SOME ASPECTS OF ANIMAL TRYPANOSOMIASIS IN SOMALIA

by

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DEDICATION

I wish to dedicate this work to my wife and children.

SUMMARY

This thesis is concerned with various aspects of animal trypanosomiasis in Somalia, with the objective of highlighting the need for further research and study. Some of the results described in this thesis constitute the first preliminary work on animal trypanosomiasis in Somalia.

The thesis consists of four chapters. The first is a general introduction and review of trypanosomiasis. In the review the different species of pathogenic trypanosomes are described together with clinical signs they cause in different species of domestic livestock. Particular emphasis is given to the main species of Trypanosoma found in Somalia, namely T. congolense, T. vivax and T. evansi. In Somalia, as in most African countries, there has been heavy reliance on use of chemotherapeutic and chemoprophylactic drugs to control trypanosomiasis. The various drugs are described and the advantages and disadvantages of their use are discussed. One important potential problem is the presence of drug-resistant strains of trypanosomes and this aspect is considered in greater detail later in the thesis.

The second chapter deals with a survey of cattle trypanosomiasis along the two main rivers in Somalia, namely the Jubba and the Shabelle. The survey encompassed the use of both questionnaires and clinical examinations, including blood sampling of individual animals. The survey's questionnaire asked

the animal owners various aspects of herd health with particular reference to trypanosomiasis and the availability and utilisation of the various available trypanocidal drugs in the field. A total of 4,152 cattle in 54 herds were examined. There was an overall infection level of 10% and the incidence of T. congolense infection showed a significantly increasing trend with the level of tsetse challenge, while the incidence of T. vivax did not show this trend. However, there was no evidence of mechanically transmitted T. vivax infection occurring outside the tsetse belt. The results of this survey showed that trypanosomiasis is widespread along the tsetse-infested riverine areas of Somalia and is regarded as a major animal health and production problem by the cattle owners.

The control of cattle trypanosomiasis in Somalia has essentially depended upon the two trypanocidal drugs, isometamidium chloride and diminazene aceturate. However, there have been persistent rumours that drug resistant strains of T. congolense exist in Somalia, although this has never been clearly demonstrated.

Chapter Three describes how two drug resistant strains of T. congolense were isolated from the lower Shabelle region. In the study a trypanosome-naive calf was transported into each study area and inoculated with blood from local cattle infected with T. congolense. It was then kept in isolation and when a parasitaemia developed it was treated with a standard dose of isometamidium chloride (0.5 mg/kg) and continually monitored for

the development of a relapse infection. The development of such an infection was taken to be indicative of the presence of a drug-resistant strain of T. congolense and blood was taken at this time and injected into groups of calves, goats and mice for subsequent trials to determine the level of drug resistance. Using these relatively unsophisticated techniques it was possible to demonstrate the existence of drug-resistant strains of T. congolense from the two sites in Somalia. Both strains were highly resistant.

A possible method of treatment for drug-resistant strains of T. congolense was investigated in Section II of Chapter Three, using intravenous as opposed to intramuscular administration of isometamidium chloride. Following the administration of isometamidium by these two routes to goats infected with a Samorin-resistant strain of T. congolense it was found that the intravenous administration of isometamidium offered no clear advantage over intramuscular administration.

Nevertheless, drug levels as measured by a newly developed ELISA assay for isometamidium indicated that by four hours post injection, intravenous administration did result in higher blood concentrations of isometamidium than intramuscular administration.

Chapter Four, Section I, presents studies on the chemotherapy of T. evansi infections in mice.

Sensitivity tests of two stabilates, T. evansi GRVPS 13 and T. evansi GRVPS 19, to six trypanocidal drugs (quinapyramine

sulphate/prosalt, isometamidium, diminazene aceturate, melarsoprol, DFMO and suramin) produced varying results. T. evansi GRVPS 13 which was known for its high degree of suramin-resistance was resistant to the maximum dose of suramin (160 mg/kg). But this strain was sensitive to all the other drugs. The isolate GRVPS 19 was resistant to diminazene aceturate but was sensitive to all the other drugs.

In a similar way, studies of the chemotherapeutic activity of a new arsenical drug Mel Cy and Trimelarsan (Mel W) against early and late infections of T. evansi GRVPS 13, indicated high sensitivity of this suramin-resistant strain to these two drugs. However studies of the prophylactic activity of Mel Cy against T. evansi GRVPS 13, infections failed to demonstrate any protection beyond three days.

In Section II the therapeutic activity of Mel Cy at a dose rate of (0.25 mg/kg) was examined in goats infected with T. evansi. Following treatment microscopic examination of the goats' blood failed to detect parasites, however, blood collected from the goats 30 days after treatment and subinoculated into irradiated mice revealed the presence of trypanosomes, indicating that this dose of drug was not curative.

Finally, when irradiated and normal groups of mice were infected with either T. evansi GRVPS 13 or T. evansi GRVPS 19 and treated with increasing doses of isometamidium it was shown that a significant decrease in drug sensitivity could develop.

Furthermore, the results of this experiment showed that under laboratory conditions, the development of drug-resistance could be promoted more readily in immunosuppressed mice than in normal animals.

CHAPTER ONE

GENERAL INTRODUCTION AND REVIEW

The Parasite

The disease caused by pathogenic trypanosomes first attracted the attention of investigators during the latter part of the 17th century and the main landmarks of the early work have been compiled by Hoare (1972) in his excellent monograph on the mammalian trypanosomes.

Parasitic organisms of the Family Trypanosomatidae are found in domestic and wild game throughout the world (Hoare 1972). The classification of trypanosomes has been discussed in different reviews by Hoare (1957a, 1964, 1966, 1970). Trypanosomes are members of the Order Kinetoplastida, Family Trypanosomatidae and Genus Trypanosoma (see Table 1). The Genus is subdivided into two sections; the Stercoria and Salivaria, on the basis of the site of development of the organisms in the insect vector that transmit them.

Stercoria trypanosomes develop in the hind gut of biting insects, following ingestion of a parasitised blood meal, and infection is transmitted to new hosts by faecal contamination of any superficial wound. This section comprises a heterogenous assemblage of a large number of non-pathogenic micro-organisms (e.g. T. theileri, T. musculi, T. lewisi, T. cruzi). T. cruzi, the only pathogen in this section, is an important parasite of man in South and Central America. It is transmitted by reduviid triatomine bugs.

The Salivarian trypanosomes, which are more homogenous, contains several species of trypanosomes of medical and veterinary importance. They are, in general, transmitted by different species of Glossina (tsetse) flies. The cyclical

Table 1

Classification of the *Trypanosoma*

TRYPANOSOMA							
SECTION	STERCORARIA			SALIVARIA			
SUBGENUS	MEGATRYPANUM	HERPETOSOMA	SCHIZOTRYPANUM	DUTTONELLA	NANNOMONAS	TRYPANOZOON	PYCNOMONAS
SPECIES	*T.(M)theileri*	*T.(H)lewisi*	*T.(S)cruzi*	*T.(D.)vivax*	*T.(N.)congolense*	*T.(T.)brucei*	*T.(P.)suis*
				T.(D.)uniforme	*T.(N.)simiae*	*T.(T).gambiense*	
					T.(N.)vanhoofi	*T.(T.)rhodesiense*	
						T.(T.)equiperdum	
						T.(T.)evansi	

(After: Stephen, 1986)

development of Salivarian trypanosomes is completed in the salivary medium of the anterior station of the insect host, hence their transmission is inoculative. Conventionally, the Salivarian trypanosomes are grouped under four subgenera accordingly to their morphological and biological similarities.

(1) Subgenus Duttonella which is represented by T. (Duttonella) vivax and T. (Duttonella) uniforme.

(2) Subgenus Nannomonas containing T. (Nannomonas) congolense and T. (Nannomonas) simiae.

(3) Pycnomonas is the least represented and has only one species, T. (Pycnomonas) suis.

(4) Subgenus Trypanozoon, contains five species, viz.

T. (Trypanozoon) brucei brucei,

T. (Trypanozoon) brucei rhodesiense,

T. (Trypanozoon) brucei gambiense,

T. (Trypanozoon) evansi, and

T. (Trypanozoon) equiperdum.

T. rhodesiense and T. gambiense are human trypanosomes while the other three are responsible for animal trypanosomiasis.

The last subgenus has considerable medical and veterinary importance and it seems to be in the midst of rapid evolution (Baker, 1969) making it difficult for systematists to divide it up neatly into distinct species. T. evansi and T. equiperdum are not cyclically transmitted while the remaining other three species are cyclically transmitted by Glossina species. Nevertheless it is now regarded by many that these two latter trypanosomes may be regarded as T.(Trypanozoon) brucei evansi and

T.(T.)b.equiperdum which have evolved from T.b.brucei.

The animal trypanosomiases caused by T. congolense, T. vivax and T.b.brucei are generally known by the name, 'Nagana' in Africa, south of the Sahara whilst 'Surra' is caused by T. evansi in North Africa and Asia, and 'Dourine', due to T. equiperdum.

Whilst, in the course of infection there are differences among trypanosome species (Losos and Ikede, 1972; Maxie, Losos and Tabel, 1979), there is also an underlying similarity in the pathogenesis of trypanosomiasis.

The problems imposed by animal and human trypanosomiasis in Africa are immense and well documented (Hoare, 1970). It is estimated that most of the fertile land in Africa is held captive by tsetse flies and the trypanosomes which they transmit (Wilson, Morris, Lewis and Krog, 1963). In Somalia, for example, the tsetse infested zone is along the two rivers Shabelle and Jubba. This area is a fertile and productive zone, but the tsetse fly had prohibited development of mixed farming in that area.

The importance of African trypanosomiasis has been recognised for many years (Harris, 1921; Bruce, 1895), yet the disease remains endemic in the animal and human populations across vast expanses of Africa. Evans (1881) described trypanosomes (T. evansi) in the blood of Indian camels and equines. In 1895, Bruce reported the pathogenic organism, Trypanosoma brucei in livestock and showed that the disease could be transmitted by tsetse flies. However, there is still a lack of concise data relating to the occurrence of trypanosomiasis in

both human and animal populations (Henson and Noel, 1979). There is often an underestimate of unknown magnitude since many of the countries where the disease is endemic do not have available the accurate data necessary for determining the concise occurrence and distribution of the disease.

The disease is seen in many forms from peracute to subclinical, in all hosts. There is an acute phase of the disease soon after infection, when a slight pyrexia usually occurs before trypanosomes are detectable in the blood. Pyrexia is more severe after trypanosomes are established in the blood stream or tissues of the host within three to twenty or more days after inoculation. In extreme cases, the host animal may die within a few days of infection, with no obvious signs of disease except those associated with pyrexia.

Host-parasite relations

The severity of the disease varies according to the degree of susceptibility of the hosts, strain of parasite and local epizootiological conditions. Although tsetse transmitted trypanosomiasis is regarded as most important in cattle, other species of domestic livestock can also be affected. Horses are very susceptible while sheep and goats are known to be susceptible under laboratory conditions, although the incidence of natural infection appears to be less than that of cattle.

T.congolense and T.vivax

Soon after the causation of trypanosomiasis was established, it was recognised that these two parasites were chiefly responsible for the death of cattle from the disease. For many years before the advent of effective trypanocidal drugs, T. vivax

was the most common pathogenic trypanosome in West Africa (Unsworth, 1953; Mornet, 1954), while T. congolense caused more damage to livestock in East Africa (Ford, 1971). However, with the widespread use of drugs in the years up to the 1939-1945 war, there was some evidence that the relative prevalence of the major species of pathogenic organisms changed. After long observation, Killick-Kendrick and Godfrey (1963) suggested that homidium, widely used in northern Nigeria was more effective against T. vivax than T. congolense and therefore reduced the prevalence of the former in the cattle population faster than the latter. The use of quinapyramine in East Africa probably favoured the survival of T. vivax (Ford, 1964).

Preliminary investigations revealed that there was some evidence for the existence of separate morphologically identifiable strains of varying pathogenicity within the species (Fairbairn, 1953; Lewis, 1954). Godfrey (1961) showed that similar variations existed with T. congolense and he distinguished longer, more virulent and shorter less virulent strains.

The virulence of different strains of the same species may vary considerably in similar conditions of infection. Krampitz (1970) gave an example of the variable response of cattle to the same strain of T. congolense, when acute and chronic infections were induced by similar infecting doses of trypanosomes. T. vivax in West Africa is often very virulent causing acute disease in cattle (Unsworth, 1953), while in East Africa this species is generally of lower pathogenicity (Hudson, 1944).

Trypanosomiasis in sheep and goats has received relatively little attention, but results from experimental infections indicate that they are susceptible to all the common tsetse transmitted pathogenic trypanosomes including T. simiae. Opasina and Onyeka (1982) reported studies on the incidence of trypanosomiasis in West African dwarf sheep and goats in South West Nigeria. Of 44 sheep and 38 goats monitored, 18% and 34% respectively were positive for trypanosomiasis, due predominantly to T. vivax infection. In Somalia, the species of trypanosomes which are naturally pathogenic for ovine and caprine are T. vivax, T. congolense and T. brucei (Di Domizio, 1925; Croveri, 1936).

In a review of trypanosomiasis in sheep and goats, Griffin (1978) indicated that many of the accounts of naturally occurring trypanosomiasis in sheep and goats came from East Africa and were caused by T. congolense. Studies on the economic effects of natural T. congolense infection in sheep and goats in Kenya indicated significant financial losses in both species over a 35 week period (Griffin and Allonby, 1979a).

Early reports have mentioned T. congolense in camels (Broden, 1906; Leese, 1933). The fact that camels are highly susceptible to T. simiae, which has some characteristics in common with the congolense organism, may indicate that the dromedary would also be quite susceptible.

T.brucei

Camels are highly susceptible to infection with T. brucei.

Horses, donkeys and mules are also susceptible to infection with T. brucei and death occurs within a fortnight to three

months if the animals are untreated (Stephen, 1970). T. brucei is usually an acute disease for dogs and if untreated almost invariably ends fatally. In T. brucei infections of ruminants the parasitaemia is often low. Cattle in all regions of tropical Africa exhibit a degree of resistance to T. brucei and while infections can become established in cattle, clinical signs of disease are seldom observed, even when there is high parasitaemia. Exotic breeds, however, are much more susceptible (Stephen, 1970).

According to Bradford and Plimmer (1902) the pig shows T. brucei in the blood very rarely and in very small numbers. Laveran and Mensil (1912) have described the course of infection in a piglet experimentally inoculated with T. brucei. The animal lived for 94 days and during the first half of the infection no signs of illness were seen. Thus it appears that experimental infections with T. brucei in pigs run a chronic course. Stewart (1935) observed that when pigs were challenged by tsetse flies known to be infected with T. congolense, T. vivax and T. brucei they only developed an infection with T. brucei.

Van Hoof (1947) reported a natural infection of T. brucei in the pig and commented that such occurrences were exceptional. He further stated that the blood was positive only at rare intervals, and that spontaneous cures occurred between one year and 15 months.

T.simiae

Various authors have observed that T. simiae produces a very acute disease with high parasitaemia in the pig (Isoun, 1968; Van

Dijk, Zwart and Leefland, 1973; Janssen and Wijers, 1974), and possibly in previously uninfected warthogs (Roberts, 1971).

Of all the pathogenic trypanosomes found in Somalia, T. simiae was the only one which could not be experimentally transmitted to cattle (Pellegrini, 1948). In addition Roberts (1971) appears to have demonstrated quite conclusively that cattle are not susceptible to infection by T. simiae. Earlier, Di Domizio (1929) reported that the camel in Italian Somaliland (Somalia) was highly susceptible to T. simiae and this was later confirmed by Pelligrini (1948) who described the disease in the dromedary as being hyperacture, and in his opinion the camel was, after the pig, the most susceptible animal to T. simiae infection whether by fly or syringe transmission.

T.evansi

Surra affects a wide range of diverse mammalian species, including equines, bovines, dog, elephant, deer etc., but the camel is its principal host. It is interesting to note that two other species of the family Camildae (Lama pasos - the alpaca, and Lama glama - the llama) are not affected. Cattle are slightly affected by Surra and horses appear to be refractory, at least in Somalia and Northern Kenya (Hoare and Bennet, 1939). However, in North Africa camels and horses are thought to be equally susceptible. In the absence of camels, horses are the principal hosts of Surra.

However, in India camels were found to be the most susceptible to Surra followed by equines and bovines (Srivastava, 1973).

Hoare (1956) suggested that there is evidence that the mean lengths of equine strains of T. evansi increase progressively from Western Morocco to the East Philippines. It would seem that this graduation is correlated with the pathogenicity of the equine strains, which could explain why the disease of horses in East Africa is apparently symptomless but extremely acute and fatal in Indochina and the Philippines.

Mohan (1968) reviewed the literature related to Surra in buffaloes and cattle in the Far East and stated "the infection is generally latent or subclinical in buffaloes as more important reservoir hosts than cattle". The authors survey indicated that Surra is prevalent and widespread throughout India, Indonesia, Philippines, Indochina, Thailand and Taiwan.

In U.S.S.R., Petrovski (1974) was unable to observe any appreciable difference in Surra infections between camels, equines and dogs, but Kunichkin and Eskaliev (1975) reported a severe outbreak of T. evansi in Kalakhstan which killed many infected camels and caused abortions to many pregnant and infected female camels. It would appear that the effect of the parasite varies from region to region and depends also on the observer.

In North Vietnam, buffaloes were reported to be highly susceptible (Wells, 1982) with a very high mortality.

Transmission of Trypanosomes

Trypanosomes are fascinating parasites which possess a considerable capacity for adapting to inhospitable environments (Clarkson, 1980).

It is widely accepted that different species of tsetse flies

(Glossina spp) can transmit several species of the Salivarian trypanosomes.

After the tsetse fly selects a site on the host, it disengages the labium from between the palps. The freed labium is directed to the skin of the host and immediately after penetration the vector starts to feed. During the process of feeding a relatively minute jet of saliva is being intermittently discharged from the tip of the hypopharynx and this saliva prevents the clotting of the blood. If the fly is infected, infective metacyclic trypanosomes injected with the saliva will be distributed in the haemorrhagic areas and in the intervening tissues. Some may enter directly into the lumen of torn capillaries. The metacyclic trypanosomes will undergo a period of adaptation from the poikilothermic environment of the fly to the relatively homeothermic conditions in the host.

Gray and Roberts (1971b) transmitted T. vivax from sheep to sheep using G. morsitans and G. tachinoides which had emerged from pupae in the laboratory, and the infection rate in both species of flies was 80%. Whitnall (1932) studied trypanosome infection rates of wild caught flies of G. pallipides. The ratio of T. vivax : T. congolense type infections was 1.8 : 1. This account is interesting because in Somalia the ratio of T. vivax : T. congolense in infected animals of the survey on cattle trypanosomiasis (see later section) was similar. It also indicates that G. pallipides is probably the vector of T. vivax in the surveyed area.

Using data from all sources of information available to him,

including his own studies, Jordan (1961) reported that a large number of different species of Glossina, each with its own individual characteristics, is capable of permitting the cyclical development of both T. congolense and T. vivax to some stage of maturity.

Harley (1966) reported that the majority of trypanosome infections, at all seasons, in G. pallidipes were of the T. vivax-type, T. congolense-type were much less common, and those of T. brucei-type were very rare. In case of G. brevipalpis, Harley (1966) reported that T. congolense infections although low were relatively constant and occasionally higher than T. vivax, and T. brucei were very rare. It is known that G. brevipalpis takes a large part of its diet from bush pig (Weitz, 1970).

In their experimental studies Wilson, Dar and Paris (1972) reported that G. fuscipes appeared to be a more common vector of T. vivax to cattle than G. pallidipes, and that G. pallidipes was a much better transmitter of T. congolense than G. swynnerton.

Many workers (Desowitz and Watson, 1953; Stephen and Gray, 1960; 1961; Stephen, 1962) have mentioned that pigs can be infected with T. simiae by the bites of G. morsitans sub-morsitans. Later Roberts and Gray (1972) also reported that wild caught G.m.sub-morsitans produced T. simiae infections in pigs. An absolute proof that G. tachinoides was capable of transmitting T. simiae was provided by Roberts (1971), after feeding G.m.sub-morsitans and G. tachinoides, which had emerged from puparia, on sheep and pigs.

In the pleomorphic trypanosomes the relationship is much

less clear. The extremely low parasite infection rates found in the field allow little opportunity for comparisons to be made. However, in his experimental work Duke (1930) failed many times to transmit T. gambiense by cyclically infected G. palpalis, likewise Carson (1932) also failed to transmit T. rhodesiense by G. palpalis, in East Africa. However, in contrast to the latter worker, Van Hoof, Henrard and Peel (1937) successfully transmitted T. gambiense cyclically from man to various pigs and back to man by G. palpalis.

A series of experiments carried out by a number of workers (Wilson et al, 1972; Moloo, 1973; Lewis and Langridge, 1947) suggested that T. rhodesiense readily establishes infections that proceed to the stage where metacyclic trypanosomes occur in the saliva in G. morsitans, G. palpalis, and G. pallidipes in East Africa. T. gambiense on the other hand is capable of developing cyclically in G. palpalis, but there is little experimental evidence that G. tachinoides is a particularly suitable vector and G. morsitans sub-morsitans in West Africa may be refractory. In his experimental studies Jordan (1965) postulated that most T. brucei infections were the result of heavy fly challenge by G.m.sub-morsitans. This experiment supports the idea that T. brucei (Stephen, 1966) develops readily in G.m.sub-morsitans.

In contrast to the above mentioned tsetse transmitted pleomorphic trypanosomes, there are other groups of trypanosomes which have lost their tsetse fly transmissibility. This phenomenon is illustrated by Trypanosoma evansi and Trypanosoma equiperdum. These two species of trypanosomes are

widely distributed throughout the world, beyond the limits of the tsetse fly.

In T. evansi no cyclical transmission occurs and the infection is transmitted between hosts by mechanical transmission effected by biting flies, particularly horseflies (Tabanus) and stable flies (Stomoxys). This method of dissemination depends on the movement of a fly with trypanosome-contaminated mouthparts to a new host within a short time, so the parasites in the mouthparts remain infective. Such transmission depends on an interrupted feed as it is necessary for the fly to continue to probe and feed when it has moved to another host. Thus the shorter the interval between feeds or attempted feeds, the greater the chance of transmissions. The abundance of tabanids is also important and there is an apparent link between these biting flies and the seasonal occurrences of outbreaks of T. evansi in camels both in Sudan (particularly Tabanus taeniola) and in Somalia.

Although feeding preferences of those species which may convey Surra seem to have escaped attention, it appears that some of them prefer some host species over others. In the presence of camels these flies will not attack sheep or goats and prefer to puncture the thick skins of camels, equines and bovines.

During the heat of the day, the animals crowd together under the shade of the trees, the tabanids swarm on them and keep up a perpetual motion thus creating extremely favourable conditions for mechanical transmission (Hornby, 1952).

In Somalia, Pangoma species of fly are the agents of transmission (Peck, 1936) and it appears that the prevalence of

T. evansi infections is highest during the two rainy seasons, (April-June) and (October-December) which correspond to the highest density of the population of a biting fly known locally as "Dhug" which is believed to be the main transmitter of Surra.

Donatien and Lestoguard (1923) observed that dogs in stables occupied by infected camels became infected through the bites of Stomoxys.

The possible role of ticks was first investigated by Cross (1923) who transmitted T. evansi from camel to rabbits by means of Ornithodoros crossi and O. labrornsis. Another two authors Kirmse and Taylor-Lewis (1978) using the same species of tick that Cross used, recorded that ticks are not efficient transmitters of T. evansi. In contrast to the other findings Kasim (1984) has isolated T. evansi from Saudi Arabian camels and incriminated the transmission of the disease by adult Hyaloma anotoliathm.

The vampire bat (Desmodus rotundus) of the American tropics is another vector of Surra trypanosomes.

The undoubted efficiency of vampire bats had overshadowed the role played by haematophagous arthropods of America. In his studies Dunn (1932) showed that vampire bats (Desmodus rotundus) could become infected with T. evansi by feeding on infected animals, such as horses, mules and guinea-pigs. Later Clark and Dunn (1933) stated that the incubation period in the bat is six or eight days. This author also stated that if unmolested the bats feed for one to two hours, voiding urine at frequent intervals. This system of consuming large quantities of blood

enables the bat to imbibe trypanosomes from even a lightly infected host.

Moreover, Wells (1984) considers that, after feeding on infected blood, bats may contract the disease which may last up to one month before spontaneous recovery or carrier status results. The trypanosomes taken in with the infected meal penetrate the oral mucosa and cause a generalised blood infection and then return to the buccal cavity where they are subsequently transmitted in the saliva.

Direct transmission is also probable through skin abrasions or bites in camels (Leese, 1927). This sort of transmission could possibly occur between old male camels which usually fight for territorial integrity. In addition carnivores can be infected by consuming the blood and tissues of diseased animals which have died from the disease through lesions of the skin or mucous membranes (Hutyara and Marek, 1938; Dejesus, 1951). Recently Raina, Rakesh Kumar, Rajora, Sridhar and Singh (1985) have conducted an attempt to transmit T. evansi orally in dogs and mice by allowing them to feed on infected meat and blood. The findings of the authors demonstrated the possibility of oral transmission of T. evansi in both these species. These authors concluded that beside the hypothesis of regarding the entry of organisms through breaks in the buccal mucous membranes there may also be the possibility of tissue invasion by T. evansi.

Direct transmission to the foetus in pregnant camels was reported by Sergent, Sergent and Lhertier (1919) and Sergent, Sergent and Donatien (1920) especially during the acute phase of the infection when circulating parasitaemias are maximal.

Compared to other trypanosome species, T. equiperdum is a specific and unique trypanosome of equines. The organism shows a remarkable tropism for the mucosa of the genital organs, the subcutaneous tissues and the central nervous system.

Dourine is transmitted from the stallion to the mare or from mare to stallion during the act of copulation, and so far as is known no other natural means of transmission exists.

The mode of transmission of Dourine may be more complex than is generally thought even amongst equines. Blood obtained from a horse infected with Dourine (T. equiperdum) rarely produces disease when injected into healthy subjects according to Watson (1920) unless it is taken during a very active stage early in the infection. In a more recent study Barrowman (1976) attempted to transmit trypanosomes from naturally infected horses to rats and rabbits, in citrated whole blood, in the eluate from an ion exchange column, and in cerebrospinal fluids containing 10 to 40 trypanosomes per ml without success.

In South America strains of T. vivax have lost the ability to develop in tsetse, and there is evidence that even in Africa in areas beyond the tsetse belt, the trypanosome develops poorly or not at all in flies that are considered to be good vectors.

Pathogenesis of trypanosome infections in domestic animals

Generally, the pathological changes subsequent to trypanosome infections and the underlying causes have not been fully elucidated and many aspects remain controversial (Moulton, Coleman and Thompson, 1974). One reason for the controversy is possibly the wide variety of diseases caused by different

trypanosomes. The precise nature of each disease depends not only upon the species of host but also upon the particular strain of trypanosome involved (Losos and Ikede, 1972) and in each case there may be acute, subacute and chronic forms. This is well illustrated by the differences in virulence for susceptible hosts which are found with the different strains of the same parasite (Fiennes, 1950a). Despite this variability many of the fundamental pathological processes in the various forms of animal trypanosomiasis appear to be similar (Fiennes, 1970; Moulton et al, 1974).

Despite considerable study, it is still not clear exactly how the trypanosomes injure their hosts and the cause of death in many cases remains an enigma. Various theories concerning the mechanism by which pathogenic trypanosomes damage their hosts have been proposed. These include toxin production, hypoglycaemia, vascular spasm, agglutination of parasites in the blood vessels and vascular change (Kliger, 1929; Andrew, Johnson and Dormal, 1930; Hoppe and Chapman, 1947; Boreham and Goodwin, 1967; Goodwin, 1974).

However, in his studies, Von Brand (1951) discussed several theories as to how the trypanosomes may affect metabolism of the host tissues, although he concluded that anaemia due to erythrophagocytosis was the most important factor. According to several reports, there may be an immunological basis to the disease. At each crisis parasites are destroyed in massive numbers and the host is permeated with dead trypanosome protein which may act as anaphylactogen (Ormerod, 1970).

