# The susceptibility of *Trypanosoma congolense* isolated in Zambézia Province (Mozambique) to isometamidium chloride, homidium chloride and diminazene aceturate

**by**

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**Master of Science (Veterinary Science)**
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# Declaration

I hereby declare that this dissertation, submitted by me to the University of Pretoria for the degree Master of Science has not previously been submitted for a degree at any other University.

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Suzana Augusta José Jamal

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# Abbreviations

DINAP - National Directorate of Livestock

FAO - Food and Agricultural Organization

INIVE - National Veterinary Research Institute

MCT - Missão de Combate as Tripanosomiases

PCV - Packed Cell Volume

RTTCP - Regional Tsetse & Trypanosomosis Control Programme

UEM - Universidade Eduardo Mondlane

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## Abstract

Bovine trypanosomosis is a serious constraint to livestock development in large parts of Mozambique. In most areas where tsetse flies are present, the disease in livestock is controlled using curative and prophylactic trypanocidal drugs. Those drugs have been used for many years and new drugs are unlikely to become available in the near future. As a result, trypanosomes have developed resistance against the currently available trypanocidal compounds. Drug resistance has been detected in various African countries and is a serious impediment to the control of livestock trypanosomosis.

A study was initiated to determine whether drug resistant trypanosome strains are present in Zambézia Province of Mozambique. The aim of this study was to determine the sensitivity of *Trypanosoma congolense* isolates from Chinde, Nicoadala and Maganja da Costa Districts to diminazene aceturate, isometamidium chloride and homidium chloride. To assess the effect of the farming system and the intensity of drug regimens on the development of drug resistance, trypanosome isolates were collected from cattle from subsistence, semi-subsistence and commercial livestock production systems. Drug-use practices in each of the production systems were determined using a questionnaire. The methodology used to assess

the level of drugs resistance in the trypanosome isolates was the standardized method described by Eisler *et al.* (2001).

Seven isolates were selected for resistance testing. For each of the seven isolates, five different doses varying between 0.01-20 mg/kg body weight for isometamidium chloride, 0.01-10 mg/kg body weight for homidium chloride and 1-30 mg/kg body weight for diminazene aceturate were used. For each dose rate six mice were treated intraperitoneally with the appropriate quantity of the drug dissolved in 0.2 ml of sterile distilled water 24 hours after the inoculation of the blood containing the trypanosomes. The control mice (six mice per trypanocidal drug) received the same amount of water without the drug.

In four of the seven isolates high levels of multiple drug resistance (diminazene aceturate and isometamidium chloride) were detected. One isolate had a low level of multiple (diminazene aceturate and isometamidium chloride) drug resistance. Two isolates were susceptible to both diminazene aceturate and isometamidium chloride. One of those was highly susceptible to isometamidium chloride even at the lowest dose rate. The observed levels of drug resistance could in most cases be correlated to the drug-use practices in the particular livestock production system.

The results obtained from homidium chloride treatment are not conclusive, because most the mice cured after receiving 10 mg/kg body weight of the drug. Hence more research is required to establish the homidium threshold in mice.

The results of this study should be useful to define the strategy of disease control in places where resistance of trypanocide were been reported.

*Key words*: production system, treatment practices, diminazene aceturate, drug resistance, *Glossina*, homidium choride, isolate, Isometamidium choride, mice, parasite, parasitaemia, trypanosomosis, *Trypanosoma congolense*, Trypanocide.

# Chapter 1 : Introduction

## Background

Livestock trypanosomosis, transmitted by tsetse flies (*Glossina* spp.), has been and still is a considerable constraint to livestock production in Mozambique. About 75 percent of Mozambique is occupied by four *Glossina* species, namely *Glossina morsitans, G. brevipalpis, G. austeni* and *G. pallidipes.*

Efforts to control the disease were initiated in 1910. In 1945 the "Missão de Combate as Trypanosomiases" (MCT) was created specifically to control tsetse and trypanosomosis. The main objective of the MCT was to determine the distribution of the tsetse fly and to conduct both tsetse and trypanosomosis control. Responsibility for tsetse and trypanosomosis control was transferred to the livestock services (Directorate of Veterinary Services) in 1973. The large commercial and subsistence cattle herds were kept under prophylactic and curative treatment regimens in tsetse-infested areas mainly in the north and central part of Mozambique.

After independence and during the civil war, this system of intensive treatment and good management could not be maintained. Cattle became infected and mortality rates were high. After the civil war (1996), restocking programmes were instituted to reintroduce cattle in areas where smallholders traditionally kept livestock.

In Zambézia Province large herds were established by the private sector. Since tsetse control is not conducted, cattle are protected using prophylactic and curative trypanocides. Those drugs have now been used extensively for several years and their effectiveness needs to be evaluated.

## Objective of the Study

The aim of the study was to determine the susceptibility of *Trypanosoma congolense* isolates from Chinde, Nicoadala and Maganja da Costa districts of Zambézia Province to diminazene

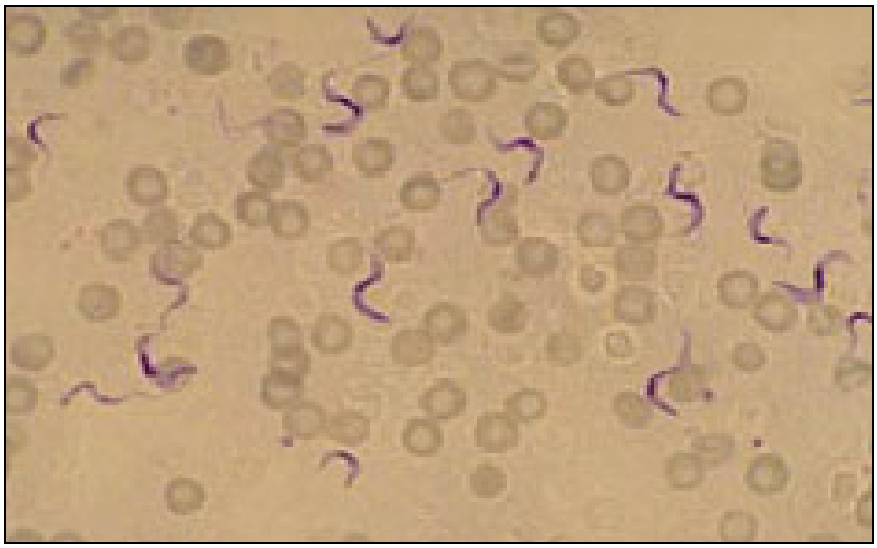
aceturate, isometamidium chloride and homidium chloride. The methodology used in this study was the standardized method described by Eisler *et al.* (2001).

# Chapter 2 : Literature review

## The parasite (trypanosomes)

Trypanosomes are protozoan parasites of the genus *Trypanosoma*, order Kinetoplastida, and have as characteristic organelles, a kinetoplast and flagellum. The genus *Trypanosoma* is divided into two sections, Salivaria and Stercoraria, which differ in their modes of transmission. Salivarian trypanosomes either undergo cyclic development in the insect before being transmitted via the saliva, or are transmitted mechanically. Stercorarian trypanosomes also undergo development in the insects, but the infective forms are deposited in the faeces of the vector (Kettle 1995; Connor 1994; Seifert 1996). The division of trypanosomes between the Sections Salivaria and Stercoraria is shown in Table 1.

**Figure 1**: Trypanosomes on a stained bloodsmear



Trypanosomes occur throughout the world and in different species or all classes of vertebrates: fish, frogs, reptiles, birds and mammals (Losos 1986; Connor 1994). Most are well adapted to their host and cause no appreciable harm. A group of trypanosomes that parasitize mammals is less well adapted, and they commonly cause disease (Losos 1986; Connor 1989; Connor 1994).

**Table 1**: Characteristics of the Sections Stercoraria and Salivaria of mammalian trypanosomes (Seifert 1996).

| Characteristic | *Stercoraria* | *Salivaria* |
|---|---|---|
| Free flagellum | Always present | Present or missing |
| Kinetoplast | Big, non-terminal | Terminal or subterminal |
| Multiplication in the mammalian host | Periodical, typically as an epi- or amastigote form (except *T. cruzi*) | Always in the trypomastigote form |
| Pathogenicity | Low or none | Pathogenic |
| Development in the gut of the vector | Hindgut (except *T. rangeli*) | Frontal part (except *T. evansi T. equinum, T. equiperdum)* |
| Subgenera | *Megatrypanum* | *Duttonella, Nanomonas, Pycnomonas, Trypanozoon* |

**The vector (tsetse flies)**

African animal trypanosomes are transmitted by tsetse flies of the genus *Glossina* and, to a lesser extent, other biting insects (Tabanids). The genus *Glossina* is restricted to sub-Saharan Africa. It is only genus in the family Glossinidae (Smith 1973; Phelps and Lovemore 1994; Kettle 1995).

**Figure 2**: The tsetse fly

In sub-Saharan Africa there are 23 species of *Glossina*, each adapted to its particular habitat and range of hosts (Short 1963, Kettle 1995). Within the genus three main groups of tsetse

are recognised, namely the riverine or *palpalis* group, the forest or the *fusca* group and the savannah or *morsitans* group (da Silva 1969, Phelps and Lovemore 1994; Kettle 1995).

**Trypanosomosis**

Trypanosomoses are diseases of humans and domestic animals that result from infection with several species of protozoa (ILRAD 1988; Connor 1994). The most important species responsible for the disease complex, commonly known as nagana in livestock, include *Trypanosoma brucei*, *T. congolense* and *T. vivax*. They are usually transmitted by tsetse flies. In pigs *T. simiae* is responsible for an acute form of disease (ILRAD 1988; Cox 1993; Kettle 1995; Anne, Onah and Nawa 2001).

In Africa, Asia, the Middle East and South America, *T. evansi*, the causative agent of surra, is known to be very important especially in draught and transport animals. The parasite is transmitted mechanically by biting flies such as *Tabanus* and *Stomoxys* spp. (Kettle 1995). *Trypanosoma brucei gambiense* and *T. b. rhodesiense* are the most important human pathogens responsible for African sleeping sickness (Rafael 1973; ILRAD 1988; Connor 1994; Kettle 1995).

**Clinical signs of trypanosome infections in livestock**

Infections of African trypanosomes are characterized by successive waves of parasitaemia. Trypanosome infections take a variable course depending on factors associated with both the host and the parasites, but are characterised in most instances by the intermittent presence of parasites in the blood and intermittent fever.

Infection of African trypanosomes may run an acute, subacute or chronic course (Murray *et al.* 1983).

In the acute case, anaemia and general loss of body condition are the first clinical signs when trypanosomes invade and multiply in the bloodstream of the infected animals. About one or two weeks later, the sick animals usually have recurrent fevers for up to three months. After the first bout of fever, which occurs normally within 12 days, the number of parasites in the circulation declines.

**Figure 3:** A cow suffering from trypanosomosis

The animals continue to be anaemic and lose body condition resulting in reduced productivity and frequently high mortality rates (Murray *et al.* 1983; Connor 1994; Kettle 1995). Infections with *T. brucei* and *T. evansi*, can result in the invasion of the brain, eyes and skin, leading to nervous signs, discharges from the eyes and oedematous swellings under the skin (Kettle 1995).

In the subacute or chronic course, the disease lasting many month or even years is more common (Kettle 1995).

**Impact of tsetse-transmitted trypanosomosis**

Trypanosomosis constitutes a major constraint to livestock development in Africa. Over 10 million $km^2$ involving 37 countries are infested by tsetse flies (Kinabo and Bogan 1988; Connor 1991; Boyt 1991). In Africa, livestock are of great economic importance, not only as a source of food, draught power and money, but also for the important role they can play in cultural affairs (Phelps and Lovemore 1994).

Annual losses in meat production are estimated at US$5 billion. This economic loss is exacerbated by losses in milk production, traction power, waste products that provide natural fuel and fertilizer. Furthermore, about 50 million people in Africa are exposed to the risk of contracting sleeping sickness (WHO 1979; ILRAD 1988; Anne, Onah and Nawa 2001).

Most governments in Southern Africa recognize bovine trypanosomosis as a serious constraint to development and a serious threat to the agricultural sector. In Zambia, bovine trypanosomosis is listed as a disease of national importance. In Mozambique, trypanosomosis is considered a serious threat to the cattle-restocking programme. In Zimbabwe, the financial implications for the communal and commercial farming sector of tsetse reinvading cleared areas is enormous and substantial efforts are made to maintain artificial barriers to tsetse reinvasion (Shereni 1990).

Despite the importance of bovine trypanosomosis in the Southern African economy, the actual impact of the disease has hardly been quantified (Connor 1989).

**Control of tsetse-transmitted trypanosomosis**

Controlling the vector or the parasite or a combination of the two usually controls tsetse-transmitted trypanosomosis.

*Parasite control* -- The African trypanosome species are able to change their surface coat antigens continuously, blocking the efficiency of the specific host antibodies. Due to this characteristic of the pathogen, the prospects of developing a protective vaccine against trypanosomes are extremely poor (Seifert 1996).

In most African countries trypanocidal drugs remain the principal method of controlling animal and human sleeping sickness (ILRAD 1988; Geerts and Holmes 1998). The drugs can be classified as therapeutic and/or prophylactic. The main therapeutic drugs for cattle include diminazene aceturate, homidium chloride and homidium bromide. The prophylactic drugs for cattle include homidium chloride, homidium bromide and isometamidium chloride. Suramin, a member of the group of acid naphthylamine drugs, is used to treat human sleeping sickness. Most of these drugs have been on the market for more than 40 years (Geerts *et al*. 2001).

Diminazene aceturate became available and was used to overcome quinapyramine-resistance. It is administered by the intravenous or intramuscular route at the doses 3,5 mg/kg body weight for all trypanosome species except *T. brucei*. *Trypanosoma brucei* infections are treated at 7 mg/kg body weight. Quinapyramine was withdrawn from the market in 1977

because of the emergence of resistance among trypanosomes in cattle. It was reintroduced in 1985 mainly to treat *T. evansi* infections in camels and horses (Connor 1989; Kettle 1995).

Homidium chloride was first marketed in 1955. Due to the emergence of resistant strains of trypanosomes it was withdrawn. Despite of this, the drug is still available in many countries (Connor 1989).

Isometamidium chloride was introduced in 1961 and is still in great demand today. The recommended route of administration is intramuscular or slowly intravenous at a dose of 1 mg/kg body weight. It was the last trypanocidal drug to be developed (Connor 1989).

*Vector control* -- Another effective control method is to control the vectors (Smith 1973). Vector control methods include insecticides spraying on the tsetse's habitat or the tsetse's host, the use of insecticide-treated artificial baits, the destruction of tsetse habitat and alteration of vegetation (Short 1963; Smith 1973). According to Shereni (1990), vector-control methods are expensive and require a high level of management, organization and specialist expertise.

The high cost of vector-control operations and the high costs of protecting tsetse-free areas from reinfestation have lead to widespread dependence on trypanocides.

**Development of resistance to trypanocidal drugs**

In the absence of an effective vaccine, trypanocidal drugs, mainly isometamidium choride, diminazene aceturate and homidium salts, are the most commonly used veterinary products in sub-Saharan Africa. Geerts and Holmes (1998) estimated that about 35 million doses of trypanocidal drugs are administered each year. Consequently, trypanocidal drug resistance has become a serious problem in recent years.

The factors contributing to the development of resistance to anti-trypanosomal compounds are still not entirely known.

Underdosing is probably one of the major causes of the resistance development. Underdosing can be the result of underestimating the weight of animals when they are treated (Boyt 1986).

Furthermore, since trypanocidal drugs are expensive, farmers have the tendency to overdilute the drug and hence underdose (Geerts and Holmes 1998).

According to ILRAD (1992), drugs administered in the field have sometimes expired, inducing parasite-population tolerance to the drug.

