UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

DEPARTMENT OF PUBLIC HEALTH

TRYPANOCIDE USAGE AND ASSOCIATED FACTORS AMONG CATTLE FARMERS IN ITEZHI TEZHI, ZAMBIA

By

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A Dissertation submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in Epidemiology

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MAY, 2014

DECLARATION

I, **Njelembo Joshua Mbewe**, hereby declare that this thesis, submitted by me to the University of Zambia for the degree of Master of Science in Epidemiology has not been submitted at any other University.

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CERTIFICATE OF APPROVAL

This dissertation of **Njelembo Joshua Mbewe** has been approved as fulfilling part of the requirements for the award of Master of Science in Epidemiology by the University of Zambia

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Examiner I.....Sign.....Date.....

Examiner II.....Sign.....Date.....

Examiner III.....Sign.....Date.....

ABSTRACT

Background: The trypanocides isometamidium (ISM) and diminazene aceturate (DA) play an important role in the control of tsetse transmitted African animal trypanosomiasis (AAT) now and in the near future. The drugs are mostly administered by farmers without any veterinary supervision leading to misuse and under dosing of medication, factors that promote trypanocidal drug resistance (TDR) development. Given the devastating nature of AAT, affecting rural development, livestock and human health, the Food Agriculture Organisation (FAO) recommended guideline on trypanocide use to delay TDR development. However factors associated with trypanocide usage among farmers with regard to the FAO guidelines remain under reported in literature. Against this background a survey was undertaken to determine the factors which were associated with the predominant trypanocide usage practice among farmers with reference to the FAO guidelines in Itezhi tezhi district, central Zambia.

Methodology: A questionnaire was used to collect socio-economic data and trypanocide usage practices from 90 farmers who used trypanocides in Itezhi tezhi. The animal handling facilities (AHFs) which the farmers use were stratified according to location, whether in the game management area (GMA) or non-GMA. At each AHF, blood samples from 613 randomly selected cattle were collected to determine trypanosome species prevalence, AAT status and DA resistance in *Trypanosoma congolense* isolates. All trypanosome positive samples upon microscopic examination of thick and thin blood smears and those with packed cell volumes (PCV) of 26% and less were subjected to polymerase chain reaction (PCR) for trypanosome isolation, identification and DA resistance testing.

Results: The results showed that only 25.6% of the farmers adhered to guidelines by FAO on trypanocide use. From a total of 60 samples that were subjected to PCR 23% (14/60) were *T.congolense* savannah type positive, 3% (2/60) were *T.vivax* and 2% (1/60) had mixed infection. Seven percent (4/60) had the non-pathogenic trypanosome *T.theileria* and the rest of the 65% (39/60) were negative for trypanosomes. None of the 14 *T. congolense* isolates showed any genes associated with DA resistance. Further none of the socio-economic factors under investigation were significantly associated with trypanocide usage. However location of and AAT status at the AHF as environment factors were significantly associated with adherence to FAO guidelines on trypanocide use ($P<0.05$).

Conclusion: DA resistance is not a problem in Itezhi tezhi despite the high levels of misuse of trypanocides suggesting a complex relationship between trypanocide usage and TDR development which requires further investigations. Additionally *T.congolense* accounted for the majority

trypanosomal infection. Furthermore location and AAT status of the AHF were associated with adherence to FAO guidelines on trypanocide use among cattle farmers in Itezhi tezhi. We therefore recommend farmer education on DA use as well as the use of an integrated approach to control AAT especially in the GMA.

DEDICATION

This dissertation is dedicated to my wife Matildah Mulenga, my son Dalitso W. Mbewe and my parents for their understanding, love, support and encouragement during the period of study.

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LIST OF ACRONYMES AND ABREVIATIONS

AAT	African Animal Trypanosomiasis
AHF	Animal Handling Facility
DA	Diminazene Aceturate
FAO	Food and Agricultural Organisation
FMD	Foot and Mouth Disease
GIS	Geographical Information System
GMA	Game Management Area
HAT	Human African Trypanosomiasis
ISM	Isometamidium
NTDs	Neglected Tropical Diseases
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction – Restriction Fragment Length Polymorphism
PCV	Packed Cell Volume
SAT	Sequential Aerosol Technique
SIT	Sterile Insect Technique
TAE	Tris/Acetic Acid/EDTA
TBE	Tris/Boric Acid/EDTA
TDR	Trypanocidal Drug Resistance
VSG	Variable Surface Glycoprotein
WHO	World Health Organisation

1.0 CHAPTER ONE: BACKGROUND

1.1 Introduction

African trypanosomiasis is a disease caused by unicellular parasites called trypanosomes (Protozoa, Kinetoplastida) and transmitted by arthropod vectors, the tsetse flies (*Glossina spp.)*, affecting humans, livestock and wildlife. The disease is endemic in 37 African countries south of the Sahara between latitude 14°N and 29°S following the distribution of the tsetse fly (Figure 1). Human African trypanosomiasis, HAT, also referred to as sleeping sickness is a deadly disease caused by two subspecies of *Trypanosma brucei* namely *T.b.gambiense* causing the chronic form common in West Africa while *T.b. rhodesiense* causes the acute form common in East and Southern Africa. HAT is among the Neglected Tropical Diseases (NTDs) and due to the uncertainties between reported cases and actual field situation, from the 30,000 reported cases, it is estimated that 300,000 infected individuals remain ignored in the field (Hotez and Kamath, 2009).

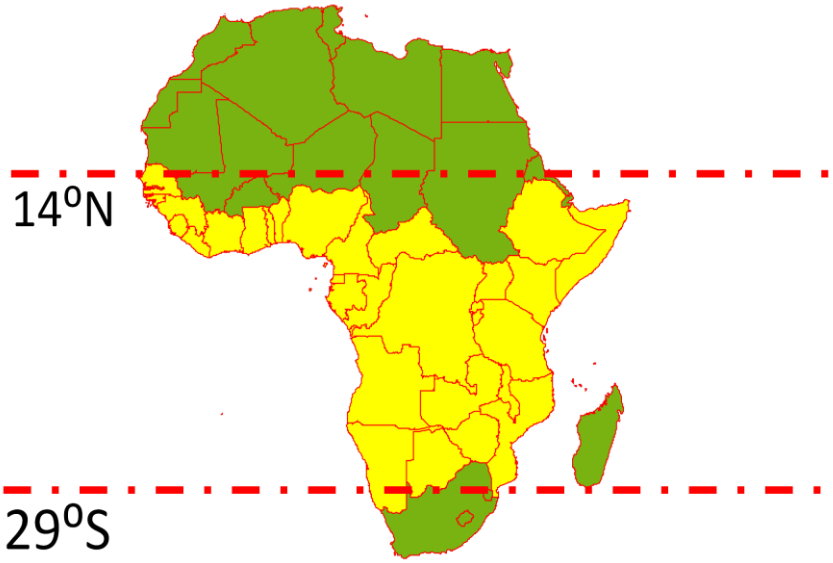

Figure 1: Map showing the distribution of the tsetse fly in Africa adopted from FAO (FAO., 1992)

Animal African trypanosomiasis, AAT, also known as nagana is a disease complex caused by *T. brucei brucei*, *T. conglense* and *T. vivax* in cattle and other livestock and *T. simiae* in pigs. AAT is characterized by a slow progressive loss of condition accompanied by increasing weakness leading to extreme emaciation, collapse and death (FAO., 1998). It is estimated that 50 to 70 million animals are at risk of AAT (Holmes et al., 2004).

The tsetse fly is the main determinant of the distribution of AAT and the only primary vector that has the competence of transmitting trypanosomes cyclically (FAO., 1992). A total of thirty one species and subspecies of tsetse have so far been identified. The species are divided into three subgenera which include the morsitans, palpalis and fusca groups based on morphological features of the adult genitalia (FAO., 1992). This classification was confirmed by comparative gene sequence analysis and geometric wing morphometry (Leak et al., 2008).

AAT represents a severe constraint to the development of the infested areas and causes losses in the agricultural sector estimated at about US$1.3 billion annually in sub Saharan Africa (Maitima et al., 2007). Control of AAT previously focussed on the suppression of the abundance and distribution of the vector but the gains attained through these control efforts were short-lived due to resurgence of the fly population and disease prevalence (Maitima et al., 2007). As a result, trypanocides which are drugs that kill trypanosomes play a vital role in the control of AAT. For over 35 years now, the trypanocides Isometamidium and Diminazene salts are among the three commonly used trypanocides in Africa for chemo-therapeutic and prophylactic treatment of AAT (FAO., 1998, Geerts et al., 2001).

These trypanocides are mostly administered by farmers without any veterinary advice leading to misuse and under dosage of the medications, practices that have been considered as major factors in development of trypanocidal drug resistance (TDR) (Chitanga et al., 2011, Onono et al., 2013). Drug resistance is the heritable loss of sensitivity of a micro-organism to an antimicrobial chemical agent to which it was sensitive to before (FAO., 1998).

Trypanosoma vivax, T. congolense and *T.brucei brucei* are responsible for AAT in Zambia. *T. brucei rhodesiense* is not known to cause AAT, but animals act as its reservoir and therefore it is zoonotic. These trypanosomes are transmitted by four species of tsetse flies which include *Glossina morsitans spp, G.pallidipes, G.fuscipes* and *G.brevipalpis.* About 40% of the arable land in Zambia is infested with tsetse flies forming fly belts in and around game parks. In some cases these fly belts merge from one game park to another. Geographical information system, GIS, which is a computer based information system that can capture, manage, manipulate, analyse and present geographically referenced data was used to produce figure 2, which shows the various fly belts. Geometric wing morphomentric analysis has demonstrated that the tsetse population structures from the same species within these fly belts differ in some cases (Mbewe et al., 2013).

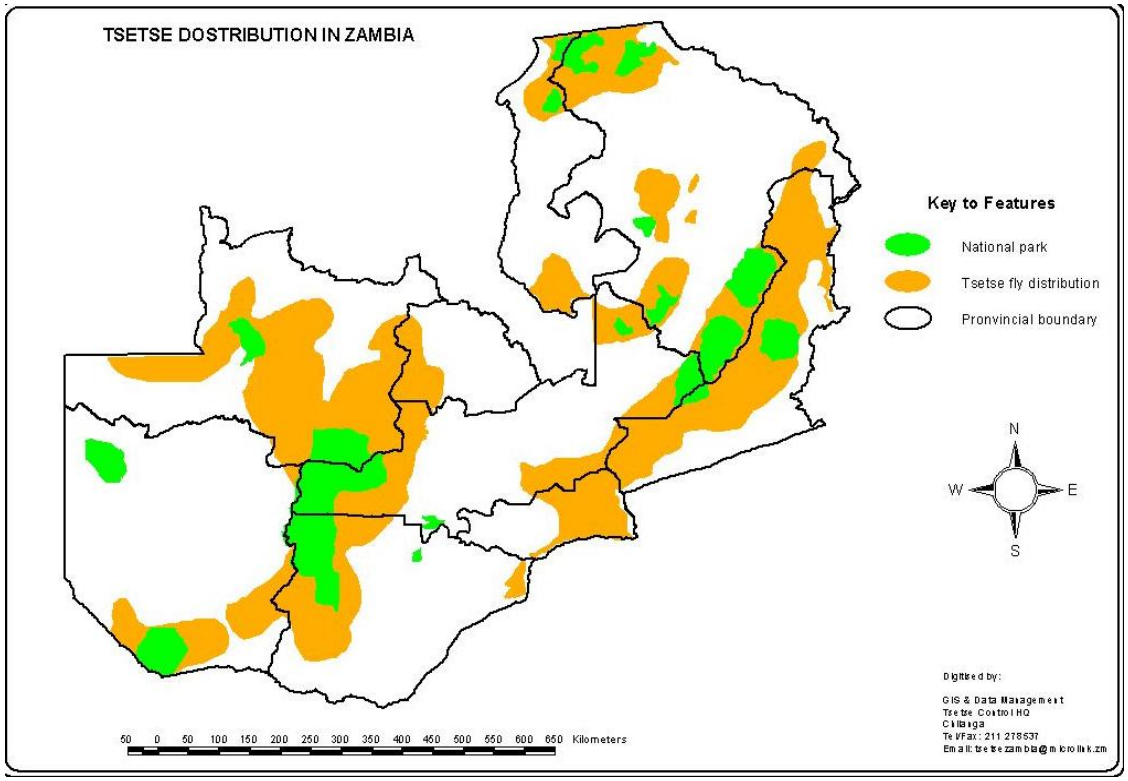

Figure 2: Tsetse fly Distribution in Zambia (TTCS, 2009)

1.2 Historical review

David Livingstone (1813-1875) a Scottish missionary and explorer was the first to suggested that nagana was caused by the bite of a Tsetse fly (Steverding, 2008) . About 40 to 50 years later, trypanosomes were identified as the causative agents of nagana and sleeping sickness. It was a Scottish pathologist and microbiologist, David Bruce (1855-1931) in 1895 who discovered that *T.brucei* was the cause of cattle trypanosomiasis (Steverding, 2008). A German military surgeon Friedrich Karl Kleine (1869-1951) in 1909 demonstrated the cyclic transmission of *T.brucei* in tsetse flies (Steverding, 2008).