On the other hand, T. brucei also releases intracellular proteases which are potent inducers of blood coagulation and platelet aggregation (Steiger, Opperdoes and Bontemps, 1980; Lonsdale-Eccles and Mpimbaza, 1986). Very recently Nwagwu, Okenu, Olusi and Molokwu (1988) observed in infected mice an extracellular protease released by T. brucei.

It has also been hypothesised that antigen-antibody (Ag-Ab) complexes formed between successive variants and their specific antibodies could produce pathological manifestations similar to those seen in immune-complex diseases such as systemic lupus erythema and chronic serum sickness (WHO, 1977). Moreover Tizard, Hay and Wilkie (1978) explained that it is only relatively recently that the disease caused by the parasite has been extensively studied with relatively sophisticated techniques. It is now realised that the diseases caused by different trypanosomes, although they have certain features in common tend to be highly variable and possibly differ widely in their pathogenesis. In his studies Urquhart (1980) based the pathogenesis of trypanosomiasis on three main factors, (a) anaemia, (b) tissue lesions such as myocarditis and myositis and (c) immunosuppression. There is increasing evidence to support the view that anaemia, a common feature of all trypanosome infections, may be immunologically mediated (Assouku, 1975; Jennings, 1976; Holmes, 1983).

Anaemia

Anaemia is recognised as the most significant factor in the disease process in naturally occurring and experimentally induced animal trypanosomiasis (Murray, 1974). The anaemia can be

divided into three types depending on (1) the species and numbers of parasites, (2) response to trypanocidal therapy (3) the clinical and pathological findings (Fiennes, 1954; Naylor, 1971; Losos, Paris, Wilson and Dar, 1973; Wellede, Duxbury, Sadun, Lanbehn, Lotzsch, Deindl and Warui, 1973).

The anaemia in hyperacute cases generally associated with T. vivax infection has an extremely rapid onset and is very severe. The anaemia in these cases results from widespread haemorrhages and haemolysis. Death usually occurs within a few days.

The onset and severity of anaemia associated with the acute/subacute forms of trypanosomiasis is directly related to the development and the level of parasitaemia. During this stage of the infection the anaemia is haemolytic and is usually normochromic and normocytic (occasionally macrocytic) in type (Murray, 1979; Valli, Forsberg and McSherry, 1978). The anaemia in this form of trypanosomiasis usually responds rapidly to treatment with trypanocidal drugs (Holmes and Jennings, 1976). In cattle which remain infected for several months, it may pass into a chronic form of anaemia. This form has not been adequately investigated under experimental conditions but is reported to be associated with low or absent parasitaemia. An important characteristic of this form of trypanosomal anaemia is that it persists despite the apparent absence of parasites and affected animals usually respond very poorly to trypanocidal therapy (Morrison, Murray, Sayer and Preston, 1981).

Assouku (1975) suggested that anaemia due to T. evansi has

an immunological basis. Other reports of trypanosome infections, such as T. brucei in mice and rabbits (Herbert and Inglis, 1973; MacKenzie and Boreham, 1971; Jenkins et al, 1980), T. congolense in sheep (MacKenzie and Cruikshank, 1973) and T. rhodesiense in rats (Rickman and Cox, 1979; 1980) have provided some evidence of immunological mechanisms as the probable cause of the accompanying anaemias of the respective trypanosome infection.

On the other hand, Fiennes (1954; 1970) attributed the development of anaemia to reduced red cell synthesis in cattle trypanosomiasis, Dargie, Murray, Murray, Grimshaw and McIntyre (1979) suggested that dyshaemopoiesis may be a complicating factor in the long-standing infections.

Initial investigations of the pathogenesis of the trypanosomal anaemia employing radioisotopic techniques were conducted by Jennings, Urquhart and Murray (1972) in rodents infected with T. brucei. A similar study was later carried out by Mamo and Holmes (1975) in Zebu cattle experimentally infected with T. congolense. The results from these studies strongly indicated that the anaemia in trypanosomiasis is due to accelerated cell loss. Despite the use of radiolabels in trypanosomal anaemia, the cause of this red cell loss is still a subject of considerable debate (Holmes, 1983). Several authors have claimed that haemodilution is important in the aetiology of the anaemia seen in trypanosomiasis. Fiennes (1954) was one of the first to suspect this condition in animal trypanosomiasis. He reported the development of hydraemia, or haemodilution by a vital-red dilution technique. It was stated that in T. vivax infections the plasma volume was expanded, but total blood

volumes were unaltered because of decreases in the number of circulating red cells (Rees and Clarkson, 1967; Clarkson, 1968). Later, markedly expanded plasma and normal total red cell volumes were reported (Naylor, 1971; Holmes and Jennings, 1976) after studying calves infected with T. congolense.

Destruction of red blood cells

The exact mechanism in which aged red cells are removed from the circulation is not exactly known. It appears however that the main modes of red cell destruction in health probably results from changes in the physiological properties of the surface membrane (Schalm, Jain and Carroll, 1975). Normal membrane function is therefore essential to the survival of the red cell (Brain, 1982). The latter author proposed that disorders of membrane functions, by virtue of the role the membrane plays in determining cell size, shape and deformability, are the underlying cause of all disorders which lead to a diminished red cell survival. Fiennes (1954) who had a vast experience of ruminant trypanosomiasis particularly T. congolense infections, in East Africa observed erythrophagocytosis and stated, "it is evident that the lymphatic glands were concerned in removal and destruction of red blood cells in company with the spleen and haemolymph glands".

There was considerable renewed interest in this subject in the 1970's. MacKenzie and Cruickshank (1973) demonstrated the occurrence of erythrophagocytosis in sheep infected with T. congolense, and to a lesser extent leucophagocytosis was also seen. MacKenzie, Boyt, Emslie, Lander and Swanepoel (1975), in

an endeavour to determine the cause of the phenomenon of erythrophagocytosis, took washed and unwashed red blood cells from sheep infected with T. congolense and tested them for the presence of trypanosomal antigen attached to the surface. They employed a direct immunoflourescent antibody technique (IFAT) to detect the antigen. No antigen was detected on the surface of red cells in any of the infected sheep until after the initial peak of parasitaemia, probably as a result of trypanolysis and release of antigens which, in effect, converted the red cells into a foreign protein, in the immunological sense and therefore primed them for phagocytosis. Other workers had also reported phagocytosis of erythrocytes in T. vivax infections in cattle and goats (Isoun and Esuruoso, 1972; Van den Ingh, Zwart, Van Miert and Schotman, 1976). Later, erythrophagocytosis in the bone marrow in goats infected with T. vivax was reported by Saror (1980), and Mwongela, Kovatch and Fazil (1981) observed erythrophagocytosis in the spleen of cattle infected with an acute form of T. vivax. In contrast to the latter workers, Masake (1980) did not mention any erythrophagocytosis in her study of the pathological changes occurring in the mononuclear phagocytic system.

Huan, Webb, Lambert amd Meischer (1975) have reported that T. congolense, T. vivax and T. brucei spp yield a haemolytic factor or factors.

Tissue Lesions

The lesions provoked by the various animal trypanosomiases are very similar. Most tissues and organs are damaged during the course of infection, although some are more consistently and

severely affected than others. In addition to the effects of emaciation, anaemia and cachexia there may be yellow gelatinous infiltration of the subcutis, small haemorrhages in the serous and mucous membranes, serous exudation in the pericardium and abdominal cavity, a variable degree of enlargement of the spleen and swelling of lymph glands. The underlying factors causing cellular infiltrates and possibly the mechanisms involved in cell injury would appear to depend on the difference in tissue invasiveness between species of trypanosomes (Morrison et al, 1981).

The heart is one of the organs which all the animal trypanosomes consistently damage. However, the histopathological changes observed in the heart could be an example of what occurs in other tissues and organs but to a greater degree. In tsetse transmitted trypanosomiasis, obvious and more severe heart lesions are observed in cattle with chronic disease while obvious lesions may be absent in the hearts of small ruminants dying of acute T. vivax infections. There is often a marked degeneration of myocardial fibres in cattle which are separated by interstitial and perivascular oedema.

In their study on T. congolense infected animals, Valli and Forsberg (1979) found an increase in the number and size of splenic follicles, but the parafollicular area around the splenic arterioles was reduced. The later authors also found a definite interstitial thickening in the lungs, alveolar size was unchanged, but there was more free cells in the alveoli and a thickening of lobular septa and alveolar wells.

Anosa and Isoun (1983) stated that haemolymph nodes were two to three times normal size in sheep and goats infected with T. vivax. The authors also noted congestion of the subcapsular, cortical and medullary sinuses, with proliferation of macrophages in the sinuses and cords. Anosa (1983) describes the testicular pathology of ruminants as including "degeneration of the seminiferous tubules, with disappearance of spermatozoa and spermatids and less commonly spermatocytes, resulting in a reduction in the mean seminiferous tubular diameter and epithelial thickness, there was also spermatid giant cell formation and calcification". There is a striking absence of severe pathognomic lesions in acute T. vivax infections despite the poor state of the animals prior to death (Anosa and Isoun, 1983).

The pathology of T. brucei infections in domestic animals, was documented by Ikede and his colleagues (Ikede, 1974; Ikede and Losos, 1972a, b, c, d; Ikede and Losos 1975a, b). The gross pathological lesions seen at post-mortem in T. brucei infections of domestic animals differ only marginally from those seen in T. vivax and T. congolense infections that have run a chronic course. Oedema, serous effusions, fatty degeneration and ocular lesions are however more noticeable and the heart shows greater evidence of damage. The lesions in the liver and kidney are similar to those caused by T. congolense and T. vivax, but possibly are more severe.

Ikede, Lule and Terry (1977) studied the effects of chronic T. brucei infections on the testicles of rams and described the inflammatory and degenerative changes that lead to total

aspermatogenosis. Morrison et al (1981) have provided a careful and well illustrated study on the pathogenesis of T. brucei in the dog induced with blood forms of the organism by intravenous inoculation. They concluded that infection of dogs with T. brucei resulted in high levels of parasitaemia and the presence of numerous trypanosomes in the tissues and in the afferent lymph.

Raisinghani, Yadov, and Lodha (1981) studied the pathological changes in experimentally T. evansi infected camels. Amongst other things, they reported a pronounced myocarditis. Verma and Guatama (1978) investigated the histopathological lesions subsequent to Surra infection in bovines. Later Seiler, Omar and Jackson (1981) studied the histopathology lesions in horses that died of T. evansi infections in Malaysia. The author found lymphoid hyperlasia in the spleen and lymphohistiocytic cells were found in most livers. Slight scattered, non-suppurative myocarditis was observed in two horses.

Lesions of the Central Nervous System

The pathological importance of trypanosomes in the cerebrospinal fluid (CSF) is not clear. The mere presence of the parasites in the CSF without an accompanying increase in total protein or leucocytes indicates that the trypanosomes are present without any demonstrable pathological change in the cerebrospinal fluid (Waitumbi, Brown, Jennings and Holmes, 1988).

In view of the current concepts of the pathogenesis, it does not seem to be of essential importance whether the parasites enter the brain or not (de Raadt and Seed, 1977). It may

however, have serious implications for the treatment of animal trypanosomiasis since no trypanocides exist which are specifically marketed for use in animals with cerebrospinal fluid trypanosomiasis (Murray and Jennings, 1983), although the arsenical, melarsoprol is used in late-stage human trypanosomiasis.

Brain lesions in ruminant animals dying of T. vivax and T. congolense infections are probably of little consequence in the pathogenesis of trypanosomiasis in these animals. The lesions observed in horses and dogs infected with T. brucei are of considerable interest to the comparative pathologist, but any attempt to attach great significance to such changes in ruminants would be a distraction from the lesions of the other tissues (Stephen, 1986).

Clinical observations of infected animals have provided some evidence of invasion of the central nervous system by T. evansi. Ataxia commonly occurs in T. evansi infections, particularly in horses. In the first description of the disease in India, Evans (1881) mentioned locomotory disturbances in fore and hind limbs. Later Steel (1886) reported oedema in the brain and spinal cord of T. evansi-infected mules.

Greenfield (1958) described lesions in sections of the forebrain, midbrain and cerebellum caused by T. gambiense in man, and, at post-mortem examination of dogs infected with T. evansi. Maychew (1968) reported that the animals showed similar lesions. Recently Seiler et al (1981) observed a few scattered petechiae in the meninges of the cerebellum and brain stem of horses infected with T. evansi. Histologically the meningoencephalitis

affecting the grey and white matter of some of these horses was more serious in some than others.

Masake, Nantulya, Akol and Musoke (1984) isolated T. congolense from the brain of a calf inoculated with T. brucei one year after infection with what was presumed to be a pure T. congolense infection. The calf developed both clinical and histological evidence of cerebral trypanosomiasis. Recently Zweygarth and Rottcher (1987) detected T. simiae in the cerebrospinal fluid of experimentally infected pigs in Kenya.

Immunosuppression

Animals and humans infected by trypanosomiasis frequently suffer from immunosuppression. The earliest experimental evidence was provided by Goodwin, Green, Gray and Voller (1972). Sheep erythrocytes were used as an antigen in the studies on Trypanosoma brucei-infected rabbits and mice and it was demonstrated that after infection the haemagglutin responses were significantly reduced.

Immunosuppression in experimentally infected mice was further investigated by Murray (1974a) and Murray, Jennings, Murray and Urquhart (1974b).

MacKenzie et al (1975) infected sheep in Zimbabwe by blood inoculation and then challenged them with the bacterium Vibrio foetus. Their results suggested immunosuppression by T. congolense occurred in the sheep, and that an organism which would normally be controlled by the host's immune defence system could show greater pathogenicity. However, efforts to demonstrate immunosuppression in large domestic animals have not

been unequivocal and most attempts to do so have involved ruminants infected in the first place by syringe inoculation of trypanosomes, and then inoculated with a known viral or bacterial antigen. A practical aspect of this subject concerns the possibility that vaccination programmes could be seriously over valued if a vaccine such as rinderpest attenuated virus failed to protect cattle in immunologically-deficient trypanosome-infected herds. Holmes, Mamo and Thomson (1974) mentioned this possibility when they noted that the secondary immune response to a polyvalent clostridial vaccine was lower in cattle infected with T. congolense, than in clean control animals. Scott, Pegram, Holmes, Ray, Knight, Jennings and Urquhart (1977) in Ethiopia, studied the immune response to a commercial trivalent foot and mouth disease (FMD) vaccine in cattle naturally infected with T. congolense. Some of the cattle in the study also received a polyvalent clostridial vaccine to confirm the results obtained by Holmes et al (1974). Their results showed distinct evidence of trypanosome-induced suppression of the immune system to foot and mouth disease vaccine, as well to the clostridial agent.

Whitelaw, Scott, Reid, Holmes, Jennings and Urquhart (1979) tested the antibody response of trypanosome infected cattle to Louping Ill virus vaccine. Three groups of cattle were employed and they were infected by blood inoculations with T. vivax, T. congolense and T. brucei before being vaccinated. In cattle infected with T. vivax and T. congolense the antibody response to the vaccine was only 10% of that in clean control animals which received the vaccine only. Contrasting with this, the response

of the cattle infected with T. brucei was not significantly reduced. They also showed that the immunosuppressive effects of trypanosomiasis could be greatly diminished by giving trypanocidal therapy at the time of vaccination.

Although there is clear evidence of suppressed antibody responses to foreign antigens in both animals and humans infected with trypanosomes, the underlying mechanisms remain obscure and much of the evidence is conflicting. Nevertheless it would seem that the investigations collectively suggested that the aetiology of the immunological hyporesponsiveness induced by trypanosome infections is multifactorial.

Many of the observations can be used in support of the hypothesis that the B cell system of the host with African trypanosomiasis undergoes generalised activation and thus proceeds to a stage of differentiation which limits the extent of additional responses to antigenic stimulation. Consistent with this idea are the observations of a B cell mitogen (Assouku and Tizard, 1978; Esuruoso, 1976; Greenwood and Whittle, 1976 and Mansfield, Craig and Stelzer, 1976), the occurrence of a polyclonal antibody response (Henderson-Begg, 1946) and the macroglobulinaemia (Terry, 1976) which are so typical of the disease. Other authors (Mansfield, 1978) have suggested that the major defect is within the T lymphocyte sub-population. The loss of T-dependent areas, histologically favour this idea. Furthermore, a deficiency in suppressor T-cells would favour an abnormally high proliferation of B-cells, which is also observed histologically and could result in elevated immunoglobulin

production. This combined with a deficiency in T-helper cells, which would be expected to retard the switching from Igm to IgG synthesis, would provide sufficient explanations for the observed macroglobulinaemia.

Other workers (Askonas, Corsini and Clayton, 1979) emphasise the generalised dysfunction of lymphoid tissue, involving cell sub-populations of a variety of functional types, including T- and B-memory cells, and T-cells, responsible for mixed lymphocyte reactivity.

More recently, Sileghem et al (1986) showed that lymph node cells from T. brucei infected mice suppressed the secondary proliferation of reactive antigen primed T cells as well as the IL-2 production by mitogen stimulated T lymphocytes. Their results suggest that trypanosome-induced immunosuppression is mediated by a suppressive cell which interferes at the level of IL-2 secretion.

According to Holmes (1980), the veterinary impact of immunosuppression may fall into three categories:

(1) the animals may be rendered more susceptible to secondary infections, e.g. secondary complications affecting the respiratory, gastrointestinal and nervous systems may occur (Cross, 1921b; Rutter, 1967).

(2) infected animals may give diminished responses to bacterial and viral vaccines e.g. Holmes et al (1974) showed T. congolense can actively suppress the responses of Zebu cattle to immunisation with a polyvalent clostridial vaccine.

(3) the parasitised host may have a reduced capacity to mount an effective response against the trypanosome infection.

Chemotherapy (General)

Escalating costs of initiating and maintaining tsetse control campaigns, together with the non-availability of a vaccine, have led to the livestock industries in the vast tsetse-infested areas of Africa being almost completely reliant on the use of trypanocidal drugs to both treat and prevent trypanosomiasis.

It is acknowledged that the early evolution of drugs owes much to the preoccupation, about the turn of the century, with the control of trypanosomiasis (Davey, 1957 and Williamson, 1970). Leach and Roberts (1981) have described briefly, the history of the chemotherapy of animal trypanosomiasis. In their review they mentioned that although the initial chemotherapeutic endeavours were at first directed towards the human species, this overlapped with the developments in drug treatment of animal trypanosomiasis and inevitably occurred simultaneously at a number of points. A good example where the chemotherapeutic susceptibility of both human and animal parasites converged is the production of the drug, suramin.

Trypanocidal drugs can be divided into two groups: those used to prevent infections (prophylactics) and those used to cure infected animals (therapeutics). The ideal compound should have broad-spectrum trypanocidal activity, no toxic side effects and should not induce drug resistance. Unfortunately, no such ideal drug exists. Despite the need and demand for effective trypanocides, no new drug has been produced for commercial use in the last 25 years. The few available drugs are widely used by

livestock owners and veterinarians throughout Africa and the risk of development of parasite resistance is increasing. To prevent the situation deteriorating further, greater economic support is needed on testing (Leach and Roberts, 1981). Lack of funds to implement control programmes, well trained veterinary graduates and other trained staff, the lack of an infrastructure required for drug administration are other obstacles encountered in the applications of trypanocides (Holmes and Scott, 1982). Because of these problems there is an increasing gap between treatment demand and the treatment actually administered (MacLennan, 1980).

Chemotherapy and chemoprophylaxis can be effective if managed in an efficient way (Blaser, Jibbo and McIntyre, 1979; Leach and Roberts, 1981). Unless the incidence of trypanosomiasis and the stock carrying capacity are both high, the protection of cattle with trypanocides is preferable to tsetse control programmes which usually are relatively costly (Finelle, 1976).

In places where the tsetse flies have been eradicated, wide use of therapeutic drugs will play a role in clearing the disease from any infected animals and future mechanical transmission will be reduced (Gray, 1983).

Chemotherapy

Those trypanocidal drugs, which are currently in use are either prophylactics or therapeutics. The chemical and proprietary names, dosage and efficacy of drugs currently used in the field are described in Table 2.

Table 2

Commonly used trypanocidal drugs and their indications

Group	Compound	Trade Name (Manufacturer)	Dosage (mg/kg)	Highly Active on	Less Active on	Possible Poor Tolerance	Duration of Efficacy
Phenanthridine	Isometamidium chloride	Samorin (RMB) Trypamidium (Specia)	0.25-1.0 (im)	vivax congolense	brucei	camels	2-6 months
Naphthalene	Suramin sodium	Naganol (Bayer) Moranyl (Specia)	10 (iv)	evansi brucei			about 2 months
Quinoline	Quinapyramine sulphate	Trypacide sulphate (RMB)	5.0 (sc)	evansi brucei	congolense vivax	horses	curative
	Quinapyramine Sulphate + Chloride	Trypacide Prosalt (RMB)					
Phenanthridine	Homidium bromide, Homidium chloride	Ethidium (FBC), Novidium (RMB)	1.0 (im)	vivax congolense		horses	1 month
Diamidine	Diminazene aceturate	Berenil (Hoechst)	3.5 (im)	vivax congolense	evansi congolense	horses camels	curative
	Quinapyramine-Suramin complex		40 (im)	simiae			3 months

im Intramuscular
iv Intravenous
sc Subcutaneous

(Modified from ILRAD Report, April 1987)

Suramin

Of the drugs currently in use suramin was the first to be introduced. (Bayer 205(R), Germanin (R), Antrypol (R), Belganyl(R), Fourneau 309(R), Moranyl(R), Naphuride(R), Naganol(R)). This sulphonated naphthylamine derivative is a white, cream-coloured or faintly pink hydroscopic powder, readily soluble in water to yield a neutral solution.

Suramin is employed for both human and animal trypanosomiasis. It has been recorded to be ineffective against T. congolense and T. vivax infections in cattle (Ruchel, 1975) but Stephen (1966b) observed some therapeutic activity towards T. simiae infections of pigs. It is strongly ionic and firmly binds with plasma proteins (Bournsnell, Dangerfield and Wormall, 1939; Bournsnell and Wormall, 1939; Dewey and Wormall, 1946). The drug may provoke delayed toxicity, including pronounced nephritis (Morcos, 1931). This author advised against treatment with suramin in cases where albuminuria is prominent. Suramin is efficaceous against infections with trypanosomes of the subgenus Trypanozoon, in horses, donkeys and camels (Leach, 1961).

In this respect it compliments the more recently introduced drugs. Since it is less toxic than quinapyramine, it is particularly useful in the treatment of equine Surra. Suramin has been in constant use in the field for more than 60 years and it remains the drug of choice for the treatment of early infections. Suramin is administered intravenously as it can generate local reactions when given intramuscularly or subcutaneously. Several attempts to improve suramin's trypanocidal activity and to lessen its toxicity to the host

animals were made without success (Spinks, 1947; Adams, Ashley and Bader, 1956).

A single dose of 5g effected a 100% cure in camels (Mahamoud and Abdel-Latif, 1958). In ponies affected by Surra a dosage of 10mg/kg bodyweight is not only curative, but also protects these animals for a period of 30 days (Ruchel, 1975). In the case of bovine Surra, a dosage of 1g/animal produced good results (Fernandez, Rico and Dumag, 1965).

Suramin complexes

Williamson (1957) has described in detail the ability of suramin to be combined with basic substances to form relatively insoluble complexes, known as suraminates. It has been recorded that the complexes considerably reduce the toxicity of cationic drug constituents and produce an increase in their prophylactic activity (Williamson, 1957).

Suramin produces complexes with quinapyramine (Antrycide$^{(R)}$), homidium (Ethidium$^{(R)}$ Novidium$^{(R)}$), pyrithidium (Prothidium$^{(R)}$), isometamidium (Samorin$^{(R)}$) and diminazene aceturate (Berenil$^{(R)}$). Williamson (1956) reported that the highest precipitation of these salt complexes has a stoichiometric basis dependent on the number of available ionised groups in the combining molecules. Williamson and Desowitz (1956) found a maximum decrease of five to three fold in the toxicity of suramin complexes of Antrycide and Berenil respectively.

Suramin complexes conferred protection in cattle, against T. vivax and T. congolense, when administered subcutaneously to

cattle. The prophylactic action could be dependant upon the accumulation of these relatively insoluble complexes at the injection site and the formation of a depot from which small amounts of the drug are released into the blood circulation over a long period of time. The quinapyramine dimethylsulphate-suraminate (Williamson, 1957) was very effective as a prophylactic against tsetse transmitted T. simiae infections in pigs in West Africa (Stephen and Gray, 1960).

Mode of action of Suramin

Suramin is a polybasic anion and binds strongly to proteins. This bonding with proteins could account for its long retentions and prophylactic activity and may also facilitate its entry into the cell (Questel, 1931). The probability that this drug enters trypanosome lysomes is strengthened by the demonstrations of its lysosomotropic properties in other cells (Davies, Lloyd and Beck, 1971; Buys, Elferink, Bourna, Gruber and Niewenhuis, 1973; Jacques, Huybrechts-Godbin and Smeesters, 1975; Hart and Young, 1975). Suramin may also interfere with ribosomal and lysosomal functions of trypanosomes (Williamson and MacAdam, 1965). Previously MacAdam and Williamson (1964) proposed that the drug induced release of lysosomal acid ribonuclease might be responsible for the disappearance of the ribosomes.

There is no experimental evidence that lysosomal enzymes damage trypanosomes (Leach and Roberts, 1981) and anyway it is not clear whether the lysosomal enzymes actually kill or merely digest organisms inactivated by other processes. One point worthy of note is that exploitation of drug lysosomotropism has a potential for the selective delivery, toxicity reductions and the

controlled release for prophylaxis (Williamson, 1976b). The correlations of the properties of suramin with its trypanocidal actions are not clear. Nonetheless, lysosome phagosome interference is unlikely to be of any relevance in this respect since demethylated suramin is as active in the macrophage system as suramin, but totally inactive as a trypanocide (Williamson, 1976a).

Quinapyramine dimethylsulphate/chloride (Prosalt)

Quinapyramine is available in two forms: on the one hand as quinapyramine dimethylsulphate, a water soluble, yellowish-white, crystalline powder with a curative effect and on the other hand as quinapyramine chloride, a light yellow compound which is very sparingly soluble and this accounts for its prophylactic activity. The mixture (three parts dimethylsulphate plus two parts chloride) i.e. Prosalt was introduced in the early fifties after a series of field trials in Africa (Davey, 1950). The drug has a broad spectrum of activity, being active against both mechanically and cyclically transmitted trypanosomiasis (Curd and Davey, 1950; Leach, 1961; Davey, 1950; 1957).

The drug is absorbed slowly as it forms a deposit after subcutaneous injection (Davey, 1950). After treatment with quinapryamine prosalt, the depot of unabsorbed drug may be surrounded by a fibrous capsule which may calcify. This prevents the slow absorption of the active agent, thus reducing the period of protection although an appreciable amount of the drug may still be present in the depot (Whiteside, 1961). Because it is absorbed so much more quickly and completely, the methylsulphate

is more toxic than the chloride (Davey, 1950).

In the initial experimentation in Africa, Davey (1950) showed that the methylsulphate at 5mg/kg could effect a cure in animals whereas the chloride at a similar dose exerted a prophylactic effect for at least two months. With 10mg/kg the sparingly soluble chloride gave four months protection. Rottcher (1982) published the findings of an experiment involving five groups of camels in different locations of Kenya which were used in chemotherapy field trials using quinapyramine, (Antrycide$^{(R)}$), suramin, (Naganol$^{(R)}$) and isometamidium, (Samorin$^{(R)}$) for a period of 3 - 18 months. The overall results showed that Antrycide was the most successful drug for both treatment and chemoprophylaxis.

Toxicity in equidae may be prevented by dividing the dose in two and injecting the second half 5 - 6 hours after the first (Leach and Roberts, 1981).

