EPIDEMIOLOGICAL STUDIES INTO THE IMPACT OF TRYPANOCIDAL DRUG RESISTANCE ON THE CONTROL OF TRYPANOSOMIASIS IN COASTAL KENYA

By

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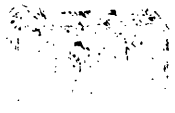
I can do all things

through Christ Jesus who strengthens me

Philippians 4: 13



SUMMARY

This thesis describes epidemiological studies into the impact of trypanocidal drug resistance on the control of trypanosomiasis in coastal Kenya.

General introduction into the objectives and location of the studies are described in Chapter 1.

Previously published work with emphasis on the use of chemotherapy and chemoprophylaxis in the control of trypanosomiasis, factors associated with development of trypanocidal resistance including existing methods available for determination of drug resistance are reviewed in Chapter 2.

Chapter 3 describes the General Material and Methods that were common to all laboratory and field studies reported in the later chapters of this thesis.

Chapter 4 describes longitudinal studies into the influence of trypanocidal drug resistance on the efficacy of chemoprophylaxis and chemotherapy in control bovine trypanosomiasis on Galana Ranch in the Coast province of Kenya. Isometamidium prophylaxis studies were conducted over a three-year period between December 1994 and March 1997. In each year, Boran cattle were divided into two groups, isometamidium-treated and sentinel group. There was significant variation of trypanosome challenge over the three-year period. There was evidence that early breakthrough infections particularly *T. vivax* had some degree of resistance to isometamidium. However, isometamidium prophylaxis was efficacious during the year with relatively low trypanosome challenge. In addition isometamidium prophylaxis was cost effective in spite of drug resistance.

Observational studies on trypanosomiasis control using chemoprophylaxis in small holder's dairy cattle are described in Chapter 5. A study covering three-prophylactic periods of three months each was carried out from November 1995 to August 1996 in the Kenyan coast north and south of Mombasa on zero grazing dairy cattle. There was evidence of isometamidium prophylaxis failure, which was attributed to drug resistance, genetic susceptibility or resistance of cattle breed, nutritional status and individual animal variations.

Chapter 6 describes longitudinal studies carried out in four different sites in Kwale District from February 1997 to October 1998 to assess the influence of trypanocidal drug resistance on efficacy of chemoprophylaxis and the factors contributing to the efficacy of trypanocidal drugs. In each study site cattle were grouped into two groups, isometamidium-treated and sentinel. There were no significant differences in trypanosome challenge between sites. There was evidence of under dosing and incorrect administration by farmers and trained veterinary personnel. This was associated with trypanocidal drug failure and development of resistance observed in the District.

In vivo drug sensitivity tests in mice of *T. congolense* and in cattle of *T. vivax* are described in Chapter 7. Factors associated with the viability of stabilates and their infectivity were investigated. The stabilates had been obtained during the studies described in Chapters 4, 5 and 6. Isometamidium treatment had no significant effect on viability and infectivity for mice of trypanosome stabilates. In cattle the route of inoculation of *T. vivax* had a significant effect on the infectivity of stabilates. Sensitivity tests in mice demonstrated that 87.2 % of *T. congolense* stabilates from Kwale District expressed multiple resistance to isometamidium chloride and diminazene aceturate. Evidence of *T. congolense* populations in Kwale District that expressed multiple drug resistance corresponded to trypanocidal drug failure observed in the longitudinal and observational studies reported in early chapters.

Finally, Chapter 8 discusses the overall findings of the studies described in this thesis with an emphasis on course of action necessary to minimise the spread and further development of drug resistance. Isometamidium prophylaxis was more efficacious against *T. congolense* than *T. vivax* infections. Factors associated with isometamidium prophylaxis failure observed included trypanosome challenge, trypanosome species drug resistance genetic susceptibility or resistance of cattle breed, drug administration, drug dose used and nutrition status.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

μ	Mu
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immuno-sorbent assay
ESG	EDTA saline glucose
HRP	Horseradish peroxidase
ILRI	International Livestock Research Institute
ILRAD	International Laboratory for Research on Animal Diseases
i.m	Intramuscular
i.v	Intravenous
KETRI	Kenya Trypanosomiasis Research Institute
kg	kilogram(s)
mg	milligram(s)
ml	millilitre(s)
mm	millimetre(s)
NDDP	National Dairy Development Programme
ng	nanogram(s)
OD	Optical density
PBS	Phosphate buffered saline
PCV	Packed cell volume
TMB	Tetramethylbenzidine
VAT	Variable antigen types
VSG	Variable surface glycoprotein

DECLARATION

The work presented in this thesis is original and was carried out solely by the author, except where collaboration with others has been acknowledged.

I also hereby certify that no part of this thesis has been submitted previously for the award of a degree to any University

Raymond Ellie Mdachi

August 1999

DEDICATION

This thesis is dedicated to:

My wife, Ruth Mulwale Mdachi

Children: Shaleen, Sara and Sheela

for their love, support, perseverance and understanding.

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Chapter 1
General Introduction

1 General Introduction

1.1 Trypanosomiasis

Trypanosomiasis is a disease caused by haemo-protozoan parasites of the genus *Trypanosoma*. In sub-Saharan Africa, the parasites are mainly transmitted by tsetse flies of the genus *Glossina*. Biting flies particularly *Tabanus* and *Stomoxys* are also vectors of the disease in camels. Approximately ten million km^2 of Africa are infested with tsetse flies, covering 37 countries (FAO, 1982). The disease affects both humans and animals. The severity, duration and outcome of infection are variable but generally have serious consequences.

In humans, the disease known as sleeping sickness causes fever, signs associated with central nervous system involvement and eventually death. Sleeping sickness is endemic in 36 countries of sub-Saharan Africa. Although the disease can be treated, early diagnosis is of paramount importance for effective treatment. Involvement of the central nervous system in the late stage of the disease and the toxicity of the arsenical drug used for treatment at that stage, complicate the treatment of the disease.

It is estimated that 50 million people are at risk of acquiring the disease (FAO, 1982). The annual reported figure of 25,000 new patients is most probably a gross underestimate because surveillance in the approximately 200 known endemic foci is difficult. Reporting of data is often inadequate. Shortage of skilled personnel, financial constraints on public health services and the presence of other major communicable diseases in Africa have led to sleeping sickness control programs receiving little attention.

In spite of the very wide distribution of tsetse flies in Kenya, sleeping sickness is endemic only in areas around Lake Victoria, where the rhodesiense form of human trypanosomiasis is found. Cases have been reported in South Nyanza, Busia and Siaya districts.

Animal trypanosomiasis presents a serious veterinary problem in most parts of the developing world, nowhere more so than in sub-Saharan Africa where it is arguably the most important animal disease. While many other animal diseases of major economic importance have been successfully controlled during this century, trypanosomiasis continues to present a major constraint to animal production in sub-Saharan Africa (Holmes and Torr, 1988). In livestock, the disease is manifested by loss of body condition, anaemia, pyrexia and death if not treated.

In Africa it is estimated that out of a total cattle population of 174 million, only about 30 million are found in the 7 million km^2 of tsetse infested savannah and light woodland areas (KETRI, 1996) that might otherwise be highly suitable for cattle production. Trypanosomiasis has therefore led to considerable under-exploitation of natural resources, and to lower levels of animal productivity. Africa produces 5% of the world's beef while it has 13% of the world's cattle. It is estimated that over 50 million cattle and 100 million goats die annually from the disease (KETRI, 1996).

Control of trypanosomiasis is based on controlling the vector and the disease. Previously ground and aerial spraying with insecticides were used, but currently the vector is controlled by use of traps and targets. Use of targets involves impregnating suitable fabric with insecticides that may not be hazardous to the environment (immobile targets) or treating the animal with an insecticidal formulation as a pour-on, dip or spray (mobile targets).

The disease is controlled by use of a very small number of chemoprophylactic and chemotherapeutic drugs. Unfortunately, due to the cost involved in development of new drugs and the limited market for trypanocides, most of the current drugs have been in circulation for over forty years. Traditionally trypanocides were provided to farmers by Government veterinary services. With a recent trend towards privatisation, Government veterinary services are frequently no longer providing trypanocidal drugs. However, private veterinary services are not usually an option for resource-poor farmers in tsetse-infested areas. Moreover, the high demand for trypanocides and the unavailability of adequate trained personnel has led to administration of the few available trypanocides by farmers, often incorrectly. In addition, a lack of effective policies regarding registration and use of

veterinary drugs in most countries of Africa has led to availability of sub-standard drugs to the farmers. This has resulted in ineffectiveness of the drugs in the field, either through failure to use the correct products at the recommended doses, or through development of drug resistance in pathogenic trypanosomes.

In Kenya, 60% of the rangeland is infested by tsetse flies (FAO 1982). In these areas, losses of up to 30% are experienced. Coast Province, an area of about 83,000 km^2 is one of the tsetse and trypanosomiasis endemic areas of Kenya. There are seven administrative districts with an estimated human population of 2,342,299 (from 1989 population census). Major resources in the province include beaches along the Indian Ocean coast, wildlife, minerals, livestock and huge plantations of various crops such as coconut and sisal.

Another important feature of the area is Kaya forests, which are traditionally sacred indigenous forests where local inhabitants used to or are still offering sacrifices to their ancestors. The Kaya forests in Kwale and Kilifi districts, including the Shimba Hills Game Reserve, are foci of tsetse habitat. The random distribution and large number of these forests within the province complicates the control of tsetse fly by use of traps and targets (Snow and Tarimo 1983). Consequently, strategic use of integrated control measures using available technologies may be the only answer.

Farming systems in Coast Province range from large-scale ranches and plantation, to small-scale farmers. The types of cattle kept in the coast region are mainly Boran, Sahiwal and Zebu breeds. There are about 900,000 head of beef cattle. In areas where conditions are favourable, large scale and small-scale farmers keep dairy cattle. In total, there are 61,000 dairy cattle. Breeds popularly kept are Friesians, Ayrshire, Guernsey, Jersey and various crosses. Other animals found in the region are goats (approx.800, 000), sheep (approx.600, 000), pigs (approx.3, 000), donkeys (approx.19, 000) and camels (approx. 40,000). All these livestock are at risk of acquiring trypanosomiasis.

According to the Department of Veterinary Services, trypanosomiasis is the most important endemic diseases in Coast Province. In 1996, 7019 cases of trypanosomiasis were reported in the province. However, this is probably an underestimate, as many cases are never reported, most of the affected areas are

inaccessible, and lack of financial support has made surveillance in known foci difficult.

In addition to direct costs associated with trypanosomiasis, a large proportion of farmers income is expended in the purchase of trypanocides. Studies in the region (KETRI, unpublished data) have indicated that Novidium®/Ethidium® ,homidium chloride/bromide and Veriben®/Norotryp® , diminazene aceturate are commonly used for treatment while Samorin® , isometamidium chloride is used for prophylaxis. According to estimates from the Veterinary Department, the cost of trypanocidal drugs used in the province amounted to Ksh.15 million (US$200,000) in 1994 and Ksh. 30 million (US$400,000) in 1996. The estimated average cost per animal was Ksh.166 ($2.30) for Samorin®, Ksh. 133 ($1.80) for Veriben® and Ksh.24 ($0.3) for Ethidium®. This is despite the use of traps and bush clearing to control the vector. Sources of these drugs are unevenly distributed with high concentration being found in urban areas and some areas having none.

As discussed earlier, ready availability of trypanocides to untrained persons can easily result in misuse leading to treatment failure and inducement of drug resistance. Treatment and prophylaxis failure at the coast has led to the Veterinary Department recommendation of higher doses of diminazene e.g. 7.0 mg/kg b.w. and a shorter prophylactic period of 45 days for isometamidium (Maloo, 1993). In view of this, it was important to investigate factors that may lead to or cause trypanocidal treatment failure and to determine the extent of resistance in a tsetse and trypanosomiasis endemic area (the coastal region of Kenya). The aim of this study therefore was to:

- Evaluate the efficacy of isometamidium prophylaxis under different management systems.
- Investigate the factors leading to isometamidium prophylaxis failure
- Determine the sensitivity of breakthrough/relapse infections to isometamidium and diminazene
- Evaluate and determine the presence and extent of isometamidium and diminazene resistance in Kwale district.
- Evaluate if possible the pathogenicity of drug resistant trypanosomes.

To meet the above objectives, several studies involving isometamidium block treatment were carried out in the Coast Province. In each study, trypanosome infections were monitored by parasitological methods, productivity data were obtained, and circulating isometamidium concentrations in the sera of treated cattle were determined by ELISA. The studies were as follows:

- A study of the efficacy of drugs in the control of trypanosomiasis in a ranch situation. This work was done in collaboration with the staff at the KETRI field station on Galana Ranch.
- Observational studies of trypanosomiasis control in small holder's zero-grazed dairy cattle at the Kenyan coast north and south of Mombasa.
- Field assessment of factors associated with drug resistance. Detailed longitudinal studies were carried out to assess drug resistance in trypanosomes and factors related to drug failure and resistance at four sites in Kwale District of Coast Province.
- Laboratory assessment of drug resistance in isolates collected during the field prophylactic trials. These studies involved sensitivity testing of trypanosomes in mice and cattle

These studies are described in detail in the following chapters.

Chapter 2
Literature Review

2 Literature review

2.1 The Trypanosome

Trypanosomes belong to the order Kinetoplastida (Vickerman, 1994), suborder Trypanosomatina, family Trypanosomastidae and genus *Trypanosoma*. They are kinetoplastid flagellates with a single locomotory flagellum, either free or attached to an undulating membrane. They contain a second flagellum presented by a barren basal body and a kinetoplast relatively small and compact with DNA fibrils. Parasites in the trypanosomastid family change their configuration during their life cycles, particularly when they change hosts from vertebrates to invertebrates and vice versa. To distinguish between the different morphological forms of trypanosomastid in a given host, Hoare and Wallace (1966) introduced a rational nomenclature. This nomenclature refers to the arrangement of the flagellum; it's starting point, it's course either inside or outside the body of the parasite, and its point of emergence to the exterior of the organism. The generally accepted descriptions and definitions of the configuration of trypanosomastid flagellates are as follows.

- *Promastigote*: Elongated form with ante-nuclear kinetoplast, with flagellum arising near it and emerging from the anterior end of the body.
- *Opisthomastigote*: Elongated form with post-nuclear kinetoplast, with flagellum arising near kinetoplast and passing through the body and emerging from the anterior end.
- *Epimastigote*: Elongated form with juxta-nuclear kinetoplast.
- *Trypomastigote*: Elongated form with post-nuclear kinetoplast, flagellum arising near it and emerging from the side of the body to run along the surface to form an undulating membrane.
- *Choanomastigote*: 'Barley corn' form with ante-nuclear kinetoplast, flagellum arising from a wide funnel- shaped reservoir and emerging anteriorly.

- *Amastigote*: Round, oval, or elongated forms devoid of flagellum.
- *Paramastigote*: Form with kinetoplast close to the nucleus, intermediate between Pro- and Opisthomastigote, flagellum emerges from anterior end and reservoir extends through body to point where flagellum and kinetoplast are close.
- *Sphaeromastigote*: Rounded form with a free flagellum, which represents a transitional stage between an Amastigote and Mastigote form.

Not all of these mastigotes occur in the genera of Trypanosomastid. There are about nine genera of trypanosomastid family. These include *Leptomonas, Crithidia, Herpetomonas*, *Blastocrithidia*, *Rhynchoidomonas*, *Endotrypanum*, *Phytomonas*, *Leishmania* and *Trypanosoma*.

Trypanosoma is characterised by its parasitic mode of life. Trypanosomes are very variable in size from 10 μm to over 1200 μm, but generally these spindle shaped organisms are 15 - 100 μm depending on the subgenus and species. The characteristic morphological forms are trypomastigote, found in the blood and tissues of vertebrate hosts and the epimastigote forms found in the invertebrate vector. However, other morphological forms are found, such as amastigotes and more rarely, promastigotes. The infective forms produced in the vector are known as metacyclic and are trypomastigote in configuration. Transmission to the vertebrate hosts is by the bite of an infected vector or by contamination of the skin or mucous membrane by faecal materials containing the infective metacyclic trypanosomes. In vertebrate hosts, parasites are found in the blood and also localised in a variety of tissues. Reproduction or multiplication of trypanosomes in the vertebrate host is by cell division. However, sexual processes, have been observed in some Trypanosomastids (Vickerman, 1994).

There are various subgenera of the genus *Trypanosoma* that occur in mammals. These are divided into two sections; the Stercoraria and Salivaria, based on the site of development of the organisms in the insect hosts that transmit them. Stercorarian trypanosomes develop in the hindgut of biting insects following ingestion of a blood meal containing parasites. Transmission of infection to new hosts is by faecal contamination. Salivarian trypanosomes develop in the mouthpart

of tsetse flies (*Glossina* species), and are transmitted from host to host by inoculation.

2.1.1 The Stercoraria

These are trypanosomes in which a free flagellum is always present. The kinetoplast is large and not terminal, and the posterior end of the body is pointed. Multiplication in the mammalian host is discontinuous, typically taking place in epimastigote or amastigote stages. Development in the vector is completed in the posterior station and transmission is by contamination. These types of trypanosomes are typically non-pathogenic.

2.1.1.1 Subgenus Megatrypanum

This is a rather heterogeneous group of large mammalian trypanosomes whose kinetoplast is typically situated near the nucleus and far from the posterior extremity of the body. These trypanosomes may be regarded as the most primitive representatives of the genus *Trypanosoma* because they show affinities with some corresponding parasites of amphibians and reptiles. These trypanosomes are parasitic to a wide range of animals including mammals. The most typical species of this subgenus is *Trypanosoma theileri* that was first found in cattle by Theiler (Wells *et al.*, 1965). *Trypanosoma theileri* is a common parasite of domestic cattle (*Bos Taurus*) throughout the world. In addition to this host, it has been found in Zebu (*Bos indicus*) in different parts of the world (Keymer, 1969). The incidence of infection with *T. theileri* varies considerably in different countries according to early reports. Incidence of infection of up to 55% was reported in Britain (Wells *et al.*, 1965) and up to 100 % in Nigeria (Gray and Nixon 1967). When first discovered in cattle affected by gall sickness by Theiler, he regarded this trypanosome as a caustic agent for gall sickness, but not pathogenic (Wells *et al.*, 1965). However, association of *T. theileri* with certain diseases is accompanied by unusually high parasitaemia. This was more pronounced with rinderpest (Curasson, 1925), and piroplasm (Reichenow 1940).

2.1.1.2 Subgenus Herpetosoma

This is a homogeneous subgenus (Hoare, 1964) comprising about 45 named species. These trypanosomes are parasites of rodents. The most important species is *Trypanosoma lewisi,* which is a common blood parasite of black and brown rats (*Rattus rattus* and *R. norvegicus*). Like all the species of subgenus *Herpetosoma*, *T. lewisi* is non-pathogenic. The common vector for this parasite is a rat flea; *Nospsyllus fasciatus* in temperate zones and *Xenopsylla cheopis* in tropical and sub-tropical zones.

2.1.1.3 Subgenus Schizotrypanum

These trypanosomes are relatively small and typically C - shaped. They have a voluminous kinetoplast situated very near the short pointed posterior end of the body. Multiplication in the mammalian host is intracellular in the amastigote stage. The most important species is *Trypanosoma cruzi*, restricted to South and Central America. This is the causative agent of Chagas disease in humans and is transmitted by reduviid triatomine bugs.

2.1.2 The Salivaria

The trypanosomes in this section may or may not have a free flagellum. The kinetoplast may be terminal or sub terminal, and the posterior end is usually blunt. Multiplication in mammalian hosts is continuous and typically in a trypomastigote stage. Development in the vector (*Glossina*) is completed in the anterior station (except in mechanical vectors) and transmission is by inoculation. These trypanosomes are pathogenic.

2.1.2.1 Subgenus Trypanozoon

The major species of this subgenus is *Trypanosoma brucei*. This species is transmitted by tsetse flies (*Glossina* species). Three main sub-species are *Trypanosoma brucei brucei*, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. *Trypanosoma brucei brucei* infects livestock while *T. b. rhodesiense* and *T. b. gambiense* infect humans causing the disease known as sleeping sickness. *Trypanosoma b. rhodesiense* is found in East and Central Africa extending to the Okavango region of Botswana in the south and as far north as

Ethiopia. *Trypanosoma b. gambiense* overlaps with the distribution of *T. b. rhodesiense* in the region around Lake Victoria. Countries west of those infected with *T. b. rhodesiense* and south of the southern limit of the Sahara have all reported cases of *T. b. gambiense*, sleeping sickness.

All members of the *Trypanozoon* sub-genus transmitted cyclically by tsetse flies have similar life cycles. The first part of the midgut phase involves transformation of the bloodstream parasites into slender procyclic trypomastigote forms. These forms multiply in the ectoperitrophic space, between the peritrophic membrane and the midgut. They then pass anteriorly through the food canal and then invade the salivary glands via the salivary duct or hypopharynx. In the hypopharynx the parasites attach themselves to the mechano-receptors in the proximial part of the labium. This affects the probing behaviour of the flies and causes infected flies to probe more frequently and more vigorously than uninfected ones. Apparently this increases the chances of transmission of the trypanosome to the host.

The parasites transform to epimastigote forms in the salivary glands and massive multiplication occurs followed by parasite attachment to the microvilli. The parasites are then transformed to the metacyclic trypomastigote forms that are infective to mammals. Transformation occurs while the epimastigote forms are attached; the metacyclic forms are free in the lumen of the gland. Around 15-35 days elapse from the time the fly feeds on infected blood to the time the fly can be infective to mammalian hosts. The complexity of the life cycle of *T. brucei* in *Glossina* may in part be responsible for the low infection rates observed in the field. However, the efficiency of transmission and interference with normal behaviour may account for the survival of the parasite despite the low infection rates.

In the subgenus *Trypanozoon*, non-tsetse transmitted species are *Trypansoma evansi* and *Trypanosoma equiperdum*. *Trypanosoma evansi* is primarily an infection of equines (horses, donkeys and mules) and camels. It is transmitted between hosts mechanically by biting flies, particularly horse flies (*Tabanus*) and stable flies (*Stomoxys*). This method of transmission depends on the movement of a fly with trypanosome contaminated mouth parts to a new host within a reasonably short time, so that the parasites in the mouth parts remain infective. Such transmission depends on uninterrupted feeding, as it is necessary for the fly to continue to probe and feed

when it has moved to another host. *Trypanosoma evansi* does not survive in the gut of biting flies, even in *Glossina*, being destroyed within hours of ingestion. In the bloodstream, *T. evansi* usually occurs as mono-morphic trypomastigotes 15 - 36 μm. There is a small sub-terminal kinetoplast in organisms from populations that are not dyskinetoplastic. Division is by longitudinal binary fission. However, genetic exchange in *T. brucei*, which appears to be a true sexual process involving meiosis, has been demonstrated (Gibson and Stevens, 1999). *Trypanosoma equiperdum* has the distinction of being the only trypanosome whose normal method of transmission is by coitus. Transmission occurs during coitus as a result of the presence of trypanosomes in the seminal fluid and mucus exudates of the genitals of stallions and the vaginal mucus of mares. The infection is restricted in nature to equines where it causes the disease Dourine. *Trypanosoma equiperdum* is a tissue parasite, and bloodstream forms are rarely observed. However such forms are important in the dissemination of the organism to different organs and skin. *Trypanosoma equiperdum* is morphologically indistinguishable from *T. evansi*.

2.1.2.2 Subgenus Duttonella

The trypanosomes of this subgenus are distributed throughout the tsetse belt where they are parasitic to both domestic livestock and game wildlife. The principal species in this subgenus is *Trypanosoma vivax*, which is pathogenic to domestic livestock. This pathogen is transmitted by tsetse flies and also (readily transmitted) mechanically by other biting flies, such as *Tabanus*. A trypanosome that is very closely related to *T. vivax* is *Trypananosoma uniforme*. This organism has been recognised in Angola, Uganda and Zaire. It was initially described in bovines but has been found in game animals as well.

Trypanosoma vivax was so named because of the vigour of its activity under the microscope when viewed in fresh preparations. The parasites move quickly across the field of view. The trypanosomes are 18 - 31μm in total length with a free flagellum of about 3 - 5 μm and a body length of about 15 - 26 μm. This pathogen is characterised by a large kinetoplast usually situated close to the posterior end. There is a degree of dimorphism, some forms being club-shaped posteriorly, while others are more slender. There is also a free flagellum in contrast to *Nannomonas* organisms. Multiplication is by equal longitudinal binary fission, usually in the blood

stream. All *Glossina* species of tsetse flies can act as vectors for this subgenus. The life cycle of *T. vivax* in *Glossina* is confined to the proboscis, without any stage development in the mid-gut or salivary glands. Infections in the proboscis are initially established on the labrum where epimastigotes multiply and form colonies attached firmly to the cuticle lining of the food canal. Some of the colonies detach and invade the hypopharynx, where the epimastigotes transform to trypomastigotes. The fully transformed ones are infective metacyclic forms capable of initiating an infection in the mammalian host. The time of development of *T. vivax* in *Glossina* varies with temperature. At 22 °C, 12-13 days must elapse, from the feeding of infected blood to the production of the metacylic forms (infective forms). At a higher temperature of 29 °C the cycle can be completed in five days (Desowitz and Fairbairn, 1955). The simplicity of the life cycle of *T. vivax* is believed to be the reason for relatively high infection rates in the field and under experimental conditions compared to infection rates observed in *Nannomonas* and *Trypanozoon*. Under laboratory conditions, *T. vivax* has been shown to be capable of infecting 70 % of tsetse flies, whilst field infection rates of *T. vivax* usually exceed 10% and seldom 50%. There is no relationship between the age of the fly and its susceptibility to *T. vivax* infections as is the case with *Trypanozoon* species; presumably because of the localisation of the parasites in the proboscis.

2.1.2.3 Subgenus Nannomonas

This subgenus has two important members, *Trypanosoma congolense* and *Trypanosoma simiae*. *Trypanosoma congolense* is distributed in the tsetse fly belt in sub-Saharan Africa. It is however, less easily transmitted mechanically than *T. vivax*. It is found in domestic bovines and game animals. On the other hand, *T. simiae* is an important pathogen of pigs in some areas and is normally found in wild Suids throughout Africa.

The trypanosomes in this subgenus have an unusual characteristic of having no free flagellum at any stage in the life cycle. The flagellum terminates at the anterior end of the parasite. The posterior end of the parasite is normally rounded but can be slightly pointed in some cases. The medium sized kinetoplast is usually in a marginal and sub-terminal position. The trypanosomes multiply by longitudinal binary fission. *Trypanosoma congolense* is the smallest of this subgenus with a mean

length of 12-17 μm. Recent studies have demonstrated that polymorphism of *T. congolense* exists in West Africa. *Trypanosoma simiae* is more pleomorphic in its characteristics and is slightly longer (15-19 μm) than *T. congolense*. *Nannomonas* are very active in fresh blood films seemingly vibrating in the same position; they tend to adhere to each other as well as to host tissues *in vivo*. Both *T. congolense* and *T. simiae* have similar cycles in tsetse flies (*Glossina* species) in which they develop. The life cycle of *T. congolense* in *Glossina* involves both the midgut and a proboscis stage. The initial development takes place in the endoperitrophic space of the midgut where elongated trypomastigote multiply before moving to the ectoperitrophic space. The trypomastigotes re-enter the endoperitrophic space by penetrating the soft peritrophic membrane. They then move anteriorly to the proboscis, where they transform to the smaller epimastigote forms and attach to the walls of the food canal. The parasites attach to mechano-receptive sensilla in this site affecting the probing behaviour of the fly. The epimastigotes multiply in colonies and enter the hypopharynx where the metacyclic trypomastigote, resembling the small blood stream forms are produced. These are infective to mammalian hosts.

The life cycle of *T. congolense* in tsetse flies requires a minimum of 12 days, but could take longer at lower temperatures. About 25% of the flies remain infected and infective throughout life.

2.2 Trypanosome hosts

Mammalian host species may possess natural resistance to particular species of trypanosomes. Pigs are refractory to infection with *T. vivax* (Stephen, 1966) and cattle are not likely to be infected with *T. simiae* (Roberts, 1971). Humans are capable of being infected with only two subspecies of *T. brucei*; *T. b. gambiense* and *T. b. rhodesiense*. However, human serum sensitivity tests indicate that *T. b. brucei* has potential infectivity for man (Rickman and Robson, 1970; Hawking, 1973). Dogs and cats are said to be refractory to *T. vivax* and *T. simiae* infections.

2.2.1 Trypanosoma brucei

This species seldom causes acute infection in cattle and because examination of blood films usually fails to establish the presence of this organism, it has been

thought that it infects such animals only rarely. However, improved diagnostic techniques have revealed that *T. brucei* infections in cattle are not uncommon (Killick-Kendrick, 1968). Horses are very susceptible to infection with this trypanosome and will die within one to three months if left untreated (Stephen, 1970; Kihurani *et al.*, 1994). Donkeys are somewhat less susceptible than horses. In contrast, pigs have shown considerable tolerance to *T. brucei* infections (Killick-Kendrick and Godfrey, 1963). Barnett (1947) reported fatal relapses with involvement of the central nervous system in pigs that had apparently recovered from chronic *T. brucei* infections. Dogs are highly susceptible to *T. brucei.* The infection is usually acute and generally fatal.

2.2.2 Trypanosoma congolense and Trypanosoma vivax

These trypanosomes are chiefly responsible for the losses of cattle from this disease. *T. vivax* is associated more with acute infections in cattle, whereas, *T. congolense* causes chronic infections. Sheep and goats are less susceptible to *T. vivax* than cattle (Ilemobade *et al.*, 1975). Earlier, Stephen (1970) observed that both sheep and goats usually develop chronic, progressive disease when experimentally infected with *T. congolense* or *T. vivax.*

In equines, infection with *T. congolense* generally results in mild, chronic disease and tends to lead to spontaneous recovery (Stephen, 1962), but Kimberling and Ewing (1973) recorded a severe case in a thoroughbred. Many natural *T. vivax* infections in horses have been described as relatively harmless (Stephen, 1970). However, earlier, Stephen and Mackenzie (1959) recorded an experimental infection with a tsetse-borne strain that resulted in a weight loss of 34 kg together with marked clinical signs. Recent findings in Kenya have shown that *T. congolense* and *T. vivax* were the main pathogens found in horses at a commercial farm (Kihurani *et al.*, 1994).

Camels are also susceptible to infection with *T. congolense* and *T. vivax*, although very few authenticated cases have been reported. *Trypanosoma congolense* can cause rapid infections in dogs; severe anaemia, loss of condition and weakness are the principal symptoms. In contrast, cats are less susceptible to *T. congolense* infections than dogs.

2.2.3 Trypanosoma evansi

This species has shown a very wide host range that varies with locality (Hoare, 1965). In Africa, horses and camels are susceptible to acute infection by these trypanosomes (Hoare, 1970). However, sheep, goats and antelopes have been shown to be carriers of *T. evansi*. African cattle appear to be rarely infected with *T. evansi* (Godfrey *et al.*, 1965). In Asia there are records of *T. evansi* from tigers, foxes, Indian elephants and orang-utans. Cattle and Asiatic buffalo (*Bubalus bubalus*) are readily infected with *T. evansi*, although they seldom show symptoms (Verma *et al.*, 1973). They are considered important reservoir in Asia from which susceptible species such as horses and camels may be infected. Dogs are occasionally infected with *T. evansi*, and the course of the disease resembles that in *T. brucei* infections.

2.2.4 Trypanosoma simiae

The course of infection with this organism in pigs is often dramatic, death frequently occurring within forty-eight hours of appearance of clinical signs. Indigenous pigs are usually capable of withstanding trypanosome infections. Whereas, exotic pigs are more susceptible. Goats are susceptible to *T. simiae*; goats experimentally infected with this organism by wild caught flies developed an acute or sub-acute infection terminating fatally (Leach and Roberts, 1981). Recently, *T. simiae* was identified as the cause of a disease outbreak in dromedary camels (*Camelus dromedarius*) introduced to Tsavo East National Park, confirming the susceptibility of camels to this pathogen (Mihok *et al.*, 1994). *Trypanosoma simiae* has also been isolated from a new host, the white rhinoceros (*Ceratotherium simum*) through xenodiagnosis with a susceptible tsetse species (*Glossina morsitans centralis*). A white rhinoceros showed some evidence of anaemia and lymphopaenia when harbouring *T. simiae*, but did not suffer any long-term health effects (Mihok *et al.*, 1994).

2.3 The vector (*Salivaria*)

All tsetse flies are similar in appearance and are placed in the genus *Glossina* that comprises of twenty-two species. The smallest and the largest species are about

7.5 mm and 14 mm respectively. They have a mottled brown grey thorax usually with darker bands on the abdominal segments. This confers an effective protective coloration making it difficult to see the flies when perched on trees or rocks. When at rest both wings lie horizontally over the abdomen, giving the fly the distinctive elongated appearance. In the middle of the wing is a closed cell of distinctive shape that looks as a butcher's cleaver. This 'hatchet cell' is a useful diagnostic character for identifying the *Glossina.*

The male flies are on average smaller than the females, but the largest males are bigger than the smallest females. The males are readily distinguished by the presence of the hypopygium centrally at the tip of the abdomen.

Glossina species totally depend on the blood sucking habit. However, different species have different food hosts for sources of food. This host preference is important in the epidemiology of trypanosomiasis. *Glossina* is larviparous. A single female that usually mates only once, at the time of its first blood meal, a few days after emergence will produce approximately 8-12 larvae during its life span of 3-5 months. The incubation period of the developing larva is approximately 9-12 days depending on the temperature. The females require regular blood meals for the development of the larvae. Any reduction in the frequency of blood meals will adversely affect larval development by inducing abortion or underweight larvae. On larvae positioning, it burrows into the moist soil or sand usually under fallen logs. The puparium, usually black in colour (6 - 8 mm in length), remains in the ground for a period of 30 days, or longer if the temperatures are low, until the adult fly emerges as a teneral fly. Teneral flies are soft, more lightly coloured and have an averted ptilinum - a sac on the head that aids the emergence from the puparium.

Three marked groups of the genus *Glossina* have been recognised (Mulligan, 1970). These are the *palpalis* group, the *morsitans* group and the *fusca* group. The division into these groups is based on the morphology of their genitalia. In addition, this grouping corresponds generally with their habitat. The *palpalis* group is associated with riverine vegetation, the *morsitans* group with woodland or savannah and the *fusca* group with more humid forested areas. However this categorisation into a species group infesting a particular habitat is not absolute. As the dry season advances, accompanied by high temperatures, there is reduction of humidity and

reduction in the availability of suitable habitats. This causes the *G. morsitans* population to be restricted to the densely vegetated riverine areas, at the extremes of their range. The *G. palpalis* group flies, normally restricted to a humid habitat, become widely distributed in the savannah during the rainy season. This group has also become associated with peri-domestic habitats in some parts of West Africa, living and breeding in close proximity to human. Invasion of the peri-domestic habitat in East Africa by *G. fuscipes*, normally confined to lake shores and riverine vegetation, was aided by the invasion of villages by *Euphorbia* and *Lantana camara* that produces thicket type vegetation and an ideal habitat for flies.

Although *Glossina* species have characteristically occupied these traditional habitats, they have recently shown their capability to invade vegetation planted by humans. In Kenya, *G. pallidipes* and *G. austeni* have been shown to invade mango and coconut plantations at the coast forming peri-domestic populations (Snow and Tarimo, 1983). In West Africa, *G. palpalis* group species can be found in mango, oil palm, banana, cocoa and sugarcane plantations.

2.3.1 Transmission

Factors that affect transmission are not completely understood. Whiteside (1962) related it to the fly infection rate and the apparent density (the number of non-teneral flies captured per 10, 000 yards on a fly round). Baylis and Nambiro, (1993) observed that feeding success of *G. pallidipes* on infected cattle was 75% greater than on non-infected cattle. In addition, the feeding success of *G. pallidipes* on infected cattle depended on the infecting trypanosome species. *Glossina pallidipes* fed more successfully on oxen infected with *T. congolense* than on uninfected individuals or oxen infected with *T. vivax* (Baylis and Mbwabi, 1995). Many field observations have suggested that the species of the fly is an important factor in transmission (Baylis, 1997). Harley and Wilson (1968) showed that experimentally, *Glossina pallidipes* and *Glossina morsitans* are better transmitters of *Trypanosoma congolense* to mice than *Glossina fuscipes*. More recently, Moloo (1992) concluded that *G. pallidipes* has a lower vector competence than *G. m. centralis* for *T. congolense* and *T. b. brucei*. However both tsetse species had equal competence for *T. vivax*.

The behaviour and activity of *Glossina* are an important aspect in relation to disease transmission. In the laboratory, peak spontaneous activities of *G. morsitan* have been recorded during early morning and evening with reduced activity during the middle of the day when in nature the temperatures would be appreciably high. Out in the field, there are marked differences in activity largely limited to ambient temperatures above 13-17 °C. In the northern part of *Glossina* distribution, peak activity occurs in the middle of the day since during early mornings and evenings the temperatures are too cool.

At the coastal areas where mid day temperatures are above 30 °C the fly activity is confined to early morning and evenings (Baylis, 1997). *Glossina* has been shown to respond to visual and olfactory stimuli both in the laboratory and in the field. At shorter distances of 10-30 m the flies respond to visual stimuli while at longer distances response to olfactory stimuli is predominant. Thus, odour stimulates fly activity but visual stimuli orientates the fly to the target. Ox breath odour has been found to be a powerful attractant.

2.3.2 Feeding habits

A well-defined feeding pattern exists for *Glossina*, which may be modified according to host availability. The development of accurate test to determine the sources of *Glossina* blood meals, has contributed immensely to the knowledge of their feeding habits. The most important test that enables the host to be identified to the species, is the haemo-agglutination inhibition test. A highly sensitive complement fixation test has also been identified. A wide range of mammals, some birds and reptiles have been identified as hosts of *Glossina*. Species of *Glossina,* can be grouped into those that take their meals predominantly from Suidae, such as *G. swynnertoni* and *G. austeni*; from Bovidae, *G. pallidipes, G. longipalpis* and *G. fusca*; and the *G. morsitans* subspecies that take meals predominantly from Suidae and Bovidae (Wilson *et al.*, 1972). The *G. palpalis* group species feed on human and any available hosts. These may be pigs, bushbucks or reptiles.

Some species of *Glossina* depend consistently on certain species of hosts for food; G. *brevipalpis* feed predominantly on hippopotamus and *G. longipennis* on rhinoceros (Wilson *et al.*, 1972). Recently, however, analysis of bloodmeal samples

from tsetse caught in two different sites on the Galana Ranch in Kenya showed that *G. longipennis* preferentially feeds on suids, bovids and hippopotamus (Makumi *et al.*, 1996).

2.3.3 Resting sites

The resting site of *Glossina* varies with the sex, species and season and time of day (between day and night). During the day *G. morsitans* usually rests mainly on tree trunks and lower branches, particularly underside of horizontal branches of trees. At night the flies rest in the upper foliage of trees. *Glossina swynnertoni* rests on the underside of small branches, 1.3 - 3 m above the ground. Riverine flies of the *G. palpalis* group rest closer to the ground. When in the proximity of water, they are usually found within 1-2 m of the water edge and resting by day, on twigs and small woody parts. At night they move to the lower surfaces of leaves where the stomata provide a more humid microclimate. However, there are considerable variations in the resting behaviour of a given species in different areas and vegetation type.

2.3.4 Tsetse control

Control of *Glossina* has been and continues to be undertaken by a variety of methods. These include pesticide application, traps and targets, genetic control.

2.3.4.1 Ground spraying

Ground application uses either knapsack sprayers or vehicle sprayers to apply deposit of residual insecticide where it remains lethal to tsetse beyond the maximum pupal period of 60 days. DDT, dieldrin and endosulfan are the pesticides that have been used in many eastern and southern Africa countries. More recently, the use of alternative pesticides such as synthetic pyrethroids has been enhanced (Shereni and Pope, 1993). The residual effect of deltamethrin after application by the ground spraying technique has been shown to last within four months of application in North-west Zimbabwe (Shereni and Pope, 1993). Ground spraying was the only proven method of large-scale tsetse control; the technique was used to eradicate tsetse from 200,000 km^2 of Nigeria (Jordan, 1986). However, the logistics involved such as; a) developing adequate access for the spraying team, b) maintaining a large

fleet of vehicles and spraying equipment and c) planning and supervising large numbers of spray teams, have led to aerial spraying.

2.3.4.2 Aerial spraying

Aerial spraying involves the use of helicopters and fixed wing aircraft (Lee *et al.*, 1978). In aerial spraying, very low doses of insecticides (usually 20-30 per cent endosulfan emulsifiable concentrate) are applied as aerosol from an aircraft, killing tsetse by direct contact. The spray interval varies according to the rate of larval development, which is temperature dependent. Usually five applications are sufficient to cover the maximum pupal period. This technique is more successful in areas of flat open terrain with light winds and a constant inversion at night. The selectivity of the technique, despite the blanket of insecticide, arises from the high susceptibility of tsetse fly to endosulfan. Although aerial spraying requires skilled pilots, sophisticated navigation equipment, and carefully controlled and monitored application, it has more logistical advantages over ground spraying because of reduced labour demands (Holmes and Torr, 1988).

2.3.4.3 Insecticide use on cattle

2.3.4.3.1 Dipping

Initially, dipping of cattle in insecticide wash was used for controlling ticks. Observations in Zambia using deltamethrin in dips for tick control indicated that dipping reduced the incident of trypanosomiasis in cattle by 40 - 50% (Luguru *et al.*, 1993). Elsewhere, at Mkwaja Ranch, Tanga region of Tanzania, where 8000 cattle were affected chiefly by *Trypanosoma congolense*, a trial to determine the efficacy of deltamethrin cattle dip on tsetse flies was conducted (Fox *et al.*, 1993). All cattle except unweaned calves were dipped in deltamethrin wash every two weeks for one year. After ten months of dipping, the populations of *G. pallidipes*, *G. m. morsitan* and *G. brevipalpis* were reduced by 90, 100 and 70% respectively. In addition, disease mortality decreased by 66% and a range of productivity measures such as calving percentages and weaning weights were raised to levels above those prevailing before the decline in the herd health (Fox *et al.*, 1993). The success of the trial allowed intervals between isometamidium prophylaxis treatments to be extended

from 5 weeks to 3-4 months. Similar studies carried out in an area with a high risk of trypanosomiasis in Uganda demonstrated that dipping of cattle regularly in a deltamethrin wash for six months, caused a reduction of 96.9% in the tsetse population (Okello-Onen *et al.*, 1994). Within a year of dipping they were able to achieve 100% reduction in the tsetse population (Okello-Onen *et al.*, 1994).

2.3.4.3.2 Pour on

With the development of non-residual synthetic pyrethroids new techniques involving use of pesticides have been developed. In one of these, the pyrethroid is poured on the host animal at various points on the back. The pesticide spreads through the sebum layer over the rest of the animal. When the flies come to feed they pick up a lethal dose of the pesticide. This technique is effective for all biting insects. Bauer *et al.* (1995) reported successful application of deltamethrin pour-on to cattle in a campaign against *Glossina* spp. in a high tsetse density area in Burkina Faso. Successful use of cypermethrin pour-on to cattle in controlling tsetse flies and other biting flies in Ethiopia (Ghibe Valley) has been described by Swallow *et al.*, (1993). Similar successes with flumethrine pour-on application to cattle in high tsetse density areas have been reported in Kenya (Lohr *et al.*, 1991) and in Burkina Faso (Bauer *et al.*, 1995).

Pyrethroid impregnated ear tags have also been used in tsetse control. Dolan *et al.* (1988) observed that cattle with impregnated ear tags had significantly lower trypanosome infections when exposed to natural challenge.

2.3.4.3.3 Traps and Targets

Traps of various types have been developed to reduce *Glossina*. The bi-conical trap was developed in the early 1970's originally for use against the riverine species such as *G. palpalis palpalis* in west Africa (Challier and Laveissiere, 1973). This trap has also been effectively used for *G. pallidipes* in East Africa. Several attractants such as 1-octen-3-ol, p-cresol, acetone and 3-n-propylphenol have been identified for use in conjunction with traps, targets and screens (Holmes and Torr, 1988). The use of attractants with the traps has increased the catch with these traps by 20 fold (Vale *et al.*, 1988).

Targets, insecticide impregnated cloth screens, have been used together with traps in tsetse control programs. In this technique, when the flies come into contact with the target, impregnated with a solution of deltamethrin suspension concentrate, they pick up a lethal dose of the insecticide. Incorporation of a UV absorber into the insecticidal formulation has been found to extend the effective life of the target (Opiyo *et al.*, 1993). In Zimbabwe the target achieved a 99.99% control within a year (Vale *et al.*, 1988).

2.3.4.4 Genetic control

Sterile male technique has been advocated for the control of *Glossina* following the success of attacks on other Diptera such as screw worm and Mediterranean fruit fly using mass release techniques. This technique involves sterilising male flies by using radiation or chemosterilants. These males are then released to mate with wild females and inseminate them with non-viable sperm (Vale and Hargrove, 1979).

The success of this technique depends on effective mass rearing and mass release of the male flies. This requires expensive sterilising equipment and large efficient rearing facilities; if several species are vectors in an area, all species need to be bred for control to be effective. In addition, these sterile males produced have to be capable of competing for the wild females for mating as effectively as wild males. This method has been used successfully in Burkina Faso where *G. p. gambiense* occupying an area of 100 Km^2 was eradicated within 16 –24 months (Holmes and Torr, 1988).

2.4 Animal Trypanosomiasis

2.4.1 Pathology and Pathogenesis

In sub- Saharan Africa, the disease syndrome in cattle has been associated with tsetse bite. A few days after being bitten cattle were seen to develop an oculonasal discharge, occasional ventral abdominal swelling, loss of appetite, dullness and progressive emaciation. In cattle the disease is mainly caused by *T. congolense* and *T. vivax*. In addition, mixed trypanosome infections of *T. vivax*, *T. congolense* and *T. brucei* have been observed in the field (Mwangi *et al.*, 1998).

When considering pathology and pathogenesis of bovine trypanosomiasis, Murray and Morrison (1978) recognised three areas, which included early events following the tsetse fly bite and subsequent changes in lymphoid system, anaemia and specific organ damage.

2.4.1.1 Chancre

Following a successful tsetse bite in domestic animals, a local skin reaction of variable intensity develops usually after several days. This lesion, referred to as the 'chancre', is associated with establishment of metacyclic trypanosomes (Emery *et al.*, 1980). After proliferation at the chancre the trypanosomes invade the blood stream and tissues via the lymph stream. It has been observed that the development of the chancre and the enlargement of the draining lymph nodes occur at the same time (Akol and Murray, 1982). The development and distribution of trypanosomes from the chancre is species dependent. When goats were bitten by infected *G. m. centralis*, trypanosomes in the skin multiplied, reaching maximum numbers when the chancre attained its maximum size (Dwinger *et al.*, 1988). In goats infected with *T. vivax* and *T. brucei* trypanosomes were observed in the blood before the peak of the chancre, while in *T. congolense* infected goats parasites were found in the blood only during the decline of the chancre (Dwinger *et al.*, 1988).

Chancres have been reported to occur in all domestic ruminants following tsetse-transmitted infections with pathogenic trypanosome species.

In cattle, chancres have been described following experimental infection with *T. congolense* (Murray *et al.*, 1980; Akol and Murray, 1982,1983), *T. vivax* (Emery *et al.*, 1980) and *T. brucei* (Akol and Murray, 1983).

In goats, chancres have been observed following experimental infection *with T. congolense* and *T. vivax* (Emery and Moloo, 1981) and *T. brucei* (Emery and Moloo, 1980).

In addition to tsetse inoculation of metacyclic trypanosomes, needle inoculation of metacyclic trypanosomes has been shown to cause chancre development (Luckins *et al.*, 1981; Akol and Murray, 1982;). Later, Dwinger *et al.* (1987) using metacyclic forms of *T. congolense* propagated *in vitro*, was able to elicit skin reactions in goats similar to the chancre induced by the bite of infected tsetse fly

by intradermal inoculation. The onset, size and duration of these chancres were dose dependent (Dwinger *et al.*, 1987).

2.4.1.2 Anaemia

The most common clinical manifestation associated with bovine trypanosomiasis is that of progressive anaemia. The onset of anaemia is associated with the first wave of parasitaemia, while the rate of development and severity of anaemia reflects the intensity and duration of parasitaemia (Murray and Dexter, 1988). Anaemia in trypanosomiasis has been reported as being haemolytic (Holmes, 1976; Murray and Dexter, 1988). Haemodilution is also an important feature of the developing anaemia, especially in the early stages of the disease (Holmes, 1976; Murray and Dexter, 1988). In a study to determine the influence of energy intake on the pathophysiology of *T. congolense* in sheep, Katunguka-Rwakishaya *et al.* (1997a) attributed the severe anaemia observed in low energy intake sheep to destruction of red blood cells and haemodilution.

Rate of recovery from anaemia of infected animals appeared to be dependent on their nutritional status. Trypanosome infected goats supplemented with cotton seed cake showed a higher rate of recovery from anaemia than those without dietary supplementation (Katunguka-Rwakishaya *et al.*, 1997b).

2.4.1.3 Pyrexia

A state of pyrexia often occurs during trypanosome infections. The elevated temperatures may contribute toward red cell damage. Periods of pyrexia in trypanosome infections have been associated with periods of parasitaemia (Murray and Dexter 1988; Mutayoba *et al.*, 1995a).

In *T. congolense* infections, persistent low level pyrexia has been described in acute infections, while intermittent low level pyrexia and occasional pyrexia have been observed in sub-acute and chronic disease respectively (Mutayoba *et al.*,1995a). Contrary, *T. vivax* infections in cattle are normally characterised by pyrexia (Obi and Anosa, 1980; Mwongela *et al.*, 1981).

2.4.1.4 Acute Haemorrhagic syndrome

Acute haemorrhagic syndrome in which death occurs 2 to 4 weeks after infection has been observed in East Africa. This syndrome has been associated with certain isolates of *T. vivax* (Mwongela *et al.*, 1981; Wellde *et al.*, 1983, Dolan *et al.*, 1992). In Kenya, acute *T. vivax* syndromes in cattle have been reported mainly at the coastal region, where death occurred after a brief illness (Njogu *et al.*, 1985; Dolan *et al.*, 1992; Mwongela *et al.*, 1981). In Ethiopia, an outbreak of acute *T. vivax* infections has also been reported (Roeder *et al* 1984).

The disease is associated with pronounced pyrexia, persistent levels of parasitaemia, and blood-stained diarrhoea. Gross pathological changes including generalised petechial and ecchymotic haemorrhages on mucosa and serosae have been observed (Mwongela *et al.*, 1981). Extended prothrombin times and elevated fibrinogen levels have been reported (Wellde *et al.*, 1983). According to Ellis *et al.* (1987), leukopenia may be a contributory factor to the development of the disease and thrombocytopenia has been recognised in experimental and natural *T. vivax* infections (Wellde *et al.*, 1983).

2.4.1.5 Reproduction disorders

Reproductive disorders are a common occurrence in animal trypanosomiasis (Ikede *et al.*, 1988; Mutayoba *et al.*, 1995a). The effect of trypanosomiasis on reproduction have been studied in sheep, goats and cattle (Sekoni *et al.*, 1990; Okuna *et al.*, 1993; Mutayoba *et al.*, 1995b). Reproduction disorders include degeneration of hypothalamus, pituitary glands and gonads with consequent disruptions in secretions and plasma concentrations of the hormones necessary for normal reproductive processes in both sexes (Sekoni, 1993). In infected female animals, irregular oestrous cycles, infertility, anoestrus, abortion, premature deliveries, cystic degeneration of ovaries and genital lesions have been observed (Luckins *et al.*, 1986; Mutayoba *et al.*, 1996; El Hassan *et al.*, 1994, 1995). Similarly, in male animals infected with haematic trypanosomes, damaged germinal epithelium, spermatozoa morphological abnormalities and aspermia have been observed (Sekoni, 1993). Most of these reproductive impairments are reversible after treatment, though it takes a long period if the abnormalities are severe (Ikede *et al.*, 1988; Omeke and Onuora, 1992). However, Sekoni *et al.*, (1992) observed that Novidium® chemotherapy was not

effective at 12 weeks post treatment in reducing the elevated values of spermatozoa morphological abnormality in Zebu bulls. In addition, genital lesions persisted after chemotherapy (Sekoni, 1990)

2.4.1.6 Production losses

Subsequent decrease in production performance is an important feature in trypanosome infected cattle. Anene *et al.* (1991) attributed reproduction wastage and poor lactational performance of Friesian dairy cattle introduced to a tsetse endemic area, to severe trypanosomiasis diagnosed in the herd. Agyemang *et al.* (1991) observed a 25% decline in milk yield of trypanosome infected cattle. Mutayoba *et al.* (1995a) described reduced growth rates and general loss of body condition in Scottish Blackface rams infected with *T. congolense.*

2.4.2 Immune responses associated with bovine trypanosomiasis

Following infection of animals with trypanosomes there is activation of the immune system. The host begins to generate IgM and IgG against the variable surface gycloprotein (VSG), the primary immunogenic protein covering the trypanosome surface. This antibody response successfully eliminates the majority of the parasites. Despite the largely successful immune response, a small number of the parasites, one out of every 10^5 to 10^6 parasites, will undergo a switch at the genetic level and begin to express a new VSG. These parasites will not be eliminated and will replicate unchecked until a new immune response is generated. This pattern is repeated again and again resulting in a fluctuating chronic parasitaemia (Hudson and Terry, 1979). This fluctuation is also seen symptomatically in the form of a relapsing and recurring fever (Taylor and Mertens, 1999). At the genomic level, VSG switching occurs by two mechanisms. VSG expression sites are located at the telomeric ends of chromosomes. Individual genes can be copied and then displace the currently expressed copy of the VSG, or alternatively the active telomeric site can be silenced, and another activated. Only one expression site, and thus only one VSG is expressed on a trypanosome at a time.

On the parasite surface, the VSGs are physically switched both spontaneously and by immune pressure. The switch is mediated by phospholipase C, which rapidly cleaves the glycophosphoinositol (GPI) anchor that binds the VSG to the parasite and

allows another VSG to take the former's place. The rapid switching of VSGs and the large number of different VSGs, postulated to be over a 1000 unique types, plus variants resulting from recombination of genes, allow the trypanosome to constantly evade the immune response. The antigenic switching found in bovine trypanosomiasis makes vaccine development very challenging (Taylor and Mertens, 1999).

2.4.3 Immuno-depression associated with trypanosomiasis

Goodwin *et al.* (1972) reported on the immunodepressive effect of trypanosomiasis in laboratory animals. While immunosuppression during trypanosome infections in cattle is less marked it is significant (Ilemobade *et al.*, 1982). Mice infected with *T. brucei* have been observed to suffer severe immuno-suppression. Immunodepression has been associated with altered macrophage activity and generation of suppressor T-cells (Grosskinsky and Askonas, 1981). In both infected trypanosusceptible and trypanotolerant breeds of cattle T cell and macrophage/monocyte responses are depressed (Taylor and Mertens, 1999).

2.4.4 Acquired immunity associated with trypanosomiasis

Cattle receiving regular treatment and exposed to natural tsetse challenge slowly develop immunity. This was indicated by reduction in the number of infections from five to just one in a year following diminazene treatment of infected animals exposed to natural tsetse challenge (Whiteside, 1962; Wellde *et al.*, 1989). Wilson *et al.* (1975a, 1975b, 1976), in a series of experiments in cattle concluded that;

- It is necessary to allow development of trypanosomiasis in a host before drug-treatment to induce any degree of immunity
- Immunity depends on the level of trypanosome challenge.

In a controlled experiment, cattle infected by tsetse harbouring a cloned derivative of *T. congolense* and treated with diminazene aceturate 3-4 weeks following infection were immune when infected with homologous clones 3-5 weeks later (Akol and Murray, 1985).

2.4.5 Diagnosis of the disease

2.4.5.1 Overview

The diagnosis of trypanosomiasis rests ultimately on the demonstration and identification of the parasite in the host (Nantulya, 1990). However, a presumptive diagnosis of trypanosome infection may often be made on epidemiological, serological or clinical grounds. It is important that accurate diagnosis of the disease is carried out in order to understand the epidemiology of animal trypanosomiasis in any location and for assessing the efficacy of treatment.

2.4.5.2 Clinical diagnosis

Epidemiological evidence of recent contact with tsetse flies is an important factor in considering the presence of trypanosomiasis. However, chronic trypanosomiasis and the wasting processes associated with it may take many months to manifest itself, and the animal may have been removed from the source of infection some months before symptoms develop. In addition, clinical symptoms in chronic trypanosomiasis may be difficult to distinguish clinically from malnutrition or severe intestinal helminthiasis. Similarly, the acute form of trypanosomiasis may easily be confused with other diseases such as ECF, babesiosis and rinderpest. Nevertheless, clinical diagnosis still remains the most commonly used method in the field. It is imperative that the presence of trypanosomes be demonstrated in infections before considering any form of treatment. However, this may not always be possible because of lack of availability of adequate facilities, equipment and transport to carry out parasitological diagnosis.

2.4.5.3 Parasitological diagnostic techniques

These techniques depend on demonstration of the trypanosomes in the blood. The only detection methods available for many years were direct methods (Molyneux, 1975). These methods include wet, thick and thin blood smears, inoculation of blood into susceptible hosts and haemo- concentration.

Trypanosomes in peripheral blood can be detected by microscopic examination of wet film or Giemsa-stained thick and thin blood smears. The most useful method of thick smear preparation for examination of trypanosome is that of

MacLennan (1957). Thick smears have the disadvantage that identification of the species of trypanosome is sometimes difficult because of distortion during the staining process. Fiennes (1952) considered the thin smear to be 120 times less sensitive than thick smear for detection of trypanosomes.

The sensitivity of direct microscopy has been improved through simple concentration methods. These consists of haematocrit centrifugation (Woo, 1970), the dark ground/ phase contrast (DG) technique (Murray *et al.*, 1977) and the miniature anion exchange technique (Lumsden *et al.*, 1979). Centrifugation of unclotted blood in micro-haematocrit capillary tubes concentrates the trypanosomes in the buffy coat. The trypanosomes are inspected through the walls of the tube, or by breaking the tube and making smears of the interface material (Woo, 1970; Murray *et al.*, 1977). This increased the sensitivity over the earlier methods.

In addition, the DG buffy coat technique (Murray *et al.*, 1977) allows the estimation of intensity of parasitaemia and identification of trypanosome species (Paris *et al.*, 1982). The DG technique is widely used as the diagnostic method to estimate the prevalence of trypanosomiasis in domestic livestock. It is also useful in determining individual trypanosome cases for treatment. Nevertheless, the sensitivity of this method is limited when detecting low or intermittent parasitaemia as in chronic *Trypanosoma congolense* infections.

2.4.5.4 Mouse Sub-inoculation

This method is used to multiply the number of trypanosomes in the blood from a low parasitaemic animal by infecting a laboratory mouse with this blood.

Intra-peritoneal inoculation of suspected blood into laboratory animals and examination of blood from inoculated animals thereafter has proved to be a valuable diagnostic aid. This is especially useful for *Trypanosoma brucei* infections. However, it is not useful for other species of trypanosomes, such as the East African *T. vivax* and some strains of *T. congolense*, that do not infect laboratory rodents (Paris *et al.*, 1982). Wider application of this method is limited since inoculated rodents have to be examined for subsequent one or two months before being ruled out as non-infected. Consequently routine diagnosis is usually done using a

combination of the above methods as any one alone is not good enough to detect all parasitaemic cases.

2.4.5.5 Immunological Techniques

2.4.5.5.1 Antigen enzyme linked immunosorbent assay (ELISA)

Antigen detection ELISAs for species-specific diagnosis of trypanosomes were developed at the International Livestock Research Institute (ILRI). The assay can be used to detect circulating antigens of *T. congolense* and *T. vivax* (Nantulya and Lindqvist 1989; Masake and Nantulya, 1991). These assays are referred to as antigen-trapping ELISAs.

The assays make use of species-specific monoclonal antibodies to capture the circulating trypanosome antigens in the serum of infected animals. The same antibody labelled with horseradish peroxidase is then introduced and binds to free epitopes of the immobilised antigen. The chromogen changes colour due to activity of the peroxidase in the presence of substrate and is used to detect the labelled antibody.

Comparisons of the Antigen ELISA technique with the buffy coat/dark ground technique (BCT) for the diagnosis of two subsequent experimental trypanosome infections in different breed of cattle have been done (Mattioli and Faye, 1996). The overall trypanosome percentage of positive cases detected by buffy coat (BCT) in blood samples was significantly higher in comparison with that obtained by Ag-ELISA in tested serum samples of the three cattle breeds (Mattioli and Faye, 1996). In addition, Ag-ELISA was less than 50 % sensitive in detecting circulating antigens during the first 2 months of the primary infection. However, it showed a high and stable sensitivity throughout the second trypanosome infection.

The Ag-ELISA has been adapted as a diagnostic kit by scientists at the Animal production and Health Unit, International Atomic Energy Agency (IAEA). Investigations into the sensitivity and specificity of these kits showed that while overall specificities were high, sensitivities were poor (Eisler *et al.*, 1998).

Consequently, Ag-ELISA is a useful tool that can be used in epidemiological surveys where prevalence of trypanosomiasis in given locations needs to be estimated, but needs to be combined with BCT to provide reliable results.

2.4.5.5.2 Antibody Enzyme linked immunosorbent assay (ELISA)

In this technique, circulating antibodies against trypanosome infections in cattle are detected rather than the antigen. However, the assay may fail to detect antibodies in early infections as the host immune response in the early stages of infection may be below their sensitivity level. Furthermore, antibody-detection system is unable to distinguish between current and past infections (Luckins *et al.*, 1979). Persistent antibody levels in animals after curative treatment, have been observed for 40 days after diminazene aceturate treatment (Luckins *et al.*, 1979).

An adaptation of the indirect antibody ELISA technique for use with dried blood spots on filter paper was evaluated in Zambia (Hopkins *et al.*, 1998). Sensitivity and specificity for sera were 86.1 and 95.2 % respectively. Whereas, for bloodspots sensitivity and specificity were 96.8 and 95.7 % respectively (Hopkins *et al.*, 1998).