By 1904, Paul Ehrlich (1854-1915), another German scientist had become interested in the chemotherapy of trypanosomiasis and had developed the dye, trypan red, which proved to be both curative and prophylactic for *Trypanosoma equinum* (causes Mal de Caderas in horses of Central and South America) in mice but not for *T. brucei* (Vickerman, 1997). From the 1950s, several drugs have become available for chemotherapy of animal trypanosomiasis. These include phenanthridine derivatives homidium bromide and isometamidium chloride (ISM), the amino-quinaldine derivative quinapyramine and aromatic diamidine diminazene aceturate (DA) (Kinabo, 1993). However trypanocidal drug resistance development to all the chemotherapeutic agents mentioned above has since been reported (Delespaux and de Koning, 2007).

1.3 Scientific review

The presence of trypanosomes, tsetse flies and hosts (both reservoir and definitive) in the same physical environment are important in sustaining the prevalence of African trypanosomiasis.

1.3.1 African Trypanosomes

The causative agents of African trypanosomiasis (HAT and AAT) belong to the following taxonomic classification (http://www.vet.uga.edu/vpp/gray_book/Handheld/aat.htm):

Kingdom: Protista

Subkingdom: Protozoa

Phylum: Sarcomastigophora

Class: Zoomastigophora

Order: Kinetoplastida

Family: Trypanosomtidae

Section: Salivaria

Genus: *Trypanosoma*

Subgenus: Nannomonas (*T.congolense),* Duttonella (*T. vivax)* and Trypanozoon *(T.brucei spp)*

African Trypanosomes that complete their life cycle by passing through the tsetse fly and vertebrate hosts are referred to as salivarian. Salivarian trypanosomes for example, *T. congolense, T.vivax* and *T. brucei* are transmitted through the oral route by the bite of tsetse flies. The tsetse fly transmits the infective metacyclic trypanosomes (figure 3). In the vertebrate host, the metacyclic blood form trypanosomes convert between long slender and stumpy form trypomastigotes (Peacock et al., 2012). The blood stream trypomastigotes multiply by binary fission in various body fluids which include blood, lymph and spinal fluid. The parasitemia fluctuates owing to the evasion of the host immune response by antigenic variation. The variable Surface Glycoprotein (VSG) is an important feature of pathogenic trypanosomes which helps them evade the hosts' immune response. The VSG forms a dense coat on the trypanosome surface. When the host mounts an effective immune response against the trypanosomes with a specific VSG coat, removing these but not other trypanosomes that have changed to a new VSG coat that is temporarily unrecognisable. It is these variants that form the next wave of infection. This

phenomenon of antigenic variation of the surface protein coat is unique to trypanosomes and is the basis of the pathological feature of intermittent parasitemia, fluctuating fever, disease chronicity, long, largely asymptomatic disease and failure by the host to develop effective post infection immunity (FAO., 2002). When the tsetse fly ingests blood containing both the slender and stumpy forms of trypanosomes, in its midgut the stumpy forms convert to procyclic stage. It is the procyclics that invade the ectoperitrophic space at the end of the peritrophic membrane near the hind gut where they continue dividing before migrating to anterior to colonize the proventriculus where they seize division (Peacock et al., 2012). Development of trypanosomes of subgenus Duttonella to produce infective metacyclic trypomastigotes is restricted to the proboscis of the fly. Development of the subgenus Nannomonas occurs in the midgut and proboscis while development of the Trypanozoon subgenus takes place in the mid gut and salivary glands (Peacock et al., 2012).

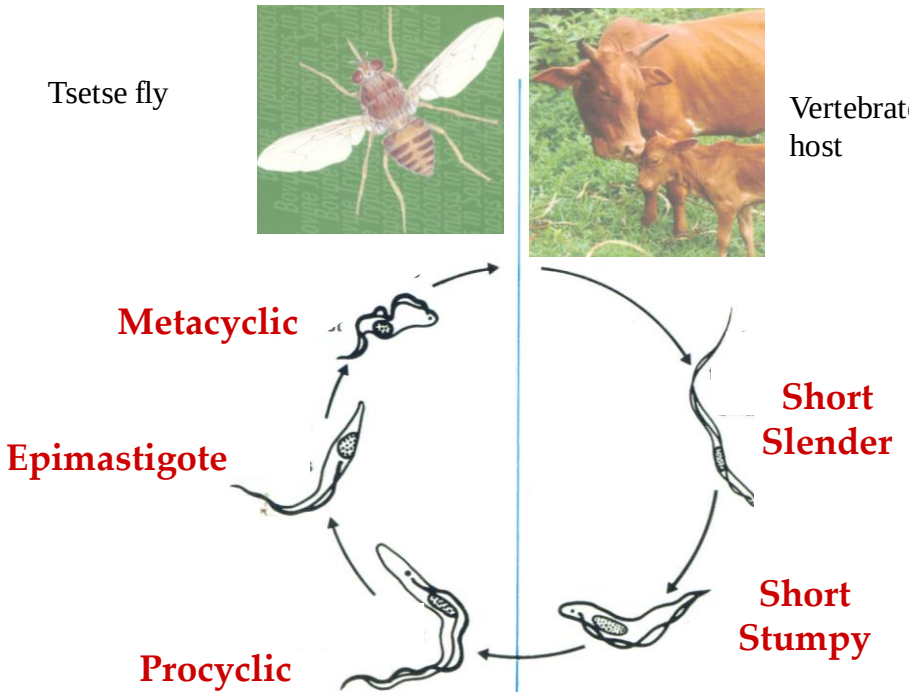

Figure 3: Trypanosoma life cycle stages adapted from Peacock et. al (Peacock et al., 2012)

Progression of HAT is characterized by two distinct stages. The first or early stage of the disease known as the haemolyphatic phase is characterized by the restriction of trypanosomes to the blood and lymph system (Fe`vre et al., 2008). The symptoms of this stage include fever, headaches, joint pains and itching. The second or late stage known as the neurological phase is defined by the presence of the trypanosomes in the cerebrospinal fluid (Fe`vre et al., 2008). At this stage the typical signs of the disease occur and they include confusion, disturbed sleep pattern, sensory disturbances, extreme lethargy, poor condition and comma. Without any treatment patients die within months when infected *T.b. rhodesiense* or within years when infected with *T.b.gambiense.*

The pathology of AAT can be categorized into haematic (*T.vivax, T. congolense)* and humoral (*T. brucei)* with the former associated with anaemia and the latter with tissue degeneration and inflammation. However it is often difficult to clinically differentiate the disease caused by different trypanosomes species and mixed infections are common (FAO., 2002). The majority of AAT infections are due to *T. congolense* (Simukoko et al., 2007). The severity of AAT in cattle varies from acute to chronic with the latter being more common in endemic areas. The signs of AAT typically present as a combination of fever, anaemia, lymphadenopathy, dull and dirty coat, piloerection, change of hair colour, hair loss, weight loss (figure 4), lacrimation, chancre, fatigue, anorexia, pica, abortion, salivation, nasal discharge, arched back, laboured respiration and jugular pulse (FAO., 2002).

Figure 4: An oxen suffering from AAT

1.3.2 The Tsetse fly

There are two features that distinguish Tsetse flies (*Glossina)* from other Diptera. The first feature is the discal cell of the wing which is like a cleaver and referred to as the hatchet cell. The second feature is the presence of secondary branches in the hairs of the arista of the antenna (FAO., 1992).

Tsetse flies primarily feed on blood and in the act of piercing the skin and drawing blood, the flies pass on the blood parasite *trypanosoma*. Therefore, they are the main determinant of the distribution of African trypanosomiasis. When the fly feeds, the blood is prevented from clotting by the anticoagulant that is contained in the fly's saliva. This helps the trypanosomes to stay alive

throughout the digestive process and later develop in mature infective metacyclics in the tsetse fly (FAO., 1992).

Tsetse females give birth to live offspring which is unusual for insects and mortality is low. Their longevity, mobility and frequent feeding enable tsetse flies to be highly efficient vectors (Hargrove, 2003). However their low rate of population growth exposes them to population decline and even extinction with small increases in mortality.

1.3.3 The Trypanosome vertebrate host

Trypanosomes have wide range of vertebrate hosts including reptiles, birds, amphibian, fish and mammals (Hamilton et al., 2004). Among the mammals, some serve as reservoirs while others serve as definitive host. In reservoir hosts, usually wild animals, African trypanosomes become established but do not cause any disease because both the trypanosome and host have evolved for many years that a balanced relationship exits (Aksoy et al., 2003, Namangala, 2011) The reservoir hosts play a very important role in the four epidemiological situations of transmission of African trypanosomiasis which include the sylvatic, sylvatic/domestic, domestic and sylvatic cycle at the game/livestock interface shown in figure 5 (Van den Bossche, 2001).

In the definitive hosts which are usually humans and their domesticated animal, trypanosomes cause disease because the host/parasite relationship has not developed fully. Wild and sometimes domestic animals usually play a major role as parasite reservoirs for human infections with trypanosomes (http://www.vet.uga.edu/vpp/gray_book/Handheld/aat.htm, Van den Bossche, 2001).

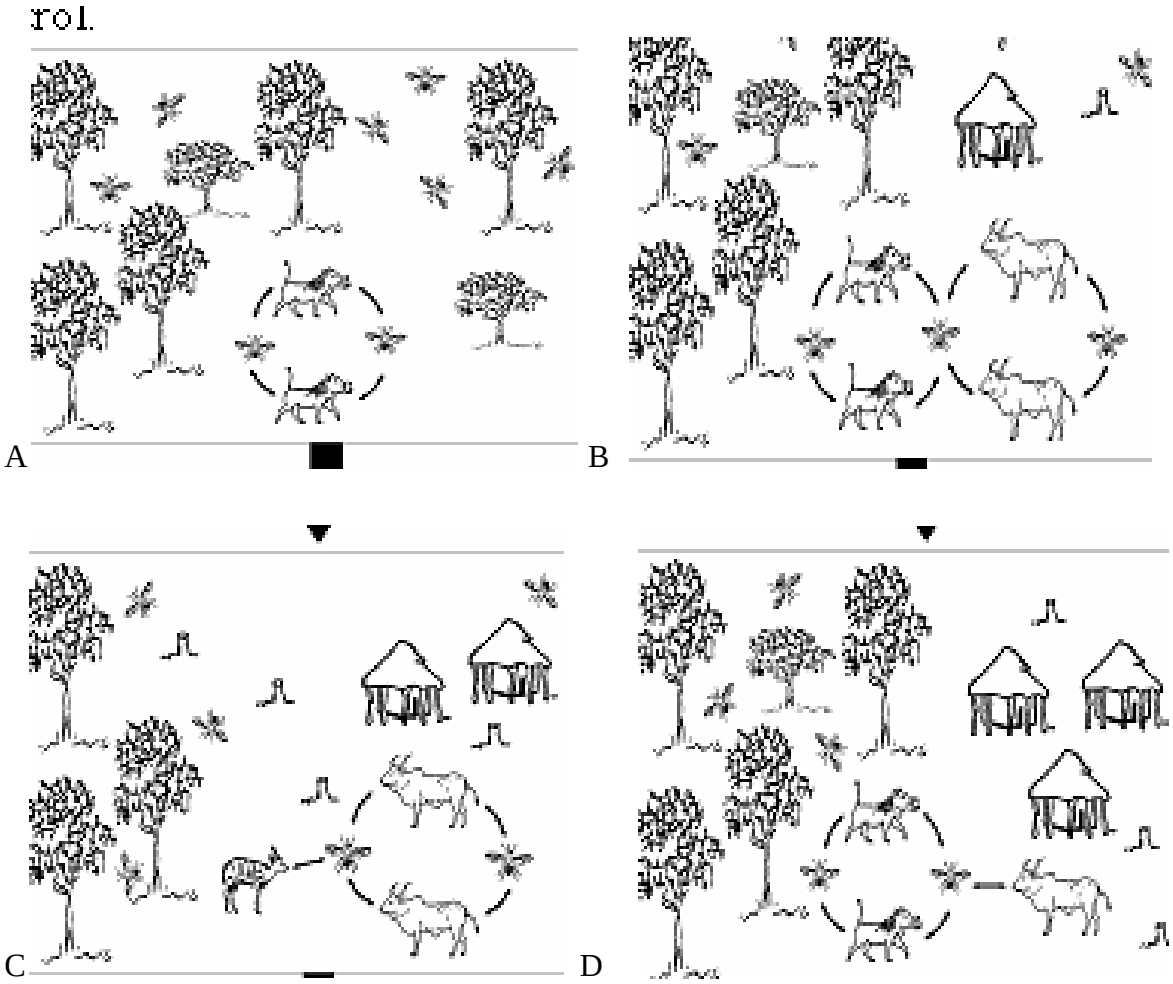

Figure 5: Adopted from Van den Bossche (Van den Bossche, 2001) showing the four epidemiological situations in the transmission of AAT. (A) Sylvatic cycle; transmission of occurs between wild game and tsetse flies (B) Sylvatic/Domestic cycle; transmission occurs among tsetse, wild game and domestic livestock (C) Domestic cycle; transmission occurs between tsetse and domestic livestock and (D) Sylvatic cycle at the game/livestock interface.

1.3.4 Diagnosis of AAT

Diagnosis of AAT is important in the management of the disease. A number of diagnostic tests are available for the detection of trypanosomes. Direct parasitological diagnostic test depend on seeing the trypanosomes in wet blood films, blood smears or buffy coat preparations with the aid of a microscope (http://www.vet.uga.edu/vpp/gray_book/Handheld/aat.htm). However these methods are not the most sensitive and a proportion of infections are usually missed (especially where there is low parasitemia) but as long as an adequate sample of animals is examined, the diagnosis of AAT can be reached (OIE, 2013).