Mode of Action

The drugs which affect the kinetoplast are cations known to bind with DNA. Ormerod and Shaw (1963) observed that cationic drugs enter the kinetoplast within minutes of treatment. However, MacAdam and Williamson (1972; 1974a) and Williamson, MacAdam and Dixon (1975) showed that quinapyramine attacked the kinetoplast DNA core specifically. This selective binding may be attributed to the circularity of the kinetoplast DNA combined with a lack of histones and the preponderance of the adenine-thymine content in kinetoplast DNA, in contrast to nuclear DNA (MacAdam and Williamson, 1974a). When administered to cultures of Crithidia oncopelti, quinapyramine inhibited RNA synthesis at

concentrations that did not affect DNA synthesis (Newton, 1964). The drug also caused aggregation of isolated ribosomes.

Diminazene Aceturate

Diminazene aceturate, (Berenil $^{(R)}$), is a diamidine and is a stable, yellow, odourless powder. Since its introduction diminazine aceturate has been extensively used against African trypanosomes, for example T. vivax, T. congolense, T. brucei but there are few reports of its success in the treatment of T. evansi infections (Razzaque and Mishra, 1977). The drug also appeared ineffective against T. simiae. Diminazene aceturate is highly active against Babesia spp in cattle which is an important additional advantage in African conditions.

Diminazene aceturate is noteworthy among trypanocidal drugs in being rapidly excreted, almost all of a parenterally administered dose being cleared through the kidneys within 24 hrs. Although there is evidence that some of the drug must be retained in the tissues (Lumsden, Herbert and Hardy, 1965), this characteristic of quick clearance is thought to lessen the risk of resistance developing through prolonged exposure of parasites to a waning concentration of the drug and also reduces opportunities for the occurrence of chronic toxicity. Recently, Kellner, Eckert and Volz (1985) have studied experimentally the blood concentrations of diminazene aceturate, its routes and rates of excretion and total tissue residues of healthy calves, injected intramuscularly. These workers found lower concentrations of diminazene in skeletal muscle and fat and higher concentration of diminazene in organs with excreting

functions such as the liver and kidneys. This finding is in accordance with Fairclough (1963), who reported that the compound is not rapidly and completely metabolised as assumed. Because of the lack of widespread resistance occurring, diminazene aceturate has replaced homidium and quinapyramine as the curative trypanocide of choice.

Early reports described an experiment in which three groups of cattle, six animals in each group, all infected with T. vivax, were treated respectively with 1, 2, or 3mg/kg intramuscularly (Bauer, 1955a). As a result of this experiment the standard dose of 3mg/kg intramuscularly was decided upon. Later the standard dose was increased up to 3.5mg/kg. However, the recommended dose of T. brucei is 7.0mg/kg.

In a toxicity study Homeida, Amin, El Adam and Mohamoud (1981) found that 10mg/kg of Berenil was highly toxic to camels. The main signs of Berenil poisoning were hyperaesthesis, salivation, intermittent convulsions, frequent urinations and defaecation, itching and sweating. In other studies, camels showed severe toxic reaction to 7.0mg/kg (Leach, 1961).

Mode of action

Diminazene aceturate appears to be actively transported into trypanosomes against a concentration gradient(Damper and Patton, 1976a, b). As in many trypanocidal drugs, Berenil interacts specifically with kinetoplast DNA. It inhibits the replication of the kinetoplast and induces dyskinetoplasty in T. brucei (Killick-Kendrick, 1964; Newton and LePage, 1967; Newton, 1975). In marked contrast to homidium, Berenil does not intercalate into the DNA helix (Waring, 1970; Festy and Daune, 1973) but binds by

external attachment across the small groove astride the two complementary strands and slightly distorts the helical structure (Festy, Sturm and Daune, 1975).

In addition, this drug appears to bind kinetoplast DNA at four equidistant sites which also bind RNA polymerase (Brack and Delain, 1975). It also affects cytoplasmic membranes and lysosomes (MacAdam and Williamson, 1972). Inhibition of phospholipid synthesis (Gutteridge, 1969), basic amino acid transport (Gutteridge, 1966) and oxygen uptake (Hill and Hunter, 1968) have also been reported.

Phenanthridium Drugs

The effectiveness of this class of drugs against tsetse-borne trypanosomes has been established (Mahamoud and Gray, 1980). The earliest compound of the phenanthridine series to be described was phenidium chloride (Browning, Morgan, Robb and Walls, 1938). Local reactions at the injection sites and its poor therapeutic activity were obvious drawbacks to its usage (Hornby, Evans and Wilde, 1943).

Dimidium bromide was immediately synthesised and was first tried in Uganda against T. congolense (Bell, 1945), and the results were most promising. They indicated that the drug had a relatively wide margin of safety, and that a dose of 1mg/kg, subcutaneously was sufficient to cure T. congolense. However, Randall and Beveridge (1946) reported that in the first 187 cattle treated with 2mg/kg subcutaneously "nearly all developed symptoms of photosensitisation some six weeks after administration of the drug, and 41 subsequently died". In

addition, severe local reactions at the site of injection were of frequent occurrence, resulting in sloughing of areas of skin up to several inches in diameter. Because of these adverse reactions the dose was reduced to 1mg/kg and the injections made intramuscularly (Davey, 1957). Further experimental work confirmed in general that photosensitisation was a serious hazard in the use of dimidium bromide (Randall and Beveridge, 1946).

Homidium

Homidium was produced by substituting an ethyl for a methyl group on the quaternary nitrogen heteroatom of dimidium (Watkins and Woolfe, 1952). The compound was first produced as Ethidium(R) (bromide salt) and later as Novidium(R) (homidium chloride).

Novidium which is equally active therapeutically, is soluble in cold water (Wragg, 1955). The drug is injected intramuscularly, as it can cause severe reactions if given subcutaneously. Homidium has been shown to have a selective effect on T. vivax infections in cattle (Mwambu, 1971) and has been used successfully for treating this parasite in horses. The compound was also found active against T. congolense (Mwambu, 1966). However, a study conducted by Srivastava and Ahluwalia (1973) demonstrated that homidium bromide was ineffective against T. evansi infections in Asian dogs.

Isometamidium

Isometamidium (Samorin(R)) was first introduced in 1958. Wragg, Washbournik, Brown and Hill (1958) had combined the diazotized p-aminobenzamide moiety of the diminazene molecule with homidium chloride in the presence of sodium acetate. This

yielded two isomeric derivatives that were both trypanocidal. The two isomers are one red and one purple. The red isomer was named isometamidium chloride and was marketed under the name of Samorin(R).

The manufacturers recommended that for cattle trypanosomiasis (T. congolense and T. vivax) a therapeutic dosage of 0.25 to 0.5mg/kg be used for sensitive strains, 0.5mg - 1.0mg/kg for less sensitive strains and for prophylaxis. Intramuscular injection of isometamidium can cause severe local reactions and extensive fibrosis (Boyt, 1971). These lesions are usually very obvious in cattle where tsetse challenge is high and drug treatments are very frequent. It is advisable to divide the dose so that not more than 15ml is given at one injection site (Finelle, 1973a). To circumvent this difficulty, Toure (1973) studied the effect of intravenous administration, three calves treated with 0.5mg/kg were inoculated with T. brucei thirty-four days later and their blood films remained negative during the subsequent two months. Isometamidium is also the drug of choice for T. congolense infections of dogs (Aliu, 1981). Isometamidium has successfully treated T. evansi infections of camels and donkeys at 1.0mg/kg and 2.0mg/kg respectively (Petrovski, 1974; Chand and Singh, 1970).

Some workers have made attempts to decrease the extensive ulceration and necrosis of isometamidium administration by complexing isometamidium with dextran sulphate (Aliu and Chineme, 1980; Fluck, 1987), and the complex provoked no gross changes.

Balis and Richard (1977) carried out trials in guinea pigs

and dromedaries infected with T. evansi in Ethiopia with isometamidium chloride. They found that it was curative in guinea pigs at dose rates ranging from 0.25 to 2.0mg/kg body weight. A dose of 2.0mg/kg caused in camels a pronounced shock with collapse, salivation, diarrhoea and increased pulse rate. In addition, intramuscular injections of 2.0mg/kg were only partially effective with trypanosomes reappearing in the blood after one to two months, and at the time of injection extensive pain was noted with consequent development of lesions. The authors concluded that isometamidium chloride is only moderately active against T. evansi.

Chemoprophylaxis

There are only two situations where treatment with prophylactic drugs should be encouraged, firstly when animals are to be exposed to tsetse challenge for a specific and limited period of time, which is shorter than the protective period of the drug concerned; and secondly when the animals are sedentary and are available throughout the year for regular inspection and treatment. MacLennan (1970) summarised the problems attending control by chemoprophylaxis.

Quinapyramine prosalt which was the first prophylactic drug used in the field conferred protection for two months to cattle in close contact with tsetse fly (Fiennes, 1953). Earlier workers have recorded that pyrithidium bromide (Prothidium$^{(R)}$) would protect cattle for 4 - 8 months (Marshall, 1958; Lyttle, 1960; Finelle and Lacotte, 1965). Watkins and Woolfe (1956) introduced the later drug as a prophylactic against cattle trypanosomiasis. The drug has been shown to be superior to

quinapyramine as a prophylactic (Smith and Brown, 1960). The usage of pyrithidium declined later because of its toxicity problems and the development of resistance and it was withdrawn from the market in late 1970s.

Suramin injected intravenously into camels in doses of about 3.0mg/kg body weight affords protection of 2 - 4 months, while quinapyramine (Antrycide Prosalt$^{(R)}$) (4.0mg/kg) administered subcutaneously protects animals such as ponies, camels and bovines for 2 - 3 months (Woo, 1977).

In T. evansi infections of domestic animals the period of protection conferred by the two drugs used for treatment (suramin and quinapyramine prosalt) is of short duration rarely exceeding three months or at the most four months (Wilson, 1984). The susceptibility of animals to trypanosomes and the duration of protection can be affected by seasonal variations in relation to fly density, food availability etc. The effect of intercurrent diseases on chemotherapy and chemoprophylaxis is well recognised (Leach and Roberts, 1981; Holmes and Scott, 1982). In addition, experience has shown that stock owners accept the treatment of their animals only when clinical symptoms or parasitaemia become evident. Therefore good collaboration between animal owners and veterinarians is vital (Holmes and Scott, 1982).

During the last two decades isometamidium has been the only prophylactic drug used in the tsetse-infested areas of Africa (Williamson, 1976a; Connor, 1984). The other commonly used trypanocides, homidium chloride and diminazene aceturate have little if any prophylactic properties. However, diminazene

aceturate is commonly used as a sanative treatment for animals in which trypanosomes have been observed before the next prophylactic treatment of isometamidium (Finelle, 1973b).

Many workers from different parts of Africa have reported the economic return associated with using isometamidium prophylaxis in trek cattle, ranch cattle, and work oxen (Na'Isa, 1969; Wilson, Le Roux, Paris, Davidson and Gray, 1975a; Wilson, Njogu, Gatuta, Mgutu, Alushula and Dolan, 1981; Bourn and Scott, 1978; Logan, Goodwin, Tembely and Craig, 1984; Trail, Sones, Jibbo, Durkin, Light and Murray, 1985).

There are fewer reports to the use of Samorin in dairy cattle. However Samorin was systematically used for several years in 200 breeding females of grade cattle (mainly Friesian type). The cattle were maintained in an area in Kenya where the challenge of G. pallidipes, G. austini and G. brevipalpis was moderate to heavy. The increased productivity, associated with the therapeutic and prophylactic use of isometamidium chloride in dairy cattle, resulted in an improved economic return (Fazil, 1987; cited by Murray and Moloo, personal communication).

Isometamidium chloride is normally recommended for prophylaxis at a dose of 0.5 to 1.0mg/kg body weight, (Fairclough, 1963a; Pinder and Authie, 1984), and at these levels livestock owners have recorded protection against trypanosomiasis lasting from two weeks to 14 weeks. The period of prophylaxis conferred by isometamidium to cattle after deep intramuscular injection varies widely.

In their extensive review of chemotherapy and chemoprophylaxis of animal trypanosomiasis, Leach and Roberts

(1981) have suggested that in circumstances where reliance is placed on drug treatment for control of trypanosomiasis, a choice must be made between chemotherapy and chemoprophylaxis. The slow elimination of prophylactic drugs which is required if they are to afford protection over an extended period implies that eventually a concentration insufficient to prevent infection is reached. When this happens, trypanosomes may have an opportunity to multiply in the presence of the drug and are likely to develop some degree of resistance to it. Hence, regular repetition of treatments within the protection period of the drugs concerned is the cornerstone of successful chemoprophylaxis. In evaluating the prospects of success for such an approach, it is therefore necessary to consider such logistical restraints as the continuing availability of trained staff and of reliable transport, as well as certainty of access to the animals involved. It follows that nomadic and semi-nomadic livestock are seldom suitable targets in attempts to control trypanosomiasis by chemoprophylaxis.

Conversely, it may provide an economic solution for stock under ranch management, as the previous authors (Leach and Roberts, 1981) indicated. One important economic factor in consideration of chemoprophylactic programmes is that the period between treatments should be as long as possible yet it must also be adjusted to correspond to the severity of risk and the sensitivity of the infecting strains to the drug in use (MacLennan, 1970).

Lyttle (1960) stated that the prophylactic period conferred

by a drug depends on the number of tsetse bites received per unit and the virulence of the trypanosomes. Other workers have discovered that Trypanosoma brucei can re-emerge from tissues or organ especially the central nervous system (CNS), which are inaccessible to most trypanocides, after the animals have shown a period of apparent cure (Jennings, Whitelaw, Holmes, Chizyuka and Urquhart, 1979). Immunity acquired by animals treated with trypanocidal drugs may increase the prophylactic period (Wilson, Paris, Luckins, Dar and Gray, 1976; Bourn and Scott, 1978).

An experiment was conducted by Whitelaw, Bell, Holmes, Moloo, Hirumi, Urquhart and Murray (1986) to study isometamidium chloride prophylaxis against Trypanosoma congolense challenge and the development of immune responses in Boran cattle. Twenty-four Boran cattle were injected with isometamidium chloride (1 mg/kg bodyweight) to investigate the duration of drug-induced prophylaxis against infection by metacyclic forms of Trypanosoma congolense and to determine if specific antibody responses to the organism were mounted by animals under chemoprophylactic cover. Complete protection against either single challenge by five tsetse flies infected with T. congolense, or repeated challenge at monthly intervals by five tsetse flies, lasted for five months. Six months after treatment, two-thirds of the cattle were still resistant to challenge, irrespective of whether subjected to single or multiple challenge with trypanosome-infected tsetse flies, or titrated doses of in vitro-cultured metacyclic forms of T. congolense (from 500 to 500,000 organisms), inoculated intradermally. No animal which resisted infection developed

detectable skin reactions at the site of deposition of metacyclic trypanosomes or produced trypanosome-specific antibodies. The authors concluded that drug residues effectively limited trypanosome multiplication at the site of deposition in the skin, thus preventing subsequent parasitaemia or priming of the host's immune response.

Ercoli (1978) stated that the prophylactic period conferred by chemoprophylaxis consists of true prophylactic period and false prophylactic period; the treated animals are initially able to resist the challenge but during the false prophylactic period the animals are not completely resistant and infections develop, although the onset of parasitaemia is extended, giving false indication of the prophylactic period.

Drug resistance

There is no compound currently in use for the treatment of animal trypanosomiasis against which drug resistance has not arisen.

Zakrezewskie (1973) has described some of the fundamental mechanisms which may be responsible for the emergence of drug resistance (a) altered drug uptake (b) altered metabolism or, (c) inactivation of the drug. Most of the investigators are inclined to support the first mechanism (Hawking, 1934; 1937; von Brand, 1951; Williamson and Rollo, 1959).

Whiteside (1961) discussed several ways in which drug resistance may emerge, especially in the tsetse-borne species, and experience has shown that they are equally applicable to mechanically transmitted trypanosomes e.g. T. evansi. First,

underdosing due to incorrect estimation of body weight. Secondly the combined effect of a high incidence of trypanosomiasis and the use of therapeutic and prophylactic drugs may result in the emergence of the drug resistant strains. Thirdly, irregular treatment with prophylactic drugs or stopping their use altogether while animals are still at risk can produce resistant strains of trypanosomes. The belief in the field, that drug resistant trypanosomes may often be of comparatively low pathogenicity (Gobel, Ferrell and Steiglitz, 1959; Stephen, 1962) is encouraging, even though this needs further confirmation.

Since trypanosomiasis occurs in developing countries mostly with inadequate veterinary services, the misuse of the drugs by the animal owners almost certainly contributes to the emergence of resistance strains.

Sergent and Donatien (1922) observed long periods during infection of T. evansi in the camel, when the parasites could not be detected in the circulation. Similarly after drug treatment, it is common for trypanosomes to be absent from the peripheral circulation for a time only to be followed by their reappearance. Although this behaviour may be due to drug resistance, sometimes the relapsed population retains the same sensitivity as the earlier infection. Jennings, Whitelaw and Urquhart (1977b) showed, at least in T. brucei infections, that these relapses were not due to drug-resistance, as the relapsed trypanosomes remained as sensitive to the treatment as the parent inoculum. Instead such relapses were due to trypanosomes occurring in sites which were inaccessible to drug penetration. Later Jennings et al (1979) and other workers demonstrated that the brain was

the main cryptic focus of infection.

In the literature the development of drug-resistance is usually attributed to the use of chemoprophylactic drugs. Of the chemoprophylactics, widespread resistance to quinapyramine led to the withdrawal of its usage for bovine trypanosomiasis, such as T. congolense and T. vivax (Whiteside, 1960a; Cockbill, 1967). Wilson (1949), mentioned how readily a single treatment with quinapyramine produced resistance in bovine trypanosomiasis. In contrast, many workers have recorded that resistance to isometamidium does not appear to develop readily when repeated treatments are administered (Folkers, 1966; Lewis and Thomson, 1974). Later, other workers found putative resistance in the field as shown by early breakthroughs, using isometamidium as a prophylactic drug (Kupper and Wolfers, 1983; Pinder and Authie, 1984).

There are limitations to the detection of resistance strains in small laboratory animals since it has been suggested that these animals are often not susceptible to infections with resistant strains (Williamson and Stephen, 1960).

The literature concerning drug-resistance by T. evansi in the field is not adequately available (Leach and Roberts, 1981). However, the disparity in the response to drug treatment reported from various countries may be largely attributed to resistance developed by the parasite as a result of prolonged exposure to the drug, especially where chemotherapy is extensively applied in the control of this disease in domesticated animals (Yutuc, 1940; Abebe, Jones and Boid, 1983).

Granouillet and Do-van-Vien (1938) have registered the failure of suramin (Naganol) treatments, in a spontaneous outbreak of T. evansi in horses and dogs. The authors proposed that the passage of trypanosomes through horses, may enhance their virulence for dogs, cattle, buffaloes and also reduce their drug sensitivity. Mice infected with three stocks of T. evansi isolated from camels required 60mg/kg suramin compared with only 10mg/kg needed to cure infection with normally susceptible stock.

Several years ago Gitaha (1981) described an experiment in which four isolates of T. evansi, isolated from camels in Kenya were tested for drug resistance in mice. All four isolates were resistant to diminazene aceturate at 3.5, 7.0 and 10.5mg/kg; they were also resistant to different concentrations of isometamidium chloride, homidium bromide and homidium chloride.

Strains resistant to suramin and quinapyramine have been shown to be fully susceptible to Berenil (Gill, 1971a, b). It is therefore likely that those isolates which did not respond to three times the standard dose of Berenil are naturally resistant to this drug. The risk of developing drug-resistant parasites is less with Berenil than with other trypanocidal drugs which persist in the body for a relatively long period of time. Indeed some workers were unable to experimentally induce Berenil resistance (Fussganger and Bauer, 1960; Bauer, 1962). Although others were successful (Whiteside, 1963; de Raadt, van Hoeve, Hart and Grainger, 1965).

It is important, however to note that although Berenil is rapidly removed from the circulation, there is some evidence for the existence of deep tissue depots, most notably expounded by

Klatt and Hadju (1976). This may account for the prophylactic activity reported to have been afforded by Berenil against T. brucei (Van Hoeve and Cunningham, 1964). Perhaps the slow development of Berenil resistance observed in the field is accounted for by the drug's tissue depot.

Homidium - resistant T. congolense have been described in many parts of Africa, such as Sudan, Nigeria, Ethiopia and Kenya (Jones-Davies and Folkers, 1966c; Na'Isa, 1967; Folkers, van Hoeve and Buys, 1968; Williamson, 1970; Gadir, Tahir, Razig and Osman, 1972; Scott and Pegram, 1974; Gitaha, 1981). But in contrast, Ilemobade and Buys (1970) did not mention any homidium-resistance in their work. However, Williamson and Stephen (1960) suggested that T. congolense has a higher level of innate resistance to homidium than T. vivax, and T. vivax has a higher level of innate resistance to diminazene aceturate than T. congolense (Williamson, 1960).

Relapse of Infection

When an infected animal is injected with a trypanocidal drug, the treatment initially appears successful in eliminating trypanosome from the peripheral blood, but relapse infections in the blood occur later. According to Jennings and Gray (1983), if an animal with a T. brucei infection is treated with a trypanocidal drug in the field and is subsequently shown to be still infected, one or more of three causes are normally incriminated; reinfection, underdosage of the drug, or drug resistance. The first cause is said to be determined by the time between treatment and re-examination and the intensity of fly

challenge. If, however, the drug fails to clear the parasites from the bloodstream, underdosage or resistance is implicated.

Relapses following treatment usually results from inadequate dosage or waning concentrations of prophylactic drug and may eventually give rise to resistant stocks. Gill and Malhotra (1971) have observed relapse infections of T. evansi following prophylaxis in horses. These infections were characterised by low grade sub-microscopic parasitaemia. However, as the relapse strains showed loss of virulence to laboratory animals, the atypical character of the infections was attributed to the residual effects of the drugs and development of some degree of immunity.

Very recently, a series of experiments were conducted in Kenya by Sones, Njogu and Holmes (1988). The sensitivities of three strains of Trypanosoma congolense to isometamidium chloride (Samorin) were determined in mice and cattle, with the objective of evaluating sensitivity testing in mice as a means of predicting curative doses in cattle. A comparison of effective dose and curative dose for mice with minimum curative dose values for cattle demonstrated a wide variation between strains, with mice requiring from one to more than 100 times the cattle dose. It was concluded that although a mouse test may give a broad indication of whether a strain is sensitive or resistant, it cannot be used accurately to predict curative doses for cattle.

In his work Abebe et al (1983) illustrated that the use of sub-curative doses of suramin to treat T. evansi generates relapse infections. Moreover, Jennings and his colleagues (1977a, b) suggested that the timing of the treatment relative to

the primary infection may be crucial in the development of the relapse infections. However, there are records of both experimental and natural infections of T. brucei which indicate that whenever strong nervous symptoms are evident, the effect of chemotherapy is bound to fail (May Chew, 1968; Ng and Vanslow, 1978).

Care must be taken in all therapeutic drug sensitivity studies to ensure that treatment occurs sufficiently soon after infection to rule out the possibility of relapses occurring from sites inaccessible to drugs (Jennings et al, 1977a, b; 1979). However, the occurrence of the relapse phenomenon in the field is difficult to evaluate since conditions precluding reinfection are difficult, if not possible to achieve.

Stability of Drug Resistance

Trypanosomes become resistant against many drugs but the speed with which the resistance develops and the tenacity with which it is retained varies with different compounds (Von Brand, 1951). In tsetse-borne species, it was shown that drug resistance can be stable and transmissible (Gray and Roberts, 1971a, b). A series of experiments conducted by the latter authors demonstrated that it can persist up to 29 months, in the absence of drug treatment, while being transmitted through tsetse and cattle. The persistence of drug-resistant stocks, even after passage through tsetse fly, has also been shown by Finelle (1973a). On the other hand, Whiteside (1963) has shown that T. congolense resistant to diminazene aceturate appeared to be unstable during repeated bovine passages. In fact Gray and

Roberts (1971b) pointed out that the disappearance of drug-resistant trypanosomes in the field does not result from reduced infectivity for animals or tsetse or from loss of drug-resistance. They suggested that the apparent disappearance may be caused by a reduction in the proportion of drug-resistant trypanosomes in the total trypanosome population in the reservoir hosts to a level where they are not easy to detect. Moreover, Gray and Roberts (1971a) demonstrated the stability of a T. congolense strain resistant to various trypanocidal drugs (diminazene aceturate at 7mg/kg, homidium chloride at 2mg/kg and isometamidium chloride at 0.5mg/kg) and a T. vivax strain resistant to diminazene aceturate at 7mg/kg and quinapyramine at 5mg/kg, after repeated cyclical transmission.

One important factor which may operate against propagation of resistant trypanosomes was their apparent inability to transfer resistance to either parasites of the same or different strain (Amrein, 1957; Amrein and Fulton, 1959; Amrein, 1965). However, the transfer of genetic information between trypanosomes has recently been demonstrated during cyclical passage through tsetse flies and this may permit transfer of resistance (Hawking, 1963).

Whiteside (1960) suggested that, if cattle infected with drug-resistant T. congolense or T. vivax are moved from the original area into another area and the use of the trypanocidal drug in question is stopped, drug-resistant strains will tend to disappear from tsetse in approximately nine months. In his observations, Hawking (1963) suggested that loss of resistance was presumably due to overgrowth by sensitive individuals which

have muliplied more rapidly than the resistant ones.

Chemotherapy and Immunity

A number of field studies have suggested that there may be a relationship between drugs and immunity in cattle exposed to trypanosome challenge. It was Bevan (1928) who apparently first suggested that "tolerance" might be produced in cattle by giving a trypanocidal drug after infection.

He frequently observed that, though trypanosomes were often present, treated animals improved markedly in condition. However, when infected blood was injected into susceptible untreated animals, the parasites were highly pathogenic. This indicated to the author that the host had acquired a degree of tolerance or non-sterile immunity to trypanosomes as a result of drug therapy.

Following these valuable observations a series of experiments were carried out in East Africa in the 1970s. Initially in Uganda, Wilson et al (1975a) studied the performance of a breeding herd introduced and maintained for two years in an area of heavy trypanosome challenge. Chemotherapy using a short-acting curative drug, diminazene aceturate was only given to animals on an individual basis whenever their haematocrit fell below 20% or if they were clinically ill. During the two years of observation period each animal required an average of eight treatments, and became parasitaemic again generally about 35 days after each treatment. Under this regimen the number of live calves born increased and subsequent calf mortality decreased, the incidence of abortion was also reduced.

In an area of medium trypanosome challenge in Kenya, Wilson et al (1975b; 1976), obtained better results using a similar system of chemotherapy and the same drug.

Non-sterile immunity to trypanosomiasis has been successfully exploited in many commercial situations in Africa but it has been rarely monitored and reported. However the remaining parasitaemias may act as a constant source of infection for the vector (Ruchel, 1975).

The mechanisms of non-sterile immunity still requires elucidation; there are several possibilities. It may be that there is a limited number of serodemes in a particular area and extended maintenance of cattle in such areas under a drug umbrella will allow them to develop a degree of immunity to the limited range of variant antigenic types (VATs). Alternatively, repeated use of drugs may permit the build-up of drug residues in cattle which confer protection by restricting parasite replication. The development of immunity in cattle in the field has been assessed indirectly, by observing the changing needs for trypanocidal drug treatment, the ability to maintain normal blood values and the ability to gain weight in face of virulent trypanosome challenge when drug treatment is discontinued. It has also been assessed by the changes in serum immunoglobulin levels and the immunofluorescent antibody test as indices of the immune status on a herd basis (Wilson et al, 1975a; Wilson, 1976).

Experimentally, sterile immunity to homologous T. congolense and T. brucei challenge has been readily conferred by a strategy of infection and cure (Wilson, Cunningham and Harley, 1969; Akol

and Murray, 1983). The resultant and protective antibody response has been shown to last up to seven months following treatment (Wilson and Cunningham, 1972).

Tsetse control

Successful control of tsetse flies only became possible when their unique life history and habitat requirements were properly understood (Allsop, 1984). Tsetse control can be either non-chemical control which consists of game destruction, bush clearing or chemical control which consists of using insecticides by means of either ground spraying or aerial spraying or modern targets impregnated with insecticide and chemical attractants.

