AN EVALUATION OF SURVEILLANCE AND CONTROL MEASURES FOR AFRICAN TRYPANOSOMIASIS IN REMOTE AREAS OF EASTERN ZAMBIA

A PhD thesis submitted by Gloria M. Mulenga (MPH, BSc), on the 26th of January 2023, to the College of Public Health, Medical and Veterinary Sciences (CPHMVS) at James Cook University to fulfil the requirements for the completion of a PhD (Health)

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DECLARATION OF ETHICS

All activities reported in this thesis were conducted in line with the human and animal ethics regulations with clearances obtained from James Cook University (H7226 and A2498) and the Zambian Ethics Committee-ERES Converge IRB (Ref. No. 2018-Oct-001). The research was approved by the Zambia National Health Research Authority. Local requirements, rules and regulations were observed.

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ABSTRACT

African trypanosomiasis is a debilitating disease affecting both man and his livestock. The disease has been the major draw-back to food security in Africa including most parts of Zambia. The devastating effects of trypanosomiasis among poor remote communities has resulted to the extensive use of various control strategies and financial burdens among affected communities. Small-scale livestock farmers in trypanosomiasis endemic areas spend large amounts of money and resources to protect their animals from the effects of tsetse and trypanosomiasis while profits based on their choice of control method remain unquantified. Therefore, this study evaluated and identified different trypanosomiasis control strategies and measures that are cost effective in the detection and control of African trypanosomiasis in endemic areas of Eastern Zambia. Opportunities that exist for establishing a One Health approach system in Zambia were explored.

The project was structured into five specific objectives which formed the basis of the five field studies conducted for the research. The first specific objective sought to address the knowledge gap in literature on the control and management of African trypanosomiasis in Zambia particularly from a One Health perspective. The second specific objective explored how sensitivity and specificity of detecting trypanosome infection in cattle varied between laboratory techniques when performed under field conditions. Through a prospective cohort study of trypanosomiasis incidence in cattle, the third specific objective, evaluated trypanosomiasis control strategies for their cost effectiveness in the control and detection of trypanosomes in resource poor remote communities of Eastern Zambia. The fourth specific objective determined and compared the prevalence of *Sodalis* and *Wobachia* in tsetse species found in the Luangwa valley tsetse belt as a basis for alternative control strategy for trypanosomiasis. The fifth and last specific objective explored the impact of the Zambian government policies on animal and human disease reporting and management, and on One Health opportunities that can be considered for the control of African trypanosomiasis.

The research was conducted in Mambwe, a rural district in Eastern Zambia, between 2019 and 2020. Firstly, an in-depth analysis was conducted on 18 articles selected from a total of 2238 articles that were screened, with application of the search engines PubMed and PubMed Central. Secondly, the effectiveness of four trypanosomiasis control treatments commonly used in Eastern Zambia (Berenil, Samorin, Cyfluthrin pour-on and Cypermethrin targets) were evaluated through a prospective cohort study of trypanosomiasis incidence in cattle. During baseline sampling, blood samples were collected from 227 cattle and tested for

infection with trypanosomes using microscopy and Ribosomal RNA Internal Transcribed Spacers (ITS)-PCR while 278 tsetse flies were analysed in a prevalence study of trypanosomes and tsetse symbionts. A stochastic partial budget analysis was applied to quantify the economic impact of the four trypanosomiasis control treatments studied. The distribution functions for the net returns of each control treatment calculated in the partial budget were then modelled using the software programme @RISK 8.2. Lastly, an in-depth review and analysis of strengths, weaknesses, opportunities, and threats in the existing policies and reporting structures in the departments responsible for Veterinary Services, Health, and Wildlife, was conducted in the context of One Health.

According to our findings, trypanosomiasis remains an important disease for communities living in tsetse infested areas of Zambia. Through field studies conducted, the research study illustrated limitations and complexities in the application of molecular and parasitological diagnostic tests when applied under field conditions. In addition, findings indicated that all trypanosomiasis control strategies studied yielded a positive net return but varying net values. The Samorin inoculation group showed the greatest return, but the Cypermethrin target group showed the greatest impact on incidence. According to our findings, Samorin inoculation was found to be a better control option for small scale livestock farmers in remote areas of Eastern Zambia while the use of Cypermethrin targets would work best for government sponsored programmes. Further, findings indicated limitations in the application of *Sodalis* as an alternative biological control option to inhibit vector competence in tsetse species in the Luangwa valley tsetse belt. Finally, the research study reviewed sub-optimal implementation of existing policies related to the control of zoonotic diseases, and as such, the study suggests measures and strategies that could be adopted in the effective control of trypanosomiasis and other zoonotic diseases in remote poor communities of Eastern Zambia, and other regions affected by tsetse and trypanosomiasis in Africa.

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LIST OF ABBREVIATIONS AND ACRONYMS

AAT: Animal African Trypanosomiasis

AUC: Area under curve

CAT: Canine Animal Trypanosomiasis

CSF: Cerebrospinal Fluid

CSO: Central Statistics Office-Zambia

CVR: Central Veterinary Research Institute

DNA: Deoxyribonucleic acid

FAO: Food and Agriculture Organization

FP: Filter Paper

GDP: Gross domestic product

GMA: Game Management Area

GPS: Global Positioning System

HAT: Human African Trypanosomiasis

ITC: Insecticide Treated Cattle

ITS: Internal transcribed spacers

ITT: Insecticide Treated Targets and Traps

LAMP: Loop Mediated Isothermal Amplification

MLF: Ministry of Livestock and Fisheries, Zambia

MOH: Ministry of Health, Zambia

NPV: Negative Predictive Value

NTD: Neglected Tropical Disease

PAAT: Programme Against African Trypanosomiasis

PATTEC: Pan African Tsetse and Trypanosomiasis Eradication Campaign

PCR: Polymerase Chain Reaction

PCV: Packed Cell Volume

PPV: Positive Predictive Value

RHC: Rural Health Centre

RIME: Repetitive Insertion Mobile Element

ROC: Receiver Operator Curve

RTTCP: Regional Tsetse and Trypanosomiasis Control Programme

SAS/SAT: Sequential Aerial Spraying

SRA: Human Serum Resistance Associated

TDRC: The Tropical Diseases Research Centre

WBC: White Blood Cell

WHO: World Health Organization

ZNPHI: Zambia National Public Health Institute

ZMW: Zambian Kwacha

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

For centuries, the devastating effects of African trypanosomiasis has continued to be a burden for affected poor communities and their livelihoods. African trypanosomiasis has remained on most affected countries zoonosis control agendas for over 60 years with more recognisable impacts being made towards eliminating the disease. The Luangwa valley in Zambia has not been spared from the economic burden of trypanosomiasis as it supports high densities of tsetse flies and wildlife reservoirs. The tourism reputation that the wildlife reservoirs has created over the years, the presence of the tsetse vector and the increasing human settlements has left the valley to be recognized as a historical Trypanosomiasis focal area (Swallow, 2000; Mwanakasale et al., 2013; Boulangé et al., 2022; WHO, 2022a).

1.1.1. The disease

African Trypanosomiasis is a debilitating vector-borne disease mainly occurring in sub-Saharan Africa. The disease is mostly transmitted to the mammalian hosts by the bite of an infected tsetse fly causing African Animal trypanosomiasis (AAT) commonly known as nagana in cattle and Human African trypanosomiasis (HAT) or sleeping sickness in humans (Swallow, 2000; Franco et al., 2022; WHO, 2022b). African trypanosomiasis is caused by protozoa belonging to the genus *Trypanosoma* transmitted by tsetse flies (Diptera: *Glossinidae*). *Trypanosoma brucei brucei, T.congolense* and *T.vivax* are the trypanosome species responsible for livestock and production loss. *Trypanosoma congolense* and *T. brucei* are the major cause of AAT in Eastern and Southern Africa whilst *Trypanosoma vivax* (together with *T. congolense*) are important in the cause of the cattle disease in West Africa (Shaw et al., 2014; Shereni et al., 2021; Percoma et al., 2022). The two species of the human infective trypanosomes are *Trypanosoma brucei gambiense* (found in West and Central Africa) which accounts for over 97% of reported cases and *Trypanosoma brucei rhodesiense* (found in Eastern and Southern parts of Africa, including Zambia) which only accounts for about 3% of reported cases (Meyer et al., 2016; WHO, 2022b). Wild animals such as lions, buffalos, hippopotamuses, etc. are the main reservoirs for the trypanosome parasite (Anderson, 2011). Domestic animals have also been reported to harbour human infective trypanosomes raising a lot of concern especially for livestock farmers living in the peripherals of tsetse infested game areas (Lisulo et al., 2014; Laohasinnarong et al., 2015; Mulenga et al., 2021b).

According to the 2018 livestock and aquaculture Census, the livestock population in Zambia stood at 3.7 million cattle, 3.5 million goats, 170 thousand sheep and 1.1 million pigs (Ministry of Livestock and Fisheries and Central statistics Office, 2019) (CSO, 2019). Cattle dominate the livestock sector both among the commercial and traditional farmers in Zambia. The prevalence of trypanosomiasis in livestock and particularly in cattle has continued reporting alarming figures in affected areas (Simukoko, 2007; Ruiz, 2015; Mulenga et al., 2021b). Disease prevalence in Zambia ranges between 1% and 90% (Richter et al., 2012). Previously collected data on AAT prevalence in cattle stood at 3.8% for Mambwe district of Eastern Zambia (Kakumbi, 2017). Trypanosomiasis occurrence has mainly been associated with the presence of the tsetse vector, host, and parasite. About five eighths (5/8) of Zambia is estimated to be infested with tsetse flies (**Error! Reference source not found.**).

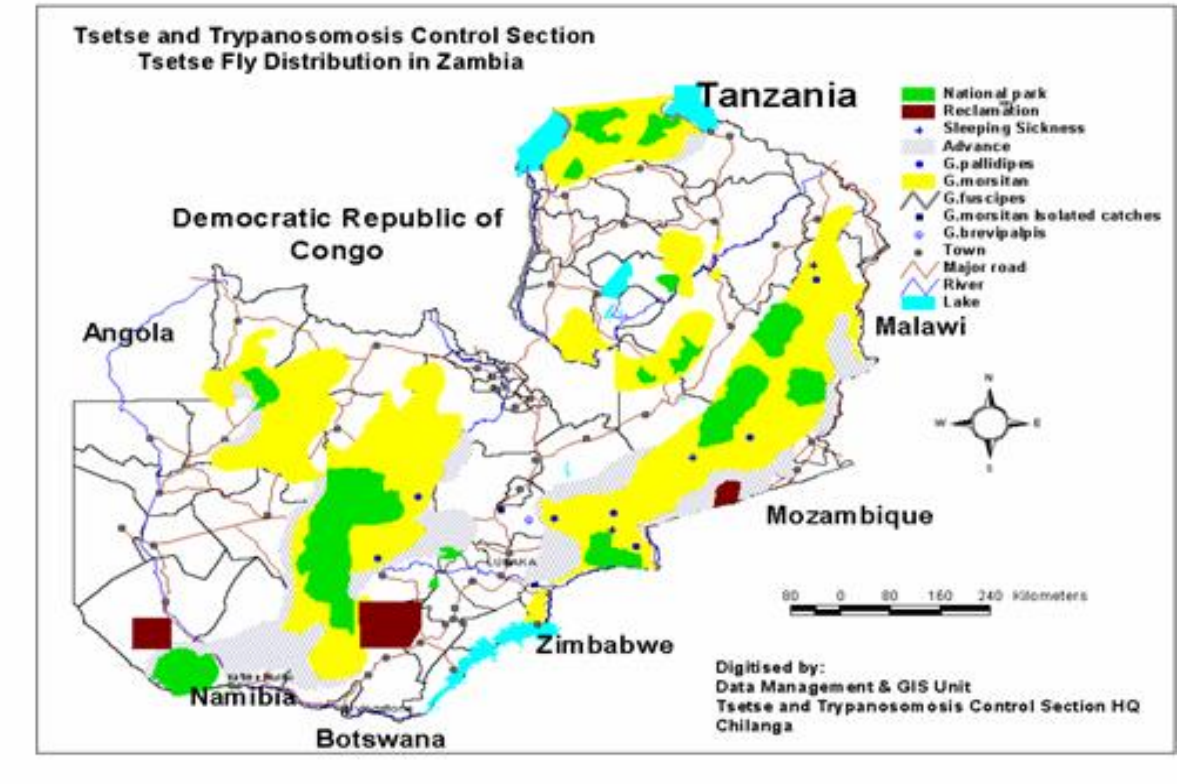

Figure 1. 1: Tsetse and trypanosomiasis distribution in Zambia. Source: Ministry of Fisheries and Livestock. Lusaka: Department of Veterinary services; 2018.

According to Food and Agriculture Organisation estimates, 50 million head of cattle are at risk of AAT, with 3 million cattle deaths recorded per year. Loss in cattle production alone is estimated at US$1.0-1.2 billion per year and US$4.5 billion per year on Agriculture gross

domestic products (Swallow, 2000; PAAT, 2022). Trypanosomiasis may not seem important on the world stage as diseases such as east coast fever, contagious bovine pleural pneumonia, malaria, and tuberculosis. Nevertheless, trypanosomiasis is an important disease, responsible for a considerable degree of suffering and mortality in Sub-African countries where it is endemic (Hide, 1999; Mwiinde, 2017). Besides death, the other outcome for untreated trypanosomiasis livestock and human victims, are its effects on the community's quality of life (Swallow, 2000; Engels, 2006; Bukachi, 2009).

The World Health Organisation (WHO, 2022b) estimates that (i) in the 36 tsetse infested African countries, approximately 65 million people, the majority of whom live in remote rural areas, are at risk of catching Human African Trypanosomiasis (HAT), (ii) an estimated 200,000 people are infected with the disease and reported among affected populations, and that (iii) about 50,000 people die from the disease every year with the situation rapidly deteriorating and increasingly more new cases being registered every year. Despite the WHO projection of over 65 million people at risk in Africa, only a fraction of that population is under surveillance and relatively few cases are diagnosed and reported annually (Engels, 2006; Franco et al., 2022; WHO, 2022b). The WHO Expert Committee on HAT Control and Surveillance reports that sustained control efforts have reduced the number of new cases. In 2022 the number of HAT cases reported dropped below 10 000 for the first time in 50 years, and there were 992 and 663 cases reported in 2019 and 2020, respectively (WHO, 2022b). With that, the WHO has targeted elimination of HAT as a public health problem by the year 2030.

According to WHO (Franco et al., 2020a), Zambia reports less than 100 cases of HAT per year. Accordingly, 102 cases of HAT have been reported between the years 2000-2013 (Franco et al., 2020a). In the recent years (between 2013 to date) parts of the Luangwa valley has seen an increase in cases reported with Rufunsa, Chama, and Mambwe districts reporting 9, 8 and 5 cases respectively (Personal Communications-University of Zambia and Kakumbi research station reports (Kakumbi, 2014)). There is, therefore, a likelihood that non-reporting districts located along the Central Luangwa valley of Eastern Zambia, could be highly under-reporting HAT (Mwanakasale, 2011).

1.1.2. Diagnosis

Diagnosis of African trypanosomiasis is based on the combination of both clinical and investigative data. Diagnosis in humans follows a three-step pathway: screening, diagnostic

confirmation, and staging. Most vector control techniques and disease control programs rely on active case detection through mass population screening. Screening tools and techniques therefore need to be sensitive, practical, quick, and cheap.

Clinical diagnosis

The clinical presentations of *T. b. gambiense* and *T. b. rhodesiense* HAT are remarkably different. While *T. b. gambiense* HAT is generally a chronic illness that lasts for years, *T. b. rhodesiense* HAT usually presents as an acute febrile illness that is fatal within weeks or months of infection (Fevre, 2001; WHO, 2022b). The typical features of the disease are fever, headache, general malaise, and enlargement of lymph nodes, particularly the posterior cervical glands, and oedema of the face (MacLean, 2010). Diseases such as malaria, enteric fever, tuberculosis meningitis and HIV infection can mimic or even coexist with HAT. Clinical presentation in a geographical location where the disease is known to be endemic simply provides a diagnostic clue. However, the non-specific nature of many clinical features makes it imperative to exclude other infections like tropical fevers hence the need for laboratory diagnosis (Blum, 2006; Frean et al., 2018). Animals presented with AAT due to *T. congolense* and *T. vivax* infection often exhibit (non-specifically); Anaemia, intermittent fever, loss of weight, loss of appetite, abortions, staring coat, discharges from eyes (commonly in *T. vivax* infections), loss of draught power and infertility (FAO, 2017; PAAT, 2022).

Microscopic diagnosis

Most patients and sick animals are diagnosed by microscopic examination (Figure 1.2) of trypanosomes of a giemsa stained thin or thick blood smear, wet blood slide, and quantitative buffy coat (QBC) (Legros, 2002). Wet blood slide is cheap, simple and gives immediate results. It is particularly used for *T. b. rhodesiense* but not useful for *T. b. gambiense* because blood parasite levels are usually high in *T. b. rhodesiense* infections compared to the later which is usually associated with low parasitaemia especially in early stages of infection (Chappuis, 2004).

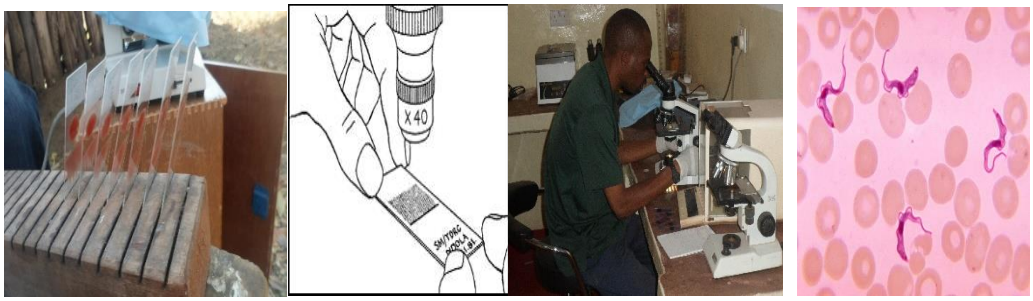

Figure 1. 21.1**: Microscopic examination of Trypanosomes (Pictures taken by Mulenga G; Unknown authors)**

To improve the accuracy of detecting trypanosomes, a thin or thick giemsa stained blood film is used for both AAT and HAT diagnosis. The method is simple, cheap and can also detect other parasites (microfilaria and plasmodium). Disadvantages of the method include limited sensitivity and requires more time for preparation and examination (Odiit., 2005). A QBC is also used for trypanosome detection where blood is concentrated in heparinised capillary tubes and examination of the buffy coat junction (a specific level in the capillary tube where trypanosomes can be found) is done under the microscope at 40 x 10 resolutions for the presence of mobile trypanosomes. The technique has improved sensitivity, is relatively rapid and can be used for the diagnosis of other parasites but it is sophisticated, materials used are fragile, and it is expensive (Van Meirvenne, 1992; Katsidzira, 2010).

Due to the low parasitaemia commonly exhibited in *T.b. gambiense* sleeping sickness, the mAECT (mini-Anion Exchange Centrifugation Technique) has been adapted for field diagnosis. mAECT is based on a purification technique first described by Lanham et al. and later adapted for diagnosis of sleeping sickness. Trypanosomes are separated from blood by anion exchange chromatography and concentrated at the bottom of a transparent tube by low-speed centrifugation (3000rpm), after which the tip of the transparent tube is examined under the microscope for the presence of mobile trypanosomes (Lumsden et al., 1979; Buscher et al., 2009).

Serological techniques

The diagnostic confirmation and staging of the Gambian HAT are based on the same methods as Rhodesian HAT. Confirmation relies on the finding of trypanosomes in the blood, lymph nodes, or cerebrospinal fluid (CSF). The Card Agglutination Test for Trypanosomiasis (CATT) currently is used for diagnosis of *T. b. g*ambiense in most areas of endemic infection (Robays, 2004). Unfortunately, it is estimated that 20 to 30% of patients are missed by the standard

parasitological techniques if the blood parasite levels are low (Robays, 2004). The LATEX agglutination test has been developed as a field alternative to the CATT. It has showed a higher specificity but lower or similar sensitivity (Busher, 1999). ELISA has also been employed in detecting specific antibodies, but the sophisticated equipment required limits its use for remote testing of samples collected in the field (Truc, 1999). Staging of the disease is a key step that allows classification of the patient into first (hemolymphatic) or second (meningoencephalitic) stage of the disease. In the absence of reliable blood tests able to detect CNS (Central nervous system) invasion by the parasite, HAT staging relies on the CSF examination of CSF (Welburn, 2002).

Molecular techniques

Molecular techniques such as polymerase chain reaction (PCR) have significantly improved the sensitivity and accuracy of trypanosome diagnosis compared to the traditional parasitological methods (Thumbi, 2008). Molecular tests differentiate between trypanosome species and subspecies using specific primers (Cox, 2005). The serum resistance-associated (SRA) PCR has been used to differentiate *Trypanosoma brucei brucei* from *T. b. rhodesiense* because the SRA gene is exclusively found in *T. b. rhodesiense* (Balmer, 2011). The human infective trypanosomes in animal reservoirs While PCR is the method of choice for the detection of both AAT and HAT, its use in the field is limited by cost implications and the requirements for highly trained personnel (Truc, 1999; Solano, 2002). The invention of the loop-mediated isothermal amplification (LAMP) method a decade ago has given new impetus towards development of point of care diagnostic tests based on amplification of pathogen DNA, a technology that has been the precinct of well-developed laboratories (Wastling, 2010). LAMP, a highly sensitive, specific, and yet simple diagnostic technique for parasite detection is currently being used for trypanosome diagnosis (Njiru, 2012). An advantage of LAMP over PCR is that; it is less expensive, Rapid, Sensitivity is equal to or higher to that of classical PCR targeting the same gene, Robust, higher specificity, allows visual detection and amplification at isothermal conditions (low heat required, hence water bath and exothermal chemical units are sufficient) (Wastling, 2010; Njiru, 2012).

1.1.3. Treatment and control

Governments of trypanosomiasis endemic areas are overwhelmed with the costs attached to the sustainable control of trypanosomiasis therefore, making its control difficult. The absence

of an effective vaccine against trypanosomiasis has made disease control even more difficult (Richard, 2006; WHO, 2022b). As such, trypanosomiasis is currently controlled by either (i) directly targeting the parasite by means of chemotherapy, or (ii) by targeting the tsetse vector including the use of bait technology (odour baited insecticide treated targets and animal bait), aerial or ground spraying with non-residual insecticides, Sterile Insect Technique (SIT), and bush clearing (Lutumba, 2005; Kamba Mebourou et al., 2020; Lord et al., 2020). Recent studies have explored the use of endosymbionts in the control of vector borne diseases with successes reported in diseases like dengue fever (John., 2008; Ricci, 2012; Utarini et al., 2021). In Zambia, AAT has been managed through constant use of trypanocides by individual livestock farmers. Treatment and/or management of trypanosomiasis in humans has been negatively affected by several factors that include, late case detection that tends to result in tragic consequences (death) associated with adverse effects of the administered drugs in the late stage (Mbewe et al., 2015; Kazumba et al., 2018).

The Zambian government has generally made some notable strides in the control of African trypanosomiasis particularly through tsetse control. However, the government's inability to put in place active surveillance systems, and the lack of adequate resources to effectively sustain control efforts, have contributed to limitation of success associated with tsetse re-invasion and resurgence of African trypanosomiasis in areas where the disease had earlier been brought under control. In the case of HAT, lack of active surveillance systems has historically hindered progress towards the goal of eliminating African trypanosomiasis as a public health problem in Zambia (Mwanakasale and Songolo, 2011; Franco et al., 2020b; Boulangé et al., 2022).

Control targeting the parasite

Chemotherapy and chemoprophylaxis are the most used option in the control of trypanosomiasis. The control method is based on screening of and treating of hosts found positive. The drugs of choice mainly used for AAT are Diminazene aceturate (Berenil) and Isometamidium chloride (Samorin). Berenil is usually used as a curative drug for the treatment of AAT whilst Samorin is used as a prophylaxis (Giordani et al., 2016). The two drugs have been reported to be very effective against strains of *T. congolense* and *T. vivax* (Jordan, 1986). Trypanocides continue to play an important role in the control of AAT. Administration of any drug regime requires good standard of supervision. Limited veterinary assistance and qualified health professionals, however, has made this ideal concept impossible

in most rural settings of Africa. Drugs are mostly administered by livestock farmers without any veterinary supervision leading to misuse and under dosing of medication which could promote drug resistance (Mbewe, 2015; T. Tekle., 2018). Under such present circumstances, treatment is carried out based on clinical presumption and with the availability of the drug (Maclennon, 1981; Mulenga, 2015)

Early diagnosis and access to prompt treatment are key components of current strategies for HAT control. Although treatable, millions of HAT cases in endemic areas have gone undiagnosed and resulted in death due to delayed action by either the health facility or the affected individuals (WHO, 2022b). According to Odiit (Odiit., 2005), it is estimated that about 3% of HAT cases who enter the health system die undiagnosed. Unfortunately, detection of HAT in its early stage is not easily achievable in many affected rural communities of Africa as initial symptoms of the disease are usually mistaken for other endemic febrile diseases such as malaria, TB, HIV/AIDS (Blum, 2006; Frean et al., 2018). After several visits to the rural health centres which in most cases have limited or no facilities for HAT diagnosis, affected individuals usually resort to other treatment options and in most cases associate the disease to witchcraft. The general inability of local healthcare personnel to diagnose HAT in its early stage leads to cases to resorting to alternative health care options (Bukachi, 2009). Since HAT affects rural remote communities, whose common trend is not to seek medical attention when sickness strikes, but instead go for traditional options, usually HAT patients are presented late at the health facilities. Failure to receive a prompt and accurate diagnosis causes delays in the initiation of treatment and an increased financial burden on the patients and their families (Bukachi, 2009).

Suramin is used for early-stage HAT treatment while Melarsoprol is the drug of choice for late-stage HAT treatment. However, both drugs have been associated with side effects which includes; Hypersensitivity, pains in the soles of the feet, skin rush/burns-for Suramin treatment while fever, headache, tremors, convulsions, coma and an estimated 5% mortality rate have been associated with melarsoprol treatment (WHO, 2022c). Due to the adverse side effects of the current treatment options for HAT, scientists have sought for safer, practical, and easier to administer treatment options. The efficacy of nifurtimox-eflornithine combination therapy (NECT) for second stage treatment of HAT has been assessed and proved to be non-inferior to that of eflornithine monotherapy (Priotto., 2009; Franco et al., 2012; Kazumba et al., 2018).

Control targeting the tsetse vector

Current methods for tsetse control include Insecticidal methods and non-insecticidal. Chemical control depends upon sufficient contact between the tsetse fly and the insecticide for the fly to pick up lethal dose (Vreysen et al., 2013). The use of insecticides (Figure 1.3) was engineered in several ways such as: ground spraying, aerial spraying, sequential aerosol technique, or in more localized areas using hand-held or vehicle-mounted fogging machines, and artificial and live-bait technique (Stich, 2003; Vale, 2015).

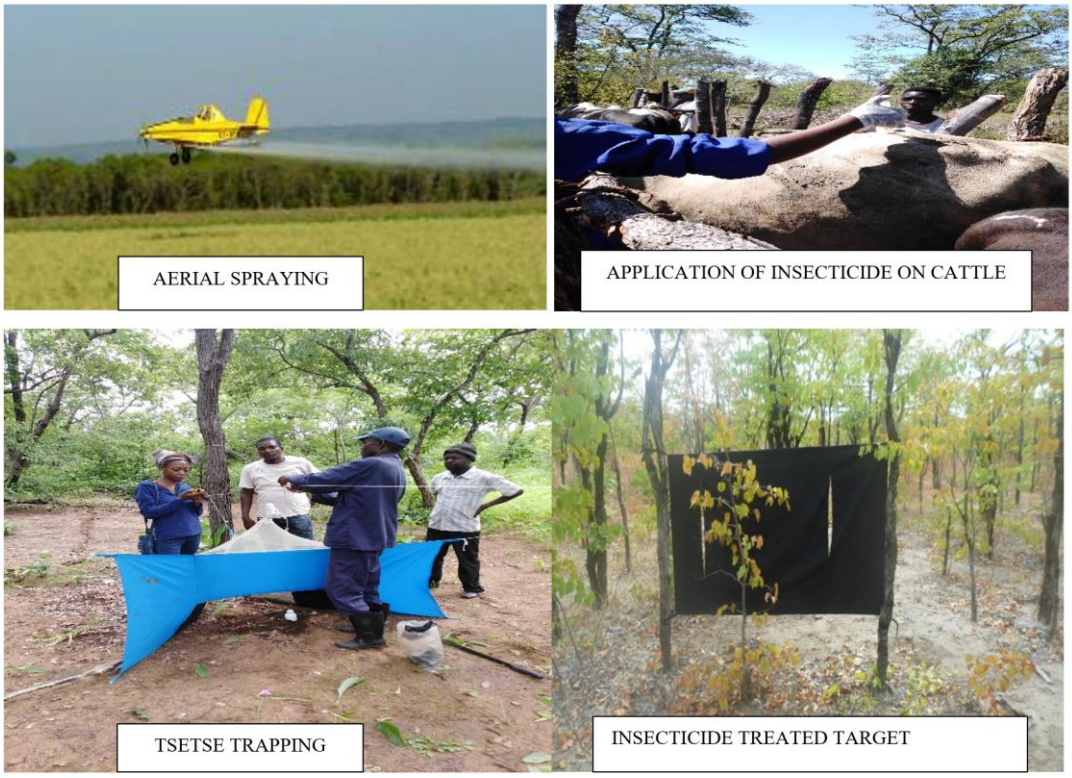

Figure 1. 3: Showing tsetse control techniques commonly used in Zambia (Pictures by Mulenga G; adopted from WHO 2009)

Methods like aerial spraying have proved to be successful in countries like Botswana (Kgori, 2006) but the costs involved has limited its use in many areas. A simpler and cheaper device involves a suspended screen of blue and black cloth (tsetse target) impregnated with an insecticide provide satisfactory results. Tsetse flies are attracted to the blue segments and land on the black segment, quickly succumbing to the insecticide (Engels, 2006; Vale., 2015; Kamba Mebourou et al., 2020). The effect of targets on the tsetse fly population depends largely on the mobility of the flies. These attributes will enable one to determine at what density and pattern targets should be deployed. Target deployment is the placement of artificial bait

devices called targets in tsetse habitats for purposes of control, eradication or blocking further advance of tsetse flies. Traps can also be used for the same purposes. Research findings into the behaviour of tsetse particularly on their responses to smells, colour, size, landing, and mobility of objects led into the development of the bait technology which exploits their behaviour in its mode of action. These devises can attract large numbers of tsetse flies from a range of distances (e.g.,100m). Further research led into treatment of these devises with insecticides so that as the tsetse fly is attracted and land on them, they pick up the insecticide which knocks and kills them after a short while (Vale, 1993; Mweempwa C., 2015)

In Zambia the most economically important tsetse species is *Glossina morsitans* followed by *Glossina pallidipes*. These species either exist alone or together. According to the Ministry of Fisheries and Livestock-Tsetse Control section operating protocols, it has been studied and observed that if suppression is expected within 6 - 9 months or a year, where *G. pallidipes* exists alone, targets baited with butanone or acetone together with octenol, 3-n-propyl phenol and 4-methyl phenol can be deployed at a density of 2/km^2 because this is a very mobile fly. Where *G. morsitans* exist alone, targets baited with butanone or acetone with octenol can be deployed at a density of 4/km^2. However, where the two species co-exist, the target density for *G. morsitans* is adopted but odours of *G. pallidipes* are used (Vale., 2015; Kamba Mebourou et al., 2020; Rayaisse et al., 2020)

Non-insecticidal tsetse control methods can be classified in two forms: Ecological and Biological methods (Torr, 2007). Ecological methods include (i) Evacuation of populations by moving people from tsetse infested areas to tsetse free areas, (ii) Bush clearing and agriculture practices by destruction of essential habitat of tsetse and, (iii) Game destruction. The latter methods were severely criticized and abandoned because of their environmental implications (Engels, 2006). Biological control relies on the existence of some pathogens of tsetse. One form of biological control of vector borne diseases is the use of insect symbionts that have attracted attention for their potential use as mode for expression of anti-parasitic gene products in arthropod disease vectors (Ricci, 2012; Utarini et al., 2021).

Symbionts influence several aspects of tsetse's physiology including reproduction, nutrition, and vector competence. Tsetse harbours three distinct populations of endogenous symbionts; *Wigglesworthia, Sodalis* and *Wolbachia* (Dale, 2001; Pais, 2001; Wamiri, 2013)**.** *Wigglesworthia;* the first resides intracellular within the bacteriocytes forming a bacteriome found in the mid gut while the second population is found extracellular in the milk gland secretions (Rio, 2012).

The bacterium provides two benefits to its tsetse host: nutritional and immunological. In the absence of this bacterium, intrauterine larval development is stunted, and progeny aborted (Pais, 2001). *Wigglesworthia*'s contracted genome encodes an unusually high number of putative vitamin biosynthesis pathways, this genotypic factor supports the theory that *Wigglesworthia* supplements its tsetse host with nutritious metabolites that are naturally present in low titres in vertebrate blood (Pais, 2001; Rio, 2012; Weiss, 2013).

Unlike *Wigglesworthia, Sodalis* can be found both intra- and extra-cellular in various tissues including mid gut, fat body, milk gland, salivary glands and hemocoel (Toju, 2010; Balmand, 2013). *Sodalis'* genome exhibits a low coding capacity and an unusually high number of pseudo genes (over 600 genes) and contains features associated with pathogenic lifestyles, including 3 type three secretion systems (TTSS) which function during tsetse's juvenile developmental stages (Toh, 2006). *Sodalis* can be cultured in cell free medium and is usually absent in several natural tsetse populations. However, studies indicate that *Sodalis* may play a role in tsetse's ability to vector pathogenic trypanosomes (Welburn., 1993; Dale, 2001).

Wolbachia is a widespread alpha-proteo bacteria endosymbiont infecting approximately 70% insects. It manipulates the reproductive biology of its host mechanisms which include cytoplasmic incompatibility (CI), male killing, feminization and parthenogenesis (Dale, 2001; Wamiri, 2013). *Wolbachia* infected females can mate with uninfected males or with a male infected with the same strain and produce viable off springs while infected males mating with uninfected females causes developmental arrest during embryogenesis. Unlike *Sodalis* and *Wigglesworthia* which are transmitted via milk gland, *Wolbachia* is transmitted via germ line cells (Balmand, 2013).

1.1.4. One Health approach to controlling African trypanosomiasis

African trypanosomiasis affects both man and his livestock, making it an idea disease for the application of a One Health approach (Boulangé et al., 2022; WHO, 2022a). Availability of hosts (man, wildlife, and livestock) in the environments habited by tsetse flies contributes to the risk of infection. Poor and marginalised communities living in the peripheral areas of national parks inhabited by tsetse and wildlife reservoirs are the most affected by trypanosomiasis (Rostal, 2018). The global health security agenda specifically identifies One Health as an integral to achieving health security against infectious disease threats. One Health is an integral approach that recognises links between human and animal health, and

the environment. One Health brings together a range of stakeholders to find common grounds of collaboration and help strengthen health and ecosystems (WHO, 2022a). Multisectoral approach for the prevention and control of vector-borne diseases including trypanosomiasis will strengthen human and animal health capacities in improving the understanding of disease epidemiology dynamics and inform risk mitigation or control measures. Resources could be saved and re-allocated to other activities through combining human and animal activities e.g., concurrent surveillance, risk assessments and evaluations of both human and animal subjects, cross-training of animal and human health staff, laboratory diagnosis, community awareness and engagement programmes, and sharing of common facilities and infrastructure (WHO, 2022a). One Health approach may in the long run improve resource efficiency and reduce duplication. Despite strong overall interest in One Health, country, local, and project level implementation remains limited likely due to the lack of pragmatic and tested operational methods for implementation and metrics for evaluation (Baum, 2017).

1.2 JUSTIFICATION OF THE STUDY

AAT has been associated with very serious economic consequences such as reduced productivity and fertility, livestock death, increased abortion in livestock and high treatment costs (Bealby, 1996; Mwiinde, 2017; Anne Meyer., 2018). Subsequent disappearance of wild animals which are the preferred host for tsetse have forced tsetse to feed on livestock and man who have encroached into game management areas, with little known about the incidence/prevalence of trypanosomes that cause HAT and AAT in domestic animals. Poor diagnostic facilities and the method of disease detection have negatively affected control efforts (Rostal, 2018; Mulenga et al., 2021b). Treatment of HAT in infected individuals has severe consequences (death) due to adverse effects of drugs (WHO, 2022b). Treatment in livestock reservoirs hosts may be a better option but has not been explored. Because of the major importance of cattle in the African economy, most livestock owners in tsetse infested areas have resulted to extensive use of various control strategies to combat the disease resulting in financial burdens while profits based on their choice of control method remain unquantified (Van den Bossche, 2000; Ndeledje et al., 2013). Current tsetse and trypanosomiasis control methods used in Zambia are insecticide based. Other control options which include the use of vector endosymbionts have not been explored. Little is known about the prevalence of symbionts in tsetse species found in Eastern Zambia. Globally, the use of biological methods for the control of vector transmitted diseases is becoming popular (Utarini

et al., 2021). Trypanosomiasis has remained an important public health problem in Zambia due to the absence of a sustainable national control surveillance programme (Mwanakasale, 2011; Mulenga et al., 2020). The capacity to detect, control and manage emerging and re-emerging zoonotic diseases in Africa has been limited by a lack of utilisation of available reporting structures and policies to support programmes at national and local levels (Mulenga et al., 2021a).

Alternative hypothesis

1 There is no systematic review of the literature that has been conducted on the control and management of African trypanosomiasis in Zambia particularly from a One Health perspective.
2 The sensitivity and specificity of detecting trypanosomiasis infection in cattle under field conditions varies between laboratory practices.
3 The cost and efficacy of controlling trypanosomiasis in cattle varies significantly between current control strategies used in Eastern Province of Zambia.
4 Tsetse flies in Eastern Zambia carry *Wolbachia* and *Sodalis* bacteria. The presence of Tsetse endosymbionts correlates with the prevalence of trypanosome infected tsetse flies.
5 Veterinary Services, Health, and Wildlife departments are currently working independently in controlling trypanosomiasis.

Expected research benefits

Findings of this study will:

- Provide insights on how diagnosis can be improved in remote areas and help reduce cattle deaths, thus improving food security in remote Zambia.
- Help reduce deaths and severe side effects associated with human treatments by considering livestock treatments as an alternative control option for the human disease.
- Help communities select cost effective control programmes which will give them greatest returns.
- Add new knowledge to the prevalence of symbionts in tsetse species in Eastern Zambia which will help identify new innovations in the control of the tsetse vector.

- Provide a One Health model that could reduce costs through sharing of Veterinary and Medical capacity and infrastructure.

1.3 GENERAL OBJECTIVE

To evaluate and identify different trypanosomiasis control strategies and measures that are cost effective in the detection and control of African trypanosomiasis in endemic areas of Eastern Zambia.

Specific objectives

1 To review literature on the control and management of African trypanosomiasis in Zambia particularly from a One Health perspective.
2 To compare laboratory diagnostic techniques with current standard methods for detecting trypanosomiasis.
3 To determine the most cost-effective trypanosomiasis control strategy for poor communities of Eastern Zambia.
4 To establish and compare selected symbiotic host prevalence and trypanosomiasis infection in tsetse species of Eastern Zambia.
5 To explore feasibility of a One Health approach suitable for controlling African trypanosomiasis in Zambia.

1.4 SCOPE OF THE RESEARCH PROJECT

The project was structured into seven chapters (Table 1.1) of which five were drawn from the field studies conducted.

Table 1. 1: Thesis chapters and their contents

Chapter	Content	Study objective	Specific objectives
1	General introduction and literature review		

2	Scooping review study	To review literature on the control and management of African trypanosomiasis in Zambia particularly from a One Health perspective	1
3	The sensitivity and specificity of detecting trypanosome infection in cattle	To compare laboratory diagnostic techniques with current standard methods for detecting trypanosomiasis	2
4	Cost-effectiveness of trypanosomiasis control strategies	To determine the most cost-effective trypanosomiasis control strategy for poor communities of Eastern Zambia	3
5	Tsetse endosymbionts and trypanosome prevalence	To establish and compare selected symbiotic host prevalence and trypanosomiasis infection in tsetse species of Eastern Zambia	4
6	One Health approach for controlling trypanosomiasis in Zambia	To explore feasibility of a One Health approach suitable for controlling African trypanosomiasis in Zambia	5
7	General discussion and conclusion		

1.5 PUBLICATIONS ARISEN FROM THE STUDY

1. Mulenga, G.M., Henning, L., Chilongo, K., Mubamba, C., Namangala, B., Gummow, B., 2020. Insights into the Control and Management of Human and Bovine African Trypanosomiasis in Zambia between 2009 and 2019-A Review. Tropical Medicine and Infectious Diseases 5, 115. Doi:10.3390/tropicalmed5030115.
2. Mulenga, G.M., Namangala, B., Chilongo, K., Mubamba, C., Hayashida, K., Henning, L., Gummow, B., 2021. Challenges in the Diagnostic Performance of Parasitological and Molecular Tests in the Surveillance of African Trypanosomiasis in Eastern Zambia. Tropical Medicine and Infectious Diseases 6, 68. Doi: 10.3390/tropicalmed6020068. Presentation of findings: Oral presentation at the ANZCVS online scientific series and abstract forum: 08.09.20 to 15.09.20, online. Poster presentation at the Townsville health research showcase: 26.10.20 to 28.10.20, Townsville hospital. Virtual Faculty Day, University of Pretoria, South Africa. Post graduate speed session: 20.11.20, online.
3. Mulenga, G.M., Chilongo, K., Mubamba C., Gummow, B. Evaluating the financial return for controlling African animal trypanosomiasis for resource poor remote communities of Eastern Zambia. Under review in Preventive Veterinary Medicine. Presentation of findings: Oral presentation at ANZCVS science week: 23.06.22 to 25.06.22, Gold coast, Australia.
4. Mulenga, G.M., Namangala, B., Chilongo, K., Henning, L., Gummow, B., 2021. Policy and Linkages in the Application of a One Health System for Reporting and Controlling African Trypanosomiasis and Other Zoonotic Diseases in Zambia. MDPI-Pathogens 11, 1. Doi:10.3390/pathogens11010030. Presentation of findings: Oral presentation at the Veterinary Sciences Faculty – Ramp up your research presentations: 03.06.22, Townsville, Australia.
5. Mulenga, G.M., Namangala, B., Gummow, B., 2022. Prevalence of trypanosomes and selected symbionts in tsetse species of eastern Zambia. Parasitology, 1-23. Doi:10.1017/S0031182022000804. Presentation of findings: Oral presentation at the ANZCVS science week: 08.07.21 to 10.07.21, online.
6. Mulenga, G.M and Gummow, B., 2022. The detection of African trypanosomes in goats reared in tsetse infested villages of eastern Zambia. Tropical Animal Health and Production. Doi: 10.1007/s11250-022-03367-5.

1.6 REFERENCES

Anderson, N., Mubanga J, Fevre EM, Picozzi K, Eisler MC, Thomas R, Welburn SC, 2011. Characterization of the Wildlife Reservior Community for Human and Animal Trypanosomiasis in the Luangwa Valley, Zambia. Plos Neglected Tropical Diseases 5, 1-16.

Anne Meyer., H.R.H., Farikou O., Kalinga C., William G., Albane F, Chison M, Javier G, 2018. Intergrated cost-benefit analysis of tsetse control and herd productivity to inform control programmes for animal African trypanosomiasis. Parasites and Vectors.

Balmand, L.C., Aksoy S, Heddi A, 2013. Tissue distribution and transmission routes for the tsetse fly endosymbionts. J Invertebr Pathol, 116-122.

Balmer, O., Beadell, J.S., Gibson W, Caccone A, 2011. Phylogeography and Taxonomy of *Trypanosoma brucei.* Plos Neglected Tropical Diseases 5, e961. Doi: 10.1371/journal.pntd.0000961.

Baum, S., C. Machalaba, P. Daszak, RH. Salerno, WB. Karesh, 2017. Evaluating one health; are we demonstrating effectiveness? One Health, 5-10.

Bealby, C.R.J.a.R.G.J., 1996. Trypanosomiasis in Goats in Zambia. International Livestock Research Institute Nairobi.

Blum, S.C., Burri C, 2006. Clinical aspects of 2541 patients with seconf stage HAT. Acta Tropical 97, 54-64.