Indirect diagnostic methods such as the measure of Anaemia status using Packed Cell Volume (PCV) can be a reliable indicator of AAT in the absence of other anaemia causing factors such as age of the animals and other parasites. Therefore determining the PCV value can give a reliable indication of disease status of a trypanosome infected animal (Marcotty et al., 2008). The PCV is the length of the packed red blood cell column expressed as a percentage of the total volume of blood in a heparinised haematocrit capillary tube after centrifugation. In fact determination of PCV value as a diagnosis tool of AAT has been shown to have higher sensitivity and specificity compared to parasitological diagnosis such as buffy coat techniques, wet mounts and dry smears (Marcotty et al., 2008).

The most sensitive and specific diagnosis of AAT is the use of molecular based techniques like polymerase chain reaction (PCR) and Loop-mediated isothermal amplification (LAMP) that amplify parasite DNA. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) being one of these techniques is more costly than parasitological and PCV because it requires sophisticated equipment and materials. This limits its use in field veterinary laboratories in AAT endemic areas. Nevertheless PCR-RFLP permits identification of parasites at levels far below the detection of the parasitological techniques. A semi-nested qualitative PCR described by Geysen et al. (2003) was able to detect 10 animals positive for trypanosomes from 100 buffy coat samples which were previously found negative using parasitological examination. This semi-nested PCR is able to detect one trypanosome genome/ 40 microlitres of blood applied as buffy coat on filter paper (Geysen et al., 2003).

Other methods of diagnosis of AAT include serological tests like indirect fluorescent antibody test (IFAT) and antibody –detection enzyme-linked immunosorbent assay (ELISA) (OIE, 2013).

1.3.5 Control of AAT

1.3.5.1 Vector Control

Modern strategies aimed at vector control including the use of sterile insect technique (SIT) and insecticides have been very effective. SIT was used to eradicate tsetse populations in Zanzibar and suppress tsetse populations in Burkina Faso, Tanzania and Nigeria. AAT has not recurred in Zanzibar since 1997(Abd-Alla et al., 2011).

Tsetse flies are susceptible to insecticides because of their low genetic variation. Of late insecticides such as synthetic pyrethroids have been used on bait animals; odour baited traps and targets as well as applied in tsetse environments using ground spraying (e.g Zimbabwe) and

Sequential aerosol technique (SAT) (e.g Botswana, Namibia, Angola and Zambia) for control of tsetse flies. SAT has successfully been implemented in 2009 spray block in western Zambia and since 2009 no fly catches and AAT has been recorded (Chilongo, 2013).

1.3.5.2 Control of AAT using Trypanocides

Trypanocides are chemicals that kill trypanosomes and play a vital role in control of the AAT in areas where there is no vector targeted control (Geerts et al., 2001). It is estimated that 35 million doses of these drugs are administered in Africa each year (Geerts et al., 2001). Isometamidium salts (ISM) have both curative and prophylactic properties while diminazene salts only have curative properties of AAT (FAO., 1983). Diminazene aceturate (DA) is the most widely used trypanocidal drug (Vitouley et al., 2011). Curative treatments are mainly administered where disease incidence is low and only limited number of animals in a herd contract the disease (FAO., 2002). Prophylactic drugs are used mainly where the risk is so high that the health of the herds cannot be maintained by administration of curative compounds and challenge or trypanosome risk is important when considering the use of prophylactic drugs (FAO., 2002). Prophylactic drugs tend to persist longer in the host than curative ones thereby protecting the host from re-infections. Concentrations of chemoprophylactic drugs in the body fluids of the host should ideally not drop below the curative level so that the establishment of trypanosomes and development of AAT are prevented at all times as illustrated in figure 6 (FAO., 2002). The intervals between treatments are equal so that the concentration of the drug in the plasma is never permitted to drop below a level which is ineffective in controlling the parasite. The recommended doses of Isometamidium salts is 0.5mg/kg and 1mg/kg body weight for curative and prophylaxis respectively while that of DA is 3.5mg/Kg body weight (FAO., 1983). ISM is commonly presented in powder form in sachets containing 8 standard doses for cattle weighing approximately 250 kilograms. The injectable solution is prepared by dissolving the whole content of the sachet in 50 or 100 millilitres of sterile water to prepare a 2 or 1 percent solution respectively. DA is commonly presented in powder form in sachets containing a single dose for cattle weighing approximately 250 kilograms but also ready to use preparations are also available. Preparation of the injectable solution from powder is done by dissolving whole contents of a sachet in 12.5 millilitres of sterile water to make a 7 percent solution. For both ISM and DA, boiled then cooled water as well as distilled water can be used when sterile water for injection is not available (FAO., 1983).

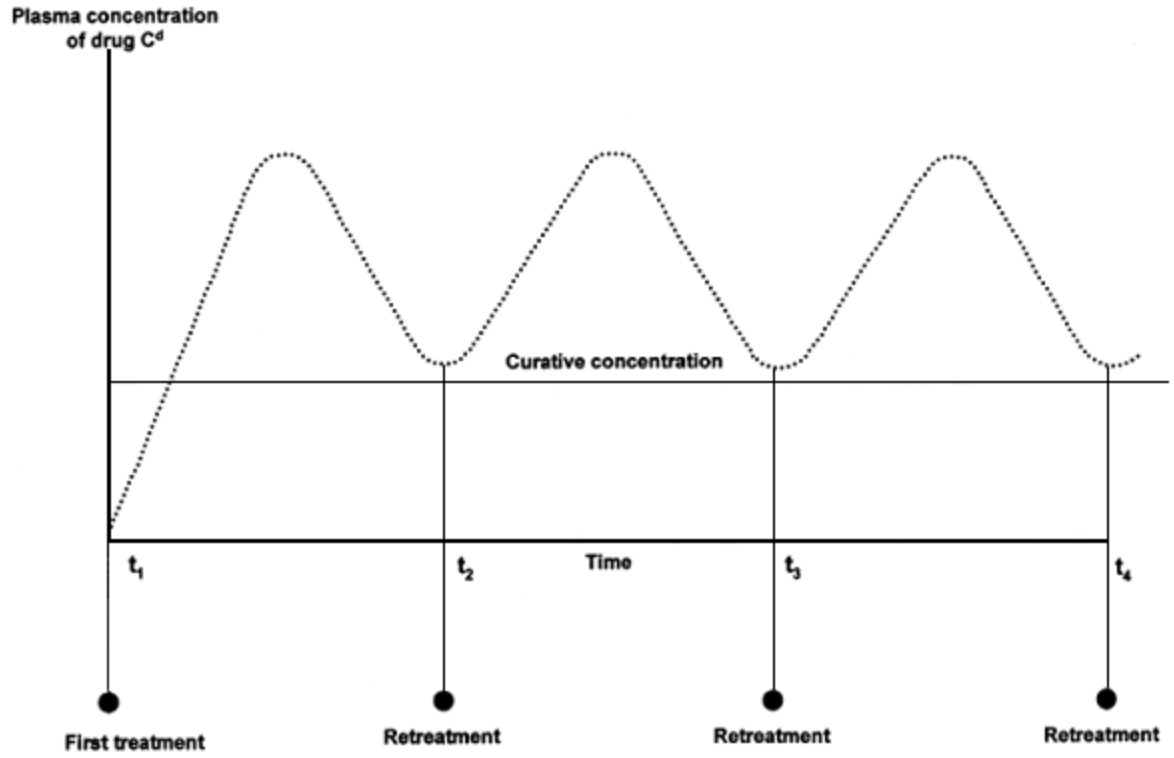

Figure 6: Diagrammatic representation of a prophylactic situation. The aim should be to maintain the concentration of the drug above subcurative level by means of a series of treatments at planned regular intervals.

1.3.5.4 Trypanocidal drug resistance

Generally, it has been argued that due to the privatization of veterinary services, farmers have had easy access to these trypanocides and this has tended to give rise to rampant misuse and under-dosage of the medications, practices that have been considered as major factors in development of trypanocidal drug resistance (Chitanga et al., 2011, Onono et al., 2013).

It has been documented that the repeated use of pesticides or chemotherapeutic agents has shown to inevitably lead to the development of drug resistance in target organisms within 10 years of the introduction of the antimicrobials, insecticides, trypanocides and anthelmintics to the market (FAO., 1998, Waller, 1994).However the major factor identified as having an influence on development of trypanocide resistance is the nature of drug use practice (FAO., 1998). With the World Health Organisation (WHO) advocating on focusing on the causes of causes (http://www.who.int/social_determinants/en) and in an attempt to delay development of trypanocide resistance the Food Agricultural Organisation, FAO, (FAO., 2002, FAO., 1998) has recommended guidelines on trypanocide usage practice as follows

i. Avoiding under-dosing which results from incorrect weight estimation, using the wrong amount of drug when making up the injectable solution or when the drug has not completely dissolved during preparation.
ii. Reducing the frequency of application of trypanocides by integrating their use with other control methods such as vector control and using insecticide treated cattle. Prophylactic treatment should be limited to a maximum of 4 treatments in a year (Dia and Desquesnes, 2005).
iii. Limiting curative treatments to individual sick animals as systematic mass treatments exert a strong selection pressure for resistance.
iv. Ban on the use of quinapyramine in cattle as it promotes cross resistance

It is assumed that adherence by trypanocide users to these recommended trypanocide usage practices would reduce selection pressure of the drug resistant trypanosomes and thereby delaying development of trypanocidal drug resistance.

Trypanocidal drug resistance is more likely to develop in environments which provide conditions where susceptible host and infectious vector can come together for long enough periods for transmission to occur. With very high tsetse challenge, transmission of AAT is also high leading to increase in prevalence of AAT (Delespaux and de Koning, 2007). High prevalence of AAT leads to high usage of trypanocides (Van den Bossche et al., 2000). This increases selection pressure and the likelihood of animals becoming infected with drug resistant trypanosomes (Geerts et al., 2001). The drug pressure then encourages such strains to establish themselves and spread rapidly (Chitanga et al., 2011).

1.3.5.6 Diagnosis of trypanocidal drug resistance

There are different techniques used for diagnosis of trypanocidal drug resistance which were reviewed by Delespaux et al. 2008 (Delespaux et al., 2008). The commonly used field techniques include block treatments and in vivo tests which present drawbacks such as problems associated with growth of some *T. congolense* strains in rodents, the long period of follow up (2 months) and the intensive use of experimental animals (Vitouley et al., 2011). In order to address these drawbacks, the Department of Animal Health of the institute of Tropical Medicine Antwerp developed a *Bcl*I-PCR_RFLP for molecular diagnosis of DA resistance in *T. congolense* in the laboratory. It is a qualitative PCR and the test is based on the detection of a single point mutation in P2-type purine transporters even though of late there has been contrary view on function of the transporter (Munday and al., 2013). It was demonstrated that correlation of the PCR-RFLP with

the single dose mouse test which is the gold standard was excellent even if the PCR-RFLP was found to be more sensitive than the single dose mouse test (Vitouley et al., 2011) . This test was further adapted for use under field conditions by adding a step of whole genome amplification using the QIAGEN REPLI-g UltraFast Mini Kit and its specificity improved by replacing the *Bcl*I (T^GATCA) enzyme with *Dpn*II (^GATC) (Vitouley et al., 2011) . In addition internal negative and positive controls are added to make certain of the absence of contamination, an effective DNA amplification and complete digestion of PCR products (Vitouley et al., 2011). The sample collection, storage and transfer are facilitated by collecting blood spots or buffy coats on filter paper (Vitouley et al., 2011).

2.0 CHAPTER TWO: RESEARCH FOCUS

2.1 Statement of the Problem

Trypanocide usage has been documented as a major factor contributing to trypanocidal drug resistance development (Chitanga et al., 2011). Despite the high usage of these trypanocides, the interest of pharmaceutical industries to invest in research for developing new products has remained low, leaving only a limited number of these existing drugs on the market (Chitanga et al., 2011, Geerts et al., 2001). With the complex debilitating and often fatal nature of AAT, emergence of trypanocidal drug resistance would have a devastating impact by contributing to loss of income and investments, food insecurity and poverty, poor human health and deterred rural development through loss of livestock (FAO., 1998) . There would also be reduction in livestock products (such as meat, milk, hides and manure) and reduced availability of draft power (FAO., 1998). The above-mentioned impacts negatively affect the socio economic status of the farmers. Socio economic status is documented as one of the fundamental determinants of human health (Link and Phelan, 1995). Trypanocide usage is therefore a matter of public health concern as it plays an important role in TDR development, which negatively affects the food production of the farmer and this has a trickledown effect on consumers, by threatening food security in the short term. In the long term TDR development negatively affects the socio economic status of the farmer and his dependants.

Additionally most studies undertaken in Zambia on trypanocidal drug resistance have only focused on trypanosomes leaving out trypanocide usage among cattle farmers. Studies that have mainly focused on the causative agent carried out in Eastern Zambia show a fivefold increase in DA resistance from 12.6% in 1996 to 63.2% in 2003 while Isometamidium chloride resistance was 34% in 1996 in *Trypanosoma congolense* isolates (Delespaux et al., 2008, Sinyangwe et al., 2004). Further, a high prevalence of the resistant genotype of the natural isolates of *T. congolense* with no history of trypanocide exposure was observed from wild animals in the Luangwa national park (Chitanga et al., 2011). Furthermore, studies that have documented trypanocide usage have only quantified and monitored it without determining the associated factors (Delespaux et al., 2002, Van den Bossche et al., 2000). With this scenario, no study has been undertaken that documents both trypanocide usage and associated factors in Zambia. Therefore Itezhi tezhi being one of the districts where trypanocides are widely used (TCES, 2009, TCES, 2010) was suitable to undertake the study. Additionally prevalence of trypanocide resistance development of ISM and DA has not been documented in Itezhi tezhi despite trypanocides having been in use for over 20 years (TCES, 2009).