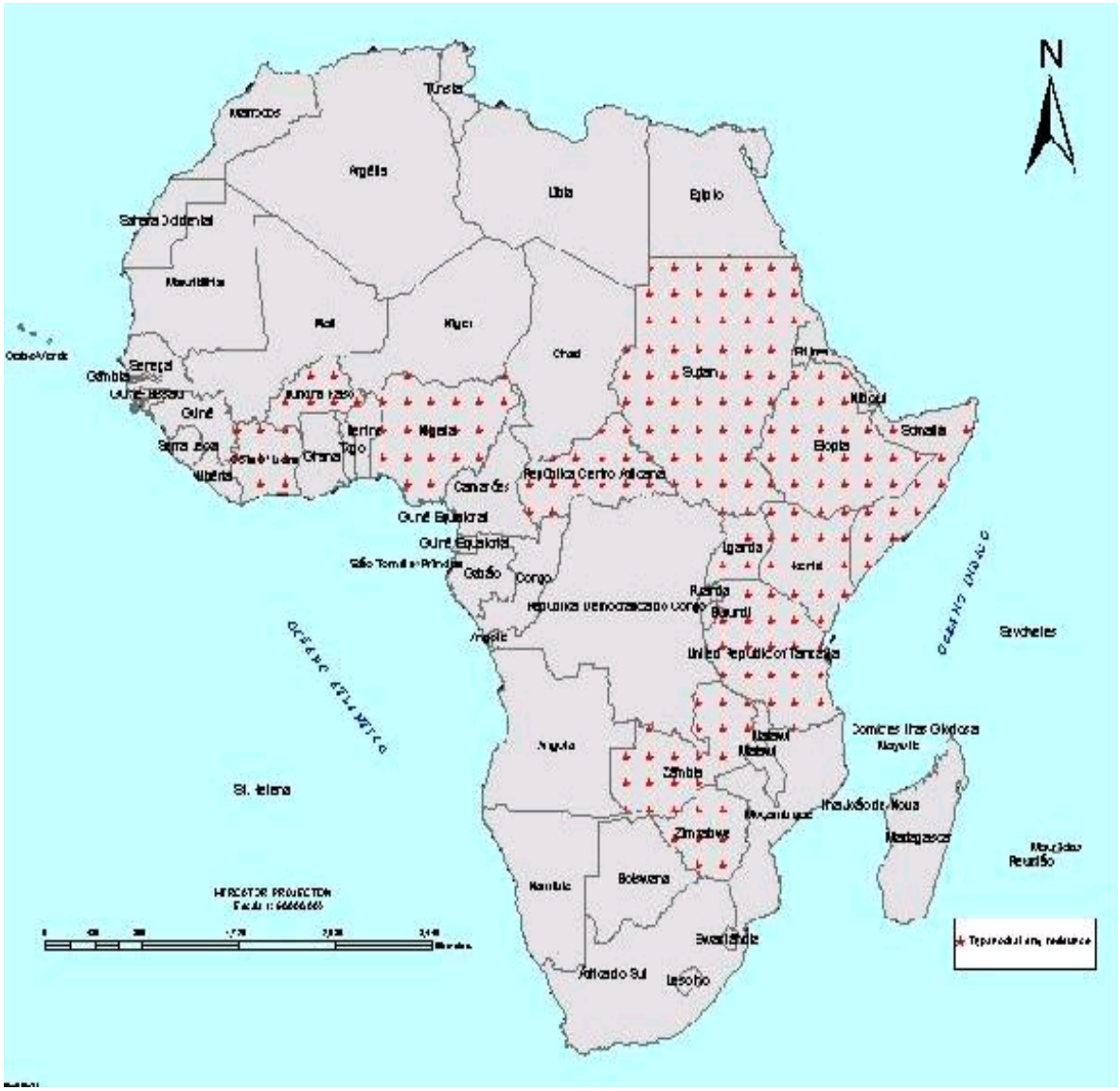
Clausen (1992, cited by Afewerk *et al*. 2000) stated that the exposure of parasites to sub-therapeutic drug concentrations results mainly from underdosing and uncontrolled use of trypanocidal drugs. He also concluded that the lack of proper diagnosis contributes significantly to increasing drug resistance throughout Africa.

Accordingly to Clausen *et al.* (1992, cited by Afewerk *et al*. 2000) and Geerts and Holmes (1998), the prolonged and frequent use of trypanocides in high challenge areas, even when applied properly, is likely to result in development of drug resistance. Large-scale drug use and the use of drugs that are eliminated slowly from the body may also contribute to development to resistance (Geerts and Holmes 1998).

The first cases of trypanocidal drug resistance in Africa were reported during the 1960s (Finelle and Yvore 1962; Fairclough 1963; Jones-Davies and Folkers 1966, cited by Geerts and Holmes 1998). At the moment, at least 13 African countries have reported resistance to one or more of the currently available trypanocidal drugs (Peregrine 1994; Finelle and Yvore 1962; Mubanga and Sinyangwe 1997).

According to Geerts and Holmes (1998), there is an underestimation of the true situation, because in several countries surveys for resistance have not been carried out or cases of resistance have not been published. Figure 2 represents the few countries where trypanocidal drug resistance has been detected.

**Figure 4:** Countries where drug resistance has been reported




**History of trypanocidal drug use and drug resistance development in the Southern African region**

*Eastern Zambia* -- The control of bovine trypanosomosis in eastern Zambia has, for the past 45 years, relied heavily on the use of chemoprophylaxis and chemotherapy. The eastern plateau was settled and reinvaded by tsetse in the mid 1950s (Vail 1977). Three-monthly block-treatment with chemoprophylactic drugs was initiated in the mid-1960s and lasted until 1989. The main trypanocide used in those campaigns was isometamidium chloride supplemented by prothidium between 1970 and 1972 (Leak 1980). Curative treatments with diminazene aceturate were also administered.

The administration of these campaigns was, however, fraught with difficulties. Lack of transport and frequent shortages of drugs resulted in prolonged treatment intervals. A cost-recovery scheme for trypanocidal drugs (isometamidium chloride and diminazene aceturate) was launched in 1990 and replaced the free-of-charge treatment campaigns. Despite their extensive use, only localised resistance to diminazene aceturate or isometamidium chloride has been reported (Chitambo and Arakawa 1991; Chitambo, Arakawa and Ono 1992).

*Zimbabwe* -- Bevan (1928) confirmed the efficacy of potassium antimony tartrate against *T. congolense* and *T. vivax* infection in cattle in Zimbabwe (then Rhodesia). Subsequently, many thousands of head of livestock were treated and saved by its use (Boyt 1967). It was replaced by dimidium bromide, which was used widely until the middle 1950s, when its use was abandoned after disastrous losses due to photosensitization in the eastern districts (Boyt 1967). Dimidium bromide was replaced by the less toxic homidium bromide or homidium chloride. The quinapyramine compounds (Antrycide), the first truly prophylactic trypanocides, were introduced in 1955 (Boyt *et al.*, 1963). They were used extensively in the Sabi Valley (Chipinge District) during the latter half of 1955 (Boyt 1979). Widespread resistance to this compound was detected in trypanosomes in 1962, however (Boyt 1971). At about the same time, diminazene aceturate was introduced and was quickly taken into general use. It was supplemented, in the mid-1960s, with isometamidium chloride. Despite its large-scale use, resistance of trypanosomes to isometamidium chloride has only been reported sporadically (Boyt 1971; Lewis and Thomson 1974). Resistance to diminazene aceturate was only recorded once (Joshua *et al*. 1995).

*Malawi* -- In Malawi (then Nyasaland) heavy reliance was initially placed on homidium bromide in the mid-1950s to early 1960s. Resistance to this compound emerged quickly, however, and campaigns were terminated in 1957 (Matson 1959). Homidium was replaced by quinapyramine, but trypanosome strains resistant to this compound soon emerged. This resistance was overcome successfully with diminazene aceturate (Connor 1989). Since the early 1970s, bovine trypanosomosis has been controlled satisfactorily by chemotherapy using diminazene aceturate and chemoprophylaxis using isometamidium. Table 2 shows an overview of drug resistance in trypanosomes in some countries of the region.

**Table 1:** Surveys for drug resistance in trypanosomes (modified from Geerts and Holmes 1998).

| Country | Tryp Species | Nº of isolates Exam | Nº of isolates Resist | % of R. Isoloates | Resist to | Reference |
|---|---|---|---|---|---|---|
| Zambia | Tc | 71 | 24 | 33.80 | I | Sinyangwe, Machila, Mubanga, Delespaux, Brandt., Geerts, Holmes and Eisler (2003) |
| | | | 8 | 11.3 | D | |
| | | | 1 | 1.4 | ID | |
| Tanzania | Tc | 17 | 17 | 29 | I | Mwambo, Ndungu, Murilla, Munga, Singwe, Machila, Holmes and Eisler (1999) |
| | | | | 65 | D.I | |
| Kenia | Tc | 7 | 2 | 29 | I | Gray,Kimarna, Peregrine and Stevenson (1993) |
| Somalia | Tv | 7 | 6 | 86 | I | Schonefeld, Rôttcher and Moloo (1987) |
| Nigéria | Tv | 19 | 12 | 63 | D,H,I | Ilemobade (1979) |
| | Tb | 12 | 2 | 17 | D,I | Kalu (1995) |
| Ethiopia | Tc | 12 | 12 | 100 | D | Afewerk, Clausen, Abebe, Tilahun and Mehlitz (2000) |
| | | 12 | 12 | 100 | D | |
| | | 12 | 11 | 92 | I | |
| Uganda | Tb | 36 | 1 | 3 | D,I | Matovu, Iten, Enyary, Schmid, Lubega, Brun and Kaminsky (1997) |
| Zimbabwe | Tc | 14 | 6 | 43 | D | Joshua, Obolo, Bwangamoi and Mandebvu (1994) |
| Sudan | Tc,Tv,Tb | 12 | 5 | 42 | H | Abdel Gadir, Osman, Abdalla, Abdel Razig (1981) |
| Burkina Faso | Tc | 12 | 9 | 75 | I | Prinder and Authié (1984) |

**D= Diminazene aceturate; H=Homidium; I= Isometamidium**

## Techniques to identify drug resistant trypanosome isolates

There are three commonly used methods to identify drug resistance: tests in ruminants, tests in mice and in vitro assays. All of them have advantages and disadvantages. Accordingly to Geerts and Holmes (1998), tests in ruminants provide direct information from studies in ruminants using recommended doses of trypanocides. The test consists of infecting a group of cattle or small ruminants with the isolate under investigation and treating them later with various doses of trypanocide, when the animals are parasitaemic. The test in ruminants is useful in situations where laboratory facilities are very limited. Furthermore, not all

trypanosome populations might grow equally well and the sensitive isolates might overgrow resistant ones when inoculated together. The advantages of studies in ruminants are that most trypanosome isolates of cattle are able to grow in the hosts and the data obtained are directly applicable in the field. The disadvantages of studies in ruminants are that purchase and maintenance of the animals is expensive and the period for detection of relapses is up to 100 days.

Tests in mice

There are two different test: single-dose test and multi-dose test. In the single-dose test, a group of 6 mice is required for each drug to be tested, while in the multi-dose test, at least 6 groups of 6 mice (1 control and 5 to be treated) are necessary for each drug to be tested (Eisler *et al.* 2001). Although a mouse test may give a broad indication of the sensitivity of a strain, it cannot be used to predict curative doses for cattle (Sones *et al.* 1988). The advantage of the mouse assay is that it is cheaper than the test in cattle (Eisler *et al.* 2001).

There are several disadvantages: *T. vivax* does not grow in mice; altough there is a reasonable correlation between drug sensitivity data in mice and in cattle, higher doses of drug must be used in mice in order to obtain comparable results to those obtained in cattle; large numbers of mice are required per isolate, for precise assessment of the degree of resistance; it takes as long as 60 days to evaluate the drug sensitivity of an isolate (Sones, Njogu and Holmes 1988; Afewerk *et al.* 2000; Eisler *et al.* 2001).

In-vitro assays

The development of an in-vitro assay to determine the drug sensitivity of trypanosomes was rewied by Kaminsky and Brun (1993, cited by Geerts and Holmes 1998). This technique utilises metacyclic or bloodstream forms instead of procyclic forms. It takes up to 40 to 50 days of in-vitro incubation to generate metacyclic trypanosomes. The advantages of this technique is that large numbers of isolates can be examined, and that tests with metacyclic trypanosomes correlate well with the field observations.

There are several disadvantages in the in vitro assays techniques (Hirumi and Peregrine 1993, cited by Geerts and Holmes 1998). In-vitro cultivation of bloodstream forms is only possible

using preadapted lines and not using isolates directly from naturally infected animals. No stocks of *T. congolense* have been reported to grow successfully in vitro as bloodstream forms directed from naturally infected animals (Gray and Peregrine 1993). Furthermore, the in-vitro assays are expensive and require good laboratory facilities and well trained staff.

**Current trypanosomosis situation in Mozambique**

In Mozambique, tsetse flies transmitting animal trypanosomosis are the major constraint to livestock production.

In 1896, the rinderpest panzootic killed most of the country's game and domestic animals. With the loss of this important source of food, the tsetse disappeared south of the Zambezi River, leaving only small tsetse foci in Southern Mozambique (da Silva 1969; FAO 1987).

Later, tsetse flies gradually invaded the previously occupied areas. Starting from the Save River, they advanced toward Southern Zimbabwe and the Kruger National Park in South Africa (FAO 1987; Davies 1982). According to work done by the MCT (1949-1960) and continued by the Food and Agricultural Organization (FAO) (1987), the National Directorate of Livestock (DINAP) (1996) and the Regional Tsetse and Trypanosomosis Control Program (RTTCP) (2000), about 876 000 $km^2$ (75%) of the country is tsetse infested (Figure 3).

The cattle population of Mozambique is estimated at 603,690 head. They are manly concentrated in areas relatively free of tsetse flies (Figure 4).

**Figure 5**: Map representing the distribution of tsetse flies in Southern Africa (from RTTCP)

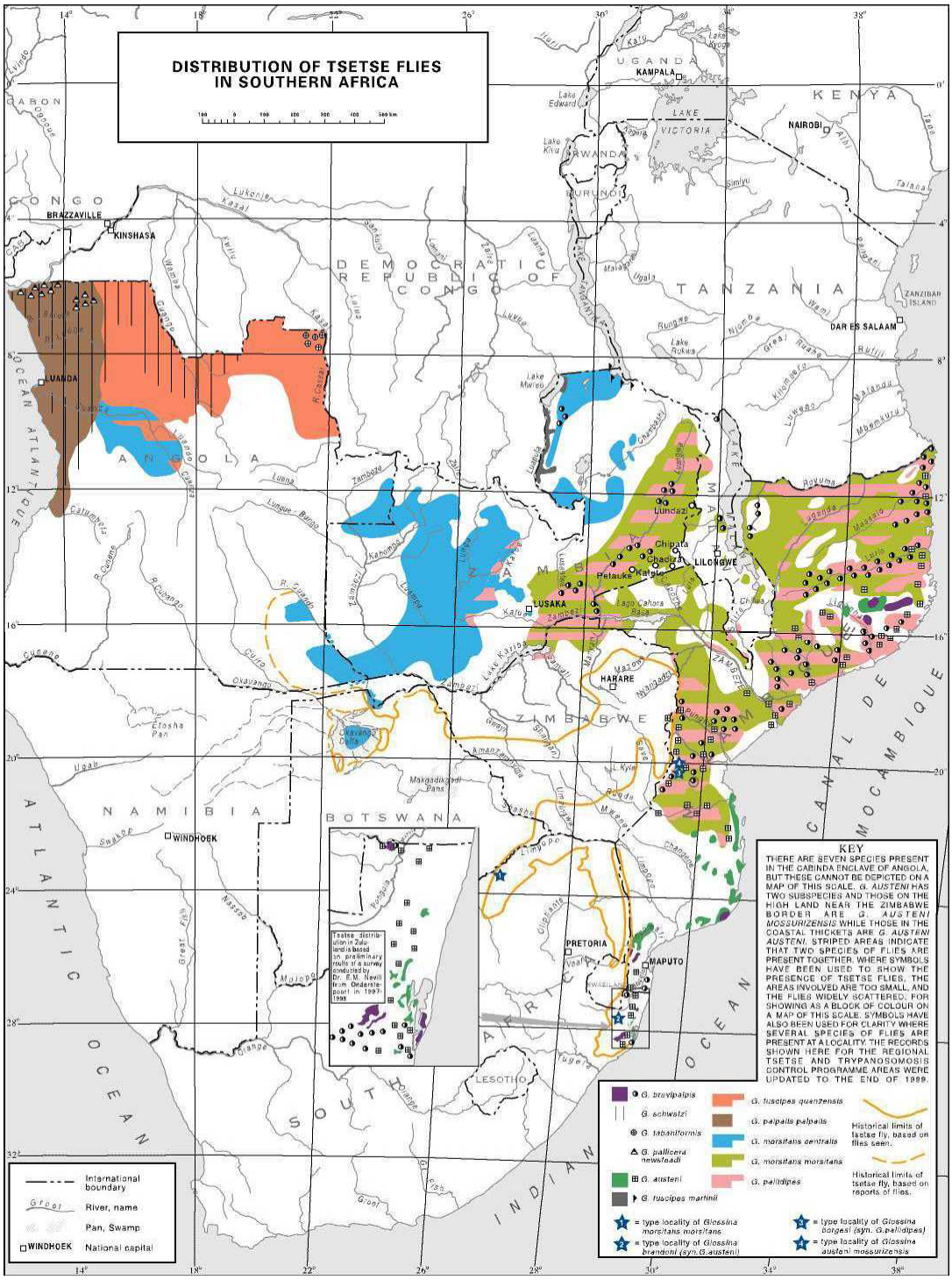
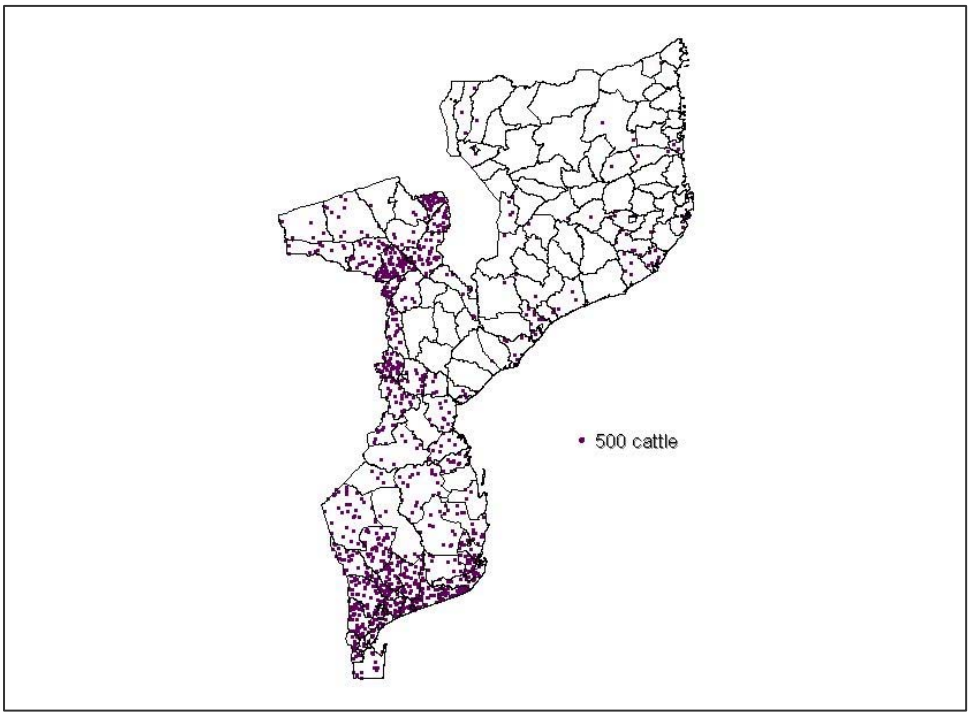