Non-chemical control

(a) Game destruction. The destruction of game is one of the processes of eliminating both the tsetse flies and the reservoir of infection. This view gained wide acceptance in the wake of the great Rinderpest epidemic of 1889-90 and 1896. Many game species disappeared at that period in north eastern Africa. Echoing Livingstone's words, Bruce, Harvey, Hamerton, Davey and Lady (1913) stated "it is self evident that wild animals should not be allowed to live in tsetse fly country, where they constitute a standing danger to the inhabitants and domestic animals".

This view proved very influential and the first experiment to rely largely on elimination of game animals was started in Zimbabwe, a similar scenario was enacted in Uganda, South Africa, Botswana, Mozambique, Tanzania, Zambia, as well as in small campaigns in other countries (Chorley, 1958; Jahnke, 1976; Dutoit, 1954; Davies, 1980b; Andrade Silva and Marques da Silva,

1960; Banage, 1979). The destruction of these animals fortunately did not encourage further slaughter of wild animals as it soon became evident that these measures were not effective. Many setbacks occurred, mainly because of game migration from outside the control areas and failure to eradicate the smaller animals which live in dense vegetation (Lovemore, 1961).

In Somalia after 1975 many species of wild animals such as elephant, giraffe, leopard and black rhinos were extensively hunted by the poachers, yet their destruction did not produce any significant effects on tsetse infestation of the riverine areas.

(b) Bush clearing. The clearance of vegetation and the use of the cleared land for pastoral or agricultural development, thus creating an adverse environment for tsetse, are long practised successful techniques. In theory the occupation of virgin territory then takes place in a controlled and optimum fashion (Jahnke, 1976; Jordan, 1979).

In Africa, the countries which carried out major clearing programmes included Uganda, Tanzania, Nigeria, Botswana and Ghana, and most African countries have attempted the technique on a more limited scale. The advantages obtained from bush clearing are difficult to quantify because the proportions of total clearing was not recorded in many cases. Bush clearing systems are not as popular as other tsetse control systems, and environmentalists have discouraged the destruction of the vegetation in zones which cannot be immediately occupied after the clearance.

Chemical control using insecticides

Game destruction and bush clearing have now been largely superseded by the use of insecticides for tsetse control (Jordan, 1974; Mulligan, 1970; Allsopp, 1984), primarily for reasons of cost effectiveness. Only three compounds have been used in large scale field programmes dichlorodiphenyl-trichloroethane (DDT), dieldrin, and endosulfan.

The persistant organochlorines, DDT became available during World War II. The first large scale use of the compounds was in Zululand by Dutoit (1954) and the use of DDT spread rapidly to all African countries. A single administration of DDT is used since the deposits can remain lethal for tsetse up to one year after application (Baldry, 1963). However, Dutoit (1954) recorded major kills of several non-target insect groups following the DDT spraying in Zululand.

Dieldrin is also a residual insecticide. Trials have shown dieldrin is better than DDT in tsetse eradication, but has many environmental side effects (Burnette, Robinson and Le Roux, 1957), particularly in places with heavy rainfall.

Endosulfan, at this moment, appears to have operational and environmental advantages over DDT and dieldrin because of its short term persistence and its good solubility in the spray solvents which are suited for aerial spraying (Hocking, Lee, Beesley and Matechi, 1966).

Detailed studies conducted by many workers (Douthwaite, Fox, Mathiessen and Russell-Smith, 1981; Muller, Nagel and Flack, 1981; Koeman, Rijksen, Smies, Na'Isa and MacLennan, 1971; Koeman, Den Boer, Feith, de Longh, Spliethoff, Na'Isa and Spielberger,

1978) have recorded the severe side effects and environmental pollution which the use of insecticides can bring. Of the above mention three insecticides endosulfan is recorded to have fewer general adverse ecological impacts (Gray, 1983). In Somalia it has been found that honey bees and little bee-eaters were at risk when dose rates exceed 18g/ha (Douthwaite, 1985).

However, the development of safer and environmentally acceptable techniques are urgently required to solve the problem.

Generally tsetse control departments do not consider the land use implications of their work, but the National Tsetse and Trypanosomiasis Control Project of Somalia is an exception, considering some of the wider issues of tsetse eradication through an Environment Section. It remains to be seen whether the timing of eradication in Somalia will be influenced by this section's findings.

Traps and Targets

Different types of traps have been designed to attract and catch tsetse flies. Currently, new traps and targets are being tested in order to improve their power of attracting and catching tsetse flies.

Many workers have recorded that the efficiency of traps could be greatly improved if a source of natural host odour (Vale, 1974; Hargrove and Vale, 1979) or a mixture of carbon dioxide and acetone (simulated host odour) (Vale, 1980) was associated with trap. Perhaps, these investigations may bring an easy way of controlling tsetse in the future.

The results obtained from the host odour attractants and target development studies carried out in Zimbabwe (Lovemore, 1986) had led to the creation of the odour-baited, insecticide treated target, in the context of tsetse eradication. It was observed that this system of odour baited, insecticide treated, target would provide a means of dealing with those seemingly intractable tsetse 'pockets' which remain from time to time after progressive operations through broken terrain. The odour-baited, insecticide-treated, targets have also been used as a means of setting up invasion barriers, using a single line of baits, spaced 100 m apart from each other and in an attempt to eliminate or at least reduce re-invasion of land recently cleared of tsetse.

Very recently this technique of using odour-attractants and targets has been used in a pilot project by Overseas Development Administration in the pockets outside the original tsetse habitat, of Middle Shabelle Region of Somalia. It was also visualised that the system may be able to identify new infested areas where the ordinary conical challier traps do not attract the tsetse.

Diagnosis of Trypanosome infection

The diagnosis of the animal trypanosomiasis is not easy. Because the signs of trypanosomiasis cover a very wide spectrum, and are often difficult to differentiate from other diseases (e.g. tick borne diseases), which have the same clinical signs such as anaemia and swelling of lymph nodes. In susceptible livestock, infection with a virulent strain of trypanosome may lead to acute disease with such signs as abortion or a

haemorrhagic syndrome.

In contrast, in animals relatively tolerant to trypanosome infections, there may be no overt signs of ill health. Furthermore, the ability of trypanosomes to vary their antigenic surface coat (VATs) is associated with intermittent presence of parasites in the blood stream. In addition, low parasitaemias are characteristic of chronic infections, and since positive diagnosis relies upon detection and identification of the parasites in the peripheral blood or lymph, many techniques such as direct microscopy, using fresh blood and thin or thick films give usually a proportion of false negative results.

Some species of the mammalian trypanosomiasis, e.g. Trypanosoma (Trypanozoon) brucei brucei can invade the cerebrospinal fluid of domestic and wild animals (McCulloch, 1967; Hornby, 1952) although examination of this fluid is seldom, if ever, used as a diagnostic aid in animals, partly no doubt due to the technical difficulties involved.

The urine of camels affected by Trypanosoma evansi, has a characteristic pungent odour, the cause of which is unknown (Hunter, 1986) and this odour is one of the methods by which camel herders of Somalia and Sudan are able to separate affected animals from the healthy ones. Furthermore, the cattle owners along the two rivers (Jubba and Shabelle) of Somalia are able to distinguish healthy cattle from the infected animals by the smell of the skin of the infected animals which has a characteristic strong putrid odour. They also claim to be able to recognise chronic infections, in spite of the emaciated clinical condition,

by the cliphair at the tip of the tail of the infected animal.

However, standard techniques for field use in the diagnosis of animal trypanosomiasis include, microscopic examination of fresh, wet blood films, dry thick and thin blood films and a wet preparation of the buffy coat of centrifuged blood microhaematocrit technique (MHCT). Less frequently, the inoculation of blood from a suspected case of trypanosomiasis, into laboratory rodents (biological test) is practiced. This latter test will only distinguish T. brucei, a proportion of T. congolense, but not T. vivax infections. However Leeflang, Buys and Blotkamp (1976) reported that T. vivax infections during the early first parasitaemia phase could infect mice; as there is almost invariably a high parasitaemia at this stage the need to use this test for diagnosis is rarely necessary.

Blood films

Blood can be examined for the presence of trypanosomes in wet (whole blood or buffy coat) or after staining with Giemsa stain.

Wet film or whole blood are mainly used when large numbers of animals are being examined daily, in particular laboratory animals in which high levels of parasitaemia are to be expected.

Wet films are prepared by placing a drop of blood on a slide, and covering it with a coverslip. The blood is then examined with a microscope for the presence of trypanosome. A total magnification of about x 400 is adequate as the movement of the trypanosome can be detected by the disturbance produced among the red blood cells, especially if examination is carried out under phase-contrast or dark-field illumination (Murray, Murray

and McIntyre, 1977). The parasitaemia is expressed as trypanosomes per field, log equivalent value (Walker, 1968) or trypanosome/ml by matching and limited counting (Herbert and Lumsden, 1976).

Fixed films of thick and thin blood smears are usually stained with Giemsa's stain, although other formulations such as Romanovsky - type stain, Fields' and Leishman's stain can be used.

Two types of stained blood films are used in trypanosome diagnosis, thick and thin films.

Thick blood films

Of the blood film techniques, thick blood films are the most efficient for detecting trypanosomes, particularly for the detection of scanty trypanosomes. A small volume of the blood is spread over a small circular area of a slide and left to dry completely. The film is then either placed directly into water or into an aqueous stain such as Giemsa's in order to stain any parasites present in the film and to bleach out the haemoglobulin in the host red cells - dehaemoglobinisation. Failure to remove the haemoglobin will result in a thick layer of stained host cells and the subsequent masking of any parasites present in the preparation. The lack of methanol fixation of thick films, however, results in poor preservation of the morphological features of the parasite making species identification difficult in such preparations. A staining modification introduced by MacLennan (1957) involving an initial one second staining in 0.5% aqueous methylene blue can improve the preservation of

trypanosome morphology in thick films.

Thin blood films allow better preservation of the morphology of any trypanosomes present and hence are useful in morphological differentiation of species. Thin blood films should consist of a single layer of cells and are fixed in methanol before staining. The fixation stage results in the preservations of the morphological characteristics of the parasite. Thin film preparations are eminently suitable for species identification. Details of trypanosome morphology are usually studied using x 100 oil-immersion objective. Species identification is based on difference in overall size, the presence of a free flagellum and the shape of the posterior end of the organisms and position of the kinetoplast. Other features such as the size and position of the kinetoplast and the distribution of cytoplasmic organelles are also useful.

In large-scale disease surveys it is more convenient to prepare thin and thick films from an animal on the same slide ensuring that the thin film is fixed in methanol. The thick film should be examined first for evidence of trypanosome infection and then the thin film can be used for species identification.

Microhaematocrit centrifugation (MHCT)

This method is designed to increase the probability of detecting trypanosomes by using larger volumes of blood than can be used in blood film examination and by concentrating any parasites present into an easily examined volume. MHCT usually involve centrifugation and the separation of the trypanosome from host cells in the samples.

It is important that blood samples are centrifuged for sufficient time to ensure the complete packing of host red cells e.g. goats blood with relatively small erythrocytes, needs to be spun for eight minutes while bovine blood requires only four minutes.

In the case of T. congolense, which is a relatively small species, blood samples may first be mixed with a diluent containing magnesium ions and glycerol to increase the specific gravity of the erythrocytes (Walker, 1972) and this improves separation. This method is also known as the capillary concentration technique.

The microhaematocrit technique can detect trypanosomes in the blood of experimentally infected animals 6-10 days earlier than by wet or thick blood film techniques. Ruckmana (1972) found the technique useful for the detection of trypanosomes in cattle and Robson and Ashkar (1972), Geigy, Mwambu and Kauffmann (1971) used the test as an aid to diagnosis of trypanosomiasis in game animals.

Recently, small, handheld, battery powered microhaematocrit centrifuges, which are useful for field use were introduced, but these tend to have a lower sample capacity in terms of capillary volume and number than conventional centrifuges. At the same time, the packed cell volume (PCV) of the sample can also be obtained which indicates the severity of the infection.

Darkground - DG

The use of darkground/phase contrast microscopy to examine the buffy coat, has been found to be sensitive in detecting trypanosome species and also in identification (Paris, Murray and

Mcodimba, 1982). The technique has been recommended by many authors including Murray et al (1977), Murray, Murray, Murray, Morrison, Pyne and McIntyre (1979) and Dillmann and Townsend (1979) who emphasised the superiority of the technique compared to other diagnostic methods, such as HCT, thick and wet films. In addition, the workers have pointed out the advantage of the method over the HCT in the detecting of T. congolense organisms because of their sluggish movement. Furthermore, darkground test overcame many constraints encountered in other diagnostic techniques by allowing identification of trypanosome species even at very low concentration (Paris et al, 1982). Each species is recognised by its size and the way in which it moved, as described by Murray et al (1977). In mixed infections, identification is more easily accomplished by the darkground. Another advantage offered by the darkground and the HCT is that a packed red cell volume (PCV) can be read prior to carrying out the parasitological examination.

Silicone Centrifugation technique:-

The silicone centrifugation technique is based on the density differences between the hosts erythrocytes and the parasites. Under the conditions used, the red cells are pelleted by centrifugation through a layer of silicone fluid (density 1.075) but the trypanosomes will not pass through this layer. Ogbunude and Magaji (1982) claim the technique gave results comparable to those obtained by the MHCT and by the miniature anion exchange chromatography technique.

Mini-anion exchange chromatography technique:-

This technique separates trypanosomes from host red cells by passing infected blood through a column of diethylamino-ethyl cellulose (DEAE). The trypanosomes pass through the column which retains the host rbc (Lanham and Godfrey, 1970). In a modification of Lanham and Godfrey's original technique, the eluate is collected in a sealed pasteur pipette which is centrifuged and the sealed end examined for trypanosomes under the microscope (Lumsden, Kimber and Strange, 1977).

Biological test (animal inoculation):-

This technique is less frequently used due to the long prepatent periods in commonly used laboratory animals and the expense. Furthermore, the technique has been limited to the use of laboratory rodents which although useful for brucei-subgroup and congolense trypanosomes usually fail to demonstrate the presence of Trypanosoma vivax.

Serological tests for diagnosis:-

Many well established tests such as the mercuric chloride test, the formol-gel test and the thymol turbidity test which rely on increases in the levels of euglobulins (gamma globulins) in infected animals are used to detect trypanosomiasis (particularly T. evansi) but these tests are not specific for trypanosomiasis and are insufficiently reliable for accurate diagnosis (Pegram and Scott, 1976).

Recently great improvement in serological techniques has been the use of labelled reagents such as the immunofluorescent antibody test (IFAT), the enzyme linked immunosorbent assay (ELISA) the capillary agglutination test (CAT) and the passive

haemagglutination test (PHT). None of these tests however, differentiates between past and present infection owing to the persistence of antibodies for up to 100 days or more after chemotherapy has cleared trypanosomes from the blood stream (Luckins, Gray and Rae, 1978). Later, a variation of the micro ELISA has been developed which enables serum to be tested for the presence of trypanosomal antigen (Rae and Luckins, 1984). This test indicates when an animal is presently infected with trypanosomes, whereas the presence of antibody indicates either current infection or infections within the previous several months. However, more accurate and specific diagnosis with highly sensitive techniques are needed urgently.

Recent developments in molecular biology offer new opportunities for more sophisticated diagnostic techniques in the future. In particular DNA probes may be used for trypanosome species identification, by using single cloned repetitive sequences. Such sequences have often been isolated from species-specific DNA bands identified after agarose gel electrophoresis of endonuclease digested genomic DNA, or from differential hybridization of radiolabelled genomic DNA from several species to replicate cloned genomic libraries. These techniques have considerable potential both to the understanding of trypanosome epidemiology and disease detection.

CHAPTER TWO

A SURVEY OF BOVINE TRYPANOSOMIASIS IN SOUTHERN SOMALIA

INTRODUCTION

A cattle trypanosomiasis survey was carried out between July, 1984 - July, 1985. The survey covered the southern half of Somalia which lies along the Shabelle and Jubba rivers. The six regions that were surveyed have an area 262,860 km^2 and comprises 41 per cent of Somalia.

According to the census of 1986 these regions are characterised by having 60 per cent of the human population, 50 per cent of the camels, 77 per cent of the cattle, 15 per cent of the sheep and 23 per cent of the goats, and make up the most economically complex part of the country.

One of the main obstacles to the development of the livestock of the regions that were surveyed is the widespread infestations of the main rivers and some of the potentially most productive ranges of the country with the tsetse fly which transmits the casual agent of trypanosomiasis.

High temperatures combined with dryness and lack of cover make conditions in most parts of Somalia unsuitable for the tsetse fly (Glossina spp) but it has been known for about 100 years that infestation is present in the riverine areas (personal communication, Abdullahi).

Generally the tsetse infestation occurs in three distinct areas, namely the Shabelle riverine area, the Jubba riverine area, and a salient extending from the Kenyan border into the drainage lines which flow into the Indian Ocean. It is also acknowledged that infestation extends outwards from these permanent foci as humidity and vegetation provide favourable habitats in the rainy seasons.

Makin and Rose Innes (1983) have described the ecological conditions and tsetse distribution of the surveyed area. Four species of tsetse fly are recognised in Somalia (see map 1) and their occurrence and characteristics are as follows:-

A. Glossina pallidipes -

The most widespread species, mostly feeds on wild animals (bush pig, bush buck, warthog and buffalo) often causing a high level of trypanosome infection. It also feeds on domestic animals and occurs on both the Jubba and Shabelle rivers.

B. Glossina longipennis -

Occurs in the Shabelle valley intermittently between Awdhiigle and Afgooye, and extends up the river to Mahaday. This species inhabits rather dry country and takes about one-fifth of its meals from bovids and buffaloes, it also feeds on rhinoceros and elephants.

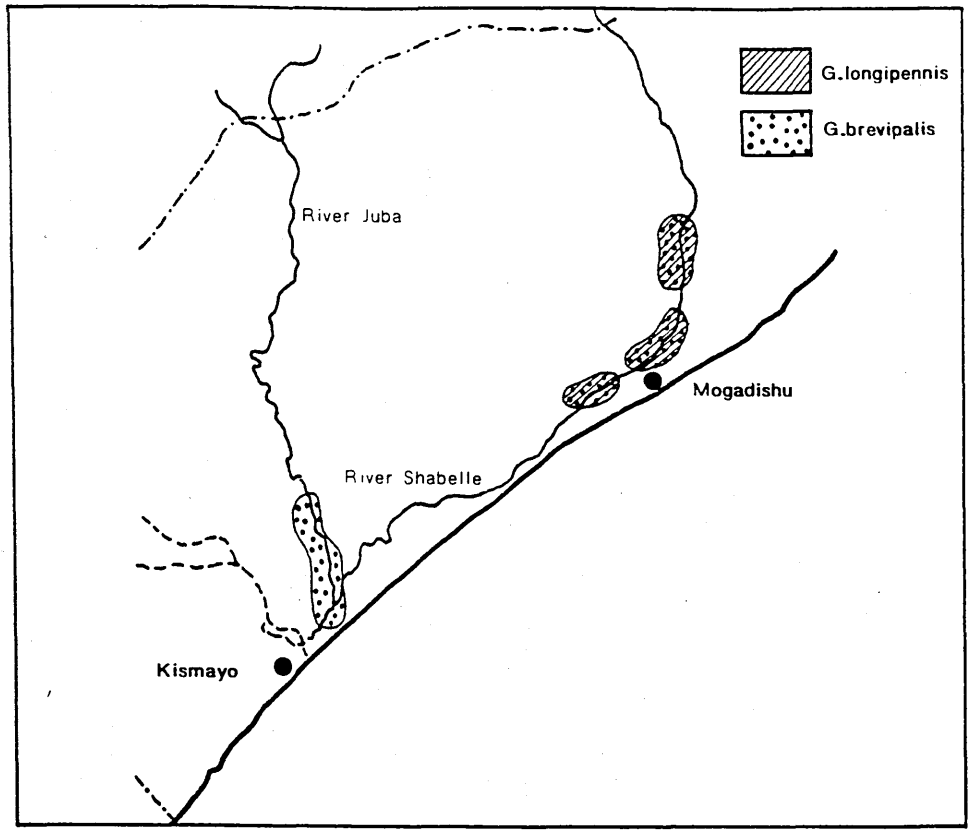
C. Glossina brevipalpis -

Occurs on the Shabelle river within the G. longipennis distribution, and on the riverine island of Alessandra in Gilib district. This species feeds mostly on bovids and bush pigs.

D. Glossina austeni -

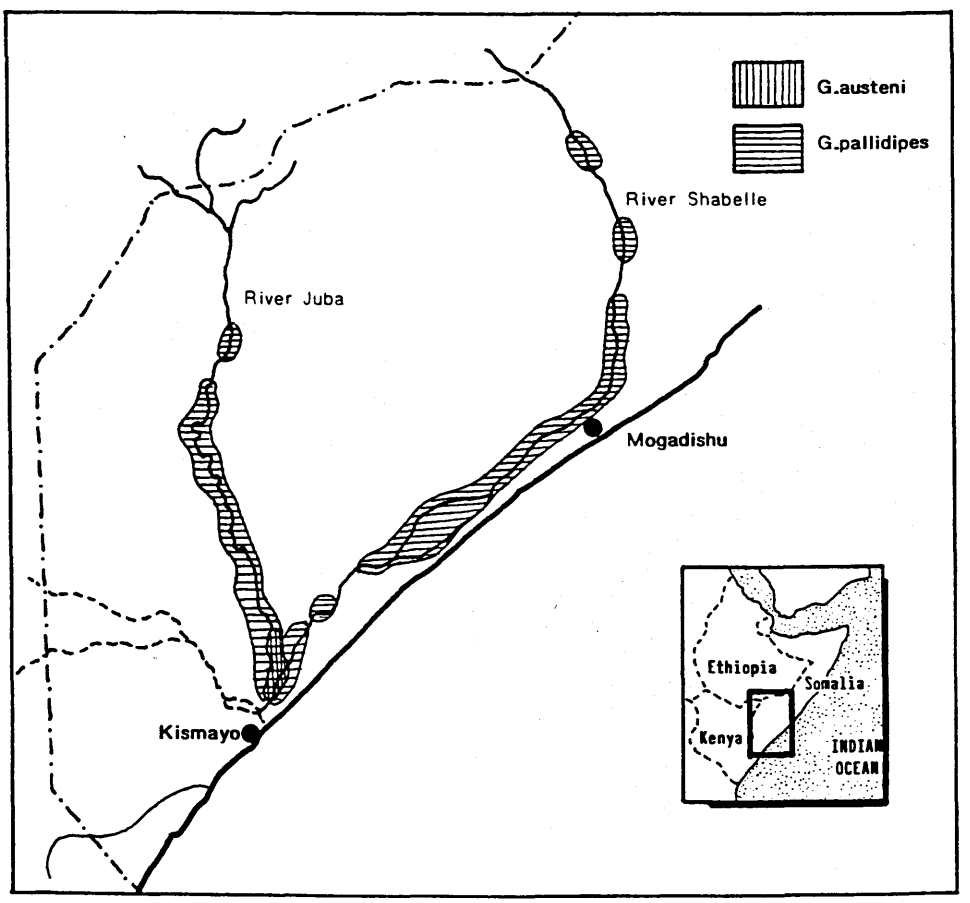
Is found in the valley of the Jubba river and within the Mahaday area of the Shabelle river. This species lives in humid evergeen thickets and feeds mostly on bovids and bush pigs.

The infestation of tsetse is mostly riverine, but varies in width according to the winding paths of the river's drainage and the presence of irrigation canals. The regeneration of Acacis arabic along the canals also encourages the infestation by



Map 1 **The distribution of Tsetse flies** (Glossina) **in Somalia**

(OAU/STRC. 1973)



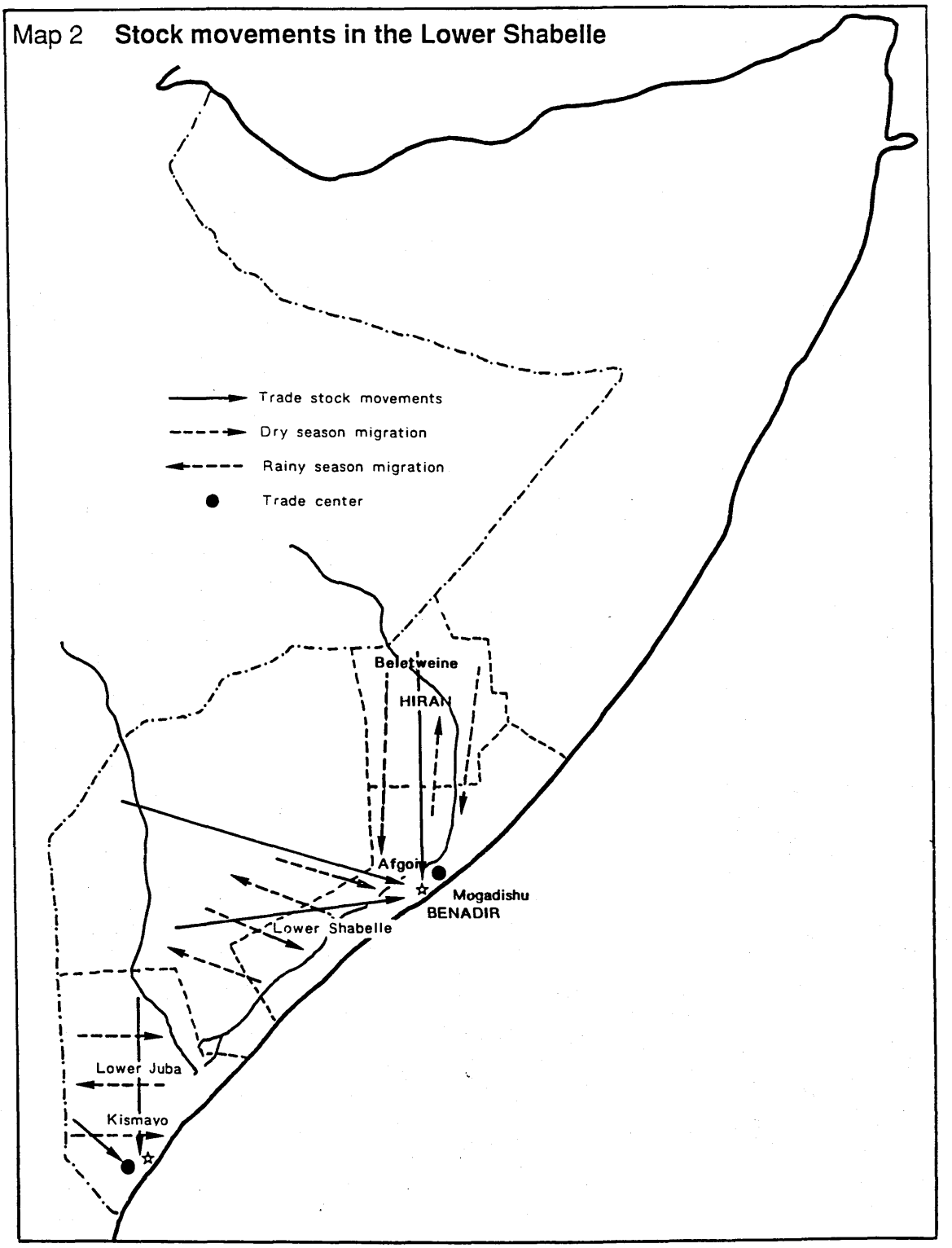
providing breeding and resting sites for the tsetse fly.

However, tsetse eradication in Somalia is feasible because of the nature of the infestation. The tsetse area of the Jubba and Shabelle regions is perhaps the only example in the whole of Africa of an isolated or near isolated tsetse population. The feasibility of eradication has encouraged the adoption of campaigns to rid Somalia of tsetse fly. The first trial was carried out using the ground-spraying technique by the application of residual insecticides, (Dielderin 15) by knapsack sprayers at Golweyn in 1976 and the spraying of non-persistent insecticide from fixed-wing aircraft in 1983 - 1984 in the middle and one part of lower Shabelle regions. Recently in 1988, a final trial of the formerly sprayed areas was initiated with the intention of total eradication of the tsetse infestation from that area.

Somali pastoralism is characterised by multi-species herds, seasonal migratory movements and split level management strategies. However most of the cattle that were sampled in the survey were nomadic.

In the dry season when there is a shortage of surface water and when grazing is in short supply and at its lowest nutritive value, cattle become dependant on the permanent rivers rather than water holes for water and so have to be moved into tsetse-infested areas along the rivers and are thereby exposed to the risk of trypanosome infection (see map 2). The cattle owners attempt to control the incidence and effects of trypanosomiasis by chemotherapy and chemoprophylactic regimes and by adopting midday grazing and watering at night.

Map 2 **Stock movements in the Lower Shabelle**



It is extremely difficult to assess the direct losses due to trypanosomiasis and the affects of these losses on the national economy due to the largely nomadic nature of livestock husbandry, the severity of periodic droughts and the presence of the intercurrent diseases. In UNDP Reports (1976) it was estimated that mortality amounted to 12 per cent of the cattle south of the $4^{o}N$ latitude.

In the present survey cattle were examined and blood sampled, and their owners were interviewed. The collected data was entered on question sheets (Table 3). The survey was designed to examine equal numbers of animals within the tsetse infested areas and outside such areas but close enough to them to be ecologically comparable.

It was against this background that investigations into the incidence of trypanosomiasis and the relative occurrence of trypanosome species in cattle were carried out in these regions. It was hoped that improved knowledge of the epidemiological pattern could form part of the basis for a possible new approach to drug usage in the control of bovine trypanosomiasis.

MATERIALS AND METHODS

Examination of Cattle

Clinical History

On the question paper the date on which animals were blood sampled, herd serial number, owner's name, animal's name and the distinguishing marks of the blood sampled animals were recorded. Also, the districts, villages and the residence or location were recorded.

Table 3

NTTCP - VETERINARY CLINICAL RECORD CLIN

Animal name _____ Herd / Animal Serial No. _____/_____

Distinguishing marks _____

Species _____ Breed _____ Age (owner's opinion) _____ yrs _____ months

Sex (M or F) _____ If female, either, not calved* ____ or, how many months -

since calved _____ or aborted _____ (include stillbirth)

now suckling* ____ lactating* ____ or dry* ____

Selection - by owner* ____ by us* ____ other* ____

Girths- chest _____ m hump _____ m

Shoulder height _____ m

Condition score _____

Incisors (no.) ____ T/____ P Corner wear score 0 - 3 _____ Hence age _____ yrs ____ months

What diseases has the animal had in the last 6 months - include the present:

Disease	Began months ago	Duration weeks	Treated with	How many times	Last time-Cost She	weeks ago	Effect* (1/T/O)

Present clinical symptoms, if any - score existence and severity 0 - 3:

Swollen nodes _____ Pallor _____ Injection scars each side of neck _____/_____

Describe _____

SAMPLES TAKEN

Ear/vein blood

___/___ Thick smear* ____ +ve* ____ Wet film - B* ____ +ve* ____ Score _____

___/___ Thin film - B* ____ BC* ____ +ve* ____ - BC* ____ +ve* ____ Score _____

___/___ Paper spot* ____ Parasites seen _____ _____

___/___ Mini* ____ or Micro* ____ -haematocrit - +ve* ____ PCV _____ %

____ for serum* ____ Decanted* ____ frozen ref: _____

Faeces* ____ Two chamber egg counts: Strong-yles _____ -yloides _____ Trichuris _____

Moniezia _____ Coccidia _____ Sedimentation:* _____

Tick count* ____ Nos. on ill-body: Nostril _____ Ear _____ Underneath _____

Anus _____ Tail _____ Feet _____ Other _____ Tick collection* ____

* - Write '1' for 'yes', positive, or 'sample taken'; 'O' for 'no', 'none' or negative; 'x' for unknown, not answered, not asked or not done; or enter information, numbers or scores, as underlined. M,F,T & P mean male, female, temporary and permanent. For 'calved' read 'given birth'.

Notes:

NTTCP - LIVESTOCK DISEASE SURVEY - HERD SUMMARY

Date _____ Enumerator _____ Herd Serial No._____

District _____ Owner's name _____

Village _____ Location _____

	CAMELS	CATTLE	SHEEP & GOATS
Approx. No. of animals present - Claimed/Seen	____/____	____/____	____/____
How many of this owner's stock are not present	____	____	____
Herd type: a)Nomadic/Semi-/Sedentary+	___/___/___	___/___/___	___/___/___
b)Home/Satellite/Complete+	___/___/___	___/___/___	___/___/___

What disease problems were seen in the last 6 months: Give the No. of animals -	CAMELS Now affected	Recovered	Dead	CATTLE Now affected	Recovered	Dead	SHEEP & GOATS Now affected	Recovered	Dead
1.CBPP / CCPP	___	___	___	___	___	___	___	___	___
2.Haemorrhagic Septicaemia	___	___	___	___	___	___	___	___	___
3.Trypanosomiasis	___	___	___*	___	___	___*	___	___	___*
4._____	___	___	___	___	___	___	___	___	___
5._____	___	___	___	___	___	___	___	___	___
6._____	___	___	___	___	___	___	___	___	___
7._____	___	___	___	___	___	___	___	___	___

How many abortions or stillbirths - Camels ____ Cattle ____ Shoats ____

* Tryps dead - no. slaughtered/sold " ____ " ____ " ____

The following movements involved - Camels+ ___ Cattle+ ___ Shoats+ ___

Movements - In what areas has this herd/flock resided in the last 12 months:

Months in area (*1)	Nearest village and/or description of locality (*2)	Tsetse ? (*3) Graze	Water	FROM MAPS: Grid Ref	kms	Fly pop.
1.___	_____	____	____	_____	___	___
2.___	_____	____	____	_____	___	___
3.___	_____	____	____	_____	___	___
4.___	_____	____	____	_____	___	___
5._____	_____	____	____	_____	___	___

(*1) No. of calendar months, area no. 1 is the present area. (*2) Show if one area is 'home'. (*3) Score owner's opinion of challenge 0 to 2.

Reasons for the above movements: Score the most important 2, others 1

	- Move to site: 1.	2.	3.	4.
1. To find grazing or fodder, or just to go home				
2. To find water	___	___	___	___
3. To avoid tsetse flies	___	___	___	___
4. _____	___	___	___	___

If camels have been sampled, how many days ago did they last drink? _____

Table 3 (Cont'd)

DRUGS - ACCESSIBILITY AND USAGE (refers to trypanocides unless stated)

1. What drugs have you bought or used in the last 6 months ? _____ _____ _____ _____ _____ _____

2. (a) Where do you go first to get drugs ? A. Nearest vet office B. Other M.L.F.R. C. Vet assistant visits D. Elsewhere

(b) Are you able to get the drugs you want from A/B/C/D - Always Usually Sometimes ?

3. (a) How many kilometers to the nearest veterinary office ? _____ km (from where you spent most of the past month)

(b) If not first choice in question 2, do you ever get drugs from the nearest veterinary office or local assistant ? Yes / No

4. Your last drug purchase was -
(a) how many weeks ago _____, (b) from where (A/B/C/D) ___,
(c) included which brands _____ _____ _____ _____ _____,
(d) in what amounts (g/ml/ds) _____ _____ _____ _____ _____,
(e) at what prices (So Shs) _____ _____ _____ _____ _____,
and (f) how much is left (g/ml/ds) _____ _____ _____ _____ _____?
(g) Was all/how much of (d) minus (f) used on present: Camels/Cattle _____ _____ _____ _____ _____,
Sheep & Goats _____ _____ _____ _____ _____?
(h) If a container is seen: Batch no. _____ _____ _____ _____ _____.
(i) If response (c) includes Samorin, did you take it in the form of bulk powder or single doses?

5. (a) Do you ever treat routinely, whether animals are sick or not ?

	Camels/Cattle		Sheep & Goats	
	Some	All	Some	All
(b) If so, how many times per year ?	____	____	____	____

6. In the average month in this area how many animals do you treat because they are sick ? (Camels (Cattle ____ (Sheep (Goats ____

7. (a) In the last 6 months how many animals died

	Camels/Cattle		Shoats
i) because a suitable drug was not available,	____		____
or ii) because correct treatment did not work?	____	____	____
(b) Which drug or drugs, if any, did not work?	____	____	____

8. Of the trypanocidal drugs you have used in the last 6 months (see question 1):

	Ant	Nag	Ber	Sam	Nov/Eth	
(a) Which work the best in: Camels/Cattle+	___	___	___	___	___	___,
Sheep & Goats+	___	___	___	___	___	___?
(b) What about the others, are they OK ? +	___	___	___	___	___	___
(c) Do you usually give - - - i) more+	___	___	___	___	___	___
than the standard dose, or ii) less+	___	___	___	___	___	___?

9. If you have used Samorin do you know how to make up standard doses from bulk powder ? Yes / No

+ - Mark '1' for yes, '0' for no, or not applicable, 'X' if a question is not asked or not answered. Circle the alternatives.

Assess reliability of responses: Score '0' to '2' for pages 1/2- ___/___

Sex: The sex of the animal (male or female) was noted. Normally the animal owners sell the males at the age of three years or more in the markets of the large cities. If the males in their herds were many in number, the owners usually castrate most for fattening. In each herd the owners usually leave uncastrated the best male for breeding purposes. For females, the general points recorded on the question papers during the interview were calving and lactations, such as whether the blood sampled animal was in lactation and suckled by the calf, or whether the animal was dry. Also the number of months in which the animal was in lactation were recorded.

Breed: The breed of every blood sampled animal was recorded on the question paper. Surgo, Dawara and Boran were the only three breeds that were met during the survey.

Surgo breed is predominently found in the area between Garyooley and Jilib districts. These animals are known for their long sharp-pointed horns. The coat colour is a mixture of white and red spots, compared with other breeds. Surgo is known for its low milk production. Many local people believe that Surgo is the most trypanotolerant breed although this has never been adequately investigated.

Dawara is found in the Hirraan and Middle Shabelle regions. This breed is short-horned with a dark red coloured coat. The animals are usually compact, and compared with other breeds, Dawara is a good milk and meat producer. One of the characteristics of most of the cows of this breed is the milk let-down in the absence of the calf, i.e. if the cow aborted in its late pregnancy or the calf died early. Because of its

production level the breed has started to spread slowly into Lower Shabelle and some parts of the Jubba area replacing the Surgo breed.

The Boran breed of cattle is located nearer the border with Kenya or Ethiopia. The breed is known to be intermediate in production level. The main owners of this breed in Kenya are the Boran tribe and the breed was named after this tribe. The colour of the hair of these animals is mostly white. Their horns are strong and medium-sized. In addition, these animals are less compact than Dawara.

Condition scoring: The condition of each blood sampled animal was recorded on the question paper. The method employed for measuring the condition of the animals was a modified Pullan's (1978) method. This depends on whether the ribs of the animals were generally visible (excluding when the animal drank water) and the profile of the hind leg behind the stifle. The condition scores were classified as follows:-

- when the animal was pathologically emaciated it scored 0
- when the animal's profile of the hind leg was concave, it scored 1
- when the animal's profile of the hind leg was approximately straight it scored 2
- if the profile of the animal's hind leg was convex it scored 3
- if the dorsum lateral to the spinous processes was not concave and the lumbar spinous processes were easily palpable, it scored 4.

- if the spinous processes were palpable with pressure it scored 5
- if the spinous processes were not palpable with pressure it scored 6

Age: The age of the animal was also recorded on the question paper. In Somalia the animal owners usually estimate the age of their animals in such a way that the animal is two years old as soon as the rainy season of the second year begins. The opinions of the owners were recorded on the question paper. In addition, the types and the numbers of the visible incisor teeth were recorded for each blood sampled animal. If the examined animal had eight permanent incisors the degree of wear of the corner teeth was scored as follows:-

- if the incisors had no visible flattening or wear of the upper surface it scored 0
- if the area of the worn, upper surface, was long and narrow it scored 1
- if the area of the worn, upper surface, was elongated oval it scored 2
- if the area of the worn, upper surface, was approximately round or the corner teeth were missing, the animal was obviously old and scored 3

Diseases: During the survey the interviewed cattle owners were asked to describe the diseases which affected their animals for the previous six months (the months before the day each herd was blood sampled). The diseases which they enumerated were, trypanosomiasis, which according to the opinion of the interviewed people appeared the most important disease. Lumpy

skin disease, an outbreak which affected almost all the cattle in the Middle and Lower Shabelle regions during the survey. Most of the owners claimed that they had not seen the disease before. Abortion was also common, especially in heavily tsetse-infested areas.

Among other diseases which the stock owners enumerated were haemorrhagic septicaemia, contagious bovine pleuropneumonia, anthrax, blackquarter ephemeral fever and other diseases which the animal owners called "unknown diseases". These diseases were sporadic, and they usually occurred at regional or village level. The district veterinary officers also mentioned that large gatherings of animals in some areas were often associated with outbreaks of these diseases as occurred for example during the dry seasons when animals gather at grazing areas in the vicinity of the two rivers. The animal owners were asked further questions in order to provide baseline data for the estimation of the effects of these diseases. In each herd, the owner stated the number of animals affected by each disease. The responses to specific questions on the numbers of animals affected and recovered, affected and still sick, or affected and died, were also recorded.

Movements: Normally nomadic herds experienced various movements per year, while semi-nomadic herds with two resident sites experienced one move between the two sites. The owners of nomadic and semi-nomadic herds were asked the areas in which their animals had resided in the last twelve months. Furthermore, the owners were asked the number of months in which

their animals reside at each site. The owners opinion of the risks of tsetse fly at the sites at which their animals grazed or drank water were recorded. The main reasons for the movements of the herds were, to find grazing, water, or to avoid tsetse flies. However some herd owners have mentioned other minor reasons, therefore the most important reasons were scored 2, others were scored 1.

Clinical examination: At the clinical examination, wet-films, thin films and thick films, prepared from blood samples collected from the jugular veins of the individual animals were examined microscopically in order to detect the presence of trypanosomes and to differentiate between trypanosome species. Scores were given to the positive wet films. The results obtained from each diagnostic technique was recorded on the question paper. In addition, the PCV of the blood sampled animals was measured and recorded after centrifuging with either a mini-centrifuge (Compur electronic) or microhaematocrit centrifuge (Hawksley).

Clean evacuated tubes were used to collect blood samples. The blood was allowed to clot and serum was drawn off by pasteur pipette into clean tubes. Thereafter, the serum was frozen for serological testing and recorded on the question papers. Similarly, for later serological testing, drops of blood were spotted on to filter papers (Whatman) and were recorded on the question papers before stored. (Serological tests were not conducted by the author and the results are therefore not included in this thesis).

All other blood parasites observed in wet-films were also recorded. The most common one was microfilariae. Faecal samples

of the animals were examined by a simplified "McMaster" technique and the eggs of various species of parasites, such as Strongloides, Trichuris, Ascaris, Moniezia, Coccidia, Paraphistomum cervi and other trematodes were recorded. Some of the sampled animals were selected systematically by the veterinary team while others were selected by the owners. During the survey, complete half-body tick counts were carried out systematically on some selected cattle and recorded according to both attachment site and identity. The sites were, nostril, ear, perineum, tail and feet. The peripheral lymph nodes were examined for signs of swelling. The pallor of each animal was recorded after the conjunctivae of both eyes and mucous membranes were examined. In addition the injection scars formed by trypanocidal drug injections on both sides of the neck of every animal were counted and recorded.

Drugs

In order to determine the availability and utilisation of trypanocidal drugs, the animal owners were asked to state the drugs that they bought and used in the last six months. Having stated the drugs that they bought, the owners were asked whether they went first to get their drugs from the nearest veterinary office or whether the veterinary assistant visited them regularly. When the owners mentioned their preference they were asked whether they got the drug and the amount that they wanted from the preferred source - always, usually or sometimes. However, in order to record the distance to the nearest veterinary office, the owners were asked to say where the nearest

veterinary office was, or where the nearest veterinary assistant, who retailed drugs, resided, and the distance from the present homestead or cattle camp was recorded.

During the interview the animal owners were asked to list the brands included in their most recent purchase of drugs, and also to state the source, the quantities acquired, the price paid and the quantities remaining unused. Furthermore, if the owners mentioned that they bought Samorin, they were asked whether they bought in bulk or in small doses and whether they knew how to make up standard doses from the bulk powder. Likewise the animal owners were asked whether they treated all of their animals routinely regardless of whether animals were sick or not or whether they treated only the sick animals. If they treated routinely how many times per year. Owners were also asked the average number of sick animals which they treat monthly in each residence area. In addition the owners were asked the total of animals which died for lack of trypanocidal drugs or when the correct treatment did not work. If any drug did not work, the owners were asked to state the drug or drugs which did not work. The owners were also asked which trypanocidal drug they considered the best, and whether they considered that the others they had used in the last six months performed satisfactorily. The owners were asked whether they customarily used either a higher or lower dose than the standard dose.

Collection of Blood Samples

Cattle owners were asked to bring all their cattle for examination. Blood samples were collected in pairs of evacuated tubes, from the jugular vein. One sample was collected into an

evacuated tube (vacutainer) containing the potassium salt of ethylenediamine tetra-acetic acid (EDTA) and the second sample in a clear tube for serum collections. The blood in the serum tube was kept in the shade until it clotted. The clotted blood was kept surrounded by ice in a cool box, and usually the serum was removed with pasteur pipettes over one, two or sometimes three consecutive days. All sera were inactivated at 56°C for 30 minutes, and then kept at -20°C in plastic ampoules after it arrived at Mogadisho.

Examination of Blood Samples

Evacuated tubes with EDTA anti-coagulant were used for immediate testing of blood samples. Standard microscope slides were used, except when making direct examinations of mini-centrifuge tubes and packed cell volume (PCV) measurements. For wet films, one drop of blood on a slide was slightly compressed under a coverslip and examined as soon as possible using semi-dark ground illumination. This was obtained by partially closing the condenser diaphragm (Killick-Kendrick, 1968).

The microscopes used were Wild M11 binocular student microscopes with 6 volt illumination in the base operated by an extension cord from one half of a Land-Rover battery.

Initially the packed cell volume (PCV) was measured after the accomplishment of the day's sampling, with a Hawksley microhaematocritic centrifuge powered by a portable generator. Later a mini-centrifuge (Compur M1101) powered by 12, 7 or 5 volts from a Land Rover battery was used.

Wet films were prepared from the buffy coat region of the

microhaematocrit tubes (Paris et al, 1982; Murray, Trail, Turner and Wissocq, 1983), usually after reading of the PCV. This was only possible when time permitted preparation of films within 4 hours of collection. When the tubes were examined directly, immediately after recording the PCV, they were put in a drop of water under a coverslip and the area of the buffy coat and adjacent supernatant was scanned at x 400 magnification. If positive, the magnification was increased to x 600 to assist species identification. The tubes were cut 1mm below the top of the red cell column and 1mm of red cells was mixed on a slide with about 4mm of the adjacent plasma and examined.

Using a modification of the method of Paris et al (1982), the wet films (whole blood or buffy coat) were given scores. The scores were classified as following; depending on the number of trypanosomes found in 80 fields.

* If only 1 trypanosome seen in 80 fields the score was 1
* If 2-9 trypanosomes seen in 80 fields the score was 2
* If 10-79 trypanosomes seen in 80 fields the score was 3
* If 1-9 trypanosomes seen per field it was scored 4
* If 10-99 trypanosomes seen per field it was scored 5
* If the trypanosomes were 100 or more per field it was scored 6

Thick smears were prepared separately, after finishing other tests, dried and haemolysed in water before staining. Later in the investigation, both thick and thin films were prepared together on the same slide in order to help identification of trypanosomes seen in other tests or if the PCV was lower than 18%, these were haemolysed in the stain. For fixation, thin

films were fixed for 30 seconds in absolute methanol before staining for 30 minutes, in a 10% dilution of Giemsa concentrate, freshly prepared each day.

In the case of stained preparations, over 80 fields were searched in order to determine whether the animals had a mixed infection (especially in animals suspected of being infected).

Sampling

Healthy animals were poorly represented, particularly in the areas with the greatest trypanosomiasis problem. Only a few owners allowed the survey team to select animals and others expected their problem animals to be examined first. Some owners expected exclusive examination of their sick animals. Because of this, the intention of examining equal numbers of sick and healthy animals was not successful. From sunrise, until the herds left the cattle pen or stable for the day's grazing, a team was able to examine 30-40 animals selected from four or five different herds in the same village or nearby area in a typical day.

Faecal samples were routinely taken from every sixth animal in sequence, and from others as requested by the owners. Faecal sampling and examination was useful in the context of public relations, providing concrete results about a disease which farmers understand. Examination of faeces was done by flotation in saturated sodium chloride solution (a simplified McMaster Method) and some were examined by sedimentation for fluke eggs.

Statistical Methods

Two statistical methods of analysis were used, namely Chi-

Square tests and Logistic regression. Chi-Square tests investigate whether there is a significant association between two unordered categorical variables.

Logistic regression is used to model how a binary variable e.g. whether an animal has or has not T. congolense, depends on an ordered variable e.g. PCV range. It assumes that for example the logarithm of the odds on an animal having T. congolense is a linear function of an explanatory variable such as PCV range. A test of goodness of fit of the model and a test of whether the slope of such a function is significantly different from zero were both carried out at a 5% significance level. ('Using and Understanding Medical Statistics' by D.E. Matthews and V. Farewell (Karger), 1985).

RESULTS

A total of 4,152 cattle in 54 herds were examined. Of these, 246 cattle were examined mainly for public relation purposes, and therefore detailed histories were not obtained from these animals, because they were isolated animals from the various herds.

Table 4 shows that the highest number of herds examined were from Qoryooley, Jilib and Jamaame districts and the lowest number of herds from Kismayo district. Of the first three districts, each was surveyed on at least two separate occasions, because various herds from different places, were concentrated in these districts during the dry season in search of fodder. It appeared a good opportunity to examine animals in the endemic areas of trypanosomiasis.

Table 4

The location and numbers of cattle examined in the survey

District	Total herds examined	Total cattle examined	No. of herds	Cattle present	Cattle examined	Mean herd size	Percent examined
Buulobarde	23	173	21	647	151	31	23
Jowhar	31	292	29	1,694	284	58	17
Balacad	21	184	21	1,293	184	62	14
Afgooye	11	100	9	657	95	73	14
Marka	64	453	57	2,801	438	48	16
Qoryooley	87	634	68	3,140	547	46	17
Sablaale	11	90	11	394	83	36	21
Baraawe	48	370	49	3,101	367	63	12
Saakow	27	268	21	1,142	239	54	21
Bu,aale	12	77	12	734	66	61	9
Jilib	61	495	59	1,668	491	28	29
Jamaame	60	426	55	1,108	397	20	36
Afmadow	8	62	8	279	62	35	22
Kismayo	2	31	2	300	31	150	10
Buur,hakeba	10	61	10	172	61	17	35
Baydhabo	32	211	28	360	206	13	57
Diinsoor	38	225	35	1,044	204	30	20

The few camel herds that were blood sampled and the flocks of goats and sheep were not included in the table. In order to establish a demarcation line between the animals in the sprayed and non-sprayed areas, six herds from the western side of Afgooye district were included in the Marka district. This was done because on the eastern side of Afgooye district the aerial spray trials were being carried out.

The herds in Kismayo and Afmadow districts were bled in the wet season, and it was not possible to obtain blood samples from sufficient numbers of cattle, except in a few herds. Therefore these herds were included with the Jamaame herds as these herds were close to the latter district.

The first four districts (see Table 4) were within an aerial spray area. The second ten districts were surveyed and it was confirmed that they were heavily infested with tsetse flies. The last three districts were all in the Bay Region which is known to be tsetse-free. These herds were blood sampled in order to determine whether mechanically transmitted T. vivax infections were occurring outside the tsetse belt. However, examination of blood films from these herds failed to detect any evidence of infection.

Trypanosome Species

Wet film examination has been demonstrated to be a fairly accurate method for the identification of a single species. However, when mixed infections occur with the two pathogenic species (T. congolense and T. vivax) the rapid movements of T. vivax may overshadow the sluggish movements of T. congolense, particularly when the parasitaemia of the latter species is low.

Inaccurate identification may also occur between T. congolense and T. brucei and a strain of T. congolense which was sent to Kenya for drug-resistance testing was eventually identified as a T. brucei strain. As no T. brucei strain was identified in the survey, it is possible that some T. brucei infections may have been missed.

A few strains of presumably T. evansi were observed from the wet films of some infected camels. These camels were brought for examination by their owners and as the camels were originally from a tsetse-free area, it is likely that Tabanidae had infected them mechanically. When the camels were blood sampled, they were in a Riverine area, but no tsetse-transmitted trypanosomes were detected in their blood. No T. simiae or T. theileri were met during the survey (although no warthogs or bushpigs were bled).

By reference to a small scale map showing the density and distribution of tsetse fly along the two rivers (prepared by Dr. Leigh Brown of NTTCP Somalia), tsetse habitat categories were classified as follows. If the working site was more than 50km away from a known tsetse habitat, it was characterised as category 0. If the working site was more than 5km but less than 50km from tsetse habitat it was characterised as category 1. If the working site was outside the tsetse habitat, but within 5km it was characterised as category 2. Sites found inside the tsetse-infested zones, were classified according to the mean number of tsetse flies caught by the traps in the last 24 hours.

(a) If the number of flies caught were less than two per trap, category 3.

(b) If the number of flies caught were between two and 20 flies per trap, category 4.

(c) If the results of the last 24 hours were more than 20 flies per trap, category 5.

The results of positive diagnosis of trypanosomiasis were compared with the various tsetse-challenge category zones of the period from two months to two weeks prior to sampling and the results are shown in Table 5.

The results show an overall infection level of 10%. No positive cases were observed in Category 0. Statistical analysis of the results in categories 1 to 5 show that the percentage of T. congolense infections show a significant increasing (linear logistic) trend with challenge category. However the percentage of T. vivax infections shows no trend with challenge category. Challenge category 5 gave unexpectedly low levels of infection possibly because the stock owners have adopted several techniques to minimising the risks of tsetse challenge, such as watering and grazing their stock at night, grazing during the midday and even zero grazing. It should also be noted from the Table that a relatively high number of unidentified trypanosomes were recorded.

Haematological tests and wet film scores

The number of tests done during the survey were restricted by the availability of manpower, however, the following tests were performed, namely wet films (whole blood or buffy coat), stained smears (thin and thick films) and direct viewing of microhaematocrit tubes.

Table 5

A comparison of positive diagnosis of trypanosomiasis with tsetse challenge category

Challenge Category	Total Cattle	Unidentified Species	T.congolense	T.vivax	Both	% Positive	Ratio T.congolense/ T.vivax
0	497					0	
1	600	3	32	13		8.0	2.5
2	901	9	41	37	1	9.8	1.1
3	1,171	16	89	39	1	12.4	2.3
4	852	17	67	34	2	14.1	2.0
5	131	1	11	5		13.0	2.2
Overall	4,152	46	240	128	4	10.1	1.9

Table 6 shows a comparison between each type of test carried out and the positive samples obtained by each test. The Table illustrates that wet film examination of whole blood and direct viewing through microhaematocrit tube showed an equal degree in sensitivity for the two pathogenic trypanosomes. The Table also indicates that direct wet film examination was the best for detection and recognition of T. congolense infections.

The number of samples in the different wet film scores, were compared with infections of T. congolense and T. vivax and the results are shown in Table 7. The wet film scores indicated the existence of higher levels of infection with T. congolense in the first three scores (1-3) and low numbers of T. congolense in score categories 4 and 5, a suggestion of lower parasitaemias in T. congolense infections.

In T. vivax infections the distribution was more evenly spread and fulminating parasitaemias were recorded with scores of 4-5. Thus in general T. vivax infections tended to have higher parasitaemias than T. congolense (Chi-Square Test).

Packed Cell Volume

Anaemia is a major feature of animal trypanosomiasis and is a reliable indicator of the progress of the disease. Table 8 compares the numbers of positive and negative samples in a range of PCV classes. The Table shows a significant (linear logistic) trend between the percentage of positive samples and PCV.

The Table also indicates that the PCV of five positive animals were above the range of 30%. However, it may be that the parasites only appeared in the blood either on the day of

Table 6

A comparison of tests for the diagnosis of trypanosomiasis on the same positive animals

Tests Performed	Positive for Trypanosome			
	Unidentified	T.congolense	T.vivax	Total
i) Wet film and	6	86	47	139
buffy coat	5	53	45	103
ii) Wet film and	8	96	46	150
through the tube	26	85	48	159

Table 7

A comparison of wet film scores for *T.congolense* and *T.vivax* infections

Range represented by each score	Wet Film Scores 1	2	3	4	5	Total Samples Scored
T.congolense	61	89	21	4	0	175
T.vivax	28	31	20	12	5	96
Total	89	120	41	16	5	271

Table 8

A comparison of the numbers of positive and negative samples for trypanosome infections in a range of P.C.V. classes

P.C.V. range	Number of positive samples	Percent positive	Number of negative samples
Over - 30	5	1.9	257
25 - 30	38	3.8	968
19 - 24	148	9.2	1,452
13 - 18	142	15.4	778
Under - 13	77	28.6	192
Total Overall	410	10.1	3,647

sampling or a few days before. The PCV level of some positive animals had fallen below 13%, an indication of severe infections or a combination of other factors.

Of the negative animals, nearly half the haemoatocrits ranged between 19-24% and indicated the existence of generalised anaemia in the cattle. The combinations of malnutrition, trypanosomiasis, tick-borne disease and helminths could have been the cause of the low PCV ranges that were observed.

Other Clinical Signs

Swollen lymph nodes

In the early stages of infection when the parasitaemia is readily detected there is intermittent pyrexia. At this time the superficial lymph nodes are palpably enlarged. The degree of swelling of the prescapular lymph nodes of the sampled animals was scored on a scale of 0, 1, 2 and 3. The last category was kept for grossly enlarged or abcessed and deformed nodes.

Tables 9A and 9B show the lymph node swelling scores according to the current challenge category of the herds and whether the animal was positive or negative on wet film examination for trypanosome infection.

Table 9A shows that the animals with higher lymph node scores tended to be in higher challenge categories. However, animals with enlarged nodes were found both in the fringes and far beyond the tsetse habitat. Of all the challenge categories the one with the highest tsetse challenge, category 5 had low lymph node scores. This may have been due to low stock density in the high tsetse challenge category 5 and also due to the fact that stock owners in these areas have adopted techniques of

Table 9 (A and B)

The degree of lymph node swelling in cattle in different categories of tsetse challenge and trypanosome infection

A. Challenge Category (2 months to 2 weeks)	Lymph node swelling score			
	0	1	2	3
0	91	345	47	4
1	114	393	84	5
2	212	508	143	15
3	241	757	129	13
4	163	500	170	16
5	34	77	18	1

B. Diagnosis	Lymph node swelling score			
	0	1	2	3
i) Trypanosome infection				
Unidentified	6	27	11	1
T.congolense	34	151	46	8
T.vivax	18	82	24	3
Both		3	1	
ii) Total of positives	58	263	82	12
iii) Total of negatives	797	2,317	509	42

conserving their animals, such as zero grazing.

Table 9B does not indicate any marked differences in the size of the lymph nodes between animals infected with T. congolense or T. vivax, but the Table does confirm the existence of a significant trend between the presence of infection and lymph node swelling.

Pallor

One of the major features of trypanosomiasis in cattle is anaemia which is seen as pallor of mucous membranes. The conjunctiva of the eyes or the mucous membranes of the animals were assessed and the degree of pallor given a score on a scale of 0 to 3. The mucous membranes of many animals that were blood sampled was pigmented and therefore pallor was unlikely to be a useful substitute for PCV in the detection of anaemia (at least in this survey).

Table 10A shows that none of the positive animals scored scale 3 on the pallor score but three of the animals which had been microscopically negative, proved to be scale 3. The scores of most of the positive animals were 0 or 1. Generally, the pallor of the positive animals did not show any marked difference depending on which of the two species of trypanosome was present. However, the table indicated a significant (linear logistic) trend with pallor and infection with both T. congolense and T. vivax. There was also a significant association between pallor and PCV range, i.e. as the PCV decreased there was a general tendency for pallor to increase (Table 10B).

Table 10 (A and B)

The degree of pallor in cattle with different categories of trypanosome infection and haematocrit value (PCV)

A. Diagnosis		Pallor Score 0	1	2	3
i)	Trypanosome infection				
	Unidentified	30	14	1	
	T.congolense	151	80	8	
	T.vivax	76	44	6	
	Both	2	1	1	
ii)	Total positives	259	139	16	
iii)	Total negatives	2,818	768	72	3
B.	P.C.V. range				
	Over -30	236	17	1	
	25 - 30	884	97	4	
	19 - 24	1,226	327	18	
	13 - 18	556	324	30	
	Under - 13	113	115	33	2

Injection scars

One of the problems with some trypanocidal drugs is the reaction to the drugs themselves, causing necrosis at the site of the injection. Accordingly the injection scars were scored to a maximum of six but sometimes when there was widespread deformities of the neck skin of the animal the extent of the deformity was scored on a scale of 0 to 3 on each side. These scores were later summated, as probably most of the lesions were several months old. The lesions represented the number of treatments experienced by the animal during its life. The majority of the scars or swelling were thought to be caused by injections of isometamidium chloride (Samorin), and this may be due to the following reasons:-

(i) Injected Samorin forms a deposit of drug at the injection site and from this deposit the drug is released over a period into the circulating blood (this is the reason that Samorin has a marked prophylactic action, in comparison with other trypanocidal drugs) and around the drug's deposit a marked tissue reaction is formed with consequential scar formation; (ii) sometimes the injection site may be contaminated by bacteria and this can happen with any drug; (iii) due to inaccuracy of deep injections the drug leaks back into a subcutaneous site.

Table 11 shows the mean of the injection scar scores of the animals found to be microscopically positive or negative in each of the tsetse challenge categories of the preceding two months. The figures in brackets represent the number of cattle from which each mean was determined.

Table 11

The severity of injection site scarring in cattle with different categories of challenge and trypanosome infection

Diagnosis	Challenge Category 1	2	3	4	5	Overall
i) Trypanosomes						
Unidentified	3.7(3)	2.4(8)	1.2(16)	1.5(17)	5.0(1)	1.8(45)
T.congolense	2.3(32)	2.4(41)	2.2(88)	2.4(67)	4.5(10)	2.4(238)
T.vivax	1.4(11)	1.6(37)	1.2(89)	1.6(34)	2.8(5)	1.5(126)
Both		1	2 (1)	1 (2)		1.2(4)
ii) All positive	2.2(46)	2.0(87)	1.8(44)	2.0(120)	4.0(16)	2.0(413)
iii) Negative	1.2(545)	1.4(789)	1.4(995)	1.8(729)	2.8(600)	1.7(3656)

Figures in parenthesis represent the number of cattle in each group

The Table shows that the injections scores were highest in challenge zone 5. It appeared that cattle recently affected by the disease had received a higher number of injections over the long term compared to the recently negative cattle. Perhaps the difference was due to injections within the time-span of the current incident of trypanosomiasis. In other words, the necrosis formed by the recent treatments were still visible. The animals infected by T. congolense had almost twice as many palpable injection scars as those animals infected by T. vivax. Moreover, the results of the Table illustrate that the mean of the mixed infections were relatively small in number.

Age Susceptibility

There are indications that young animals may be less susceptible to trypanosomiasis than adults. Table 12 shows the results of studying the possibility that T. vivax and T. congolense species could differ in the frequency of their occurrence in cattle of various ages. The Table shows that the number of animals infected with T. congolense increases as the age of the animals increase, and T. congolense is the most predominant species in infections of the older animal. In contrast there is no trend between T. vivax infection and age. In Somalia, cattle owners usually sell their oxen at early ages, therefore the increasing overall percentage of infections with T. congolense from 18 months of age may reflect the increasing percentage of female cattle in the population, and the combination of various factors such as malnutrition, pregnancy and lactation may affect the ability of these animals to build up immunity against the disease.

Table 12

The relationship between cattle age and the incidence of trypanosome infection

Diagnosis		Age range (years) 0.0-0.5	0.6-1.5	1.6-3.5	3.6-7.5	Over 7.5	Total
i)	Trypanosome infection						
	Unidentified	3	9	9	20	4	45
	T.congolense	10	22	37	121	48	238
	T.vivax	13	23	32	46	13	127
	Both			2	1	1	4
ii)	Total of positives	26	54	80	188	66	414
iii)	Negatives	299	562	624	1,196	472	3,153
iv)	Absolute % of animal infected						
	T.congolense	3.1	3.6	5.3	8.7	8.9	6.7
	T.vivax	4.0	3.7	4.5	3.3	2.4	3.6
	Overall	7.1	7.3	9.8	12.0	11.3	10.5

Treatment

Cattle owners were asked during which of the two seasons (wet or dry season) they usually gave more treatment to their cattle. The interviews were done during the dry season as no survey was carried out during the wet season due to inaccessibility of the cattle herds. Therefore, the percentage of animals was biased towards the dry season situation where semi-sedentary and nomadic herds are more in contact with tsetse flies. The need for treatment may be affected by the movement patterns. The results of the interviews are compiled in Table 13. From the Table it can be noted that there is not much difference in treatments of the two seasons of the sedentary cattle. But in the semi-sedentary or nomadic cattle, more treatments are given in the dry season than in the wet season. However, these animals move away from the tsetse belts in the wet seasons, which could save the animals from the risk of trypanosomiasis and also reduce drug costs.

Therapy of animals with symptoms

Animals affected by trypanosomiasis were usually treated by their owners as soon as they observed clinical symptoms, and there after continuing treatments are given at intervals of days or weeks until the animal recovers. The animal owners were either mixing two different trypanocidal drugs e.g. Samorin and Berenil, interchanging one with another or repeating treatment with the same drug.

Table 13

The reports of frequency of trypanocidal drug treatments in different seasons and differing herd management

Treatments	Sedentary	Semi-Sedentary	Nomadic	Overall
More in wet season	54	38	32	124
More in dry season	46	62	68	176

Table 14 shows the drugs used by the cattle owners during the preceding month and it is apparent that Berenil and Samorin were equally used by the animal owners at least for the first injections. But, in contrast to this, the animal owners took diminazene aceturate as their drug of choice in the repeated injections.

Probably, the reasons why animal owners prefer diminazine aceturate for repeated injections are that the drug does not create skin reactions at the injection sites, like isometamidium. Also the animal owners believed that Berenil does not affect milk production of the treated animal, in contrast to isometamidium. Finally the owners feel that this drug permanently cures the infections whilst isometamidium according to the animal owners often fails to clear the parasites from the blood.

Drug Preference

The animal owners were asked first of all, which trypanocidal drug they thought the best and then whether they believe the other drugs they had administered to their cattle in the previous six months had worked adequately or not.

Table 15 shows the results of their responses. The Table illustrates that diminazene aceturate and isometamidium chloride appeared to be equally popular first choices of trypanocidal drugs for treatment. On the other hand ethidium and novidium are listed in the satisfactory category. It appears that if resistance by trypanosomes is encountered by animal owners they use Berenil and Samorin alternatively. Some owners added supplementary remarks to the effect that they observed relapses

Table 14

Trypanocidal drug usage by cattle owners during the preceding month

Repeat Injections	First Injections				
	None	Berenil	Samorin	Ethidium or Novidium	Total
None	176	343	344	102	965
Berenil		27	48	12	87
Samorin		42	4	3	49
Ethidium or Novidium		19	6	11	36
Total cases	176	431	402	128	1,137

Table 15

Overall trypanocidal drug preferences by cattle owners

	Berenil	Samorin	Ethidium or Novidium	Other
First choice (or equal 1st)	166	114	55	2
Also satisfactory	59	58	72	3

after Berenil treatments, and a temporary reduction of milk production after Samorin had been given, with subsequent necrosis at the injection sites.

Dosages

In the interview, the animal owners were asked whether they gave their cattle the recommended doses, or if they commonly treated the affected animals with either an overdose or underdose. The majority of the people responded that they gave double doses of Samorin, if the animals did not respond immediately to the initial injection. A relatively small number of the people mentioned that they usually gave higher doses at the beginning. In most cases the owners stated that they used the standard dose of each drug, even in the mixed form or simultaneous injections of two different drugs. However, none of the interviewed people admitted to drug failure due to mal-administration or using reconstituted trypanocidal drugs in the field. These could have contributed to the development of drug resistance which may have gradually developed without the animal owners becoming aware that resistance was developing.

Samorin

Isometamidium chloride is known for its chemotherapy and chemoprophylactic activities, but in Somalia most of the animal owners usually use it for chemotherapy only. Those interviewed mentioned the difficulty of preparing the requisite dose of Samorin from the 10g bulk packet. However, the doses they use to treat their animals, are either prepared by veterinary assistants or by the retailers. Thus, these pre-prepared doses of Samorin may result in underdosing due to the adsorption of the drug onto

the paper in which it was wrapped. In addition, profiteering by unscrupulous retailers may also produce underdosing. Therefore the impression which animal owners have is that using these prepared doses of Samorin the actual weight of the dose which is administered to their animals is usually less than that which is recommended.

In the interviews, the animal owners reported that some of their animals died from trypanosomiasis. They were therefore asked the number of animals which had died due to the unavailability of a suitable drug at the time the sick animals required treatment. One hundred and seventy three (15%) of 1,102 deaths were attributed to the lack of drugs, by 26 out of 346 owners interviewed.

Similarly, the animal owners were asked when they obtained a suitable drug and used the correct dose level how many treatments were unsuccessful. Seventy two of the 346 owners interviewed attributed 740 deaths to the unsatisfactory performance of either Berenil or Samorin. This represents 76% of all mortality from suspected trypanosomiasis. Those animals were the ones they named "Salaaf". Therefore the results of the interviews strongly supports the existence of drug resistance.

Health status

The health status of the cattle which were blood sampled was classified by asking the owners whether trypanosomiasis had affected their animals recently or in the past six months. They were also asked if some other unrecognised sickness other than trypanosomiasis had affected their animals or had they been

continuously healthy for the past six months. The results of the interview are compiled in Table 16. The Table shows the numbers of animals sampled according to their health status and tsetse challenge category of the herd for the period from two months to two weeks before sampling. The proportions of 'allegedly healthy' cattle are significantly different across the different challenge categories but they do not follow a linear logistic model as does the corresponding data in Table 5.

Trypanotolerance

In Somalia there are three local breeds of cattle, Surgo, Boran and Dawara. Various groups of cattle owners have suggested that the Surgo breed is tolerant to trypanosomiasis. They believe this is the reason that the latter breed has become the most dominant one throughout the areas south and west of Qoryooley excluding Jamamme district. However, comparing them to the other two breeds, Surgo appears less productive in milk and meat. The question was whether or not cattle of the Surgo breed could resist or tolerate trypanosome species relatively better than cattle of the other breeds.

Table 17 shows the numbers of positive animals of these breeds according to their wet film scores and infection with trypanosomes. Statistical analysis showed that there is no significant relationship between the wet film score and breed for either *T. congolense*, *T. vivax* or the total number of positives. Thus there is no evidence from these results to support the view that the Surgo breed has lower levels of parasitaemia than the Boran and Dawara breeds.

Table 16

The opinions of cattle owners on the health status of their animals in various challenge categories

Challenge Category	Allegedly Affected by Trypanosomiasis		Allegedly Healthy	Total
	Now	Formerly		
0	0	1	479	480
1	171	22	313	506
2	422	57	308	787
3	533	99	404	1,036
4	455	87	203	745
5	63	14	33	110
Totals	1,644	280	1,740	3,664

Table 17

The incidence of trypanosome infections in three local Somali breeds of cattle

Wet Film Scores	Boran		Dawara		Surgo	
	congolense	vivax	congolense	vivax	congolense	Vivax
3 and Over	4	4	9	11	11	21
2	14	1	34	10	38	18
1	10	2	21	9	27	15
Not Available	8	5	23	10	33	15
Total	36	12	87	40	109	69
Per 100 Cattle Sampled	75	25	69	31	61	39

DISCUSSION

Trypanosomiasis in the riverine areas of Somalia is such that it is necessary to treat most of the cattle during the dry season to prevent the debilitating effects and loss of production caused by infections of T. congolense and T. vivax. The incidence of infections with T. congolense showed a significantly increasing trend with the level of tsetse challenge, whilst the incidence of T. vivax infections showed no such trend.

In Somalia, it appears that tsetse populations remain relatively stable throughout the year both as regards numbers and distribution (Leigh Brown, personal communication). However, in the wet season animals are usually away from the tsetse infested areas, but in the dry season they concentrate on the infested areas and this permits a more extensive exploitation of tsetse-infested resource during the dry period of the year, than would otherwise be the case. The increased availability of trypanocidal drugs which reduce morbidity and mortality losses, have encouraged more extensive utilisation of grazing.

During the survey, it was observed that the incidence of the disease increased towards the delta of the Shabelle river and in the vicinity of the lower parts of the Juba river. This could be assumed to be an indication of the co-existence of a high degree of tsetse infestation and wildlife hosts. It would seem that in this ecological zone where wild ungulates were common trypanosome infections are more prevalent than in areas where they were scarce (e.g. upper parts of Shabelle and Juba rivers).

The rate of trypanosome infections are high in Glossina species, mainly G. pallidipes which is the most dominant one

(Ahmed and Dirya, 1987). Bushpigs and warthogs were the predominant wild hosts since other wild game have been reduced by poaching. The warthogs were also reported to be resistant to T. vivax (Ashcroft, 1959; Lumsden, 1962).

In T. congolense species it has been confirmed that the rate of infection in Glossina usually remains the same whatever the frequency of either their suid or bovid feeds (Moloo, Kutuza and Boreham, 1979). Therefore it is conceivable that the T. congolense infections encountered in the blood sampled cattle, originated from other animals like buffaloes, which normally harbour trypanosomiasis, and warthogs, because all nomadic cattle are usually away from tsetse habitats in the wet seasons.

Both T. congolense and T. vivax were the most frequently reported pathogenic species in endemic areas of trypanosomiasis of the upper and lower Shabelle regions of Somalia in previous smaller surveys e.g. NTTCP report, 1982. That report also recorded that T. brucei was the least encountered species. While the cattle blood sampled in the present survey did not reveal T. brucei infections in wet films, an isolate sent to Kenya as a T. congolense for drug-resistance tests was found later to be T. brucei. In a recent study, the later strain has shown a high degree of multiple drug-resistance (Zweygarth and Rottcher, 1989).

Earlier work has shown a higher proportion of T. vivax infections in animals when first introduced into tsetse habitat and greater frequency of T. vivax in tsetse vectors (Wilson, Le Roux, Dar, Paris, Davidson and Gray, 1975a). Therefore, the

high proportion of T. vivax infections observed from the tsetse-infested areas could be due to the introduced animals which had been born while their dams were away from the tsetse habitat and which became highly infected when they first encountered the high density of the vector during the dry season.

The regional variations in the level of infection within the two rivers is probably due to differences in wildlife agricultural development with consequent differences in availability of tsetse-breeding sites and concentrations of the animals on non-agricultural areas, during dry season grazing.

The circulation of these different trypanosome species, via tsetse, between various hosts has perpetuated the continuous and indiscriminate use of chemotherapeutic and prophylactic drug regimes. The cost of these drugs is, however, substantial. The livestock owners are often prepared to obtain these drugs at any cost and they administer them personally to their animals. Considering the price of the drugs in Somalia it is uneconomic to use the drugs at higher dosages, but up to now this system appears to be the only solution to the problem of drug-resistant parasites.

The results obtained from the interviews with the cattle owners concerning drug preference and drug related deaths, revealed the inefficiency of trypanocidal drugs used in the field. Breakthrough infections, treated earlier with a single, double or mixed dose of trypanocidal drugs were reported by many of those interviewed. However, the subsequent isolation of two drug-resistant strains of T. congolense (see Chapter 3) and the previous isolation of a multidrug-resistant T. vivax (Schonefeld,

Rottcher and Moloo, 1987) at Buulabarde, are in agreement with the results obtained in the interviews with these animal owners.

Therefore the long standing suspicion that drug-resistance of the pathogenic cattle trypanosomes are prevalent is further substantiated. No important differences were encountered in the study on the susceptibility of the three local breeds and the suggestion that the Surgo breed is trypanotolerant was not substantiated.

Definite diagnosis of the disease was dependent ultimately on the detection of the trypanosome in blood samples from infected animals but generally the diagnosis of the animal owners and the results of wet film examinations were well correlated. This suggests that the animal owners had acquired diagnostic acumen through long experience.

Many workers have described the inferiority of the wet film method compared to the phase contrast buffy coat method (Paris et al, 1982; Robson and Ashkar, 1972), but in the present field experience the wet film was marginally superior, at least with T. congolense infections, although the time which elapsed before examination of the buffy coats may be a factor which made this method inferior.

It is believed that nutritional status of the host can influence the severity of the disease, therefore during the dry season when there is poor pasture, and the animals gather along the tsetse infested areas, those cattle in poor condition may be more susceptible to the risk of the disease. However, the low mean packed cell volume observed in allegedly healthy as well as

infected cattle, blood sampled in areas inside the tsetse habitat may be attributed to a combination of poor pasture and the effect of pathogenic trypanosomes.

Finelle (1976), Leach and Roberts (1981) and other workers have reported the severity of the swellings and abscesses at the injected sites, after the administration of isometamidium. These local reactions, the risk of subsequent necrosis and sloughing of the skin was experienced by many of the cattle probably because of poor methods of drug administration.

The animal owners were not able to measure indirect losses of the trypanosome-affected animals. However, they estimated that the annual direct loss in mortality due to trypanosomiasis was about 16%.

In conclusion, this study provides the most comprehensive information currently available on the prevalence of cattle trypanosomiasis in Southern Somalia. It is clear from the survey that T. vivax and T. congolense are the predominant pathogenic cattle trypanosomes along the riverine areas and the cause of significant losses. Moreover, many cattle owners reported the apparent inefficiency of trypanocidal treatments. It is hoped that the results of this survey may assist in the future control of bovine trypanosomiasis in Somalia.

CHAPTER THREE

STUDIES ON DRUG-RESISTANT STRAINS OF *Trypanosoma congolense*

SECTION I

THE ISOLATION OF DRUG-RESISTANT STRAINS OF *T. congolense* FROM THE LOWER SHABELLE REGION OF SOUTHERN SOMALIA

INTRODUCTION

Drug-resistance in trypanosomes has been reported from many parts of Africa (see review in Chapter I and Jones-Davies, 1967a; Graber, 1968; Mwambu and Mayende, 1971; Lewis and Thomson, 1974 and Williamson, 1979). In East Africa drug-resistant strains of T. congolense have been described in Ethiopia (Scott and Pegram, 1974) and Kenya (Gitaha, 1981). However, to date, they have not been reported from Somalia although their existence has been suspected for many years. In Southern Somalia there have been persistent rumours of drug-resistant strains of trypanosomes in local livestock and this condition has been given the name "Salaaf" by the animal owners.

The results of the survey of bovine trypanosomiasis reported in Chapter 2 confirmed these observations by cattle owners.

At the present time the most widely used trypanocidal drugs in Somalia are isometamidium chloride (Samorin, RMB) and diminazene aceturate (Berenil, Hoechst AG).

Drug resistance is usually suspected when a curative drug fails to achieve a clinical cure. However the existence of drug resistant strains of trypanosomes may be only one of several possible explanations for such failures. The animals may have been underdosed because bodyweight was underestimated or the drug may have been incorrectly prepared or kept too long before use.

Cattle weighing crates are rarely available in Somalia and similarly facilities for weighing doses of drug do not exist in the countryside. Isometamidium comes to Somalia in bulk (10g or 1g) and diminazene aceturate comes also in multiple and single doses (10 doses and single doses 3.5 mg/kg). However, animal

owners are not capable of measuring the exact doses required. This has introduced an empirical system of handling the trypanocidal drugs in the field particularly isometamidium chloride which is the most valuable drug in the country.

Lack of veterinary supervision has also complicated the problem of drug usage and injection. For example, a cattle owner admitted during the survey that he reconstituted (10g) ten grams of isometamidium chloride and kept and used the reconstituted solution in a bottle for about 48 days. The abuse of the recommended handling procedures and the improper use of the drug may play a part in producing drug resistance if these cattle owners misused the drugs in this way.

To prove that drug resistant trypanosomes are, in fact, involved it is necessary to first discount the difficulties of drug preparation, handling and administration outlined above, and secondly to demonstrate the response of the trypanosomes involved to treatment under carefully controlled conditions. Ideally this should be carried out in the host species of interest, i.e. Bos indicus cattle for strains isolated from East African Zebu cattle, although in many situations it is necessary to use small ruminants or laboratory animals because of limited finances and facilities.

In the present study the isolation of trypanosome strains from the field was attempted using a modified method of Jones-Davies and Folkers (1966c) who transported trypanosome-naive calves by lorry to treatment camps in Nigeria and subinoculated them with blood collected from cattle presented for treatment.

Large numbers of subinoculations were carried out simultaneously, up to 80 in one calf. Calves were then transported back to the laboratory and 24 hours later treated with 1mg/kg homidium chloride (Novidium). Treated animals were then monitored for the development of parasitaemia and the appearance of infection in a calf was taken as evidence of homidium resistant strains in one or more of the cattle from which blood had been inoculated.

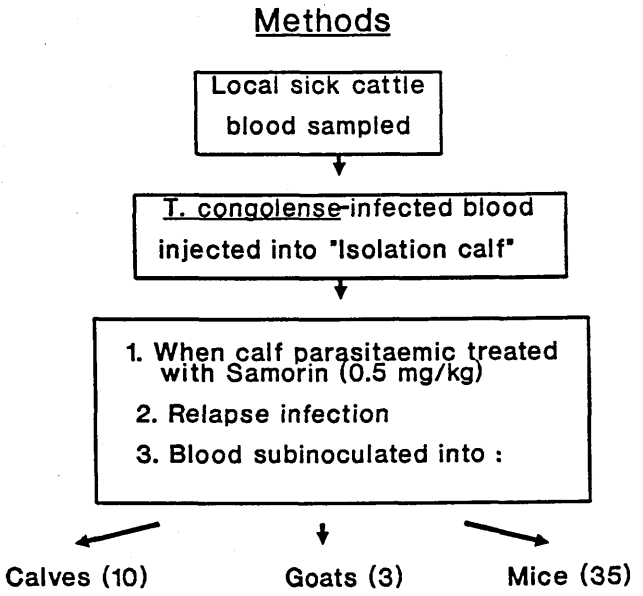
The aim of the present study was to determine whether drug-resistant strains of T. congolense are present in the livestock of villages of the Lower Shabelle Region and particularly in villages in the tsetse belt along the river bank. In addition, the strains were examined in comparative studies in cattle, goats and mice.

MATERIALS AND METHODS

Experimental design

Two studies were conducted. One in the Afgooye district and the seond in the district of Shallanbood. The basic protocol of each study is presented in Figure 1. In each study an 'isolation calf' was purchased from a tsetse-free area and transported into the study area by Landrover. In the villages the animal owners were invited to present their cattle in poor condition for blood examination on the understanding that any found to be infected with trypanosomes would be treated with a trypanocidal drug. When cattle were found which were infected with T. congolense five millitres of blood was taken by jugular venepuncture and injected subcutaneously into the 'isolation calf'. The 'isolation calf' received up to ten such injections over a one to two day period and was then transported back to the animal

Fig. 1 Protocol for isolation of drug-resistant strains of T. congolense



facility. At the animal facility the calf was carefully monitored for the presence of a trypanosome parasitaemia. After a few days of patent parasitaemia the calf was treated with 0.5 mg/kg isometamidium chloride and continually monitored for the development of a relapse infection. The development of such an infection was taken to be indicative of the presence of a drug-resistant strain of T. congolense and blood was taken at this time from the 'isolation calf' and injected into groups of calves, goats and mice for subsequent trials to determine the level of drug resistance. In each study ten calves were used and randomly allocated into three groups of three animals with the remaining single calf used as an untreated control. Following the development of patent parasitaemias, calves in groups A + E, B + F and C + G were treated with 0.5 mg, 1.0 mg and 2.0 mg/kg isometamidium respectively. It was planned to treat any subsequent relapse infections with increasing doses of diminazene aceturate (Berenil) to determine the degree of cross-resistance.

In each study three goats received blood from the isolation calf and two were treated with 0.5 mg/kg isometamidium. In addition groups of mice were infected and treated with 0 - 20 mg/kg isometamidium in order to compare the drug-resistance of the isolate of T. congolense in different hosts.