Elsewhere literature documents environmental and socio economic factors to be critically linked to drug usage practices (FAO., 2002, Geerts et al., 2001, Mugabi et al., 2010, Mugisha et al., 2008) . Nevertheless these are yet to be documented in Itezhi tezhi district. The environmental factors of interest include AAT status at reference points (animal handling facilities) and location of the animal handling facility whether in the game management area (GMA) or not representing proximity to the Kafue National Park which is the source of the vector. The socio economic factors of interest include age, Education (number of school years), sex, availability of extension services on trypanocide use and self rated competence on trypanocide use of the farmers.

In addition, information on frequency of prophylaxis treatments and limiting of curative treatments to sick animals in relation the recommended guideline by FAO is lacking for the cattle farmers in Itezhi tezhi. Furthermore, there is no information on the adherence levels to the FAO recommended guidelines on trypanocide usage practices in Itezhi tezhi.

2.2 Study Justification

Improper use of veterinary drugs wastes resources, occasion avoidable animal sickness and death, mask poor production and promote drug resistance leading to exacerbated disease in animals and humans (FAO., 2002). Given that isometamidium salts and DA remain a vital remedy for AAT control in Itezhi tezhi district of Zambia, it is important that these drugs remain effective for as long as possible. With trypanocide usage being the major contributor to TDR development, it was essential to know which environmental and socio economic factors are associated with it in reference to adherence to the FAO recommended guidelines among cattle farmers in Itezhi tezhi. Therefore this study generated new knowledge on which specific environmental and socio economic factors were associated with adherence to the recommended FAO guidelines on trypanocide usages practices among the cattle farmers in Itezhi tezhi.

This study also provided information on the current trypanocide usage practices among the cattle farmers in Itezhi tezhi. The information on the current trypanocide usage practices among the cattle farmers in Itezhi tezhi would provide an opportunity for the District veterinary office to evaluate the extension package on trypanocide usage practices for the cattle farmers so that they could plan for and design suitable extension messages. With DA being documented as the most widely used trypanocide (Vitouley et al., 2011), its resistance was determined in order to ascertain the levels of TDR prevalence to this curative drug.

The cattle farmers would eventually benefit from the extension messages that will be designed by the District veterinary office in a manner that addresses their needs on trypanocide usage practice

and thus delay development of TDR and its effects. Further, the District Veterinary Office and the cattle farmers benefitted from the diagnosis of AAT and knowing the prevalence of DA resistance from the study.

2.3 Research Question and Objective

2.3.1 Research Question

What factors are associated with Trypanocide usage among cattle farmers in Itezhi tezhi?

2.3.2 Overall objective

To determine the factors associated with Trypanocide usage among cattle farmers in Itezhi tezhi

2.3.3 Specific objectives

1. To determine the proportion of cattle farmers in Itezhi tezhi that adhered to the recommended guideline by FAO on trypanocide usage practices.
2. To establish the prevalence of trypanosome species in cattle with PCV of less than or equal to 26%.
3. To assess the prevalence of Diminazene Aceturate resistance in the *T. congolense* found in Itezhi tezhi.
4. To examine the socio economic factors that may be associated with trypanocide usage that are predominant in Itezhi tezhi
5. To assess how the location of an animal handling facility whether in the GMA or not as an environmental factor was associated with trypanocide usage practices in Itezhi tezhi.

2.4 Conceptual Framework

In seeking to answer the specific objectives, a theoretical framework by FAO (FAO., 1998) (figure 7) on the important factors influencing the development of resistance to trypanocidal drugs was modified into the conceptual framework in figure 8 which indicates how the various factors can influence trypanocide usage practices.

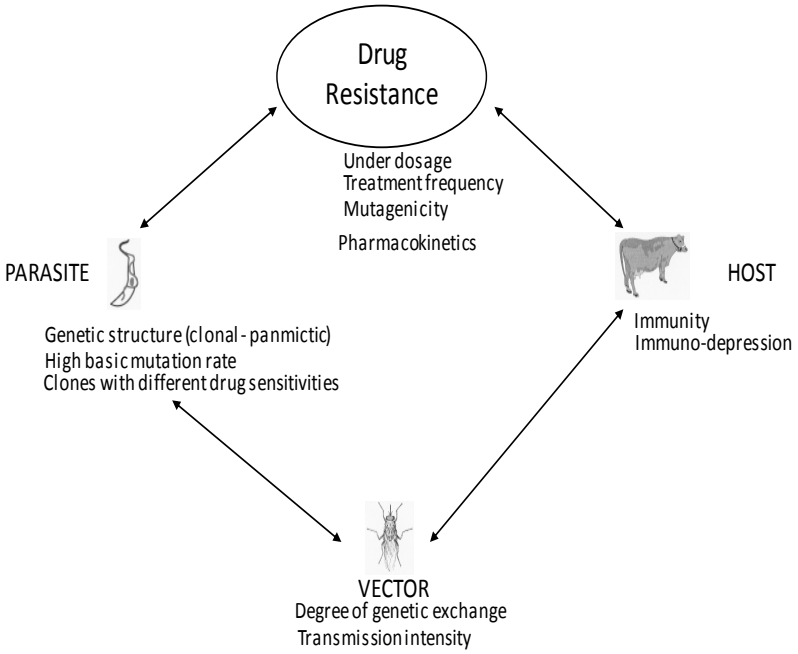

Figure 7 Biological factors influencing trypanocidal drug resistance development (FAO, 1998)

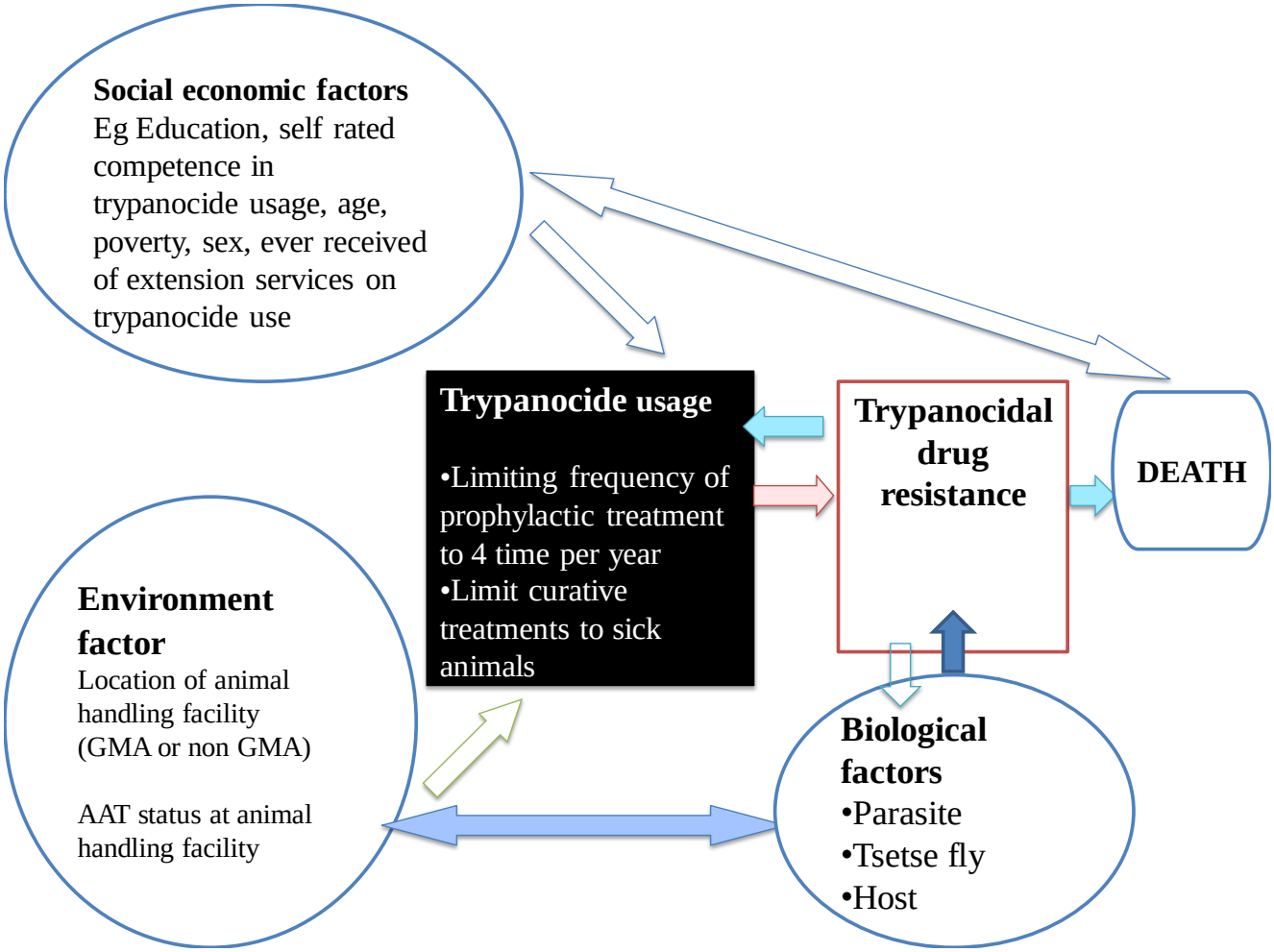

Figure 8: Conceptual framework adapted from FAO showing trypanocide usage and factors that may be associated with it. Arrows represent direction of influence (FAO., 1998)

3.0 CHAPTER THREE: METHODOLOGY

3.1 Study Setting and population

This study was conducted in Itezhi tezhi which is one of the districts of central province of Zambia at seven animal handling facility sites (Iyanda, New Ngoma, Kapulwe, Mutenda, Itumbi, Banachoongo and shinampamba) as shown in figure 9. The district is classified as rural and has a total surface area of about 13,000 square kilometres. Approximately 50% of the district is in the Kafue National Park, while the rest is either game management area (GMA) or gazette forest (DVLD., 2007). Mainly it is drained by the Kafue River. The geographical nature of the district greatly influences the population distribution of the district. Most people have resorted to settle along the Kafue River or Lake Itezhi tezhi for economic purposes that include fishing and cattle rearing (DVLD., 2007). In addition, the ecological condition of suitable temperature, vegetation, abundant blood meal source from wild and domestic animals and soils for larval deposition(FAO.b, 1992) in most of the district supports the existence and proliferation of the Tsetse Fly, *Glossina spp*, which transmits African animal trypanosomiasis (AAT) (TCES, 2009). AAT is endemic in Itezhi tezhi and eight out of ten veterinary camps have reported the disease (TCES, 2010). The two trypanocides widely used to treat AAT are ISM and DA (TCES, 2009) commonly known as samorin and berenil respectively by the users.

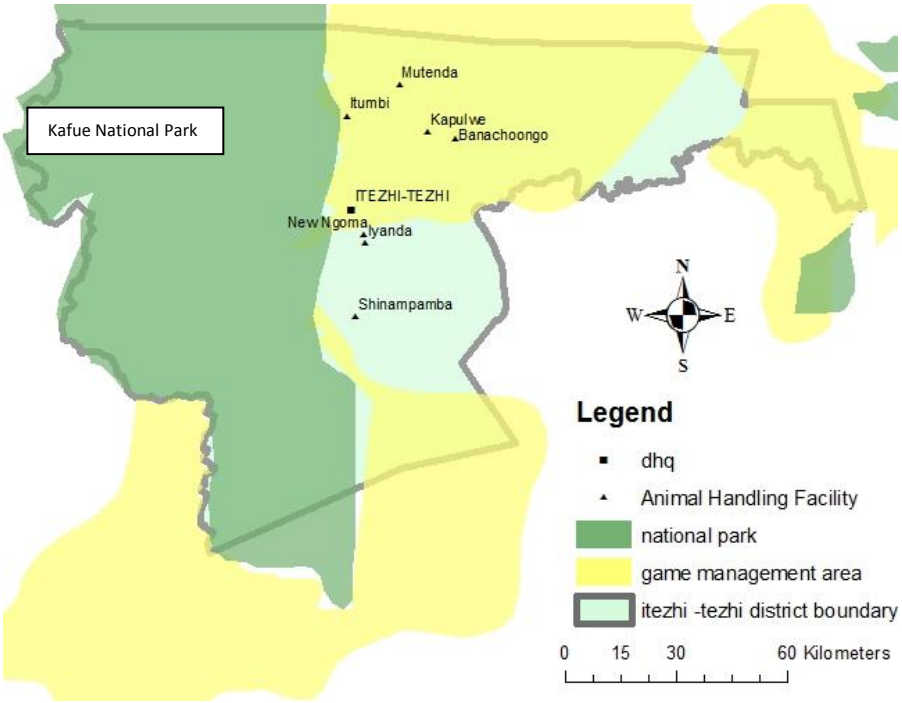

Figure 9: Location of animal handling facilities in the study area. Those in the GMA include Mutenda, Itumbi, Kapulwe and Banachoongo and in the non-GMA are New Ngoma, Iyanda and Shinampamba.

The target population for this study were farmers who used trypanocides and their cattle at animal handling facilities in camps within Itezhi tezhi district where AAT is endemic. The study populations comprised of farmers that used trypanocides and cattle from the seven animal handling facilities where the study was undertaken.