Boulangé, A., Lejon, V., Berthier, D., Thévenon, S., Gimonneau, G., Desquesnes, M., Abah, S., Agboho, P., Chilongo, K., Gebre, T., Fall, A.G., Kaba, D., Magez, S., Masiga, D., Matovu, E., Moukhtar, A., Neves, L., Olet, P.A., Pagabeleguem, S., Shereni, W., Sorli, B., Taioe, M.O., Tejedor Junco, M.T., Yagi, R., Solano, P., Cecchi, G., 2022. The COMBAT project: controlling and progressively minimizing the burden of vector-borne animal trypanosomosis in Africa. Open Research Europe 2. Doi:10.12688/openreseurope.14759.1

Bukachi, S., Wandibba S, Nyamongo IK, 2009. The Treatment Pathways followed by cases of Human African Trypanosomiasis in Western Kenya and Eastern Uganda. Ann Trop Med Parasitol 103, 200-211.

Buscher P, Mumba Ngoyi D, Kabore´ J, Lejon V, Robays J, et al. 2009. Improved Models of Mini Anion Exchange Centrifugation Technique (mAECT) and Modified Single

Centrifugation (MSC) for Sleeping Sickness Diagnosis and Staging. PLoS Negl Trop Dis 3(11): e471. doi:10.1371/journal.pntd.0000471

Busher, L.V., Magnum E, Van Meirvenne N, 1999. Improved latex agglutination test for detection of antibodies in serum and CSF of *T. b. gambiense* infected patients. Acta Trop 73, 11-20.

Chappuis, S.E., Adams K, Kidane S, Pittet A, Bovier PA, 2004. Chappuis F, Stivanello E, Adams K, Ki Card agglutination test for trypanosomisis end-dilution titer and cerebrospinal fluid cell count as predictors of human African trypanosomiasis (*T. b. r*) among serologically susp. Am J Trop Med Hyg 71, 313-317.

Cox, T.A., McOdimba F, Fyfe J, Eisler M, Hide G, Welburn S, 2005. Cox A, Tilley A, McOdimba F, Fyfe J, A PCR based assay for detection and differentiation of African trypanosome species in blood. Experimental parasitology 22, 24-29.

CSO, 2019. Preliminary Livestock and Aquaculture Census Results. Central Statistics Office-Republic of Zambia, Lusaka.

Dale, W.S., 2001. The endosymbionts of tsetse flies: Manipulating host-parasite interactions. International journal of parasitology, 628-631.

Engels, S.L., 2006. Reconsidering the underestimated burden caused by neglected tropical diseases. Trends Parasitology 22, 363-366.

FAO, 2017. Intervening against bovine trypanosomosis in eastern Africa: mapping the costs and benefits. Food and Agriculture Organization of the United Nations Rome,.

Fevre, C.P., Odiit M, Magona JW, Welburn SC, 2001. Fevre EM, Coleman PG, Odiit M, M. The origins of a new *Trypanosoma brucei rhodesiense* sleeping sickness outbreak in eastern Uganda. Lancet, 625-628.

Franco, J.R., Cecchi, G., Paone, M., Diarra, A., Grout, L., Kadima Ebeja, A., Simarro, P.P., Zhao, W., Argaw, D., 2022. The elimination of human African trypanosomiasis: Achievements in relation to WHO road map targets for 2020. PLOS Neglected Tropical Diseases 16, e0010047. doi:10.1371/journal.pntd.0010047

Franco, J.R., Cecchi, G., Priotto, G., Paone, M., Diarra, A., Grout, L., Simarro, P.P., Zhao, W., Argaw, D., 2020a. Monitoring the elimination of human African trypanosomiasis at continental and country level: Update to 2018. PLOS Neglected Tropical Diseases 14, e0008261. doi:10.1371/journal.pntd.0008261

Franco, J.R., Cecchi, G., Priotto, G., Paone, M., Diarra, A., Grout, L., Simarro, P.P., Zhao, W., Argaw, D., 2020b. Monitoring the elimination of human African trypanosomiasis at

continental and country level: Update to 2018. PLoS Negl Trop Dis 14, e0008261. doi:10.1371/journal.pntd.0008261

Franco, J.R., Simarro, P.P., Diarra, A., Ruiz-Postigo, J.A., Samo, M., Jannin, J.G., 2012. Monitoring the use of nifurtimox-eflornithine combination therapy (NECT) in the treatment of second stage gambiense human African trypanosomiasis. Research and reports in tropical medicine 3, 93-101. doi:10.2147/rrtm.s34399

Frean, J., Sieling, W., Pahad, H., Shoul, E., Blumberg, L., 2018. Clinical management of East African trypanosomiasis in South Africa: Lessons learned. Int J Infect Dis 75, 101-108. doi:10.1016/j.ijid.2018.08.012

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. doi:10.1017/S0031182016001268

Hide, G., 1999. History of Sleeping sickness in East Africa. Clin Micro Rev 12, 112-125.

John., H., Werren, Laura baldo, Michael E Clark, 2008. *Wolbachia*: Master manipulators of Invertebrate biology. Nature Reviews Microbiology, 741-751.

Jordan, A.M., 1986. Trypanosomiasis control and African rural development. Longman Inc New York.

Kakumbi, 2014. Annual Report. Tsetse and Trypanosomiasis Research Station-Ministry of Fisheries and Livestock, Mambwe, Zambia.

Kakumbi, 2017. Annual Report. Tsetse and Trypanosomiasis Research Station-Ministry of Fisheries and Livestock, Mambwe, Zambia.

Kamba Mebourou, E., Bourquin, M., Oloo, F., Vallat, A., Guerin, P.M., 2020. Deltamethrin Persistence on Textiles Used to Make Visual Targets for Tsetse Fly Control is Positively Correlated With Their Polyester Content. Journal of Medical Entomology 57, 1525-1531. doi:10.1093/jme/tjaa057

Katsidzira, F.G., 2010. KatsiPitfalls in the diagnosis of Trypanosomiasis in low endemic countries: A case Report. PLos Negl Trop Dis 4, 823.

Kazumba, L.M., Kaka, J.T., Ngoyi, D.M., Tshala-Katumbay, D., 2018. Mortality trends and risk factors in advanced stage-2 Human African Trypanosomiasis: A critical appraisal of 23 years of experience in the Democratic Republic of Congo. PLoS Negl Trop Dis 12, e0006504. doi:10.1371/journal.pntd.0006504

Kgori, M.S., Torr SJ, 2006. The use of aerial spraying to eliminate tsetse from the Okavango Delta of Botswana. Acta Tropica 99, 184-199.

Laohasinnarong, D., Goto, Y., Asada, M., Nakao, R., Hayashida, K., Kajino, K., Kawazu, S., Sugimoto, C., Inoue, N., Namangala, B., 2015. Studies of trypanosomiasis in the Luangwa valley, north-eastern Zambia. Parasites and Vectors 8, 555. doi:10.1186/s13071-015-1173-y

Legros, 2002. Treatment of human African trypanosomiasis-present situation and needs for research and development. Lancet Infectious Diseases 2, 437-440.

Lisulo, M., Sugimoto, C., Kajino, K., Hayashida, K., Mudenda, M., Moonga, L., Ndebe, J., Nzala, S., Namangala, B., 2014. Determination of the prevalence of African trypanosome species in indigenous dogs of Mambwe district, eastern Zambia, by loop-mediated isothermal amplification. Parasites and Vectors 7, 19. doi:10.1186/1756-3305-7-19

Lord, J.S., Lea, R.S., Allan, F.K., Byamungu, M., Hall, D.R., Lingley, J., Mramba, F., Paxton, E., Vale, G.A., Hargrove, J.W., Morrison, L.J., Torr, S.J., Auty, H.K., 2020. Assessing the effect of insecticide-treated cattle on tsetse abundance and trypanosome transmission at the wildlife-livestock interface in Serengeti, Tanzania. PLOS Neglected Tropical Diseases 14, e0008288. doi:10.1371/journal.pntd.0008288.

Lumsden, W. H. R., Kimber, C. D., Evans, D. A. & Doig, S. (1979). *Trypanosoma brucei*: miniature anion-exchange/centrifugation technique for detection of low parasitaemias: adaptation for field use. Transactions of the Royal Society of Tropical Medicine and Hygiene, 73, 312-317.

Lutumba, P., Robays J, Miaka C, Kande V, Molisho D, Declereq J, 2005. Trypanosomiaisis control, Democratic Republic of Congo, 1993-2003. Emerging Infectious Diseases 11, 1382-1388.

MacLean, O.M., Chisi JE, Kennedy PGE, Sternberg JM, 2010. MacLean LM, Odiit M, Chisi JE, Kenne Focus–Specific Clinical Profiles in Human African Trypanosomiasis Caused by *Trypanosoma brucei rhodesiense*. PLos Neglected Tropical Diseases 4, 906.

Maclennon, K., 1981. Tsetse transmitted trypanosomiasis in retation to rural economy in Africa: Part 2. Techniqiues in use for the control or eradication of tsetse infestations. Wld Anim. Rev, 9-19.

Mbewe, J.N., Lungowe Sitali., Boniface Namangala., Charles Michelo, 2015. Adherence to the Food and Agricultural Organization guidelines on trypanocide usage among cattle farmers in Itezhi tezhi, Central Zambia. Veterinary Parasitology, 43-49.

Mbewe, N.J., Sitali, L., Namangala, B., Michelo, C., 2015. Adherence to the Food and Agricultural Organization guidelines on trypanocide usage among cattle farmers in Itezhi tezhi, Central Zambia. Veterinary Parasitology 209, 43-49. doi:10.1016/j.vetpar.2015.02.012

Meyer, A., Holt, H.R., Selby, R., Guitian, J., 2016. Past and Ongoing Tsetse and Animal Trypanosomiasis Control Operations in Five African Countries: A Systematic Review. PLoS Neglected Tropical Diseases 10, e0005247. doi:10.1371/journal.pntd.0005247

Mulenga, G.M., Henning, L., Chilongo, K., Mubamba, C., Namangala, B., Gummow, B., 2020. Insights into the Control and Management of Human and Bovine African Trypanosomiasis in Zambia between 2009 and 2019-A Review. Tropical Medicine and Infectious Diseases 5, 115. doi:10.3390/tropicalmed5030115

Mulenga, G.M., Namangala, B., Chilongo, K., Henning, L., Gummow, B., 2021a. Policy and Linkages in the Application of a One Health System for Reporting and Controlling African Trypanosomiasis and Other Zoonotic Diseases in Zambia. MDPI-Pathogens 11, 1. doi:10.3390/pathogens11010030

Mulenga, G.M., Namangala, B., Chilongo, K., Mubamba, C., Hayashida, K., Henning, L., Gummow, B., 2021b. Challenges in the Diagnostic Performance of Parasitological and Molecular Tests in the Surveillance of African Trypanosomiasis in Eastern Zambia. Tropical Medicine and Infectious Diseases 6, 68.

Mulenga, R.N.L., Boniface Namangala, 2015. Assessing the capacity to diagnose human African tryoanosomiasis among health care personnel from Chama and Mambwe districts of eastern Zambia. Biomed Central research notes, 1-7.

Mwanakasale, V., Songolo, P., 2011. Disappearance of some human African trypanosomiasis transmission foci in Zambia in the absence of a tsetse fly and trypanosomiasis control program over a period of forty years. Transactions of the Royal Society of Tropical Medicine and Hygiene 105, 167-172. doi:10.1016/j.trstmh.2010.12.002

Mwanakasale, V., Songolo, P., Daka, V., 2013. Challenges in the control of human African trypanosomiasis in the Mpika district of Zambia. BMC Research Notes 6, 180.

Mwanakasale, V., Songolo Peter, 2011. Disapperance of some Human African Trypanosomiasis transmission foci in Zambia in the absence of tsetse fly and trypanosomiasis control program over a period of forty years. Transactions of the Royal Society of Tropical Medicine and Hygiene, 167-172.

Mweempwa C., T.M., Claudia De Pus, Penzhorn B.L., Dick A.H., Bouyer J., De Deken R., 2015. Impact of habitat fragmentation on tsetse populations and trypanosomiasis risk in eastern Zambia. Parasites and Vectors.

Mwiinde, A., Simuunza M, Namangala B, Chitalu M, Machila N, Anderson N, Welburn SC, 2017. Estimating the economic and social coonsequences for patients diagnosed with human african trypanosomiasis in Muchinga, Lusaka and Eastern provinces of Zambia (2004-2014). Infectious Disseases of Poverty, 150.

Ndeledje, N., Bouyer, J., Stachurski, F., Grimaud, P., Belem, A.M., Molele Mbaindingatoloum, F., Bengaly, Z., Oumar Alfaroukh, I., Cecchi, G., Lancelot, R., 2013. Treating cattle to protect people? Impact of footbath insecticide treatment on tsetse density in Chad. PloS one 8, e67580.

Njiru, Z., 2012. Loop-Mediated Isothermal Amplification Technology: Towards Point of Care Diagnostics. PLos Negl Trop Dis, 1572.

Odiit., C.P., Liu W, McDermolt J, Fevre E, Welburn SC, Woolhouse MEJ, 2005. Quantifying the level of under detection of *T. b. r*. sleeping sickness cases. Tropical Medicine and International Health 10, 840-849.

PAAT, 2022. Programme Against African Trypanosomiasis. Food and Agriculture Organization of the United Nations.

Pais, 2001. The obligate mutualist *wigglesworthia glossinidia*. pubmed 3, 245-254.

Percoma, L., Rayaisse, J.B., Gimonneau, G., Bengaly, Z., Pooda, S.H., Pagabeleguem, S., Ganaba, R., Sow, A., Argiles, R., Bouyer, J., Ouedraogo, M., Zhao, W., Paone, M., Sidibe, I., Gisele, O., Cecchi, G., 2022. An atlas to support the progressive control of tsetse-transmitted animal trypanosomosis in Burkina Faso. Parasites and Vectors 15, 72. doi:10.1186/s13071-021-05131-4

Priotto., G., Kasparian S, Mutombo W, Ngouama D, Ghorashian S, Arnold U, Ghabri S, Baudin E, Buard V, Kazadi-Kyanza S, Ilunga M, Mutangala W, Pohlig G, Schmid C, Karunakara U, 2009. Nifurtimox-efl ornithine combination therapy for second-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. Lancet, 56-64.

Rayaisse, J.B., Courtin, F., Mahamat, M.H., Cherif, M., Yoni, W., Gadjibet, N.M.O., Peka, M., Solano, P., Torr, S.J., Shaw, A.P.M., 2020. Delivering 'tiny targets' in a remote region of southern Chad: a cost analysis of tsetse control in the Mandoul sleeping sickness focus. Parasites & Vectors 13, 419. doi:10.1186/s13071-020-04286-w

Ricci, M.V., Ulisse Ulissi, Sara Epis, Alessia Cappelli, Guido Favia, 2012. Symbiotic Control of mosquito borne disease. Pathogens and Global Health 106, 380-385.

Richard, L., David HW, Peter FW, 2006. Tropical Infectious Diseases: Principles, pathogens and practice. Elsevier Churchill Livingstone 2, 1072-1080.

Richter, J., Gobels, S., Gobel, T., Westenfeld, R., Muller-Stover, I., Haussinger, D., 2012. A returning traveller with fever, facial swelling, and skin lesions. BMJ 344, e2092. doi:10.1136/bmj.e2092

Rio, R.E.S., Jingwen Wang, Claudia Lohs, Yi-neng Wu, Anna K.Snyder, Robert D.Bjornson, Kenshiro Oshima, Bryan S.Biehi, Nicole T.Perna, Masahira Hattori, Serap Aksoy, 2012. Insight into the Transimission Biology and Species-Specific Functional Capabilities of Tsetse (Diptera: *Glossinidae*) Obligate symbiont *Wigglesworthia*. Mbio.asm.org, 1-13.

Robays, B.M., Van der Stuyft P, Boelaert M, 2004. The effectiveness of active population screening and treatment for sleeping sickness control in the Democratic Republic of Congo. Trop Med Int Health 9, 542-550.

Rostal, K., Noam Ross, Catheerine Machalaba, Claude Cordel, Janusz T. Paweska, William B. Karesh, 2018. Benefits of a one health approach: An example using Rift Valley Fever. EcoHealth Alliance.

Ruiz, J.P., Hamisi S. Nyingilili, Geofrey H. Mbata, and Imna I. Malele, 2015. The role of domestic animals in the epidemiology of human african trypanosomiasis in Ngorongoro conservation area, Tanzania. Parasites and Vectors.

Shaw, A.P., Cecchi, G., Wint, G.R., Mattioli, R.C., Robinson, T.P., 2014. Mapping the economic benefits to livestock keepers from intervening against bovine trypanosomosis in Eastern Africa. Preventive Veterinary Medicine 113, 197-210. doi:10.1016/j.prevetmed.2013.10.024

Shereni, W., Neves, L., Argiles, R., Nyakupinda, L., Cecchi, G., 2021. An atlas of tsetse and animal African trypanosomiasis in Zimbabwe. Parasites and Vectors 14, 50. doi:10.1186/s13071-020-04555-8

Simukoko, H., Marcotty J, Phiri I, Geysen D, Vercruysee J, P. Vanden Bossche, 2007. The comparative role of cattle, goats and pigs in epidemiology of livestock trypanosomiasis on plateau of eastern Zambia. Veterinary Parasitology, 231-238.

Solano, J.V., Guessan PN, 2002. Comparison of different DNA preparation protocols for PCR diagnosis of HAT in Cote d'ivoire. Acta Trop 82, 349-356.

Stich, B.M., Krishna S, 2003. Waking up to sleeping sickness. Trends in parasitology 19, 195-197.

Swallow, B.M., 2000. Impacts of Trypanosomiasis on African Agriculture. PAAT Technical and Scientific series 2-FAO, Rome.

T. Tekle., G.T., T. Cherenet, H.Ashenafti, 2018. Aberrant use and poor quality of trypanocides: a risk for drug resistance in southern western Ethopia. BMC Veterinary Research.

Thumbi, M.F., Mosi RO, Jung'a JO, 2008. Comparative evaluation of three PCR base diagnostic assays for the detection of pathogenic trypanosomes in cattle blood. Parasites and Vectors 1, 1-46.

Toh, W.B., Perkin SAH, Yamashita A, Oshima K, Hattori M, et al, 2006. Massive genome erosion and functional adaptations provide insight into the symbiontic lifestyle of *Sodalis glossinidius* in the tsetse host. Genome Research, 149-156.

Toju, H.T., Koga R, Nikoh N, Meng XY, Kimura N, et al, 2010. "*Candidalus curculioniphilus bucheneri*" a novel clade of bacterial endocellular symbionts from weevils of the genus curculio. Applied Environmental Microbiology, 275-282.

Torr, S., Vale G, Hall D, 2007. Tsetse Biology.

Truc, J.V., Cuny G, Frezil JL, 1999. Truc P, Jamonneau V, Cuny G, anUse of polymerase chain reaction in HAT stage determination and follow-up. Bull WH 77, 745-748.

Utarini, A., Indriani, C., Ahmad, R.A., Tantowijoyo, W., Arguni, E., Ansari, M.R., Supriyati, E., Wardana, D.S., Meitika, Y., Ernesia, I., Nurhayati, I., Prabowo, E., Andari, B., Green, B.R., Hodgson, L., Cutcher, Z., Rances, E., Ryan, P.A., O'Neill, S.L., Dufault, S.M., Tanamas, S.K., Jewell, N.P., Anders, K.L., Simmons, C.P., Group, A.S., 2021. Efficacy of *Wolbachia*-Infected Mosquito Deployments for the Control of Dengue. The New England Journal of Medicine 384, 2177-2186. doi:10.1056/NEJMoa2030243

Vale, G., 1993. Visual responses of tsetse flies (*Diptera: Glossinidae*) to odour-baited targets. Bull Entomol Res 83. doi:10.1017/S0007485300034775

Vale, G.A., Hargrove J.W., Chamisa A., Grant Ian F., Stephen J. Torr, 2015. Pyrethroid treatment of cattle for tsetse control-reducing its impacts on dung fauna. PLOS Neglected Tropical Diseases.

Vale., G.A., Hargrove J.W., Michael J. Lehane., Philippe Solano., Stephan J. Torr, 2015. Optimal strategies for controlling riverine tsetse flies using targets: a modelling study. PLOS Neglected Tropical Diseases, 1-20.

Van den Bossche, P., Doran M, Connor RJ, 2000. An analysis of trypanocidal drug use in the Eastern province of Zambia. Acta Tropica, 247-258.

Van Meirvenne, N., 1992. Diagnosis of Human African Trypanosomiasis. Ann Soc Belg Med Trop 72, 53-56.

Vreysen, M.J., Seck, M.T., Sall, B., Bouyer, J., 2013. Tsetse flies: their biology and control using area-wide integrated pest management approaches. Journal of Invertebrate Pathology 112 Suppl, S15-25. doi:10.1016/j.jip.2012.07.026

Wamiri, F., Uzma Alam, Paul C Thande, Emre Aksoy, Raphael M Ngure, Serap Aksoy, Johnson O Ouma, Grace A Murilla, 2013. *Wolbachia, Sodalis* and Trypanosome co-infections in natural populations of *Glossina austeni* and *Glossina Pallidipes*. Parasites and Vectors, 232.

Wastling, P.K., Kakembo ASL, Welburn SC, 2010. LAMP for Human African Trypanosomiasis: A Comparative Study of Detection Formats. PLos Negl Trop Dis 4, 865.

Weiss, J.W., Michele A, Maltz Yineng Wu, Serap Aksoy, 2013. Trypanosome infection establishment in tsetse fly gut is influenced by microbiome-regulated host immune barriers. PLos-Pathogens.

Welburn, O.M., 2002. Recent Developments in Human African Trypanosomiasis. Curr Opin Infect Dis 15, 477-484.

Welburn., S., Arnold K, Maudlin I, Gooday GW, 1993. Rickettsia-like organims and chitinase production in relation to transmission of trypanosomes by tsetse flies. Parasitology, 141-145.

WHO, 2022a. Ending the neglect to attain the Sustainable Development Goals One Health: Approach for action againt neglected tropical diseases 2021–2030. World Health Organization, Geneva.

WHO, 2022b. Fact sheet-Human african trypanosomiais. Available online: https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness) (accessed on 30 April 2022).

WHO, 2022c. World Health Organization (sleeping sickness): Epidemiological update. WER, 71-80.

CHAPTER 2

INSIGHTS INTO THE CONTROL AND MANAGEMENT OF HUMAN AND BOVINE AFRICAN TRYPANOSOMIASIS IN ZAMBIA BETWEEN 2009 AND 2019—A REVIEW

Publication

Mulenga GM, Henning L, Chilongo K, Mubamba C, Namangala B, Gummow B. Insights into the Control and Management of Human and Bovine African Trypanosomiasis in Zambia between 2009 and 2019-A Review. MDPI Tropical Medicine Infectious Diseases. 2020; 5:3.

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Abstract: Tsetse transmitted trypanosomiasis is a fatal disease commonly known as *Nagana i*n cattle and sleeping sickness in humans. The disease threatens food security and has severe economic impact in Africa including most parts of Zambia. The level of effectiveness of commonly used African trypanosomiasis control methods has been reported in several studies. However, there have been no review studies on African trypanosomiasis control and management conducted in the context of One Health. This paper therefore seeks to fill this knowledge gap. A review of studies that have been conducted on African trypanosomiasis in Zambia between 2009 and 2019, with a focus on the control and management of trypanosomiasis was conducted. A total of 2238 articles were screened, with application of the search engines PubMed, PubMed Central and One Search. Out of these articles, 18 matched the required criteria and constituted the basis for the paper. An in-depth analysis of the 18 articles was conducted to identify knowledge gaps and evidence for best practices. Findings from this review provide stakeholders and health workers with a basis for prioritisation of African trypanosomiasis as an important neglected disease in Zambia and for formulation of One Health strategies for better control and/or management of the disease.

Keywords: trypanosomiasis; control; management; One Health; Zambia

2.1 INTRODUCTION

African trypanosomiasis is endemic to Sub-Saharan Africa and continues to threaten human health and food security. African trypanosomiasis has been a major draw-back to agriculture and economic development in affected countries, with annual losses in agricultural gross domestic product estimated at US$4.7 billion (Swallow, 2000; Holt et al., 2016). The current strategy of the Zambian government to preserve natural resources and create state protected National Parks (NPs) and Game Management Areas (GMAs) has led to an expansion of wildlife population that serve as long term reservoirs for African trypanosomiasis, and also to an increase in the population of tsetse flies that transmit the disease (Munang'andu et al., 2012). At the same time, increase in human population density and the changing climate, particularly rainfall patterns, have forced people (and their livestock) to migrate into these GMAs in search of fertile land for farming. Such uncontrolled migration of people into protected areas has brought about changes in land use patterns that threaten to alter tsetse habitat quality and patterns of African trypanosomiasis transmission due to increased tsetse-human and tsetse-livestock contacts (Muriuki et al., 2005; Anderson et al., 2011; Anderson et al., 2015).

Tourists visiting NPs and GMAs have not been spared from risks of Human African Trypanosomiasis (HAT) infections occurring through transmission from wildlife reservoir hosts (Muriuki et al., 2005). Despite cases of HAT reported from tourists after their visit to Zambia's NPs, (Richter et al., 2012; Frean et al., 2018) there are gaps in protecting tourists and international travellers from tsetse and HAT. Some tour operators have taken it upon themselves to undertake some interventions, particularly in the form of tsetse control, aimed at reducing the risk of HAT infection among tourists visiting their facilities. Such limited interventions produce very limited levels of effectiveness or success, considering that such interventions need to cover considerable large proportions of the affected areas and as such require the collective input of many key stakeholders (Kakumbi, 2017).

Tsetse flies are found in about 37% of Zambia's land area, and it is estimated that the prevalence of African animal trypanosomiasis (AAT) in cattle ranges from 1% to 90% depending on the area (Richter et al., 2012). Most of the affected areas are located in rural remote parts of the country and as such the direct negative impacts of the trypanosomiasis problem occur in communities that live in these areas. These impacts include serious economic

consequences such as reduced livestock productivity and mortality and the high cost of treating affected livestock (CSO, 2017; Mwiinde et al., 2017).

In Zambia, AAT has been managed through constant use of trypanocides by individual livestock farmers, while treatment and/or management of the disease in humans has been negatively affected by several factors that include late case detection that tends to result in tragic consequences (death) associated with adverse effects of the administered drugs in the late stage (Mbewe et al., 2015b; WHO, 2018). The Zambian government has generally made some notable strides in the control of African trypanosomiasis particularly through tsetse control. However, the government's inability to put in place active surveillance systems, and the lack of adequate resources to effectively sustain control efforts, have contributed to limitation of success associated with tsetse re-invasion and resurgence of African trypanosomiasis in areas where the disease had earlier been brought under control. In the case of HAT, lack of active surveillance systems has historically hindered progress towards the goal of eliminating African trypanosomiasis as a public health problem in Zambia (Mwanakasale and Songolo, 2011a; Franco et al., 2020).

The period between 2009 to 2019 has seen a significant number of undertakings focused largely on the parasite, transmission, and epidemiology of African trypanosomiasis. However, no systematic review of the literature has been conducted on the control and management of African trypanosomiasis in Zambia particularly from a One Health perspective. This review seeks to address this knowledge gap.

2.2 MATERIALS AND METHODS

With a focus on studies conducted on HAT and AAT control in Zambia, a systematic review (Figure A1 in Appendix) of published data was undertaken. Using three searches with three categories of key words, a cumulative total of 2238 peer reviewed articles were identified in December 2019 from the following three search engines: PubMed, PubMed Central and One Search. One Search was used because it has a wider research area while PubMed was used because it is more aligned with veterinary sciences. Using the following key words: trypanosomiasis AND control AND management AND One Health AND Zambia, 610 articles were identified. In addition, two independent searches were done using key words: trypanosomiasis AND control AND Zambia (995 articles identified), trypanosomiasis AND control AND One Health AND Zambia (633 articles identified). Duplicate articles were

removed after which remaining articles were screened by title and abstract to assess the relevance of documents. Articles related to biochemical and biological developments in tsetse and African trypanosomiasis diagnostic assays were excluded from the review as most of the articles were focused on the trypanosome agent rather than management and control. Inclusion criteria were as follows: (i) studies conducted on the control and management of African trypanosomiasis in Zambia, (ii) related to One Health, (iii) related to African trypanosomiasis diagnostic methods, (iv) published in English only, and (v) published between January 2009 and December 2019. A final full text screening from the search conducted left 18 articles that met the inclusion criteria for the review (Table B1 in Appendix). To support and supplement data from articles included in the review, published and unpublished government records and reports related to tsetse and African trypanosomiasis control for the same period were also referenced.

This review was conducted as part of a PhD project with ethical clearances from James Cook University (H7226 and A2498), Zambian Ethics Committee (Ref. No. 2018-Oct-001) and research approval from the Zambia National Health Research Authority.

2.3 RESULTS

Based on the analysis of publications included in this review (Table B1 in Appendix), results indicate that various trypanosome species circulate within a wide and diverse host community in Zambia (Anderson et al., 2011). The presence of the tsetse fly has facilitated the circulation of the parasite in the ecosystem (Laohasinnarong et al., 2015). Movement of people has led to the development of a new wildlife/livestock /human interface (Anderson et al., 2011; Alderton et al., 2016) *T. congolense* and *T. vivax* are the major causes of clinical AAT in cattle with low PCV usually an indicator of infection (Marcotty et al., 2008; Simukoko et al., 2011; Mweempwa., 2015). Infections with *T.b.r* in domestic animals remained a significant indicator that domestic animals could be reservoirs of HAT. Findings show that the impact of AAT is highest in cattle with dogs becoming a potential reservoir host for the human disease (Lisulo et al., 2014; Laohasinnarong et al., 2015).

Current diagnostic methods used in Zambia do not conform to what is now thought to be the best practice (Simukoko et al., 2011; Namangala et al., 2012; Mbewe et al., 2015a). Diagnosis of African trypanosomiasis remains a challenge in endemic areas of Zambia due to low staffing levels and non-functional laboratories (Mwanakasale et al., 2013; Mulenga et al., 2015).

Food security for communities living in tsetse-infested areas has continued to be negatively impacted (Grant et al., 2015). The impact of AAT can be reduced through use of trypanocides and application of insecticide to control tsetse flies. Cattle farmers living in African trypanosomiasis-endemic areas and GMAs have resorted to drastic use of trypanocides to combat the disease (Livestock, 2016). African trypanosomiasis control in Zambia have been focused on cattle and not humans (Grant et al., 2015), with nothing published on the control and management of the disease in other domestic animals. Wildlife trypanosomiasis hosts pose a risk to communities and tourists living near or in national parks and game reserves (Richter et al., 2012; Holt et al., 2016; Frean et al., 2018).

Despite Zambia having had three major African trypanosomiasis control programmes (aerial spraying, insecticide treated targets and trypanocide drug use), the country has recorded several disease re-occurrences in areas where control was once undertaken. New cases are being reported in new areas while some old foci are disappearing (Mwanakasale and Songolo, 2011b; Meyer et al., 2016).. Despite the evidence of the occurrence of African trypanosomiasis in both humans and livestock and the challenges faced by communities living in tsetse-infested areas, there is no One Health approach to control the disease (Grant et al., 2015; Laohasinnarong et al., 2015; Holt et al., 2016).

A weak health system is in place for the management of HAT. Knowledge of HAT management among health workers is unsatisfactory (Mulenga et al., 2015). Wide diversity of control programmes are available but lack government support (Mwanakasale and Songolo, 2011a; Mwanakasale et al., 2013; Mulenga et al., 2015; Meyer et al., 2018). Stakeholders in Zambia have competing views and beliefs regarding tsetse and African trypanosomiasis control, which is critical in developing a One Health approach for the control in both HAT and AAT. Environmentalists believe tsetse flies help keep environments wild and natural by stopping farmers encroaching protected areas. Agriculturalists feel that such moves have contributed to increased poverty as farmers are kept away from protected areas that are tsetse-infested (Grant et al., 2015).

2.4 DISCUSSION

The Luangwa and Zambezi River basins support high densities of tsetse flies and wildlife reservoirs of African trypanosomiasis (Anderson et al., 2011). This review of tsetse and African trypanosomiasis studies undertaken in Zambia clearly indicates that most of these studies

have been undertaken from or along the peripherals of the two river basins. With an estimated 37% of Zambia's land area tsetse-infested, the risk of African trypanosomiasis infection for people and livestock living in the tsetse-infested areas in the country cannot therefore be overemphasized (CSO, 2017).

An assessment by the World Health Organization (WHO) indicated that HAT usually affected people whose occupations took them into tsetse-infested areas. Categories of people so affected include among others: small scale farmers, workers under wildlife services, tsetse control workers, poachers, honey gatherers and fishermen (WHO 2018). Increased human populations and thus increased demand for land for agriculture continues to force people and their livestock into tsetse-infested areas in search for fertile land. Migration of people with their livestock into tsetse-infested areas, as highlighted in this review, has resulted in changes in the epidemiology of African trypanosomiasis. Livestock rearing in these tsetse-infested areas has thus eroded the diverse ecosystems and led to the development of a new kind of wildlife/livestock/human interface with domestic animals acting as potential link for trypanosome exchange (Anderson et al., 2011; Laohasinnarong et al., 2015; Squarre et al., 2016).

The risk of HAT infection in travellers to national parks and game reserves has however not received much attention. Despite reported cases of HAT from tourists after visiting tsetse-infested areas (Richter et al., 2012; Frean et al., 2018), there are currently no deliberate interventions in place to protect international travellers from tsetse flies and HAT. In Zambia, most tsetse interventions have been focused in areas with potential for livestock production, with little synchronization with human intervention programmes (Nyimba et al., 2015; Alderton et al., 2018)

Currently, African trypanosomiasis control in humans relies on early diagnosis and treatment. However, challenges in HAT diagnosis in rural settings of Zambia has hindered progress to the control of the disease. Most diagnostic health centres in rural Zambia depend on microscopy for diagnosis. Despite the low sensitivity associated with microscopy, the test remains the gold standard for both HAT and AAT diagnosis because it is affordable. However, the low sensitivity exhibited by microscopy makes it difficult to determine disease incidences, especially in cases where parasitaemia is low, thus stressing the need to improve field diagnosis of African trypanosomiasis (Marcotty et al., 2008; Laohasinnarong et al., 2015; Mbewe et al., 2015a; Nyimba et al., 2015).

Recent developments of molecular tools such as Polymerase chain reaction (PCR) and Loop-mediated isothermal amplification (LAMP) for detecting trypanosomiasis has provided hope for improving field diagnosis which may lead to eliminating African trypanosomiasis (Delespaux et al., 2008; Njiru et al., 2008; Njiru, 2012). LAMP has been proven to be more sensitive than microscopy in detecting infections of *T. brucei* and *T. vivax* as compared to *T. congolense.* Such findings indicate the importance of LAMP in epidemiological studies related to HAT rather than AAT. The simplicity and sensitivity of LAMP makes it an ideal diagnostic tool for HAT (Namangala et al., 2012; Laohasinnarong et al., 2015; Nyimba et al., 2015). On the other hand, multispecies PCR can identify several species of trypanosomes in a single PCR reaction, thus reducing the cost of molecular diagnosis. The main advantage of molecular tools over microscopy is for epidemiological studies and to identify different trypanosome species (Njiru et al., 2005; Picozzi et al., 2008; Ahmed et al., 2013) other than point of care diagnostic tools. Limited support from relevant authorities has negatively impacted on the use of molecular methods in Zambia. Most molecular laboratory consumables cannot be sourced locally, therefore, procurement of consumables has remained a challenge even for institutions that have implemented the use of molecular tools.

For continued efforts to control African trypanosomiasis infections, there is a need to establish strong active and passive surveillance systems in African trypanosomiasis focal point areas. In the absence of diagnostic centres as seen in most rural settings of Zambia, departments of Health and Veterinary services can share resources, diagnostic capacities and personnel for improved case detection, treatment and control of African trypanosomiasis and other zoonotic diseases. Future control efforts for HAT may also consider simultaneous control of the disease in livestock and wildlife reservoirs as a One Health approach (Mwanakasale et al., 2013; Grant et al., 2015).

Meanwhile, lack of political commitment to sustain tsetse and African trypanosomiasis control programmes (Mulenga et al., 2016) has pushed livestock farmers to constant use of trypanocides. The study conducted by Mbewe et al (Mbewe et al., 2015b) confirms that livestock farmers living in GMAs or near NPs where tsetse challenge is high have resorted to constant trypanocide use to protect their livestock, which may have serious consequences related to trypanosome resistance to trypanocides (Van den Bossche, 2000). Treatment of infected animals may seem to be the best option for most livestock farmers, but it may tend to be unsustainable and costly in the long run as AAT is largely a herd health problem (Tsetse

and Trypanosomiasis section strategic plan 2020, Zambia-unpublished Government record). Unfortunately, most farmers living in tsetse-infested areas treat their animals based on clinical signs and symptoms due to lack of access to laboratories and regular surveys from local veterinarians. In this case, most infections remain in their livestock populations and may be responsible for sustaining sporadic African trypanosomiasis incidences within their communities (Von Wissmann et al., 2011).

Earlier studies by Simukoko et al (Simukoko et al., 2011), indicate that livestock treatment with trypanocides is dependent on seasonal variations of tsetse populations and the risk of AAT infection. Such findings indicate the need for tsetse and AAT control programmes to be focused on seasonal differences in the risk of AAT infection when tsetse challenge is highest. Key stakeholders can therefore use such findings to link to biological characteristics of the tsetse vector in developing cost effective and sustainable control programmes during periods of highest challenge (Simukoko et al., 2011; Mweempwa et al., 2015). From a travel medicine perspective, such findings also highlight risk periods for travellers.

Increased focus on communicable and non-communicable disease management has pushed African trypanosomiasis off the Government's priority list. There is a need to holistically quantify the impact and cost of African trypanosomiasis again in the context of disease prioritisation within Zambia and similarly affected countries. Lack of sustainable control programmes and the absence of a national surveillance and control programme for African trypanosomiasis among others, have impacted negatively on control efforts (Mwanakasale et al., 2013; Grant et al., 2015; Mulenga et al., 2015; Mulenga et al., 2016). Breaking down barriers between social and natural scientists will help in developing a more holistic One Health approach to control tsetse flies and African trypanosomiasis in Zambia. Lessons learnt from past tsetse and African trypanosomiasis control operations can be useful in developing future cost effective and sustainable control programmes as well as informing health practitioners as to the risks travellers face in visiting these travel destinations and the in-country health support system available to them.

2.5 RECOMMENDATIONS

It is recommended that:

- Work is done to evaluate and identify African trypanosomiasis control programmes that are cost effective and sustainable in the regions where they are applied.

- Data on biological characteristics of tsetse and seasonal differences in African trypanosomiasis infection risk be considered when developing tsetse and trypanosomiasis control programmes in Zambia.
- More robust field diagnostic procedures for African trypanosomiasis be developed that consider the environmental, capacity and infrastructure constraints of working in countries like Zambia.
- Line Ministries consider sharing resources in order to improve diagnosis and treatment of African trypanosomiasis and other zoonotic diseases.
- A One Health approach be considered for the control of African trypanosomiasis in humans, livestock, wildlife, and tsetse flies.

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2.6 REFERENCES

Ahmed, H.A., Picozzi, K., Welburn, S.C., MacLeod, E.T., 2013. A comparative evaluation of PCR- based methods for species- specific determination of African animal trypanosomes in Ugandan cattle. Parasites & Vectors 6, 316. doi:10.1186/1756-3305-6-316

Alderton, S., Macleod, E.T., Anderson, N.E., Machila, N., Simuunza, M., Welburn, S.C., Atkinson, P.M., 2018. Exploring the effect of human and animal population growth on vector-borne disease transmission with an agent-based model of Rhodesian human African trypanosomiasis in eastern province, Zambia. PLoS Negl Trop Dis 12, e0006905. doi:10.1371/journal.pntd.0006905

Alderton, S., Macleod, E.T., Anderson, N.E., Schaten, K., Kuleszo, J., Simuunza, M., Welburn, S.C., Atkinson, P.M., 2016. A Multi-Host Agent-Based Model for a Zoonotic, Vector-Borne Disease. A Case Study on Trypanosomiasis in Eastern Province, Zambia. PLoS Negl Trop Dis 10, e0005252. doi:10.1371/journal.pntd.0005252

Anderson, N.E., Mubanga, J., Fevre, E.M., Picozzi, K., Eisler, M.C., Thomas, R., 2011. Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. PLoS Negl Trop Dis 5. doi:10.1371/journal.pntd.0001211

Anderson, N.E., Mubanga, J., Machila, N., Atkinson, P.M., Dzingirai, V., Welburn, S.C., 2015. Sleeping sickness and its relationship with development and biodiversity conservation in the Luangwa Valley, Zambia. Parasites & Vectors [Electronic Resource] 8, 224.

CSO, 2017. Preliminary Livestock and Agriculture Census Results. Central Statistics Office-Republic of Zambia, Lusaka.

Delespaux, V., Dinka, H., Masumu, J., Van den Bossche, P., Geerts, S., 2008. Five-fold increase in *Trypanosoma congolense* isolates resistant to diminazene aceturate over a seven-year period in Eastern Zambia. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy 11, 205-209. doi:10.1016/j.drup.2008.10.002

Franco, J.R., Cecchi, G., Priotto, G., Paone, M., Diarra, A., Grout, L., Simarro, P.P., Zhao, W., Argaw, D., 2020. Monitoring the elimination of human African trypanosomiasis at

298 continental and country level: Update to 2018. PLoS Negl Trop Dis 14, e0008261.
299 doi:10.1371/journal.pntd.0008261
300 Frean, J., Sieling, W., Pahad, H., Shoul, E., Blumberg, L., 2018. Clinical management of East
301 African trypanosomiasis in South Africa: Lessons learned. Int J Infect Dis 75, 101-108.
302 doi:10.1016/j.ijid.2018.08.012
303 Grant, C., Anderson, N., Machila, N., 2015. Stakeholder Narratives on Trypanosomiasis, Their
304 Effect on Policy and the Scope for One Health. PLoS Negl Trop Dis 9, e0004241.
305 doi:10.1371/journal.pntd.0004241
306 Holt, H.R., Selby, R., Mumba, C., Napier, G.B., Guitian, J., 2016. Assessment of animal African
307 trypanosomiasis (AAT) vulnerability in cattle-owning communities of sub-Saharan
308 Africa. Parasites & Vectors 9, 53.
309 Kakumbi, 2017. Annual Report. Tsetse and Trypanosomiasis Research Station-Ministry of
310 Fisheries and Livestock, Mambwe, Zambia.
311 Laohasinnarong, D., Goto, Y., Asada, M., Nakao, R., Hayashida, K., Kajino, K., Kawazu, S.,
312 Sugimoto, C., Inoue, N., Namangala, B., 2015. Studies of trypanosomiasis in the
313 Luangwa valley, north-eastern Zambia. Parasites and Vectors 8, 555.
314 doi:10.1186/s13071-015-1173-y
315 Lisulo, M., Sugimoto, C., Kajino, K., Hayashida, K., Mudenda, M., Moonga, L., Ndebe, J.,
316 Nzala, S., Namangala, B., 2014. Determination of the prevalence of African
317 trypanosome species in indigenous dogs of Mambwe district, eastern Zambia, by
318 loop-mediated isothermal amplification. Parasit Vectors 7, 19. doi:10.1186/1756-3305-
319 7-19
320 Marcotty, T., Simukoko, H., Berkvens, D., Vercruysse, J., Praet, N., Van den Bossche, P., 2008.
321 Evaluating the use of packed cell volume as an indicator of trypanosomal infections
322 in cattle in eastern Zambia. Preventive Veterinary Medicine 87, 288-300.
323 doi:10.1016/j.prevetmed.2008.05.002
324 Mbewe, N.J., Namangala, B., Sitali, L., Vorster, I., Michelo, C., 2015a. Prevalence of pathogenic
325 trypanosomes in anaemic cattle from trypanosomosis challenged areas of Itezhi-tezhi
326 district in central Zambia. Parasites and Vectors 8, 638. doi:10.1186/s13071-015-1260-0
327 Mbewe, N.J., Sitali, L., Namangala, B., Michelo, C., 2015b. Adherence to the Food and
328 Agricultural Organization guidelines on trypanocide usage among cattle farmers in
329 Itezhi tezhi, Central Zambia. Veterinary Parasitology 209, 43-49.
330 doi:10.1016/j.vetpar.2015.02.012

Meyer, A., Holt, H.R., Oumarou, F., Chilongo, K., Gilbert, W., Fauron, A., Mumba, C., Guitian, J., 2018. Integrated cost-benefit analysis of tsetse control and herd productivity to inform control programs for animal African trypanosomiasis. Parasites & vectors 11, 154. doi:10.1186/s13071-018-2679-x

Meyer, A., Holt, H.R., Selby, R., Guitian, J., 2016. Past and Ongoing Tsetse and Animal Trypanosomiasis Control Operations in Five African Countries: A Systematic Review. PLoS Neglected Tropical Diseases 10.