**Figure 6**: Map representing the distribution of cattle in Mozambique




According to Ministry of Health, human sleeping sickness is endemic only in the central and northern part of the country, namely in Tete, Niassa and Cabo Delgado Provinces. The disease is caused by *T. brucei rhodesiense*. Active surveillance and control is not carried out and therefore few cases of the disease are reported.

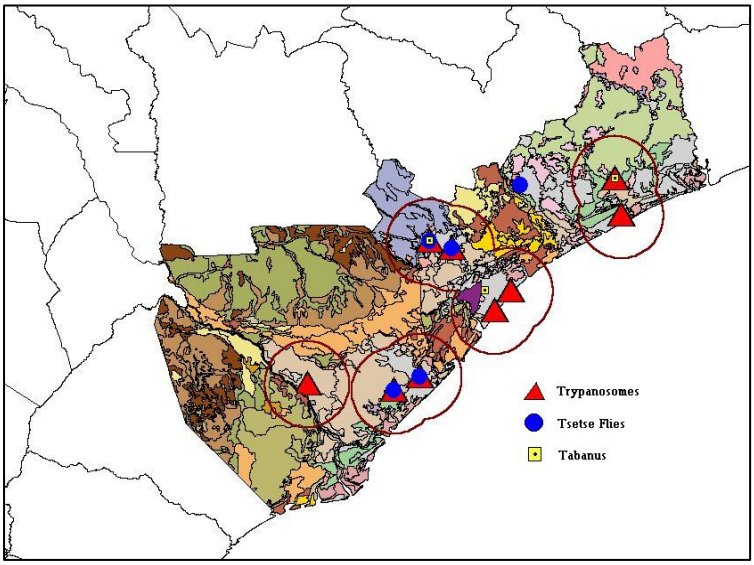
Tsetse control is not practised in Mozambique. Surveys are being carried out using the epsilon trap. Trypanosomosis in livestock is controlled by applications of diminazene aceturate as curative and isometamidium chloride as prophylactic drugs. Despite of this, five outbreaks of trypanosomosis were reported in 2002. This is probably an underestimation of the true situation, as outbreaks have not been reported for all provinces.

According to the laboratory results from previous years the cases of trypanosomosis tend to be higher in Zambézia, Manica and Tete Provinces. Especially Zambézia Province is subject to challenge by various tsetse species.

Tsetse and trypanosomosis surveys conducted by RTTCP and DINAP in Zambézia Province, Nicoadala, Chinde and Maganja da Costa Districts detected *Glossina brevipalpis*,

*G. morsitans* and *G. pallidipes*, and other flies, i.e. *Tabanus* spp. The most important trypanosome species found was *T. congolense* (Figure 5).

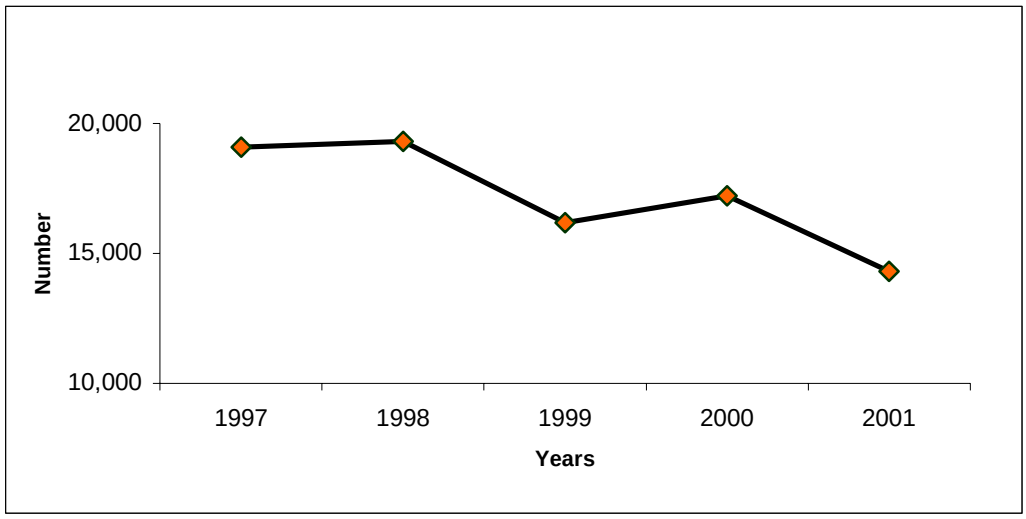
**Figure 7**: The distribution of tsetse flies, other flies (*Tabanus* spp.) and trypanosome infections in cattle in Zambézia Province




The cattle population in Zambézia Province, which had amounted to 19,099 in 1997, was distributed mainly in the palm plantations in the littoral part of the province. Since then, cattle numbers have dropped to 14,303 animals due to the trypanosomosis problem (Figure 6).

Research done by DINAP and the Veterinary Faculty of UEM in 2000 in the Botão area of Nicoadala District of Zambézia Province indicated the presence of multi-drug resistance (i.e. diminazene aceturate, isometamidium chloride and homidium chloride) in that area (Muacamule, pers. com 2000). Because of those findings, the research presented in this thesis was conducted.

**Figure 8**: Reduction of cattle numbers in Zambézia Province

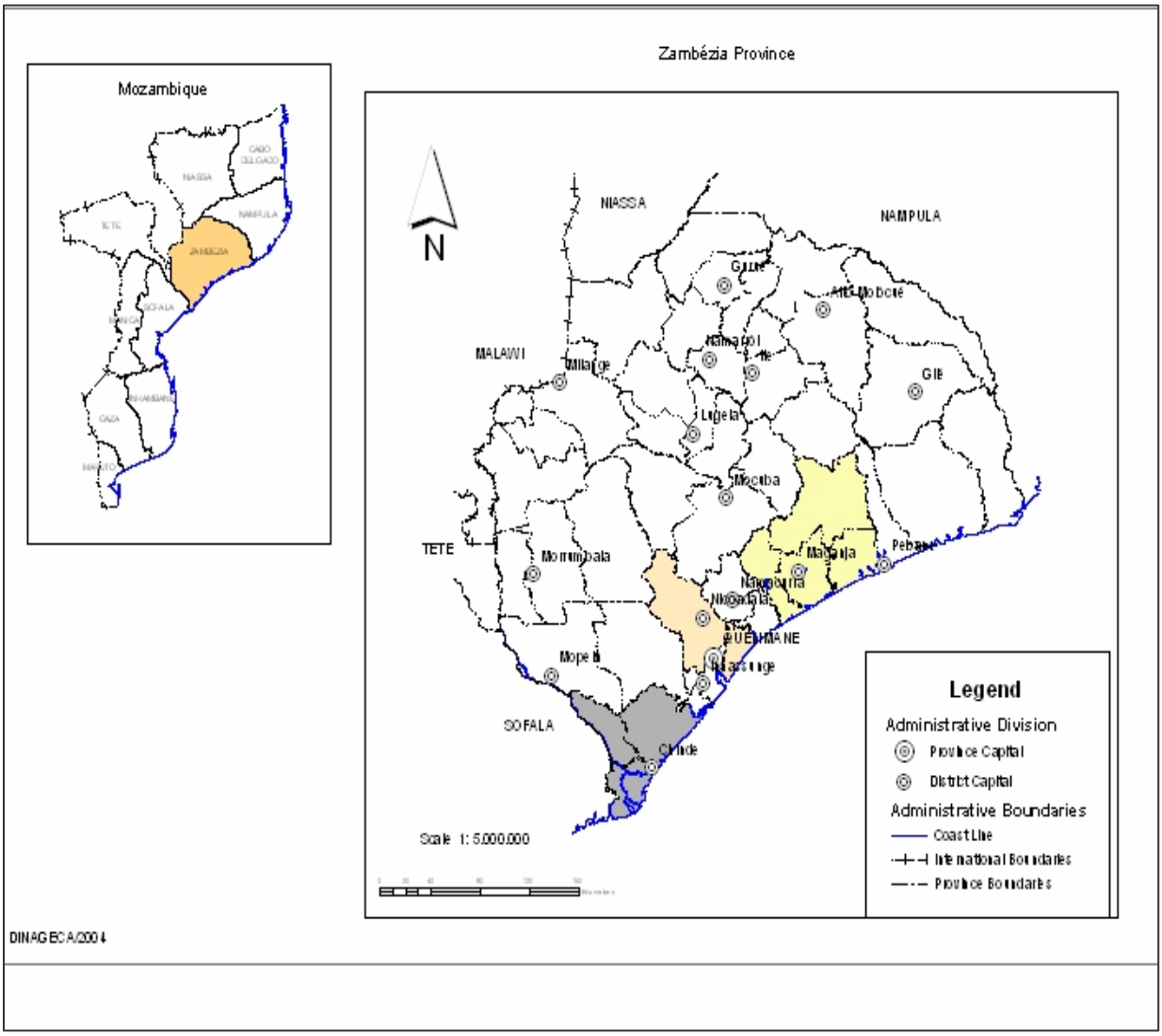


# Chapter 3 : Material and Methods

## Study area

The study was conducted in the Chinde, Nicoadala and Maganja da Costa Districts of Zambézia Province. Zambézia Province is located in the central part of the country (Figure 7). It is bordered in the north by Nampula Province, in the south by Sofala Province, in the west by Tete and Niassa Provinces and by Malawi in the northwest.

**Figure 9**: Location of Zambézia Province and the districts under study




Zambézia Province covers 103,129 km$^2$ and has 3,316,703 inhabitants and a population density of 32,2 inhabitant per km$^2$ (INE 2000).

*Vegetation* -- The littoral zone consists mainly of extensive coconut plantations. It has an extensive and excellent grazing area. Mangal, the dense littoral riverine vegetation, is found along the edges of the Macarau River. The remaining areas are covered with savannah trees of the genus *Brachystegia, Isoberlinia* and *Julbernardia*.

*Climate* -- The climate of Zambézia Province can be divided into two main seasons, a hot and dry season (May – October) and a rainy season (November – April).

**Sample collection**

Eight sampling sites were identified (Table 3).

**Table 3**: Location and geo-references of sampling sites in Zambézia Province.

| Distict | Location | Latitude | Longitude |
|---|---|---|---|
| Nicoadala | Botao | $17^0$ 38’ 07,5’’ S | $36^0$ 47’ 59,7’’ E |
| Nicoadala | Namutengurine | $17^0$ 33’ 31,2’’ S | $36^0$ 33’ 04,3’’ E |
| Nicoadala | Licuary | $17^0$ 32’ 06,0’’ S | $36^0$ 26’ 01,7’’ E |
| Chinde | Micaune | $18^0$16’ 02,5’’ S | $36^0$ 38’ 44,6’’E |
| Maganja da Costa | Cangu | $17^0$ 11’ 27,1’’ S | $37^0$ 28’ 12,0’’E |
| Maganja da Costa | Irrive | $17^0$33’ 30,2’’ S | $37^0$ 32’ 10,2’’ E |
| Maganja da Costa | Raragué 1 | $17^0$ 13’ 35,0’’ S | $37^0$ 31’ 20,7’’ E |
| Maganja da Costa | Raragué 2 | $17^0$ 11’ 52,8’’S | $37^0$ 31’ 04,5’’ E |

A total of 165 animals were selected randomly per district. The sample size was determined using the computer program Epicalculo, with an estimated trypanosomosis prevalence of 13% (according to the last surveys conducted in 2000, a precision level of 5% and a 95% confidence interval. According to DINAP 2001 annual report, the cattle population in Chinde, Nicoadala, and Maganja da Costa Districts was 3,492, 3,559 and 2,105 head, respectively.

Blood was collected from an ear vein into heparinized microhaematocrit centrifuge capillary tubes and onto glass slides as thick and thin blood smears. The capillary tubes were sealed with “Cristaseal” (Hawksley) and centrifuged immediately in a microhaematocrit centrifuge

for 5 minutes at 9000 rpm. After centrifugation, the packed cell volume (PCV) was determined. The buffy coat and the uppermost layer of red blood cells of each specimen were extruded onto a microscope slide and examined for the presence of motile trypanosomes Paris *et al.* (1980). The stained thick and stained thin smears were used for trypanosome species identification and were examined at the Veterinary Faculty of UEM.

**Isolation of trypanosomes in the field**

Outbred albino male and female mice of about 60 days of age, weighing between 30 and 35 g, were provided by the National Veterinary Research Institute (INIVE) and used to isolate the trypanosomes in the field.

The mice were kept in metallic boxes with sawdust bedding and a perforated lid to allow the circulation of air. They were fed and received water *ad libitum.*

Experimental mice were immunosuppressed 24h before injection with blood containing trypanosomes by intraperitoneal injection with 300 mg/kg cyclophosphamide (Endoxan®, Asta Medica, Cambridge, UK).

One immunosuppressed mouse was injected intraperitoneally with 1 ml of parasitaemic blood (*T. congolense*) collected from one parasitologically positive animal. The results of the inoculation are presented in Table 4.

The parasitaemia of the mice inoculated in the field was monitored twice per week using the buffy coat of tail blood. Of the mice that developed a parasitaemia, three isolates from Nicoadala, three isolates from Chinde and one isolate from Maganja da Costa were used for resistance testing (Table 4). Infected blood for resistance testing was collected by heart puncture.

Infected blood from the remainder of the mice inoculated in the field was stored in liquid nitrogen.

**Table 4**: Total number of mice inoculated, mortality 12 hours after inoculation, number of mice developing a parasitaemia and number of isolates used for resistance testing in each district.

| District | Number of mice inoculated | Mortality 12 h after inoculation | Number developing parasitaemia | Isolates used for resistance testing |
|---|---|---|---|---|
| Nicoadala | 7 | 0 | 3 | 3 |
| Chinde | 10 | 0 | 9 | 3 |
| Maganja da Costa | 10 | 7 | 1 | 1 |

**Origin of trypanosome isolates**

The isolates were collected from cattle kept under different management systems and treatment regimens.

Isolates 4, 5 and 6 originated from Micaune in Chinde District. Cattle from which the isolates were collected belonged to Madal Estates, an international commercial company based in Quelimane. Madal Estates consist of extensive coconut plantations, timber concessions and game and cattle ranches. The cattle ranch currently consists of about 3500 animals and has been functional for more than 20 years. Losses due to trypanosomosis are high. The trypanosomosis prevalence ranges between 20-40% and tsetse challenge is high. Trypanosomosis control measures included insecticide treatments of cattle and the regular treatment with trypanocides, i.e. diminazene aceturate and isometamidium chloride. The use of quinapyramine was introduced recently. Relapses after treatment are common, suggesting a high prevalence of trypanosome strains resistant to trypanocides.