The cattle farmers manage their livestock under the extensive system. Most of them administer chemotherapeutic and prophylactic drugs to their animals themselves often without veterinary supervision. They also practice transhumance. They graze their cattle in the Kafue flats during the dry months from April to October and during the wet months they graze them upland in the forests. These grazing areas also serve as grazing land for wild animals; as a result, cattle and wild animals are in constant contact creating conditions suitable for transmission of AAT from reservoir hosts to definitive hosts (cattle) by the vector as shown in figure 10 below

Figure 10: Wild Zebras co-grazing with domestic cattle in the Kafue plains in Itezhi tezhi (DVLD., 2007)

In the study area the major cattle breeds include the Angoni and crosses of the local breeds (Angoni, Ila and Tonga breeds) with exotic breeds such as the Brahaman. According to the 2013 first round foot and mouth disease report (DVS., 2013), Itezhi tezhi has a total of approximately over 85,000 herds of cattle with approximately 2,140 bulls, 49,386 cows (including heifers), 18,525 oxen and over 15,520 calves.

3.2 Study Design

A cross sectional survey was used to undertake this study targeting farmers that use trypanocides and their cattle. The variables that were of interest for the survey on farmers are shown in the table 1.

Table 1. Variables and measurements of interest

Exposure Variable		**Measurement scale**
Environmental	Location of animal handling facility (Game Management Area , GMA or Non GMA)	Nominal
	AAT status recorded at animal handling facility	Nominal
Socio economic	Education (# of school years of participants)	Interval
	age,	Interval
	sex	Nominal
	self-rated competence in trypanocide usage by participants	Ordinal
	Ever received extension services on trypanocide use	Nominal
Outcome Variable		
Adherence to FAO guidelines on trypanocide use	Avoiding exposure of the whole parasite population to the drug by limiting curative treatments to individual sick animals (Purpose of Diminazene Aceturate use either Other or Curative)	Nominal
	reducing the number of prophylactic treatments on whole herd up to a maximum of 4 times in a year by integrating drug usage with other control measures and (Frequency of ISM treatment in a year either at a maximum of 4 times or over 4 times)	Nominal

3.3 Sample selection

Multistage purposive sampling method was used to select camps and animal handing facility. Only camps and animal handling facilities that were in AAT endemic areas of Itezhi tezhi district were selected. Then farmers who use trypanocides and their cattle were selected purposively and randomly respectively at animal handling facility level.

3.3.1 Farmers who use trypanocides

The farmers were purposively selected using criterion sampling (Lisa, 2008) because the study was interested only in farmers who used trypanocides. Only farmers who used and administered trypanocides at animal handling facilities that were in AAT endemic areas were eligible to participate. Farmers who were at animal handling facilities that has less than 5 users were excluded because they could easily be identified. Since sampling of the farmers to participate in

the study was done purposively, sample size was obtained by targeting a minimum of 50% of the farmers at each animal handling facility. From the 2012 FMD report for Itezhi tezhi district which listed farmers at each animal handling facility, 50% of the farmers translated into a minimum of 82 farmers as shown in the table 2 below.

Table 2. Animal handling facilities and minimum targeted number of farmers (DVS., 2012)

Camp	**Animal Handling Facility**	**Total number of farmers (24)**	**Minimum target sample**
Itezhi tezhi central	Iyanda	21	11
Basanga	New Ngoma	17	16
Itezhi tezhi	Kapulwe	15	8
	Mutenda	17	9
	Itumbi	28	14
Lubanda	Banachoongo	24	12
Nanzhila	Shinampamba	24	12
	Total	**146**	**82**

3.3.2 Cattle population

Simple random sampling using computer generated random numbers from Microsoft excel was used to select animals at herd level for determination of trypanocide resistance prevalence in the trypanosomes infective to cattle in Itezhi tezhi. Only Cattle belonging to farmers who permitted their cattle to be part of the study qualified. Further only cattle that had not been administered with trypanocides for the last 3 months were included in the study as this is average time ISM provides protection from AAT once administered (Delespaux et al., 2002). Calves were excluded from the study because these were less likely to be exposed to AAT. The sample size for cattle was determined using Cochran's sample size formula for proportions (Cochran and Cox, 1957) shown below at 95% confidence interval and a 5% margin of error:

$$n_1 = \frac{z^2 \hat{P}\left(1-\hat{P}\right)}{e^2}$$ (Cochran and Cox, 1957)

Where z was 1.96, P which was the estimated prevalence at 50% obtained as the actual prevalence of AAT and DA resistance in Itezhi tezhi was not known and so it gave the maximum possible sample size. The margin of error e to be accepted was 0.05 therefore gave a minimum of 384 cattle to be sampled in order to obtain prevalence of DA resistance.

3.4 Data Management

3.4.1 Questionnaire and interview of farmers

A semi structured questionnaire was used to collect demographic and socio economic data as well as data points that were used in estimating the proportion of farmers that adhered to FAO recommended guidelines on trypanocide usage practices. Interviewers were trained on questionnaire handling and interview to ensure good quality of data was collected. Further, completeness and consistency of the questionnaires was monitored every day to ensure that all sections of the questionnaires had been answered appropriately. Geographical Information System (GIS) software ArcMap GIS 9.0 was used to assign animal handling facilities to either the GMA or non-GMA location after collecting their GPS coordinates. The data was then be entered in a Microsoft excel data base according to animal handling facility.

3.4.2 Cattle blood collection

A total of 613 cattle were randomly selected for blood sample collection and details of sex, age and colour of the animals were obtained. Blood samples were obtained from the ear vein of the cattle and collected in haperinzed capillary tubes of about 0.1ml volume which were then sealed at one end using Cristal seal. From each animal, two blood samples were collected in two separate capillary tubes. One capillary tube containing blood from each herd of cattle was centrifuged in a microhaematocrit centrifuge for 4 minutes at 9000 revolutions per minute. The PCV value was determined using a PCV reader. All samples with PCV values of 26 % and less were put on Whatman no4 filter paper as buffy coat spots by breaking the capillary tube just below the buffy coat region and eluting the plasma, buffy coat and part of the red blood cells onto filter paper. After air drying, all filter paper with buffy coat spots were stored in envelops at room temperature away from sun light awaiting transportation and DNA extraction (Vitouley et al., 2011). All animals with a PVC value of 26% or less were regarded as positive for AAT (Marcotty et al., 2008) and their corresponding buffy coat spots noted. From the other capillary tube the thick and thin blood smears were prepared on single frosted end glass slides. The thin smear was fixed in methanol while the thick smear was not fixed. Both types of smears were stained with Geimsa stain to improve visibility of the parasites and examined under a microscope at X1000 magnification using immersion oil. Thick smears were used to determine prevalence of AAT

while thin smears were to be used for speciation of trypanosomes. All the positive samples by either direct parasitological or PCV value diagnosis were selected for molecular isolation, identification and DA resistance testing using PCR-RFLP (Vitouley et al., 2011). Molecular isolation and identification of trypanosome DNA was done at the Bio-safety level two plus (BSL2+) laboratory, Department of Veterinary Tropical Diseases, Faculty of Veterinary science, University of Pretoria, Onderstepoort campus in South Africa, using a modified qualitative PCR method described by Geysen et al. (2003) where as DA resistance testing was done using the method described by Vitouley et al. (2011). The BSL2+ laboratory is one of the labs in the Southern Africa region recognised by the Institute of Tropical Medicine in Antwerp, Belgium that competently undertakes the molecular diagnosis of DA resistance with strong quality control measures in place to avoid contamination. It is at the Institute of Tropical medicine that this method of DA resistance diagnosis was developed.

3.4.2.1 DNA extraction

Upon arrival at the BSL2+ labs, the condition of samples was inspected to determine if they were acceptable for DNA extraction. DNA extraction was done by using the QIAamp® DNA blood mini kit (Qiagen, US). In brief, small pieces from the buffy coat spot on filter paper were cut using a sterile surgical blade and placed into a 1.5ml centrifuge tube. To that tube, 180µl of buffer ATL was added and then incubated at 85°C for 10 minutes. This was then briefly centrifuged to remove drops from inside the lid. 20µl of proteinase K stock solution was added and mixed by vortexing, then incubated for 1 hour at 56°C. The mixture was then briefly centrifuged to remove drops from inside the lid. Then 200µl of buffer AL was added to the sample and mixed by vortexing then incubated for 10 minutes at 70°C. This was then briefly centrifuged to remove drops from inside the lid. Then 200µl of absolute ethanol was added to the sample and mixed thoroughly by vortexing. This was then briefly centrifuged to remove drops from inside the lid. The mixture was then carefully applied to the QIAamp Mini spin column and added to 500µl of buffer AW1 without wetting the rim. The cup was then closed and the column centrifuged at 8000 rpm for 1 minute. The QIAamp mini spin column was then placed in a clean collection tube and the collection tube containing the filtrate was discarded. The QIAamp mini spin column was then opened carefully and 500µl of buffer AW2 was added without wetting the rim. The cup was closed and the column centrifuged at full speed of 14000 rpm for 3 minutes. The QIAamp mini column was then place in a new 2ml collection tube and the old tube was discarded. The QIAamp mini column in the new tube was then centrifuged at full speed for 1 minute. The QIAamp mini spin column was then placed in a clean 1.5ml centrifuge tube and the old collection tube with the filtrate was discarded. The QIAamp mini column was the opened carefully and 150µl of Buffer

AE was added. This was incubated at room temperature for 1 minute and then centrifuged at 8000 rpm for 1 minute. Thereafter, 75µl of the extracted DNA was aliquoted into another sterile 1.5ml centrifuge tube. Both tubes containing extracted DNA were then stored at -20°C while waiting for amplification and future use respectively. This was done for each of the 60 samples.

3.4.2.2 Primers used for identification of Trypanosoma species

The primary amplification was done on the 18S gene using the forward primer $18STnF_2$ (5'-CAACGATGACACCCATGAATTGGGGA-3') and $18STnR_3$ (5'-TGCGCGACCAATAATTGCAATAC-3') as a reverse primer. A semi nested second amplification was done using the forward primer $18STnF_2$ of the first amplification with the reverse primer 18STn R_2 (GTGTCTTGTTCTCACTGACATTGTAGTG).

3.4.2.3 DNA amplification for identification of Trypanosoma species

Standard PCR amplifications were done in 25µl reaction mixtures containing 4.5µl extracted DNA sample and 20µl of PCR mix (12.5µl DreamTaq PCR Master Mix [Thermo Scientific], 0.5µl of 10mM forward Primer, 0.5µl of 10mM reverse primer and 7µl of distilled water). The reaction mixture was then placed on a heating block of a programmable thermo cycler. After the denaturation step of 4 minutes at 95°c each of the 40 cycles consisted of 30 seconds at 95°C, 45 s at 58°C and 60 s at 72°C.

In the semi nested run, 25µl reaction mixtures containing 2.5µl amplification products from the first run and the same ingredients and concentrations except for the reverse primer which was replaced with the same volume of the nested reverse primer. The amplification programme was identical except 25 cycles were used. A negative control consisted of adding ultrapure water instead of template DNA to the PCR mixture was included in the PCR amplification.

The mixtures were examined for the presence of DNA fragments by loading 5µl of each reaction mixture with 2µl of loading buffer into the wells on 2% agarose gels. A 100 bp DNA ladder was included in every gel for fragment size determination. The samples were run for 20 min at 100 V in 2% Tris/Acetic acid/EDTA (TAE) buffer, stained with ethidium bromide for 20 min washed under running tap water and photographed under UV illumination.

3.4.2.4 Restriction Fragment Length Polymorphism for identification of Trypanosomes

6µl of the nested products were digested using the restriction enzymes *Msp*1 and *Eco*571 in restriction mixtures containing 0.3µl (0.6U/µl of nested PCR product) of *Msp*1, 0.3µl (0.6U/µl of nested PCR product) of *Eco*571, 1.5µl of Tango buffer, 1.5µl of SAM/Buffer G and 6.9µl of distilled water. The reaction was left overnight in a water bath at 37°C. Four microlitres (4µl) of the restricted sample was then mixed with 2µl of loading buffer and transferred into a 10% SDS

polyacrylamide gel. A 100bp DNA ladder for fragment size determination was also loaded in the SDS polyacrylamide gel. The DNA fragments were thereafter separated by horizontal electrophoresis in 0.5x Tris/Boric acid/EDTA (TBE) buffer at 80 V for 2.5 h. The gel was then stained using SYBRE- green dye.

3.4.2.5 Primers used for DA resistance detection

The amplification was done on the TcoAT1 gene using the forward primer Ade2F (5'-ATAATCAAAGCTGCCATGGATGAAG-3') and Ade2R (5'-ATGACTAACAATATGCGGGCAAAG-3') as a reverse primer.

3.4.2.6 DNA amplification for DA resistance detection

All samples that amplified DNA for *T.congolense* were subjected to DNA amplification for detection of DA resistance in 25µl reaction mixtures containing 5µl DNA sample and 20µl of PCR mix (12.5µl Dream Taq PCR Master Mix [Thermo Scientific], 0.5µl of 10mM forward Primer, 0.5µl of 10mM reverse primer and 7µl of distilled water). The reaction mixture was then placed on a heating block of a programmable thermo cycler. After the denaturation step of 4 minutes at 95°c each of the 40 cycles consisted of 30 seconds at 95°C, 45 s at 59°C and 60 s at 72°C.

The mixtures were examined for the presence of DNA fragments by loading 5µl of each reaction mixture with 2µl of loading buffer onto 2% agarose gels. A 100 bp DNA ladder was included in every gel for DNA fragment size determination. The samples were run for 20 min at 100 V in 2% TAE buffer, stained with ethidium bromide for 20 min washed under running tap water and photographed under UV illumination.

