1.32µm) was significantly shorter than that of drug-sensitive (12.38±1.56µm) trypanosomes (p <0.001, 95% CI=1.562-3.033). This distinction in mean body length between the drug-resistant and drug-sensitive trypanosomes was observed at day 16, 19 and 22 post-infection (Table 3.4). Otherwise, before then, both trypanosomes were similar in length.

3.3.2 The relative abundance of different morphological forms

Based on body length measurements, trypanosomes were categorized into different forms; and thereof their relative proportions compared across drug-sensitive/drug-resistant trypanosomes, hosts and time post-infection. Different morphological forms of the trypanosomes were observed; and these were short-, intermediate- and long-forms. The relative abundance of these forms varied across strains, hosts (mice and cattle) and days post-infection. Overall, the rising phase of parasitemia, the short form predominated, while at the peak parasitemia, the trypanosomes were highly pleomorphic with

considerable proportions of the other two forms. In mice, all three forms of trypanosomes were observed over time post-infection. However, the short form of trypanosomes was more abundant than the intermediate- and long-forms ($p < 0.001$; Table 3.5); except at day 3 post-infection, where the three morphological forms of drug-sensitive trypanosomes were equally abundant. Furthermore, the long form of drug-resistant trypanosomes was more abundant than the long- and intermediate-forms at day 5, 6 and 8 post-infection ($p < 0.001$; Table 3.6). In cattle, the abundance of short-form was higher in both drug-sensitive and drug-resistant strain than that of long- and intermediate-forms ($p < 0.001$; Table 3.7). Nevertheless, the drug-resistant strain did not exhibit any intermediate form of trypanosomes throughout the monitoring period. Furthermore, the long form of resistant trypanosomes was only observed at day 12 post-infection. For drug-sensitive trypanosomes, the intermediate and long forms were equally abundant at day 19, 22, 25 and 28 post-infection (Table 3.8).

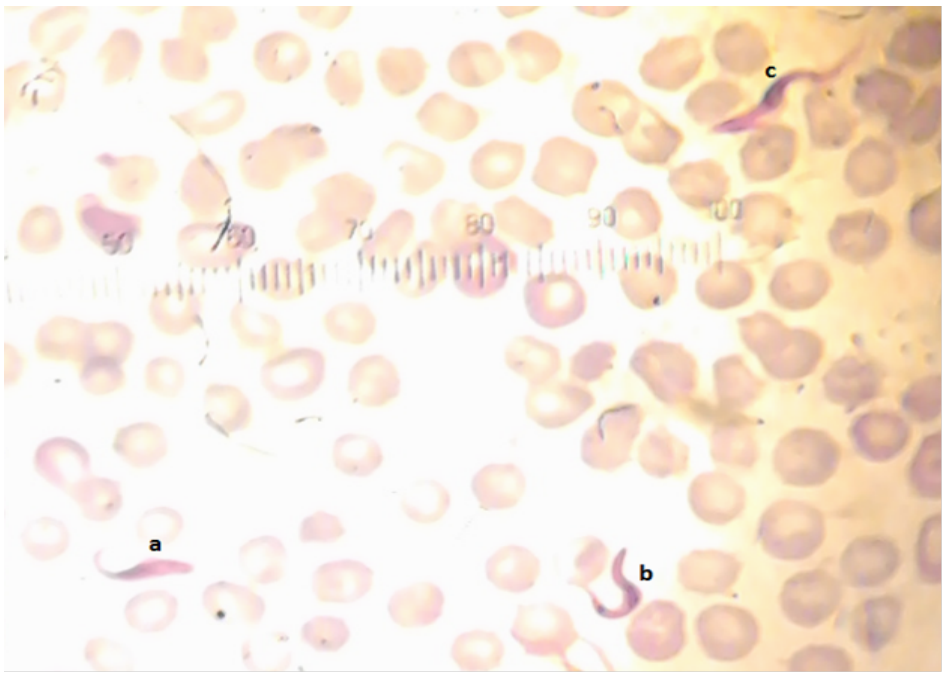

Figure 3.2: Giemsa stained thin blood smear from mice showing different morphological forms of *T. congolense*: a = short form, b = intermediate form and c = long form

Table 3. 2: Mean lengths of trypanosomes in mice infected with drug-sensitive and drug-resistant *T. congolense* strains

	Mouse I/D No.	No. of trypanosomes measured	Mean length ± std (µm)	Range (µm)
Druf-sensitive strain (2A)	1	64	12.37± 1.8	10-17
	2	44	12.70± 1.9	10-17
	3	62	12.30± 1.7	9-18
	4	52	12.11± 1.3	10-15
	5	37	12.46± 1.4	10-17
Drug-resistant strain (2B)	1	88	12.66± 2.1	9-18
	2	106	12.64± 2.3	9-18
	3	65	12.20± 1.9	9-17
	4	75	12.57± 2.1	9-17
	5	89	12.46± 1.8	9-17

Table 3. 3: Mean (Mean ± stdv) and range of lengths of drug-sensitive and drug-resistant strains of *T. congolense* **over time post infection in cohorts of mice**

Days post infection (DPI)	Drug-sensitive strain			Drug-resistant stock		
	No. of trypanosomes measured	Mean length ± std (µm)	Range (µm)	No. of trypanosomes measured	Mean length ± std (µm)	Range (µm)
2	0	-	-	13	10.54± 1.4	9-14
3	5	12.80± 1.6	10-14	49	12.80± 2.4	9-18
4	15	11.27± 1.2	9-13	17	12.35± 2.2	9-16
5	16	12.56± 1.5	10-15	50	13.22± 1.7	9-17
*6	26	12.23± 1.2	11-15	40	13.42± 2.0	10-17
*7	41	12.56± 1.5	10-16	30	11.13± 1.3	9-15
*8	36	12.88± 1.9	9-18	59	13.83± 1.7	10-18
9	37	12.43± 1.8	9-16	51	12.59± 1.4	10-15
11	17	11.47± 1.1	10-13	38	11.84± 2.1	9-16
13	30	11.90± 1.5	10-15	40	11.75± 1.9	9-15
14	36	12.78± 2.0	10-17	36	12.11± 1.6	9-15

*** = Days post-infection with significant difference between the mean length of the two stocks of *T. congolense* in mice**

Table 3. 4: Mean (Mean ± stdv) and range of lengths of drug-sensitive and drug-resistant strains of *T. congolense* over time post infection in cattle

Days post infection (DPI)[a]	Drug-sensitive stock (Steer No. 1333)			Drug-resistant stock (Steer No. 1334)		
	Number of trypanosomes measured	Mean length (µm) ± Std	Range (µm)	Number of trypanosomes measured	Mean length (µm) ± Std	Range (µm)
9	0	-	-	5	10.20 ± 1.304	9-12
12	7	11.43 ± 1.512	10-13	6	11.17 ± 1.789	9-14
*16	6	13.16 ± 0.753	12-14	5	9.40 ± 0.548	9-10
*19	8	12.00 ± 1.851	9-14	6	10.00 ± 1.095	9-12
*22	7	12.14 ± 1.952	9-14	3	9.33 ± 0.577	9-10
25	5	13.20 ± 0.837	12-14	0	-	-
28	6	13.00 ± 1.414	11-15	0	-	-
30	6	12.17 ± 1.602	10-14	0	-	-
Overall	**45**	**12.38 ± 1.56**	**9-15**	**25**	**10.08 ± 1.32**	**9 – 14**

[a] = Days post inoculation,

- = no Trypanosomes observed in a thin blood film examined

*= days post-infection with significant difference between the mean length of the two stocks of *T. congolense* in cattle

Table 3. 5: Abundance of different morphological forms, congolense type, intermediate type and dimorphon type of trypanosomes measured in cohorts of mice infected with drug-sensitive and drug-resistant *T congolense* strains

Mouse ID No.	No. of trypanosomes	Congolense type (≤	Intermediate type (>12 - <14 µm) (%)	Dimorphon type (≥

		measured	12µm) (%)		14µm) (%)
Drug-sensitive strain	1	64	53.1	20.3	26.6
	2	44	47.7	18.2	34.1
	3	62	56.5	17.7	25.8
	4	52	57.7	26.9	15.4
	5	37	48.7	35.1	16.2
	TOTAL	259	53.3	22.8	23.9
Drug-resistant strain	1	88	44.3	17.1	38.6
	2	106	48.1	11.3	40.6
	3	65	52.3	21.5	26.2
	4	75	50.7	13.3	36.0
	5	89	51.7	13.5	34.8
	TOTAL	423	49.2	14.9	35.9

Table 3. 6: Abundance of different morphological forms, congolense type, intermediate type and dimorphon type, of trypanosomes measured over time post infection of mice with drug sensitive and resistant *T congolense* **strains. Congolense type (≤ 12µm long); Intermediate type (>12 - <14 µm long); Dimorphon type (≥ 14µm long)**

DPI	Drug-sensitive strain				Drug-resistant strain			
	No. of trypanosomes measured	Congolense type[a]	Intermediate type[b]	Dimorphon type[c]	No. of trypanosomes measured	Congolense type[a]	Intermediate type[b]	Dimorphon type[c]
2	0	0	0	0	13	92.3	0.0	7.7
3	5	20.0	40.0	40.0	49	49.0	12.2	38.8
4	15	80.0	20.0	0.0	17	47.1	17.6	35.3
5	16	43.8	31.3	25.0	50	34.0	14.0	52.0
6	26	61.5	26.9	11.5	40	35.0	15.0	50.0
7	41	43.9	29.3	26.8	30	83.3	10.0	6.7
8	36	36.1	27.8	36.1	59	22.0	22.0	55.9
9	37	56.8	13.5	29.7	51	47.1	23.5	29.4
11	17	76.5	23.5	0.0	38	60.5	13.2	26.3
13	30	70.0	13.3	16.7	40	65.0	7.5	27.5
14	36	44.4	19.4	36.1	36	61.1	13.9	25.0
TOTAL	259	53.3	22.8	23.9	423	49.2	14.9	35.9

[a]Congolense type (≤ 12µm long); [b]Intermediate type (>12 - <14 µm long); [c]Dimorphon type (≥ 14µm long)

Table 3. 7: Abundance of different morphological forms, congolense type, intermediate type and dimorphon type, of trypanosomes in cattle infected with drug sensitive and drug resistant *T congolense* **strains**

T. congolsense strains	Steers	No. of trypanosomes	Congolense type	Intermediate type (>12-<14	Dimorphon type

		measured	(≤12µm)	µm)	(≥14µm)
Drug-sensitive strain	1333	45	42.22	31.11	26.67
Drug-resistant strain	1334	25	96.00	0.00	4.00

Table 3. 8: Percentage of different morphological forms, congolense type, intermediate type and dimorphon type, of trypanosomes measured over time post infection of cattle with drug sensitive and resistant *T congolense* strains

Days post infection (DPI)	Sensitive stock (in Steer No. 1333)				Resistant stock (In steer No. 1334)			
	No. of trypanosomes measured	Congolense type (≤12µm)	Intermediate type (>12-<14 µm)	Dimorphon type (≥14µm)	Trypanosomes measured	Congolense type (≤12µm)	Intermediate type (>12-<14µm)	Dimorphon type (≥14µm)
9	-	-	-	-	5	100.0	0	0
12	7	57.1	42.9	0	6	83.3	0	16.7
16	6	16.7	50.0	33.3	5	100.0	0	0
19	8	50.0	25.0	25.0	6	100.0	0	0
22	7	42.9	28.6	28.6	3	100.0	0	0
25	5	20.0	40.0	40.0	0	-	-	-
28	6	33.3	33.3	33.3	0	-	-	-
30	6	66.7	0	33.3	0	-	-	-
TOTAL	45	42.22	31.11	26.67	25	96.00	0.00	4.00

3.4 DISCUSSION

This study has established that the drug-sensitive and drug-resistant strains of *T. congolense* studied exhibit morphological variation in bloodstream forms within and between hosts. Both strains revealed pleomorphic populations of *T. congolense* strains with morphological length ranging from 9µm-18µm in mice and 9µm-15µm in cattle.

The overall mean lengths of both strains ranged from10.08 µm to 12.58 µm; and this was consistent with the range reported for *T. congolense* in previous studies [7, 9, 10, 11, 14].

Unlike in mice, where drug-sensitive and drug-resistant trypanosomes had similar length, the resistant trypanosomes in cattle exhibited shorter mean body length (10.08µm) relative to drug-sensitive trypanosomes (12.38µm). Similarly, mean body lengths of resistant trypanosomes varied significantly between hosts; whereas those in cattle were one-fold shorter; supporting the view host's internal environment influence the morphology of trypanosomes [9, 15-16]. Host-induced variation in morphological length was demonstrated in *Trypanosoma copemani* infecting wildlife in Australia [16]. Host dependent factors which may modify parasites morphological appearance and/or biomass include among others immunocompetence, diet and body temperature. Exhibition of high abundance of short-form trypanosomes could have been adopted as a mechanism to evade the effect of trypanocidal drugs and/or enhance the spread of resistant genes. Although not ascertained in trypanosomes, certain morphological presentations have been shown to render bacteria more resilient to drugs and other adverse agents [17-18]. Likewise, short-form trypanosomes are presumably not affected by trypanocidal drugs in the same way as the long forms. Infections associated with abundant short form trypanosomes persist relatively longer than those associated with long-form trypanosomes indicating an improved chance of being passed on to other susceptible hosts.

Transmissibility of trypanosomes is most efficient for sub-acute and chronic infection causing species and/or variants [19]. Moreover, the patterns of parasitaemia observed in this study conform to the behaviour of slender form and stumpy form of *T. brucei* during its acute and chronic phase of parasitaemia [20, 21].