Mulenga, G., Bonface, N., Likwa, R., 2016. Capacity and Policy Change in Managing Trypanosomiasis in Endemic Rural Health Centres of Eastern Zambia. ARC Journal of Public Health and Community Medicine, 1-8. doi:10.20431/2456-0596.01040003

Mulenga, G.M., Likwa, R.N., Namangala, B., 2015. Assessing the capacity to diagnose human African trypanosomiasis among health care personnel from Chama and Mambwe districts of eastern Zambia. BMC Research Notes 8, 433.

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. Interdiscip Perspect Infect Dis 2012, 372523. doi:10.1155/2012/372523

Muriuki, G.W., Njoka, T.J., Reid, R.S., Nyariki, D.M., 2005. Tsetse control and land-use change in Lambwe valley, south-western Kenya. Agriculture, Ecosystems & Environment 106, 99-107. doi:10.1016/j.agee.2004.04.005

Mwanakasale, V., Songolo, P., 2011a. Disappearance of some human African trypanosomiasis transmission foci in Zambia in the absence of a tsetse fly and trypanosomiasis control program over a period of forty years. Transactions of the Royal Society of Tropical Medicine and Hygiene 105, 167-172. doi:10.1016/j.trstmh.2010.12.002

Mwanakasale, V., Songolo, P., 2011b. Disapperance of some Human African Trypanosomiasis transmission foci in Zambia in the absence of tsetse fly and trypanosomiasis control program over a period of forty years. Transactions of the Royal Society of Tropical Medicine and Hygiene, 167-172.

Mwanakasale, V., Songolo, P., Daka, V., 2013. Challenges in the control of human African trypanosomiasis in the Mpika district of Zambia. BMC Research Notes 6, 180.

Mweempwa, C., Marcotty, T., De Pus, C., Penzhorn, B.L., Dicko, A.H., Bouyer, J., De Deken, R., 2015. Impact of habitat fragmentation on tsetse populations and trypanosomosis risk in Eastern Zambia. Parasites & vectors 8, 406. doi:10.1186/s13071-015-1018-8

Mweempwa., C., Tanguy Marcotty, Claudia De Pus, Penzhorn B.L., Dick A.H., Bouyer J., De Deken R., 2015. Impact of habitat fragmentation on tsetse populations and trypanosomiasis risk in eastern Zambia. Parasites and Vectors.

Mwiinde, A.M., Simuunza, M., Namangala, B., Chama-Chiliba, C.M., Machila, N., Anderson, N., Shaw, A., Welburn, S.C., 2017. Estimating the economic and social consequences for patients diagnosed with human African trypanosomiasis in Muchinga, Lusaka and Eastern Provinces of Zambia (2004-2014). Infectious Diseases of Poverty 6, 150. doi:10.1186/s40249-017-0363-6

Namangala, B., Hachaambwa, L., Kajino, K., Mweene, A.S., Hayashida, K., Simuunza, M., Simukoko, H., Choongo, K., Chansa, P., Lakhi, S., Moonga, L., Chota, A., Ndebe, J., Nsakashalo-Senkwe, M., Chizema, E., Kasonka, L., Sugimoto, C., 2012. The use of Loop-mediated Isothermal Amplification (LAMP) to detect the re-emerging Human African Trypanosomiasis (HAT) in the Luangwa and Zambezi valleys. Parasites & vectors 5, 282. doi:10.1186/1756-3305-5-282

Njiru, Z., 2012. Loop-Mediated Isothermal Amplification Technology: Towards Point of Care Diagnostics. PLos Neglected Tropical Diseases, 1572.

Njiru, Z.K., Constantine, C.C., Guya, S., Crowther, J., Kiragu, J.M., Thompson, R.C., Davila, A.M., 2005. The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. Parasitology research 95, 186-192. doi:10.1007/s00436-004-1267-5

Njiru, Z.K., Mikosza, A.S., Matovu, E., Enyaru, J.C., Ouma, J.O., Kibona, S.N., Thompson, R.C., Ndung'u, J.M., 2008. African trypanosomiasis: sensitive and rapid detection of the sub-genus Trypanozoon by loop-mediated isothermal amplification (LAMP) of parasite DNA. International Journal of Parasitology 38, 589-599. doi:10.1016/j.ijpara.2007.09.006

Nyimba, P.H., Komba, E.V., Sugimoto, C., Namangala, B., 2015. Prevalence and species distribution of caprine trypanosomosis in Sinazongwe and Kalomo districts of Zambia. Veterinary Parasitology 210, 125-130. doi:10.1016/j.vetpar.2015.04.003

Picozzi, K., Carrington, M., Welburn, S.C., 2008. A multiplex PCR that discriminates between *Trypanosoma brucei brucei* and zoonotic *T. b. rhodesiense*. Experimental parasitology 118, 41-46. doi:10.1016/j.exppara.2007.05.014

Richter, J., Gobels, S., Gobel, T., Westenfeld, R., Muller-Stover, I., Haussinger, D., 2012. A returning traveller with fever, facial swelling, and skin lesions. BMJ 344, e2092. doi:10.1136/bmj.e2092

Simukoko, H., Marcotty, T., Vercruysse, J., Van den Bossche, P., 2011. Bovine trypanosomiasis risk in an endemic area on the eastern plateau of Zambia. Research in veterinary science 90, 51-54. doi:10.1016/j.rvsc.2010.04.021

Squarre, D., Kabongo, I., Munyeme, M., Mumba, C., Mwasinga, W., Hachaambwa, L., Sugimoto, C., Namangala, B., 2016. Human African Trypanosomiasis in the Kafue National Park, Zambia. PLoS Negl Trop Dis 10, e0004567. doi:10.1371/journal.pntd.0004567

Swallow, B.M., 2000. Impacts of Trypanosomiasis on African Agriculture. PAAT Technical and Scientific series 2-FAO, Rome.

Van den Bossche, P., Doran M, Connor RJ, 2000. An analysis of trypanocidal drug use in the Eastern province of Zambia. Acta Tropica, 247-258.

Von Wissmann, B., Machila, N., Picozzi, K., Fevre, E.M., de, C.B.B.M., Handel, I.G., Welburn, S.C., 2011. Factors associated with acquisition of human infective and animal infective trypanosome infections in domestic livestock in Western Kenya. PLoS Neglected Tropical Diseases 5, e941. doi:10.1371/journal.pntd.0000941

WHO, 2018. WHO fact sheet-Human african trypanosomiasis. Available online: https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness) (accessed on 30 May 2020).

CHAPTER 3

CHALLENGES IN THE DIAGNOSTIC PERFORMANCE OF PARASITOLOGICAL AND MOLECULAR TESTS IN THE SURVEILLANCE OF AFRICAN TRYPANOSOMIASIS IN EASTERN ZAMBIA

Publication

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Abstract: African animal trypanosomiasis (AAT) control programs rely on active case detection through the screening of animals reared in disease endemic areas. This study compared the application of the polymerase chain reaction (PCR) and microscopy in the detection of trypanosomes in cattle blood in Mambwe, a rural district in Eastern Zambia**.** Blood samples were collected from 227 cattle and tested for infection with trypanosomes using microscopy and Ribosomal RNA Internal Transcribed Spacers (ITS)-PCR. Microscopy on the buffy coat detected 17 cases, whilst thin and thick smears detected 26 cases and 28 cases, respectively. In total, microscopy detected 40 cases. ITS-PCR-filter paper (FP) on blood spots stored on FP detected 47 cases, and ITS-PCR-FTA on blood spots stored on Whatman FTA Classic cards detected 83 cases. Using microscopy as the gold standard, ITS-PCR-FTA had a better specificity (SP) and sensitivity (SE) (SP = 72.2%; SE = 77.5%; kappa = 0.35) than ITS-PCR-FP (SP = 88%; SE = 60%; kappa = 0.45). The prevalence of *Trypanosoma brucei s.l*. was higher on ITS-PCR-FTA (19/227) than on ITS-PCR-FP (0/227). Our results illustrate the complexities around trypanosomiasis surveillance in rural Africa and provide evidence of the impact that field conditions and staff training can have on diagnostic results, which in turn impact the success of tsetse and trypanosomiasis control programs in the region.

Keywords: diagnosis; African trypanosomiasis; rural areas; Zambia

3.1 INTRODUCTION

Tsetse-transmitted trypanosomiasis, caused by protozoan parasites of the genus *Trypanosoma,* affects both humans and animals. While *Trypanosoma congolense, Trypanosoma vivax* and *Trypanosoma brucei s.l.* cause nagana or African animal trypanosomiasis (AAT) in livestock, the two subspecies of *T. brucei s.l.*: *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are responsible for Human African trypanosomiasis (HAT), commonly known as sleeping sickness. Countries affected by nagana have continued to suffer from economic losses in millions of dollars (Welburn et al., 2001; Simukoko et al., 2007; FAO, 2018; PAAT, 2022). The Food Agriculture Organisation (FAO) estimates that 50 million heads of cattle are at risk of AAT with 3 million cattle deaths recorded per year. Loss in cattle production alone is estimated at US$1.0−1.2 billion per year and US$4.7 billion per year in agricultural gross domestic products (FAO, 2018).

Microscopy has been traditionally regarded as the gold standard in detecting the presence of trypanosomes. Microscopic examinations of the buffy coat and wet blood films, as well as thin and thick blood smears stained with Giemsa, are the most common methods used in Africa for trypanosome detection. Microscopy is considered a good diagnostic method because it is simple, cheap and can also simultaneously detect other haemoparasites (microfilaria and *Plasmodium* spp.) (Katsidzira. and Fana, 2010). However, microscopy has a very low sensitivity, especially in detecting early infections that are associated with low parasitaemia (Chappuis, 2004; Odiit., 2005; Cox et al., 2010).

Molecular techniques such as the polymerase chain reaction (PCR) have significantly improved the level of sensitivity and accuracy in trypanosome diagnosis in comparison to traditional parasitological methods. However, most remote areas of Africa do not have the resources to facilitate the use of such molecular techniques (Thumbi, 2008; Moti et al., 2014). Molecular tests have the ability to differentiate trypanosome species and subspecies through the use of specific primers (Desquesnes et al., 2001; Cox, 2005; Njiru et al., 2008; Musinguzi et al., 2017). Ribosomal RNA Internal Transcribed Spacers (ITS)-PCR can be used for the detection of both AAT and HAT, but its use in rural settings of Africa is limited by high costs and the need for trained personnel (Solano, 2002; Njiru et al., 2005).

Understanding the capabilities of each diagnostic technique is key to the quick and accurate detection of trypanosomes in samples and is critical to disease surveillance, control and

485 eradication. Unfortunately, in most rural settings in Africa, poor detection of trypanosome
486 infections has occurred due to a poor understanding of the limitations of the diagnostic tests
487 used, which can lead to incorrect decision making. Against this background, this study
488 compared the diagnostic performance of microscopy and ITS-PCR in detecting trypanosomes
489 under common field conditions in rural Zambia.

490 ## 3.2 MATERIALS AND METHODS

491 ### 3.2.1. Study Area

492 The study was undertaken in Mambwe, a rural district in the Eastern province of Zambia from
493 February to April 2019. The district was purposively selected because it is highly tsetse
494 infested and has a high prevalence of bovine trypanosomiasis (Laohasinnarong et al., 2015).
495 Located along the Luangwa River basin, the district covers an area of 4480 km^2 and is home to
496 the South Luangwa National Park. With a human population of 92,445 belonging to 18,489
497 households, most of the local community relies on tourism and small-scale farming for their
498 livelihoods (Zambia Central Statistical Office, 2015).

499 ### 3.2.2. Study Design and Sample Size

500 The study compared two diagnostic techniques (ITS-PCR and microscopy) for the detection
501 of bovine trypanosomiasis under rural, field conditions. To facilitate the comparison under
502 field conditions, a trypanosomiasis prevalence survey was conducted using 227 cattle from
503 193 cattle-owning small-scale farmers in selected parts of the Mambwe district, i.e., located in
504 tsetse-infested parts of the district close to the South Luangwa National Park. The cattle
505 farmers were purposively selected, but their inclusion in the study was largely based on their
506 willingness to participate. Written informed consent from each farmer was required prior to
507 their participation in the survey.

508 ### 3.2.3. Sample Collection

509 From each animal, blood was drawn into three capillary tubes (Kimble Chase Life Science,
510 Vineland, NJ, USA) containing heparin (anticoagulant) after puncturing the ear vein of the
511 animal with a blood lancet. One capillary tube was sealed with a crista seal for an on-site
512 examination by buffy coat technique. About 50 μL of blood from the second capillary tube
513 was used to make thin and thick smears for a later microscopic examination at the laboratory
514 (Katsidzira. and Fana, 2010). About 50 μL of blood from the third capillary tube was applied

onto a well-labeled Whatman FTA Classic Card (GE Healthcare, Madison, WI, USA) and on Whatman® No. 1 filter paper (GE Healthcare). After air drying, both the filter paper and FTA card samples were separately packed in zip locked storage bags containing silica gel and transported to the laboratory for further processing with ITS-PCR (Ahmed et al., 2011).

3.2.4. Application of Diagnostic Tests

Microscopy:

To increase the chance of parasite detection, three slides were prepared from one animal, i.e., buffy coat, thin and thick smears. An on-site microscopic examination was conducted on cattle blood stored in heparinized capillary tubes. The sealed capillary tubes were spun on-site in a microhematocrit centrifuge for five minutes at 10,000 rpm (Chagas et al., 2020), after which packed cell volumes (PCVs) were determined using a PCV reader. The buffy coat from each sample was then placed on a microscopic slip with a cover slip and examined on site at a x400 magnification for the presence of motile trypanosomes. At the laboratory, thin and thick smears were stained with Giemsa solution and later examined by trained veterinary laboratory technicians for the presence of trypanosomes (Marcotty et al., 2008; Mbewe et al., 2015).

DNA Extraction from Whatman® No. 1 Filter Paper:

DNA from stored blood spots was extracted using the buffer technique (Morrison et al., 2007). Two discs of about 3 mm diameter were punched from each blood spot and placed in labeled 1.5 mL sterile tubes. About 66 µL of TE buffer (10 mM Tris-HCl pH 8.0 and 0.1 mM EDTA in distilled water) was added to each tube and incubated at 50 ^{o}C for 15 minutes. The discs were then pressed gently to the bottom of the tube using a new rod for each tube and heated at 97 ^{o}C for another 15 minutes to eluate the DNA. The tubes were then spun down at 10,000 rpm for 1 minute (Morrison et al., 2007).

DNA Extraction from FTA Cards:

DNA was extracted from the stored blood spots using the Chelex method (Ahmed et al., 2011). Two discs of about 3 mm diameter from each blood spot were placed in a labeled 1.5 mL sterile tube. About 200 µL of Whatman purification reagent was used to wash each disc for 15 minutes, after which the solution was carefully decanted. The discs were then washed twice with 200 µL of 1% TE buffer for 15 minutes, after which the solutions were gently decanted.

A separate rod for each sample was used during decanting to make sure that the discs did not flow over with the solutions. The discs were then left to air dry for one hour, after which 100 µL of 5% (w/v) Chelex-100 (Sigma-Aldrich Japan, Tokyo, Japan) in distilled water solution was added and mixed thoroughly. The discs containing Chelex solution were finally incubated at 90 °C for 30 minutes to elute DNA. The eluted DNA was stored at 4 °C for use within 12 hours and at −20 °C for use after 12 hours (Ahmed et al., 2011).

ITS-PCR:

ITS-PCR was undertaken in 25 µL reaction mixtures containing primers AITS-F: CGGAAGTTCACCGATATTGC and AITS-R: AGGAAGCCAAGTCATCCATC (Gaithuma et al., 2019), One Taq 2 ⊚ master mix (New England BioLabs, Ipswich, MA, USA), nuclease free water and 5 µL of extracted DNA sample. For the detection of *T. b. rhodesiense*, SRA F (5′-ATAGTGACAAGATGCGTACTCAACGC-3′) and SRA R (5′-AATGTGTTCGAGTACTTCGGCACGCT-3′) (Radwanska et al., 2002) were used (procured from Inqaba Biotec, Pretoria, South Africa). Thermocycler amplification conditions were at 94 °C for 5 minutes, followed by 40 cycles of 94 °C for 40 seconds, 58 °C for 40 seconds, 72 °C for 90 minutes and 72 °C for 5 minutes. ITS-PCR targets the internal transcribed spacer 1 of the ribosomal RNA (100−200 copies per genome), producing different sized products for different trypanosome species (Desquesnes et al., 2001; Njiru et al., 2005; Gaithuma et al., 2019). ITS-PCR products were separated by electrophoresis (95 volts for 60 minutes) in a 2% (w/v) agarose gel containing ethidium bromide. The separated products were then visualized under ultraviolet light in a transilluminator. Known positive controls of *T. congolense* (560−705 bp), *T. vivax* (226−238 bp) and *T. brucei* (415−431 bp) and a negative control were included in each reaction. All samples that were positive for *T. brucei* were subjected to a multiple PCR using a serum resistance-associated antigen (SRA) targeting primer for the detection of *T. b. rhodesiense.*

3.2.5. Data Analysis

Statistical analyses were performed in SPSS version 26 (IBM Corporation, 2019). Trypanosomiasis prevalence determined by microscopy (buffy coat, thin smears and thick smears) was used as the gold standard. The prevalence values determined by ITS-PCR were compared against this gold standard, and the sensitivity and specificity were calculated on this basis. A Chi-square test was used to determine the statistical significance between the

tests. For expected values under 5, Fisher's exact test was used. *P* values under 0.05 were considered statistically significant. The impact of the diagnostic test performance was estimated using positive and negative predictive values for each test. The usefulness and benefits between the tests were measured using the ROC, while the kappa coefficient was used to measure agreements and accuracy between tests. The area under the receiver operator curve (AUC-ROC) scores were used to distinguish between a perfect and worthless test. AUC scores were classified as follows: excellent (0.90−1), good (0.80−0.90), fair (0.70−0.80), poor (0.60−0.70) and worthless (0.50−0.60). The kappa values were classified as follows: values ≤ 0 indicated no agreement, slight agreement (0.01−0.20), fair (0.21−0.40), moderate (0.41−0.60), considerable (0.61−0.80) and perfect (0.81−1.00) Landis & Koch (1977).

3.3 RESULTS

The microscopic examination of trypanosome infection on the buffy coat detected 17/227 cases (7.5%; 95% CI = 4.1−10.9), that on thin smears detected 26/227 cases (11.5%; 95% CI = 7.3−15.6), while that on thick smears detected 28/227 cases (12.3%; 95% CI = 8.1−16.6). Combined microscopy using these three microscopic techniques in parallel recorded a total of 40/227 cases (17.6%; 95% CI = 12.7−22.6).

Out of the 227 cattle blood samples screened using ITS-PCR (Table 3. 1), the overall prevalence of trypanosomes from blood spots stored and transported on FP was 20.7% (47/227; 95% CI = 15.4−26.0), while a 36.6% (83/227; 95% CI = 30.3−42.8) prevalence was recorded from blood spots stored and transported on FTA cards. The Mean Packed Cell Volume (PCV) for trypanosome positive samples was 34.21 (95% Cl = 33.21−35.22), while that for negative samples was 35.21 (95% CI = 34.21−36.22).

Table 3. 1: Prevalence of trypanosome species in cattle (n = 227) by PCR

Trypanosome species	PCR-FP	Sample prevalence %	Confidence Interval at 95%	PCR-FTA	Sample prevalence %	Confidence Interval at 95%
T. congolense	7	3.1	0.8–5.3	14	6.2	3.0–9.3
T. vivax	39	17.2	12.3–22.1	50	22.0	16.6–27.4
T. brucei	1	0.4	−0.4–1.3	19	8.4	4.8–12.0
TOTAL	**47**	**20.7**	**15.4–26.0**	**83**	**36.6**	**30.3–42.8**
T. b. rhodesiense	0	–	–	3	1.3	−0.2–2.8

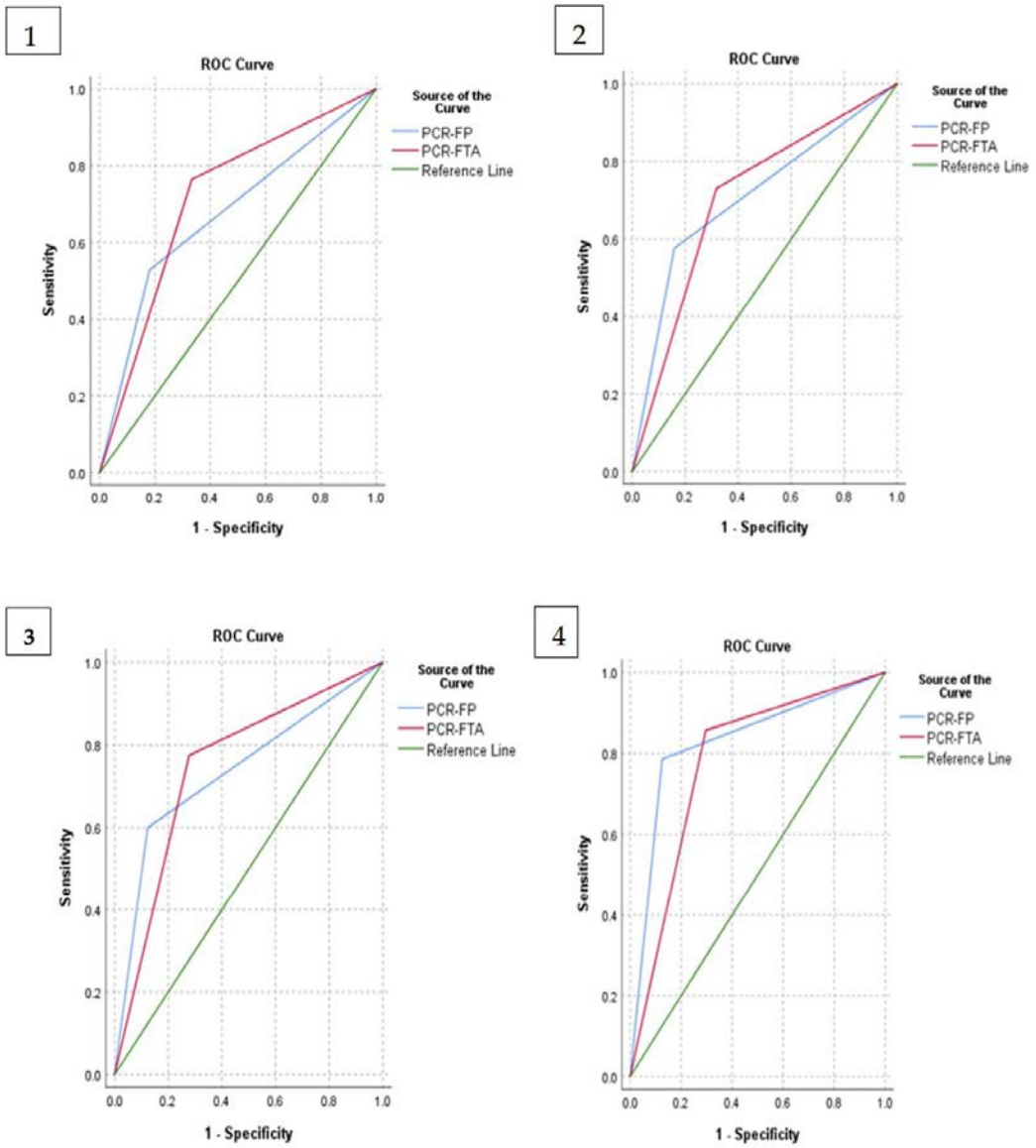
Mixed	1	0.4	−0.4–1.3	9	4.0	1.4–6.5

599

600 The diagnostic accuracy, sensitivity, and specificity of ITS-PCR on blood spots stored on filter
601 paper FP (Accuracy = 0.8; SE = 60%; SP = 87.7%, kappa = 0.45) and those of ITS-PCR on blood
602 spots stored on FTA cards (Accuracy = 0.7; SE = 77.5%; SP = 72.2%; kappa = 0.35) were
603 determined using microscopy as the gold standard**.** Agreement between the tests was
604 measured using the kappa test. The results of the comparison of ITS-PCR using FTA and FP
605 as the collection method showed an accuracy of 0.69, kappa = 0.27 and *P* value < 0.05,
606 indicating that the difference in the two collection methods was statistically significant.

607 Receiver operating characteristic (ROC) curves (Figure 3. 1) were used to compare the
608 sensitivity and specificity across a range of values, and the area under the ROC was used to
609 measure the test performance. The curves show the usefulness of ITS-PCR and its ability to
610 detect trypanosomes when compared with the buffy coat (ROC 1), thin smear (ROC 2), thick
611 smear (ROC 3) and combined microscopy (ROC 4).

612



1- ITS-PCR compared to buffy coat; 2- ITS-PCR compared to thin smear; 3- ITS-PCR compared to thick smear; 4- ITS-PCR compared to combined microscopy

Figure 3. 1: Receiver Operator Curves illustrating the diagnostic abilities of laboratory tests used

The AUC-ROC scores for ITS-PCR-FP and ITS-PCR-FTA are shown in **Table 3. 2** . The higher the AUC score, the better the test at distinguishing diseased from none-diseased individuals.

Table 3. 2: Area under the ROC curves shown in Figure 3.1

Reference		Test Result Variable(s)	AUC	Std. Error[a]	*P*-Value	AUC 95% Confidence Interval		Test performance relative to reference
						Lower Bound	Upper Bound	
(1)	Buffy coat	ITS-PCR-FP	.674	.075	.020	.528	.821	Poor
	ROC 1	ITS-PCR-FTA	.716	.063	.001	.592	.839	Fair
(2)	Thin smear	ITS-PCR-FP	.709	.060	.001	.591	.827	Fair
	ROC 2	ITS-PCR-FTA	.706	.054	.000	.601	.812	Fair
(3)	Thick smear	ITS-PCR-FP	.830	.047	.000	.738	.922	Good
	ROC 3	ITS-PCR-FTA	.780	.044	.000	.695	.866	Good
(4)	Combined Microscopy	ITS-PCR-FP	.739	.049	.000	.643	.834	Fair
	ROC 4	ITS-PCR-FTA	.748	.043	.000	.665	.832	Fair

3.4 DISCUSSION

Our study confirmed that prevalence can be underestimated by a single microscopy technique as compared to combined microscopy methods, while molecular techniques significantly improve the apparent prevalence. Differences and discrepancies in the number of cases detected from the three microscopy tests may be attributed to the climatic conditions under which these tests were conducted, the low parasitaemia of trypanosome species and the time during which observations were made. The use of the buffy coat is considered to be more sensitive than that of thick and thin smears (Florkwoski 2008), but in this case the buffy coat detected the least number of trypanosomes. An on-site low case detection on the buffy coat can occur when the field conditions do not allow for a thorough screening of samples as compared to a laboratory screening where operators take time to thoroughly screen the samples. Factors that can negatively affect case detection on the buffy coat include poor quality capillary tubes and high ambient temperatures in the study area, which could lead to a diminished motility and/or death of trypanosomes before examiners could observe trypanosome movement in the buffy coat. Other factors include examiners' inability to observe immature trypanosome movements.

To validate available molecular diagnostic techniques for AAT, ITS-PCR was employed using blood spots that were stored and transported on FTA cards and normal filter paper. ITS-PCR-FP had a low detection rate compared to ITS-PCR-FTA, which detected a higher number of trypanosomes. This result suggested that blood spots collected and stored on FTA paper were more reliable in determining trypanosome prevalence than blood spots collected and stored

642 on common filter paper (Chi-square p-value < 0.01). Such results may be attributed to the fact
643 that FTA paper, unlike common filter paper, has the ability to protect DNA from degradation
644 (Ahmed et al., 2011; Ahmed et al., 2013).

645 Unfortunately, due to costs attached to the use of FTA cards, their use may be limited as they
646 may not be readily available to most researchers in trypanosomiasis endemic areas of Africa.
647 The comparative analysis between the use of FTA and FP for blood sample storage and ITS-
648 PCR analysis did, however, show a fair agreement between the two techniques (kappa = 0.27).
649 Our data confirm that both techniques could be useful in the transport of samples for the
650 detection of African trypanosomiasis considering that the transportation of whole blood
651 samples for ITS-PCR analysis may not be feasible under remote field conditions. Our study
652 has demonstrated the convenience of using dry blood samples in areas with limited
653 refrigeration facilities. Practically, dry blood samples could be collected from selected animals
654 and stored on FTA cards or FP on a regular basis for onward analysis at diagnostic centers
655 (Cox et al., 2010; Sawitri et al., 2016). Both FTA cards and FP may, however, inhibit ITS-PCR,
656 making it less accurate compared to when DNA is extracted directly from whole blood
657 samples, which could explain why microscopically positive samples tested negative on the
658 ITS-PCR test (Ahmed et al., 2013).

659 When the ITS-PCR-FTA results were compared to microscopy, the results indicated a gradual
660 increase in both sensitivity and specificity, with the single microscopy tests reporting the
661 lowest sensitivity and specificity when compared to the combined microscopy tests, which, as
662 expected, had a relatively higher sensitivity and specificity. This pattern of a gradual increase
663 in the ability of the tests to correctly determine infected and noninfected cases was also
664 observed for the microscopy and ITS-PCR-FP comparisons. Such results indicate the need for
665 combining the buffy coat, thin and thick smear techniques when considering microscopy for
666 trypanosome case detection in remote areas of Africa due to limitations in using molecular
667 tests.

668 When using the "rule-in" and "rule-out" test, as described by Florkwoski (Florkwoski, 2008),
669 the results showed that ITS-PCR-FTA (kappa = 0.18) (high NPV and high sensitivity) was a
670 better test for identifying diseased cattle than ITS-PCR-FP (kappa = 0.30). AUC-ROC scores
671 for both ITS-PCR-FP (0.7) and ITS-PCR-FTA (0.8) were, however, within the acceptable range
672 of 0.7 to 0.9, indicating that both techniques were acceptable in trypanosome case diagnosis
673 (Cadioli et al., 2015; Laohasinnarong et al., 2015).

The sourcing, cost and transportation of molecular requirements to perform ITS-PCR was another challenge experienced in this study. Although reagents were available from regional suppliers, the cost was high due to the exchange rate and depreciation of the local currency. Since Zambia does not produce any molecular reagents, importation and transportation costs are a constraint. The use of ITS-PCR is therefore still limited, as most rural laboratories in Zambia have not yet transitioned to the use of molecular techniques for the point of care diagnosis of African trypanosomiasis and other zoonotic diseases. Findings from this study highlighted the limitations of the existing tests for African trypanosomiasis in rural areas of Africa, i.e., microscopy and ITS-PCR, which may have crucial clinical and epidemiological implications (Cox et al., 2005; Njiru et al., 2005; de Clare Bronsvoort et al., 2010). The mAECT (mini Anion Exchange Centrifugation Technique) was not used in this study area and could be a technique worth considering in the future in order to improve sensitivity (Lumsden et al., 1980).

Although previous studies suggested that *T. congolense* was the main cause of AAT and anaemia in Eastern and Southern Africa (Despommier, 2005; Cox et al., 2010; Simukoko et al., 2011; Muhanguzi et al., 2017), data from the current study demonstrated that *T. vivax* was present in most of the sampled cattle and that anaemia was not an indicator for trypanosome infection. ITS-PCR has previously been reported as being better at detecting *T. vivax* infections when compared to other trypanosome species (Njiru et al., 2005; Thumbi, 2008), which may partially explain their high prevalence in these results. The high prevalence of *T. vivax* infections may also suggest that trypanosomiasis transmission within the sites included in this study could be mechanical by other blood sucking insects, such as tabanids, prevalent in the area (Taioe et al., 2017) rather than by tsetse flies.

Finally, the detection of the human infective trypanosomes *T. b. rhodesiense* from cattle blood samples analyzed in this study highlights the risks that cattle pose to communities living in tsetse-infested areas (Welburn et al., 2001). Cattle may be potential sources of sleeping sickness when humans get bitten by tsetse after the fly has taken a blood meal from an infected animal (Namangala et al., 2013; Selby et al., 2013; Ruiz et al., 2015). Our results support the need for a more holistic approach in the control of trypanosomiasis with a focus on the control of the disease in domestic animal reservoirs.

3.5 CONCLUSIONS

This study serves as a prime example of the impact that remote field conditions and staff training can have on results that in turn impact the success of tsetse and trypanosomiasis control programs in the region. The study illustrates current challenges with AAT diagnosis using molecular and microscopy techniques in rural areas and the need for innovation in field diagnostics. However, considering that trypanosomiasis is prevalent in remote rural areas where access to diagnostic facilities is limited, FTA cards and FP should be considered for collecting, storing and transporting blood samples for analysis using ITS-PCR where the collection of whole blood is not feasible. Currently used diagnostic tests have their own advantages and limitations. ITS-PCR is a good screening test of trypanosomes causing nagana. However, the use of ITS-PCR may be limited and impractical in remote rural areas of Africa where trypanosomiasis is endemic. Microscopy could, therefore, be used for diagnosis but as a combination of the three commonly used techniques of buffy coat, thin smears and thick smears. Microscopy remains the most practical option for the diagnosis of trypanosomes in the field, but understanding its limitations is critical when using it for surveillance purposes. Better staff training in disease diagnosis, better maintenance of diagnostic equipment, a better funding model and an improvement in field quality control would help address challenges in disease diagnosis, as highlighted in this study.

Author Contributions: G.M.M. and B.G. developed, conceptualized and drafted the manuscript. B.G., B.N. and L.H. edited the manuscript, and supervised and facilitated finances for the project. K.C., K.H. and B.N. facilitated the smooth operations of both field and laboratory work. C.M. helped in data collection. All authors reviewed, read and approved the final manuscript.

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740

741 **3.6 REFERENCES**

742 Ahmed, H.A., MacLeod, E.T., Hide, G., Welburn, S.C., Picozzi, K., 2011. The best practice for
743 preparation of samples from FTA(R)cards for diagnosis of blood borne infections
744 using African trypanosomes as a model system. Parasites and Vectors 4, 68.
745 doi:10.1186/1756-3305-4-68
746 Ahmed, H.A., Picozzi, K., Welburn, S.C., MacLeod, E.T., 2013. A comparative evaluation of
747 PCR- based methods for species- specific determination of African animal
748 trypanosomes in Ugandan cattle. Parasites & Vectors 6, 316. doi:10.1186/1756-3305-6-
749 316
750 Cadioli, F.A., Fidelis Junior, O.L., Sampaio, P.H., dos Santos, G.N., Andre, M.R., Castilho, K.J.,
751 Machado, R.Z., 2015. Detection of *Trypanosoma vivax* using PCR and LAMP during
752 aparasitemic periods. Vet Parasitol 214, 174-177. doi:10.1016/j.vetpar.2015.09.001
753 Chagas, C.R.F., Binkiene, R., Ilgunas, M., Iezhova, T., Valkiunas, G., 2020. The buffy coat
754 method: a tool for detection of blood parasites without staining procedures. Parasit
755 Vectors 13, 104. doi:10.1186/s13071-020-3984-8
756 Chappuis, S.E., Adams K, Kidane S, Pittet A, Bovier PA, 2004. Chappuis F, Stivanello E,
757 Adams K, Ki Card agglutination test for trypanosomisis end-dilution titer and
758 cerebrospinal fluid cell count as predictors of human African trypanosomiasis (T. b.
759 r) among serologically susp. Am J Trop Med Hyg 71, 313-317.
760 Cox, A., Tilley, A., McOdimba, F., Fyfe, J., Eisler, M., Hide, G., Welburn, S., 2005. A PCR based
761 assay for detection and differentiation of African trypanosome species in blood.
762 Experimental parasitology 111, 24-29. doi:10.1016/j.exppara.2005.03.014
763 Cox, A.P., Tosas, O., Tilley, A., Picozzi, K., Coleman, P., Hide, G., Welburn, S.C., 2010.
764 Constraints to estimating the prevalence of trypanosome infections in East African
765 zebu cattle. Parasites & Vectors 3, 82. doi:10.1186/1756-3305-3-82
766 Cox, T.A., McOdimba F, Fyfe J, Eisler M, Hide G, Welburn S, 2005. Cox A, Tilley A, McOdimba
767 F, Fyfe J, A PCR based assay for detection and differentiation of African trypanosome
768 species in blood. Experimental parasitology 22, 24-29.
769 de Clare Bronsvoort, B.M., von Wissmann, B., Fevre, E.M., Handel, I.G., Picozzi, K., Welburn,
770 S.C., 2010. No gold standard estimation of the sensitivity and specificity of two
771 molecular diagnostic protocols for *Trypanosoma brucei spp*. in Western Kenya. PloS one
772 5, e8628. doi:10.1371/journal.pone.0008628

773 Despommier, G., Hotez Knirsch, 2005. The Protozoa. Parasitic diseases. Apple trees
774 productions, New York, 32-39.
775 Desquesnes, M., McLaughlin, G., Zoungrana, A., Davila, A.M., 2001. Detection and
776 identification of Trypanosoma of African livestock through a single PCR based on
777 internal transcribed spacer 1 of rDNA. International journal for parasitology 31, 610-
778 614.
779 FAO, 2018. World Health Organization and Food and Agriculture Organization step up
780 towards the elimination of human African trypanosomiasis to ensure health and Food
781 Security in Africa. Addis Ababa, Ethiopia.
782 Florkwoski, C., 2008. Sensitivity, Specificity, Receiver-Operating Characteristic (ROC) Curves
783 and Likelihood Ratios: Communicating the Performance of Diagnostic Test. Clinical
784 Biochemistry Rev 29, S83.
785 Gaithuma, A.K., Yamagishi, J., Martinelli, A., Hayashida, K., Kawai, N., Marsela, M.,
786 Sugimoto, C., 2019. A single test approach for accurate and sensitive detection and
787 taxonomic characterization of Trypanosomes by comprehensive analysis of internal
788 transcribed spacer 1 amplicons. PLoS Negl Trop Dis 13, e0006842.
789 doi:10.1371/journal.pntd.0006842
790 Katsidzira., Fana, G., 2010. Pitfalls in the diagnosis of Trypanosomiasis in low endemic
791 countries: A case Report. PLos Neglected Tropical Diseases 4, 823.
792 Landis, J. R. and Koch, G. G. [1977]. The Measurement of Observer Agreement for Categorical
793 Data. Biometrics 33, 159-174.
794 Laohasinnarong, D., Goto, Y., Asada, M., Nakao, R., Hayashida, K., Kajino, K., Kawazu, S.,
795 Sugimoto, C., Inoue, N., Namangala, B., 2015. Studies of trypanosomiasis in the
796 Luangwa valley, north-eastern Zambia. Parasites and Vectors 8, 555.
797 doi:10.1186/s13071-015-1173-y
798 Lumsden, W.H.R., Evans, D.A., Kimber, C.D., 1980. Miniature anion-exchange/centrifugation
799 technique for the diagnosis of microfilaraemia in the field. Transactions of the Royal
800 society society of tropical medicine and hygiene 74.
801 Marcotty, T., Simukoko, H., Berkvens, D., Vercruysse, J., Praet, N., Van den Bossche, P., 2008.
802 Evaluating the use of packed cell volume as an indicator of trypanosomal infections
803 in cattle in eastern Zambia. Preventive Veterinary Medicine 87, 288-300.
804 doi:10.1016/j.prevetmed.2008.05.002

805 Mbewe, N.J., Namangala, B., Sitali, L., Vorster, I., Michelo, C., 2015. Prevalence of pathogenic
806 trypanosomes in anaemic cattle from trypanosomosis challenged areas of Itezhi-tezhi
807 district in central Zambia. Parasites and Vectors 8, 638. doi:10.1186/s13071-015-1260-0
808 Morrison, L.J., McCormack, G., Sweeney, L., Likeufack, A.C., Truc, P., Turner, C.M., Tait, A.,
809 MacLeod, A., 2007. Use of multiple displacement amplification to increase the
810 detection and genotyping of trypanosoma species samples immobilized on FTA
811 filters. The American journal of tropical medicine and hygiene 76, 1132-1137.
812 Moti, Y., Fikru, R., Buscher, P., Van Den Abbeele, J., Duchateau, L., Delespaux, V., 2014.
813 Detection of African animal trypanosomes: the haematocrit centrifugation technique
814 compared to PCR with samples stored on filter paper or in DNA protecting buffer.
815 Veterinary Parasitology 203, 253-258. doi:10.1016/j.vetpar.2014.04.014
816 Muhanguzi, D., Mugenyi, A., Bigirwa, G., Kamusiime, M., Kitibwa, A., Akurut, G.G., Ochwo,
817 S., Amanyire, W., Okech, S.G., Hattendorf, J., Tweyongyere, R., 2017. African animal
818 trypanosomiasis as a constraint to livestock health and production in Karamoja
819 region: a detailed qualitative and quantitative assessment. BMC veterinary research
820 13, 355. doi:10.1186/s12917-017-1285-z
821 Musinguzi, S.P., Suganuma, K., Asada, M., Laohasinnarong, D., Sivakumar, T., Yokoyama, N.,
822 Namangala, B., Sugimoto, C., Suzuki, Y., Xuan, X., Inoue, N., 2017. A PCR-based
823 survey of animal African trypanosomosis and selected piroplasm parasites of cattle
824 and goats in Zambia. The Journal of veterinary medical science 78, 1819-1824.
825 doi:10.1292/jvms.16-0240
826 Namangala, B., Oparaocha, E., Kajino, K., Hayashida, K., Moonga, L., Inoue, N., Suzuki, Y.,
827 Sugimoto, C., 2013. Preliminary investigation of trypanosomosis in exotic dog breeds
828 from Zambia's Luangwa and Zambezi valleys using LAMP. Am J Trop Med Hyg 89,
829 116-118. doi:10.4269/ajtmh.13-0078
830 Njiru, Z.K., Constantine, C.C., Guya, S., Crowther, J., Kiragu, J.M., Thompson, R.C., Davila,
831 A.M., 2005. The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes.
832 Parasitology research 95, 186-192. doi:10.1007/s00436-004-1267-5
833 Njiru, Z.K., Mikosza, A.S., Matovu, E., Enyaru, J.C., Ouma, J.O., Kibona, S.N., Thompson, R.C.,
834 Ndung'u, J.M., 2008. African trypanosomiasis: sensitive and rapid detection of the
835 sub-genus Trypanozoon by loop-mediated isothermal amplification (LAMP) of
836 parasite DNA. International Journal of Parasitology 38, 589-599.
837 doi:10.1016/j.ijpara.2007.09.006

838 Odiit., C.P., Liu W, McDermolt J, Fevre E, Welburn SC, Woolhouse MEJ, 2005. Quantifying
839 the level of under detection of T. b. r. sleeping sickness cases. Tropical Medicine and
840 International Health 10, 840-849.
841 PAAT, 2022. Programme Against African Trypanosomiasis. Food and Agriculture
842 Organization of the United Nations.
843 Radwanska, M., Chamekh, M., Vanhamme, L., Claes, F., Magez, S., Magnus, E., de Baetselier,
844 P., BÃ¼scher, P., Pays, E., 2002. The serum resistance-associated gene as a diagnostic
845 tool for the detection of Trypanosoma brucei rhodesiense. The American Journal of
846 Tropical Medicine and Hygiene 67, 684-690. doi:10.4269/ajtmh.2002.67.684
847 Ruiz, J.P., Nyingilili, H.S., Mbata, G.H., Malele, II, 2015. The role of domestic animals in the
848 epidemiology of human African trypanosomiasis in Ngorongoro conservation area,
849 Tanzania. Parasites & Vectors 510. doi:10.1186/s13071-015-1125-6
850 Sawitri, D.H., Wardhana, A.H., Dewi, D.A., Ekawasti, F., Widjaja, E., 2016. Application of
851 Dried Blood Sample on Whatman Filter Paper for Detection of Trypanosoma evansi
852 from Cattle in Central Kalimantan by Internal Trascriber Spacer-1 Polymerase Chain
853 Reaction. 191-197. doi:10.14334/Proc.Intsem.LPVT-2016-p.191-197
854 Selby, R., Bardosh, K., Picozzi, K., Waiswa, C., Welburn, S.C., 2013. Cattle movements and
855 trypanosomes: restocking efforts and the spread of *Trypanosoma brucei rhodesiense*
856 sleeping sickness in post-conflict Uganda. Parasites and Vectors 6, 281.
857 doi:10.1186/1756-3305-6-281
858 Simukoko, H., Marcotty, J., Phiri, I., Geysen, D., Vercruysee, J., Vanden Bossche, P., 2007. The
859 comparative role of cattle, goats and pigs in epidemiology of livestock
860 trypanosomiasis on plateau of eastern Zambia. Veterinary Parasitology, 231-238.
861 Simukoko, H., Marcotty, T., Vercruysse, J., Van den Bossche, P., 2011. Bovine trypanosomiasis
862 risk in an endemic area on the eastern plateau of Zambia. Research in veterinary
863 science 90, 51-54. doi:10.1016/j.rvsc.2010.04.021
864 Solano, J.V., Guessan PN, 2002. Comparison of different DNA preparation protocols for PCR
865 diagnosis of HAT in Cote d'ivoire. Acta Tropica 82, 349-356.
866 Taioe, M.O., Motloang, M.Y., Namangala, B., Chota, A., Molefe, N.I., Musinguzi, S.P.,
867 Suganuma, K., Hayes, P., Tsilo, T.J., Chainey, J., Inoue, N., Thekisoe, O.M.M., 2017.
868 Characterization of tabanid flies (Diptera: Tabanidae) in South Africa and Zambia and
869 detection of protozoan parasites they are harbouring. Parasitology 144, 1162-1178.
870 doi:10.1017/S0031182017000440

871 Thumbi, M.F., Mosi RO, Jung'a JO, 2008. Comparative evaluation of three PCR base diagnostic
872 assays for the detection of pathogenic trypanosomes in cattle blood. Parasites and
873 Vectors 1, 1-46.
874 Welburn, S.C., Picozzi, K., Fevre, E.M., Coleman, P.G., Odiit, M., Carrington, M., Maudlin, I.,
875 2001. Identification of human-infective trypanosomes in animal reservoir of sleeping
876 sickness in Uganda by means of serum-resistance-associated (SRA) gene. Lancet 358,
877 2017-2019.

CHAPTER 4

EVALUATING THE FINANCIAL RETURN FOR CONTROLLING AFRICAN ANIMAL TRYPANOSOMIASIS IN RESOURCE POOR REMOTE COMMUNITIES OF EASTERN ZAMBIA

Publication under review

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Presentation of findings

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Abstract: The effectiveness of trypanosomiasis control methods has been reported in several studies with financial analyses of estimated costs of control based on retrospective data. This study was a prospective cohort study using cumulative incidence data to assess current treatments for African animal trypanosomiasis (AAT) used in Zambia and their cost effectiveness in controlling the disease in cattle. The study was undertaken between February 2019 and March 2020 in cattle (n = 227) using four treatment groups (Berenil inoculation, Samorin inoculation, Cyfluthrin pour-on and Cypermethrin treated targets) in Mambwe district of Eastern Zambia. Monthly incidence rates were calculated using ITS-PCR as a diagnostic test. The financial return for the four treatments under study, were quantified using a stochastic partial budget analysis. Endemic trypanosome prevalence rates for the Berenil inoculation (78%, n = 39, 95%CI = 66.52-89.48), Samorin inoculatin (46%, n = 23, 95%CI = 32.19-59.81), Cyfluthrin pour-on (82%, n = 41, 95%CI = 71.35-92.65) and Cypermethrin target (98%, n = 49, 95%CI = 94.12-101.88) were higher for all four treatment groups compared to incidence rates at the end of the treatment period (18%, n = 9, 95%CI = 7.35-28.65; 8%, n = 4, 95%CI 0.48-15.52; 2%, n = 1, 95%CI -1.88-5.88; 16%, n = 8, 95%CI = 5.84-26.16), respectively. The Cypermethrin target group showed a greater impact on incidence than the Cyfluthrin pour-on, Samorin inoculation, and Berenil inoculation treatment groups, respectively (p value < 0.01). The median annual net returns from the partial budget analysis showed that the Samorin inoculation group (ZMW 910.00) had a net return greater than the Cypermethrin target (ZMW 849.11) and Berenil inoculation groups (ZMW 636.36), whilst the returns for the Berenil inoculation group were greater than that of the Cyfluthrin pour-on group (ZMW 477.71). Sensitivity analysis showed that additional returns due to births from lower mortality rates had the highest effect on the financial net return for the Samorin inoculation, Berenil inoculation and Cyfluthrin pour-on groups while, costs no longer incurred due to deaths had the highest effect on the financial net return for the Cypermethrin target group. The Samorin group showed the greatest return and is therefore, the most cost-effective method for controlling AAT for small scale-farmers in resource poor communities of Eastern Zambia, but the Cypermethrin target group showed the greatest impact on incidence and may be the most appropriate option for large-scale government sponsored vector control programmes.

Keywords: African trypanosomiasis, Control, Financial returns, Incidence, Remote communities, Zambia

4.1. INTRODUCTION

African Animal Trypanosomiasis (AAT), also known as nagana, is a major constraint to livestock production in settled parts of tropical Africa (Swallow, 2000; Muhanguzi et al., 2017). In Africa alone, 50 million head of cattle are at risk of the disease. Direct losses are estimated at US$1.2 billion per year and about US$4.5 billion for overall agriculture production (Franco et al., 2020; WHO, 2022). Cattle dominate the livestock sector in Zambia, both among the commercial and traditional farmers in Zambia. According to the 2018 livestock and aquaculture Census, the livestock population in Zambia stood at 3.7 million cattle, 3.5 million goats, 170 thousand sheep and 1.1 million pigs (Ministry of Livestock and Fisheries and Central statistics Office, 2019) (CSO, 2019). The Zambian agricultural industry has not been spared from the devastating effects of AAT. Over 60% of the country's cattle population is under threat from trypanosomiasis. The prevalence of trypanosomiasis in livestock and particularly in cattle ranges between 1% and 90% depending on the location (Simukoko et al., 2011; Livestock, 2017; Mulenga et al., 2021).

Livestock rearing in tsetse infested regions has been restricted due to the drastic effects of trypanosomiasis. The disease is associated with very serious economic consequences, such as reduced productivity and fertility, livestock death, increased abortion, and high treatment costs (Shaw et al., 2014; Shaw et al., 2015). Most livestock owners in tsetse infested areas have resorted to extensive use of various treatments to combat the disease, resulting in financial burdens (Engels, 2006; Van den Bossche and Delespaux, 2011; Mulenga et al., 2020).

Common tsetse and trypanosomiasis control methods employed in Zambia, include the use of odour baited Cypermethrin targets, animal treatment with trypanocides, and dipping (Lord et al., 2020; Mulenga et al., 2020). The use of odour baited Cypermethrin treated targets involves a suspended screen of blue/black or black cloth (tsetse target) impregnated with an insecticide. Tsetse flies are attracted to the screen by the odour bait and land on the black segment, where they collect a lethal dose of the insecticide on contact and later succumb to the lethal effects of the insecticide. The use of insecticides was engineered in several ways such as ground spraying, aerial spraying, sequential aerosol technique, or in more localized areas using hand-held or vehicle-mounted fogging machines, and artificial and live-bait techniques (Vale et al., 2015; Percoma et al., 2018; Lord et al., 2020).

Chemotherapy and chemoprophylaxis in animals are the commonly used options in the control of AAT. They are based on screening of and treating of hosts found positive. The drugs of choice mainly used for AAT are Diminazene aceturate (Berenil® Dopharma Inter. Raamsodnksveer, The Netherlands) and Isometamidium chloride (Samorin® Merial Ltd, Lyon-France). Berenil inoculation is usually used as a curative drug for the treatment of AAT whilst Samorin inoculation is used as a prophylaxis. The two drugs have been reported to be very effective against strains of *T. congolense* and *T. vivax*. The interval between successive administrations of a prophylactic drug will vary between different drugs and according to the level of trypanosome challenge the animals are exposed to. Protection with Samorin inoculation has been shown experimentally to be between 3 to 6 months depending on exposure rates (Mungube et al., 2012; Fyfe et al., 2017; Hamill et al., 2017; Mulenga et al., 2017).

Affected livestock farmers in trypanosomiasis endemic areas have continued to spend large amounts of money and resources to protect their animals from the devastating effects of tsetse and trypanosomiasis whilst profits based on their choice of control method remain unquantified. Most financial assessments that have been conducted on the control of tsetse and trypanosomiasis have been based on retrospective data and focused on the cost of the control methods (FAO, 2017; Meyer et al., 2018). In this paper, we assessed the financial returns of four tsetse and trypanosomiasis control methods that are commonly used in Zambia (Berenil inoculation, Samorin inoculation, Cyfluthrin pour-on and Cypermethrin targets) through a prospective cohort study of AAT incidence in cattle.

4.2. MATERIALS AND METHODS

4.2.1 Study area and animal recruitment

The study was conducted in Mambwe district, eastern Zambia. Located along the Luangwa River basin, Mambwe district covers an area of 4,480 km^2 and includes part of the South Luangwa National Park. It has a population of 92,445 people translated into 18,489 households. Most of the local community rely on wildlife tourism and small-scale farming for their livelihoods (Zambia, Central statistics data, 2015). Communal animal grazing is a common practice for livestock farmers in the area. Tsetse transmitted trypanosomiasis is one of the major diseases occurring in Mambwe district. Affected communities have employed several control methods to combat the disease, which include the use of odour baited targets, chemotherapy and dipping (Livestock, 2017). Livestock farmers were recruited in February

2019, by field veterinary assistants based on their experience in livestock farming and their willingness to participate. Written informed consent from each farmer was required prior to their participation in the survey.

Farmers and their animals were only recruited after details on the information sheet had been read to them and consent forms signed. All cattle included in the study were ear tagged with a unique number for easy identification. Both young (weaned from their mothers) and adult animals were included in the study. Age was determined by a veterinarian who was part of the research team using the dentition method (Dyce et al., 2009)

4.2.2 Study design

A prospective cohort study of AAT incidence rates between February 2019 and March 2020 in cattle was carried out under four treatment control methods commonly used to control AAT in Eastern Zambia, i.e., Berenil inoculation, Samorin inoculation, Cyfluthrin pour-on and Cypermethrin treated targets. Four sites (Nsefu, Katemo, Chikowa, Ncheka) (Fig. 4.1) were purposively selected and matched based on climate, livestock and human populations habiting wildlife interface areas (man and his livestock are casual intruders) where the likelihood of tsetse bites by infected flies was high. All four areas were subject to similar temperature and rainfall patterns. Average daily rainfall and temperatures were recorded and collated in four-week periods corresponding with those of the chemotherapy treatments. All four groups were comprised of the local 'Agoni' cattle breed with similar age and sex patterns across the herds. Four cohort herds were created corresponding to each treatment group and followed monthly.

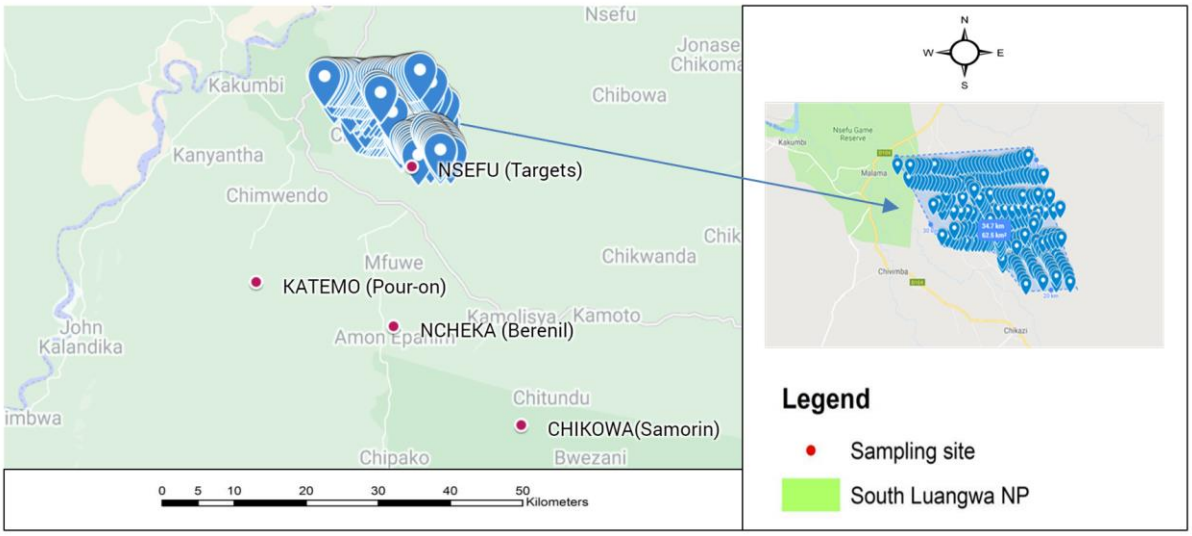

Fig. 4. 1. Map showing study sites for the four treatment groups (Insert showing area deployed with targets). Source: (Mulenga 2021-Google Maps)

4.2.3 Sample size

Using an estimated prevalence of AAT of 35% based on routine AAT surveillance conducted by the Department of Veterinary Services in non-treated areas of Mambwe district (Kakumbi tsetse and trypanosomiasis research station, annual report, 2017) and previously conducted studies (Mubamba et al., 2011), and assuming the treatments in the study would decrease the prevalence to an average of 10% then 43 cattle were needed in each treatment group (Hulley et al., 2013). To account for cattle losses due to livestock movements, slaughtering, selling and trypanosomiasis unrelated deaths, and to comply with some of the farmer herd sizes in the area, 227 cattle drawn from 34 small-scale cattle farmers (Appendix C) were finally included in the study.

Treatment groups

The selected animals were divided into four treatment groups i.e., Berenil inoculation, Samorin inoculation, Cyfluthrin pour-on and Cypermethrin targets. Two weeks before initiating the intervention strategies, all animals were treated with Berenil (3.5 mg/kg b.w. deep intramuscular injection) (Mungube et al., 2012) to clear any existing trypanosomes. The assumption was therefore that cattle were free of trypanosomes at the start of the study. Due to ethical issues, at every monthly sampling, infected animals from each treatment group were treated with Berenil (within 24hrs of testing positive) at the same dose as described above,

with the Berenil treatment being constant for all groups. All cattle in the four groups were therefore, assumed to be cleared of trypanosome infection at the start of each month, allowing for a monthly incidence rate to be calculated.

***Group 1-Berenil inoculation*:** In the Berenil inoculation group, at every monthly sampling, all cattle found positive for trypanosomes during the month of sampling were treated with Berenil at a dose of 3.5 mg/kg b.w. (Mungube et al., 2012) by deep intramuscular injection at the start of the next monthly sampling period. Berenil treatment remained constant as treatment was conducted monthly in all groups to allow for the calculation of monthly incidence rates. This group received no other treatments.

Group 2-Samorin inoculation: The Samorin inoculation group were treated every twelve weeks with Samorin by deep intramuscular injection at a dose of 0.5 mg/kg in 2% solution as described by Mungube et al. (2012) .

Group 3-Cyfluthrin pour-on: The Cyfluthrin pour-on group were applied with Cyfluthrin (Amipor-Virbac Pty, Ltd, South Africa) pour-on every eight weeks at 15 mL/100kg. Insecticide application was restricted to belly and legs (biting sites for tsetse). Restricted application reduces cattle dung contamination and costs by 40% as compared to full body application (Torr et al., 2007; Vale et al., 2015).

Group 4-Cypermethrin targets: Insecticide treated black clothes (Cypermethrin 1:9 concentrate) baited with Butanone (50 mg/h) and 1-octen-3-ol (0.5 mg/h) (AVIMA-Pty-Ltd, South Africa) were deployed in the area where animals in the target group grazed. Deployment was done at 250 m intervals over a linear distance with focus on paths that animals use for grazing or drinking water. Four Cypermethrin targets were deployed per km^2. The width of deployed Cypermethrin targets ranged between 2-5 km (Fig. 4.1). GPS coordinates were recorded for all Cypermethrin targets deployed (Kgori, 2006; Kamba Mebourou et al., 2020). However, the use of Cypermethrin targets has been associated with vandalism and destruction by wild animals. To overcome such limitations, locals were involved in the deployment (to create awareness), monitoring and maintenance conducted every three months.

4.2.4 Sampling and treatment procedure

At the beginning of the study, all enrolled animals were screened to determine their baseline prevalence rates of trypanosomiasis prior to the implementation of the treatments. All animals were then treated with Berenil to clear any existing trypanosomes after which all four treatments were initiated.

All cattle were screened for trypanosomes using blood samples collected monthly over a period of 12 months between 2019 and 2020. Blood samples were collected by puncturing animal ear veins with blood lancets and collected using two micro capillary tubes containing an anticoagulant. For each animal, about 200 μL of whole blood collected from the first capillary tube was placed on a labelled FTA® card and left to air dry out of direct sunlight. All collected samples on FTA® cards were packed in zip-locked storage bags containing silica gel and transported to the laboratory where they were stored at ambient temperature for further processing on ITS-PCR. Blood from the second capillary tube was used to make thin and thick smears for further microscopic examination after staining with Giemsa solution. Supplementary data for each animal was recorded (breed, sex, age, location, and date of sampling) and categorised as young or adult. GPS coordinates were recorded for each sampling site.

Trypanosome infection for the four treatments (Berenil inoculation, Samorin inoculation, Cypermethrin target and Cyfluthrin pour-on), was determined using both microscopy and PCR (Cox et al., 2010; Ahmed et al., 2013; Mulenga et al., 2021). Being a simple and quick test, infections detected by microscopy were used to treat infected animals during monthly follow-ups. Infected animals were treated within 24 hours using Berenil at the dose described above. Diagnosis was further improved using PCR to calculate monthly incidence rates (Mungube et al., 2012; Hassan-Kadle et al., 2020; Mulenga et al., 2021). Sensitivity of the diagnostic tests used was discussed in a separate paper Mulenga et al. (2021)

4.2.5 Laboratory analysis

Sample preparation: Samples collected on FTA® cards were prepared by puncturing two 3mm diameter discs from each card using a Harris micro-punch Tool. The discs were placed in 1.5 mL sterile tubes accordingly and labelled. The discs were then washed twice in 100 μL of Whatman purification reagent for 15 minutes followed by two washes in 100 μL of 1x-concentrate TE buffer for 15 minutes to remove any residual Whatman purification reagent.

The discs were transferred to labelled 100 μL tubes and allowed to dry at room temperature. Finally, after the discs had dried, DNA was eluted using a Chelex 100® elution protocol. The eluted DNA was stored at 4 °C for use within 12 hours and at −20 °C for use after 12 hours (Morrison et al., 2007; Anderson et al., 2011; Mulenga et al., 2021). ITS-PCR was performed as described by Njiru et al. (2005). At the same time, thin and thick smears were stained with 10% Giemsa solution and later examined microscopically for the presence of trypanosomes (Marcotty et al., 2008; Mulenga et al., 2021). Microscopy results were available within 24hrs prior of sampling.

Disease Incidence: Laboratory positive samples on ITS-PCR were used to calculate incidence rates. The endemic trypanosome prevalence rates of each treatment site was recorded prior to initiation of the control treatments and used as a baseline for the disease. Trypanosomiasis incidence rates for the four groups over the entire period of the study were standardized by subtracting the endemic prevalence from each monthly post-treatment incidence rate. This was done to account for possible differences in exposure rates between sites. Analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences in mean standardized incidence rates between the four treatment groups. In a post hoc analysis, the Bonferroni (All-Pairwise) Multiple Comparison Test was used to determine differences in means between the pairs of the treatment groups.

4.2.6 Partial budget analysis and modelling

To quantify the financial annual net return of the four tsetse and trypanosomiasis control interventions under study, a partial budget analysis was carried out using the following inputs (Lowa, 2018).

Additional returns:

Cattle sales = proportion of births * value per unit.

According to livestock sales figures at the time the study was undertaken, the average value per cattle ranged between ZMW 3000 and ZMW 5000 (Department of Livestock development, Zambia-marketing section and Livestock owners, personal communication) and was modelled as a triangular distribution function with the most likely value being ZMW 3500. Additional returns were cattle born during, and at 48 weeks of the study. The proportion of

births was modelled as a beta distribution function multiplied by the triangular distribution function for the value of an animal.

Costs no-longer incurred:

Treatment cost for deaths = Number of cattle deaths * treatment cost.

The costs no longer incurred were based on the savings in cost of treatment for every animal that died during the study. It was assumed that treatment was necessary to prevent animals dying from trypanosomiasis.

Foregone returns:

Cattle that died during the treatment period = proportion of deaths * value per unit.

Foregone returns were the proportion of deaths during the study period which was modelled as a beta distribution function. This was then multiplied by the triangular distribution function for the value of an animal as given above.

Additional costs:

Cost of treatment = deployment cost * number of deployments * frequency of deployment

'Deployment costs' was defined as the cost of treatment per animal plus the cost of fuel by Veterinary services (administrative costs).

Additional costs associated with each control strategies were defined as follows:

i. The cost of one sachet of Berenil was modelled as a triang (ZMW 3.57, ZMW 4.05, ZMW 4.64) distribution (Table 4.1) based on data from, Livestock Services Cooperative Society Ltd, Showgrounds, Lusaka. One sachet was used per animal per treatment.

ii. Each sachet of Samorin could treat on average of eight animals. The cost of one sachet was modelled as a triang (ZMW 93.89, ZMW 108.41, ZMW 122.81) (Table 1) distribution per sachet (Livestock Services Cooperative Society Ltd, Showgrounds, Lusaka). Therefore, the cost of treating one animal was modelled as a triangular (ZMW 11.86, ZMW 11.74, ZMW 13.55, ZMW 15.35) distribution (Table 4.1) divided by the number of animals treated (48 were animals treated).

iii. Black fabric material was tailored (1.5 x 1 m per piece) into 300 pieces and treated with cypermethrin at a concentration of 10%. A total of 300 treated targets, baited with butanone were deployed in an area of 62.5 km^2. The cost per treated target was modelled as a triang (ZMW 27.54, ZMW 30.60, ZMW 33.66) distribution (Table 4.1) based on data from Tradeget enterprises, Indeco House, Cairo Road, Lusaka, Safique's trading for fabrics, Kamwala, Lusaka and Uncle James best tailor and design, P. O Box 18, Mfuwe.

iv. Pour-on group; Every 5 L of Amipor was used for four months when applied to 50 animals at a dose of 15 mL/100 kg every eight weeks. The cost of pour-on applied per four months was modelled as a triang (ZMW 702.34, ZMW 810.87, ZMW 918.68) distribution (Table 4.1) based on date from Livestock Services Cooperative Society Ltd, Showgrounds, Lusaka).

To account for variability and uncertainty in the partial budget, inputs were modelled using distribution functions. Table 1 shows inputs used in the model and the distribution functions, used to model that input.

Table 4. 1: Inputs and distribution functions used to simulate a partial budget for trypanosomiasis treatments studied in Mambwe district, 2019

Input	Distribution functions (per cattle)
Samorin treatment cost (ZMW)	Triang (10.674, 11.86, 13.046)
Berenil treatment cost (ZMW)	Triang (3.57, 4.05, 4.64)
Cyfluthrin pour-on treatment cost (ZMW)	Triang (12.771, 14.19, 15.609)
Cypermethrin targets treatment cost (ZMW)	Triang (231.12, 256.8, 282.48)
Births	Beta (α, β)
Deaths	Beta (α, β)
Livestock value (ZMW)	Triang (3150, 3500, 3850)

The financial net returns for each of the treatments under study were simulated using the add-in software program @RISK 8.2 in MS Excel (Palisade company LLC). The programme was set up for 10000 iterations using the Latin Hypercube sampling technique (Vose, 2008). All unit costs were modelled as triangular distribution functions, which uses parameters, minimum value, most likely value, and maximum value. Births and deaths occurring during the study period were modelled using the Beta distribution function. The Beta distribution function uses the parameters alpha (α) and beta (β), where alpha is set to the value r + 1 and beta is set to n – r + 1. In our study, r was the number of births or deaths recorded per treatment group while n was the total number of animals present at the beginning of the treatment per group. Sensitivity analysis was then performed on the output variables using the Palisade programme TopRank 8.2, to see which of the inputs had the highest effect on the financial net return for each of the four treatment groups (Vose, 2008).

4.3. RESULTS

4.3.1 Trypanosomiasis incidence

The crude and standardized incidence rates as recorded by PCR during the study period are as shown in Table 4.2.

Table 4. 2: Crude and standardized trypanosomiasis 4 weekly incidence rates for treatments conducted in the Luangwa Valley, eastern Zambia between the years 2019 and 2020

	Parasite control				Vector control			
Week	Berenil inoculation		Samorin inoculation		Cyfluthrin pour-on		Cypermethrin targets	
	Crude	Std. inc.	Crude	Std. inc.	Crude	Std. inc.	Crude	Std. inc.
0	0.78	0.00	0.46	0.00	0.82	0.00	0.98	0.00
4	0.72	-0.06	0.36	-0.1	0.72	-0.1	0.66	-0.32
8	0.82	0.04	0.28	-0.18	0.54	-0.28	0.54	-0.44

12	0.82	0.04	0.46	0	0.28	-0.54	0.46	-0.52
16	0.58	-0.2	0.52	0.06	0.44	-0.38	0.48	-0.5
20	0.78	0	0.58	0.12	0.48	-0.34	0.4	-0.58
24	0.58	-0.2	0.26	-0.2	0.48	-0.34	0.22	-0.76
28	0.54	-0.24	0.2	-0.26	0.34	-0.48	0.36	-0.62
32	0.52	-0.26	0.3	-0.16	0.38	-0.44	0.36	-0.62
36	0.42	-0.36	0.26	-0.2	0.32	-0.5	0.12	-0.86
40	0.46	-0.32	0.04	-0.42	0.26	-0.56	0.04	-0.94
44	0.28	-0.5	0.08	-0.38	0.14	-0.68	0.2	-0.78
48	0.18	-0.6	0.08	-0.38	0.02	-0.8	0.16	-0.82
Min	0.18	-0.6	0.04	-0.42	0.02	-0.8	0.04	-0.94
Mean	0.46	-0.22	0.22	-0.18	0.29	-0.45	0.25	-0.65
Std	1.00	0.21	0.87	0.17	0.86	0.19	0.79	0.19
Max	0.82	0.04	0.58	0.12	0.72	-0.10	0.66	-0.32
Median	0.62	-0.22	0.32	-0.18	0.44	-0.45	0.42	-0.65

1177 Std. inc.: Standardized incidence rates; Crude: Crude incidence rates

1178 Endemic trypanosome prevalence rates for the treatment groups were as follows: Berenil
1179 inoculation (78%, n = 39, 95%CI = 66.52-89.48), Samorin inoculation (46%, n = 23, 95%CI = 32.19-
1180 59.81), Cyfluthrin pour-on (82%, n = 41, 95%CI = 71.35-92.65), and Cypermethrin targets (98%,
1181 n = 49, 95%CI = 94.12-101.88).

1182 Annual incidence rates after initiation of the treatments were as follows: Berenil inoculation
1183 (18%, n = 9, 95%CI = 7.35-28.65), Samorin inoculation (8%, n = 4, 95%CI 0.48-15.52), Cyfluthrin
1184 pour-on (2%, n = 1, 95%CI -1.88-5.88), and Cypermethrin targets (16%, n = 8, 95%CI = 5.84-
1185 26.16).

The results indicated a significant drop in incidence for all the treatment groups over the study period (Fig. 4.2).

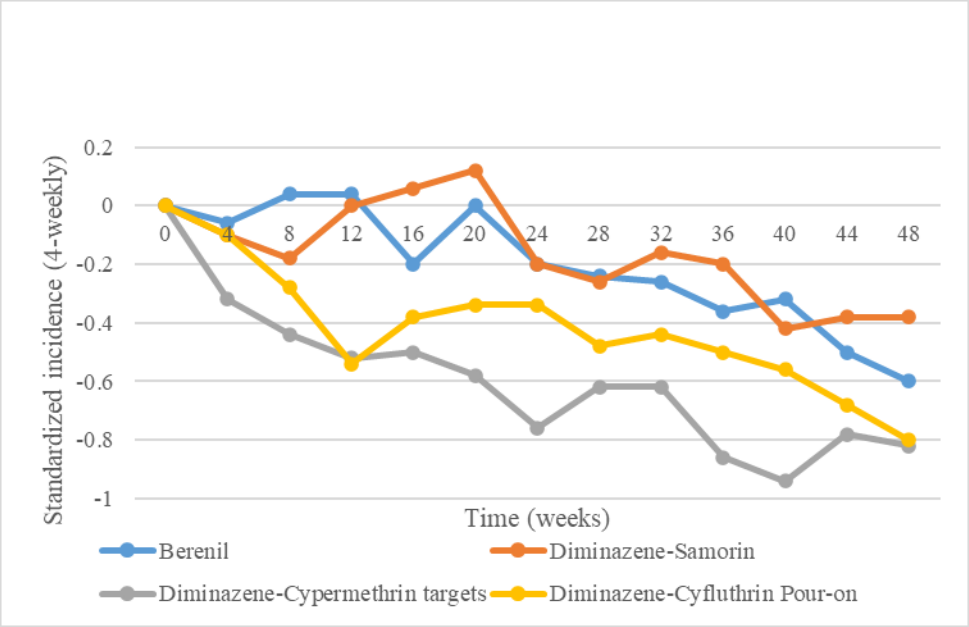

Fig. 4. 2. Chart showing change in standardized incidence rates between treatment groups during the study period conducted in the Luangwa Valley of Eastern Zambia between the 2019 and 2020.

4.3.2 Statistical significance between treatment groups

Results showed significant differences in mean incidence rates between the treatment groups (p value < 0.01, F value = 16.181) when compared using ANOVA.

The group standardized incidence rates were then compared, with the Berenil group and each other, in a Post hoc analysis using the Bonferroni (all-pairwise) multiple caparison test (Alpha = 0.050, Error Term = S(A), DF = 48, MSE = 0.046, Critical Value = 2.752). Results indicated differences between group means as shown below:

			Different From
Group	**Count**	**Mean**	**Groups**
Berenil	13	0.5753846	Samorin
Pour_on	13	0.4015385	
Samorin	13	0.2984615	Berenil

Targets 13 0.3830769

Our results were the same when the post hoc test was done using the Fisher's LSD multiple-comparison test.

4.3.3 Weather

The mean rainfall, minimum and maximum temperatures by monthly treatment period are shown in Fig. 4.3. High temperatures were seen in the middle of the rainy season at the beginning of the study. An increase in rainfall and temperature were later seen at the start of the next rainy season from month 11 to 13.

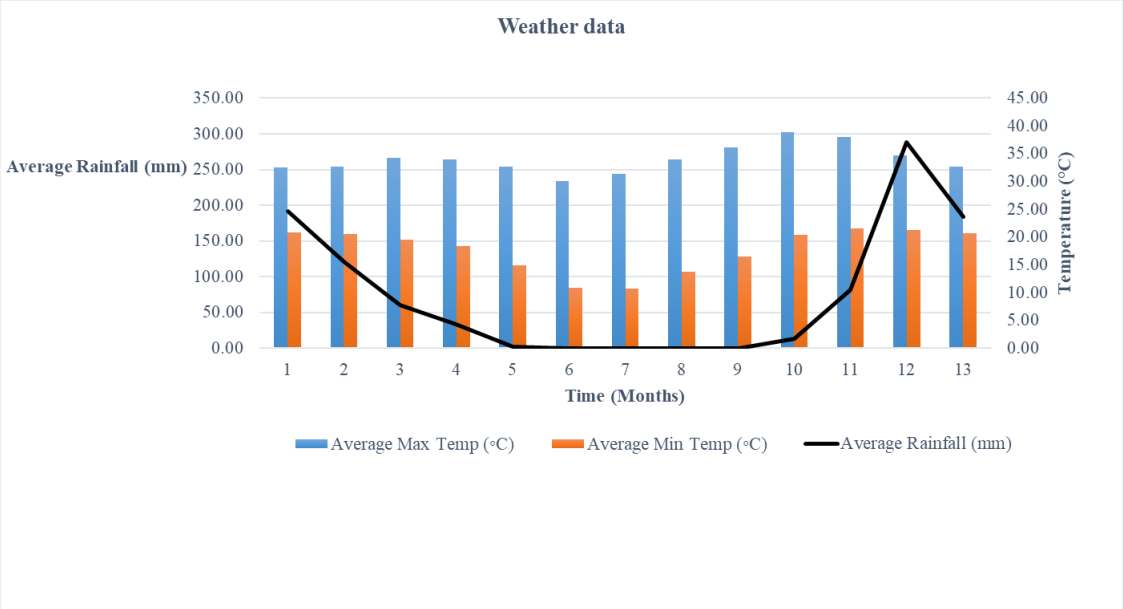

Fig. 4. 3. Chart showing rainfall and temperature data per month for each treatmen site over the treatment period conducted in the Luangwa Valley of Eastern Zambia between the 2019 and 2020.

4.3.4 Partial Budget

The median annual net returns from the distribution functions as calculated in the partial budget are shown in Table 4.3. The median return for the Berenil inoculation, Samorin inoculation, Cyfluthrin pour-on and Cypermethrin targets was ZMW 636.36, ZMW 910.00, ZMW 477.71, and ZMW 849.11 respectively.

Table 4. 3: Partial budget showing median net returns estimated by the model for the different treatment groups

	Berenil inoculation	Samorin inoculation	Cyfluthrin pour-on	Cypermethrin targets
Additional returns (ZMW)	689.39	1050.00	700.00	753.85
Costs no longer incurred (ZMW)	0.00	47.44	170.28	513.60
Foregone returns (ZMW)	53.03	140.00	350.00	161.54
Additional costs (ZMW)	0.00	47.44	42.57	256.80
NET RETURN (ZMW)	636.36	910.00	477.71	849.11

Based on the partial budget, the distribution functions for the net return estimates were produced for each of the treatment groups (Fig. 4.4).

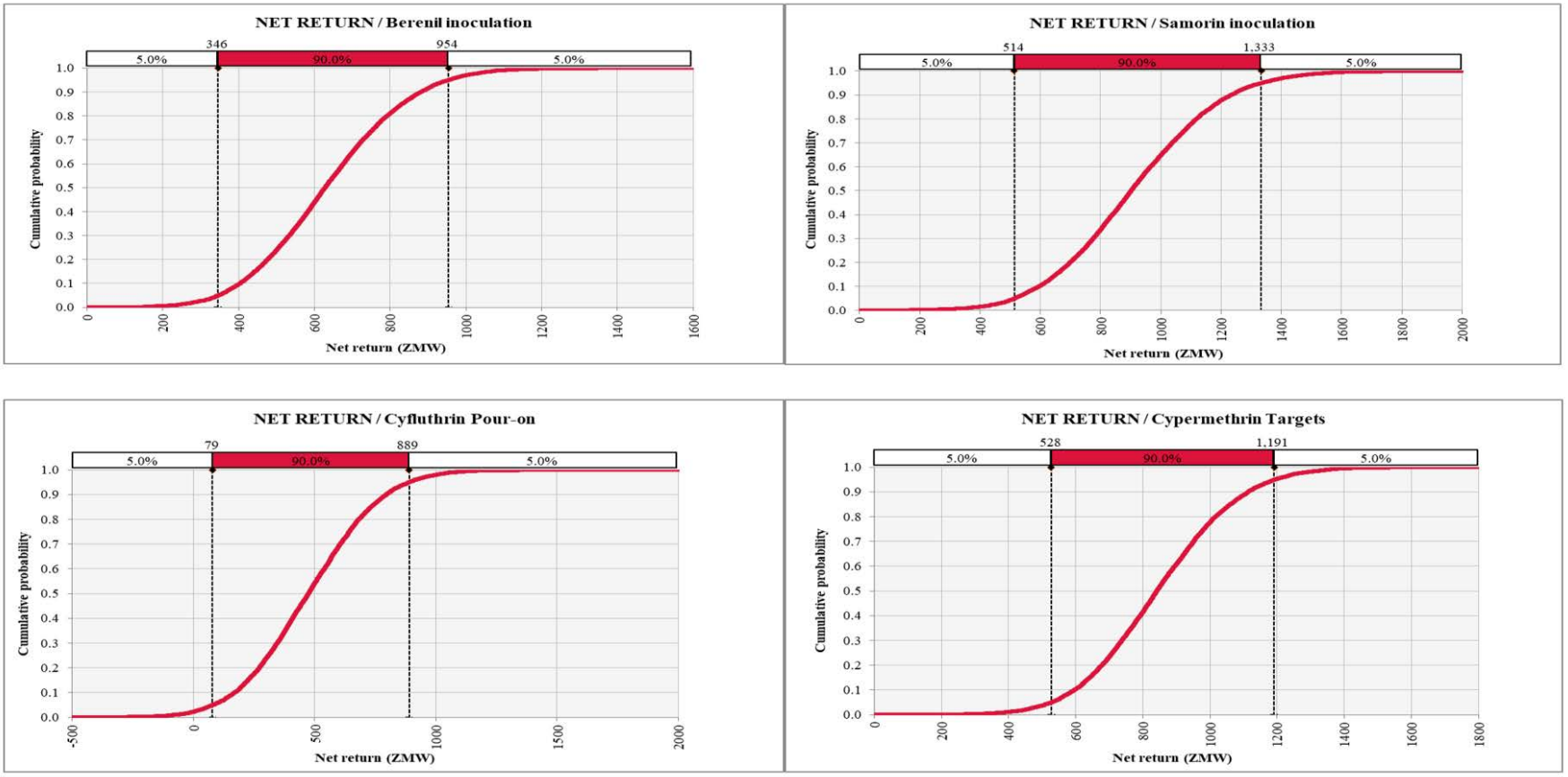

Fig. 4. 4. Distribution functions showing financial net return estimates of the four treatment groups at 5th and 90th percentiles.

The graphs show that, there is a 5% chance of the Berenil inoculation net return to be below ZMW 342, a 90% chance of being below ZMW 959. For the Samorin inoculation group, there is a 5% chance that the net return would be below ZMW 508 and a 90% chance of being below ZMW 1326. For the Cyfluthrin pour-on group, there is a 5% chance that the net return to be below ZMW 76 and a 90% chance of being below ZMW 884. While for the Cypermethrin targets group, there is a 5% chance that the net return would be below ZMW 528 and a 90% chance of being below ZMW 1185.

Sensitivity analysis (Fig. 4.5) showed that additional returns due to births from low mortality had the highest effect on the financial net returns for the Samorin inoculation, Berenil inoculation and Cyfluthrin pour-on groups while, costs no-longer incurred due to deaths had the highest effect on the financial net return for the Cypermethrin targets group.

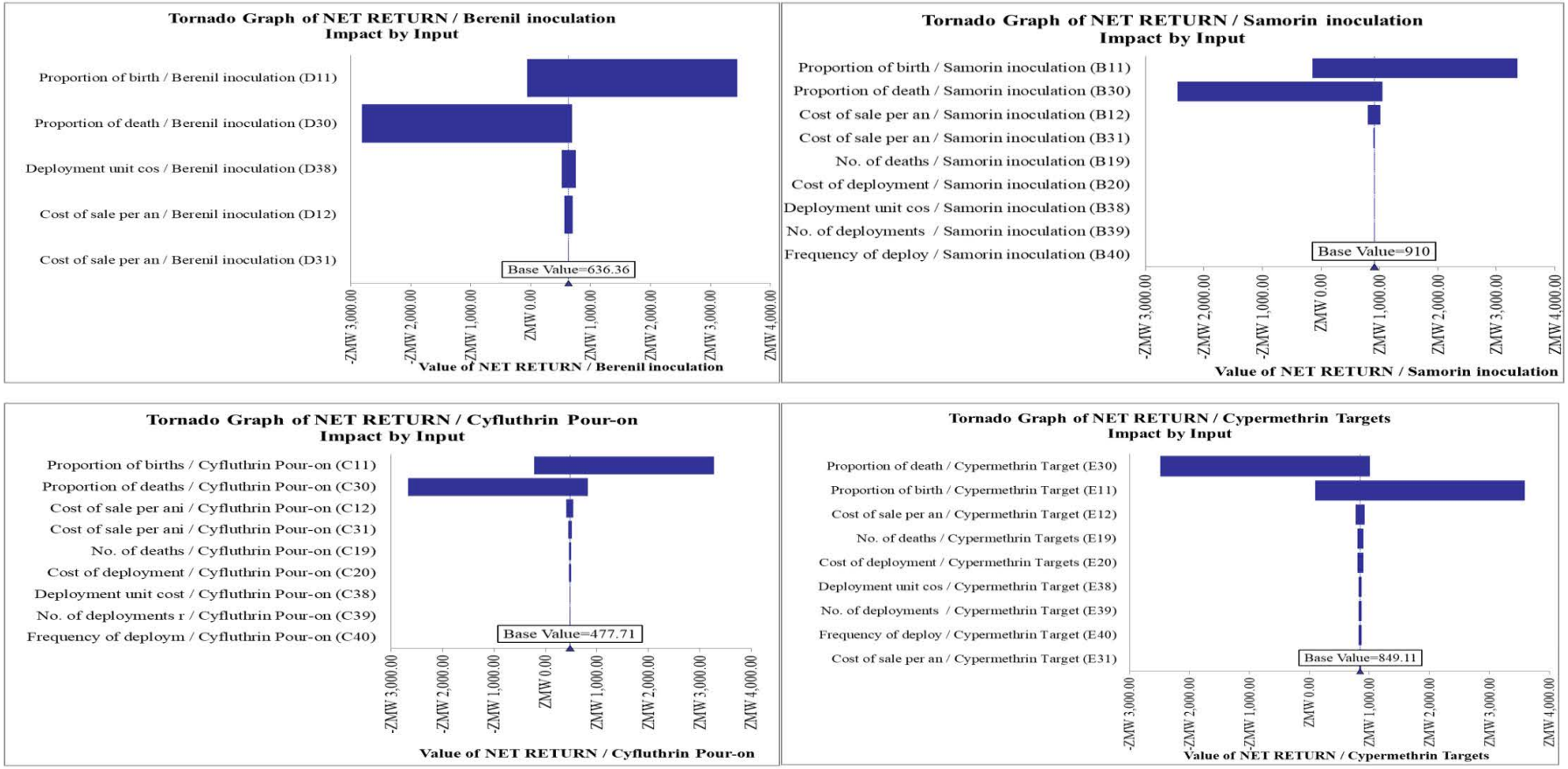

Fig. 4. 5. Sensitivity tornado graphs showing inputs for the four treatment groups ranked by effect on output means.

4.4. DISCUSSION

The results showed that baseline trypanosome prevalence rates were higher for all four treatment groups compared to incidence rates after initiation of treatments. Monthly trypanosome incidence rates however, fluctuated over time and reduced towards the end of the study period. High rainfall and temperatures were experienced at the time the baseline survey was conducted and towards the end of the survey. The wet-warm weather has been reported to favour tsetse population growth and tsetse movements resulting in increased transmission and infection rates in animals (Van den Bossche., 2010; Van den Bossche and Delespaux, 2011)..

Results indicated that the vector control groups (Cypermethrin targets and Cyfluthrin pour-on) showed a greater impact on trypanosome incidence than the parasite control groups (Samorin inoculation, and Berenil inoculation). Our findings were in agreement with observations made in other studies (Hamill et al., 2017; Kamba Mebourou et al., 2020; Lord et al., 2020; Rayaisse et al., 2020).

Animal protection from the vector control method is dependent on the chemical residual effects and technical issues related to the use of the control method (Tekle et al., 2018; Kamba Mebourou et al., 2020). The high toxicity and long residual effect of Cypermethrin insecticide in the black target materials allowed for long periods of effective vector control. This resulted in the reduction in trypanosome incidence rates in the group over time. Residual effects of Cypermethrin have been reported as effective for a period of 12 months, after which efficacy starts to reduce. Annual re-deployment of Cypermethrin targets is therefore recommended. The use of insecticide treated Cypermethrin targets has received much attention as one of the leading treatments effective in reducing tsetse populations which in turn reduces trypanosome case detection in man and livestock (Courtin et al., 2015; Kamba Mebourou et al., 2020; Rayaisse et al., 2020). The control of tsetse populations using odour baited Cypermethrin targets has been considered more effective as a suppression method and for protecting small, localised farming communities. Several technical issues have however, been associated with Cypermethrin targets which include theft, vandalism and maintenance challenges (Vreysen et al., 2013).

In our study, vector control using Cyfluthrin pour-on may have been affected by rainfall patterns experienced around week 8 (Fig. 4.3), which may have resulted in some wash off of

the active Cyfluthrin pour-on ingredients resulting in reduced efficacy as compared to the Cypermethrin targets treatment. Cattle are natural hosts for the tsetse vector, thus, baiting them with insecticide is a more logical method to protect them from tsetse bites. The application of Cyfluthrin pour-on on cattle not only offers protection against tsetse bites, but also provides control for ticks thus improving animal health and increasing meat and milk productivity to ensure food security (Kamau et al., 2000; Abro et al., 2021). Cyfluthrin pour-on is convenient and less demanding than other vector control methods. Cyfluthrin pour-on application has limited adverse effects on the environment, thus making the method, environmentally friendly. Challenges in the use of Cyfluthrin pour-on include among others, the costs associated with the treatment frequency, reduced farmer motivation to adhere to the Cyfluthrin pour-on application schedule, and risk of re-infections from other untreated animal disease reservoirs in the area (Kamau et al., 2000; Vreysen et al., 2013).

While several trypanosomiasis control methods target the tsetse vector, treatment of trypanosome infected animals with trypanocides continue to be the most widely applied control methods (Percoma et al., 2018). Our study employed two treatments which target the trypanosome parasite in livestock i.e., Berenil inoculation and Samorin inoculation. In the Berenil inoculation and Samorin inoculation groups, trypanocides are administered directly to the targeted animals and have a direct impact on the reduction of parasite levels provided the absence of drug resistance (Fyfe et al., 2017; Mulandane et al., 2018). Our findings showed that the Samorin inoculation group was more effective in reducing trypanosome incidence compared to the Berenil inoculation group. Increased infections in the Samorin inoculation group were however, observed during times when scheduled inoculations were due. This may have been due to the diminished levels of prophylaxis around that time (Tekle et al., 2018). Reports from the field indicate that the period of protection is reduced by high tsetse challenge. Experimental evidence, however, does not confirm this common observation. Even in the absence of tsetse, infected livestock can trigger infections in other livestock and humans via other vectors like tabanids (Van den Bossche., 2010; Baldacchino et al., 2014). Such findings indicate that treatment of trypanosome infected livestock using trypanocides can be used to reduce the risk of trypanosome transmission between livestock and man and may also limit spill overs from wildlife (Hamill et al., 2017; Meisner et al., 2019; Lord et al., 2020; Mulenga et al., 2021).

Furthermore, the partial budget analysis showed that all four treatment groups yielded a positive financial net return but varying net values. The median net returns from the distribution functions as calculated in the partial budget showed that the Samorin inoculation group had the greatest return followed by the Cypermethrin target group and the Berenil inoculation group. The Cyfluthrin pour-on group had the lowest return. Previous studies (Shaw et al., 2013; Shaw et al., 2015; Sutherland et al., 2017; Meyer et al., 2018) which however, focused on costs of the treatment group, suggested that Cypermethrin targets were costly but effective in reducing trypanosomiasis incidence while the use of Cyfluthrin pour-on on cattle had the lowest cost (Meyer et al., 2018; Tekle et al., 2018). Based on our findings, we can add that in as much as the use of treated Cypermethrin targets have showed to be cost-effective, their financial net yields are equally beneficial for livestock farmers, but not as much as the financial net yields the farmer would get by using Samorin inoculation. The use of treated Cypermethrin targets may therefore, be more beneficial for large-scale government sponsored vector control programmes (Rayaisse et al., 2020), while Samorin inoculation may be a better option for individual small-scale farmers affected by trypanosomiasis because it provides a better return on investment (Van den Bossche and Delespaux, 2011). The Cyfluthrin pour-on treatment, showed the lowest treatment cost in other studies (Meyer et al., 2018; Abro et al., 2021), yet provided the lowest financial net return in our study.

The 'what if' sensitivity analysis results showed that for the three treatments studied (Berenil inoculation, Samorin inoculation and Cyfluthrin pour-on), additional returns due to births from low mortality had the greatest impact on the financial net return outputs, while costs no-longer incurred due to deaths had the greatest impact on the financial net return for the Cypermethrin target treatment. Cattle births from the Samorin inoculation, Berenil inoculation and Cyfluthrin pour-on treatment, had a positive impact on the overall benefits the farmer would get as these overshadowed the set-up cost for the three treatments. The Cypermethrin target treatment was the most expensive in setting up, which meant the cost per animal increased when cattle died, and this impacted on the financial net return for the Cypermethrin target group.

Costs of parasite treatments can be reduced if farmers could conduct the treatments themselves instead of using veterinary officers. Such actions may however, come with consequences resulting from non-compliance in the use of trypanocides which may result in

trypanocide resistance (Mulandane et al., 2018), increase disease incidence rates, increased deaths and reduced financial net returns. Training and use of community livestock assistants in the administration of trypanocides may be a better option to maximize net returns realised from the parasite treatment groups. Community participation has been identified to have a positive impact in efforts made to mitigate community vulnerability to vector borne diseases as well as ensuring sustainable application of such treatments (Bardosh et al., 2017).

Financial net returns can be maximized further through integrating treatment control methods. Samorin inoculation and Cypermethrin targets groups yielded higher returns and may be better paired as parasite and vector treatments respectively, while the Berenil inoculation and Cyfluthrin pour-on may provide the second-best option as parasite and vector treatments respectively. Integration of these control methods would maximise the benefits and reduce costs of controlling trypanosomiasis in Zambia and within the region (FAO, 2017; Meyer et al., 2018).

4.5. CONCLUSIONS

The impact of a trypanosomiasis control method on AAT incidence does not determine its financial net return. The Samorin inoculation treatment is a more cost-effective method for controlling AAT for small scale farmers in remote poor resource communities of Eastern Zambia while the use of Cypermethrin targets may be a better option for large-scale government sponsored vector control programmes. The Berenil inoculation and the Cyfluthrin pour-on were equally cost effective but their financial net return should be taken into consideration if applied as control options for AAT at farm level. These findings will help communities make better decisions in the choice of trypanosomiasis control methods based on the greatest returns. This will enable better use of the limited resources, which will in turn, will protect the livelihood of communities through increased profit margins thus improving food security.

Ethical approval

Animal ethical clearances were obtained from James Cook University (A2498) and the Zambian Ethics Committee-ERES Converge IRB (Ref. No. 2018-Oct-001), and the research was approved by the Zambia National Health Research Authority.

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Availability of data and materials

All data generated or analysed during this study are available in the James Cook University data repository

Conflict of interest

The authors declare that they have no competing interests.

4.6. REFERENCES

Abro, Z., Kassie, M., Muriithi, B., Okal, M., Masiga, D., Wanda, G., Gisele, O., Samuel, A., Nguertoum, E., Nina, R.A., Mansinsa, P., Adam, Y., Camara, M., Olet, P., Boucader, D., Jamal, S., Garba, A.R.I., Ajakaiye, J.J., Kinani, J.F., Hassan, M.A., Nonga, H., Daffa, J., Gidudu, A., Chilongo, K., 2021. The potential economic benefits of controlling trypanosomiasis using waterbuck repellent blend in sub-Saharan Africa. PloS one 16, e0254558. doi:10.1371/journal.pone.0254558

Ahmed, H.A., Picozzi, K., Welburn, S.C., MacLeod, E.T., 2013. A comparative evaluation of PCR- based methods for species- specific determination of African animal trypanosomes in Ugandan cattle. Parasites & Vectors 6, 316. doi:10.1186/1756-3305-6-316

Anderson, N.E., Mubanga, J., Fevre, E.M., Picozzi, K., Eisler, M.C., Thomas, R., 2011. Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. PLOS Neglected Tropical Diseases 5, e1211. doi:10.1371/journal.pntd.0001211

Baldacchino, F., Desquesnes, M., Mihok, S., Foil, L.D., Duvallet, G., Jittapalapong, S., 2014. Tabanids: neglected subjects of research, but important vectors of disease agents! Infection, Genetics & Evolution 28, 596-615. doi:10.1016/j.meegid.2014.03.029

Bardosh, K.L., Ryan, S.J., Ebi, K., Welburn, S., Singer, B., 2017. Addressing vulnerability, building resilience: community-based adaptation to vector-borne diseases in the context of global change. Infectious Diseases of Poverty 6, 166. doi:10.1186/s40249-017-0375-2

Courtin, F., Camara, M., Rayaisse, J.B., Kagbadouno, M., Dama, E., Camara, O., Traore, I.S., Rouamba, J., Peylhard, M., Somda, M.B., Leno, M., Lehane, M.J., Torr, S.J., Solano, P., Jamonneau, V., Bucheton, B., 2015. Reducing Human-Tsetse Contact Significantly Enhances the Efficacy of Sleeping Sickness Active Screening Campaigns: A Promising Result in the Context of Elimination. PLOS Neglected Tropical Diseases 9, e0003727. doi:10.1371/journal.pntd.0003727

Cox, A.P., Tosas, O., Tilley, A., Picozzi, K., Coleman, P., Hide, G., Welburn, S.C., 2010. Constraints to estimating the prevalence of trypanosome infections in East African zebu cattle. Parasites & Vectors 3, 82. doi:10.1186/1756-3305-3-82

CSO, 2019. Preliminary Livestock and Aquaculture Census Results. Central Statistics Office-Republic of Zambia, Lusaka.

Dyce, K.M., Sack, W.O., Wensing, C.J.G., 2009. Textbook of veterinary anatomy-E-Book. Elsevier Health Sciences.

Engels, S.L., 2006. Reconsidering the underestimated burden caused by neglected tropical diseases. Trends Parasitology 22, 363-366.

FAO, 2017. Intervening against bovine trypanosomosis in eastern Africa: mapping the costs and benefits. Food and Agriculture Organization of the United Nations Rome,.

Franco, J.R., Cecchi, G., Priotto, G., Paone, M., Diarra, A., Grout, L., Simarro, P.P., Zhao, W., Argaw, D., 2020. Monitoring the elimination of human African trypanosomiasis at continental and country level: Update to 2018. PLOS Neglected Tropical Diseases 14, e0008261. doi:10.1371/journal.pntd.0008261

Fyfe, J., Picozzi, K., Waiswa, C., Bardosh, K.L., Welburn, S.C., 2017. Impact of mass chemotherapy in domestic livestock for control of zoonotic *T. b. rhodesiense* human African trypanosomiasis in Eastern Uganda. Acta Tropica 165, 216-229. doi:10.1016/j.actatropica.2016.08.022

Hamill, L., Picozzi, K., Fyfe, J., von Wissmann, B., Wastling, S., Wardrop, N., Selby, R., Acup, C.A., Bardosh, K.L., Muhanguzi, D., Kabasa, J.D., Waiswa, C., Welburn, S.C., 2017. Evaluating the impact of targeting livestock for the prevention of human and animal trypanosomiasis, at village level, in districts newly affected with T. b. rhodesiense in Uganda. Infectious Diseases of Poverty 6, 16. doi:10.1186/s40249-016-0224-8

Hassan-Kadle, A.A., Ibrahim, A.M., Nyingilili, H.S., Yusuf, A.A., Rafael, F.C.V., 2020. Parasitological and molecular detection of *Trypanosoma spp*. in cattle, goats and sheep in Somalia. Parasitology 147, 1786-1791. doi:10.1017/S003118202000178X

Hulley, S.B., Cummings, S.R., Browner, W.S., Grady, D., Newman, T.B., 2013. Designing clinical research: an epidemiologic approach. Lippincott Williams & Wilkins Philadelphia, PA.

Kamau, S.W., Omukubaa, J., Kiragua, J., Masikaa, P., Ndung'ua, J.M., Wachirab, P., Mehlitzc, D., 2000. Financial analysis of animal trypanosomiosis control using cypermethrin pour-on in Kenya. Preventive Veterinary Medicine 44, 231-246.

Kamba Mebourou, E., Bourquin, M., Oloo, F., Vallat, A., Guerin, P.M., 2020. Deltamethrin Persistence on Textiles Used to Make Visual Targets for Tsetse Fly Control is

Positively Correlated With Their Polyester Content. Journal of Medical Entomology 57, 1525-1531. doi:10.1093/jme/tjaa057

Kgori, M.S., Torr SJ, 2006. The use of aerial spraying to eliminate tsetse from the Okavango Delta of Botswana. Acta Tropica 99, 184-199.

Livestock, M.o.F.a., 2017. Annual Report. Kakumbi Tsetse and Trypanosomiasis Research Station, Mambwe, Zambia.

Lord, J.S., Lea, R.S., Allan, F.K., Byamungu, M., Hall, D.R., Lingley, J., Mramba, F., Paxton, E., Vale, G.A., Hargrove, J.W., Morrison, L.J., Torr, S.J., Auty, H.K., 2020. Assessing the effect of insecticide-treated cattle on tsetse abundance and trypanosome transmission at the wildlife-livestock interface in Serengeti, Tanzania. PLOS Neglected Tropical Diseases 14, e0008288. doi:10.1371/journal.pntd.0008288

Lowa, 2018. Partial Budgeting: A Tool to Analyze Farm Business Changes. Lowa State University, http://www.extension.iastate.edu/agdm.

Marcotty, T., Simukoko, H., Berkvens, D., Vercruysse, J., Praet, N., Van den Bossche, P., 2008. Evaluating the use of packed cell volume as an indicator of trypanosomal infections in cattle in eastern Zambia. Preventive Veterinary Medicine 87, 288-300. doi:10.1016/j.prevetmed.2008.05.002

Meisner, J., Barnabas, R.V., Rabinowitz, P.M., 2019. A mathematical model for evaluating the role of trypanocide treatment of cattle in the epidemiology and control of *Trypanosoma brucei rhodesiense* and *T. b. gambiense* sleeping sickness in Uganda. Parasite Epidemiology and Control 5, e00106. doi:10.1016/j.parepi.2019.e00106

Meyer, A., Holt, H.R., Oumarou, F., Chilongo, K., Gilbert, W., Fauron, A., Mumba, C., Guitian, J., 2018. Integrated cost-benefit analysis of tsetse control and herd productivity to inform control programs for animal African trypanosomiasis. Parasites & Vectors 11, 154. doi:10.1186/s13071-018-2679-x

Morrison, L.J., McCormack, G., Sweeney, L., Likeufack, A.C., Truc, P., Turner, C.M., Tait, A., MacLeod, A., 2007. Use of multiple displacement amplification to increase the detection and genotyping of trypanosoma species samples immobilized on FTA filters. The American journal of tropical medicine and hygiene 76, 1132-1137.

Mubamba, C., Sitali, J., Gummow, B., 2011. Trends of selected cattle diseases in eastern Zambia between 1988 and 2008. Preventive Veterinary Medicine 101, 163-172. doi:10.1016/j.prevetmed.2011.05.013

Muhanguzi, D., Mugenyi, A., Bigirwa, G., Kamusiime, M., Kitibwa, A., Akurut, G.G., Ochwo, S., Amanyire, W., Okech, S.G., Hattendorf, J., Tweyongyere, R., 2017. African animal trypanosomiasis as a constraint to livestock health and production in Karamoja region: a detailed qualitative and quantitative assessment. BMC veterinary research 13, 355. doi:10.1186/s12917-017-1285-z

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. doi:10.1007/s00436-017-5718-1

Mulenga, G., Sakala, C., Sichande, M., Mashili, J., Mwansa, P., Hanamwanza, W., Mbumwae, K., Phiri, L., 2017. Protective period for Isometamedium Chloride (samorin) against trypanosomiasis in areas with varying tsetse challenge in Mambwe district, Zambia. General Conference of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). Livingstone, Zambia.

Mulenga, G.M., Henning, L., Chilongo, K., Mubamba, C., Namangala, B., Gummow, B., 2020. Insights into the Control and Management of Human and Bovine African Trypanosomiasis in Zambia between 2009 and 2019-A Review. Tropical Medicine and Infectious Diseases 5, 115. doi:10.3390/tropicalmed5030115

Mulenga, G.M., Namangala, B., Chilongo, K., Mubamba, C., Hayashida, K., Henning, L., Gummow, B., 2021. Challenges in the Diagnostic Performance of Parasitological and Molecular Tests in the Surveillance of African Trypanosomiasis in Eastern Zambia. Tropical Medicine and Infectious Diseases 6, 68.

Mungube, E.O., Vitouley, H.S., Allegye-Cudjoe, E., Diall, O., Boucoum, Z., Diarra, B., Sanogo, Y., Randolph, T., Bauer, B., Zessin, K.-H., Clausen, P.-H., 2012. Detection of multiple drug-resistant *Trypanosoma congolense* populations in village cattle of south-east Mali. Parasites & Vectors 5, 155. doi:10.1186/1756-3305-5-155

Njiru, Z.K., Constantine, C.C., Guya, S., Crowther, J., Kiragu, J.M., Thompson, R.C., Davila, A.M., 2005. The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. Parasitology research 95, 186-192. doi:10.1007/s00436-004-1267-5

Percoma, L., Sow, A., Pagabeleguem, S., Dicko, A.H., Serdebeogo, O., Ouedraogo, M., Rayaisse, J.B., Bouyer, J., Belem, A.M.G., Sidibe, I., 2018. Impact of an integrated

control campaign on tsetse populations in Burkina Faso. Parasites & Vectors 11, 270. doi:10.1186/s13071-017-2609-3

Rayaisse, J.B., Courtin, F., Mahamat, M.H., Cherif, M., Yoni, W., Gadjibet, N.M.O., Peka, M., Solano, P., Torr, S.J., Shaw, A.P.M., 2020. Delivering 'tiny targets' in a remote region of southern Chad: a cost analysis of tsetse control in the Mandoul sleeping sickness focus. Parasites & Vectors 13, 419. doi:10.1186/s13071-020-04286-w

Shaw, A.P., Cecchi, G., Wint, G.R., Mattioli, R.C., Robinson, T.P., 2014. Mapping the economic benefits to livestock keepers from intervening against bovine trypanosomosis in Eastern Africa. Preventive Veterinary Medicine 113, 197-210. doi:10.1016/j.prevetmed.2013.10.024

Shaw, A.P., Wint, G.R., Cecchi, G., Torr, S.J., Mattioli, R.C., Robinson, T.P., 2015. Mapping the benefit-cost ratios of interventions against bovine trypanosomosis in Eastern Africa. Preventive Veterinary Medicine 122, 406-416. doi:10.1016/j.prevetmed.2015.06.013

Shaw, A.P.M., Torr, S.J., Waiswa, C., Cecchi, G., Wint, G.R.W., Mattioli, R.C., Robinson, T.P., 2013. Estimating the costs of tsetse control options: An example for Uganda. Preventive Veterinary Medicine 110, 290-303. doi:https://doi.org/10.1016/j.prevetmed.2012.12.014

Simukoko, H., Marcotty, T., Vercruysse, J., Van den Bossche, P., 2011. Bovine trypanosomiasis risk in an endemic area on the eastern plateau of Zambia. Research in veterinary science 90, 51-54. doi:10.1016/j.rvsc.2010.04.021

Sutherland, C.S., Stone, C.M., Steinmann, P., Tanner, M., Tediosi, F., 2017. Seeing beyond 2020: an economic evaluation of contemporary and emerging strategies for elimination of *Trypanosoma brucei gambiense*. The Lancet Global Health 5, e69-e79. doi:10.1016/s2214-109x(16)30237-6

Swallow, B.M., 2000. Impacts of Trypanosomiasis on African Agriculture. PAAT Technical and Scientific series 2-FAO, Rome.

Tekle, T., Terefe, G., Cherenet, T., H.Ashenafti, 2018. Aberrant use and poor quality of trypanocides: a risk for drug resistance in southern western Ethopia. BMC veterinary research 14, 4.

Torr, S.J., Maudlin, I., Vale, G.A., 2007. Less is more: restricted application of insecticide to cattle to improve the cost and efficacy of tsetse control.

1527 Vale, G.A., Hargrove JW, Chamisa A, Grant IF, Torr, S.J., 2015. Pyrethroid treatment of cattle
1528 for tsetse control-reducing its impacts on dung fauna. PLOS Neglected Tropical
1529 Diseases 9, e0003560.
1530 Van den Bossche, P., Delespaux, V., 2011. Options for the control of tsetse-transmitted
1531 livestock trypanosomosis. An epidemiological perspective. Veterinary Parasitology
1532 181, 37-42. doi:10.1016/j.vetpar.2011.04.021
1533 Van den Bossche., P., Stephane de La Rocque, Guy Hendrickx, Jeremy Bouyer, 2010.
1534 Achanging environment and the epideiomigy of tsetse-transmitted livestock
1535 trypanosomiasis. Trends in parasitology, 236-243.
1536 Vose, D., 2008. Risk Analysis: A quantitative guide. John Wiley & Sons, Ltd Chichester, UK.
1537 Vreysen, M.J., Seck, M.T., Sall, B., Bouyer, J., 2013. Tsetse flies: their biology and control using
1538 area-wide integrated pest management approaches. Journal of Invertebrate Pathology
1539 112 Suppl, S15-25. doi:10.1016/j.jip.2012.07.026
1540 WHO, 2022. Ending the neglect to attain the Sustainable Development Goals One Health:
1541 Approach for action againt neglected tropical diseases 2021–2030. World Health
1542 Organization, Geneva.
1543

CHAPTER 5

PREVALENCE OF TRYPANOSOMES AND SELECTED SYMBIONTS IN TSETSE SPECIES OF EASTERN ZAMBIA

Publication

Gloria M. Mulenga, Boniface Namangala, and Bruce Gummow. Prevalence of trypanosomes and selected symbionts in tsetse species of Eastern Zambia (2022). Parasitology. 1-5

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Abstract: Insect symbionts have attracted attention for their potential use as anti-parasitic gene products in arthropod disease vectors. While tsetse species of the Luangwa valley have been extensively studied, less is known about the prevalence of symbionts and their interactions with the trypanosome parasite. Polymerase chain reaction was used to investigate the presence of *Wolbachi*a and *Sodalis* bacteria, in tsetse flies infected with trypanosomes (*Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei*). Out of 278 captured tsetse flies in Eastern Zambia, 95.3% (n = 265, 95% CI = 92.8–97.8) carried endosymbionts: *Wolbachia* (79.1%, 95% CI 73.9–83.8) and *Sodalis* (86.3%, 95% CI 81.7–90.1). Overall, trypanosome prevalence was 25.5% (n = 71, 95% CI = 20.4–30.7), 10.8% (n = 30, 95% CI 7.1–14.4) for *T. brucei*, 1.4% (n = 4, 95% CI = 0.4–3.6) for both *T. congolense* and *T. vivax*, and 0.7% (n = 2, 95% CI 0.1–2.6) for *T. b. rhodesiense*. Out of 240 tsetse flies that were infected with *Sodalis*, trypanosome infection was reported in 40 tsetse flies (16.7%, 95% CI = 12.0–21.4) while 37 (16.8%, 95% CI 11.9–21.8) of the 220 *Wolbachia* infected tsetse flies were infected with trypanosomes. There was 1.3 times likelihood of *T. brucei* infection to be present when *Wolbachia* was present and 1.7 likelihood of *T. brucei* infection when *Sodalis* was present. Overall findings suggest absence of correlation between the presence of tsetse endosymbionts and tsetse with trypanosome infection. Lastly, the presence of pathogenic trypanosomes in tsetse species examined provided insights into the risk communities face, and the importance of African trypanosomiasis in the area.

Key words: Trypanosome; Tsetse; Symbiont; Prevalence; Zambia

5.1 INTRODUCTION

African trypanosomiasis, caused by protozoa belonging to the genus *Trypanosoma*, is a vector-borne disease endemic in sub-Saharan Africa. African trypanosomes are transmitted to the mammalian hosts by the bite of an infected tsetse fly (Diptera: *Glossinida*e) causing a fatal disease commonly known as *Nagana* in cattle and Sleeping sickness in humans (WHO, 2017; Franco et al., 2020; Franco et al., 2022). *Trypanosoma congolense* is the major cause of animal African trypanosomiasis (AAT) in Eastern and Southern Africa whilst *Trypanosoma vivax* (together with *Trypanosoma congolense*) is a more important cause of AAT in cattle in West Africa (Cox et al., 2010; Laohasinnarong et al., 2015; Mulenga et al., 2021). The two human-infective trypanosome sub-species are *Trypanosoma brucei gambiense* (found in West and Central Africa) which accounts for over 98% of reported cases of sleeping sickness, and *Trypanosoma brucei rhodesiense* (found in Eastern and Southern parts of Africa, including Zambia) which only accounts for less than 2% of reported cases (Nakamura et al., 2019; Franco et al., 2020).

Tsetse flies host the following three endogenous symbionts: *Wigglesworthia glossinidia*, *Sodalis glossinidius* and *Wolbachia* (Wamiri, 2013; Makhulu et al., 2021). *Wigglesworthia,* found in all tsetse flies, provides nutritional and immunological benefits to its tsetse host. In the absence of this bacteria, intrauterine larval development is stunted, and progeny aborted (Weiss and Aksoy, 2011). *Wigglesworthia*'s contracted genome, encodes an unusually high number of putative vitamin biosynthesis pathways, which support the theory that *Wigglesworthia* supplements its tsetse host with nutritious metabolites that are naturally present in low titres in vertebrate blood (Wang J, 2009; Rio, 2012). *Sodalis* on the other hand can be found both intra- and extra-cellular in various tissues of tsetse flies, including midgut, body fat, milk gland, salivary glands and hemocoel (Doudoumis et al., 2017). *Sodalis* contains features associated with pathogenic lifestyles, including secretion systems which function during the tsetse's juvenile developmental stages (Dennis et al., 2014). *Sodalis* can be cultured in cell free medium, and, unlike *Wigglesworthia*, it is usually absent in several natural tsetse populations. Lastly, *Wolbachia* is a wide-spread bacteria endosymbiont infecting approximately 70% of surveyed insects. It manipulates the reproductive biology of its host mechanisms which include cytoplasmic incompatibility (CI), male killing, feminization and parthenogenesis (Wamiri, 2013).

Symbiotic interactions are widespread in insects (as well as animals and plants) and may provide an avenue for disease control. The use of biological methods for the control of vector transmitted diseases is becoming popular globally (Ricci, 2012; Utarini et al., 2021). Symbionts influence several aspects of the tsetse's physiology, including reproduction, nutrition, and vector competence. Several studies have suggested the involvement of insect microbiota in the ability of insect disease vectors to transmit pathogens (Geiger et al., 2007; Ricci, 2012; L. et al., 2013; Hamidou Soumana et al., 2014; Makhulu et al., 2021) thus providing hope in the potential use of symbionts to control African trypanosomiasis (Medlock et al., 2013). The presence of tsetse microbiota in Zambia's tsetse flies has been described in studies conducted by Mbewe et al. (2015) and Dennis et al. (2014) on wild tsetse flies. While the earlier study observed significant association between present endosymbiont and trypanosome infection, the later study found it difficult to establish if some tsetse microbiota could play a role in the susceptibility of tsetse flies to trypanosomiasis infection. Little is known about the presence of symbionts in tsetse species found along the Luangwa tsetse belt of the Eastern province of Zambia and the role that tsetse endosymbionts may play in the transmission and control of trypanosomiasis. Thus, the potential use of endosymbionts in trypanosomiasis control seems attractive because trypanocide based management of *Nagana* has proven to be costly and not sustainable. Furthermore, increasing resistance of trypanosomes to the available trypanocides has also been seen to threaten the efficacy of current control approaches. The study was therefore conducted to establish the prevalence of *Sodalis* and *Wolbachia* in tsetse species found in the Eastern province of Zambia, and to determine the relationship that exists between these symbionts and trypanosomiasis infected tsetse flies.

5.2 MATERIALS AND METHODS

Study area and sample collection

Polymerase chain reaction (PCR) was used in a survey of tsetse symbionts and trypanosomes in tsetse species of Eastern Zambia. Taking into consideration tsetse characteristics, Epsilon traps baited with 3-n-prophyphenol and 1-octe-3-nol released at 5g/h from open bottles and 0.5g/h from polythene sachets, respectively, were used for collecting tsetse flies. In areas where fly density was low, flies trapped within a moving vehicle in the trapping site was used as a supplementary method to maximize catches. Traps were deployed within, and along peripheral known tsetse affected villages (Katemo, Ncheka, Nsefu, Chilanga, Chinzombo,

Malama and Chikowa) of Mambwe district in Zambia's Eastern Province between the years 2019 and 2020, during the dry-hot and wet-hot seasons. Deployment of traps was determined by the availability of suitable environments to maximise tsetse catches. Each trapping site was given a unique identifier and global positioning system (GPS) coordinates recorded and maintained for cross-referencing purposes. Milking of traps was done 24hours after deployment.

Sample preparation and storage

Tsetse samples collected were stored as whole flies in well labelled bottles containing ethanol. Each bottle contained all tsetse samples captured from one trapping site. Tsetse flies caught from supplementary techniques (e.g., moving vehicle) were stored together with samples captured from the nearest possible trapping site. Prior to storage, identification data was recorded (date of collection, location, numbers captured, sex and species). During sample preparation, captured flies were removed from ethanol storage, blotted with tissue paper towel, and left to air dry overnight at room temperature. Unique identifiers given during sample collection were maintained.

Laboratory analysis

Total genomic deoxyribonucleic acid (DNA) was extracted from individual flies after removing wings and legs. Manufacturer's instructions on DNA extraction kits (QIAamp® DNA mini kit) were followed during the extraction process. Extracted DNA was stored in 1.5mL tubes, labelled with unique trapping numbers related to where they were trapped. The eluted DNA was stored at 4◦C for use within 12 hours and at -20◦C for use after 12hours.

The presence of symbionts from the extracted DNA was determined using a symbiont species-specific PCR amplification assay as described by Pais et al. (2008). Four nanograms of the extracted DNA template was used for each PCR. For identification of *Sodalis*, HemF (ATGGGAAACAAACCATTAGCCA) and HemR (TCAAGTGACAAACAGATAAATC) primers (Pais et al., 2008) were used to amplify the 650-bp fragment of the hemolysin gene (accession no. AP008232). The presence of *Wolbachia* was detected by the amplification of a 610-bp fragment of the wsp gene with primers 81F (TGGTCCAATAAGTGATGAAGAAAC) and 691R (AAAAATTAAACGCTACTCCA) (Pais et al., 2008). For DNA quality control, the *G. morsitans morsitans tubulin* gene (accession no. DQ377071) were amplified with primers

GmmTubF (TAGTTCTCTTCAACTTCAGCCTCTT) and GmmTubR (TCGTTGACCATGTCTGGTGT) (Pais et al., 2008). Bacteria-specific PCR amplification conditions consisted of initial denaturation at 94°C for 2 minutes, followed by 30 cycles of 94°C for 30 sec, 54°C for 40 sec, and 72°C for 1 min with a final elongation at 72°C for 7 min. For *gmmtub* amplification, an annealing temperature of 60°C was used. The amplification products were analysed by agarose gel electrophoresis using ethidium bromide and visualised using a transilluminator (Pais et al., 2008).

ITS-PCR was undertaken in 25 μL reaction mixtures containing primers AITS-F: CGGAAGTTCACCGATATTGC and AITS-R: AGGAAGCCAAGTCATCCATC (Gaithuma et al., 2019), One Taq 2 @ master mix (New England BioLabs, Ipswich, MA, USA), nuclease free water and 5 μL of extracted DNA sample. For the detection of *T. b. rhodesiense*, SRA F (5′-ATAGTGACAAGATGCGTACTCAACGC-3′) and SRA R (5′-AATGTGTTCGAGTACTTCGGTCACGCT-3′) (Radwanska et al., 2002) were used (procured from Inqaba Biotec, Pretoria, South Africa). Thermocycler amplification conditions were at 94 ^{o}C for 5 minutes, followed by 40 cycles of 94 ^{o}C for 40 seconds, 58 ^{o}C for 40 seconds, 72 ^{o}C for 90 minutes and 72 ^{o}C for 5 minutes. ITS-PCR targets the internal transcribed spacer 1 of the ribosomal RNA (100−200 copies per genome), producing different sized products for different trypanosome species (Desquesnes et al., 2001; Njiru et al., 2005; Gaithuma et al., 2019). ITS-PCR products were separated by electrophoresis (95 volts for 60 minutes) in a 2% (w/v) agarose gel containing ethidium bromide. The separated products were then visualized under ultraviolet light in a transilluminator. Known positive controls of *T. congolense*, *T. vivax*, *T. b. rhodesiense* and *T. brucei* and a negative control were included in each reaction. All samples that were positive for *T. brucei* were subjected to a multiple PCR using a serum resistance-associated antigen (SRA) targeting primer for the detection of *T. b. rhodesiense* (Welburn et al., 2001; Radwanska et al., 2002; Gaithuma et al., 2019).

Statistical analysis

The prevalence data of trypanosome and symbiont infection from captured tsetse flies were summarised as frequencies and percentages and analysed using descriptive statistics in Epi-info 7.2. Odds ratios were used as measures of association. A Chi-square test was used to determine statistical differences between proportions. For expected values under 5, Fisher's exact test was used. Statistical significance was acceptable at $P < 0.05$. Pearson correlation test

was used to see if the presence of symbionts correlated with the presence of trypanosomes. Scores were used to determine the degree of correlation present. The scale of correlation coefficients were classified as follows: negative values (negative association), positive values (positive association), no association (0.00), very low (0.00-0.19), low (0.20-0.39), moderate (0.40-0.69), high (0.70-0.89), very high (0.90) (Schober et al., 2018).

5.3 RESULTS

The combined prevalence for *Sodalis* and *Wolbachia* in captured tsetse flies was 95.3% (n = 278, 95%CI = 92.8-97.8) while the overall trypanosome prevalence in captured tsetse flies was 25.5% (n = 278, 95%CI = 20.4-30.7). Trypanosome prevalence was 10.8% (n = 30, 95%CI = 7.1-14.4) for *T. brucei*, 1.4% (n = 4, 95%CI = 0.0-2.8) for both *T. congolense* and *T. vivax,* and 0.7% (n = 2, 95%CI = -0.3-1.7) for *T. b. rhodesiense*.

Out of 278 tsetse flies that were captured for the study, a total of 237 (85.3%) flies belonged to the group of *Glossina pallidipes* while 41 (14.8%) were *G. morstitans morsitans*. Total symbiont infections in *G. pallidipes* were 94.9% (n = 225, 95%CI = 92.2-97.7) while in *G. m. morsitans* was 97.6% (n = 40, 95%CI = 92.8-102.3), Trypanosome infections in *G. pallidipes* was 26.6% (n = 63, 95%CI = 21.0-32.2) while in *G. m. morsitans* was 19.5% (n = 8, 95%CI = 7.4-31.6). No significant difference was observed in both symbiont ($P = 0.46$) and trypanosome ($P = 0.34$) infections in the two tsetse species sampled. The prevalence of symbionts and trypanosomes in the two tsetse species detected by PCR was summarized (Table 5.1).

Table 5. 1: Prevalence (%) of symbionts and trypanosomes in tsetse species captured in the Luangwa valley, Eastern Zambia

	Symbionts		**Trypanosomes**					
Tsetse species	*Sodalis*	*Wolbachia*	*T. brucei*	*T. b. brucei*	*T. vivax*	*T. congolense*	*T. b. rhodesiense*	*Mixed infections*
G. m. morsitans **Prevalence (95%CI)**	85.4% (74.6-96.2)	80.5% (68.4-92.6)	19.5% (7.4-31.6)	19.5% (7.4-31.6)	0	0	0	0

G. pallidipes **Prevalence (95%CI)**	86.5% (82.2-90.9)	78.9% (73.7-84.1)	12.7% (8.4-16.9)	8.2% (4.6-11.5)	1.7% (0.1-3.3)	1.7% (0.1-3.3)	0.8% (-0.3-2.0)	1.7% (0.1-3.3)

The likelihood of female flies harbouring *Sodalis* (OR = 1.9, 95%CI 0.8-4.4) and *Wolbachia* (OR = 1.3, 95%CI 0.7-2.5) was higher than in male flies (Table 5.2).

Table 5. 2: Symbiont and trypanosome infection in relation to the sex of caught tsetse flies in the Luangwa valley, Eastern Zambia

	Symbionts		**Trypanosomes**			
	Sodalis	*Wolbachia*	*T. brucei*	*T. vivax*	*T. congolense*	*T. b. rhodesiense*
Female	158	146	17	2	1	1
Male	82	74	17	2	3	1
Odds ratio	1.9	1.3	2.3	2.1	6.4	2.1

Of the 240 tsetse flies that were positive for *Sodalis,* the prevalence of *T. brucei* was 12.9% (95%CI 8.7-17.2) while that of *T. congolense* was 1.7% (95%CI 0.1-3.3), *T. vivax* 1.3% (95%CI -0.2-2.7) and *T. b. rhodesiense* 0.8% (95%CI -0.3-2.0). Similarly, of the 220 tsetse flies that were positive for *Wolbachia*, trypanosome prevalence for *T. brucei* was 12.7% (95%CI 8.3-17.1) while that of *T. congolense* was 1.8% (95%CI 0.1-3.6), *T. vivax* 1.4% (95%CI -0.2-2.9) and *T. b. rhodesiense* 0.9% (95%CI -0.4-2.2).

Analysis of the association between trypanosomes and endosymbiont infection in the caught tsetse flies (Table 5.3) found a 1.3 (95%CI 0.5-3.2) times likelihood of *T. brucei* infection when *Wolbachia* is present and 1.7 (95%CI 0.5-6.0) likelihood of *T. brucei* infection when *Sodalis* is present. Similarly, results indicate a 0.8 (95%CI 0.1-7.7) likelihood of *T. vivax* infection when

Wolbachia is present and a 0.5 (95%CI 0.0-4.6) likelihood of *T. congolense* infection when *Sodalis* is present.

Table 5. 3: Measures of association between trypanosome and symbiont infection in tsetse flies caught in the Luangwa valley, Eastern Zambia

	Trypanosome Infection	*Wolbachia* Infection		Odds ratio	Confidence interval at 95%	*Sodalis* Infection		Odds ratio	Confidence interval at 95%
		Present	Absent			Present	Absent		
T. brucei	Present	192	52			209	35	1.7	0.5-6.0
	Absent	28	6	1.3	0.5-3.2	31	3		
T. congolense	Present	216	58	-	-	237	37	0.5	0.0-4.6
	Absent	4	0			3	1		
T. vivax	Present	217	57	0.8	0.1-7.7	236	38	-	-
	Absent	3	1			4	0		
T. b. rhodesiense	Present	218	58	-	-	238	38	-	-
	Absent	2	0			2	0		

Analysis of the correlation between the presence of tsetse endosymbionts and trypanosome infection showed no correlation (Table 5.4).

Table 5. 4: Correlations between trypanosome and symbiont infection in tsetse flies caught in the Luangwa valley, Eastern Zambia

	T. brucei	*T. vivax*	*T. congolense*	*T. b. rhodesiense*

	Pearson correlation	Sig. (2-tailed)	Pearson correlation	Sig. (2-tailed)	Pearson correlation	Sig. (2-tailed)	Pearson correlation	Sig. (2-tailed)
Sodalis	0.05	0.38	0.05	0.43	-0.04	0.51	0.03	0.57
Wolbachia	0.03	0.62	-0.01	0.84	0.06	0.30	0.04	0.47

5.4 DISCUSSION AND CONCLUSIONS

The tsetse fly has established symbiotic associations with bacteria which influence its reproduction, nutrition, and vector competence. Symbiotic interactions are widespread in insects (and also animals and plants) and may provide an avenue for disease control (Ricci, 2012; Wamiri, 2013). The current study provided the prevalence of selected tsetse symbionts and trypanosomes in *Glossina* tsetse species from Eastern Zambia. Results showed no statistical difference in the prevalence of both symbionts and trypanosomes in the two tsetse species (*G. m. morsitans* and *G. pallidipes*) analysed. No association was either observed between symbiont and trypanosome infection in the two tsetse species., suggesting that endosymbionts play no role in tsetse vector competence and reproduction in the area. These data are in agreement with those obtained by Dennis et al. (2014) but disagree with those by Farikou *et al*. and Mbewe *et al.* (Farikou et al., 2010; Mbewe et al., 2015) who established the existence of a relationship between tsetse bacteria and trypanosomes and the potential role of endosymbionts in tsetse vector competence and reproduction. The later studies however, where conducted in different geographical areas with different species of tsetse flies (*G. p. palpalis* and *G. m. Centralis* respectively)

Tsetse symbionts (*Wolbachia* and *Sodalis)* were detected in about 95% of the tsetse samples examined with varying prevalence within tsetse species. Both symbionts were found in relative abundance in the two tsetse species examined, with *Sodalis* prevalence slightly higher than *Wolbachia*. This agrees with findings from similar studies on tsetse symbionts though with varying levels of infection rates which may be attributed to differences in the sensitivity of the screening methods (Doudoumis et al., 2012; Dennis et al., 2014; Doudoumis et al., 2017).

The low numbers of *Wolbachia* have been associated with low sensitivity of the standard PCR assay (Wamiri, 2013) which was also used in our laboratory analysis of tsetse samples. The presence of *Sodalis* and *Wolbachia* infection in the tsetse population sampled re-affirms the presence of tsetse bacteria in tsetse species found in Zambia and particularly the Luangwa valley (Doudoumis et al., 2012; Dennis et al., 2014; Mbewe et al., 2015).

The overall trypanosome prevalence in the captured tsetse flies (25.5%) were similar to what was found by Nakamura *et al.* (Nakamura et al., 2021). The identification of *T. congolense, T. brucei* and *T. vivax* from tsetse samples analysed confirms the presence of AAT in the community (Mekata et al., 2008; Laohasinnarong et al., 2015; Mulenga et al., 2021; Nakamura et al., 2021). The presence of *T. b. rhodesiense* further indicated the circulation of the human-infective trypanosomes in the area, responsible for sleeping sickness and the importance of the tsetse species in trypanosomiasis transmission. Taken together, the presence of pathogenic trypanosomes in tsetse species examined provide insights to the risk of contracting sleeping sickness and AAT by the local communities and their livestock (Mekata et al., 2008; Djohan et al., 2015; Auty et al., 2016).

In agreement with (Mekata et al., 2008), high infections of both symbionts and trypanosomes were reported in the *G. pallidipes* species compared to *G. m. morsitans*. However, unlike observations from the current study, Doudoumis et al. (2012) found *G. m. morsitans* to be more likely to habour *Wolbachia* than *G. pallidipes*. On the other hand, current study findings were in concordance with findings obtained elsewhere, where *G. pallidipes* was captured with other tsetse species other than *G. morsitans* (Wamiri, 2013). Further, the high prevalence of female *G. pallidipes* found agree with findings by Laohasinnarong *et al.* (Laohasinnarong et al., 2015). Overall, both symbiont and trypanosome prevalence were, however, higher in female tsetse flies than in male tsetse flies and were associated with the host tsetse species as previously reported (Wamiri, 2013; Dennis et al., 2014). Such findings prompt for further research in the importance of *G. pallidipes* tsetse species with regards to host genetic diversity and vectoral capacity in areas where other tsetse species are present.

The weak relationship between tsetse symbiont prevalence and trypanosome prevalence shown in the current study does not support the synergistic role between symbiont and trypanosomiasis transmission in the surveyed area. However, the low number of tsetse flies infected with trypanosomes could explain the poor correlation observed, which suggest the

need for further work on the importance of *Sodalis* in tsetse species in the Luangwa valley tsetse belt. Understanding insect-parasite-symbiont interactions is necessary in establishing opportunities for biologically based trypanosomiasis control strategies (Boulanger et al., 2002). The importance of understanding this relationship is emphasised by the urgent need for environmentally friendly methods for both tsetse and trypanosomiasis control. The high prevalence of *Wolbachia* in female flies need to be investigated further as a possible basis for environmentally sustainable tsetse population control for *Glossina* species.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

GM developed, conceptualized, and drafted the manuscript. GM conducted specimen collection and analysis. BG offered guidance during specimen collection and contributed to the development of the manuscript. BG and BN were involved in supervision and project administration. All authors reviewed, read, edited the draft and final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethical Standards

Human and animal ethical clearances were obtained from James Cook University (H7226 and A2498) and the Zambian Ethics Committee (Ref. No. 2018-Oct-001), and the research was approved by the Zambia National Health Research Authority.

5.5 REFERENCES

Auty, H., Morrison, L.J., Torr, S.J., Lord, J., 2016. Transmission Dynamics of Rhodesian Sleeping Sickness at the Interface of Wildlife and Livestock Areas. Trends Parasitology 32, 608-621. doi:10.1016/j.pt.2016.05.003

Boulanger, N., Brun, R., Ehret-Sabatier, L., Kunz, C., Bulet, P., 2002. Immunopeptides in the defense reactions of *Glossina morsitans* to bacterial and *Trypanosoma brucei brucei* infections. Insect Biochem Mol Biol 32, 369-375. doi:10.1016/S0965-1748(02)00029-2

Cox, A.P., Tosas, O., Tilley, A., Picozzi, K., Coleman, P., Hide, G., Welburn, S.C., 2010. Constraints to estimating the prevalence of trypanosome infections in East African zebu cattle. Parasites & Vectors 3, 82. doi:10.1186/1756-3305-3-82

Dennis, J.W., Durkin, S.M., Horsley Downie, J.E., Hamill, L.C., Anderson, N.E., MacLeod, E.T., 2014. *Sodalis glossinidius* prevalence and trypanosome presence in tsetse from Luambe National Park, Zambia. Parasit Vectors 7, 378. doi:10.1186/1756-3305-7-378

Desquesnes, M., McLaughlin, G., Zoungrana, A., Davila, 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.

Djohan, V., Kaba, D., Rayaisse, J.B., Dayo, G.K., Coulibaly, B., Salou, E., Dofini, F., Kouadio Ade, M., Menan, H., Solano, P., 2015. Detection and identification of pathogenic trypanosome species in tsetse flies along the Comoe River in Cote d'Ivoire. Parasite 22, 18. doi:10.1051/parasite/2015018

Doudoumis, V., Blow, F., Saridaki, A., Augustinos, A., Dyer, N.A., Goodhead, I., Solano, P., Rayaisse, J.B., Takac, P., Mekonnen, S., Parker, A.G., Abd-Alla, A.M.M., Darby, A., Bourtzis, K., Tsiamis, G., 2017. Challenging the *Wigglesworthia, Sodalis, Wolbachia* symbiosis dogma in tsetse flies: Spiroplasma is present in both laboratory and natural populations. Sci Rep 7, 4699. doi:10.1038/s41598-017-04740-3

Doudoumis, V., Tsiamis, G., Wamwiri, F., Brelsfoard, C., Alam, U., Aksoy, E., Dalaperas, S., Abd-Alla, A., Ouma, J., Takac, P., Aksoy, S., Bourtzis, K., 2012. Detection and characterization of *Wolbachia* infections in laboratory and natural populations of different species of tsetse flies (*genus Glossina*). BMC Microbiol 12 Suppl 1, S3. doi:10.1186/1471-2180-12-S1-S3

Farikou, O., Njiokou, F., Mbida Mbida, J.A., Njitchouang, G.R., Djeunga, H.N., Asonganyi, T., Simarro, P.P., Cuny, G., Geiger, A., 2010. Tripartite interactions between tsetse flies, *Sodalis glossinidius* and trypanosomes--an epidemiological approach in two historical human African trypanosomiasis foci in Cameroon. Infect Genet Evol 10, 115-121. doi:10.1016/j.meegid.2009.10.008

Franco, J.R., Cecchi, G., Paone, M., Diarra, A., Grout, L., Kadima Ebeja, A., Simarro, P.P., Zhao, W., Argaw, D., 2022. The elimination of human African trypanosomiasis: Achievements in relation to WHO road map targets for 2020. PLOS Neglected Tropical Diseases 16, e0010047. doi:10.1371/journal.pntd.0010047

Franco, J.R., Cecchi, G., Priotto, G., Paone, M., Diarra, A., Grout, L., Simarro, P.P., Zhao, W., Argaw, D., 2020. Monitoring the elimination of human African trypanosomiasis at continental and country level: Update to 2018. PLOS Neglected Tropical Diseases 14, e0008261. doi:10.1371/journal.pntd.0008261

Gaithuma, A.K., Yamagishi, J., Martinelli, A., Hayashida, K., Kawai, N., Marsela, M., Sugimoto, C., 2019. A single test approach for accurate and sensitive detection and taxonomic characterization of Trypanosomes by comprehensive analysis of internal transcribed spacer 1 amplicons. PLoS Negl Trop Dis 13, e0006842. doi:10.1371/journal.pntd.0006842

Geiger, A., Ravel, S., Mateille, T., Janelle, J., Patrel, D., Cuny, G., 2007. Vector competence of *Glossina palpalis gambiensis* for *Trypanosoma brucei s.l.* and genetic diversity of the symbiont *Sodalis glossinidius*. Mol Biol Evol 24, 102-109. doi:10.1093/molbev/msl135

Hamidou Soumana, I., Loriod, B., Ravel, S., Tchicaya, B., Simo, G., Rihet, P., Geiger, A., 2014. The transcriptional signatures of *Sodalis glossinidius* in the *Glossina palpalis gambiensis flies* negative for *Trypanosoma brucei gambiense* contrast with those of this symbiont in tsetse flies positive for the parasite: possible involvement of a *Sodalis*-hosted prophage in fly *Trypanosoma* refractoriness? Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases 24, 41-56. doi:10.1016/j.meegid.2014.03.005

Weiss, B. L., Wang, J., Maltz, M.A., Wu, Y., Aksoy, S., 2013. Trypanosome infection establishment in the tsetse fly gut is influenced by microbiome-regulated host immune barriers. PLoS pathogens 9, e1003318. doi:10.1371/journal.ppat.1003318

1893 Laohasinnarong, D., Goto, Y., Asada, M., Nakao, R., Hayashida, K., Kajino, K., Kawazu, S.,
1894 Sugimoto, C., Inoue, N., Namangala, B., 2015. Studies of trypanosomiasis in the
1895 Luangwa valley, north-eastern Zambia. Parasites and Vectors 8, 555.
1896 doi:10.1186/s13071-015-1173-y
1897 Makhulu, E.E., Villinger, J., Adunga, V.O., Jeneby, M.M., Kimathi, E.M., Mararo, E., Oundo,
1898 J.W., Musa, A.A., Wambua, L., 2021. Tsetse blood-meal sources, endosymbionts and
1899 trypanosome-associations in the Maasai Mara National Reserve, a wildlife-human-
1900 livestock interface. PLoS Neglected Tropical Diseases 15, e0008267.
1901 doi:10.1371/journal.pntd.0008267
1902 Mbewe, N.J., Mweempwa, C., Guya, S., Wamwiri, F.N., 2015. Microbiome frequency and their
1903 association with trypanosome infection in male *Glossina morsitans* centralis of Western
1904 Zambia. Veterinary Parasitology 211, 93-98. doi:10.1016/j.vetpar.2015.04.027
1905 Medlock, J., Atkins, K.E., Thomas, D.N., Aksoy, S., Galvani, A.P., 2013. Evaluating
1906 paratransgenesis as a potential control strategy for African trypanosomiasis. PLoS
1907 Neglected Tropical Diseases 7, e2374. doi:10.1371/journal.pntd.0002374
1908 Mekata, H., Konnai, S., Simuunza, M., Chembensofu, M., Kano, R., Witola, W.H., Tembo, M.E.,
1909 Chitambo, H., Inoue, N., Onuma, M., Ohashi, K., 2008. Prevalence and source of
1910 trypanosome infections in field-captured vector flies (Glossina pallidipes) in
1911 southeastern Zambia. The Journal of veterinary medical science 70, 923-928.
1912 Mulenga, G.M., Namangala, B., Chilongo, K., Mubamba, C., Hayashida, K., Henning, L.,
1913 Gummow, B., 2021. Challenges in the Diagnostic Performance of Parasitological and
1914 Molecular Tests in the Surveillance of African Trypanosomiasis in Eastern Zambia.
1915 Tropical Medicine and Infectious Diseases 6, 68.
1916 Nakamura, Y., Hayashida, K., Delesalle, V., Qiu, Y., Omori, R., Simuunza, M., Sugimoto, C.,
1917 Namangala, B., Yamagishi, J., 2021. Genetic Diversity of African Trypanosomes in
1918 Tsetse Flies and Cattle From the Kafue Ecosystem. Frontiers in Veterinary Science 8,
1919 599815. doi:10.3389/fvets.2021.599815
1920 Nakamura, Y., Yamagishi, J., Hayashida, K., Osada, N., Chatanga, E., Mweempwa, C.,
1921 Chilongo, K., Chisi, J., Musaya, J., Inoue, N., Namangala, B., Sugimoto, C., 2019.
1922 Genetic diversity and population structure of *Glossina morsitans morsitans* in the active
1923 foci of human African trypanosomiasis in Zambia and Malawi. PLoS Neglected
1924 Tropical Diseases 13, e0007568. doi:10.1371/journal.pntd.0007568

1925 Njiru, Z.K., Constantine, C.C., Guya, S., Crowther, J., Kiragu, J.M., Thompson, R.C., Davila,
1926 A.M., 2005. The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes.
1927 Parasitology research 95, 186-192. doi:10.1007/s00436-004-1267-5
1928 Pais, R., Lohs, C., Wu, Y., Wang, J., Aksoy, S., 2008. The obligate mutualist *Wigglesworthia*
1929 *glossinidia* influences reproduction, digestion, and immunity processes of its host, the
1930 tsetse fly. Applied and Environmental Microbiology 74, 5965-5974.
1931 doi:10.1128/AEM.00741-08
1932 Radwanska, M., Chamekh, M., Vanhamme, L., Claes, F., Magez, S., Magnus, E., de Baetselier,
1933 P., Buscher, P., Pays, E., 2002. The serum resistance-associated gene as a diagnostic
1934 tool for the detection of *Trypanosoma brucei rhodesiense*. The American Journal of
1935 Tropical Medicine and Hygiene 67, 684-690. doi:10.4269/ajtmh.2002.67.684
1936 Ricci, M.V., Ulisse Ulissi, Sara Epis, Alessia Cappelli, Guido Favia, 2012. Symbiotic Control of
1937 mosquito borne disease. Pathogens and Global Health 106, 380-385.
1938 Rio, R.E.S., Jingwen Wang, Claudia Lohs, Yi-neng Wu, Anna K.Snyder, Robert D.Bjornson,
1939 Kenshiro Oshima, Bryan S.Biehi, Nicole T.Perna, Masahira Hattori, Serap Aksoy,
1940 2012. Insight into the Transimission Biology and Species-Specific Functional
1941 Capabilities of Tsetse (*Diptera: Glossinidae*) Obligate symbiont *Wigglesworthia*.
1942 Mbio.asm.org, 1-13.
1943 Schober, P., Boer, C., Schwarte, L.A., 2018. Correlation Coefficients: Appropriate Use and
1944 Interpretation. Anesthesia & Analgesia 126, 1763-1768.
1945 doi:10.1213/ane.0000000000002864
1946 Utarini, A., Indriani, C., Ahmad, R.A., Tantowijoyo, W., Arguni, E., Ansari, M.R., Supriyati,
1947 E., Wardana, D.S., Meitika, Y., Ernesia, I., Nurhayati, I., Prabowo, E., Andari, B.,
1948 Green, B.R., Hodgson, L., Cutcher, Z., Rances, E., Ryan, P.A., O'Neill, S.L., Dufault,
1949 S.M., Tanamas, S.K., Jewell, N.P., Anders, K.L., Simmons, C.P., Group, A.S., 2021.
1950 Efficacy of *Wolbachia*-Infected Mosquito Deployments for the Control of Dengue. The
1951 New England Journal of Medicine 384, 2177-2186. doi:10.1056/NEJMoa2030243
1952 Wamiri, F., Uzma Alam, Paul C Thande, Emre Aksoy, Raphael M Ngure, Serap Aksoy,
1953 Johnson O Ouma, Grace A Murilla, 2013. *Wolbachia, Sodalis* and Trypanosome co-
1954 infections in natural populations of *Glossina austeni* and *Glossina Pallidipes*. Parasites
1955 and Vectors, 232.

Wang J, W.Y., Yang G, Aksoy S. , 2009. Interactions between mutualist *Wigglesworthia* and tsetse peptidoglycan recognition protein (PGRP-LB) influence trypanosome transmission. Proc Natl Acad Sci 106, 12133-12138.

Weiss, B., Aksoy, S., 2011. Microbiome influences on insect host vector competence. Trends in parasitology 27, 514-522. doi:10.1016/j.pt.2011.05.001

Welburn, S.C., Picozzi, K., Fevre, E.M., Coleman, P.G., Odiit, M., Carrington, M., Maudlin, I., 2001. Identification of human-infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (SRA) gene. Lancet 358, 2017-2019.

WHO, 2017. Managing zoonotic public health risks at the human–animal–ecosystem interface. [Online]. Available at https://www.who.int/foodsafety/ about/flyer_zoonoses.pdf?ua=1 (Accessed 26 December 2020)

CHAPTER 6

POLICY AND LINKAGES IN THE APPLICATION OF A ONE HEALTH SYSTEM FOR REPORTING AND CONTROLLING AFRICAN TRYPANOSOMIASIS AND OTHER ZOONOTIC DISEASES IN ZAMBIA

Publication

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Presentation of findings

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Abstract: The capacity to detect, control and manage emerging and re-emerging zoonotic diseases in Africa has been limited by a lack of utilisation of available reporting structures and policies to support programmes at national and local levels. This study explored the impact of the Zambian government policies on animal and human disease reporting and management and on One Health opportunities. An in-depth review and analysis of strengths, weaknesses, opportunities, and threats in the existing policies and reporting structures in the departments responsible for Veterinary Services, Health, and Wildlife, was conducted. According to our findings, sub-optimal implementation of existing policies related to the control of zoonotic diseases was impacting disease reporting, and reporting structures play an important role in effective and sustainable reporting of zoonotic diseases. Further, the study explored capacities and strategies in trypanosomiasis control as a case study that could prompt effective adoption of a One Health approach, and as such, the study suggests measures that could help to assess the performance of a One Health system in the control of African trypanosomiasis and other zoonotic diseases.

Keywords: One Health; African trypanosomiasis; reporting structures; zoonotic diseases

6.1 INTRODUCTION

Occurrence of human and animal African trypanosomiasis is associated with the presence of tsetse flies. Mammalian wild animals such as lions, buffalos, hippopotamuses, and elephants are the main reservoirs for the tsetse-transmitted trypanosome parasites (Anderson et al., 2011; Munang'andu et al., 2012). However, an influx of people into tsetse infested areas has tended to increase the importance of humans and domestic animals as reservoirs of trypanosomes, and this is increasing reason for concern (Anderson et al., 2015; Haji et al., 2015; Auty et al., 2016). The World Health Organisation (WHO) and Food and Agriculture Organisation (FAO) project that, in Africa, over 65 million people and 50 million head of cattle, respectively, are at risk of exposure to infection with African trypanosomiasis. However, due to poor and/or nonexistence of active surveillance for Human African trypanosomiasis (HAT) and Animal African trypanosomiasis (AAT) in the affected countries, few cases of the diseases are diagnosed and reported annually (Katsidzira. and Fana, 2010; FAO, 2018; WHO, 2018).

With about five eighths (5/8) of Zambia estimated to be infested with tsetse flies, overall, AAT prevalence in Zambia remains unknown. Based on trypanosomiasis studies conducted in different regions of the country, AAT prevalence in livestock ranges from 1% to as high as 90%. Over 60% of the country's cattle population is under threat from trypanosomiasis with about 80% of the livestock owned by traditional farmers. The prevalence of trypanosomiasis in livestock and particularly in cattle has continued reporting alarming figures in affected areas (Simukoko et al., 2011; Mbewe et al., 2015; Mulenga et al., 2021). According to data collected during a trypanosomiasis survey conducted in Mambwe district of Eastern Zambia, AAT prevalence in cattle stood at 3.8% (Kakumbi, 2014). Meanwhile, the prevalence of HAT in Zambia as provided in the latest update for 2018 stood at 8.3%, higher than that of Malawi (5.7%) and the Democratic Republic of Congo (5.9%) (Franco et al., 2020). Zambia, like many HAT endemic countries, face several challenges in the successful implementation of HAT elimination programs. These include among others, shortage of trained health workers in some areas, inadequate diagnostic and treatment centres, lack of more sensitive laboratory diagnostic techniques and shortage of trypanocides for effective treatment, which need to be instituted early enough to minimize serious drug reactions and mortality (Mwanakasale et al., 2013; Mulenga et al., 2015; Franco et al., 2020).

Most developing countries in Africa are faced with poor policy support with regard to animal disease surveillance (FAO, 2015). Poor and/or inadequate policies and, weak and unsustainable reporting structures limit capacity to detect and control emerging and re-emerging zoonotic diseases such as African trypanosomiasis in developing countries (Gummow, 2013; FAO, 2018). In sub-Saharan Africa, diagnostic capacity for many of the zoonotic diseases that are endemic in these countries is generally poor. According to studies conducted in Zambia, there is limited laboratory capacity for diagnosis of such diseases in literally all the provinces in the country (Mwanakasale et al., 2013; Mulenga et al., 2015; Mulenga et al., 2021), thus, lack of effective diagnostic capacity and under diagnosis has been a critical weakness regarding effective treatment and control of infectious diseases. This challenge is mainly based on inadequate laboratory facilities and associated equipment and skilled laboratory personnel. This has affected the countries' capacity and efforts to deal with re-emerging and emerging zoonotic diseases such as trypanosomiasis (Mwanakasale et al., 2013; Mpanya et al., 2015; Mulenga et al., 2015; Mulenga et al., 2021).

The global health security agenda specifically identifies One Health as an integral part of efforts to achieve health security against the threat of infectious diseases and other public health emergencies. Analysis by the World Bank suggests that given the high economic and health burden of zoonotic diseases, strengthening human and veterinary health capacity, to facilitate One Health approaches to disease prevention and control at country level, could yield high returns on investment, averaging $30 billion per year (Bank, 2012). Despite strong overall interest in the One Health approach, implementation at country, local, and project level in Zambia remains limited.

This study compared and examined the policies and reporting structures in departments of Veterinary Services, Health, and Wildlife, in Zambia, in the context of the existing strengths, weaknesses, opportunities and threats, with the aim of gaining some insights into prospects for effective multi-sectoral and coordinated surveillance systems for zoonotic diseases. The study also examined existing opportunities and capacities among personnel and available operational provisions, in the department of Veterinary Services, Health and Wildlife, that could be used in a One Health approach to better manage and control trypanosomiasis and other zoonotic diseases.

6.2 MATERIALS AND METHODS

The provisions in the Zambian public health Act, Animal health Act and Wildlife Act were evaluated in the context of providing the key elements on national policies and on animal disease reporting systems in Zambia. Organizational structures of each of the departments responsible for Veterinary Services, Health, and Wildlife, were also examined in relation to disease reporting systems (Figure 6.1). An online search was conducted in December 2020 using One search hosted by James Cook University, Townsville Australia, using the following Key words: “National Policy” AND, OR “Animal Disease Reporting” AND, OR “Human Disease Reporting” AND, OR “Zambia”. A systematic review of reporting structures for the departments of Veterinary Services, Health, and Wildlife in Zambia, was conducted. Analysis of the strengths, weaknesses, opportunities, and threats (SWOT analysis) in existing reporting structures and policies with respect to trypanosomiasis was applied for the departments under review.

In Zambia, personnel that undertake disease diagnosis are of various training/professional backgrounds of human and animal health service providers. An interview-based questionnaire study targeting all officers based in Mambwe in Zambia was conducted within the Veterinary department (Zambia, 1995a), Wildlife department (Zambia, 1995c), and Health department (Zambia, 1995b). The interviews looked at the management and control of African trypanosomiasis in their respective departments. To conduct the study, written informed consents were obtained from all respondents before administering the questionnaires. Participation in the study was voluntary and participants were free to withdraw from the study without giving any reasons. Information sheets (Appendix D) were provided for each recruited participant explaining the aims, benefits of the study, and possible risks. The focus of the questionnaire (Appendix E) was on the departments' ability to detect African trypanosomiasis and any of the other zoonotic diseases known to be prevalent in the area. Included were questions on funding provisions for trypanosomiasis control and management in their departments, and on any existing collaboration with other government departments or with other organisations/institutions in general and the reporting structure/system in the respective departments in relation to occurrence of trypanosomiasis in the area.

Data analysis

We conducted a SWOT analysis (Tukana et al., 2018) of policies and reporting structures in each of the departments. The data from the interviews were stored in MS Excel file and later exported to IBM SPSS Statistics 27 where it was summarized as frequencies and percentages and analysed using descriptive statistics. The Chi square test was used to compare proportions between departments. For each analysis, p values <0.05 were considered statistically significant. Fisher's exact test was used to compare proportions between districts where expected values or responses were less than five.

Ethical clearances were obtained from James Cook University (H7226 and A2498) and the Zambian Ethics Committee (Ref. No. 2018-Oct001), and the research was approved by the Zambia National Health Research Authority

6.3 RESULTS

Reporting structure in relation to occurrence of animal diseases

Control of animal diseases falls under the jurisdiction of the department of Veterinary Services in the Ministry of Livestock and Fisheries (MLF). The ministry is headed by a Minister who undertakes sourcing of funds and oversees allocation of resources to the various programmes and activities in the ministry (**Error! Reference source not found.**a). The controlling officer in the ministry is the Permanent Secretary (PS), under whom directors fall – and among these is the Director in the department of Veterinary Services (DVS) that has two branches each headed by a deputy director – i.e., 'Veterinary Field Services' and 'Veterinary Research, Epidemiology, and Information'. Each of the branches has two units each headed by a 'Chief Officer'. Under the 'Veterinary Field Services' branch, there is the 'Veterinary Field Services Unit' (VFSU) and 'Tsetse and Trypanosomiasis Control Unit' (TTCU). In the 'Veterinary Research, Epidemiology and Information Branch', there is the 'Veterinary Research and Diagnostics Unit' and the 'Epidemio-surveillance and Information Unit'. In each of the units, there are 'Principal Officers' that report to the 'Chief Officers'. With regard to the positions that fall below the 'Principal officer', it is only in the Veterinary Field Services Unit where there is a structure with officers stationed at the ministry's offices in each province and district, and also at veterinary camp level in each district – i.e., Senior Veterinary/Tsetse Officers (at province level), District Veterinary/Tsetse Officers and Livestock officers (LOs) (district level) and, Veterinary Assistants (VAs) and Tsetse Control Assistants (TCAs) (at camp level). In the other units, 'Senior Officers' and 'District Officers' are strategically deployed only in selected provincial and district offices. In the case of TCAs and other personnel under the TTCU in the camps and districts, they are expected to also relay information (reports) on occurrence of trypanosomiasis directly through the unit's line of reporting.

Within the structure, it is the VAs and the TCAs that interact routinely with farmers and hence with livestock, and as such it is these personnel that constitute the front-line workers and the first and most important sources of information on disease occurrence, and also as the first line of defence in the control and management of livestock diseases – i.e. they are expected to be the first to see indicators of disease (clinical signs), take the first possible/recommended interventions where feasible, and relay the necessary information (reports) urgently to their supervising officers (at district level) on suspected disease outbreaks and also routinely (e.g. monthly) on the general disease situation in their areas (camps) of jurisdiction (i.e. at district level). Some districts have trained Community Livestock Assistants (CLAs) who help report cases of animal disease that occur in their communities to the VAs (Personal communication,

Mambwe District Fisheries and Livestock, 2019) (Zambia, 1995a, b). Thus, the structure/system is such that information flow (reporting) on occurrence of a disease such as trypanosomiasis, is expected to start at camp level (where there is routine interaction between farmers and the department's camp personnel), and flow upwards to the district officers and then to the provincial officers (for scrutiny/evaluation and quality control), and later transmission to the Chief Officers in the Veterinary Field Services and Epidemio-surveillance and Information units of the directorate of Veterinary Services.

However, regarding animal trypanosomiasis, active surveillance is carried out intermittently by the TTCU, and this information is made available through the specific reports.

Reporting structure in relation to occurrence of human diseases

The Ministry of health (MOH) is responsible for reporting all human diseases occurring in Zambia. Just like the MLF, the MOH is also headed by a Minister (**Error! Reference source not found.**b). Under the Minister, there are two Permanent Secretaries (Administration and technical services). Each Permanent secretary also has several directors and chief staff herein referred to as the directorates. The directorate gathers all reports from the provinces. The ten directorates at MOH are Clinical Care, Public Health, Finance, Human Resources, Policy and Planning, Infectious Disease, Monitoring and Evaluation, Nursing, and Quality Improvement and Performance. The provinces are headed by the Provincial health directors (PHDs), who also have principal and senior officers under them. Under the PHDs are District health directors (DHDs) who receive all reports from the hospitals, health centres, health posts, other government departments and other non-government organisations (NGOs) operating in their districts. Some districts also have trained community health workers and, in some places, community health assistants who work hand in hand with their local health centres or health posts (Zambia, 1995b; Mandyata et al., 2017). Similarly, with the MLF, the structure also allows the flow of information from communities through interactions with community structures.

Reporting structure in relation to occurrence of wildlife diseases

The department of Wildlife and National Parks formally known at Zambia wildlife authority (ZAWA) falls under the Ministry of tourism and arts (**Error! Reference source not found.**c). The department is supervised through the Head office, Regional offices, and the Area Management Units. The Head office basically provides supervisory roles and backstopping

services to the Regional Offices and the Area Management Units. The Regional Offices also supervise the Area Management Units under their jurisdiction and implement some activities. The Area Management Units mainly implement the department's activities throughout the country. At the Head Office which falls under the Permanent secretary, there is a management structure which is headed by a Director General who has the overall responsibility for the day-to-day management of the department. There is a line management of six Directorates namely, Conservation and Management, Research, Planning and Information, Commercial Services, Game Management Areas, Finance and Corporate Services, and Legal Counsel. These together with Administration and Human Resources Manager, Head Intelligence and Investigations, Chief Internal Auditor and Projects Coordinator comprise the senior management structure of the department. The department of wildlife is run under a decentralized system, with four (4) geographical regions (Eastern, Western, Northern and Southern and with their offices in Mfuwe, Mumbwa, Kasama and Mazabuka, respectively). Each region is headed by a Regional Manager who is assisted by an Area Warden, a Regional Accountant, an Extension Officer, Park Ranger and Senior Investigations Officer. Although the organizational structure is said to be decentralized most of the management decisions (i.e. procurement, disbursement of funds, etc.) are still very much Centralized at headquarters where most decisions are made (Zambia, 1995c, 2006). The department also has community structures which allows the flow of information from the communities to the regional offices and management.

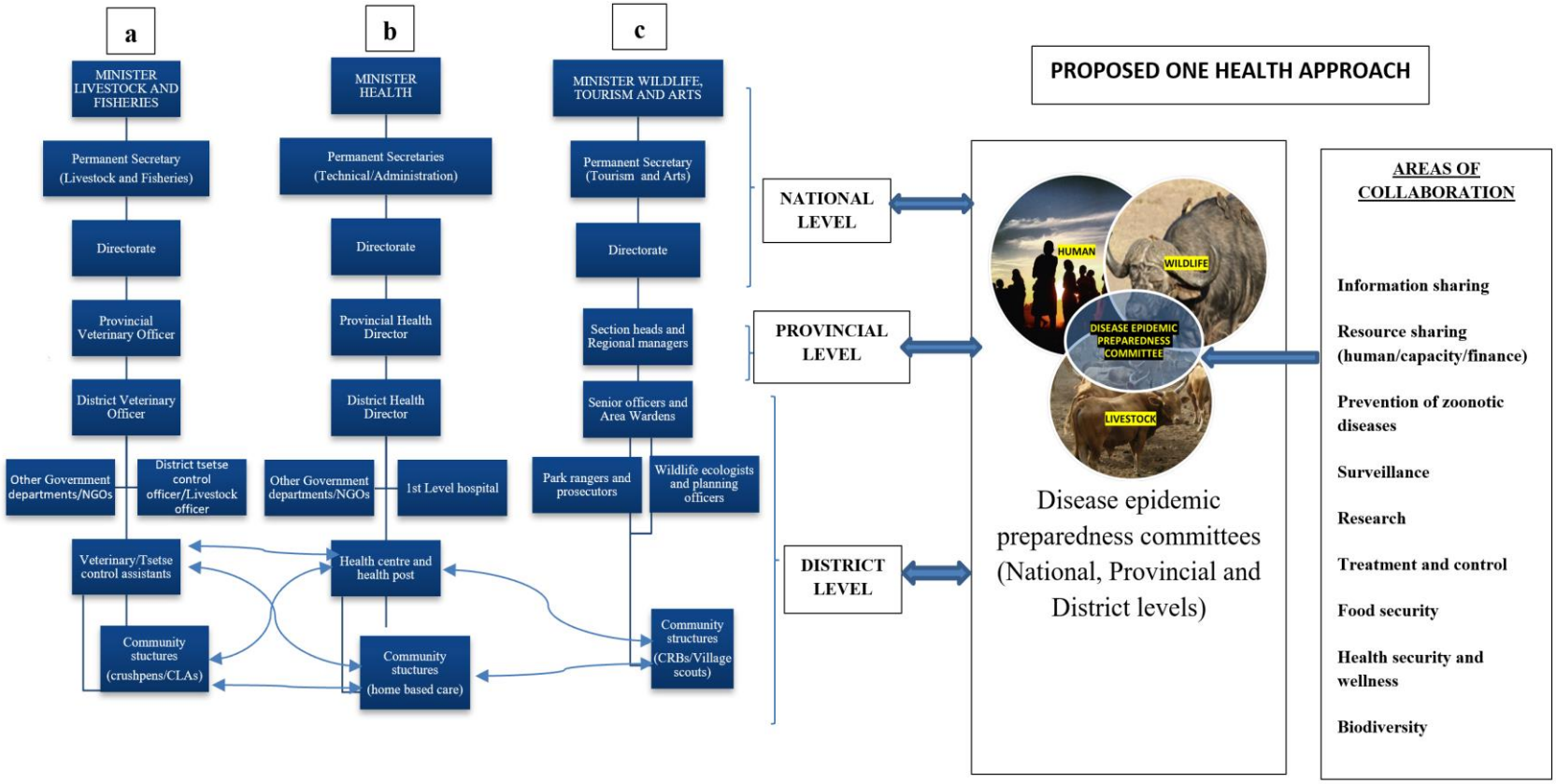

Figure 6. 1: Zambian reporting structures and conceptual framework for the Ministries of Health, Livestock and Fisheries and Wildlife, Tourism and Arts showing areas where the One Health approach can be applied for the control of African trypanosomiasis and other zoonotic diseases.

Horizontal bold double arrows indicate areas where the One Health approach at that level can be applied. Curved double arrows indicate areas where officers can brief each other on disease situation and response taken. (a): Reporting structure for Ministry of Fisheries and Livestock (b): Reporting structure for Ministry of Health (c): Reporting structure for Ministry of Wildlife, Tourism and Arts, respectively.

SWOT analysis - Reporting structure on occurrence of animal and human diseases

The results of the SWOT analysis are as shown in Table C1 (Appendix C). In the reporting structures, there were clear similarities between the department of Veterinary Services and Health, compared to the department of National Parks and Wildlife, while some elements were common in all three government institutions under review.

Questionnaire survey

In total, 21 health centres and health posts from Mambwe district were involved in our survey, namely, Kamoto, Chilanga, Mphata, Kakumbi, Airport, Masumba, Nyamaluma, Kasamanda, Ncheka, St. Lukes, Malama, Kamubaba, Chikowa, Nyakatokoli, Lupande, Lusamdwa South, Chipako, Jumbe, Chisengu, Jumbe and Mphomwa (**Error! Reference source not found.**). Only healthcare personnel that were currently working and were present at the centres were interviewed. Due to low staffing levels from the department of Veterinary services, all key personnel present in the district were interviewed while interviews from the department of national parks and wildlife were focused on officers from Chinzombo offices in Mfuwe. The numbers of key personnel for disease control available at the centres visited (Table 6.1) were as reported by personnel manning the centres. It is important to note that only officers that agreed to be interviewed were involved in the study.

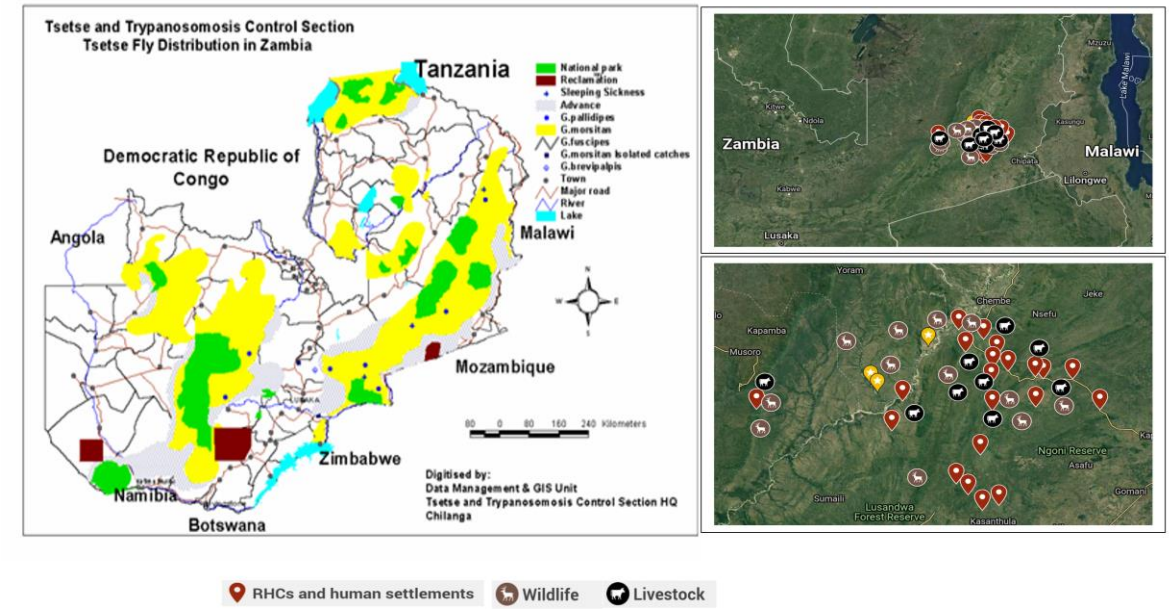

Figure 6. 2: Tsetse and Trypanosomiasis distribution in Zambia. Source: (Tsetse control Section-Zambia 2018). Inserts showing locations of Rural Health Centres (RHCs) visited and distribution of Wildlife and Livestock in study area. Source: (Mulenga 2021-Google Maps)

Table 6. 1: Demographics of key personnel involved in managing trypanosomiasis and other zoonotic diseases in Mambwe district of Eastern Zambia in February 2020.

	Profession	Qualification	Positions present at time of study
Health	Medical officers	Degree	4
	Clinical officers	Diploma	19
	Nurses	Diploma	95
	Environmental health technicians	Certificate	20
	Laboratory technicians	Diploma	13
Veterinary Services	Veterinary officers	Degree	0
	Biologists	Degree	1

	Livestock officers	Diploma	1
	Livestock technicians	Diploma	1
	Veterinary assistants	Certificate	6
	Laboratory technicians	Diploma	0
Wildlife and National Parks	Veterinarians	Degree	1
	Ecologists	Degree	1
	Laboratory technicians	N/A	0

During interviews, responses on the availability of parasitological and molecular tools (Table 6.2) that could be useful for both passive and active surveillance of trypanosomiasis in man and animals were recorded and are indicated below.

Table 6. 2: Results on availability of laboratory tools that could be used to diagnose trypanosomiasis and other zoonotic diseases.

Diagnostic tool	Health	Veterinary	Wildlife and National parks
Microscopy	Present	Present	Present
Rapid test kits	Absent	Absent	Absent
PCR	Absent	Present	Absent
LAMP	Absent	Absent	Absent

(Abbreviations: PCR: Polymerase chain reaction; LAMP: Loop-mediated iso-thermal amplification).

Respondents from the Veterinary department indicated they received financial support for the control of trypanosomiasis while respondents from the departments of Health and Wildlife reported no financial support for trypanosomiasis control (Table 6.3). In the same manner, the Veterinary department reported undertaking more surveys and surveillance for tsetse and trypanosomiasis (T & T) as compared to their Health and Wildlife counterparts (Chi-square, $p = 0.001$) (Table 6.3). On the other hand, the department of Wildlife indicated

that they collaborated more with other departments and NGOs than the Health and Veterinary departments (Chi-square, $p = 0.04$) (Table 6.3).

Table 6. 3: Results of responses on tsetse and trypanosomiasis control and management and options for collaboration.

	Health (*n* = 21)			Veterinary (*n* = 9)			Wildlife and National Parks (*n* = 15)		
	Yes	**No**	**Do not know**	**Yes**	**No**	**Do not know**	**Yes**	**No**	**Do not know**
Does the centre receive financial support for trypanosomiasis?	0	19 (90.5%)	2 (9.5%)	8 (88.9%)	1 (11.1%)	0	0	12 (80%)	3 (20%)
Does your department undertake trypanosomiasis surveys/surveillance?	6 (28.6%)	12 (57.1%)	3 (14.3%)	9 (100%)	0	0	2 (13.3%)	11 (73.3)	2 (13.3)
Does your department work with other GRZ/NGOs on trypanosomiasis issues?	4 (19%)	17 (81%)	0	4 (44.4%)	4 (44.4%)	1 (11.1%)	12 (80%)	2 (13.3)	1 (6.7%)

(Abbreviations: GRZ: Government of the Republic of Zambia; NGO: Non-Government Organizations).

Type of collaboration required as indicated by the departments of Veterinary, Health and Wildlife during the survey included the following: Staff training and capacity building, disease awareness and management and disease diagnosis.

6.4 DISCUSSION

The results of the review of reporting structures for the Zambian departments of Veterinary Services, Health, and National Parks and Wildlife (Table C1), indicate opportunities that may exist for improved disease reporting and management. The study identified existing links in reporting systems (Figure 6.1) that could be used to provide a more holistic response to emerging and re-emerging livestock, human and wildlife diseases (Zambia, 1995a, b, c; Lorusso, 2021). The Zambian departments of Health and Veterinary Services have been using the Public Health Act and Animal Health Act of the Laws of Zambia as major policies to guide the provision of human and animal health care services, respectively (Zambia, 1995a, b). These acts have clear statements on the reporting procedures of notifiable diseases. The study also

found similarities between the organizational structures of Veterinary and Health (Figure 6.1) that could be utilized in disease reporting and the adoption of a One Health system. On the other hand, the Zambian Wildlife Act has no mention of reporting notifiable diseases despite most zoonotic diseases having a wildlife origin (Auty et al., 2016). The Wildlife Act has instead focused on management and protecting wildlife areas whilst overlooking wildlife disease management (Zambia, 1995c).

Findings from the survey indicate limited government financial support for the three government departments to undertake surveys/surveillance for the control of trypanosomiasis and the need to strengthen collaboration between sectors for disease control and management. A previous study (Mulenga et al., 2015) conducted in the area re-affirms the absence of financial support to manage trypanosomiasis whilst similar diseases like malaria, HIV/AIDS and tuberculosis remain on the government's funding priority list. Such limited priorities in areas of livestock/wildlife disease support from local authorities has a negative impact on zoonotic disease response as infection rates in either domestic or wild animals can be early predictors of transmission risks to humans (Jones et al., 2008; Welburn and Maudlin, 2012; Mulenga et al., 2020). According to findings by Mandyata *et. al.* (Mandyata et al., 2017), several challenges, including human resources, poor infrastructure and coordination, hamper effective response to re-surging diseases. Our study confirms these gaps in human/financial resources and laboratory tools that could be used in an ideal setting for trypanosomiasis management and other zoonotic diseases. Sharing human capacities and collaboration on trypanosomiasis awareness and management as suggested by respondents from our survey could help achieve health security against the threat of other infectious diseases (Mandyata et al., 2017; Lorusso, 2021).

Governments of trypanosomiasis endemic areas are however, overwhelmed with the costs attached to the sustainable control of trypanosomiasis thus making its control difficult (Grant et al., 2015). The high cases of HAT in Zambia compared to neighbouring Malawi and the Democratic Republic of Congo, could be mainly related to spill overs from wildlife and livestock reservoirs which dwell within human settlement areas as observed from our survey map in Figure 6.2. Reinforced passive surveillance, scaling up of active surveillance and sustained control efforts, backed-up by an adequate surveillance system in Malawi and the Democratic Republic of Congo has resulted in the reduction of HAT cases (Franco et al., 2020).

Such lessons learnt from Zambia's neighbouring countries can be adopted to improve the HAT and AAT situation locally.

The Zambian government has made efforts to control tsetse and trypanosomiasis, but due to financial limitations and other disease burdens, trypanosomiasis control programmes have not been sustained (Meyer et al., 2016; Meyer et al., 2018). This has resulted in tsetse re-invasions and disease flareups even in areas where control was once undertaken. From the time of colonial British rule through independence to date, Zambia has used several approaches to try and combat trypanosomiasis. These include ground spraying, occasional use of sequential aerosol technique (SAT), the use of curative and prophylactic trypanocides, the use of odour baited targets, traps, and live baits (Meyer et al., 2016; Franco et al., 2020; Abro et al., 2021).

Currently, in consideration of past lessons learnt and in adopting the approach of the African Union's Pan African Tsetse and Trypanosomiasis Eradication Campaign (AU-PATTEC), Zambia has adopted the principle of an area wide integrated pest management which is based on interventions against trypanosomiasis (Meyer et al., 2016). However, the control of the tsetse vector in protected areas and game reserves could be more complicated due to conservationist, ecological, and environmental considerations (Kabasa, 2007). Current methods for tsetse control include non-insecticidal (bush clearing, Sterile Insect Technique, and the use of insect symbionts), and insecticidal methods (odour baits, SAT, and ground spraying). Tsetse infested areas are categorised as low, medium, and high priority areas to determine the type of intervention to be employed (Franco et al., 2020; Mulenga et al., 2020; Muyobela et al., 2021).

Through review of reporting structures for the departments of Veterinary Services, Health, and Wildlife as shown in Table C1 (Appendix C), we identified areas through which departments could maximize resources, share information, and collaborate. These areas exist at National, Provincial and District levels as indicated by bold horizontal lines in Figure 6.1. Literature also revealed the existence of epidemic preparedness committees at National, Provincial and District levels (Mandyata et al., 2017) (Ministry of Livestock and Fisheries reports). The committees comprise members from government departments which include among others, Health, Veterinary, Wildlife, Agriculture Lands and Natural resources, Education, Community development and partnering non-government departments. These

committees, if utilised effectively provide a good platform for reporting zoonotic diseases and their status. We advocate that committees can also be used to promote and drive One Health strategies that will promote biodiversity, food security, safe environment, information and resource sharing, human and animal health as well as to strengthen the collaboration and coordination between sectors in order to improve the prevention and control of zoonotic diseases (WHO, 2017; Lorusso, 2021) (Figure 6.1). The chairpersons for these committees, who are Provincial and District administrative officers, respectively, can spear-head and direct solutions for the implementation of a One Health approach in their respective areas.

Analysis of reporting structures for Veterinary, Wildlife, and Health reveals that each department had their strengths, weaknesses, opportunities, and threats as indicated in Table C1 (Appendix C). However, some issues were common across the departments studied. All three reporting structures allowed for interactions between senior and junior officers but the culture of not bypassing immediate supervising officers created a challenge in the timely reporting of disease incidences. The limited and low levels of key personnel for disease diagnosing and surveillance as indicated from the survey data created a gap in the reporting system. The non-availability of senior personnel especially at district level limits reporting capacities of junior officers who may not be experienced enough. Our results indicate that against a population of over 96000 (Zambia, Central statistics projections 2019), Mambwe district had 20 health posts and a hospital with a bed capacity of 170 (personal communication). The district is managed by only 4 doctors who are overwhelmed with work, thus impacting negatively on their capacities to service delivery (Mulenga et al., 2015). In the same manner, the Veterinary department was the worst hit in terms of staffing levels. The department had no Veterinary officer and only six veterinary assistants who are field officers to cover and manage livestock diseases in a district with an area size of 4480km squared and over 18,000 households (Zambia, Central statistics data-Mambwe district, 2015). The absence of key personnel responsible for disease surveillance and reporting at grass root level could threaten the effective reporting of trypanosomiasis and other animal and human diseases.

The reporting structure for the Wildlife department (Figure 6.1) is long, which may impact on timely reporting, especially if some positions are vacant. The tough training and military culture incorporated in the management of National Parks and Wildlife may also contribute to the rigidness of the structure, thus affecting the processing time of reports and information

sharing. There is a need to shorten the reporting structure which will promote interaction and information sharing, thus increasing efficiency of disease reporting.

In general, the study identified levels as indicated in Figure 6.1 within reporting structures under review, that can be platforms for supporting more collaboration, information and capacity sharing between departments. Interactions at National, Provincial and District levels may also allow for the development of policies that will promote a collective approach in the management and control of zoonotic diseases (FAO, 2015; Tukana et al., 2018; Lorusso, 2021). To overcome the resource challenge as indicated from our findings, resources could be saved and re-allocated to other activities through combining human and livestock/wildlife activities e.g., concurrent sampling of both human and animal subjects, sharing of diagnostic capacities and cross-training of Veterinary, Wildlife, and human Health staff. Introducing more holistic approaches and policies for cross reporting within systems may be a more sustainable approach towards achieving a One Health approach. The recent creation of the Zambia National Public Health Institute (ZNPHI), under the Zambian MOH (ZNPHI, 2015) has been a step further into improving reporting systems of diseases that are of public health interest. The Tropical Diseases Research Centre (TDRC), an initiative of the WHO, also under the Zambian MOH and the Central Veterinary Research Institute (CVRI) under the Zambian MLF are other institutions that can be strengthened for effective management of zoonotic diseases by incorporating a multi-sectoral coordination approach. Broadening the capacities of these existing Institutions will be a better approach towards effective management and control of zoonotic diseases.

6.5 CONCLUSIONS

Coordinated surveillance systems within available organizational structures could play a key role in disease reporting and have the potential to impact the reporting of emerging and re-emerging diseases. A better One Health system could be applied in Zambia and other countries in the region and beyond by strengthening links for collaboration and coordination at National, Provincial and District levels between sectors (Health, Veterinary, Wildlife and Natural resources) and by creating improved reporting links within available reporting structures that will promote interactions and provide for a more holistic response to disease control. This can be done through already existing institutions like CVRI, ZNPHI, TDRC as well as through epidemic preparedness committees. With a slight shift in focus to include

zoonotic diseases, the ZNPHI could provide the platform for disease reporting between partnering departments.

More efficient use of existing capacity by implementing a One Health approach is possible between sectors. For example, in areas where Veterinary Services has laboratory capacity, samples from Health and Wildlife can be sent to veterinary facilities for analysis and vice versa. In addition, community awareness programmes for zoonotic diseases and collaborated staff training/upscaling of skills related to veterinary, health and wildlife can be collaborative to save resources. The digitalization of records for information sharing through national, provincial and district epidemic preparedness committees could be carried out in partnership to create a more efficient response system. To support sustainable zoonotic disease control approaches that can be implemented at national, provincial and district levels, new policies will however, need to be developed in the future.

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6.6 REFERENCES

Abro, Z., Kassie, M., Muriithi, B., Okal, M., Masiga, D., Wanda, G., Gisele, O., Samuel, A., Nguertoum, E., Nina, R.A., Mansinsa, P., Adam, Y., Camara, M., Olet, P., Boucader, D., Jamal, S., Garba, A.R.I., Ajakaiye, J.J., Kinani, J.F., Hassan, M.A., Nonga, H., Daffa, J., Gidudu, A., Chilongo, K., 2021. The potential economic benefits of controlling trypanosomiasis using waterbuck repellent blend in sub-Saharan Africa. PloS one 16, e0254558. doi:10.1371/journal.pone.0254558

Anderson, N.E., Mubanga, J., Fevre, E.M., Picozzi, K., Eisler, M.C., Thomas, R., 2011. Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. PLOS Neglected Tropical Diseases 5, e1211. doi:10.1371/journal.pntd.0001211

Anderson, N.E., Mubanga, J., Machila, N., Atkinson, P.M., Dzingirai, V., Welburn, S.C., 2015. Sleeping sickness and its relationship with development and biodiversity conservation in the Luangwa Valley, Zambia. Parasites & Vectors [Electronic Resource] 8, 224.

Auty, H., Morrison, L.J., Torr, S.J., Lord, J., 2016. Transmission Dynamics of Rhodesian Sleeping Sickness at the Interface of Wildlife and Livestock Areas. Trends Parasitology 32, 608-621. doi:10.1016/j.pt.2016.05.003

Bank, T.W., 2012. The World Bank, Development Indicators 2012. Washington.

FAO, 2015. A national policy for efficient Veterinary Laboratory services-Kenya as a pilot country. FAO Regional Office for Asia and the Pacific, 39 Phra Athit road, Bangkok.

FAO, 2018. World Health Organization and Food and Agriculture Organization step up towards the elimination of human African trypanosomiasis to ensure health and Food Security in Africa. Addis Ababa, Ethiopia.

Franco, J.R., Cecchi, G., Priotto, G., Paone, M., Diarra, A., Grout, L., Simarro, P.P., Zhao, W., Argaw, D., 2020. Monitoring the elimination of human African trypanosomiasis at continental and country level: Update to 2018. PLOS Neglected Tropical Diseases 14, e0008261. doi:10.1371/journal.pntd.0008261

Grant, C., Anderson, N., Machila, N., 2015. Stakeholder Narratives on Trypanosomiasis, Their Effect on Policy and the Scope for One Health. PLoS Neglected Tropical Diseases 9, e0004241. doi:10.1371/journal.pntd.0004241

2434 Gummow, B., Hedlefs, R., Kapo, N., Kenneally, G., Yomba, A, 2013. An approach to
2435 sustainable disease reporting in developing countries. European College of
2436 Veterinary Public Health. Turin, Italy.
2437 Haji, I.J., Sugimoto, C., Kajino, K., Malele, I., Simukoko, H., Chitambo, H., Namangala, B.,
2438 2015. Determination of the prevalence of trypanosome species in cattle from Monduli
2439 district, northern Tanzania, by loop mediated isothermal amplification. Tropical
2440 Animal Health Production 47, 1139-1143. doi:10.1007/s11250-015-0840-5
2441 Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., Daszak, P., 2008.
2442 Global trends in emerging infectious diseases. Nature 451, 990-999.
2443 Kabasa, J., 2007. Public-private partnership works to stamp out sleeping sickness in Uganda.
2444 Trends Parasitol 23, 191-192.
2445 Kakumbi, 2014. Annual Report. Tsetse and Trypanosomiasis Research Station-Ministry of
2446 Fisheries and Livestock, Mambwe, Zambia.
2447 Katsidzira., Fana, G., 2010. Pitfalls in the diagnosis of Trypanosomiasis in low endemic
2448 countries: A case Report. PLos Neglected Tropical Diseases 4, 823.
2449 Lorusso, V., 2021. Parasitology and One Health—Perspectives on Africa and Beyond.
2450 Pathogens 10. doi:10.3390/pathogens10111437
2451 Mandyata, C.B., Olowski, L.K., Mutale, W., 2017. Challenges of implementing the integrated
2452 disease surveillance and response strategy in Zambia: a health worker perspective.
2453 BMC Public Health 17, 746. doi:10.1186/s12889-017-4791-9
2454 Mbewe, N.J., Namangala, B., Sitali, L., Vorster, I., Michelo, C., 2015. Prevalence of pathogenic
2455 trypanosomes in anaemic cattle from trypanosomosis challenged areas of Itezhi-tezhi
2456 district in central Zambia. Parasites and Vectors 8, 638. doi:10.1186/s13071-015-1260-0
2457 Meyer, A., Holt, H.R., Oumarou, F., Chilongo, K., Gilbert, W., Fauron, A., Mumba, C., Guitian,
2458 J., 2018. Integrated cost-benefit analysis of tsetse control and herd productivity to
2459 inform control programs for animal African trypanosomiasis. Parasites & Vectors 11,
2460 154. doi:10.1186/s13071-018-2679-x
2461 Meyer, A., Holt, H.R., Selby, R., Guitian, J., 2016. Past and Ongoing Tsetse and Animal
2462 Trypanosomiasis Control Operations in Five African Countries: A Systematic Review.
2463 PLoS Neglected Tropical Diseases 10, e0005247. doi:10.1371/journal.pntd.0005247
2464 Mpanya, A., Hendrickx, D., Baloji, S., Lumbala, C., da Luz, R.I., Boelaert, M.L., P., 2015. From
2465 health advice to taboo: community perspectives on the treatment of sleeping sickness

in the Democratic Republic of Congo, a qualitative study. PLoS Neglected Tropical Diseases 9, e0003686. doi:10.1371/journal.pntd.0003686

Mulenga, G.M., Henning, L., Chilongo, K., Mubamba, C., Namangala, B., Gummow, B., 2020. Insights into the Control and Management of Human and Bovine African Trypanosomiasis in Zambia between 2009 and 2019-A Review. Tropical Medicine and Infectious Diseases 5, 115. doi:10.3390/tropicalmed5030115

Mulenga, G.M., Likwa, R.N., Namangala, B., 2015. Assessing the capacity to diagnose human African trypanosomiasis among health care personnel from Chama and Mambwe districts of eastern Zambia. BMC Research Notes 8, 433.

Mulenga, G.M., Namangala, B., Chilongo, K., Mubamba, C., Hayashida, K., Henning, L., Gummow, B., 2021. Challenges in the Diagnostic Performance of Parasitological and Molecular Tests in the Surveillance of African Trypanosomiasis in Eastern Zambia. Tropical Medicine and Infectious Diseases 6, 68.

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. Interdiscipline Perspective Infectious Diseases 2012, 372523. doi:10.1155/2012/372523

Muyobela, J., Pirk, C.W.W., Yusuf, A.A., Mbewe, N.J., Sole, C.L., 2021. A novel vehicle-mounted sticky trap; an effective sampling tool for savannah tsetse flies *Glossina morsitans morsitans* Westwood and Glossina morsitans centralis Machado. PLoS Neglected Tropical Diseases 15, e0009620. doi:10.1371/journal.pntd.0009620

Mwanakasale, V., Songolo, P., Daka, V., 2013. Challenges in the control of human African trypanosomiasis in the Mpika district of Zambia. BMC Research Notes 6, 180.

Simukoko, H., Marcotty, T., Vercruysse, J., Van den Bossche, P., 2011. Bovine trypanosomiasis risk in an endemic area on the eastern plateau of Zambia. Research in veterinary science 90, 51-54. doi:10.1016/j.rvsc.2010.04.021

Tukana, A., Hedlefs, R., Gummow, B., 2018. The impact of national policies on animal disease reporting within selected Pacific Island Countries and Territories (PICTs). Tropical Animal Health and Production 50, 1547-1558. doi:10.1007/s11250-018-1594-7

Welburn, S.C., Maudlin, I., 2012. Priorities for the elimination of sleeping sickness. Advanced Parasitology 79, 299-337. doi:10.1016/B978-0-12-398457-9.00004-4

WHO, 2017. Managing zoonotic public health risks at the human–animal–ecosystem interface.

WHO, 2018. WHO fact sheet-Human african trypanosomiasis. World Health Organization.

Zambia, G.o.t.R.o., 1995a. Republic of Zambia Animal Health act. 2010. Zambia National Assembly, Lusaka, Zambia.

Zambia, G.o.t.R.o., 1995b. Republic of Zambia Public Health Act. 2010. Zambia National Assembly, Lusaka, Zambia.

Zambia, G.o.t.R.o., 1995c. Republic of Zambia Wildlife Act, 2015. Zambia National Assembly, Lusaka, Zambia.

Zambia, G.o.t.R.o., 2006. Current protected areas categories and their management institutions. Ministry of Tourism, Environment and Natural resources, Lusaka, Zambia.

ZNPHI, 2015. Zambia National Public Health Institute. About Us - Zambia National Public Health Institute (webworxzambia.com). Date accessed: 26.12.20.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

7.1 DISCUSSION

The effectiveness of trypanosomiasis control methods has been reported in several studies (Meyer et al., 2018; Abro et al., 2021). However, gaps remain on the financial and environmental implications of such control methods on poor affected communities. The main objective of the current study was to evaluate and identify different trypanosomiasis control strategies and measures that are cost-effective in the detection and control of African trypanosomiasis in endemic areas of Eastern Zambia. The study also explored the feasibility of a One Health approach suitable for the control of trypanosomiasis through surveys of Veterinary, Health and Wildlife personnel capacities and reporting systems of Zambia. The project was structured into five subcomponents to look at the detection and control of trypanosomiasis in areas of Eastern Zambia. The first area of research sought to address the knowledge gap in literature on the control and management of African trypanosomiasis in Zambia particularly from a One Health perspective. The second study explored how sensitivity and specificity of detecting trypanosomiasis infection in cattle varied between laboratory techniques when performed under field conditions. Through a prospective cohort study of trypanosomiasis incidence in cattle, the third study, evaluated tsetse and trypanosomiasis control strategies for their cost effectiveness in the control and detection of trypanosomiasis in resource poor remote communities of Eastern Zambia. While the fourth study explored the use of an alternative control strategy for trypanosomiasis through the use of the symbionts *Sodalis* and *Wobachia* infection in tsetse species found in the Luangwa valley tsetse belt. The final study explored the impact of the Zambian government policies on animal and human disease reporting and management, and whether a One Health approach to controlling trypanosomiasis was feasible with the current Zambian system.

The study demonstrates that trypanosomiasis is a threat to both human and animal health and remain an important disease for communities living in tsetse infested areas of the Luangwa valley. Migration of people with their livestock into tsetse infested areas due to increased demand for Agricultural land has resulted in changes in the epidemiology of African trypanosomiasis therefore, increasing risks to public health and global health security. Livestock rearing in these tsetse-infested areas has led to the development of a new kind of wildlife/livestock/human interface with domestic animals acting as potential link for trypanosome exchange. The detection of the human infective trypanosomes *T. b. rhodesiense*

from cattle blood samples analysed in this study reaffirms these statements. However, most tsetse and trypanosomiasis control efforts have been focused on livestock with very little attention on human intervention programmes. Treatment of livestock reservoirs for *T. b. rhodesiense* maybe a better option for the control of sleeping sickness (Anderson et al., 2011; Richter et al., 2012; Laohasinnarong et al., 2015; Mulenga et al., 2020; Gashururu et al., 2021).

Despite reported cases of HAT from tourists after their visit to tsetse infested National Parks and game reserves (Richter et al., 2012), the risk of HAT infection in travellers has however not received much attention. With increasing numbers of tourists in the South Luangwa National Park, there is need for developing deliberate interventions to protect tourists from tsetse and trypanosomiasis. Some tour operators in Eastern Zambia have taken it upon themselves to undertake some interventions, particularly in the form of tsetse control, aimed at reducing the risk of HAT infection among tourists visiting their facilities. Such limited interventions produce very limited levels of effectiveness or success, considering that such interventions need to cover large proportions of the affected areas and as such require the collective input of many key stakeholders. In addition to vector control, awareness can be undertaken through production of information flyers highlighting precautions (e.g., wearing of long sleeve shirts and use of insect repellents) to be taken as tourists head for the National Parks, which can be made available at ports of entry, at National Parks entry points, and by tour operators. Unfortunately, increasing focus on communicable and non-communicable disease management has pushed African trypanosomiasis off the government's priority list (Richter et al., 2012; Kakumbi, 2014; Mulenga et al., 2020).

Currently, African trypanosomiasis control in humans relies on early diagnosis and treatment. Active case detection through screening of both man and animals in tsetse endemic areas is an effective step towards the control of trypanosomiasis. Challenges in trypanosomiasis diagnosis in rural settings of Zambia has however, hindered progress to the control of the disease. This study serves as a prime example of the impact that remote field conditions and staff training can have on results that in turn impact the success of tsetse and trypanosomiasis control programs in the region. However, considering that trypanosomiasis is prevalent in remote rural areas where access to diagnostic facilities is limited, for surveillance purposes, FTA cards and FP should be considered for collecting, storing, and transporting blood samples for analysis using ITS-PCR or other molecular techniques where the collection of whole blood

is not feasible. Currently used diagnostic tests have their own advantages and limitations. ITS-PCR is a good screening test of trypanosomes causing nagana. Since Zambia does not produce any molecular reagents, importation and transportation costs related to the use of molecular techniques was one of the study constraints. The use of ITS-PCR is therefore still limited, as most rural laboratories in Zambia have not yet transitioned to the use of molecular techniques for the point of care diagnosis of African trypanosomiasis and other zoonotic diseases (Njiru et al., 2005; Thumbi, 2008; Moti et al., 2014; Mulenga et al., 2021b).

Microscopy could, therefore, be used for diagnosis but as a combination of the three commonly used techniques of buffy coat, thin smears, and thick smears. The use of the buffy coat is considered to be more sensitive than that of thick and thin smears, but in this case the buffy coat detected the least number of trypanosomes. An on-site low case detection on the buffy coat can occur when the field conditions do not allow for a thorough screening of samples as compared to a laboratory screening where operators take time to thoroughly screen the samples. Factors that can negatively affect case detection on the buffy coat include the quality of capillary tubes and ambient temperatures in the study area, which could affect motility and/or death of trypanosomes before examiners could observe trypanosome movement in the buffy coat. Other factors include examiners' ability to observe immature trypanosome movements (Florkwoski, 2008; Mulenga et al., 2021b). Microscopy remains the most practical option for the diagnosis of trypanosomes in the field, but understanding its limitations is critical when using it for surveillance purposes. Microscopy has been traditionally regarded as the gold standard in detecting the presence of trypanosomes because it is simple, cheap and can also simultaneously detect other haemoparasites. However, the low sensitivity exhibited by microscopy makes it difficult to determine disease incidences, especially in cases where parasitaemia is low, thus stressing the need to improve field diagnosis of African trypanosomiasis (Laohasinnarong et al., 2015; Mulenga et al., 2015; Nyimba et al., 2015).

Trypanosomiasis surveillance in livestock can be strengthened by building diagnostic capacities in field veterinary officers and equipping them with diagnostic kits that will allow them to collect blood samples routinely and forward them to district laboratories for analysis. Routine interactions between farmers and field officers will improve relationships as well as improve reporting of emerging and re-emerging zoonotic diseases within communities. There

is need to re-vamp and strengthen crush pen committees under the department of Veterinary Services to help improve relationships and feedback loops between livestock farmers and field officers. This can only be achieved through improving operational support for field officers which seems to be the current biggest challenge for field officers in remote areas. Better staff training in disease diagnosis, better maintenance of diagnostic equipment, a better funding model and an improvement in field quality control would help address challenges in disease diagnosis, as highlighted in this study (Mulenga et al., 2021b; Boulangé et al., 2022; WHO, 2022).

Because tsetse flies are largely found in remote rural areas, the impact of the disease is mainly on poor rural populations relying on small scale farming for their livelihood. Cattle farmers have therefore, resorted to the drastic use of various control strategies to control tsetse and trypanosomiasis. The treatment and control costs have negatively impacted on food security and the livelihoods of the poor livestock farmers while returns based on their choice of control method remain unquantified. Using field experimental trials, this study was the first to demonstrate that, the returns of controlling trypanosomiasis in cattle varies significantly between control strategies used in Eastern Zambia (Bouyer et al., 2013; Ramirez, 2017; Lord et al., 2020). The study highlighted that the Samorin inoculation treatment was the most cost-effective method for controlling AAT which can be employed by small scale farmers in remote poor resource communities of Eastern Zambia while the use of Cypermethrin targets may be a better option for government sponsored tsetse and trypanosomiasis control programmes. The Berenil inoculation and Cyfluthrin pour-on were equally cost effective but their low financial net returns should be taken into consideration if applied as control options for AAT (Mulenga et al., 2022-under review).

Costs of parasite treatments can be reduced if farmers could conduct the treatments themselves instead of using veterinary officers. Such actions may however, come with consequences resulting from non-compliance in the use of trypanocides which may result in trypanocide resistance (Mulandane et al., 2018), increase disease incidence rates, increased deaths and reduced financial net returns. Training and use of community livestock assistants in the administration of trypanocides may be a better option to maximize net returns realised from the parasite treatment groups. Community participation has been identified to have a positive impact in efforts made to mitigate community vulnerability to vector borne diseases

as well as ensuring sustainable application of such treatments (Bardosh et al., 2017). Financial net returns can be maximized further through integrating treatment control methods. Samorin inoculation and Cypermethrin targets groups yielded higher returns and may be better paired as parasite and vector treatments respectively, while the Berenil inoculation and Cyfluthrin pour-on may provide the second-best option as parasite and vector treatments respectively. Integration of these control methods would maximise the benefits and reduce costs of controlling trypanosomiasis in Zambia and within the region (FAO, 2017; Meyer et al., 2018).

Additionally, this study showed that establishing opportunities for biologically based trypanosomiasis control strategies using tsetse endosymbionts is currently not feasible in the surveyed area due to the weak relationship between tsetse symbionts and trypanosomes observed (Mulenga et al., 2022). However, the low number of tsetse flies infected with trypanosomes could explain the absence of correlation observed, which suggest the need for further work on the importance of *Sodalis* in tsetse species in the Luangwa valley tsetse belt. The importance of understanding this relationship is emphasised by the urgent need for environmentally friendly methods for both tsetse and trypanosomiasis control as the application insect-symbiont interactions for the control of vector-borne diseases is becoming popular globally (Boulanger et al., 2002; Ricci, 2012; Utarini et al., 2021). The presence of *Sodalis* and *Wolbachia* infection in the tsetse population sampled re-affirms the presence of the tsetse bacterium in tsetse species found in the Luangwa valley (Doudoumis et al., 2012; Dennis et al., 2014; Mbewe et al., 2015). The high prevalence of *Wolbachia* in female flies (Laohasinnarong et al., 2015) need to be investigated further as a possible basis for environmentally sustainable tsetse population control for *Glossina* species.

Stakeholders in Zambia have competing views and beliefs regarding tsetse and African trypanosomiasis control, which is critical in developing a One Health approach for the control in HAT and AAT. Environmentalists believe tsetse flies help keep environments wild and natural by stopping farmers encroaching protected areas. Agriculturalists feel that such moves have contributed to increased poverty as farmers are kept away from protected areas that are tsetse-infested which has led to uncoordinated control approaches between stakeholders (Grant et al., 2015; Okello et al., 2015). Furthermore, this study demonstrated that coordinated surveillance systems within available organizational structures could play a key role in disease reporting and have the potential to impact the reporting of emerging and re-emerging

zoonotic diseases. A better One Health system could be applied in Zambia and other countries in the region and beyond by strengthening links for collaboration and coordination at national, provincial and district levels between sectors (Veterinary, Health, Wildlife and Natural resources). This could in turn, reduce costs through sharing of capacities and infrastructure by sectors, including non-health sectors e.g., Education and Finance. Clear stakeholder roles need to be agreed and adhered to meet set targets (Mulenga et al., 2021a).

More efficient use of existing capacities by implementing a One Health approach is possible between sectors. Human and animal treatments for the control of animal diseases which do not have an impact on livestock production but pose a threat to human health can be combined to avoid programme duplication and save resources. This includes collaborating in community awareness programmes for zoonotic diseases and collaborated staff training/upscaling of skills (Bordier et al., 2020; Ghai et al., 2022; WHO, 2022). In the same manner, the Wildlife department need to be open to collaboration in the control of wildlife diseases which pose a threat to human and domestic animal health but do not directly impact wildlife. This will not only make environments safe for communities but will also promote the tourism industry and increase Gross Domestic Product (GDP) for the country and region. The digitalization of records for information sharing through national, provincial and district reporting links could be carried out in partnership to create a more efficient response system. Development of new policies that will promote the establishment of a Central public health department specifically under ZNPHI for NTDs, that will collaborate between all sectors and stakeholders, collect data from human, livestock, wildlife, and environment may be a more sustainable way of achieving One Health goals with regards to NTDs at country level (WHO, 2020; Boulangé et al., 2022; Franco et al., 2022).

7.2 CONCLUSIONS

1. Trypanosomiasis remain an important disease for communities living in tsetse infested areas of Eastern Zambia.

2. The use of PCR as point of care diagnosis is still limited and impractical in remote rural areas. Microscopy remains the most practical option for the diagnosis of trypanosomes in the field, but understanding its limitations is critical when using it for surveillance purposes. Microscopy should be used as a combination of the three commonly used techniques i.e., buffy coat, thin smears, and thick smears.

3. The use of Samorin (Isometamidium Chloride) is the most cost-effective trypanosomiasis control strategy for small scale farmers in Eastern Zambia. The use of insecticide treated Cypermethrin targets is a better option for government sponsored control programmes.

4. The application of *Sodalis* as a biological control option for tsetse and trypanosomiasis is limited in the Luangwa valley of Zambia.

5. Sub-optimal implementation of existing policies related to the control of zoonotic diseases in Zambia is impacting on disease reporting and application of a One Health approach.

7.3 RECOMMENDATIONS

Based on the findings from this study, the following recommendations are made:

1. More robust field diagnostic procedures for African trypanosomiasis be developed that consider the environmental, capacity and infrastructure constraints of working in countries like Zambia.
2. Better staff training in disease diagnosis, accreditation training programmes for practising technicians, and the establishment of an external quality assurance scheme, better maintenance of diagnostic equipment, a better funding model and an improvement in field quality control would help address challenges in disease diagnosis, as highlighted in this study.
3. Work be conducted to evaluate and identify African trypanosomiasis control programmes that are cost effective and sustainable in the regions where they are applied.
4. Data on biological characteristics of tsetse to be considered when developing tsetse and trypanosomiasis control programmes.
5. A better One Health system could be applied in Zambia and other countries in the region and beyond by:

    - strengthening links for collaboration and coordination within already existing organizational structures for the departments of Health, Veterinary and Wildlife. Improved links will promote interactions and provide a more holistic response to disease control.

- Digitalizing records for information sharing between sectors to create a more efficient response system.
- Sharing capacities between sectors to save resources (staff, laboratory, coordinated activities).
- Developing new policies to support sustainable zoonotic disease control approaches that can be implemented at different reporting levels.

7.4 REFERENCES

Abro, Z., Kassie, M., Muriithi, B., Okal, M., Masiga, D., Wanda, G., Gisele, O., Samuel, A., Nguertoum, E., Nina, R.A., Mansinsa, P., Adam, Y., Camara, M., Olet, P., Boucader, D., Jamal, S., Garba, A.R.I., Ajakaiye, J.J., Kinani, J.F., Hassan, M.A., Nonga, H., Daffa, J., Gidudu, A., Chilongo, K., 2021. The potential economic benefits of controlling trypanosomiasis using waterbuck repellent blend in sub-Saharan Africa. PloS one 16, e0254558. doi:10.1371/journal.pone.0254558

Anderson, N.E., Mubanga, J., Fevre, E.M., Picozzi, K., Eisler, M.C., Thomas, R., 2011. Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. PLOS Neglected Tropical Diseases 5, e1211. doi:10.1371/journal.pntd.0001211

Bardosh, K.L., Ryan, S.J., Ebi, K., Welburn, S., Singer, B., 2017. Addressing vulnerability, building resilience: community-based adaptation to vector-borne diseases in the context of global change. Infectious Diseases of Poverty 6, 166. doi:10.1186/s40249-017-0375-2

Bordier, M., Uea-Anuwong, T., Binot, A., Hendrikx, P., Goutard, F.L., 2020. Characteristics of One Health surveillance systems: A systematic literature review. Prev Vet Med 181, 104560. doi:10.1016/j.prevetmed.2018.10.005

Boulangé, A., Lejon, V., Berthier, D., Thévenon, S., Gimonneau, G., Desquesnes, M., Abah, S., Agboho, P., Chilongo, K., Gebre, T., Fall, A.G., Kaba, D., Magez, S., Masiga, D., Matovu, E., Moukhtar, A., Neves, L., Olet, P.A., Pagabeleguem, S., Shereni, W., Sorli, B., Taioe, M.O., Tejedor Junco, M.T., Yagi, R., Solano, P., Cecchi, G., 2022. The COMBAT project: controlling and progressively minimizing the burden of vector-borne animal trypanosomosis in Africa. Open Research Europe 2. doi:10.12688/openreseurope.14759.1

Boulanger, N., Brun, R., Ehret-Sabatier, L., Kunz, C., Bulet, P., 2002. Immunopeptides in the defense reactions of *Glossina morsitans* to bacterial and *Trypanosoma brucei brucei* infections. Insect Biochem Mol Biol 32, 369-375. doi:10.1016/S0965-1748(02)00029-2

Bouyer, J., Bouyer, F., Donadeu, M., Rowan, T., Napier, G., 2013. Community- and farmer-based management of animal African trypanosomosis in cattle. Trends Parasitol 29, 519-522. doi:10.1016/j.pt.2013.08.003

Dennis, J.W., Durkin, S.M., Horsley Downie, J.E., Hamill, L.C., Anderson, N.E., MacLeod, E.T., 2014. *Sodalis glossinidius* prevalence and trypanosome presence in tsetse from Luambe National Park, Zambia. Parasit Vectors 7, 378. doi:10.1186/1756-3305-7-378

Doudoumis, V., Tsiamis, G., Wamwiri, F., Brelsfoard, C., Alam, U., Aksoy, E., Dalaperas, S., Abd-Alla, A., Ouma, J., Takac, P., Aksoy, S., Bourtzis, K., 2012. Detection and characterization of *Wolbachia* infections in laboratory and natural populations of different species of tsetse flies (genus *Glossina*). BMC Microbiol 12 Suppl 1, S3. doi:10.1186/1471-2180-12-S1-S3

FAO, 2017. Intervening against bovine trypanosomosis in eastern Africa: mapping the costs and benefits. Food and Agriculture Organization of the United Nations Rome,.

Florkwoski, C., 2008. Sensitivity, Specificity, Receiver-Operating Characteristic (ROC) Curves and Likelihood Ratios: Communicating the Performance of Diagnostic Test. Clinical Biochemistry Rev 29, S83.

Franco, J.R., Cecchi, G., Paone, M., Diarra, A., Grout, L., Kadima Ebeja, A., Simarro, P.P., Zhao, W., Argaw, D., 2022. The elimination of human African trypanosomiasis: Achievements in relation to WHO road map targets for 2020. PLOS Neglected Tropical Diseases 16, e0010047. doi:10.1371/journal.pntd.0010047

Gashururu, R.S., Githigia, S.M., Gasana, M.N., Habimana, R., Maingi, N., Cecchi, G., Paone, M., Zhao, W., Masiga, D.K., Gashumba, J., 2021. An update on the distribution of *Glossina* (tsetse flies) at the wildlife-human-livestock interface of Akagera National Park, Rwanda. Parasit Vectors 14, 294. doi:10.1186/s13071-021-04786-3

Ghai, R.R., Wallace, R.M., Kile, J.C., Shoemaker, T.R., Vieira, A.R., Negron, M.E., Shadomy, S.V., Sinclair, J.R., Goryoka, G.W., Salyer, S.J., Barton Behravesh, C., 2022. A generalizable one health framework for the control of zoonotic diseases. Scientific reports 12, 8588. doi:10.1038/s41598-022-12619-1

Grant, C., Anderson, N., Machila, N., 2015. Stakeholder Narratives on Trypanosomiasis, Their Effect on Policy and the Scope for One Health. PLoS Neglected Tropical Diseases 9, e0004241. doi:10.1371/journal.pntd.0004241

Kakumbi, 2014. Annual Report. Tsetse and Trypanosomiasis Research Station-Ministry of Fisheries and Livestock, Mambwe, Zambia.

Laohasinnarong, D., Goto, Y., Asada, M., Nakao, R., Hayashida, K., Kajino, K., Kawazu, S., Sugimoto, C., Inoue, N., Namangala, B., 2015. Studies of trypanosomiasis in the

Luangwa valley, north-eastern Zambia. Parasites and Vectors 8, 555. doi:10.1186/s13071-015-1173-y

Lord, J.S., Lea, R.S., Allan, F.K., Byamungu, M., Hall, D.R., Lingley, J., Mramba, F., Paxton, E., Vale, G.A., Hargrove, J.W., Morrison, L.J., Torr, S.J., Auty, H.K., 2020. Assessing the effect of insecticide-treated cattle on tsetse abundance and trypanosome transmission at the wildlife-livestock interface in Serengeti, Tanzania. PLOS Neglected Tropical Diseases 14, e0008288. doi:10.1371/journal.pntd.0008288

Mbewe, N.J., Mweempwa, C., Guya, S., Wamwiri, F.N., 2015. Microbiome frequency and their association with trypanosome infection in male *Glossina morsitans* centralis of Western Zambia. Veterinary Parasitology 211, 93-98. doi:10.1016/j.vetpar.2015.04.027

Meyer, A., Holt, H.R., Oumarou, F., Chilongo, K., Gilbert, W., Fauron, A., Mumba, C., Guitian, J., 2018. Integrated cost-benefit analysis of tsetse control and herd productivity to inform control programs for animal African trypanosomiasis. Parasites & Vectors 11, 154.

Moti, Y., Fikru, R., Buscher, P., Van Den Abbeele, J., Duchateau, L., Delespaux, V., 2014. Detection of African animal trypanosomes: the haematocrit centrifugation technique compared to PCR with samples stored on filter paper or in DNA protecting buffer. Veterinary Parasitology 203, 253-258. doi:10.1016/j.vetpar.2014.04.014

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. doi:10.1007/s00436-017-5718-1

Mulenga, G.M., Henning, L., Chilongo, K., Mubamba, C., Namangala, B., Gummow, B., 2020. Insights into the Control and Management of Human and Bovine African Trypanosomiasis in Zambia between 2009 and 2019-A Review. Tropical Medicine and Infectious Diseases 5, 115. doi:10.3390/tropicalmed5030115

Mulenga, G.M., Likwa, R.N., Namangala, B., 2015. Assessing the capacity to diagnose human African trypanosomiasis among health care personnel from Chama and Mambwe districts of eastern Zambia. BMC Research Notes 8, 433. doi:10.1186/s13104-015-1403-6

Mulenga, G.M., Namangala, B., Chilongo, K., Henning, L., Gummow, B., 2021a. Policy and Linkages in the Application of a One Health System for Reporting and Controlling

2829 African Trypanosomiasis and Other Zoonotic Diseases in Zambia. MDPI-Pathogens
2830 11, 1. doi:10.3390/pathogens11010030
2831 Mulenga, G.M., Namangala, B., Chilongo, K., Mubamba, C., Hayashida, K., Henning, L.,
2832 Gummow, B., 2021b. Challenges in the Diagnostic Performance of Parasitological and
2833 Molecular Tests in the Surveillance of African Trypanosomiasis in Eastern Zambia.
2834 Tropical Medicine and Infectious Diseases 6, 68. doi: 10.3390/tropicalmed6020068.
2835 Mulenga, G.M., Namangala, B., Gummow, B., 2022. Prevalence of trypanosomes and selected
2836 symbionts in tsetse species of eastern Zambia. Parasitology, 1-23.
2837 doi:10.1017/S0031182022000804
2838 Njiru, Z.K., Constantine, C.C., Guya, S., Crowther, J., Kiragu, J.M., Thompson, R.C., Davila,
2839 A.M., 2005. The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes.
2840 Parasitology research 95, 186-192. doi:10.1007/s00436-004-1267-5
2841 Nyimba, P.H., Komba, E.V., Sugimoto, C., Namangala, B., 2015. Prevalence and species
2842 distribution of caprine trypanosomosis in Sinazongwe and Kalomo districts of
2843 Zambia. Veterinary Parasitology 210, 125-130. doi:10.1016/j.vetpar.2015.04.003
2844 Okello, A., Welburn, S., Smith, J., 2015. Crossing institutional boundaries: mapping the policy
2845 process for improved control of endemic and neglected zoonoses in sub-Saharan
2846 Africa. Health Policy & Planning 30, 804-812.
2847 Ramirez, B., 2017. Support for research towards understanding the population health
2848 vulnerabilities to vector-borne diseases: increasing resilience under climate change
2849 conditions in Africa. Infect Dis Poverty 6, 164. doi:10.1186/s40249-017-0378-z
2850 Ricci, M.V., Ulisse Ulissi, Sara Epis, Alessia Cappelli, Guido Favia, 2012. Symbiotic Control of
2851 mosquito borne disease. Pathogens and Global Health 106, 380-385.
2852 Richter, J., Gobels, S., Gobel, T., Westenfeld, R., Muller-Stover, I., Haussinger, D., 2012. A
2853 returning traveller with fever, facial swelling, and skin lesions. BMJ 344, e2092.
2854 doi:10.1136/bmj.e2092
2855 Thumbi, M.F., Mosi RO, Jung'a JO, 2008. Comparative evaluation of three PCR base diagnostic
2856 assays for the detection of pathogenic trypanosomes in cattle blood. Parasites and
2857 Vectors 1, 1-46.
2858 Utarini, A., Indriani, C., Ahmad, R.A., Tantowijoyo, W., Arguni, E., Ansari, M.R., Supriyati,
2859 E., Wardana, D.S., Meitika, Y., Ernesia, I., Nurhayati, I., Prabowo, E., Andari, B.,
2860 Green, B.R., Hodgson, L., Cutcher, Z., Rances, E., Ryan, P.A., O'Neill, S.L., Dufault,

S.M., Tanamas, S.K., Jewell, N.P., Anders, K.L., Simmons, C.P., Group, A.S., 2021. Efficacy of *Wolbachia*-Infected Mosquito Deployments for the Control of Dengue. The New England Journal of Medicine 384, 2177-2186. doi:10.1056/NEJMoa2030243

WHO, 2020. Multisectoral Approach to the Prevention and Control of Vector-Borne Diseases: A conceptual framework. Geneva.

WHO, 2022. Ending the neglect to attain the Sustainable Development Goals One Health: Approach for action againt neglected tropical diseases 2021–2030. World Health Organization, Geneva.

APPENDICES AND SUPPLEMENTARY DATA

Appendix A

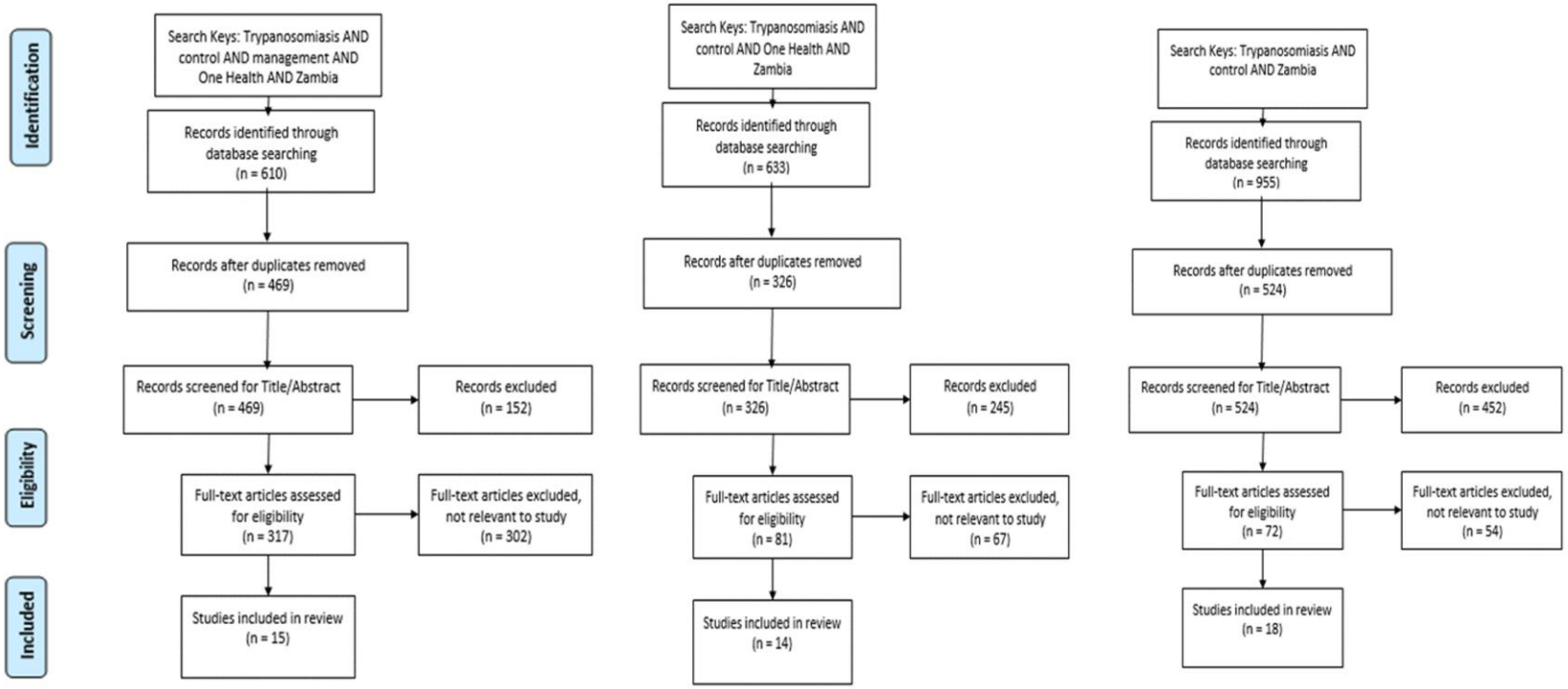

Figure A1. PRISMA diagrams showing three categories of searches conducted and selection criteria of literature included in the review.

2874 **Appendix B**

2875 **Table B1.** Articles meeting selection criteria on trypanosomiasis control in Zambia between January 2009 and December 2019 and a summary of key findings.

Author year	Study Aim	Study Design	Sample and Participation	Study Findings	Needs Domain
(Simukoko et al., 2011)	To assess the monthly risk of bovine trypanosomiasis in cattle kept in tsetse-infested Eastern plateau of Zambia.	Longitudinal study of bovine trypanosomiasis incidence in cattle	Eighty-five herds of cattle that grazed together were selected for a 19-month follow-up study	-The risk of trypanosome infection varied significantly between months with the higher risk recorded between December and February. -PCVs of infected and un-infected cattle did not differ significantly -*Trypanosoma congolense* and *T. vivax* were detected in 92.3% and 4.5% of the infected cattle, respectively. Mixed infections were detected in 3.2% of positive samples. -Overall, 155 infections were detected using PCR while microscopy detected 85 infections.	1. More effort in optimizing Animal African Trypanosomiasis (AAT) control during periods of highest challenges. 2. Accuracy of AAT incidence using parasitological diagnosis stresses need for more sensitive diagnostic tools to improve field diagnosis.

(Mwanakasale and Songolo, 2011)	-To identify districts in Zambia that were still reporting cases of Human African Trypanosomiasis (HAT). -To compare the occurrence of HAT cases before and after year 2000.	-Cross sectional survey of districts located close to national parks. -Literature review of occurrence of HAT in Zambia in the 1960s to 1990s.	-Conducted in nine provinces of Zambia except for Lusaka district. -Used google search, PubMed and world health organisation HINARI access to obtain data on HAT occurrence. Only articles with data on	-Chama, Mpika and Chipata districts were still reporting HAT cases. Seven districts that used to report HAT no longer had cases after January 2000. -All surveyed districts had no existing tsetse control programs. -In all surveyed health institutions, giemsa stain thick smear microscopy was the routine diagnostic method to detect HAT. Only Chilonga mission hospital used microhaematocrit centrifuge method to detect HAT. -Six of the surveyed hospitals had stocks of suramin but none had melarsoprol. -Findings from literature survey show a significant difference in HAT reporting foci from 1960s to 1990s and	1. Districts reporting HAT 2008 to date 2. Data on Agriculture practices between 2000 to 2007 and compared with 1960s to 1990s to confirm if agriculture practices may have contributed to reduced tsetse flies in previously tsetse-infested areas and thus the drastic reduction of HAT cases. 3. Current data on human activities occurring in game management areas (GMAs) as they may be responsible for persistent HAT transmission and tsetse-human contacts. 4. Human animal contacts as animals may carry trypanosomes with them 5. Poaching as game destruction was once used to eliminate wildlife reservoirs 6. Under diagnosing of HAT due to increased focus on management of HIV/AIDS and malaria

			HAT distribution, epidemics, treatments and control of HAT before 2000 were reviewed.	2000 to 2007 with some old foci disappearing whilst new ones emerged or re-emerged	
(Anderson et al., 2011)	To characterise the nature of the reservoir community for trypanosomiasis in the absence of influence from	A cross-sectional survey of trypanosome prevalence in wildlife hosts. Conducted in the Luangwa valley from 2005 to 2007	A total of 418 wild animals were examined for the presence of trypanosomes	-Overall prevalence in all species was 13.9% with infection likely to be detected in waterbuck, lion, kudu and bushbuck, respectively. -Bushbuck indicated to be important hosts for *T. brucei s.l* with bushbuck, greater Kudu, and Lion to be important hosts for *T. congolense* while *T. vivax* was frequently detected in waterbuck.	1. Tsetse blood meal preference was identified as a risk factor for trypanosome infection. 2. Difficulties in sampling wildlife and method used to sample in this study limited ability to investigate age as a risk factor in trypanosome infection 3. Infection of *T. b. rhodesiense* in buffalo raises concern on possibility of infection been established in cattle populations not far from sampling area i.e., Mambwe district of the

	domesticated hosts			-*T. b. rhodesiense* were first identified in African buffalo and *T. brucei s.l* in leopard -First use multispecies PCR for the diagnosis of samples collected from free ranging wildlife which offers improved diagnostic specificity and sensitivity compared to traditional techniques. -Results indicated the ability of trypanosomes to survive in a wide variety of wildlife hosts.	Eastern province of Zambia. This is because buffalos move over large distances with potential to disseminate infection to other species. 4. Trypanosome reservoir in wildlife hosts maybe wider that estimated in this study 5. Influx of people with their livestock and land use may have an impact on the epidemiology of African trypanosomiasis.
(Namangala et al., 2012)	To evaluate the performance of repetitive insertion mobile	Case study	Four male patients from Luangwa and Zambezi river basins	-Both RIME-LAMP and SRA-LAMP were able to detect *T. b. rhodesiense* in patients' blood and in cerebrospinal fluid (CSF). -LAMP results correlated with microscopy results but they do not	1. Need for a detailed study with larger sample size to evaluate potential of LAMP to be used as a bedside diagnostic test for HAT and for making therapeutic decisions.

	element (RIME)– loop mediated isothermal amplification (LAMP) and human serum resistance associated (SRA)–LAMP against microscopy in HAT diagnosis			confirm the standard staging criteria using microscopy and white blood cell (WBC) in CSF.	2. Need for both active and passive surveillance of HAT and community sensitisation in HAT old foci.
(Namangala et al., 2013)	To evaluate the performance of LAMP against	Cross sectional survey of trypanosomiasis in exotic dogs	Six exotic dogs naturally infected with trypanosomes from Zambia's	-Results indicated first report of canine animal trypanosomiasis (CAT) in Zambia -All cases initially diagnosed by microscopy and later confirmed by	1. Further investigation on SRA gene isolated from two dogs in this communication. 2. Scanty parasitaemia sometimes pose challenges caused by weak fluorescence signal thus need to quantify the fluorescence intensity

	microscopy to detect CAT in exotic dogs		South Luangwa National Park and Chiawa GMA.	LAMP, showing good correlation between the two methods. -Three dogs reported infection with *T. congolense* according to CON2-LAMP -All SRA-LAMP positive cases were also RIME-LAMP positive indicating similar sensitivity.	and consider samples to be positive after subtracting the background fluorescence of the negative control. 3. Dogs as potential source of HAT infections 4. Need to investigate performance of LAMP in CAT diagnosis among locally bred dogs in tsetse-infested GMAs and National parks.
(Mwanakasale et al., 2013)	To assess current health delivery system in the management of HAT.	Cross sectional survey of health institutions using structured questionnaires	Nine health institutions from Mpika district of Zambia were involved in the study	-The general knowledge on HAT of health staff from surveyed health institutions was unsatisfactory for proper management of the disease -Study revealed gross understaffing of essential staff to clinically diagnose and manage HAT -No staff from the surveyed institutions had received specific	1. Need for refresher courses to be conducted every two years for health personnel in districts at risk of HAT transmission in Zambia. 2. Need for awareness on HAT for health policy makers so that they understand the need for refresher courses and trainings on disease management. 3. Need to motivate in kind health staff at the frontline of identifying suspected cases and

				training on HAT diagnosis and treatment. -There was only one treatment centre (Chilonga mission hospital) from the surveyed health institutions -Erratic supply of trypanocides at the only treatment centre in the district -Only 2 of the surveyed institutions has functional laboratories with qualified personnel. Both institutions used less-sensitive methods to diagnose HAT -Distances between rural health centres (RHCs) and treatment centres and non-availability of transport to ferry suspected HAT patients.	encourage them to refer such cases to diagnostic and treatment centres 4. Need to establish Mpika district hospital as an additional treatment centre to decongest Chilonga mission hospital and improve health service delivery at both hospitals. 5. Ministry of health to ensure that drugs for both stages of HAT are always in stock. 6. Need for Ministry of health to equip and capacitate health institutions with laboratories and personnel as well as more sensitive diagnostic tools. 7. Need for a proper referral system for HAT suspected cases to diagnostic treatment to ensure they reach their designated centres.
	To evaluate the performance	Cross sectional survey of Canine	A total of 237 indigenous dogs from 47	-Fourteen cases of trypanosomes were detected using microscopy.	1. Diagnostic accuracy of LAMP against microscopy suggested that its use in CAT

(Lisulo et al., 2014)	of LAMP in determining trypanosome prevalence in indigenous dogs.	African Trypanosomiasis	villages within five chiefdoms of Mambwe district of Zambia	-LAMP detected an additional 6 cases indicating higher sensitivity and specificity than microscopy. -Adult dogs were more likely to acquire CAT as they are involved in hunting. -CAT was significantly related to corneal opacity -Dogs are potential links for trypanosome exchange between livestock and humans.	diagnosis could improve disease management in African trypanosomiasis in endemic areas. 2. Results from study can trigger a One Health approach towards control of HAT through disease intervention in livestock. 3. Need for continuous surveillance of African trypanosomiasis in tsetse-infested regions using more user friendly and sensitive tests such as LAMP. 4. Need to sensitise locals in GMAs potential dangers of keeping dogs that are left to scavenge without receiving veterinary services. 5. Dogs may harbour other zoonoses apart from *T. b. rhodesiense* with potential serious implications to human health.
	To determine the prevalence and species	Cross-sectional cluster survey of AAT in goats	Overall, 422 goats from Kalomo and Sinazongwe	-One goat was found infected on microscopy while 100 goats reported positive for AAT on LAMP.	1. Need for improved staffing to enhance disease prevention and containment.

(Nyimba et al., 2015)	distribution of caprine trypanosomiasis		districts of Southern province of Zambia	-Infection rate for Sinazongwe district was 22.4% while that for Kalomo district was 24.7% -*Trypanosoma brucei, T. vivax* and *T. congolense* were detected in 82.0%, 31.0% and 23.0% of the infected goats, respectively. Mixed infections were detected in 33.0% of positive samples. - Study results indicate the re-emergence of AAT in study areas were aerial spraying was once conducted by the government.	2. Need for refresher courses for frontline Veterinary staff in order to improve service delivery. 3. Need for sustainable control operations to avoid tsetse re-invasions and re-occurrence of disease in areas where control was once a success story.
	To examine the presence of different trypanosome species in cattle, goats	Cross sectional survey of trypanosomes in cattle, goats and tsetse flies.	In total, 243 cattle, 36 goats and 546 tsetse flies were examined for the presence of	-Microscopy exhibited relatively low sensitivity than PCR and LAMP -There was poor agreement among test methods. For instance, failure of PCR and LAMP to detect microscopically positive samples.	1. Need to establish if trypanosome DNA detected from cattle, goats and tsetse were active infections or residual DNA from dead trypanosomes picked from blood meals or treated animals.

(Laohasinnarong et al., 2015)	and tsetse using a combination of microscopy, PCR and LAMP		trypanosomes. Study conducted from Petauke, Chama and Isoka districts of Zambia.	-KIN PCR was found to be sensitive for detecting *T. congolense* -TviCatL-PCR and PFL-LAMP were better for detecting *T. Vivax* and *T. b. rhodesiense*, respectively. -The presence of *T. b. rhodesiense* in tsetse samples indicates its ability to take blood meal from multiple hosts (wildlife, humans and domestic animals), facilitating the circulation of the parasite in the ecosystem. -Infection in cattle and goats was highest with *T. congolense* and lEast with *T. vivax*	2. Need for a One Health approach towards the control of HAT through disease intervention in livestock, wildlife and tsetse.
	To investigate health personnel's and health centre's	Cross sectional survey using structured questionnaires.	A sample of 101 health personnel drawn from 12 and nine	-Staffing levels from both districts were extremely low with most health centres manned by one trained staff -Staff had basic knowledge to identify HAT with staff from Chama districts	1. Need for authorities to train and post more health staff in rural areas and to come up with deliberate policies that provide incentives to attract and motivate health workers in rural areas

(Mulenga et al., 2015)	capacity to diagnose Human African trypanosomiasis		health centres from Chama and Mambwe districts, respectively	more likely to identify a case compared to their Mambwe counterparts. -Only Chama district had functional laboratories. Most health centres surveyed reported frequent use of rapid test kits for diagnosing mainly malaria parasites thus reducing diagnosis of other blood parasites that can be detected by microscopy including HAT.	2. Need for capacity building and refresher trainings for health staff with regards to HAT diagnosis. 3. Need for health centres located in HAT foci to be equipped with at lEast microscopes to enable them more easily identify cases when they occur. Further, referral or district hospitals can also be equipped with more sensitive laboratory tools like PCR and LAMP. 4. Need for HAT national surveillance and control programmes to be enhanced.
(Mbewe et al., 2015b)	To examine how socio-economic and environmental factors are associated with adherence to	Cross sectional survey using a structured questionnaire.	Farmers interviewed from five veterinary camps from Itezhi tezhi district of Central	-Of the interviewed farmers, 25.6% adhered to FAO guidelines on trypanocide use; (i) reducing the number of treatments on whole herd up to a maximum of four times in a year by integrating drug usage with other control measures and (ii) avoiding exposure of the	1. Need for an integrated approach of measure to control AAT in the GMA of Itezhi tezhi to lessen overuse of trypanocides by farmers. 2. Need to investigate if household income may influence farmer's adherence to FAO guidelines of trypanocide use as defined in this study.

	the recommended guidelines on trypanocide use	province of Zambia	whole parasite population to the drug by limiting treatments to individual sick animals. -None of the socio-economic factors (age, education, cattle herd size, competence in trypanocide use and access to extension on trypanocide use) were associated with a farmer's adherence to FAO guidelines. -Low adherence to recommended FAO guidelines on trypanocide use was associated with the location of crush pen, whether in GMA or not, as an environmental factor. Farmers in GMAs were less likely to adhere to FAO guidelines than those in non-GMA.	3. Need to investigate if household income may influence control of vector borne diseases.

(Mbewe et al., 2015a)	-To investigate the prevalence of animal trypanosomiasis in anaemic cattle	Cross sectional survey of AAT in cattle	A total of 564 Anaemic cattle from Itezhi tezhi district of Zambia	-Out of 564 cattle screened, 58 (10.3 %) had anaemia. PCR-RFLP results showed that 17 (29.3 %) anaemic cattle were positive for pathogenic trypanosomes compared to 1 (1.7 %) on parasitological examination using thick smears. -Infections were caused by *Trypanosoma congolense* and *Trypanosoma vivax*.	1. Need to investigate other anaemia causing factors in animal trypanosomiasis endemic areas of Itezhi tezhi district of Zambia.
(Grant et al., 2015)	-To examine the narratives on African trypanosomiasis in Zambian policy. -To explore relationships	Case study of key informant interviews	Twenty participants from international organisations, research organisations and local activists.	-Environmentalists believed tsetse stop farmers encroaching protected areas thus keeping areas natural and wild. -Increased poverty because tsetse keeps farmers away from productive areas. -The Zambian government has other diseases of priority other than African	1. Need for cross-sector, interdisciplinary decision making to stop rival narratives leading to competing actions. 2. Need for a One Health approach to break down the barriers between social scientists, natural scientists and the expertise of the community.

	between human, animal and environmental sectors	trypanosomiasis and does not have funds to keep areas tsetse free. -Major focus of African trypanosomiasis control is emphasised on cattle and not humans. -The need to undertake tsetse control using the best methods have been identified but with no financial resources to support the plan. -Tsetse-infested forests that have been cleared for cotton growing have disrupted tsetse habitats due to chemicals used. -Current conservation strategies have sustained the preservation of tsetse flies and African trypanosomiasis.

(Mweempwa et al., 2015)	To establish the impact of habitat fragmentation on the physiological and demographic parameters of tsetse flies in order to enhance the understanding of the relationship between fragmentation and AAT risk	Longitudinal study of tsetse age, abundance and trypanosome infection in areas of varying degrees of habitat fragmentation in Eastern Zambia.	-A set of 3200 *Glossina morsitans morsitans* were caught using black screen fly rounds. -Overall, 577 female tsetse flies were dissected for ovarian age estimation. -A sentinel herd of 40 cattle was established at each of the four sites of	-Results indicated a significant increase in tsetse age as fragmentation increased. -Tsetse density was lower in most fragmented areas whilst the proportion of female flies increased significantly as fragmentation reduced. -AAT incidence in cattle was determined using buffy coat method. Infection rate in both cattle and tsetse flies was higher in highly fragmented areas.	1. Need to develop models that link biological characteristics of tsetse flies with habitat conditions. Such models maybe helpful in planning tsetse control interventions.

			Katete and Mambwe districts.		
(Meyer et al., 2016)	A literature review of past and on-going tsetse and African trypanosomiasis programmes	Systematic literature review of tsetse and African trypanosomiasis programmes between 1980 and 2015	Five African countries including Zambia. 68 documents plus 12 structured questionnaires reviewed.	-Twenty-three major Tsetse and Trypanosomiasis control programmes recorded from the five countries. Three control programmes conducted in Zambia during the stated period include the following: - Insecticide treated targets and traps (ITT) + trypanocidal drugs (TRY) in Western province under government services for tsetse elimination (1987–1989). - Sequential aerial spraying (SAS) + ITT in Eastern province under Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) for tsetse control (1989–1994)	1. Need for evaluation of the control programmes recorded. 2. Need for standardised protocols to conduct such evaluations of control programmes

				-SAS + ITT in Kwando Zambezi belt under Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) for tsetse elimination (2008 onwards)	
(Alderton et al., 2016)	To develop an agent-based model (ABM) for investigating *Trypanosoma brucei rhodesiense*	-Mixed methods	-ABM comprised of human/animal trypanosomiasis and tsetse ecological survey data obtained along the 75km transect in the Luangwa valley of Zambia.	-ABM produced output that could not be readily generated by other techniques. On average there were 1.99 (S.E. 0.245) human infections and 1.83 (S.E. 0.183) cattle infections per 6-month period. -The model output identified that the approximate incidence rate (per 1000 person-years) was lower amongst cattle owning households (0.079, S.E. 0.017), than those without cattle (0.134, S.E. 0.017). - Immigrant tribes (e.g., Bemba I.R. = 0.353, S.E.0.155) and school-age	1. The ABM can be used as a tool for scenario testing at an appropriate spatial scale to allow the design of logistically feasible mitigation strategies suggested by model output. This is of importance where resources are limited, and management strategies are often pushed to the local scale.

			-Ethnicity, age and gender data were also incorporated.	children (e.g., 5–10-year-old I.R. = 0.239, S.E. 0.041) were the most at-risk for acquiring infection.	
(Holt et al., 2016)	To assess AAT vulnerability in cattle owing communities	Cross sectional survey of cattle owners using questionnaire interviews.	210 households from Lundazi and Mambwe districts of Zambia	-AAT was constant with seasonal pattern, some trypano-tolerant breeds and communal grazing, small/moderate herd size with crops and mixing farming as primary income source, losses to draft reported, slightly higher mortalities and moderate costs diagnosing and treating, less likely to report treatment failure, low/good knowledge of control and tsetse traps/targets reported. -moderate AAT challenge, some concerns with resistance reported and	1. Need to integrate novel treatments with new and existing diagnostic and control programmes with findings of the study to develop tailored recommendations for AAT control and the reduce its impact in vulnerable communities.

				most likely to keep pigs while some keep sheep and goats.	
(Meyer et al., 2018)	To propose a framework for conducting a cost benefit analysis of possible AAT control analysis	A literature review of AAT of cattle production, herd management, impact of AAT on productivity, incidence and mortality	Two districts from Cameroon and Zambia (Mambwe district)	-For Zambia, the 10-year impact of tsetse elimination on the net value of cattle production was calculated as benefit–cost ratios using a discount rate of 5% and indicated the following: - 2.3 (1.8–2.7) Targets, insecticide treated cattle (ITC) barrier -2.0 (1.6–2.4) Targets, barrier traps -2.8 (2.3–3.3) Aerial spraying, ITC barrier -2.5 (2.0–2.9) Aerial spraying, barrier trap -The use of SAT as elimination method for Mambwe district yielded a higher benefit–cost ratio than the use of targets.	1. Need for barriers to be maintained and monitoring activities conducted continuously unless sequential elimination of the entire tsetse belt is achieved. 2. Cost–benefit studies should be supported by recent estimates of key parameters such as frequency of trypanosome infection and impact, livestock and tsetse demographics. 3. Model generated in study combined data from different locations and from studies conducted years ago, there is need to validate the model using current data from same locations. 4. Need to use existing control programmes for designing future control programmes.

		-The model estimated the total discounted control costs at 3.8 million USD and benefits at 10.5 million USD for Mambwe district if SAT was used as tsetse elimination method

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Appendix C

Table C1: Demographic data

	No. of farmers	Cattle enrolled	Female cattle	Male cattle	Young cattle	Adult cattle	Cattle births	Cattle deaths
Berenil	3	64	31	33	12	52	12	0
Samorin	3	48	24	24	14	34	14	1
Cyfluthrin Pour-on	17	48	22	26	8	40	9	4
Cypermethrin target	11	67	30	37	9	58	13	2

Appendix D

Table D1. Summary of SWOT analysis for reporting structures for the Departments of Veterinary Services, Health and National Parks and Wildlife.

Internal		**External**	
Strengths	**Weaknesses**	**Opportunities**	**Threats**
Veterinary Services (Figure 6.1a) 1. The structure was flexible and allowed for interactions among officers—for example, VAs and TCAs could report directly to DVOs. In the same manner, LOs could report directly to the senior veterinary officers in the province. 2. The TTCU is mandated to specifically oversee all	1. Extension officers cover very large areas, and this made it difficult to achieve timely reporting of disease occurrence. 2. The flexibility of reporting to senior veterinary officers directly creates gaps in knowledge among immediate staff.	1. Some veterinary camps could be divided into smaller units per VA or TCA to facilitate more effective coverage by the Vas and TCAs efficiently. **2. A network of reporting and information sharing needs to be enhanced between**	1. Employment of extension personnel at VA/TCA and related levels occurs, and this diminishes prospects for effective/beneficial interactions with farmers regarding collection of

tsetse and trypanosomiasis control and management programmes in the country. 3. All heads of units in the Department of Veterinary Services interact frequently, and this facilitates sharing of information on the occurrence of livestock diseases. 4. Directors from the livestock sector at the national level also interact frequently and share information.	3. The process of replacing deceased/retired officers is slow, resulting in vacant positions. 4. The structure does not allow for position funding to ensure timely filling of vacancies. 5. Shortage of staff makes work overwhelming for available officers.	**staff (including staff from Health and Wildlife Departments) and the community by creating better extension methodologies.** **3. Additional positions could be created in the structure to improve on the effectiveness of reporting on trypanosomiasis and other zoonotic diseases occurrences.**	information on disease occurrence. 2. Some districts such as Mambwe do not have DVOs, which makes gaps in reporting and decision making. 3. Slow recruitment of staff and non-availability of funds has led to prolonged

5. Personnel at district and provincial levels interact routinely with their colleagues from other ministries and NGOs, and this allows for information sharing	4. Levels of funding to the TTCU could be improved, increasing capacity for the unit to undertake collection of relevant data/information more routinely and more effectively on tsetse and trypanosomiasis in the country. **5. The mandate/role of the TTCU could be increased/enhanced to include more elements in the One Health approach to addressing the**	vacancies in key positions. 4. Shortage of staff makes work overwhelming for available officers. 5. Prolonged poor funding for the ministry. 6. Lack of operational funds for the T & T has created a group of unmotivated staff who must perform other

		trypanosomiasis problem and other zoonotic diseases. 6. Improvement of salaries and other working conditions for personnel in the department could encourage/enhance better performance of personnel in the department.	duties of the department. 7. Crushing economy and stagnant salaries. 8. Reduced political will.
Health (Figure 6.1b) 1. The structure is flexible and allows interactions between junior and senior officers.	1. Some health centres/posts have a large coverage therefore reduce effective service delivery.	**1. The building of more health posts across the country will enhance service delivery and increase points of**	1. Some health workers are new and inexperienced. 2. Majority of experienced

2. Department and section heads interact frequently, enabling disease information sharing 3. Permanent secretaries and directors at the national level interact frequently and share information. 4. The ministry is the secretariat for epidemic preparedness meetings at national, provincial and district levels. 5. Staff at both district and provincial levels interact with their colleagues from other ministries and	2. The flexibility of reporting to senior officers directly creates gaps in knowledge among immediate staff. 3. Some districts such as Mambwe do not have a district hospital, which has overwhelmed the mission hospital that has limited bed and staff capacity. 4. Epidemic preparedness committees prioritise and are driven to	**contact and interaction. This will also create opportunities for active epidemiological assessments of zoonotic diseases.** **2. Creation of district hospitals and converting some hospitals at the province to level 2 or 3 hospitals could provide relief for health centres/posts. District hospitals could provide common public**	health workers have fled the country in search of greener pastures. 3. Slow recruitment of staff and non-availability of funds has led to prolonged staff vacancies in key positions at health facilities. 4. Crushing economy and

NGOs through the epidemic preparedness, which allows for networking and information sharing. 6. Strong interaction between health workers and communities through health centre committees at local levels.	report on diseases of political interest and affect the majority, while diseases that affect the voiceless poor, remote communities are not given much attention. 5. Shortage of staff makes work overwhelming for available officers. 6. Focus areas are determined at the national level, which may not be priorities in all districts.	**health services conveniently.** **3. Data collection takes place at all levels, which can enhance information sharing between line ministries and departments with emphasis on zoonotic diseases.** 4. **Opening of more health training facilities, both public and private, will provide person power to fill key positions/vacancies**.	stagnant salaries. 5. Reduced political will. 6. Erratic and inadequate funding to health resulting in poor performance of the sector. 7. Inadequate laboratory equipment and reagents to detect disease outbreaks early.

		5. Improved salaries and attractive conditions of service for medical personnel will motivate and encourage them to join the public sector.	
National Parks and Wildlife (Figure 6.1c) 1. The department operates under a decentralised structure. 2. The department works hand in hand with other wildlife conservation organisations enhancing the control, management,	1. Though the structure is decentralised, decisions and allocation of financial resources is still conducted at headquarters. 2. The department, which was formally an authority and	1. Decision making and financial allocation of funds should be decentralised. 2. Improved funding will strengthen operations and enhance control, management, and	1. Recruitments are slow and politically driven. 2. Limited funding has created a lot of vacancies and weakened operations.

conservation and administration of national parks, bird sanctuaries, wildlife sanctuaries and Game Management Areas (GMAs). 3. Through partnership with local communities (CRBs), responsibilities of management in game management areas are shared. 4. Community networks allows sensitisation and education of the public on the necessity of wildlife conservation, and the	managed by a board, is now a government department and operations are now dependant on irregular government funding. 3. Poaching has negatively impacted on the department's operations and is a threat to tourism. 4. Corruption when dealing with recruitments, prosecutions, and issuance of licenses.	conservation of wildlife. **3. Improved funding will also strengthen active surveillance of zoonotic diseases originating from wildlife.** **4. More smart partnerships need to be created with other government departments, NGOs and the private sector.** **5. Community awareness and sensitisations need**	3. Reduced political will 4. Crushing economy and stagnant salaries. 5. The large structure is a challenge to timely reporting	

importance of wildlife to foster appreciation of the economic value of wildlife. 5. Networking and information sharing is enhanced through staff interaction at all levels of management	5. The process of replacing deceased/retired officers is slow, resulting in vacant positions. 6. The structure is large.	**to be enhanced and be undertaken in collaboration with other government departments and NGOs.** 6. Community empowerment programmes to be created to reduce poaching. 7. Improved salaries for wildlife officers. **8. Create policy to retain key positions.** **9. Create sections within the**

	department that could be responsible for addressing zoonotic diseases, including trypanosomiasis, thereby promoting the One Health approach.

Abbreviations: VAs: Veterinary Assistants; DVOs: District Veterinary Officers; LOs: Livestock Officers; TCAs: Tsetse Control Assistants; TTCU: Tsetse and Trypanosomiasis Control Unit; T & T: Tsetse and Trypanosomiasis; CRBs: Community Resources Boards). Bold and highlighted 'opportunities' indicate areas where a One Health approach can be applied.

Appendix D

INFORMATION SHEET

PROJECT TITLE: **The control of Bovine and Human African Trypanosomiasis and role tsetse endosymbionts play in disease transmission in endemic areas of Zambia**.

You are invited to take part in a research project that aims at evaluating and identifying strategies and measures that are economically important in the control of African trypanosomiasis. The study will help in improving detection of Nagana (AAT) in cattle and sleeping sickness (HAT) in people in endemic areas of Zambia. This will help authorities to treat infected people and cattle more rapidly.

The study will also help in our knowledge of the role and importance different control strategies play in the control of Animal Trypanosomiasis so that we can identify what will be the most cost-effective way of controlling this disease in your region of Zambia. This could help make the control of the disease more affordable in your region.

The study is being conducted by **Gloria Mulenga** and will contribute to the attainment of a **Doctor of Philosophy Degree (PhD)** in **Epidemiology** at James Cook University in Australia.

If you agree to be involved in the study, you will be invited to be interviewed through a questionnaire. The interview, with your consent, should only take approximately 45 min of your time. The interview will be conducted at your home, or a venue of your choice. The questionnaire that you will be requested to complete, asks you about your personal details including the level of your education. It also asks you questions on farm structure and income, trading practices and interaction.

Taking part in this study is completely voluntary and you can stop taking part in the study at any time without explanation or prejudice.

Your responses will be non-identifiable so no one will know who gave the information to us. The data from the study will be used in research publications and reports to be published by the principal investigator and other collaborators through **James Cook University**, **University of Pretoria**, **Zambian Government and University of Zambia**. You will not be identified in any way in these publications.

If you have any questions about the study, please contact—**Gloria Mulenga (Principal Investigator) and Bruce Gummow (Supervisor).**

Principle Investigator:

Gloria Mulenga

Department of Veterinary Services

Ministry of Fisheries and Livestock

Republic of ZAMBIA

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Australian Investigator:

Name: Bruce Gummow

College of Public Health, Medical and Veterinary Science

James Cook University, AUSTRALIA

Phone:

Mobile:

Email: bruce.gummow@jcu.edu.au

If you have any concerns regarding the ethical conduct of the study, please contact:

Human Ethics, Research Office

James Cook University, Townsville, Qld, 4811

Phone: (07) 4781 5011 (ethics@jcu.edu.au)

(Chairperson), ERES CONVERGE IRB; 33 Joseph Mwilwa Road Rhodes Park; LUSAKA; Tel: ; Mobile: ; E. Mail:

Appendix E

QUESTIONNAIRE SURVEY

THE CONTROL OF AFRICAN TRYPANOSOMIASIS AND THE ROLE TSETSE ENDOSYMBIONTS PLAY IN DISEASE TRANSMISSION IN ENDEMIC AREAS OF EASTERN ZAMBIA

QUESTIONAIRE ID: .....

NAME OF RHC: .....

GPS COORDINATES: .....

INSTRUCTIONS:

No name should appear on/and or in this questionnaire.

Answer all the questions.

Tick √ in the space provided next to your choice.

Write in provided space wherever appropriate.

Use a pen/pencil in the questionnaire.

SECTION A: DEMOGRAPHIC DATA FOR OFFICIAL USE

Occupation/position of the respondent at the center (In-charge)

[1] Medical Officer

[2] Clinical Officer

[3] Nurse

[4] Laboratory Technician

[5] Environmental health technician

[6] Other (specify)

Highest level of education of respondent

[1] Degree

[2] Diploma

[3] Certificate

[4] Other (specify)

Indicate numbers of staff at the clinic with the following occupations

(Write in the space provided)

[1] Doctors

[2] Clinical Officer

[3] Nurses

[4] Laboratory Technician

[5] Environmental health technicians

[6] Other (Specify)

For how long have you being working in this district?

[1] Less than 5 years

[2] Between 5 and 10 years

[3] More than 10 years

SECTION B: CAPACITY TO MANAGE SLEEPING SICKNESS/AAT

Is tsetse transmitted sleeping sickness/AAT a problem within your community?

[1] Yes [2] No [88] Don't know

What do you think plays a role in sleeping sickness/AAT transmission?

[1] Wildlife [2] domestic animals [3] Tsetse [88] Don't know

From your knowledge, what signs and symptoms does a suspected sleeping sickness patient/AAT animal present themselves with?

[1] Sleeping disorder

[2] General body pains/extreme fatigue/severe headache

[3] Chancre-swelling at site of tsetse bite

[4] Puffy swollen face

[5] Severe fever/history of taking anti-malarial drugs with no relief

[6] Skin rash

[7] Loss of weight/appetite

[8] Anaemia

[9] Others

Have you ever encountered a case of sleeping sickness/AAT at this health center?

[1] Yes [2] No

If NO skip to Question 9

From hospital/station records, how many cases of sleeping sickness/AAT has your Centre reported? (Ask to see records if available)

[1] Last 12 months [2] Last 5 years [3] Last 10 years

Does your department undertake surveys/surveillances for sleeping sickness/AAT within your catchment areas?

[1] Yes [2] No [88] Don't know

If yes, how often?

[1] Quarterly [2] Annually [3] Whenever resources available

Has any officer at this Centre received any special training on sleeping sickness/AAT diagnosis/management?

[1] Yes [2] No [88] Don't know

Does the Centre have a laboratory equipped with the following facilities?

[Tick √ in the box next to your choice]

[1] Standard diagnosis equipment/materials

(Microscope, centrifuge, Giemsa stain, slides, capillary tubes)

[2] Rapid test kits

[3] Molecular technique facilities

(PCR, LAMP)

[4] None of the above

Does the centre receive any financial support specifically for the management of sleeping sickness/AAT?

[1] Yes [2] No [88] Don't know

If yes, tick below

[1] GRZ [2] Private Sector [3] Other

SECTION C: KNOWLEDGE AND COLLABORATION

Are you aware of the occurrence of AAT in your area?

[1] Yes [2] No [88] Don't know

Do you think AAT is a problem in your area?

[1] Yes [2] No [88] Don't know

Does your centre work with officers from the Veterinary/Health/Wildlife department on issues related to sleeping sickness and AAT?

[1] Yes [2] No [88] Don't know

If yes, which area of collaboration below?

[Tick √ in the box next to your choice]

[1] Community awareness

[2] Staff training and capacity building

[3] Laboratory diagnosis

[4] Other (Specify)

Does your department in your catchment area work with officers from the other government/non-governmental departments on issues related to sleeping sickness and AAT?

[1] Yes [2] No [88] Don't know

If yes, which area of collaboration below?

[Tick √ in the box next to your choice and indicate name of organization]

[1] Community awareness

[2] Staff training and capacity building

[3] Laboratory diagnosis

[4] Other (Specify)

If no, would you want collaboration?

[1] Yes [2] No

2903 THE END

2904 THANK YOU FOR YOUR COOPERATION

2905 NOTE the following differences in the questionnaire for the Department of Veterinary
2906 Services:

SECTION A: DEMOGRAPHIC DATA FOR OFFICIAL USE

Occupation/position of the respondent

[1] Veterinary Officer	[2] Tsetse control Officer	[3] Laboratory Technician	[4] Other (specify)

Indicate numbers of staff at the facility with the following occupations

(Write in the space provided) SKIP Question if you are the only staff

[1] Doctors

[2] Biologists/Scientist

[3] Field assistants

[4] Laboratory Technician

[5] Other (Specify)

2907 NOTE the following differences for the questionnaire for the Department of National Parks
2908 and Wildlife:

SECTION A: DEMOGRAPHIC DATA FOR OFFICIAL USE

Occupation/position of the respondent

[1] Veterinary Officer [2] Ecologist [3] Laboratory Technician [4] Other (specify)

Indicate numbers of staff at the facility with the following occupations

(Write in the space provided) SKIP Question if you are the only staff

[1] Doctors

[2] Ecologist/Scientist

[3] Field assistants

[4] Laboratory Technician

[5] Other (Specify)

Appendix F

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2911 **Appendix G**

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2913 **Appendix H**

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2915 **Appendix I**

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Appendix J

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Appendix K

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SUPPLEMENTARY DATA

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THE DETECTION OF AFRICAN TRYPANOSOMES IN GOATS REARED IN TSETSE INFESTED VILLAGES OF EASTERN ZAMBIA

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Abstract

Control programmes for African animal trypanosomiasis (AAT) in livestock have been mainly focused on cattle with very little focus on goats, an important reservoir for the disease. Using the polymerase chain reaction (PCR), this study investigated trypanosome infection in village goats in Mambwe, a rural District in Eastern Zambia**.** Filter paper blood spots were collected from 326 goats and tested for infection with *Trypanosoma congolense, Trypanosoma vivax* and *Trypanosoma brucei s.l*. using Ribosomal RNA Internal Transcribed Spacers (ITS)-PCR. The frequency of trypanosomes from the sampled goats was 4.6% (95% CI = 2.3-6.8). Results indicated significantly high infections with *Trypanosoma vivax* (4.0%; 95% CI = 1.9-6.1) than *T. congolense* (0.6%; 95% CI = -0.2-1.5), and *T. brucei* (0.0%), $P = 0.04$. Findings show the circulation of trypanosomes that causes AAT in goats and that they may pose serious threats to not only goats but also to other livestock reared alongside goats.

Keywords: trypanosomiasis; goats; prevalence; Zambia

Introduction

Tsetse transmitted trypanosomiasis is an important disease in Sub-Saharan Africa and has continued to threaten food security (FAO, 2018; Franco et al., 2022). While crop farming is a major economic activity in Zambia, livestock farming is also practiced by a number of small-scale farmers who depend on livestock rearing for their livelihood (Lysholm et al., 2020). Trypanosomiasis in small ruminants has increasingly become important especially with an increase in human encroachment into tsetse and wildlife interface areas (Kebede et al., 2009). For remote rural districts like Mambwe, small ruminants play an economically important role for small scale farmers who are unable to keep large animals such as cattle. Apart from providing meat, milk, manure and skin for famers, goats provide liquid assets and are also a source of household savings. (Kebede et al., 2009; Von Wissmann et al., 2011). The control and management of both human and animal trypanosomiasis through treatment in livestock reservoirs has been evaluated using cattle but has not been explored in small ruminants including goats. Previously goats have been considered to be tolerant to trypanosome infection and that they play a minimum role in the transmission of trypanosomiasis and have thus, not been targeted for control programmes (Hamill et al., 2017). This study was therefore, conducted to investigate *T. brucei, T. congolense* and *T. vivax* infections in village goats using Internal Transcribed Spacer-Polymerase Chain Reaction (ITS-PCR) due to the ability of the test to detect mixed trypanosomes from field samples. Primers ITS1 CF and ITS1 BR, have been evaluated for use in a universal diagnostic test for all pathogenic trypanosomes because of its highly conserved flanking regions and size variability among trypanosomes species and subgroups (Desquesnes et al., 2001; Njiru et al., 2005).

Materials and methods

Using an estimated prevalence of 60% (Ruiz et al., 2015), error margin of 5%, 326 goats were sampled from 193 livestock-owning small-scale farmers of Mambwe District, Eastern Zambia. Livestock farmers were drawn from four villages located about 50km from each other: Nsefu, Katemo, Chikowa and Ncheka. Using a micro-capillary tube, about 200 μL of blood was drawn from each selected animal after puncturing the ear veins of the animals with a blood lancet. From each goat sample, blood spots were applied on Whatman® No. 1 filter paper (GE Healthcare) and air dried before packing in a zip locked storage bag containing silica gel. DNA from stored blood spots was extracted using the buffer technique as described by Morrison et

al. (2007). PCR was undertaken in 25 μL reaction mixtures containing primers ITS1 CF (5'-CCGGAAGTTCACCGATATTG-3') and ITS1 BR (5'-TTGCTGCGTTCTTCAACGAA-3'), One Taq 2X master mix, (New England BioLabs, Ipswich, MA, USA), nuclease free water and 5 μL of extracted DNA sample, all reagents procured from Inqaba Biotec, Pretoria, South Africa (Radwanska et al., 2002; Njiru et al., 2005; Mulenga et al., 2021).

Results and discussion

Demographics of trypanosome infection as recorded from the four study sites were as follows: (Nsefu = 80 sampled, 3 infected; Katemo = 80 sample, 0 infected; Chikowa = 81 sampled, 11 infected, Ncheka = 85 sampled, 1 infected).

The frequency of *Trypanosoma vivax* as detected by ITS-PCR was 4.0% (13/326) and that of *T. congolense* was 0.6% (2/326). No *T. brucei* nor mixed infections were reported in this study. Sampled goats were significantly more infected with *T. vivax* infections than *T. congolense* (t-test = 2.87, *P*-value = 0.04) (Table 1).

Table 1: Proportion of goats sampled in the Luangwa valley, Eastern Zambia with trypanosomes in the year 2019

Trypanosome species	No. positive	Sample prevalence %	Confidence Interval at 95%
T. congolense	2	0.6	-0.2-1.5
T. vivax	13	4.0	1.9-6.1
T. brucei	0	0	0
Mixed	0	0	0
Total	**15**	**4.6**	**2.3-6.8**

Our results indicate that trypanosomiasis is prevalent and widely spread among goat farmers in Mambwe District of the Eastern Province of Zambia. Most livestock farmers in Eastern Zambia, rear goats, and other small ruminants alongside cattle with livestock treatments, exclusively carried out in cattle. This poses a great threat to livestock health and production

(Laohasinnarong et al., 2015). Results obtained in this study showed similar trypanosome infection levels (< 5%) as those obtained by Kebede et al. (2009) and Simukoko et al. (2007)), which were much lower than findings obtained from Nyimba et al. (2015), 23.7%. This may be attributed to difference in sensitivities of the methods used, and trypanosomiasis challenge in the study area. However, the use of ITS-PCR as a universal PCR-based test, adds value to the collection of epidemiological data on trypanosomiasis, while easing the cost of running several PCRs, especially in the endemic zones of Africa (Njiru et al., 2005; Von Wissmann et al., 2011).

Despite our study reporting no cases of *T. brucei* from the goats sampled, indicating that *T. brucei* was not circulating in the goats sampled. Our findings were consistent with observations made by Kebede et al. (2009) and Van den Bossche et al. (2010) but disagreed with findings from other studies were *T. brucei* was found to be highly prevalent (Von Wissmann et al., 2011; Hassan-Kadle et al., 2020). The absence of *T. brucei* in our study may have been attributed to the inability of the sample collection technique i.e., Filter paper, to preserve enough DNA to be detected by PCR. Filter paper, however, inhibit ITS-PCR, making it less accurate compared to when DNA is extracted directly from whole blood samples (Ahmed et al., 2013). The frequency in trypanosome species, *T. congolense* and *T. vivax* distribution were similar with other findings (Kebede et al., 2009; Von Wissmann et al., 2011; Maganga et al., 2020), where goats were found to be highly prevalent in *T. vivax* as compared to cattle which is highly prevalent in *T. congolense* (Hassan-Kadle et al., 2020; Mulenga et al., 2021). In livestock, *T. congolense* and *T. vivax* are the most prevalent under natural infections while *T. brucei* is the least prevalent (Van den Bossche and Delespaux, 2011; Maganga et al., 2020). The frequency of trypanosomes in goats indicates that goats are important reservoirs of trypanosomes that causes AAT and should be considered when undertaking AAT treatment control programmes in livestock.

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References

Ahmed, H.A., Picozzi, K., Welburn, S.C., MacLeod, E.T., 2013. A comparative evaluation of PCR- based methods for species- specific determination of African animal trypanosomes in Ugandan cattle. Parasites & Vectors 6, 316. doi:10.1186/1756-3305-6-316

Desquesnes, M., McLaughlin, G., Zoungrana, A., Davila, 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.

FAO, 2018. World Health Organization and Food and Agriculture Organization step up towards the elimination of human African trypanosomiasis to ensure health and Food Security in Africa. Addis Ababa, Ethiopia.

Franco, J.R., Cecchi, G., Paone, M., Diarra, A., Grout, L., Kadima Ebeja, A., Simarro, P.P., Zhao, W., Argaw, D., 2022. The elimination of human African trypanosomiasis: Achievements in relation to WHO road map targets for 2020. PLOS Neglected Tropical Diseases 16, e0010047. doi:10.1371/journal.pntd.0010047

Hamill, L., Picozzi, K., Fyfe, J., von Wissmann, B., Wastling, S., Wardrop, N., Selby, R., Acup, C.A., Bardosh, K.L., Muhanguzi, D., Kabasa, J.D., Waiswa, C., Welburn, S.C., 2017. Evaluating the impact of targeting livestock for the prevention of human and animal trypanosomiasis, at village level, in districts newly affected with T. b. rhodesiense in Uganda. Infectious Diseases of Poverty 6, 16. doi:10.1186/s40249-016-0224-8

Hassan-Kadle, A.A., Ibrahim, A.M., Nyingilili, H.S., Yusuf, A.A., Rafael, F.C.V., 2020. Parasitological and molecular detection of Trypanosoma spp. in cattle, goats and sheep in Somalia. Parasitology 147, 1786-1791. doi:10.1017/S003118202000178X

Kebede, N., Fetene, T., Animut, A., 2009. Prevalence of Trypanosomosis of small ruminants in Guangua district of Awi Zone, northwestern Ethiopia. Journal of Infection in Developing Countries 3, 245-246. doi:10.3855/jidc.45

Laohasinnarong, D., Goto, Y., Asada, M., Nakao, R., Hayashida, K., Kajino, K., Kawazu, S., Sugimoto, C., Inoue, N., Namangala, B., 2015. Studies of trypanosomiasis in the Luangwa valley, north-eastern Zambia. Parasites and Vectors 8, 555. doi:10.1186/s13071-015-1173-y

Lysholm, S., Johansson Wensman, J., Munyeme, M., Fischer, K., 2020. Perceptions and practices among Zambian sheep and goat traders concerning small ruminant health and disease. PloS one 15, e0233611-e0233611. doi:10.1371/journal.pone.0233611

Maganga, G.D., Boundenga, L., Ologui-Minkue-Edzo, E.J., Kombila, L.B., Mebaley, T.G.N., Kumulungui, B., Mavoungou, J.F., 2020. Frequency and diversity of trypanosomes in sheep and goats from Mongo County in South Gabon, Central Africa. Veterinary World 13, 2502-2507. doi:10.14202/vetworld.2020.2502-2507

Morrison, L.J., McCormack, G., Sweeney, L., Likeufack, A.C., Truc, P., Turner, C.M., Tait, A., MacLeod, A., 2007. Use of multiple displacement amplification to increase the detection and genotyping of trypanosoma species samples immobilized on FTA filters. The American journal of tropical medicine and hygiene 76, 1132-1137.

Mulenga, G.M., Namangala, B., Chilongo, K., Mubamba, C., Hayashida, K., Henning, L., Gummow, B., 2021. Challenges in the Diagnostic Performance of Parasitological and Molecular Tests in the Surveillance of African Trypanosomiasis in Eastern Zambia. Tropical Medicine and Infectious Diseases 6, 68.

Njiru, Z.K., Constantine, C.C., Guya, S., Crowther, J., Kiragu, J.M., Thompson, R.C., Davila, A.M., 2005. The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. Parasitology research 95, 186-192. doi:10.1007/s00436-004-1267-5

Nyimba, P.H., Komba, E.V., Sugimoto, C., Namangala, B., 2015. Prevalence and species distribution of caprine trypanosomosis in Sinazongwe and Kalomo districts of Zambia. Veterinary Parasitology 210, 125-130. doi:10.1016/j.vetpar.2015.04.003

Radwanska, M., Chamekh, M., Vanhamme, L., Claes, F., Magez, S., Magnus, E., de Baetselier, P., BÃ¼scher, P., Pays, E., 2002. The serum resistance-associated gene as a diagnostic tool for the detection of Trypanosoma brucei rhodesiense. The American Journal of Tropical Medicine and Hygiene 67, 684-690. doi:10.4269/ajtmh.2002.67.684

Ruiz, J.P., Nyingilili, H.S., Mbata, G.H., Malele, II, 2015. The role of domestic animals in the epidemiology of human African trypanosomiasis in Ngorongoro conservation area, Tanzania. Parasites & Vectors 510. doi:10.1186/s13071-015-1125-6

Simukoko, H., Marcotty, J., Phiri, I., Geysen, D., Vercruysee, J., Vanden Bossche, P., 2007. The comparative role of cattle, goats and pigs in epidemiology of livestock trypanosomiasis on plateau of eastern Zambia. Veterinary Parasitology, 231-238.

Van den Bossche, P., Delespaux, V., 2011. Options for the control of tsetse-transmitted livestock trypanosomosis. An epidemiological perspective. Veterinary Parasitology 181, 37-42. doi:10.1016/j.vetpar.2011.04.021

Van den Bossche., P., Stephane de La Rocque, Guy Hendrickx, Jeremy Bouyer, 2010. Achanging environment and the epideiomigy of tsetse-transmitted livestock trypanosomiasis. Trends in parasitology, 236-243.

Von Wissmann, B., Machila, N., Picozzi, K., Fevre, E.M., de, C.B.B.M., Handel, I.G., Welburn, S.C., 2011. Factors associated with acquisition of human infective and animal infective trypanosome infections in domestic livestock in Western Kenya. PLoS Neglected Tropical Diseases 5, e941. doi:10.1371/journal.pntd.0000941