2.4.5.6 Detection of trypanosome deoxyribonucleic acid (DNA)

Developments in molecular biology and advances in recombinant DNA technology have created new avenues for major improvements in parasite detection and characterisation

2.4.5.6.1 Deoxyribonucleic acid probes

In the diagnosis of infection, a specific nucleic acid probe has been used to hybridise the complementary pathogen-specific nucleic acid species directly in the specimens immobilised on solid phases (Majiwa and Webster, 1987; Gibson *et al.*, 1988; Majiwa and Otieno, 1990). Probes are labelled with a radio-nucleotide of biotin to permit detection of specific hybrids. Massamba and Williams (1984) first described how DNA probes can be used in a simple test to distinguish *Trypanozoon* species from other trypanosome species. Kukla *et al.* (1987) refined these techniques to identify *Trypanosoma brucei*, *Trypanosoma vivax* and two types of *Trypanosoma*

congolense in experimentally infected flies. Using the DNA probe therefore, the morphological identification of all trypanosome species, particularly those in tsetse have been possible (Majiwa *et al.*, 1986; McNamara *et al.*, 1989).

2.4.5.6.2 Polymerase chain reaction (PCR)

The inception of polymerase chain reaction (PCR) has enabled amplification of minute amounts of parasite DNA that can then be exposed to a hybridised probe (Moser *et al.*, 1989). Amplification of 10% of the DNA in a single parasite of *Trypanosoma congolense* or *Trypanosoma brucei* produced sufficient amplified product to be visible as a band in an agarose gel stained with homidium bromide. This level of detection that does not depend on the use of radioactivity, is about 100 times more sensitive than detection methods based on radioactive DNA probes (Moser *et al.*, 1989). Amplification of DNA from the blood of animals infected with *T. congolense* and/or *T. brucei* permits the identification of parasite levels far below that detectable by microscopic inspection. Furthermore, Masiga *et al.* (1992) using species or subspecies-specific oligonucleotide primers in the polymerase chain reaction (PCR) were able to identify *Trypanosoma simiae*, *Trypanosoma congolense* (savannah, forest and Kenya coast subgroup), *T. brucei* and *T. vivax* accurately. By incorporating more than one set of primers in a single reaction it was possible to detect mixed infections (Masiga *et al.*, 1992).

Evaluation of PCR technique when applied directly to cattle sera for diagnosis of trypanosome infections was carried out in French Guyana (Desquesnes, 1997) and in Uganda (Clausen *et al.*, 1998). The PCR was able to detect active *T. vivax* infection in sera samples when parasitaemia was over 10^3 trypanosomes /ml (Desquesnes, 1997).

The application of these molecular diagnostic tests, provides useful new tools not only for the understanding of the complexity of the taxonomy of the trypanosomes, but also as sensitive techniques for conducting epidemiological surveys. However, the disadvantage of such tests is their practical use in the field where appropriate facilities are lacking.

2.5 Disease control

2.5.1 Evolution of chemotherapy

2.5.1.1 Antimony derivatives

The use of compounds with trypanocidal activity started as early as 1908, when Plimmer and Thomson showed that infections with *T. brucei* and *T. evansi* in laboratory rodents could be eliminated with potassium antimony tartrate (tartar emetic) or its sodium analogue. Tartar emetic was subsequently introduced for the control of trypanosomiasis. However, because it provoked severe tissue reactions, the drug was given intravenously at a dose rate of 1.0 - 1.5 g in a 5 per cent aqueous solution, repeated at daily or weekly intervals. Bevan (1928) confirmed the efficacy of potassium antimony tartrate against most *T. congolense* and *T. vivax* infections in cattle. However, the compound was not effective against *T. brucei* in cattle.

Other derivatives of antimony also proved to possess trypanocidal activities. These were antimosan, an analogous sulfonated pyrocatechol, and stilbophen, its sodium salt. These drugs could be given intramuscularly or subcutaneously. Parkin (1935) found antimosan to be effective against *T. congolense* and *T. vivax*, but less against *T. brucei*. However it required repeated doses of 3-6 g per 300-400 kg b.w., given at four weekly intervals (Parkin, 1935).

2.5.1.2 Napthylamine derivatives

2.5.1.2.1 Suramin

A synthetic compound suramin (Germanin®, Naganol®, Bayer 205®), developed in Germany during the 1914-18 war became available in 1920. Suramin is the oldest of the trypanocides used in domestic livestock. It is a sulphonated napthhylamine and has been shown to be effective against experimental infections with *T. equiperdum* and also in the treatment of naturally occurring *T. evansi* infections in camels and in other species (Edwards *et al.*, 1956). Consequently suramin has been the drug of choice for the treatment of *T. evansi* infections in camels and horses for many years (Zhang *et al.*, 1991). It is also considered as the drug of choice for early stages of African human trypanosomiasis especially, *T. b.*

rhodesiense infections. Suramin is strongly ionic and binds readily to plasma proteins (Fairlamb and Bowman, 1980); traces may be found up to three months after intravenous administration, thus accounting for its prophylactic activities (Gutteridge, 1985). The drug may cause delayed toxicity including nephritis and has been shown by Smeesters and Jacques (1968) to aggregate in lysosomes. Suramin has been used to treat *T. evansi* infections in cattle and buffalo at doses of approximately 3 g per animal. However, the drug is ineffective against *T. vivax* and *T. congolense* infections (Leach and Roberts, 1981). Suramin is only administered intravenously as it can cause severe local reactions (Williamson, 1970), as such, routine administration of the drug requires a high level of veterinary management (Bennett, 1933).

After more than 60 years of use there is increasing evidence of drug resistance (Rottcher and Schillinger, 1985; El Rayah *et al.*, 1999). Unfortunately, the future availability of Suramin is currently in question since manufacture of the compound has been discontinued (Otsyula *et al.*, 1992).

2.5.1.3 Quinoline derivatives

2.5.1.3.1 Surfen C

During the same period that suramin was being developed, attention was also directed to the styrlquinolines, some of which were found to be active trypanocides against organism of the *Trypanozoon* subgenus (Browning *et al.*, 1926). However, styrlquinolines did not act rapidly on trypanosomes and were systematically toxic, producing severe reactions at the site of injection. Surfen-C, a 4 aminoquinoline derivative, was shown to have had marked activity against *T. congolense*, but in field trials the drug produced unacceptable toxic reactions.

2.5.1.3.2 Quinapyramine

Synthesis of quinapyramine, a quinoline pyrimidine was carried out by restructuring Surfen-C molecule (Barrett *et al.*, 1953). The activity of the soluble quinapyramine dimethosulfate, marketed as (Antrycide sulphate®), against most of the trypanosomes pathogenic to livestock was demonstrated experimentally (Curd and Davey, 1949, 1950), and in field trials (Davey, 1950). The drug has marked

prophylactic effect and is well tolerated by cattle, although some animals show symptoms of post-injection stress. A formulation containing a mixture of quinapyramine dimethosulfate (3 parts) and the insoluble quinapyramine chloride (2 parts) was marketed in 1958 as Antrycide pro-salt® for prophylactic purposes.

Whiteside (1960) showed that this formulation was effective and could protect animals in Kenya for two months in medium fly challenge. The compound was widely used in the rest of Africa between the 1950s and 1970s as a therapeutic and prophylactic drug against *T. congolense*, *T. vivax* and *T. brucei* infections in cattle sheep and goats (Leach and Roberts, 1981). *Trypanosoma evansi* and *Trypanosoma equiperdum* infections have been treated effectively using quinapyramine (Leach and Roberts, 1981). Because of problems with drug toxicity and ease with which drug resistance appeared to develop (Ndoutamia *et al.*, 1993), the manufacture of the drug was stopped in 1974 (Schillinger and Rottcher, 1984). However, the withdrawal of the compound compromised the control of trypanosomiasis in camels. As such, quinapyramine was re-introduced into the market, under two different names, but to be use only in camels, horses and donkeys (Schillinger and Rottcher, 1984). One of the products, Tribexin Prosalt® (quinapyramine sulphate: quinapyramine chloride, in ratio 3:4; Indian Drugs and Pharmaceuticals Ltd, India) is recommended for use in *T. evansi* infections in donkeys and camels (Suryanarayana *et al.*, 1985). Another product, Trypacide® (May and Baker, UK) is available in two forms for field use. Trypacide sulphate® is recommended for subcutaneous treatment for clinical cases and Trypacide Pro-salt® (quinapyramine sulphate: quinapyramine chloride, in the ratio of 3:2) is recommended for prophylaxis.

The compound is no longer recommended for use in cattle because of previously observed problems with drug resistance and particularly because induction of resistance to quinapyramine appeared to be associated with cross-resistance to each of the other trypanocides that are used in cattle (Whiteside, 1960).

2.5.1.4 Phenanthridine derivatives

2.5.1.4.1 Phenidium chloride

Further improvement in drug development led to a report by Browning *et al.* (1938) that phenidium chloride was active against the *Trypanozoon* subgenus as well as *T. congolense.* This marked an important advance in development of phenanthridines as trypanocides. However, the solubility of phenidium chloride was low and it had a narrow therapeutic index. This led to the introduction of dimidium bromide

2.5.1.4.2 Dimidium bromide

Dimidium bromide was readily soluble and was shown to be active against *T. congolense* in the field by Carmichael and Bell (1944); a dose of 1 mg/kg b.w. administered subcutaneously would eliminate the majority of infections. The drug was used for mass treatment campaigns in East and Central Africa, but drug resistance had become widespread by 1952 and the use of higher doses resulted in severe toxicity. Randall and Beveridge (1947) observed that photosensitization occurred in all cattle treated with 2 mg/kg b.w., and reported numerous cases of photosensitization and also local reactions when cattle received 1 mg/kg b.w. subcutaneously.

2.5.1.4.3 Homidium bromide/chloride

Watkins and Woolfe (1956) reported development of a new amino-phenanthridinium compound, homidium bromide from dimidium chloride. This drug proved to be effective against *T. congolense* and *T. vivax* at 1 mg/kg b.w as dimidium bromide and was considerable less toxic (Ford *et al.*, 1953a,b). The introduction of homidium and quinapyramine dimethosulfate made possible for the first time, the safe mass treatment of cattle in contact with tsetse. The number of animals injected increased dramatically. However, homidium-resistant strains of *T. congolense* began to be identified and by 1966, it was reported that they were becoming widespread Jones-Davis and Folkers (1966).

2.5.1.4.4 Pyrithidium bromide

Watkins and Woolfe (1956) reported the synthesis of a new trypanocidal drug pyrithidium bromide, by substituting the pyrimidyl moeity of quinapyramine into a phenanthridine resembling phenidium. Watkins (1958) showed this compound to have a marked therapeutic and prophylaxis properties. Whiteside (1960) found that 0.2 - 0.4 mg/kg would cure *T. congolense* infections in cattle and was able to protect cattle for 4-6 months in an area of high tsetse challenge. The drug produced severe local reactions when given subcutaneously at doses over 2.5 mg/kg (Stephen, 1962b) and intramuscular injections of 5 mg/kg were considered to be responsible for deaths (Leach and El Karib, 1960). Resistance to pyrithidium tends to occur readily and often involves cross-resistance to quinapyramine and homidium.

2.5.1.4.5 Isometamidium chloride

The synthesis from homidium of a compound named metamidium was reported by Wragg *et al.* (1958). This consisted of two isomers; one red and the other purple. The red isomer was considerably more water soluble and active than the purple isomer. The red isomer was subsequently isolated in pure form (Berg, 1963) and marketed as isometamidium. It was recommended that the drug should be administered intramuscularly at a dose of 0.5 mg/kg b.w. for drug sensitive infections 1.0 mg/kg b.w. for drug resistant infections and 2.0 mg/kg b.w. for prophylaxis (Berg, 1963).

2.5.1.5 Diamidine derivatives

2.5.1.5.1 Diminazene aceturate

Bauer (1955) and Fussganger (1955) reported trials with a new aromatic diamidine, diminazene aceturate. A dose of 3.5 mg/kg b.w was claimed to cure *T. congolense* and *T. vivax* infections in cattle, but a higher dose was necessary to eliminate *T. brucei* infections, and the drug was not effective against *T. simiae.* It had a considerable wider therapeutic index than other trypanocidal drugs then available. Fairclough (1963) reported the safe use in Kenya of subcutaneous doses of up to 21 mg per kg b.w. in cattle. When diminazene was first introduced it was suggested that

its rapid excretion might reduce the risk of resistance developing since trypanosomes would not be exposed to extend periods of low concentrations.

2.5.1.6 Arsenical derivatives

2.5.1.6.1 Melarsenoxide cysteamine

Melarsenoxide cysteamine, a melaminyl thioarsenite is the most recently introduced amongst the trypanocide used in domestic livestock (Raynaud *et al.*, 1989). The molecule was patented in 1985 by E. Freidheim and was synthesised by combining melarsen oxide with cysteamine. In addition to its brand name (Cymelarsan®, Rhone Merieux, Toulouse, France), it is known as Mel Cy or RM 110. The drug product is a white powder highly soluble in water. It is active against members of the *Trypanozoon* subgenus (Raynaud *et al.*, 1989), particularly *T. evansi*. Trials with this drug in camels experimentally infected with *T. evansi* have shown therapeutic activity over the dose range 0.3 – 1.25 mg/kg b.w. (Tager-Kagan *et al.*, 1989; Zelleke *et al.*, 1989). Efficacy against natural infections with *T. evansi* has been demonstrated in camels in Kenya, over the dose range 0.2-1.2 mg/kg b.w. (Otsyula *et al.*, 1992) and in buffaloes in China over the dose range 0.25-3.0 mg per kg b.w. (Lun *et al.*, 1991). The drug has also been reported to exhibit activity against *Trypanosoma brucei* (Raynaud *et al.*, 1989). However, the compound has no prophylaxis properties (Raynaud *et al.*, 1989). Because the drug has satisfactory efficacy in the lower dose ranges, but has local and systematic toxicity at the higher doses, it is recommended that a standard dose of 0.25 mg/kg b.w should be used for treatment of acute, sub-acute and chronic *Trypanosoma evansi* infections in camels.

Following subcutaneous administration, maximum concentrations in cattle, horses and camels were 86, 120 and 98 ng/ml respectively (Raynaud *et al.*, 1989). These concentrations were attained at about 30-min post treatment. The maximum plasma concentration observed after intramuscular administration of the drug in cattle was slightly higher than that found after subcutaneous administration. The half-life for drug in camels was 32.58 h. However, the half-life reported appears to have been calculated on the basis of the entire disposition curve, as such it may not be the correct value for the elimination half life of the drug in camels (Kinabo, 1993).

Absolute bioavailability in camels was 70-80% (Raynaud *et al.*, 1989). The recommended withdrawal time for meat and dairy animals is two weeks.

The therapeutic index (lethal dose/therapeutic dose) of Mel Cy is greater than 10 indicating that the drug is reasonably safe for use at the recommended dose. After subcutaneous administration, the drug has been observed to induce a limited swelling and oedema in cattle and some camels. No effects observed were observed in both mutagenic and embryotoxic studies. Thus, the drug is not contraindicated for use in pregnant animals (Raynaud *et al.*, 1989).

However, resistance to the drug has been reported in *T. evansi* and *T. brucei* population in laboratory studies (Osman *et al.*, 1992; Pospichal *et al.*, 1994).

Table 1: Chemotherapeutic and chemoprophylaxis compounds used for animal trypanosomiasis

Compound	Trade name	Dose (mg/kg)	Route	Use	Activity against	Animal
Diminazene aceturate	Berenil®	3.5 - 7.0	i.m.	T	*T. congolense*	Cattle
	Veriben®				*T. vivax*	small ruminants
	Norotryp®				*T. brucei**	dogs!
	Ganaseg®				*T. evansi**	equine!
Homidium chloride	Novidium®	1.0	i.m.	T/P[a]	*T. congolense*	Cattle
Homidium bromide	Ethidium®				*T. vivax*	small ruminants
					T. brucei	dogs
					T. evansi	equine
Isometamidium chloride	Samorin®	0.25 - 0.5	i.m.	T	*T. congolense*	Cattle
	Trypamidium®	0.5 - 1.0	i.m.	P	*T. vivax*	Small ruminants
					T. brucei	Equine
					T. evansi	camels
Quinapyramine dimethylsuphate	Trypacide sulphate®	3.0 - 5.0	s.c.	T	*T. congolense*	Camels
					T. vivax	equine
Quinapyramine dimethyl sulphate: chloride (3:2 w/w)	Trypacide Pro-salt®	3.0 - 5.0[b]	s.c.	P	*T. brucei*	Pigs
					T. evansi	dogs
					T. equinum	
					T. simiae	
Suramin	Naganol®	7.0 - 10.0[c]	i.v.	T(P)	*T. evansi*	Camels
						Equine
Melarsomine	Cymelarsen	0.25	s.c./i.m	T	*T. evansi*	Camels

T therapeutic

P prophylaxis

* limited activity

! small therapeutic index

a Prophylaxis observed in areas of low tsetse challenge

b Dosage of sulphate

c Grams per animal

2.5.2 Trypanocides currently in use

Chemotherapy and chemoprophylaxis for trypanosomiasis in domestic livestock currently depend upon the salts of six compounds.

Table 1 lists the dosage regimens for each compound, their spectra of activity, the animal species in which they are used and the routes by which the compounds should be administered. Diminazene, homidium and isometamidium are primarily used for treatment and prophylaxis of trypanosomiasis in cattle, sheep and goats.

2.5.2.1 Diminazene aceturate

Diminazene (Berenil®, Veriben®, Norotryp®, Ganaseg®) is described as an aromatic diamidine and is marketed in combination with phenyldimethyl pyrazole (antipyrine) in ratio 44.5:55.5 w/w. This stabilises and prolongs the activity of the drug in aqueous solutions (Fairclough, 1963). Sensitive populations of *T. congolense* and *T. vivax* can be eliminated by intramuscular treatment of the host with diminazene aceturate at the dose rate of 3.5 mg per kg b.w.. However, higher doses may be required to eliminate *T. brucei* infections (Fussganger and Bauer, 1960). Although well tolerated by most domestic species, severe toxic reactions occur in camels given 7 mg/kg b.w. (Leach, 1961).

Diminazene is now probably the most commonly used therapeutic agent for trypanosomiasis in livestock in sub-Saharan Africa. This has been due to a number of factors such as; a) activity against trypanosomes that are resistant to most other trypanocides (Williamson, 1970), b) the low incidence of resistance that had been detected as a result of using the compound and c) a higher therapeutic index in most animal species than other trypanocides (Fairclough, 1963; Williamson, 1970).

However, in recent years, there has been increasing reports of field isolates resistant to diminazene aceturate from Zambia (Joshua and Sinyangwe, 1991; Chitambo and Arakwa, 1992), Zimbabwe (Joshua *et al.*, 1995), Ethiopia (Codjia *et al.*, 1993; Rowland *et al.*, 1993; Mulugeta *et al.*, 1997) and Somalia (Ainanshe *et al.*, 1992).

2.5.2.2 *Homidium bromide/chloride*

Homidium belongs to the phenanthridinium class of compounds. It is manufactured as both the bromide (Ethidium®) and the chloride salts (Novidium®), which are equally active *in vivo* (Leach and Roberts, 1981). Both salts are recommended for use as therapeutic agents administered intramuscularly at a dose of 1.0 mg/kg b.w.. It is rapidly absorbed from the injection site, and peak blood concentration of about 268.9 ng/ml is attained after 15 min. (Murilla *et al.*, 1996a). Bio-availability of the intra-muscular dose of homidium is 62.5% and 57.8% in non-infected and trypanosome infected cattle, respectively and approximately 90 % of the total dose is excreted within 14 days (Murilla *et al.*, 1996a). Homidium is active against *T. congolense* and *T. vivax* infections in cattle, sheep and goats (Watkins and Woolfe, 1956; Dolan *et al.*, 1992; Murilla *et al.*, 1996a).

Homidium was extensively used but its usefulness has been greatly reduced due to widespread resistance (Scott and Pegram, 1974; Na'Isa, 1967). Homidium bromide has been used as a prophylactic (Whiteside, 1962; Mwambu, 1971). Prophylactic periods of up to 17 weeks have been reported in a ranch situation in Kenya (Dolan *et al.*, 1990, 1992). This could be due to high level of homidium observed at the injection site, possibly acting as a depot for slow release of the drug, and the persisting high levels in the organs acting as secondary drug depots (Gilbert and Newton, 1982; Murilla *et al.*, 1996a). Variation in homidium susceptibility and the level of trypanosome challenge was indicated as factors that influenced the duration of prophylaxis. According to Dolan *et al.*, (1992), one year of continued use reduces the effectiveness of the drug three-fold.

2.5.2.3 *Isometamidium chloride*

2.5.2.3.1 *Drug composition*

Isometamidium is a phenanthridinium-aromatic amidine formed by combining homidium with the diazotised p-aminobenzamide moiety of diminazene (Berg, 1963). The drug product of isometamidium marketed as Samorin® and Trypamidium® contains 70% isometamidium and the remaining fraction (30 %) is a mixture of its two isomers, a small proportion of a bis-compound and homidium (RMB Animal Health).

No studies have addressed the speculation that these isomers may account for the observed differences in sensitivity of Kenyan *T. vivax* populations to the prophylactic and therapeutic activities of isometamidium in Boran cattle (Peregrine *et al.*, 1991).

2.5.2.3.2 Drug administration and drug elimination

The manufacturer recommends intramuscular injection of 0.5 mg/kg for curative treatment of sensitive strains, and a dose range of 0.5-1.0 mg/kg b.w. for prophylaxis purposes. Following intramuscular administration, isometamidium is rapidly detected in plasma, and maximum plasma concentrations are attained within 1 hr after drug administration (Kinabo and McKellar, 1990; Kinabo *et al.*, 1991; Murilla *et al.*, 1996b; Eisler *et al.*, 1996). The bioavailability of the drug is very low according to Kinabo and McKellar (1990), about 58% in non-infected cattle (Murilla *et al.*, 1996b). It is agreed that the drug forms a depot at the injection site at which the drug is slowly released to exert its prophylactic activity (Hill and McFadzean, 1963; Kinabo and Bogan, 1988; Murilla *et al.*, 1996b).

More recently, in a controlled study, using an isometamidium enzyme-linked immunosorbent assay, the drug could be detected up to 140 days post-treatment after intramuscular administration of isometamidium at 0.5 mg/kg b.w. to *T. congolense* infected cattle (Geerts *et al.*, 1999). In addition, after a subcutaneous implantation of a sustained release device (SRD) containing isometamidium , traces of the drug were detectable until 330 days after treatment (Geerts *et al.*, 1999).

2.5.2.3.3 Mechanism of action

The primary mode of action is blockade of nucleic acid synthesis through intercalation between DNA base pairs, inhibition of RNA and DNA polymerase (Marcus *et al.*, 1982) and incorporation of nucleic acid precursors into DNA and RNA (Lantz and Van Dyke, 1972). This is currently considered to account for the molecular mechanisms of anti-trypanosomal activity of isometamidium.

However, there are a number of biochemical reactions that may account partly to its effect. These include, selective cleavage of kinetoplast DNA mini circles

(Shapiro and Englund, 1990), lipid metabolism, ATP metabolism, membrane transport and modulation of glycoprotein biosynthesis (Casero *et al.*, 1982).

2.5.2.3.4 Mechanism of resistance

The mechanism of resistance to isometamidium, however, is less clear. Decreased levels of drug accumulation have been observed in drug-resistant populations of *T. congolense* (Sutherland *et al.,* 1991) and later work found indirect evidence of an increased efflux of drug from resistant trypanosomes (Sutherland and Holmes, 1993). Recently, Mulugeta *et al.* (1997) showed that the maximal uptake rates (V_{max}) of isometamidium in resistant *T. congolense* were significantly lower than in sensitive populations. It remains to be shown whether this is caused by a decreased number of protein transporters of isometamidium in the plasma membrane and/or by changes in the balance between influx and efflux.

The role of nucleoside transporters in resistance to isometamidium by *T. congolense* remains to be examined, although changes in these transporters have been associated with resistance to arsenical drugs in *T. brucei* (Carter and Fairlamb, 1993; Carter *et al.*, 1995; Ross and Barns, 1996; Barrett and Fairlamb, 1999). In addition, Wilkes *et al.* (1997) has demonstrated changes in mitochondrial electrical potential in isometamidium-resistant *T. congolense.*

2.5.2.3.5 Duration of isometamidium prophylaxis

Isometamidium has been used successfully to maintain the productivity of Zebu cattle in both small holder and ranch management systems in East Africa (Trail *et al.*, 1985).

The period of prophylaxis reported would depend on the definition of "duration of prophylaxis" used by the various authors. Some of the definition that have been used are;

- The average duration of prophylaxis within a group of a number of cattle.
- Time taken for 10 % of the group to become infected
- The period until the first animal in a group becomes infected. This definition would be an indication of the minimum period of prophylaxis to be expected

within a group of animals and the frequency of drug administration required to prevent trypanosome patency within the group.

- Where a sentinel group was present, the time taken for 20% of the sentinel herd to be detected positive.

Nevertheless, whatever definition of prophylaxis used, considerable variation in prophylactic activity of isometamidium has been observed. A dose rate of 1 mg/kg has been shown to confer prophylaxis to cattle for 2-22 weeks (Kirkby, 1964; Pinder and Authie, 1984; Whitelaw *et al.*, 1986; Peregrine *et al.*, 1991). Consequently, various factors have been indicated to influence duration of prophylaxis. These include, drug dosage (Peregrine *et al.*, 1988), tsetse challenge or trypanosomiasis risk (Whiteside, 1962; Dolan *et al.*, 1990, 1992), relapses from tissue impenetrable by the drug (Jennings *et al.*, 1977b), plane of nutrition and stress (Boyt *et al.*, 1962; Ogunyemi and Illemobade, 1989), acquisition of immunity following the use of trypanocides (Bourn and Scott, 1978; Wellde *et al.*, 1989), differing levels of drug sensitivity between trypanosome populations (Peregrine *et al.*, 1991) and drug formulation (Geerts *et al.*, 1997, 1999). Most of these factors have been demonstrated in either field situation or under controlled conditions.

In field trials it has been observed that duration of prophylaxis is related to intensity of tsetse challenge (Whiteside, 1962; Dolan *et al.*, 1990). In studies, at Mkwaja Ranch in Tanzania cattle kept in parts of the ranch with high tsetse challenge required frequent isometamidium treatments than those kept in parts of the ranch with low fly density (Trail *et al.*, 1985). In Kenya, at Galana Ranch in years of low tsetse challenge prophylaxis period of 8-17 weeks was observed whereas, under high tsetse challenge, periods of less than four weeks have been observed (Dolan *et al.*, 1990, 1992). Similar observations were reported in Mozambique where protection conferred by prophylactic drugs was shorter when trypanosomiasis risk was high (Takken *et al.*, 1988).

Studies in experimental animals have indicated that duration of prophylaxis is a function of dose rate, but is not dependent on the intensity of metacyclic *T. congolense* challenge (Peregrine *et al.*, 1988). A dose of 1 mg/kg of isometamidium conferred complete protection for four months while at 0.5 mg/kg b.w. isometamidium protected cattle for three months (Peregrine *et al.*, 1988). In a similar

experiment, Boran cattle treated intramuscularly with 1.0 mg/kg isometamidium chloride and challenged either once or repeatedly at monthly interval with five tsetse flies infected with *T. congolense* were completely protected for 5 months (Whitelaw *et al.*, 1986).

Under experimental conditions, studies have shown vast differences between prophylactic and therapeutic effect of isometamidium chloride at 0.5 mg/kg b.w. on fly transmitted Kenyan *T. vivax* infections in cattle (Peregrine *et al.*, 1991). These findings demonstrated that the two *T. vivax* populations expressed different levels of resistance to the prophylactic action of the drug, but were all sensitive to the therapeutic action of the drug. The results also suggested that variation in drug susceptibility between different trypanosome populations plays an important role in determining the duration of prophylaxis (Peregrine *et al.*, 1991).

The effect of drug formulation on period of prophylaxis was demonstrated in a controlled and a field study. In an experiment under controlled conditions, two groups of cattle were treated with 0.5 mg/kg isometamidium either as a SRD or intramuscularly (i.m.), and exposed at monthly intervals to *G. m. morsitans* infected with *T. congolense.* The average protection period was at least 24 months in the SRD treated against 5.7 months in the i.m. treated group (Geerts *et al.*, 1999).

In a similar trial in the field using isometamidium at 1 mg/kg b.w. the incidence of trypanosomiasis was significantly lower in the SRD treated than in the i.m. treated cattle 12 months after treatment (Geerts *et al.*, 1999).

2.5.3 Alternative options and possible new drugs

2.5.3.1 Vaccination

Immunisation as a method of trypanosomiasis control is not foreseen in the near future due to the substantial antigenic variation that is displayed by trypanosome populations. The rapid switching of VSGs and the large number of different VSGs plus the variants that result from recombination of genes, allow the trypanosome to constantly evade the immune response. This antigenic switching makes vaccine development difficult. Despite these limitations, however, there are a few other hopeful options.

2.5.3.1.1 Flagellar pocket antigens

The flagellar pocket of the trypanosome is an area where receptors are used in specific host macromolecule uptake, and thus it is postulated that these antigens are highly conserved amongst flagellated protozoans (Borst, 1991). For this reason a vaccine against the flagellar proteins of *T. brucei* may also confer protection against many other parasites. Borst, (1991) observed that in chronic trypanosome infections, antibodies are produced against a specific flagellar pocket protein , Trypanosomal transferrin-binding protein (TFBP), whose gene is present in multiple versions. He proposed that a vaccine stimulating antibody production against the repertoire of TFBPs may hinder trypanosome proliferation. He also suggested that several other proteins were present in the flagellar pocket, and they may also serve as targets for vaccines.

Recently, in a field study in Kenya, Mkunza *et al.* (1995) used a flagellar pocket antigen from *T. b. rhodesiense*, with bovine serum albumin as the carrier and alum as the adjuvant, to inoculate 90 cattle under natural disease exposure. The rate of infection was reduced from 13% (in the control herd) to 0.9% (in the immunised herd).

2.5.3.1.2 Cysteine protease (congopain)

A second focus of vaccine research has been on attempting to eliminate the pathogenicity of the parasite, not the infection itself. Cysteine proteases of micro-organisms can degrade host proteins such as immuno-globulins and complement factors. They can also modulate cytokine activities, and are suspected of interfering with antigen presentation and processing (Authie, 1994). In addition, it is known that some cattle species in Africa are more resistant to infection than others (Murray *et al*, 1991). Consequently, studies have shown that a *T. congolense* cysteine protease (congopain) may play a role in the different levels of tolerance (Authie, 1994). The more resistant cattle generate a stronger IgG response to congopain than the less resistant cattle. Attempts are being made to use congopain antigens in a vaccine, which would generate antibodies capable of neutralising the enzyme activity.

2.5.3.1.3 Intracellular antigens

A final strategy in vaccine development may involve intracellular antigens. Members of the Trypanosomatidae family have subpellicular microtubules cross-linked to each other and to the plasma membrane by unique trypanosomal microtubule-associated proteins (MAPs). The trypanosomal MAP (p52) has been used in an antigenic preparation with the enzymes aldolase and GAPDH (which the protein copurifies with) as a vaccine in mice and rats (Balaban *et al.*, 1995). The p52 was isolated from *T. b. brucei*, and when the animals were immunised three times over a period of three weeks, 100% protection was achieved. In addition, the serum from immunised animals also cross reacted with *T. b. rhodesiense* and *T. b. evansi*, giving hope for possibility of cross protection amongst trypanosome species, if not the whole Trypanosomatidae family (Balaban *et al.*, 1995).

2.5.3.1.4 Glycolysis inhibitor as an antiparasitic drug

Glycolysis in the bloodstream form of *Trypanosoma brucei* provides a convenient context for studying the prospects for using enzyme inhibitors as antiparasitic drugs. Recently, it has been suggested that metabolism, and the metabolic effects of drugs, should be considered as one of the steps when designing a drug for vaccines.

The glycolytic pathway is an attractive target because the predominant bloodstream form of *T. brucei* has no energy resources. Once *T. brucei.* enters the bloodstream the parasite relies entirely on rapid glycolysis for its energy supply. So the ability to kill trypanosomes by halting glycolysis may prove to be important in the development of a drug against trypanosomiasis (Eisenthal and Cornish, 1998).

Two basic metabolic methods of killing an organism have been identified as either the flux through an essential metabolic pathway can be decreased to the point where life is no longer possible, or the metabolic concentration can be increased to toxic levels (Bakker *et al.*, 1999). In their model for trypanosomal glycolysis, Bakker *et al.* (1999) demonstrated that there are two possible ways of altering glycolysis of trypanosomes in the bloodstream;

- The first, inhibition of glucose transport was found to have the highest degree of control over trypanosome growth.

- The second, inhibition of pyruvate transport is an alternate strategy to decrease the carbohydrate flux and increase metabolite concentration to catastrophic levels.

However, when the stoichiometric constraints are taken into account, the glycerol transport inhibition can be eliminated due to the fact that under aerobic condition, it has such a low rate of efflux that some authors have doubted whether it occurs at all. Fortunately, the remaining candidate, pyruvate transport has proved to respond to inhibition well. It is expected to have powerful anti-trypanosomal activity. If the metabolic pathway of blood stream trypanosomes can be targeted it may prove to be more successful than inhibition during transcription or translation (Eisenthal and Cornish-Bowden, 1998)

2.5.3.1.5 Phenothiazine Inhibitors as Anti-trypanosomal Drugs:

Glutathione is responsible for many cellular protection activities including those against free radicals and oxygen-derived species. In the course of this action glutathione disulfide is formed from glutathione reductase (GR). Trypanosomes do not contain GR but rather an analogous enzyme, trypanothione reductase (TR). The mutual substrate exclusivity indicated that selective ligand design should be possible, making TR an important potential target for drug design against parasitic diseases involving trypanosomes. Effective inhibition of TR would compromise the parasites' redox defences, making them more sensitive to redox-damage drugs, such as nifurtimox. A trypanothione reductase inhibitor might be an effective anti-trypanosomal drug in its own right, or with the redox-active drug such as nifurtimox (Chan *et al*, 1998).

2.5.3.2 Trypanotolerant breeds of cattle

Wildlife has been known to have genetic resistance to trypanosomiasis. In domestic livestock, certain breeds seem to be less susceptible to trypanosomiasis than others (Murray *et al.*, 1982). Trypanotolerance has been extensively investigated in West Africa. Field and laboratory experiments have led to the identification of taurine cattle breeds namely the N'Dama and the West African shorthorn which possess a high degree of resistance to trypanosomiasis. Trypanotolerance is measured by the ability to survive and be productive under sustained trypanosomiasis challenge

(Murray *et al.*, 1991). Several studies comparing the performance of the N'Dama and the more susceptible breeds have shown the superiority of the N'Dama to the other cattle such as the Borans in terms of packed cell volume and productivity (Kora and Bojang, 1992). Under laboratory challenge with *T. congolense*, *T. brucei* and *T. vivax*, the N'Dama was observed to develop low intensity of parasitaemia compared with breeds such as Zebu, Boule and Boran (Akol *et al.*, 1986; Paling *et al.*, 1991). Under natural challenge a significant higher intensity and longer duration of *T. vivax* parasitaemia in Zebu than N'Dama was reported by Murray *et al.* (1981).

In east Africa, generally, less attention has been paid to the question of trypanotolerance and it has been assumed that the *Bos indicus* cattle in this region are all highly susceptible to trypanosomiasis. However, there has been evidence that some Zebu cattle have acquired some resistance to the disease. In Kenya, at Galana Ranch, the Orma Boran has been shown to be less susceptible to trypanosome infections than the Galana Boran (Njogu *et al.*, 1985). In addition following experimental infection, the Orma Boran has shown significant tolerance to trypanosome infections compared to the Galana Boran. (Ismail *et al*, 1985; 1988).

The performance of the Orma Boran and the Galana Boran under varying tsetse challenge in a different environment was carried out in Kajiado District (Mwangi *et al.*, 1998). The two breeds from Galana Ranch were compared with the Maasai Zebu which is indigenous to Kajiado district. All animals in the three breeds became infected. However, significant differences were observed among the Maasai Zebu, Orma Boran and Galana Boran cattle in the prepatent periods, incidence of trypanosome infection, degree of anaemia, treatment requirements, growth rates and mortality with the Galana Boran showing the most severe effects of infection. (Mwangi *et al.*, 1998). It was concluded that the superior resistance of the Maasai Zebu and Orma Boran over the Galana Boran has a significant genetic basis and the breed should be considered as part of integrated trypanosomiasis control programmes for livestock development in tsetse-infested areas of east Africa.

Trypanotolerance has been observed in sheep and goats (Osaer *et al.*, 1994, 1998). Interactions between *T. congolense* and nutritional status were studied in 42 Djallonke ewes, bred at the peak of parasitaemia after synchronisation of oestrus.It was observed that the most pronounced effect of *T. congolense* was a negative

influence on establishment of pregnancy, with nutritional supplementation unable to overcome this effect but having a beneficial influence on maintenance and successful outcome of pregnancy. However, individual exceptions indicate that some ewes cope better with the negative effects of infection and poor nutrition (Osaer *et al.*, 1998).

Finally, although well-controlled studies have been carried out on productivity and general performance of the trypanotolerant breeds, most of these studies involve breeds from west Africa. Breeds of cattle, sheep and goats in east Africa have not been exploited enough. This needs to be done, so that they can be incorporated in animal livestock production and tsetse control programmes in Eastern Africa.

2.6 Drug resistance

2.6.1 Overview

Drug resistance is the ability of a parasite to survive, what was previously determined to be, lethal concentrations of a toxic drug. This has become a problem, both in medical and veterinary parasitology and has provoked much interest in the field of research.

So far, resistance to one or more of the three trypanocides used in cattle has been reported in at least thirteen countries in sub-Saharan Africa (Geerts and Holmes, 1998). This is probably an underestimation since in many countries surveys have not been carried out or if they have, have not been reported. In addition most of the available information on drug resistance does not give any indication of the prevalence of resistance in a region or country as organised surveys have not been done. Furthermore, the methodologies and criteria used for determining the drug resistance varies between countries and investigators involved (Geerts and Holmes, 1998).

There is need therefore to have systematic surveys, using standardised methodologies concerning number of representative samples, collection and storage, sensitivity testing and data analysis. These types of surveys would provide more reliable data and help to identify factors influencing sensitivity or resistance to trypanocidal drugs. Consequently, such surveys could be incorporated in longitudinal or cross-sectional epidemiological studies on other aspects of trypanosomiasis, being carried out within the various countries by the different departments or institutions. This would cut down on the cost involved and avoid replication of data.

It is also important to understand that drug resistance does not imply an end to chemotherapy. Recent observations have shown that resistant trypanosome populations vary greatly in the sensitivity of individual trypanosomes in that population. In addition, the response of resistant trypanosome populations to chemotherapy is dependent on the concentration of trypanosomes in the blood and the timing of treatment. (Burudi *et al.*, 1994; Mamman *et al.*, 1995a, 1995b).

2.6.2 Potential mechanisms of drug resistance

Little is known about the mechanisms involved in drug resistance, and much of what is known has been based on studies on bacteria. The possible mechanisms of drug resistance in parasites are as follows;

- Conversion of the drug to an inactive form by an enzyme.
- Modification of a drug sensitive site.
- Increased efflux or decreased influx
- Alternative pathway to bypass inhibited reaction.
- Increased production of drug sensitive enzymes.
- Increase in the amount of an enzyme substrate (i.e. to compete with the drug).
- Decrease requirement for product of inhibited reaction.
- Failure to activate the drug.

A number of these mechanisms have been observed in parasites such as alteration in cell permeability, modifications of drug sensitive sites and increased quantities of the target enzyme. These modifications can arise in a population of parasites by a number of mechanisms such as;

- Physiological adaptations
- Differential selection of resistant individuals from a mixed population of susceptible and resistant individuals.
- Spontaneous mutations followed by selection.
- Changes in gene expression (gene amplification)

Drug resistance is particularly a problem in veterinary helminthiasis, more specifically the resistance to benzimidazole drugs by Trichostrongylid nematodes (Cabaret *et al.*, 1995). These drugs bind to free tubulin preventing assemblage of microtubules, and microtubules are associated with many cellular functions. It is believed that the genes, which confer resistance to a particular drug, are already within the parasite populations, but prior to exposure to the drug, are present at low

frequencies. Exposure to the drug selects these resistant individuals and their frequency within the population increases rapidly. The appearance of resistance within a population has been observed to occur within 5-50 generations (Grant and Mascord, 1996).

In trypanosomiasis the mechanism of resistance is poorly understood. However drug resistance seems to be acquired when trypanosomes are exposed to sub-therapeutic levels, thereby facilitating selection. Studies to elucidate the underlying mechanisms of resistance to isometamidium have shown that resistance is associated with reduced accumulation of the drug in the parasite (Sutherland and Holmes, 1993). Mulugeta *et al.* (1997) suggested that in resistant isolates the number of isometamidium transporters in the plasma membrane may be lower or their turn-over rate for isometamidium may be significantly reduced. Nevertheless, molecular mechanisms responsible for the accumulation of the drug in the cells have not been extensively characterised.

2.6.3 Factors associated with drug resistance

Drug resistance has been associated with numerous factors such as, prolonged under-dosing due to incorrect body weight estimation, failure to calculate adequate dosage, deliberate under dosing, poor drug preparation and administration, high incidence of trypanosomiasis and erratic treatments with prophylactics among others (Davey, 1957; Whiteside, 1962).

2.6.3.1 Extensive drug use

From its earliest days, development of chemotherapy for bovine trypanosomiasis has been accompanied by development of drug resistance (Williamson, 1979). The extensive use of trypanocidal drugs for the control of bovine trypanosomiasis, has resulted in appearance of drug resistant trypanosomes in parts of Africa, and elsewhere (Leach and Roberts, 1981; Kupper and Wolters, 1983; Pinder and Authie, 1984; Rottcher and Schillinger, 1985; Rowlands *et al.*, 1993).

In addition, diminazene, homidium, isometamidium quinapyramine and suramin have been used in the field for over 30 years. In some areas, drug resistance has become so prevalent that the drugs concerned have been withdrawn for use in

some animal species (Schillinger and Rottcher, 1984). Early breakthrough trypanosome infections following prophylactic treatment with isometamidium chloride in the field are usually attributed to drug resistance. Such observations have been reported in Zimbabwe (Lewis and Thomson, 1974), in Ethiopia (Scott and Pegram,1974; Codjia *et al*, 1993; Mulugeta *et al.* (1997), in Cote d'Ivoire (Kupper and Wolters, 1983), in Burkina Faso (Pinder and Authie, 1984) in Nigeria (Na'Isa, 1967; Gray and Roberts, (1971), in Sudan (Mohamed- Ahmed *et al.*, 1992) and in Kenya (Dolan *et al.*, 1992; Munstermann *et al.*, 1992).

At Mkwaja Ranch in Tanga, Tanzania where isometamidium has been used prophylactically for over thirty years, problems of drug resistance were not encountered for the first twenty years, probably due to good management and the use of correct drug dosage (Trail *et al.*, 1985). However, in 1988/89 the interval of treatment with Samorin at 1 mg/kg was reduced to five weeks when positive cases of trypanosomiasis were confirmed in blood films four weeks after treatment with isometamidium (Fox *et al.*, 1993). These early breakthrough infections were attributed to development of resistance (Fox *et al.*, 1993).

In Kenya, at Galana Ranch, isometamidium prophylaxis has been used routinely and effectively for a long time. However, in 1984, presence of *T. vivax* infections expressing multiple drug resistance to isometamidium at 2.0 mg/kg b.w., diminazene at 3.5 mg/kg b.w. and homidium at 1.0 mg/kg b.w. was confirmed (Rottcher and Schillinger, 1985; Njogu and Heath, 1986).

2.6.3.2 Use of sub-therapeutic doses

Exposure of trypanosomes to sub-therapeutic drug levels, thereby favouring the selection of drug-resistant populations has been attributed to development of resistance (Leach and Roberts, 1981). Furthermore, Nyeko *et al.* (1988) demonstrated that long-term exposure of infected flies to trypanocides leads to development of resistance. Repeated sub-curative treatment of *T. congolense* infected mice with isometamidium increased resistance to the drug 94-fold (Peregrine *et al.*, 1997).

Drug resistance is likely to occur with trypanocides that have prophylactic activity rather than those that have therapeutic activity only (Holmes and Torr,

1988). This is attributed to persistence of the drug at diminishing levels thereby exposing the trypanosomes to sub-curative levels of drug leading to selective elimination of the sensitive ones at that level. Eventually, the very resistant population will survive. This suggestion was supported by the absence of resistance to diminazene aceturate until 1960s (Holmes and Torr, 1988).

However since then, a number of reports have described the appearance of diminazene resistant isolates in cattle. *Trypanosoma vivax* isolates resistant to diminazene aceturate from eastern Africa, have been reported by various authors (Rottcher and Schillinger, 1985; Schonefeld *et al., 1987;* Mbwambo *et al,* 1988*).* Fewer cases of resistance in field isolates of *T. congolense* to diminazene aceturate at 3.5 mg per kg and at 7.0 mg/kg have also been reported (Codjia *et al.*, 1993; Rowland *et al.*, 1993; Mulugeta *et al.*, 1997).

Attempts to induce resistance to diminazene in trypanosomes by using sub-curative doses have resulted in contradicting results. Fussganger and Bauer (1960) did not succeed in inducing resistance to diminazene in infected mice and cattle. In contrast, Hawking (1963) was able to increase resistance of a *T. congolense* population to diminazene by 50-fold over a 40 month period. Recently, it was demonstrated that *T. congolense* can acquire resistance to diminazene aceturate, following lengthy passages and sub-curative treatments (Peregrine and Mamman, 1993).

2.6.3.3 Immunological status of the host

Immunological status of the host has been considered as factor contributing to development of induced resistance (Osman *et al.*, 1992). Frequent passage of clones of *T. evansi* in immunosuppressed mice given sub-curative doses of isometamidium led to rapid development of high level of resistance. However, using similar protocol in normal immunocompetent mice infected with the same parent *T. evansi* clone did not lead to development of drug resistance (Osman *et al.*, 1992). They concluded that the immunological status of the host animal rather than the trypanosome species appeared to be the main factor determining the rate of development of resistance

2.6.4 Cross Resistance

Cross-resistance is a term used to describe a situation where a strain of trypanosomes resistant to one trypanocide is also observed to be resistant to a second chemically unrelated trypanocide even though it might not have been exposed.

The most extensive work on the development of cross- resistance in cattle was carried out by Whiteside (1960; 1962) in Kenya. He concluded that induction of resistance to quinapyramine results in cross-resistance to diminazene aceturate at a higher dose and to curative dose of homidium and metamidium. As a result of these conclusions, Whiteside (1960) proposed that homidium and diminazene, and isometamidium and diminazene should be used together as 'sanative' pairs of drugs. These are pairs of drugs that do not induce resistance to each other and can be used alternatively in the field when resistance to either drug appears. Sanative combinations of drugs are therefore recommended for use in the field and have not been associated with development of resistance (Trail *et al.*, 1985). However, Codjia *et al* (1993) demonstrated that 11 out of 12 stabilates collected from cattle in Ghibe valley, Ethiopia, expressed resistance to diminazene aceturate, isometamidium chloride, and homidium chloride at 7.0, 0.5 and 1.0 mg/kg b.w. respectively. Furthermore, when five clones were derived from one of these isolates, each clone expressed high levels of resistance to all the three trypanocides when characterised in mice (Codjia *et al.*, 1993).

Amongst the trypanocides that were evaluated by Whiteside (1962), quinapyramine was associated with the greatest problems. Induction of resistance to this compound under field conditions appeared to result in cross-resistance to homidium, isometamidium and diminazene aceturate. These findings have been confirmed in a laboratory study in which resistance to quinapyramine was induced in a clone of *T. congolense* (Ndoutamia *et al.*, 1993). It was therefore indicated that quinapyramine should not be used in cattle.

However, cross- resistance in *T. evansi* infections did not seem to occur between quinapyramine and suramin (Gill, 1971). In addition, *in vitro* studies (Zweygarth and Kaminsky, 1990) have shown quinapyramine and suramin - resistance population of *T. evansi* to be susceptible to treatment with melarsomine. Though induction of resistance to melarsomine does not result in cross- resistance to

suramin, it does appear to result in cross-resistance to diminazene aceturate (Osman *et al.*, 1992).

Recently, resistance to isometamidium was increased 94-fold in a clone of *Trypanosome congolense* (IL 1180) by repeated sub-curative treatment of infected mice for 11 months. This was associated with 3.4-, 33-, and 4.2-fold increases in resistance to diminazene, homidium and quinapyramine, respectively (Peregrine *et al.*, 1997).

2.6.5 Stability of resistance

Instability of resistance to trypanocidal compounds in trypanosome populations has been reported (Whiteside 1963; Peregrine *et al.*, 1991; Mamman *et al.*, 1993). Mulugeta *et al.* (1997), however, showed that the phenotype of multiple drug-resistant *T. congolense* remained stable over a period of four years. *Trypanosome congolense* isolates collected at random from cattle at Ghibe, Ethiopia expressed multiple resistance to diminazene aceturate, isometamidium chloride and homidium chloride at doses of 7.0 mg/kg b.w., 0.5 mg/kg b.w. and 1.0 mg/kg b.w. respectively. Individual trypanosome clones from the same *T. congolense* isolates also expressed multiple resistance to the three drugs. In addition *T. congolense* isolates collected at the same location three years earlier had expressed multiple resistance to all the three trypanocides used in cattle at individual trypanosome level (Codjia *et al.*, 1993). This would indicate that over the three years the resistance of the *T. congolense* phenotype in Ghibe valley, Ethiopia remained stable.

2.6.6 Pathogenicity of drug resistant trypanosomes

Pathogenicity of drug resistant trypanosomes has not really been established yet. Decrease in virulence and loss of fitness in drug resistance trypanosomes has been reported (Whiteside, 1962; Stephen, 1962c; Silayo and Marandu, 1989; Berger, *et al.*, 1995; Mutugi, *et al.*, 1995). However, recent studies using four populations of *T. congolense*, ranging from extremely sensitive to strongly resistant to isometamidium, demonstrated no differences in virulence between them, except the most resistant one showed a reduced infectivity, i.e. it took longer to establish parasitaemia than the other three (ILRI, 1996). The loss of fitness in other drug-resistant parasites is well known and may probably also be present in trypanosomes

2.6.7 Methods of identifying drug resistance;

Several methods have been described to identify drug resistance in trypanosomes (Peregrine, 1994; Geerts and Holmes, 1998). At present, three types of technique are commonly used to identify drug resistance: tests in ruminants; tests in mice; and *in vitro* assays. None of these is, however, an ideal test and other tests are still in the phase of development or validation. The advantages and disadvantages of each of the different techniques are discussed.

2.6.7.1 Longitudinal field data

Cross-sectional studies on prevalence of trypanosomiasis in the field situations do not provide informative data about the prevalence of drug resistance. However, data from longitudinal studies can be used for this purpose. Rowland *et al.* (1993) applied a model that could distinguish the incidence of new infections from recurrent ones, to parasitological data collected on monthly basis from cattle treated with only diminazene aceturate. He showed that the mean prevalence of resistant infections increased significantly from 6% in 1986 to 14% in 1989. The occurrence of drug-resistant populations from this area was confirmed by Codjia *et al.* (1993).

The advantage of these kinds of data is of course that they are directly applicable to the field. The disadvantages are;

- The true prevalence of drug-resistant infections seems to be underestimated, it is retrospective by at least six months (Geerts and Holmes, 1998)
- The technique is quite expensive, especially if a longitudinal study is not carried out for other purposes (Geerts and Holmes, 1998).

2.6.7.2 Trypanocidal drug enzyme linked immunosorbent assay (ELISA)

The use of trypanocidal drug enzyme-linked immunosorbent assays (ELISAs) in combination with parasite detection tests has given promising results for the detection of resistant trypanosomes. A competitive ELISA that allowed the detection of small amounts of isometamidium in the serum of cattle was first described by Whitelaw *et al.* (1991). This technique was further improved by Eisler *et al.* (1993) and Eisler, *et al.*, (1996a) and has been validated in cattle under experimental and

field conditions (Eisler, 1996; Eisler *et al.*,1994; 1996b; 1997b). The test is both sensitive, detecting sub-nanogramme concentrations, and specific. It allows the monitoring of drug levels over extended periods and the evaluation of factors influencing drug disappearance rates from the plasma.

The available data indicate that there is a considerable individual variation after intramuscular injection of isometamidium in cattle (Eisler, 1996). It appears that the drug disappears more rapidly in animals challenged and acquiring infection with drug-resistant trypanosome isolates than in those challenged but protected against infection with sensitive trypanosomes (Eisler *et al.*, 1994). Observations showed that the presence of trypanosomes in animals with an isometamidium concentration > 0.4 ng/ml suggests resistance; the higher the drug level detected the greater the degree of resistance that could be inferred (Eisler *et al.*, 1997a). Further research is necessary, however, in order to confirm these results in a larger number of animals. Similar drug ELISAs have been developed for the detection of sub-nanogramme amounts of homidium bromide (Murilla, 1996) and a similar test for diminazene is in development (Karanja personal communication).

The advantage of the isometamidium ELISA is that large numbers of sera can be tested within a relatively short time. The technique may also provide information on drug usage in an area of investigation. The disadvantage is that further studies are required to confirm the correlation between protection against tsetse challenge with various trypanosome populations and the isometamidium concentration in the serum. It is not yet possible to draw firm conclusions on the sensitivity or resistance of a trypanosome population at the level of the individual animal. The ELISA should, however, give some indication of the resistance situation at the level of the herd.

A further disadvantage is that, while the ELISA may indicate the level of drug withstood by a trypanosome population, it does not provide information about the level required for protection in field studies.

2.6.7.3 Tests in large animals

For many years the drug resistance phenotype of trypanosome populations has been characterised in small and large ruminants (Gray and Roberts, 1971;

Gitatha, 1979; Codjia *et al.*, 1993). Tests in ruminants provide direct information from studies done in ruminants using recommended doses of trypanocide.

The tests commonly consist of infecting a group of cattle or small ruminants with the isolate under investigation and later, when the animals are parasitaemic, treating them with various levels of trypanocide. The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the effective dose (ED), i.e. the dose that clears the parasites from the circulation, and the curative dose (CD), i.e. the dose that provides a permanent cure (Sones, *et al.*, 1988). For these studies, the cattle or small ruminants must be kept in fly-proof accommodation or in a non-tsetse area in order to eliminate the risk of re-infection during the study.

Ainanshe, *et al.*, (1992) used a variation of this technique in Somalia to examine a group of isolates from a district. Blood from a group of infected cattle was inoculated into a single recipient calf, which was monitored, and later, when it was parasitaemic, treated with trypanocide at the recommended dose. A breakthrough infection, indicative that one of the inoculated trypanosome populations was drug-resistant, was inoculated into groups of calves and mice to determine the level of drug resistance.

This technique is useful in situations where laboratory facilities are very limited but it only allows a qualitative assessment and does not indicate how many of the isolates inoculated into a single calf were resistant. Further constraints to this technique are that not all populations might grow equally well and that sensitive isolates might overgrow resistant ones when inoculated together (Sones, *et al.*, 1989). However this is not a consistent observation (Burudi *et al.*, 1994). A useful indication of the level of resistance can be obtained from studies in ruminants (and mice) by recording the length of time between treatment and the detection of breakthrough populations of trypanosomes. The shorter the period, the greater the level of resistance (Ainanshe, *et al.*, 1992; Williamson and Stephen, 1960).

The advantages of studies in ruminants are that most trypanosome isolates of cattle are able to grow in these hosts and that the data obtained are directly applicable to the field. The disadvantages are the long duration (a follow-up of 100 days is necessary to allow the detection of relapses) and the cost (purchase and maintenance

of the animals are expensive). Furthermore, if only one isolate per animal is tested, it is usually impractical and too expensive to examine a large number of isolates.

2.6.7.4 Tests in mice

Because of expensive and logistical problems incurred when using large animals, many workers have used mice and rats to characterise the drug resistance of trypanosome populations. Such systems reduce experimental costs and reduce the time taken to characterise to approximately 2 months (Peregrine, 1994).

After expansion of an isolate in a donor mouse, groups of five or six mice are inoculated with trypanosomes. Twenty-four hours later, or at the first peak of parasitaemia, each group except the control group is treated with a range of drug doses. Thereafter, the mice should be monitored three times a week for 60 days. The ED_{50} or ED_{95} (the effective dose that gives temporary clearance of the parasites in 50 or 95 percent of the animals, respectively) can be calculated, as can the CD_{50} or CD_{95} (the curative dose that gives complete cure in 50 or 95 percent of the animals, respectively). Sones *et al.*, (1988) used groups of five mice, which allowed an easy calculation of ED80 and CD80 values (one out of five mice was not cleared or cured).

These figures should be compared with those obtained using reference-sensitive trypanosome strains. The advantage of the mouse assay is that it is cheaper than the test in cattle. There are several disadvantages;

- Most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice
- There does not appear to be a consistent relationship between drug sensitivity data obtained in small animals and that obtained in large animals (Sones *et al.*, 1988). This necessitates use of higher doses of drug in mice, ten times higher in order to obtain comparable results to those obtained in cattle because of the vast difference in metabolic rates (Sones *et al.*, 1988).
- Accurate assessment of the degree of resistance needs a large number of mice per isolate, which makes it a labour-intensive test. However, identification of a discriminatory dose for each drug, above which an isolate should be considered

as resistant, could drastically reduce the number of mice and the amount of work to be carried out.

- Finally, it takes as long as 60 days to evaluate the drug sensitivity of an isolate.

2.6.7.5 In vitro test (Tissue culture)

More recently, methods have been developed to assess drug sensitivities in trypanosomes using in vitro techniques. This approach became feasible with the advent of tissue culture techniques for the *in vitro* propagation of blood stream form trypanosomes, in feeder cell free systems (Baltz *et al.*, 1985; Duszenko *et al.*, 1985; Hirumi and Hirumi, 1991; Zweygarth *et al.*, 1991, 1992). Kaminsky and Brun (1993) have reviewed various in vitro drug sensitivity systems for blood stream from trypanosomes. These are categorised according to whether the systems require in vitro adaptation of trypanosomes or they do not. The selection of the system to be used depends largely on the specific aims of the study, on the trypanosome stocks available and on the equipment of the laboratory. Ideally, the sensitivity should be determined using trypanosomes directly from the host. However, in most cases the parasitaemia in the host is too low to yield a sufficient number of organisms for drug sensitivity testing.

Compared to drug-sensitivity studies carried out *in vivo*, such systems are usually cheaper and may be quicker. Nevertheless logistically, it is not possible to screen large numbers of isolates with such systems. It is not yet possible to use drug sensitivity data derived in vitro to predict the drug sensitivity of trypanosome populations in definitive hosts (Kaminsky *et al.*, 1990). However, Gray *et al.* (1993) have observed a consistent relationship between the in vitro sensitivity of *Trypanosoma congolense* metacyclic trypanosomes to diminazene, homidium and isometamidium, and the sensitivity of the same populations to these trypanocides in cattle.

Further clarification of the relationship between drug sensitivity data obtained *in vitro* and *in vivo* will help determine the future application of in vitro characterisation systems (Peregrine, 1994). In addition, it will be necessary to standardise the assay by generating reference trypanosome clones with known drug sensitivity profiles. This will allow data on drug sensitivity of unknown

trypanosome isolates to be related to drug-sensitive or drug-resistant trypanosome populations (Kaminsky and Brun, 1993)

2.6.7.6 Drug incubation Glossina *infectivity test (DIGIT)*

A test to assess the sensitivity of trypanosomes to drugs relying on infectivity to tsetse flies has been described (Clausen *et al.*, 1999). In this method, the stabilate to be tested is inoculated into a susceptible host. When an infection has been established in this experimental host, blood is then removed and incubated at 37 °C for a period of time in the presence of various concentrations of trypanocidal drugs. The trypanosome /blood/drug/suspension is then fed to tsetse flies using an *in vitro* feeding system. After about 20 days the tsetse flies are dissected and examined for presence of trypanosomes in labrum, hypopharynx and midgut. Presence of trypanosomes in the tsetse would suggest that the trypanosomes survived the incubation with the particular drug at a certain drug concentration.

This could be a useful tool in assessing susceptibility of trypanosome population. However, it needs standardisation of the range of doses for each drug necessary to characterise the resistant trypanosome populations effectively.

2.6.8 Possible causes and types of relapse infections

Generally, failure of a therapeutic drug to achieve cure or a prophylactic drug to confer protection for the expected period, when the drug was administered correctly, the usual conclusion is that a drug resistant strain of trypanosome has been encountered. However, this is not necessarily the case

2.6.8.1 Cryptic foci

Jennings *et al.* (1977a,b) demonstrated the importance of 'privileged sites' in experimental infection in mice; that is locations in the body that can be invaded by trypanosomes but are inaccessible to drugs. MacLennan (1971) demonstrated a period of aparasitaemia in cattle infected with *T. vivax* and treated with diminazene aceturate, during which sub-inoculation of blood into naive cattle failed to establish infections, suggesting the existence of a site inaccessible to drug.

2.6.8.2 *Time before treatment of infection*

The timing of treatment relative to primary infection might be crucial in the development of a relapse infection. Treatment of *T. brucei* and *T. congolense* infected mice within 3-7 days post infection resulted in a permanent cure, whereas if treatment was delayed until 14 days post infection, all mice relapsed 20 - 60 days later. It was shown that neither the survival of drug resistant trypanosomes nor the numbers of parasites inoculated were responsible for the relapse infections (Jennings *et al.*, 1977b).

Evans *et al.* (1977) has provided further evidence of the possible importance of time of treatment relative to infections in determining the effect of treatment. Infections in mice with *T. brucei* were eliminated when treatment was carried out within one day of infection, but were not eliminated when treatment was delayed.

Recently, Silayo *et al.* (1992) demonstrated in goats infected with *T. congolense* IL 3274, that when treatment was administered 24 hrs after infection the parasites were eliminated. However, treatment failed when administered after the goats were found to be parasitaemic.

Field observations, however, indicate that the relapse phenomenon described above may not be easily explicable. Smith and Scott (1961) found that the length of time cattle have been infected with trypanosomes before chemotherapy did not affect the result of treatment. Gray and Roberts (1971) showed that periods during which drug resistant trypanosomes were present in animals before treatment had only minor effects on the length of time between treatment and relapse.