3.4.2.7 Restriction Fragment Length Polymorphism for DA resistance detection

6µl of the PCR products were digested using the restriction enzyme DnpII (Mbol) in restriction mixtures containing 0.3µl (0.6U/µl of PCR products) DnpII, 1.5µl Tango buffer and 7.2 µl of distilled water. The reaction was left for 5 minutes in a water bath at 37°C. 4µl of the restricted sample was then mixed with 2µl of loading buffer and transferred into a 10% SDS polyacrylamide gel together with a 100bp DNA ladder for fragment size determination. The DNA fragments were thereafter separated by horizontal electrophoresis in 0.5x TBE buffer at 80V for 2.5 h. The gel was then stained using 10µl of SYBRE- green for 30 minutes. The gel images were then taken using ChemiDoc^{TM} MP System imager.

3.4.2.8 Source of reagents and Primers

The QIAamp DNA Mini Kit for DNA extraction kit was sourced from Whitehead Scientific (Pty) Ltd, Cape Town, South Africa while the rest of the reagents and primers were obtained from Inqaba Biotechnical Industries (Pty) Ltd, Hatfield-Pretoria, South Africa.

The diagnosis results using microscopy and PCR as well as PCV values obtained from cattle blood were recorded and then entered in a Microsoft excel database with details of sex, age and colour according to animal handling facility. All animal handling facilities were coded appropriately depending on whether they recorded any case of AAT or not upon microscopy and/or PCR diagnosis.

3.4.3 Statistical analysis

The data from the farmers and that from cattle were merged and linked using the AAT status at the animal handling facility in a Microsoft excel data base then was exported to STATA IC version 11 for management and analysis. The outcome variable, adherence to FAO guidelines on trypanocide use, was created by combining the variables purpose of DA use and frequency of ISM treatment in a year (table 1). Then multiple Logistic regression was used to determine associations between adherence to FAO guidelines and particular independent variables while holding others fixed. Odds ratios were used as the measures of effect while proportions were measures of frequency.

3.5 Ethics

All information concerning this study was given to the participants. Informed consent was obtained from the participants. Anonymity and confidentiality of the participants' identity and details respectively was maintained. Further the study posed no physical or psychological risks to the participants.

Permission was sought from the cattle owners before collection blood samples. In addition, Permission to conduct this study was obtained from the Department of Veterinary Services Directorate. Further the animals were handled with respect and in a humane way during blood collection. In addition, pain and distress were minimized during blood collection by using standard methods undertaken by qualified veterinary personnel who were research assistants from the district veterinary office. The only risk posed was a small puncture on the ear vein the size of the diameter of a lancet in order to collect 0.2ml of blood however the benefits outweighed the

risk by providing data on AAT status as well as diminazene aceturate resistance thereby providing information to be used by the district veterinary office to devise AAT control strategies that would prevent losses of cattle through deaths. Furthermore all animals that were diagnosed with AAT by the field techniques of microscopy and PCV value were immediately administered with the correct dose of Diminazene Aceturate according to their weight.

The GPS coordinates for each animal handling facility were solely used for the purpose of the study and nothing more. Further all the diagnostic results of AAT, DA resistance and recommendations made from the study were communicated to the District Veterinary Office and Department of Veterinary Services for their information and action. Ethical clearance for the study was sought from Excellence in Research Ethics and Science (IRB number 00005948).

4.0 CHAPTER FOUR: RESULTS

4.1 Population characteristics

4.1.1 Farmers

The total number of farmers eligible for interview at the seven animal handling facilities was 146 out of which 90 were interviewed. Fourteen (14) out of 17 (82.3%) farmers were interviewed at Mutenda, nine out of 28 (32.1%) at Itumbi, 14 out of 15 (93.3%) at Kapulwe and 18 out of 21 (85.7%) at Iyanda. Further 12 out of 17 (70.5%) farmers were interviewed at New Ngoma, six out of 24 (25.0%) at Shinampamba and 17 out of 24 (70.8%) at Banachoongo.

All the 90 farmers interviewed were male of which 25.6% were aged between 17 and 30 years, 33.2% aged between 31 and 40 years, 25.6% aged between 41 and 50 years and those between 51 to 78 (maximum age) were 15.6%. Further 32.2% of the farmers had attended between zero and six school years while 36.7% attended seven school years and 31.1% attended eight and above school years. In addition, 60% of the interviewed farmers were from animal handling facilities in the game management area while 40% were from non-GMA (table 3).The trypanocides ISM and DA were the only ones being used by the farmers who participated in the study. Ninety six percent (96%) of the farmers diagnosed AAT from the body condition of the animals. On average 70% of the farmers reported having been using trypanocides for a period of one to 10 years while 30% reported over 10 years use. Ninety four percent (94%) of the farmers sourced the trypanocides from shops while 6% sourced them from Veterinary Officers, briefcase business men and other sources. Eighty four farmers reported having used both ISM and DA to manage trypanosomosis in their herds while 6 used only DA for that purpose. The majority (90%) of the farmers reported that they either used sterile or boiled water for preparation of the injectable trypanocide solutions of ISM and DA. About 75% of the DA users used 10 millilitres of water for DA preparation while 25% used the volume of 12.5millilitres as recommended by the manufacturers for a sachet. For ISM, 89% of its users used the recommended volumes of 50 and 100millilitresof water to prepare 2% and 1% injectible solutions of ISM respectively while 11% used volumes less than those recommended.

Table 3. Characteristic of farmers

	No in Non GMA (%)	No in GMA (%)	Total No. of farmers Interviewed (%)
Sample size	36(40.0)	54 (60.0)	90 (100.0)
Sex			
Male	36(40.0)	54(60.0)	90 (100.0)
Female	0(0.0)	(0.0)	0 (0)
Age in years			
17-30	10(27.8)	13(24.1)	23 (25.6)
31-40	12 (33.3)	18(33.3)	30 (33.2)
41-50	9 (25.0)	14(25.9)	23 (25.6)
≥51	5 (13.9)	9(16.7)	14 (15.6)
Mean age (95%CI)	37.7(33.4-41.9)	39.3(36.3-42.3)	38.6(36.2-41.0)
Education in years			
0-6	14(38.9)	15(27.8)	29 (32.2)
7	11(30.6)	22(40.7)	33 (36.7)
≥8	11(30.6)	17(31.5)	28 (31.1)
Mean education in years (95%CI)	6.9(6.1-7.7)	6.9(6.2-7.6)	6.9(6.4-7.4)
Cattle herd size			
1-10	12()33.3)	26(48.2)	38 (42.2)
11-20	10(27.8)	14(25.9)	24 (26.7)
21-30	4(11.1)	6(11.1)	10 (11.1)
≥31	10(28.8)	8(14.8)	18 (20.0)
Mean cattle herd size (95%CI)	38.2(14-62)	16.4(12-20)	25.1(15.4-34.8)

4.1.2 Cattle

From the 1, 227 cattle presented for blood sample collection, 613 were randomly sampled. Table 4 summarises the characteristics of the cattle sampled.

Table 4. Characteristics of cattle sampled

	No. Non-GMA (%)	No. GMA (%)	Total No. (%)
Sample size	198 (32.3)	415(67.7)	613 (100)
Sex			
Male (Bulls and Oxen)	55(27.8)	121(29.2)	176 (28.7)
Female (Heifer, Cows)	143(72.2)	294(70.8)	437 (71.3)
Age in years			
0-5	111(56.0)	232(55.9)	343(55.9)
≥6	72(36.4)	181(43.6)	253(41.3)
Missing	15(7.6)	2(0.5)	17(2.8)
Mean age (95% CI)	5.4 (5.0-5.8)	5.7 (5.4-6.0)	5.6 (5.4-5.9)

4.2 Adherence levels

Thirty two percent (32%) of the farmers that use DA, used it as a curative drug and limited its use only for sick cattle as recommended by FAO. About 95.6% of those that use Isometamidium Chloride, used limited the frequency of treatments on whole herd to a maximum of 4 times in a year. The outcome variable adherence to recommended FAO guidelines on trypanocide used was arrived at by combining two outcome variables which were; reducing the number of prophylactic treatments on whole herd up to a maximum of 4 times in a year by integrating drug usage with other control measures and avoiding exposure of the whole parasite population to the drug by limiting curative treatments to individual sick animals Overall only 25.6% of the trypanocide users adhered to the FAO guidelines on trypanocide use. Table 5 gives proportions of adherence according to location of the animal handling facilities.

Table 5. Proportion of farmers that adhere to FAO guidelines on trypanocide use

Outcome variable	**%Proportion in Non-GMA (95% CI)**	**% Proportion in GMA (95CI %)**	**Overall Proportion in % (95% CI)**
Purpose for DA use	n=36	n=48	n=84
Non curative	50.0 (32.8-67.2)	81.2 (69.8-92.7)	67.8 (57.7-78.1)
Curative	50.0 (32.8-67.2)	18.7 (7.3-30.2)	32.2 (21.9-42.3)
Frequency of ISM treatment	n=36	n=54	N=90
Over 4 times	2.8 (0.0-8.4)	5.6 (0.0-11.9)	4.4 (0.1-8.8)
Up to a maximum of 4 times	97.2 (91.6-100)	94.4 (88.1-100)	95.6 (91.2-99.9)
Adherence to FAO guidelines	n=36	n=54	N=90
No	52.8 (35.6-69.9)	88.9 (80.2-97.5)	74.4(65.3-83.6)
Yes	47.2 (30.1-64.4)	11.1 (2.5-19.8)	25.6(16.4-34.7)

CI is Confidence Interval

4.3 Diminazene Aceturate resistance

4.3.1 Microscopy

All the 613 blood samples were examined using microscopy and only two 2 were positive for AAT. The positive samples came from a GMA location.

4.3.2 PCV

Sixty (60) out of the 613 samples cattle sampled had a PCV value of 26 or less. The samples that were positive on microscopy had a PCV value of less than 26%. Table 6 summarizes the results. A logistic regression performed on the 60 samples that had a PCV of ≤26% , with PCR diagnosed AAT status as an outcome variable and PCV, sex of the cattle, age in years and environment

(GMA or non-GMA) as explanatory variables, showed that PCV had the strongest association (P<0.000) than the other explanatory variables.

Table 6. AAT prevalence upon diagnosis using microscopy and PCV value.

Diagnostic Method	**Non GMA (%)** n= 198	**GMA (%)** n=415	**Total (%)** N =613
Microscopy			
Negative	198 (100.0)	413 (99.5)	611 (99.7)
Positive	0 (0)	2 (0.5)	2 (0.3)
PVC			
Negative (>26)	182 (91.9)	337(81.2)	519 (84.7)
Positive (≤26)	16 (8.1)	44 (10.6)	60 (9.8)
Missing	0 (0)	34 (8.2)	34 (5.5)

4.3.3 PCR

Positive samples for trypanosome DNA in the semi-nested PCR amplification gave products of between 600 and 700 base pairs (figure 11). From the 60 samples that had PCV value of 26% and less, 44 samples were from the GMA and 16 were from the non GMA. Seventeen (17) samples were positive for pathogenic animal trypanosomal DNA while 6.7% (4/60) were positive for *T. theileria* which is non pathogenic. The other 39 samples were negative. From the 60 samples 23.3% (14/60) [95% CI 12.3-34.4%] were *T.congolense* savannah type infection, 3.3% (2/60) [95% CI 0.0-8.0%] were *T.vivax* and 1.7% (1/60) [95%CI 0.0-5.0%] were mixed infection as shown on SDS-PAGE gel images in figure 12. The PCR results when stratified according to the location of the animal handling facility showed significantly different proportions of trypanosomal infections between GMA and non-GMA facilities (Table 7)

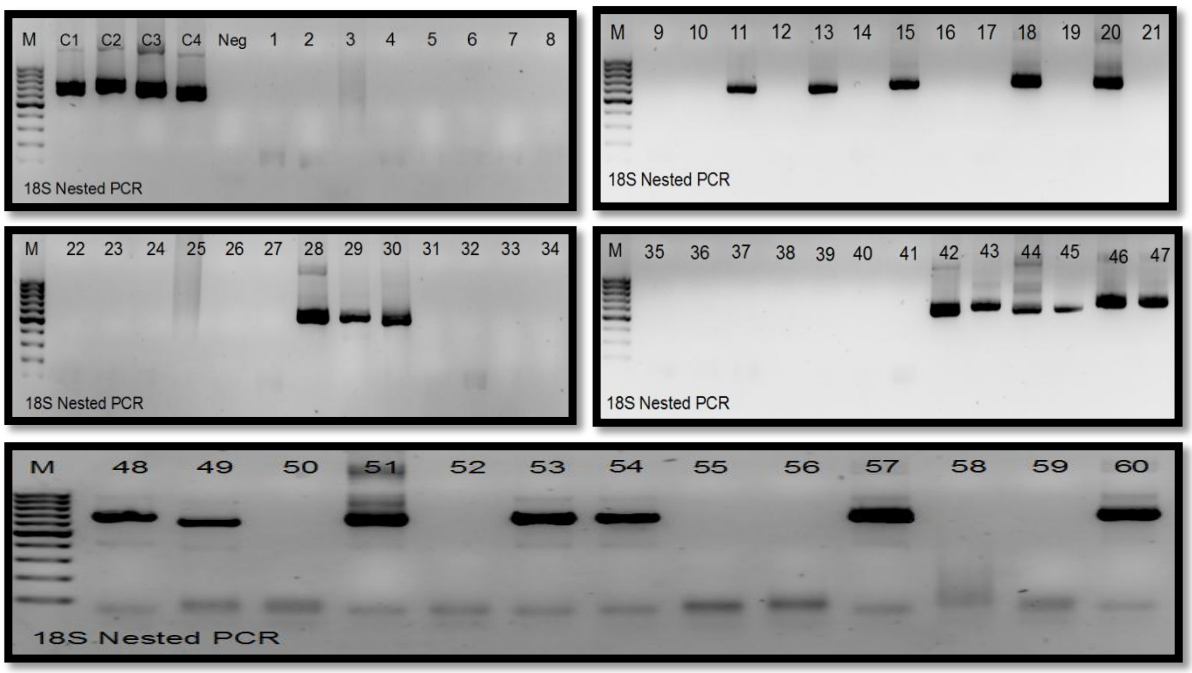

Figure 11: The products of the semi-nested PCR using the primers 18ST nF_2 and 18STnR_2. M is the 100bp DNA ladder, C1 is positive control *T.brucei,* C2 is positive control for *T. congolense kilifi,* C3 is positive control for *T.c. savannah,* C4 is positive control for *T.theileria,* Neg is the negative control and 1-60 are the samples