Trypanosome Isolation

In the first study in Afgooye district a total of 35 cattle were blood sampled and examined by wet films. Ten of these animals were found to be infected with T. congolense. The other infected animals were either positive with T. vivax or had mixed infections of T. congolense and T. vivax. The animals were from

six different herds, two of them from the first and second villages and the other herds were from the third and fourth villages.

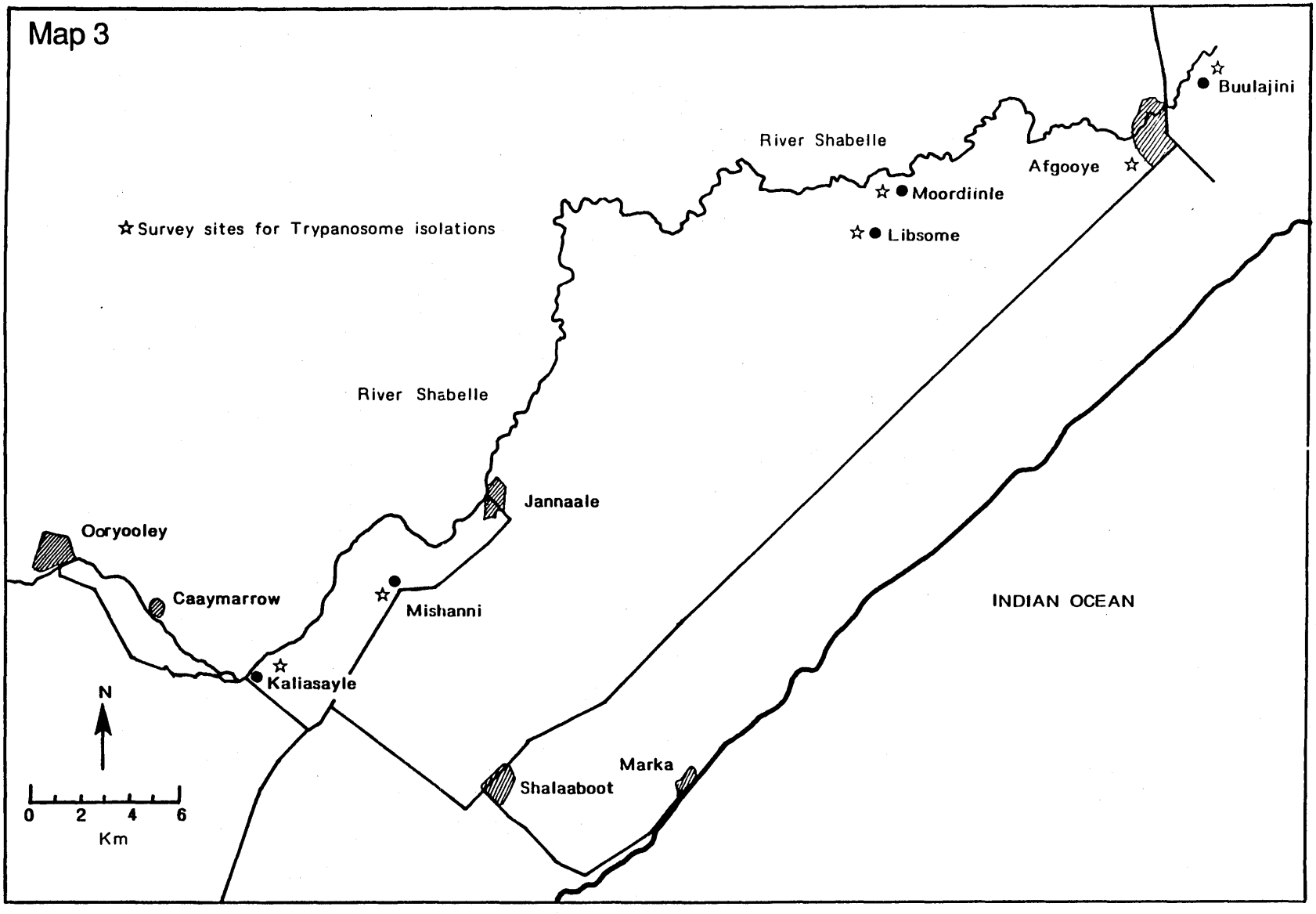
Each group or herd belonged to different individuals resident in these villages. 5 ml of blood was taken from every animal that was only infected with T. congolense. The blood from ten different animals (each 5 ml) was inoculated subcutaneously into the isolation calf at different sites along each flank.

The villages, where the animals were sampled included Moordiinle, Buulajini, Afgooye and Libsome (see map 3). The environs of these villages were highly infested with tsetse flies and the most predominant species was Glossina pallidepes (Leigh Brown, personal communication, entomologist in the Tsetse Department). However, the first isolation was done during the wet season and the flies had dispersed into the Acacia thickets approximately 2-4km away from the river, in these particular areas. Ahmed and Dirie (1987) have reported an infection rate of G. pallidepes of 2.6% and 1.5% during the wet and dry seasons respectively in an area 3km south-west of Afgooye district.

The isolate from Afgooye district was entitled AFI.

The second isolation was carried out in two villages of the Shallanbood district, Mishaani and Kaliasayle villages (see map). It was winter time (March, 1987) and most of the animals which were found in these two villages were nomadic and had come near the river in search of grass and water.

A total of 30 animals were blood sampled and the wet blood films examined by light microscopy. Five animals (four cows and


Map 3
Buulajini
River Shabelle
Afgooye
Moordiinle
Survey sites for Trypanosome isolations
Libsome
River Shabelle
Jannaale
Ooryooley
Caaymarrow
Mishanni
INDIAN OCEAN
Kaliasayle
N
Marka
Shalaaboot
0 2 4 6
Km

a female camel) were found to be infected with T. congolense and 5 ml of blood from each animal was injected subcutaneously into a second 'isolation calf'.

The infected animals which were blood sampled had previously been treated with isometamidium chloride, homidium or diminazene aceturate by their owners several days before blood samples were collected from them. Most of the treatments were either with a single dose of one trypanocidal drug or a combination of two doses of two different drugs e.g. one dose of isometamidium chloride and one of diminazene aceturate.

The trypanosome isolate from Shallanbood was entitled SH2.

Calves

The calves that were used in these experiments were Zebu type. Their ages ranged from 13-27 months in the first study and in the second study from 6-12 months. They were all bought from Mogadishu animal market. Originally they were from Aliyaale, Warsheekh, inside Mogadishu or Waharocade. The areas from which these animals came are considered to be tsetse-free. A total of 22 calves were used for the two experiments. Eleven for the first study of which three of them were females and eight were males.

The other eleven calves were used for the second study, two of them were females and the other nine were steers.

The distribution of the calves and their treatments are presented in Table 18.

The animals were brought to the cattle pens at Afgooye at an ex-dairy farm by means of a lorry and they did not pass through any tsetse infested territory. All the animals were identified

Table 18

Calf numbers and trypanocidal drug treatments

Group	Calf no. First Study AF1	Drug treatments (mg/kg) Samorin	Berenil	
A	45301 18840 45303	0.5	3.5	7.0
B	45302 45304 45306	1.0	3.5	7.0
C	45305 45307 45308	2.0	3.5	7.0
D	18839	0	3.5	

Group	Calf no. Second Study SH2	Drug treatments (mg/kg) Samorin	Berenil		
E	260 261 262	0.5	3.5	7.0	
F	263 264 265	1.0	3.5	7.0	10.5
G	266 267 268	2.0	3.5	7.0	10.5
H	269	0	3.5	7.0(x2)	10.5

with an eartag and weighed on their arrival at the cattle pen at Afgooye. Blood sampling and faeces examination was also carried out before they were infected with trypanosomes.

Goats

The goats were white Galla goats. They were six in number, three for the first study and the other three for the second study. All of them were brought from Mogadishu animal market. Originally, they were from the tsetse-free region of Aliyaale and Warsheekh. Their ages ranged from 3.5-5 years.

Maintenance of the calves and goats

The main food for these animals was hay, silaged maize and sorgham stalks. Ground maize grains were supplied when the above mentioned items were in short supply in the dry season. Sliced cassava roots were supplied to the calves for one month (June) as a minimal supplementary feed. Sodium chloride dissolved into the drinking water was also given to these animals. The animals were allowed free access to the drinking water but they were not allowed to graze, in order to avoid contact with the tsetse fly. The cattle pen was located in a tsetse-free area.

Mice

Two groups of healthy white Swiss mice (one group locally bred) of both sexes were used in these studies. The first group (the locally bred), kindly supplied by the Serum and Vaccine Institute at Mogadishu, were 9-10 weeks of age and 28-37g in weight. The second group, kindly supplied by the International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, was six weeks old and 20-26g in bodyweight. The mice were kept at the animal house of the Serum and Vaccine Institute at Mogadishu.

They were kept in clean plastic cages and allowed free access to drinking water. They were given ground sorghum grains, carrots and salad ad libitum.

Trypanocidal Drugs

Isometamidium chloride (Samorin, RMB) was administered to the calves by deep intramuscular injection into the neck, and in the goats into the hind limb. Mice were given the drug by intraperitoneal injection.

In the case of the calves diminazine aceturate (Berenil, Hoechst) was given by intramuscular injection.

Other drugs

The other drugs that were given to calves were sulphadimidine tablets which were ground into powder and used for spreading on the wounds caused by foot rot. The tablets were also given for control of diarrhoea. The calves were sprayed every thirty days with an acaracide (Bacdip) to control ticks.

An anthelmintic (Panacur, Hoechst) was given to the calves before the trypanosome infection in order to remove existing nematode infections.

Several injections of oxytetracycline were administered intramuscularly to one calf (45304) which had received a bruise on the lower part of a front leg.

Blood Sampling and Examination

Blood samples were collected three times per week from the infected calves, goats and mice for parasitological examination. Fixed thin smears of blood were also collected once per week and stained by Giemsa's method. Blood was collected from the

ruminants by jugular venepuncture into evacuated tubes containing EDTA and centrifuged in capillary tubes in a microhaematocrit centrifuge. After reading the haematocrit value the buffy coat was placed on a slide and examined for the presence of trypanosomes.

Blood was collected from the mice by tail snip venesection and placed directly on a slide for wet film microscopic examination.

Immunosuppressive Treatment of Mice

In the first study the mice had to be immunosuppressed in order to allow a patent parasitaemia to develop. This was achieved by the intraperitoneal injection of 0.2 ml (4mg/mouse) of cyclophosphamide (Sigma) in saline.

Body Weight

Periodically throughout the studies and immediately prior to trypanocidal drug treatment the calves and goats were weighed using a weighing crate.

The mice were weighed before treatment on a top-pan balance.

RESULTS

First Isolation - AFI

Cattle

Parasitaemia

The isolation calf became parasitaemic seven days after the subinoculation of blood from the village cattle. Following treatment with 0.5mg/kg isometamidium on day 18 it became aparasitaemic until day 32. On day 35 infected blood was taken from the isolation calf and inoculated into ten calves, three goats and 35 mice (see experimental design).

The ten calves in Groups A, B, C and D all became parasitaemic on day 11 post inoculation (see Figures 2, 4, 6). On day 29 the calves in Groups A, B and C were treated with 0.5, 1.0 and 2.0mg/kg isometamidium respectively. Following treatment there was a period of aparasitaemia before relapse infections developed. In Group A treated with 0.5mg/kg isometamidium relapses occurred on days 44 and 46, and one calf (18840) died on day 44. In Group B relapse infections developed on days 53-58, and in Group C, treated with 2.0mg/kg relapses were delayed until days 66-67.

The control calf (18839) Group D was not treated with isometamidium and by day 44 had a high parasitaemia and died despite treatment that day with diminazine aceturate (Berenil) at 3.5mg/kg.

Following the development of relapse infections after isometamidium treatment in Groups A, B and C, the calves were treated with diminazine aceturate first at a dose of 3.5mg/kg and later with 7.0mg/kg.

Following the first treatment with diminazine aceturate the calves became aparasitaemic for approximately 30 days (range 29-33 days) before trypanosomes were again detectable in the blood. Following the second diminazine aceturate treatment, this time at 7.0mg/kg the period of aparasitaemia was relatively short in all the groups, namely 19 days (range 14-25 days) and all the calves developed moderately high levels of parasitaemia by termination of the experiment on day 149 post infection.

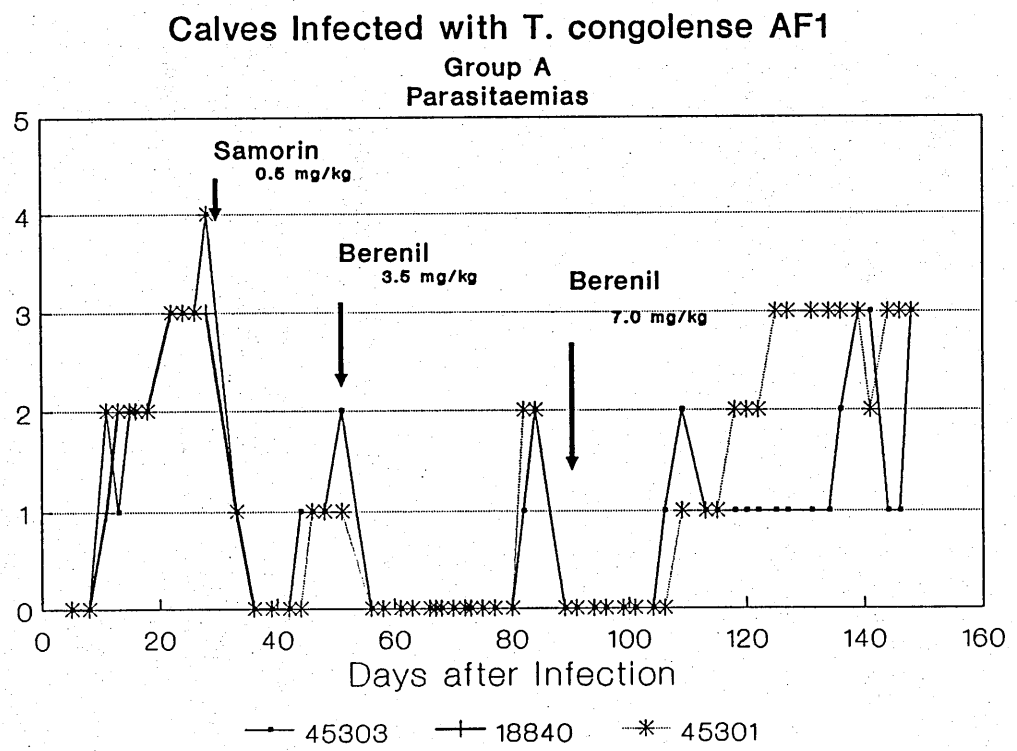


Fig. 2 Parasitaemias of calves infected with T. congolense AF1

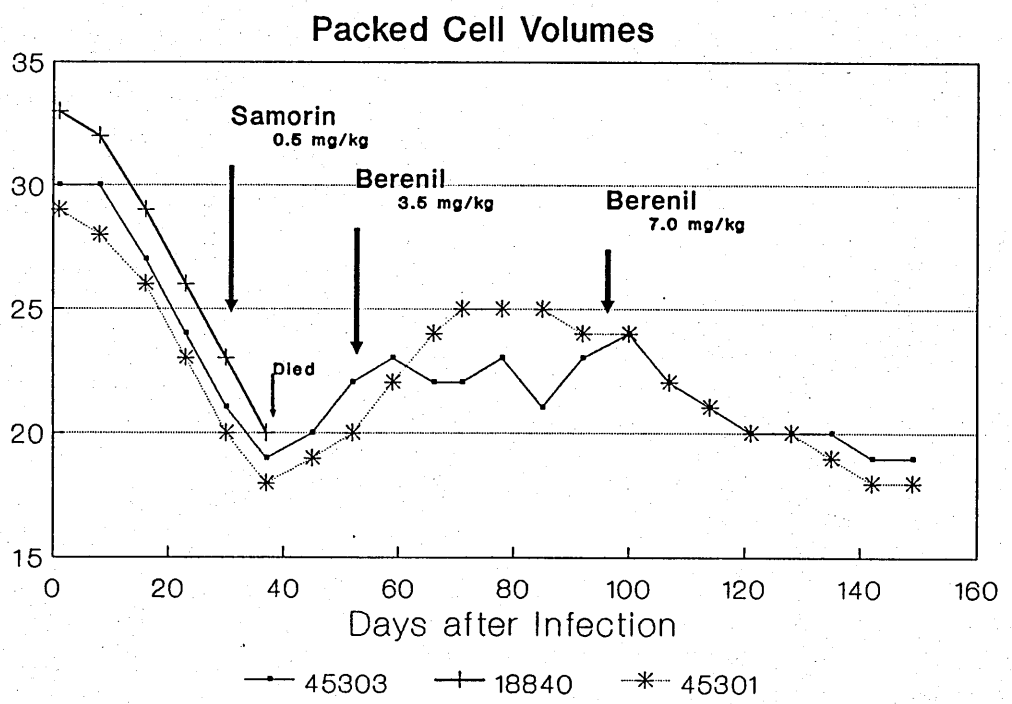


Fig. 3 Packed cell volumes of calves infected with T. congolense AF1

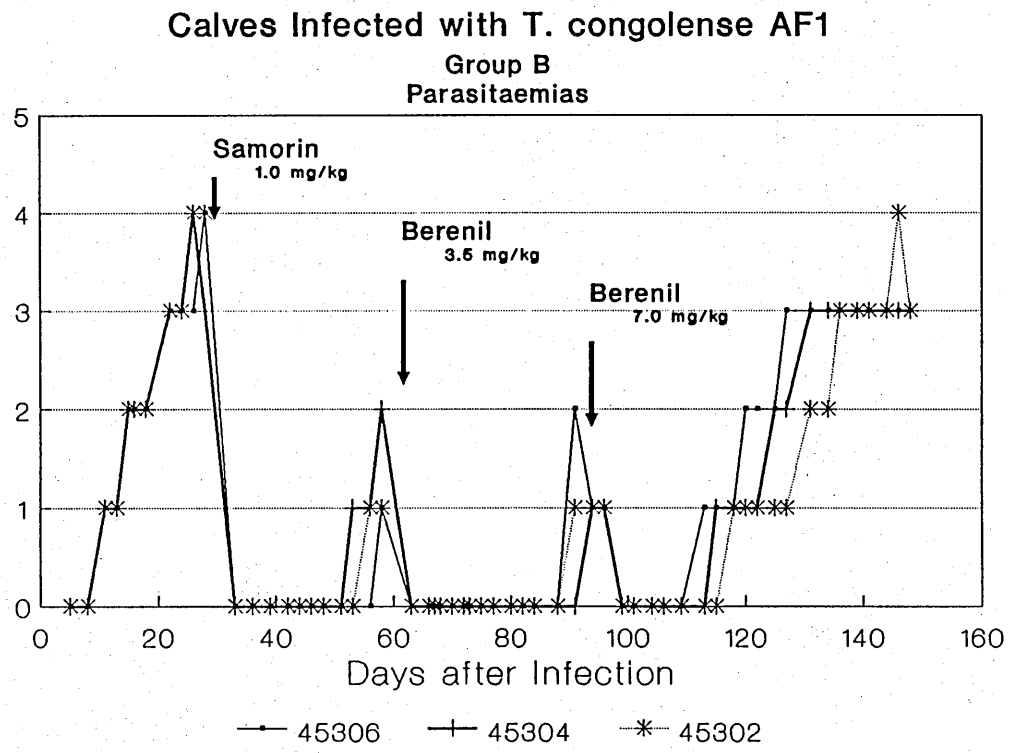


Fig. 4 Parasitaemias of calves infected with T. congolense AF1

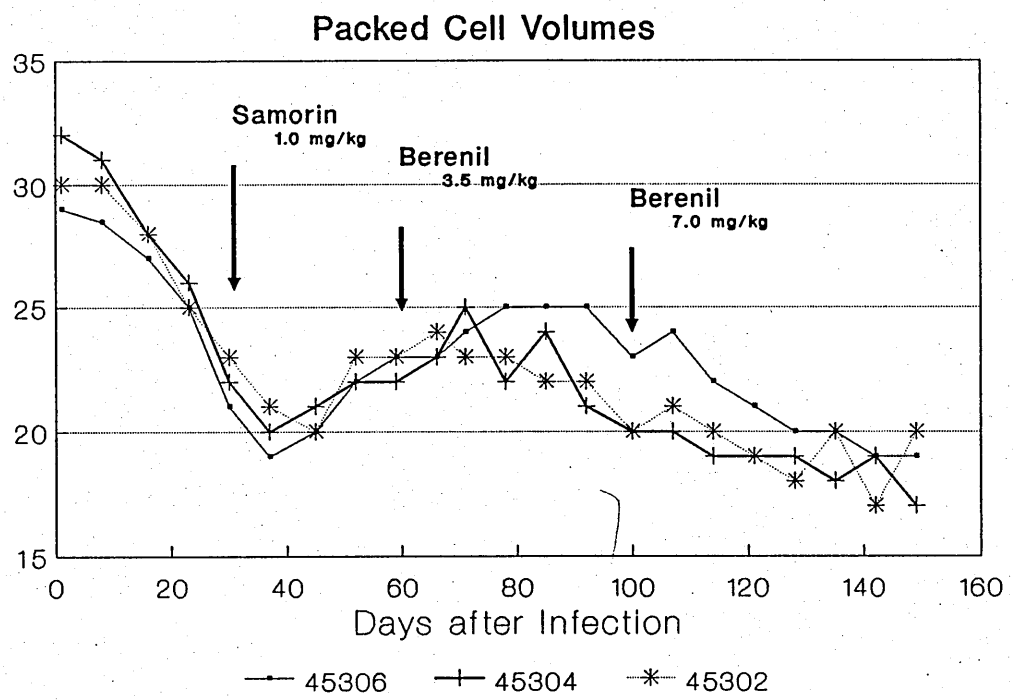


Fig. 5 Packed cell volumes of calves infected with T. congolense AF1

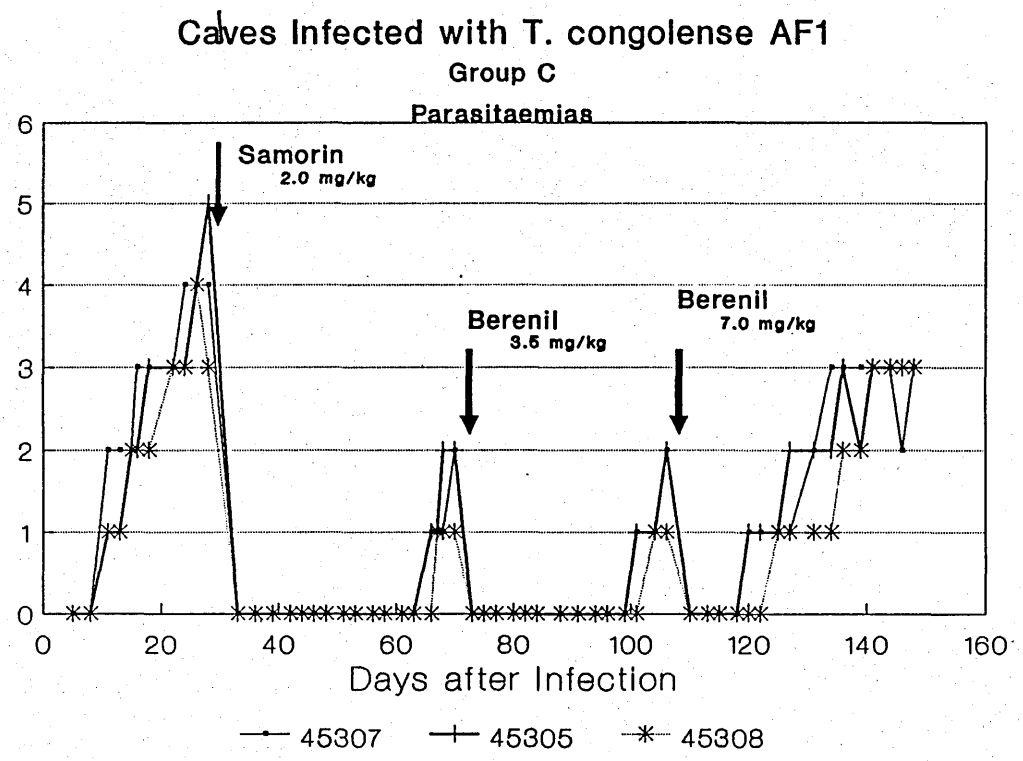


Fig. 6 Parasitaemias of calves infected with T. congolense AF1

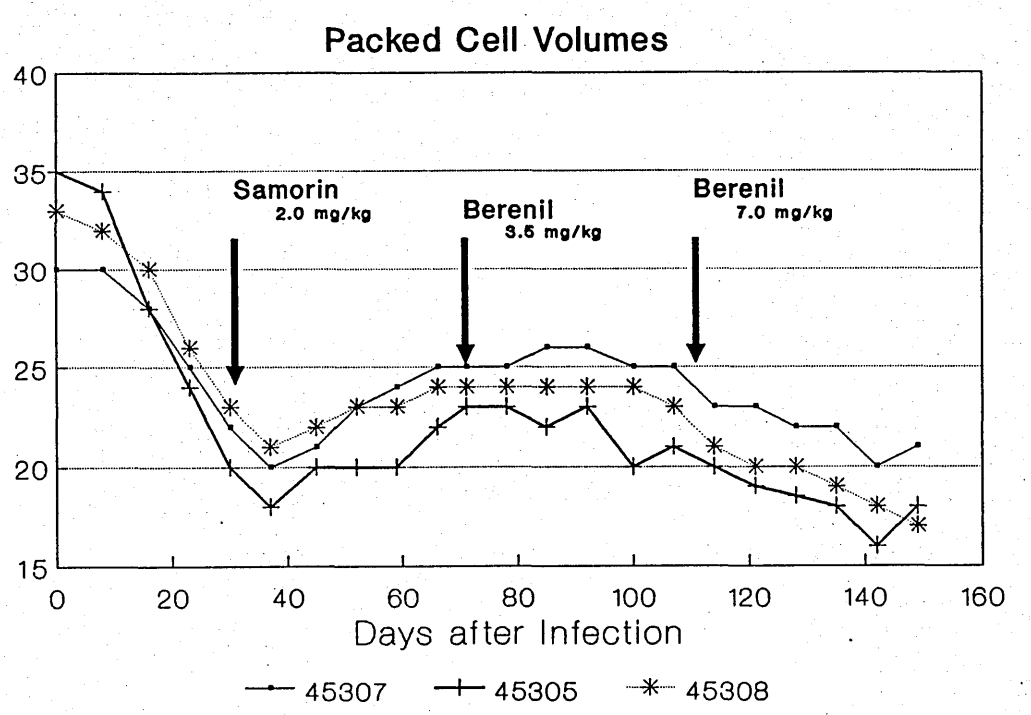


Fig. 7 Packed cell volumes of calves infected with T. congolense AF1

Anaemia

The haematocrit values of the calves in Groups A, B and C are presented in Figures 3, 5 and 7.

It is apparent that following infection all the calves developed anaemia and by day 40 the haematocrit values had been reduced to approximately 20%. In the two calves that died by day 45 (namely 18840 of Group A and 18839, the control (Group D) both had haematocrit values of 20% immediately prior to death. In the surviving calves treatment with Samorin was followed by some improvement in haematocrit values to approximately 25%. However the development of subsequent relapse infections were followed by falls in haematocrit value to below 20%.

Body Weights

All the calves lost weight (Figures 8, 9 and 10) as a result of the infection despite chemotherapy.