Infections with drug-sensitive strain both in the murine and bovine model were initially characterized by low parasitaemia, however as infection progressed in cattle, we noted a remarkable increase in parasitaemia suggesting high replication rate of the trypanosomes

in cattle over time post-infection. The drug-resistant strain demonstrated descending parasitaemia in cattle, presumably implying an increased rate of differentiation of such strain. This could be due to a high density in the bloodstream during its early parasitaemia phase. Similar mean body lengths of the sensitive trypanosomes indicated a much-controlled differentiation thus giving the consistent morphological appearance, and this variation in parasitaemic profiles is consistent with those in BALB/c mice infected with *T. brucei* [22]. Furthermore, variation in parasitaemia which corresponded with morphological length variations highlights variation in the rate of differentiation between sensitive and resistant strain. In this context, resistant strain exhibited typical behaviour of short-form trypanosomes population of not replicating thus limiting parasitaemia as parasites numbers became low and eventually prolonging host survival as in the case of the non-dividing stumpy form of *T. brucei* [22, 23-27]. As such, drug-resistant trypanosomes in the preferential host (cattle) demonstrated the law of diminishing return as they progressively became scant and eventually disappeared in blood over time post-infection. This could be attributed to the low rate of antigenic variation switching following syringe passage through mice [28], thus allowed transmission and survival of short forms parasites in a new host, of which previous observation reported to be more infective [10] but less pathogenic [12] to the mammalian host. Similar infectivity phenomenon was observed in the transmissible short stumpy form of *T. brucei* with arrested growth and multiplication in the bloodstream [25].Moreover, in contrast to parasitaemia in mice, parasitaemia of resistant trypanosomes in cattle was maintained by high abundance of short forms population indicating the ability of trypanosomes of different morphological forms to infect new mammalian host; and the same was previously described in cyclical transmission with the stumpy form of *T. brucei* [25].

Despite the afore-explained dominance of short-form trypanosomes, the abundance of different forms varied over time post-infection suggesting morphological transition across the animal models. The different morphological forms observed represented different life stages of the trypanosomes within hosts' bloodstream.

3.5 CONCLUSION

Findings of this study suggest a correlation between the trypanosomes' therapeutic responses and their morphological appearance (morphometry). The drug-resistant strain, bloodstream trypanosomes in cattle were noticeably short than in drug-sensitive strain. This is worth worrying because of the view that short forms trypanosomes, through less pathogenic, are easily transmissible across preferential hosts, which eventually precipitate the occurrence of the disease among the animal population and use of more costly drugs. However, these conclusions need to be strengthened by additional studies involving fine-scale measurements of other determinant morphological and/or behavioural features (kinetoplast, nucleus, organelle, flagella, pathogenicity and transmissibility).

Ethics and consent statement

Ethical approval was granted by the Ethics Review Committee of Sokoine University of Agriculture (SUA), Morogoro, Tanzania. All experiments were conducted with strict adherence to the principles of animal care.

Acknowledgements

We deeply thank the late Prof. A.E. Kimambo for providing a fly-proof facility where experimental cattle were kept. This research was funded by the government of Tanzania through the Commission for Science and Technology (COSTECH).

Author's contributions

Both authors made substantial contributions to the conception and design, data collection, or analysis and interpretation of data; took part in drafting the article and revising it; and gave final approval of the version to be published and agree to be accountable for all aspects of the work.

REFERENCES

1. Auty H, Torr SJ, Michoel T, Jayaraman S, Morrison LJ. Cattle trypanosomosis: the diversity of trypanosomes and implications for disease epidemiology and control. *Rev Sci Tech Off Int Epiz.* 2015;34(2):587-598.
2. Vickerman K. The fine structure of *Trypanosoma congolense* in its bloodstream phase. *J Protozool*. 1969;16:54-69.
3. Vickerman K. Developmental cycles and biology of pathogenic trypanosomes. *Br Med Bull.* 1985;41(2):105-114.
4. Peacock L, Cook S, Ferris V, Bailey M, Gibson W. The life cycle of *Trypanosoma* (Nannomonas) *congolense* in the tsetse fly. *Parasit & Vectors.* 2012;5:109.
5. Gibson W. Species concepts for trypanosomes: from morphological to molecular definitions? *Kinetoplastid Biol Dis.* 2003;2(1):210.
6. Young JC, Godfrey GD. Enzyme polymorphism and the distribution of *Trypanosoma congolense* isolates. *Ann Trop Med Parasit*. 1983;77(5):467-481.

7. Uilenberg G, Boyt WP. A field guide for the diagnosis treatment and prevention of African Animal Trypanosomosis. Food and Agriculture Organization of the United Nations. Rome 1998.
8. McNamara JJ, Mohammed G and Gibson WC. *Trypanosoma* (Nannomonas) *godfrey* isp.nov.from tsetse flies in The Gambia: Biological and biochemical characterization. *Parasitology.* 1994;109:497-509.
9. Godfrey DG. Types of *Trypanosoma congolense* I. Morphological differences. *Ann Trop Med Parasit.* 1960;54(4):428-438.
10. Nantulya VM, Doyle JJ, Jenni L. Studies on *Trypanosoma* (Nannomonas) *congolense* I. On morphological appearance of the parasite in the mouse. *Acta Tropica*. 1978;35(4):329-337.
11. Hoare CA. Morphological and taxonomic studies on mammalian trypanosomes. IX: Revision of Trypanosoma dimorphon. *Parasitology.* 1959;49:210-231.
12. Godfrey DG. Types of *Trypanosoma congolense* II. Differences in the courses of infection. *Ann Trop Med Parasit*. 1961;55:2:154-166.
13. Lumsden WHR, Herbert WJ and McNeillage GJC. Techniques with Trypanosomes 1973, Churchill Livingstone Edinburgh & London.
14. Fairbairn H. Measurements of strains of *Trypanosoma congolense*. *Ann Trop Med Parasit.* 1962;56:218-221.
15. Desquesnes M, Holzmuller P, Lai D, Dargantes A, Lun Z, Jittaplapong S. *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *Biomed Res Int.* 2013;194176
16. Thompson KC, Botero A, Wayne FA, Godfrey SS, Lumbery JA, Thompson ARC. Morphological polymorphism of *Trypanosoma copemani* and description of the genetically diverse *T. vegrandis* sp.nov.from the critically endangered Australian

potoroid, the brush-tailed bettong *Bettongia penicillata* (Graty1837). *Parasit & Vectors*. 2013;6:121.

17. Spalding C, Keen E, Smith DJ, Krachler A-M, Jabbari S. Mathematical modelling of the antibiotic-induced morphological transition of *Pseudomonas aeruginosa*. PLoS Comput Biol 2018;14(2): e1006012.
18. Chakravarty D, Banerjee M, Bihani SC, Ballal A. Novelmolecularinsightsintotheanti-oxidativestressresponseandstructure-functionofasalt-induciblecyano-bacterialMn-catalase. PlantCellEnviron.2019;42:2508–2521.
19. Gitonga PK, Ndung'u K, Murilla GA, et al. Differential virulence and tsetse fly transmissibility of *Trypanosoma congolense* and *Trypanosoma brucei* strains. *Onderstepoort J Vet Res*. 2017;84(1):e1-e10.
20. Turner CMR, Aslam N, Dye C: Replication, differentiation, growth and the virulence of *Trypanosoma brucei* infections. *Parasitology.* 1995;111(3):289–300.
21. Masumu J, Akoda K, Van de Bossche P. Transmissibility by *Glossina morsitans morsitans* of *Trypanosoma congolense* strains during the acute and chronic phases of infection. *Acta Trop*. 2010;113(2):195-198.
22. Tyler MK, Higgs GP, Matthews RK, Gull K. Limitation of *Trypanosoma brucei* parasitaemia results from density-dependent parasite differentiation and parasite killing by the host immune response. *Proc R Soc Lond B.* 2001;268(1482):2235-2243.
23. Shapiro ZS, Naessens J, Liesegang B, Moloo KS, Magondu J. Analysis by flow cytometry of DNA synthesis during the life cycle of African trypanosomes. *Acta Tropica.* 1984;41:313–323.
24. McGregor P, Savill JN, Hall D, Matthews RK. Transmission stages dominate trypanosome within-host dynamics during chronic infections. *Cell Host Microbe.* 2011; 9(4):310–318.

25. Rico E, Rojar F, Mony MB, Szoor B, MacGregor P, Matthews RK. Bloodstream form pre-adaptation to the tsetse fly in *Trypanosoma brucei*. *Front Cell Infect Microbiol.* 2013;3:78.
26. Matthews KR, McCulloch R, Morrison LJ. The within-host dynamics of African trypanosome infections. *Phil Trans R Soc B.* 2015;370(1675):2014-288.
27. Silvester E, McWilliam RK, Matthew RK. The cytological events and molecular control of life cycle development of *Trypanosoma brucei* in the mammalian bloodstream. *Pathogens.* 2017;6(3):29.
28. Turner CM: The rate of antigenic variation in fly-transmitted and syringe-passaged infections of *Trypanosoma brucei*. *FEMS Microbiol Lett.* 1997;153:227-31.

CHAPTER FOUR

4.0 Pathogenicity and transmissibility of trypanosomes in relation to drug sensitivity: comparative studies on two *Trypanosoma congolense* strains in bovine and murine models

A.F. Ngumbi[1,2*] and L.L. Mnyone[3]

[1]Livestock Training Agency, P.O. Box 603, Morogoro, Tanzania

[2]Department of Veterinary Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3019, Chuo Kikuu, Morogoro, Tanzania

[3]Sokoine University of Agriculture, Pest Management Centre, P.O. Box 3110, Chuo Kikuu, Morogoro, Tanzania

Email: AFN: ngumbianna0@gmail.com

Submitted: Research and Reports in Tropical Medicine

Abstract

Background: Tsetse transmitted animal trypanosomosis remains a major animal disease affecting livestock production in Sub-Saharan Africa. *Trypanosoma congolense (T. congolense)* is the most pathogenic and prevalent causative agent of the disease in cattle in most of sub-Saharan Africa. Misuse and indiscriminate exposure of *T. congolense* and other predominant *Trypanosoma species* to drugs have led to emergence of resistant strains, which consequently impede control efforts against the disease. Despite several reports of resistance, whether or not such trait is positively or negatively correlated to pathogenicity and transmissibility in several trypanosomes has not been unraveled. This study assessed the relationship between pathogenicity and drug sensitivity in two putatively drug-sensitive and resistant *T. congolense* stocks/strains using bovine and murine model.

Methodology: Mice and steers were infected with the drug-sensitive and resistant *T. congolense* strain, 0.2 ml and 5 ml of PBSG diluted blood containing 5×10^6trypanosomes/ml respectively, treated with different doses of isometamidium chloride and diminazene aceturate either drugs and thereafter monitored for parasitaemia, prepatent period and packed cell volume (PCV) for over 14 days. Parasitaemia was monitored via microscopic examination of wet smear of blood from the experimental animals.

Results: Diminazene aceturate was 40% effective at the dose of 5.25 mg/kg and 80% at the dose of 7.0 mg/kg in mice infected with *T. congolense* Mikese strain. In mice infected with *T. congolense* Mbagala strain, trypanosomes persisted treatment with all doses of diminazene aceturate; 3.5, 5.25 and 7 mg/kg body weight. All mice infected with *T. congolense* Mikese strain, except one, were cleared of trypanosomes after treatment with 2.5 mg/kg body weight of Isometamidium chloride (ISMM). The duration of clearance was reduced to about two days post treatment with 5 – 10 mg/kg body weight of ISMM. However, all mice treated with high dose all died 11 days later; and this could be due to drug intoxication. All mice infected with *T. congolense* Mbagala resisted treatment with all test doses of ISMM; 2.0, 5.0 and 10 mg/kg body weight. Apparently, these mice remained parasitological positive throughout the observation period. The prepatent period in mice was 2.8 ± 1.3 days for resistant strain and 4.6 ± 1.5 days for sensitive strain with no significant difference ($p=0.08$) whereas the mean parasitaemic levels in mice were 6.5 ± 0.8 and 7.5 ± 0.8 for *T. congolense*–sensitive and *T. congolense*–resistant strain respectively. Parasitaemia was significantly higher in *T. congolense*–resistant strain [$p<0.001$, 95% CI= -1.28 to -0.68]. Mean rectal temperature was significantly higher in mice infected with *T. congolense*–sensitive strain [$p=0.049$, 95% CI= 0.003 - 1.13].

Mice infected with *T. congolense*–resistant strain appeared dull and lethargic compared to mice infected by *T. congolense*–sensitive strain. The level of decline in PCV was

significantly higher in steer infected with *T. congolense*–sensitive stock than the steer infected with *T. congolense*–resistant stock [*p=0.041*, (95% CI, -6.97 to -0.17)]

Conclusion: Transmission of infection to murine and bovine hosts was quite high for *T. congolense* SIO-210 Mbagala which is resistant to both DA and ISMM. Besides, *T. congolense* SIO-210 Mbagala demonstrated high pathogenic potentials in mice than in bovine, thus decoupling transmissibility with pathogenicity in the presence of host defenses.

Keywords: Syringe passage, drug sensitivity, pathogenicity, transmissibility, *T. congolense*

4.1 INTRODUCTION

Tsetse-transmitted animal trypanosomosis causes devastating socio-economic impact in sub-Saharan Africa as a result of hindrance to livestock productivity. The disease is caused by extracellular protozoan parasites belonging to the genus *Trypanosoma*. The main pathogenic species in cattle include *Trypanosoma congolense*, *T. vivax* and *T. brucei* to a lesser extent. Of these, *T. congolense* is the most prevalent and pathogenic species in Eastern and Southern Africa (Gillingwater *et al.*, 2010).

Animal trypanosomosis can affect a wide range of livestock; however, their susceptibility to trypanosome infection varies across species. This variation is influenced by parasite and host-related variables, the most important of which include species and strains of trypanosomes as well as breed, age, behavior, previous exposure and health status of the host species (Murray, 1989). Similarly, these parasite and host-related variables influence the pathogenicity of trypanosomes (Taylor and Authie, 2004; Van den Bossche *et al.*, 2011).

In cattle, the most consistent clinical manifestations are intermittent fever, anaemia and weight loss (Holmes, 2005). Other manifestations include reduced productivity, infertility

and abortion. Death in some animals may also occur often as a result of congestive heart failure due to anaemia, myocarditis and circulatory disturbances. Unfortunately, there are no specific manifestations and/or signs that are pathognomonic to trypanosomosis. However, in susceptible cattle, the development of anaemia is a cardinal sign of trypanosomosis and is used as a key diagnostic feature for the disease (Noyes *et al.*, 2009). Anaemia in animal trypanosomosis is due to multiple aetiological factors associated with the level and duration of parasitaemia such as lashing actions of flagella, undulating pyrexia, toxins and metabolites from trypanosomes which disrupt erythrocytes membrane continuity and/or permeability (Mbaya *et al.*, 2012). Certain breeds of cattle such as N'Dama calves (*Bos Taurus*) have been reported to possess a resistance trait that limits anaemia (Naessens, 2006).