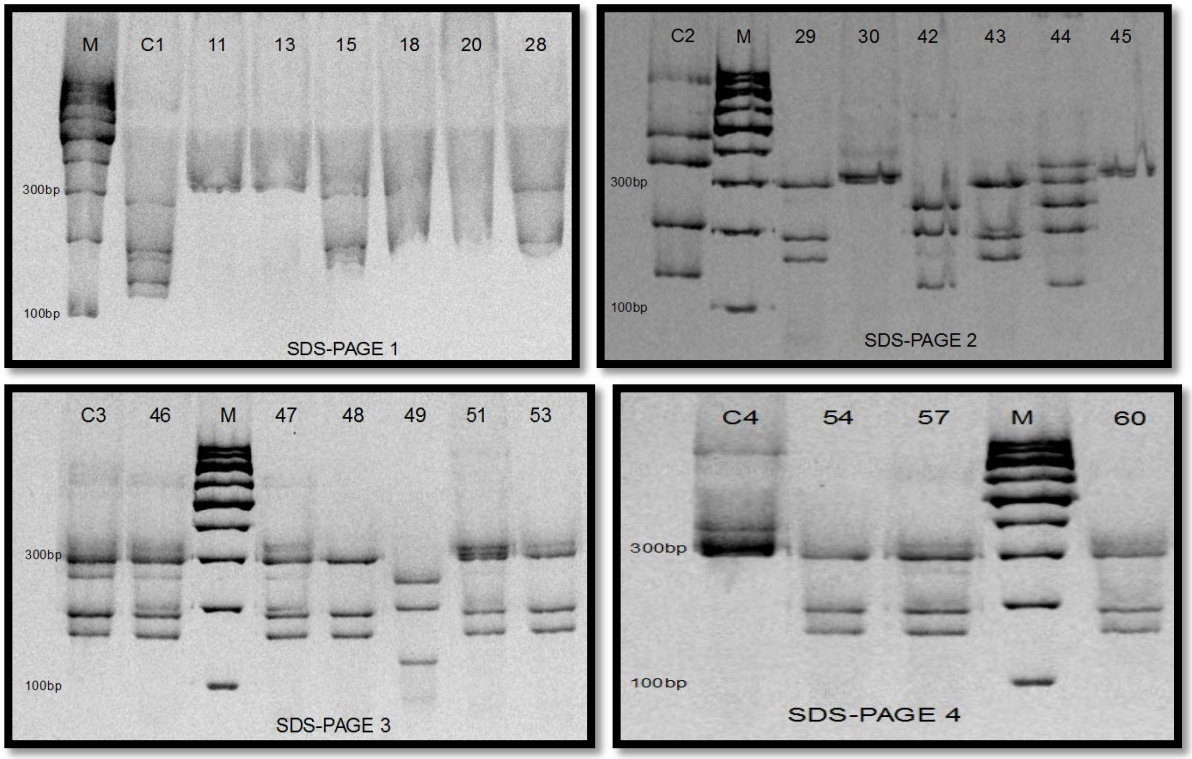

Figure 12: SDS PAGE image: Trypanosome DNA fragments obtained after subjecting the semi nested PCR products to the restriction enzymes MboII and Eco571 on SDS PAGE . M is the 100bp DNA ladder, C1 is positive control *T.brucei,* C2 is positive control for *T. congolense kilifi,* C3 is positive control for *T.c. savannah* and C4 is positive control for *T.theileria*

Table 7. Proportion of pathogenic African trypanosomes according to Location of animal handling facility

PCR diagnosis of Pathogenic trypanosomes	**Non GMA (95% CI)** n= 16	**GMA (95% CI)** n=44	**Overall Proportion in % (95% CI)**
Negative	93.7 (80.4-100)	63.6 (48.8-78.4)	71.7(59.6-83.8)
Positive	6.2 (0.0-19.6)	36.4 (21.6-51.2)	28.3(16.2-40.4)

CI is Confidence Interval

4.3.4 DA resistance detection

None of the 14 *T.congolense* isolates showed any genes associated with DA resistance.

4.4 Determinants of:

4.4.1 Diminazene Aceturate (DA) use

None of the socio-economic variables under investigation showed any significant associations with the purpose for DA use. Nevertheless, the environment variable showed a significant association with purpose for DA use ($P<0.05$) with an odds ratio of 0.23 at Univariate analysis but was not significant at multivariate analysis as shown in table 8. The AAT status at the animal handling facility using PCR was significantly associated with DA use at both Univariate and multivariate analysis.

Table 8. Purpose for DA use by socio economic and environment characteristics

Explanatory variable	UOR (95% CI)	P-Value	AOR (95% CI)	P-Value
Total (n=84)				
Age in years				
17-30	1	-	1	-
31-40	0.93(0.29-3.03)	0.914	0.71(0.14-3.49)	0.676
41-50	0.75 (0.21-2.69)	0.659	0.41(0.07-2.28)	0.308
51-maximium age	0.83 (0.19-3.58)	0.806	1.14(0.15-8.50)	0.896
Education in years				
0-6	1	-	1	-
7	0.68(0.23-1.97)	0.480	1.00(0.23-4.26)	0.996
8-maximum	0.34(0.09-1.19)	0.092	0.36(0.07-1.82)	0.216
Cattle herd size				
1-10	1	-	1	-
11-20	1.93(0.60-6.24)	0.273	3.25(0.68-15.53)	0.140
21-30	3.37(0.78-14.67)	0.105	6.58 (0.94-45.91)	0.057
31- maximum cattle herd size	1.84(0.52-6.55)	0.346	2.43(0.42-13.97)	0.320
Self rated competence in DA usage				
Poor	1	-	1	-
Fair	0.47(0.11-1.95)	0.292	0.72(0.13-4.09)	0.708
Good	0.72(0.2-2.67)	0.628	0.73(0.11-4.89)	0.749
Excellent	-	-	-	-
Ever received extension on DA usage				
Yes	1	-	1	-
No	1.48(0.59-3.72)	0.408	3.15(0.80-12.48)	0.102
Location of Animal handling facility				-
Non-GMA	1		1	
GMA	0.23(0.09-0.61)	**0.003**	0.33(0.10-1.16)	0.085
PCR AAT				
Negative	1	-	1	
Positive	0.18(0.05-0.58)	**0.004**	0.12(0.02-0.63)	**0.011**

UOR is unadjusted odds ratio, AOR is adjusted odds ratio and CI is Confidence Interval

4.4.2 Isometamidium Chloride (ISM) use

None of the socio economic and environment characteristics of the farmers were significantly associated with the frequency of ISM use (P>0.05)

4.4.3 Adherence to FAO guidelines

The status of a farmer ever accessed extension on trypanocide use was significantly associated with adherence to FAO guidelines on trypanocide use (P=0.019). Further the location of the animal handling facility used by the farmers as an environment variable was significantly associated with adherence (P=0.024). In addition the AAT status at the animal handing facility

was also significantly associated with adherence to FAO guidelines (P=0.003). Table 9 gives a summary in detail.

Table 9. Adherence by socio economic and environment characteristics

Explanatory variable	UOR (95% CI)	P-Value	AOR (95% CI)	P-Value
Total (n=90)				
Age in years				
17-30	1	-	1	-
31-40	1.54(0.44-5.45)	0.500	1.76(0.31-9.87)	0.520
41-50	1.00 (0.25-4.06)	1.000	0.71(0.10-5.27)	0.739
51-maximium age	1.44(0.31-6.62)	0.639	2.40(0.24-24.29)	0.460
Education in years				
0-6	1	-	1	-
7	0.83(0.28-2.50)	0.745	1.46(0.30-7.23)	0.642
8-maximum	0.48(0.14-1.68)	0.253	0.61(0.10-3.72)	0.596
Cattle herd size				
1-10	1	-	1	-
11-20	1.82(0.55-6.07)	0.328	4.98(0.79-31.45)	0.088
21-30	1.90(0.39-9.23)	0.427	4.03(0.44-36.94)	0.218
31- maximum cattle herd size	2.21(0.62-7.95)	0.223	3.86(0.51-29.27)	0.192
Self rated competence in trypanocide usage				
Poor	1	-	1	-
Good	1.11(0.28-4.58)	0.889	1.37(0.18-10.25)	0.758
Ever received extension on trypanocide usage				
Yes	1	-	1	-
No	2.09(0.80-5.46)	0.134	7.18(1.39-37.14)	**0.019**
Environment				
Non-GMA	1		1	-
GMA	0.14(0.05-0.41)	**0.000**	0.20(0.05-0.81))	**0.024**
PCR AAT status at animal handling facility				
Negative	1	-	1	
Positive	0.09(0.02-0.40)	**0.002**	0.04(0.01-0.36))	**0.003**

UOR is unadjusted odds ratio, AOR is adjusted odds ratio and CI is Confidence Interval

5.0 CHAPTER FIVE: DISCUSSION

Clearly from the results of this study, most of the farmers reported low adherence to the guidelines recommended by FAO on trypanocide use for delaying of TDR development. This supports the assertion by Chitanga et. al (2011) that the privatization of veterinary services has lead to misuse of veterinary drugs. The misuse was more prominent with farmers that were from animal handling facilities located in the game management area than those outside game management area. This could be partly due to the lack of integration of other AAT control measures as indicated in the FAO guidelines. These are indications that trypanocide usage differ between farmers in the GMA and those in the non-GMA. This situation is different from what was observed by Van den Bossche et al (2000) in Eastern Zambia where trypanocide usage was not different between an area that had high tsetse challenge and the one that was cleared of tsetse flies. That situation was attributed to the treatment strategy before tsetse control which focused on treatment of clinically sick animals rather than only on animals infected with trypanosomes. But for this study the plausible explanation could be linked to high tsetse and AAT challenge as it is expected that in the GMA which harbours the reservoir hosts as well as the vector creates suitable conditions for efficient transmission of the disease. Therefore it could actually support the argument by Van den Bossche (2001) that different epidemiological circumstances are triggered by the encroachment of people and their livestock into tsetse infested area. Further there is an indication that DA was mostly used for other purposes instead of curative purposes as recommended by FAO. However most of the farmer used ISM as recommended. These findings on the frequency of ISM use appear to be similar to those reported by Delespaux et al (2002) on knowledge of ISM use in Petauke and Katete districts. The findings further seem to indicate that trypanocide users in this study were more familiar with ISM than DA use, demonstrating that farmers' usage practices on these drugs were independent. This also indicates that usage practices of anyone of these drugs cannot be generalized to the other.

It is possible that non participation by some farmers due to not coming for interviews after invitation to participate in this study could have biased our estimates. Nevertheless we are confident that the results are valid for trypanocide usage among Itezhi tezhi cattle farmers as we were able to interview over 60% of the trypanocide users at animal handling facilities and all those that turned up for the interview.