The isolate from Cangu in Maganja da Costa District (Isolate 7) was collected from an animal belonging to a commercial herd of approximately 300 animals. The owner has been keeping cattle in the area for more than 15 years. He treats his herd once per year with diminazene aceturate (3.5 mg/kg) and isometamidium chloride (1 mg/kg) and treats the sick animals with diminazene aceturate at 3.5 mg/kg. Despite this treatment regimen, high cattle mortalities due to trypanosomosis were observed in 2002.

In Namutengurine one trypanosome strain (Isolate 1) was collected from cattle belonging to a subsistence farmer. Although the total herd size is large (about 200 animals), animals are only sold when cash needs arise. The owner has been keeping cattle in the area for about 4 years. He obtained the majority of his cattle from Tete Province. About 20 animals originated from Madal Estates. Tsetse challenge in the grazing areas of the herd is high. To protect his cattle, the owner treats all his animals three times per year with diminazene aceturate (3.5 mg/kg) and isometamidium chloride (1 mg/kg).

Isolate 3 was collected from an animal in Botão belonging to a commercial herd of about 300 animals. Tsetse challenge is high and wildlife is present in the area. Animals are treated once a year with diminazene aceturate at 3.5 mg/kg followed by a treatment with isometamidium chloride at 1 mg/kg. Sick animals are treated with diminazene aceturate or quinapyramine.

Isolate 2 was collected in Licuary from an animal belonging to a small-scale farmer who had been operating in the area for about 20 years. His total herd size was 26 animals. Tsetse challenge is high but treatments with trypanocides are irregular and only sick animals are treated with diminazene aceturate at 3.5 mg/kg.

**Table 5:** Origin and number of isolates used in the resistance study.

| District | Location | Isolate number |
|---|---|---|
| Nicoadala | Namutengurine | 1 |
| Nicoadala | Licuare | 2 |
| Nicoadala | Botao | 3 |
| Chinde | Micaune | 4 |
| Chinde | Micaune | 5 |
| Chinde | Micaune | 6 |
| Maganja da Costa | Cangu | 7 |

**Drug-use survey**

All cattle owners in each district were interviewed and their trypanocidal drug-use practices determined. A uniform questionnaire was developed and used (Annex 1). The questionnaire was pre-tested on a pilot basis (Van den Bossche *et al*. 2000). It was revised to clarify

specific questions and ensure that the average time taken to interrogate the respondents was not more than 45 minutes. Questions were posed on herd structure, trypanocidal drug preference, treatment rationale, reason for treatment, method of treatment and treatment frequency. The information obtained was entered into a database. Analyses were conducted using the Tad Info Software.

**Trypanosome isolate resistance testing**

Testing for resistance was done according to the multi-dose protocol described by Eisler *et al*. (2001). For each of the seven isolates (Table 6), five different doses varying between 0.01-20 mg/kg body weight for isometamidium chloride, between 0.01-10 mg/kg body weight for homidium chloride and between 1-30 mg/kg body weight for diminazene aceturate were used. The exact dose rates for each of the drug are presented in Table 6.

For each dose rate 6 mice were treated intraperitoneally with the appropriate quantity of the drug dissolved in 0.2 ml of sterile distilled water 24 hours after the inoculation of the blood containing the trypanosomes. The control mice (six mice per trypanocidal drug) received the same amount of water without the drug.

Trypanocides used in the studies were purchased from DINAP. The drug used were:

- Diminazene aceturate (Nozomil$^{R}$), 3771 NH Barneveld-Holland, the Netherlands.
- Isometamidium chloride (Samorin$^{R}$), Merial, Lyon, France, lot R259971.
- Homidium chloride (Novidium$^{R}$), Merial, Lyon, France, lot R 123461$^{A}$.

**Table 6**: Number of mice treated and dose rates of diminazene aceturate (DMZ), isometamidium chloride (ISM) and homidium chloride (HC).

| Dose of DMZ (mg/kg) | Number of mice treated | Dose of ISM (mg/kg) | Number of mice treated | Dose of HC (mg/kg) | Number of mice treated |
|---|---|---|---|---|---|
| 1.0 | 6 | 0.01 | 6 | 0.01 | 6 |
| 3.0 | 6 | 0.1 | 6 | 0.1 | 6 |
| 10 | 6 | 0.5 | 6 | 0.5 | 6 |
| 20 | 6 | 3.0 | 6 | 3.0 | 6 |
| 30 | 6 | 20.0 | 6 | 10.0 | 6 |
| Control (0) | 6 | Control (0) | 6 | Control (0) | 6 |

Mice treated with diminazene aceturate at 60 mg/kg body weight died soon after treatment. High mortality in mice occurred also when mice were treated with homidium chloride at 20 mg/kg body weight. Both doses were excluded from the experiment.

**Inoculation of trypanosomes for resistance testing**

Mice were infected intraperitoneally with blood containing $1x10^5$ trypanosomes of one of the seven isolates 24 hours before treatment with trypanocides (see above). After inoculation tail blood of each individual mouse was examined twice weekly. Blood was collected from the tail tip into a heparinized capillary tube, centrifuged and the buffy coat was examined. Blood of the trypanocide-treated mice was examined until a relapse occurred or until 60 days post treatment. Parasitaemia of the mice that relapsed was estimated using the method described by Murray *et al.* (1983) (Table 7).

**Table 7**: Estimation of the parasitaemia according to the method of Murray *et al.* (1983).

| Number of trypanosomes per field (magnification x 250) | Score | Estimated parasitemia (Tryps/ml blood) |
|---|---|---|
| >100 | 6+ | $>5x10^6$ |
| >10 | 5+ | $>5x10^5$ |
| 1-10 | 4+ | $10^4$-$5x10^5$ |
| 1 per 2 fields –1 per 10 fields | 3+ | $5x10^3$-$5x10^4$ |
| 1-10 per slide | 2+ | $10^3$-$10^4$ |
| 1 per slide | 1+ | $10^2$-$10^3$ |

Parasitologically positive control mice were euthanized.

**Criterion of susceptibility or resistance of an isolate**

The trypanosome isolates were considered as drug-sensitive if at least 5 out of 6 treated mice were cured, i.e. they remained aparasitaemic until the end of the 60-day observation period. If fewer than 5 mice were cured, the isolate was considered resistant to the dosage used. If one test mouse died prior to detection of parasitaemia, the isolate was considered as drug-sensitive if at least 4 out of the remaining 5 treated mice were cured; if fewer than 4 of remaining 5 mice were cured, the isolate was considered resistant to the dosage used.

# Chapter 4 : Results

## The prevalence of trypanosome infections at the study sites

In Chinde District 21/166, in Nicoadala District 10/179 and in Maganja da Costa District, 49/172 cattle were found infected with trypanosomes. The results are summarised in Table 8.

**Table 8**: Results of the parasitological trypanosomosis survey conducted in Nicoadala, Chinde and Maganja da Costa Districts.

| District | Location | Number sampled | Number positive | | | |
|---|---|---|---|---|---|---|
| | | | *T. c.* | *T. v.* | *T. b.* | Mixed |
| Nicoadala | Botao | 100 | 6 | 1 | - | 1 |
| Nicoadala | Namutengurine | 72 | 1 | - | - | - |
| Nicoadala | Licuari | 07 | 1 | - | - | - |
| Chinde | Micaune | 166 | 13 | 3 | - | 5 |
| Maganja da Costa | Cangu | 100 | 15 | 19 | 2 | 12 |
| Maganja da Costa | Irrive | 48 | - | - | - | - |
| Maganja da Costa | Rarague 1 | 10 | - | - | - | - |
| Maganja da Costa | Rarague 2 | 14 | - | - | - | 1 |

*T.c. = T. congolense*

*T.v. = T. vivax*

*T.b. = T. brucei*

Mixed = *T. congolense and T. vivax*

Other haemoparasites that were detected included *Anaplasma marginale* and *Theileria* spp. (Table 9).

The average PCVs (± SD) of the trypanosomosis positive and the trypanosomosis negative animals are summarised in Table 10.

**Table 9**: Parasitic infections with blood parasites of animals sampled in Nicoadala, Chinde and Maganja da Costa Districts.

| District | *Anaplasma marginale* | *Theileria* spp. |
|---|---|---|
| Nicoadala | 17 | - |
| Chinde | 4 | 5 |
| Maganja da Costa | 16 | 3 |

**Table 10**: Average PCV (± SD) of the parasitologically positive and negative animals in each of the districts.

| District | Average PCV (± SD) in % | |
|---|---|---|
| | Positive | Negative |
| Nicoadala | 26.8 ± 6.7 | 34.6 ± 5.2 |
| Chinde | 23.7 ± 5.7 | 36.3 ± 5.8 |
| Maganja da Costa | 28.5 ± 4.9 | 34.0 ± 4.6 |

**Trypanocide drug-use practices**

A total of 46 cattle owners were interviewed in the three districts. Their drug-use practices are summarised in Table 11. All cattle owners recognise trypanosomosis with others diseases. The cattle owners purchased their trypanocides at the offices of the Provincial Veterinary Services or bought them from private pharmacies in Maputo.

None of cattle owners that was interviewed treated their calves. Diminazene aceturate was used frequently to treat clinically sick animals. On an annual basis a total of 2 to 3 curative treatments are given to sick animals in a herd. The majority of the treatments were administered in November and in May.

**Table 11:** Proportion of cattle owners in three districts using trypanocidal drugs and their drug preference over the last year.

| District | Number of cattle owners | Cattle owners using trypanocides (in %) | Trypanocide used (in %) | | |
|---|---|---|---|---|---|
| | | | Diminazene aceturate | Isometamidium chloride | Both drugs |
| Nicoadala | 28.0 | 60.7 | 39.2 | - | 25.0 |
| Chinde | 9.0 | 22.2 | - | - | 22.2 |
| M. da Costa | 9.0 | 100.0 | 55.5 | - | 44.4 |

The reasons for trypanocidal drug use (i.e., diminazene aceturate and isometamidium chloride) are summarised in Table 12.

**Table 12:** Reasons for diminazene aceturate and isometamidium chloride use in each district.

| Reason for use | Nicoadala | | Chinde | | M. da Costa | |
|---|---|---|---|---|---|---|
| | DMZ % | ISM % | DMZ % | ISM % | DMZ % | ISM % |
| Trypanosomosis diagnosed | 5.8 | | 100.0 | | | |
| To prevent trypanosomosis | | 85.7 | | 100.0 | | 100.0 |
| Trypanosomosis suspected | 41.3 | 14.3 | | | 11.1 | |
| Animal sick | 52.9 | | | | 63.6 | |
| Combination | | | | | | |
| Other reason | | | | | 25.3 | |

An analysis of the drug-use survey showed that the high treatment frequency (in Isolates 4, 5 and 6 from Chinde District), mass treatment (in Isolate 1, from Namutengurine, Isolates 4, 5, 6 from Chinde, and Isolate 7 from Maganja da Costa District), the use of quinapyramine (in Isolate 3 from Nicoadala District and Isolates 4, 5 and 6 from Chinde District) and probably underdosing (mainly in the Cangu area, Maganja da Costa District) were the most important causes of the observed level of drug resistance (Table 13).

**Table 13**: Factors promoting resistance, according to Geerts and Holmes (1998).

| Factor promoting resistance | Isolate | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| High treatment frequency | - | - | - | + | + | + | - |
| Underdosing | ? | - | ? | ? | ? | ? | ? |
| Mass treatment | + | - | | + | + | + | + |
| Use of quinapyramine | | - | + | + | + | + | - |

**Drug sensitivity tests**

*Diminazene aceturate*

The parasitological results of the sensitivity testing of each of the seven isolates for various doses of diminazene aceturate are presented in Tables 14 to 20.

**Table 14**: Sensitivity to various doses of diminazene aceturate of a *T. congolense* isolate (Isolate 1) from Namutengurine in Nicoadala District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 1 | - | - | - | 1+ | 1+ | 1+ | 2+ | 4+ | 6+ | 6+ | X | | | | | | | | | |
| 2 | 1 | - | 1+ | 1+ | 2+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 3 | 1 | - | 1+ | 2+ | 2+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 4 | 1 | - | 1+ | 1+ | 3+ | 5+ | 5+ | 5+ | 6+ | 6+ | X | | | | | | | | | | |
| 5 | 1 | - | 1+ | 1+ | 2+ | 2+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 6 | 1 | - | 1+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 1 | 3 | - | - | - | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | X | | | | | | | | | | |
| 2 | 3 | - | 1+ | 1+ | 3+ | 3+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 3 | 3 | - | 2+ | 2+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | 5+ | 5+ | 5+ | 5+ | 4+ | 4+ | X | | | | |
| 4 | 3 | - | 3+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | | | |
| 5 | 3 | - | 2+ | 2+ | 5+ | 5+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 6 | 3 | - | - | - | 1+ | - | 2+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 1 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 10 | - | 1+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 3 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 10 | - | - | - | 2+ | 4+ | 4+ | 4+ | 3+ | 3+ | 2+ | 6+ | X | | | | | | | | |
| 6 | 10 | - | 1+ | 1+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 1 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |
| 4 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 5 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |
| 6 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 15**: Sensitivity to various doses of diminazene aceturate of a *T. congolense* isolate (Isolate 2) from Licuari in Nicoadala District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 1 | - | 1+ | 2+ | 2+ | 2+ | 2+ | 3+ | 3+ | 6+ | 6+ | X | | | | | | | | | |
| 2 | 1 | - | 1+ | 2+ | 2+ | 3+ | 3+ | 4+ | 4+ | X | | | | | | | | | | | |
| 3 | 1 | - | - | 2+ | 3+ | 3+ | 3+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | |
| 4 | 1 | - | - | 4+ | 4+ | 5+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 5 | 1 | - | - | - | - | 2+ | 2+ | 2+ | 4+ | 4+ | X | | | | | | | | | | |
| 6 | 1 | - | 2+ | - | 1+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 1 | 3 | - | 1+ | 2+ | 2+ | 2+ | 4+ | 6+ | 6+ | 6+ | 3+ | 3+ | X | | | | | | | | |
| 2 | 3 | - | 1+ | 2+ | 2+ | 2+ | 1+ | 4+ | 5+ | 6+ | X | | | | | | | | | | |
| 3 | 3 | - | 1+ | 1+ | 1+ | 3+ | 3+ | 3+ | 6+ | 6+ | X | | | | | | | | | | |
| 4 | 3 | - | - | 3+ | 4+ | 4+ | 5+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 5 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 3 | - | - | 2+ | 2+ | 2+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 1 | 10 | - | - | - | 1+ | 1+ | 2+ | 2+ | 2+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 2 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 10 | - | 2+ | 1+ | 2+ | 2+ | X | | | | | | | | | | | | | | |
| 4 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 10 | - | - | 1+ | 1+ | 3+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 6 | 10 | - | - | 1+ | 3+ | 4+ | 4+ | 4+ | 6+ | 6+ | X | | | | | | | | | | |
| 1 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 20 | - | 1+ | 1+ | 2+ | 2+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | |
| 1 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 30 | - | 1+ | 2+ | 2+ | X | | | | | | | | | | | | | | | |
| 1 | 0 | - | | | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | | | | | | | | | | | | | | | | | | | |
| 3 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |
| 4 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |
| 5 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |
| 6 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 16**: Sensitivity to various doses of diminazene aceturate of a *T. congolense* isolate (Isolate 3) from Botao in Nicoadala District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 1 | 1+ | 1+ | 4+ | 5+ | 6+ | X | | | | | | | | | | | | | | |
| 2 | 1 | 1+ | 1+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 3 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 1 | - | 1+ | 5+ | 5+ | 6+ | X | | | | | | | | | | | | | | |
| 5 | 1 | 1+ | 4+ | 5+ | - | | | | | | | | | | | | | | | | |
| 6 | 1 | - | - | 4+ | 5+ | 6+ | X | | | | | | | | | | | | | | |
| 1 | 3 | - | - | - | 5+ | 6+ | 6+ | 6+ | 4+ | 3+ | 3+ | 3+ | 4+ | 4+ | X | | | | | | |
| 2 | 3 | - | - | - | 1+ | 5+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 3 | 3 | - | - | - | 2+ | 5+ | 5+ | 6+ | 6+ | 6+ | 5+ | 5+ | X | | | | | | | | |
| 4 | 3 | - | - | 1+ | 5+ | 5+ | X | | | | | | | | | | | | | | |
| 5 | 3 | - | - | - | 1+ | 3+ | 5+ | 6+ | 6+ | X | | | | | | | | | | | |
| 6 | 3 | - | - | 2+ | 4+ | 5+ | 6+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | X | | | | | | | |
| 1 | 10 | - | - | - | - | 1+ | 1+ | 3+ | 3+ | 4+ | 6+ | X | | | | | | | | | |
| 2 | 10 | - | 1+ | 2+ | 3+ | 5+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 3 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 10 | - | - | - | - | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 5 | 10 | - | - | 2+ | 2+ | 3+ | 4+ | 6+ | 6+ | 5+ | 5+ | X | | | | | | | | | |
| 6 | 10 | - | - | 1+ | 2+ | 2+ | 2+ | 4+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | |
| 1 | 20 | - | 1+ | 2+ | 2+ | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 2 | 20 | - | - | 1+ | 3+ | 3+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | |
| 3 | 20 | - | - | - | 1+ | 2+ | 2+ | 2+ | 4+ | 4+ | X | | | | | | | | | | |
| 4 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 20 | - | - | 2+ | 3+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 6 | 20 | - | - | 2+ | 2+ | 3+ | 4+ | 4+ | 5+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 1 | 30 | - | 1+ | 1+ | 1+ | 2+ | 3+ | 4+ | 4+ | 4+ | - | | | | | | | | | | |
| 2 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | 1+ | X | | | | | | | | | | | | | | | | | | |
| 2 | 0 | 1+ | X | | | | | | | | | | | | | | | | | | |
| 3 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 4 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 5 | 0 | - | 2+ | X | | | | | | | | | | | | | | | | | |
| 6 | 0 | 1+ | 2+ | X | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 18**: Sensitivity to various doses of diminazene aceturate of a *T. congolense* isolate (Isolate 5) from Micaune in Chinde District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 1 | 2+ | 2+ | 3+ | 6+ | X | | | | | | | | | | | | | | | |
| 3 | 1 | 2+ | 2+ | 4+ | 5+ | 6+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 6+ | X | |
| 4 | 1 | - | 2+ | 3+ | 2+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | X | | | | | | |
| 5 | 1 | - | 2+ | 3+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 3+ | 1+ | 1+ | X | | | | |
| 6 | 1 | - | 4+ | 3+ | 5+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 1 | 3 | 1+ | 1+ | 2+ | 3+ | 4+ | 5+ | 6+ | X | | | | | | | | | | | | |
| 2 | 3 | - | 1+ | 2+ | 4+ | 4+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 3 | 3 | - | 1+ | 1+ | - | 2+ | 4+ | 4+ | 6+ | X | | | | | | | | | | | |
| 4 | 3 | - | 1+ | 3+ | 5+ | 6+ | 6+ | 6+ | 5+ | 6+ | 6+ | 6+ | 4+ | 4+ | 3+ | 3+ | 6+ | X | | | |
| 5 | 3 | - | 1+ | - | 2+ | 4+ | 6+ | 5+ | 5+ | 6+ | 6+ | X | | | | | | | | | |
| 6 | 3 | - | 1+ | 3+ | 5+ | X | | | | | | | | | | | | | | | |
| 1 | 10 | - | 1+ | - | - | 3+ | 4+ | 5+ | 6+ | 6+ | 6+ | 4+ | X | | | | | | | | |
| 2 | 10 | - | 1+ | 1+ | 1+ | 3+ | 5+ | 4+ | X | | | | | | | | | | | | |
| 3 | 10 | - | 1+ | 2+ | 1+ | 4+ | 5+ | 6+ | X | | | | | | | | | | | | |
| 4 | 10 | - | - | 1+ | 1+ | 4+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | |
| 5 | 10 | - | 1+ | 1+ | - | 2+ | 5+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 6 | 10 | - | 1+ | 1+ | 3+ | 5+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 1 | 20 | - | - | - | - | 1+ | - | 1+ | 3+ | 6+ | 6+ | 4+ | 4+ | 4+ | 3+ | 3+ | 4+ | 4+ | X | | |
| 2 | 20 | 1+ | 1+ | 2+ | 3+ | 5+ | X | | | | | | | | | | | | | | |
| 3 | 20 | - | - | 1+ | 2+ | 3+ | 3+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 3+ | 4+ | 4+ | 3+ | X | |
| 4 | 20 | - | - | 1+ | 2+ | 3+ | 5+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 5 | 20 | - | - | 1+ | 3+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 6 | 20 | - | - | 1+ | 3+ | 4+ | 5+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 1 | 30 | - | - | - | - | 1+ | 1+ | 1+ | 3+ | 3+ | 6+ | 6+ | 6+ | X | | | | | | | |
| 2 | 30 | - | - | 1+ | 2+ | 2+ | 2+ | 4+ | 4+ | 6+ | 6+ | X | | | | | | | | | |
| 3 | 30 | - | - | 2+ | 2+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 4 | 30 | - | - | - | 1+ | 4+ | X | | | | | | | | | | | | | | |
| 5 | 30 | - | - | - | 2+ | 4+ | 6+ | 6+ | 6+ | 4+ | 2+ | 2+ | 6+ | X | | | | | | | |
| 6 | 30 | - | 2+ | 4+ | 6+ | X | | | | | | | | | | | | | | | |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 4 | 0 | - | - | - | - | - | - | - | - | - | - | 1+ | X | | | | | | | | |
| 5 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 6 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 19**: Sensitivity to various doses of diminazene aceturate of a *T. congolense* isolate (Isolate 6) from Micaune in Chinde District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 1 | - | 2+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 2 | 1 | 1+ | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 3 | 1 | 1+ | 3+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 4 | 1 | 1+ | 1+ | 5+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 5 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 1 | - | 1+ | 2+ | 5+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 1 | 3 | 1+ | - | 1+ | 2+ | 5+ | 6+ | 6+ | 6+ | 4+ | 6+ | X | | | | | | | | | |
| 2 | 3 | 1+ | 1+ | 2+ | 2+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 3 | 3 | - | - | 1+ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 3 | - | 1+ | 3+ | 4+ | 5+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 5 | 3 | 1+ | 3+ | 4+ | 5+ | 6+ | 6+ | X | | | | | | | | | | | | | |
| 6 | 3 | - | - | 1+ | 2+ | 2+ | 4+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 1 | 10 | 1+ | 3+ | 3+ | 3+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | X | | | | | | | |
| 2 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 10 | - | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 4 | 10 | 1+ | 3+ | 4+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 5 | 10 | - | 1+ | 1+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 6 | 10 | - | - | - | - | - | - | - | 1+ | 1+ | 3+ | 4+ | 6+ | 6+ | X | | | | | | |
| 1 | 20 | - | 1+ | 2+ | 4+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 2 | 20 | 1+ | 1+ | 5+ | X | | | | | | | | | | | | | | | | |
| 3 | 20 | - | 1+ | 2+ | 4+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 4 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 20 | - | 1+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 6 | 20 | - | - | 1+ | 3+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 1 | 30 | - | 1+ | 1+ | 3+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 2 | 30 | - | - | - | 1+ | 1+ | 1+ | 2+ | 4+ | 4+ | 4+ | 4+ | X | | | | | | | | |
| 3 | 30 | - | 1+ | 2+ | 2+ | 4+ | 4+ | 3+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | X | | | | | | |
| 4 | 30 | - | 1+ | 4+ | 6+ | X | | | | | | | | | | | | | | | |
| 5 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 30 | - | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 1 | 0 | - | 2+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | 1+ | X | | | | | | | | | | | | | | | | | | |
| 3 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 5 | 0 | - | 2+ | X | | | | | | | | | | | | | | | | | |
| 6 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 20**: Sensitivity to various doses of diminazene aceturate of a *T. congolense* isolate (Isolate 7) from Cangu in Maganja da Costa District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 1 | - | - | - | 1+ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 1 | - | 1+ | 1+ | 1+ | 2+ | 2+ | 2+ | 6+ | 6+ | X | | | | | | | | | | |
| 3 | 1 | - | - | - | - | - | 6+ | 6+ | 6+ | 4+ | 4+ | X | | | | | | | | | |
| 4 | 1 | - | - | - | - | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 5 | 1 | - | - | 1+ | 3+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 6 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 3 | - | - | - | 1+ | 1+ | 1+ | 2+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | |
| 2 | 3 | - | - | - | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 3 | 3 | - | - | 1+ | 2+ | 2+ | 2+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | X | | | | | | |
| 4 | 3 | - | - | - | 1+ | 1+ | 3+ | 3+ | 6+ | 6+ | 5+ | 4+ | 4+ | 4+ | X | | | | | | |
| 5 | 3 | - | - | - | - | 1+ | 5+ | 3+ | 1+ | 1+ | 6+ | X | | | | | | | | | |
| 6 | 3 | - | 1+ | 2+ | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | |
| 1 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 10 | - | - | - | - | - | 1+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | X | | | | |
| 3 | 10 | - | - | - | - | - | 1+ | 3+ | 3+ | 3+ | 3+ | 4+ | 4+ | 2+ | X | | | | | | |
| 4 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 10 | - | - | - | - | ´- | 1+ | 1+ | 4+ | 6+ | 6+ | 4+ | 4+ | 4+ | X | | | | | | |
| 6 | 10 | - | - | - | - | 1+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | X | | | | | | | |
| 1 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 20 | - | - | - | - | - | 1+ | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | X | | | |
| 5 | 20 | - | - | - | - | - | - | - | - | - | - | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X |
| 6 | 20 | - | - | - | - | - | 1+ | - | ´- | ´- | ´- | ´- | ´- | ´- | ´- | ´- | ´- | ´- | ´- | ´- | ´- |
| 1 | 30 | - | - | - | - | - | 1+ | 3+ | 3+ | 4+ | 4+ | 2+ | 2+ | 2+ | X | | | | | | |
| 2 | 30 | - | - | - | 1+ | 1+ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 30 | - | - | - | ´- | 1+ | 1+ | 3+ | 3+ | 5+ | 5+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | X | | |
| 5 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |
| 4 | 0 | - | 1+ | | | | | | | | | | | | | | | | | | |
| 5 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |
| 6 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

*Isometamidium chloride*

The parasitological results of the sensitivity testing of each of the seven isolates for various doses of isometamidium chloride are presented in Tables 21 to 27.

**Table 21**: Sensitivity to various doses of isometamidium chloride of a *T. congolense* isolate (Isolate 1) from Namutengurine in Nicoadala District. The figures in the table represent the *parasitaemia* estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | 1+ | 6+ | 6+ | X | | | | | | | | | | | | | | | |
| 2 | 0.01 | - | - | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 3 | 0.01 | - | 1+ | 6+ | X | | | | | | | | | | | | | | | | |
| 4 | 0.01 | - | 2+ | 4+ | 6+ | 6+ | X | | | | | | | | | | | | | | |
| 5 | 0.01 | - | 2+ | 4+ | 6+ | X | | | | | | | | | | | | | | | |
| 6 | 0.01 | - | 1+ | 6+ | 6+ | X | | | | | | | | | | | | | | | |
| 1 | 0.1 | - | 1+ | 4+ | 4+ | 6+ | X | | | | | | | | | | | | | | |
| 2 | 0.1 | - | 1+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | |
| 3 | 0.1 | - | - | 1+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 4 | 0.1 | - | - | - | 1+ | 6+ | 6+ | X | | | | | | | | | | | | | |
| 5 | 0.1 | - | 1+ | 4+ | 4+ | 6+ | 6+ | 4+ | X | | | | | | | | | | | | |
| 6 | 0.1 | - | 1+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 1 | 0.5 | - | - | 1+ | - | | | | | | | | | | | | | | | | |
| 2 | 0.5 | - | 1+ | 1+ | 2+ | 4+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 3 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | 1+ | X | | | | | | | | | | | | | | | | |
| 3 | 0 | - | 2+ | 2+ | X | | | | | | | | | | | | | | | | |
| 4 | 0 | - | - | 4+ | X | | | | | | | | | | | | | | | | |
| 5 | 0 | - | - | 2+ | X | | | | | | | | | | | | | | | | |
| 6 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 22**: Sensitivity to various doses of isometamidium chloride of a *T. congolense* isolate (Isolate 2) from Licuari in Nicoadala District. The figures in the table represent the *parasitaemia* estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 5 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 6 | 0 | 2+ | X | | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 23**: Sensitivity to various doses of isometamidium chloride of a *T. congolense* isolate (Isolate 3) from Botao in Nicoadala District. The figures in the table represent the *parasitaemia* estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | - | - | - | 1+ | 6+ | X | | | | | | | | | | | | - |
| 2 | 0.01 | - | - | - | - | 1+ | 1+ | 4+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 6+ | 4+ | 4+ | 4+ | 6+ | 4+ | - |
| 3 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 0.01 | - | - | 1+ | 2+ | 2+ | 4+ | 4+ | 6+ | X | | | | | | | | | | | - |
| 5 | 0.01 | - | - | - | - | - | 1+ | 1+ | 2+ | 4+ | 4+ | 6+ | 6+ | X | | | | | | | - |
| 6 | 0.01 | - | - | - | - | - | 1+ | 4+ | 4+ | 6+ | 6+ | 6+ | 4+ | X | | | | | | | - |
| 1 | 0.1 | - | - | 1+ | 3+ | 4+ | X | | | | | | | | | | | | | | - |
| 2 | 0.1 | - | - | - | - | 2+ | 4+ | 4+ | 4+ | 6+ | 4+ | X | | | | | | | | | - |
| 3 | 0.1 | - | - | - | 1+ | 2+ | 6+ | 4+ | 4+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | - |
| 4 | 0.1 | - | - | - | 1+ | 2+ | 4+ | 4+ | X | | | | | | | | | | | | - |
| 5 | 0.1 | - | - | - | 4+ | 4+ | 6+ | X | | | | | | | | | | | | | - |
| 6 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.5 | - | - | 1+ | - | 4+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | - |
| 3 | 0.5 | - | - | - | - | - | 1+ | 4+ | 4+ | X | | | | | | | | | | | - |
| 4 | 0.5 | - | - | - | - | - | 1+ | 6+ | 4+ | 6+ | X | | | | | | | | | | - |
| 5 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.5 | - | - | - | - | 1+ | 4+ | 4+ | 6+ | 4+ | X | | | | | | | | | | - |
| 1 | 3 | - | - | 1+ | 2+ | 4+ | 6+ | 6+ | 6+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | - |
| 2 | 3 | - | - | - | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | - |
| 3 | 3 | - | - | - | - | - | 4+ | 4+ | 1+ | 6+ | X | | | | | | | | | | - |
| 4 | 3 | - | - | - | - | - | - | - | 1+ | 6+ | X | | | | | | | | | | - |
| 5 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | 1+ | X | | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 4 | 0 | 1+ | X | | | | | | | | | | | | | | | | | | |
| 5 | 0 | - | 2+ | X | | | | | | | | | | | | | | | | | |
| 6 | 0 | 1+ | X | | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 24**: Sensitivity to various doses of isometamidium chloride of a *T. congolense* isolate (Isolate 4) from Micaune in Chinde District. The figures in the table represent the *parasitaemia* estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | - | 1+ | 4+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 2 | 0.01 | - | - | - | 1+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 3 | 0.01 | - | - | - | 1+ | 3+ | 4+ | 2+ | 1+ | 6+ | X | | | | | | | | | | |
| 4 | 0.01 | - | - | 1+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | |
| 5 | 0.01 | - | - | 1+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | |
| 6 | 0.01 | - | - | - | 1+ | 4+ | 6+ | 6+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | |
| 1 | 0.1 | - | - | - | 1+ | 4+ | 6+ | 6+ | 4+ | 3+ | 3+ | 6+ | 6+ | X | | | | | | | |
| 2 | 0.1 | - | - | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 3 | 0.1 | - | - | 1+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 4 | 0.1 | - | - | 2+ | 2+ | 6+ | 4+ | 4+ | 4+ | 4+ | X | | | | | | | | | | |
| 5 | 0.1 | - | - | 1+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 6 | 0.1 | - | - | 1+ | 3+ | 6+ | 6+ | 6+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 1 | 0.5 | - | - | 2+ | 6+ | 6+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | |
| 2 | 0.5 | - | - | - | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 3 | 0.5 | - | 1+ | 4+ | 4+ | 6+ | X | | | | | | | | | | | | | | |
| 4 | 0.5 | - | 1+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 5 | 0.5 | - | 1+ | 1+ | 1+ | 1+ | 4+ | 6+ | X | | | | | | | | | | | | |
| 6 | 0.5 | - | 1+ | 1+ | 4+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | |
| 1 | 3 | - | 1+ | 3+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 2 | 3 | - | 4+ | 3+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 3 | 3 | - | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | |
| 4 | 3 | - | 1+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 5 | 3 | - | 2+ | 4+ | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | |
| 6 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 20 | - | - | - | 1+ | 2+ | 2+ | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 3 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 20 | - | - | - | 1+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 5 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 4 | 0 | 1+ | X | | | | | | | | | | | | | | | | | | |
| 5 | 0 | 1+ | X | | | | | | | | | | | | | | | | | | |
| 6 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 25**: Sensitivity to various doses of isometamidium chloride of a *T. congolense* isolate (Isolate 5) from Micaune in Chinde District. The figures in the table represent the *parasitaemia* estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | - | - | - | 1+ | 4+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 2 | 0.01 | - | 1+ | 4+ | 6+ | X | | | | | | | | | | | | | | | |
| 3 | 0.01 | - | 1+ | 1+ | 3+ | 3+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | |
| 4 | 0.01 | - | 1+ | 6+ | X | | | | | | | | | | | | | | | | |
| 5 | 0.01 | - | 1+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 6 | 0.01 | - | 1+ | - | - | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 1 | 0.1 | - | 4+ | 6+ | X | | | | | | | | | | | | | | | | |
| 2 | 0.1 | - | - | - | 4+ | 4+ | 4+ | 6+ | X | | | | | | | | | | | | |
| 3 | 0.1 | - | 1+ | 3+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 4 | 0.1 | - | - | - | - | 3+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | |
| 5 | 0.1 | - | - | - | 4+ | 6+ | 6+ | 6+ | 4+ | 3+ | 3+ | 6+ | 6+ | 6+ | X | | | | | | |
| 6 | 0.1 | - | 1+ | 1+ | 3+ | 6+ | X | | | | | | | | | | | | | | |
| 1 | 0.5 | - | 4+ | 6+ | X | | | | | | | | | | | | | | | | |
| 2 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 0.5 | - | - | - | 3+ | - | 6+ | 6+ | X | | | | | | | | | | | | |
| 5 | 0.5 | - | 1+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 6 | 0.5 | - | - | 4+ | 4+ | 2+ | 6+ | X | | | | | | | | | | | | | |
| 1 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 3 | - | 1+ | 4+ | 4+ | 6+ | 6+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 3 | 3 | - | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 4 | 3 | - | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 5 | 3 | - | 4+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | X | | |
| 6 | 3 | - | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 1 | 20 | - | - | 1+ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 20 | - | - | - | 1+ | 4+ | 4+ | 4+ | 4+ | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 3 | 20 | - | - | 1+ | - | - | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 20 | - | - | 1+ | 4+ | 4+ | 3+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 6 | 20 | - | - | - | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 3 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 4 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 5 | 0 | - | - | - | 1+ | X | | | | | | | | | | | | | | | |
| 6 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 26**: Sensitivity to various doses of isometamidium chloride of a *T. congolense* isolate (Isolate 6) from Micaune in Chinde District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | - | 1+ | 4+ | 6+ | 6+ | 6+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | | |
| 2 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 0.01 | - | - | 4+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 6+ | 4+ | 6+ | X | | | | | | |
| 4 | 0.01 | - | - | 4+ | 6+ | X | | | | | | | | | | | | | | | |
| 5 | 0.01 | - | 4+ | 4+ | 6+ | 6+ | X | | | | | | | | | | | | | | |
| 6 | 0.01 | - | - | 3+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 0.1 | - | - | - | 2+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 2 | 0.1 | - | - | 1+ | 2+ | 4+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 3 | 0.1 | - | - | - | - | 4+ | 6+ | 6+ | 4+ | 6+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 0.1 | - | - | 2+ | 4+ | 6+ | 6+ | X | | | | | | | | | | | | | |
| 5 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0.5 | - | - | 2+ | 2+ | 6+ | 4+ | 4+ | 4+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 2 | 0.5 | - | - | 6+ | 6+ | 6+ | x | | | | | | | | | | | | | | |
| 3 | 0.5 | - | 2+ | 6+ | 6+ | X | | | | | | | | | | | | | | | |
| 4 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0.5 | - | 2+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 6 | 0.5 | - | - | 2+ | 6+ | X | | | | | | | | | | | | | | | |
| 1 | 3 | - | - | 1+ | 4+ | 6+ | 6+ | 6+ | 4+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 2 | 3 | - | - | - | 1+ | 6+ | 6+ | 6+ | 3+ | 4+ | 4+ | X | | | | | | | | | |
| 3 | 3 | - | 2+ | 2+ | 4+ | 6+ | 6+ | 6+ | 3+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 3 | - | 2+ | 2+ | 6+ | 6+ | 4+ | 6+ | 3+ | 6+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 5 | 3 | - | - | 1+ | 6+ | 6+ | 6+ | 6+ | 3+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 6 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 20 | - | - | - | - | 1+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 3+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 2 | 20 | - | - | - | 2+ | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 20 | - | - | - | - | - | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 6 | 20 | - | - | - | - | 1+ | 2+ | 2+ | 4+ | 4+ | 4+ | 6+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 2 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 3 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 4 | 0 | - | - | - | 1+ | X | | | | | | | | | | | | | | | |
| 5 | 0 | - | - | - | 1+ | X | | | | | | | | | | | | | | | |
| 6 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 27**: Sensitivity to various doses of isometamidium chloride of *a T. congolense* isolate (Isolate 7) from Cangu in Maganja da Costa District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | 1+ | 4+ | 6+ | 6+ | 4+ | 4+ | 3+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | X |
| 2 | 0.01 | - | - | 1+ | 1+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 4+ | X | |
| 3 | 0.01 | - | - | 1+ | 6+ | 6+ | X | | | | | | | | | | | | | | |
| 4 | 0.01 | - | - | - | - | 1+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | X | | | | | | | | |
| 5 | 0.01 | - | - | 1+ | 1+ | 4+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 6 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0.1 | - | 1+ | 6+ | 6+ | X | | | | | | | | | | | | | | | |
| 2 | 0.1 | - | - | - | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 3 | 0.1 | - | - | 6+ | 6+ | X | | | | | | | | | | | | | | | |
| 4 | 0.1 | - | - | - | 4+ | 6+ | X | | | | | | | | | | | | | | |
| 5 | 0.1 | - | - | 1+ | 4+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 6+ | 6+ | X | | | | | | | |
| 6 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0.5 | - | - | 4+ | 6+ | 6+ | 6+ | 4+ | 4+ | 6+ | 6+ | X | | | | | | | | | |
| 2 | 0.5 | - | 1+ | 4+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ |
| 3 | 0.5 | - | 1+ | 4+ | 4+ | 2+ | 3+ | 3+ | 4+ | 4+ | 6+ | 4+ | 4+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 6+ | 6+ |
| 4 | 0.5 | - | - | 1+ | 4+ | 6+ | 6+ | X | | | | | | | | | | | | | |
| 5 | 0.5 | - | - | 1+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 6+ | X | | | | | | | |
| 6 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 3 | - | 4+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | | |
| 3 | 3 | - | 1+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 1+ | 1+ | X | | |
| 4 | 3 | - | 1+ | 4+ | 4+ | 6+ | 6+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | |
| 5 | 3 | - | - | 1+ | 3+ | 6+ | 4+ | 6+ | 4+ | 4+ | 4+ | 4+ | 1+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 6 | 3 | - | - | 1+ | 4+ | 6+ | 6+ | 4+ | 6+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 6+ | 6+ |
| 1 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 20 | - | - | - | - | 1+ | 4+ | 1+ | 1+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 3 | 20 | - | - | - | - | 1+ | 2+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 4 | 20 | - | 1+ | 4+ | 4+ | 4+ | 4+ | 4+ | 2+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 3+ | 4+ | 4+ | 4+ |
| 5 | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 20 | - | - | 1+ | - | 4+ | 6+ | 4+ | 1+ | 4+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | - | - | - | 1+ | X | | | | | | | | | | | | | | | |
| 4 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 5 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 6 | 0 | - | - | - | 1+ | X | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

*Homidium chloride*

The parasitological results of the sensitivity testing of each of the seven isolates for various doses of homidium chloride are presented in Tables 28 to 34.

**Table 28**: Sensitivity to various doses of homidium chloride of a *T. congolense* isolate (Isolate 1) from Namutengurine in Nicoadala District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.01 | - | 1+ | 1+ | 2+ | 2+ | 4+ | 6+ | X | | | | | | | | | | | | |
| 3 | 0.01 | - | - | - | 1+ | 1+ | 2+ | 4+ | 6+ | 6+ | 6+ | 4+ | 4+ | 6+ | X | | | | | | |
| 4 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | | | | | | | |
| 5 | 0.01 | - | - | - | 1+ | 1+ | 1+ | 4+ | 4+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 6+ | X | | | |
| 6 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.1 | - | - | 1+ | 1+ | 1+ | 3+ | 3+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ |
| 3 | 0.1 | - | - | - | - | - | - | 1+ | 1+ | 1+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 0.1 | - | - | - | 1+ | 1+ | 2+ | 2+ | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | X | | | | | | |
| 5 | 0.1 | - | - | - | 1+ | 2+ | 1+ | 1+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | |
| 6 | 0.1 | - | - | - | - | 2+ | 2+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | |
| 1 | 0.5 | - | - | - | - | - | - | 1+ | 1+ | 1+ | 1+ | 3+ | 3+ | 4+ | 6+ | 6+ | 6+ | X | | | |
| 2 | 0.5 | - | - | - | 1+ | 1+ | 1+ | 1+ | 2+ | 2+ | 1+ | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 3 | 0.5 | - | - | - | - | 1+ | 1+ | 2+ | 2+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ |
| 4 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 3 | - | - | 1+ | 1+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | |
| 2 | 3 | - | - | - | 1+ | 1+ | 1+ | 2+ | 2+ | X | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 3 | - | - | 1+ | 1+ | 1+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | X | - | - | - | - | - | - | - | - |
| 5 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 3 | - | - | - | - | 1+ | 1+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | - | - | - | - | - | - |
| 1 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | -- | - | - | - |
| 3 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | -- | - | - | - | - | - | - |
| 6 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | | | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | | | 1+ | X | | | | | | | | | | | | | | | | |
| 4 | 0 | | 1+ | X | | | | | | | | | | | | | | | | | |
| 5 | 0 | | 1+ | X | | | | | | | | | | | | | | | | | |
| 6 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 29**: Sensitivity to various doses of homidium chloride of a *T. congolense* isolate (Isolate 2) from Licuari in Nicoadala District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | 1+ | 1+ | 1+ | 2+ | 2+ | 2+ | 3+ | 3+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | |
| 2 | 0.01 | - | - | 1+ | 1+ | 4+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 3 | 0.01 | - | - | - | 1+ | 2+ | 2+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 4 | 0.01 | - | - | - | 1+ | 1+ | 1+ | 1+ | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | 4+ | X | | | | |
| 5 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.01 | - | 1+ | 1+ | 1+ | 2+ | 2+ | 2+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | |
| 1 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 0.1 | - | - | - | 1+ | 1+ | 2+ | 4+ | 6+ | 6+ | 6+ | X | - | - | - | - | -- | - | - | - | - |
| 4 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0.1 | - | 1+ | 1+ | 1+ | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 6 | 0.1 | - | - | - | - | - | - | 1+ | 1+ | 1+ | 1+ | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 1 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.5 | - | 1+ | 1+ | 1+ | 2+ | 2+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | X | - | - | - | - |
| 3 | 0.5 | - | - | - | 1+ | 1+ | 4+ | 4+ | X | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0.5 | - | 1+ | 2+ | 2+ | X | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | -- | - | - | - |
| 3 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 4 | 0 | 1+ | X | | | | | | | | | | | | | | | | | | |
| 5 | 0 | - | - | 1X | | | | | | | | | | | | | | | | | |
| 6 | 0 | 1X | | | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 30**: Sensitivity to various doses of homidium chloride of a *T. congolense* isolate (Isolate 3) from Botao in Nicoadala District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | 2+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 2 | 0.01 | - | 1+ | 2+ | 5+ | X | | | | | | | | | | | | | | | |
| 3 | 0.01 | - | 1+ | 2+ | 4+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 4 | 0.01 | - | 2+ | 2+ | 5+ | 6+ | 6+ | 4+ | 4+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 5 | 0.01 | - | 1+ | 3+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 6 | 0.01 | - | 3+ | 3+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 1 | 0.1 | - | 1+ | 2+ | 5+ | X | | | | | | | | | | | | | | | |
| 2 | 0.1 | - | 2+ | 2+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 3 | 0.1 | - | 1+ | 2+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 3+ | 6+ | 6+ | 6+ |
| 4 | 0.1 | - | 2+ | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 5 | 0.1 | - | 1+ | 2+ | 6+ | X | | | | | | | | | | | | | | | |
| 6 | 0.1 | - | 1+ | 2+ | 2+ | 3+ | 4+ | 4+ | 4+ | 6+ | X | | | | | | | | | | |
| 1 | 0.5 | - | 2+ | 2+ | 6+ | 6+ | X | | | | | | | | | | | | | | |
| 2 | 0.5 | - | 3+ | 2+ | 5+ | 5+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 3 | 0.5 | - | 1+ | 2+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | | |
| 4 | 0.5 | - | 1+ | 3+ | 5+ | X | | | | | | | | | | | | | | | |
| 5 | 0.5 | - | 3+ | 3+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 6 | 0.5 | - | 2+ | 3+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 3+ | 3+ | 3+ | 3+ | X | | | | |
| 1 | 3 | - | - | - | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 2 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 3 | - | - | - | - | 2+ | 4+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 10 | - | - | - | 3+ | 3+ | 3+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 3 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 4 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 5 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 6 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 31**: Sensitivity to various doses of homidium chloride of a *T. congolense* isolate (Isolate 4) from Micaune in Chinde District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | 2+ | 4+ | 6+ | X | | | | | | | | | | | | | | |
| 2 | 0.01 | - | 1+ | 3+ | 4+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 3 | 0.01 | - | - | - | - | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 4 | 0.01 | - | - | - | - | 3+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | |
| 5 | 0.01 | - | - | 2+ | 4+ | 4+ | 6+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | |
| 6 | 0.01 | - | - | 2+ | 6+ | 4+ | 1+ | X | | | | | | | | | | | | | |
| 1 | 0.1 | - | - | - | - | 2+ | 4+ | 6+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 2 | 0.1 | - | - | 2+ | 2+ | 4+ | 4+ | 6+ | X | | | | | | | | | | | | |
| 3 | 0.1 | - | - | 1+ | 4+ | 6+ | 4+ | 4+ | 4+ | 4+ | X | | | | | | | | | | |
| 4 | 0.1 | - | - | 1+ | 4+ | 6+ | 6+ | 4+ | 6+ | 1+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 5 | 0.1 | - | - | 2+ | 6+ | 6+ | X | | | | | | | | | | | | | | |
| 6 | 0.1 | - | - | - | - | 1+ | 2+ | 6+ | 4+ | 1+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 0.5 | - | - | 2+ | 4+ | 4+ | 6+ | 4+ | X | | | | | | | | | | | | |
| 2 | 0.5 | - | - | 1+ | 6+ | 6+ | 4+ | 6+ | X | | | | | | | | | | | | |
| 3 | 0.5 | - | - | 3+ | 4+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 0.5 | - | - | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | X | | | | | | | | |
| 5 | 0.5 | - | - | 1+ | 4+ | 6+ | 4+ | 6+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 6 | 0.5 | - | - | 1+ | 4+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 3 | - | - | - | - | 1+ | 4+ | 6+ | X | | | | | | | | | | | | |
| 2 | 3 | - | - | - | 2+ | 1+ | 5+ | X | | | | | | | | | | | | | |
| 3 | 3 | - | - | - | - | 1+ | 2+ | 4+ | 6+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 3 | - | - | - | 2+ | 6+ | 4+ | 6+ | X | | | | | | | | | | | | |
| 6 | 3 | - | - | - | 2+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 4 | 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 6 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 32**: Sensitivity to various doses of homidium chloride of a *T. congolense* isolate (Isolate 5) from Micaune in Chinde District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | 1+ | 1+ | 4+ | 4+ | 4+ | 5+ | 5+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 2 | 0.01 | - | 1+ | 2+ | 2+ | 2+ | 2+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 3 | 0.01 | - | 1+ | 1+ | 1+ | 1+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 0.01 | - | 2+ | 3+ | 3+ | 3+ | 6+ | X | | | | | | | | | | | | | |
| 5 | 0.01 | - | 1+ | 1+ | 1+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | |
| 6 | 0.01 | - | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | X | | | | |
| 1 | 0.1 | - | 1+ | 1+ | 1+ | 5+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 2 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 0.1 | - | 1+ | 1+ | 3+ | 3+ | 3+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | |
| 5 | 0.1 | - | - | 1+ | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | |
| 6 | 0.1 | - | 1+ | 3+ | 3+ | 3+ | 3+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.5 | - | - | 2+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 3 | 0.5 | - | - | 2+ | 4+ | X | | | | | | | | | | | | | | | |
| 4 | 0.5 | - | - | 1+ | 1+ | 2+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 5 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.5 | - | - | - | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 3 | - | 1+ | 1+ | 1+ | 4+ | X | | | | | | | | | | | | | | |
| 2 | 3 | - | - | 1+ | 2+ | 2+ | 2+ | 2+ | 6+ | X | | | | | | | | | | | |
| 3 | 3 | - | - | - | - | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ |
| 4 | 3 | - | - | - | - | 1+ | 1+ | 4+ | 4+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ |
| 5 | 3 | - | - | - | - | 1+ | 1+ | 1+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 6 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | - | - | 1+ | X | | | | | | | | | | | | | | | |
| 3 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 4 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 5 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 6 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 33**: Sensitivity to various doses of homidium chloride of a *T. congolense* isolate (Isolate 6) from Micaune in Chinde District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.01 | - | 1+ | 2+ | 2+ | 2+ | 4+ | 4+ | 6+ | X | | | | | | | | | | | |
| 3 | 0.01 | - | - | - | 1+ | 2+ | 5+ | 5+ | 5+ | 6+ | 6+ | X | | | | | | | | | |
| 4 | 0.01 | - | 1+ | 1+ | 3+ | 3+ | 6+ | X | | | | | | | | | | | | | |
| 5 | 0.01 | 1+ | 1+ | 1+ | 2+ | 2+ | 3+ | 2+ | X | | | | | | | | | | | | |
| 6 | 0.01 | - | - | 1+ | 1+ | 2+ | 2+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | |
| 1 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 0.1 | - | - | 1+ | 2+ | 2+ | 4+ | 6+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 3 | 0.1 | - | - | 3+ | 3+ | 6+ | 6+ | X | | | | | | | | | | | | | |
| 4 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0.1 | - | - | 1+ | 2+ | 2+ | 2+ | 4+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 6 | 0.1 | - | - | 2+ | 2+ | 5+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 1 | 0.5 | - | - | 1+ | 1+ | 1+ | 3+ | 3+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 2 | 0.5 | - | 1+ | 2+ | 2+ | 4+ | 4+ | X | | | | | | | | | | | | | |
| 3 | 0.5 | - | - | 2+ | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 0.5 | - | - | 1+ | 1+ | 2+ | 2+ | 6+ | X | | | | | | | | | | | | |
| 5 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 0.5 | - | 1+ | 2+ | 6+ | 6+ | X | | | | | | | | | | | | | | |
| 1 | 3 | - | - | - | 1+ | 1+ | 2+ | 4+ | 6+ | 6+ | 4+ | 6+ | X | | | | | | | | |
| 2 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 3 | - | - | - | 2+ | 2+ | 2+ | 2+ | 4+ | X | | | | | | | | | | | |
| 5 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 10 | - | - | - | - | 1+ | 1+ | 1+ | 1+ | 2+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 2 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 4 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 5 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 6 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

**Table 34**: Sensitivity to various doses of homidium chloride of a *T. congolense* isolate (Isolate 7) from Cangu in Maganja da Costa District. The figures in the table represent the parasitaemia estimated according to the Murray method (Table 7).

| Mouse | Dose (in mg/kg) | Score of the parasitaemia on various days post-treatment | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 |
| 1 | 0.01 | - | 2+ | 3+ | 3+ | 3+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 2 | 0.01 | - | 1+ | 5+ | 6+ | 6+ | X | | | | | | | | | | | | | | |
| 3 | 0.01 | - | 1+ | 1+ | 5+ | 6+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | |
| 4 | 0.01 | - | - | 1+ | 1+ | 2+ | 2+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 5 | 0.01 | - | 1+ | 5+ | X | | | | | | | | | | | | | | | | |
| 6 | 0.01 | - | 1+ | 4+ | 4+ | 6+ | X | | | | | | | | | | | | | | |
| 1 | 0.1 | - | 2+ | 3+ | 3+ | 6+ | 6+ | 4+ | 4+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 2 | 0.1 | - | 4+ | 1+ | 1+ | 3+ | 6+ | X | | | | | | | | | | | | | |
| 3 | 0.1 | - | 3+ | 4+ | 4+ | 6+ | 6+ | 6+ | X | | | | | | | | | | | | |
| 4 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 0.1 | - | - | - | 2+ | 2+ | 6+ | 6+ | 4+ | 4+ | 4+ | 3+ | 1+ | X | | | | | | | |
| 6 | 0.1 | - | 1+ | 1+ | 2+ | 2+ | X | | | | | | | | | | | | | | |
| 1 | 0.5 | - | 1+ | 4+ | 4+ | 6+ | X | | | | | | | | | | | | | | |
| 2 | 0.5 | - | 2+ | 4+ | 6+ | X | | | | | | | | | | | | | | | |
| 3 | 0.5 | - | 1+ | 1+ | 2+ | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 0.5 | - | 2+ | 4+ | 4+ | 4+ | 6+ | 6+ | 1+ | 6+ | X | | | | | | | | | | |
| 5 | 0.5 | - | 1+ | 4+ | 5+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 6 | 0.5 | | 2+ | 4+ | 3+ | 6+ | X | | | | | | | | | | | | | | |
| 1 | 3 | - | - | 1+ | 6+ | 6+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| 2 | 3 | - | - | 1+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | X | | | | | | | | | | |
| 3 | 3 | - | - | 1+ | 2+ | 3+ | 4+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 3 | - | - | 1+ | 6+ | X | | | | | | | | | | | | | | | |
| 6 | 3 | - | - | 1+ | 1+ | 4+ | 3+ | 3+ | 3+ | 3+ | 6+ | X | | | | | | | | | |
| 1 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 10 | - | - | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 4+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ | 6+ |
| 4 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 2 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 3 | 0 | - | - | 1+ | X | | | | | | | | | | | | | | | | |
| 4 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 5 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |
| 6 | 0 | - | 1+ | X | | | | | | | | | | | | | | | | | |

X = mouse died or euthanised (control)

The results of the resistance tests are summarised in Table 35.

**Table 35**: Number of mice (out of a total of six) that relapsed after treatment with a trypanocide at various doses for each of the seven isolates. The highlighted cells indicate resistance according to the parameters proposed by Eisler *et al*. (2001).

| Drug | Dose (in mg/kg) | Number of mice positive per isolate | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| Diminazene aceturate | 1 | 6 | 6 | 5 | 5 | 5 | 5 | 5 |
| | 3 | 6 | 5 | 6 | 6 | 6 | 6 | 6 |
| | 10 | 3 | 4 | 5 | 4 | 6 | 5 | 4 |
| | 20 | 0 | 1 | 5 | 2 | 6 | 5 | 3 |
| | 30 | 0 | 1 | 1 | 2 | 6 | 5 | 3 |
| | 0 (control) | 6 | 4 | 6 | 6 | 5 | 5 | 6 |
| Isometamidium chloride | 0.01 | 6 | 0 | 5 | 6 | 6 | 5 | 6 |
| | 0.1 | 6 | 0 | 5 | 6 | 6 | 4 | 5 |
| | 0.5 | 2 | 0 | 4 | 6 | 4 | 5 | 5 |
| | 3 | 0 | 0 | 4 | 5 | 5 | 5 | 5 |
| | 20 | 0 | 0 | 0 | 2 | 5 | 4 | 4 |
| | 0 (control) | 6 | 5 | 6 | 6 | 6 | 6 | 6 |
| Homidium chloride | 0.01 | 3 | 5 | 6 | 6 | 6 | 5 | 6 |
| | 0.1 | 5 | 3 | 6 | 6 | 3 | 4 | 5 |
| | 0.5 | 3 | 3 | 6 | 6 | 4 | 5 | 6 |
| | 3 | 4 | 0 | 2 | 5 | 5 | 2 | 5 |
| | 10 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| | 0 (control) | 6 | 6 | 6 | 5 | 6 | 6 | 6 |

All seven isolates were resistant to DMZ at doses between 1 to 10 mg/kg body weight. At the highest dose (30 mg/kg) resistance was observed in Isolates 4, 5, 6 and 7. For isometamidium the results were similar with the exception of Isolate 2, which was highly susceptible to isometamidium at all doses used in the experiment. Treatment with homidium chloride resulted in relapses of almost all isolates at doses between 0.01 and 3 mg/kg body weight.

# Chapter 5 : Discussion

Results from the trypanosomosis survey indicated that bovine trypanosomosis is prevalent in the three districts under investigation. Parasitological prevalence was highest in Maganja da Costa District and lowest in Nicoadala. A high proportion of the infections were due to *T. congolense*. This is in accordance with observations made in the region (Sigauque, personal communication) and other countries of Southern Africa (Van den Bossche 2001). *Trypanosoma vivax* infections were detected in all three districts but the prevalence was very high at Cangu in Maganja da Costa District. The reason for such a high prevalence of *T. vivax* is difficult to explain but could be attributed to the presence of a high density of mechanical vectors. The observed prevalence of trypanosome infections in cattle could be an underestimate of the true prevalence of infection. The low PCV recorded in cattle in Chinde District should be attributed not only to trypanosomosis but also to other factors like helminths or ticks and tick-borne diseases.

The outcome of the trypanocidal drug resistance tests in mice clearly shows the presence of trypanosome strains that have developed resistance to one or several of the currently available trypanocides. The susceptibility to the trypanocidal compounds differs between strains. Extrapolating results from trypanocidal drug resistance tests in mice to susceptibility in cattle is not straightforward. Hawking (1963) indicated that drug sensitivity tests in mice give a broad indication of the response of *T. congolense* in cattle. Mwambo *et al.* (1988, cited by Joshua 1994) showed that *T. congolense* that was resistant to 14 mg/kg body weight of diminazene aceturate in cattle was successfully treated with 56 mg/kg body weight of diminazene aceturate in mice. For the purpose of this work, trypanosome strains showing resistance in mice to diminazene aceturate or isometamidium chloride at a dose of at least 20 and 1 mg/kg, respectively, were considered resistant to the normal dose of the drugs (3.5 mg/kg diminazene aceturate or 1 mg/kg isometamidium chloride) used in cattle (Eisler *et al*. 2001). Hence, Isolates 3 to 7 or five out of the seven isolates collected from Zambézia Province are strains that are expected to show resistance in cattle to a normal dose of diminazene aceturate or isometamidium chloride. Isolate 1 obtained at Namutengurine in Nicoadala District was susceptible to a normal dose of diminazene aceturate or isometamidium chloride but the bioassay in mice indicated that low levels of resistance may be developing to both drugs. Isolate 2 also isolated in Nicoadala District, on the other hand, seems to be highly susceptible to isometamidium chloride. Interpreting the results obtained

after homidium chloride treatment is difficult. The susceptibility of the different isolates differs little and it is not clear whether the trypanosome strains can be considered susceptible or resistant. However, most of the mice appear negative at dose of 10 mg/kg body weight. This may suggest that homidium chloride could be used in some areas where the prevalence of resistant strains to both diminazene aceturate and homidium chloride is high. Nevertheless, more research is required to establish the homidium threshold in mice and in cattle. When evaluating the efficacy of Cylence [Bayer (Pty) Ltd, Cyfluthrin 1% m/v] to control tsetse, Emslie (2003) also observed resistance to trypanocidal drugs in trypanosomes isolated from cattle kept at Madal Estate (Quelimane, Zambézia Province). A study conducted at the Veterinary Faculty of the UEM also showed the presence of resistant trypanosome strains in isolates from Nicoadala District. Tests in mice showed relapses after treatment with diminazene aceturate, isometamidium chloride and homidium chloride at doses of 7.0 mg/kg and 1.0 mg/kg respectively (Macuamule, personal communication).

Various factors have been identified that may promote the development of resistance (Geerts & Holmes 1998). They are:

- high selection pressure on the parasite population because of high number of treatments;
- underdosing of the trypanocide;
- high selection pressure on the parasite population because of mass treatments;
- use of quinapyramine.

With the exception of the trypanosome strain isolated in Licuary (Isolate 2), at least one of the factors promoting the development of resistance in trypanosomes was present in all the other cases (see Table 13).

It is thus not surprising that levels of resistance were low in the isolate from Licuary. Since isometamidium chloride is not used in the herd inspected in Licuary, the high susceptibility to this trypanocidal drug may be explained.

The regular mass treatments with diminazene aceturate and isometamidium chloride conducted in the herd from Namutengurini (Isolate 1) seem to have induced a certain level of resistance to both drugs. The level of resistance is lower than in the isolates from Micaune, however. The high level of multiple drug resistance observed in Isolates 4, 5 and 6 is not

surprising considering the high treatment frequency and the use of quinapyramine. The use of quinapyramine in Ghibe Valley of Ethiopia also caused a multiple drug resistance problem. After artificial induction of resistance to quinapiramine in *T. congolense*, multiple resistance to isometamidium chloride, homidium chloride and diminazene aceturate was expressed at the level of the trypanosome which could be transmitted by tsetse flies (Ndoutamia *et al.* 1993). The high levels of multiple drug resistance in Isolate 7 cannot be explained easily. The owner does conduct mass treatment but only once per year compared to Namutengurine where treatments are conducted three times per year but the level of resistance is lower. Underdosing could be a factor that has contributed to the development of the observed level of resistance.

The impact of multiple drug resistance was not investigated in this study. In the Ghibe area, north-western Ethiopia, isometamidium and diminazene aceturate failed to cure *T. congolense* infections in mice. To verify whether the double-resistance phenotype observed was associated with mixed or single infections, three clones were derived from one of the isolates and characterised in mice for their sensitivity to isometamidium and diminazene. Each clone expressed high levels of resistance to both trypanocides, comparable to the parental isolate. According to Codjia *et al.* (1993), if such resistance at the clonal level was highly this would indicate that chemotherapeutic agents would not control trypanosomosis in the Ghibe area.

The observed, often high level of multiple drug resistance in some areas in Mozambique is cause for serious concern, especially since trypanocidal drugs are and will for the foreseeable future continue to be the major tool to control the disease in livestock. Furthermore, some of the areas where multiple drug resistance was detected are considered quarantine areas for the ongoing restocking exercise. It is plausible that during the quarantine period animals become infected with resistant trypanosome strains that circulate in the area. Those strains may spread easily into areas where resistance is absent when cattle are distributed.

The development of resistance to trypanocides in trypanosomes is probably one of the major threats to the control of livestock trypanosomosis in Mozambique and elsewhere in Africa. Mechanisms should be put in place to monitor the prevalence of resistant strains, the degree of resistance and the number of drugs towards which resistance has developed. The information obtained through such mechanisms should be included in local trypanosomosis control strategies.

# Chapter 6 : Conclusions

The outcome of the trypanocidal drug resistance tests in mice shows the presence of trypanosome strains that have developed resistance to one or several of the currently available trypanocides (diminazene aceturate, isometamidium chloride and homidium chloride)

The high treatment frequency, mass treatment, the use of quinapyramine and probably underdosing could be factors that have contributed to the development of the observed level of resistance.

The results obtained from homidium are inconclusive.

# Chapter 7 : Recommendations

The findings of this study show the impact of trypanocidal drug-use practices on the development of resistance against those drugs. Moreover, our study confirms the recommendation made by Geerts and Holmes (1998) with regard to type and combination of drug(s) used and number and frequency of treatments to retard the development of drug resistance.

Considering the important level of drug resistance in some areas it is important to avoid the distribution of animals infected with those resistant trypanosome strains to other areas where drug resistance is absent or not considered to be a problem.

Further monitoring of the prevalence of trypanocidal drug resistance in Mozambique is required.

Livestock from areas where multiple drug resistance was detected should not be used for the ongoing restocking exercise.

More research is required to establish the normal susceptibility of *T. congolense* in mice to homidium chloride.

Finally, it is recommended that derived clones from each isolate are used to verify whether multi resistance is associated with mixed or single infections.

# Reference list

ABDEL GADIR, F., OSMAN, O.M., ABDALLA, H.S., ABDEL RAZIG, M.T. 1981 Ethidium bromide-resistant trypanosomes in Southern Darfur. *Sudan Journal of Veterinary Research* 3: 63-65

AFEWERK, Y., CLAUSEN, P.H., ABEBE, G., TILAHUN, G., MEHLITZ, D. 2000. Multiple drug resistant *Trypanosoma congolense* populations in village cattle of Metekel district, north-west Ethiopia. *Acta Tropica* 76: 231-238

ANNE, B.M., ONAH, D.N., NAWA, Y. 2001. Drug resistance in pathogenic African trypanosomes: What hopes for the future? *Veterinary Parasitology* 96: 83 –100

BEVAN, E.W. 1928. A method of inoculating cattle against trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 22: 147-156

BOYT, W.P. 1967. The veterinary contribution to the control of trypanosomiasis. *Proceedings and Transactions of the Rhodesian Scientific Association* 52: 16-20

BOYT, W.P. 1971. Trypanosomiasis control in Rhodesia. *Bulletin Office Internationale des Epizooties* 76: 301-306

BOYT, W.P. 1979. Trypanosomiasis in Zimbabwe. *Rhodesian Veterinary Journal* 10: 54-63

BOYT, W.P. 1986. Guide pratique pour le diagnostic, le traitement et la prévention de la trypanosomiase animale africaine. *Food and Agricultural Organization (FAO)*, Rome, 281 pp

BOYT, W.P. 1991. Tripanosomoses Animais em Africa. Diagnostico, Tratamento e Prevenção, 1st ed. CTA, Lisbon, Portugal

BOYT, W.P., LOVEMORE, D.F., PILSON, R.D., SMITH, I.D. 1963. A preliminary report on the maintenance of cattle by various drugs in a mixed *G. morsitans* and *G. pallidipes* fly belt. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 9th meeting, Conakry, pp. 71 –79

CHITAMBO, H., ARAKAWA, A. 1991. Therapeutic effect of Berenil and Samorin in mice infected with four trypanosome populations isolated from Zambian cattle. *Veterinary Parasitology* 39: 42-52

CHITAMBO, H., ARAKAWA, A., ONO, T. 1992. In vivo assessment of drug sensitivity of African trypanosomes using the akinetoplastic induction test. *Research in Veterinary Science* 52: 243-249

CODJIA, V., MULATU, W., MAJIWA, P.A.O., LEAK, S.G.A., ROWLANDS, G.J., AUTJIE, E., d'IETEREN, G.D.M., PEREGRINE, A.S. 1993. Epidemiology of bovine trypanosomiasis in the Ghibe valley southwest Ethiopia. *Acta Tropica* 53: 151-163

CONNOR, R.J. 1989. Final report of the Regional Trypanosomiasis Expert. Regional tsetse and trypanosomosis control program. FGU, West Germany

CONNOR R.J. 1991. Report on diagnosis, treatment and prevention of animal trypanosomiasis under field conditions. RTTCP, Harare (Unpublished report)

CONNOR, R.J. 1994. African Animal Trypanosomiasis. In: Coetzer, J.A.W., Thomson, G.R., Tustin, R.C. (eds.) *Infectious diseases of livestock with special reference to Southern Africa*, Oxford University Press, Cape Town, pp. 166-203

COX, F.E.G. 1993. *Modern Parasitology - A Textbook of Parasitology*, 2nd ed. Oxford: Blackwell Scientific Publications, pp 62-65

da SILVA, J.M. 1969. O fomento pecuário de Moçambique e as tripanosomiases. *Anais dos Serviços Veterinaria Moçambique* 15: 50-60

DAVIES, M.I.V. 1982. Tripanosomiase humana em Moçambique, Breve história e sitruação actual. *Revista médica de Moçambique* 3: 87-96

DIRECÇÃO NACIONAL DE PECUÁRIA (DINAP). 1996. The current situation of tsetse and trypanosomiasis in Mozambique, Maputo. (Unpublished report)

DIRECÇÃO NACIONAL DE PECUÁRIA (DINAP). 2001 Relatório Anual de Pecuária, Maputo. (Unpublished report)

EISLER, M.C., BRANDT, J., BAUER, B., CLAUSEN, P.H., DELESPAUX, V., HOLMES, P.H., ILEMOBADE, A. MACHILA, N., MBWAMBO, H., McDERMOTT, J., MEHLITZ, D. MURILLA, G., NDUNGU, J.M., PEREGRINE, A.S., SIDIBE, I., SINYANGWE, L., GEERTS, S. 2001. Standardised test in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle. *Veterinary Parasitology* 97: 171-182

EMSLIE, R.F. 2003. Results of Mozambique field evaluation of two trypanosomosis control strategies. *Newsletter on Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV)* 8: 14-17

FAIRCLOUGH, R. 1963. Observation on the use of berenil against trypanosomiasis of cattle in Kenya. *Veterinary Research* 75: 1107-1112

FINNELE, P., YVORE, P. 1962. Quelques observations sur la chimioresistance. *Proceedings of the 9th International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) Meeting*, Conakry, Guinea, CCTA 88: 107-110

FOOD AND AGRICULTURE ORGANIZATION (FAO). 1987. Trypanosomiasis and Tsetse Fly Control. AG:DP/Moz/81/019, Rome

GEERTS, S., HOLMES, P.H, 1998. *Drug management and parasite resistance in animal trypanosomiasis in Africa*. Programme Against African Trypanosomiasis (PAAT) Technical and Scientific Series 1. Food and Agriculture Organization of the United Nations (FAO), Rome

GEERTS, S., HOLMES, P.H., DIALL, O., EISLER, M.C. 2001. African bovine trypanosomiasis: the problem of drug resistance. *Trends in Parasitology* 17: 25-28

GEERTS, S, NDUNG'U, J.M., MURILLA, G.A., MBWAMBO, H., SINYANGWE, L., MACHILA, N., DELESPAUX, V., BRANDT, J., PEREGRINE, A.S., McDERMOTT, J.J., HOLMES, P.H., EISLER, M.C. 1999. A simplified cost-effective method for area-wide testing of trypanocidal drug sensitivity. *Proceedings of the 25th International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) Meeting*, Mombasa, Kenya, pp 175-178

GEERTS, S., NDUNG´U, J.M., MURILLA, G.A., MBWAMBO, H., SINYANGWE, L., MACHILA, N., DELESPAUX, V., BRANDT, J., PEREGRINE, A.S., McDERMOTT, J.J., HOLMES, P.H., EISLER, M.C. 2000. In vivo test for detection of resistance to trypanocidal drugs: Test in mice and in ruminant. *Newsletter on Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV)* 2: 1-6

GRAY, M.A., KIMARUA, R.W., PEREGRINE, A.S., STEVENSON, P. 1993. Drug sensitivity screening in vitro of population of *Trypanosoma congolense* originating from cattle and tsetse flies at Nguruman, Kenya. *Acta Tropica* 55: 1-9

GRAY, M.A., PEREGRINE, A.S. 1993. An in vitro assay for drug sensitivity of trypnosoma congolense using in vitro-derived metacyclic trypanosomes. *Acta Tropica* 54: 291-300

HAWKING, F. 1963. Drug-resistance of *Trypanosoma congolense* and other trypanosomes to quinapyramine, phenanthridines, Berenil and other compounds in mice. *Annas of Tropical Medicine and Parasitology* 57: 262-282

ILEMOBADE, A.A. 1979. Drug sensitivity of mouse infective *Trypanosoma vivax* isolates in cattle and sheep. In: *Proceedings of the 16th International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) Meeting.* Yaounde, Cameroon, OAU/STRC Publ. No 111, 251-253

ILRAD. 1988. *Annual Report of International Laboratory for Research on Animal Diseases.* Nairobi, Kenya

ILRAD. 1992. How TRypanosomes tolerate drugs that once killed them. *ILRAD Reports* 10: 4, Nairobi, Kenya

INSTITUTO NACIOANAL DE ESTATÍSTICA (INE). 2000. *Anuario Estatístico da Província da Zambézia*, 2000

JOSHUA, R.A., OBWOLO, M.J., BWANGAMOI, O., MANDEBVU, E. 1994. Resistance to diminazene aceturate by *Trypanosoma congolense* from cattle in the Zambezi Valley of Zimbabwe. *Veterinary Parasitology* 60: 1-6

KALU, A.U. 1995. Sensitivity of animal-derived Trypanozoon stocks from sleeping sickness endemic foci of Nigeria to trypanocides and human plasma. *Revue d'Elevage et Médecine Vétérinaire des Pays Tropicaux* 48: 139-144

KETTLE, D.S. 1995. *Medical and veterinary entomology*, 2nd ed. CADI, Wallingford, pp 225-227, 612, 627

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

KINABO, L.D.B., BOGAN, J.A. 1988. The pharmacology of isometamidium. *Journal of Veterinary Pharmacology and Therapeutics* 11: 233-245

LEAK, S.G.A. 1980. *A review of the tsetse situation in Petauke and Katete Districts of Eastern Province over the ten-year period 1970-1979.* Department of Veterinary and Tsetse Control Services, Chipata, Zambia. 14 pp

LEWIS, A.R., THOMSON, J.R. 1974. Observations on an isometamidium resistant strain of *Trypanosoma congolense* in Rhodesia. *Rhodesian Veterinary Journal* 4: 62-67

LOSOS, G.J. 1986. *Infectious tropical diseases of domestic animals.* Longman Scientific & Technical, in association with the International DevelopmentReserch Centre, Canada, pp 183-212

MATOVU, E., ITEN, M., ENYARY, J.C.K., SCHMID, C., LUBEGA, G.W., BRUN, R., KAMINSKY, R. 1997. Susceptibility of Ugandan *Trypanosoma brucei rhodesiense* isolated from man and animal reservoirs to diminazene, isometamidium and melarsoprol. *Tropical Medicine International Health* 2: 13-18

MATSON, B.A. 1959. *An investigation into the livestock problems of the Lower Shire Valley of Nyasaland with particular reference to the disease trypanosomiasis.* Department of Veterinary Services and Animal Industry, Blantyre, Nyasaland, 13 pp

MBWAMBO, H., NDUNG'U, J.M., MURILLA, G.A., MUNGA, L., SINYANGWE, L., MACHINA, N., HOLMES, P.H., EISLER M.C. 1999. Trypanocidal drug resistance in Tanzania. *International Scientific Council for Trypanosomiasis Research and Control* (ISCTRC), Mombasa, Kenya

MISSÃO DE COMBATE ÀS TRIPANOSSOMIASES. Annual Reports 1949-1960

MUBANGA, J., SINYANGWE, L. 1997. STD3 Project (unpublished report)

MURRAY, M., TRAIL, J.C.M., TURNER, D.A., WISSOC, Y. 1983. *Livestock productivity and trypanotolerance*. ILCA, Addis Ababa, Ethiopia

NDOUTAMIA, G., MOLOO, S.K., MURPHY, N.B., PEREGRINE, A.S. 1993. Derivation and characterization of a quinapyramine-resistant clone of *Trypanosoma congolense*. *Antimicrobial Agents and Chemotherapy* 37: 1163-1166

PARIS, J., MURRAY, M., MCODIMBA, F. 1982. A comparative evaluation of the parasitological techniques currently available for the diagnosis of African Trypanosomiasis in cattle. *Acta Tropica* 39: 307-316

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

PHELPS, R.J., LOVEMORE, D.F. 1994. Tsetse flies. In: Coetzer, J.A.W., Thomson, G.R., Tustin, R.C. (eds.). *Infectious diseases of livestock with special reference to Southern Africa.* Oxford University Press, Cape Town, pp. 25-52

PRINDER, M., AUTHIE, E. 1984. The appearance of isometamidium-resistant *Trypanosoma congolense* in West Africa. *Acta Tropica* 41: 247-252

RAFAEL, A.G. 1973. Tripanosomiases animais. *Anais dos Serviços de Veterinaria Maputo*

REGIONAL TSETSE AND TRYPANOSOMIAIS CONTROL PROGRAMME, (RTTCP). 2000. *An update of the distribution of bovine trypanosomosis in Mozambique.* Harare, Zimbabwe

SCHONEFELD, A., ROTTCHER, D., MOLOO, S.K. 1987. The sensitivity to trypanocidal drugs of *Trypanosoma vivax* isolated in Kenya and Somalia. *Tropical Medicine and Parasitology* 38: 177-180

SEIFERT, S.H. 1996. *Tropical animal health*, 2nd ed. Kluwer Academic Publishers, Wageningen, the Netherlands

SHERENI, W. 1990. Strategic and tactical development in tsetse control in Zimbabwe. *Insect Scientific Application* 11(3): 1-11

SHORT, J.R.T. 1963. *Introduction to applied entomology.* Longmans, London, pp. 122-123

SINYANGWE, L., DELESPAUX, V., BRANDT, J., GEERTS, S., MUBANGA, J., MACHILA, N., HOLMES, P.H., EISLER, M.C. 2003. Trypanocidal drug resistance in the eastern province of Zambia. *Veterinary Parasitology* 119: 125-135

SMITH, K.G.V. 1973. *Insects and other arthropods of medical importance*, 2nd ed. Trustees of the British Museum (Natural History), London

SONES, K.R., NJOGU, A.R., HOLMES, P.H., 1988. Assessment of sensitivity of trypanosoma congolense to isometamidium chloride: a comparison of test using cattle and mice. *Acta Tropica* 45: 153-164

WORD HEALTH ORGANIZATION. 1979. *The African trypanosomiasis*. Report of joint WHO/FAO expert consultation, Rome 1976. WHO, Geneva

VAN DEN BOSSCHE, P., DORAN, M., CONNOR, R.J. 2000. An analysis of trypanocidal drug use in the Eastern Province of Zambia. *Acta Tropica* 75: 247-258

VAN DEN BOSSCHE, P. 2001. Some general aspects of the distribution and epidemiology of bovine trypanosomosis in Southern Africa. *International Journal for Parasitology* 31: 592-598

VAIL, L. 1977. Ecology and history: the example of eastern Zambia. *Journal of Southern African Studies* 3: 129-155

# Annex 1 : Drug use survey questionnaire

**DRUG USE SURVEY QUESTIONNAIRE**

Name of enumerator:
Date of interview:

**Information details**

Name of cattle onwer
Village
District
Village Grid reference/GPS reading
S
E

Are you directely envolving in the manangent of the cattle

yes No

**Number of cattle**

Herd struture data

0-1 year males
0-1 year females
1-4 year old males
1-4 year old females
Cows
Bulls
Oxen

2.Interview with the cattle onwers or informer

Ask the cattle onwer or informer to list the most important problems that afect the catlle.

| Disease | Cattle |
|---|---|
| Tic&Tic born disease | |
| Gastro intestinal parasites | |
| Brucelosis | |
| Trypanosomosis | |
| Lumpy skin disease | |
| Dermatophilosis | |
| Tuberculosis | |
| Others diseases | |

The cattle onwers is able to destinguish trypanosomosis with others diseases?

yes ☐ No ☐

**Diminazene aceturate use**

Have you used diminazene aceturate to treat your cattle?

yes ☐ No ☐

Since what time ? ☐

Why did you treat your cattle with diminazene aceturate (Berenil)?

Because I througth that the cattle were sick with tryps ☐

Because I througth that the cattle were sick with ECF ☐

Because the cattle were sick but I didnot know the reason ☐

Because the Veterinary assintance diagnosed tryps ☐

Any other reason ☐

How many cattle did you have treat with diminazene aceturate (Berenil) since the last year until now? ☐

*The cattle onwers must give the total number of treatments and the season that cattle were been treating*

When you tret your cattle with diminazene aceturate do you give the same doses
to calves (0-1) Young cattle (1-4) mature cattle (older than 4 years)

Yes ☐ No ☐

How many adults cattles did you has been treated with 1 sacheta of Berenil? ☐

How many calves (0-1 years) would you normally treat with one sachet of diminazene aceturate? ☐

How many sachets of diminazene aceturate did you use to treat your cattle since last year until now? ☐

**Isometamidium chloride use**

Have you used isometamidium chloride to treat your cattle?

yes ☐ No ☐

Since what time ? ☐

Why did you treat your cattle with Isometamidium chloride (Trypamidium)?

Because I wanted to prevent them from getting sick ☐

Because the cattle were sick with tryps ☐

Because the cattle were sick but I didnot know the reason ☐

Any other reason ☐

How many cattle did you treat with isometamidium chloride (trypamidium) since the last year until now?

*The cattle onwers must guive the total number of treatments and the season that cattle were been treating*

When you tret your cattle with isometamidium chloride do you give the same doses
to calves (0-1) Young cattle (1-4) mature cattle (older than 4 years)

Yes ☐ No ☐

How many adults cattles would you normally treat with 1 sacheta of isometamidium chloride? ☐

How many calves (0-1 years) would you normally treat with one sachet of isometamidium chloride? ☐

How many sachets of isometamidium chloride did you use to treat your cattle sice last year until now? ☐

**Treatments (General)**

When you use diminazene aceturate (Berenil) or Isometamidium chloride (trypamidium) to treat your cattle, do you dilue the powder with

1. Water that you have boiled

2. Water straight from a river or dam

3. Water straight from a well

4. Water straight from a borehole

5.Other

Who treats (injects) your cattle with diminazene aceturate(berenil) or isometamidium (trypamidium)

1. I treat them my self
2.A veterinary officer treats them
3.A veterinary assistant treats them
4.Any combination of the above
5.Other