It is possible though, that primary infections of cattle that are detectable between one and two weeks after infection may already have developed past the stage of complete susceptibility to trypanocides by the time of treatment. These infections would therefore be thought to be drug-resistant upon relapse (Leach and Roberts, 1981)

2.6.8.3 *Pathogenicity of relapse infections*

Field observations have shown that relapse infections may be less pathogenic than primary infection in some circumstances. This has been attributed to

enhancement of the hosts immune response. Stephen (1962c) showed that newly acquired infections may also be less pathogenic in animals previously treated with prophylactic drugs than in untreated control animals.

2.6.8.4 Host immunosuppression

Suppression of the host immune system could also lead to resurgence of an infection, which in a normal functioning immune system would not be established. (Osman *et al.*, 1992). Thus, animals under stress, probably from diseases, constant probing and bites of tsetse flies and other biting flies, agricultural work, constant milking or improper feeding may succumb to frequent relapse infections.

2.6.9 Possible measures necessary to alleviate drug resistance in the field

Geerts and Holmes (1998) have outlined several measures necessary to reduce and/or delay drug resistance in the field. These measures include;

- Reducing the number of treatments by integrating drug usage with other control measures. It is widely agreed that the most efficient way to delay the development of drug resistance remains the reduction of selection pressure by the drugs, i.e. decreasing the number of treatments. This is of particular importance in areas of high tsetse challenge, which are commonly associated with reduced periods of chemoprophylaxis (Whiteside, 1960; 1962). In such situations the treatment frequency is commonly increased and drug resistance often emerges as a constraint to further drug usage.

- Ensuring use of correct dose and proper drug administration. Underdosing is one of the major causes of resistance development. Sub-therapeutic drug concentrations exert a strong selective pressure for the emergence of resistant clones that pre-exist in the trypanosome population. Unfortunately, underdosing occurs very frequently. Farmers have the tendency to underestimate the weight of their animals when they have to treat them (Besier and Hopkins, 1988). Sometimes generic products are used, which have a reduced efficacy, as has been shown in the field of anthelmintics (Van Wyk *et al.*, 1997). In addition, since in many countries unskilled persons have access to trypanocides and thus

administer drugs themselves (qualified veterinary personnel are usually not available for one reason or another), errors easily occur in calculating the correct doses for the treatment of the animals. Packaging of isometamidium as a one-dose treatment – similar to diminazene – would undoubtedly help to reduce this problem. Furthermore, as the drugs are relatively expensive there is a temptation to overdilute the drug and hence underdose.

- Avoiding exposure of the whole trypanosome population to a drug. As explained earlier, animal trypanosomiasis has commonly been controlled with mass treatments which can be highly successful over many years (Trail *et al.*, 1985). However, this form of treatment exerts a strong selection pressure on the trypanosome population. The higher the proportion of the trypanosome population exposed to the drug and the lower the trypanosomes present in the fly population or in other hosts, the higher the selection pressure.

- Banning completely, use of quinapyramine in cattle. After use for more than twenty years in cattle, in 1967 quinapyramine was withdrawn from sale for cattle use because of problems with toxicity and resistance development. It is still available for use in camels, however, and it is likely that it is still mistakenly used in cattle in some situations in Africa. In addition, the use of quinapyramine was the suggested cause of the multiple drug-resistance problem in the Ghibe valley of Ethiopia

2.7 Trypanosomiasis at the Kenyan coast

Trypanosomiasis is endemic in most parts of the Coast Province of Kenya. However, the incidence of the disease varies from area to area depending on a number of factors that influence the distribution of tsetse flies (Heckalau, 1986). One of the factors is the rapidly increasing human population, particularly in the vicinity of the major urban centres, causing disruption of the tsetse habitat due to settlement (Heckalau, 1986). As a result, tsetse distribution along the coastal region is limited to the sparsely populated regions, areas of and adjacent to national game reserves and the preserved original forest areas. *Glossina pallidipes*, *Glossina austeni*, *Glossina brevipalpis* and *Glossina longipennis* are the most common widespread tsetse species throughout the region (Heckalau, 1986).

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2.7.1 Prevalence of Trypanosomiasis

Along the northern Kenyan coast (Lohr *et al.*, 1991), at Witu, Lamu district, relative tsetse densities during 1989 ranged from 53-163 flies per trap per day. The weekly trypanosome prevalence ranged from 17% to 34 % in Boran/Orma cattle. Munstermann *et al.* (1992) recorded weekly prevalence of 11% - 18% in cattle, from a nearby ranch in Kipini. In these cattle, 60% - 75% of the infections were *T. congolense*, just fewer than 20% were *T. vivax* and the rest were mixed *T. congolense* and *T. vivax* infections.

Along the coastal strip, south of Lamu district, in Kilifi district, tsetse population of *Glossina pallidipes* and *Glossina austeni* occur in varying intensities. In a ranch near the Kilifi creek, trypanosome prevalence of 0.8% was observed during a nine month study period (Paling *et al.*, 1987). A small isolated focus of *Glossina austeni* was responsible for transmitting trypanosomiasis in the dairy cattle managed in this ranch. In a sentinel herd of twenty mixed Ayrshire, Sahiwal, Brown Swiss cross bred dairy cattle, exposed for 182 days in the same ranch, all cattle became infected with *T. congolense* (Paling *et al.*, 1987). These infections responded to treatment with diminazene aceturate.

Further south of the creek, on a commercial ranch at Vipingo, *G. pallidipes* was the only species trapped in the area (Dowler *et al.*, 1989). In 1985, the monthly trypanosome prevalence was 1.9 - 22% in 3000 Boran cattle that were regularly blood tested on the ranch. The cattle that were detected parasitaemic were treated using isometamidium intravenously at 0.6 mg/kg b.w. (Dowler *et al.*, 1989).

In Kwale district on the southern Kenyan coast, trypanosomiasis was responsible for major losses in productivity. In an investigation carried out on the farms, 30% of the sampled cattle were positive for *T. vivax* (Mwongela *et al.*, 1981). In another study carried out in 1988 - 1989, weekly monitoring of the dairy herd on one of the farms revealed prevalence ranging from 5 to 54%. Most of the infections coincided with the onset of rains. *Trypanosoma congolense* accounted for 13% of the infection, 79% of the infections were *T. vivax* and 8% were mixed infections (Gaturaga *et al.*, 1990).

A lower prevalence was observed in east African Zebu cattle kept under traditionally managed herds at Muhaka in Kwale district. In a baseline study where trypanosome infections were monitored monthly, the prevalence of infection was 2 – 8%. Infection rates of tsetse flies ranged from 2 % to 10 % (Maloo *et al.*, 1985). There was a rise in fly density immediately after the onset of rains. Although the tsetse density was relatively low, trypanosome infections were detected in all months, indicating that transmission of the disease occurred throughout the twelve months study period (Maloo *et al.*, 1985). In prophylactic treated dairy cattle overall prevalence was 12.9 % and varied from 16.5 % to 18.1 % at Kubo and Matuga respectively, and 2 % at Msambweni (Maloo, 1993)

2.7.1.1 Use of trypanocides

Control of trypanosomiasis in the province depends mainly on the use of trypanocidal drugs. The importance of the disease is reflected on the large quantity of trypanocides used to combat the disease. Due to the varying degree of trypanosomiasis risk within the coastal belt, cattle are maintained either by prophylaxis with isometamidium, by chemotherapy with curative trypanocides or by a combination of both.

Livestock owners keeping local Zebu cattle in low to medium challenge areas generally rely on curative treatments, though prophylactic cover using isometamidium is occasionally used. A study to assess the effectiveness of prophylaxis and therapeutic program, in traditionally managed east African Zebu cattle was conducted at Muhaka, in the southern Kenyan coast. Two-thirds of some 700 cattle within 17 herds were treated with isometamidium (0.5 mg/kg b.w.), at three monthly intervals for a period of 30 months. Data collected monthly, showed that prophylactically treated cattle, had 39% fewer detectable trypanosome infections, required 64% less diminazene therapeutic treatments and gave 24% more extracted milk. This resulted in productivity increase of 20 % (Maloo *et al.*, 1988b). The increase in productivity attained by the implementation of a chemoprophylaxis regime, was considered highly cost effective (Itty *et al.*, 1988).

In the northern Kenyan coast, in an area of relatively low tsetse challenge, a series of abortions observed were eventually associated with trypanosomiasis. To prevent losses in Kilifi plantation, a chemotherapeutic strategy was initiated whereby

regular blood testing of the dairy herd was done four to five times a year. Cattle with parasitaemia or PCV less than 30% were treated with diminazene aceturate at 3.5 mg per kg or homidium at 1.0 mg per kg. This strategy was successful in resolving the trypanosomiasis problem (Wissocq *et al.*, 1983; Paling *et al.*, 1987). Using this control method, it was possible to achieve an average lactation yield of 2,833 kg and a calving interval of 402 days. This resulted in a mean annual milk yield of 2,589 kg (Murray and Trail, 1982).

When a therapeutic approach using diminazene aceturate, failed to control trypanosomiasis on a beef ranch at Vipingo, a prophylactic regime of isometamidium at 1.0 mg/kg b.w.was introduced. In order to control the chronic trypanosomiasis cases observed, individual trypanosome infected cattle were treated with isometamidium. The injection was administered intravenously at 0.6 mg/kg b.w. (Dowler *et al.*, 1989), to avoid the tissue reaction normally observed after intramuscular administration. By weekly blood testing and treating parasitaemic cases over a period of more than two years, the number of trypanosome-infected cattle declined ten-fold. Continuous presence of tsetse was observed during this period. The early detection and treatment of infected cattle may possibly have led to the reduction of cattle reservoirs of trypanosome infections. This may in turn have led to reduced infection rates in flies, thus lowering the risk of trypanosomiasis (Dowler *et al.*, 1989; Munstermann, *et al.*, 1992).

Although the intravenous administration of isometamidium for therapeutic effect resulted in improved control of trypanosomiasis, implementation of this regime requires a competent and well-organised team. This criterion will limit its application elsewhere (Dowler *et al.*, 1989).

In the high challenge areas of Lamu district, despite routine prophylaxis treatments, effective control of trypanosomiasis could not be achieved. Consequently cattle in poor body condition with low PCV values were frequently observed on these ranches. In a ranch at Kipini, with 4,500 beef cattle, a study incorporating two groups of 50 Boran weaner cattle was conducted, to evaluate the efficacy of isometamidium administered at 1.0 mg/kg b.w., either intramuscularly as a prophylactic, or intravenously to individual infected animals. Another group of 50 weaners kept as control was treated with diminazene aceturate upon detection of

trypanosome infection (Munstermann *et al.*, 1992). Curative intravenous application of isometamidium at 1.0 mg/kg b.w. seems to be more effective in comparison to the same dosage given i.m prophylactically. There was reduction in drug costs and over 30% higher weight gains in the 30-week study period in the intravenously treated group. In addition, weekly infection rates declined over time even though the tsetse catches were constantly high (Munstermann *et al.*, 1992).

The development of toxicity, following prolonged use of this approach should be of concern, more so in the event of acute systematic reactions that seem to occur after intravenous administration of isometamidium (Schillinger *et al.*, 1985; Dowler *et al.*, 1989).

These studies concluded that trypanosomiasis control was difficult regardless of the method used. The high tsetse challenge and the probability of drug resistant strains could account for the detection of early breakthrough observed in the prophylactic group (Munstermann *et al.*, 1992).

Evidence of drug resistance to *T. vivax* isolates from coastal regions of Kenya and Somalia has been reported by Schonefeld *et al.* (1987), while drug resistance to *T. congolense* isolates collected from Shimba hills, Kwale district has been described by Gitatha, (1979). There has been increasing evidence of trypanocidal drug failure to control trypanosomiasis, especially in the high tsetse challenge areas of the Coast Province, Kenya (Rottcher and Schillinger, 1985; Schonefeld *et al.*, 1987; Dolan *et al.*, 1992; Munstermann *et al.*, 1992).

With no new trypanocides available now or in the near future, the necessity to re-examine existing compounds with the view of introducing strategic use of trypanocides, integrated with alternative control measures directed towards the vector cannot be over emphasised. Use of odour-baited deltamenthrin impregnated targets significantly reduced populations of *Glossina* species in the suppression zone on Galana Ranch (Opiyo *et al.*, 1987). Recently, the use of synthetic pyrethroids 'pour on' preparations on beef cattle under high tsetse challenge areas of Galana and Witu resulted in significant decreases in the incidence of trypanosomiasis and in tsetse catches (Lohr *et al.*, 1991).

Due to the varying risk of trypanosomiasis on the Kenyan coast, no single control strategy can resolve the problem of trypanosomiasis. In areas where the risk is high, effective control can be achieved with an integrated approach where combinations of suitable cost-effective methods targeted towards both the vector and the parasite need to be implemented. However, in the low to medium challenge areas strategic intervention with trypanocides in individual cattle or on herd basis can control trypanosomiasis. For any effective control, the epidemiology of the disease must be understood at the local or regional level. In addition, the incidence and distribution of drug resistance in the area should be thoroughly investigated, and factors associated with the incidence determined.

Chapter 3
Materials and Methods – General

3 General Materials and Methods

3.1 Study areas

In this chapter, methodologies that were common to all the studies carried out are discussed. Detailed procedures unique to the particular studies are discussed later in the relevant chapters.

Methods were standardised with those used under the EU INCO-DC project No IC18 CT95 0006 at the Kenya Trypanosomiasis Research Institute (KETRI) which was conducted in close association with this work.

The field studies in cattle were carried out in the northern and southern coastal parts of the Coast Province of Kenya, where trypanosomiasis challenge is considered among the highest in the Country. The study sites are shown in Figure 1. Climatic conditions in this Province vary from fairly wet in the Taita Hills to arid in Tana River District. Average annual rainfall for the Coast province ranges from between 900 - 1500 mm at the coastal belt to below 500 mm in the Tana River district (KETRI 1996). In Kilifi district, the average annual rainfall ranges from 400mm in the hinterland (rangeland zone; including Galana Ranch) to over 1200mm at the coastal belt. The narrow coastal belt from Malindi to Kilifi Town receives an average annual rainfall of about 900 mm to 1100 mm. To the north in the hinterland towards Tana River district there is a marked decrease in the rainfall intensity. In Kwale district, amount of rainfall diminishes from the coastline to the hinterland with the average annual rainfall varying from 900-1500 mm along the coastline to 500-600 mm in the hinterland with 60 % reliability (Kenya Web, 1998).

Traditionally, the long rains are expected from March to June while the short rains are from October to December. However, there may be considerable variation between years, with periods of severe drought interspersed with years of unusually heavy rains, such as those associated with the El-Nino phenomenon in October 1997-February 1998. Over this period the usual rainfall pattern was drastically altered consequently changing the tsetse transmission dynamics of bovine trypanosomiasis, with far reaching socio-economic consequences. Initially, the studies were intended

to cover the traditionally dry and rainy seasons, when trypanosomiasis challenge is expected to be low and high respectively. However, during the study period of 3 years the weather pattern changed significantly from the expected.

The field studies involved:

- Prophylactic block treatment of groups of cattle with the trypanocidal drug isometamidium chloride.
- Use of sentinel groups of untreated, susceptible cattle for monitoring trypanosome incidence.
- Regular examination of blood samples for trypanosome infection.
- Regular collection of trypanosome isolates from infected cattle.
- Regular monitoring of productivity parameters.
- Regular collection of blood samples from the block treatment group for isometamidium determinations.

Laboratory analyses of the samples generated from the field experiments were carried out at the Kenya Trypanosomiasis Research Institute. These analyses were:

- Determination of isometamidium levels in serum of cattle in the block treatment groups. The technique used is based on the enzyme-linked immunosorbent assay.
- Drug sensitivity tests of the trypanosome isolates in mice and cattle. This methodology will be discussed later in chapter 7.

Figure 1: Map of Kenya showing the study area at the Coast Province

3.2 Trypanocidal Drugs

Two trypanocidal drugs were used in these studies, the prophylactic trypanocide isometamidium chloride (Samorin®, Merial Lyon, France), and the therapeutic trypanocide diminazene aceturate (Veriben®, Sanofi, Lyon France).

3.3 Definitions of Terms

The definitions of terms and abbreviations used in describing the field prophylaxis studies are shown in Table 2.

Table 2: Definitions used in the isometamidium prophylaxis field studies at the coast province of Kenya

ISMM	Isometamidium chloride
DIM	Diminazene aceturate
Covered by ISMM	Cattle having received a prophylactic dose of isometamidium at the beginning of the trial period under consideration
Covered by DIM	Cattle treated with diminazene within the last three weeks
Animals at risk	Cattle not covered by either isometamidium or diminazene
Isometamidium group	Cattle covered by isometamidium
Sentinel group	Cattle not covered by ISMM
Prophylaxis period	A period of three months when cattle are covered by ISMM
Block treatment	Treatment of a group or groups of cattle with a trypanocidal drug on the same occasion
New infections	Infections acquired by cattle at risk or covered by ISMM, that were uninfected during the previous 3 weeks
Breakthrough infections	Infection acquired by cattle despite prophylactic trypanocide treatment
Ongoing infections	Infections ongoing in cattle, after either trypanocidal drug treatment or no treatment, that were infected the previous week
Remission	Infected cattle becoming aparasitaemic after either treatment with a trypanocidal drug or no treatment
Relapse infection	Parasitaemia detected in cattle within 3 weeks of remission

3.4 General outline of field studies methodology

3.4.1 Block treatments with isometamidium chloride

Groups of cattle were treated prophylactically with a 2% solution of isometamidium chloride (Samorin®) intramuscularly in the rump at a dose of 1 mg/kg body weight. The Samorin® solution was prepared by dissolving 1 g in 50 ml of distilled water and allowing 15 minutes for the powder to dissolve.

3.4.2 Individual treatments with diminazene aceturate

Diminazene aceturate (Veriben®) was administered at 7.0 mg/kg body weight intramuscularly to individual trypanosome-infected cattle. The Veriben® solution was prepared by dissolving one sachet (1.02 g) of the drug in 15 ml of distilled water.

3.4.3 Experimental design

Animals were usually divided into two groups; the isometamidium block treated group and the sentinel group. Cattle in either group were usually treated with Veriben® when trypanosomes were detected. However, in some studies infected cattle were treated only when there were signs of ill health associated with the infection, such as PCV less than 23 %. The two groups were monitored as follows:

- Monitoring for trypanosome infections and packed cell volume was conducted weekly
- Serum samples for isometamidium-ELISA were collected weekly
- Trypanosome isolates were collected from all parasitaemic cattle
- Body weight and condition score were determined monthly

3.5 Monitoring of packed cell volume and trypanosome infections

3.5.1 Packed cell volume determination

Blood from a marginal ear vein, punctured using a sterile lancet (Unilet® JBMSL Ltd. Hamilton, Scotland) or a needle was drawn into a heparinised capillary tube. The capillary tube was filled to about 5 mm from the top of the tube. It was then sealed on one end with Cristaseal (Labpak Ltd., Coventry, England). The tube was placed in the Microhaematocrit centrifuge (Hawksley, Lancing, West Sussex, England) and spun for 10 min at 12,000g. The packed cell volumes were determined as a ratio of red cell to plasma volumes using a PCV reader (Hawksley)

3.5.2 Buffy-coat/Haematocrit Centrifugation Techniques for trypanosome detection

The blood from a punctured ear vein was collected into a heparinised capillary tube as described above in section 5.1. The capillary tube was sealed on one end and spun in a microhaematocrit centrifuge at 12,000g for 10 min. After centrifugation, the capillary tube was cut just below the buffy coat with a diamond pencil (Merck, Lutterworth, England). The buffy coat was expressed onto a slide, mixed and examined for trypanosomes with a light Leica Galen III® microscope (Fisher Scientific, Loughborough, England) under dark-background illumination according to Murray *et al* (1977). Parasitaemia was quantified by counting the number of parasites in 50 fields using a tally counter (Fisher Scientific).

3.5.3 Stained thick and thin blood smears for trypanosome detection

3.5.3.1 Thick blood smear

A drop of blood was placed on a clean microscope slide and a thick film prepared by using the corner of another slide to produce a rounded smear of about 1 cm in diameter. The slide was then rapidly air-dried and then placed in distilled water for 5 minutes. The smear was later stained with a 10 % solution of Giemsa (Merck). The stained slides, following addition of a drop of immersion oil Microil® (Merck), were examined under a microscope using a 100-times magnification oil-immersion lens (MacLennan, 1957). All fields were examined.

3.5.3.2 Thin blood smear

A drop of blood was placed at one end of a clean grease-free microscope slide and a thin film prepared by spreading the drop along the length of the slide with a straight edge of another slide, quickly air dried and fixed for a few minutes in methanol. The film was then stained with a 10 % solution of Giemsa followed by examination for parasites under oil immersion using a 100-times magnification oil-immersion lens (Fiennes, 1952). All fields were examined.

3.6 Collection and preparation of trypanosome stabilates

Two different methods were used to collect parasitaemic blood for stabilate preparation. One method involved collecting the blood from an ear vein and the other from the jugular vein.

3.6.1 Collection of blood from an ear vein

- The ear of infected cattle was cleaned with distilled water and dried
- Blood (1 ml) from a punctured ear vein was collected from its skin surface into a 2-ml syringe containing 1 ml of buffered EDTA (Sigma, Poole, England) with 20% glycerol and mixed well.
- The blood buffer mixture (1:1) was then put in 2-ml cryovials (Greiner Labortechnik, Stonehouse, England).

3.6.2 Collection of blood from the jugular vein

- Blood from the jugular vein of trypanosome-infected cattle was collected in 5 ml heparinised Vacutainer tubes (Labpak Ltd)
- 1.8 ml of heparinised blood was then added to a cryovial containing 0.2 ml glycerol and mixed well

With both methods several cryovials were prepared. The cryovials were then suspended in liquid nitrogen vapour for 2-12 hrs in an insulated bottle and then stored in liquid nitrogen

3.7 Body weight and condition scoring

3.7.1.1 Body weight measurement

The cattle's body weights were measured by either a weighing bridge or estimated using a weighing band (Wool Growers, Glasgow, Scotland). Estimation of body weight with a weighing band involved measuring the heart girth using a calibrated tape. The animal was restrained and allowed to stand in a straight posture. The tape was then placed round the girth just behind the shoulders and the two ends of the tape allowed to cross, at the animals back. The tape was then tightened slightly to press on the body and the weight was estimated using the pointer marked on one end of the tape (Nicholson and Sayers, 1987).

3.7.1.2 Body condition score estimation

The body condition of the cattle was estimated using the method according to Nicholson and Butterworth (1986). In this method, nine scores were used in which the three main conditions (fat [F], medium [M] and lean [L] were subdivided into three categories. The scores were abbreviated as F+, F, F-; M+, M, M-; L+, L, L-. Each scoring was given a number from 1 (L-) to 9 (F+). In a borderline case a half point was added to the lower score, such that a cow described as M-/L+ was scored as 3.5. The following anatomical parts were used in determining the condition score:

- Tail-head; brisket and hump
- Transverse processes of the lumbar vertebrae; hips (trochanter major) and ribs
- The shape of the muscle mass between the *tuber coxae* and *tuber ischii*.

For consistency the same person carried out all condition scoring and weight estimates. In addition the scores were estimated early in the morning before the animals had access to food or water that day.

3.8 Serum collection and preparation

Blood was removed from the jugular vein using a plain Vacutainer tube with a 19-gauge needle. In the field, the blood was kept in a cool box containing ice

packs after initially allowing for clotting at ambient temperature, protected from direct sunlight. The clotted blood was later refrigerated at 4 °C overnight.

Serum was separated the following day by centrifugation at 3000g for 10 min. It was then aliquoted into 2-ml storage tubes (Alpha Labs, Eastleigh, England), and stored at -20 °C.

3.9 Isometamidium ELISA

The isometamidium levels in serum were determined by an enzyme-linked immunosorbent assay technique developed by Eisler *et al* (1996a), with subsequent modification as described by Mubanga (1996).

3.9.1 Materials

All chemicals and reagents used were pure (Analar grade). All incubations were carried out in an orbital shaker-incubator, Varishaker® (Dynex Technologies, Billingshurst, England). Serum dilutions were carried out in 8 x 12 racks of polypropylene tubes in microtitre plate format with strip-caps. Dispensing of the pre-diluted sera and the assay reagents on the microtitre plates were carried out using 100 µl precision pipette tips (C20, Anachem Luton, England) fitted on an eight-channel pipette (pipetman®, Anachem). All buffer salts (NaCl, Na_2HPO_4, KH_2PO_4, $NaHCO_3$, Na_2CO_3) were supplied by University of Glasgow. All washings were carried out using a 12-channel hand washer (catalogue no., 7337-6201 BDSL, Kilmarnock, Scotland).

3.9.1.1 Hyper-immune rabbit anti-isometamidium serum

The hyper-immune rabbit anti-isometamidium serum was provided by the Division of Veterinary Physiology, Department of Veterinary Pre-Clinical Studies at the University of Glasgow, Scotland. The anti-isometamidium serum had been prepared as follows:

- Two rabbits were primarily immunised by subcutaneous injection of isometamidium-thyroglobulin conjugate emulsified in Freud's complete adjuvant (FCA) at two sites (right side of the neck and the right flank).

- Three booster immunisations of isometamidium-thyroglobulin conjugate emulsified in Freud's incomplete adjuvant (FICA) were given 21/2, 7 and 18 weeks later
- Twelve days after the final booster, blood was collected and the hyper-immune serum the separated. This was stored at -20 °C until required.

3.9.1.2 Coating buffer

Carbonate/bicarbonate buffer 0.1 M, pH 9.2

The preparation procedure was as follows:

- Sodium bicarbonate, $NaHCO_3$ (8.4 g) was dissolved in 1 L distilled/deionised water.
- Sodium carbonate, Na_2CO_3 (2.65 g) was dissolved in 250 ml distilled/deionised water.
- The pH of 900 ml of the bicarbonate solution was adjusted to 9.2 by slow addition of the carbonate solution. The buffer was then stored at 4° C for up to 2 weeks

3.9.1.3 Microtitre plate coating

Immulon 4 (Dynex Technologies) microtitre plates were coated with 1/10000 dilution of hyper-immune rabbit anti-isometamidium serum in carbonate/bicarbonate buffer 0.1M, pH 9.2 as follows:

- The coating buffer and the hyper-immune rabbit anti-isometamidium serum were pre-warmed to room temperature.
- A 1/10000 dilution of hyper-immune rabbit anti-isometamidium serum was prepared and 100 µl of the reagent dispensed in each well of a 96-well microtitre plate. Sufficient quantity of the diluted hyper-immune rabbit anti-isometamidium serum was prepared to coat at least ten microtitre plates at any one time.
- The plates were sealed with plate sealers (Dynex Technologies) and incubated overnight at 4° C. The following day the plates were store at -20° C until required.

3.9.1.4 Phosphate buffered saline pH 7.4

Sodium chloride, NaCl (40.0g), disodium hydrogen phosphate, Na_2HPO_4 (7.4g) and potassium dihydrogen phosphate, KH_2PO_4 (2.15g) were dissolved together in 5 L distilled/de-ionised water

3.9.1.5 Diluent buffer

Phosphate buffered saline (PBS) pH 7.4 containing 0.05 % Tween 20 (PBST)

3.9.1.6 Washing buffer

PBS diluted 1/5 in distilled/de-ionised water, containing a final concentration of 0.05% Tween 20 (Sigma)

3.9.1.7 Isometamidium-HRP conjugate

Isometamidium conjugated to horseradish peroxidase (Boehringer Manheim, Germany) via 1-ethyl-3- (3-dimethylaminopropyl carbodiimide/n-hydroxy succinamide linkage (Sigma). Supplied in 50% glycerol (Sigma) and stored at -20° C. This conjugate was provided by the Division of Veterinary Physiology, Department of Veterinary Pre-Clinical Studies at the University of Glasgow, Scotland. The preparation procedure had been as follows:

- A solution of 2 mg N-hydroxy succinamide dissolved in 100 µl 1 mM sodium acetate was added to a solution of 10 mg of isometamidium chloride dissolved in 250 µl 1 mM sodium acetate containing 125 µl pyridine (Sigma).
- The resultant solution was mixed well and allowed to stand at room temperature for 2 minutes.
- A solution of 10 mg 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide dissolved in 250 µl of 1mM sodium acetate was added and mixed well.
- Aliquots (100 µl) of the reaction mixture prepared above were added at room temperature to a solution of 20 mg horseradish peroxidase in 1 ml of 1 mM sodium acetate solution, over a period of 15 minutes with constant mixing.

- The solution was incubated at 37° C for 20 hours, then dialysed against phosphate-buffered saline, pH 7.4.
- Passing the solution through a Sephadex PD-10 column (Pharmacia, U.K) purified the resulting conjugate further.
- This was followed by mixing the coloured portion of the volume for 15 minutes with 0.5 ml of a 0.25 % (w/v) suspension of activated charcoal (Sigma) in distilled water containing 0.025 % (w/v) dextran T70 (Pharmacia).
- The mixture was centrifuged at 14,000 rpm, the supernatant removed, mixed with an equal volume of glycerol and stored at –20° C

3.9.1.8 Substrate-chromogen (TMB)

Two components of horseradish peroxidase substrate containing 3,3,5,5 tetramethyl-benzidine and hydrogen peroxide (Vetoquinol, Bicester England). Equal volumes of the two components were mixed before use.

3.9.1.9 Stopping solution

One molar (1M) orthophosphoric acid, H_3PO_4 (Fisher Scientific)

3.9.2 Sequential saturation assay

The procedure of the assay was as follows:

- All microtitre plate volumes were 100 µl /well
- Serum from isometamidium treated cattle was diluted 1/10 in diluent buffer.
- The coated plates previously stored at –20 °C were left to thaw on the bench at room temperature then washed five times with washing buffer, and blotted dry on a thin sponge (Spontex®)
- The pre-diluted serum was incubated overnight at +4 ° C in coated microtitre plates.
- The serum was discarded and the plate washed 5-times with washing buffer.

- Isometamidium-HRP conjugate diluted 1/128000 in PBST was added and the plate incubated at 37° C for 15 minutes.
- The excess conjugate was discarded and the plate washed 5-times.
- The mixture of the two components of the substrate-chromogen system were pre-warmed to 37° C, added to the microtitre plate and incubated for a further 10 min at 37 ° C.
- Stopping solution was then added to all the wells.
- A multi- channel microtitre plate reader (Bio-Tek EL-311SX , Bio-Tek Instruments Inc. Vermont, USA) with a desktop computer (Compaq Pressario series 5000, USA), online via the RS232C serial connection read the optical densities of the samples using a 450 nm filter

3.9.3 Calibration curves and Quality Assurance

To calculate calibration curves and to carry out quality assurance on the assay, spiked isometamidium standards and treated quality controls had to be prepared respectively. In addition, control sera from untreated cattle were screened.

3.9.3.1 Preparation of isometamidium standards

Isometamidium-spiked bovine serum standards were prepared by serial dilution of a stock isometamidium solution of concentration 500μg/ml. The stock solution was prepared by adding distilled/de-ionised water to carefully weighed 50 mg of Samorin® powder to a final volume of 100ml. The stock solution was then diluted 1/10 in distilled/ de-ionised water. This was further diluted by adding 45 μl of the 1/10 diluted solution into 1455 μl pooled normal bovine serum (control serum) to give a concentration of 1.5 μg/ml Samorin®. The subsequent 1:3 serial dilutions were all carried out in control serum. The final standards obtained were at the following concentration in ng/ml

- 55.55, 18.52, 6.17, 2.06, 0.686, 0.229, 0.076, 0.025

All standards contained at least 99% serum by volume.

3.9.3.2 Preparation of Quality controls (QC)

Quality controls were prepared from bovine serum from isometamidium chloride treated cattle (Eisler, *et. al*, 1993). The serum was diluted 1/10 and 1/2 in pooled untreated sera to give $QC_{1/10}$ and $QC_{1/2}$ respectively. The third QC was pooled normal bovine serum (control serum) of untreated cattle.

3.9.3.3 *Isometamidium-ELISA plate layout*

The layout of the plate for the assay was as described by Eisler *et al.* (1993). Each plate contained 34 samples, 8 isometamidium standards and six quality controls, 3 before and 3 after the samples (see below). The first half of the plate was duplicated in the second half (shaded).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S10	QC_0	6	14	22	30	S10	QC_0	6	14	22	30
B	S9	$QC_{1/10}$	7	15	23	31	S9	$QC_{1/10}$	7	15	23	31
C	S8	$QC_{1/2}$	8	16	24	32	S8	$QC_{1/2}$	8	16	24	32
D	S7	1	9	17	25	33	S7	1	9	17	25	33
E	S6	2	10	18	26	34	S6	2	10	18	26	34
F	S5	3	11	19	27	QC_0	S5	3	11	19	27	QC_0
G	S4	4	12	20	28	$QC_{1/10}$	S4	4	12	20	28	$QC_{1/10}$
H	S3	5	13	21	29	$QC_{1/2}$	S3	5	13	21	29	$QC_{1/2}$

Serum isometamidium concentrations were determined by fitting four-parameter logistic regression curves of calibration standards and calculating the concentrations of the unknown samples. This was carried out using the ELISA data analysis Eiaquik program (© M.C Eisler, 1995).

The results of the plate were considered not valid if the calibration standards did not closely fit the four-parameter logistic model, or the quality assurance samples did not give the expected results (QC0: 0 - 0.15 ng/ml; QC1/10: 1.875 - 3.125 ng/ml; QC1/2: 0.375 - 0.625 ng/ml). Similarly, any individual samples for which the coefficient of variation (CV) of the two duplicate wells was greater than 12 % were re-tested.

Each serum sample was tested in two assays. The mean drug concentrations and the CV (between assays) of the repeats were then calculated. If the CV (between assays) of the two repeat samples was greater than 25 % and the mean concentration was greater than 0.2 ng/ml, the sample was re-tested.

B/Bo values were calculated where B was the optical density of the sample and Bo, extrapolated from the calibration curve fitting was the expected optical

density at zero concentration. Samples that had very low B/Bo values (< 5%) were re-tested following dilution in pooled normal bovine serum used to prepare the assay standards.

3.9.3.4 Screening of pre-treatment sera

Pre-treatment sera from cattle that had not been treated with isometamidium before in the studies were collected and the optical density (OD) of the individual sera determined. Each individual sample was tested twice. The confidence interval (CI) was taken as mean OD ± 2standard deviation (SD). Sera with OD outside the CI were discarded and the rest pooled together as control sera. This was used for preparation of the assay standards and for quality controls (QCs).

3.10 Data Analysis

3.10.1 Parasitological data

3.10.1.1 Weekly trypanosome infection rates

In calculating infection rates, data from cattle treated with diminazene within the last three weeks were excluded from the calculations. This was done in order to take into account the brief prophylactic effect (14 days) of diminazene aceturate and the pre-patent period of infection (7-10 days). The infection rates were then calculated as number of infections in cattle considered to be at risk.

3.10.1.2 Survival analysis

The field infection data were analysed using survival analysis techniques. Survival, S is the probability of individuals with a specific disease remaining alive for a specified length of time. In this case, Survival was considered as the probability of individual cattle at risk in a trypanosomiasis endemic area not acquiring an infection for the duration of the prophylaxis period. Cattle that had trypanosome infections and/or were treated with diminazene aceturate three weeks before the beginning of a prophylactic period or isometamidium treatment, were not included in the survival analysis. The method applied was life table analysis where hazard rate and survival function was determined. In essence the survival function is the

proportion of cattle that remain uninfected during a particular period of time. Hazard rate, the force of infection is a theoretical measure of the risk of occurrence of disease at a point in time and is estimated by the disease incidence rate. The hazard rate and the survival function in the life tables were calculated as follows:

If, n_k = number of animals at risk at the start of a weekly monitoring period k,

p_k = number of new infections in that period,

q_k = the number of animals remaining at risk at the end of the period, and

c_k = number of animals censored (animals that were lost to follow during the prophylactic period) in period k

Then;

$q_k = n_k - p_k - c_k$

$n_k = n_{k-1} - p_{k-1} - c_{k-1} = q_{k-1}$

Hazard function $f(H)_k = p_k/q_k$

Survivor function $f(S)_k = (1 - p_k/q_k) \times f(S)_{k-1} = (1 - f(H)_k) \times f(S)_{k-1}$

Where k= 1.....13

The baseline hazard was calculated as the average weekly hazard rates of sentinel cattle for the first eight weeks of the prophylactic period. The hazard rate between groups was compared using the Log Rank test and the Wilcoxon test. The statistical tests were carried out using the Lifetest and Lifereg procedures of the SAS/STAT software system (SAS Institute Inc., Cary, North Carolina USA).

3.10.2 Assessment of efficacy of isometamidium prophylaxis

Three simple measures relating to the efficacy of isometamidium prophylaxis were also considered for each study site:

- The proportion of sentinel cattle infected by the 8th week of each study period as indicated by the survival function in Life-tables

- The proportion of isometamidium-treated cattle infected by the 8th week of each study period as indicated by the survival function in Life-tables.
- The ratio between the average of the hazard functions of the sentinel and isometamidium-treated groups over the first 8 weeks of each study period.

It is postulated that these measures may be interpreted as follows, and the data were appraised in this way to assess the validity of these postulates:

- If fewer than 25% of sentinel cattle are infected by the 8th week of a study period, then trypanosome challenge is insufficient to indicate use of isometamidium prophylaxis.
- If more than 25% of isometamidium-treated cattle are infected by the 8th week of a study period, this is evidence of treatment failure and or isometamidium resistance, depending on the reliability of drug administration.
- If the ratio between the average of the hazard functions of the sentinel and isometamidium-treated groups over the first 8 weeks of a study period is less than two-fold, efficacy of prophylaxis is insufficient to indicate its continued use.

3.10.3 Serum isometamidium concentration data

Descriptive statistics were calculated using Microsoft Excel 97 software (Microsoft Corporation).

Natural logarithm-transformed isometamidium concentration data were analysed as the dependent variable in generalised linear regression models using days post treatment as the independent variable, and study site and study period as co-variates. Modelling of isometamidium concentration was limited to data from cattle prior to the detection of the first trypanosome infection following prophylactic administration of the drug. This was because there is evidence that isometamidium elimination is faster in cattle with trypanosome parasitaemia resulting from breakthrough infections (Eisler *et al.*, 1994).

Regression modelling was performed using Genstat 5 Release 3.2 for Windows, 2nd Edition, Lawes Agricultural Trust IACR, Rothampstead.

3.10.4 Viability and mice infectivity data

The most important factors considered in the analysis of these data were the effect of isometamidium treatment, time after treatment and the drug concentration. Site and year were considered as co-variates and were included in the mixed models used. Mixed models were analysed using the Mixed procedure of the SAS/STAT software system (SAS Institute Inc., Cary, North Carolina USA).

3.10.5 Modelling

A linear mixed model is a linear model with both fixed and random effects. Fixed effects refer to terms in the model, which can only take certain specified levels, for example a drug treatment group in an experiment. Random effects refer to terms in the model in which the levels to be modelled are considered to be a random selection from a larger number of possible levels, for example particular study herds of cattle in a region containing a large number of cattle herds. Traditional regression models are usually fixed effects models and usually only allow for one source of error, the random error term associated with each observation. Random effects, such as herd in the example just given, are thus treated as fixed effects, and the inferences from the model are applicable only to the herds under study, rather than all herds in the region.

The mixed model methodology allows the inclusion of any combination of fixed effects, random effects and their interactions. This is useful in situations where normally analysis of variance might be used but the data are unbalanced, or where linear regression might normally be used, but there is more than one source of variation in the data. Hence, mixed models are widely applicable in a wide variety of situations and can be used to obtain information on sources and sizes of variability in data sets. This can be of interest where the relative size of different sources of variability must be assessed. Mixed models also provide efficient estimates of treatment effects in unbalanced designs with more than one source of error. They can be used to provide estimates of treatments effects that combine information from all the strata of a partially balanced design, or to combine information over similar experiments conducted at different times or in different places. This allows estimates to be obtained making use of the information from all the experiments, as well as the

separate estimates from each individual experiment (Genstat 5 Release 3.2, second edition).

Chapter 4

Longitudinal studies into the influence of trypanocidal drug resistance on the efficacy of chemoprophylaxis and chemotherapy in the control of bovine trypanosomiasis on Galana Ranch in Coast Province, Kenya

4 Longitudinal studies on Galana Ranch

4.1 Introduction

Studies were conducted over a three-year period between December 1994 and March 1997 at Galana Ranch, a commercial, extensively reared beef cattle enterprise in Coast Province Kenya under heavy seasonal tsetse and trypanosome challenge. The aims of these studies were to assess the efficacy of trypanocidal chemoprophylaxis and chemotherapy in controlling the disease, and to investigate the role of trypanocidal drug resistance.

4.1.1 Galana Ranch

The studies described in this Chapter were conducted at Galana Ranch in the south-eastern part of Kenya. The ranch consists of some 6,000 km^2 lying between 2° S and 3° S and 39° E and 40° E in the hinterland of Coast Province of Kenya. Part of the ranch is in the north-western part of Kilifi District and the southern part of Tana River District at an average altitude of 270 m above sea level. The Galana River forms the southern border of the ranch. The mean annual rainfall increases from 250 mm in the west of the ranch to 625 mm nearer the coast. The bulk of this precipitation occurs in two rainy seasons, the first of which occurs between March and April and the second, usually with heavier rainfall (40% of the yearly rainfall), between October and December. The position of the ranch is shown in Figure 1 in Chapter 3.

Most of the ranch is semi-arid with thin and scattered vegetation, consisting largely of Acacia thorn scrub. However, some parts of the ranch, especially the wetter eastern parts, have vegetation which is thick with thorny scrub bushes. The ranch lies in the Nyika plateau topographical region that is characterised by low rainfall and poor soil for agricultural activities. Some parts of the area are suitable for extensive cattle rearing. The ranch is populated with different species of wild (lions, gazelles, warthogs, buffaloes, rhinos etc) and domestic animal life (King *et al.*, 1977).

4.1.1.1 Brief history of Galana Ranch

The ranch is traditionally a commercial beef ranch. First owned by Galana Game and Ranching Company and later by a parastatal company, Agricultural Development Corporation (K). The main constraints to beef production on the ranch are predators, trypanosomiasis and lack of water (Njogu *et al.,* 1985). To alleviate the water problem, water tanks were built at strategic sites. Kenya Trypanosomiasis Research Institute, mandated to combat trypanosomiasis by research in Kenya, has maintained a field station at the location of one of them, Tank E, since 1979. This field station is used as a base from which field trials are conducted on the ranch cattle.

4.1.1.2 Tsetse infestation on Galana Ranch

The distribution of the main tsetse species on Galana Ranch is shown in Figure 2. Although the tsetse distribution map of Kenya shows almost the entire ranch as being tsetse infested, approximately the whole of the western area of the ranch is free of tsetse.

Galana Ranch is infested with *G. longipennis* in the centre and toward the south. In the wetter eastern zone where there is thick vegetation, the ranch is infested with large numbers of *G. pallidipes* (Baylis, 1997) which is the major vector of *T. congolense* and T. *vivax* on the ranch. Dakabuku, one of the sites of this study has predominantly *G. pallidipes*, while Kapangani, the other site of this study situated at the western periphery of the *G. pallidipes* zone is also infested with *G. longipennis* (Makumi *et al.,* 1996). Other tsetse species have been caught on the ranch. Challier and Laveissiere (1973) reported catches of *G. austeni* near Galana River at Kasiki.

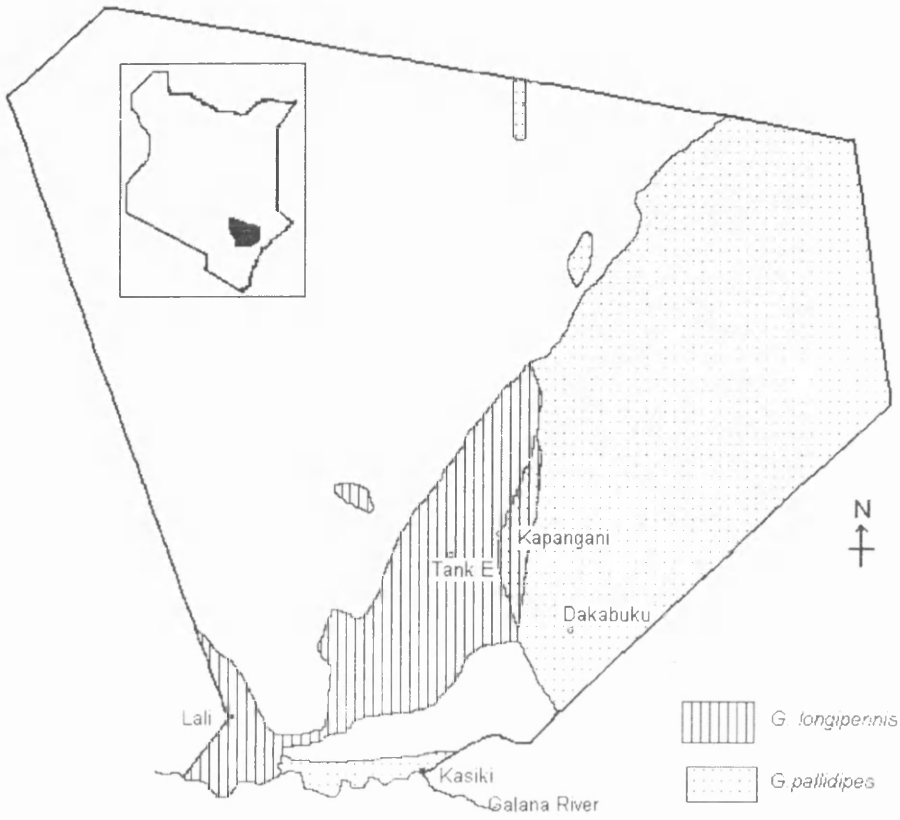

Figure 2: Tsetse and trypanosomiasis distribution on Galana Ranch in the coastal region of Kenya

4.1.1.3 Cattle breeds

Two varieties of large zebu Boran (*Bos indicus*) cattle are used on the ranch (Mason and Maule, 1960), the Galana Boran, and the Orma Boran.

The Galana Borans derived from 16,000 range-bred Boran cattle were first introduced to Galana Ranch in 1978 from Laikipia District in the tsetse-free highlands of the Central Province of Kenya (Njogu *et al.,* 1985) they had undergone 70 years of selection for beef production. On Galana Ranch these cattle reach 400 kg at three and half years of age.

Orma Boran steers are purchased from the Orma tribe of lower Tana River basin at the age of 12-18 months and brought to the ranch where they reach 400 kg at four years of age (Njogu *et al.*, 1985). The Tana basin where the Orma people have traditionally selected their cattle for milk yield for several centuries, is a tsetse and trypanosomiasis endemic area (Ford and Katondo, 1973). This may explain the trypanotolerant trait that they possess (Wilson *et al.*, 1986).

4.1.1.4 Cattle husbandry practices

During the day, cattle are grazed in herds of about 250 head, under the control of locally recruited herdsmen. At night they are kept in thorn bush fenced enclosures for protection against predators. In recent years the ranch has not been fully stocked, and the management policy has been to graze cattle on the western part of the ranch where there is very little tsetse challenge.

4.1.1.5 Seasonality of tsetse and trypanosomiasis challenge

Seasonal variability of tsetse and trypanosomiasis has been observed on the ranch. At Kapangani and Kasiki areas (Figure 2) a major peak in tsetse and trypanosome challenge was indicated by the Berenil index between December and February, with a minor peak between May and July (Njogu *et al.*, 1985). At Dakabuku (Figure 2) two trypanosome challenge peaks were observed in cattle between December 1986 and January 1987, and between May and June 1997 (Dolan *et al.*, 1990). In addition, trypanosome infection rates observed in trapped tsetse flies

suggested that heifers received highest number of *T. congolense* and *T. vivax* infected bites per day during January and the least number of infected bites during May (Baylis, 1997).

4.1.1.6 Impact of trypanosomiasis on Galana Ranch

In the study of the epidemiology of bovine trypanosomiasis on the ranch (1980-1981), potential losses in beef production due to trypanosomiasis in ranch population at risk were estimated at approximately KSh. 8,900/km^2, when the stocking rate was 14.2 Tropical Livestock units per km^2 (Wilson *et al.*, 1986). The estimated potential losses in the total population at risk were approximately KSh. 5 million (around US$ 700, 000 at 1981 value). In 1982 however, a combination of treating only those animals under trypanosome risk at an opportune time, and the increased utilisation of trypano-tolerant Orma Borans resulted in an annual saving of about US$ 1,100,000 in control costs and an increased land usage of approximately 5% (Wilson *et al.*, 1986, Dolan, 1999)

4.1.1.7 Control of trypanosomiasis

Control of trypanosomiasis in the tsetse-infested areas of Galana Ranch has traditionally been through chemotherapy, and chemoprophylaxis using homidium and isometamidium. For many years, cattle were given prophylactic cover with five doses of isometamidium chloride at 0.5 mg/kg b.w. annually and two additional doses of diminazene aceturate at 3.5 mg/kg b. w. annually.

However, the efficacy of chemoprophylaxis appears to be dependent on trypanosome challenge. For instance, in a year of low to medium challenge, homidium administered twice to Boran cattle in a year, provided protection for periods of 8 and 17 weeks (Dolan *et al.*, 1990). However, the following year, when the tsetse challenge was exceptionally high, breakthrough infections were detected two weeks after isometamidium treatment. Ninety-seven percent of these infections were caused by *T. vivax* (Dolan *et al.*, 1992).

Multiple drug resistance was first confirmed on the ranch in 1984 (Rottcher and Schillinger, 1985; Njogu and Heath, 1986). During a period of high tsetse challenge in 1984, high numbers of *T. vivax* breakthrough infections were observed.

Many of these infections were of the acute haemorrhagic type that had been observed earlier in other parts of Kenya (Mwongela *et al.*, 1981). The *T. vivax* infections responded to treatment with diminazene at 7.0 mg/kg. *Trypanosoma vivax* stabilates prepared from 11 cattle , two of which had shown the haemorrhagic signs of the disease, were pooled together and used to infect a clean zebu steer at the Veterinary Research Laboratory, Kabete (Rottcher and Schillinger, 1985). The established *T. vivax* infection from the donor steer was used to infect another 39 steers with 1.72 x 10^5 trypanosome/kg b.w. All animals became positive within 3 days and were treated with four drugs i.m at three different doses each. Three cattle were left untreated as controls. The drugs were; isometamidium chloride (0.5, 1.0, 2.0 mg/kg b.w.), diminazene aceturate (1.75, 3.5, 7.0 mg/kg b.w.), homidium chloride (0.5, 1.0, 2.0 mg/kg b.w.) and quinapyramine (1.5, 3.0, 5.0 mg/kg b.w. The pooled *T. vivax* stabilate was resistant to all four drugs at 2.0 mg/kg b.w. for isometamidium, 1.0 mg/kg b.w. for homidium, 5.0 mg/kg b.w. for quinapyramine and 3.5 mg/kg b.w. for diminazene.

However, when seven of the original eleven *T. vivax* stabilates were individually tested in cattle, those that established a patent infection were susceptible to isometamidium at 1 mg/kg b.w. (Njogu and Heath, 1986). In response to the findings of the sensitivity testing, KETRI established two herds of cattle from March 1985 on eight-weekly isometamidium prophylaxis. No further evidence of resistance was detected (Njogu and Heath, 1986). The observed variation of efficacy of isometamidium with season and level of challenge indicates that it would be more appropriate to use isometamidium prophylaxis only during the high challenge season as opposed to year around routine prophylaxis.

In an attempt to reduce early breakthrough infections in a situation of very high trypanosome challenge, increasing the frequency of treatment (reducing the recommended period of prophylaxis) has been commonly used (Fox *et al.*, 1991). However, previous experience on Galana Ranch shows that this should not be advocated. Stevenson *et al.* (1990) reported a phenomenon manifested by progressive emaciation and characteristic odour emanating from the animal's coat leading to death. This phenomenon, which occurred in 1985 on Galana Ranch in a group of cattle under chemoprophylaxis, was associated with frequent use of isometamidium. High mortality and loss of production were observed (Dolan *et al.*,

1992). An attempt to recreate the syndrome under typical pastoralist conditions in a semi-arid area of Ngurumani in south-western Kenya demonstrated that the phenomenon was associated with hepatotoxicity due to repeated treatment with isometamidium in conjunction with diminazene (Eisler *et al.*, 1997b).

From previous studies in Galana Ranch it is clear that trypanosomiasis risk or level of challenge is an important determinant of the prophylactic period of a drug. Whether an increase in tsetse challenge leads to an increase in the resistant population or increase in the metabolism of the drug leading to more rapid elimination of the drug from the system has not been addressed by previous studies carried out at Galana Ranch. It was important then to establish factors that may contribute to seasonally efficacy of isometamidium observed at Galana Ranch.

4.1.2 Objectives

4.1.2.1 To establish how effective isometamidium prophylaxis is in controlling bovine trypanosomiasis at Galana Ranch.

4.1.2.2 To investigate variation in isometamidium efficacy at Galana Ranch in years with different levels of trypanosome challenge.

4.1.2.3 To assess the effect of diminazene treatment at Galana Ranch as an alternative chemotherapeutic strategy to isometamidium prophylaxis.

4.1.2.4 To determine the impact of prophylaxis failure at Galana Ranch in terms of animal health-related losses and treatment costs.

4.2 Material and methods

4.2.1 Description of the study sites

4.2.1.1 Dakabuku area of Galana Ranch

This area is considered to be one of high tsetse challenge, on the basis of several years monitoring of tsetse and trypanosomiasis by KETRI. The predominant tsetse species is *G. pallidipes* (Figure 2). The 1994/95 and 1995/96 studies were conducted at this site.

4.2.2 Kapangani area of Galana Ranch

In the 1996/97 experiment, the study was moved to Kapangani of Galana Ranch for logistical reasons. This area though close to Dakabuku, is considered to have an intermediate tsetse density (Baylis, 1997). The site is situated on the western fringe of the *G. pallidipes* zone, where it overlaps with the *G. longipennis* zone (Figure 2).

4.2.3 Experimental design

As discussed earlier, tsetse challenge at Galana Ranch involving primarily *Glossina pallidipes* is highly seasonal. Therefore, the studies described here were timed to coincide with the season normally associated with peak tsetse challenge (December – March). Studies were carried out over this peak challenge period for three consecutive years, 1994/1995, 1995/1996 and 1996/1997. In each year, a herd of cattle was divided into two groups, one of which was treated with isometamidium, while the other sentinel group remained untreated. Each study lasted approximately three months. The treatment and number of cattle involved is shown in Table 3.

In each year, the isometamidium-treated group received a prophylactic block treatment with Samorin® (isometamidium chloride) by intramuscular injection into the middle third of the neck at a dose rate of 1.0 mg/ kg b.w. (see Chapter 3 section 4.1.). Thereafter, all cattle were monitored for trypanosome infections on a weekly basis and all trypanosome-positive cattle in both the isometamidium-treated and sentinel groups were treated with diminazene aceturate at a dose rate of 7.0 mg/kg

b.w. by intramuscular injection (see chapter 3 section 4.1). In addition, during the three-year study, cattle were treated with antihelmintic, (albendazole, Valbazen® Beecham) every three months and dipped in acaricide fortnightly.

Prior to the beginning of the first study in December 1994, 120 cattle had been kept at "Tank A", a low tsetse challenge area of Galana Ranch, for three months. These cattle were moved to Dakabuku on the evening of 20th December, but were kept close to the crush where tsetse challenge is low, until after trypanocidal drug treatment, two days later.

In the 1995/96 studies two herds of Galana Boran steers were used:

- Herd 1 consisted of 114 of the cattle that had been used for the isometamidium prophylaxis study (isometamidium-treated and untreated sentinel groups) the previous year.
- Herd 2 consisted of 111 cattle that had been used for a similar Ethidium prophylaxis study (Murilla, 1996) the previous year.

These two herds were exposed to the similar tsetse and trypanosome challenge. They were grazing together. For the purpose of analysis the two herds were combined together as shown in Table 3, on the basis of preliminary results which showed no difference between the two.

In 1996/97, 160 cattle were purchased by Galana Ranch from Kenya's North Eastern Province (Tana River area) about 3-6 months before the study. Thereafter they were kept on the western part of the ranch where tsetse challenge is very low, until they were transferred to Kapangani, an area of intermediate trypanosome challenge (Baylis, 1997) about 2 weeks before the study. The number of cattle in each treatment group is shown in Table 3.

Table 3: Details of isometamidium prophylaxis studies on Galana Ranch, December 1994 – April 1997

Study site	Year of study	Group[1]	No. of cattle	Breed
Dakabuku	1994/95	ISMM	80	Galana Boran
		Sentinel	40	Galana Boran
	1995/96	ISMM	149	Galana Boran
		Sentinel	76	Galana Boran
Kapangani	1996/97	ISMM	80	Orma Boran
		Sentinel	80	Orma Boran

[1]ISMM: isometamidium. Isometamidium group cattle were treated prophylactically with the drug at a dose rate of 1.0 mg/kg b.w at the beginning of each study.

4.2.3.1 Parasitological monitoring

Trypanosome infections were monitored weekly in the isometamidium-treated and the sentinel cattle, using the buffy-coat phase-contrast technique and Giemsa stained blood films, according to Chapter 3 section 5.2 and 5.3.

4.2.3.2 Serum samples

Blood samples were collected from the jugular veins of all cattle in the two groups before isometamidium treatment and thereafter only from isometamidium-treated cattle, weekly during the 1994/95 study, and fortnightly during the 1995/96 and 1996/97 studies. The methodology used is described in Chapter 3 section 9.

4.2.3.3 Body weights and condition scoring

Body weights were measured monthly using a weighing bridge (see Chapter 3 section 7.1.1).

Body condition score was estimated monthly during the prophylaxis period of 1996/97 (see Chapter 3 section 7.1.2)

4.2.4 Data analysis

4.2.4.1 Parasitological data

Weekly prevalence of trypanosome infections was calculated as infections detected in that week divided by the animals at risk on that week according to Chapter 3 section 10.1.

Life tables of time to first trypanosome infection over the first eight weeks of each study were drafted and the difference in hazard rates between groups and between trypanosome species were tested using Log Rank and Wilcoxon tests as described in Chapter 3 section 10.1.

4.2.4.2 Packed cell volume data

Descriptive statistics of packed cell volume in each group was calculated using Microsoft Excel 97 software (Microsoft Corporation) and graphs of variation of mean packed cell volumes with days post treatment in each group and year of study plotted.

Mixed modelling to determine the effect of groups within year and baseline hazard on packed cell volumes over the prophylaxis period was done using SAS/STAT software system (SAS Institute inc., Cary, North Carolina USA)

4.2.4.3 Body weights and condition score data

Descriptive statistics on the weight gain in isometamidium treated and sentinel cattle over the prophylaxis period were calculated using Microsoft Excel 97 software (Microsoft Corporation) and graphs of variation of body weights with time in days plotted.

4.2.4.4 Serum isometamidium concentrations data

Descriptive statistics were derived according to Chapter 3 section 10.3. Log – linear regression of the drug concentration was done using Microsoft Excel 97 software (Microsoft Corporation) and elimination rate constants and half-lives calculated from the slope of the regression line as follows;

Slope (coefficient of regression) = k_{el} (elimination rate constant).

$t_{1/2}$ (half-life) = $0.693/k_{el}$

4.2.4.5 Assessment of efficacy of isometamidium prophylaxis

The measurements relating to assessment of efficacy of isometamidium were based on proportion of cattle in the isometamidium group that were infected by the eighth week of the prophylactic period (see Chapter 3. section 10.2). Ratios of mean hazard function over eight weeks of the prophylactic period of sentinel cattle to isometamidium treated cattle were calculated based on life- tables (see Chapter 3. section 10.2).

4.3 Results

4.3.1 Trypanosome infections during isometamidium block treatment studies

Trypanosome infections were detected in cattle in both the isometamidium-treated and sentinel cattle in all three-study years. A summary of the number of trypanosome infections detected over the three years of the study is shown in Table 4. Trypanosome infection rates over the three study years are shown in Table 5.

4.3.1.1 Year 1994/95

In the sentinel group there was a total of 51 infections. Of these, 51.0% were *T. congolense* and 49.0% were *T. vivax*. The overall infection rate in the 520 samples collected from the sentinel group was 9.8% (Table 4). Between two and ten infections were detected every week from the first week to the tenth week of the study (Table 5).

In contrast, in the isometamidium-treated group there were only six breakthrough infections, all *T. vivax*. The two earliest breakthrough infections were detected in the third week after isometamidium treatment, with two infections occurring in the fifth week. Three further *T. vivax* infections were detected one each in the eighth, tenth and twelfth weeks.

The overall *T. vivax* infection rate (4.8%) in samples from sentinel cattle was approximately seven times the overall *T. vivax* infection rate (0.7%) in samples from isometamidium-treated cattle.

4.3.1.2 Year 1995/96

Breakthrough infection in the isometamidium groups occurred as early as the second week after treatment. Breakthrough infections occurred weekly throughout the experimental period of 13 weeks. Two hundred and fifteen infections were detected in 2,352 samples from 149 isometamidium-treated cattle. Of these infections, 21.6% were T. congolense. The remaining 78.4% were *T. vivax*.

In the sentinel group, 183 infections were observed in 1,216 samples from 76 cattle. Of the infections, 42.1% were *T. congolense* and 67.9% were *T. vivax*.

The overall *T. vivax* infection rates in samples from isometamidium-treated and sentinel cattle were similar (8.0% and 8.7% respectively). However, the overall *T. congolense* infection rates in samples (6.3%) from sentinel cattle was approximately six times the overall *T. congolense* infection rate in samples (1.1%) from isometamidium-treated cattle (Table 4).

4.3.1.3 Year 1996/97

In 1996/97, the two earliest breakthrough infections occurred in the third week in the isometamidium-treated cattle, rather than the sentinel group (Table 5). A further seven infections were detected the following week, five in the isometamidium-treated cattle and only two in the sentinel cattle. However, between the fifth and ninth weeks there were fewer infections in the isometamidium-treated cattle (n = 1) than in sentinel cattle (n = 8).

In total, nine infections were detected in 1,200 samples from 80 isometamidium-treated cattle. Of these infections, three (33.3%) were *T. congolense* and six (66.7%) were *T. vivax* (Table 4).