The diagnostic results show alarming differences in the diagnostic capabilities of microscopic detection of trypanosome on thick and thin smears and amplification of trypanosome DNA using

semi nested PCR. In this study microscopy was able to miss 88% (15 out of 17) of the positive trypanosomal infections. This discrepancy raises a lot of concern especially that microscopic diagnosis based methods are mainly used for routine diagnosis of AAT. These findings are consistent with results from other studies (Marcotty et al., 2008, Simukoko et al., 2007). Additionally the results show a significant difference in the proportion of positive AAT samples between the GMA and non-GMA (table 7). This indicates high AAT challenge in the GMA than in the non-GMA supporting the earlier assertion that low adherence to FAO guidelines on trypanocide use observed in the GMA could be due to high AAT challenge. The results also show that the majority of AAT infections were caused by *T. congolense* which is consistent with other field observations (Simukoko et al., 2007). Furthermore the RLFP-PCR used to detect DA resistance in the *T.congolense* isolates did not amplify any DA resistance associated nucleic acid indicating that there may be no drug resistance in the study sites. This could be because most (70%) of the farmers had reported having been using trypanocides for a period of between 1 and 10 years whereas it is documented that antimicrobial resistance inevitably develops if they are used for more than 10 years (FAO., 1998). We are confident that the results of the PCR are valid because its resultant products were consistent with those documented by the developers of the approaches (Delespaux et al., 2008, Geysen et al., 2003, Vitouley et al., 2011)

In many studies of public health, socio economic variables have been shown to be significantly associated with health state (http://www.who.int/social_determinants/en). The findings from the study show that not all the socio economic variables under investigation where significantly associated ($P<0.05$) with adherence to FAO guidelines on the trypanocide use. Adherence was significantly associated with the status of ever having received extension on trypanocide use. It is surprising that those that never received extension services on trypanocide usage had a 7 fold increase of adhering to the FAO guidelines than those that had received (table 9). There is need to find out why that is the case. Nevertheless the location and AAT status of the animal handling facility as environment factors were significantly associated with adherence. None of the socio-economic factors were significantly associated with the purpose of DA use; however, only AAT status at the animal handling facility as an environment variable was associated with purpose of DA use. The association showed that there was an 88% reduction in the odds of farmers in the

GMA to use DA for curative purposes than farmers in the non-GMA and the reduction can be as high as 98% and as low as 37% at 95% CI while holding other explanatory variables fixed. The frequency for ISM treatment was not significantly associated with any of the explanatory variables under investigation ($P<0.05$). This was probably due lack of variability as most of the farmers (95.6%) reported having used it as recommended**.** It is possible that the creation of the outcome variable adherence could have biased our estimates as we only considered two from the four recommendations on the FAO guidelines. Nevertheless we are confident that the results are valid because we see that from the preparation of the injectible trypanocide solution, there was a tendency to over dose if farmers did not use the recommended volumes of water. That ruled out under dosing resulting from wrong volumes during trypanocide preparation. Further none of the farmers reported having used quinapyramine indicating the ban of its use did not influence the adherence to the recommended guidelines.

In the study, location of the animal handling facility (GMA or Non GMA) as an environmental variable was used as a proxy for tsetse and AAT challenge. To support that assertion, the results from PCR indicate that there was a significantly higher AAT challenge in the GMA than the non-GMA. The results show that location of animal handling facility whether in the GMA or non-GMA could be a good estimate of AAT burden and could further be argued to contribute towards the epidemiology of AAT. The findings of this study are also consistent with other findings elsewhere indicating that PCV is a strong predictor of AAT status especially in areas where the disease is more prominent than others such as tick borne that may also lower the PCV (Marcotty et al., 2008). Further the findings from this study seem to indicate that even though other haemo-parasites like *Theileria spp* and *Anaplasma spp* associated with anaemia are reported in Itezhi-tezhi (DVLD., 2007), PCV still remains a strong predictor of AAT.

6.0 CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion Diminazene Aceturate resistance is not a problem in Itezhi tezhi despite the majority of farmers misusing the drug suggesting a complex relationship between trypanocide usage and TDR development which requires further investigations. In addition the majority of farmers used ISM appropriately which is commendable. Further, *T.congolense* accounted for the majority trypanosomal infection in cattle in the study area. In addition location and AAT status of the animal handling facility is associated with trypanocide usage among cattle farmers in Itezhi tezhi.

6.2 Recommendations

- Clearly the findings from this study indicate that microscopic based diagnosis can miss up to 88% of AAT infections therefore we recommend that the use of PCR based diagnosis be encouraged in AAT investigations.
- Further we recommend that PCV should be used as a routine screening test for all AAT diagnosis as it can give an indication of the animals infected and limit the number of samples for confirmatory diagnosis using more sensitive and specific tests like PCR.
- With the high levels of trypanocide misuse, we also recommend that the Department of Veterinary Services should develop an extension package on trypanocide use which focuses and demonstrates FAO guidelines on trypanocide use as soon as possible so that trypanosomes are not given an opportunity to develop resistance.
- Furthermore we recommend that surveillance on trypanocide resistance be under taken from time to time by the Department of veterinary services to ensure that its emergence is captured early
- Given that the farmers in the GMA less likely to adhere to FAO guidelines than those in the non-GMA, we recommend that an integrated approach control measures of trypanosomosis in the GMA of Itezhi tezhi to lessen overuse of trypanocides by the farmers.

ANNEX I: REFERENCES

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ANNEX II: LETTER OF PERMISSION

All correspondence should be addressed to:
The Director Livestock Development
Tel: 256679

In reply please quote

No:.....

VTS/10591

REPUBLIC OF ZAMBIA

MINISTRY OF AGRICULTURE AND LIVESTOCK

DEPARTMENT OF VETERINARY SERVICES
MULUNGUSHI HOUSE
P.O. BOX 50060
15100 RIDGEWAY
LUSAKA-ZAMBIA

10TH June, 2013

Mr. Njelembo Joshua Mbewe
Department of Veterinary Services
P.P BOX 630065
CHOMA

RE: PERMISSION TO CONDUCT A STUDY IN ITEZHI TEZHI ON TRYPANOCID USAGE PATTERNS AND ASSOCIATED FACTORS AMONG FARMERS

Reference is made to the above subject matter.

I am hereby informing you that permission has been granted to you to conduct a study on Trypanocide usage patterns and associated factors among farmers in Itezhi Tezhi.

Before you proceed with the study make sure you obtain permission from Ethics Committee.

Dr. Joseph Mubanga
Director
Department Of Veterinary Services
MINISTRY OF AGRICULTURE AND LIVESTOCK

ANNEX III: QUESTIONNAIRE

Questionnaire number……Name of Interviewer:……..

Camp: ……. Animal Handling facility: .....

Date: .....

SOCIO CHARACTERISTICS

Age (*Indicate age at last birth day)* ☐

Sex 1= Female 2= Male ☐

Number of School years (*Indicate completed school years)* ☐

Farmer's herd size……

II. TRYPANOCIDE USE REGIMEN

Name three most problematic cattle diseases in this area (*Specify)*

a).....

b).....

c).....

Of these diseases mentioned in 4. above which one is the most problematic? (*Specify)*

.....

Name three most commonly used drugs on cattle in the farmer's herd (*Specify)*

a).....

b).....

c).....

Of these drugs mentioned in 6 above which one is the most commonly used? (*Specify)*

.....

Which trypanocide(s) does the farmer use?

1. Berenil® (Diminazene) only ☐
2. Samorin® (Isometamidium) only
3. Samorin® and Berenil®
4. Other (*Specify*).....

How often do you use Berenil® (Diminazene) in a year? (*Specify number of times in a year)*

.....

Why did you use Berenil to treat your animals? ☐

1. Because I wanted to prevent them from getting sick with AAT
2. Because the animals were sick with AAT
3. Because the animals were sick and I did not know the reason
4. Any other reason (*Specify)*……

Do you use it on the whole herd or individual animals? ☐

1. Whole herd
2. Individual sick animals
3. Other (*Specify)*……..

How often do you use Samorin (Isometamidium) in one year? (S*pecify number of times in a year)*
.....

Why did you use Samorin to treat your animals? ☐

1. Because I wanted to prevent them from getting sick with AAT
2. Because the animals were sick with AAT
3. Because the animals were sick and I did not know the reason
4. Any other reason *(Specify)*……..

Do you use it on the whole herd or individual animals? ☐
1. Whole herd
2. Individual sick animals
3. Other (*Specify)*……..

For how long have you been using trypanocides? (*Specify number of years)*
.....

What is the source of trypanocides? ☐

1. Shops (Chemist or other specify)
2. Veterinary Officers
3. Brief-case businessmen
4. Fellow farmers
5. Other (*Specify)*……..

How do you tell that the animal has trypanosomiasis? ☐

1. Body condition (clinical)
2. Blood examination
3. Just know it is trypanosomiasis
4. Other (specify)…….

SOLUTION PREPARATION OF TRYPANOCIDES;

What kind of water do you use to make solutions of Diminazene or Isometamidium to treat your animals

1. Sterile water
2. Boiled water
3. Water straight from a river or dam
4. Water straight from a well
5. Water straight from a bole hole
6. Other source (*Specify)*……..

What do you use to measure the quantity of water? (*Specify)*
1. Measuring jar
2. Graduated syringe
3. Un-graduated syringe
4. Other (*Specify)* ……..

What quantity of water do you use for a Diminazene (Berenil ®) sachet?
1. 12.5 milliliters
2. Other (*Specify)*…….

What quantity of water do you use for an Isometamidium (Samorin®) sachet?

1. 50 milliliters
2. 100 milliliters
3. Other (*Specify)*……

What quantity of powder do you use to make the solution?

1. Whole sachet
2. Part of sachet

How do you determine the amount of solution (drug) to administer to a particular animal?

1. Instrument measured weight of the animal
2. Visual estimated weight of the animal
3. Other specify…….

How do you rate your competence on correctness use of Diminazene (Berenil®) use?

1. Poor
2. Fair
3. Good
4. Excellent

How do you rate your competence on correctness use of Isometamidium (Samorin®) use?

1. Poor
2. Fair

3. Good
4. Excellent

Do you read the manufacturer's instructions on the Berenil®/Samorin® sachets?

1. Yes (*if yes go to question 26*) ☐
2. No (*if no proceed to question 25*)

Why don't you read the manufacturer's instructions?

1. I do not know how to read ☐

Other (*Specify)*……

AVAILABILITY OF EXTENSION SERVICES ON TRYPANOCIDE USE

Are extension services on trypanocide use available in this area?

Yes ☐
No

Have you ever received any extension service on trypanocide use?

Yes ☐
No (*If no end interview)*

When did you last receive any extension service on trypanocide use? (*Specify year)*

……

Who offered the extension service? ☐

Veterinary Officer
Community Livestock Assistant
NGO
Fellow Farmer
Other (*Specify)*……

THANK YOU VERY MUCH FOR PARTICIPATION IN THIS INTERVIEW

END OF INTERVIEW

ANNEX IV: INFORMATION SHEET / CONSENT FORM

Introduction

My name is Njelembo Joshua Mbewe. I work for the Ministry of Agriculture and Livestock as a Senior Tsetse Control Biologist in Choma and I am currently pursuing a Master of Science in Epidemiology at the University of Zambia, School of Medicine. As a requirement for this programme, I am expected to conduct a research study of my choice. My research topic is "Trypanocide usage practice and associated factors among cattle farmers in Itezhi tezhi, Zambia"

Objective of the study

The main objective of the study is to determine the factors and perceptions that are associated practices of trypanocide usage among cattle farmers in Itezhi tezhi because trypanocides are vital in control of African animal Trypanosomiasis in the district.

Procedure

Information will be collected using a semi structured questionnaire. The questionnaire has questions which you are encouraged to answer to enable me collect the necessary information for the study.

Confidentiality and Anonymity

As participants in this study, you are not obliged to reveal your identity. No name or any form of identity will appear on the form and the information obtained will be treated with the highest confidentiality it deserves at all levels of the study

Voluntary participation

Participation in the study is voluntary. You are free to participate or not. Once you have decided to participate, in the study you have the right to withdraw or seek clarification about the study. My contact details have been given below.

Risks/Benefits

The benefit for this study is that it will help identify gaps in your knowledge of trypanocide use and make recommendations to the department of veterinary services for planning and designing extension messages for correct use of the trypanocides. This will help in keeping the limited number of trypanocide effective against African animal trypanosomiasis for a longer period.

This study poses no risk of any physical or psychological harm.

In case of any clarifications that you may require, you can contact me on the following

Name : Njelembo Joshua Mbewe

Department of Veterinary Services
P.O Box 630065
Choma,
Southern Province

Email: njelembombewe@yahoo.com

Cell: +260977433470 or +260966132068

Principal Investigator: Njelembo Joshua Mbewe Sign:

If you are willing to participate in this study, kindly sign the declaration in the space provided below

Declaration.

I have read and understood the information provided above and I do hereby consent to participate in this study.

Date: Sign:

Witness

Date: Sign:

ANNEX V: CATTLE RECORD SHEET

Sheet___ of ___

Camp:_____ Date:_____ Animal Handling Facility name:_____

Recorder:_____ Latitude:_____ ^{o} S Longitude:_____ ^{o} E

S/N	Animal identity	Thick Smear (+or-)	Thin smear Tc Tv Tb	PCV (%)	Age	Sex	Colour	Name of Owner
Total								

ANNEX VI: ETHICS CLEARANCE

33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
F.W.A. No. 00011697

9th September, 2013

Ref. No. 2013-June-003

The Principal Investigator
Mr. Njelembo Joshua Mbewe
P.O. Box 630065,
CHOMA.

Dear Mr. Mbewe,

RE: Trypanocide usage pattern and associated factors in Itezhi tezhi, Zambia.

Reference is made to your corrections dated 14th August, 2013. Noting that you addressed all concerns raised the IRB resolved to approve this study and your participation as Principal Investigator for a period of one year.

Review Type	Ordinary	Approval No. **2013-June-003**
Approval and Expiry Date	Approval Date: 9th September, 2013	Expiry Date: 8th September, 2014
Protocol Version and Date	Version-Nil	8th September, 2014
Information Sheet, Consent Forms and Dates	• English.	8th September, 2014
Consent form ID and Date	Version-Nil	8th September, 2014
Recruitment Materials	Nil	8th September, 2014
Other Study Documents	Questionnaire, Interview Guides.	8th September, 2014
Number of participants approved for study	166	8th September, 2014

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON

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I.R.B. No. 0000594
F.W.A. No. 0001169

10th January, 2014

Ref. No. 2013-June-003

The Principal Investigator
Mr. Njelembo Joshua Mbewe
P.O. Box 630065,
CHOMA.

Dear Mr. Mbewe,

RE: Request for amendment: Trypanocide usage pattern and associated factors in Itezhi tezhi, Zambia.

Reference is made to your letter dated 6th January, 2014 requesting to amend the research protocol.

The amendments are approved as submitted.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON