Resistance of Trypanosome species isolated from cattle populations in Lambwe Valley, Kenya, to diminazene aceturate

by

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Submitted in partial fulfilment for the degree of

MASTER OF GLOBAL ONE HEALTH

in the

Department of Veterinary Tropical Diseases

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

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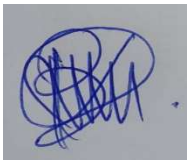
10 November 2023

DECLARATION

I hereby declare that this dissertation, which I hereby submit for the Master of Science degree in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, to be my own work and has not been previously submitted by me for degree purposes at another tertiary institution.



Boscoh O. Kimathi

ACKNOWLEDGEMENTS

I would like to express my utmost gratitude to Belgian Directorate-General for Development Co-operation Framework Agreement (FA5 DGD-ITM2022-2026) through the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria for the bursary to fund my study and research.

I greatly thank the University of Pretoria, my supervisors; Prof. Luis Neves and Ms. Ilse Vorster and administration staff (Mrs. Rina Serfontein and Prof. Darshana Morar-Leather). Am forever indebted to you.

I deeply acknowledge Dr. Seth Onyango the Acting C.E.O of Kenya Tsetse and Trypanosomiasis Eradication Council (KENTTEC) for his support during the study. Thank you to Dr. Masiga for facilitating laboratory space and resources at the International Centre for Insect Physiology and Ecology (ICIPE) and for his technical assistance. I appreciate the laboratory technicians Mr. Julius Ogumbo, Johnmark Makwata and Oscar for their invaluable time and technical expertise.

I want to deeply thank my wife and children for their support, love and understanding during the period of study. I love you very much.

ABSTRACT

Resistance of Trypanosome species isolated from cattle populations in Lambwe Valley, Kenya, to diminazene aceturate

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Trypanosomosis is a parasitic disease of humans and animals that occurs mainly in sub-Saharan Africa where it negatively affects livelihoods. The control of trypanosomosis in animals has for decades relied on the use of trypanocidal drugs that have increasingly reported resistance. A cross-sectional study was conducted in Kigoto, Wiga and Gendo villages of Lambwe Valley in South-West Kenya to determine the point prevalence of trypanosomosis and to investigate the presence and level of resistance to diminazene aceturate (DA), a commonly used trypanocidal drug in the study area. Three hundred and ninety-five cattle were microscopically screened for trypanosomosis using the buffy coat technique (BCT). To test treatment efficacy, trypanosome positive cattle were recruited into a block treatment experimental design, with DA at 3.5mg/Kg body weight. They were

monitored on days 7 and 28 and screened using the BCT and internal transcribed spacer 1-polymerase chain reaction (ITS1-PCR). Data were entered in Microsoft Excel 2016, coded and cleaned. Statistical analysis was carried out using statistical package for social sciences (IBM SPSS) version 2020. The results were presented as mean with their standard deviations (mean ± SD). The T-test was used to compare differences in packed cell volume (PCV) between infected and non-infected cattle while the Pearson Chi-square was used to compare statistical differences in trypanosome infection based on villages, sex and age categories. Analysis of variance (Ivanova *et al.*) provided statistical differences in mean PCVs across the treatment group. The study did not find any significant statistical difference on the prevalence of trypanosomosis across villages, cattle ages and sexes. On day 0, 4.94% (19/395) of the cattle tested positive for one or more species of trypanosomes. *Trypanosoma vivax* was the most prevalent species at 73.6% (N=19) followed by *Trypanosoma congolense* at 24.4%. There was however no significant difference in prevalence between the *Trypanosoma* species isolated. On day 7, no cattle tested positive on both BCT and ITS1-PCR. On day 28, 3 cattle tested positive by BCT while on PCR, 4 tested positive. The relapses in cattle 4111, 4116 and 4118 encountered on day 28 were either a result of new infections or probable resistant parasites that were not detected in the initial days. The *T. vivax* of animal 4102 isolated on day 28 could be a relapse due to a possible resistance or appearance of parasites previously sequestered in parts of the body that are not easily accessible by DA such as Central Nervous System, adipose tissue and eye globe. The findings from this study suggest a likelihood of resistance to diminazene aceturate by *Trypanosoma* species in cattle populations of Lambwe Valley a finding that could not be absolutely confirmed. Further molecular analysis of day 28 infections or drug efficacy experimental trials in goats are therefore recommended to confirm/rule out resistance. Incorporating pyrethroid insecticide treatment of cattle in block treatment program, monitoring on day 14 and extension of monitoring beyond day 28 would improve outcomes for future research deploying block treatment. Community training and sensitization on appropriate use of trypanocides, insecticides and other veterinary drugs to avert the development of resistance against veterinary drugs are recommended.

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1. INTRODUCTION

1.1 Background and general introduction

Trypanosomosis is a parasitic disease of humans and animals caused by protozoa of the genus *Trypanosoma*. It is mainly transmitted by tsetse flies; however, mechanical transmission is also possible through other biting flies such as horseflies and stable flies. Transmission may also occur by iatrogenic means (Reid, 2002), ingestion of fresh infected blood or organs in carnivores (Moloo *et al.*, 1973), through bites of infected vampire bats (Hoare, 1965) and by venereal means as in the case of *Trypanosoma equiperdum* (Brun *et al.*, 1998). Trypanosomosis affects domestic animals such as cattle, sheep, goats, camels, dogs, cats and pigs (Mossaad *et al.*, 2020; Giordani *et al.*, 2016). It's worth noting that there exist differences between infections caused by the various species and strains of trypanosomes in different host species (Nantulya, 1990). Surra in Camelidae and Equidae presents with intermittent fever, haemolytic anaemia, anorexia, emaciation, production loss, cachexia, nervous symptom, abortions and death (Evum, 2015; Gutierrez *et al.*, 2005). In goats and sheep, trypanosomosis is largely mild or asymptomatic. However, infected sheep may have superficial corneal ulceration and/or retinochoroiditis (Evum, 2015). In susceptible cattle, trypanosomosis presents in acute or chronic states generally characterized by intermittent fever, loss of condition, lymphadenopathy, dehydration, lacrimation, anaemia, inappetence, corneal opacity and death if not treated.

Trypanosomosis presents a major constraint to health and livelihoods in Africa due to its widespread occurrence. It's estimated that African Animal Trypanosomosis (Staats *et al.*) is endemic in at least 37 African countries with 150 million livestock at risk of contracting the disease and with an estimated loss (direct and indirect) of USD 4 billion per year in Gross Domestic Product (https://www.fao.org/3/ca3887en/ca3887en.pd). Human African Trypanosomiasis (HAT) is classified by the World Health Organization (WHO) as a Neglected Tropical Disease (NTD) (https://www.who.int/health-topics/neglected-tropical-diseases) as it is related to impoverishment. At the start of the century, HAT prevalence

rose to epidemic proportions in countries such as Angola, the Democratic Republic of Congo, Uganda, and Sudan (Organisation Mondiale de la Santé, 2021). Moreover, countries such as Cameroon, Congo, Côte d'Ivoire, Central African Republic, Guinea, Mozambique, Tanzania, and Chad have registered increased incidences and prevalence of HAT in the recent past (Venturelli *et al.*, 2022). In Kenya, epidemics of the Rhodesian sleeping sickness caused by *Trypanosoma brucei rhodesiense,* occurred in the Western parts of the country up until the year 2009 when the last clinical case was reported. The country is therefore on course for elimination of HAT and is currently in the process of certification as free of the disease by the WHO in line with the set guidelines (file:///C:/Users/Admin/Desktop/KENYA_NTD_MASTER_PLAN_2023_2027.pdf).

Appreciating the need for a concerted continent-wide effort to address the Tsetse and Trypanosomosis problem, the African heads of state and governments meeting in Lome, Togo, in the year 2000 adopted a declaration (Decision AHG/156(XXXVI) of the 36th assembly of the heads of state and government) to free Africa of trypanosomosis. This was implemented through the Pan African Tsetse and Trypanosomosis Campaign (PATTEC) project which saw a significant reduction in trypanosomosis burden on the continent.

Tsetse transmitted trypanosomosis is unique to sub-Saharan Africa affecting 9 million Km^2 (Allsopp, 2001). The distribution of trypanosomosis in Africa largely coincides with that of its principal biological vector the tsetse flies and its prevalence changes with changing tsetse densities. *Trypanosoma* species and subspecies are specific to certain regions and have distribution patterns determined by the type of *Glossina* species and susceptible host animal species. For example, the human infective *T. brucei gambiense* is found in Western and Central Africa while *T. brucei rhodesiense* occurs in Eastern and Southern Africa (Uilenberg, 1998). Among the existing *Trypanosoma* species causing AAT, *Trypanosoma vivax*, *T. congolense* and *T. b. brucei* have the largest economic significance and relevance in livestock in parts of Africa where the disease is a menace (Gebre *et al.*, 2022; Percoma *et al.*, 2022; Ngari *et al.*, 2020; Diarra *et al.*, 2019). Wildlife

conservation areas in Africa play an important role in the epidemiology of trypanosomosis by providing suitable ecological niche for tsetse flies and blood meal for their sustenance (Munang'andu *et al.*, 2012). Furthermore, wildlife act as reservoirs of trypanosomes for both humans and domestic animals living in areas surrounding wildlife conservancies (Kasozi *et al.*, 2021).

Tsetse flies in the genus, *Glossina*, are involved in the cyclic transmission of trypanosomes to susceptible host species. Tsetse flies exclusively feed on blood and are confined to the sub-Saharan Africa (Krafsur, 2009). Tsetse flies get infected by bloodstream forms of trypanosomes called trypomastigotes when feeding on parasitaemic animals. The trypomastigotes thereafter, undergo morphological and physiological development in the gut of the fly to change into the long forms, the epimastigotes which multiply to give rise to the infective metacyclic trypanosomes that occur either in the biting mouthparts or salivary glands. Different trypanosome species develop in different regions of the gut of the fly. *Trypanosoma vivax* develops in the proboscis, *T. congolense* in the midgut and the proboscis and the *T. brucei* group in the midgut and salivary glands (Uilenberg, 1998). Infection of hosts occur during feeding as the fly penetrates the skin with its proboscis, rupturing blood vessels and injecting saliva with infective metacyclic trypanosomes into the blood stream (Adam *et al.*, 1979; Uilenberg, 1998). This fly bite triggers a local inflammatory reaction leading to the development of a swelling called a chancre at the site. The metacyclic trypanosomes multiply in the chancre giving rise to blood forms that spread to the lymphatic system and bloodstream (Pays *et al.*, 2023).

In Kenya, tsetse flies infest about 23% of the landmass which is equivalent to 138,000 Km^2 (of 582,646 km^2) (FORD J, 1977; KETRI, 1996). On average, Kenya loses about US$ 0.2 billion per year in GDP to tsetse and trypanosomosis (Onyango, 2020). The economic losses are attributed to direct losses in production including decreased milk production, decreased weight gain, abortions, loss of draught power and death of affected animals and indirect losses through increased cost of animal treatment and cost of vector

control (Gamba *et al.*, 2021; Onyango, 2020). There exists eight species of tsetse flies in Kenya including *Glossina brevipalpis, G. fuscipleuris, G. longipennis, G. fuscipes, G. austeni, G. morsitans, G. swynnertoni,* and *G. pallidipes* spread across seven tsetse belts/zones (Bourn *et al.*, 2001) as illustrated in Figure 1.

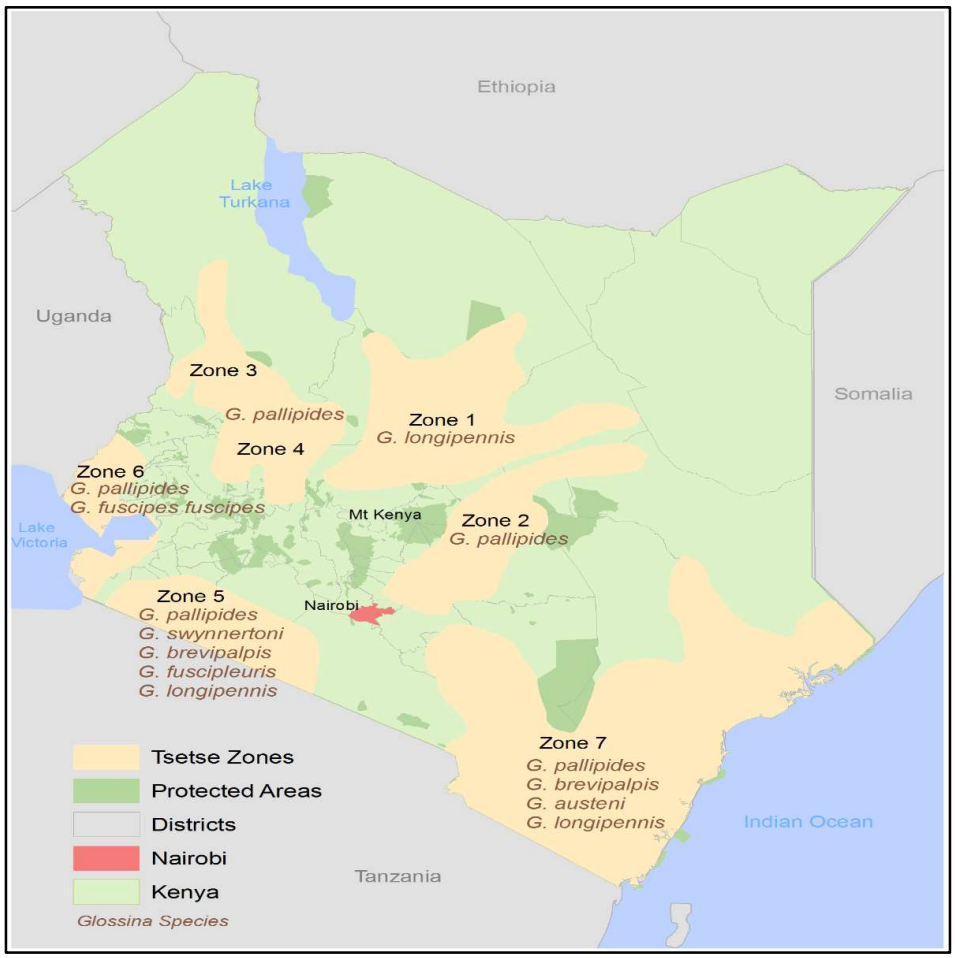

Figure 1 Tsetse habitats (Zones 1–7) and Glossina spp., Kenya, 1999. (Data Source: International Livestock Research Institute, Kenya 2009).

The most common species in Kenya is *G. pallidipes (87%),* followed by *G. brevipalpis (8%); G. fuscipes fuscipes (4%); G. longipennis (<1%)* (Ngari *et al.*, 2020). About 8% of the Kenya's land mass is protected area for wildlife conservation (Onditi *et al.*, 2021). These areas embrace various types of ecosystems namely: forests, wetlands, savannah, marine, arid and semi-arid that provide suitable tsetse habitats and host wildlife that act

as reservoirs of human and livestock pathogenic trypanosomes. The Ruma national park, Masai national reserve, the Meru National Park, Tsavo national Park, Shimba Hills national reserve are examples of protected areas in Kenya infested with tsetse flies.

In Kenya, AAT distribution aligns with that of tsetse flies across the seven tsetse belts/zones with an exception of the arid and semi-arid areas of the Northern parts of the country where other biting flies such as *Hippobosca camelina*, *Stomoxys calcitrans, Tabanus* spp. and *Pangonia rueppellii* predominate in mechanical transmission of trypanosomes (Getahun *et al.*, 2022). *Trypanosoma vivax* is the most isolated trypanosome species in the country followed by *T. congolense* and *T. brucei brucei* (Ngari *et al.*, 2020).

Over the years, the control of trypanosomosis has relied on the use of trypanocidal drugs, vector control and the breeding of trypanotolerant livestock (Van den Bossche and Delespaux, 2011). Up to the year 2005, tsetse control in Kenya was undertaken by the Department of Veterinary Services (DVS) as part of animal disease control. Thereafter in 2005, the PATTEC Kenya launched its Tsetse and Trypanosomosis eradication activities with funding from the African Development Bank. The Kenya Tsetse and Trypanosomosis Eradication Council (KENTTEC) was later established via a legal notice number 77 of July 2012 under the state corporations act (Cap 446) to succeed the PATTEC project. KENTTEC implements tsetse and trypanosomosis control activities in collaboration with stakeholders such the Directorate of veterinary services, County governments, Kenya Wildlife Services (KWS), research institutions and communities living in tsetse infested areas. Some of the techniques deployed by the KENTTEC to control tsetse and trypanosomosis include the use of traps and targets, livestock spraying, installation of livestock protective netting and exploration of trypanotolerant cattle breeds (Gamba *et al.*, 2021). Dwindling resources owing to reduced government support over the years has negatively impacted the effective, area wide and sustained implementation of these strategies for the control of tsetse and trypanosomosis (McCord *et al.*, 2012; Gamba *et al.*, 2021; Ngari *et al.*, 2020; Onyango, 2020). Furthermore, KENTTEC faces challenges

in the coordination of tsetse control in the devolved administrative units in the current constitutional dispensation and across borders with neighbouring countries where tsetse and trypanosomosis is a menace (https://www.kenttec.go.ke/wpcontent/uploads/2019/08/Draft-KENTTEC-Strategic-Plan.pdf).

Drug resistance occurs when disease pathogens such as viruses, bacteria, parasites and fungi develop an ability to thrive in the presence of medications that previously had a destructive impact on them (Founou *et al.*, 2017; Dadgostar, 2019). This occurrence decreases the options for treatment and increases costs, thus causing a negative impact on livestock general health and production. Due to its significance, the Food and Agriculture Organization (FAO), World Organization of Animal Health (WOAH) and World Health Organization (WHO) in 2015 in a tripartite, developed the Global Action Plan (Agrawal *et al.*, 2003) to combat antimicrobial resistance (AMR) (Organization, 2015). The GAP identifies "Strengthening the knowledge and evidence base through surveillance and research" as its second objective thus emphasizing the need for active surveillance for AMR. Consequently, various countries including Kenya have developed and are implementing their National Action Plans (NAP) in the fight against drug resistance.

Treatment with trypanocidal drugs remains the most widely used method of controlling bovine trypanosomosis in many parts of Africa where the disease is a constraint (Machila *et al.*, 2003). In Kenya, treatment of AAT is dependent on the use of Diminazene Aceturate (DA) and Homidium and Isometamidium Chloride (ISM) (Leach and Roberts, 1981), compounds that have been used for more than six decades without the introduction of any new one (Connor, 1992). Various reports from different countries have demonstrated the presence of resistance by trypanosomes to each of these molecules (Mapenay and Maichamo, 2006; Gray and Roberts, 1971; Gitatha, 1979; Pinder and Authie, 1984; Chitanga *et al.*, 2011) to various degrees. Geerts and Holmes (1998) estimated that approximately 35 million doses of trypanocides are administered every year in sub-Saharan Africa with ISM and DA representing 40% and 33% respectively.

The privatization of clinical veterinary services in Kenya (Okwiri, 2006; Chema and Gathuma, 2004) resulted to an inadequacy of trained veterinary professionals especially in rural areas where 50% of the livestock are (Chema and Gathuma, 2004). Most farmers therefore have resorted to self-treat their animals (Okello *et al.*, 2022), a practice that has resulted in drug misuse and under-dosing hence contributing to the development of resistance (Ozturk *et al.*, 2019). There is scanty information on the prevalence of DA resistance in Kenya despite its widespread use and misuse (Makau *et al.*, 2022; Machila *et al.*, 2007; Irungu *et al.*, 2007).

The Lambwe Valley rangeland in South West Kenya has been the focus of Tsetse and Trypanosomosis (T&T) activities since the beginning of the 20th Century. Efforts on control of T&T in the valley by various stakeholders over the years have seen a marked decline in its burden (Okello *et al.*, 2022; Opiyo *et al.*, 1990; Muriuki *et al.*, 2005). The historical threat posed by trypanosomosis in the area, has resulted in the extensive and widespread use of trypanocides. A study by Okello *et al.* (2022) in the Lambwe Valley established that ISM is the most used trypanocide followed by DA. The study further asserts that most farmers self-treat their animals and that infections are reported to be higher in herds of farmers that self-treat their livestock. It is therefore speculated that the historical widespread use and misuse of trypanocides in the area has resulted in the development of resistance by the circulating *Trypanosoma* species to the commonly used trypanocides. This study sought to reject the null hypothesis that there exists no resistance to DA by *Trypanosoma* species isolated from cattle populations in Lambwe Valley and also to accept the alternate hypothesis that trypanosomosis is prevalent in the selected villages of Lambwe Valley.

2. LITERATURE REVIEW

2.1 Drugs used for the treatment and prophylaxis of trypanosomosis

2.1.1 Phenanthridine (Homidium (or Ethidium Bromide), Isometamidium Chloride

The antitrypanosomal activity of Phenanthridium compounds was discovered more than 9 decades ago (Browning *et al.*, 1938). **Homidium bromide or ethidium bromide/chloride** was approved for treatment of AAT caused by *T. congolense* and *T. vivax* (Solomon and Workineh, 2018) in 1952 (Wainwright, 2010) and has been widely used in Africa (Giordani *et al.*, 2016) in spite of its proven mutagenic and probable carcinogenic potential as a DNA escalator (Sutcliffe *et al.*, 2014). It is used as a curative drug though it also possesses prophylactic properties albeit lower as compared to Isometamidium. Homidium is administered deep intramuscularly at a dosage of 1 mg/kg body weight (bw) (Peregrine, 1994). Its relatively excreted fast as its serum concentration declines rapidly over 24 hours following administration (Murilla *et al.*, 2002). Its half-life in cattle ranges from 178h to 488h following IM administration however, it can persist in the circulatory system at low levels for 8 to 17 weeks when administered intramuscularly and offer prophylaxis (Dolan *et al.*, 1990; Whiteside, 1962; Mwambu, 1971).

Isometamidium chloride hydrochloride is a phenanthridine with amphiphilic and cationic properties synthesized by combining homidium with the diazotized *p*-aminobenzamide moiety of diminazene, modified with the amidine group in the *meta* position (Sutcliffe *et al.*, 2014). It's got both prophylactic and curative properties against *T. congolense* and *T. vivax* but low activity against *T. brucei* and *T. evansi* (Giordani *et al.*, 2016). For cure, Isometamidium is administered in cattle as a single dose at 0.25-1.0 mg/kg bw while for prophylaxis its given at 0.5-1 mg/kg bw (Leach and Roberts, 1981). The duration of prophylactic activity following intramuscular administration in cattle depends on the formulation, dosage used, parasite strain, susceptibility of the cattle breed and general health status (Toro *et al.*, 1983). Eisler

(1996), in a study indicated that Isometamidium has a half-life of approximately 9 to 19 days. It exerts it prophylactic activity by accumulating in the organs such as liver, kidneys, spleen and at injection sites, from where it gradually released into plasma (Kinabo and BOGAN, 1988).

2.1.2 Diminazene Aceturate (DA)

Diminazene aceturate is an aromatic diamidine compound that was developed in the research laboratories of Fabwerke Hoechst in 1944 (Fussgänger, 1995). It is marketed as a diaceturate salt consisting of two aminodinophenyl moieties linked by a triazene bridge and is chemically described as 4,4'-(1-Triazene-1,3-diyl)bis (benzenecarboximidamide) (Wien, 1943). As an antitrypanosomal drug, DA acts by binding to the AT-rich regions of nucleic acid duplexes via complexion into the minor grooves of the AT-rich domains of the DNA double helices. It can bind to both DNA and RNA duplexes. The binding unwinds negative supercoils in plasmids and interferes with activities of the eukaryotic type II topo-isomerase enzymes (Portugal, 1994; Miller, 2006). It is administered intramuscularly (IM) at a dosage of 3.5-7.0 mg/KG body weight and is excreted in urine within 20 days together with two metabolites: *p*-amino benzamidine and *p*-aminobenzamide (Aliu *et al.*, 1993a; Peregrine and Mamman, 1993; Kellner *et al.*, 1985a). Following its use, it has been established that IM administration of DA at a dose of 3.5mg/kg body weight eliminates *T. congolense* and *T. vivax* infections in cattle. However, *T. brucei* infections require a higher dosage of 7mg/kg body weight (Fussgänger, 1995).

DA is rapidly excreted and has little prophylactic activity. It is therefore recommended for use as a therapeutic drug (Bauer, 1958). However, some studies have established that DA can have trypanocidal activity lasting between 2 to 21 days after administration (Fairclough, 1963; Van Hoeve and Cunningham, 1964). The elimination half-life values following intramuscular administration varies from 11-19 hours in sheep and goats, 74 to >200 hours in cattle (Peregrine and Mamman, 1993).

In Kenya, DA is currently marketed as Veriben® and Berenil® for the treatment of Babesiosis and Trypanosomosis in livestock (Bauer, 1955). Currently, it is the most widely used trypanocide in cattle, sheep and goats due to its activity against both *T. congolense* and *T. vivax* and its relatively low toxic effects (Giordani *et al.*, 2016).

2.2 Trypanocidal drug Resistance

The impact of drug resistance in animal farming is associated with its negative effects on livestock health and the potential public health consequence of transfer of resistant pathogens to humans (Bengtsson and Greko, 2014). Antimicrobials are needed for the effective treatment of animals and for prophylaxis against various diseases. Resistance robs them of this ability leading to therapy failures that result in production losses, increased cost of treatment, animal suffering, distress and death. Over the years, the control of trypanosomosis has relied on the use of trypanocidal drugs, vector control and the breeding of trypanotolerant livestock (ILRAD, 1994). Furthermore, the mechanism behind the development of resistance by trypanosomes to DA has not been clearly defined. While some studies associate the resistance with the loss of the transporter gene P2/AT1 in *T. b. brucei* (Matovu et al., 2003), *T. equiperdum* (Barrett et al., 1995) and *T. evansi* (Witola *et al.*, 2004), other studies dissociate its *T. congolense* orthologue TcoATI from the uptake of DA (Munday *et al.*, 2013).

The mechanism behind the development of resistance by *T. congolense* to isometamidium is associated with diminished mitochondrial membrane potential which in turn diminishes the accumulation of the drug in the mitochondrion and a net result of reduced uptake at the plasms membrane (WILKES *et al.*, 1997). Other studies suggest active extrusion by plasma membrane transporter as the cause of resistance (Sutherland and Holmes, 1993).

Most trypanocidal drug resistance tests make use of mice or ruminants (Eisler *et al.*, 2001), and although labour intensive, results obtained from tests in these species have been reported to be consistent with one another (Peregrine et al., 1991, Ainanshe et al., 1992, Codjia et al., 1993, Ndoutamia et al., 1993). However, likely due to variations in metabolic size, the precise curative dose for a particular isolate in cattle cannot be inferred

directly from the outcomes in mice (Sones et al., 1988). Therefore, for this study, trypanocidal drug resistance in cattle was evaluated by means of a block treatment to investigate resistance of trypanosome species to DA. This method is reliable, easy, fast and does not require the isolation of the parasites (Eisler *et al.*, 2001; Mulandane *et al.*, 2018; McDermott *et al.*, 2003).

3. MATERIALS AND METHODS

3.1 Ethics and approval

This study obtained ethical approval (KALRO-VSRI/ISERC031/22032023) (Appendix 4) from the Institutional Scientific and Ethical Review Committee (ISERC) of the Kenya Agriculture and Livestock Research Organization (KALRO), The University of Pretoria, Faculty of Veterinary Science Research Ethics Committee (REC) - REC147-23 (Appendix 5) and research license No. NACOSTI/P/23/26760 (Appendix 3) from the National Commission for Science, Technology & Innovation (NACOSTI).

3.2 Study area

This cross-sectional study was conducted in Kigoto, Kigwa and Gendo Villages of the Lambwe Valley in Homabay County, Kenya. The villages were selected based on their proximity to Ruma National Park with the closest preferred.