In the sentinel group, 10 infections were detected in 1,187 samples from 80 cattle. Of these infections seven (70%) were *T. congolense* and three (30%) were *T. vivax*.

The proportion of *T. vivax* in samples from isometamidium-treated cattle (0.5%) was approximately one and a half times higher than the proportion of *T. vivax* samples from sentinel cattle (0.3%). In contrast, the proportion of *T. congolense* in samples from isometamidium-treated cattle (0.3%) was approximately half the proportion of *T. congolense* in samples from sentinel cattle (0.6 %).

Table 4: Summary of trypanosome infections detected in cattle during the 3-year prophylaxis study on Galana Ranch

Year of Study	Group	No. of cattle	No. of samples	No. of Tc[2] infections	No. of Tv[3] infections	Tc%[4]	Tv%
1994/5	ISMM[1]	80	1040	0	7	0.0	0.7
	Sentinel	40	520	26	25	5.0	4.8
1995/6	ISMM	149	2352	27	188	1.1	8.0
	Sentinel	76	1216	77	106	6.3	8.7
1996/7	ISMM	80	1200	3	6	0.3	0.5
	Sentinel	80	1187	7	3	0.6	0.3

[1]ISMM: isometamidium-treated

[2]Tc: *Trypanosoma congolense*

[3]Tv: *Trypanosoma vivax*

[4]%: percentage of samples with *T. congolense* or *T. vivax*

Table 5: Trypanosome infection rates in isometamidium-treated and sentinel cattle on Galana Ranch

Year	1994 - 1995		1995 - 1996		1996 - 1997	
Weeks:	ISMM[1]	Sentinel	ISMM	Sentinel	ISMM	Sentinel
1	0.0%	5.0%	0.00%	22.65%	0.0%	0.0%
2	0.0%	7.9%	3.30%	27.25%	0.0%	0.0%
3	2.5%	25.7%	2.75%	45.10%	2.5%	0.0%
4	0.0%	42.3%	8.60%	47.20%	6.4%	2.5%
5	2.6%	29.4%	20.40%	53.10%	1.4%	2.6%
6	0.0%	46.7%	16.55%	33.25%	0.0%	2.6%
7	0.0%	11.8%	18.30%	41.50%	0.0%	2.7%
8	1.3%	34.6%	35.85%	45.65%	0.0%	1.4%
9	0.0%	4.6%	14.25%	20.80%	0.0%	1.3%
10	1.3%	7.1%	21.90%	11.70%	0.0%	0.0%
11	0.0%	0.0%	9.35%	15.85%	0.0%	0.0%
12	1.3%	0.0%	15.95%	24.00%	0.0%	0.0%
13	0.0%	0.0%	13.85%	21.65%	0.0%	0.0%

[1]ISMM: Isometamidium-treated cattle

4.3.2 Survival analysis of isometamidium block treatment studies

Plots of the survival functions for cattle in the isometamidium-treated and sentinel groups for the three years of study (1994/95, 1995/96 and 1996/97) are shown in Figures 3, 4 and 5. The proportions of cattle that experienced at least one infection during the first eight weeks of each prophylaxis study are shown in Table 6. The mean hazard rates and hazard ratios over the first eight weeks of each prophylactic study are shown in Table 7.

4.3.2.1 Year 94/95

The plot of survival function with time during the first year of study is shown in Figure 3. There was a significant difference ($p < 0.001$) in the survival functions between isometamidium-treated and sentinel cattle. Whereas 87.5% of sentinel cattle had experienced at least one trypanosome infection by week eight of the study, only 6.3% of isometamidium-treated cattle experienced an infection over the same period (Table 6). The hazard ratio calculated over the first 8 weeks was 27 (Table 7).

4.3.2.2 Year 95/96

The plot of survival function with time during the second year of study is shown in Figure 4. There was a significant difference ($p < 0.05$) in the survival functions between isometamidium-treated and sentinel cattle. In this study year, the number of cattle experiencing at least one trypanosome infection by week eight (Table 6) was over 60% in both the sentinel group (61.5%) and the isometamidium group (96.6%). The hazard ratio on these eight weeks was 3.0 (Table 7).

4.3.2.3 Year 96/97

The plot of survival function with time during the third year of study is shown in Figure 5. During this year of study there was no significant ($p=0.56$) difference in the survival function between the two groups. In this study year, the number of cattle experiencing at least one trypanosome infection by week eight (Table 6) was fewer than 12% in either the sentinel group (10.0%) or the

isometamidium group (11.3%). The hazard ratio for the two groups over this period was only 1.2 (Table 7).

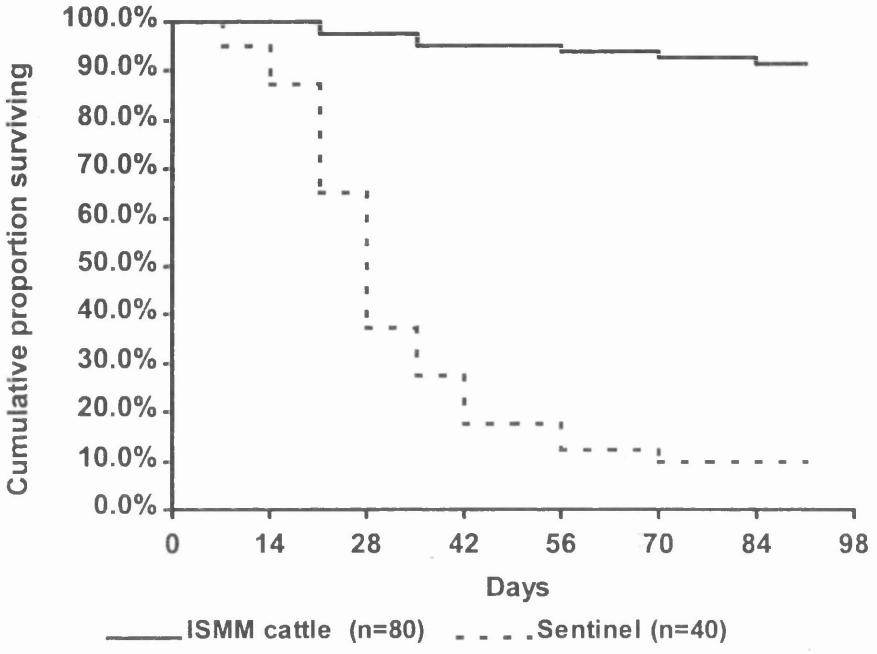

Figure 3: Cumulative proportion of isometamidium treated and sentinel cattle surviving (i.e. remaining uninfected by trypanosomes) during the prophylactic period in 1994/95 on Galana Ranch

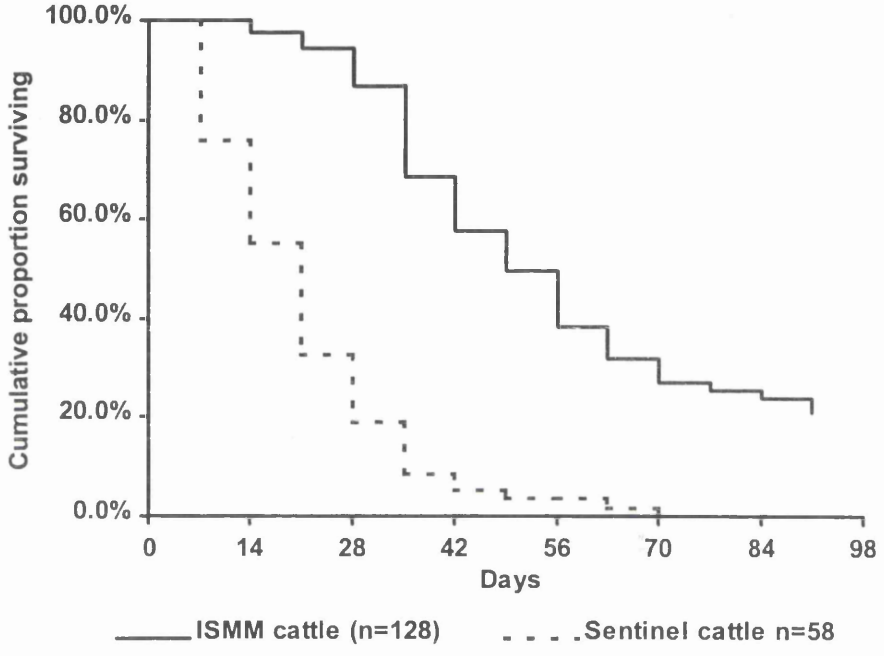

Figure 4: Cumulative proportion of isometamidium treated and sentinel cattle surviving (i.e. remaining uninfected by trypanosomes) during the prophylactic period in 1995/96 on Galana Ranch

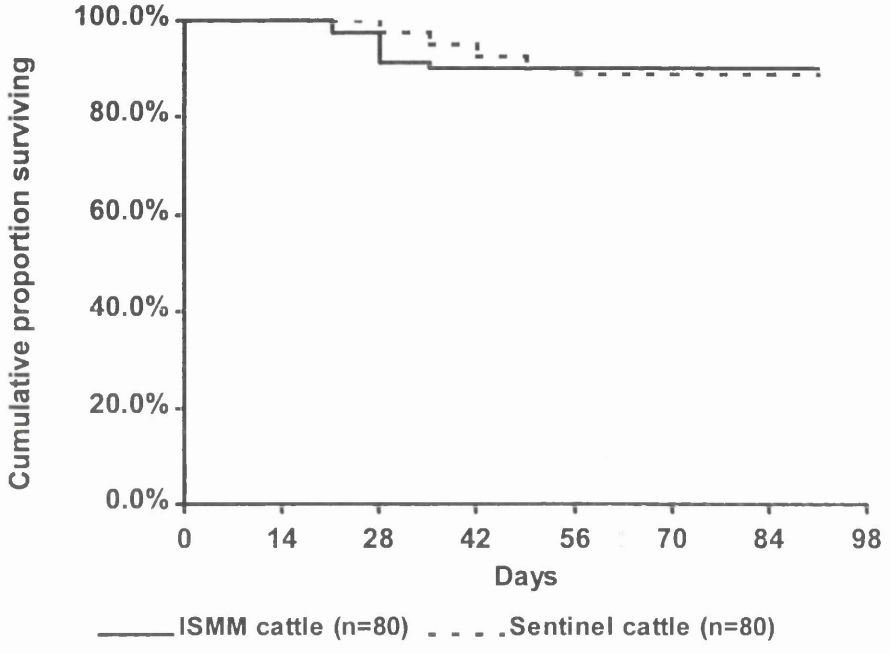

Figure 5: Cumulative proportion of isometamidium-treated and sentinel cattle surviving (i.e. remaining uninfected by trypanosomes) during the prophylactic period in 1996/97 on Galana Ranch

Table 6: Proportions of cattle experiencing at least one infection during the first eight weeks of each prophylaxis study

Year of study	Group	Proportion of cattle[1] (%)
94/95	ISMM[2]	6.3
	Sentinel	87.5
95/96	ISMM	61.5
	Sentinel	96.6
96/97	ISMM	10.0
	Sentinel	11.3

[1]Proportion of cattle in the group experiencing at least one infection during the first eight weeks of the prophylaxis study

[2]ISMM: isometamidium-treated

Table 7: Mean hazard rates and hazard ratios over the first eight weeks of each prophylactic study

Year of study	Group	Mean hazard[1]	Hazard ratio[2]
94/95	ISMM	0.0080	
	Sentinel	0.2156	27.0
95/96	ISMM	0.1089	
	Sentinel	0.3275	3.0
96/97	ISMM	0.0129	
	Sentinel	0.0147	1.2

[1] Mean hazard function over 8 weeks of the study period
[2] Ratio of sentinel group hazard function to isometamidium group hazard function

4.3.3 Health and productivity indices for isometamidium and sentinel groups

4.3.3.1 Packed cell volume

4.3.3.1.1 Variations in packed cell volumes

The mean packed cell volumes (PCV) of isometamidium-treated and sentinel group cattle for the three years of study are shown in Figure 6. Packed cell volumes were variable in all three years of study, but some general trends could be observed. In the 1994/1995 and 1995/1996 studies, PCVs of isometamidium-treated cattle were generally higher than PCVs of sentinel cattle. In the 1996/1997 study, the converse appeared to be true, and PCVs of isometamidium-treated cattle were generally lower than PCVs of sentinel cattle.

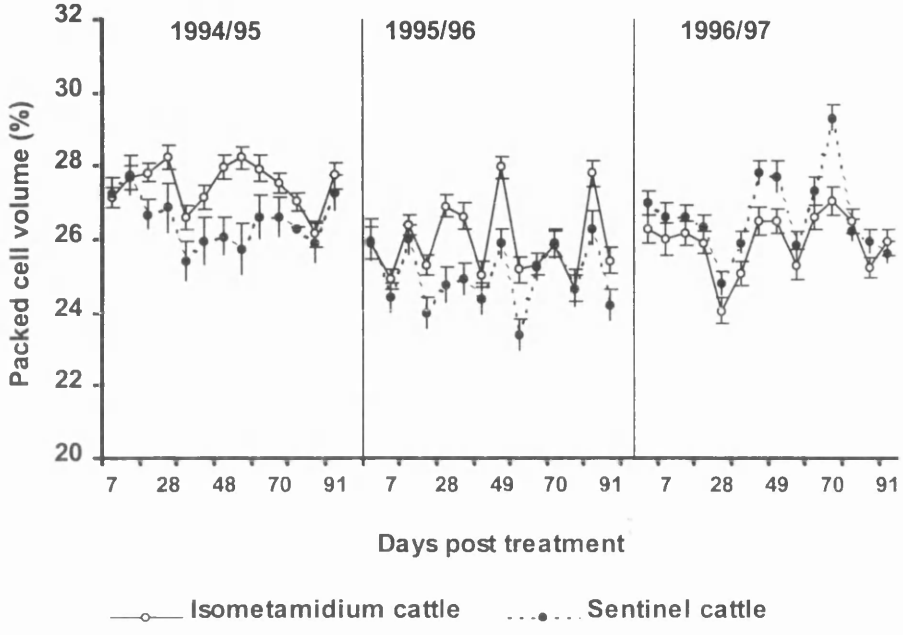

Figure 6: Mean packed cell volumes of isometamidium treated and sentinel cattle in each year of the study on Galana Ranch

4.3.3.1.2 Effect of isometamidium treatment on packed cell volumes

To determine the effect and significance of isometamidium treatment on packed cell volumes (PCV), the data were fitted to a mixed model. In the model, drug-treatment group within year was fitted as a fixed effect, and animal within year was fitted as a random effect. Because there was no evidence for a particular trend in PCV over time, days post-treatment within year was treated as a class variable rather than a continuous variable, and was fitted as a random effect. The model specification was as follows:

$$Y_{ijkl} = \mu + \delta_{ij} + a_{jk} + t_{lj} + e_{ijkl}$$

Where, for $i = 1,2; j = 1,\ldots,3; k = 1,\ldots,325; l = 1,\ldots,30$

Y_{ijkl} is the measured PCV

μ is the overall mean PCV

δ_{ij} is the fixed effect of treatment group i within year j: isometamidium treatment (δ_1) or sentinel (δ_2)

a_{jk} is the random effect of animal k within year j

t_{lj} is the random effect of day post treatment l within year j

e_{ijkl} is the random error associated with measurement l on animal k in group i within year j

The least square mean PCVs predicted by the model for isometamidium treated and sentinel cattle within each year are shown in Table 8.

The least square mean PCVs of sentinel cattle in 1994/95 (26.48%) and 1996/97 (26.62%) were not significantly different (difference 0.135%; $p = 0.804$). However, the least square mean PCV (26.55%) of sentinel cattle in these two years was significantly higher (difference 1.54%; $p < 0.001$) than the least square mean PCV (25.01%) of sentinel cattle in 1995/96.

Within each year, isometamidium treatment had a significant ($p = 0.001$) effect on PCVs in cattle. Isometamidium-treated cattle had significantly higher PCVs than sentinel cattle in 1994/95 (difference = 0.99%; $p = 0.0267$) and 1995/96 (difference = 0.94%; $p = 0.0037$). In 1996/97 isometamidium treated cattle had

lower PCVs than sentinel cattle. However, the difference (0.69%) was of marginal significance ($p = 0.057$).

Table 8: Least square mean packed cell volumes for isometamidium-treated and sentinel cattle over the three years of study

Effect	Year	Group	LS Means[2]	SE[3]
Group(year)	94/95	ISMM[1]	27.47	0.344
		Sentinel	26.48	0.429
	95/96	ISMM	25.95	0.284
		Sentinel	25.01	0.338
	96/97	ISMM	25.93	0.338
		Sentinel	26.62	0.338

[1]ISMM: Isometamidium treated cattle

[2]LS Means: Least Squared Means

[3]SE:Standard error

4.3.3.1.3 The effect of baseline hazard on packed cell volumes

The effect of baseline hazard on packed cell volumes of isometamidium-treated and sentinel cattle was investigated using mixed models. Baseline hazard is the hazard rate calculated from the life table for the sentinel cattle for the first eight week of each prophylactic period. The model specification was as follows:

$$Y_{ijkl} = \mu + \beta_0 + \beta_{i1}\tau_j + a_{ijk} + t_{jl} + e_{ijkl}$$

Where, for $i = 1,2$; $j = 1,2,3$; $k = 1,\ldots,325$; $l = 1,\ldots,30$

Y_{ijkl} is the PCV measurement

μ is the overall mean PCV

β_0 is the intercept of the regression line

β_{i1} is the slope of the regression line for group i

τ_j is the baseline hazard in year j

a_{ijk} is the random effect of animal k in group i in year j

t_{jl} is the random effect of day post treatment l in year j

e_{ikl} is the random error of measurement on day l on animal k in group i in year j

In this model, the effect of baseline hazard within group was significant ($p = 0.0015$). The regression line for the sentinel cattle had a significant negative slope (co-efficient = -3.69; SE = 1.44; $p = 0.0121$), reflecting a significant decrease in PCV with increasing baseline hazard. The regression line for isometamidium-treated cattle (co-efficient = -0.550; SE = 1.35) had a much smaller negative slope, which was not significant ($p = 0.68$). Hence for isometamidium-treated cattle, there was no significant decrease in PCV with increasing baseline hazard. The difference (3.14; SE = 0.894) between the regression co-efficients for sentinel and isometamidium-treated cattle was highly significant ($p = 0.0005$). PCVs predicted by this model are shown in Figure 7.

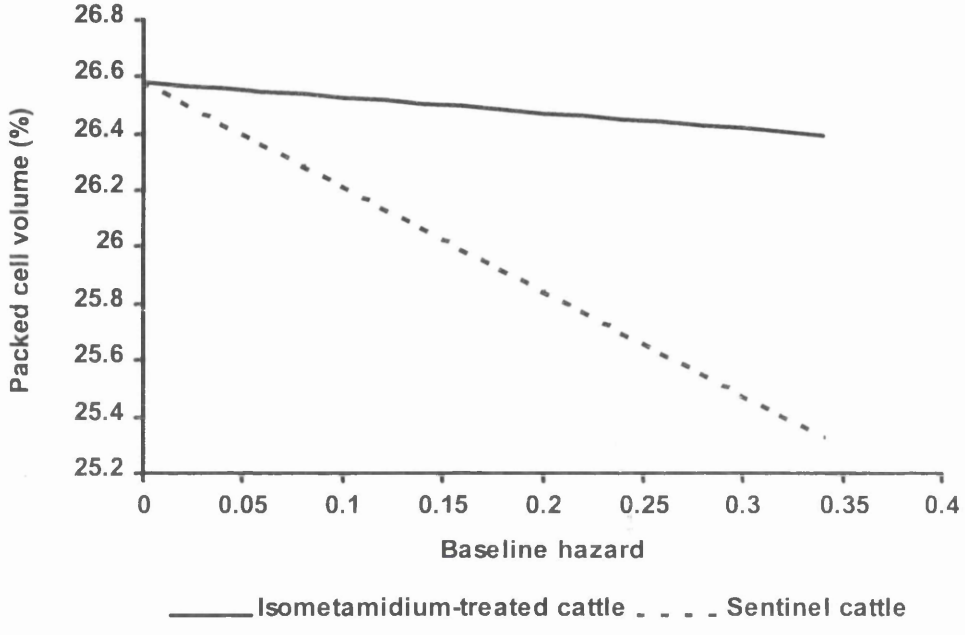

Figure 7: Modelled changes in packed cell volumes of isometamidium-treated and sentinel cattle with baseline hazard during the prophylaxis studies on Galana Ranch

4.3.4 Body Weights and condition scores

4.3.4.1 Changes in body weight

The mean monthly body weights (kg) for the studies in the years 1994/95, 1995/96 and 1996/97 are shown in Figure 8.

In 1994/95, there was a similar rapid increase in body weight of both isometamidium-treated and sentinel cattle up to the eighth week, from a mean of approximately 180kg to 200kg. Thereafter, over the next four weeks, there was only a slight increase in mean body weight of just 0.5 kg.

In 1995/96, generally the bodyweights of sentinel cattle were slightly lower than the body weights of isometamidium cattle over the prophylactic period. Most of the weight gain occurred in the first six weeks. Thereafter there was a steady increase in both groups to the end of the prophylaxis period.

In 1996/97, the study was conducted on a younger group of cattle of lower body weight. In this year, there was a steady increase in body weights in both isometamidium treated and sentinel cattle to the seventh week (Figure 8). During the remaining five weeks of the study, both groups of cattle lost weight.

4.3.4.2 Condition scores

Body condition scores were measured only during the 1996/97 study. The mean condition scores of the isometamidium treated and sentinel cattle variation with days post treatment in this study are shown in Figure 9. Although there was little difference in condition score between the two groups of cattle initially, thereafter, throughout the prophylactic period, the condition scores of sentinel cattle were appreciably higher than the condition scores of isometamidium treated cattle. In both groups, there was an initial increase in body condition to the third week of the prophylactic period followed by a steady decrease to the twelfth week.

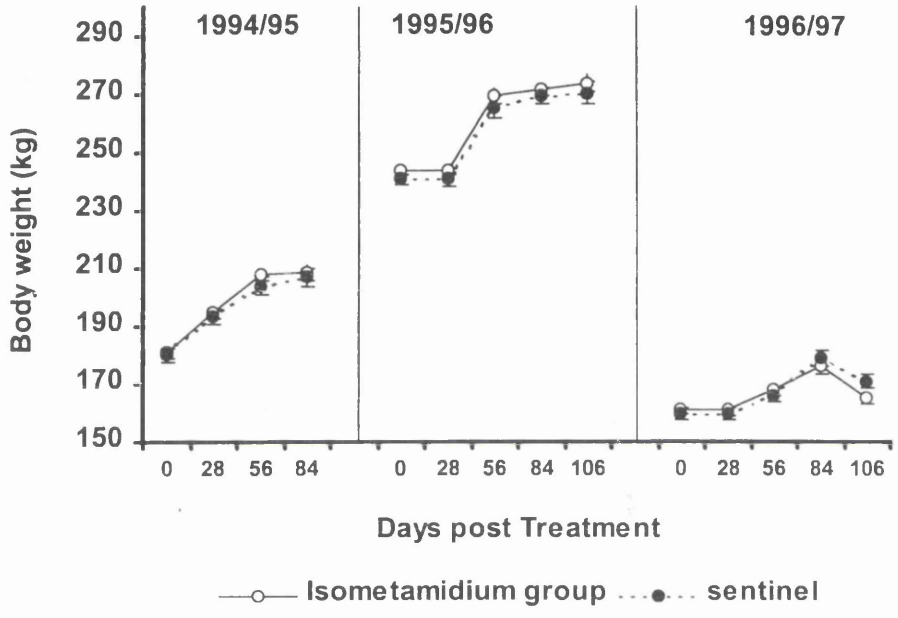

Figure 8: Monthly mean body weights of isometamidium treated and sentinel cattle during prophylaxis studies on Galana Ranch

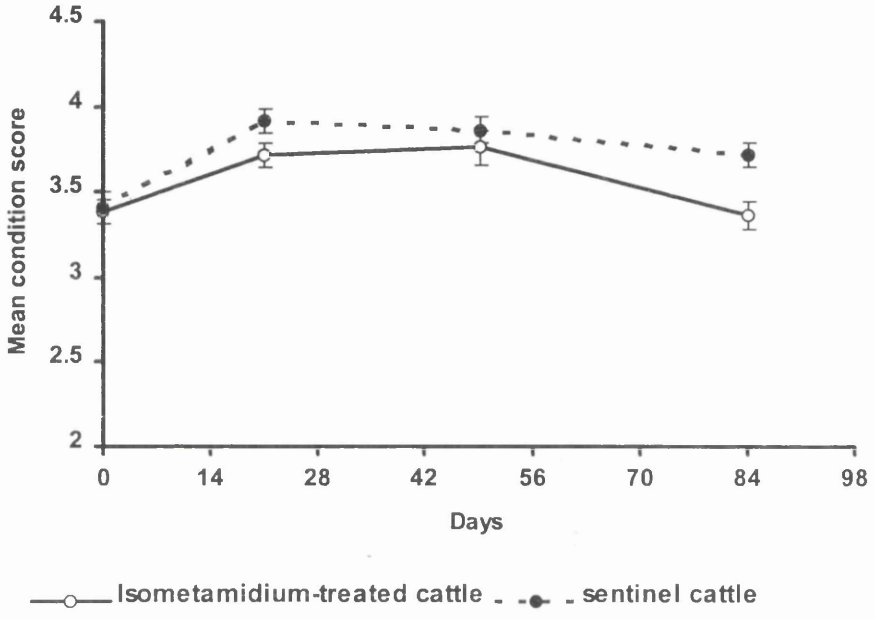

Figure 9: Monthly means body condition scores of isometamidium treated and sentinel cattle during the prophylactic period in 1996/97 on Galana Ranch

4.3.4.3 The effect of isometamidium treatment on weight gain

To determine the effect of isometamidium treatment on body weight gain within each year, the body weight data was fitted to a mixed model. The year and the interaction between year and number of days post treatment were fitted as fixed effects, giving an average intercept and slope for each year. The animal within year was fitted as repeated measure, allowing a random intercept and slope for each animal. The model specification was as follows:

$$Y_{ijkl} = \mu + ß_0 + ß_{ij1}\tau_l + a_{ijk} + b_{ijk}\tau_k + e_{ijkl}$$

Where, for $i = 1,2$; $j = 1,2,3$; $k = 1,\ldots,91$; $l = 1,\ldots,325$

Y_{ijkl} is the measurement of body weight

μ is the overall mean body weight

$ß_{j0}$ is the average intercept of the linear regression line in year j

$ß_{ij1}$ is the average slope of the linear regression line for group i in year j

τ_l is the number of days post treatment of measurement l

a_{ijk} is the random intercept of the linear regression line for animal k in group i in year j

b_{ijk} is the random slope of the linear regression line for animal k in group i in year j

e_{ijkl} is the random error of measurement l on animal k in group i in year j

The covariance structure of the model assumed that the a_{ijk}, b_{ijk} and e_{ijkl} are independently and identically distributed, and that both a_{ijk} and b_{ijk} are independent from e_{ijkl}, but that a_{ijk} and b_{ijk} are possibly correlated.

Unsurprisingly, the model showed there were significant differences ($p = 0.0001$) between the three years in initial body weights. More importantly, it showed significant differences in growth rates between the isometamidium and sentinel cattle. There were no significant differences in body weight gain between isometamidium treated and sentinel cattle in 1994/95 (difference 0.0192 kg/day; $p = 0.69$) and 1995/96 (difference -0.0008 kg/day; $p = 0.97$). However in 1996/97, the growth rate in isometamidium treated cattle (0.0616 kg/day; SE 0.0202), was less

than half that of sentinel cattle (0.145 kg/day; SE 0.0204), the difference (0.0830 kg/day; SE 0.0285) being highly significant (0.0036).

4.3.4.4 The effect of isometamidium treatment on body condition score

To determine the effect of isometamidium treatment on body condition score during 1996/97, the condition score data was fitted to a mixed model. Drug treatment group was fitted as a fixed effect. Days post treatment was fitted as a class variable and a random effect, because there was no clear trend in increasing condition score with time. Animal was fitted as a random effect. The model specification was as follows:

$$Y_{ijk} = \mu + \delta_i + a_{ik} + t_j + e_{ijk}$$

Where, for $i = 1,2$; $j = 1,\ldots,91$; $k = 1,\ldots, 325$

Y_{ijk} is the measurement of condition score

δ_i is the fixed effect of treatment group i: isometamidium treatment (δ_1) or sentinel (δ_2)

a_{ik} is the random effect of animal k in group i

t_j is the random effect of day j post treatment

e_{ijk} is the random error associated with animal k in group i on day j

In the one study year in which condition score was measured, 1996/97, isometamidium treatment had a significant effect on condition score. Isometamidium-treated cattle (least squared mean = 3.53) had significantly lower ($p = 0.025$) condition scores than sentinel cattle (least squared mean = 3.71)

4.3.5 Factors associated with the efficacy of isometamidium prophylaxis

4.3.5.1 Disposition of isometamidium

Isometamidium could be detected by ELISA in the sera of all cattle that were treated with the drug. However, in all three years of the study, there were wide variations in isometamidium levels in individual cattle in any given week. Log-linear plots of mean isometamidium concentrations against time are shown in Figure 10. In

all the 3 prophylaxis periods (1994/5, 1995/6/ and 1996/7) isometamidium concentrations in serum declined exponentially. Similar drug elimination rates and half-lives were seen in all three years (Table 9).

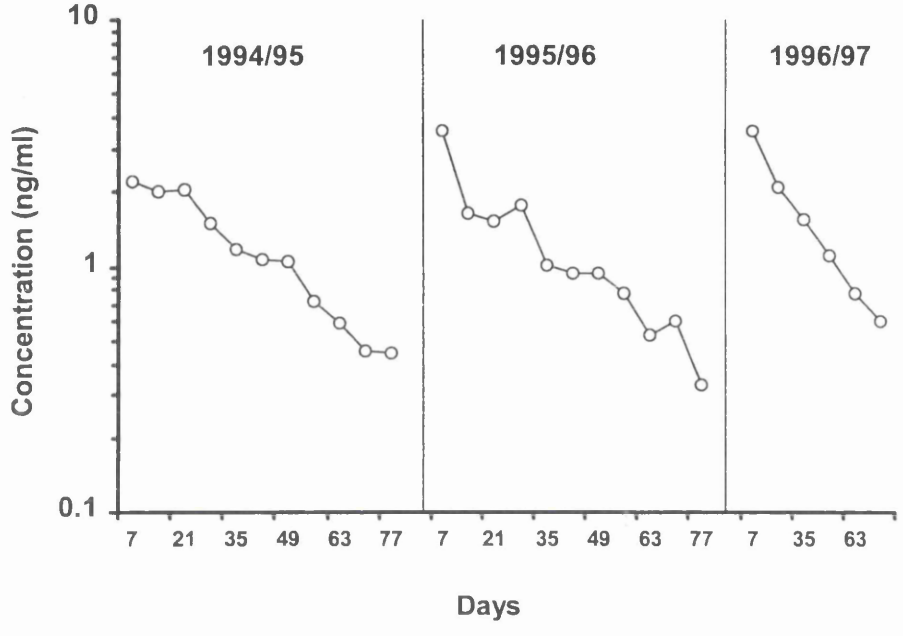

Figure 10: Log-linear plots of mean isometamidium concentrations against time in days during the prophylaxis studies on Galana Ranch

Table 9: Elimination rates and half-lives of isometamidium

Year of Study	Elimination rate constant (hr^{-1})	$t_{½}$ (hr)[1]
1994/5	-0.00110	631
1995/6	-0.00114	580
1996/7	-0.00105	663

[1]Elimination half life

During 1995/96 and 1996/97 mean isometamidium concentrations were similar one week post-isometamidium treatment (3.47 ± 0.14 ng/ ml and 3.51 ± 0.15 respectively).

Descriptive statistics of isometamidium concentrations in the isometamidium-treated cattle during the 3-year prophylaxis studies at Galana Ranch are shown in Appendix 1.

4.3.5.1.1 Year: 1994/95

Ten days after isometamidium treatment, isometamidium concentrations (median = 2.19 ng/ml) varied from a minimum of 0.99 ng/ml to a maximum of 3.82 ng/ml. By the twelfth week after treatment the isometamidium levels (median = 0.30 ng/ml) varied from a minimum of undetectable (less than 0.2 ng/ml) to a maximum of 0.96 ng/ml.

4.3.5.1.2 Year: 1995/96

One week after isometamidium treatment, isometamidium concentrations (median = 3.11 ng/ml) varied from a minimum of 0.94 ng/ml to a maximum of 15.30 ng/ml (Appendix 1). By the eleventh week after treatment isometamidium levels (median = 0.27 ng/ml) varied from a minimum of undetectable (less than 0.2 ng/ml) to a maximum of 1.22 ng/ml.

4.3.5.1.3 Year: 1996/97

One week after isometamidium treatment, isometamidium concentrations (median = 3.38 ng/ml) varied from a minimum of 1.28 ng/ml to a maximum of 8.41 ng/ml. By the eleventh week after treatment, isometamidium levels (median = 0.57 ng/ml) varied from a minimum of undetectable levels (less than 0.2 ng/ml) to a maximum of 1.37 ng/ml

The mean drug concentrations determined in all three years of the study (1994/95, 1995/96 and 1996/97) were similar.

4.3.5.2 *Isometamidium concentrations in sera obtained from cattle at the time of detection of breakthrough infections.*

Trypanosomes detected in isometamidium-treated cattle in which serum determinations of the drug showed concentrations greater than 0.4 ng/ml were considered to have some degree of resistance. The number of *T. congolense* and *T. vivax* infections in which serum isometamidium concentrations were found to be above or below this level are shown in Table 10. Serum samples for isometamidium determination were not always available on the exact days on which breakthrough infections were first detected. In such cases approximate isometamidium concentrations were estimated by interpolation of the nearest available concentration data points, as described below.

4.3.5.2.1 Year: 1994/95

In this year of study, there were a total of seven breakthrough infections in isometamidium-treated cattle, all *T. vivax* (Table 10). In one of these cases, isometamidium concentrations were at least three times greater than 0.4 ng/ml at the time the breakthrough infection was detected. In another two cases, interpolation of concentrations determined one-week prior and one and two weeks respectively following the detection of breakthrough infections suggested that the concentration was at least three times greater than 0.4 ng/ml at the time the breakthrough was detected. In these three cases, the infecting *T. vivax* populations were considered to exhibit a degree of drug resistance. In another case, isometamidium concentration was at least two times greater than 0.4 ng/ml at the time the breakthrough infection was detected. In this case too, the infecting *T. vivax* populations were considered to exhibit a degree of drug resistance. In a further two cases, isometamidium concentrations were at just above 0.4 ng/ml at the time the breakthrough infection was detected. Similarly in another case, interpolation of concentrations determined one week prior to and one week following the detection of the breakthrough infection suggested that the concentration was just above the 0.4 ng/ml level. In these last three cases, there was no sufficient evidence to consider that the infecting *T. vivax* populations exhibited drug resistance.

4.3.5.2.2 Year: 1995/96

During this year of study, breakthrough infection with *T. vivax* was detected no less than 79 times in cattle with circulating isometamidium concentrations greater than 0.4 ng/ml (Table 10). Five breakthrough infections with *T. congolense* were detected in cattle with drug concentrations above this level. These *T. vivax and T. congolense* populations were therefore considered to exhibit a degree of isometamidium resistance. In 26 further cases of *T. vivax* infection and eight further cases of *T. congolense* infection occurring in isometamidium-treated cattle, isometamidium concentrations had fallen below the 0.4 ng/ml level by the time of breakthrough. In these cases, there was no evidence therefore that the infecting trypanosome populations expressed resistance to the drug.

4.3.5.2.3 Year: 1996/97

During this year of study, isometamidium levels were above 1.0 ng/ml in eight cases of breakthrough infection, five cases with *T. vivax* and three with *T. congolense* (Table 10). In all but one of these cases, interpolation of data was required. In all eight cases, the infecting trypanosome populations were considered to exhibit drug resistance. In one further case of *T. vivax* breakthrough, the drug concentration was 0.72 ng/ml at the last available determination four weeks prior to the detection of infection. This was not considered sufficient evidence that the infecting parasite population expressed resistance to the drug.

Table 10: Isometamidium concentrations in treated cattle at the time of detection of breakthrough trypanosome infections

	T. congolense		*T. vivax*	
Year of Study	**n[1]**	**>0.4 ng/ml[2]**	**n**	**>0.4 ng/ml**
1994/5	0	0	7	4
1995/6	13	5	105	79
1996/7	3	3	6	5

[1]Number of trypanosome infections

[2]Number of trypanosome infections isolated from cattle in which isometamidium concentrations were above 0.4 ng/ml

4.3.5.3 Infecting trypanosome species

To determine the effect of trypanosome species on the efficacy of isometamidium treatment comparisons were made between survivor functions *for T. congolense* and *T. vivax* in the isometamidium-treated and sentinel cattle.

4.3.5.3.1 Year: 1994/95

The plot of survival function for *T. congolense* and *T. vivax* in the isometamidium-treated and sentinel cattle during the first year of the study (1994/5) is shown in Figure 11. By the eighth week of this prophylaxis period 23 (57.5%) sentinel cattle had been infected with *T. congolense* and 19 (48.0%) cattle had been infected with *T. vivax*. The difference between the survival functions for the two trypanosome species was not significant ($p > 0.05$).

Five isometamidium-treated cattle (6.2%) were infected *with T. vivax* by the eighth week of this prophylaxis period. However there was no *T. congolense* breakthrough infection in this group of cattle.

4.3.5.3.2 Year: 1995/96

The plot of survival function for *T. congolense* and *T. vivax* in the isometamidium-treated and sentinel cattle during the second year of the study (1995/96) is shown in Figure 12.

In the sentinel cattle, the survivor functions for *T. congolense* and *T. vivax* were not significantly ($p > 0.05$) different. By the eighth week, 32 (54.2%) sentinel cattle had been infected with *T. congolense* and 44 (58.7%) cattle had been infected with *T. vivax*.

In the isometamidium-treated cattle, the survivor functions for *T. congolense* and *T. vivax* were significantly different ($p < 0.001$). While only seven (5.1%) of these cattle had been infected with *T. congolense* by the eighth week, 80 (59.1%) of the isometamidium-treated cattle had been infected with *T. vivax* over the same period.

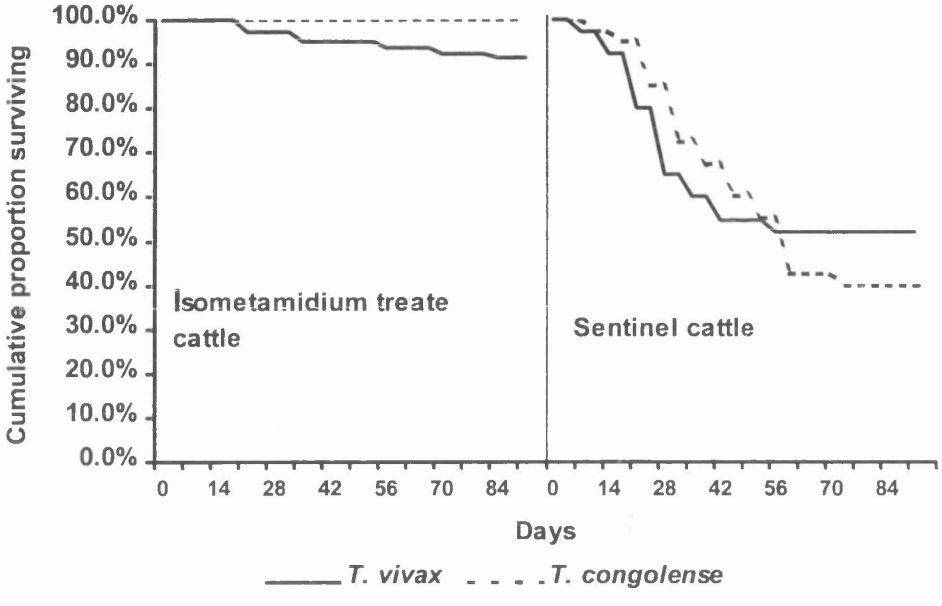

Figure 11: Cumulative proportion of isometamidium-treated and sentinel cattle that survived from *T. congolense* and *T. vivax* infections during the prophylactic period in 1994/95 on Galana Ranch

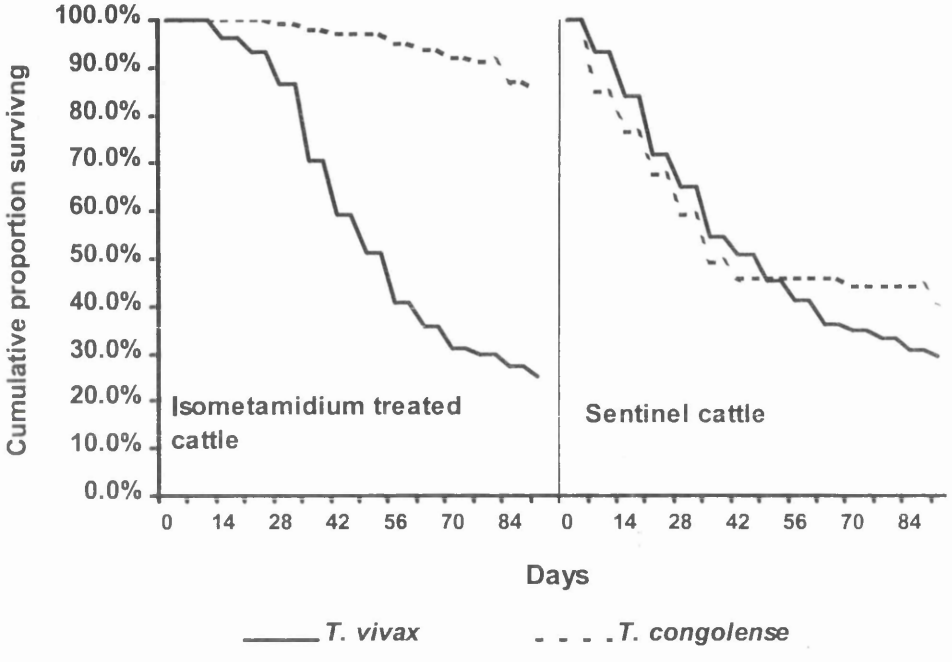

Figure 12: Cumulative proportion of isometamidium treated and sentinel cattle that survived from *T. congolense* and *T. vivax* infections during the prophylactic period in 1995/96 on Galana Ranch

4.3.5.3.3 Year:1996/97

The plot of survival function for *T. congolense* and *T. vivax* in the isometamidium-treated and sentinel cattle during the third year of the study is shown in Figure 13. Neither in isometamidium-treated nor sentinel cattle were there significant ($p > 0.05$) differences between survivor functions for *T. vivax* and *T. congolense*. In the sentinel group, 7 (8.8%) of the cattle were infected with *T. congolense* and 2 (2.5%) were infected with *T. vivax* by the eighth week. In the isometamidium-treated group, 3 (3.8%) cattle were infected with *T. congolense* and 5 (6.3%) cattle were infected with *T. vivax* over the first eight weeks.

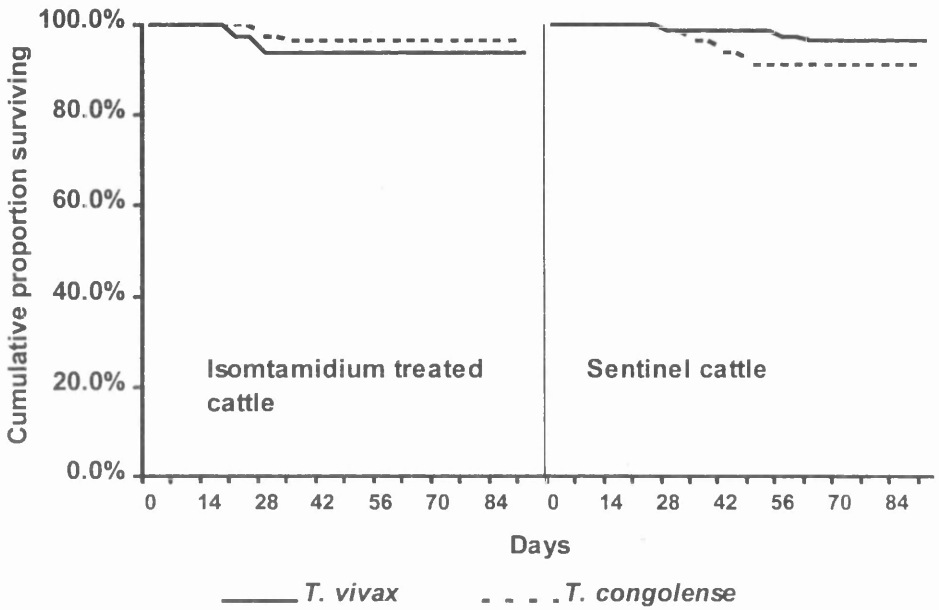

Figure 13: Cumulative proportion of isometamidium treated and sentinel cattle that survived from *T. congolense* and *T. vivax* infections during the prophylactic period in 1996/97 on Galana Ranch

4.3.5.4 Effect of pre-trial infection on the incidence of infection

To determine the effect of pre-trial infection on the incidence of infection in the isometamidium-treated and sentinel cattle during a prophylaxis period at Galana Ranch, the survivor functions for trypanosome infection in cattle with and without pre-treatment infections were compared. The comparison was done for the year 1995/96 when there was a high prevalence of trypanosome infections in cattle before the prophylaxis period.

In the isometamidium-treated cattle, survivor functions were not significantly different ($p = 0.092$) between cattle with and without pre-treatment trypanosome infections as shown in Figure 14. However there were significantly fewer trypanosome infections among sentinel cattle that were infected at the start of the trial than among those that were not (Figure 14). Fourteen (77.8%) sentinel cattle with pre-trial infections were infected with trypanosomes by the eighth week of the prophylaxis period while 56 (96.5%) of the sentinel cattle without pre-trial infections were infected by the eighth week of the prophylaxis period.

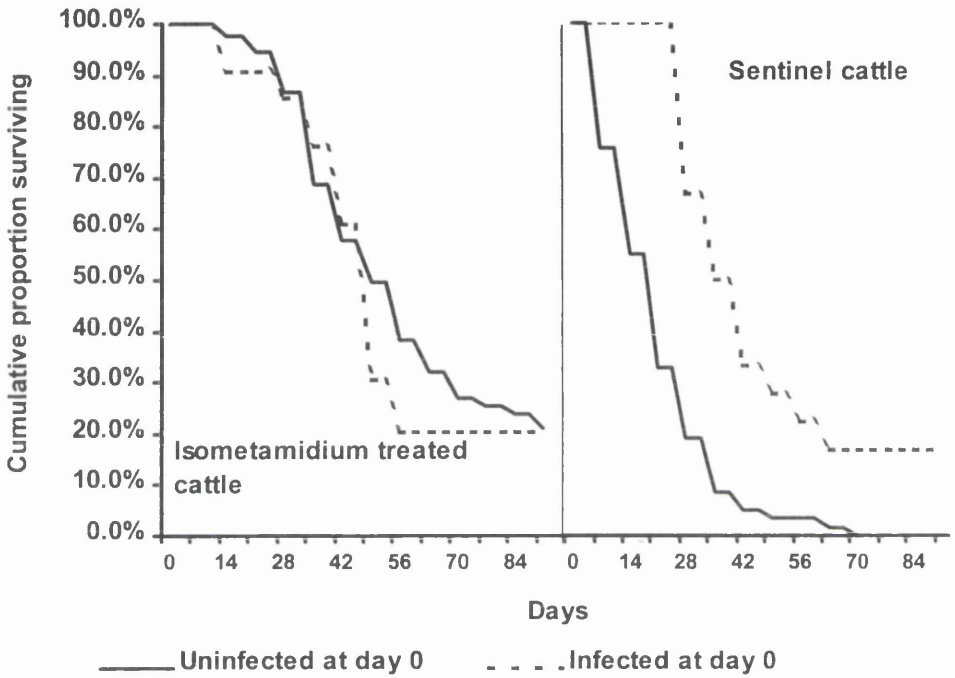

Figure 14: Cumulative proportion of isometamidium treated and sentinel cattle with or without trypanosome infections on day 0 that survived trypanosome infections during the prophylactic period in 1995/96 on Galana Ranch

4.3.6 Efficacy of chemotherapy of trypanosome infections using diminazene aceturate

The efficacy of diminazene treatment was determined by establishing the proportion of first infections in the sentinel cattle that were cured after treatment. First infections were considered as the first infection detected in each sentinel animal from the beginning of the prophylaxis period (day 0) in each year. These infections were assumed to have been cured if cattle became aparasitaemic after treatment with diminazene for eight consecutive weeks with PCV > 26% in each of those weeks. The calculations were done for *T. congolense* and *T. vivax* infections. The cure rate was calculated as the proportion of the new infection cured over thirteen weeks of the prophylaxis period. The proportion of first *T. congolense* and *T. vivax* infections cured during each year of the study is shown in Table 11.

In 1994/95, 91.7% of *T. congolense* infections and 73.7% of *T. vivax* infections respectively were cured after treatment with diminazene. In the following year of the study (1995/96) 53.8% *T. congolense* and 23.6% *T. vivax* infections were cured after treatment with diminazene (Table 11). The cure rates of *T. congolense* and *T. vivax* were thus reduced by 41% and 68% respectively. In the third year of the study (1996/97) when trypanosome challenge was very low, 5 (71.4%) *T. congolense* and 1 (33.3%) *T. vivax* infections detected in the sentinel cattle were cured after treatment with diminazene at 7.0 mg/kg b.w. The cure rates for both strains in 1994/95 were significantly higher ($p < 0.05$) than the cure rates for the following two years of the study. However, the cure rates for 1995/96 and 1996/97 were not significantly different ($p=0.158$ for *T. congolense* and $p = 0.679$ for *T. vivax*)

Table 11: Proportions of new infections in sentinel cattle that were cured after treatment with diminazene at 7.0 mg/kg b.w. during the three year prophylaxis study

Year of study	new Tc[1] infections	new Tv[2] infections	No. of Tc cured	No. of Tv cured	% Tc cured	% Tv cured
1994/95	24	19	22	14	91.7	73.7
1995/96	52	55	28	13	53.8	23.6
1996/97	7	3	5	1	71.4	33.3

[1] *T. congolense*
[2] *T. vivax*

4.3.7 Financial analysis of prophylaxis studies

The costs of isometamidium and diminazene treatments were calculated from the doses of isometamidium and diminazene at 1.0 mg/kg b.w. and 7.0 mg/kg b.w. respectively. Estimated benefits from productivity (body weight gains) were calculated using the current price of 1 kg live-weight of Ksh. 45.0. Costs of mortality losses due to trypanosomiasis were calculated by multiplying the last body weight measured with cost of 1kg live-weight. The costs are presented in US dollar using the exchange rate of Ksh. 71.00 per dollar. The financial analysis of the benefits and costs incurred during prophylaxis studies are shown in Table 12.

The net financial benefit per animal in both isometamidium treated and sentinel cattle decreased over the three years of study (Table 12). In both groups of cattle the highest decrease in net financial benefit per animal was observed from 1995/96 to 1996/97. In 1996/97 there was a net financial loss per animal US$ -0.3) in isometamidium treated cattle. In sentinel cattle the net financial benefit per animal (US$5.7) in 1996/97 was less than half the value of net financial benefit per animal (US$ 12.3) in 1995/96.

In 1994/95, in isometamidium treated cattle the net financial benefit per animal was approximately nine times higher than the cost of trypanocidal treatment per animal. Whereas in sentinel cattle during this year, the net financial benefit per animal was approximately six times higher than the cost of trypanocidal treatment per animal.

In 1995/96, in both isometamidium treated and sentinel cattle, the net financial benefit per animal was approximately two times higher than the cost of trypanocidal treatment per animal (Table 12).

In 1996/97, in isometamidium treated cattle there was a net financial loss. Whereas, in the sentinel cattle, the net financial benefit per animal was approximately twenty eight times higher than the cost of trypanocidal treatment per animal (Table 12).

Table 12: Financial impact of a three-year prophylaxis study on Galana Ranch including benefit from productivity, mortality costs and trypanocidal treatment costs

Year of study	Group	Number of cattle	Benefit from productivity per animal[1] (US$)	Cost of losses per animal[2] (US$)	Net benefit per animal[3] (US$)	Cost of drugs per animal[4] (US$)
94/95	ISMM[5]	80	17.6	0	15.9	1.7
	Sentinel	40	16.6	0	14.2	2.4
95/96	ISMM	149	18.8	3.2	10.3	5.3
	Sentinel	76	18.5	0	12.3	6.2
96/97	ISMM	80	2.5	0	-0.3	2.8
	Sentinel	80	7.2	1.3	5.7	0.2

[1]Total benefit per animal from overall weight gains calculated as weight gain x price of live-weight (Ksh 45)

[2]cost of mortality losses due to trypanosomiasis (body weight at time of death x Ksh.45)

[3]Net profit from total benefit - losses - drug cost per animal

[4]Total cost of treatment with diminazene and isometamidium per animal

[5]ISMM: Isometamidium treatment

4.4 Discussion

In the work described in this chapter, the incidence of trypanosome infection and the efficacy of isometamidium prophylaxis were investigated in three month studies conducted at Galana Ranch in Kenya's Coast Province over three consecutive years.

The effects of isometamidium treatment on production indices including, packed cell volumes, body weights and condition scores over three years were assessed. In addition, the variation of packed cell volumes with trypanosome challenge was investigated.

Circulating isometamidium concentrations were related to occurrence of trypanosome infections in cattle under natural tsetse challenge at the ranch and the number of breakthrough infections that may have been caused by drug resistant trypanosome populations were considered.

Finally, the efficacy of diminazene treatment trypanosome infections and impact of prophylaxis failure in terms of animal health-related losses and treatment costs was assessed

The earliest breakthrough infections were observed by the second week after prophylactic isometamidium treatment in 1995/96 and by the third week in 1994/95 and 1996/97. All of the earliest breakthrough infections were *T. vivax* infections.

Similar early breakthrough infections in isometamidium-treated cattle were observed two weeks after isometamidium treatment on Galana Ranch (Dolan *et al.*, 1992). In a year of relatively high trypanosome challenge, 97% of breakthrough infections were attributed to *T. vivax* (Dolan *et al.*, 1992). Elsewhere in Ngurumani, Kajiado District, breakthrough infections mainly *T. vivax* were observed as early as two weeks post isometamidium treatment (Stevenson *et al.*, 1995).

During the study period the baseline hazards (the mean hazard rate of sentinel cattle over 8 weeks of each prophylactic period) in 1994/95, 1995/96 and 1996/97 were 21.6 %, 32.8 % and 1.5 % respectively. The baseline hazard is an indicator of

the force of infection. Isometamidium treatment reduced the hazard rate by a factor of 27, 3 and 1.2 in 1994/95, 1995/96 and 1996/97 respectively.

Evidently, isometamidium prophylaxis was highly efficacious in the year 1994/95, when the baseline hazard was high (21.6%), but the hazard was greatly reduced by a factor of 27. In the year 1995/96, when baseline hazard was at its highest (32.8%), isometamidium prophylaxis appeared to be less efficacious and reduced the hazard by a smaller but still significant factor of 3. The tendency of the efficacy of isometamidium prophylaxis to depend on trypanosome challenge, i.e. baseline hazard, has been reported previously (Whiteside 1962). At Galana Ranch, Stevenson *et al.* (1990) and Dolan *et al.* 1992 associated high tsetse and trypanosome challenge with apparent isometamidium prophylaxis failure.

In the present study, during 1996/97 the hazard rates in isometamidium treated and sentinel cattle were similar (hazard ratio of 1.2). The fact that isometamidium prophylaxis was not beneficial in this year was probably due to the very low baseline hazard in this year (1.5%). Several factors may be considered as contributing to the low baseline hazard in this year. Firstly, the Orma Boran cattle used in this year are considered to show a greater degree of trypanotolerance than the improved Galana Boran breed (Njogu *et al.*, 1985; Mwangi *et al.*, 1998). This breed has been observed to show trypanosome infection rates reduced by 30 - 70 % compared to the Galana Boran when left untreated under natural trypanosome challenge in Galana Ranch (Wilson *et al* 1986) and in Kajiado district (Mwangi *et al.*, 1998). In addition it was observed that isometamidium treatment had less effect on the performance of Orma Borans than on that of Galana Borans (Wilson *et al* 1986).

Secondly, the predominant tsetse species in the site of study during this year (1996/97) was *G. longipennis* . Earlier studies in this same site have shown that the feeding success of *G. longipennis* to be 13-17 % compared to 36-65 % for *G. pallidipes* (Makumi *et al.*, 1996; Baylis, 1997).

Therefore it is likely that the poor transmission efficiency of the flies coupled by the trypanotolerant trait of the cattle resulted in the low trypanosome prevalence observed in the present study during 1996/97. However, it may be interesting to

investigate the performance of Orma Boran under isometamidium prophylaxis in the area where the vector has higher transmission efficiency for trypanosomes.

The protection, judged by the cumulative proportion of cattle that had not been infected by the eighth week of the prophylactic period, afforded by isometamidium prophylaxis against *T. congolense* was consistent over the three years of study, while the protection afforded against *T. vivax* decreased with trypanosome challenge. Protection conferred by isometamidium against *T. congolense* over three years varied from 100 % in 1994/95 to 94.9 % and 93.2 % in 1995/96 and 1996/97, respectively. However, the protection conferred against *T. vivax* infections varied from approximately 94 % in 1994/95 and 1996/97 to just 41.0 % in 1995/96. There appears to be appreciable consistency in the efficacy of isometamidium prophylaxis of *T. congolense* infections, in spite of variation in trypanosome challenge over the three years. This would suggest that the distribution of resistant/sensitive *T. congolense* population did not vary significantly over the 3 years. Whereas, the efficacy of isometamidium prophylaxis on *T. vivax* infections was very much dependent on challenge. This observation is consistent with variation in proportions of sensitive and/or resistant strains in trypanosome populations with increase in trypanosome density.

The occurrence of acute, haemorrhagic *T. vivax* infection (Mwongela *et al.*, 1981; Wellde *et al.*, 1983) has been observed on Galana Ranch. This acute haemorrhagic form of the disease was normally observed during periods of unusually high trypanosome challenge when prophylaxis appeared to fail (Rottcher and Schillinger 1985; Njogu and Heath, 1986; Stevenson *et al.*, 1990). During one such period when the haemorrhagic syndrome had occurred in Galana Ranch, *T. vivax* stabilates that expressed multiple resistance to isometamidium at 2 mg/kg and diminazene at 3.5 mg/kg were isolated from infected cattle (Rottcher and Schillinger 1985). However, it was not evident whether the resistant isolates were the actual strain responsible for the haemorrhagic syndrome (Njogu and Heath 1986).

In the present study, the acute haemorrhagic form of trypanosomiasis was observed in cattle during 1995/96, when three isometamidium treated cattle died from *T. vivax* infections. However, this acute form of the disease was not observed in any of the other years. This would suggest that the general increase in

trypanosome challenge in 1995/96 was accompanied by an increase in the pathogenicity of some strains. Consequently, isometamidium appears to be less effective to some strains of *T. vivax*, especially those associated with the acute form of trypanosomiasis. In case of such strains therapeutic treatment using diminazene would be the preferred strategy to adopt (Njogu and Heath, 1986; Dolan *et al.*, 1992).

Packed cell volumes were significantly higher in isometamidium-treated cattle than sentinel cattle in the first two years of the study. However in 1996/97, the packed cell volumes of isometamidium-treated cattle were marginally lower than packed cell volumes of sentinel cattle. This important observation suggests that when trypanosome challenge is moderate or high, the drug has a beneficial effect on PCV, whereas when challenge is minimal, as in 1996/97, the drug may even have a deleterious effect.

In addition, the packed cell volumes of isometamidium-treated cattle in 1995/96 and 1996/97 (25.95 ± 0.28 % and 25.93 ± 0.34 % respectively) were comparable in spite of significant differences in the level of challenge as indicated by baseline hazards (32.8 % and 1.5 % respectively) between the two years. However, in sentinel cattle, the packed cell volumes in 1995/96 were significantly ($p<0.001$) lower than packed cell volumes in 1996/97.

Wilson *et al.* (1986) observed that there were no differences in packed cell volumes in isometamidium-treated cattle between Orma Boran and Galana Boran cattle under the same natural trypanosome challenge. However, in untreated cattle they observed that the packed cell volumes of Orma Boran cattle were significantly higher than packed cell volumes of Galana Boran cattle (Wilson *et al.* (1986). Recently in Kajiado District, Mwangi *et al.* (1998) demonstrated that under high trypanosome challenge, untreated Orma Boran maintained a significantly higher packed cell volumes than untreated Galana Boran under the same trypanosome challenge.

In the present study, similar packed cell volumes in sentinel cattle in 1995/96 and 1996/97 would therefore suggest that the prevailing trypanosome challenge in 1996/97 was higher than that observed in sentinel cattle but was reduced by the trypanotolerance expressed by the Orma Boran breed. The low packed cell volumes of isometamidium-treated cattle in comparison to sentinel cattle in 1996/97 may be

attributed to detrimental effect of isometamidium especially when challenge is low and grazing is poor.

Packed cell volumes of sentinel cattle decreased significantly with increase in trypanosome challenge. In isometamidium-treated cattle, the decrease of packed cell volumes with trypanosome challenge was minimal. The significant decrease of packed cell volumes with increase of trypanosome challenge (baseline hazard) in sentinel cattle is expected as low packed cell volumes are normally associated with high parasitaemia and development of anaemia due to high trypanosome challenge (Murray *et al.*, 1980). The marginal decrease of packed cell volumes of isometamidium treated cattle with trypanosome challenge further demonstrate efficacy of isometamidium in protecting cattle from becoming anaemic.

These findings are contrary to earlier observations on Galana Ranch by Dolan *et al.* (1992). During their study there were no differences in the lowering of packed cell volume by trypanosome challenge between isometamidium-treated and sentinel cattle. This was attributed to the frequent administration of isometamidium according to the treatment regime that was used, and the unusually high trypanosome challenge observed (Dolan *et al.*, 1992).

Isometamidium treatment had no significant effect on body weight gain in the first two years of the study. However, in 1996/97 the body weight gain in isometamidium-treated cattle was significantly less than in sentinel cattle. This trend of body weights was consistent with what was observed with packed cell volumes during the same year. The absence of a difference in body weight gain in the two years, 1994/95 and 1995/96, when there was a significant trypanosome challenge, and when there were significantly more infections among sentinel cattle may be explained by the fact that all infections detected in either treatment group were immediately treated with the therapeutic trypanocide diminazene aceturate.

Loss of body weight in isometamidium-treated cattle has been observed in Galana (Dolan *et al.*, 1992). When grazing was poor, under high tsetse challenge, isometamidium-treated cattle were observed to loose weight. This was accompanied by noticeable loss of body condition. However this was not observed in the sentinel cattle (Dolan *et al.*, 1992). This general loss of weight and body condition was

attributed to toxic effects of frequent treatment with isometamidium and diminazene (Dolan *et al.*, 1992; Eisler *et al.*, 1997b).

In the present study no toxic manifestations were observed. However, the low growth rate of isometamidium-treated cattle observed in 1996/97 may be attributed to; firstly, the relatively, poor grazing conditions during 1996/97. In addition, inconsistent provision of water experienced in Kapangani during this year resulted in cattle travelling, longer distances to the next functioning water tank. This led to losses in body condition and body weights. It would therefore appear that during high trypanosome challenge, nutrition (quality of the grazing) is an important determinant of efficacy of isometamidium prophylaxis in terms of increase in productivity (Otesile *et al.*, 1992). Secondly, previous observation on the ranch showed that isometamidium treatment did not have an effect on body weights of Orma Boran cattle under natural trypanosome challenge (Wilson *et al.*, 1986).

Isometamidium could be readily detected by the ELISA in the sera of treated cattle, in all three years of the experiment, and concentrations could be measured for up to 3 months following treatment. The mean isometamidium concentrations in the prophylaxis groups were similar in all three years of the study, as were the mean elimination rates and half-lives. During any given week after treatment, considerable variation was observed between individual cattle. These findings were similar to that obtained in a study conducted in a high tsetse challenge area of the Zambezi valley, Zimbabwe (Eisler *et al.*, 1996b), in which the isometamidium ELISA was shown to be capable of quantifying drug levels in 20 out of 23 cattle for at least 70 days after treatment, but drug levels in individual cattle were found to be quite variable.

In the present studies, isometamidium levels were followed up to 60 days after treatment but in most animals the levels were still above the detection limit of 0.2 ng/ml (Mubanga, 1996). The elimination half-lives were between 24 days and 26 days, which was similar to the value (23 days) obtained in the Zimbabwe study (Eisler *et al.*, 1996b).

Isometamidium concentrations greater than 0.4 ng/ml in trypanosome-infected cattle were considered to provide evidence of drug resistance in the infecting parasite population. This threshold was chosen for two reasons. Firstly, this was the lowest measurable drug concentration that could be shown to confer protection

against experimental tsetse challenge of cattle with an isometamidium-sensitive clone of *T. congolense* (Eisler *et al.* 1997a). Secondly, based on the results obtained with pre-treatment sera (data not shown), the drug could be detected at this level with a reasonable degree of confidence ($p < 0.005$).

On this basis, evidence of drug-resistant trypanosome infections was obtained in all three years of the study In 1994/95, four (36.4 %) of the *T. vivax* infections were considered to be resistant. In 1995/6 seventy-nine (66.9 %) of the *T. vivax* and five (4.0 %) of the *T. congolense* infections were considered to be resistant and in 1996/97, five (55.5 %) of *T. vivax* and three (33.3 %) of *T. congolense* infections were considered to be resistant. The increase in the proportions of *T. vivax* and *T. congolense* resistant to isometamidium from 1994/95 to 1995/96 is co-incident with an observed increase in trypanosome challenge in the two years. This is consistent with the suggestion of Dolan *et al.* (1992) that drug resistance increases with increasing challenge.

In this year (1995/96), sentinel cattle that had trypanosome infections on day 0 of the prophylactic period were treated on the same day. By the eighth week of the prophylactic period a significantly lower proportion (77.8%) of these cattle than the proportion (96.5%) of cattle that did not have trypanosome infections on day 0 had been infected. In addition, this phenomenon is evidence that diminazene is efficacious in treating and briefly preventing infection, and there is no evidence of diminazene resistance.

The efficacy of diminazene in sentinel cattle varied with the year and the infecting trypanosome species. During 1995/96 the cure rates for both *T. congolense* and *T. vivax* infections was the lowest at 53.8% and 23.6% respectively. This was a significant drop from the year 1994/95 when the cure rate were 91.7% and 73.7% for *T. congolense* and *T. vivax* respectively. The cure rate for *T. congolense* and *T. vivax* did not change significantly from 1995/96 (53.8% and 23.6% respectively) to 1996/97 (71.4% and 33.3% respectively). However, there was an appreciable decrease in the rate of diminazene treatment failure from 1995/96 to 1996/97. This would indicate a slight decrease though not significant in the trypanosome populations resistant to diminazene in the two years.

In the present studies, the variation of diminazene treatment failure with year of study is an indication of the variation in drug resistant population with challenge. As in the case of isometamidium, it appears that the *T. vivax* population on Galana Ranch were less sensitive to diminazene than *T. congolense* population. The presence of multiple drug resistant population in Galana Ranch cannot be ruled out (Njogu and Heath, 1996). Field isolates expressing multiple resistance have been observed in Ethiopia (Codjia *et al.*, 1993; Mulugeta *et al.*, 1997) and Somalia (Ainanshe *et al.*, 1992)

The net financial benefit per animal in both isometamidium treated and sentinel cattle was significantly higher than the cost of treatment per animal in 1994/95 and 1995/96. However in 1996/97 there was a net financial loss in isometamidium-treated cattle. While in sentinel cattle the net financial benefit was 28 times higher than the cost of treatment with diminazene

In 1994/95, 1995/96 and 1996/97 the net financial benefits per animal of sentinel cattle over the prophylactic period were US$ 13.9,12.3 and 5.7 respectively. The corresponding net financial benefits per animal from isometamidium-treated cattle were US$ 15.9, 10.3 and -0.3. There was a decrease in net financial benefit per animal in both isometamidium-treated and sentinel cattle from 1994/95 to 1995/96. The decrease was higher in isometamidium-treated cattle (35.2%) than in sentinel cattle (11.5%).

The decrease in net financial benefits per animal observed in the two years corresponds to the observed increase in trypanosome challenge in the two years. In addition, the higher decrease of the net financial benefits per animal of isometamidium-treated cattle reflects the mortality due to trypanosomiasis that occurred in the isometamidium-treated cattle.

In 1996/97 isometamidium prophylaxis was not profitable. There was a net financial loss per animal from isometamidium-treated cattle during this year. In contrast, there was a net financial benefit per animal (US$ 5.7) in maintaining cattle under diminazene therapy during the same year. This was less than half the net financial benefits per animal (US$ 12.3) obtained in 1995/96.

This is an indication of the lower productivity (weight gains) of the Orma Boran cattle in 1996/97, probably due to the poor grazing during this year. Nevertheless, the net financial benefit of the sentinel cattle in 1996/97 was 28 times more than the cost per animal of trypanocidal treatment used during this year. This is evidence of high productivity of Orma Borans under natural trypanosome challenge with little input in terms of chemotherapy in spite of evidence of drug resistant trypanosome populations.

Similar findings of a benefit-cost analysis that showed profitable cattle production is possible in a problem area with high prevalence of drug resistant trypanosomes have been reviewed (Geerts and Holmes, 1998)

In conclusion, in all three years of the study, there was evidence that breakthrough infections, particularly *T. vivax*, had some degree of resistance to isometamidium. In addition trypanosome populations resistant to diminazene may have contributed to treatment failure observed in sentinel cattle. It is likely that multiple drug resistance observed earlier by Rottcher and Schillinger, (1985) still exists in Galana Ranch. Nevertheless profitable cattle production is still very possible. Furthermore it was evident that these multiple resistant trypanosome populations depend on the level of challenge and season (but are normally relatively uncommon) (Mamman *et al.*, 1995b).

In spite of the presence of drug resistance, isometamidium prophylaxis can still be used effectively in low challenge seasons. However, during seasons of high trypanosome challenge, when the population of *T. vivax* responsible for the acute form of the disease is likely to be high, chemotherapy using diminazene would be appropriate.

It is evident that the Orma Borans are more productive during high trypanosome challenge when maintained under chemotherapy with diminazene than under isometamidium prophylaxis. However their productivity is affected more by poor grazing than the more trypano-susceptible Galana Borans.

Finally, it is recommended that the seasonal variation of resistant *T. vivax* populations on Galana Ranch requires use of both isometamidium and diminazene strategically. During rainy seasons, when the whole ranch has sufficient grazing

areas, the more susceptible breed of Borans could be moved to the areas with low tsetse challenge and maintained on isometamidium prophylaxis while the more trypano-tolerant breed is moved to the high tsetse challenge area with lush vegetation, and maintained on monthly block treatment with diminazene. During the drier season when the tsetse challenge is low in the more productive tsetse zone, the Galana Borans at the now drier tsetse free areas can be moved to this tsetse zone and be maintained on chemoprophylaxis with little supervision. In addition, the frequency of diminazene block treatment on the Orma breed can be reduced during the drier season. This pattern can be repeated again with season to enhance productivity and reduce mortality losses.

Chapter 5

Observational studies on Trypanosomiasis control in small holder dairy farms

5 Observational studies

5.1 Introduction

5.1.1 Overview

This chapter describes a series of observational studies carried out on semi-zero/zero-grazing cross-bred dairy cattle. The performance of dairy cattle under isometamidium prophylaxis was investigated. These studies were initiated after veterinary officers at the Veterinary Investigation Laboratories, Ukunda in Kwale District observed increasing breakthrough infections in zero-grazing dairy cattle under isometamidium prophylaxis in Kwale. Initially, the studies were to be carried out in Kwale district in two administrative divisions, Matuga and Kubo where most of the zero-grazing units are located. In an attempt to establish the extent of prophylaxis failure reported, small holder zero-grazing farmers within the Mtwapa area of Kilifi District were also included in the observational studies. The zero-grazing units in Kwale were established under the National Dairy Programme where a group of farmers were in charge of the animals in the individual zero-grazing units. In contrast, in Kilifi, the zero-grazing units had been set up by individual farmers who decided what control strategy they would adopt for their own cattle. Most of the zero-grazing units in Kilifi had more than five cattle, while in Kwale all units had less than three animals. The animals in these studies were mixed crosses of exotic breeds with local zebu cattle. In Kwale district a total of three prophylactic periods of about 3 months each were carried out in 1996. In Kilifi one prophylactic period of three months was carried out, however no trypanosome infections were detected over this period.

5.1.2 South coast

5.1.2.1 Area of study

The zero-grazing units were located in Kubo and Matuga Divisions of Kwale District. In Matuga the study units were located between longitude 39° 45' and 39°

58'E and between latitude 4° 15' and 4° 20'S covering the coastal plain and foot plateaux topographical zones. In Kubo the study units were located between longitude 39° 30'- 39° 54'E and latitude 4° 32' - 4° 40'S in the foot plateaux topographical zone. The zero-grazing units in Kubo and Matuga lie in the agro-ecological Zone L3 – coconut-cassava (Jaetzold and Schmidt, 1983) with an average annual rainfall between 1000-1230 mm. The rainfall is bimodal with long rains starting between March and April and continuing until July. Sixty percent of the annual rain falls during this period. The short rains are usually in October and November.

5.1.2.1.1 Brief history of the cattle in the Study

The animals in the study had been acquired under the National Dairy Development Programme (NDDP) of the Ministry of Livestock Development of Kenya, which was initiated in 1991. The aim of the programme was to improve the living standard of poor farmers by encouraging them to keep dairy cattle in zero-grazing unit where the individual would benefit from the income generated from milk sales. Groups of five to ten farmers were provided with dairy cattle, assisted in setting up the zero-grazing units and provided with drugs and artificial insemination services which they would pay for later from the milk sales and group contributions. The female calves would be allocated to members of a group to take care of, by casting lots. This trend will continue for subsequent female calves that are produced until each member of the group has a dairy animal. Male calves would be sold and the proceeds used to offset their debt from the NDDP. The dairy animal provided under the programme to the organised groups would belong to an individual farmer but, the group the farmer belongs to, has the authority to withdraw the animal in case of mismanagement and give it to another member. This occurred on several occasions during this observational study thus re-locating the animal to another farm with a different level of tsetse challenge

The animals were purchased mainly from Kilifi plantation. The health services were provided by the Veterinary Department under the co-ordination and guidance of the District Veterinary Officer (DVO).

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79 % of the infections while the remainder were *T. congolense* (13 %) and mixed infections (8%) (Gaturaga *et al.*, 1990).

5.1.3 North coast

5.1.3.1 Area of Study

The study was carried out approximately 15 km north of Mombasa along the coast within Mtwapa area. Mtwapa is located at longitude 39° 44'E, latitude 3° 46'S at about 15m above sea level (Figure 1). This area is in the coconut-cassava agro-ecological zone (CL3) of the coastal strip (Jaetzold and Schmidt, 1983).

The mean annual rainfall is about 1,100 mm with peak rainfall between April and June. Only January, February and March have an average rainfall less than 50 mm rain with wide variation recorded between years in the annual precipitation and its distribution. Daily temperatures are high with mean monthly maxima ranging between 27 °C and 32 °C and mean monthly minima between 20 °C and 24 °C. Relative humidity rarely falls below 70 per cent.

5.1.3.1.1 Husbandry practices of the farmers

All the farms in the study were small-holder dairy farms. Four out of the eight farmers were practising true zero-grazing, while the other four were restricting grazing such that animals are allowed to free graze part of the time within the farm or outside the farm. Mtwapa is highly populated with many small scale farmers keeping poultry. Most of the farms are about 2-5 acres. But the acreage is intensively utilised. Besides keeping dairy cattle (a minimum of five and a maximum of 15 cattle) and poultry, most of the farmers produce green vegetables for commercial purpose.

5.1.3.1.2 Tsetse and Trypanosomiasis prevalence

Along the coastline north of Mombasa, tsetse have been observed since 1961, and a high prevalence of trypanosome infections has been observed in cattle (Paling *et al.*, 1987; Dowler *et al*,. 1989). The predominant tsetse species along the coastline is *G. austeni.* At the Kenya Agricultural Research Institute's field station in Mtwapa, trypanosomiasis has been one of the major constraints in the maintenance of the

Research herd. This necessitated the use of isometamidium prophylaxis three times a year (Maloo, 1993). In a dairy herd at nearby Vipingo plantation, a high monthly trypanosome prevalence of 22 % has been observed (Dowler *et al.*, 1989).

5.1.3.1.3 Trypanosomiasis control strategy by small holder dairy farmers at Mtwapa

Most of the farmers had been using isometamidium prophylaxis for more than ten years. These farmers would seek the services of a private veterinarian or in most cases to cut down on the high cost involved, they would use the Government's agricultural extension field officers who under the Government's policy of cost-sharing would charge much less. In recent years, increasing numbers of clinical cases were associated with trypanosomiasis (Maloo, 1993). Consequently, field veterinary personnel were able to convince some farmers to stop using isometamidium and to revert to chemotherapy using diminazene as an alternative. Other farmers were advised to use "pour-on" insecticidal treatments as a tsetse control measure. Therefore the control strategy used by individual farmers was dependent on the extension field officers serving them. In this study it was important to determine how the use of prophylaxis by the various farmers would affect the observed efficacy of isometamidium.

5.1.4 Objectives

5.1.4.1 To determine the efficacy of isometamidium prophylaxis of trypanosomiasis in zero-grazed dairy cattle at the Kenya coast

5.1.4.2 To investigate factors associated with isometamidium prophylaxis failure in zero-grazed dairy cattle at the Kenya coast

5.2 Material and Methods

5.2.1 South coast

5.2.1.1 The study site

The study was carried out in two administrative divisions of Kwale district mainly, Kubo and Matuga. The Shimba Hills Game Reserve occupies the upper western parts of the two divisions.

5.2.1.2 Study Animals

The animals used were dairy cattle maintained under zero/semi zero-grazing system. The dairy cattle are produced by crossing the local Boran zebu cattle with a mixture of the exotic breeds such as the Friesian, Sahiwal and Ayrshire. These animals were provided to local farmers under the National Dairy Development Programme.

5.2.1.3 Experimental design

Twenty-seven mixed cross-bred dairy cattle were selected from two divisions of Kwale district. The cattle were observed for three consecutive isometamidium prophylaxis cycles, commencing November 1995. Twelve animals were located in Kubo division; each animal belonged to an individual farmer. Fifteen animals were located in Matuga Division (Table 13). These belonged to seven owners. Two owners had 3 and 4 cattle each, 3 owners had 2 cattle each and 2 owners had one animal each. The number of animals and the zero-grazing units, and the dates of the prophylaxis periods are shown in Table 13.

The cattle were maintained under the individual farmers' zero-grazing system and therefore exposed to the localised tsetse challenge within the smallholding. The grazing units were covered with either iron sheets or woven palm leaves. The sides of the units were open such that the animals were accessible to the tsetse flies or any other biting flies. The number of cattle in a unit varied from one to four. The feed included elephant grass, cassava leaves, Napier grass and any weeds found around the homestead. All the animals were given water *ad libitum*. The area has many wells

with clean water. None of the farmers included mineral supplements in the animals' diets. The trial was conducted from late November, 1995 to mid August 1996.

Table 13: Number of cattle, zero-grazing units and dates of the prophylactic periods in Kubo and Matuga in Kwale District

Site	Number of cattle	No. of zero-grazing units	1st prophylactic period	2nd prophylactic period	3rd prophylactic period
Kubo	13	13	Nov. 95 - Feb. 96	Feb. - May 1996	May - Aug. 1996
Matuga	15	7	Nov. 95 - Feb. 96	Feb. - May 1996	May - Aug. 1996

5.2.1.4 Experimental protocol

5.2.1.4.1 Isometamidium treatment

A week before the first isometamidium prophylaxis period, cattle were parasitologically screened and pre-treatment serum collected. Any animals found to have trypanosome parasitaemia were treated with diminazene aceturate at a dose rate of 7.0 mg per kg body weight. Cattle were block treated with isometamidium at a dose rate of 1.0 mg/kg b.w. in accordance with to Chapter 3, section 4.1. However, in cattle with trypanosome parasitaemia, isometamidium treatment was deferred until two weeks after diminazene treatment. The treatments of cattle in Kubo and Matuga were done on different days.

At the start of the second and third prophylactic periods, the animals were again block treated with isometamidium. The animals in the experiment were given 3 consecutive isometamidium treatments at a maximum interval of 84 days. The following baseline data was collected; body weight, pregnancy, lactational status and general health.

5.2.1.4.2 Parasitological and packed cell monitoring

The cattle were monitored weekly for trypanosome infections and determination of packed cell volumes. Blood samples were collected directly into heparinised capillaries from an ear vein punctured using a sterile lancet. The presence of trypanosomes was determined by using the microhaematocrit centrifugation and buffy coat /dark ground techniques. The procedures used are described in Chapter 3 section 5.

5.2.1.4.3 Serum sampling

Serum samples were collected from the treated animals on the same occasions as parasitological monitoring, i.e. weekly. Jugular venous blood samples were collected into plain vacutainers, allowed to clot and kept at ambient temperature

out of direct sunlight until transfer to the laboratory. The protocol was according to Chapter 3 section 8.

5.2.1.4.4 Trypanosome stabilate collection

Stabilates of trypanosome populations causing breakthrough infections were obtained from the ear vein according to the method in Chapter 3 section 6.1

5.2.1.4.5 Body weights and Body condition

Body weights were estimated using a weighing band at the beginning of each prophylactic period according to Chapter 3 section 7.1. Body conditions were measured visually and graded as good, fair or poor.

5.2.1.4.6 Treatment of breakthrough trypanosome infections:

1st prophylactic period: Any animals found to be infected with trypanosomes were treated with diminazene aceturate at 7 mg/kg body weight.

2nd and 3rd prophylactic periods: The animals found to be infected with trypanosomes were treated only when there were signs of ill health associated with infection or when the packed cell volume dropped below 23 %.

5.2.2 North coast

5.2.2.1 The study sites

The farms included in the study were located along the Mombasa-Malindi road at Mtwapa, Kikambala, Mtepeni and Tezo. They were all in the Bahari south division of Kilifi except the farm at Tezo that was in Bahari north several kilometres from Kilifi Township.

5.2.2.2 Experimental design

The number of cattle involved in each area and the time of the study and treatment doses used are shown in Table 14. Cattle were monitored weekly for trypanosome infection and determination of packed cell volumes according to Chapter 3 section 5. The livestock animal health assistants, according to their normal practice carried out isometamidium block treatment.

Table 14: Number of cattle in the study including period of study, type of cattle isometamidium dose rates and the person administering the drug

Site	No. of cattle	Prophylactic period	Type of cattle	Isometamidium dose	Who treats
Mtwapa	61	March – June 1996	Cross-bred dairy cattle	According to normal practice ≈ 0.5 mg/kg b.w.	LAHA[1]
Kikambala	12	March – June 1996	Cross-bred dairy cattle	According to normal practice ≈ 0.5 mg/kg b.w.	LAHA
Mtepeni	6	March – June 1996	Cross-bred dairy cattle	According to normal practice ≈ 0.5 mg/kg b.w.	LAHA
Tezo	6	March – June 1996	Cross-bred dairy cattle	According to normal practice ≈ 0.5 mg/kg b.w.	LAHA

[1]LAHA: livestock animal health assistant

5.2.3 Data Analysis:

5.2.3.1 Parasitaemia data

Weekly infection rates were calculated as number of infections detected in a week divided by the number of animals at risk in that week according to Chapter 3 section 10.1.1.

Life tables were calculated based on the day an animal acquired its first trypanosome infection and the survival analysis conducted according to Chapter 3 section 10.1.2, wherein "survival" refers to an animal remaining uninfected by trypanosomes.

5.2.3.2 Packed cell volume data

Descriptive statistics of weekly packed cell volumes were calculated using Excel 97 software (Microsoft). Mean packed cell volumes were plotted against days post treatment. To determine differences between periods a paired students t-test was performed on the packed cell volumes.

5.2.3.3 Serum isometamidium concentrations data

Descriptive statistics were derived according to Chapter 3 section 10.3. Log – linear regression of the drug concentration was done using Microsoft Excel 97 software (Microsoft Corporation) and elimination rate constants and half-lives calculated from the slope of the regression line as follows;

Slope (coefficient of regression) = k_{el} (elimination rate constant).

$t_{1/2}$ (half-life) = $0.693/k_{el}$

5.2.3.4 Assessment of efficacy of isometamidium prophylaxis

The measurements relating to assessment of efficacy of isometamidium were based on proportion of cattle in the isometamidium group that were infected by the eighth week of the prophylactic period (see Chapter 3. section 10.2). Ratios of mean

hazard function over eight weeks of the prophylactic period of sentinel cattle to isometamidium treated cattle were calculated based on life- tables (see Chapter 3. section 10.2).

5.3 Results

5.3.1 South coast

5.3.1.1 Trypanosome infections during isometamidium block treatment studies

Trypanosome infections were detected in cattle during all three prophylactic periods. A summary of the number of trypanosome infections detected during the prophylaxis studies is shown in Table 15. The weekly infection rates are shown in Figure 15.

5.3.1.1.1 Period 1:November 1995 – February 1996

At Kubo, there were a total of 8 infections. Of these 25 % were *T. congolense* and 75 % were *T. vivax*. The overall infection rate in 89 samples was 9.0 % (Table 15). The earliest breakthrough infection in Kubo was detected on the second week of the prophylactic period. It was a *T. vivax* infection. Four animals out of 12 had breakthrough infections. Of these, two had a single breakthrough infection each and the other two, had two and three infections respectively over the prophylactic period

In Matuga, of the 124 were samples collected, 11 (8.9 %) had trypanosome infections. Of these 45.5 % were *T. congolense* and 54.5 % were *T. vivax*. The earliest breakthrough infection was detected on the second week of the prophylactic period. It was a *T. congolense* infection. More infections were detected in the subsequent weeks. Eight of the 15 animals at Matuga became infected during this period. Of these, six had a single breakthrough trypanosome infection each and two animals had two and three breakthrough infections respectively over the prophylactic period.

5.3.1.1.2 Period 2:February – May 1996

In Kubo, 17 trypanosome infections were detected. Of these 23.5 % were *T. congolense* whilst 76.5 % were *T. vivax.* The overall infection rate in 136 samples was 12.5%. The first breakthrough infections were detected as early as 2 weeks post treatment. This was *T. vivax* . The earliest *T. congolense* breakthrough infection was detected in the eighth week of the prophylactic period. Five of the 12 cattle had breakthrough infections. Of these, one had a single breakthrough infection and the rest had between two and eight infections during the prophylactic period.

In Matuga, 10 trypanosome infections were detected. Of these 60 % were *T. congolense* while 40 % were *T. vivax.* The overall infection rate in 159 samples was 6.3 %. The earliest breakthrough was detected on the first week of the prophylaxis period. It was a *T. congolense* infection. Six of the 15 cattle had breakthrough trypanosome infections during this prophylactic period. Of these, 4 had a single breakthrough infection each and two had two and four infections respectively during the prophylactic period.

5.3.1.1.3 Period 3: May – August 1996

In Kubo, 9 trypanosome infections were detected. Of these 88.9 % *were T. congolense* while 11.1 % were *T. vivax.* The overall infection rate in 108 samples was 8.33 % (Table 15). The earliest breakthrough infection was detected in the fourth week of the prophylactic period. It was a *T. congolense* infection. Three of the nine cattle in the study at Kubo had breakthrough infections. All three had more than two breakthrough infections.

In Matuga, 16 trypanosome infections were detected. Of these 81.3 % were *T. congolense* while 18.7 % were *T. vivax.* The earliest breakthrough infection was detected in the third week of the prophylactic period. It was a *T. congolense.* The earliest *T. vivax* breakthrough infection was detected on the sixth week of the prophylactic period. Six of the 14 cattle had breakthrough infections. Of these, 3 had a single breakthrough infection each (two *T. vivax* and one *T. congolense*), and the rest had between three and five infections during the prophylactic period.

Table 15: Summary of trypanosome infections detected in cattle during the observational studies in Kwale District

Site	Prophylactic period	No. of samples	No. of Tc[1]	No. of Tv[2]	Tc %[3]	Tv %[3]
Kubo	1	89	2	6	2.25	6.74
	2	136	4	13	2.94	9.56
	3	108	8	1	7.41	0.93
Matuga	1	124	5	6	3.23	4.84
	2	159	6	4	3.77	1.89
	3	149	13	3	8.72	2.01

[1]Tc:Trypanosoma congolense

[2]Tv: *Trypanosoma vivax*

[3]%: percentage of samples with *T. congolense* or *T. vivax*

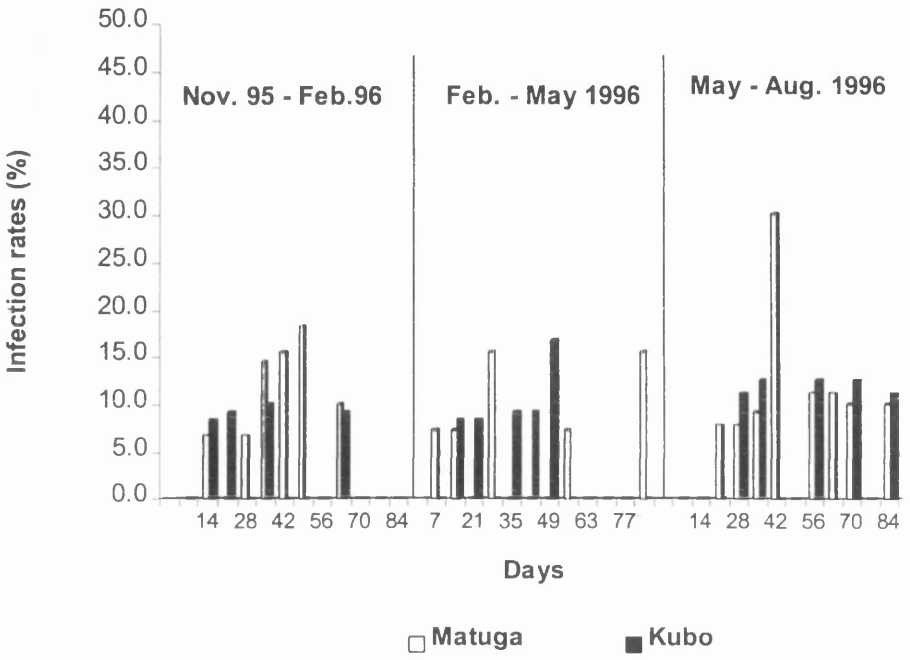


Figure 15: Weekly prevalence of trypanosome infections at Kubo and Matuga during the observational studies in Kwale

5.3.1.2 Survival analysis of isometamidium block treatment studies

Plots of survival functions for the cattle treated with isometamidium in Kubo and Matuga for the three prophylactic periods are shown in Table 16. The proportions of cattle that experienced at least one infection during the first eight weeks of each prophylactic period are shown in Table 16.

5.3.1.2.1 Period 1: November 1995 – February 1996

On the basis of log-rank and Wilcoxon tests, there was no significant difference ($p = 0.60$) in the survival function between the two sites. In Matuga, 46.7 % of cattle experienced at least one trypanosome infection by week eight of the study while in Kubo 35.2% of cattle experience at least one trypanosome infection over the same period.

5.3.1.2.2 Period 2: February – May 1996

On the basis of log-rank and Wilcoxon tests, there was no significant difference ($p = 0.94$) in the survival function between the two sites

In Matuga, 35.7% of cattle experienced at least one trypanosome infection by week eight of the study, while in Kubo 41.7 % of cattle experienced at least one trypanosome infection over the same period.

5.3.1.2.3 Period 3: May – August 1996

On the basis of log-rank and Wilcoxon tests, there was no significant difference ($p = 0.98$) in the survival function between the two sites. During this period the proportion of cattle that experienced at least one trypanosome infection by week eight at Matuga was 30.8 % and at Kubo was 33.3 %.

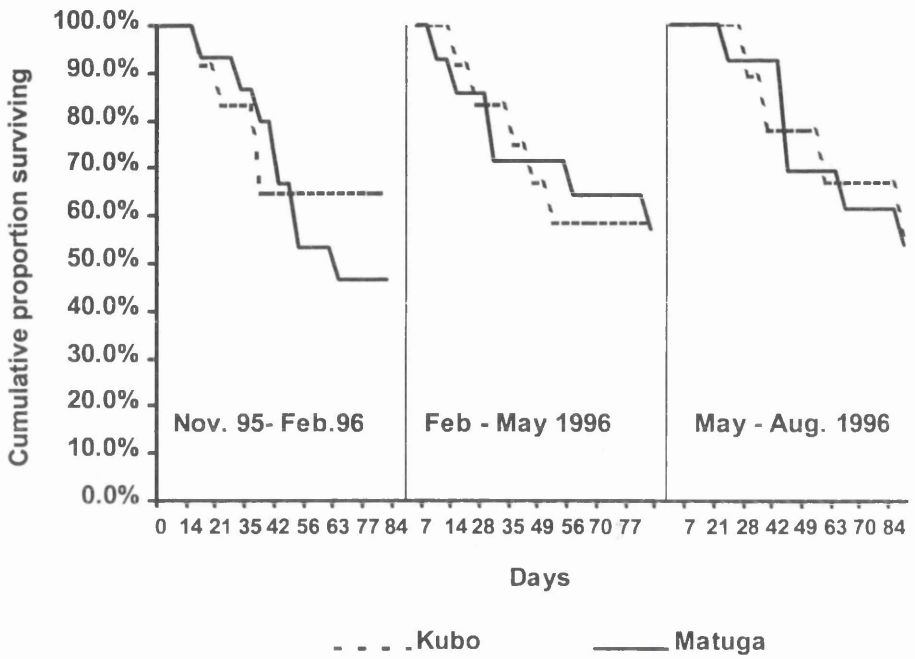


Figure 16: Cumulative proportions of isometamidium treated cattle surviving (i.e. remaining uninfected by trypanosomes) during the observational studies at Kwale in 1996

Table 16: Proportions of cattle experiencing at least one infection during the first eight weeks of each prophylactic study

Site	Prophylactic period	Proportion of cattle (%)
Matuga	Nov. 95 – Feb. 1996	46.7
	Feb. – May 1996	35.7
	May – August 1996	30.8
Kubo	Nov. 95 – Feb. 1996	35.2
	Feb. – May 1996	41.7
	May – August 1996	33.3

[1]Proportion of cattle experiencing at least one infection during the first eight weeks of the prophylaxis study.

5.3.1.3 Efficacy of isometamidium treatment in preventing infection by different trypanosome species

The relative efficacy of isometamidium treatment in preventing infection by different trypanosome species was determined by comparisons of proportions of cattle that experienced at least one *T. congolense* or *T. vivax* infection by the eighth week of each prophylaxis period. The plots of survival function with time for *T. congolense* and *T. vivax* infections during the observational studies at Kubo and Matuga are shown in Figures 17 and 18. The proportions of cattle that experienced at least one trypanosome infection are shown in Table 17.

5.3.1.3.1 Period 1: November 1995 – February 1996

There was a significant difference ($p < 0.001$) in survival functions for *T. congolense* and *T. vivax* in Kubo. By the eighth week, 9.1 % cattle had been infected with *T. congolense* and 35.2 % had been infected with *T. vivax* (Table 17).

There was a significant difference ($p = 0.024$) in survival functions for *T. congolense* and *T. vivax* in Matuga. By the eighth week, 13.3 % cattle had been infected with *T. congolense* and 33.3 % had been infected with *T. vivax* (Table 17).

5.3.1.3.2 Period 2: February – May 1996

There was a significant difference ($p < 0.001$) in survival functions for *T. congolense* and *T. vivax* in Kubo. By the eighth week, 8.3 % cattle had been infected with *T. congolense* and 41.7 % had been infected with *T. vivax* (Table 17).

There were no significant differences $p > 0.05$) in survival functions for *T. congolense* and *T. vivax* in Matuga. By the eighth week, 14.3 % cattle had been infected with *T. congolense* and 21.4 % had been infected with *T. vivax* (Table 17).

5.3.1.3.3 Period 3: May – August 1996

There was a significant difference ($p < 0.01$) in survival functions for *T. congolense* and *T. vivax* in Kubo. By the eighth week, 33.3 % cattle had been infected with *T. congolense* and 11.1 % had been infected with *T. vivax* (Table 17).

There were no significant differences $p > 0.05$) in survival functions for *T. congolense* and *T. vivax* in Matuga. By the eighth week, 15.4 % cattle had been infected with *T. congolense* and *T. vivax* respectively (Table 17).

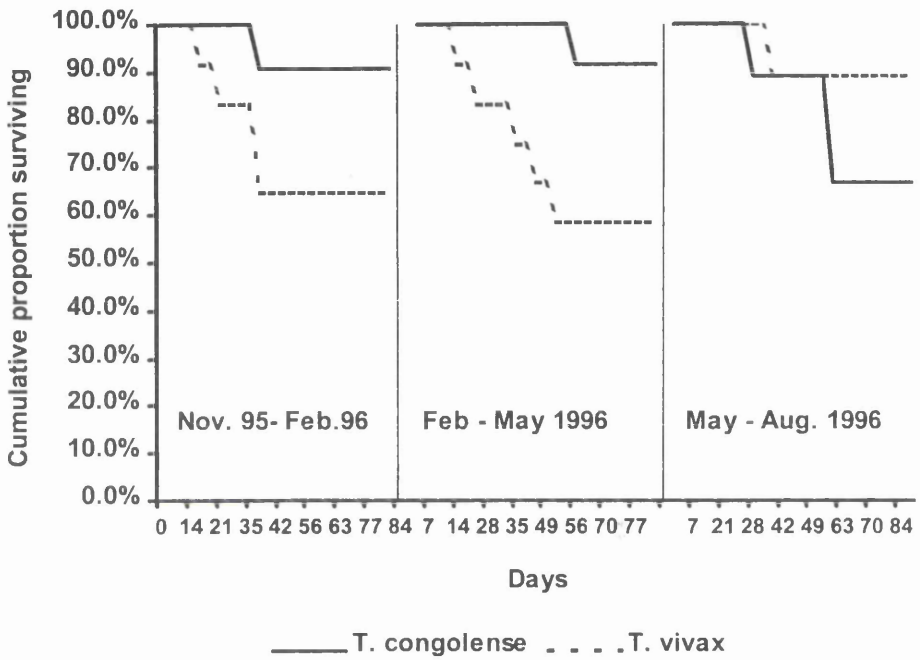


Figure 17: Cumulative proportion of dairy cattle at Kubo surviving (i.e. remaining uninfected by *T. congolense* and *T. vivax*) during the observational studies in Kwale

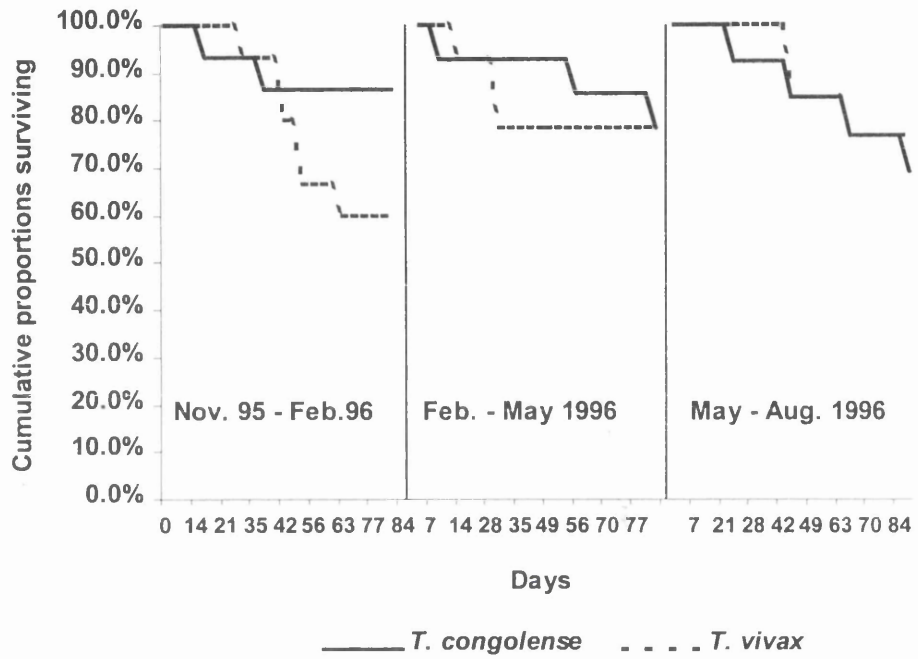


Figure 18: Cumulative proportion of dairy cattle at Matuga surviving (i.e. remaining uninfected by *T. congolense* and *T. vivax*) during the observational studies at Kwale

Table 17: Proportion of cattle experiencing at least one *T. congolense* and *T. vivax* infection during the first eight weeks of each prophylactic period at Kubo and Matuga

Site	Prophylactic period	Proportion of cattle infected with *T.congolense*[1] (%)	Proportion of cattle infected with *T. vivax*[2] (%)
Kubo	Nov. 1995 – Feb. 1996	9.1	35.2
	Feb. – May 1996	8.3	41.7
	May – August 1996	33.3	11.1
Matuga	Nov. 1995 – Feb. 1996	13.3	33.3
	Feb. – May 1996	14.3	21.4
	May – August 1996	15.4	15.4

[1]Proportion of cattle experiencing at least one *T. congolense* infection during the first eight weeks of the prophylaxis study.

[2]Proportion of cattle experiencing at least one *T. vivax* infection during the first eight weeks of the prophylaxis study.

5.3.1.4 Packed cell volumes

The means of the weekly packed cell volumes of the cattle in Kubo and Matuga plotted against days post treatment are shown in Figure 19.

5.3.1.4.1 Period 1: November 1995-February 1996

Before isometamidium treatment the packed cell volumes of cattle in Kubo were significantly higher than packed cell volumes of cattle in Matuga. Thereafter there was a steady decline in the packed cell volumes of cattle in Kubo during the prophylactic period. In Matuga, after isometamidium treatment, there was an increase in packed cell volumes over the prophylactic period (Figure 19).

5.3.1.4.2 Period 2: February – May 1996

In Kubo the mean packed cell volumes decreased from 30.3 ± 1.2 % before treatment to a minimum (26.8 ± 1.3%) in the seventh week. Thereafter the packed cell volumes increased slightly but remained below 30% to the end of the prophylactic period.

In Matuga, the mean PCV was fairly constant at around 27 - 28% for most of this prophylactic period, with an exception on the ninth week when the mean packed cell volumes dropped to 25.2 ± 1.3 % (Figure 19).

5.3.1.4.3 Period 3:May - August 1996

In this period the packed cell volumes of cattle in Matuga were generally lower than the packed cell volumes of cattle in Kubo. On a few occasions the difference was significant (based on the standard errors) especially between the third week and ninth week of the prophylactic period.

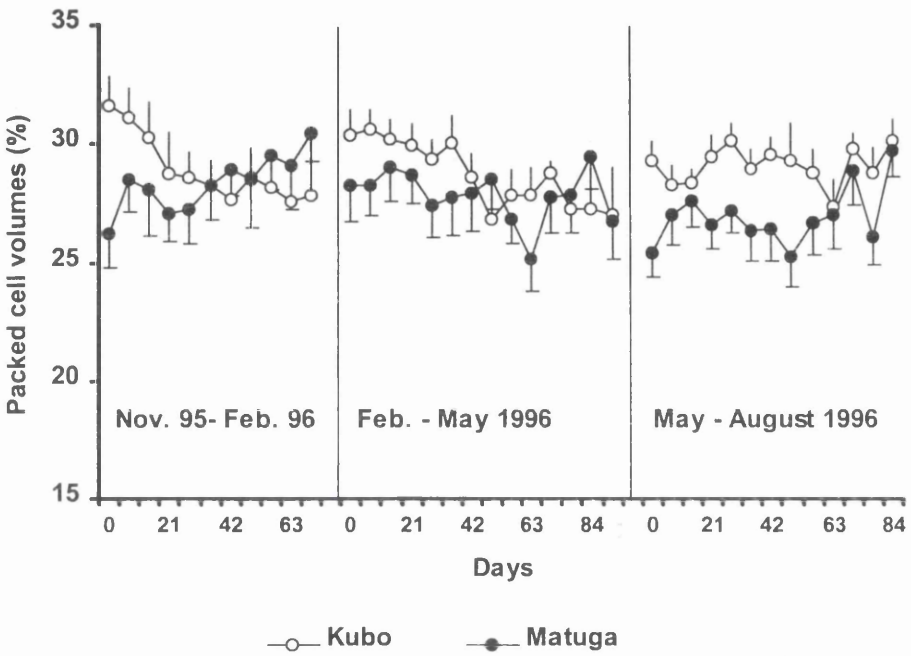


Figure 19: Weekly mean packed cell volumes of dairy cattle at Kubo and Matuga during the observational studies in Kwale

5.3.1.5 Packed cell volumes at time of detection of trypanosome infections

During the observational studies at Matuga and Kubo the criteria of treatment of breakthrough infections was either that the animal was clinically sick with detectable parasitaemia or that the packed cell volumes at detection of parasitaemia was less than 23 %. In this section the *T. congolense* and *T. vivax* breakthrough infection are thus categorised. The number of breakthrough infections that occurred when packed cell volumes were below 23 % is shown in Table 18.

5.3.1.5.1 Period 1: November 1995 – February 1996

In Kubo, there were six *T. vivax* breakthrough infections. Of these, 3 were detected in cattle with PCVs between 17% and 19%. Both of two *T. congolense* breakthrough infections were detected in cattle with PCVs above 23 % (Table 18).

In Matuga, there were six *T. vivax* breakthrough infections. Of these 3 were detected in cattle with PCVs of 15 %, 17% and 21%. Two *T. congolense* breakthrough infections were detected in cattle with PCVs of 17 % and 22 % (Table 18).

5.3.1.5.2 Period 2: February – May 1996

In Kubo, of the 13 *T. vivax* breakthrough infections, one was detected in an animal with a packed cell volume of 21 %. All *T. congolense* breakthrough infections were detected when PCVs were above 23 % (Table 18).

In Matuga, 4 of the 6 breakthrough infections were detected when PCVs were between 17% and 22 %. All four *T. vivax* breakthrough infections were detected PCVs were above 23 %. (Table 18).

5.3.1.5.3 Period 3: May – August 1996

In Kubo, during this prophylactic period, in all eight *T. congolense* and one *T. vivax* breakthrough infections were detected in cattle with PCVs above the 23 % level.

In Matuga, for nine of the 13 *T. congolense* breakthrough infections, the PCVs of the hosts at the time of detection were between 17 % and 22 %. For two of the three *T. vivax* breakthrough infections detected, the hosts' PCVs were 19 % and 20 % (Table 18).

Table 18: Number of *T. congolense* and *T. vivax* breakthrough infections detected in cattle at Kubo and Matuga when packed cell volume was below 23 %

		T. congolense		*T. vivax*	
Site	**Prophylactic period**	**n[1]**	**PCV< 23%[2]**	**n**	**PCV< 23%**
Kubo	Nov 1995- Feb 1996	2	0	6	3
	February-May 1996	4	0	13	1
	May- August 1996	8	0	1	0
Matuga	Nov 1995- Feb 1996	5	2	6	3
	February-May 1996	6	4	4	1
	May- August 1996	13	9	3	2

[1]Number of trypanosome infections

[2]Number of trypanosome infections isolated from cattle in which PCVs were below 23%.

5.3.1.6 Disposition of isometamidium in dairy cattle

Isometamidium could be detected in the sera of all cattle examined in Matuga and Kubo. In the three prophylactic studies there were wide variations in isometamidium levels in individual cattle in any given week. Log- linear plots of mean isometamidium concentration against time are shown in Figure 20. In all the three prophylactic periods isometamidium concentrations in serum from Kubo and Matuga declined exponentially. Similar drug elimination rates and half-lives were seen in Kubo and Matuga in all three prophylactic periods (Table 19).

Descriptive statistics of isometamidium concentrations in dairy cattle during the 3 prophylaxis periods at Kubo and Matuga are shown in Appendix 2.

5.3.1.6.1 Period 1: November 1995- February 1996

In Kubo, a week after isometamidium treatment, isometamidium concentration (mean = 2.81ng/ml) varied from a minimum of 1.44 ng/ml to a maximum of 4.45 ng/ml. By the tenth week after treatment, the isometamidium levels (mean 0.602 ng/ml) varied from a minimum of 0.442 ng/ml to a maximum of 0.782 ng/ml.

In Matuga, one week after isometamidium treatment, isometamidium concentrations (mean = 3.50 ng/ml) varied from a minimum of 0.55 ng/ml to a maximum of 6.18 ng/ml. By the tenth week, the isometamidium levels (mean = 0.47 ng/ml varied from a minimum of undetectable (less than 0.2 ng/ml) to a maximum of 0.68 ng/ml.

5.3.1.6.2 Period 2: February-May 1996

In Kubo, one week after isometamidium treatment, isometamidium concentrations (mean = 2.53 ng/ml) varied from a minimum of 0.59 ng/ml to a maximum of 4.78 ng/ml. By the twelfth week, the isometamidium levels (mean:0.58 ng/ml) varied from a minimum of undetectable (less than 0.2 ng/ml) to a maximum of 1.22 ng/ml.

In Matuga, one week after isometamidium treatment, isometamidium concentrations (mean = 3.18 ng/ml) varied from a minimum of 0.40 ng/ml to a maximum of 5.19 ng/ml. By the twelfth week, the isometamidium levels (mean = 0.47 ng/ml) varied from a minimum of 0.25 ng/ml to a maximum of 0.70 ng/ml.

5.3.1.6.3 Period 3: May – August 1996

In Kubo, one week after isometamidium treatment, isometamidium concentrations (mean = 3.84 ng/ml) varied from a minimum of 2.64 ng/ml to a maximum of 6.14 ng/ml. By the eleventh week, the isometamidium levels (mean = 0.53 ng/ml) varied from a minimum of 0.23 ng/ml) to a maximum of 0.77 ng/ml.

In Matuga, one week after isometamidium treatment, isometamidium concentrations (mean = 4.32 ng/ml) varied from a minimum of 1.316 ng/ml to a maximum of 7.48 ng/ml. By the tenth week, the isometamidium levels (mean = 0.42 ng/ml) varied from a minimum of 0.34 ng/ml to a maximum of 0.50 ng/ml.

Table 19: Elimination rates and half-lives of isometamidium in dairy cattle

Site	Prophylaxis period	Elimination rate constant (hr^{-1})	$t_{1/2}$ (hr)
Kubo	Nov 95 – Feb 1996	-0.00122	568.164
	Feb. – May 1996	-0.00105	658.075
	May – August 1996	-0.00117	590.30
Matuga	Nov 95 – Feb 1996	-0.0013	532.90
	Feb. – May 1996	-0.00111	624.04
	May – August 1996	-0.00141	491.02

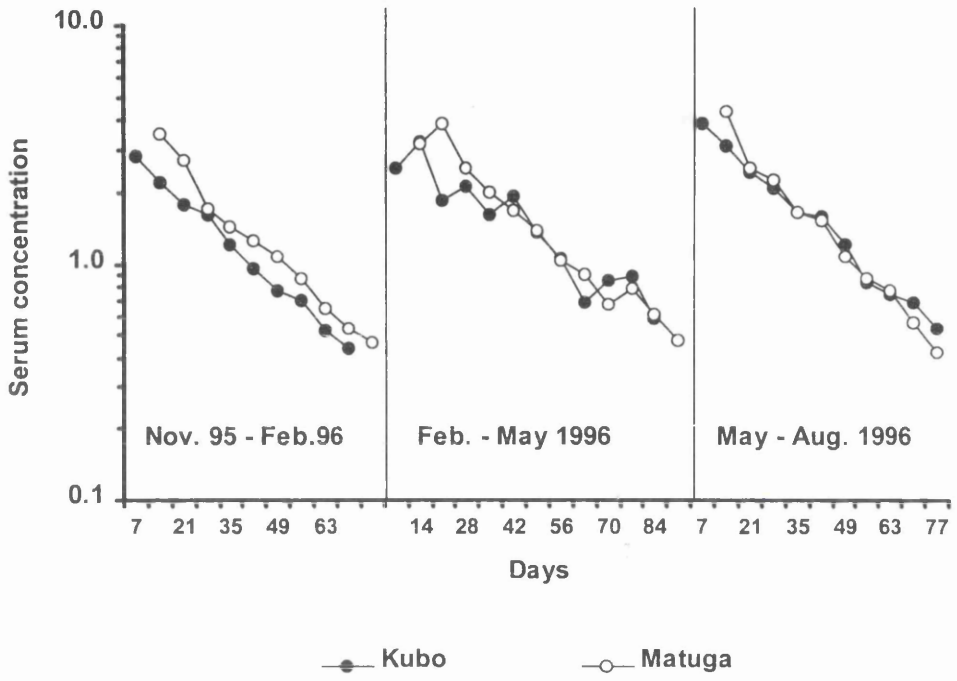


Figure 20: Log-Linear plots of isometamidium concentration in dairy cattle at Kubo and Matuga during the observational studies in Kwale District

5.3.1.7 Isometamidium concentrations in sera obtained from cattle at the time of detection of breakthrough infections.

Trypanosomes detected in isometamidium-treated cattle in which serum determinations of the drug showed concentrations greater than 0.4 ng/ml were considered to have some degree of resistance. The number of *T. congolense* and *T. vivax* infections in which serum isometamidium concentrations were found to be above or below this level are shown in Table 20. Serum samples for isometamidium determination were not always available on the exact days the breakthrough infections were first detected. In such cases approximate isometamidium concentrations were estimated by interpolation of the nearest available data points, or extrapolation of the last available data points

5.3.1.7.1 Period 1: November-February 1996

In Kubo during this period there were a total of eight breakthrough infections, two *T. congolense* and six *T. vivax* (Table 20). In the case of the *T. congolense* breakthrough infections isometamidium concentrations were just above 0.4 ng/ml at the time the infections were detected. In these two cases there was equivocal evidence to consider that the infecting *T. congolense* populations exhibited drug resistance. In the case of one of the *T. vivax* breakthrough infections, isometamidium concentration was approximately 3.2 ng/ml (eight times greater than the detection limit of 0.4 ng/ml) at the time the breakthrough infection was detected. For another three *T. vivax* infections, isometamidium concentrations in the host's sera were about three times greater than 0.4 ng/ml at the time the breakthrough infections were detected. In these four cases, the infecting *T. vivax* populations could be considered to exhibit some degree of drug resistance. A further two *T. vivax* breakthrough infections were detected when isometamidium concentrations were just above 0.4 ng/ml (Table 20). In these two cases there was equivocal evidence to consider the infecting *T. vivax* populations exhibited drug resistance.

In Matuga, during this prophylactic period, a total of eleven breakthrough infections were detected. Three *T. congolense* and two *T. vivax* breakthrough infections were detected when isometamidium concentrations were just above 0.4

ng/ml. In these five cases there was equivocal evidence to consider that the infecting trypanosome populations exhibited drug resistance. In another four cases, of two *T. congolense* and two *T. vivax* infections, isometamidium concentrations were above 1.0 ng/ml at the time of detection of these infections. These four cases were considered to exhibit a degree of drug resistance.

5.3.1.7.2 Period 2: February – May 1996

In Kubo during this prophylactic period, seven *T. vivax* breakthrough infections were detected when isometamidium concentrations were above 2.0 ng/ml (Table 20). These infections were considered to exhibit some degree of drug resistance. In another five *T. vivax* and two *T. congolense* breakthrough infections, isometamidium concentrations were just above the 0.4 ng/ml level. In these seven cases there was equivocal evidence to suggest that the infecting *T. vivax* populations exhibited drug resistance. Similarly in three cases of breakthrough infections (one *T. vivax* and two *T. congolense*), extrapolation of isometamidium concentrations determined a week prior to detection of the breakthrough infections suggested that the concentrations were just close to the 0.4 ng/ml level. In these last three cases, there was not sufficient evidence to consider that the infecting *T. vivax* and *T. congolense* populations exhibited drug resistance.

In Matuga, during this period, in one *T. vivax* breakthrough infection, isometamidium concentration was at least seven times greater than 0.4 ng/ml at the time the breakthrough infection was detected. In other two *T. vivax* breakthrough infections, isometamidium concentration was about 1.0 ng/ml at the time the breakthrough infection was detected. In these three cases, the infecting *T. vivax* populations were considered to exhibit a degree of drug resistance. In one *T. congolense* breakthrough infection, isometamidium concentration was two times greater than 0.4 ng/ml at the time the infection was detected (Table 20). In this case, the infecting *T. congolense* population was considered to exhibit some degree of drug resistance. In 6 further cases of breakthrough infection (one *T. vivax* and five *T. congolense*) isometamidium concentrations had fallen below the 0.4 ng/ml level by the time of breakthrough. In these cases there was insufficient evidence that the infecting trypanosome populations expressed resistance to the drug.

5.3.1.7.3 Period 3: May – August 1996

In Kubo, during this prophylactic period, in three breakthrough infections (two *T. congolense* and one *T. vivax*), isometamidium concentrations were just about 1.0 ng/ml at the time the infections were detected. In these cases the infecting trypanosome populations were considered to exhibit some degree of resistance. In five *T. congolense* breakthrough infections, isometamidium concentrations were just above the 0.4 ng/ml level (Table 20). In these cases there was not sufficient evidence to suggest that the infecting *T. congolense* population exhibited some degree of drug resistance. In two last cases of *T. congolense* breakthrough infections, isometamidium concentrations had fallen below the 0.4 ng/ml level. In these cases therefore there was no evidence that the infecting *T. congolense* populations expressed resistance to the drug

In Matuga, in two cases of *T. congolense* breakthrough infections, isometamidium concentrations were at least three times greater than the 0.4 ng/ml level. In another two cases of *T. vivax* and *T. congolense* breakthrough infections respectively, isometamidium concentrations were at least two times greater than 0.4 ng/ml level. In these four cases, the infecting trypanosome populations were considered to express some degree of drug resistance. In a further four breakthrough infections (three *T. congolense* and one *T. vivax*), isometamidium concentrations were just above the 0.4 ng/ml levels. In another two cases of *T. congolense* breakthrough infections, extrapolation of isometamidium concentration determined one week prior to the detection of the breakthrough infections, suggested that the concentrations were just above 0.4 ng/ml level. In these five cases there was not sufficient evidence to consider the infecting trypanosome populations as exhibiting drug resistance. In the last five cases of breakthrough infections (four *T. congolense* and one *T. vivax*) determination and extrapolation of isometamidium concentrations suggested the concentrations at the time of detection of these infection had fallen below the 0.4 ng/ml levels. In these last cases there was no evidence that the infecting trypanosome populations exhibited resistance to the drug.

Table 20: Isometamidium concentrations in dairy cattle at the time of detection of breakthrough trypanosome infections at Kubo and Matuga.

		T. congolense		*T. vivax*	
Site	**Prophylactic period**	**n[1]**	**> 0.4 ng/ml[2]**	**n**	**> 0.4 ng/ml**
Kubo	Nov. 1995- Feb. 1996	2	0	6	4
	February-May 1996	4	0	13	7
	May- August 1996	8	2	1	1
	Sub-Total	14	2	20	12
Matuga	Nov 1995- Feb 1996	5	1	6	4
	February-May 1996	6	1	4	3
	May- August 1996	13	3	3	1
	Sub-Total	24	5	13	8
	Grand Total	38	7	33	20

[1]Number of trypanosome infections

[2]Number of trypanosome infections isolated from cattle in which isometamidium concentrations were above 0.4 ng/ml

5.3.2 North coast

During the prophylactic period at the four sites on the north of Mombasa no trypanosome infections were detected in all the animals over the 12 weeks of the study. A tsetse survey by the technical team of the Kenya Trypanosomiasis Research Institute (KETRI) showed that the tsetse challenge in the area was very low. Furthermore, in most of the farms in the study pour-on insecticide was being used on calves that were not in the study within the farms.

5.4 Discussion

In this study the efficacy of isometamidium prophylaxis in zero-grazed cattle at the Kenya coast south of Mombasa was assessed. Factors that would influence isometamidium prophylaxis failure in zero-grazed dairy cattle at the same area were in addition investigated.

Trypanosome infections were detected in cattle in both study sites during all the three prophylactic periods. These were *T. congolense* and *T. vivax.* No other trypanosome species was detected. This is contrary to earlier observation where *T. brucei* was detected in dairy cattle in the same area under prophylaxis (Maloo, 1993) and in the longitudinal studies in Kwale (Chapter 6).

At both sites during every prophylactic period between 30% and 50% of cattle had acquired an infection within the first eight weeks following isometamidium prophylaxis. This is evidence of prophylaxis failure which contrasts a successful prophylaxis situation such as in Galana Ranch during 1994/95 where only 6% of cattle under prophylaxis were infected over the same period compared to 88% of sentinel cattle (Chapter 4).

In the present study, there were no sentinel cattle to monitor trypanosome challenge, however, the present study area is a known trypanosomiasis endemic area (Snow, 1979; Snow *et al,* 1988). Thus the high proportion of breakthrough infections observed is a typical example of isometamidium prophylaxis failure under high trypanosome challenge (Whiteside, 1962).

Similar occurrence of prophylaxis failure has been observed in Kwale (Maloo, 1993). Cases of early breakthrough infections in dairy cattle were reported by field extension offices which were attributed to the development of drug resistance (Maloo, 1993).

In the present study isometamidium could be detected in all sera examined from treated cattle in the two study sites (Kubo and Matuga) and there was no evidence that breakthrough trypanosome infection were associated with low concentration of drug. This suggests that most of the breakthrough infections occurred when circulating

isometamidium concentrations were at the inhibitive level for sensitive trypanosome (Eisler *et al*, 1997a). Similar findings were observed in the longitudinal studies on Galana Ranch (Chapter 4).

The trypanosome species responsible for early breakthrough infections differed in the two sites. During all three prophylactic periods early breakthrough infections in one site (Kubo) were *T. vivax*, whereas in the other study site (Matuga) early breakthrough infections were *T. congolense*.

The observation in Kubo is similar to those reported previously in Kwale (Gaturaga *et al*,1990) and Galana Ranch (Dolan *et al*,1992) and during the longitudinal studies in 1994/95 on Galana Ranch (Chapter 5), where more than 97 % of breakthrough infections were *T. vivax*. In contrast, the preferential *T. congolense* breakthrough infections observed in Matuga may probably be explained by the observations in the present study that in the three prophylactic periods the earliest breakthrough infections in both sites were detected repeatedly in the same animals.

In Matuga three animals had repeated *T. congolense* infections. Two of the animals were in the same zero-grazing unit. The three animals had very poor body condition and severe anaemia (PCV < 20 %) during the study period. The chronic *T. congolense* infection in these cattle was not curable by diminazene aceturate at 7.0 mg/kg b.w., but temporary remissions were observed on some occasions after diminazene aceturate treatment. One of these animals died 18 days after isometamidium treatment during the second prophylaxis period (there were no detectable trypanosomes) having excessive liver damage. Probably due to toxicity as a result of frequent treatment of the chronic *T. congolense* infections with trypanocidal drugs (Eisler *et al*, 1997b).

Three early *T. vivax* and one *T. congolense* breakthrough infections following isometamidium prophylaxis in four different animals were cured without further treatment. Similar observations were reported in cattle experimental infected with *T. vivax* isolates from Likoni at the Coast of Kenya (Wellde *et al*, 1989). Boran cattle infected with *T. vivax* isolates self cured the infections without treatment and thereafter remained immune to further tsetse fly challenge with homologous trypanosome stock for long periods (Wellde *et al*, 1989). In the present study, similarly, the four animals that were self cured were immune to further trypanosome challenge for the entire study period.

Eight of the 28 cattle under prophylaxis in the two sites were never infected during the 3 prophylactic periods. All were Sahiwal crosses. In addition, these animals belonged to zero grazing units in which other animals had acquired breakthrough infections. There is evidence suggesting that the Bos *indicus* breed of cattle in East Africa acquire some degree of resistance to trypanosomiasis when maintained under chemotherapy (Wilson *et al*, 1976; Murray *et al*, 1982). Resistance of Sahiwal crosses to trypanosome infection was observed in Kilifi District (Paling *et al*, 1987). Murray *et al*, (1982) presented evidence of genetic resistance in the Sahiwal cross bred cattle on Kilifi Plantation. In the present study the Sahiwal cross-breed were among the dairy cattle obtained from Kilifi Plantation by the NDDP zero-grazing programme in Kwale. Thus the above findings provide further evidence of genetic resistance in the Sahiwal cross breeds in an area of relatively higher trypanosome challenge.

During the 3 prophylactic periods similar proportions of *T. congolense* (39.4%) and *T. vivax* (30.3%) breakthrough infections were detected in host cattle with PCV values below 23%.. All PCV values below 23 % were observed in cattle with recurrent infections. This may be an indication of the degree of pathogenicity of these recurrent and or relapse infections.

In the present study, 9 of the *T. congolense* breakthrough infections that were detected in cattle with PCV values below 23% expressed multiple resistance to isometamidium chloride and diminazene aceturate in mice at 10mg/kg b.w. and 40mg/kg b.w., respectively (Chapter 7). The only *T. vivax* breakthrough infection detected in cattle with PCV values below 23% that was tested for drug sensitivity in cattle expressed resistance to isometamidium at 0.5mg/kg b.w and diminazene aceturate at 3.5 mg/kg (Chapter 7). Although the pathogenicity of drug resistant trypanosomes still remains a controversial issue (Geerts and Holmes, 1998), it is evident in the present study that the infecting *T. congolense* populations that expressed multiple resistance in mice caused severe anaemia in host cattle.

Finally, during the 3 prophylactic periods, the significantly higher proportion of *T. vivax* breakthrough infections than *T. congolense* breakthrough infections detected in cattle with isometamidium concentrations above 0.4 mg/ml, indicates that a larger proportion of the *T. vivax* population than *T. congolense* in the two sites exhibit some degree of drug resistance. Similar findings were observed in the longitudinal studies at

Galana Ranch (Chapter 4). In the present study, these findings reflect the higher proportion of *T. vivax* than *T. congolense* breakthrough infection that were previously observed in dairy cattle in Kwale District (Gaturaga, *et al.,* 1990).

In conclusion, there was evidence of prophylaxis failure. However, it may not be possible to fully determine the efficacy of isometamidium prophylaxis with the absence of sentinel cattle to monitor trypanosome challenge.

Several factors were identified that appeared to influence isometamidium prophylaxis failure. These include drug resistance, genetic susceptibility or resistance of cattle breed, nutritional status of the animal and individual animal variation.

Finally, It is recommended that the Sahiwal crossbreed, which appeared more resistant to infection, should be made available to dairy farmers in trypanosome endemic areas.

Chapter 6

Longitudinal studies to assess influence of trypanocidal drug resistance on efficacy of chemoprophylaxis and chemotherapy in control of bovine trypanosomiasis in Kwale District

6 Longitudinal studies in Kwale District

6.1 Introduction

6.1.1 Overview

This chapter describes a series of longitudinal studies carried out in Kwale District of Coast Province in Kenya. These studies involved monitoring cattle under natural tsetse and trypanosomiasis challenge. The performance of cattle under a trypanocidal prophylaxis regimen was investigated and a comparison made with cattle without prophylactic protection in the same areas. In addition, a comparison was made between administration of trypanocides by qualified and unqualified persons. Four study sites were selected that are located in the Msambweni Division of Kwale District (Figure 21). The sites were within the coastal plain and foot plateau, two of the four major topographical zones of the Coast Province. In each site, cattle were randomly assigned to one of two groups. One group was block-treated with isometamidium chloride while the other group was not. Cattle in either group were treated with diminazene aceturate when trypanosome infections and associated clinical signs were detected. Three of the study sites were farms owned by individuals or a company. The other study site comprised a group of individual farmers each contributing a number of cattle, ranging from 2-8 animals. The animals, mainly of the local Zebu breed and crosses with exotic breeds were under free range grazing. Isometamidium was administered at the start of five prophylactic periods each of 3 months. These spanned a period of two years from February 1997 to October 1998. The first three prophylactic periods were carried out in 1997, and the last two were carried out in 1998. There was a break from mid September 1997 to the end of March 1998 due to the ethnic tension compounded with flooding caused by unusually high rainfall, which made the study areas inaccessible.

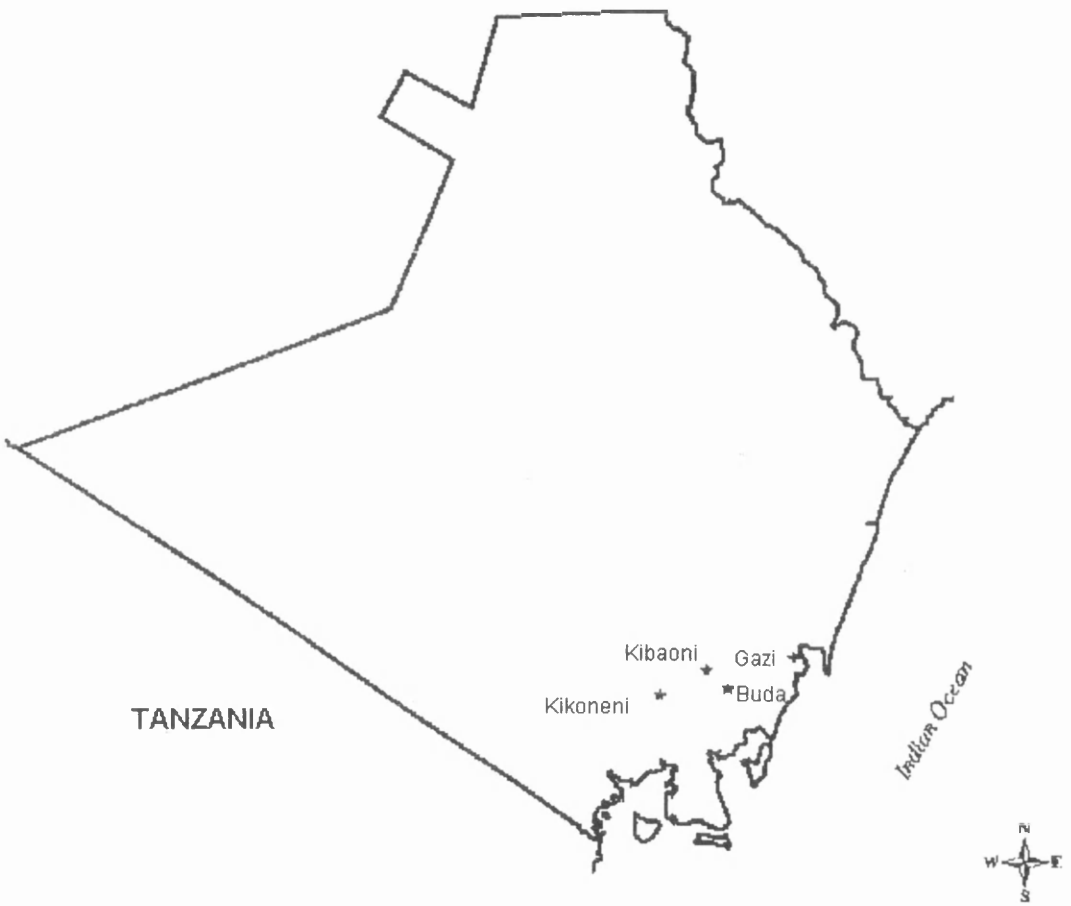


Figure 21: The map of Kwale District showing the location of the study sites

6.1.2 Tsetse and trypanosomiasis in the area of study

Trypanosomiasis is one of the major, possibly the major, disease constraint to cattle rearing in this agro-ecological zone. The disease is responsible for major loss in productivity in cattle. Prevalence rates of 30 % for *T. vivax* have been observed (Mwongela *et al.*, 1981). Furthermore, in a dairy herd monitored weekly between 1988-1989 trypanosome infection rates ranging from 5 % to 54 % was observed. The highest infection rates in these cattle were observed after the onset of rains (Maloo, 1993).

The main tsetse species in this area are *G. pallidipes* and *G. brevipalpis* (Figure 22). Trypanosome infection rates in tsetse flies in this area range from 2 % to 10 % (Maloo, 1993). The proximity of traditional Kaya forests (see Chapter 1) to the study sites that appears to be the foci of the flies, ensures that cattle in these areas are infected through the year. These forests are inhabited by bush pigs, warthogs, bushbucks and baboons that act as reservoirs and a source of blood meals for the flies (Snow *et al.*, 1988). Studies on cattle grazing around these forests estimated that each cow received from *G. pallidipes* one infective inoculum of *T. congolense* and *T. vivax* every 5-5.8 days and 3.2-79.1 days respectively (Snow and Tarimo 1983).

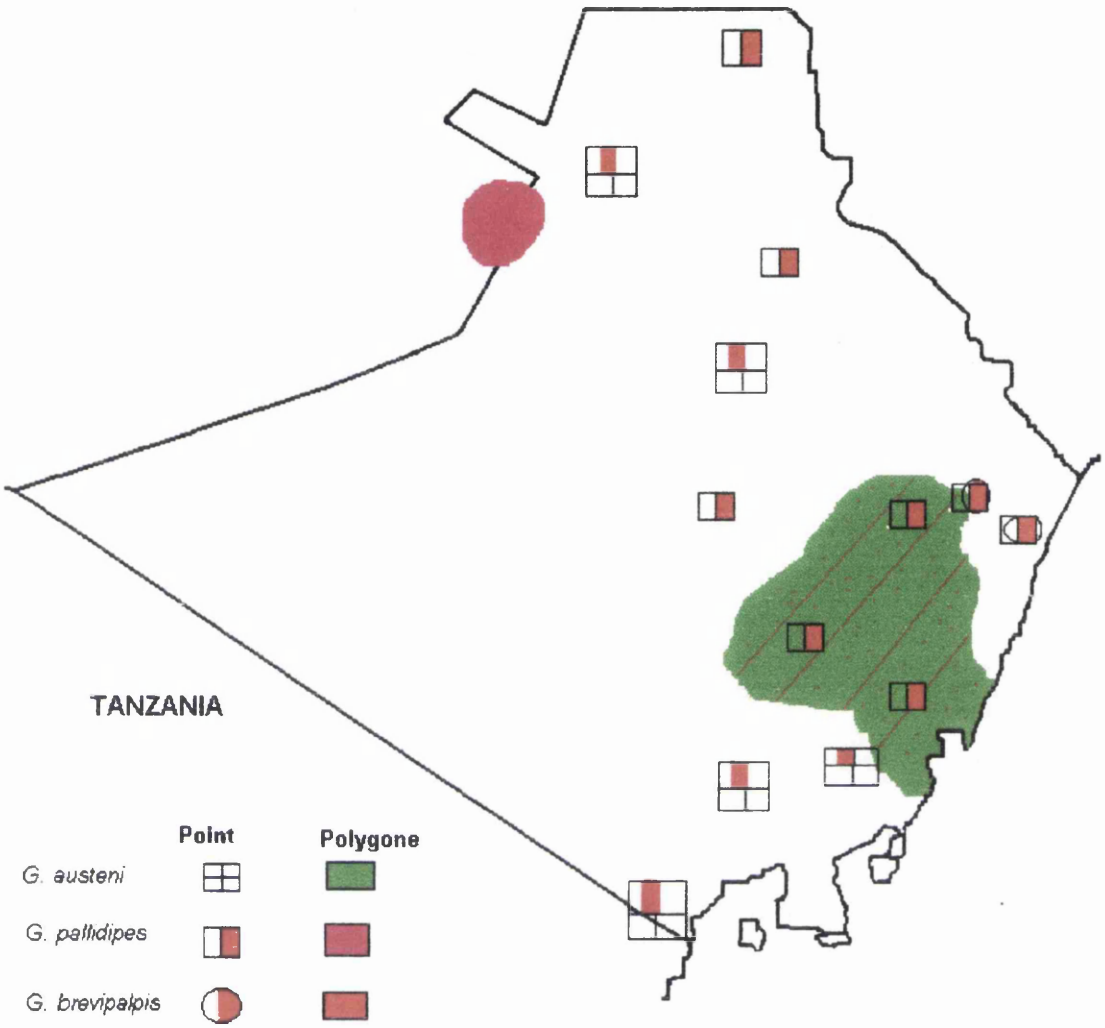


Figure 22: Map of Kwale District showing distribution of various tsetse fly species

From proceedings of the National Workshop to Revise the Tsetse Distribution Map of Kenya (1997)

6.1.3 Objectives

6.1.3.1 To establish how effective isometamidium prophylaxis is at controlling bovine trypanosomiasis in Kwale District.

6.1.3.2 To investigate variation in isometamidium efficacy in Kwale District with sites, type of cattle and trypanosome challenge in a trial period.

6.1.3.3 To investigate sources of variation of drug efficacy

- To assess the contribution of drug administration and drug resistance to drug efficacy
- To assess factors influencing drug administration and drug resistance for isometamidium and diminazene aceturate

6.1.3.4 To determine impact of prophylaxis failure in Kwale District in terms of animal health-related losses and treatment costs

6.2 Materials and methods

6.2.1 Longitudinal isometamidium prophylaxis study sites

Longitudinal isometamidium prophylaxis studies were conducted at four sites in Msambweni Division of Kwale District in Kenya's Coast Province.

Kwale District lies within the coastal lowlands (CL) zone group with mean annual temperatures higher than 24 °C and mean maximum temperature lower than 31 °C. The main crops of this zone group are cashewnuts and coconuts. The sites of the study lie in the agro-ecological zone CL3 with temperatures between 26.3 – 26.6 °C (Jaetzold and Schmidt 1983). The description of the sites and farming practices are shown in Table 21.

The locations of the four study sites Buda, Kibaoni, Kikoneni, and Gazi in Kwale District are shown in Figure 21. The sites are between 20 km to 70 km apart. Gazi lies within the coastal plain topographical zone by the ocean, while Buda, Kibaoni and Kikoneni are in the foot plateau between altitudes of 70-91m above sea level. These areas experience a monsoon-type climate, which is hot and dry from January to April/May. The rainfall is bimodal with the long rains usually starting around March/April and continuing until July. The short rains are in October and November. The annual precipitation is between 900-1500 mm (Kenya web, 1998).

Table 21: Longitudinal study sites in Msambweni Division, Kwale District, including description, location, cattle breed and farming practices

Site	Location	Latitude & Longitude	Owner	Type of cattle	Type of Farm	Agro-Ecological Zone
Buda	Bordering Buda forest, Kwale District, South coast	S04.46841, E039.40328	Dr. Gathua	Mixed crosses of zebu and exotic dairy cattle	Orchard and cattle rearing	Coconut-Cassava zone L3[1]
Gazi	At Gazi along Ukunda-Lungalunga highway, bordering Gogoni forest, Kwale District, South coast	S 04.42336, E 039..50092	Msambweni Development company	Local zebu dairy and beef cattle	Coconut plantation and cattle rearing	Coconut-Cassava zone L3[1]
Kibaoni	Mafisini location at Kibaoni, Kwale District, South coast	S 04.44326, E 039..37455	Individual farmers	Mixture of male and female local zebu cattle	Small scale subsistence	Coconut-Cassava zone L3[1]
Kikoneni	At Kikoneni, Kwale District	S 04.47616, E 039..31214	P. Kiambo	Mixed cross dairy and Boran/Zebu beef cattle	Dairy and beef production	Coconut-Cassava zone L3[1]

[1]Jaetzold and Schmidt 1983

6.2.1.1 Herd health practices including trypanocidal drug treatment history at longitudinal study sites

Herd health practices prior to the longitudinal isometamidium prophylaxis studies were ascertained by conducting key-informant interviews with the livestock owners or keepers at each study site.

6.2.1.1.1 Buda

Prior to the longitudinal isometamidium prophylaxis studies, lactating animals at Buda were routinely treated with isometamidium chloride (Samorin®) every three months in accordance with the manufacturers recommendation. Clinically affected animals were usually treated with diminazene aceturate (Veriben® or Berenil®) at a dose of one sachet dissolved in 15 ml boiled and cooled water for an adult animal and half the amount for a calf.

However, at the start of the longitudinal studies in February 1997, no animals had been treated with isometamidium in the previous six months. There had been a deficiency of isometamidium at the local pharmaceutical companies in Mombasa. Where the drug was available, it was sold at an exorbitant price which the farmer was not willing to pay.

6.2.1.1.2 Gazi

At Gazi, the whole herd was covered every three months with isometamidium chloride (Samorin®). The animals were de-wormed every three months and dipped in acaricide every two weeks. Animals showing clinical signs were treated with 10 mls of an antibiotic (tetracycline) and one sachet of diminazene aceturate (Veriben®, Berenil® or Norotryp®). The animal attendant, a qualified Livestock Animal Health Assistant carried out drug administration.

However, when the longitudinal studies started in April 1997, isometamidium treatment was one month overdue (lack of availability of Samorin®). In addition, animals had not been dipped for over two months, because of lack of water due to a drought since December 1996.

6.2.1.1.3 Kibaoni

The individual farmers treated their animals with the available trypanocides (diminazene aceturate, homidium chloride/bromide or isometamidium chloride) and antibiotics whenever clinical signs of dullness, licking of sand or swollen lymph nodes were observed. Treatment with homidium bromide (Ethidium®), homidium chloride (Novidium®), isometamidium chloride (Samorin®) or diminazene aceturate (Berenil®, Veriben® or Norotryp®) was administered as follows. One tablet of either Ethidium® or Novidium® would be dissolved in 10 mls measured using a syringe. The entire volume was given to an adult animal or half the volume to a calf. Alternatively, a pinch of Samorin® powder would be dissolved in 10 mls of water (pre-boiled) and administered to the sick animal by intramuscular injection. Finally, when diminazene was used, one sachet of either Berenil®, Veriben® or Norotryp® would be dissolved in 10 ml of water and the whole content given to an adult animal or half the content given to a calf. Treatment would be given preferentially to bulls, which are used for ploughing and carrying water from boreholes and rivers and are considered to be at greater risk of acquiring trypanosomiasis.

6.2.1.1.4 Kikoneni

Animals were treated with diminazene or homidium when clinically sick subject to availability of drugs. Isometamidium had been used on the farm about 5 years earlier, but its use had been discontinued because lack of availability and because the farmer believed that trypanosome challenge had decreased significantly.

6.2.1.2 Tsetse challenge at longitudinal study sites

6.2.1.2.1 Buda

The predominant tsetse species are *G. pallidipes and G. brevipalpis.* Because of the close proximity of the Buda forest the area was considered a medium to high challenge area according to the Veterinary Department, Kwale District.

6.2.1.2.2 Gazi

The farm is a coconut plantation with intense undergrowth of *Lantana camara* bushes. The Gogoni forest lies to the west of the farm. The area is considered a moderately high challenge area by the Veterinary Department, Kwale District. The predominant tsetse species are *G. pallidipes, G. brevipalpis* and *G. austeni*. However, tsetse and trypanosomiasis challenge decrease towards the sea that borders the farm to the east.

6.2.1.2.3 Kibaoni

The area borders Buda forest on the eastern side. It is considered a moderately high challenge area. The predominant tsetse species are *G. pallidipes* and *G. brevipalpis*

6.2.1.2.4 Kikoneni

According to the local Veterinary Department offices in Kwale District tsetse and trypanosomiasis challenge in the area is considered to be moderate. The predominant tsetse species is *G. pallidipes*. The farmer has reduced the challenge by systematic clearing of the forest that previously occupied the farm leaving a few scattered bushes. In the south the farm bordered grassland area that had previously been part of the Ramisi sugar plantation.

6.2.2 Study design

In selection of the experimental cattle, if one sex was predominant in an area , the animals were all selected from this sex. For example at Buda and Kikoneni where the majority of herd were dairy cattle, no bull was included in the experiment. However in the other two sites where there was no particular sex dominating, both bulls and cows were included the experiment. All cattle selected were above two years old. Animals in each site were divided in two groups, the isometamidium-treated and sentinel. The isometamidium-treated cattle were given block treatment (see Chapter 3, Section 3.1) according to the number of prophylactic periods as shown in Table 22. The numbers of cattle in each group at each site is shown in

Table 23. The treatment protocol for the two groups in each site was as shown in Table 24

The cattle in each group were monitored weekly for trypanosome infections and determination of packed cell volumes. The procedures used are described in Chapter 3 section 4. Blood samples for serum and trypanosome stabilate preparation were collected weekly as indicated in sections 5 and 7 respectively. Body weights and condition scores were determined monthly as described in Chapter 3 section 6.

Isometamidium concentrations in serum samples were determined using the isometamidium-ELISA as described in Chapter 3, section 9.

Table 22: Isometamidium prophylaxis studies at sites in Kwale District

Study no.	**1**	**2**	**3**	**4**	**5**
Period	Feb.-May 1997	May-Aug 1997	Aug.-Sept 1997	April-Jul 1998	July-Oct. 1998
Site:					
Buda	+[1]	+	+	+	+
Gazi	-[2]	+	+	+	+
Kibaoni	-	+	+	+	+
Kikoneni	+	+	+	-	-

[1] + indicates that studies were conducted at site during the period

[2] - indicates that studies were not conducted at site during the period

Table 23: Number of cattle in each drug treatment group at each site during the longitudinal studies in Kwale District

Site	Isometamidium group	Sentinel group
Buda	30	30
Gazi	40	40
Kibaoni	40	40
Kikoneni	30	30

Table 24: Trypanocidal drug treatments during the longitudinal studies in Kwale District, including dose rates, treatment criteria and the person administering the drugs

Site	Group	Isometamidium dose	Diminazene dose	Who treats	Treatment Criteria
Buda	ISMM	1 mg/kg body wt.	7 mg/kg body wt.	VO/ RO	+ve cattle treated with diminazene when PCV< 22% or clinically sick
	sentinel	Not treated	7 mg/kg body wt.	VO/ RO	Treated with diminazene when trypanosomes detected
Gazi	ISMM	According to normal practice ≈ 0.5 mg/kg body wt.	According to normal practice ≈ < 3.5 mg/kg b.w.	LAHA	+ve cattle treated with diminazene when PCV< 22% or clinically sick during 1st and 2nd prophylactic periods,
	sentinel	Not treated	According to normal practice ≈ 3.5 mg/kg b.w.	LAHA	Treated with diminazene when trypanosomes detected
Kibaoni	ISMM	According to normal practice ≈ 0.5 mg/kg body wt	According to normal practice ≈ 0.5-1 mg/kg b.w.	2 selected farmers	Treated with diminazene when trypanosomes detected
	sentinel	Not treated	According to normal practice ≈ 3.5-7 mg/kg b.w.	2 selected farmers	Treated with diminazene when trypanosomes detected
Kikoneni	ISMM	1 mg/kg body wt.	1 mg/kg b.w.	VO/ RO	+ve cattle treated with diminazene when PCV< 22% or clinically sick
	Sentinel	Not treated	7 mg/kg b.w.	VO/ RO	Treated with diminazene when trypanosomes detected

VO: Veterinary Officer

RO: Research Officer

LAHA: Livestock Animal Health Officer

ISMM: isometamidium

6.2.3 Data Analysis

6.2.3.1 Survival analysis of parasitological data

Survival analysis of the trypanosome infection data was carried out using the life-table technique as described in Chapter 3 section 10.1.2.

6.2.3.2 Packed cell volume data

Descriptive statistics of packed cell volume in each group were calculated using Microsoft Excel 97 software (Microsoft Corporation) and graphs of variation of mean packed cell volumes with days post treatment in each group and year of study plotted.

Mixed modelling to determine the effect of groups within year and baseline hazard on packed cell volumes over the prophylaxis period was done using SAS/STAT software system (SAS Institute inc., Cary, North Carolina USA)

6.2.3.3 Body weights and condition score data

Descriptive statistics on the weight gain in isometamidium treated and sentinel cattle over the prophylaxis period were calculated using Microsoft Excel 97 software (Microsoft Corporation) and graphs of variation of body weights with time in days plotted.

6.2.3.4 Serum isometamidium concentration data

Descriptive analysis of isometamidium data was performed as explained in Chapter 3, section10.3. In addition factors influencing isometamidium levels were determined by fitting the data in a linear regression model (see Chapter 3 section 10.3)

6.2.3.5 Assessment of efficacy of isometamidium prophylaxis

The measurements relating to assessment of efficacy of isometamidium were based on the proportion of cattle in the isometamidium group that were infected by

the eighth week of the prophylactic period (see Chapter 3. section 10.2). Ratios of mean hazard function over eight weeks of the prophylactic period of sentinel cattle to isometamidium treated cattle were calculated based on life- tables (see Chapter 3. section 10.2).

6.3 Results

6.3.1 Trypanosome infection prevalence at sites in Kwale District prior to the longitudinal studies

The trypanosome infection prevalence in cattle at the individual site prior to the longitudinal studies in 1997 and 1998 are shown in Table 25. The only trypanosome species detected prior to these studies were *T. congolense* and *T. vivax*.

In 1997, the overall mean prevalence of trypanosome infections at the four sites (Buda, Kibaoni, Kikoneni, and Gazi) was 12.5%. Prevalence at individual study sites varied between 1.7% at Kikoneni to 25.9% at Gazi. *Trypanosoma vivax* was the predominant trypanosome species accounting for 60% of infections, the remaining 40% being *T. congolense*.

In 1998, the overall mean prevalence at the three sites remaining in the study (Buda, Kibaoni, and Gazi) was 28.2%. This represented an almost two-fold increase over the equivalent 1997 figure of 15.5% calculated excluding the low prevalence site Kikoneni. At two sites, Buda and Kibaoni, prevalence increased strikingly (3.5 and 4.5 fold respectively) between 1997 and 1998, whereas at Gazi prevalence fell by half over the same period. The trypanosome species proportion also changed considerably in 1998. *Trypanosoma congolense* accounted for 84% of infections the remaining 16% being *T. vivax*

Table 25: Prevalence of trypanosome infections at the study sites prior to longitudinal studies in 1997 and 1998 in Kwale District

Site	Year	No. of cattle	No. of Infections	Prevalence (%)	Tc[1]	Tv[2]	Tc %[3]	Tv %
Buda	1997	59	7	11.9	5	2	8.5	3.4
Gazi		81	21	25.9	5	16	6.2	19.8
Kibaoni		80	6	7.5	4	2	5.0	2.5
Kikoneni		60	1	1.7	0	1	0.0	1.7
Overall		280	35	12.5%	14	21	5.0	7.5
Buda	1998	59	25	42.4	24	1	40.7	1.7
Gazi		81	10	12.3	7	3	8.6	3.7
Kibaoni		80	27	33.8	21	6	26.3	7.5
Overall		220	62	28.2%	52	10	23.6	4.5

[1]Tc: *Trypanosoma congolense*

[2]Tv: *Trypanosoma vivax*

[3]%: percentage of samples with *T. congolense* or *T. vivax*

6.3.2 *Trypanosome infections during isometamidium block treatment studies*

Trypanosome infections detected during the longitudinal study Periods 1 to 3 in 1997 are summarised in Table 26. Trypanosome infections detected during the longitudinal study periods 4 and 5 in 1998 are summarised in Table 27.

6.3.2.1 *Period 1: February – May 1997*

During Period 1, studies were conducted at two sites, Buda and Kikoneni.

6.3.2.1.1 *Sentinel cattle*

Thirty-eight trypanosome infections were detected in seven hundred samples from 59 sentinel cattle at the two study sites. The overall infection rate in these samples was 5.0 %. 71.1% of infections were *T. congolense*, 21.1 % were *T. vivax* and the remaining 7.8 % were *T. theileri.*

6.3.2.1.2 *Isometamidium-treated cattle*

Eighteen trypanosome infections were detected in seven hundred and eight samples from 60 isometamidium-treated cattle at the two study sites. The overall infection rate in these samples was 15.6 %. Of the infections 38.9 % were *T. congolense*, 50.0 % were *T. vivax*, 5.6 % were *T. brucei* and the remaining 5.5 % were *T. theileri.*

6.3.2.2 *Period 2: May – August 1997*

During Period 2, studies were conducted at all four sites, Buda, Kibaoni, Kikoneni, and Gazi.

6.3.2.2.1 *Sentinel cattle*

Two hundred and eighty three trypanosome infections were detected in 1,675 samples from 140 sentinel cattle at the four study sites. The overall infection rate in

these samples was 16.9 %. Of the infections 54.1 % were *T. congolense*, 41.3 % were *T. vivax*, 1.4 % were *T. brucei* and the remaining 3.2 % were *T. theileri*

6.3.2.2.2 Isometamidium-treated cattle

One hundred and eighty three trypanosome infections were detected in 1,698 samples from 140 isometamidium-treated cattle at the four study sites. The overall infection rate in these samples was 10.8 %. Of the infections 35.5 % were *T. congolense*, 61.7 % were *T. vivax*, 1.1 % were *T. brucei* and the remaining 1.7 % were *T. theileri*

6.3.2.3 Period 3: August – September 1997

During Period 3, studies were conducted at all four sites, Buda, Kibaoni, Kikoneni, and Gazi.

6.3.2.3.1 Sentinel cattle

One hundred and ten trypanosome infections were detected in four hundred and ninety six samples from 140 sentinel cattle at the four study sites. The overall infection rate in these samples was 22.2 %. Of the infections, 67.3 % were *T. congolense*, 29.1% were *T. vivax* and the remaining 3.6 % were *T. theileri*

6.3.2.3.2 Isometamidium-treated cattle

Sixty three trypanosome infections were detected in four hundred and eighty five samples from 140 isometamidium-treated cattle at the four study sites. The overall infection rate in these samples was 13.0 %. Of the infections, 66.7 % were *T. congolense*, 31.7% were *T. vivax* and the remaining 1.6 % were *T. brucei*

6.3.2.4 Period 4: April – July 1998

During Period 4, studies were conducted at three sites, Buda, Kibaoni, and Gazi.

6.3.2.4.1 Sentinel cattle

Two hundred and sixty four trypanosome infections were detected in 1,380 samples from 110 sentinel cattle at the three study sites. The overall infection rate in these samples was 19.1%. Of the infections, 87.9 % were *T. congolense*, 9.9 % were *T. vivax,* 1.5 % were *T. brucei* and the remaining 1.7 % were *T. theileri*

6.3.2.4.2 Isometamidium-treated cattle

One hundred and fifty three trypanosome infections were detected in 1333 samples from 110 isometamidium-treated cattle at the three study sites. The overall infection rate in these samples was 11.5%. Of the infections, 92.8 % were *T. congolense*, 6.5 % were *T. vivax* and the remaining 0.7 % were *T. brucei*

6.3.2.5 Period 5: July – October 1998

During Period 5, studies were conducted at three sites, Buda, Kibaoni, and Gazi.

6.3.2.5.1 Sentinel cattle

Two hundred and fifty one trypanosome infections were detected in 1,379 samples from 110 sentinel cattle at the three study sites. The overall infection rate in these samples was 18.2 %. Of the infections, 82.9 % were *T. congolense*, 15.1 % were *T. vivax,* 0.4 % were *T. brucei* and the remaining 1.6 % were *T. theileri*

6.3.2.5.2 Isometamidium-treated cattle

One hundred and twenty eight trypanosome infections were detected in 1317 samples from 100 isometamidium-treated cattle at the three study sites. The overall infection rate in these samples was 9.7%. Of the infections, 89.8 % were *T. congolense*, 7.8 % were *T. vivax,* 0.8 % were *T. brucei* and the remaining 1.6 % were *T. theileri*

Table 26: Summary of trypanosome infections detected during the longitudinal studies in 1997 in Kwale District

Site	Group	Prophylactic period	No. of samples	Tc	Tv	Tb	Tt	%Tc	%Tv	%Tb	%Tt
Buda	ISMM	1	359	6	6	0	0	1.7	1.7	0.0	0.0
	Sentinel	1	358	27	6	0	0	7.5	1.7	0.0	0.0
Kikoneni	ISMM	1	349	1	3	1	1	0.3	0.9	0.3	0.3
	Sentinel	1	342	0	2	0	3	0.0	0.6	0.0	0.9
Sub-total	ISMM	1	708	7	9	1	1	1.0	1.3	0.1	0.1
	Sentinel	1	700	27	8	0	3	3.9	1.1	0	0.4
Buda	ISMM	2	347	13	13	0	0	3.7	3.7	0.0	0.0
	Sentinel	2	352	38	21	1	0	10.8	6.0	0.3	0.0
Gazi	ISMM	2	555	26	46	0	0	4.7	8.3	0.0	0.0
	Sentinel	2	562	53	33	0	0	9.4	5.9	0.0	0.0
Kibaoni	ISMM	2	468	22	20	1	0	4.7	4.3	0.2	0.0
	Sentinel	2	433	50	21	2	0	11.5	4.8	0.5	0.0
Kikoneni	ISMM	2	328	4	34	1	3	1.2	10.4	0.3	0.9
	Sentinel	2	328	12	42	1	9	3.7	12.8	0.3	2.7
Sub-total	ISMM	2	1698	87	113	2	3	5.1	6.7	0.1	0.2
	Sentinel	2	1675	153	117	4	9	9.1	7.0	0.2	0.5
Buda	ISMM	3	79	2	2	0	0	2.5	2.5	0.0	0.0
	Sentinel	3	81	13	2	0	0	16.0	2.5	0.0	0.0
Gazi	ISMM	3	189	25	10	0	0	13.2	5.3	0.0	0.0
	Sentinel	3	185	29	17	0	0	15.7	9.2	0.0	0.0
Kibaoni	ISMM	3	161	11	3	1	0	6.8	1.9	0.6	0.0
	Sentinel	3	156	27	7	0	1	17.3	4.5	0.0	0.6
Kikoneni	ISMM	3	56	4	5	0	0	7.1	8.9	0.0	0.0
	Sentinel	3	74	5	7	0	2	6.8	9.5	0.0	2.7
Sub-total	ISMM	3	485	42	20	1	0	8.7	4.1	0.2	0.0
	Sentinel	3	496	74	33	0	3	14.9	6.7	0.0	0.6
Grand Total	ISMM	1997	2891	114	142	4	4	3.9	4.9	0.1	0.1
	Sentinel	1997	2871	254	158	4	15	8.9	5.5	0.1	0.5

Tc = *Trypanosoma congolense*

Tv = *Trypanosoma vivax*

Tb = *Trypanosoma brucei*

Tt = *Trypanosoma theileri*

ISMM = Isometamidium

Table 27: Summary of trypanosome infections detected during the longitudinal studies 1998 in Kwale District

Site	Group	Prophylactic period	No. of samples	Tc	Tv	Tb	Tt	%Tc	%Tv	%Tb	%Tt
Buda	ISMM	4	345	35	1	0	0	10.1	0.3	0.0	0.0
	Sentinel	4	365	68	1	3	1	18.6	0.3	0.8	0.3
Gazi	ISMM	4	507	37	5	1	0	7.3	1.0	0.2	0.0
	Sentinel	4	526	40	15	1	0	7.6	2.9	0.2	0.0
Kibaoni	ISMM	4	481	70	4	0	0	14.6	0.8	0.0	0.0
	Sentinel	4	489	124	10	0	1	25.4	2.0	0.0	0.2
Sub-total	ISMM	4	1333	142	10	1	0	10.7	0.8	0.1	0.0
	Sentinel	4	1380	232	26	4	1	16.8	1.9	0.3	0.1
Buda	ISMM	5	333	24	0	0	0	7.2	0.0	0.0	0.0
	Sentinel	5	353	54	4	1	1	15.3	1.1	0.3	0.3
Gazi	ISMM	5	507	37	6	0	0	7.3	1.2	0.0	0.0
	Sentinel	5	533	51	24	0	0	9.6	4.5	0.0	0.0
Kibaoni	ISMM	5	477	54	4	1	2	11.3	0.8	0.2	0.4
	Sentinel	5	493	103	10	0	3	20.9	2.0	0.0	0.6
Sub-total	ISMM	5	1317	115	10	1	2	8.7	1.0	0.1	0.2
	Sentinel	5	1379	208	38	1	4	15.1	2.8	0.1	0.4
Total	ISMM	1998	2650	257	20	2	2	9.7	0.8	0.1	0.1
	Sentinel	1998	2759	440	64	5	6	16.0	2.3	0.2	0.2

Tc = *Trypanosoma congolense*

Tv = *Trypanosoma vivax*

Tb = *Trypanosoma brucei*

Tt = *Trypanosoma theileri*

ISMM = Isometamidium

6.3.3 Survival analysis of isometamidium block treatment studies

6.3.3.1 Period 1

The plot of survivor function of the trypanosome infections in cattle in the isometamidium and sentinel groups during the first prophylactic period (Feb.- May 1997) in Kwale District is shown in Figure 23. In this period the studies were carried out in only two sites, Buda and Kikoneni. By the 8th week post-trial, approximately 27 % of the cattle in the isometamidium group at Buda had been infected (Table 28). By the same week, about 59 % of the cattle in the sentinel group had been infected. The differences in the proportions of the cattle infected between isometamidium and sentinel were significant different ($p< 0.05$). No infections were detected in the two groups at Kikoneni by the 8th week of the first prophylactic period.

6.3.3.2 Period 2

The plot of survivor function of the trypanosome infections in cattle in the isometamidium and sentinel groups during the second prophylactic period (May – August 1997) in Kwale District is shown in Figure 24. During this period the trial was carried out in four sites Buda, Gazi, Kibaoni and Kikoneni. In Buda, approximately 27 % of the cattle were infected by the 8th week of the second prophylactic period (Table 28). In the sentinel group at Buda a significantly higher ($p < 0.05$) proportion (74 %) of cattle were infected by the 8th week of the second prophylactic period.

During the second prophylactic period, approximately 36 % of the cattle in the isometamidium group at Kikoneni were infected by the 8th week post-treatment. In the sentinel group at Kikoneni, 75 % of the cattle were infected by the 8th week of the prophylactic period. The difference between the two groups was just significant ($p=0.054$).

In Gazi and Kibaoni, isometamidium treatment did not have a significant ($p > 0.05$) effect on the number of cattle infected during the second prophylactic period (Figure 24). Approximately 48 % and 71 % of cattle in the isometamidium and sentinel groups respectively were infected at Gazi by the 8th week of the second

prophylactic period. Similarly, in Kibaoni about 37 % and 54 % of the cattle in the isometamidium and sentinel groups respectively were infected by the 8th week of the second prophylactic period (Table 28).

6.3.3.3 Period 3

The plot of survivor function of the trypanosome infections in cattle in the isometamidium and sentinel groups during the third prophylactic period (August-Sept., 1997) in Kwale District is shown in Figure 25. In this period the cattle were followed up to maximum of 6 weeks. The measure of isometamidium efficacy was considered as the proportion of cattle infected by the 4th week of this prophylactic period. There were significant differences ($p < 0.01$) in the numbers of cattle that were infected by the 4th week between the isometamidium and sentinel groups at Buda, Kikoneni and Gazi (Table 28). In contrast, the proportions of cattle infected (18.8 % and 35.3 % respectively) in the isometamidium and sentinel groups at Kibaoni by the 4th week of the third prophylactic period were not significantly different ($p > 0.05$).

6.3.3.4 Period 4

The plot of survivor function of the trypanosome infections in cattle in the isometamidium and sentinel groups during the fourth prophylactic period (April - July 1998) in Kwale District is shown in Figure 26. During this prophylactic period, only three sites were included in the study, Buda, Gazi and Kibaoni. In all three sites (Buda, Gazi and Kibaoni) there were significant differences ($p< 0.001$, $p <0.05$ and $p < 0.001$ respectively) in the proportions of cattle infected between the isometamidium and the sentinel groups during the fourth prophylactic period. Approximately 30 %, 42 % and 53 % of the cattle in the isometamidium groups at Buda, Gazi and Kibaoni respectively were infected by the 8th week of the fourth prophylactic period (Table 28).

6.3.3.5 Period 5

The plot of survivor function of the trypanosome infections in cattle in the isometamidium and sentinel groups during the fifth prophylactic period (July - October 1998) in Kwale District is shown in Figure 27. In all the three sites there

were significant differences ($p < 0.01$) in the proportion of cattle infected between the isometamidium and the sentinel groups.

Survivor functions of trypanosome infections in the isometamidium groups were not significantly different ($p > 0.05$) between sites. However, during the fourth prophylactic period the proportion of cattle in the isometamidium group that acquired trypanosome infections by the 8^{th} week at Buda was significantly lower ($p < 0.001$) than the proportion of cattle infected at Gazi and Kibaoni (Table 28). In addition, at Buda, hazard ratios in all the five prophylactic periods were greater or equal to two (Table 29). Whereas, in Gazi and Kibaoni hazard ratios were greater or equal to two only during the 4^{th} and 5^{th} prophylactic periods (Table 29).

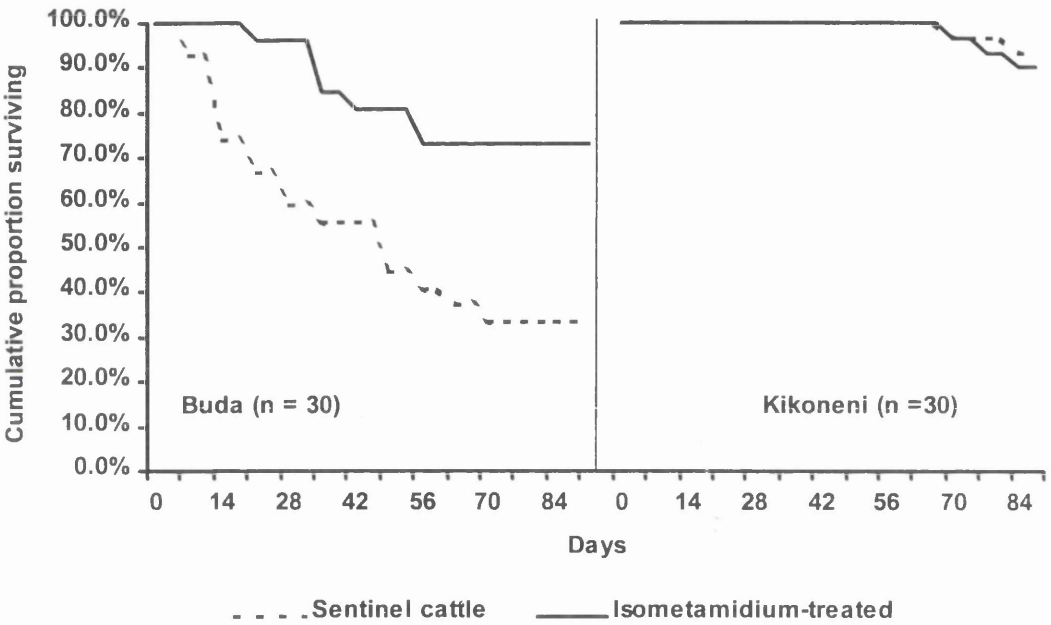
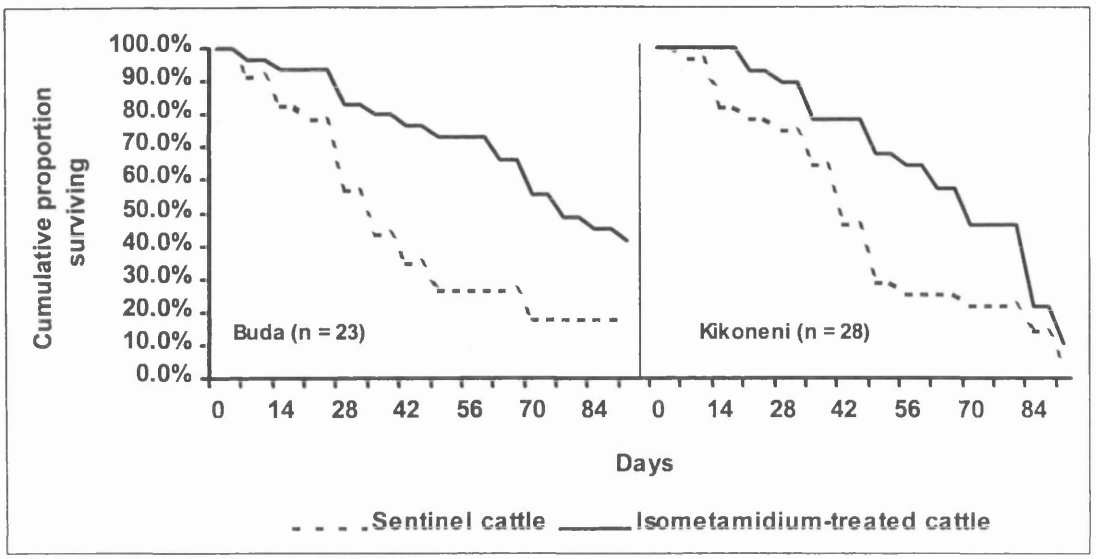


Figure 23: Cumulative proportion of isometamidium-treated and sentinel cattle surviving (i.e. remaining uninfected by trypanosomes) during the first prophylactic period (February - May, 1997) in Kwale District



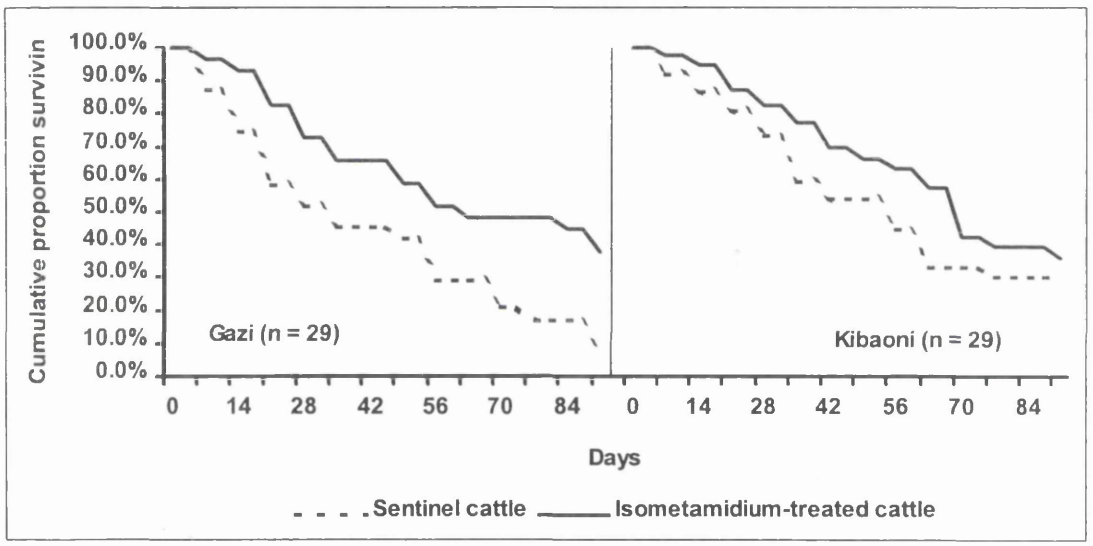
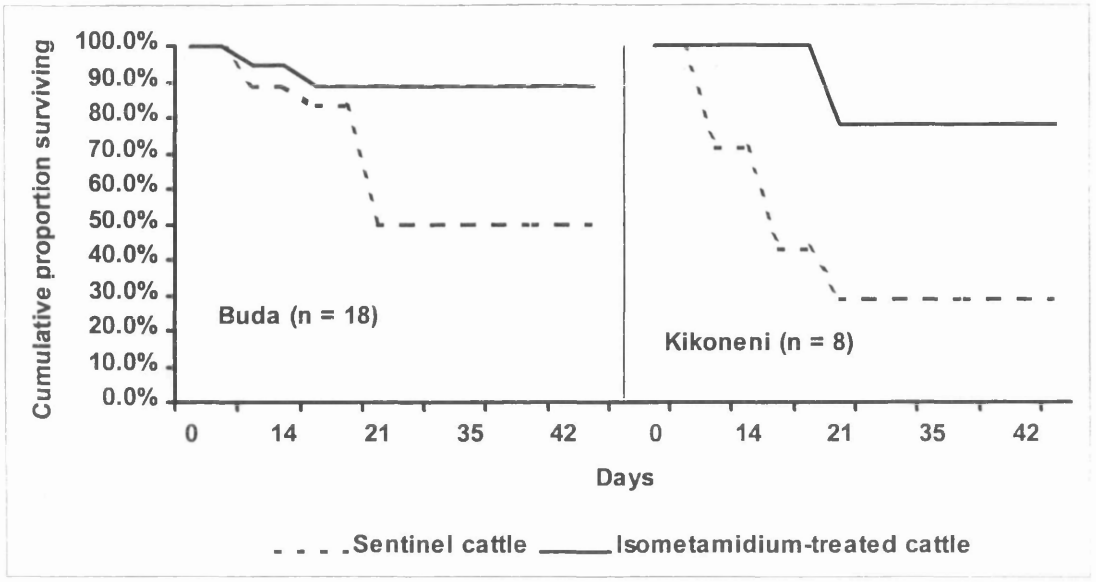


Figure 24: Cumulative proportion of isometamidium-treated and sentinel cattle surviving (remaining uninfected by trypanosomes) during the second prophylactic period (May - August, 1997) in Kwale District



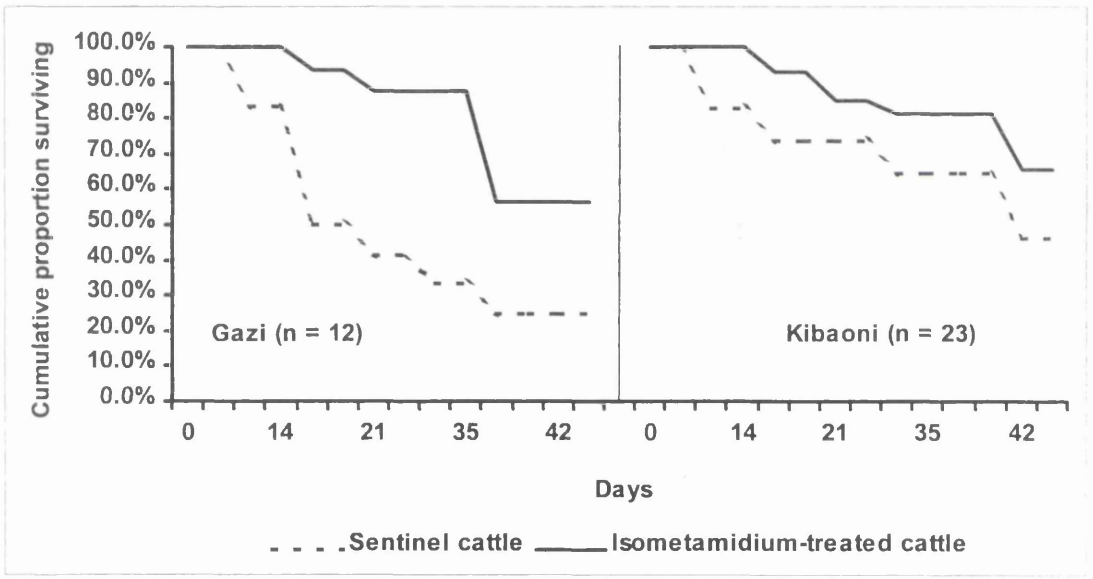


Figure 25: Cumulative proportion of isometamidium-treated and sentinel cattle surviving (remaining uninfected by trypanosomes) during the third prophylactic period (August - September, 1997) in Kwale District

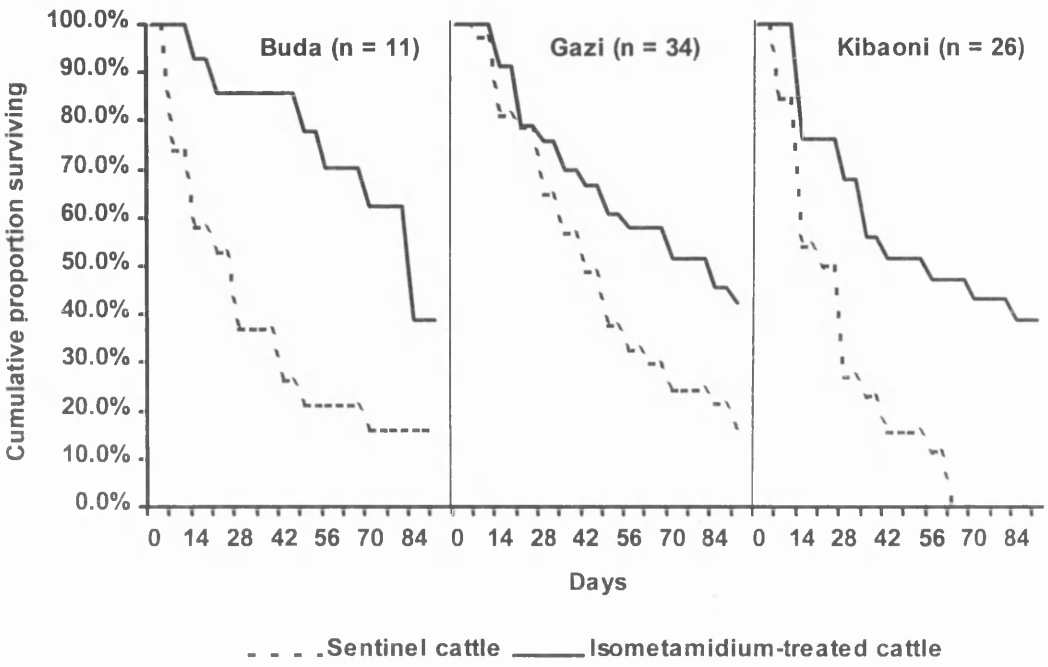


Figure 26: Cumulative proportion of isometamidium-treated and sentinel cattle surviving (remaining uninfected by trypanosomes) during the fourth prophylactic period (April - July, 1998) in Kwale District

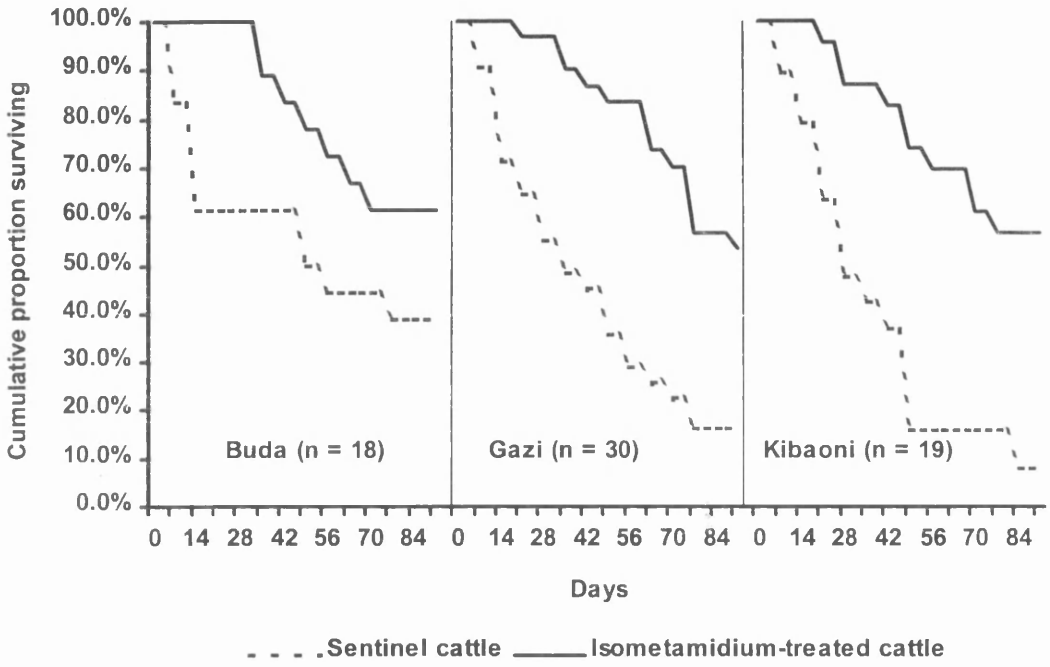


Figure 27: Cumulative proportion of isometamidium-treated and sentinel cattle surviving (remaining uninfected by trypanosomes) during the fourth prophylactic period (July - October, 1998) in Kwale District

Table 28: Proportion of cattle that were infected by the 8th week of the prophylactic period in the isometamidium and sentinel groups, during the longitudinal studies in Kwale District

		1997			1998	
		Prophylactic period:				
Site	Group	1	2	3	4	5
Buda	ISMM	26.9	27.1	11.1	29.9	27.8
	Sentinel	59.3	73.9	50	79	55.6
Kikoneni	ISMM	0	35.7	22.2	nd	nd
	Sentinel	0	75	88.1	nd	nd
Gazi	ISMM	nd[1]	48.3	12.5	42.3	16.7
	Sentinel	nd	71	66.7	67.6	71
Kibaoni	ISMM	nd	36.6	18.8	52.6	30.4
	Sentinel	nd	54.9	35.3	88.5	84.2

[1]nd studies not done

Table 29: Mean hazard functions and hazard ratios for isometamidium block treatment studies

		1997			1998	
		Prophylactic period:				
Site	**Group**	**1**	**2**	**3**	**4**	**5**
Buda	ISMM	0.037[1]	0.038	0.046	0.042	0.045
	Sentinel	0.104	0.149	0.143	0.169	0.091
Hazard Ratio[2]		2.8	3.9	3.1	4.0	2.0
Kikoneni	ISMM	0.000	0.057	0.044	nd[4]	nd
	Sentinel	0.000	0.161	0.208	nd	nd
Hazard Ratio		na[3]	2.8	4.7	nd	nd
Gazi	ISMM	nd	0.078	0.123	0.066	0.027
	Sentinel	nd	0.139	0.123	0.129	0.142
Hazard Ratio		nd	1.8	1.6	2.0	5.2
Kibaoni	ISMM	nd	0.061	0.061	0.089	0.066
	Sentinel	nd	0.099	0.111	0.222	0.185
Hazard Ratio		nd	1.6	1.8	2.5	2.8

[1]Mean hazard function over first 8 weeks of study period

[2]Ratio of sentinel group hazard function to isometamidium group hazard function

[2]na: not applicable

[1]nd: study not done

6.3.4 Health and productivity indices for isometamidium and sentinel groups

6.3.4.1 Packed cell volumes

6.3.4.1.1 The effect of isometamidium treatment on the packed cell volume

To determine the effect of isometamidium treatment, and trypanosome infections on packed cell volume, a mixed model was used to describe the PCV findings. The response variable was taken as the difference between packed cell volume at the beginning of the prophylaxis period and at the end. The percentage variance accounted for in this model was 18.0 %.

The model used was as follows:

$$Y_{ijk} = \mu + \delta_i + h_{ij} + \rho_{ijk} + e_{ijkl} \qquad (1)$$

Y_{ijl} is PCV_{diff}, the difference between packed cell volumes, PCV_0 before isometamidium treatment and packed cell volumes, PCV_1 at the end of the prophylaxis period given by; $PCV_{diff} = PCV_1 - PCV_0$

Where, for $i = 1,2; j = 1....4; k = 1,...,5; l = 1,....,n_{ijkl}$

μ is the overall mean

δ_i is the effect of isometamidium treatment

h_{ij} is the effect of site to which the isometamidium and sentinel groups are located

ρ_{ijl} is the effect of the prophylactic period

e_{ijkl} is the random error of animal l in group i at site j during prophylactic period k

The fixed effects parameters are δ_i, and ρ_{ijl}, whereas h_{ij}, and e_{ijklm} are random effects.

Changes in packed cell volumes from the beginning of the prophylaxis periods to the end were significantly different ($p = 0.014$) between isometamidium treated cattle and sentinel cattle . There were significant changes ($p < 0.001$) in packed cell volume between the five prophylactic periods. Within sites, changes in packed cell volumes were not significantly different ($p = 0.312$) between Buda, Kikoneni and Kibaoni. However, changes in packed cell volume at Gazi were significantly higher ($p = 0.008$) than changes in packed cell volumes at either Buda or Kikoneni.

6.3.4.1.2 The effect of baseline hazard on packed cell volume

To determine the overall effect of trypanosome infections on packed cell volumes, baseline hazard (the hazard rate for sentinel cattle calculated as described in Chapter 3, section 10.1 for each prophylaxis period) was included in the above Model 1 for packed cell volume change as an additional variable.

The baseline hazard lowered the changes in packed cell volumes of both groups significantly ($p < 0.001$). For a given baseline hazard, isometamidium treated cattle had a lesser reduction in packed cell changes. In addition the effect of hazard (infections) on packed cell volume changes in isometamidium treated cattle was significantly less ($p < 0.001$) than on packed cell changes in sentinel cattle. Using the baseline hazard as the explanatory variable in the model, in both isometamidium treated and sentinel cattle there was increase in packed cell volumes changes with increasing hazard.

6.3.4.1.3 Packed cell volumes at time of detection of trypanosome infections

The means of packed cell volumes of samples at detection of *T. congolense* and *T. vivax* infections are shown in Table 30.

During the first prophylaxis period, samples from isometamidium treated cattle with *T. congolense* (27.6 ± 1.38 %) infections had higher packed cell volumes than samples with *T. vivax* (24.8 ± 1.45%). However the difference was not significant. In sentinel cattle, samples with *T. vivax* had a significantly higher ($p < 0.01$) packed cell volumes than samples with *T. congolense*.

During the second prophylaxis period, in isometamidium-treated cattle there were no differences in packed cell volumes between samples with *T. congolense* (25.9 ± 0.54 %) and *T. vivax* (26.5 ± 0.43 %). However, in sentinel cattle samples with *T. vivax* (26.8 ± 0.42%) had significantly higher ($p< 0.01$) packed cell volumes than samples with *T. congolense* (23.8 ± 0.36 %). In both groups of cattle, samples with mixed infections (*T. congolense* and *T. vivax*) had lower packed cell volumes than samples with either *T. congolense* or *T. vivax* alone (Table 32).

During the third prophylaxis period, in isometamidium treated cattle, samples with *T. congolense* had significantly higher ($p<0.05$) packed cell volumes than samples with *T. vivax* infections. In addition, samples with no trypanosome infections from isometamidium treated cattle had significantly higher packed cell volumes than samples with *T. congolense* (28.1 ± 0.19 % and 25.2 % respectively) but similar to samples with *T. vivax* infections (28.1 ± 0.19 % and 27.3 ± 0.94 % respectively). In sentinel cattle, packed cell volumes of samples with either *T. congolense* or *T. vivax* were significantly lower than packed cell volumes of samples without trypanosomes. In addition, packed cell volumes of samples with *T. congolense* (24.4 ± 0.56 %) and *T. vivax* (25.2 ± 0.81 %) infections were similar. Samples with mixed infections from either group had significantly lower packed cell volumes than samples with either *T. congolense* or *T. vivax* alone (Table 32).

During the fourth prophylaxis period, samples without trypanosome infections (27.4 ± 0.12 %) from isometamidium treated cattle had significantly higher packed cell volumes than samples with *T. congolense* (24.8 ± 0.33 %) but, significantly lower packed cell volumes than samples with *T. vivax* (29.8 ± 1.46 %) infections. In sentinel cattle, samples without trypanosomes had significantly higher ($p <0.001$) packed cell volumes than samples with *T. congolense*, but had similar packed cell volume to samples with *T. vivax* infections. The difference in packed cell volumes between samples with *T. congolense* (24.2 ±0.26 %) and *T. vivax* (25.8 ± 0.79 %) was not significant ($p = 0.09$).

During the fifth prophylaxis period, packed cell volumes of samples without trypanosome infections from isometamidium treated samples were significantly higher ($p < 0.001$) than packed cell volumes of samples with either of the trypanosome species. Between species, packed cell volumes of samples from

isometamidium treated cattle were similar (26.5 ± 0.3 % for *T. congolense* and 26.7 ± 0.73 % for *T. vivax*). In sentinel cattle, samples without trypanosomes had significantly higher ($p<0.001$) packed cell volumes than samples with *T. congolense* but, not significant ($p =0.05$) to samples with *T. vivax*.

Overall isometamidium treatment did not have a significant effect ($p=0.17$) on packed cell volumes of samples with *T. vivax*. However, isometamidium treatment had a significant effect ($p < 0.0001$) on packed cell volumes of samples with *T. congolense* infections

Table 30: Mean packed cell volumes of samples from isometamidium treated and sentinel cattle at detection of *T. congolense* and *T. vivax* infections during the longitudinal studies

Prophylaxis period	Trypanosome species	Group	No. of samples	Mean PCV (%)	Standard error
1	No Infection	ISMM	672	29.3	0.17
		Sentinel	658	28.6	0.18
	T. congolense	ISMM	7	27.6	1.38
		Sentinel	27	23.9	0.76
	T. vivax	ISMM	9	24.8	1.45
		Sentinel	8	27.0	1.74
2	No infection	ISMM	1451	28.6	0.12
		Sentinel	1353	27.4	0.12
	Mixed	ISMM	1	22.0	
		Sentinel	6	23.3	2.36
	T. congolense	ISMM	64	25.9	0.54
		Sentinel	147	23.8	0.36
	T. vivax	ISMM	111	26.5	0.43
		Sentinel	111	26.8	0.42
3	No infection	ISMM	425	28.1	0.19
		Sentinel	390	27.1	0.23
	Mixed	ISMM	2	20.0	0.00
		Sentinel	2	22.5	1.50
	T. congolense	ISMM	40	25.2	0.59
		Sentinel	72	24.4	0.56
	T. vivax	ISMM	18	27.3	0.94
		Sentinel	31	25.2	0.81
4	No infection	ISMM	1181	27.4	0.12
		Sentinel	1122	26.7	0.12
	T. congolense	ISMM	142	24.8	0.33
		Sentinel	232	24.2	0.26
	T. vivax	ISMM	10	29.8	1.46
		Sentinel	26	25.8	0.79
5	No infection	ISMM	1192	28.8	0.12
		Sentinel	1134	27.8	0.11
	Mixed	Sentinel	1	24.0	
	T. congolense	ISMM	115	26.5	0.30
		Sentinel	207	25.4	0.28
	T. vivax	ISMM	10	25.9	1.11
		Sentinel	37	26.7	0.73

[1]Isometamidium treated cattle

6.3.4.1.4 Effect of isometamidium and diminazene treatment on pre-treatment packed cell volumes

To determine the beneficial effect of isometamidium treatment on PCV, a Model that utilises information on PCV values of cattle before treatment was considered. The following model was fitted to the PCV data.

$$Y_{ijklm} = \mu + \beta_i \times (PCV0_{ijk}) + \delta_i + h_{ij} + \tau_k + \rho_{ijl} + e_{ijklm} \quad (2)$$

Y_{ijklm} is the PCV after isometamidium treatment

Where, for i = 1,2; j = 1....4; k = 1,2; l = 1....5; m = 1,....,n_{ijkl}

PCV0 is the PCV before isometamidium treatment/or the beginning of the prophylactic period.

β_i is the fixed effect of PCV0 before the start of the prophylactic period on group *i*

all other parameters having the same meaning as in Model 1

The effect of initial packed cell volumes (PCV_0) on PCV after isometamidium treatment for the isometamidium and sentinel groups is shown in Figure 29. There was a significant effect ($p < 0.001$) of the PCV measured in both groups at the beginning of the prophylactic period, on the PCV values post treatment. In both groups, the higher the packed cell volume at the start of the prophylactic period, the lower its increase in response to treatment.

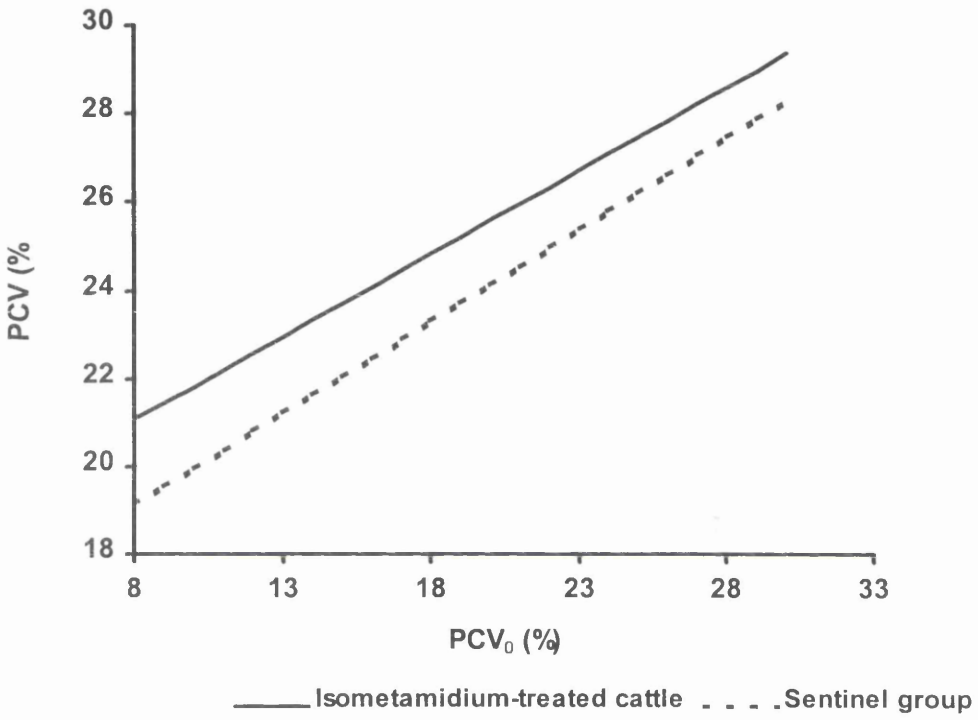


Figure 28: Effect of pre-treatment packed cell volumes (PCV_0) on the packed cell volumes after isometamidium in the isometamidium-treated and sentinel cattle during the longitudinal study in Kwale District

6.3.4.2 Body Weights

The body weights presented here are from cattle in Buda, Gazi and Kibaoni. Student's un paired t-test was used to determine difference between isometamidium-treated and sentinel cattle. Overall mean body weights gain during the prophylactic periods in 1997 are shown in Table 31

6.3.4.2.1 Buda

In all the prophylactic periods there was no significant difference ($p = 0.69$) in body weights gain between isometamidium treated and sentinel cattle. During the 3 prophylactic periods in 1997 there was a net weight gain in both groups.

The monthly mean body weights during the fourth and fifth prophylactic periods are shown in Figures 29 and 30. In the fourth period there was an increase of mean body weights of isometamidium treated cattle from the beginning to the eighth week followed by slight drop to the end of the prophylactic period (Figure 29). However, in sentinel cattle the mean body weights remained on level with a slight increase from the eighth week to the end of the prophylactic period.

Over the prophylactic period the mean body weights of isometamidium-treated cattle were higher though not significant. In the 5^{th} prophylactic period, there was an increase in the mean body weight of isometamidium treated cattle from the beginning to the end of the period. In sentinel cattle there was no change in body weight over the prophylactic period (Figure 30).

6.3.4.2.2 Gazi

During all the prophylactic periods there were no significant differences in body weight gains between isometamidium-treated and sentinel cattle. In the fourth prophylactic period, there was an initial weight loss during the first four weeks in both groups followed by an increase to the end of the period (Figure 29).

6.3.4.2.3 Kibaoni

During the prophylactic periods of 1997 there was mean weight loss in both isometamidium-treated and sentinel cattle (Table 31). During the 4th and 5th prophylactic periods the mean body weights of isometamidium treated cattle did not change significantly over the prophylactic periods. However, in sentinel cattle there was a significant increase in mean body weights over the 4th prophylactic periods (Figure 29).

Table 31: Mean body weight gains over 13 weeks prophylactic periods of isometamidium-treated and sentinel cattle during the prophylactic periods in 1997 in Kwale District

		Isometamidium-treated cattle		Sentinel cattle	
Site	**Prophylactic period**	**Mean B W gains(kg)[1]**	**S E[2]**	**Mean B W gains (kg)**	**S E**
Buda	1	7.5	4.3	11.7	6.7
	2	2.8	3.2	2.6	7.9
Gazi	2	19.5	3.6	19.6	4.3
	3	4.3	6.7	-0.1	5.6
Kibaoni	2	-1.5	3.4	-1.3	4.7
	3	3.8	3.4	-0.7	2.5
Kikoneni	1	3.3	4.9	37.4	7.2
	2	-0.3	7.1	-13.3	5.1

[1]Mean body weight gains over 13-week prophylactic period

[2]Standard error

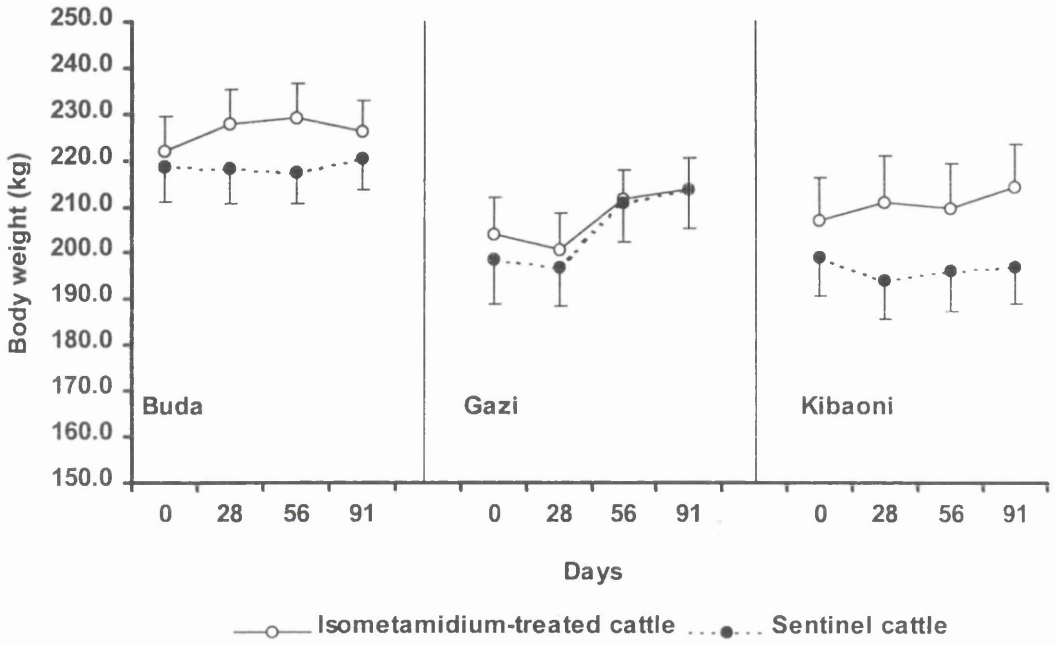


Figure 29: Monthly mean body weights (kg) of isometamidium-treated and sentinel cattle during the fourth prophylactic period (April - July 1998) of the longitudinal studies in Kwale District (error bars represents standard error).

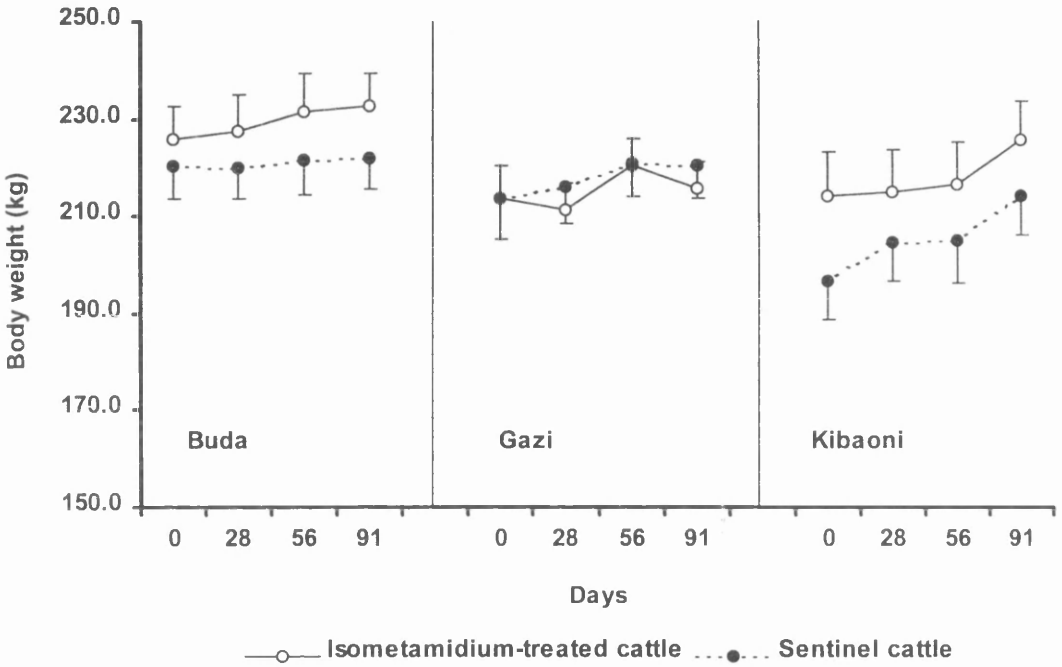


Figure 30: Monthly mean body weights (kg) of isometamidium-treated and sentinel cattle during the fifth prophylactic period (July - October 1998) of the longitudinal studies in Kwale District (error bars represents standard error).

6.3.4.3 Body condition Score

During the prophylactic periods of 1997 there was loss of body condition in both isometamidium-treated and sentinel cattle in Buda and Kibaoni. However in Gazi there was an increase in condition scores during 1997.

During the 4th and 5th prophylactic periods the body condition scores of both isometamidium-treated and sentinel cattle in Gazi and Kibaoni were higher than condition scores in Buda though not significant (Figure 31). Fluctuations of mean condition scores over the prophylactic periods were observed in both groups at all the sites. (Figure 32)

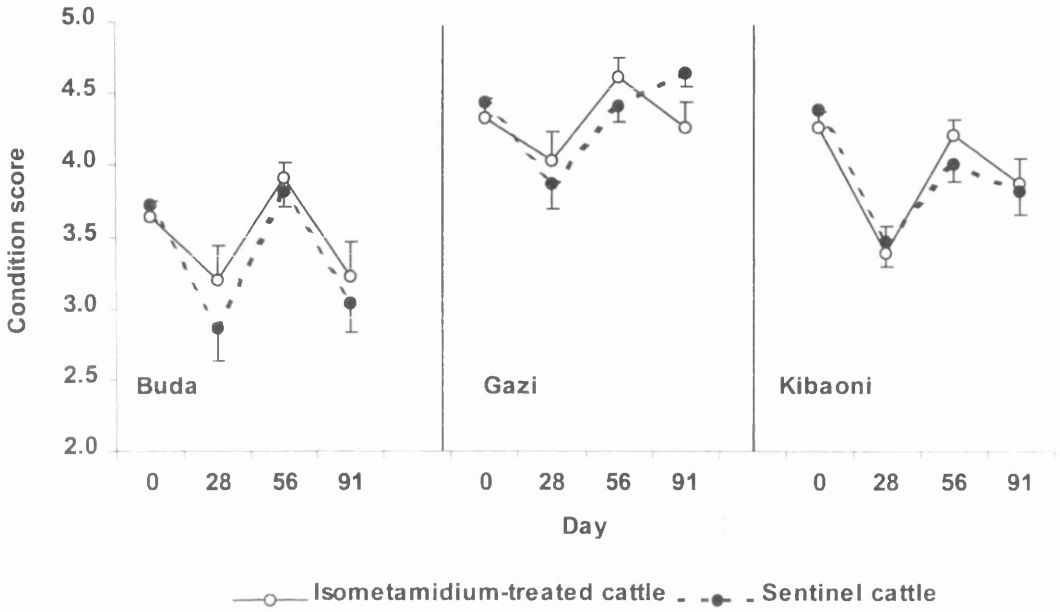


Figure 31: Monthly mean body condition scores of isometamidium-treated and sentinel cattle in the fourth prophylactic period (April - July 1998) during the longitudinal studies in Kwale District (error bars represents standard error)

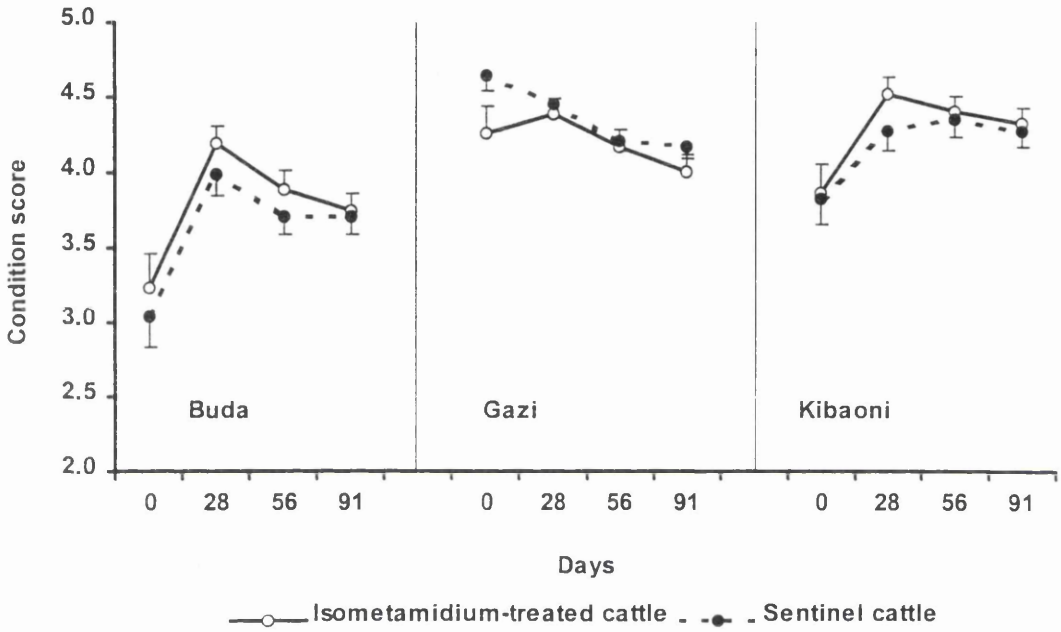


Figure 32: Monthly mean body condition scores of isometamidium-treated and sentinel cattle in the fifth prophylactic period (July – October 1998) during the longitudinal studies in Kwale District (error bars represent standard error).

6.3.5 Factors associated with the efficacy of isometamidium

6.3.5.1 Disposition of isometamidium

The Log-linear elimination curves of isometamidium in cattle in each of the five prophylactic periods in the longitudinal studies carried out in Kwale are shown in Figures 33 and 34. There was an exponential decline of isometamidium in serum during all the prophylaxis periods.

The models investigated are shown in Table 32.

The model selected for the analysis of the isometamidium data was model 4 (Table 32), which includes the main effects of site and prophylactic period, but no interaction terms. The value of r^2 adjusted for degrees of freedom for this model was 67.1 %, this being the percentage of overall variance in log isometamidium concentration accounted for by the model. Models 5 -12 incorporating interaction terms were more complex, but none accounted for a sufficiently greater percentage of overall variance to warrant its use. The maximal model (model 9, Table 32) accounted for only a slightly higher percentage of overall variance (71.3%), in spite of inclusion of three additional (interaction) terms.

In all the prophylactic periods during the longitudinal studies, serum isometamidium concentrations in cattle declined exponentially from 7 days to 84 days post-treatment (Figures 33 and 34). Log-linear regression of the isometamidium concentrations was used in the model described in Section 1.6.4. There were significant differences ($p < 0.001$) in serum isometamidium concentrations between sites (Figure 35). Cattle from Buda had significantly higher isometamidium concentrations than any of the other sites. Gazi had the lowest serum isometamidium concentrations. In addition, serum isometamidium concentration in cattle varied with prophylactic period. During the first prophylactic period, isometamidium concentrations in cattle were significantly higher ($p < 0.001$) than during the fourth and fifth prophylactic periods (Figure 36). There were no significant differences ($p > 0.05$) in serum isometamidium concentration in cattle between the prophylactic periods carried out in 1997

Trypanosome infections had a significant effect on the levels of isometamidium in cattle (Figure 37). Serum isometamidium concentrations before cattle were infected were significant higher ($p < 0.001$) than after the animals were infected.

Whether cattle were infected before isometamidium treatment or not did not have a significant ($p > 0.5$) effect on the disposition of isometamidium in cattle during the trials.

Table 32: Linear models used in the analysis of isometamidium concentrations of cattle in longitudinal prophylaxis studies

No.	Model description	*d.f. adjusted r^2
1.	$\text{Log } Y = \mu + t + e_k$	47.6%
2.	$\text{Log } Y = \mu + \rho_j + t + e_{jk}$	59.7%
3.	$\text{Log } Y = \mu + s_i + t + e_{ik}$	62.2%
4.	$\text{Log } Y = \mu + s_i + \rho_j + t + e_{ijk}$	67.1%
5.	$\text{Log } Y = \mu + s_i + t + s_i.t + e_{ik}$	62.6%
6.	$\text{Log } Y = \mu + s_i + \rho_j + t + s_i.t + e_{ijk}$	67.4%
7.	$\text{Log } Y = \mu + s_i + \rho_j + t + s_i.t + \rho_j.t + e_{ijk}$	68.8%
8.	$\text{Log } Y = \mu + s_i + \rho_j + t + s_i.t + \rho_j.t + s_i.\rho_j + e_{ijk}$	70.9%
9.	$\text{Log } Y = \mu + s_i + \rho_j + t + s_i.t + \rho_j.t + s_i.\rho_j + s_i.\rho_j.t + e_{ijk}$	71.3%
1	$\text{Log } Y = \mu + s_i + \rho_j + t + \rho_j.t + s_i.\rho_j + e_{ijk}$	70.8%
1	$\text{Log } Y = \mu + s_i + \rho_j + t + s_i.t + s_i.\rho_j + e_{ijk}$	69.4%
1	$\text{Log } Y = \mu + s_i + \rho_j + t + \rho_j.t + e_{ijk}$	68.6%
1	$\text{Log } Y = \mu + \rho_j + t + \rho_j.t + e_{jk}$	61.5%

*r^2 adjusted for degrees of freedom
≡ proportion of overall variance accounted for by model

Where, for $i = 1,\ldots,4$, $j = 1,\ldots,5$, $k = 1,\ldots,n_{ijk}$,

Y is the isometamidium concentration

μ is the overall mean

s_i is the main effect of study site Buda (s_1), Kibaoni (s_2), Kikoneni (s_3) or Gazi (s_4)

ρ_j is the main effect of prophylactic periods $1,\ldots,5$ ($\rho_{1,\ldots,5}$)

t is the number of days post-treatment

$s_i.t$ is the interaction of site and days post-treatment

$s_i.\rho_j$ is the interaction of site and prophylactic period

$\rho_j.t$ is the interaction prophylactic period and days post-treatment

$s_i.\rho_j.t$ is the interaction of site, prophylactic period and days post-treatment

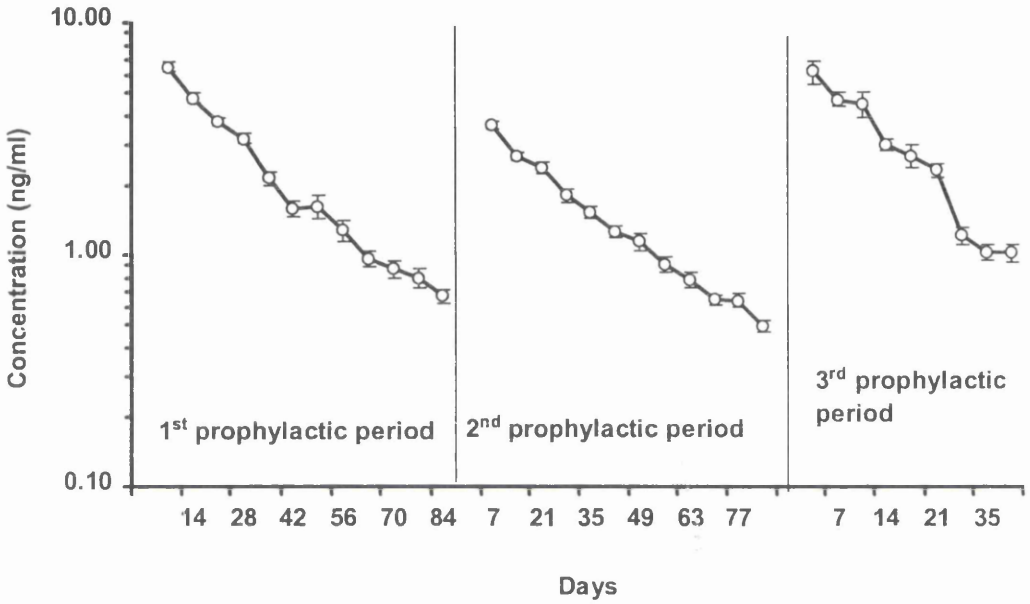


Figure 33: Weekly mean isometamidium concentrations in isometamidium-treated cattle in the three prophylactic periods during the longitudinal study in 1997 in Kwale District (error bars represents standard error).

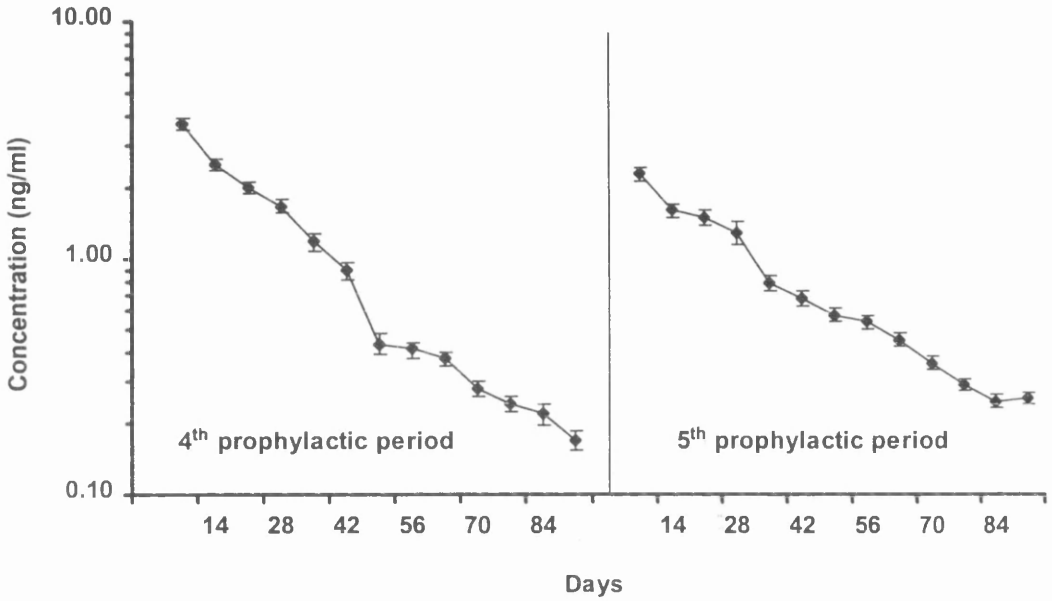


Figure 34: Weekly mean isometamidium concentrations in isometamidium-treated cattle in the two prophylactic periods during the longitudinal studies in 1998 in Kwale District (error bars represents standard error)

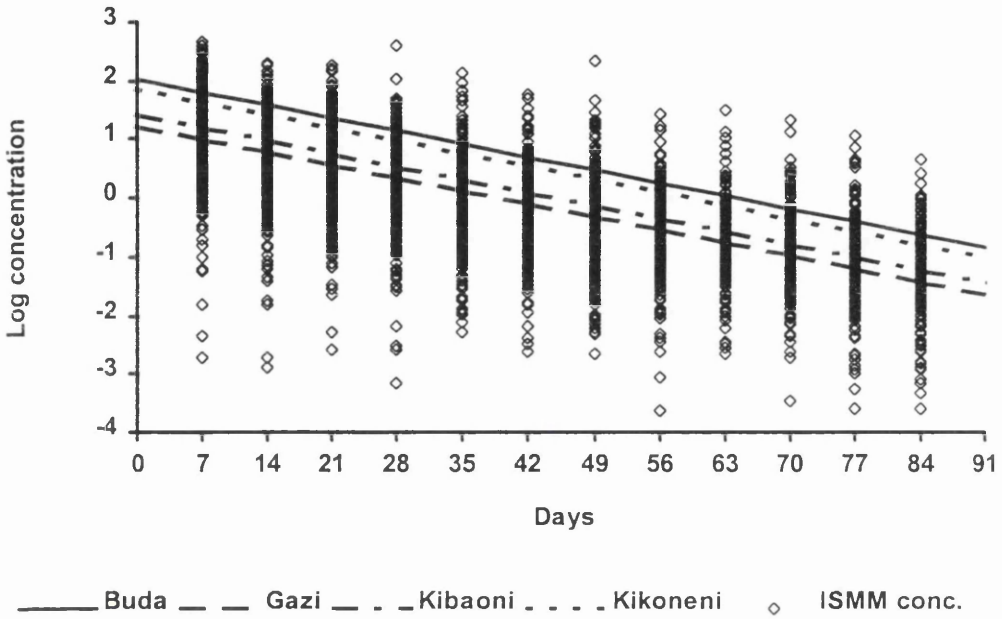


Figure 35: Log-linear regression curves of isometamidium concentrations in cattle with study sites during the longitudinal studies in Kwale District

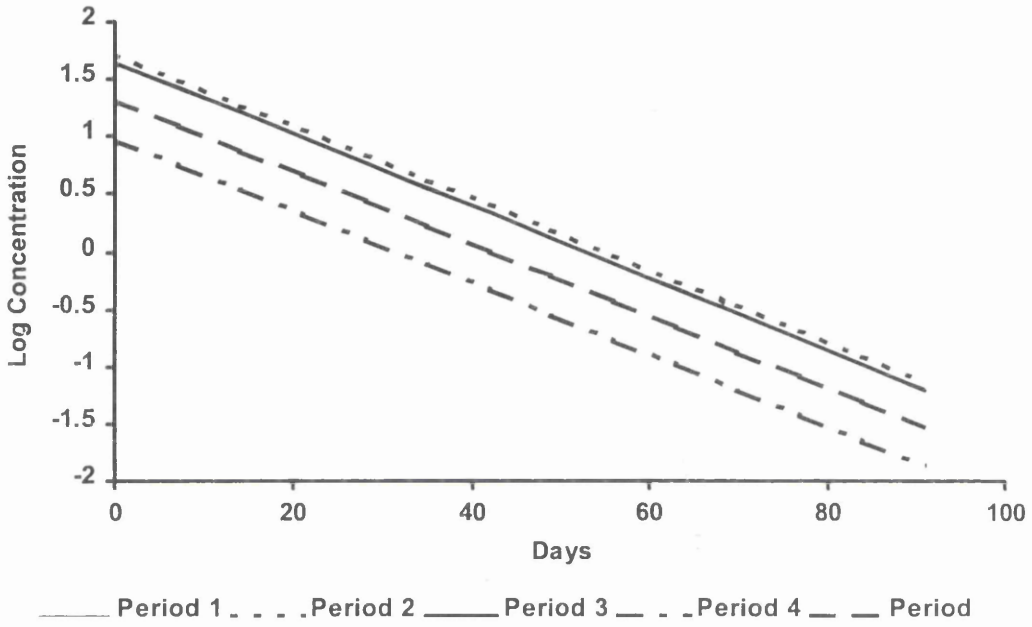


Figure 36: Log-linear regression curves of isometamidium concentrations in cattle with prophylactic periods during the longitudinal studies in Kwale District.

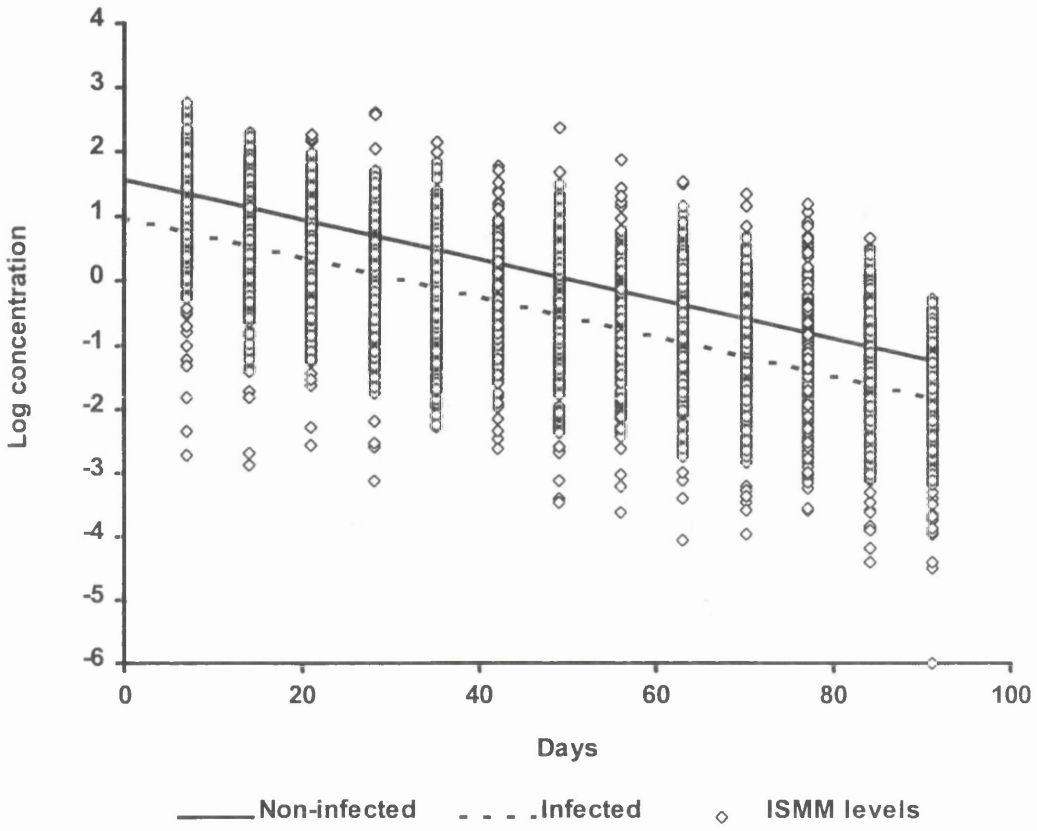


Figure 37: Log-linear regression curves of isometamidium concentrations in cattle before and after acquiring trypanosome infections during the longitudinal studies in Kwale District

6.3.5.2 Drug administration

Doses of isometamidium administered to cattle in Buda, Gazi and Kibaoni were estimated from the volumes of a 2% solution of isometamidium chloride administered and the estimated body weights. Comparison of estimated doses of isometamidium used in cattle in the three sites was carried out. Students t-test was used to determine whether there were any differences in the mean doses used at each site and prophylactic period. The mean doses of isometamidium administered in each site are shown in Table 33.

The mean doses of isometamidium administered at Gazi in all sites were significantly lower than the doses of isometamidium administered in the other two sites. In addition, there were significance differences in the doses of isometamidium administered to cattle between Buda and Gazi. At Gazi, doses of isometamidium administered to individual cattle varied from a minimum of 0.24 mg/kg body weight to a maximum of 0.73 mg/kg body weight. Similarly a wide range of isometamidium doses administered to cattle at Kibaoni were observed. The minimum and maximum doses observed at Kibaoni were 0.426 mg/kg b.w. and 1.79 mg/kg b.w. respectively. At Buda the range of isometamidium doses given to individual cattle was from 0.94 mg/kg body weight to 1.29 mg/kg body weight.

Table 33: Mean isometamidium doses administered to cattle at each site during the five prophylactic periods in Kwale

Site	Prophylactic period	No. of cattle treated	Mean dose[1] (mg/kg) b.w	Standard error
Buda	1	34	1.063	0.011
	2	28	1.029	0.007
	3	29	1.026	0.011
	4	30	1.019	0.010
	5	26	1.002	0.017
Gazi	2	40	0.494	0.014
	3	38	0.446	0.015
	4	39	0.456	0.011
	5	35	0.431	0.013
Kibaoni	2	39	0.883	0.029
	3	39	0.878	0.025
	4	39	0.967	0.045
	5	37	0.917	0.232

[1] Calculated from the volume of a 2 % isometamidium solution administered and the body weight estimated before treatment.

6.3.5.3 *Infecting trypanosome species*

A comparison of the survivor functions for *T. congolense* and *T. vivax* infections in the isometamidium groups was carried out. From the life-tables the measure of isometamidium efficacy against the different trypanosome species was determined by considering the proportion of cattle that acquired either *T. congolense* or *T. vivax* infections by the 8th week of each prophylactic period (Table 34).

In all prophylactic periods carried out in 1997, there were no significant differences ($p > 0.05$) in the proportions of cattle infected between *T. congolense* and *T. vivax* at the three sites. An exception was observed during the second prophylactic period at Gazi. A significantly higher ($p < 0.001$) proportion of cattle were infected by *T. vivax* (41.4 %) than *T. congolense* (17.2 %) by the 8th week post isometamidium treatment (Table 34)

In addition, there was no significant difference ($p > 0.05$) between the prophylactic periods (first and second) in the proportions of cattle infected by the 8th week post isometamidium treatment at Buda (Table 34).

During the prophylactic periods, four and five carried out in 1998, the proportions of cattle that acquired *T. congolense* infections were significantly higher ($p < 0.01$) than the proportion of cattle that acquired *T. vivax* at all three sites (Table 34).

Table 34: Percentages of cattle that acquired *T. congolense* and *T. vivax* infections by the eighth week of the prophylactic period in the isometamidium groups during the longitudinal studies in Kwale District

Sites	Species	1st prophylactic period	2nd prophylactic period	3rd prophylactic period	4th prophylactic period	5th prophylactic period
Buda	Tc[1]	19.2	14.3	8.7	36.4	32.0
	Tv[2]	19.2	17.9	8.7	9.1	0
Gazi	Tc	ns[3]	17.2	12.5	39.3	16.7
	Tv	ns	41.4	12.5	8.9	0
Kibaoni	Tc	ns	16.1	7.4	52.6	30.4
	Tv	ns	23.1	11.3	16.0	13.0

[1]Tc: Trypanosoma congolense

[2]Tv: Trypanosoma vivax

[3]ns the studies had not started during this time

6.3.5.4 Breed, sex and age

Comparison of time to first infection between cattle of different breed, sex and age that had been treated with isometamidium was carried out.

The proportion of cattle infected by the eighth week post isometamidium treatment was used to determine the effect of breed, sex and age on the efficacy of isometamidium prophylaxis. Chi-squared statistics was applied to determine differences between breed, sex and age.

The proportions of cattle infected by the eighth week of the prophylactic period are shown in Table 35.

There were significant differences ($p < 0.05$) between breeds in the proportions of cattle infected by the eighth week after isometamidium treatment. A significantly higher proportion of the crossbreed had been infected by two months after isometamidium treatment than the local breed. This was observed during all prophylactic periods (Table 35). Sex of the animals had a significant ($p < 0.05$) effect on the proportions of cattle infected during the studies. In Kibaoni, a significantly higher ($p < 0.05$) proportion of bulls were infected than cows (Table 35). However in Gazi, the opposite was true. In the second and third prophylactic period significantly ($p < 0.05$) higher proportion of cows were infected than bulls (Table 35).

Age did not have a significant effect ($p > 0.05$) on the proportion of cattle infected during the prophylactic periods.

Table 35: Proportion (%) of cattle in the isometamidium treated group that acquired trypanosome infections by the eighth week post treatment in terms of breed, sex and age during the five prophylactic periods in Kwale

Site	Prophylactic period	Sex		Age		Breed	
		Bull	Cow	Calf[1]	Adult	Local	Cross
Buda	1	na[2]	30.0	na	30.0	15.4	41.2
	2	na	30.0	na	30.0	30.8	47.1
	3	na	14.3	na	14.3	0.0	23.5
	4	na	39.3	50.0	39.3	28.6	50.0
	5	na	30.8	50.0	28.6	33.3	35.7
Gazi	2	57.1	64.5	64.3	62.5	63.2	na
	3	14.3	45.2	78.6	45.8	39.5	na
	4	46.2	40.7	42.1	42.9	42.5	na
	5	23.1	22.2	42.1	23.8	22.5	na
Kibaoni	2	32.1	33.3	33.3	32.4	33.3	na
	3	40.0	23.1	40.0	33.3	34.2	na
	4	77.8	60.0	63.6	70.4	44.7	na
	5	61.1	36.8	36.4	53.8	48.6	na
Kikoneni	1	na	0.0	na	0.0	0	0
	2	na	3.3	na	36.7	0	39.3
	3	na	31.0	na	31.0	1	25.9

[1] includes all cattle between one and two years.

[2] no cattle of this sex, breed or age were included in the studies

6.3.5.5 Isometamidium concentrations in sera obtained in cattle at the time of detection of breakthrough infections

Trypanosomes detected in isometamidium-treated cattle in which serum determinations of the drug showed concentrations greater than 0.4 ng/ml were considered to have some degree of resistance.

The numbers of *T. congolense* and *T. vivax* breakthrough infections in which serum isometamidium concentrations were found to be below this level are shown in (Table 36).

6.3.5.5.1 Buda

Of 75 *T. congolense* breakthrough infections, 54.6 % were detected when isometamidium concentrations were above 0.4 ng/ml, while 48.5 % of the 33 *T. vivax* breakthrough infections in five prophylactic periods were detected when isometamidium levels were above 0.4 ng/ml.

6.3.5.5.2 Gazi

Of 117 *T. congolense* breakthrough infections, 37.6 % were detected when isometamidium concentration were above 0.4 ng/ml, while 39.3 % of 61 *T. vivax* breakthrough infections during four prophylactic periods were detected when isometamidium levels were above 0.4 ng/ml.

6.3.5.5.3 Kibaoni

Of 8 *T. congolense* breakthrough infections, 75 % were detected when isometamidium concentration were above 0.4 ng/ml, while 84.2 % of 38 *T. vivax* breakthrough infections in four prophylactic periods were detected when isometamidium levels were above 0.4 ng/ml.

6.3.5.5.4 Kikoneni

Of 151 *T. congolense* breakthrough infections, 21.9 % were detected when isometamidium concentration were above 0.4 ng/ml, while 55.6 % of 27 *T. vivax*

breakthrough infections in two prophylactic periods were detected when isometamidium levels were above 0.4 ng/ml.

Table 36: Isometamidium concentrations in cattle at the time of detection of breakthrough trypanosome infections during the longitudinal studies in Kwale District

		T. congolense		*T. vivax*	
Site	**Prophylactic period**	**Number of infections**	**> 0.4 ng/ml[1]**	**Number of infections**	**> 0.4 ng/ml**
Buda	1	5	3	6	6
	2	13	13	13	7
	3	2	2	2	2
	4	34	14	2	1
	5	21	9	0	0
Gazi	2	21	9	40	13
	3	25	21	10	8
	4	37	12	5	3
	5	34	2	6	0
Kibaoni	2	21	10	16	9
	3	7	4	3	2
	4	69	11	4	3
	5	54	8	4	1
Kikoneni	2	4	2	33	17
	3	4	4	5	5

[1] Number of trypanosome infections isolated from cattle in which isometamidium concentrations were above 0.4 ng/ml (Eisler *et al.*, 1997a)

6.3.6 Efficacy of diminazene in the sentinel groups

The efficacy of diminazene treatment on the sentinel group was determined by considering the number of first infections that were cured after treatment with diminazene aceturate. Infections caused by *T. congolense* and *T. vivax* were considered.

Proportions of *T. congolense* infections cured after treatment with diminazene aceturate are shown in Table 37.

Except at Kibaoni during the fourth and the fifth prophylaxis periods where 9 (24.3 %) and 4 (12.5%) *T. congolense* infections were cured respectively, the cure rate of *T. congolense* infections at the other sites was above 30 %. At Kikoneni, cure rate of *T. congolense* infections of 75% and above were observed. During the five prophylaxis periods, the cure rates for *T. vivax* by diminazene at all sites were above 40 %.

Proportions of *T. vivax* infections cured after treatment with diminazene aceturate are shown in Table 38. Except at Buda, Gazi and Kikoneni during the second prophylaxis period, all *T. vivax* infections detected were cured when treated with diminazene.

Table 37: Number of *T. congolense* infections in the sentinel groups, including the proportion cured after treatment with diminazene during the longitudinal studies in Kwale

Site	Prophylaxis period	No of *T. congolense* infections[1]	No of *T. congolense* infections cured[2]	%Cured
Buda	1	18	9	50.0
	2	21	11	52.4
	3	11	9	81.8
	4	23	6	36.1
	5	20	6	33.3
Gazi	2	26	15	57.7
	3	18	11	61.1
	4	36	14	38.9
	5	31	15	48.3
Kibaoni	2	19	6	33.33
	3	19	13	68.4
	4	37	9	24.3
	5	32	4	12.5
Kikoneni	2	8	6	75.0
	3	5	5	100.0

[1] number of cattle that were infected with *T. congolense* during that prophylaxis period

[2]number of cattle that acquired only one *T. congolense* infection during the prophylaxis period.

Table 38: Number of *T. vivax* infections in the sentinel group including the proportion cured after treatment with diminazene aceturate during the longitudinal studies in Kwale

Site	Prophylaxis period	No of *T. vivax* infections[1]	No of *T. vivax* infections cured[2]	% Cured
Buda	1	6	5	83.3
	2	12	5	41.7
	3	2	2	100
	4	1	1	100
	5	4	3	75.0
Gazi	2	23	10	43.5
	3	11	11	100
	4	14	11	78.6
	5	20	17	85
Kibaoni	2	12	9	75
	3	7	7	100
	4	12	11	91.7
	5	9	8	88.9
Kikoneni	1	2	2	100
	2	25	15	60.0
	3	7	7	100

[1] number of cattle that were infected with *T. congolense* during that prophylaxis period

[2] number of cattle that acquired only one *T. congolense* infection during the prophylaxis period.

6.3.7 Cost of drug treatment

The cost implication of trypanocidal treatment during the longitudinal studies was assessed. The cost of the treatments were based on the market prices in Kenya shillings of the drugs at the time of the studies.

The total cost incurred and the cost per animal treated with isometamidium during the prophylaxis trials in Kwale is shown in Table 39. At Buda, Kibaoni and Kikoneni, the costs incurred of isometamidium per animal were significantly higher $p < 0.01$) than the cost incurred of isometamidium per animal at Gazi during all the prophylaxis periods.

The costs of diminazene treatment incurred in the isometamidium treated and sentinels groups during the prophylaxis periods are shown in Table 40. There were significant differences ($p < 0.001$) in the total diminazene cost incurred between the isometamidium treated and sentinel groups. The total diminazene cost (US$ 436.5) incurred in the isometamidium groups were about half the diminazene cost (US$ 852.2) incurred in the sentinel groups.

For both isometamidium and diminazene treatments respectively, there was no significant ($F = 7.89$; $p > 0.05$) relationship between the number of treatments in a group during the prophylaxis period and the body weights. However, an exception was observed at Kibaoni. The mean body weights decreased significantly ($F = 135.56$; $p < 0.01$) with the number of diminazene treatments administered.

Table 39: Number of cattle treated with isometamidium in each prophylaxis period at each site, including total cost incurred and cost per animal treated during the longitudinal studies in Kwale

Site	Prophylaxis period	No. of cattle treated	Isometamidium used in gms	Total Cost (US$)	Cost per animal treated (US$)
Buda	1	30	6.44	54.4	1.8
	2	26	6.08	51.4	2.0
	3	28	6.45	54.5	1.9
	4	30	6.76	57.1	1.9
	5	26	6	50.7	2.0
Gazi	2	40	3.72	31.4	0.8
	3	38	3.94	33.3	0.9
	4	40	3.68	31.1	0.8
	5	35	3.48	29.4	0.8
Kibaoni	2	42	8.2	69.3	1.6
	3	38	7.4	62.5	1.6
	4	40	7.4	62.5	1.6
	5	37	6.86	58.0	1.6
Kikoneni	1	30	9.46	79.9	2.7
	2	28	8.9	75.2	2.7
	3	27	8.48	71.7	2.7

Table 40: Number of cattle treated with diminazene in each prophylaxis period including total cost incurred and cost per animal during the longitudinal studies in Kwale

Site	Group	Prophylaxis period	No. of cattle treated	Diminazene used in gms	Total Cost (US$)	Cost per animal treated (US$)
Buda	ISMM	1	8	14.9	12.3	1.5
		2	17	30.9	25.5	1.5
		3	4	7.4	6.1	1.5
		4	38	58.8	48.4	1.3
		5	24	35.7	29.4	1.2
	Sentinel	1	19	30.8	25.4	1.3
		2	39	66.6	54.9	1.4
		3	20	34.4	28.3	1.4
		4	73	114.0	94.0	1.3
		5	62	95.4	78.6	1.3
Gazi	ISMM	1	56	50.5	41.6	0.7
		2	11	11.5	9.5	0.9
		4	38	36.8	30.3	0.8
		5	33	32.5	26.8	0.8
	Sentinel	2	57	51.7	42.6	0.7
		3	32	30.9	25.5	0.8
		4	57	48.6	40.1	0.7
		5	71	67.9	56.0	0.8
Kibaoni	ISMM	2	37	51.6	42.5	1.1
		3	16	18.1	14.9	0.9
		4	66	66.2	54.6	0.8
		5	58	59.2	48.8	0.8
	Sentinel	2	50	63.8	52.6	1.1
		3	42	48.8	40.2	1.0
		4	144	142.6	117.6	0.8
		5	121	124.4	102.5	0.8
Kikoneni	ISMM	2	18	39.2	32.3	1.8
		3	7	16.1	13.3	1.9
	Sentinel	2	26	59.1	48.8	1.9
		3	22	54.8	45.2	2.1

6.4 Discussion

In the work described in this chapter, the efficacy of isometamidium prophylaxis in controlling trypanosomiasis in Kwale District was determined.

Variation of isometamidium efficacy with sites, type of cattle and trypanosome challenge in a trial period were also investigated. Furthermore, sources of variation of drug efficacy including drug administration and drug resistance were determined.

Finally, impact of prophylaxis failure in Kwale District in terms of animal health related losses and treatment was assessed.

Five prophylactic periods of 3 months each were carried out in four sites in Kwale District. In two sites Buda and Kikoneni isometamidium chloride and diminazene aceturate were administered at the recommended doses of 1.0mg/kg b.w. and 7.0 mg/kg b.w. respectively. In Gazi and Kibaoni drug administration was carried out according to the farmer's normal practice.

Breakthrough trypanosome infection was detected in both isometamidium and sentinel cattle in all study sites during the five prophylactic periods. In the isometamidium-treated cattle, 371 *T. congolense*, 162 *T. vivax*, 6 *T. brucei* and 6 *T. theileri* infections were detected during the study period, With the exception of Kikoneni, there was no significant difference in trypanosome challenge between the three sites and between prophylactic periods. The three study sites, Buda, Gazi and Kibaoni are located in close proximity to forested areas that are a foci for tsetse (Snow and Tarimo, 1983). In Kikoneni, during the first prophylactic period very few trypanosome infections were detected in both isometamidium-treated and sentinel cattle towards the end (from week 11) of the prophylactic period.

In Buda, in all prophylactic periods isometamidium prophylaxis reduced the force of infection (trypanosome challenged) by a factor of greater than two. An indication that isometamidium was efficacious when used at 1 mg/kg b.w. In

Kibaoni and Gazi, isometamidium prophylaxis had no effect on trypanosome challenge in the prophylactic period 2 and 3 as indicated by the hazard ratios of 1.6-1.8. However, in the fourth and fifth prophylactic periods isometamidium treatment had a significant effect on trypanosome infections. The prophylaxis failure observed in Gazi and Kibaoni during the second and third prophylactic periods is an indication that the dose of isometamidium administered was inadequate to confer protection. However, during the fourth and fifth prophylactic periods several factors may have influenced the efficacy of isometamidium prophylaxis in Gazi and Kibaoni. Firstly, the infecting species was predominantly *T. congolense* during the fourth and fifth periods. *Trypanosoma congolense* was observed to be more sensitive to prophylaxis activity of isometamidium on Galana Ranch (Chapter 4) and Kubo (Chapter 5). Secondly, following an education phase on how to administer drugs correctly after the end of the third prophylactic period at Gazi and Kibaoni, the dose rate of isometamidium administered by the farmers at Kibaoni in the fourth and fifth periods was similar to the recommended dose of 1 mg/kg b.w.(Table 33).

Estimations of doses of isometamidium used provided evidence of significant under-dosing during isometamidium administration at Gazi and Kibaoni. Estimated doses of isometamidium administered by the farmers at Kibaoni and the Animal Health assistant at Gazi were significantly lower than the dose of 1 mg/kg b.w. administered in cattle at Buda. Under-dosing is considered to be the major cause of resistance development (Geerts and Holmes, 1998). It is suggested that sub-therapeutic drug concentrations exert a strong selective pressure for the emergence of resistance-clones that pre-exist in the trypanosome population (Geerts and Holmes, 1998)

In the present study, under-dosing in these two sites may have occurred because of the tendency to under-estimate the weight of the animals when treating (Besier & Hopkins, 1988)

Interestingly, the Livestock Animal Health Assistant, a qualified field extension officer who treated the animals at Gazi administered a significantly ($P<0.001$) lower dose (mean: 0.4mg/kg) than the seemingly untrained farmers (mean: 0.8mg/kg).

Isometamidium could be detected in all sera collected during the five prophylactic periods. The circulating isometamidium concentrations were significantly higher in Buda than in Gazi and Kibaoni. This provides further evidence of misuse of trypanocidal drugs by trained and untrained personnel alike. Exposure of trypanosome to sub-therapeutic drug levels has been attributed to development of drug resistance (Leach and Roberts, 1981).

In the present study, the under-dosing leading to sub-therapeutic circulating drug levels observed in Gazi and Kibaoni, may have led to the development of high level resistance expressed by *T. congolense* and *T. vivax* infections in mice and cattle respectively obtained from these sites (Chapter 7).

Trypanosome infections had a significant effect on the levels of isometamidium in cattle. Serum isometamidium concentrations in cattle before acquiring infections, were significantly higher than after the animals were infected.

Eisler *et al.* (1994) observed that the drug disappears more rapidly in animals which are challenged and becoming infected with drug resistant trypanosome isolates than in those challenged but protected against infections with sensitive trypanosomes.

In the present study, the isometamidium ELISA showed that between 37.6 % and 75 % of breakthrough infections in the four sites were expected to exhibit some degree of drug resistance. It is likely therefore that the low isometamidium concentrations observed after cattle became infected is an indication of the resistant nature of the infecting trypanosomes.

In conclusion, evidence of misuse of trypanocidal drugs by farmers and trained personnel was observed. This is probably the cause of development of drug resistance in Kwale leading to prophylaxis failure. However, when administered correctly, isometamidium prophylaxis is still effective.

It is therefore recommended that there is a need to re-train the field extension personnel in the correct dosage and use of trypanocidal drugs in tsetse endemic areas in view of fast development

Chapter 7

Laboratory assessment of drug resistance

7 Laboratory assessment of drug resistance

7.1 Introduction

Despite their shortcomings, *in vitro* and *in vivo* systems for testing drug sensitivity have been used effectively to assess drug resistance in trypanosome isolates obtained in cattle exposed to natural tsetse challenge in the field (Sones *et al.*, 1988; Peregrine, 1994). Although *in vitro* systems may be quicker, the prohibitive costs of suitably equipping laboratories, and the difficulties involved in adapting the trypanosomes to in vitro culture make *in vivo* systems preferable for use in African laboratories. In addition, it is not easy to use drug sensitivity data derived from *in vitro* systems to predict the drug sensitivity of trypanosome isolates in definitive hosts.

To interpret effectively the field observations of early breakthrough infections and treatment failure during the prophylaxis studies described in Chapter 6, it was imperative that the drug sensitivity of trypanosomes associated with these infections be characterised. Furthermore, the *in vivo* sensitivity testing of trypanosome stocks collected from the field would provide greater insight into the level of sensitivity of trypanosome populations in a given area. This would form a basis for recommendations on the effective use of trypanocides used in the study areas.

Ideally, cattle would be used for *in vivo* trypanocidal drug sensitivity testing, however tests in mice can be a valuable alternative (Sones *et al.*, 1988). In these studies, mice were used for the drug sensitivity testing of *T. congolense* isolates because of the much lower costs of purchasing and maintaining these animals. However, because *T. vivax* isolates from Eastern Africa do not generally grow in mice (Hawking 1963), *T. vivax* isolates were tested in cattle.

Drug sensitivity tests in mice and cattle are only possible if the isolated trypanosomes are able to infect and maintain detectable parasitaemia in the experimental host animal. This requires the trypanosomes not only to be viable in terms of motility, but also to be capable of replicating in the host species.

In the work described in this chapter, the levels of resistance to isometamidium chloride and diminazene aceturate of field isolates of *T. congolense* and *T. vivax* collected during isometamidium prophylaxis studies in Kwale district were determined. In addition, factors associated with the viability and infectivity of the isolates were investigated in mice (*T. congolense*) and cattle (*T. vivax*).

Ideally, trypanocidal drug sensitivity testing of a trypanosome isolate *in vivo* should include determination of the dose required to cure 50% or 80% of infected animals (CD_{50} or CD_{80}, respectively). However, this requires the use of a number of different trypanocidal drug doses, and a correspondingly large number of experimental animals. In order to minimise the number of experimental mice used in the sensitivity testing of *T. congolense*, only three doses of each drug were used to classify the levels of resistance of the isolates. These doses were used to treat groups of mice infected with a defined number of parasites obtained from immuno-suppressed donor mice, which were used to expand the trypanosome populations.

Because of the significant costs involved, individual cattle were used for the investigation of trypanocidal drug resistance in *T. vivax*. Isometamidium was the drug initially used for sensitivity testing in cattle. If relapses to isometamidium occurred, diminazene treatment was then administered, to ascertain whether the infecting population showed multiple resistance.

7.1.1 Objectives

7.1.1.1 To assess factors associated with viability and infectivity in mice of T. congolense *stabilates collected from the field.*

7.1.1.2 To assess factors associated with viability and infectivity in cattle of T. vivax *stabilates collected from the field.*

7.1.1.3 To assess in vivo *sensitivity of trypanosome isolates to trypanocidal drugs collected during observational and longitudinal studies in Kwale district*

7.1.1.4 To compare in vivo *trypanocidal drug sensitivity of trypanosome isolates to field data collected during observational and longitudinal studies in Kwale district*

7.2 Materials and Methods

7.2.1 Drug sensitivity testing of *T. congolense* in mice

7.2.1.1 Experimental animals

White Swiss mice about 6 weeks old from the small animal unit in KETRI were maintained 5 per cage and provided with mice pellets (Unga Ltd., Nairobi, Kenya) and water *ad libitum*.

Donor mice used for expanding trypanosome populations were either γ-irradiated with a sub-lethal dose of 650 rads or treated intraperitoneally 24 hrs before infection with cyclophosphamide, Endoxana® (ASTA MEDICA Cambridge England), at a dose rate of 300 mg/kg.

7.2.1.2 Inoculation and monitoring of mice

Frozen trypanosome-infected blood stabilates obtained from cattle in the field (see Chapter 3, 6.2) were removed from liquid nitrogen and thawed at room temperature for 15 – 30 minutes. The stabilates were examined for viability under a microscope using the wet-film method (described in detail in Chapter 2). Stabilates were considered viable if motile trypanosomes were observed. Two (donor) mice per isolate were each inoculated intraperitoneally with 0.5 ml of stabilated, infected blood containing the viable trypanosomes. The level of parasitaemia was monitored on a daily basis by examining wet films of tail blood.

7.2.1.3 Harvesting trypanosomes from mice for sub-inoculation or stabilate preparation.

The steps followed after collection of the stabilate from the field to drug sensitivity testing in mice and cattle are shown in Figure 38. At the first peak of parasitaemia, donor mice were euthanised using chloroform. Cardiac blood was removed into 1 ml syringes containing EDTA (8 mM). The trypanosome density of the pooled blood from the donor mice was determined with a Neubauer haemocytometer (Labpak Ltd) as described by Lumsden *et al.* (1973). Appropriate

dilution in phosphate buffered saline glucose (PBSG) to produce a parasite concentration of 5 x 10^5 trypanosome/ml was carried out. The final volume after dilution should be above that required to infect 35 experimental mice. In addition, secondary stabilates from the donor mice were prepared before dilution as described in the next section 2.1.4.