Several trypanocidal drugs have been developed and are widely employed for treatment and control of animal trypanosomosis for many years (Holmes *et al.*, 2004). Most of such drugs are available and affordable to farmers. However, the efficiency of those drugs is increasingly constrained by development of resistance in trypanosomes, largely as a consequence of their indiscriminate use in many endemic countries (Geerts *et al.*, 2001). Resistance to commonly used trypanocides has been reported in many countries including Tanzania (Mbwambo *et al.*, 1988; McDermott *et al.*, 2003; Delespaux and Koning, 2007; Achenef and Bekele, 2013). Similarly, experimental studies in mice and cattle have indicated varying degrees of resistance in many endemic countries (Mungube *et al.*, 2012; Hagos *et al.*, 2014).

However, whether or not resistance to trypanocides is positively or negatively correlated to pathogenicity in several trypanosomes remains indeterminate. Observations made by a number of authors indicated substantial differences in virulence of isolates of

trypanosomes stocks between subgroups within trypanosome species (Bengaly *et al.*, 2002a,b; Motloang *et al.*, 2014) and strains within subgroups of trypanosome species (Masumu *et al.*, 2006; Van den Bossche *et al.*, 2011). Despite the resistant trait in some trypanosomes, several authors observed a loss of virulence and/or loss of fitness in drug resistant trypanosomes (FAO, 1998). Trypanosome virulence or pathogenicity, at one extreme has been defined as a measure of the impact of parasite on the fitness of its host (Brown *et al.*, 2000). At the other end, Bengaly *et al.* (2002b) defined parasite virulence as the capacity of parasites to damage and cause disease to its host, while parasite's pathogenicity being a combination of its infectivity [ability to multiply and be maintained in a given host] and its virulence. Lipsitch and Moxon (1997) linked the fitness of a pathogenic microorganism to its transmission from host to host, whereas defined pathogenicity as the potential for a pathogen to infect or to damage a hosts.

Furthermore transmissibility and pathogenicity determines succession of pathogen to its host, thus determines how a host and its parasite interact in a given area. This study therefore sought to determine the correlation between drug sensitivity, transmissibility and the pathogenicity of putatively drug-sensitive and resistant *T. congolense* strains.

4.2 MATERIALS AND METHODS

4.2.1 Experimental animals

Two sets of experimental animals were used in this study: indigenous Zebu cattle and Swiss Albino mice. The indigenous cattle used in this study were Shorthorn zebu steers, 6-8 months old, weighing 120-140 kg. These cattle were purchased from the Maasai herds in Lugala village, Morogoro, Tanzania, which was known to be tsetse free. They were ear-tagged and maintained under room temperature in a fly proof pen at DAARS farm at SUA. They were acclimatized for four months before the experiments commenced. During this

period, they were also screened for trypanosomes, other haemoparasites and worms. Furthermore, these animals were regularly dewormed throughout the study using Levamisole at the dose of 1ml per 10 kg body weight; and were also injected with Imidocarb 3mg/kg body weight to prevent them from other haemoparasites. The body weight of these animals was measured using a weigh-band (Rondo®, Hyperdrug, UK). Packed cell volume (PCV) was also examined and recorded as baseline data. The animals were fed on cut grass and supplemented with hay and concentrates. Water and a mineral lick (farmers superlick®) were provided *ad libitum* throughout the study. The Swiss albino mice used in this study, 8-12 weeks old males, weighing 20-30 g, were obtained from the stocks bred at the Small Animals Breeding Unit, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania. These mice were kept inside clean cages and maintained under room temperature within well-ventilated fly-proof experimental animal house. The individual mice were identified accordingly with picric acid as described by Lumsden *et al.* (1973). They were fed on broiler mash commercial preparation (in lieu of specific mouse feed formula). Water was provided *ad libitum*, and wood chippings were used as bedding material. The wood chippings were frequently changed to ensure dry micro-habitats.

4.2.2 Trypanosomes

Stocks of two strains of *T. congolense* namely, *T. congolense*-Mikese (a putatively drug-sensitive strain) and *T. congolense*-SIO-201 Mbagala (a putatively drug-resistant strain) were used in this study. The former and latter strains were isolated from cattle at Mikese, Morogoro region and Mbagala, Dar es Salaam region respectively. These stocks are maintained through serial passages in mice at the Small Animals Breeding Unit, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture,

Morogoro, Tanzania. Before the current study, the strains had undergone a minimum of 20 passages.

4.2.3 Trypanocidal drugs

The trypanocidal drugs used were Diminazene aceturate (Diminakel®), a product of KELA N.V., Sint Lenaartseweg 48, 2320 Hoogstraten/Belgium, reg No TAN 06, 074PO1X KEL with batch number 21558 and isometamidium chloride (Trypamidium-Samorin®), a product of MERIAL- 4 chemin du calquet – 31057 TOULOUSE- FRANCE/ MERIAL 29 avenue Torry Garnier - 69007 LYON-FRANCE with batch number TF J657A. The drugs were purchased locally from veterinary pharmacy (Makanyaga Vet Agro) in Morogoro, Tanzania. The drugs were administered after being freshly diluted in sterile distilled water as per manufacturer's recommendations.

4.2.4 Inoculation of experimental mice with trypanosomes

Infected Swiss albino donor mice were examined three times per week until a high degree of parasitaemia, approximately 1×10^8 as estimated by the method of Herbert and Lumsden. (1976) was reached before inoculation into the experimental mice. The parasitaemia was determined by examining wet films of tail blood. At least 20 microscopic fields at ×400 magnification were examined before declaring a sample negative. Before inoculation, tail-blood drop was diluted in phosphate buffered saline with glucose (PBSG) to attain a concentration of 5×10^6 parasites in 0.2 ml. As such, each mouse in the treatment groups was inoculated intraperitoneally (IP) with 0.2 ml of either *T. congolense*-Mikese or *T. congolense*-SIO-201 Mbagala. Control groups (CG) of mice were similarly inoculated with 0.2 ml of PBSG.

4.2.5 Drug sensitivity of *T. congolense* stocks

The sensitivity of drug sensitive and resistant *T. congolense* strain against isometamidium chloride and diminazene aceturate was tested in vivo using 40 male mice in groups of five (5). They were allocated into sixteen (16) groups of five (5) mice, of which seven (7) each were treated with either diminazene aceturate or isometamidium chloride and the remaining two (2) served as untreated groups. As described elsewhere in this chapter, the individual mice in the treatment and control groups were inoculated with 2ml of blood from donor mice at a concentration 5×10^6 trypanosomes/ml and 2 ml of PBSG respectively. The freshly prepared stock solution of each drug was serially diluted in accordance to targeted dose rate such that a mouse weighing 20 g received 0.2 ml of drug solution.

For drug sensitive *T. congolense* strain, 8 groups of 5 mice each, were treated with either diminazene aceturate (DA) at a dose rate of 1 mg/kg, 1.75 mg/kg, 3.5 mg/kg, 5.25 mg/kg and 7 mg/kg or isometamedium chloride hydrochloride (ISMM) at a dose rate of 2.5 mg/kg, 5 mg/kg and 10 mg/kg. As such, a total of 25 mice were tested for diminazene aceturate and 15 mice were tested for isometamidium.

For drug resistant *T. congolense* strain, 6 groups of 5 mice each were treated with either DA at a dose rate of 3.5 mg/kg, 5.25 mg/kg and 7 mg/kg or similar doses of ISMM. As such, a total of 30 mice were used for the two drugs.

All mice in the above groups were confirmed for parasitaemia before treatment with respective doses of DA and ISMM solutions intraperitoneally. Overall, day four post infection all mice in the treatment groups showed parasitaemia and got treated thereof. Mice were examined for parasitaemia 24 hrs, 48 hrs, and then twice a week for up to 60 days post treatment. Mice which were parasitaemic after 60 days post treatment were declared cured.

4.2.6 Pathogenicity testing in mice

The mice were divided into 2 treatment groups and one control group, 5 mice each. The mice in treatment group one were inoculated with drug-sensitive *T. congolense* strain; referred herein as the drug-sensitive *T. congolense* group (TCSG). The mice in treatment group two were inoculated with drug-resistant *T. congolense* strain; referred herein as the drug-resistant *T. congolense* group. The mice in group three were inoculated with PBSG; referred herein as the control group (CG).

The mice in all three groups were monitored for parasitaemia daily starting from day 2 to day 14 post infection. This was done through microscopic examination of wet tail blood films of individual mouse per procedures described by Herbert and Lumsden. (1976). additionally, the pre-patent period (PP), death and survival time of these mice were monitored daily for over 30 days post-infection. The body temperature of individual mouse was also measured by rectally-placed thermometer and recorded. At the end of the post-infection monitoring, the strains were categorized as acute infection causing (mean survival time [MST] ≤ 10 days), sub-acute infection causing (MST > 10 days but < 30 days) and chronic infection causing (MST ≥ 30 days) (Bengaly *et al.*, 2002a).

4.2.7 Pathogenicity testing in cattle

Three steers were used in this experiment. The first steer named BIS1 was inoculated with the drug sensitive *T. congolense* strain (BIS1-S) and the second steer named BIS2 was inoculated with drug resistant *T. congolense* strain. The third steer named BIS3 was inoculated with PBSG and served as control. The *T. congolense* sensitive and resistant strains inoculated in steers were obtained from the previously infected donor mice. The individual treatment steers were infected with the trypanosomes via the intravenous injection with 5 ml diluted blood from donor mice at a concentration of

5×10^6trypanosomes/ml (Herbert and Lumsden, 1976). The control steer received a 5 ml intravenous injection of PBSG.

After infecting the steers, the pathogenicity of individual *T. congolense* strains was assessed by comparing and contrasting the pre-patent period, level of parasitaemia, development of anaemia and development of clinical signs and death rate across the treatment and control steers. These parameters were assessed through wet blood films and buffy coat microscopic examination, packed cell volume (PCV) and visual examination of the steers. These animals were examined three times a week and blood samples were collected from jugular vein into vacutainer tubes with EDTA. The wet blood films and buffy coat were examined for trypanosomes under microscope at 400× magnification. The collected blood was also used to assess anaemia through estimation of PCV using micro-haematocrit centrifugation technique (Woo, 1969). The monitoring of different pathogenicity indicators examined herein, continued for 14 days after detection of test trypanosomes in the bloodstream.

4.2.8 Data analysis

The data obtained from all experiments were entered and managed in excel. The student t-test was in SPSS version 16 was employed to analyse the data. The proportions of treatment and control animals in respect of pre-patent period, level of parasitaemia, development of anaemia and development of clinical signs and death were compared over time post-infection or treatment with test drugs. The rate of drug resistant infections was calculated as the number of parasitaemic animals on the days of observation divided by the total number of animals treated. Interpretation of the drug sensitivity results was made according to the descriptions given by Eisler *et al.* (2001). *P-values* < 0.05 were considered significant.

4.3 RESULTS

4.3.1 Drug sensitivity in mice

For *T. congolense* Mikese (drug-sensitive strain) the effect of diminazene aceturate (DA) on infected mice increased the drug dosage. At the dose of 3.5 mg/kg body weight, DA did not reduce parasitaemia in all groups of infected mice. At the dose of 5.25mg/kg body weight, DA reduced parasitaemia in 40% of infected mice. At the dose of 7.0mg/kg body weight, 80% of infected mice remained parasitological negative following treatment with DA (Table 4.1). Based on these results, the curative dose CD_{80} of DA was 7.0 mg/kg body weight.

For *T. congolense* SIO-210 Mbagala (drug resistant strain), trypanosomes reappeared in all groups of infected mice within 16 days to the end of monitoring period irrespective of the dose of DA used, 3.5, 5.25 and 7 mg/kg body weight (Table 4.2).

Table 4. 1: Proportions of mice that were parasitaemic for *T. congolense* Mikese (drug-sensitive stock/strain) over time post treatment (carried out at day-4 post infection) with different doses of diminazene aceturate

	Dose (mg/kg) **Number of parasitaemic mice/total mice treated**					
Days post treatment	**0.00 mg/kg**	**1 mg/kg**	**1.75 mg/kg**	**3.5 mg/kg**	**5.25 mg/kg**	**7.0 mg/kg**
1	5/5	4/5	3/5	4/5	4/5	3/5
2	4/4	5/5	4/5	0/5	0/5	0/5
5	3/3	5/5	5/5	3/5	2/5	4/5
8	3/3	5/5	5/5	5/5	2/5	4/5
11	3/3	5/5	5/5	4/5	3/5	4/5
14	3/3	5/5	5/5	5/5	2/5	0/5
17	3/3	5/5	5/5	5/5	3/5	2/5
20	3/3	5/5	5/5	5/5	3/5	1/5
58	2/2	4/4	5/5	3/3	2/ 3	1/5
61	2/2***	4/4*	5/5	3/3**	2/ 3**	1/5

Table 4. 2: Proportion of mice that were parasitaemic for *T. congolense* SIO-201 Mbagala (drug resistant stock/ strain) over time post treatment (carried out at day-4 post infection) with different doses of diminazene aceturate

Days post treatment	**Dose (mg/kg) Number of parasitaemic mice/total mice treated**			
	0.00 mg/kg	**3.5 mg/kg**	**5.25 mg/kg**	**7.0 mg/kg**
0	5/5	5/5	4/5	4/5
1	5/5	0/5	0/5	2/5
2	5/5	0/5	0/5	1/5
3	5/5	0/5	0/5	2/5
4	5/5	2/5	2/5	3/5
7	5/5	3/5	4/5	3/5
10	5/5	4/5	4/5	3/5
13	5/5	4/5	5/5	5/5
16	5/5	5/5	5/5	5/5
58	2/2	2/2	3/3	3/3
61	2/2***	2/2***	3/3**	3/3**

0.00mg/kg-Untreated control, *-One mouse died, **Two mice died, ***Three mice died

For *T. congolense* Mikese (drug-sensitive strain), trypanosomes disappeared in all, except one, infected mice after treatment with ISMM at the lowest dose used, 2.5 mg/kg body weight throughout the monitoring period (Table 4.3). This suggested a curative dose CD_{80} of 2.5 mg/kg ISMM. At the higher doses used, 5 and 10 mg/kg body weight, trypanosomes disappeared in all groups of infected mice by day-2 post treatment. All five mice treated with ISMM at a dose of 10 mg/kg died by day-11 post-treatment necessitating declining this dose from the experiment.

For *T. congolense* SIO-210 Mbagala (drug resistant strain), trypanosomes reappeared in all groups of infected mice even after treatment with ISMM irrespective of the dose used, 2.5 mg/kg, 5 mg/kg and 10 mg/kg (Table 4.4).

Table 4. 3: Proportions of mice that were parasitaemic for *T. congolense* Mikese (drug-sensitive stock/strain) over time post treatment (carried out at day 4 post infection) with different doses of isometamedium chloride

Days post treatment	Dose (mg/kg) Number of parasitaemic mice/total mice treated			
	0.00 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
1	5/5	2/5	0/5	1/5
2	4/4	1/5	0/4	0/5
5	3/3	0/5	0/3	0/4
8	3/3	2/5	0/3	0/3
11	3/3	2/5	0/3	-
14	3/3	1/5	0/3	-
17	3/3	1/5	0/3	-
20	3/3	1/5	0/3	-
58	2/2	1/3	0/3	-
61	2/2***	1/3**	0/3**	-

Table 4. 4: Proportion of mice that were parasitaemic for *T. congolense* SIO-201 Mbagala (drug resistant stock/ strain) over time post treatment (carried out at day 4 post infection) with different doses of isometamidium chloride

Days post treatment	Dose (mg/kg) Number of parasitaemic mice/total mice treated			
	0.00 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg
0	5/5	5/5	5/5	4/5
1	5/5	0/5	0/5	0/5
2	5/5	0/5	0/5	0/5
3	5/5	0/5	0/5	0/5
4	5/5	0/5	1/5	0/5
7	5/5	1/5	2/5	2/5
10	5/5	3/5	4/5	3/5
13	5/5	5/5	4/5	3/5
58	2/2	3/3	3/3	2/2
61	2/2***	3/3**	3/3**	2/2***

0.00mg/kg-Untreated control, **Two mice died, ***Three mice died, All dead

4.3.2 Pathogenicity of trypanosomes

The mean prepatent period (time-lapse post-infection to parasite detection in blood) in mice infected with sensitive *T. congolense* strain (2.8 ± 1.3 days) was similar to that of mice infected with resistant *T. congolense* strain (4.6 ± 1.5 days) [p=0.079]. Parasitaemia

in mice infected with resistant strain (7.5 ± 0.8 trypanosomes/ml) was significantly higher than in mice infected with sensitive strain (6.5 ± 0.8 trypanosomes/ml) [p<0.001, 95% CI= -1.28 to -0.68]. Furthermore, parasitaemia increased with time in both strains, however, parasitaemia in mice infected with resistant strain stabilized at $1\times10^{7.5}$ - $^{8.1}$trypanosomes/ml from day 8 post infection to the end of observation period (Figure 4.1).

Rectal temperatures for mice infected with sensitive and resistant strain were elevated above 37ºC normal temperature (http: //www.informatics .jax.org/mgihome/other/mouse_ facts1.shtml) in the first three days post infection. Temperature declined to subnormal, 36.5ºC, at day six and stabilized at day eight post-infection correlating to stabilization of parasitaemia in *T. congolense* – resistant strain. In *T. congolense* sensitive strain, episodes of temperature elevations continued to occur for the rest of the observation period (Figure 4.2). The mean rectal temperature was significantly higher in mice infected with sensitive strain (37.6 ± 0.6ºC) than in mice infected with resistant strain and (37.0 ± 1.3ºC) [p=0.049, 95% CI= 0.003 - 1.13]. Mice infected with resistant strain appeared dull and lethargic compared to mice infected by sensitive strain, which remained active over the entire period of observation. Mortalities were recorded from day 9 to 30 for mice infected with resistant strain, whereas those infected with sensitive strain survived for more than 30 days post infection.

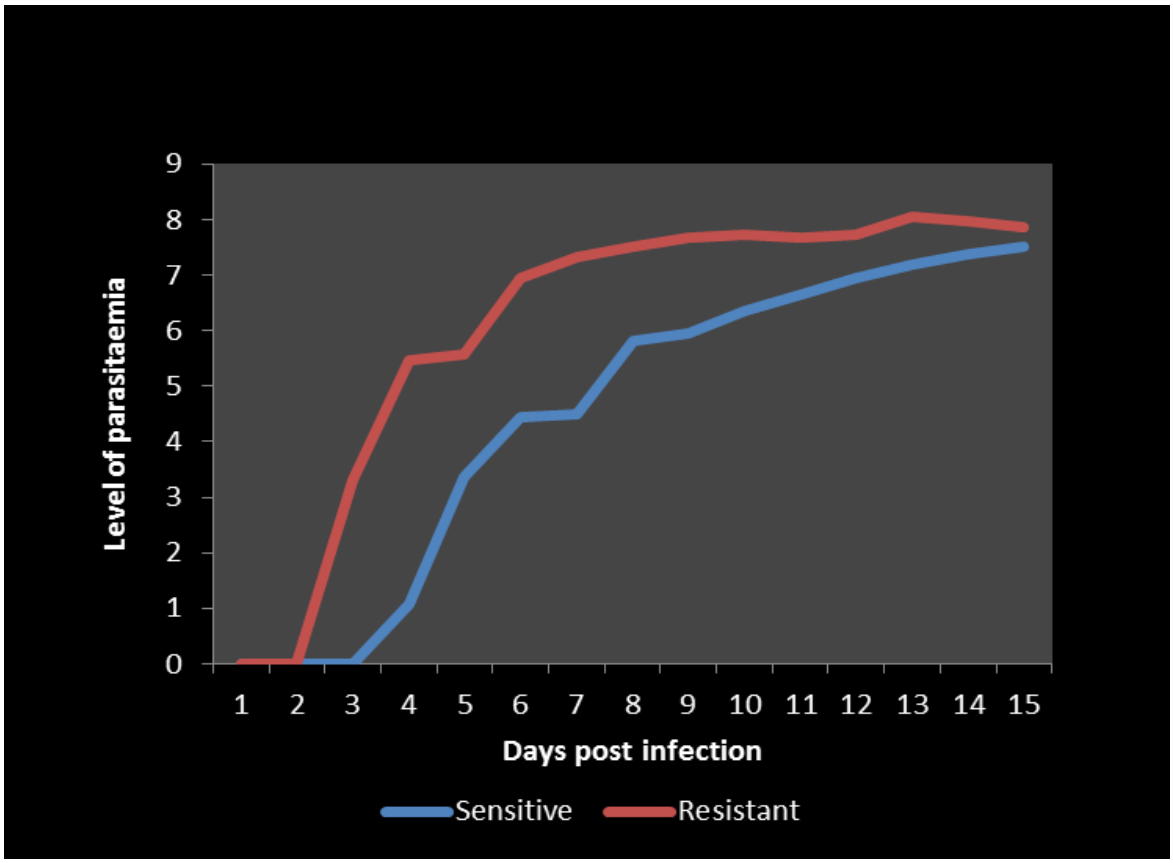

Figure 4. 1: Parasitaemia level (10^xparasites/ml) in mice post infection with *T. congolense* drug-sensitive and drug-resistant stocks/ strains

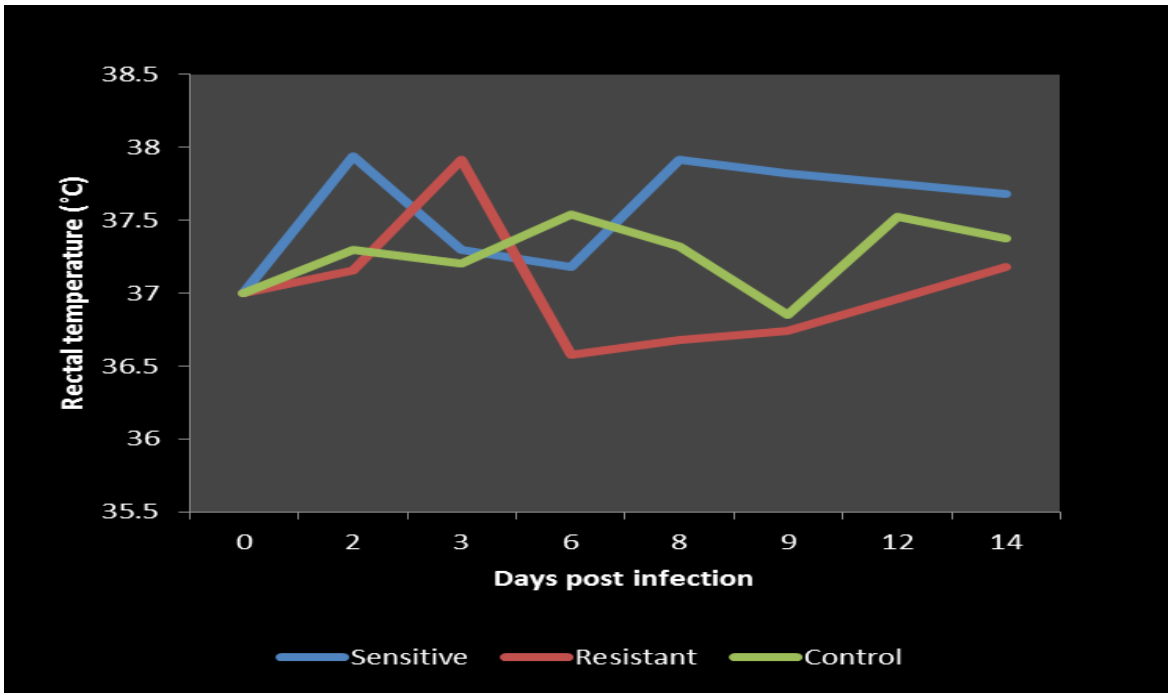

Figure 4. 2: Body temperature (°C) of mice post infection with *T. congolense* drug-sensitive and drug-resistant stocks/ strains

In experimentally infected steers; the mean prepatent period (time-lapse post-infection to parasite detection in blood) was 9 days for steers infected with resistant strain and 7 days for steers infected with sensitive strain. The mean PCV was 18.1 ± 2.2 for steers infected with sensitive strain and 21.7 ± 3.5 for steers infected with resistant strain. Generally, the PCV declined for experimental steers compared to control steer. However, the decline in PCV was significantly higher in the steer infected with sensitive strain than the steer infected with resistant strain [$p=0.041$, (95% CI, -6.97 to -0.17)] (Figure 4.3).

Parasitaemia in the steer infected with resistant strain decreased to $1\times10^{5.4}$ trypanosomes/ml, with increase in days post- infection and the steer was observed to be asymptomatic. The steer infected with sensitive strain presented with poor body condition, dullness and reluctance to eat. Finally, this animal went down in sternal recumbence as parasitaemia increased to $1\times10^{7.5}$ trypanosomes/ml on day 26, and eventually it died in the course of treatment 41 days post infection. The animal had been cleared of the parasites before death.

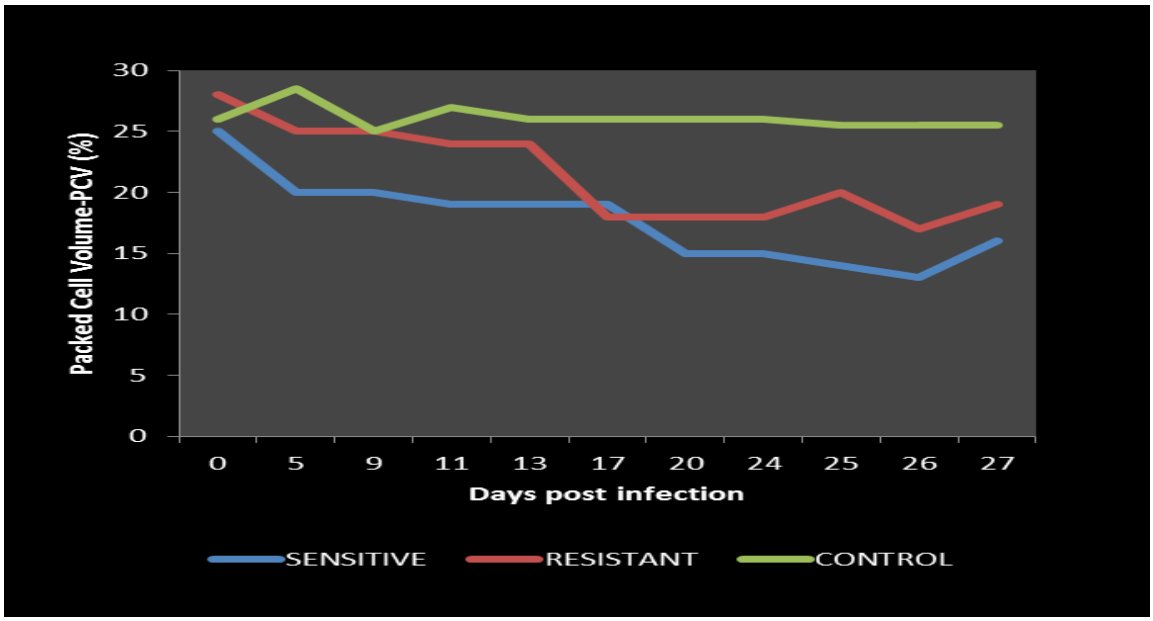

Figure 4. 3: Packed cell volume (%) of steers following infection with *T. congolense* stocks/ strains

4.4 DISCUSSION

This study was meant to assess the relationship between pathogenicity and drug sensitivity in two putatively drug-sensitive and resistant *T. congolense* stocks/strains. The results have shown continued occurrence of DA and ISMM sensitive and resistant populations in stocks of *T. congolense* Mikese and *T. congolense* SIO-210 Mbagala respectively. *T. congolense* sensitive stock previously was highly sensitive to DA at a very low dose rate 2 mg/kg body weight as studied earlier by Mkumbukwa and Silayo (Unpublished data). However in present experiment, susceptibility of this trypanosome stock to DA decreased to as high dose rate as 5.25 to 7.0 mg/kg in mice. ISMM 2.5 mg/kg was shown to effect 80% cure of *T. congolense* Mikese infected mice. However, the levels of susceptibility of *T. congolense* Mikese to DA and ISMM observed in this experiment are relatively low as compared with susceptibility to normal recommended dose rate 3.5 mg/kg for DA and 1.0 mg/kg for ISMM which becomes practically important in the field. This suggests cautious use of drug curative dosage rates obtained for mice in predicting those for cattle as these two hosts (bovine and murine) obviously differ in body size, pharmacokinetics and metabolism of drugs (Sones *et al*, 1988). Resistance of *T. congolense* SIO-210 Mbagala strain to DA at dose rate up to 7 mg/kg persisted. Similar resistance trend was shown previously with this trypanosome stock on its discovery. Resistance to ISMM was also retained at dose rate of up to 10 mg/kg. In line with this, high degree of resistance of resistant stock was previously observed by Mushule and Silayo (Unpublished) following single dose treatment with DA and ISMM at dose rate 7 mg/kg to 84 mg/kg and 1.0 mg/kg to 20 mg/kg respectively.

The present studies aimed to compare trypanocidal resistance and its impact to trypanosomes infection development in animals (cattle and mice) infected with two stocks of *T. congolense* namely, *T. congolense* Mikese, putatively drug sensitive and

T congolense Mbagala putatively drug resistant. It was shown that the prepatent periods of infections with these differed slightly. *Trypanosoma congolense* Mbagala appeared to have a relatively shorter prepatent period in cattle and mice than the case for *T. congolense* Mikese. Despite this finding being in agreement with the findings of Tesfaye *et al.* (2012) in goats, theoretically, it is the sensitive stock which should have had a shorter prepatent period, meaning that the drug sensitive individuals within a population tend to multiply faster. This theory coincides with the proliferation and differentiation phenomenon of actively dividing slender form of trypanosomes, *T. brucei* in particular (Matthews *et al.*, 2015). In this study, the shorter prepatent period indicated the higher ability of the resistant strain to greatly multiply and establish in new hosts as compared to sensitive strain resulting in appearance of parasitaemia within a very short period post inoculation as evidenced in mice and steers. This could be due to selection pressure created by extensive syringe passage, that tend to select for parasite with higher replication rates during rapid syringe passages, thus transmission been in a parasite density-dependent manner. Resistant stock (*T. congolense* Mbagala) was shown to have higher pathogen fitness than the sensitive stock (*T. congolens*e Mikese) in mice. The fitness was demonstrated by the significantly higher parasitaemia in the case of *T. congolense* Mbagala in contrast to the case with *T. congolense* Mikese. However, infections in mice with both stocks parasitaemia increased with increased time post- inoculation. The ability of the resistant strains to more rapidly multiply in the host than the sensitive strains could be explained by their ability to survive a die-off phenomenon owing to host's immunological responses and possible rapid expression of Variable Surface Glycoprotein (VSG) by these resistant stock (Godfrey, 1961) enough to increase their capacity for successful uptake and establishment between experimental hosts. The results obtained in this study can also be coupled with reports of serial passage of *T. congolense* not resulting in diminishing of drug resistance (Silayo and Marandu, 1991).

However, it is worth noting that even with short prepatent period, very low parasitaemia was observed with *T. congolense* Mbagala a resistant stock infection in cattle as days post infection progressed, the same pattern also observed with packed cell volume. This differs prominently with observed parasitaemia of the same stock in mice, thus suggesting the role of host immune response in limiting the increase of parasitaemia and anaemia. In this context, it is worth to recognize the existence of trypanotolerant breeds of cattle. Other studies had found little or no difference in parasitaemia between trypanosuceptible and trypanotolerant cattle at early stages of infection. Naessens (2006) defined trypanotolerance in bovine as the better capacity to control anaemia throughout the infection and eventually control parasite numbers in the blood during the later stages of infection. Similarly low parasitaemia, moderate anaemia and non-disease state was observed in this study in steer infected with resistant strain of *T. congolense*. Marcotty *et al.* (2008) also explained more parasite detection at early stages of infection even before anaemia developed. Additionally, decreased parasitaemia shown by resistant stock in cattle was implicated with low rate of switching of variant surface glycoprotein (VSG) coat of trypanosomes associated with serial syringe passaged infection (Turner, 1997).

Anaemia is a well recognised detrimental consequence of trypanosomosis in bovine, measured by determining the packed cell volume (PCV). In this study, PCVs in the animal infected with the sensitive stock were significantly lower than the PCVs of the steer infected with the resistant stock. This observation affirmed that there was a difference in the level of anaemia between the two stocks as evaluated by PCVs. Anaemia was also reported by Mbaya *et al.* (2012) as the key feature of disease caused by mechanical injury of erythrocytes due to lashing movement of trypanosome during parasitaemia. Similarly, Mbaya *et al.* (2009a) observed severe anaemia which was associated with high parasitaemia in gazelles concurrently infected with *T. congolense* and *Haemonchus*

contortus. This is also corroborated by the present studies in that the severity of anaemia correlated with parasite load. Despite several studies associating parasitaemia with anaemia, Noyes *et al.* (2009) study indicated that there were no relationship between parasitaemia and severity of anaemia.

Scanty or absence of parasitaemia has been a characteristic feature in chronic form of *T. congolense* infections in cattle. This is normally accompanied by severe illness and death. Contrary was observed in this study, that parasitaemia in steer infected with *T. congolense* resistant stock decreased with time resulting into asymptomatic host. However, the situation was different in steer infected with *T. congolense*–sensitive stock which demonstrated significantly low PCV, relatively higher parasitaemia, which resulted into development of the disease and eventually death of the host within 31 days of observation. The animal died in the course of treatment. Cattle mortalities in 4-7 weeks post infection as a result of *T. congolense* strains infection were also reported by Bengaly *et al.* (2002b). Drug–sensitive *Trypanosoma congolense* strain in cattle was observed to be more pathogenic than the drug-resistant strain and resulted to severe decline in PCV, clinical manifestation and death of the animal.

The characteristic fluctuation in the rectal body temperature was also observed in the present study. There was increase in rectal body temperature in both groups in the second and third day post infection for sensitive and resistant strains infected mice. Despite both groups showing rise in rectal temperature, the rise was significantly higher ($p=0.049$) in mice infected with sensitive strains. The fluctuation in rectal temperature was observed throughout the parasitaemic phase and these observations were consistent with those reported by Mbaya *et al.* (2009b) following *T. brucei* infection in gazelles.

In the course of this study it was observed that mice infected with *T. congolense*–resistant strain appeared dull with reduced activities in a way that made them easy to handle as compared to those infected with drug-sensitive *T. congolense* strain. Mortality was observed in drug-resistant *T. congolense* strain as early as nine days post-infection and hardly struggled to survive for 30 days since inoculation. In view of the observations mentioned above drug-resistant *T. congolense* was categorized as highly pathogenic stock and drug-sensitive *T. congolense* as moderately pathogenic strain in mice and this was in accordance to the categorization by Bengaly *et al.* (2002a). These observed signs and animal death could be owing to the facts that highly virulent parasites tends to cause a severe damage as they multiply rapidly and reach a peak of parasitaemia within a short time after their introduction to a host

The two strains of *T. congolense* behave contrariwise in cattle and mice. This could be due to variations of these hosts in terms of species, their body size, daily and metabolic activities and their immunological response. However, the observed high and low transmissibility and pathogenicity of *T. congolense*–resistant stock in mice and cattle respectively could also be attributed to pathogen defense against the hosts reactions and transmission cycle between hosts as this involved serial syringe passage of the stocks in mice prior to infection in cattle. Van Den Bossche *et al.* (2011) suggested that susceptible host infected by highly virulent trypanosome stocks will display a severe disease and leading to either treatment and/or death, leading therefore to a decrease in dispersion of highly virulent trypanosome as compared to its less pathogenic competitor resulting in a relatively low fitness. This complements the observed difference in pathogenicity of resistant stock in mice and cattle in this study.

Additionally variation in the ability of these hosts to handle infection of these two trypanosome stocks suggests difference in pathogenicity of trypanosomes stocks, since parasites pathogenicity is influenced by a number of hosts - related variables. Matthews *et al.* (2015) revealed that neither parasite antigen switching nor developmental progression to transmission stages is driven by host, instead host contribute to the infection dynamic. Whereas Tyler *et al.* (2001) indicated that level at which primary peak of parasitaemia is reached is host specific, similarly Frank and Barbour. (2006) explained host immunity to many variants cause drop in antigenic switching rate as infection progresses. In consistence with this study, a number of studies correlated ability of controlling anaemia with maintenance of low level of parasitaemia and thus signify trypanotolerance in cattle (Taylor *et al.,* 1999; Naessens, 2006; Toya, 2010). In contrast, trypanotolerance in mice associated with parasitaemia control as well as maintaining longer survival time (Morrison *et al.,* 2010). As true this may be, results obtained in mice should cautiously be extrapolated in cattle, as may not be a true reflection of the same stock characteristics in cattle (Eisler *et al.,* 2001).

4.5 CONCLUSIONS AND RECOMMENDATIONS

This study showed variation in syringe passaged transmissibility and pathogenicity between drug-sensitive and resistant *T. congolense* in murine and bovine hosts. The drug-resistant *T. congolense* strain was highly transmissible than drug-sensitive strain. Parasite pathogenicity is usually taken to be interrelated with its transmissibility. Consequently, this study expected to find interplay between those two important epidemiological aspects with respect to drug sensitivity of *T. congolense* strain. Surprisingly, the pathogenicity of these strains differed in cattle and in mice in that, the pathogenicity of drug-resistant *T. congolense*–strain was high in mice but mild in cattle. The reverse was observed for drug-sensitive *T. congolense* strain that it was less pathogenic in mice and highly

pathogenic in cattle. As these variations observed, the present study therefore emphasize on the pathways by which the trypanosomes act upon the host. Hence, it is recommended that, with a large number of experimental animals; cattle in particular, various pathways can then be studied to represent the field situation. Nevertheless, it would be worthwhile to take into consideration findings of this study when planning for control operations particularly with the use of diminazene aceturate and isometamidium chloride.

AKNOWLEDGEMENTS

I would like to thank the Commission for Science and Technology (COSTECH) for awarding me the grant to pursue PhD studies. Also, I extend my sincere thanks to my supervisors for their constructive and tireless guidance. Deeply thanks to Prof. A.E. Kimambo for providing the fly-proof maintenance facility and other forms of support. I am indebted to staff at the Animal Breeding Unit and Department of Veterinary Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, as well as Department of Animal, Aquaculture and Range Sciences farm, College of Agriculture, for their technical and logistical assistance.

3.3 REFERENCES

Achenef, M. and Bekele, B. (2013). Drugs and Drug Resistance in African Animal Trypanosomosis: A review. *European Journal of Applied Sciences* 5 (3): 84-91.

Bengaly, Z., Sidibe, I., Boly, H., Sawadogo, L. and Desquesnes, M. (2002b). Comparative pathogenicity of three genetically distinct Trypanosoma congolense-types in inbred Balb/c mice. *Veterinary Parasitology* 105: 111-118.

Bengaly, Z., Sidibe, I., Ganaba, R., Desquesnes, M., Boly, H. and Sawadogo, L. (2002a). Comparative pathogenicity of three genetically distinct types of *Trypanosoma*

congolense in cattle: clinical observations and haematological changes. *Veterinary Parasitology* 108: 1-19.

Brown, M. J. F., Loosli, R. and Schmid-Hempel, P. (2000). Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos* 91: 421-427.

Delespaux, V. and de Koning, H. P. (2007). Drugs and drug resistance in African trypanosomiasis. *Drug resistance updates* 10 (1 suppl 2): 30-50.

Eisler, M. C., Brandt, J., Bauer, B., Clause, P. H., Delespaux, V., Holmes, P. H, Ilemobade, A., Machila, N., Mbwambo, H., McDermott, J., Mehlitz, D., Murilla, G., Ndung'u, J. M., Peregrine, A. S., Sidibe, I., Sinyangwe, L. and Geerts, S. (2000). Standardized tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle. *Veterinary Parasitology* 97(3): 171-182.

FAO. (1998). Drug management and parasite resistance in Bovine trypanosomiasis; Pathogenicity of drug-resistant parasites and the impact on livestock productivity. Viale delle Terme di Caracalla, 00100 Rome, Italy.

Frank, S. A. and Barbour, A. G. (2006). Within host dynamics of antigenic variation, *Infection, Genetics and Evolution* 6: 141-146.

Geerts, S., Holmes, P. H., Diall, O. and Eisler, M. (2001) African bovine trypanosomiasis: the problem of drug resistance. *Trends in Parasitol*ogy 17: 25-28.

Gillingwater, K., Mamabolo, M. V. and Majiwa, P. A. O. (2010). Prevalence of mixed *Trypanosoma congolense* infections in livestock and tsetse in KwaZulu-Natal, South Africa. *Journal of the South African Veterinary Association* 81: 219–223.

Godfrey, D. G. (1961), Types of *Trypanosoma congolense*. II. Differences in the courses of infection. *Annals of Tropical Medicine and Parasitology* 55:154-66.

Hagos, A., Gewado, A. and Yacob, H. T. (2014). Sensitivity of *Trypanosoma congolense* field isolates in experimentally infected calves in Konso district, Southern Ethiopia to isomethamidium and diminazene *Journal of Veterinary Medicine and Animal Health* 6(1): 44-47.

Herbert, W. J. and Lumsden, W. H. R. (1976). *Trypanosoma brucei*. A rapid 'matching' method for estimating the host's parasitaemia. *Experimental Parasitology* 40: 427-431.

Holmes, P. H. (2005). Trypanosomiasis, in the Merck Veterinary Manual. Kahn C, Line S, Aiello S (Eds). MERCK & CO., INC, Whitehouse station, N.J., U.S.A, 9th: 32- 35.

Holmes, P. H., Eisler, M. C. and Geerts, S. (2004). Current chemotherapy of animal trypanosomiasis. In the trypanosomiases. Maudlin I, Holmes P H and Miles M A (Eds).CABI Publishing. London, UK pp.431 – 444.

Lipsitch, M. and Moxon, E. R., (1997). Virulence and transmissibility of pathogens: what is the relationship?.*Trends in Microbiology* 5(1): 31-37.

Lumsden, W. H. R., Herbert, W. J. and McNeillage, G. J. C. (1973). Techniques with Trypanosomes. Churchill Livingstone Edinburgh & London, pp 95-100.

Marcotty, T., Simukoko, H., Berkvens, D., Vercruysse, J., Praet, N. and Van den Bossche, P. (2008). Evaluating the use of packed cell volume as an indicator of trypanosomal infections in cattle in eastern Zambia. *Preventive veterinary medicine* 87: 288-300.

Masumu, J., Marcotty, T., Geysen, D., Geerts, S., Vercruysse, J., Dorny, P. and Van den Bossche, P. (2006). Comparison of the virulence of *Trypanosoma congolense*

strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. *International Journal for Parasitology* 36: 497-501.

Matthews, K. R., McCulloch, R. and Morrison, L. J. (2015). The within-host dynamics of African trypanosome infections. *Philosophical Transactions of the Royal Society B* 370: 10 pp.

Mbaya, A. W., Aliyu, M. M., Nwosu, C. O. and Egbe-Nwiyi, T. (2009a). The relationship between parasitaemia and anaemia in concurrent *Trypanosoma brucei* and *Haemonchus contortus* infections in red fronted gazelles (*Gazella rufifi frons*). *Journal Veterinarski Arhiv* 79: 451-460.

Mbaya, A. W., Aliyu, M. M., Nwosu, C. O., Taiwo, V. O. and Ibrahim, U. I. (2009b). Effects of melarsamine hydrochloride (Cymelarsan®) and diaminazene aceturate (Berenil®) on the pathology of experimental *Trypanosoma brucei* infection in red fronted gazelles (Gazella rufifrons). *Veterinary Parasitology* 163(1-2): 140-143.

Mbaya, A., Kumshe, H. and Nwosu, C. (2012). The Mechanisms of Anemia in Trypanosomosis: A Review. In: Silverberg, D. (Ed.): Anemia. Shanghai: InTech. pp. 269-282.

Mbwambo, H. A., Mella, P. N. P. and Lekaki, K. A. (1988). Berenil (Diminazene aceturate) - resistant *Trypanosoma congolense* in cattle under natural tsetse challenge at Kibaha, Tanzania. *Acta Tropica* 45: 239-244.

McDermott, J., Woitag, T., Sidibe, I., Bauer, B., Diarra, B., Ouedraogo, D., Kamuanga, M., Peregrine, A. S., Eisler, M. C., Zessin, K. H., Mehlitz, D. and Clausen, P. H. (2003). Field studies of drug-resistant cattle trypanosomes in Kenedougou Province, Burkina Faso. *Acta Tropica* 86: 93-103.

Morrison, L. J., McLellan, Sweeney L, Chan, C. N, MacLeod, A., Tait, A. and Turner, C. M. R. (2010). Role for parasite genetic diversity in differential host responses to *Trypanosoma brucei* infection. *American Society for Microbiology* 78(3): 1096-1108.

Motloang, M. Y., Masumu, J., Mans, B. J. and Latif, A. A., (2014). Virulence of *Trypanosoma congolense* strains isolated from cattle and African buffaloes *(Syncerus caffer*) in KwaZulu-Natal, South Africa. *Onderstepoort Journal of Veterinary Research* 81(1): 7 pp.

Mungube, E. O., Vitouley, H. S., Allegye-Cudjoe, E., Diall, O., Boucoum, Z., Diara, B., Sanogo, Y., Randolph, T., Bauer, B., Zessin, K. and Clausen, P. (2012). Detection of multiple drug resistant *Trypanosoma congolense* populations in village cattle of South-East Mali. *Parasites and Vectors* 5(155); 9 pp.

Murray, M. (1989). Factors affecting duration and intensity of trypanosome infection of domestic animals. *Annales de la Société Belge de Médecine Tropicale* 1: 189-196.

Naessens, J. (2006) Bovine trypanotolerance: A natural ability to prevent severe anaemia and haemophagocytic syndrome? *International Journal for Parasitology*, 36: 521-528.

Noyes, H. A., Alimohammadian, M. H., Agaba, M., Brass, A., Fuchs, H., Gailus-Durner, V., Hulme, H., Iraqi, F., Kemp, S., Rathkolb, B., Eckard W., de Angelis, M. H., Roshandel, D. and Jan, N. (2009). Mechanisms Controlling Anaemia in *Trypanosoma congolense* Infected Mice. *PLoS ONE*, 4(4): 13pp.

Silayo, R. S. and Marandu, W. P. (1991). The stability of drug resistance and relative pathogenicity of a strain of *Trypanosoma congolense* maintained in mice. In: *The International Scientific Council for Trypanosomiasis Research and Control*,

Twentieth Meeting, Mombasa, Kenya, 9 - l4 April, l989, OAU/STRC, Nairobi, Kenya, Publication No. 115, pp.382-388.

Sones, K. R., Njogu, A. R. and Holmes, P. H. (1988). Assessment of sensitivity of *Trypanosoma congolense* to isometamidium chloride: a comparison of tests using cattle and mice. *Acta Tropica* 45: 153-164.

Taylor, K. A. and Mertens, B. (1999). Immune response of cattle infected with African trypanosomes. *Memórias do Instituto Oswaldo Cruz* 94(2): 239-244.

Taylor, K. and Authié, E. M. L. (2004). 'Pathogenesis of animal trypanosomiasis', in I. Maudlin, P.H. Holmes & M.A. Miles (eds.), The Trypanosomaises, pp. 331–353, CABI Publishing, Wallingford.

Tesfaye, B., Getachew, A., Hagos, A. and Tolossa, Y. H. (2012). Comparative study on the pathogenic effects of diminazine aceturate sensitive and resistant isolates of *Trypanosoma congolense* in goats. *Ethiopian Veterinary Journal* 16(1): 59-69.

Toya, N. B. (2010). Immunobiology of African Trypanosomes: Need of Alternative Interventions. Journal of Biomedicine and Biotechnology D 389153: 24pp

Turner, M. C. R. (1997). The rate of antigenic variation in fly-transmitted and syringe-passaged infections of *Trypanosoma brucei*. *FEMS Microbiology Letters* 153: 227-231.

Tyler, K. M., Higgs, P. G., Matthews, K. R. and Gull, K. (2001). Limitations of *Trypanosoma brucei* parasitaemia results from density-dependent parasite differentiation and parasite killing by the host immune response. *Proceedings of the Royal Society B* 268: 2235-2243.

Van den Bossche, P., Chitanga, S., Masumu, J., Marcotty, T. and Delespeux, V., (2011). Virulence in *Trypanosoma congolense* Savannah subgroup. A comparison between strains and transmission cycles. *Parasite Immunology* 33: 456-460.

Woo, P. T. K. (1969). The haematocrit centrifuge for the detection of trypanosomes in blood. *Canadian Journal of Zoology* 47: 921-923

CHAPTER FIVE

5.0 Molecular characterization of drug-sensitive and drug-resistant strains of *Trypanosoma congolense* isolated from Tanzania

A.F. Ngumbi[1,2] and L.L. Mnyone[3]

[1]Livestock Training Agency, P.O. Box 603, Morogoro Tanzania

[2]Department of Veterinary Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3019, Chuo Kikuu, Morogoro, Tanzania

[3]Sokoine University of Agriculture, Pest Management Centre, P.O. Box 3110, Chuo Kikuu, Morogoro, Tanzania

Email: ngumbianna0@gmail.com

Published: Entomology and Zoology Studies2020;8(4):1754-1759.

Abstract

Like in any other disease-causing agents, intra-species variation in trypanosomes influences how individual subspecies interact with their hosts, vectors and external environment. We conducted genomic characterization and phylogenetic analysis of two stocks of trypanosomes originating from different parts of Tanzania: *Trypanosoma congolense* Mikese, a putatively drug-sensitive strain and *T. congolense* SIO-201 Mbagala, a putatively drug-resistant strain. These strains were isolated and maintained in the laboratory for several years; and have been distinguished from other members of the group and regarded as different strains based on morphometric measurements only. Polymerase Chain Reaction (PCR) using species-specific primers confirmed that indeed the two strains belong to *T. congolense*. Sequencing and phylogenetic analysis revealed

further that the two strains are genetically distinct and are related to genotypes described elsewhere in the region. The *T. congolense* drug-resistant strain was identified as *T. congolense* savannah whereas the sensitive strain was identified as *T. congolense* Kilifi. These findings warrant further studies to establish prevalence, distribution and drug sensitivity status of these two genotypes across Tanzania to inform the development of effective control and surveillance strategies.

Keywords: drug-resistant, drug-sensitive, genotype, *Trypanosoma congolense*

5.1 INTRODUCTION

The African Animal Trypanosomosis (AAT) remains one of the major causes of poverty and food insecurity in Africa [1]. The AAT causes approximately 3 million cattle deaths annually. The AAT related deaths and other costs related to prevention and treatment correspond to direct annual loss of approximately 1-4 billion US dollars worldwide [2].

The transmission of AAT in Tanzania and elsewhere in Africa is primarily transmitted by tsetseflies [3]. Tsetseflies and the disease are widely distributed in Tanzania. Nevertheless, the epidemiological map of this devastating disease is expanding drastically. Many species of trypanosomes are capable of causing AAT in cattle and other animals; however, *Trypanosoma congolense*, *T. vivax* and *T. brucei* play the most significant role. Of these, *T. congolense* remains the most pathogenic and widespread species [4].

Trypanosoma congolense belongs to the subgenus Nannomonas. Other important species under this subgenus are *T. simiae* and *T. godfreyi* [5]. Their pathogenicity similar to members of other subgenera varies with species of susceptible host animals.

T. congolense causes the most severe disease in cattle and other susceptible domestic animals other than pigs. *Trypanosoma simiae* and *T. godfreyi* cause acute and sub-actute

diseases in pigs [5, 6, 7]. Generally, these three species are quite similar morphologically. However, they can provisionally be separated by means of their morphometric measurements and isoenzyme electrophoresis. Trypanosoma congolense is the smallest (9.0–22µm in length), followed by *T. godfreyi* (9.1–21.8µm) and *T. simiae* (12–24µm). Furthermore, all the three species are morphometrically smaller than other trypanosomes of animals for instance *T. vivax* (18-26µm) and (*T. brucei* 17–30µm) [8]. The two closely related of the three species, *T. congolense* and *T. simiae* can be distinguished further through isoenzyme electrophoresis [9]. However, this method is incapable of differentiating subspecies/genotypes within *T. congolense* and *T. simiae*. Molecular techniques are increasingly becoming desirable in addressing such and other prevailing challenges. Polymerase Chain Reaction (PCR) has made explicit identification of subspecies and/or sub-species possible, thus allowing characterization of trypanosomes to the lowest level possible. Intriguingly, genomic sequencing has rendered it possible to identify and/or characterize novel trypanosomes. Through PCR, two biochemically and genetically distinct clades have been demonstrated within the subgenus Nannomonas: *T. congolense* and *T. simiae* clade [9, 10]. The *T. congolense* clade presently contains three recognized subspecies or genotypes namely *T. congolense* Savannah, *T. congolense* Kilifi and *T. congolense* Forest [6, 11]. The *T. simiae* clade presently contains two recognized genotypes or subspecies namely *T. simiae and T. simiae Tsavo* [12].

Intra-species genetic variations in trypanosomes influence how individual subspecies or genotypes interact with their hosts, vectors and external environment. These and other kinds of variations eventually affect their geographical distribution, pathogenicity, transmissibility and control options.

Studies have repeatedly emphasized variations on transmissibility, pathogenicity and virulence among subspecies of trypanosomes [13-19]. We conducted molecular

characterization of two stocks of trypanosomes originating from different parts of Tanzania: *T. congolense* Mikese (isolated from cattle in Mikese village, Morogoro region, Tanzania), a putatively drug-sensitive stock and *T. congolense* SIO-201 Mbagala (isolated Mbagala ward, Dar es Salaam region, Tanzania) a putatively drug resistant strain. These strains were compared with subspecies or genotypes described elsewhere in the region. These strains were isolated and maintained in the laboratory for several years; and have been regarded as subspecies, rather tentatively, without confirmatory molecular characterization.

5.2 MATERIALS AND METHODS

5.2.1 Blood sample collection

Blood samples from infected mice were collected via cardiac puncture using 23 gauge needle and kept in EDTA- coated tubes. Blood samples from infected steers were collected from jugular vein into 5-ml EDTA-coated vacutainer tubes. The blood samples were labeled accordingly and preserved at 4^{o}C while awaiting molecular analysis.

5.2.2 Oligonucleotides

The oligonucleotide primer sequences and expected band size used in this study [20] were purchased from Inqaba Biotec East Africa Ltd (Africa’s Genomics Company).

Table 5. 1: **Oligonucleotide primer sequences and their expected product size**

Primer specificity	Oligonucleotide sequence	Expected product size (bp)	Reference
African Trypanosomes	18STnF2-5'-CAA CGA TGA CAC CCA TGA ATT GGG GA-3' 18STnR3-5'-TGC GCG ACC AAT AAT TGC AAT AC-3'	750	[21]
T. congolense Savannah	ILO344F-5,-CGA GCG AGA ACG GGC AC-3' ILO345R-5'-GGG ACA AAC AAA TCC CGC-3'	320	[22]
T. congolense Kilifi	TCK1F-5'-GTG CCC AAA TTT GAA GTG AT-3' TCK2R-5'-ACT CAA AAT CGT GCA CCT CG-3'	294	[23]
T. congolense Riverine-Forest	TCF1F-5'-GGA CAC GCC AGA AGG TAC TT-3' TCF2R-5'-GTT CTC GCA CCA AAT CCA AC-3'	350	[23]
T. congolense Tsavo	ILO892F-5'-CGA GCA TGC AGG ATG GCC G-3' ILO893R-5'-GTC CTG CCA CCG AGT ATG C-3'	400	[12]
T. simiae	TSM1F-5'-CGG TCA AAA ACG CAT T-3' TSM2R-5'-AGT CGC CCG GAG TCG AT-3'	437	[22]

5.2.3 DNA extraction

DNA extraction from 100µl of the whole blood samples obtained from infected mice and steers was done using extraction kit (Quick – gDNA™ Blood MiniPrep kit) as per manufacturer's instructions, with exception of final elution, whereby, 100 µl DNA elution buffer was added to elute the DNA. The supernatants containing DNA were stored at -20°C or used directly for PCR.

5.2.4 DNA amplification

Identification of trypanosomes was done in three (3) phases: The first phase confirmed success of trypanosome DNA extraction and allowed for identification of African trypanosomes. In this phase, the extracted DNAs were used to amplify 750 bp fragment of

the 18S ribosomal RNA gene of trypanosome using the primers 18ST nF2 and 18ST nR3 (Table 5.1) specific for African trypanosomes. The second phase was carried out using primers specific to Nannomonas (Table 5.1). In this phase a multiplex PCR was done to identify trypanosomes under the subgenus Nannomonas to species level. After multiplex PCR, DNA samples were further tested in third phase using specific primers designed to detect two *T. congolense* subgroups ILO 344F/345R and TCK 1F/2R so as to identify specific trypanosome species to subgroup level. Selected primers were those of a molecular weight corresponding to that of tested DNA fragments. Each PCR round was performed in a final volume of 25µl reaction mixture containing 12.5 µl 1X One Taq Master Mix with standard buffer (20mM Tris-HCl [pH 8.9], 22mM KCl, 1.8mM $MgCl_2$, 22mM NH_4Cl, 0.2mM of deoxynucleotide triphosphate (dNTPs), 5% glycerol, 0.06% IGEPAL® CA-630, 0.05% Tween® 20,25units/ml of One Taq DNA polymerase), 0.6 µl of each primer (forward and reverse) at 10µM, 6.3 µl Nuclease free water with 5µl of DNA template. Positive and negative controls were included in each PCR reaction. The reaction mixtures were subjected to a programmable heating block (TaKaRa PCR thermal cycler). The amplification conditions were identical in all phases, and involved initial denaturation at 94ºC for 3 minutes, followed by 35 amplification cycles each consisting of denaturation at 94ºC for 1 minute, annealing at 60ºC for 2 minutes and extension at 72ºC for 30 seconds, then final extension at 72ºC for 7 minutes.

5.2.5 Gel electrophoresis

PCR products obtained were scored as positive for *T. congolense* subgroup after electrophoresis. One percent agarose gel containing 3µl ethidium bromide in 1x TBE buffer was prepared. The electrophoresis chamber was filled with electrophoresis buffer solution until the prepared gel submerged. One microliter of the loading dye was added and mixed with 8 µl of each amplified product. 9µl of each amplification product and

DNA ladder of 100bp (9µl) were loaded into the agarose gel wells. The chamber was connected to a power supply of 60 volts and amplification products allowed to migrate for 55-60 minutes. The amplified DNA products size were detected under ultraviolet (UV) illumination and photographed.

5.2.6 Sequence and phylogenetic analysis

Sequencing was carried out to confirm the correct identification and characterization of trypanosome stocks. After agarose gel electrophoresis, the PCR products were purified from the gel using a commercial kit (Illustra GFX^{TM} PCR purification kit) as per manufacturer's protocol. Prior to sequencing, purified DNA fragments were amplified by cyco-sequencing and followed by ethanol precipitation to remove cyco-sequencing unused components. The amplified DNA fragments were sequenced with the forward and reverse primers using programmed sequencing machine (Capillary Sequencer [ABI 3730]). Sequences were recorded, aligned, edited and assembled using Geneious software. The sequence results obtained were subjected to BLAST analysis and compared to the sequences available on the NCBI database to identify the highly similar sequences. Phylogenetic analyses were conducted using MEGA7 Software to understand the relationship with other ancestral *Trypanosoma* species and a neighbor-joining tree was constructed.

5.3 RESULTS

5.3.1 Identification of the Nannomonas group

DNA for each sample was successfully extracted as revealed by PCR amplification of 18S rRNA. The DNA was further tested for detection of Subgenus Nannomonas trypanosomes. The multiplex PCR with Nannomonas species specific primers showed that both tested samples carried subgroups of *Trypanosoma congolense* under the subgenus Nannomonas (Figure 5.1).

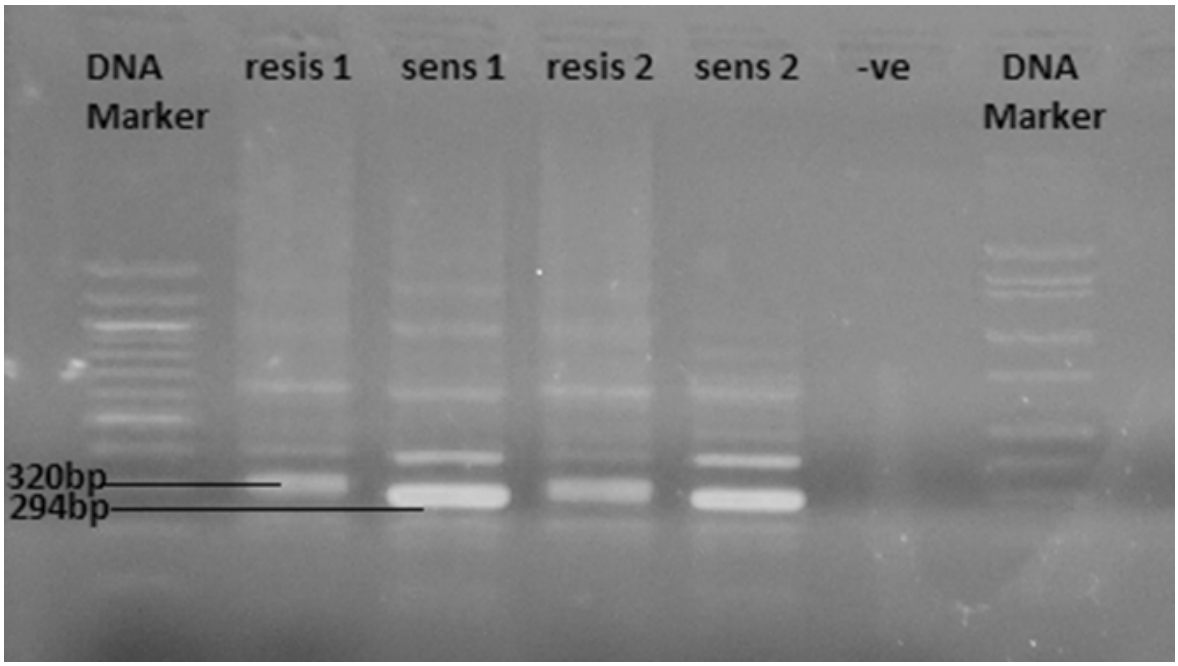

Figure 5. 1: Gel electrophoresis profile of drug-resistant stock/ strains lanes 2 and 4 and drug-sensitive stock/ strains lanes 3 and 5 and a negative control lane 6; lane 1 and 7 is a 100bp DNA ladder

5.3.2 Identification of *T. congolense* subgroups

Results were further confirmed by amplification of *T. congolense* to subgroup level using two subgroup specific primers with product size that appeared to correspond with the tested samples. Thus two trypanosomes stocks used in this study were identified as *Trypanosoma congolense*. Of these, the *T. congolense* trypanocide resistant stock was shown to contain *T. congolense* savannah genotype while the *T. congolense* trypanocide-sensitive stock contained *T. congolense* Kilifi genotype (Figure 5.2).

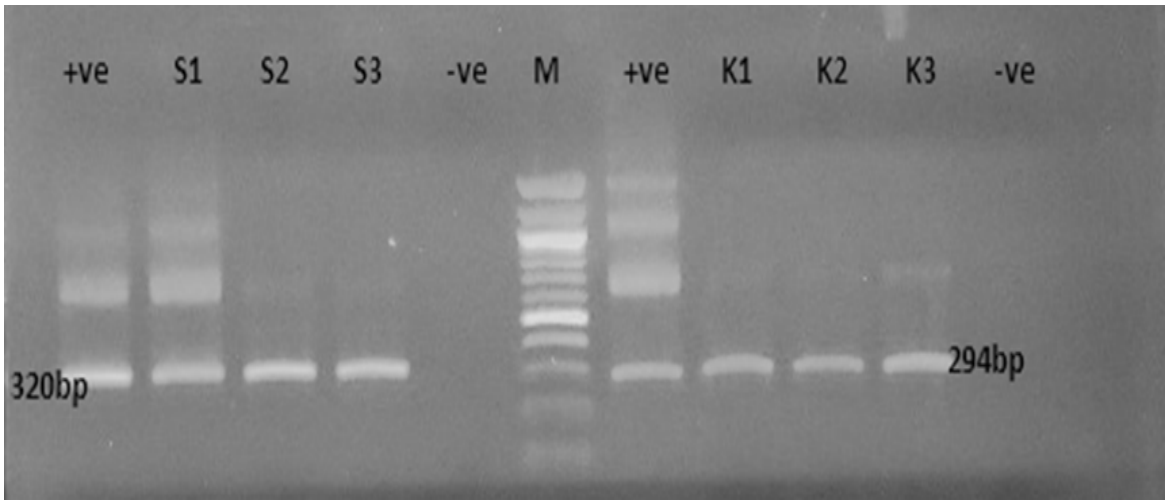

Figure 5. 2: Gel electrophoresis profile showing amplification of a positive control for *T. congolense* savannah subgroup in lane 1, drug-resistant stock/ strains S1-3 in lanes 2,3 and 4 and positive control for *T. congolense* Kilifi subgroup in lane 7 and drug-sensitive stock/ strains K1-3 in lanes 8,9 and 10; lanes 6 was a 100bp ladder and lane 5 and 11 were negative controls.

5.3.3 Phylogenetic relatedness of the *T. congolense* strains

Blast analysis of *T. congolense* trypanocide-sensitive and resistant gene sequences confirmed that close matches were found with *T. congolense* Savannah and *T. congolense* Kilifi respectively. Sequences generated in this study were included in the phylogenetic analysis (Figure 5.3). *Trypanosoma congolense* stocks were grouped into three clusters: I, II and III. Cluster I and II comprised *T. congolense* stocks belonging to the Savannah subgroup while cluster III comprised *T. congolense* belonging to Kilifi sub-group. Phylogenetic results showed that, the *T. congolense* trypanocide-resistant stock obtained from cattle at Mbagala Dar es Salaam shared 100% identity with the *T. congolense* IL3000 (savannah type) reference sequences from cluster I and shared 99% identity with *T. congolense* DNA fragment reference sequence from cluster II at the nucleotide level. The *T. congolense* stock obtained from cattle from Mikese shared 99% identity with two *T. congolense* Kenya coast (Kilifi) reference sequences from cluster III.

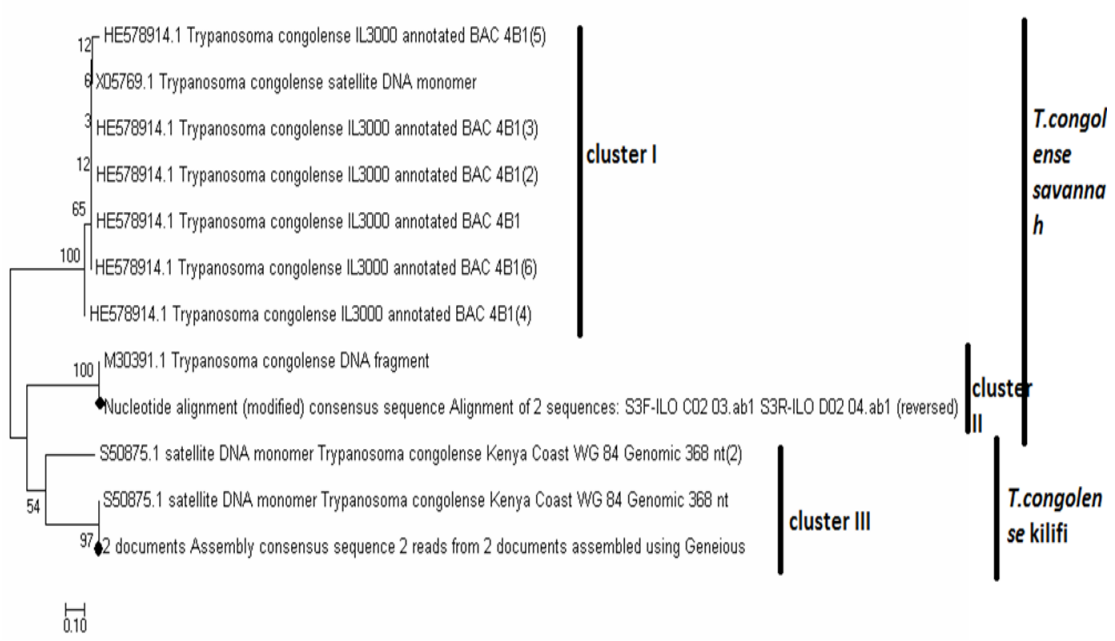

Figure 5. 3: Phylogenetic tree showing relationships of sequences generated from this study shown with black dots and sequences retrieved from database shown with their accession numbers.

5.4 DISCUSSION

This study has confirmed that the drug sensitive and resistant trypanosomes employed in this and other previous studies were all *T. congolense*. These isolates were originally classified as *T. congolense* only based on their small size which is one of the basic characteristics of members of the subgenus Nannomonas [24, 25]. Furthermore, the resistant strain of *T. congolense* Mbagala had a band size of 320bp which was the expected band size of *T. congolense* Savannah type. Phylogenetic analysis of the resulting sequence showed 100% similarity with the Gene Bank reference strain IL3000 which was *T. congolense* Savannah originating from Transmara region in Kenya [10]. It also showed 99% similarity to *T. congolense* DNA fragment. Similar trypanocidal resistant *T. congolense* was also reported in cattle at Kibaha district, Tanzania [26]. Kibona et al. [27] reported drug resistance in north-western Tanzania in mouse infected with human pathogenic trypanosome, *Trypanosoma brucei rhodesiense*. The development and spread

of resistant trypanosomes against commonly used trypanocides, isometamidium and diminazene aceturate is quickly developing and/or spreading in Tanzania consequent to indiscriminate use of these drugs [28]. The emergence of single- and multi-drug resistant trypanosome strains is considered a serious concern in AAT control in over 21 African countries, possibly more, including Tanzania [31-32].

Considering that *T. congolense* is the most common species in Tanzania, presence of *T. congolense* Savannah, as suggested in this study, will increasingly spread and impede livestock productivity in the country; more so due to lack of interest by national and international pharmaceutical industries to invest in developing alternative or complementary trypanocidal drugs.

A number of studies have associated the occurrence of resistant trypanosomes with indiscriminate use of currently available trypanocidal drugs; such as under dosage and excessive frequency of treatment [2, 32, 33-34]. Arguably, the resistant *T. congolense* Savannah, described in this study, has been associated with the under dosage and frequent exposure of cattle to trypanocidal drugs. These malpractices, under dosage of, and frequent exposure to, trypanocidal drugs have been reported in many parts of Tanzania. The *T. congolense* Savanna was also identified in wildlife species from the Serengeti National park in Tanzania [35] however its response to commonly used trypanocides was not examined.

The *T. congolense* drug sensitive strain was 97% related to *T. congolense* Kilifi type. The *T. congolense* Kilifi strain was obtained from the coast of Kenya (Kilifi), thus given a synonym Kenya Coast [36].

The Mikese strain was morphologically small, 9-12 micrometer long, with amplified band size of 294 which was the expected band size in reference to *T. congolense* Kilifi [20]. Despite its wide distribution in East and Southern Africa, less prevalent infections with *T. congolense* Kilifi has been reported in many areas [37]. In Tanzania, *T. congolense* Kilifi has been identified in cattle within human-livestock-wildlife interfaces of Mikumi National Park [38], and tsetse flies from Tarangire and Serengeti National parks respectively [39] as well as tsetse flies from the farming areas of Rufiji district in Coast region [40]. None of these studies indicated correlation between presence of the trypanosome in tsetse vectors and infection in cattle.

The molecular characterization has confirmed the existence of two different strains of *T. congolense*, each with its genotype and with variable degrees of sensitivity to trypanocidal drugs. These strains are related to *T. congolense* Kilifi and *T. congolense* Savannah respectively. Equally, the previously observed variation in their morphological length, pathogenicity, transmissibility and therapeutic responses are justified.

5.5 CONCLUSION

This is the first study confirming that indeed the two strains are genetically distinct and relate with subspecies or genotypes described elsewhere in the region. This warrants subsequent studies on their prevalence, distribution and drug sensitivity status in different parts of Tanzania.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We extend our heartfelt gratitude to Prof. P. Gwakisa and Mr. G. Makingi at the Genome Science Centre, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Tanzania, for the laboratory facilities, advice and technical assistance. We are also indebted to staff at the Wellcome Laboratory, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture for the support and technical assistance during sequencing. This study was funded by the government of Tanzania through the Commission for Science and Technology (COSTECH).

REFERENCES

1. Mersha C, Dulecha A, and Basaznew B. Socio-Economic assessment of the impacts of Trypanosomiasis on cattle in Girja District, Southern Oromia Region, Southern Ethiopia. Acta Parasitologica Globalis. 2013; 4:80-85.
2. Assefa S, and Shibeshi W. Drug resistance in African animal trypanosomes; A review. African Journal of Microbiology Research. 2018; 12:380-386.
3. Firesbhat A, and Desalegn C. Epidemiology and impacts of Trypanosomiasis in cattle. EJAS 2015; 7.
4. Auty H, Torr SJ, Michoel T, Jayaraman S, Morrison LJ. Cattle trypanosomosis: the diversity of trypanosomes and implications for disease epidemiology and control. Revue scientifique et technique (International Office of Epizootics). 2015; 34:587-598.
5. McNamara JJ, Mohammed G and Gibson WC. *Trypanosoma (Nannomonas) godfreyi* sp.nov.from tsetse flies in The Gambia: Biological and biochemical characterization. Parasitology .1994; 109:497-509.
6. Garside LH and Gibson WC. Molecular characterization of trypanosome species and subgroups within subgenus Nannomonas. Parasitology. 1995; 111:301-312.

7. Sturn RN, Murthy VK, Garside L, Campbell DA. The mini-exon gene of Trypanosoma (Nannomonas) simiae: sequence variation between isolates and a distinguishing molecular marker. Acta Tropica. 1998; 71:199-206.
8. Uilenberg G, Boyt WP. A field guide for the diagnosis treatment and prevention of African Animal Trypanosomosis. Food and Agriculture Organization of the United Nations, Rome 1998.
9. Gashumba JK, Gibson WC and Opiyo EA. A preliminary comparison of *Trypanosoma simiae* and *Trypanosoma congolense* by isoenzyme electrophoresis. Acta Tropica. 1986; 43:15 -19.
10. Gibson W. Species-specific probes for the identification of the African tsetse-transmitted trypanosomes. Parasitology. 2009; 136:1501-1507.
11. Majiwa PAO, Hamers R, Van Meirvrnne N and Matthyssens G. Evidence for genetic diversity in *Trypanosoma* (Nannomonas) congolense. Parasitology. 1986; 93:29–304.
12. Majiwa PAO, Maina M, Waitumbi JN, Mihok S, Zweygarth E. *Trypanosoma* (Nannomonas) *congolense*: molecular characterization of a new genotype from tsavo, Kenya. Parasitology. 1993; 106:151-162.
13. Bengaly Z, Sidibe I, Ganaba R, Desquesnes M, Boly H, Sawadogo L. Comparative pathogenicity of three genetically distinct types of *Trypanosoma congolense* in cattle: clinical observations and haematological changes. Veterinary Parasitology. 2002a; 108:1–19.
14. Bengaly Z, Sidibe I, Boly H, Sawadogo L, Desquesnes M. Comparative pathogenicity of three genetically distinct *Trypanosoma congolense*-types in inbred Balb/c mice. Veterinary Parasitology. 2002b; 105:111–118.
15. Masumu J, Marcotty T, Ndeledje N, Kubi C, Geerts S, Vercruysse J, Dorny P, Van den Bossche P. Comparison of the transmissibility of *Trypanosoma congolense* strains

isolated in a trypanosomiasis endemic areas of eastern Zambia, by *Glossina morsitans morsitans*. Parasitology. 2006; 133:331-334.

16. Van de Bossche P, Akoda K, Kubi C, Marcotty T. Transmissibility of *Trypanosoma congolense* seems to be associated with its level of resistance to isometamidium chloride. Veterinary Parasitology. 2006; 135:365–367.
17. Masumu J, Akoda K, Van de Bossche P. Transmissibility by *Glossina morsitans morsitans* of *Trypanosoma congolense* strains during the acute and chronic phases of infection. Acta Tropica. 2010; 113:195-198.
18. Van den Bossche P, Chitanga S, Masumu J, Marcotty T, Delespaux V. Virulence in *Trypanosoma congolense* Savannah Subgroup. A comparison between strains and transmission cycles. Parasite Immunology. 2011; 33:456–460.
19. Motloang MY, Masumu J, Mans BJ, Latif AA. Virulence of *Trypanosoma congolense* strains isolated from cattle and African buffaloes (*Syncerus caffer*) in KwaZulu-Natal, South Africa. Onderstepoort Journal of Veterinary Reseasrch. 2014; 81:679.
20. Reinfenberg JM, Solano P, Duvallet G, Cuisance D, Simpore J, Cuny G: Molecular characterization of trypanosome isolates from naturally infected domestic animals in Burkina Faso. Veterinary Parasitology. 1997; 71:251-262.
21. Geysen D, Delespaux V, Geerts S. PCR-RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of Trypanosoma species in cattle. Veterinary Parasitology. 2003; 110:171–180.
22. Majiwa PA, Otieno LH. Recombinant DNA probes reveal simultaneous infection of tsetse flies with different trypanosome species. Molecular Biochemistry and Parasitology. 1990; 40(2):245-253.
23. Masiga DK, Smyth AJ, Hayes P, Bromidge TJ, Gibson WC. Sensitive detection of trypanosomes in tsetse flies by DNA amplification. International Journal of Parasitology. 1992; 22:909–918.

24. Godfrey DG. Types of *Trypanosoma congolense* I. Morphological differences. Annals of Tropical Medicine and Parasitology. 1960; 54:428-438.

25. Nantulya VM, Doyle JJ, Jenni L. Studies on *Trypanosoma (Nannomonas) congolense* I. On morphological appearance of the parasite in the mouse. Acta Tropica. 1978; 35:329-337.

26. Mbwambo HA, Mella PNP, Lekaki KA. Berenil (Diminazene aceturate)- resistant *Trypanosoma congolense* in cattle under natural tsetse challenge at Kibaha, Tanzania. Acta Tropica. 1988; 45:239–244.

27. Kibona SN, Matemba L, Kaboya JS, Lubega GW. Drug resistance of *Trypanosoma b. rhodesiense* isolates from Tanzania. Tropical Medicine and International Health. 2006; 11:144-155.

28. Ngumbi AF and Silayo RS. A cross sectional study on the use and misuse of trypanocides in selected pastoral and agropastoral areas of eastern and northeastern Tanzania. Parasites & Vectors. 2017; 10:2544-3.

29. McDermott J, Woitag T, Sidibe I, Bauer B, Diarra B, Ouedraogo D, Kamuanga M, Peregrine AS, Eisler MC, Zessin KH, Mehlitz D, Clausen PH. Field studies of drug-resistant cattle trypanosomes in Kenedougou Province, Burkina Faso. Acta Tropica. 2003; 86:93-103.

30. Mungube EO,Vitouley HS, Allegye-Cudjoe E, Diall O, Boucoum Z, Diara B, Sanogo Y, Randolph T, Bauer B, Zessin K, Clausen P. Detection of multiple drug resistant Trypanosoma congolense populations un village cattle of South-east Mali. Parasites & Vectors. 2012; 5:155.

31. Tsegaye B, Dagnachew S, Terefe G. Review on drug resistant animal trypanosomes in Africa and Overseas. AJBAS 2015; 7:73-83

32. Dagnachew S, Tsegaye B, Awukew A, Tilahun M, Ashenafi H, Rowan T, Abebe G, Barry DJ, Terefe G, Goddeeris MB. Prevalence of bovine trypanosomosis and

assessment of trypanocidal drug resistance in tsetse infested and non-tsetse infested areas of Northwest Ethiopia. Parasite Epidemiology and control. 2017; 2:40-49.

33. Geerts S, Holmes PH, Diall O and Eisler M. African bovine trypanosomiasis: the problem of drug resistance. Trends Parasitology. 2001; 17:25-28.
34. Delespaux V, Geysen D, Van den Bossche P, Geerts S. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends in Parasitology. 2008; 24:236-242.
35. Auty H, Anderson NE, Picozzi K, Lembo T, Mubanga *et al*. Trypanosome diversity in wild life species from the Serengeti and Luangwa valley ecosystems. PloS Neglected Tropical Diseases. 2012; 6.
36. Masake RA, Nantulya VM, Musoke AJ, Moloo SK and Nguli K. Characterization of *Trypanosoma congolense* serodemes in stocks isolated from cattle introduced onto a ranch in Kilifi Kenya. Parasitology. 1987; 94:349-357.
37. Manangwa O, Ouma JO, Malele I, Mramba F, Msangi A, Nkwengulila G. Trypanosome prevalence in the *Glossina fuscipes fuscipes* (tsetse) and cattle along the shores of Lake Victoria in Tanzania. Livestock Research and Rural Development. 2016; 28:147.
38. Nhamitambo NL, Kimera SI, Gwakisa PS. Molecular identification of trypanosome species in cattle of the Mikumi human/livestock/wildlife interface areas, Tanzania. Journal of Infectious Disease Epidemiology. 2017; 3:029.
39. Adams ER, Hamilton PB, Malele II, Gibson WC. The identification, diversity and prevalence of trypanosomes in field caught tsetse in Tanzania using ITS-1 primers and fluorescent fragment length barcoding. Infection Genetics and Evolution. 2008; 8: 439-444.

40. Malele II, Magwisha HB, Nyingilili HS, Mamiro KA, Rukambile EJ, Daffa JW, Lyaruu EA, Kapange LA, Kasilagila GK, Lwitiko NK, Msami HM, Kimbita EN. Multiple trypanosoma infections are common amongst *Glossina* species in the new farming areas of Rufiji district, Tanzania. Parasites & Vectors 2011; 4:217.

CHAPTER SIX

6.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study provided overview concerning current control practices against tsetse and trypanosomiasis in the selected areas of Tanzania, where most of the farmers administered trypanocides themselves as a consequence of poor veterinary service delivery in pastoral areas, thus leading to haphazard use of trypanocides a factor which promoting development of resistance to trypanocides in trypanosomes. Moreover, different criteria (morphological length, transmissibility, pathogenicity and molecular) were used to compare the characteristics of drug sensitive and drug resistant trypanosome stocks previously isolated from cattle and maintained through passages in mice. A marked correlation between trypanosomes' therapeutic responses and their morphometry was observed in this study that, drug resistant stock bloodstream trypanosomes in a normal bovine host were noticeably short than sensitive stock. Though appeared mild pathogenic but short forms trypanosomes as mostly shown by resistant stock in this study were capable of being easily transmitted between hosts and be carried on life cycle with low parasitaemia. Furthermore, results from this study confirm that indeed *T. congolense* is circulating amongst livestock in Tanzania, and that there is great genetic diversity between the two trypanosome stocks of one species. Observations in this study, therefore suggest existence of two different genotypes of *T. congolense* among the two laboratory maintained stocks each with its degrees of sensitivity to trypanocides. The *T. congolense* Kilifi type shown susceptibility to drugs while *T. congolense* Savannah type resist.

6.2 Recommendations

It is recommended that more studies be carried out on the issue of veterinary service delivery especially in transhumant pastoral situations where private veterinary services delivery faces difficulties because of livestock movements and lack of permanent settlements. Furthermore, farmers' education to sensitize on importance of veterinary extension services concerning livestock diseases and their management, as well as farmer's awareness on consequences of not adhering to drug's indications both economically and for public health concerns is important. Moreover, further study on trypanosomes' characteristics is recommended through cyclical transmission of the two of *T. congolense* in a bovine - normal host of trypanosomes to represent the field situation. Additionally, further study is recommended to establish the prevalence of trypanosome species, subspecies or strain in a particular area with regard to drug response so as to consider it when planning measures for effective disease control.