3.3 Sampling

Sample size of 385 cattle determined according to formula by Cannon and Roe (1982) with an assumed AAT prevalence of 50%, desired absolute precision of 5%, confidence level of 95%.

$$n=Z^2P(1-P)/E^2$$

Where:

n *is the required sample size.*

Z *is the Z-score corresponding to the desired confidence level.*

P *is the estimated prevalence of the condition in the population.*

E *is the desired margin of error.*

Blood samples for microscopic examination and molecular analysis were collected between 4th July 2023 and 4th August 2023.

3.4 Study design

3.4.1 Determination of parasitological prevalence of trypanosomosis in cattle

The number of cattle sampled per village and herd were proportional to total number of cattle presented per village/herd. Cattle belonging to one household were considered a herd resulting in sampling of 65 herds. Systemic random sampling of cattle was deployed where selection was done regardless of age, sex or clinical status and with the sampling every odd numbers up to the N^{th} animal. Blood samples were collected from selected cattle through the ear vein prick into heparinized capillary tubes, sealed on one end with plasticine then centrifuged at 12000 rotations per minute (rpm) for five minutes. The packed cell volume (PCV) for each sample was determined using the Hawksley's Micro Haematocrit Reader (Hawksley, Lancing, UK).

The blood was centrifuged at 12000 rpm for 5 minutes using the microhematocrit centrifuge (Hawksley®, Lancing, UK). The contents of the buffy coat were then transferred onto a microscope slide then viewed for motile trypanosomes under a compound microscope (Leica Microsystems, Wetzlar, Germany) at a magnification of × 400 (Murray et al., 1977). Trypanosoma species were differentiated based on their morphology and motility patterns as illustrated by (Adam *et al.*, 1979). *Trypanosoma congolense* is a small trypanosome with a blunt posterior end, less prominent undulating membrane, lacks a free flagellum but tapered towards the anterior end. Its movement is detected by moving cells around it in a microscopic field. *Trypanosoma vivax* has a rounded posterior end, long free flagellum and moves rapidly across the field.

Trypanozoon are slender and tapered at both ends, have a free and long flagellum, moves relatively fast but not as fast at *T. vivax* and are relatively larger than *T. vivax*.
Point prevalence of trypanosomosis was expressed as a percentage of the number of cattle that tested positive for trypanosomiasis against the number of cattle sampled per village.

3.4.2 Determination of resistance to diminazene aceturate

Seventeen (17) cattle, that tested positive on BCT, were traced and four millilitres of blood drawn from them through the jugular/coccygeal vein into Ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. The blood was then mixed with a cryopreservation agent (EDTA Saline Glucose buffer) in equal ratios. Two millilitres of the mixture were transferred into cryovials and suspended in liquid nitrogen cylinders for 24 hours before immersion into liquid nitrogen cylinder for preservation of the blood for further molecular analysis and transportation to the laboratory.
The 17 BCT positive cattle were ear tagged then recruited into a block treatment regime. Their body weights were estimated using the livestock weighing band by measuring the heart girth and converting the measurement to fairly accurate estimate of the animal' body weights. They were treated on day zero with DA at 3.5 mg/kg of body weight then monitored on days 7 and 28 for infection status with BCT (Field) and using the Internal Transcribed Spacer 1 (ITS1)-PCR. The PCR was performed as described by Njiru *et al.* (2005) using the following forward (ITS1-CF) and reverse (ITS1-BR) primers. These primers hybridize in the18S and 5.8S rDNA, therefore the PCR amplifies ITS1 gene and is used to identify the three *Trypanosoma congolense* types (savannah, forest and Kenya Coast), with distinction among themselves and from the subgenus *Trypanozoon* (*T. brucei spp., T. evansi* and *T. equiperdum*), *T. vivax, Trypanosoma simiae* and *Trypanosoma theileri* (Desquesnes *et al.*, 2001).

3.4.3 Molecular detection of *Trypanosoma* spp.

The BCT *Trypanosoma* positive samples were retrieved from -80°C freezer and thawed on ice before starting DNA extraction. DNA was extracted from a uniformly mixed 200 µL of each blood sample using the Isolate II Bioline Genomic DNA extraction kit (Bioscience) according to the manufacturer's instructions and the end products (elutes) subjected to PCR for amplification of trypanosomes using internal transcribed spacer 1 (ITS1) primers developed by Njiru *et al.* (2005). A 20 µL PCR mixture entailing 10 µL of nuclease free water, 4 µL HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu, Estonia), 1 µL of 10 µM reverse (ITS1-BR) and forward (ITS1-CF) primers, and 4 µL of the DNA template was performed in a thermocycler (Proflex PCR System Applied Biosystems by Life technologies Holdings Pte Ltd, Marsiling Industrial Estate Road 3, Singapore). The cycling conditions for amplification were an initial enzyme activation step set at 95°C for 15 minutes followed by 35 cycles of DNA denaturation at 95°C for 20 s, annealing at 62°C for 30 s, and extension at 72°C for 30 s, followed by a final extension step at 72°C for 7 minutes. Upon completion of the PCR the amplicons were infinitely held at 8°C before analysis by agarose gel electrophoresis. Then 10 µL of the PCR products was analysed by running on 1.5% agarose gel at 95 V for 1 hour before visualization on the gel doc imager (Kodak Gel Logic 200 Imaging System, SPW Industrial, Laguna Hills, CA, USA). Due to the reported low sensitivity of ITS1 primers in amplifying trypanosomes, we resorted to cross-check the presence or absence of Trypanosoma spp. using species-specific primers: TCS for detection of *Trypanosoma congolense Savannah*, TCF for *T. congolense Forest*, and TCK for *T. congolense Kilifi* subtypes, TBR for *Trypanosoma brucei*, and TVW for screening *Trypanosoma vivax* West Africa. The sequences of the primers used are shown in Table 1 below.

Table 1 Species specific primer sequences used for molecular analysis of trypanosomes in cattle.

Primer name	Gene	Primer sequence (5'-3')	Band size	Reference
ITS1 – CF	*Trypanosoma* spp.	CCGGAAGTTCACCGATATTG	250 – 710	(Njiru *et al.*, 2005)
ITS1 – BR		TTGCTGCGTTCTTCAACGAA		
TCF – 1	*T. congolense* Forest	GGACACGCCAGAAGGTACTT	350	(Masiga *et al.*, 1992)
TCF – 2		GTTCTCGCACCAAATCCAAC		
TCS – 1	*T. congolense*	CGAGAACGGGCACTTTGCGA	316	(Masiga *et al.*, 1992)
TCS – 2	Savannah	GGACAAACAAATCCCGCACA		
TCK – 1	*T. congolense* Kilifi	GTGCCCAAATTTGAAGTGAT	294	(Masiga *et al.*, 1992)
TCK – 2		ACTCAAAATCGTGCACCTCG		
TVW1	*T. vivax*	CTGAGTGCTCCATGTGCCAC	150	(Masiga *et al.*, 1992)
TVW2		CCACCAGAACACCAACCTGA		
TBR – 1	*T. brucei*	GAATATTAAACAATGCGCAG	177	(Masiga et al., 1992)
TBR – 2		CCATTTATTAGCTTTGTTGC		

4. RESULTS

4.1 Parasitological prevalence of trypanosomosis

Out of 395 cattle screened using the BCT from all the three villages, 19 tested positive for trypanosomosis translating to a mean prevalence of 4.54%. Most of the infections (73.6%) were of *Trypanosoma vivax* followed by *Trypanosoma congolense* (26.4%). There were no members of the subgenus *Trypanozoon* detected. Kigoto village registered the highest prevalence (5.02%) followed by Wiga (4.9%) and Gendo (3.7%). The total prevalence of trypanosomosis per village is summarized in Table 2. Please see appendix 1 for a summary of all the samples collected for this study.

Table 2 Prevalence of trypanosomosis by BCT in the three villages in Lambwe Valley (Kenya), before treatment (July 2023).

Village	Number of cattle Sampled	BCT results				Prevalence (%) of trypanosomosis
		Tc	Tv	Tb	Total positive	
Wiga	102	1	4	0	5	4.9
Kigoto	239	3	9	0	12	5.02
Gendo	54	1	1	0	2	3.7
TOTAL	395	5	14	0	19	4.54 (N=395)
Prevalence (%) N=19		26.4	73.6	0		

Key: Tc - *Trypanosoma congolense,* Tb - *Trypanosoma brucei,* Tv - *Trypanosoma vivax*

No significant statistical difference in prevalence was noted across the villages ($X_{(2, N=395)}^2$ = 0.805, p = 0.668) *T. congolense* and *T. vivax* cross tabulation did not reveal any significant difference in prevalence between species ($X_{(1, N=19)}^2$ = 3.69, p = 0.056). In terms of sex, 54.7% of the cattle sampled were females while males constituted 45.3%. On the other hand, 58.85% cattle that tested positive (N=17) were female while males were 41.17%. No significant statistical difference was however noted in infectivity across sexes ($X_{(1, N=17)}^2$ = 0.123, p = 0.726). The animals were divided into ages categories (< 2

years = calves, 2 to 4 years = young adults and > 4 years = adults). Of the animals sampled, 12.3% were calves, 52.2% were young adults while 35.5% were adults. 21.4% of the animals that tested positive were calves, 57.14% were young adults and 21.4% were adults. No significant difference was noted in infectivity across ages ($X_{(2, N=17)}^2$ = 1.88, p = 0.389).

4.2 PCV Trends across the monitoring days and between infected and non-infected animals

The mean PCV on day 0 (treatment day) for the cattle that tested positive was 19.03%. This rose to 23.39% on monitoring day 7 and to 26.8% on day 28. Figure 2 below illustrates the changes in mean PCV across the monitoring days. Whereas there is there is a clear upward trend in PCVs across the days, Analysis of Variance (Ivanova *et al.*) comparison of the means revealed no significant difference across the monitoring days (F-statistic = 2.2045, df = 2, p = 0.1268). The comparison of PCV of infected and non-infected animals revealed a significant statistical difference with Infected animals having a significantly lower PCV (19.29±4.07) compared to non-infected animals (24.09±5.07) (t = 3.844, p = 0.000).

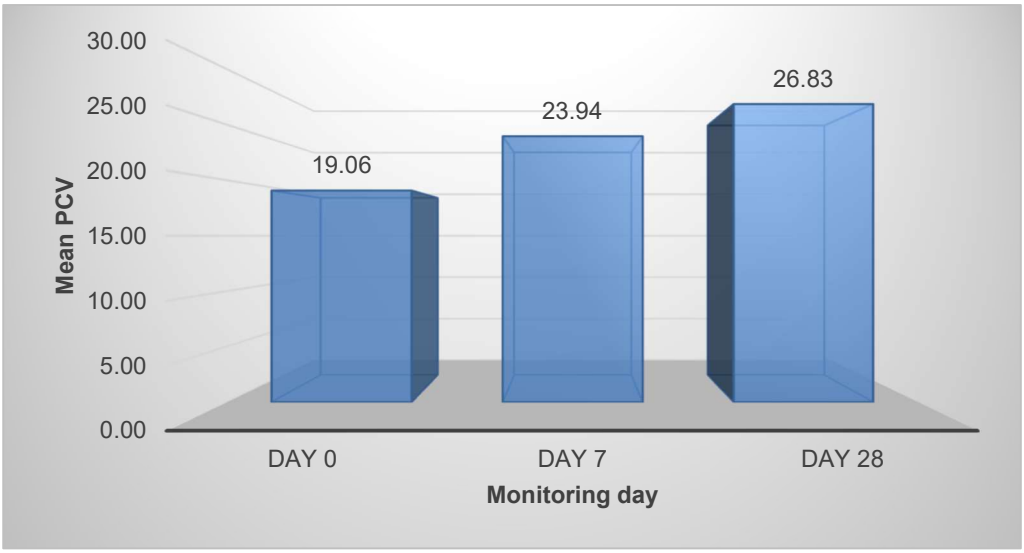

Figure 2 PCV trends across monitoring day 0, 7 and 28.

Table 3 Summary screening results across the monitoring days on BCT and PCR.

Trypanosoma species	Day 0		Day 7		Day 28	
	BCT	PCR	BCT	PCR	BCT	PCR
T. congolense	5	6	0	0	1	2
T. vivax	11	7	0	0	2	2
Mixed *T. vivax* / *T. congolense*	1	2	0	0	0	0
Negative	0	2	17	17	9	8
Total	17	17	17	17	12	12

Of the 19 positive cases isolated on day zero through the BCT, one was a mixed infection of *T. vivax* and *T. congolense* while one animal went missing after sample collection and therefore considered drop out. Only 12 animals were presented for screening on day 28 as five dropped out. No animal tested positive on day seven on both BCT and PCR while 3 tested positive on day zero upon BCT and 4 upon PCR. The screening results across the monitoring days are summarized in Table 3. Please see appendix 2 for a summary of the PVC, BCT and PCR results for the positives samples across all monitoring days.

Trypanosoma congolense subtypes revealed that majority were *T. congolense Savanna*. Only one was *T. congolense Kilifi* while one mixed *T. congolense Savanna* and *T. congolense Kilifi*. There was no *T. congolense Forest* detected.

All the cattle tested negative for trypanosomiasis on both BCT and ITS1-PCR on day 7 after treatment with DA. Figures 3, 4 and 5 illustrates the molecular test results for day 0 before treatment, day 7 and day 28 after treatment, respectively.

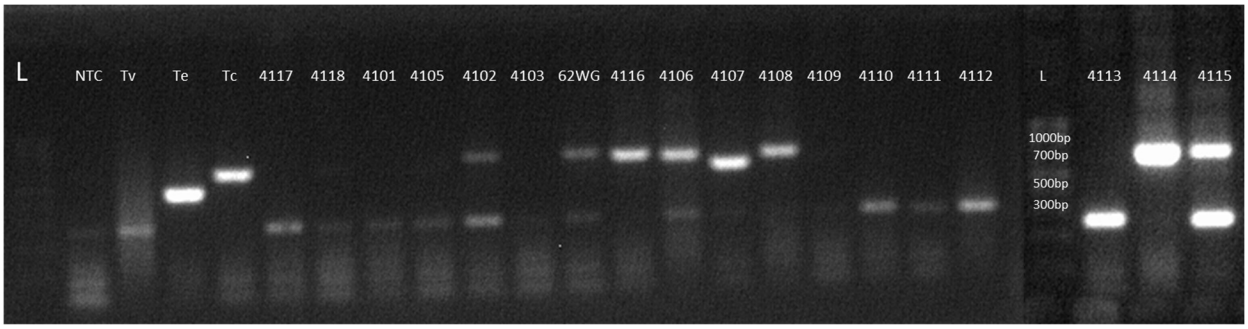

Figure 3 Results of PCR for amplification of DNA from trypanosomes isolated on day 0 using internal transcribed spacer 1 (ITS1) primers. L – DNA ladder, NTC – no template control, Tc - *T. congolense* (4105, 4106, 4107, 4108, 4114 and 4116), Tv - *T. vivax* (4110, 4111, 4112, 4113, 4117 and 418) and mixed *T. vivax* and *T. congolense* (4102 and 4115).

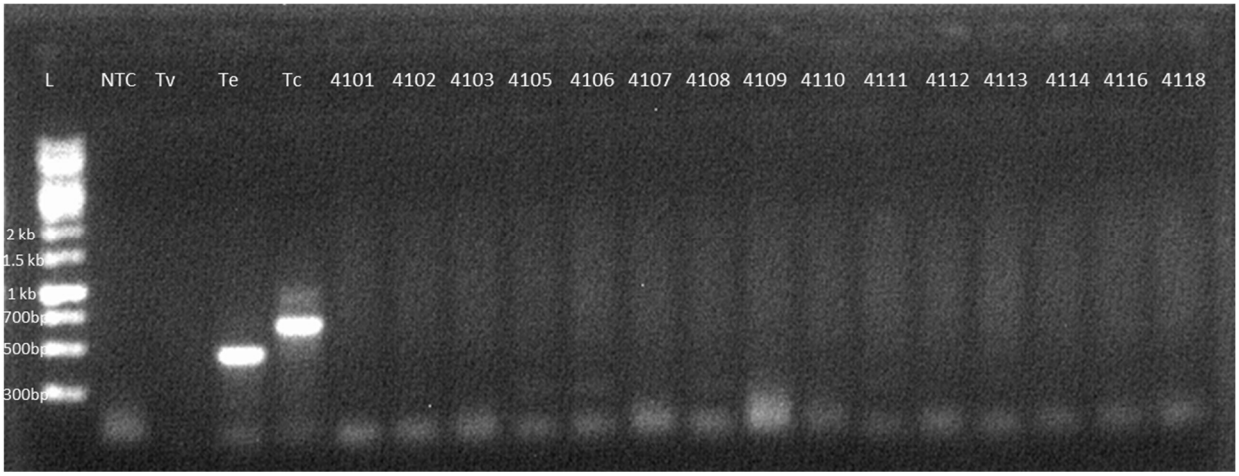

Figure 4 PCR amplification results using ITS1 primers for samples collected on day 7 after treatment with diminazene aceturate. No trypanosome DNA was amplified.

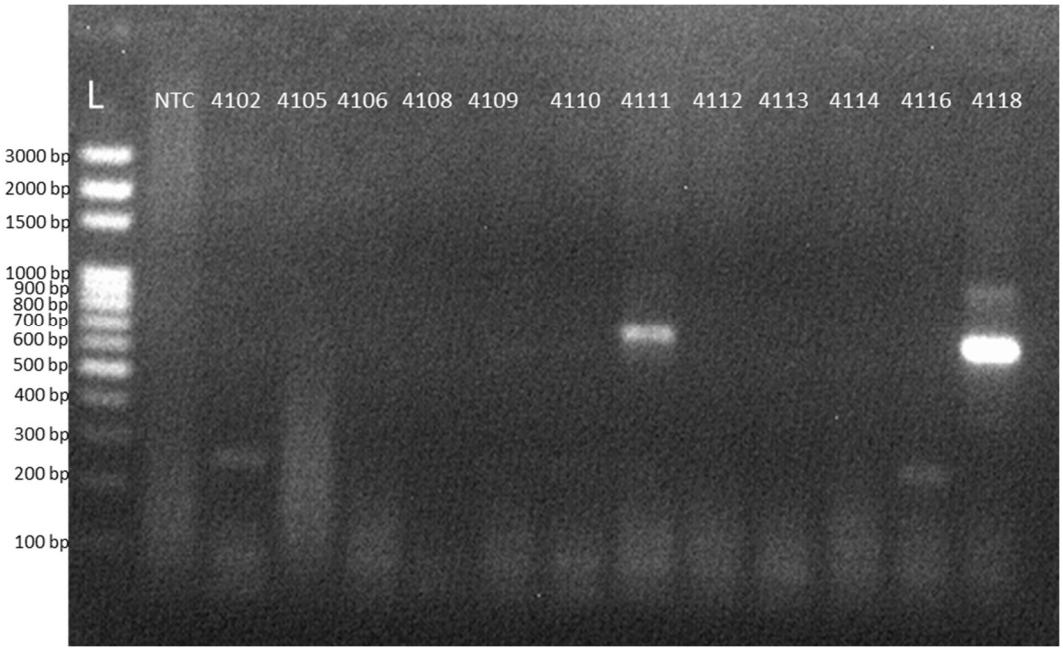

Figure 5 Results of PCR for amplification of DNA from trypanosomes isolated on day 28 using internal transcribed spacer 1 (ITS1) primers. *T. congolense* (4118), *T. vivax* (4102,4111 and 4116).

On day 28 after the treatment, three animals tested positive on BCT while on PCR-ITS1, four animals tested positive. Three of these animals tested positive for different species of trypanosomes from those initially detected on the first day of screening (before treatment with DA). One animal that had a mixed infection of *T. vivax* and *T. congolense* on day 0 had *T. vivax* infection on day 28. A summary of the PCR results is shown in Table 4 below.

Table 4 Summary of molecular results for animals that tested positive on day 0 and day 28.

Sample ID	4102	4111	4116	4118
PCR Results: Day 0	Tv, Tc	Tv	Tc	Tv
PCR Results: Day 28	Tv	Tc	Tv	Tc

Key*:* Tc - *Trypanosoma congolense,* Tb - *Trypanosoma brucei,* Tv - *Trypanosoma vivax*

5. DISCUSSION

Tsetse transmitted trypanosomosis is a major constraint to livestock production, human health and settlements in the Lambwe Valley. Despite control interventions by the government and various stakeholders, trypanosomiasis remains a challenge in the Lambwe Valley with this study reporting a prevalence of 4.54% (BCT) in the study area. Previous studies conducted in the area by Okello *et al.* (2022) and Okoth *et al.* (2019) registered average prevalences of 3.44% and 9.2% respectively . The prevalence of the disease in the study area is slightly higher than the estimated national prevalence of the disease (Ngari *et al.*, 2020). The persistence of the disease in this area can be partly attributed to the surrounding Ruma National Park which hosts wild animals, that act as reservoirs of the disease (Anderson *et al.*, 2011), and also provides a good habitat for tsetse flies, the primary vectors of trypanosomiasis. *Trypanosoma vivax* is the most common trypanosome species present in the Lambwe Valley at 73.6% followed by *Trypanosoma congolense* at 23.4% which is in agreement with findings from other studies conducted in the area (Okello *et al.*, 2022). This study did not isolate any members of the subgenus *Trypanozoon* which is a significant finding for the surveillance and control of HAT in the country. The last incident of sleeping sickness in Kenya was reported in the year 2009 and the country is in the process of validation for HAT free status by the WHO (file:///C:/Users/Admin/Desktop/KENYA_NTD_MASTER_PLAN_2023_2027.pdf). This study, failing to isolate any circulating HAT causative trypanosomes in cattle populations, provides a further reference to possible absence of the parasite in circulation.

Packed Cell Volume (PCV) and other haematological parameters provide baseline information on physiology, nutrient and health status of animals (Daramola *et al.*, 2005). There exists a great range of values for normal PCV for animals which is accounted for by variation in age, sex, breed or strain, blood sampling technique and testing methodology. The normal range for cattle for instance is 24-48 (Etim *et al.*, 2014). The animal's hydration status at the time of sampling can also affect the PCV level as noted by Abdelatif *et al.* (2010). The low mean PCV observed in trypanosomosis positive animals in this study compared to the negative ones ($t = 3.844$, $P = 0.000$) is a result of

anaemia through general destruction of infected red blood cells, accelerated destruction of the red blood cells by the immune system, the suppression of the bone marrow response by cytokines, massive gastrointestinal haemorrhage and increased spleen Clarence of infected red blood cells by trypanosomes (Rufa'i *et al.*, 2021).

This study did not absolutely confirm the presence or absence of resistance by Trypanosoma species to DA. The positive cases encountered in cattle 4111, 4116 and 4118 on day 28 could either be new infections or they were parasites that were present in the animals during the initial screening on day 0 but were not detected on day 0/7, consequently indicating possible resistance. The prepatent period of animal trypanosomosis in cattle range between 4 to 21 days (Grootenhuis *et al.*, 1990; Dargie *et al.*, 1979), thus allowing sufficient time for the establishment of new infections and parasitaemia. Moreover, various studies (Klatt and Hajdu, 1976; Kellner *et al.*, 1985b; Aliu *et al.*, 1993b) document the elimination half-life of DA to be between 40 to 205 hours further suggesting that there was not enough DA in the plasma, beyond day 10 after treatment, to prevent re-infection. The BCT and PCR-ITS1 do not have absolute sensitivity in detection of *Trypanosoma* species and sub species illustrating the possibility of missed detection of some parasites on day 0 and day 7. Furthermore, the *T. vivax* of animal 4102 isolated on day 28 could be because of relapse due to possible resistance or appearance in the bloodstream of parasites that were previously sequestered in parts of the body that are not easily accessible by trypanosomes such at the central nervous system (CNS), eye globe, skin and adipose tissue. The positive cases recorded on day 28 were likely due to new infections, except the *T. vivax* infection of animal 4102 that could most probably be attributed to a relapse of the primary infection detected on day 0. It is probable that some parasites had escaped DA by invading various parts of the body which DA does not reach levels high enough to eliminate them, such as the central nervous system, eye globe, skin and adipose tissue (Bastos *et al.*, 2020). Similar results have been recorded by other studies for both natural and experimental infections (Bassi *et al.*, 2018; Bastos *et al.*, 2017; Batista *et al.*, 2007).

Block treatment provides a reliable alternative for assessing drug resistance, however, it can be improved by incorporating pyrethroid insecticide treatment for the study animals to prevent new infections during the monitoring period. Further, monitoring on day 14 and extension of monitoring to 60 days can improve outcomes on possible resistance to DA. This study recommends the continued application of the integrated approaches of controlling tsetse and trypanosomosis in the Lambwe Valley together with community training and sensitization on appropriate use of trypanocides, insecticides and other veterinary drugs to avert the development of AMR.

ACKNOWLEDGEMENTS

I would like to acknowledge the funding and support from the Belgium Development Cooperation (DGD), the Kenya Tsetse and Trypanosomosis Eradication Council (KENTTEC), University of Pretoria, the International Centre for Insect Physiology and Ecology (ICIPE), The county Government of Homabay and the Community Mobilization About Tsetse Control (CMATCO) community group in the Lambwe Valley.

REFERENCES

ABDELATIF, A. M., ELSAYED, S. A. & HASSAN, Y. M. 2010. Effect of state of hydration on body weight, blood constituents and urine excretion in Nubian goats (Capra hircus). *World J. Agric. Sci,* 6**,** 178-188.

ADAM, K. M. G., PAUL, J. & ZAMAN, V. 1979. *Medical and veterinary protozoology. An illustrated guide*, Churchill Livingstone.

AGRAWAL, A., LINGAPPA, J., LEPPLA, S. H., AGRAWAL, S., JABBAR, A., QUINN, C. & PULENDRAN, B. 2003. Impairment of dendritic cells and adaptive immunity by anthrax lethal toxin. *Nature,* 424**,** 329-334.

ALIU, Y., MAMMAN, M. & PEREGRINE, A. 1993a. Pharmacokinetics of diminazene in female Boran (Bos indicus) cattle. *Journal of Veterinary Pharmacology and Therapeutics,* 16**,** 291-300.

ALIU, Y. O., MAMMAN, M. & PEREGRINE, A. S. 1993b. Pharmacokinetics of diminazene in female Boran (Bos indicus) cattle. *J Vet Pharmacol Ther,* 16**,** 291-300.

ALLSOPP, R. 2001. Options for vector control against trypanosomiasis in Africa. *Trends in parasitology,* 17**,** 15-19.

ANDERSON, N. E., MUBANGA, J., FEVRE, E. M., PICOZZI, K., EISLER, M. C., THOMAS, R. & WELBURN, S. C. 2011. Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. *PLoS neglected tropical diseases,* 5**,** e1211.

BARRETT, M. P., ZHANG, Z. Q., DENISE, H., GIROUD, C. & BALTZ, T. 1995. A diamidine-resistant Trypanosoma equiperdum clone contains a P2 purine transporter with reduced substrate affinity. *Molecular and biochemical parasitology,* 73**,** 223-229.

BASSI, P. B., DE ARAÚJO, F. F., GARCIA, G. C., VINÍCIUS DA SILVA, M., OLIVEIRA, C. J. F., BITTAR, E. R., DE SOUZA GOMES, M., RODRIGUES DO AMARAL, L., COSTA E SILVA, M. F., NASCENTES, G. A. N., RODRIGUES JUNIOR, V., MARTINS-FILHO, O. A., ARAÚJO, M. S. S. & BITTAR, J. F. F. 2018. Parasitological and immunological evaluation of cattle experimentally infected with Trypanosoma vivax. *Experimental Parasitology,* 185**,** 98-106.

BASTOS, T. S. A., FARIA, A. M., DE ASSIS CAVALCANTE, A. S., DE CARVALHO MADRID, D. M., ZAPA, D. M. B., NICARETTA, J. E., CRUVINEL, L. B., HELLER, L. M., COUTO, L. F. M., SOARES, V. E., CADIOLI, F. A. & LOPES, W. D. Z. 2020. Comparison of therapeutic

efficacy of different drugs against Trypanosoma vivax on experimentally infected cattle. *Preventive Veterinary Medicine,* 181**,** 105040.

BASTOS, T. S. A., FARIA, A. M., MADRID, D. M. D. C., BESSA, L. C. D., LINHARES, G. F. C., FIDELIS, O. L., SAMPAIO, P. H., CRUZ, B. C., CRUVINEL, L. B. & NICARETTA, J. E. 2017. Primeiro surto e casos subsequentes de Trypanosoma vivax no Estado de Goiás, Brasil. *Revista Brasileira de Parasitologia Veterinária,* 26**,** 366-371.

BATISTA, J. S., RIET-CORREA, F., TEIXEIRA, M. M. G., MADRUGA, C. R., SIMÕES, S. D. V. & MAIA, T. F. 2007. Trypanosomiasis by Trypanosoma vivax in cattle in the Brazilian semiarid: Description of an outbreak and lesions in the nervous system. *Veterinary Parasitology,* 143**,** 174-181.

BAUER, F. 1955. Trypanosomen-und Babesienerkrankungen in Afrika und ihre Behandlung mit dem neuen Präparat Berenil. *Z. Tropenmed. Parasitol,* 6**,** 129-140.

BAUER, F. 1958. The Mode of Action of Berenil (4, 4'-Diamidino-Diazoaminobenzol) on Trypanosoma congolense. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abt. I (Originale),* 172**,** 605-20.

BENGTSSON, B. & GREKO, C. 2014. Antibiotic resistance—consequences for animal health, welfare, and food production. *Upsala Journal of Medical Sciences,* 119**,** 96-102.

BIOSCIENCE, M. Nucleic Acid
Isolation Guide.

BOURN, D., REID, R., ROGERS, D., SNOW, B. & WINT, W. 2001. Environmental change and the autonomous control of tsetse and trypanosomosis in sub-Saharan Africa: case histories from Ethiopia, The Gambia, Kenya, Nigeria and Zimbabwe. *Environmental change and the autonomous control of tsetse and trypanosomosis in sub-Saharan Africa: case histories from Ethiopia, The Gambia, Kenya, Nigeria and Zimbabwe.*

BROWNING, C., MORGAN, G., ROBB, J. & WALLS, L. 1938. The Trypanocldal Action of Certain Phenanthridinium Compounds. *Journal of pathology and bacteriology,* 46.

BRUN, R., HECKER, H. & LUN, Z.-R. 1998. Trypanosoma evansi and T. equiperdum: distribution, biology, treatment and phylogenetic relationship (a review). *Veterinary parasitology,* 79**,** 95-107.

CHEMA, S. & GATHUMA, J. 2004. Kenya: the development of private services and the role of the Kenya Veterinary Association. *Revue scientifique et technique-Office international des épizooties,* 23**,** 331-340.

CHITANGA, S., MARCOTTY, T., NAMANGALA, B., VAN DEN BOSSCHE, P., VAN DEN ABBEELE, J. & DELESPAUX, V. 2011. High prevalence of drug resistance in animal trypanosomes without a history of drug exposure. *PLoS neglected tropical diseases,* 5**,** e1454.

CONNOR, R. 1992. The diagnosis, treatment and prevention of animal trypanosomiasis under field conditions. *Programme for the control of African animal trypanosomiasis and related development: ecological and technical aspects***,** 1-38.

DADGOSTAR, P. 2019. Antimicrobial Resistance: Implications and Costs. *Infection and Drug Resistance,* 12**,** 3903-3910.

DARAMOLA, J., ADELOYE, A., FATOBA, T. & SOLADOYE, A. 2005. Haematological and biochemical parameters of West African Dwarf goats. *Livestock research for rural development,* 17**,** 95.

DARGIE, J. D., MURRAY, P. K., MURRAY, M. & MCINTYRE, W. I. 1979. The blood volumes and erythrokinetics of Ndama and Zebu cattle experimentally infected with Trypanosoma brucei. *Research in veterinary science,* 26**,** 245-7.

DESQUESNES, M., MCLAUGHLIN, G., ZOUNGRANA, A. & DÁVILA, A. M. 2001. Detection and identification of Trypanosoma of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. *International journal for parasitology,* 31**,** 610-614.

DIARRA, B., DIARRA, M., DIALL, O., BASS, B., SANOGO, Y., COULIBALY, E., SYLLA, M., ZHAO, W., PAONE, M. & CECCHI, G. 2019. A national atlas of tsetse and African animal trypanosomosis in Mali. *Parasites & Vectors,* 12**,** 466.

DOLAN, R., OKECH, G., ALUSHULA, H., MUTUGI, M., STEVENSON, P., SAYER, P. & NJOGU, A. 1990. Homidium bromide as a chemoprophylactic for cattle trypanosomiasis in Kenya. *Acta Tropica,* 47**,** 137-144.

EISLER, M. C. 1996. Pharmacokinetics of the chemoprophylactic and chemotherapeutic trypanocidal drug isometamidium chloride (Samorin) in cattle. *Drug metabolism and disposition,* 24**,** 1355-1361.

EISLER, M. C., BRANDT, J., BAUER, B., CLAUSEN, 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., SIDIBÉ, I., SINYANGWE, L. & GEERTS, S. 2001. Standardised tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle. *Veterinary Parasitology,* 97**,** 171-183.

ETIM, N. N., WILLIAMS, M. E., AKPABIO, U. & OFFIONG, E. E. 2014. Haematological parameters and factors affecting their values. *Agricultural science,* 2**,** 37-47.

EVUM, U. P. C. V. V. 2015. Insight into trypanosomiasis in animals: various approaches for its diagnosis, treatment and control: a review. *Asian Journal of Animal Sciences,* 9**,** 172-186.

FAIRCLOUGH, R. 1963. Observations on the use of Berenil against trypanosomiasis of cattle in Kenya. *Vet. rec,* 75**,** 07.

FAO. 2023. *Programme against African trypanosomosis (PAAT)* [Online]. Available: https://www.fao.org/paat/the-programme/the-disease/en/ [Accessed 20 March 2023].

FORD J, K. K. 1977. Maps of tsetse fly (Glossina) distribution in Africa. *Bulletin of Animal Health and Production in Africa,* 15**,** 187-193.

FOUNOU, R. C., FOUNOU, L. L. & ESSACK, S. Y. 2017. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. *PloS one,* 12**,** e0189621.

FUSSGÄNGER, R. 1995. Berenil in veterinary medicine: report from the chemotherapeutical Institute of Faberwerke Hoechst. *AG, Germany*.

GAMBA, D., OLET, P., MAICHOMO, M., KORIR, S. & KITETO, I. 2021. Role of Kenya Tsetse and Trypanosomiasis Eradication Council (KENTTEC) in Control of African Animal Trypanosomiasis (AAT)/Nagana.

GEBRE, T., KAPITANO, B., BEYENE, D., ALEMU, D., BESHIR, A., WORKU, Z., KIFLE, T., SELAMU, A., DEBAS, E., KALSA, A., ASFAW, N., ZHAO, W., PAONE, M. & CECCHI, G. 2022. The national atlas of tsetse flies and African animal trypanosomosis in Ethiopia. *Parasites & Vectors,* 15**,** 491.

GEERTS, S. & HOLMES, P. H. 1998. Drug management and parasite resistance in bovine trypanosomiasis in Africa. *Drug management and parasite resistance in bovine trypanosomiasis in Africa.*

GETAHUN, M. N., VILLINGER, J., BARGUL, J. L., MUEMA, J. M., ORONE, A., NGIELA, J., AHUYA, P. O., SAINI, R. K., TORTO, B. & MASIGA, D. K. 2022. Molecular characterization of pathogenic African trypanosomes in biting flies and camels in surra-endemic areas outside the tsetse fly belt in Kenya. *International Journal of Tropical Insect Science,* 42**,** 3729-3745.

GIORDANI, F., MORRISON, L. J., ROWAN, T. G., DE KONING, H. P. & BARRETT, M. P. 2016. The animal trypanosomiases and their chemotherapy: a review. *Parasitology,* 143**,** 1862-1889.

GITATHA, S. 1979. T. congolense (Shimba Hills) resistant to various trypanocidal drugs. *Proc. 16th Meet. int. sci. Counc. for Trypanosomiasis Research and Control, Yaoundé, Cameroon*, 257-263.

GRAY, A. & ROBERTS, C. 1971. The cyclical transmission of strains of Trypanosoma congolense and T. vivax resistant to normal therapeutic doses of trypanocidal drugs. *Parasitology,* 63, 67-89.

GROOTENHUIS, J. G., DWINGER, R. H., DOLAN, R. B., MOLOO, S. K. & MURRAY, M. 1990. Susceptibility of African buffalo and Boran cattle to Trypanosoma congolense transmitted by Glossina morsitans centralis. *Veterinary parasitology,* 35, 219-31.

GUTIERREZ, C., CORBERA, J., JUSTE, M., DORESTE, F. & MORALES, I. 2005. An outbreak of abortions and high neonatal mortality associated with Trypanosoma evansi infection in dromedary camels in the Canary Islands. *Veterinary Parasitology,* 130, 163-168.

HOARE, C. A. 1965. Vampire Bats as Vectors and Hosts of Equine and Bovine Trypanosomes. *Acta Tropica,* 22, 204-16.

ILRAD, H. A. 1994. TOWARDS INCREASED USE OF TRYPANOTOLERANCE: CURRENT RESEARCH AND FUTURE DIRECTIONS.

IRUNGU, P., BETT, B., MBOGOH, S., NYAMWARO, S., MURILLA, G. & RANDOLPH, T. 2007. Evidence of improper usage of veterinary drugs in cattle in Maasailand, Kenya. *Bull Anim Health Prod Afr,* 55, 210-225.

IVANOVA, N. V., ZEMLAK, T. S., HANNER, R. H. & HEBERT, P. D. 2007. Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes,* 7, 544-548.

KASOZI, K. I., ZIRINTUNDA, G., SSEMPIJJA, F., BUYINZA, B., ALZAHRANI, K. J., MATAMA, K., NAKIMBUGWE, H. N., ALKAZMI, L., ONANYANG, D. & BOGERE, P. 2021. Epidemiology of trypanosomiasis in wildlife—implications for humans at the wildlife interface in Africa. *Frontiers in Veterinary Science,* 8, 621699.

KELLNER, H., ECKERT, H. & VOLZ, M. 1985a. Studies in cattle on the disposition of the anti-trypanosomal drug diminazene diaceturate (Berenil). *Tropical Medicine and Parasitology: Official Organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ),* 36, 199-204.

KELLNER, H. M., ECKERT, H. G. & VOLZ, M. H. 1985b. Studies in cattle on the disposition of the anti-trypanosomal drug diminazene diaceturate (Berenil). *Tropical medicine and parasitology : official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ),* 36, 199-204.

KETRI 1996. Tsetse Distribution in Kenya Showing Tsetse Belts and Conservation Areas.

KINABO, L. & BOGAN, T. L. J. 1988. The pharmacology of isometamidium. *Journal of Veterinary Pharmacology and Therapeutics,* 11**,** 233-245.

KLATT, P. & HAJDU, P. 1976. Pharmacokinetic investigations on diminazene and rolitetracycline in comparison to a combination of both. *The Veterinary Record,* 99**,** 372-374.

KRAFSUR, E. S. 2009. Tsetse flies: Genetics, evolution, and role as vectors. *Infection, Genetics and Evolution,* 9**,** 124-141.

LEACH, T. M. & ROBERTS, C. J. 1981. Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the eastern hemisphere. *Pharmacology & therapeutics,* 13**,** 91-147.

MACHILA, N., EMONGOR, R., SHAW, A. P., WELBURN, S. C., MCDERMOTT, J., MAUDLIN, I. & EISLER, M. C. 2007. A community education intervention to improve bovine trypanosomiasis knowledge and appropriate use of trypanocidal drugs on smallholder farms in Kenya. *Agricultural Systems,* 94**,** 261-272.

MACHILA, N., WANYANGU, S. W., MCDERMOTT, J., WELBURN, S. C., MAUDLIN, I. & EISLER, M. C. 2003. Cattle owners' perceptions of African bovine trypanosomiasis and its control in Busia and Kwale Districts of Kenya. *Acta tropica,* 86**,** 25-34.

MAKAU, D. N., SLIZOVSKIY, I., OBANDA, V., NOYES, N. R., JOHNSON, J. R., OAKES, M., TRAVIS, D., VANDERWAAL, K. & OMONDI, G. P. 2022. Factors influencing usage of antimicrobial drugs among pastoralists in Kenya. *Tropical Animal Health and Production,* 54.

MAPENAY, I. & MAICHAMO, M. 2006. Epidemiology of Trypanocidal Drug Resistance in the Transmara District of Kenya. *Kenya Veterinarian,* 30**,** 57-61.

MASIGA, D. K., SMYTH, A. J., HAYES, P., BROMIDGE, T. J. & GIBSON, W. C. 1992. Sensitive detection of trypanosomes in tsetse flies by DNA amplification. *International journal for parasitology,* 22**,** 909-918.

MATOVU, E., STEWART, M. L., GEISER, F., BRUN, R., MÄSER, P., WALLACE, L. J., BURCHMORE, R. J., ENYARU, J. C., BARRETT, M. P. & KAMINSKY, R. 2003. Mechanisms of arsenical and diamidine uptake and resistance in Trypanosoma brucei. *Eukaryotic cell,* 2**,** 1003-1008.

MCCORD, P. F., MESSINA, J. P., CAMPBELL, D. J. & GRADY, S. C. 2012. Tsetse Fly Control in Kenya's Spatially and Temporally Dynamic Control Reservoirs: A Cost Analysis. *Appl Geogr,* 34**,** 189-204.

MCDERMOTT, J., WOITAG, T., SIDIBÉ, I., BAUER, B., DIARRA, B., OUÉDRAOGO, D., KAMUANGA, M., PEREGRINE, A., EISLER, M. & ZESSIN, K.-H. 2003. Field studies of drug-resistant cattle trypanosomes in Kenedougou Province, Burkina Faso. *Acta Tropica,* 86**,** 93-103.

MILLER, D. B. 2006. *The Pharmacokinetics of Diminazene aceturate after intramuscular and intravenous administration in the healthy dog.* University of Pretoria.

MOLOO, S. K., LOSOS, G. J. & KUTUZA, S. B. 1973. Transmission of Trypanosoma brucei to cats and dogs by feeding on infected goats. *Annals of Tropical Medicine and Parasitology,* 67**,** 331-4.

MOSSAAD, E., ISMAIL, A. A., IBRAHIM, A. M., MUSINGUZI, P., ANGARA, T. E., XUAN, X., INOUE, N. & SUGANUMA, K. 2020. Prevalence of different trypanosomes in livestock in Blue Nile and West Kordofan States, Sudan. *Acta tropica,* 203**,** 105302.

MULANDANE, F. C., FAFETINE, J., VAN DEN ABBEELE, J., CLAUSEN, P.-H., HOPPENHEIT, A., CECCHI, G., OOSTHUIZEN, M., DELESPAUX, V. & NEVES, L. 2018. Resistance to trypanocidal drugs in cattle populations of Zambezia Province, Mozambique. *Parasitology research,* 117**,** 429-436.

MUNANG'ANDU, H. M., SIAMUDAALA, V., MUNYEME, M. & NALUBAMBA, K. S. 2012. A Review of Ecological Factors Associated with the Epidemiology of Wildlife Trypanosomiasis in the Luangwa and Zambezi Valley Ecosystems of Zambia. *Interdisciplinary Perspectives on Infectious Diseases,* 2012**,** 372523.

MUNDAY, J. C., LÓPEZ, K. E. R., EZE, A. A., DELESPAUX, V., VAN DEN ABBEELE, J., ROWAN, T., BARRETT, M. P., MORRISON, L. J. & DE KONING, H. P. 2013. Functional expression of TcoAT1 reveals it to be a P1-type nucleoside transporter with no capacity for diminazene uptake. *International Journal for Parasitology: Drugs and Drug Resistance,* 3**,** 69-76.

MURILLA, G. A., PEREGRINE, A. S., NDUNG'U, J. M., HOLMES, P. H. & EISLER, M. C. 2002. The effects of drug-sensitive and drug-resistant Trypanosoma congolense infections on the pharmacokinetics of homidium in Boran cattle. *Acta tropica,* 81**,** 185-195.

MURIUKI, G. W., NJOKA, T., REID, R. S. & NYARIKI, D. 2005. Tsetse control and land-use change in Lambwe valley, south-western Kenya. *Agriculture, ecosystems & environment,* 106**,** 99-107.

MWAMBU, P. 1971. The effect of a block-treatment regimen, using ethidium, on cattle trypanosomiasis in an endemic area. *East African Agricultural and Forestry Journal,* 36**,** 414-418.

NANTULYA, V. 1990. Trypanosomiasis in domestic'altimals: the problems of diagnosis. *Revue Scientifique et Technique–Office International des Epizooties***,** 357-367.

NGARI, N. N., GAMBA, D. O., OLET, P. A., ZHAO, W., PAONE, M. & CECCHI, G. 2020. Developing a national atlas to support the progressive control of tsetse-transmitted animal trypanosomosis in Kenya. *Parasites & vectors,* 13**,** 1-12.

NJIRU, Z., CONSTANTINE, C., GUYA, S., CROWTHER, J., KIRAGU, J., THOMPSON, R. & DÁVILA, A. 2005. The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. *Parasitology research,* 95**,** 186-192.

OKELLO, I., MAFIE, E., EASTWOOD, G., NZALAWAHE, J., MBOERA, L. E. & ONYOYO, S. 2022. Prevalence and associated risk factors of african animal trypanosomiasis in cattle in lambwe, kenya. *Journal of Parasitology Research,* 2022.

OKOTH, W. O., MICHAEL, O. G., KENNEDY, O., THEDEUS, O. O., AWINO, B., OYIEKO, W., KHAYEKA-WANDABWA, C. & WILSON, O. 2019. Human and animal trypanosomiasis in Lambwe Valley Foci, Kenya–current situation and latent trypanotolerance.

OKWIRI, F. 2006. Privatization of veterinary services in Kenya.

ONDITI, K. O., LI, X., SONG, W., LI, Q., MUSILA, S., MATHENGE, J., KIOKO, E. & JIANG, X. 2021. The management effectiveness of protected areas in Kenya. *Biodiversity and Conservation,* 30**,** 3813-3836.

ONYANGO, S. O. 2020. *Impact of Choice and Integration of Tsetse Fly and Trypanosomiasis Control Methods on Household Income in Lamu County, Kenya.* University of Nairobi.

OPIYO, E., NJOGU, A. & OMUSE, J. 1990. Use of impregnated targets for control of Glossina pallidipes in Kenya. *International Journal of Tropical Insect Science,* 11**,** 417-425.

ORGANISATION MONDIALE DE LA SANTÉ, W. H. O. 2021. Elimination of Human African Trypanosomiasis as public health problem–Élimination de la trypanosomiase humaine africaine comme problème de santé publique. *Wkly. epidemiol. rec***,** 196-196.

ORGANIZATION, W. H. 2015. Global action plan on antimicrobial resistance.

OZTURK, Y., CELIK, S., SAHIN, E., ACIK, M. N. & CETINKAYA, B. 2019. Assessment of Farmers' Knowledge, Attitudes and Practices on Antibiotics and Antimicrobial Resistance. *Animals,* 9**,** 653.

PATTEC 2000. Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC). A continental plan of action for the eradication of tsetse and trypanosomosis.

PAYS, E., RADWANSKA, M. & MAGEZ, S. 2023. The pathogenesis of african trypanosomiasis. *Annual Review of Pathology: Mechanisms of Disease,* 18**,** 19-45.

PERCOMA, L., RAYAISSÉ, J. B., GIMONNEAU, G., BENGALY, Z., POODA, S. H., PAGABELEGUEM, S., GANABA, R., SOW, A., ARGILÉS, R., BOUYER, J., OUEDRAOGO, M., ZHAO, W., PAONE, M., SIDIBÉ, I., GISELE, O. S. & CECCHI, G. 2022. An atlas to support the progressive control of tsetse-transmitted animal trypanosomosis in Burkina Faso. *Parasites & Vectors,* 15**,** 72.

PEREGRINE, A. & MAMMAN, M. 1993. Pharmacology of diminazene: a review. *Acta tropica,* 54**,** 185-203.

PEREGRINE, A. S. 1994. Chemotherapy and delivery systems: haemoparasites. *Veterinary Parasitology,* 54**,** 223-248.

PINDER, M. & AUTHIE, E. 1984. The appearance of isometamidium resistant Trypanosoma congolense in West Africa. *Acta tropica,* 41**,** 247-252.

PORTUGAL, J. 1994. Berenil acts as a poison of eukaryotic topoisomerase II. *FEBS letters,* 344**,** 136-138.

REID, S. A. 2002. Trypanosoma evansi control and containment in Australasia. *Trends in parasitology,* 18**,** 219-224.

RUFA'I, F. A., ZAKARI, A. I., UMAR, A., SHUAIBU, M. & SANI, A. A. 2021. Clinical signs and pathogenesis of trypanosomal infection in human and animals. *Asian Journal of Pharmaceutical Research and Development,* 9**,** 57-61.

SOLOMON, A. & WORKINEH, S. 2018. Drug resistance in African animal trypanosomes: A review. *African Journal of Microbiology Research,* 12**,** 380-386.

STAATS, H. F., ALAM, S. M., SCEARCE, R. M., KIRWAN, S. M., ZHANG, J. X., GWINN, W. M. & HAYNES, B. F. 2007. In vitro and in vivo characterization of anthrax anti-protective antigen and anti-lethal factor monoclonal antibodies after passive transfer in a mouse lethal toxin challenge model to define correlates of immunity. *Infection and immunity,* 75**,** 5443-5452.

SUTCLIFFE, O., SKELLERN, G., ARAYA, F., CANNAVAN, A., SASANYA, J., DUNGU, B., VAN GOOL, F., MUNSTERMANN, S. & MATTIOLI, R. 2014. Animal trypanosomosis: making quality control of trypanocidal drugs possible. *Rev Sci Tech,* 33**,** 813-830.

SUTHERLAND, I. & HOLMES, P. 1993. Alterations in drug transport in resistant Trypanosoma congolense. *Acta tropica,* 54**,** 271-278.

TORO, M., LEON, E., LOPEZ, R., PALLOTA, F., GARCIA, J. & RUIZ, A. 1983. Effect of isometamidium on infections by Trypanosoma vivax and T. evansi in experimentally-infected animals. *Veterinary Parasitology,* 13**,** 35-43.

UILENBERG, G. 1998. *A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis.*

VAN DEN BOSSCHE, P. & DELESPAUX, V. 2011. Options for the control of tsetse-transmitted livestock trypanosomosis. An epidemiological perspective. *Veterinary Parasitology,* 181**,** 37-42.

VAN HOEVE, K. & CUNNINGHAM, M. 1964. Prophylactic activity of Berenil against trypanosomes in treated cattle. *Vet. Rec,* 76**,** 260.

VENTURELLI, A., TAGLIAZUCCHI, L., LIMA, C., VENUTI, F., MALPEZZI, G., MAGOULAS, G. E., SANTAREM, N., CALOGEROPOULOU, T., CORDEIRO-DA-SILVA, A. & COSTI, M. P. 2022. Current treatments to control african trypanosomiasis and one health perspective. *Microorganisms,* 10**,** 1298.

WAINWRIGHT, M. 2010. Dyes, trypanosomiasis and DNA: a historical and critical review. *Biotechnic & Histochemistry,* 85**,** 341-354.

WHITESIDE, E. 1962. Interactions between drugs, trypanosomes and cattle in the field. *Interactions between drugs, trypanosomes and cattle in the field.*

WHO 2012. Anthrax vaccines to humans. Information sheet: Observed rate of vaccine reactions. World Health Organization, Geneva. https://www.who.int/vaccine_safety/initiative/tools/Anthrax_Vaccine_rates_information_sheet.pdf?ua=1.

WIEN, R. 1943. The pharmacological actions of certain aromatic diamidines possessing trypanocidal activity. *Annals of Tropical Medicine & Parasitology,* 37**,** 1-18.

WILKES, J. M., MULUGETA, W., WELLS, C. & PEREGRINE, A. S. 1997. Modulation of mitochondrial electrical potential: a candidate mechanism for drug resistance in African trypanosomes. *Biochemical Journal,* 326**,** 755-761.

WITOLA, W. H., INOUE, N., OHASHI, K. & ONUMA, M. 2004. RNA-interference silencing of the adenosine transporter-1 gene in Trypanosoma evansi confers resistance to diminazene aceturate. *Experimental Parasitology,* 107**,** 47-57.

Appendix 1: Summary of samples collected for this study from Gendo, Kigoto and Wiga villages (in Homabay, Gwasi East, Kenya).

Gendo Village (samples collected on 06/07/2023)

Cattle number	Breed	Sex	Age (years)	Ear tag number / Cattle ID	PCV (%)	BCT results		
						T. congolense	*T. vivax*	*T. brucei*
1	Zebu	Female	2		18	Negative	Negative	Negative
2	Zebu	Male	2	4117	14	Negative	**Positive**	Negative
3	Zebu	Female	4		16	Negative	Negative	Negative
4	Zebu	Female	8		14	Negative	Negative	Negative
5	Zebu	Female	1.5	4118	24	**Positive**	Negative	Negative
6	Zebu	Female	8		12.5	Negative	Negative	Negative
7	Zebu	Female	10		20	Negative	Negative	Negative
8	Zebu	Female	8 months		35	Negative	Negative	Negative
9	Zebu	Female	10		30	Negative	Negative	Negative
10	Zebu	Female	4		29	Negative	Negative	Negative
11	Zebu	Male	5		21	Negative	Negative	Negative
12	Zebu	Female	3		25	Negative	Negative	Negative
13	Zebu	Female	1		30	Negative	Negative	Negative
14	Zebu	Male	2		22	Negative	Negative	Negative
15	Zebu	Female	2		24	Negative	Negative	Negative
16	Zebu	Male	4		29	Negative	Negative	Negative
17	Zebu	Male	5		25	Negative	Negative	Negative
18	Zebu	Female	5		24	Negative	Negative	Negative
19	Zebu	Female	3		29	Negative	Negative	Negative
20	Zebu	Female	5		29	Negative	Negative	Negative
21	Zebu	Male	3		21	Negative	Negative	Negative
22	Zebu	Male	3		30	Negative	Negative	Negative
23	Zebu	Female	3		28	Negative	Negative	Negative
24	Zebu	Female	0.5		26	Negative	Negative	Negative
25	Zebu	Male	7 months		27	Negative	Negative	Negative
26	Zebu	Male	2		14	Negative	Negative	Negative
27	Zebu	Male	2.5		25	Negative	Negative	Negative
28	Zebu	Male	2.5		25	Negative	Negative	Negative
28	Zebu	Female	2		23	Negative	Negative	Negative
30	Zebu	Male	3		20	Negative	Negative	Negative
31	Zebu	Female	5		20	Negative	Negative	Negative
32	Zebu	Male	3		17	Negative	Negative	Negative
33	Zebu	Male	2		24	Negative	Negative	Negative
34	Zebu	Female	4 Months		24	Negative	Negative	Negative

35	Zebu	Male	1.5		21	Negative	Negative	Negative
36	Zebu	Female	3		22.5	Negative	Negative	Negative
37	Zebu	Male	4		24	Negative	Negative	Negative
38	Zebu	Female	1		26	Negative	Negative	Negative
39	Zebu	Male	2		34	Negative	Negative	Negative
40	Zebu	Female	8		22	Negative	Negative	Negative
41	Zebu	Female	4		30	Negative	Negative	Negative
42	Zebu	Female	7		15	Negative	Negative	Negative
43	Zebu	Male	4		25	Negative	Negative	Negative
44	Zebu	Female	6		30	Negative	Negative	Negative
45	Zebu	Male	4		17	Negative	Negative	Negative
46	Zebu	Female	10		35	Negative	Negative	Negative
47	Zebu	Male	1		26	Negative	Negative	Negative
48	Zebu	Female	3 months		26	Negative	Negative	Negative
49	Zebu	Female	5 months		15	Negative	Negative	Negative
50	Zebu	Male	3 months		26	Negative	Negative	Negative
51	Zebu	Female	2 months		30	Negative	Negative	Negative
52	Zebu	Male	5		22	Negative	Negative	Negative
53	Zebu	Male	3 months		24	Negative	Negative	Negative
54	Zebu	Male	5 months		10	Negative	Negative	Negative

Kigoto Village (samples collected on 07/07/2023)

Cattle number	Breed	Sex	Age (years)	Ear tag number / Cattle ID	PCV (%)	BCT results		
						T. congolense	***T. vivax***	***T. brucei***
1	Zebu	Female	3		20	Negative	Negative	Negative
2	Zebu	Female	3	4116	19	**Positive**	Negative	Negative
3	Zebu	Female	3		28	Negative	Negative	Negative
4	Zebu	Female	3		32	Negative	Negative	Negative
5	Zebu	Male	8		27	Negative	Negative	Negative
6	Zebu	Male	4		26	Negative	Negative	Negative
7	Zebu	Male	3		26	Negative	Negative	Negative
8	Zebu	Male	3		19	Negative	Negative	Negative
9	Zebu	Female	2		27	Negative	Negative	Negative
10	Zebu	Male	5		26	Negative	Negative	Negative
11	Zebu	Female	6		26	Negative	Negative	Negative
12	Zebu	Male	4		29	Negative	Negative	Negative
13	Zebu	Male	3		34	Negative	Negative	Negative
14	Zebu	Male	2		26	Negative	Negative	Negative
15	Zebu	Female	3		29	Negative	Negative	Negative
16	Zebu	Male	5		24	Negative	Negative	Negative
17	Zebu	Male	3		25	Negative	Negative	Negative

18	Zebu	Female	4		27	Negative	Negative	Negative
19	Zebu	Female	3		25	Negative	Negative	Negative
20	Zebu	Male	1		29	Negative	Negative	Negative
21	Zebu	Female	4		28	Negative	Negative	Negative
22	Zebu	Male	2		44	Negative	Negative	Negative
23	Zebu	Female	4		29	Negative	Negative	Negative
24	Zebu	Female	4		22	Negative	Negative	Negative
25	Zebu	Female	3		28	Negative	Negative	Negative
26	Zebu	Female	4	4106	15	**Positive**	Negative	Negative
27	Zebu	Female	5		28	Negative	Negative	Negative
28	Zebu	Female	4		24	Negative	Negative	Negative
29	Zebu	Male	2		25	Negative	Negative	Negative
30	Zebu	Female	3		29	Negative	Negative	Negative
31	Zebu	Female	1.5		19	Negative	Negative	Negative
32	Zebu	Male	2		22	Negative	Negative	Negative
33	Zebu	Female	2		28	Negative	Negative	Negative
34	Zebu	Male	3		17	Negative	Negative	Negative
35	Zebu	Female	5		30	Negative	Negative	Negative
36	Zebu	Female	5		26	Negative	Negative	Negative
37	Zebu	Female	1		29	Negative	Negative	Negative
38	Zebu	Male	1		19	Negative	Negative	Negative
39	Zebu	Female	1.5		13	Negative	Negative	Negative
40	Zebu	Female	2		23	Negative	Negative	Negative
41	Zebu	Female	3		19	Negative	Negative	Negative
42	Zebu	Female	2		28	Negative	Negative	Negative
43	Zebu	Female	2		29	Negative	Negative	Negative
44	Zebu	Female	2		18	Negative	Negative	Negative
45	Zebu	Female	1		24	Negative	Negative	Negative
46	Zebu	Male	6		33	Negative	Negative	Negative
47	Zebu	Female	4		28	Negative	Negative	Negative
48	Zebu	Male	3		24	Negative	Negative	Negative
49	Zebu	Female	6		20	Negative	Negative	Negative
50	Zebu	Female	4		27	Negative	Negative	Negative
51	Zebu	Female	3		29	Negative	Negative	Negative
52	Zebu	Male	3		17	Negative	Negative	Negative
53	Zebu	Female	6		27	Negative	Negative	Negative
54	Zebu	Male	2		19	Negative	Negative	Negative
55	Zebu	Male	1.5		31	Negative	Negative	Negative
56	Zebu	Female	4		31	Negative	Negative	Negative
57	Zebu	Male	2		36	Negative	Negative	Negative
58	Zebu	Male	4		19	Negative	Negative	Negative
59	Zebu	Male	6		34	Negative	Negative	Negative

60	Zebu	Female	3		27	Negative	Negative	Negative
61	Zebu	Female	2		27	Negative	Negative	Negative
62	Zebu	Female	5		22	Negative	Negative	Negative
63	Zebu	Female	4		26	Negative	Negative	Negative
64	Zebu	Female	3		20	Negative	Negative	Negative
65	Zebu	Female	6		30	Negative	Negative	Negative
66	Zebu	Male	4		24	Negative	Negative	Negative
67	Zebu	Male	2		24	Negative	Negative	Negative
68	Zebu	Female	5		22	Negative	Negative	Negative
69	Zebu	Male	3		20	Negative	Negative	Negative
70	Zebu	Female	5		22	Negative	Negative	Negative
71	Zebu	Male	3		19	Negative	Negative	Negative
72	Zebu	Female	1		18	Negative	Negative	Negative
73	Zebu	Female	6		30	Negative	Negative	Negative
74	Zebu	Male	2		25	Negative	Negative	Negative
75	Zebu	Female	1.5		26	Negative	Negative	Negative
76	Zebu	Female	2		15	Negative	Negative	Negative
77	Zebu	Male	5		31	Negative	Negative	Negative
78	Zebu	Male	5		23	Negative	Negative	Negative
79	Zebu	Female	6		29	Negative	Negative	Negative
80	Zebu	Female	5		32	Negative	Negative	Negative
81	Zebu	Male	3		25	Negative	Negative	Negative
82	Zebu	Female	1		29	Negative	Negative	Negative
83	Zebu	Female	2		24	Negative	Negative	Negative
84	Zebu	Female	2		26	Negative	Negative	Negative
85	Zebu	Female	3		31	Negative	Negative	Negative
86	Zebu	Male	4		25	Negative	Negative	Negative
87	Zebu	Male	5		29	Negative	Negative	Negative
88	Zebu	Female	3		17	Negative	Negative	Negative
89	Zebu	Male	4	4107	23	Negative	**Positive**	Negative
90	Zebu	Male	3		13	Negative	Negative	Negative
91	Zebu	Male	4		19	Negative	Negative	Negative
92	Zebu	Male	5		16	Negative	Negative	Negative
93	Zebu	Female	5		26	Negative	Negative	Negative
94	Zebu	Female	5		29	Negative	Negative	Negative
95	Zebu	Male	6		24	Negative	Negative	Negative
96	Zebu	Female	6		20	Negative	Negative	Negative
97	Zebu	Female	3		21	Negative	Negative	Negative
98	Zebu	Female	2	4108	17	**Positive**	Negative	Negative
99	Zebu	Male	4		24	Negative	Negative	Negative
100	Zebu	Female	3		24	Negative	Negative	Negative
101	Zebu	Male	4		21	Negative	Negative	Negative

102	Zebu	Female	3		23	Negative	Negative	Negative
103	Zebu	Male	5		21	Negative	Negative	Negative
104	Zebu	Male	4		24	Negative	Negative	Negative
105	Zebu	Female	6	4109	20	Negative	**Positive**	Negative
106	Zebu	Female	2		17	Negative	Negative	Negative
107	Zebu	Male	6		33	Negative	Negative	Negative
108	Zebu	Female	4		19	Negative	Negative	Negative
109	Zebu	Female	5		25	Negative	Negative	Negative
110	Zebu	Female	6		24	Negative	Negative	Negative
111	Zebu	Male	2	4110	22	Negative	**Positive**	Negative
112	Zebu	Female	7		31	Negative	Negative	Negative
113	Zebu	Male	9		18	Negative	Negative	Negative
114	Zebu	Male	5		22	Negative	Negative	Negative
115	Zebu	Male	3		25	Negative	Negative	Negative
116	Zebu	Female	10		23	Negative	Negative	Negative
117	Zebu	Male	6		26	Negative	Negative	Negative
118	Zebu	Female	7		28	Negative	Negative	Negative
119	Zebu	Male	5		29	Negative	Negative	Negative
120	Zebu	Male	6		24	Negative	Negative	Negative
121	Zebu	Female	8		25	Negative	Negative	Negative
122	Zebu	Male	8		17	Negative	Negative	Negative
123	Zebu	Male	2		21	Negative	Negative	Negative
124	Zebu	Male	3		23	Negative	Negative	Negative
125	Zebu	Male	5		27	Negative	Negative	Negative
126	Zebu	Male	4		19	Negative	Negative	Negative
127	Zebu	Female	2		24	Negative	Negative	Negative
128	Zebu	Male	3		19	Negative	Negative	Negative
129	Zebu	Female	4		24	Negative	Negative	Negative
130	Zebu	Female	3		23	Negative	Negative	Negative
131	Zebu	Female	3		21	Negative	Negative	Negative
132	Zebu	Female	4		21	Negative	Negative	Negative
133	Zebu	Female	5		31	Negative	Negative	Negative
134	Zebu	Male	3		26	Negative	Negative	Negative
135	Zebu	Male	6		19	Negative	Negative	Negative
136	Zebu	Female	2	4111	28	Negative	**Positive**	Negative
137	Zebu	Male	2		22	Negative	Negative	Negative
138	Zebu	Male	1.5	4112	22	Negative	**Positive**	Negative
139	Zebu	Female	2		18	Negative	Negative	Negative
140	Zebu	Male	1.5	4113	18	Negative	**Positive**	Negative
141	Zebu	Male	2		21	Negative	Negative	Negative
142	Zebu	Female	2		19	Negative	Negative	Negative
143	Zebu	Male	5		33	Negative	Negative	Negative

144	Zebu	Male	3		28	Negative	Negative	Negative
145	Zebu	Female	4		28	Negative	Negative	Negative
146	Zebu	Male	3		12	Negative	Negative	Negative
147	Zebu	Female	3	4114	12	Negative	**Positive**	Negative
148	Zebu	Female	2		25	Negative	Negative	Negative
149	Zebu	Male	2		29	Negative	Negative	Negative
150	Zebu	Male	2		29	Negative	Negative	Negative
151	Zebu	Female	3		29	Negative	Negative	Negative
152	Zebu	Female	5	4115	16	Negative	**Positive**	Negative
153	Zebu	Female	2.5		26	Negative	Negative	Negative
154	Zebu	Male	3		19	Negative	Negative	Negative
155	Zebu	Female	4		24	Negative	Negative	Negative
156	Zebu	Female	2		29	Negative	Negative	Negative
157	Zebu	Female	2		20	Negative	Negative	Negative
158	Zebu	Female	3		27	Negative	Negative	Negative
159	Zebu	Male	2		21	Negative	Negative	Negative
160	Zebu	Female	4		25	Negative	Negative	Negative
161	Zebu	Male	2		19	Negative	Negative	Negative
162	Zebu	Female	3		20	Negative	Negative	Negative
163	Zebu	Male	1		20	Negative	Negative	Negative
164	Zebu	Female	5 months		27	Negative	Negative	Negative
165	Zebu	Female	8		17	Negative	Negative	Negative
166	Zebu	Female	6		30	Negative	Negative	Negative
167	Zebu	Male	5		23	Negative	Negative	Negative
168	Zebu	Female	10		24	Negative	Negative	Negative
169	Zebu	Male	6		23	Negative	Negative	Negative
170	Zebu	Male	10		24	Negative	Negative	Negative
171	Zebu	Female	5		24	Negative	Negative	Negative
172	Zebu	Male	5 months		25	Negative	Negative	Negative
173	Zebu	Male	1		25	Negative	Negative	Negative
174	Zebu	Male	1		27	Negative	Negative	Negative
175	Zebu	Female	1		23	Negative	Negative	Negative
176	Zebu	Female	15		12	Negative	Negative	Negative
177	Zebu	Female	12		27	Negative	Negative	Negative
178	Zebu	Female	3		30	Negative	Negative	Negative
179	Zebu	Female	10		16	Negative	Negative	Negative
180	Zebu	Female	11		27	Negative	Negative	Negative
181	Zebu	Male	4		27	Negative	Negative	Negative
182	Zebu	Female	6		24	Negative	Negative	Negative
183	Zebu	Female	11		24	Negative	Negative	Negative
184	Zebu	Female	2		30	Negative	Negative	Negative
185	Zebu	Male	12		28	Negative	Negative	Negative

186	Zebu	Female	11	32	Negative	Negative	Negative
187	Zebu	Male	12	29	Negative	Negative	Negative
188	Zebu	Female	7.5	27	Negative	Negative	Negative
189	Zebu	Male	7.5	30	Negative	Negative	Negative
190	Zebu	Female	10	30	Negative	Negative	Negative
191	Zebu	Female	6	19	Negative	Negative	Negative
192	Zebu	Female	5	20	Negative	Negative	Negative
193	Zebu	Female	4	27	Negative	Negative	Negative
194	Zebu	Female	6	24	Negative	Negative	Negative
195	Zebu	Female	6.5	29	Negative	Negative	Negative
196	Zebu	Female	7	26	Negative	Negative	Negative
197	Zebu	Female	3	20	Negative	Negative	Negative
198	Zebu	Female	4	26	Negative	Negative	Negative
199	Zebu	Female	3	22	Negative	Negative	Negative
200	Zebu	Female	5	26	Negative	Negative	Negative
201	Zebu	Female	10	19	Negative	Negative	Negative
202	Zebu	Female	8	17	Negative	Negative	Negative
203	Zebu	Female	6	27	Negative	Negative	Negative
204	Zebu	Male	5	15	Negative	Negative	Negative
205	Zebu	Male	5	22	Negative	Negative	Negative
206	Zebu	Male	9	22	Negative	Negative	Negative
207	Zebu	Male	6	30	Negative	Negative	Negative
208	Zebu	Female	3	30	Negative	Negative	Negative
209	Zebu	Male	3	23	Negative	Negative	Negative
210	Zebu	Female	11	27	Negative	Negative	Negative
211	Zebu	Male	9	27	Negative	Negative	Negative
212	Zebu	Male	5	19	Negative	Negative	Negative
213	Zebu	Male	5	30	Negative	Negative	Negative
214	Zebu	Female	3	23	Negative	Negative	Negative
215	Zebu	Female	3	24	Negative	Negative	Negative
216	Zebu	Male	5	21	Negative	Negative	Negative
217	Zebu	Female	2	19	Negative	Negative	Negative
218	Zebu	Male	2.5	24	Negative	Negative	Negative
219	Zebu	Male	3	18	Negative	Negative	Negative
220	Zebu	Female	2	24	Negative	Negative	Negative
221	Zebu	Female	2.5	24	Negative	Negative	Negative
222	Zebu	Male	5	28	Negative	Negative	Negative
223	Zebu	Female	9	24	Negative	Negative	Negative
224	Zebu	Female	4	28	Negative	Negative	Negative
225	Zebu	Male	3.5	25	Negative	Negative	Negative
226	Zebu	Female	7	28	Negative	Negative	Negative
227	Zebu	Male	7	19	Negative	Negative	Negative

228	Zebu	Male	5		24	Negative	Negative	Negative
229	Zebu	Female	3		24	Negative	Negative	Negative
230	Zebu	Male	7		29	Negative	Negative	Negative
231	Zebu	Male	4		24	Negative	Negative	Negative
232	Zebu	Female	4		15	Negative	Negative	Negative
233	Zebu	Male	3		24	Negative	Negative	Negative
234	Zebu	Female	9		24	Negative	Negative	Negative
235	Zebu	Female	7		27	Negative	Negative	Negative
236	Zebu	Female	5	NA	20	Negative	**Positive**	Negative
237	Zebu	Female	7 months		26	Negative	Negative	Negative
238	Zebu	Female	3		28	Negative	Negative	Negative
239	Zebu	Female	1		37	Negative	Negative	Negative

Wiga Village (samples collected on 06/07/2023)

Cattle number	Breed	Sex	Age (years)	Ear tag number / Cattle ID	PCV (%)	BCT results		
						T. congolense	***T. vivax***	***T. brucei***
1	Zebu	Male	N/A		21	Negative	Negative	Negative
2	Zebu	Male	N/A		27	Negative	Negative	Negative
3	Zebu	Male	N/A		25	Negative	Negative	Negative
4	Zebu	Female	N/A		22	Negative	Negative	Negative
5	Zebu	Female	N/A		18	Negative	Negative	Negative
6	Zebu	Female	N/A		18	Negative	Negative	Negative
7	Zebu	Female	N/A		28	Negative	Negative	Negative
8	Zebu	Female	N/A		19	Negative	Negative	Negative
9	Zebu	Male	N/A		20	Negative	Negative	Negative
10	Zebu	Male	N/A		20	Negative	Negative	Negative
11	Zebu	Male	N/A		18	Negative	Negative	Negative
12	Zebu	Male	N/A		25	Negative	Negative	Negative
13	Zebu	Female	N/A		20	Negative	Negative	Negative
14	Zebu	Female	N/A		26	Negative	Negative	Negative
15	Zebu	Female	N/A		23	Negative	Negative	Negative
16	Zebu	Female	N/A		21	Negative	Negative	Negative
17	Zebu	Female	N/A		26	Negative	Negative	Negative
18	Zebu	Male	N/A		20	Negative	Negative	Negative
19	Zebu	Female	N/A		30	Negative	Negative	Negative
20	Zebu	Male	N/A		22	Negative	Negative	Negative
21	Zebu	Male	N/A		29	Negative	Negative	Negative
22	Zebu	Female	N/A		27	Negative	Negative	Negative
23	Zebu	Male	N/A	4101	23	**Positive**	**Positive**	Negative
24	Zebu	Male	N/A		19	Negative	Negative	Negative
25	Zebu	Male	N/A		20	Negative	Negative	Negative

26	Zebu	Female	N/A		24	Negative	Negative	Negative
27	Zebu	Male	N/A		26	Negative	Negative	Negative
28	Zebu	Female	N/A		26	Negative	Negative	Negative
29	Zebu	Female	N/A		21	Negative	Negative	Negative
30	Zebu	Male	N/A		20	Negative	Negative	Negative
31	Zebu	Male	N/A		19	Negative	Negative	Negative
32	Zebu	Male	N/A		16	Negative	Negative	Negative
33	Zebu	Male	N/A		19	Negative	Negative	Negative
34	Zebu	Male	N/A		24	Negative	Negative	Negative
35	Zebu	Male	N/A		26	Negative	Negative	Negative
36	Zebu	Female	N/A		21	Negative	Negative	Negative
37	Zebu	Female	N/A		25	Negative	Negative	Negative
38	Zebu	Male	N/A		22	Negative	Negative	Negative
39	Zebu	Male	N/A		31	Negative	Negative	Negative
40	Zebu	Female	N/A		25	Negative	Negative	Negative
41	Zebu	Male	N/A		24	Negative	Negative	Negative
42	Zebu	Female	N/A		22	Negative	Negative	Negative
43	Zebu	Male	N/A		24	Negative	Negative	Negative
44	Zebu	Male	N/A	4105	17	Negative	**Positive**	Negative
45	Zebu	Female	N/A		21	Negative	Negative	Negative
46	Zebu	Male	N/A		16	Negative	Negative	Negative
47	Zebu	Female	N/A		18	Negative	Negative	Negative
48	Zebu	Male	N/A		35	Negative	Negative	Negative
49	Zebu	Female	N/A		25	Negative	Negative	Negative
50	Zebu	Male	N/A		23	Negative	Negative	Negative
51	Zebu	Male	N/A		20	Negative	Negative	Negative
52	Zebu	Male	N/A		23	Negative	Negative	Negative
53	Zebu	Male	N/A		26	Negative	Negative	Negative
54	Zebu	Female	N/A		25	Negative	Negative	Negative
55	Zebu	Female	N/A		20	Negative	Negative	Negative
56	Zebu	Male	N/A		16	Negative	**Positive**	Negative
57	Zebu	Female	N/A		19	Negative	Negative	Negative
58	Zebu	Female	N/A	4103	18	Negative	**Positive**	Negative
59	Zebu	Male	N/A		24	Negative	Negative	Negative
60	Zebu	Female	N/A		16	Negative	Negative	Negative
61	Zebu	Male	N/A		19	Negative	Negative	Negative
62	Zebu	Female	N/A		19	Negative	**Positive**	Negative
63	Zebu	Female	N/A		19	Negative	Negative	Negative
64	Zebu	Female	N/A		20	Negative	Negative	Negative
65	Zebu	Male	N/A		16	Negative	Negative	Negative
66	Zebu	Male	N/A		24	Negative	Negative	Negative
67	Zebu	Male	N/A		24	Negative	Negative	Negative

68	Zebu	Female	N/A	21	Negative	Negative	Negative
69	Zebu	Female	N/A	20	Negative	Negative	Negative
70	Zebu	Female	N/A	29	Negative	Negative	Negative
71	Zebu	Female	N/A	25	Negative	Negative	Negative
72	Zebu	Male	N/A	22	Negative	Negative	Negative
73	Zebu	Female	N/A	29	Negative	Negative	Negative
74	Zebu	Male	N/A	38	Negative	Negative	Negative
75	Zebu	Male	N/A	15	Negative	Negative	Negative
76	Zebu	Male	N/A	15	Negative	Negative	Negative
77	Zebu	Male	N/A	35	Negative	Negative	Negative
78	Zebu	Male	N/A	24	Negative	Negative	Negative
79	Zebu	Male	N/A	24	Negative	Negative	Negative
80	Zebu	Male	N/A	50	Negative	Negative	Negative
81	Zebu	Female	N/A	24	Negative	Negative	Negative
82	Zebu	Female	N/A	18	Negative	Negative	Negative
83	Zebu	Male	N/A	34	Negative	Negative	Negative
84	Zebu	Female	N/A	25	Negative	Negative	Negative
85	Zebu	Male	N/A	20	Negative	Negative	Negative
86	Zebu	Female	N/A	25	Negative	Negative	Negative
87	Zebu	Male	N/A	26	Negative	Negative	Negative
88	Zebu	Male	N/A	20	Negative	Negative	Negative
89	Zebu	Male	N/A	22	Negative	Negative	Negative
90	Zebu	Male	N/A	26	Negative	Negative	Negative
91	Zebu	Male	N/A	23	Negative	Negative	Negative
92	Zebu	Female	N/A	23	Negative	Negative	Negative
93	Zebu	Female	N/A	24	Negative	Negative	Negative
94	Zebu	Female	N/A	26	Negative	Negative	Negative
95	Zebu	Female	N/A	24	Negative	Negative	Negative
96	Zebu	Female	N/A	23	Negative	Negative	Negative
97	Zebu	Male	N/A	29	Negative	Negative	Negative
98	Zebu	Male	N/A	30	Negative	Negative	Negative
99	Zebu	Female	N/A	21	Negative	Negative	Negative
100	Zebu	Female	N/A	20	Negative	Negative	Negative
101	Zebu	Female	N/A	19	Negative	Negative	Negative
102	Zebu	Male	N/A	18	Negative	Negative	Negative

Appendix 2: Summary of PCV, BCT and PCR results for day 0, day 7 and day 28.

Cattle	Day 0			Day 7			Day 28		
	PCV (%)	BCT	PCR	PCV (%)	BCT	PCR	PCV (%)	BCT	PCR
4101	23	Tc and Tv	Tv	25	Negative	Negative	N/A	N/A	N/A
4102	16	Tv	Tc, Tv	23	Negative	Negative	31	Tv	Tv
4103	18	Tv	Negative	26	Negative	Negative	N/A	N/A	NA
4105	17	Tc	Tc	19	Negative	Negative	26	Negative	Negative
4106	15	Tc	Tc	24	Negative	Negative	22	Negative	Negative
4107	23	Tv	Tc	21	Negative	Negative	N/A	N/A	N/A
4108	17	Tc	Tc	22	Negative	Negative	27	Negative	Negative
4109	20	Tv	Negative	28	Negative	Negative	30	Negative	Negative
4110	22	Tv	Tv	28	Negative	Negative	34	Negative	Negative
4111	28	Tv	Tv	29	Negative	Negative	24	Negative	Tc
4112	22	Tv	Tv	24	Negative	Negative	31	Negative	Negative
4113	18	Tv	Tv	25	Negative	Negative	25	Negative	Negative
4114	12	Tv	Tc	25	Negative	Negative	30	Negative	Negative
4115	16	Tv	Tv &Tc	16	Negative	Negative	N/A	NA	N/A
4116	19	Tc	Tc	26	Negative	Negative	20	Tv	Tv
4117	14	Tv	Tv	27	Negative	Negative	N/A	N/A	N/A
4118	24	Tc	Tv	19	Negative	Negative	22	Tc	Tc

Appendix 3: Research Permit – National Commission for Science, Technology and Innovation (NACOSTI).

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **469349**

Date of Issue: **22/June/2023**

RESEARCH LICENSE

This is to Certify that Dr.. Boscoh Odhiambo Kimathi of University of Pretoria, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Homabay on the topic: RESISTANCE TO TRYPANOCIDAL DRUGS IN CATTLE POPULATIONS IN HOMABAY COUNTY, KENYA for the period ending : 22/June/2024.

License No: **NACOSTI/P/23/26760**

469349

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

Appendix 4: Ethical Approval

KENYA AGRICULTURAL AND LIVESTOCK RESEARCH ORGANIZATION (KALRO)
VETERINARY SCIENCES RESEARCH INSTITUTE
P.O. Box 32, KIKUYU 00902, KENYA
TEL: 020 – 2519769, 2524616, 2020512
Email: director.vsri@ kalro.org

Date: 22nd March, 2023

When replying quote:
Our ref: COMTE/45/ (110)

Dr. Bosco Kimathi,
Kenya Tsetse and Trypanosomiasis Eradication Council (KENTTEC),
P.O. Box 66290 – 00800,
Wetlands.

RE: Approval of your research project on Resistance to trypanocidal drugs in cattle populations of Homabay County, Kenya

The Institute Scientific and Ethical Review Committee (ISERC) of Kenya Agricultural and Livestock Research Organization (KALRO) - Veterinary Science Research Institute (VSRI), Muguga North, met on 22nd March, 2023 and evaluated your research work presented. The committee established that the work met the requirements needed to comply with ethical considerations, animal welfare and use during its implementation. This committee therefore gave you approval to undertake the study as stipulated with a provision that you include a local supervisor from Kenyan university or research organization.

The approval Code No. given is: **KALRO-VSRI/ ISERC031/22032023.**

This statement of KALRO-VSRI ISERC approval must be included in all publications and any other work as may be required **"This research was approved by the Institute Scientific and Ethical Review Committee of KARLO-Veterinary Science Research Institute, Muguga North upon compliance with all provisions vetted under and coded : KALRO-VSRI/ ISERC031/22032023".**

Yours faithfully,

Dr. J.M. Nginyi, PhD
Chairperson, KALRO-VSRI, Institute Scientific and Ethical Review Committee (ISERC)

Appendix 5: Research Ethical Approval from the University of Pretoria (REC147-23).

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Research Ethics Committee

21 December 2023

LETTER OF APPROVAL

Ethics Reference No	**REC147-23**
Protocol Title	**Resistance of Trypanosome species isolated from cattle populations in Lambwe Valley, Kenya, to diminazene aceturate(DA)**
Principal Investigator	**Dr BO Kimathi**
Supervisors	**Prof LCBGD Neves**

Dear Dr BO Kimathi,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC147-23) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

Approved.

Based on the communication as on 8 December 2023, after the matter was discussed with the Dean (and captured in the review section for record purposes).

Please note: If a student is based at an institution in another country, and that institution gave permission for the animal ethics-related part of the study, no AEC approval will be needed at UP. However, on condition that, should the study be published, it must be clearly stated that the animal ethics approval was granted by the relevant institution (and not the UP AEC).

100 YEARS

Room 3-0, Arnold Theiler Building
University of Pretoria, Faculty of Veterinary Science
Private Bag X04, Onderstepoort, 0110, South Africa
Tel +27 (0)12 529 3090
Email: [illegible]
www.up.ac.za

Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Disaense tsa Bongakadinaka

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

Room 6-6, Arnold Theiler Building
University of Pretoria, Faculty of Veterinary Science
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Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Disaense tša Bongakadiruiwa