Phosphate buffered saline glucose was prepared by adding 1.5 g of glucose to every 100 ml of PBS (see Chapter 3 section 9.1.4)

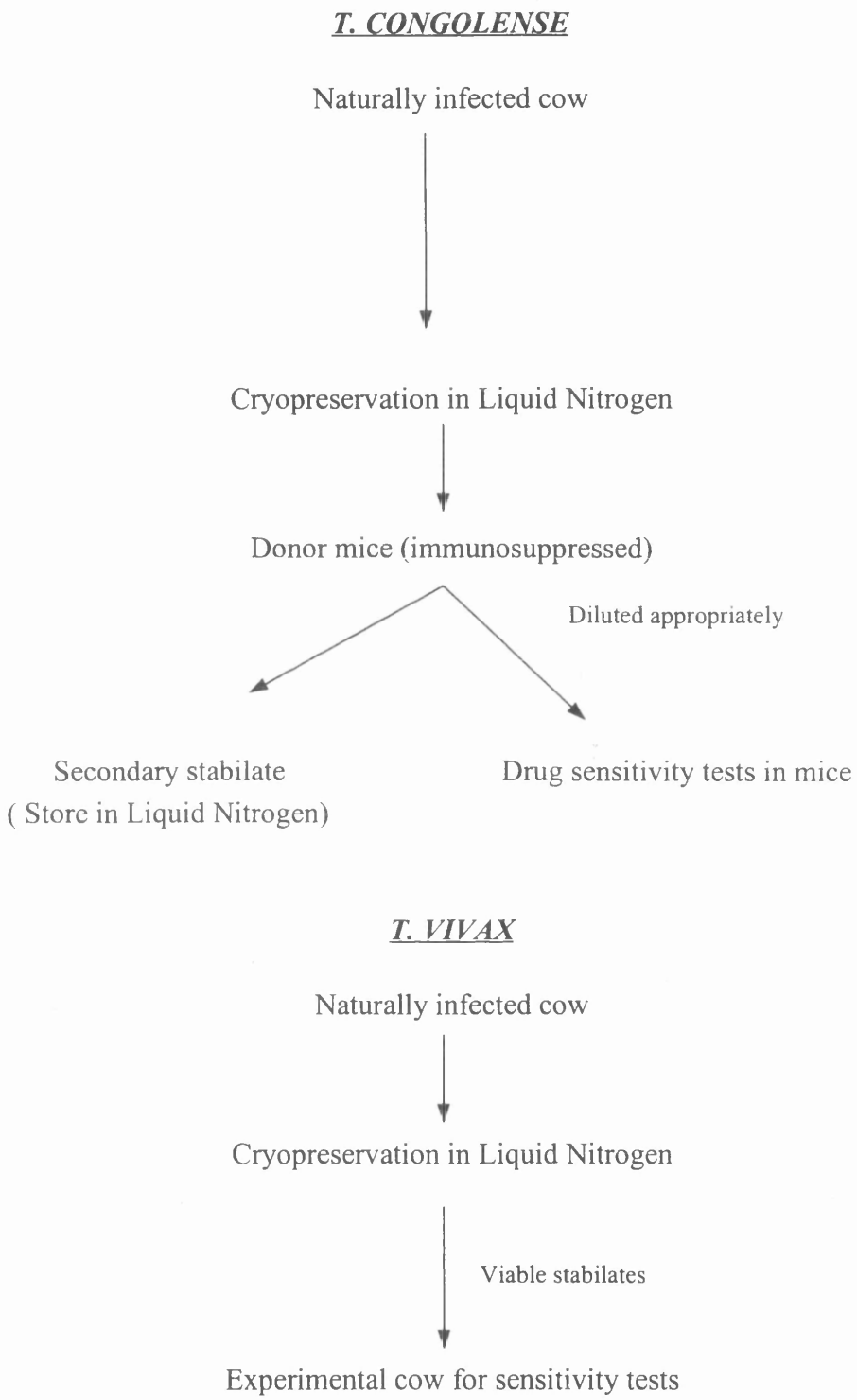


Figure 38: Systematic procedure followed during sensitivity testing in mice and cattle

7.2.1.4 Cryopreservation of secondary trypanosomes stabilates from donor mice

Secondary stabilates from immunosuppressed mice that had been inoculated with trypanosome-infected blood from the field were prepared for cryopreservation (Figure 38).

Infected donor mice blood was removed in a 10ml tube containing EDTA. The stabilates were prepared as follows:

- To each cryovial (2 in total) containing 0.2ml glycerol, 1.8ml of blood was added and mixed well by inversion.
- About 20 plain capillaries were filled with the infected blood from one of the cryovials and sealed on both ends with Cristaseal®. The sealed capillary tubes were placed in 5ml cryovials (5-8 capillaries per vial).
- After allowing for the glycerol to equilibrate with the trypanosomes, all the cryovials were inserted into a large screw cap plastic bottle insulated with a 1cm thick layer of plasticine.
- The insulated bottle was suspended on a piece of string in the vapour phase of the liquid nitrogen container, and allowed to cool for 2 hours.

After cooling, the cryovials were transferred from the cooling device to a storage canister in the liquid phase of the nitrogen.

7.2.1.5 Drug sensitivity testing protocol in mice

The experimental design of the procedure for the sensitivity testing in mice is shown in Table 41. The testing procedure was as follows:

- The experimental mice were infected intraperitoneally with 5 x 10^5 *Trypanosoma congolense* and treated intraperitoneally 24 hr later with the appropriate drug at the doses shown in Table 41.

- The mice were monitored thrice weekly for presence of trypanosomes using the wet film method (see Chapter 2) for 60 days, using blood obtained by snipping the tail tip.
- If trypanosomes were detected during the follow-up period, the species of the relapsing population was determined and the mice were removed from the experiment.

Table 41: Description of dose, number of mice and number of trypanosomes used in the sensitivity testing of stabilates collected during the prophylaxis trials in Kwale district

	Isometamidium chloride			Diminazene aceturate			Controls
Dose (mg/kg b.w.)	0.1	1.0	10.0	1.0	20.0	40.0	Not treated
Number of Trypanosomes (10^5)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Number of mice	5	5	5	5	5	5	5

7.2.2 Drug sensitivity testing of *T. vivax* in cattle

7.2.2.1 Experimental animals

Twenty six Boran cattle between 6 - 12 months old weighing between 150-200 kg from Rumuruti, a tsetse-free area, in the Central region of Kenya were used. These animals were bought in two groups of 10 and 16, at different times. Initially the first ten steers were purchased and after the first phase of the experiment the next group of sixteen were later purchased from the same area.

The animals were kept in a fly proof barn and fed with hay and a protein diet supplement (Ranch cubes, Unga Ltd., Nairobi Kenya) at 500 g per animal per day. Water, hay and mineral salt lick were given *ad libitum*. Before housing, the cattle were sprayed with an acaricide solution containing 12.5%w/v amitraz (Triatix®, Cooper Kenya Ltd Nairobi Kenya). Once housed, the animals were weighed and treated with long acting tetracycline (Terramycin LA®, Pfizer Ltd., Sandwich, Kent England) at a dose rate of 20 mg/kg body weight and diminazene aceturate, (Veriben®) at a dose rate of 3.5 mg/kg body weight. Blood smears were prepared on clean microscopic slides, stained with Giemsa and examined microscopically for haemoprotozoan infection. Faecal samples were examined for nematode infection. The animals were given orally a de-worming drug formulation containing 3.0 % oxyclozanide BP and 1.5 % levamisole hydrochloride BP (Nilzan® Plus with Cobalt, Cooper Kenya Ltd.) at the dose rate of 0.5 ml per kg body weight. Packed cell volume was monitored daily starting two weeks before inoculation of trypanosomes.

7.2.2.2 Drug sensitivity testing protocol

- The *T. vivax* stabilates were removed from liquid nitrogen and left on the bench to thaw for 15-30 minutes.
- After confirming the viability of a trypanosome isolate by microscopic examination, the stabilate (2 ml) was inoculated into a jugular vein of the calf (or subcutaneously where stated). For the first 10 calves, approximately 1ml each from five stabilates (7 calves) or 3 stabilates (3 calves) were mixed together,

then used to inoculate one calf into the jugular vein. All the five (5-Buda, 5-Kibaoni, 15-Gazi, 10-Kikoneni) or three (3-Kibaoni, 3-Gazi, 3-Kikoneni) stabilates pooled were obtained from the same study site. The next 16 calves were each inoculated with one stabilate (2-Buda, 5-Kibaoni, 2-Gazi, 1-Kubo).

- Thereafter and continuing until the end of the experiment, PCV and parasitaemia were monitored at least twice a week. The parasitaemia was assessed by the buffy-coat phase-contrast technique (Murray *et al.,* 1977).
- At the first peak of parasitaemia, the calf was weighed and treated intramuscularly, on the same day, with a 2 % solution of isometamidium chloride at a dose of 0.5 mg/kg b.w.
- If a relapse was detected later, the PCV and parasitaemia were determined and monitored daily thereafter.
- If the PCV dropped by 20% of the value determined at the time of relapse, the calf was weighed and treated with a 7 % solution of diminazene aceturate at 3.5 mg/kg b.w.
- If no relapse was detected after 100 days monitoring, the trypanosome isolate was defined as sensitive to the dose of isometamidium chloride or diminazene aceturate used.

7.2.3 Analysis of viability and infectivity of stabilates

The viability of a field stabilate of *T. congolense* or *T. vivax* after storage in liquid nitrogen was defined in terms of whether or not motility was observed when a wet film was examined microscopically using phase contrast or dark-ground. The infectivity of a field stabilate of *T. congolense* or *T. vivax* after storage in liquid nitrogen was defined in terms of whether or not it established infection in an immuno-suppressed mouse (*T. congolense*) or in a calf (*T. vivax*).

7.2.4 Factors influencing viability and infectivity of trypanosome stabilates from Kwale District

The factors influencing viability and infectivity of trypanosome stabilates from longitudinal studies at Buda, Kibaoni, Kikoneni and Gazi in Kwale District

were investigated using linear mixed models as described by Duchateau *et al.* (1998). Isometamidium treatment and days post treatment were fitted as fixed effects. Study site was fitted as a random effect.

7.2.5 Analysis of in-vivo trypanocidal drug sensitivity data for *T. congolense* tested in mice

Mice that went into remission following trypanocidal drug treatment and remained aparasitaemic for the remainder of the 60-day follow up period were regarded as cured. Mice that did not go into remission, or that relapsed were regarded not to be cured. For each stabilate, the number of mice that were cured with each trypanocidal drug dose used was determined and expressed as a percentage of number of mice in each dose group. The percentage of infected mice cured was used to estimate whether or not a particular drug dose was greater than or equal to the dose required to cure 80% (CD_{80}). If 80% or more of the mice in a treatment group were cured, the drug dose used to treat the group was considered to be greater than or equal to the CD_{80} for the stabilate in question.

The classification of level of drug sensitivity or resistance of *T. congolense* stabilates is shown in Table 42. Stabilates of *T. congolense* for which 80 % or more mice were cured by the lowest dose of isometamidium (0.1 mg/kg b.w.) or diminazene (1.0 mg/kg b.w.) were classified as highly sensitive (SS) to the drug used. Stabilates for which 80 % or more mice were cured by the intermediate dose of isometamidium (1.0 mg/kg b.w.) or diminazene (20 mg/kg b.w.), but not by the lowest dose were classified as sensitive (S) to the drug used. Stabilates for which 80 % or more mice were cured by the highest dose of isometamidium (10 mg/kg b.w.) or diminazene (40 mg/kg b.w.), but not by the intermediate dose were classified as resistant (R) to the drug used. Stabilates for which fewer than 80% of mice were cured by the highest dose of isometamidium (10 mg/kg b.w.) or diminazene (40 mg/kg b.w.) were classified as highly resistant (RR) to the drug used.

Table 42: Classification of drug resistance testing of *T. congolense* in mice

	Dose (mg/kg b.w.)		
Isometamidium chloride	0.1	1.0	10
Diminazene aceturate	1.0	20	40
Classification:			
Highly sensitive (SS)	≥ CD80[1]	≥ CD80	≥ CD80
Sensitive (S)	< CD80[2]	≥ CD80	≥ CD80
Resistant (R)	< CD80	< CD80	≥ CD80
Highly Resistant (RR)	< CD80	< CD80	< CD80

[1] ≥ CD80: at least 80% of mice cured
[2] < CD80: fewer than 80% of mice cured

7.3 Results

7.3.1 Trypanosome stabilates

A total of 665 trypanosome stabilates were prepared during the observational studies at Kubo and Matuga (1996) and the longitudinal studies at Buda, Gazi, Kibaoni, and Kikoneni (1997 – 1998). Four hundred and twenty two stabilates (63.5%) were prepared from cattle infected with *T. congolense*, 227 stabilates (34.1 %) were prepared from cattle infected with *T. vivax* and sixteen stabilates (2.4 %) were prepared from cattle with mixed *T. congolense* and *T. vivax* infection. Consequently, a total of 437 (65.7 %) of the 665 (65.7 %) stabilates contained *T. congolense* and a total of 243 (36.5 %) contained *T. vivax* trypanosomes (Table 43).

7.3.2 Viability of *T. congolense* and *T. vivax* stabilates

Two hundred and nineteen of the 665 stabilates (32.9 %) were found to be viable on the basis of motility (Table 43). Three of the viable stabilates had a mixture of motile *T. congolense* and *T. vivax* . Very similar proportions of *T. congolense* stabilates (142/ 438; 32.4%) and *T. vivax* stabilates (80/243; 32.9%) were viable.

7.3.3 Infectivity of trypanosome stabilates for mice and cattle

7.3.3.1 Infectivity of T. congolense *stabilates for mice*

Of the 437 *T. congolense* stabilates collected, only 39 (8.9 %) established infections in mice (Table 43). This represented 27.5 % of the *T. congolense* stabilates (142) that were viable in terms of motility. Slight differences in infectivity for mice were observed between *T. congolense* stabilates from different sites, but the differences were not significant ($p > 0.1$).

7.3.3.2 Infectivity of T. vivax *stabilates for cattle*

Sixty *T. vivax* stabilates that were viable in terms of motility were inoculated into 26 calves. Seven calves were each inoculated with a different pool of five stabilates, and 3 calves were each inoculated with a different pool of 3 stabilates. Each pool comprised stabilates from a single site.

Very few *T. vivax* were found to be infective for cattle. Parasitaemia was detected in only 4 (15.4%) of the 26 calves. Of these parasitaemias, 3 were *T. vivax* and one was *T. congolense.* All four of these calves had been inoculated with a single stabilate, each from a different study site. One additional stabilate inoculated intravenously resulted in a parasitaemia that was observed on days one and three, but never again for the 100 days of follow up. None of the pooled stabilates resulted in a detectable parasitaemia.

Table 43: Summary of trypanosome isolates from Kwale District, including viability and infectivity for mice

	No. of stabilates	No. viable[1]	% viable	No. infective in mice	% infective in mice[2]
T. congolense	422	139	32.9	39	27.5
T. vivax	227	77	33.9	NA[3]	NA
Mixed infection	16	3	18.7	0	0
Total	665	219	32.9	39	27.5

[1]Stabiliates were classed as viable if normal motility was observed in a wet film
[2] Percentage of viable *T. congolense* isolates found to be infective for mice
[3]NA: not attempted

7.3.4 Factors influencing viability and infectivity of trypanosome stabilates from Kwale District

7.3.4.1 Effect of trypanosome species on viability and infectivity of trypanosome stabilates

The viability of stabilates of the two trypanosome species, *T. congolense* (32.6 %) and *T. vivax* (32.9%) were very similar, and the difference was not significantly different ($\chi^2 = 4.25$; $p>0.1$).

7.3.4.2 Effect of isometamidium treatment on viability and infectivity of trypanosome stabilates

The effect of isometamidium treatment and number of days after treatment on the viability of stabilates and infectivity of stabilates for mice were investigated using mixed models. The treatment group (isometamidium-treated or sentinel) of the ox from which the stabilate was obtained, and the number of days since the most recent isometamidium treatment were fitted as fixed effects. The study site was fitted as a random effect. The analyses were conducted for the four sites where longitudinal studies were conducted, Buda, Kibaoni, Kikoneni and Gazi. Data from stabilates collected on days of isometamidium treatment were excluded from the models.

7.3.4.2.1 Viability results

Eighty-eight out of two hundred and fifty six (33.9 %) stabilates from isometamidium-treated cattle were viable. While 32% (121/369) stabilates from sentinel cattle were viable.

Neither the effects of treatment group ($p = 0.51$) and days post treatment ($p = 0.27$), nor their interaction ($p = 0.59$) were significant. There was a slight increase in the viability of stabilates from isometamidium-treated cattle with increasing numbers of days post treatment, but this was not significant ($p = 0.59$).

7.3.4.2.2 Infectivity results

Twenty out of one hundred and seventy (11.8 %) *T. congolense* stabilates from isometamidium-treated cattle established an infection in mice. While 7.1 % (19/267) *T. congolense* stabilates from sentinel cattle established an infection in mice.

Neither the effects of treatment group ($p = 0.91$) and days post treatment ($p = 0.95$), nor their interaction ($p = 0.54$) had any significant effects on infectivity of stabilates for mice. There was a slight decrease in the infectivity of stabilates from isometamidium-treated cattle with increasing numbers of days post treatment, but this was not significant ($p = 0.54$).

7.3.4.3 Effect of isometamidium concentration on viability and infectivity of trypanosome stabilates

The influence of isometamidium concentration on the viability and infectivity of *T. congolense* stabilates were investigated using a logistic regression model. Isometamidium concentration and trypanosome species was fitted as the explanatory variables.

Isometamidium concentration had a marginal effect ($p=0.05$) on the viability of stabilates. The viability of stabilates in term of motility decreased with increase in isometamidium concentration. There was no significant difference ($p> 0.05$) on effect of isometamidium concentration between *T. congolense* and *T. vivax.* However, there appears to be a greater loss of viability with increasing isometamidium concentration for *T. vivax* than for *T. congolense.*

7.3.4.4 Effect of route of inoculation on infectivity of T. vivax stabilates for cattle

Of the 16 cattle that were inoculated with a single *T. vivax* stabilate, 3 were inoculated subcutaneously, one was inoculated both intravenously and subcutaneously, and the remainder ($n = 12$) were inoculated intravenously. All stabilates that were inoculated subcutaneously or both subcutaneously and intravenously resulted in parasitaemia that persisted until treated with isometamidium at 0.5 mg/kg b.w., whereas none of the stabilates injected only by the

intravenous route resulted in persistent parasitaemia. The difference in outcome depending on whether or not the subcutaneous route of inoculation was used was highly significant (Fisher's exact test: $P < 0.001$).

7.3.5 *In vivo* sensitivity of trypanosome isolates to trypanocidal drugs

7.3.5.1 Sensitivity of T. congolense *isolates from Kwale District to trypanocidal drugs in mice*

Thirty nine *T. congolense* stabilates from the two observational study areas in Kwale District, Kubo and Matuga, and from the four longitudinal study sites in Kwale District, Buda, Kibaoni, Kikoneni and Gazi were tested in mice for sensitivity to isometamidium chloride and diminazene aceturate (Tables 44 and 45).

Thirty-six (92.3%) of the 39 stabilates showed evidence of resistance to isometamidium chloride in mice. Twenty-three (59%) were resistant to the highest dose of isometamidium chloride (10 mg/kg b.w.), and hence were classified as highly resistant (RR). A further 13 stabilates were resistant to the intermediate dose of isometamidium chloride (1.0 mg/kg b.w.) and hence were classified as resistant (R). The remaining 3 stabilates were sensitive to the lowest dose of isometamidium chloride (0.1 mg/kg b.w.), and hence were classified as highly sensitive (SS).

Isometamidium treatment did not have a significant ($\chi 2 = 12.01$; $p = 0.151$) effect on the level of resistance to isometamidium expressed by the isolates.

Thirty-four (87.2%) of the 39 stabilates showed evidence of resistance to diminazene aceturate in mice. Thirty-two (82.1%) were resistant to the highest dose of diminazene aceturate (40 mg/kg b.w.), and hence were classified as highly resistant (RR). A further 2 stabilates were resistant to the intermediate dose of diminazene aceturate (20 mg/kg b.w.) and hence were classified as resistant (R). Three stabilates were resistant to only the lowest dose of diminazene aceturate (1.0 mg/kg b.w.), and hence were classified as sensitive (S). The remaining 2 stabilates were sensitive to the lowest dose, and hence were classified as highly sensitive (SS). Isometamidium treatment did not have a significant ($\chi^2 = 2.81$; $p = 0.946$) effect on the level of resistance to diminazene expressed by the isolates.

Table 44: Sensitivity of *T. congolense* isolates from Kwale District to isometamidium chloride in mice

Level of resistance	Isometamidium-treated cattle		Sentinel cattle		All cattle	
SS[1]	0	(0)[5]	3	(15.8)	3	(7.7)
S[2]	0	(0)	0	(0)	0	(0)
R[3]	3	(15)	10	(52.6)	13	(33.3)
RR[4]	17	(85)	6	(31.6)	23	(59.0)
Total	20	(100)	19	(100)	39	(100)

[1]SS: Highly sensitive: sensitive to 0.1 mg/kg b.w.
[2]S: Sensitive: sensitive to 1.0 mg/kg b.w. (resistant to 0.1 mg/kg b.w.)
[3]R: Resistant: resistant to 1.0 mg/kg b.w.; sensitive to 10 mg/kg b.w.
[3]R: Highly resistant: resistant to 10 mg/kg b.w.
[5]Figures in parentheses are percent of total for each category of cattle.

Table 45: Sensitivity of *T. congolense* isolates from Kwale District to diminazene aceturate in mice

Level of resistance:	Isometamidium-treated cattle		Sentinel cattle		All cattle	
SS[1]	0	(0)[5]	2	(10.5)	2	(5.1)
S[2]	1	(5)	2	(10.5)	3	(7.7)
R[3]	1	(5)	1	(5.3)	2	(5.1)
RR[4]	18	(90)	14	(73.7)	32	(82.1)
Total	20	(100)	19	(100)	39	(100)

[1]SS: Highly sensitive: sensitive to 1.0 mg/kg b.w.
[2]S: Sensitive: sensitive to 20 mg/kg b.w.; resistant to 1.0 mg/kg b.w.
[3]R: Resistant: resistant to 20 mg/kg b.w.; sensitive to 40 mg/kg b.w.
[3]R: Highly resistant: resistant to 40 mg/kg b.w.
[5]Figures in parentheses are percent of total for each category of cattle.

7.3.5.2 *Multiple resistance of* T. congolense *isolates from Kwale District to trypanocidal drugs in mice*

Resistance to the highest doses of both drugs was observed in stabilates from all 6 study areas in Kwale District, and in both isometamidium-treated and sentinel cattle (Tables 44, 45 and 46).

Thirty-four of the thirty-nine (87.2%) *T. congolense* stabilates tested showed resistance to the intermediate doses of both isometamidium (1.0 mg/kg b.w.) and diminazene (20 mg/kg b.w.). Of these 34 *T. congolense* stabilates, 21 (53.8% of the total of 39) showed resistance to the highest doses of both isometamidium (10 mg/kg b.w.) and diminazene (40 mg/kg b.w.). Only 2/39 (5.1%) stabilates were sensitive to the lowest doses of both drugs (0.1 mg/kg b.w. isometamidium and 20 mg/kg b.w. diminazene).

There was a significant association between isometamidium resistance and diminazene resistance in *T. congolense* isolates from all cattle ($\chi^2 = 22.1$, $p < 0.001$), and in *T. congolense* isolates from sentinel cattle ($\chi^2 = 13.4$, $p < 0.001$).

Table 46: Multiple drug resistance in *T. congolense* isolates from Kwale District

Level of resistance	Isometamidium-treated cattle		Sentinel cattle		All cattle	
SS[1]	0	(0)[6]	2	(10)	2	(5.1)
≤S[2]	0	(0)	3	(15)	3	(7.7)
≥R[3]	19	(100)	15	(75)	34	(87.2)
RR[4]	15	(78.9)	6	(30)	21	(53.8)
Total[5]	19	(100)	20	(100)	39	(100)

[1]SS: Highly sensitive: sensitive to both 0.1 mg/kg b.w. isometamidium and 1.0 mg/kg b.w. diminazene

[2]≤S: Sensitive: sensitive to both 1.0 mg/kg b.w. isometamidium and 20 mg/kg b.w. diminazene

[3]≥R: Resistant: resistant to both 1.0 mg/kg b.w. isometamidium and 20 mg/kg b.w. diminazene

[4]RR: Highly resistant: resistant to both 10 mg/kg b.w. isometamidium and 40 mg/kg b.w. diminazene

[5]Total: the total number of stabilates tested for each category of cattle (NB. This is not the total for the column.)

[6]Figures in parentheses are percent of total for each category of cattle.

7.3.5.3 *Sensitivity of* T. congolense *isolates from observational study areas in Kwale District to trypanocidal drugs in mice*

7.3.5.3.1 Kubo Division

All four *T. congolense* isolates from Kubo that were tested in mice were resistant to both trypanocides. Three were highly resistant (RR) to both, and one was highly resistant (RR) to diminazene aceturate.

7.3.5.3.2 Matuga Division

All five *T. congolense* isolates from Matuga that were tested in mice were highly resistant (RR) to both trypanocides.

7.3.5.4 *Sensitivity of T. congolense isolates from longitudinal study sites in Kwale District to trypanocidal drugs in mice*

7.3.5.4.1 Buda

All 7 *T. congolense* isolates from Buda tested in mice were from sentinel group cattle. One of these isolates was sensitive (S) to both drugs, and two were highly sensitive (SS) to both drugs. The remaining 4 isolates were resistant to both drugs, one being highly resistant (RR) to both, and the other 3 resistant (R) to isometamidium chloride, and highly resistant (RR) to diminazene aceturate.

7.3.5.4.2 Kibaoni

Fourteen *T. congolense* isolates from Kibaoni were tested in mice. All fourteen isolates were resistant to both drugs. Seven isolates were highly resistant (RR) to both drugs. Three of the remaining seven were highly resistant (RR) to isometamidium chloride, and three others were highly resistant (RR) to diminazene aceturate.

7.3.5.4.3 Kikoneni

The one *T. congolense* isolate from Kikoneni that was tested in mice was highly resistant (RR) to both trypanocides.

7.3.5.4.4 Gazi

Eight *T. congolense* isolates from Gazi were tested in mice. Of these eight isolates, two were sensitive (S), but not highly sensitive, to diminazene aceturate. One of these two isolates was resistant (R), and the other highly resistant (RR) to isometamidium chloride. The other six isolates from Gazi were resistant to both drugs. Four of these six were highly resistant (RR) to both drugs, one was highly resistant (RR) to isometamidium chloride, and one was highly resistant (RR) to diminazene aceturate.

7.3.5.5 Sensitivity of T. vivax *isolates from Kwale District to trypanocidal drugs in cattle*

Four stabilates grew in cattle and were treated with isometamidium at a dose of 0.5 mg/kg b.w. The sensitivity to isometamidium and diminazene of *T vivax* is shown in Table 47. One stabilate was sensitive to isometamidium at a dose of 0.5 mg/kg b.w. Three stabilates demonstrated resistance to isometamidium at 0.5 mg/kg b.w. Out of these three one also demonstrated resistance to diminazene at 7.0 mg/kg b.w. The remaining two were sensitive to diminazene at 7.0 mg /kg b.w. In addition, one of the two stabilates demonstrated resistance to diminazene at 3.5 mg/kg b.w.

Table 47: Sensitivity of *T. vivax* isolates from Kwale District to isometamidium and diminazene in cattle

Site	Route of infection	Pre-patent period (days)	ISMM Treatment (0.5 mg/kg b.w)	DIM Treatment (mg/kg b.w)
Buda	s.c[1]	10	-ve 3 (2 DPT)	NA
Kibaoni	s.c	10	Relapsed (22 DPT)	3.5 mg/kg b.w. [4]
Gazi	s.c/i.v[2]	18	Relapsed (55 DPT)	7.0 mg/kg b.w[5]
Kubo	s.c	18	Relapsed (67 DPT)	7.0 mg/kg b.w[6]

[1]s.c : subcutaneous

[2]i.v : intravenous

-ve [3] : the animal became aparasitaemic two days after isometamidium treatment at 0.5 mg/kg b.w. and did not relapse during the follow up period

[4]The trypanosomes were resistant to diminazene at a dose of 3.5 mg/kg b.w., but the animal was cured at 7.0 mg/kg b.w

[5]This animal relapsed after treatment with diminazene at a dose of 7.0 mg/kg

[6]This animal was sensitive to diminazene at a dose of 7.0 mg/kg

DPT: Days post isometamidium treatment

ISMM: Isometamidium

DIM: diminazene

7.3.5.6 *Factors influencing sensitivity of* T. congolense *isolates from Kwale District to trypanocidal drugs in mice*

The factors influencing sensitivity of *T. congolense* isolates from Kwale District to trypanocidal drugs in mice were investigated using mixed models. Isometamidium treatment and days post treatment were fitted as fixed effects. Study site was fitted as a random effect. Serum isometamidium concentrations corresponding to the various levels of resistance is shown in Figure 39. There was a tendency for the highly resistant isolates to be associated with higher isometamidium concentrations. However the difference between the resistant (R) and the highly resistant (RR) was not significant.

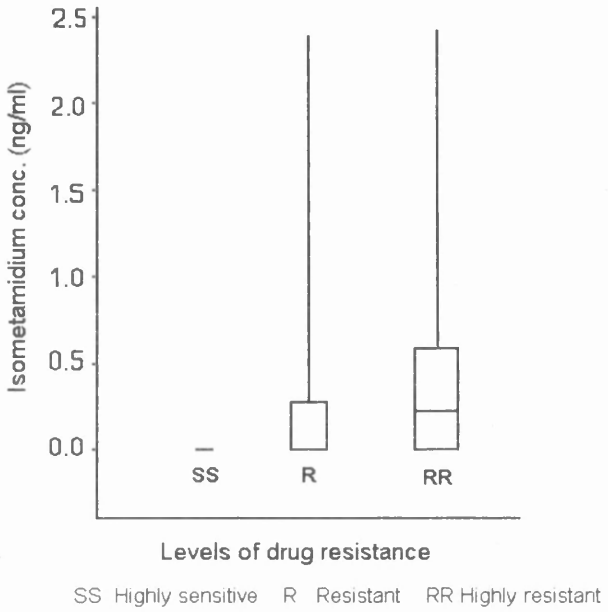


Figure 39: Serum isometamidium concentrations in cattle from which *T. congolense* with various levels of resistance to isometamidium were isolated in Kwale District (In this box-and whisker diagram, there is a box that spans the inter-quartile range of the values in the variate, with a line indicating the median. Whiskers are drawn beyond the ends of the box as far as the minimum and maximum values)

7.4 Discussion

In the work described in this chapter, the viability and infectivity of 665 *T. congolense* and *T. vivax* field stabilates collected during observational and longitudinal studies in Kwale District were assessed. Thereafter, the infectivity of viable *T. congolense* stabilates was assessed in mice, and the infectivity of viable *T. vivax* field stabilates was assessed in cattle. The *in vivo* sensitivities of trypanosome isolates to trypanocidal drugs were also determined in the same hosts.

Factors associated with viability and infectivity of *T. congolense* field stabilates in mice and of *T. vivax* field stabilates in cattle were assessed. Finally, *in vivo* trypanocidal drug sensitivities of trypanosome isolates were compared to field data collected during observational and longitudinal studies in Kwale district.

Of the 665 stabilates prepared 32.9 % were viable in terms of motility. These stabilates were collected over 3 years. There appeared to be difference in viability of the stabilates between years. This difference may have been caused by several factors including, variations in trypanosome populations over the years and methods of stabilate preparations.

Ideally the *T. congolense* stabilates to be tested in mice would have been selected to represent equally, populations collected from isometamidium-treated and sentinel cattle at each study site during the early and late post-treatment period but because of the low viability of many of the trypanosome stabilates, this was not possible.

Of the viable *T. congolense* stabilates (142), 27.5 % produced infections in mice. In cattle, four of the 60 viable *T. vivax* stabilates established parasitaemia.

Infectivity of *T. congolense* isolates from Kilifi for mice was observed to be 90.1 % (ILRAD, 1986).

In the present study the low infectivity for mice compared to that of stabilates from Kilifi may be due to the fact the stabilates from Kilifi were inoculated in mice before cryopreservation. In this case the infectivity for mice is mainly dependent on the trypanosome population being biologically capable of infecting mice. However,

when looking at the infectivity for mice or cattle of viable stabilates (after cryopreservation), as was the case in this study, the following influencing factors would be considered;

Firstly, viability (motility) which means that the trypanosomes are alive, but they may not be capable of reproducing in any host, i.e. they may no longer be infective. Moreover, the trypanosome population may theoretically be infective for a host e.g. mice, and the stabilate may be viable. Nevertheless, it may have lost the ability to reproduce as a result of damage sustained during the stabilisation and storage process. Probably this is what occurred to the *T. vivax* that had been isolated from cattle in the field in the present study. Although the *T. vivax* obtained from the field were infective for cattle, after the process of stabilisation and storage they were not capable of sustaining parasitaemia in cattle

Secondly, if a stabilate does infect mice, it means that the trypanosomes are normal and that the population is infective for mice. If it does not infect mice, it may be either not infective for mice as a host species, or no longer capable of replication, or both. This was observed when *T. congolense* infected blood was left in a vacutainer at room temperature for a week, the trypanosomes were viable (normal motility) for 6 days, but were only infective for mice on the first three days.

In carrying out sensitivity studies in mice, an important assumption is that the trypanosome population established is similar to the population that occurred in the bovine host. Consequently, it is assumed that the levels of resistance attributed to the tested isolates reflect accurately the levels of resistance of the original isolates.

Thirty four (87.2 %) of 39 *T. congolense* stabilates expressed multiple resistance to both isometamidium and diminazene in mice. In addition, one of the stabilates that established an infection in cattle was resistant in that host to both isometamidium and diminazene at 0.5 and 7.0 mg/kg b.w respectively.

The high resistance expressed by *T. congolense* isolates to isometamidium and diminazene in mice represents strong evidence of multiple drug resistance in Kwale District. Similar evidence of multiple drug resistance *T. congolense* in Kwale was observed by Gitatha, (1979). He reported on *T. congolense* stabilates from Shimba Hills that expressed resistance to both isometamidium at 2.0 mg/kg b.w. and

diminazene at 10.5 mg/kg b.w. in cattle. In addition, Schonefeld *et al.* (1987) has described existence of multiple drug resistance of *T. vivax* strains along the coast of Kenya and Somalia.

Elsewhere, persistence of multiple drug resistant populations of *T. congolense* has been reported (Mulugeta *et al.*, 1997). In the Ghibe valley in Ethiopia multiple drug resistant infections at clonal level were still present after a four-year period. (Codjia *et al.*, 1993; Mulugeta *et al.*, 1997). The use of sanative pair of drugs at Ghibe Valley is therefore not expected to work, requiring the use of other strategic control measures.

In the present study, it would appear therefore, that multiple drug resistant populations of *T. congolense* are still widespread in Kwale. However, this resistance did not appear to be at individual trypanosome level. In the multiple resistant *T. congolense* obtained from Shimba Hills, isometamidium chloride was considered as "sanative" at 0.4 mg/kg, while diminazene aceturate was considered as "sanative" at large doses when used repeatedly (Gitatha 1979). This raises hopes of being able to use the "sanative pair" of drugs for chemotherapy and chemoprophylaxis on resistant trypanosome populations in Kwale. However, a more appropriate solution to delay or stop further development of drug resistance would be to reduce the number of drug treatments used, by integrating drug use with other control measures (Geerts and Holmes, 1998).

The significant ($p < 0.001$) association observed between isometamidium resistance and diminazene resistance in *T. congolense* isolates may indicate that the multiple resistance of the isolate is due to cross resistance. Peregrine *et al.* (1997) observed that a 94-fold increase in induced resistance to isometamidium in *T. congolense* clone (Clone IL1180) was associated with a 3.4-, 33-, and 4.2 –fold increases in resistance to diminazene, homidium and quinapyramine respectively. In addition, when multiple resistant isolates were first observed in Kwale, drugs that had been used in cattle in that area were quinapyramine, homidium bromide and diminazene aceturate, isometamidium had never been used before yet isolates obtained from that area expressed resistance to isometamidium at 1 mg/kg b.w. (Gitatha, 1979).

In the present study, the evidence of drug resistance obtained would suggest that drug resistance is a significant contributing factor to the trypanocidal treatment failure that has been observed in Kwale district.

Maloo (1993) reported failure of isometamidium prophylaxis in zero grazing dairy cattle in Muhaka and Matuga, and recommended reduction of the recommended prophylaxis period to 45 days. He concluded that drug resistance might be one of the factors.

On the relationship between isometamidium concentrations circulating at the time trypanosome infection was detected and the trypanocidal drug sensitivity in mice of the infecting population, the following was observed; Firstly, all trypanosome populations found to be sensitive to isometamidium in mice were isolated from cattle without evidence of detectable circulating concentrations of the drug. Secondly, all trypanosome populations isolated from cattle with detectable isometamidium concentration were resistant to the drug in mice. Finally, trypanosome populations isolated from cattle with the highest drug concentrations tended to show the highest levels of drug resistance.

These findings, further confirm the hypothesis behind the isometamidium ELISA technique (Eisler, 1994, 1996a). The drug ELISA technique may not identify the sensitive stabilates because at the low level < 0.4 ng/ml (Eisler, 1996) both drug sensitive and drug resistance trypanosomes may exist. However, trypanosomes detected at circulating isometamidium levels greater than 0.4 ng/ml would indicate that infecting populations exhibit some degree of resistance. Thus drug ELISA could be used to select the stabilates to be used in mice and cattle sensitivity tests (Eisler, 1996a).

Trypanosoma congolense stabilates obtained from five of the six sites expressed multiple resistance of the highest level to isometamidium (10 mg/kg b.w.) and diminazene (40 mg/kg b.w.) in more than 50 % the stabilates tested in mice. These sites are between 20 and 70 Km apart. It is apparent that there exist various pockets of *T. congolense* resistant populations within Kwale District. These pockets are localised within the scattered Kaya forests (see Chapter 1) which previously formed part of the greater Shimba Hill forest (Snow and Tarimo 1983). With the increase in human population, parts of the forest were cleared for human settlements

leaving isolated pockets of forest which are foci for semi-isolated tsetse populations (Snow *et al* 1988). The sites from which the stabilates tested for drug sensitivity were obtained are each situated in the proximity of these isolated forests.

Buda was the only site where highly sensitive *T. congolense* stabilates (3 out of 39 stabilates) to both drugs were found alongside resistant stabilates. In addition, the only *T. vivax* isolate that was sensitive to isometamidium at 0.5 mg/kg b.w. in cattle was obtained from this site. Furthermore, in the longitudinal studies, Buda was the site that showed evidence of correct drug administration (see chapter 6). It would appear that the resistant isolates observed in Buda might have developed after many years of drug use at sub-curative level before the beginning of this study (Nyeko, 1988).

Prior to the intervention of this study at the farm in Buda, the animal attendant had been treating the animals with isometamidium at approximately 0.5 mg/kg b.w. Body weights were estimated by eye, as is the common practice of farmers and animal attendants in the area. However, during the two-year study period isometamidium was used at 1 mg/kg b.w. and diminazene at 7.0 mg/kg b.w. This may have selectively reduced the trypanosome population infecting the cattle that were resistant to 3.5 mg/kg b.w. and 0.5 mg/kg b.w. of diminazene and isometamidium respectively to very low levels (Mamman *et al.*, 1995a).

In, Gazi and Kibaoni, the high level of resistance expressed in mice and cattle by *T. vivax* and *T. congolense* stabilates could be as a result of prolonged incorrect drug use at sub-therapeutic doses. Results from the longitudinal studies showed that trypanocidal drugs at these two sites were administered at doses lower that that recommended. (see Chapter 6). Likewise, at Kubo and Matuga, the highly resistance *T. congolense* and *T. vivax* population from these sites could have developed over the years due to prolonged drug use Maloo, (1993).

The following factors that might have influenced the viability of stabilates collected from the field were studied namely, isometamidium treatment and the resulting circulating concentrations of the drug and the trypanosome species.

No significant differences in viability were observed between stabilates from isometamidium-treated and sentinel cattle. Since isometamidium is trypanocidal, an

increase in the viability of stabilates from isometamidium-treated cattle might be expected with increasing numbers of days post treatment and falling isometamidium concentrations. The viability of stabilates from untreated sentinel cattle might also be expected to be higher than that of stabilates from isometamidium-treated cattle. However this was not observed, suggesting that either isometamidium was not correctly administered or the trypanosomes were resistant to the drug.

The length of time after isometamidium treatment is not the only determinant of circulating concentrations. Individual variation among cattle in terms of serum isometamidium concentration has been observed previously (Eisler *et al.*, 1996b), and in the present work, one week after isometamidium treatment, drug concentrations varied between 8.41ng/ml and 1.28 ng/ml in Galana Borans and between 1.44 ng/ml and 4.45 ng/ml in mixed breed dairy cattle. The viability of trypanosome stabilates, in terms of motility, decreased with increased circulating isometamidium concentration at the time of stabilate preparation, although this effect was not statistically significant.

The infecting trypanosome species had no effect on the viability of stabilates. This was in contrast to the large difference in infectivity of *T. congolense* for mice and *T. vivax* for cattle.

There was no significant difference in the infectivity for mice between stabilates obtained from isometamidium-treated and sentinel cattle. Probably the fact that only viable stabilates were inoculated in mice masked the effect of isometamidium treatment.

Infectivity for cattle of the *T. vivax* stabilates was very poor. None of ten cattle infected via the intravenous route with pools of 3 or 5 stabilates became parasitaemic. Out of the 26 cattle inoculated with *T. vivax*, an infection was established in only four. These were the only four cattle of 16 inoculated with individual *T. vivax* stabilates to be inoculated using the subcutaneous route. Although the numbers involved were small, the effect of using this route of inoculation was highly significant ($p < 0.001$). These findings agree with what happens in a natural tsetse transmitted infection. Trypanosomes are inoculated into the skin with the formation of a chancre from where multiplication takes place followed by distribution to the blood stream and tissues (Emery *et al.*, 1980).

It appears that while the *T. vivax* may be infective to cattle of poor immune status in the field, they may not be able to readily infect an experimental animal in good condition.

In conclusion, evidence of *T. congolense* and *T. vivax* populations that expressed multiple resistance to isometamidium and diminazene in Kwale was obtained. In addition, when carrying out drug sensitivity tests of *T. vivax* in cattle the subcutaneous inoculation of the viable stabilates was observed to be the route that would give better results.

Finally, it is recommended that; firstly, whenever field studies or surveys are carried out to assess distribution and incidence of drug resistance, the cryopreservation of stabilate should be conducted meticulously. In addition, the factors associated with viability and infectivity discussed above should always be taken into consideration when planning such studies.

Secondly, the methodology for sensitivity tests in mice used in this study should be the adopted for use in later surveys for determination of drug resistance in field isolates.

Thirdly, the isolates that expressed multiple resistance in the present study should to be cloned to ascertain whether the multiple resistance observed is expressed at the level of the individual trypanosome. The results obtained herewith can be used to make decisions of the best control strategy for trypanosomiasis in Kwale District.

Fourthly, with the current situation of drug resistance, proper policies and guidelines regarding drug availability and use in the field should be put in place.

Lastly, research findings and recommendations from National and International institutions concerning current trends and measures in disease management should be made available to the various Agricultural and Veterinary advisory boards and committees (which should incorporate committed scientists) of the affected countries.

Chapter 8

General Discussion, Recommendations and Future Work

8 General Discussions

8.1 Discussion

In this thesis a series of epidemiological studies into the impact of trypanocidal drug resistance on the control of trypanosomiasis in the coastal area of Kenya are described. These studies were aimed at evaluating the efficacy of chemoprophylaxis and chemotherapy of the drugs used most commonly in endemic areas for the control of bovine trypanosomiasis namely, isometamidium chloride and diminazene aceturate. In addition, factors leading to, or associated with, prophylaxis failure and/or treatment failure were investigated. Finally, the various studies reported in this thesis attempted to assess the extent to which trypanocidal drug resistance influences chemoprophylaxis and chemotherapy under different management systems.

One of the most important findings from the work conducted was that drug failure was observed in many of the prophylaxis studies at the Kenyan coast However, isometamidium and diminazene were effective in controlling bovine trypanosomiasis under some circumstances.

Failure of prophylaxis or treatment can usually be attributed to improper drug administration and especially, under-dosing because of inability to estimate body weight accurately and/or the use of generic or counterfeit products, which may have reduced efficacy (Geerts and Holmes, 1998).

Ultimately, the development of drug resistance is the final result of mis-management of chemoprophylaxis and chemotherapy. Factors responsible for the development of resistance are not well described. However, the exposure of parasites to sub-therapeutic drug concentrations has been considered as an important factor for development of resistance (Whiteside, 1962).

The findings in the present study are consistent with these ideas. In the longitudinal studies in Kwale evidence of under-dosing leading to sub-therapeutic isometamidium levels was provided in cattle treated by untrained farmers (at

Kibaoni) and a trained Livestock Animal Health Assistant (at Gazi). In addition, evidence of prophylaxis failure was demonstrated by the inability of isometamidium to confer adequate protection against trypanosome challenge in cattle at these two sites. Furthermore, drug sensitivity tests in mice of *T. congolense* isolates from Gazi and Kibaoni demonstrated multiple drug resistance at the highest doses of isometamidium (10 mg/kg b.w.) and diminazene (40mg/kg b.w.) used. Similar tests in cattle showed that a *T. vivax* isolate and a *T. congolense* isolate from Kibaoni and Gazi respectively expressed resistance to isometamidium at 0.5 mg/kg b.w. and diminazene at 3.5 (*T. vivax*) and 7.0 (*T. congolense*) mg/kg b.w.

Three of the methods described by Geerts and Holmes, (1998) for the detection of drug resistance, namely sensitivity tests in ruminant (cattle), tests in mice and drug ELISA were used in the present studies to establish the presence of drug resistance in the coastal region of Kenya.

The isometamidium ELISA described by Eisler *et al.*, (1993, 1996a) was used to relate circulating isometamidium levels at the time of parasite detection to evidence of the absence or presence of drug resistance. At all the sites described in this thesis the majority of trypanosome breakthrough infections were considered to exhibit some degree of resistance. Mice and cattle sensitivity tests (Chapter 7) were able to support the findings of isometamidium ELISA, particularly in Kwale District. Approximately 87% of *T. congolense* isolates from five sites in Kwale expressed multiple drug resistance in mice. No drug sensitive isolate was obtained from an animal in which significant levels of isometamidium were present. Similarly, all isolates found in cattle in which significant quantities of the drug were detected were resistant to it in mice. Finally, there was a tendency for the isolates from cattle with the highest drug concentrations to show the greatest level of resistance in mice.

At many of the sites covered in the present study drug resistance to a single or multiple drugs had been previously suggested to be the likely cause of treatment failure. At Galana Ranch, Rottcher and Schillinger, (1985) described *T. vivax* infections that expressed high level of multiple drug resistance to isometamidium, diminazene aceturate, homidium and quinapyramine. At the same location, Dolan *et al.* (1992) attributed failure of isometamidium prophylaxis against *T. vivax* to drug resistance. In addition, reduced sensitivity to isometamidium of a stock of *T. vivax*

from Galana Ranch was demonstrated in experimental fly challenge studies where the drug afforded protection for 1 month or less (Peregrine *et al.*, 1991).

At Kubo and Matuga, Maloo, (1993) attributed early breakthrough infection in isometamidium treated dairy cattle to drug resistance.

At Buda, Kikoneni, Gazi and Kibaoni no previous work on sensitivity of infections to trypanocidal drugs has been reported. However, reports from nearby areas have demonstrated the presence of *T. congolense* isolates expressing resistance to isometamidium, diminazene, homidium and quinapyramine (Gitatha, 1979) and *T. vivax* isolates expressing resistance to prophylactic activities of isometamidium at 1.0 mg/kg b.w. in cattle (Schonefeld *et al.*, 1987).

The findings of the present study and earlier studies at the Kenya coast on the presence of *T. vivax* and *T. congolense* populations that express multiple resistance to the trypanocides currently used in cattle raises concern on the future role of chemotherapy and chemoprophylaxis of trypanosomiasis in the region and indeed in other trypanosomiasis endemic areas. There is hope however, in that the proportion of the total trypanosome population that are resistant forms may be small (Mamman *et al.*, 1995a). Moreover, when multiple drug resistance occurs in mixed infections, rather than at the clonal level, administration of various drugs to which sub-populations are sensitive, may eliminate the whole trypanosome population (Mulugeta *et al.*, 1997). Furthermore, the impact of resistant populations is only felt during periods of exceptionally high tsetse and trypanosome challenge when treatment failure and increasing mortality cases are observed (Chapters 5 and 7; Dolan *et al.*, 1992).

In the coastal region of Kenya, at the moment, evidence of multiple resistance shows that drug resistance is at the population level, and therefore the use of the "sanative pair" of drugs to alleviate the problem may still be applicable. Previously, isometamidium and diminazene were used effectively as "sanative" drugs on multiple resistant *T. congolense* isolates from Shimba Hills (Gitatha 1979). It should be determined in further work whether or not multiple trypanocidal drug resistance detected in isolates from the present study is expressed at the clonal level. This would indicate whether the use of "sanative" drugs is still feasible or whether other control measures would have to be included, as was the case in Ghibe valley,

Ethiopia (Swallow *et al.*, 1993). In *T. congolense* isolates from Ghibe, multiple resistance was expressed at the level of the individual trypanosome (Codjia *et al.*, 1993; Mulugeta, *et al.*, 1997). In that case, integration of tsetse control using insecticide treated targets with continued drug use was found to provide a satisfactory solution.

The drug resistance situation at the coast of Kenya observed in the present study is not unique. According to Geerts and Holmes (1998), resistance to one or more of the three trypanocidal drugs used in cattle is present in at least 13 other countries in sub-Saharan Africa. The number is expected to be higher since in several countries surveys for resistance have not yet been conducted. In addition, most of the currently available information was derived from studies that did not use standardised methodologies to diagnose drug resistance. Finally, in very few studies has the extent of the drug resistance problem been quantified as extensively as in the present work.

An additional observation of the present work, was that isometamidium prophylaxis, which is widely used in Coast Province may have deleterious effects on both PCV and body weight when administered to cattle in the absence of significant tsetse challenge (Chapter 4). At the north coast study sites in Kilifi District, trypanosomiasis is considered to be an important disease and most of the farmers had been using isometamidium prophylaxis for more than ten years (Chapter 5). However, in the present studies no infection was detected over a twelve-week period (Chapter 5). It is likely, therefore, that in this area, unwarranted isometamidium prophylaxis was having a more deleterious effect on animal health than the disease itself.

8.2 Recommendations

8.2.1 Disease control strategy at coastal Kenya

Chemotherapy and chemoprophylaxis still remain the principal effective methods of bovine trypanosomiasis control in Africa. In areas where seasonal or yearly variation of trypanosome challenge is prominent such as Galana, isometamidium prophylaxis could still be used effectively during periods of medium

to high challenge. When trypanosome challenge is low, the use of isometamidium prophylaxis is not cost effective, since the drug itself may have adverse effects on PCV and weight gain. In this case use of chemotherapy of individual cases using diminazene aceturate whenever clinical signs are observed would be more appropriate.

In areas where trypanosome challenge is high such as Kwale District integrated use of tsetse control methods and chemoprophylaxis should be encouraged for grazing cattle. For zero-grazed dairy cattle, fly proofing of enclosures is recommended. This should obviate the need for chemoprophylaxis and enhance productivity. At one of the Kwale study sites, Buda, pure-bred Friesian dairy cattle were housed in a zero-grazing unit enclosed with a net. These animals never became infected with trypanosomes throughout the two years of study at that farm.

Use of isometamidium-sustained-release devices, has been shown to provide a significantly longer prophylactic period, and may decrease the chances of over diluting the drug and underdosing. Other advantages over the use of the existing drug formulation are no requirement of sterile water, and no potential toxic residues or lesions at intramuscular injection sites. Although they are not commercially available it would be useful to test such devices in high challenge areas at the Kenyan coast, where drug resistance is known to occur. Finally the implications of their use for development of drug resistance and for public health (e.g. isometamidium residues in milk) should be examined.

8.2.2 Drug resistance control

At the Kenyan coast and throughout sub-Saharan Africa the most important measure that can be undertaken to control drug resistance is regular monitoring of drug usage and effectiveness. Where field evidence indicates drug failure e.g. from survival analysis, measures should be implemented to determine the extent and nature of possible drug resistance in the trypanosome population using the techniques described in this thesis

8.2.2.1 Determination of drug resistance

The isometamidium-ELISA used in the present study to assess infecting trypanosome populations that express a degree of resistance proved to be effective and reliable and should be incorporated in epidemiological studies on trypanosomiasis and drug resistance surveys in other parts of Kenya and Africa as a whole. Drug-ELISA might then be used to select the number of stabilates that need to be tested *in vivo*, thereby reducing the costs involved.

In situations where analysis of the field data suggests that drug resistance is a problem, *in vivo* sensitivity studies in mice should be conducted. However, for *T. vivax*, which does not grow adequately in laboratory animals, ruminants will have to be used. In addition, the poor infectivity of *T. vivax* stabilates in cattle observed in the present studies should be investigated further, in particular the effect of the route of inoculation.

8.2.2.2 Avoiding incorrect use of trypanocidal drugs

The most important measure required to control drug resistance is to avoid misuse of trypanocidal drugs. The results of the present study shows that incorrect use of trypanocidal drugs in Kwale was associated with the presence of trypanosome populations expressing a high level of resistance.

Educating farmers and livestock personnel through community-based seminars on how to use trypanocidal drugs appropriately is recommended, since it would undoubtedly help reduce under-dosing and improper drug administration.

This was proved to be effective during the longitudinal studies in Kwale (Chapter 6). When farmers involved in the study at Kibaoni requested to be advised on how to administer drugs effectively at the end of the first three prophylactic periods in 1997, their request was granted. Prior to this farmer education, during 1997 the doses of isometamidium used were significantly lower than the recommended dose of 1.0 mg/kg b.w. Following the education phase the estimated doses of isometamidium used by these same farmers were not significantly different from 1.0 mg/kg b.w.

8.2.2.3 Avoiding exposing the whole trypanosome population to the drugs.

Although block treatment of cattle en masse in the control of trypanosomiasis can be used successfully over many years (Trail *et al.*, 1985), strong selection pressure on the trypanosome population can be exerted. This selection pressure increases with the proportion of trypanosome population exposed to the drug. This occurs mainly when block treatment is based on the infection prevalence in a herd or is undertaken on a regular basis without prior diagnosis.

Limiting treatment to individual clinical cases and identified trypanosome – infected cattle would greatly reduce whole population exposure. However, this would require availability of reliable and cheap on-site diagnostic tests.

8.2.2.4 Banning use of quinapyramine in Livestock

After a period of being unavailable on the market, quinapyramine was recently re-introduced for use in camels only. However, its availability to untrained personnel could easily result in the use of this drug in cattle. It is known that multiple resistance to the trypanocides used in cattle can occur when trypanosome populations develop resistance to quinapyramine (Ndautomia *et al.*, 1993). In addition, in Kwale District, earlier findings demonstrated resistance to isometamidium expressed by trypanosomes from cattle that had never before been treated with isometamidium, but had been treated with quinapyramine for many years (Gitatha, 1979). It is recommended that total removal of quinapyramine from the market would further reduce development of drug resistance. However, suitable alternative trypanocides for use in camels would have to be made available.

8.3 Follow up studies to be carried out in future

The nature of the multiple resistant population in Kwale should be investigated. Cloning of the multiple resistant *T. congolense* and *T. vivax* isolates should be carried out and the sensitivity of a representative number of clones determined, preferably in cattle.

The importance of the subcutaneous route of inoculation of *T. vivax* isolates in cattle would be investigated further in comparison to intravenous route. In

addition, other factors that may influence infectivity of *T. vivax* stabilates for cattle should be assessed so that more effective screening of *T. vivax* isolates can be conducted.

Every effort should be made to encourage the regular monitoring of the prevalence and incidence of trypanosomiasis in cattle at the Kenyan coast, as well as the drug-resistance situation. In this way, integrated control measures for both the disease and its vector can be recommended appropriately for both the level of challenge and the drug sensitivity phenotypes of the prevailing challenge populations of trypanosomes.

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APPENDICES

Appendix 1: Descriptive statistics of isometamidium concentrations in isometamidium treated cattle during the three year prophylaxis study at Galana ranch

Study Year	Days post treatment	Mean Conc (ng/ml)	Standard error	Mininum conc. (ng/ml)	Maximum conc. (ng/ml)
94/95	10	2.22	0.08	0.99	3.82
94/95	14	2.01	0.11	0.53	4.30
94/95	21	2.05	0.11	0.69	4.81
94/95	28	1.49	0.10	0.39	4.00
94/95	35	1.17	0.07	0.20	2.63
94/95	42	1.06	0.16	0.22	12.43
94/95	49	1.04	0.14	0.14	10.72
94/95	56	0.73	0.04	0.16	1.99
94/95	63	0.58	0.04	0.12	1.67
94/95	70	0.45	0.03	0.08	1.21
94/95	77	0.45	0.05	0.10	3.66
94/95	84	0.32	0.02	0.05	0.96
95/96	0	0.08	0.01	0.00	0.42
95/96	7	3.55	0.14	0.94	15.30
95/96	14	1.64	0.20	0.84	2.88
95/96	21	1.53	0.07	0.14	5.43
95/96	28	1.77	0.34	0.25	3.74
95/96	35	1.01	0.05	0.21	4.60
95/96	42	0.94	0.21	0.17	2.83
95/96	49	0.93	0.06	0.20	8.51
95/96	56	0.78	0.17	0.32	3.43
95/96	62	0.53	0.03	0.05	2.62
95/96	70	0.60	0.14	0.16	1.68
95/96	77	0.33	0.02	0.02	1.22
96/97	0	0.05	0.01	0.00	0.38
96/97	7	3.51	0.15	1.28	8.41
96/97	26	2.11	0.12	0.63	7.33
96/97	36	1.56	0.07	0.60	4.10
96/97	50	1.11	0.05	0.26	2.44
96/97	65	0.78	0.04	0.19	2.13
96/97	78	0.59	0.03	0.14	1.37

Appendix 2a: Descriptive statistics of serum isometamidium concentrations in dairy cattle at Kubo and during the observational studies in Kwale

Prophylactic period	Site	Days post treatment	Mean Conc. (ng/ml)	Standard error	Mininum Conc. (ng/ml)	Maximum Conc. (ng/ml)
1	Kubo	7	2.810	0.295	1.439	4.448
1	Kubo	14	2.199	0.266	0.710	3.869
1	Kubo	21	1.783	0.236	0.547	2.851
1	Kubo	28	1.608	0.310	0.389	3.443
1	Kubo	35	1.194	0.238	0.473	2.725
1	Kubo	42	0.955	0.197	0.421	2.079
1	Kubo	49	0.770	0.153	0.257	1.807
1	Kubo	56	0.702	0.176	0.369	1.299
1	Kubo	63	0.521	0.090	0.230	0.847
1	Kubo	70	0.442	0.075	0.080	0.580
2	Kubo	7	2.526	0.349	0.586	4.777
2	Kubo	14	3.229	0.355	0.537	4.932
2	Kubo	21	1.845	0.280	0.266	3.316
2	Kubo	28	2.129	0.392	0.577	4.069
2	Kubo	35	1.620	0.335	0.416	3.759
2	Kubo	42	1.903	0.408	0.429	4.749
2	Kubo	49	1.359	0.506	0.370	4.615
2	Kubo	56	1.052	0.250	0.387	2.105
2	Kubo	63	0.684	0.121	0.275	1.376
2	Kubo	70	0.845	0.211	0.265	2.105
2	Kubo	77	0.889	0.190	0.207	1.754
2	Kubo	84	0.582	0.321	0.188	1.218
3	Kubo	7	3.844	0.526	2.636	6.139
3	Kubo	14	3.140	0.311	2.946	5.010
3	Kubo	21	2.443	0.287	2.008	3.916
3	Kubo	28	2.057	0.299	1.583	3.695
3	Kubo	35	1.6405	0.3705	1.27	2.011
3	Kubo	42	1.582	0.49515	1.09	2.0803
3	Kubo	49	1.212	0.192	1.02	1.404
3	Kubo	56	0.8375	0.0715	0.766	0.909
3	Kubo	63	0.7345	0.1315	0.603	0.866
3	Kubo	70	0.682	0.218	0.464	0.9
3	Kubo	77	0.5325	0.2325	0.3	0.765

Appendix 2b: Descriptive statistics of serum isometamidium concentrations in dairy cattle at matuga and during the observational studies in Kwale

Prophylactic period	Site	Days post treatment	Mean Conc (ng/ml)	Standard error	Mininum conc. (ng/ml)	Maximum conc. (ng/ml)
1	Matuga	7	3.499	0.389	0.549	6.183
1	Matuga	14	2.704	0.241	1.662	4.080
1	Matuga	21	1.716	0.168	0.414	2.663
1	Matuga	28	1.444	0.098	0.917	1.873
1	Matuga	35	1.256	0.170	0.301	2.898
1	Matuga	42	1.069	0.115	0.397	1.888
1	Matuga	49	0.870	0.128	0.524	1.828
1	Matuga	56	0.642	0.075	0.190	1.278
1	Matuga	63	0.535	0.069	0.346	0.820
1	Matuga	70	0.468	0.063	0.129	0.684
2	Matuga	7	3.181	0.370	0.404	5.187
2	Matuga	14	3.840	0.363	2.106	6.223
2	Matuga	21	2.536	0.272	1.138	4.547
2	Matuga	28	1.998	0.256	0.683	3.660
2	Matuga	35	1.676	0.187	0.621	3.179
2	Matuga	42	1.384	0.142	0.674	2.510
2	Matuga	49	1.021	0.133	0.301	1.992
2	Matuga	56	0.899	0.125	0.340	1.970
2	Matuga	63	0.667	0.081	0.273	1.142
2	Matuga	70	0.781	0.119	0.388	1.613
2	Matuga	77	0.612	0.081	0.294	1.184
2	Matuga	84	0.474	0.064	0.246	0.703
3	Matuga	7	4.320	0.483	1.316	7.478
3	Matuga	14	2.535	0.260	1.079	4.837
3	Matuga	21	2.231	0.482	1.094	4.291
3	Matuga	28	1.642	0.279	0.832	2.475
3	Matuga	35	1.524	0.036	1.488	1.560
3	Matuga	42	1.068	0.133	0.935	1.200
3	Matuga	49	0.869	0.079	0.789	0.948
3	Matuga	56	0.765	0.094	0.671	0.858
3	Matuga	63	0.564	0.020	0.543	0.584
3	Matuga	70	0.420	0.081	0.339	0.501