Goats

Three goats (Nos. 1, 2 and 3) were infected with the T. congolense isolate AF1 by inoculation of parasitaemic blood from the first isolation calf (see experimental design). Following inoculation the goats developed a patent parasitaemia on days 23, 19 and 19 respectively. By day 34 all three goats had high parasitaemias (Figure 11) and goats 1 and 2 were treated with 0.5mg/kg Samorin, whilst goat 3 remained untreated. Following treatment goats 1 and 2 became aparasitaemic until days 46 and 47 respectively. On day 43 goat 3 and on day 51 goats 1 and 2 were treated with diminazine aceturate (3.5mg/kg). Following this treatment the goats became aparasitaemic and remained so until termination of the experiment on day 62,

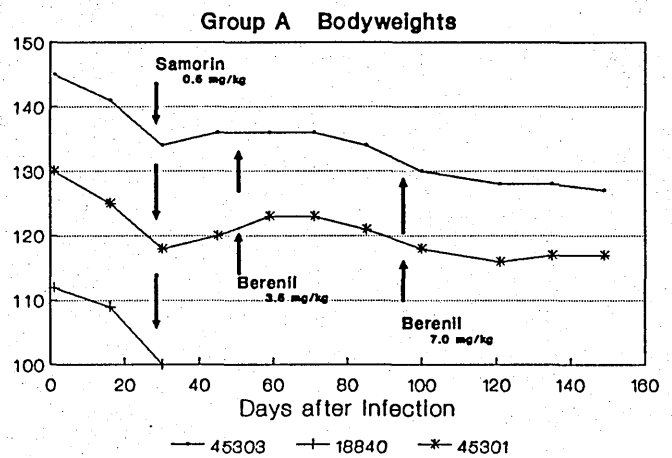


Fig. 8 Bodyweights of calves infected with T. congolense AF1

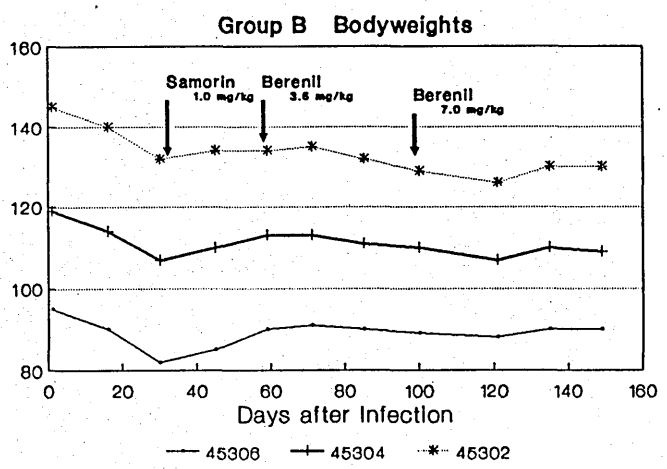


Fig. 9 Bodyweights of calves infected with T. congolense AF1

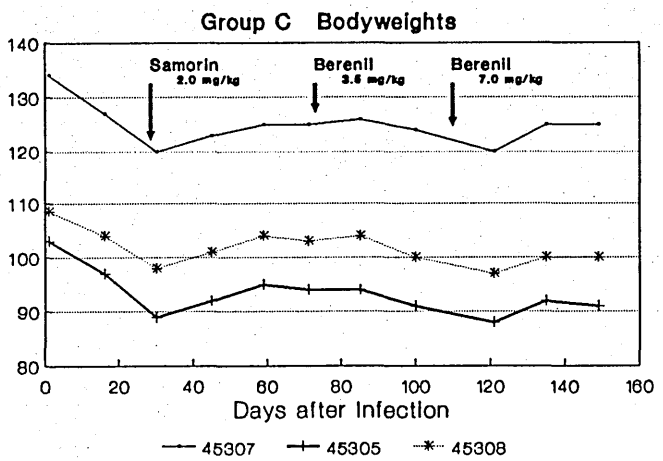


Fig. 10 Bodyweights of calves infected with T. congolense AF1

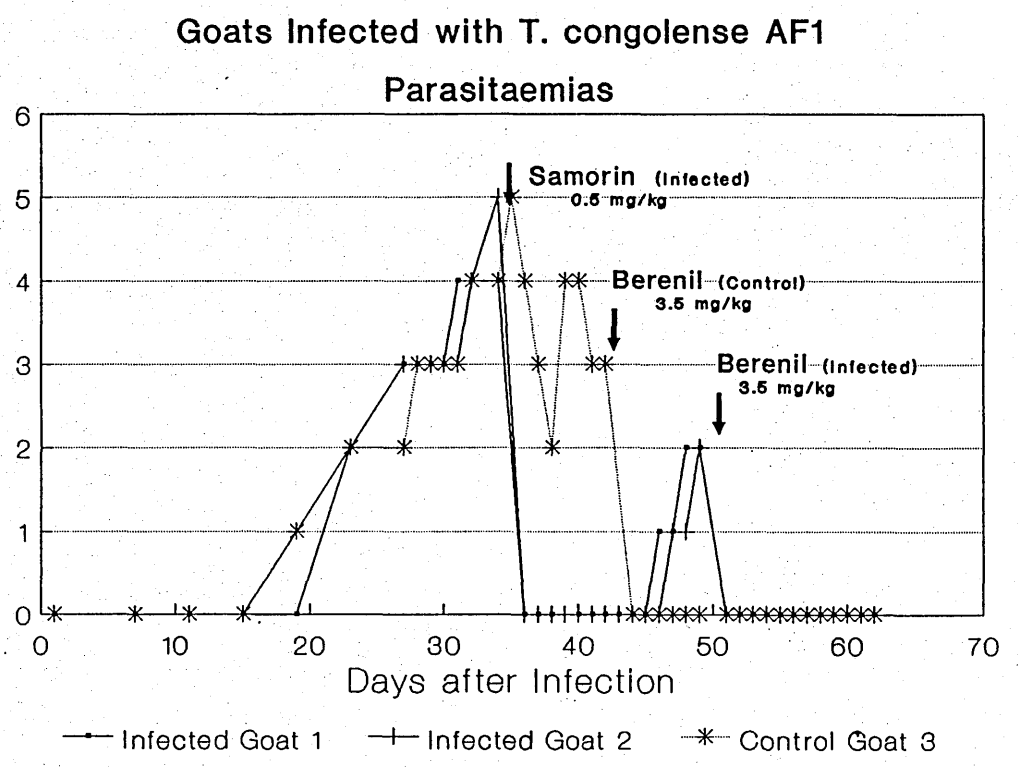


Fig. 11 Parasitaemias of goats infected with T. congolense AF1

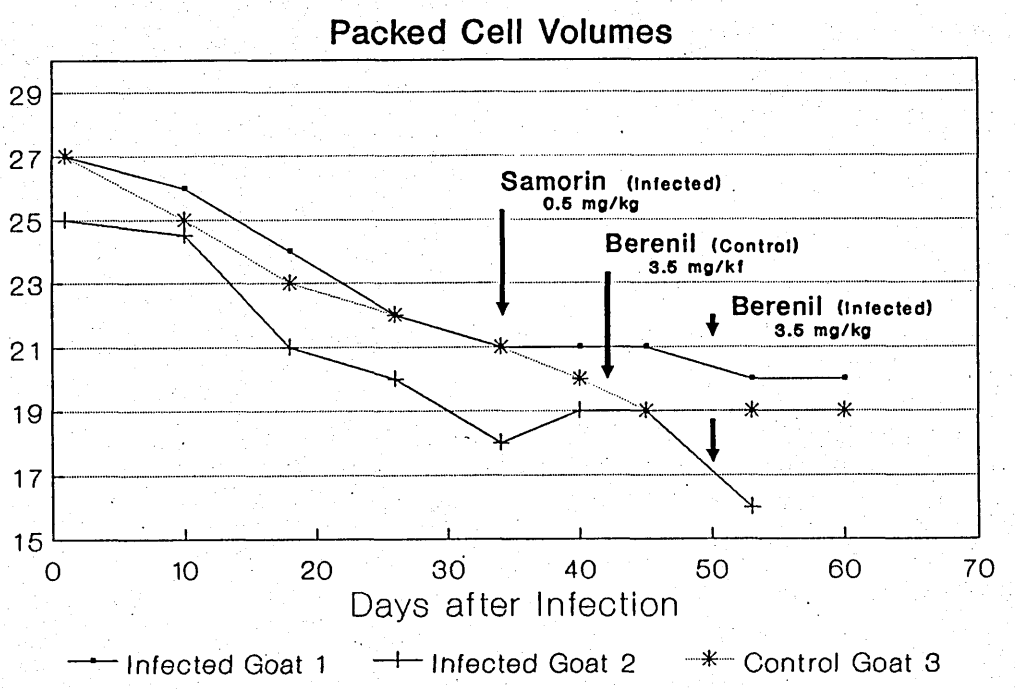


Fig. 12 Packed cell volumes of goats infected with T. congolense AF1

although goat 2 died suddenly of unknown causes on day 57.

Infection was associated with a decrease in haematocrit values in the three goats (Figure 12). By day 34 the values of goats 1, 2 and 3 were 21%, 18% and 21% respectively and despite treatment with isometamidium and/or diminazine aceturate they remained at 20% or below until termination of the experiment. Goat 2 which died on day 57 had an haematocrit value of 16% immediately prior to death.

Mice

A total of 35 mice were infected with T. congolense isolate AF1 and divided into seven groups of five mice (Groups A - G). Following the development of patent parasitaemias they were treated with isometamidium at doses between 0.5 - 20mg/kg. One group (G) remained as untreated controls. The mice were monitored regularly for the development of relapse infections and these occurred in Groups B, C, D, E and F (see Table 19). In Group F, four of the five mice remained parasitaemic following treatment. There was a correlation between the time of relapse, the incidence of relapse and the dose of drug such that mice treated at lower doses showed a shorter time to relapse and a greater incidence of relapse than mice treated at higher doses. Mice in Group A, treated with 20mg/kg Samorin did not relapse during the period of observation (60 days).

Second Isolation - SH2

Cattle

Parasitaemia

The isolation calf became parasitaemic nine days after the subinoculation of blood from the infected village cattle.

Table 19

The development of relapse infections in mice infected with *T. congolense* isolate AF1 and treated with various doses of isometamidium

Group	Treatment	Days to relapse	No. relapsed/ No. treated	Comments
A	20 mg/kg Samorin	No relapse	0/5	
B	10 mg/kg Samorin	42, 51	2/5	
C	5 mg/kg Samorin	25, 38, 25, 25, 31	5/5	
D	2 mg/kg Samorin	10, 14, 14, 18, 18	5/5	
E	1 mg/kg Samorin	8, 10, 10, 14, 18	5/5	
F	0.5 mg/kg Samorin	10	5/5(1/5)	4 of 5 mice remained positive after treatment
G	No treatment	-	-	

Following treatment with 0.5mg/kg isometamidium on day 22 it became aparasitaemic until day 40. On day 44 infected blood was taken from the isolation calf and inoculated into ten calves, three goats and 35 mice (see experimental design).

The ten calves in Groups E, F, G and H became parasitaemic on days 14-18 and by day 36 all had developed moderate to heavy parasitaemias (Figures 13, 15 and 17). On day 39 the calves in Groups E, F and G were treated with 0.5, 1.0 and 2.0mg/kg isometamidium respectively. Following treatment there was a period of aparasitaemia which varied in duration with the dose of drug. In Group E treated with 0.5mg/kg isometamidium relapses occurred within 7-9 days i.e. 46-49 days post infection.

In Group F relapse infection developed within 14 days i.e. days 52-53 post infection, whilst in Group G, treated with 2.0mg/kg isometamidium relapses occurred by 24 days post treatment. The control calf 269 (Group H) which did not receive isometamidium maintained a moderate parasitaemia until it was treated with diminazine aceturate (3.5mg/kg) on day 49. Following the development of relapse infections after isometamidium treatment in Groups E, F and G, the calves were treated with diminazine aceturate, first with 3.5mg/kg and later with higher doses.

Following the first treatment with Berenil all the calves became aparasitaemic for approximately 16 days (range 13 - 22 days) and then relapsed although two calves in Group E (261, 262) died eight and ten days after treatment respectively. The second treatment with Berenil was given at a dose of 7.0mg/kg. In Group E the remaining calf (260) died 25 days later, on day 88

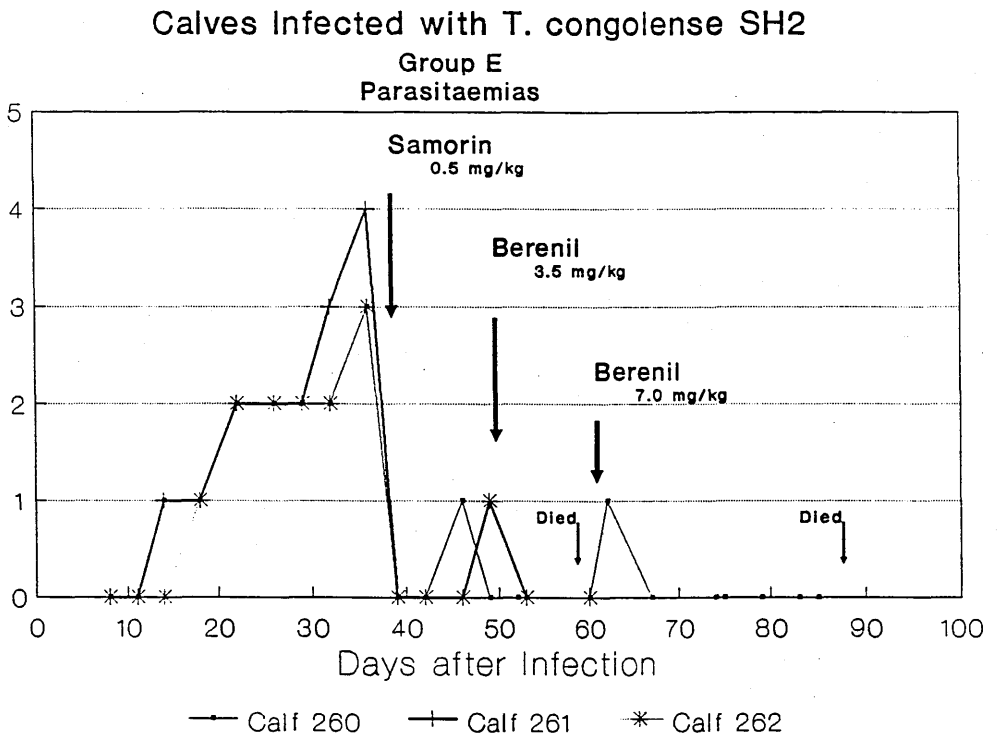


Fig. 13 Parasitaemias of calves infected with T. congolense SH2

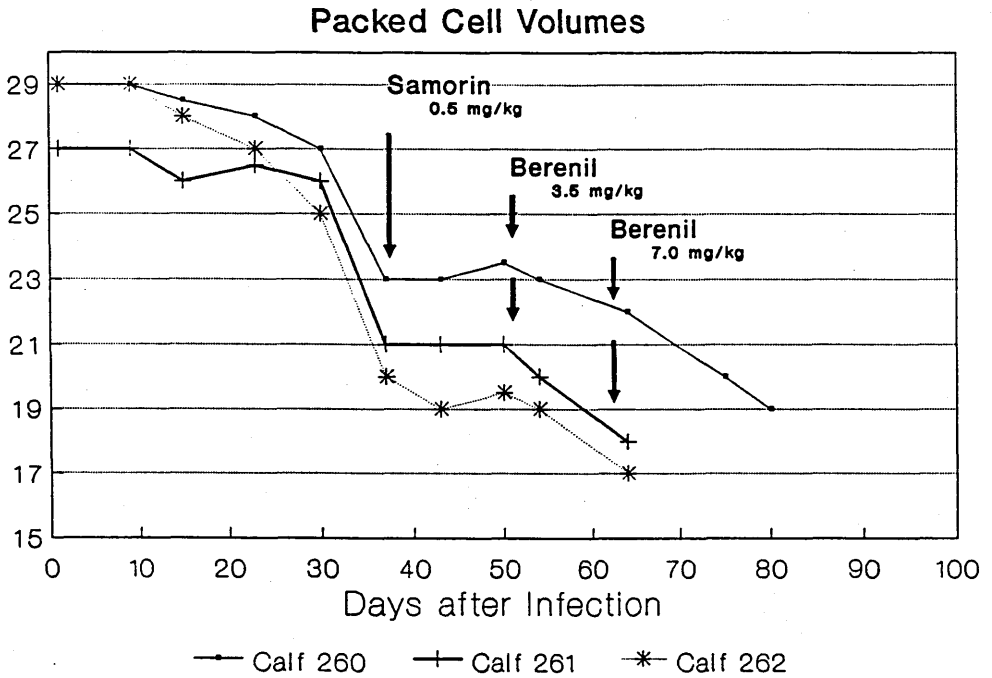


Fig. 14 Packed cell volume of calves infected with T. congolense SH2

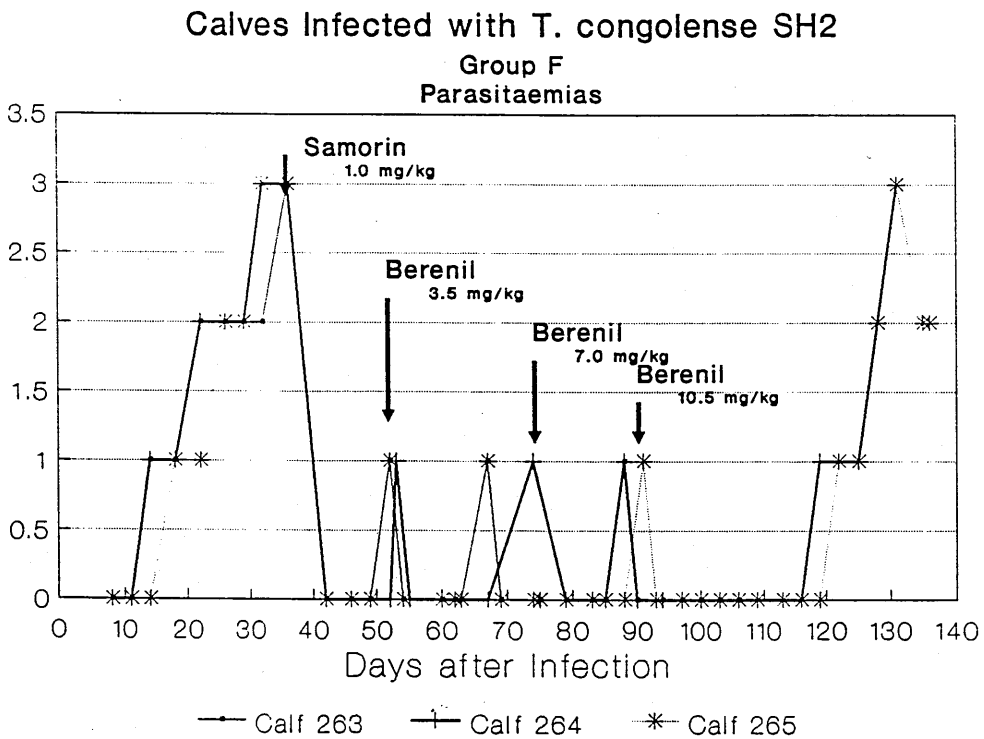


Fig. 15 Parasitaemias of calves infected with T. congolense SH2

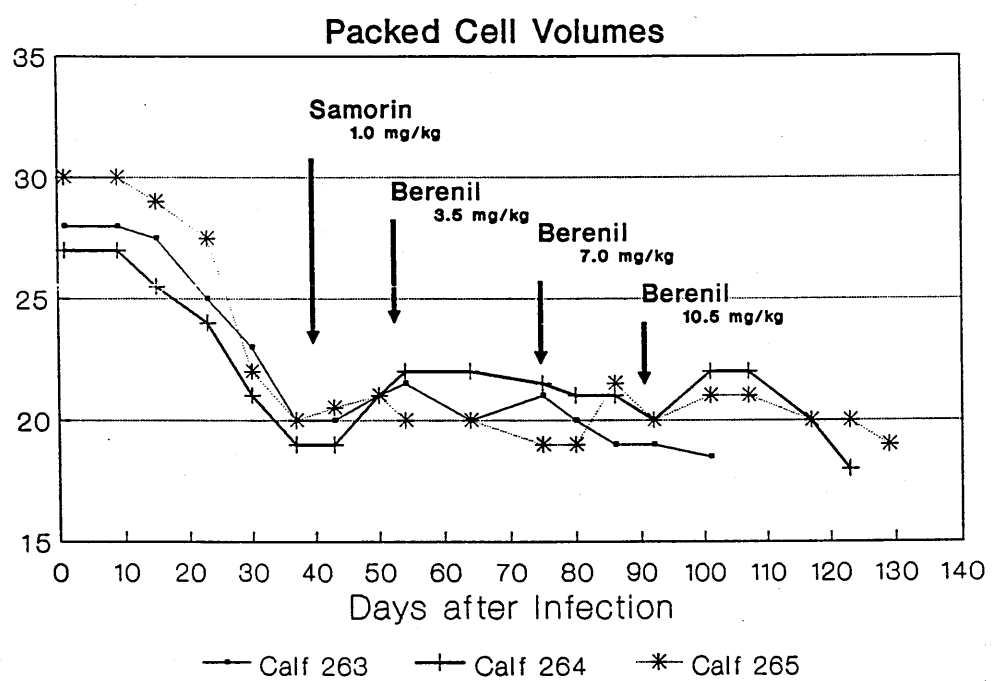


Fig. 16 Packed cell volumes of calves infected with T. congolense SH2

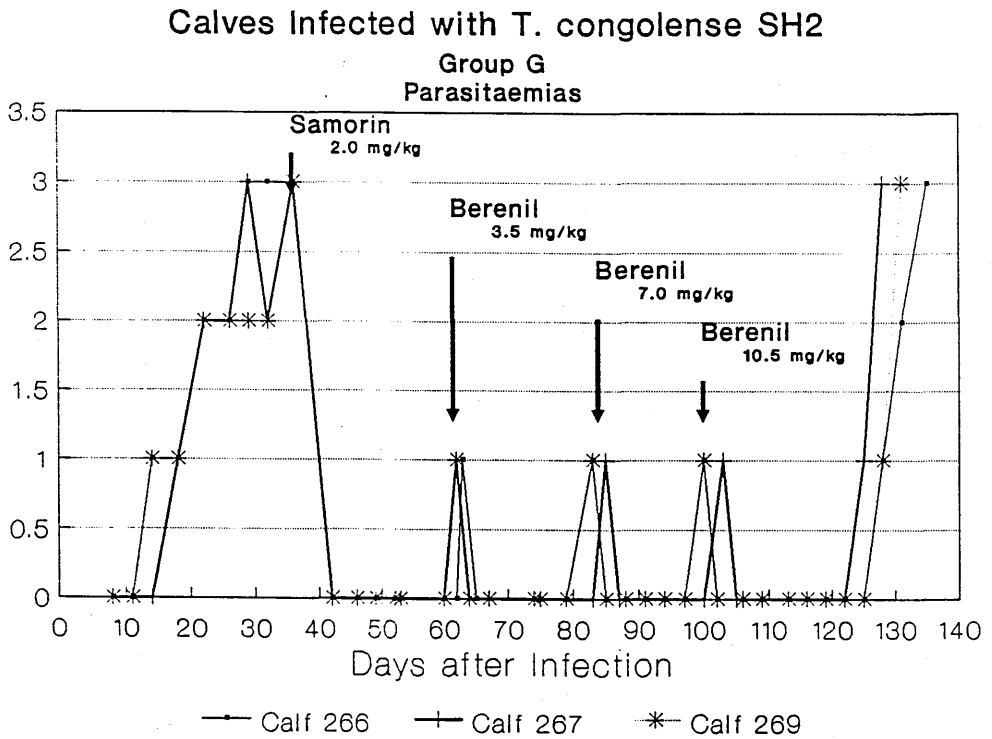


Fig. 17 Parasitaemias of calves infected with T. congolense SH2

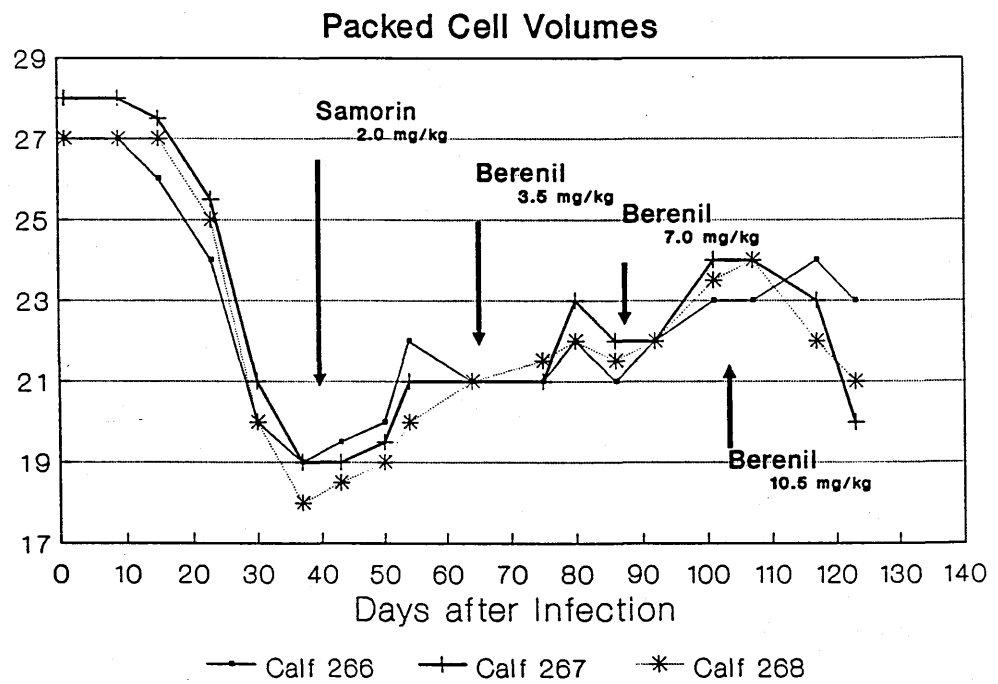


Fig. 18 Packed cell volumes of calves infected with T. congolense SH2

post infection. In Group F relapse infections developed 13, 14 and 20 days after treatment. Similarly the calves in Group G relapsed 15 days after treatment with 7.5mg/kg. The calf in Group H (269) received two treatments at this dose and relapsed at 21 days and 18 days after each treatment. Finally the surviving calves in each group were treated with three times the normal dose, i.e. 10.5mg/kg Berenil. In Group F, one calf (263) died 18 days later on day 109 post infection whilst the remaining two calves relapsed 28 days later. In Group G relapses occurred 25, 19 and 25 days post treatment. The calf in Group H relapsed 22 days after treatment. Three of the remaining six calves died before termination of the experiment at 136 days after infection.

Anaemia

The haematocrit values of the calves in Groups E, F and G are presented in Figures 14, 16 and 18.

Prior to infection the values ranged between 27 - 30% and following infection they decreased to between 18 and 24% on the day of Samorin treatment (day 39). Following this treatment the haematocrit values stabilised in Groups E and F before declining further later in the experiment. In Group G the haematocrit values showed some increase following the Samorin and subsequent Berenil treatments before falling during the final relapse infection.

Body Weights

The calves in the second study were younger and had lower bodyweights than in the first experiment. As in the first study all the calves lost weight as a result of the trypanosome infection.

Goats

Three goats (Nos. 4, 5 and 6) were infected with the T. congolense isolated SH2 (see experimental design). Following infection the goats developed a patent parasitaemia on days 8, 9 and 8 respectively. By day 18 all three goats had high parasitaemias and two goats (4 and 5) were treated with 0.5mg/kg Samorin, whilst the third goat (No.6) remained untreated. Following treatment goats 4 and 5 became aparasitaemic and remained so for 10 and 11 days respectively. On day 24 goat 6 and on day 31, goats 4 and 5 were treated with Berenil (3.5mg/kg). Between 14 and 16 days later all three goats developed relapse infections. A subsequent Berenil treatment at 7mg/kg on day 50 (goats 4 and 5) or day 41 (goat 6) resulted in a period of aparasitaemia in goats 4 and 5 until termination of the experiment on day 57, whilst goat 6 died nine hours after treatment.

Infection was associated with a decrease in haematocrit from values of 27 - 30% at the beginning of the experiment to values of 23 - 25% by the time of the isometamidium treatment. The haematocit values continued to decline and the values of goats 4, 5 and 6 at termination of the experiment or death in the case of goat 6 were 17, 21 and 15% respectively.

Mice

A total of 35 mice were infected with T. congolense isolate SH2 and divided into seven groups of five mice (Groups H - N). Following the development of patent parasitaemias they were treated with isometamidium at doses between 0.5 - 20mg/kg. One

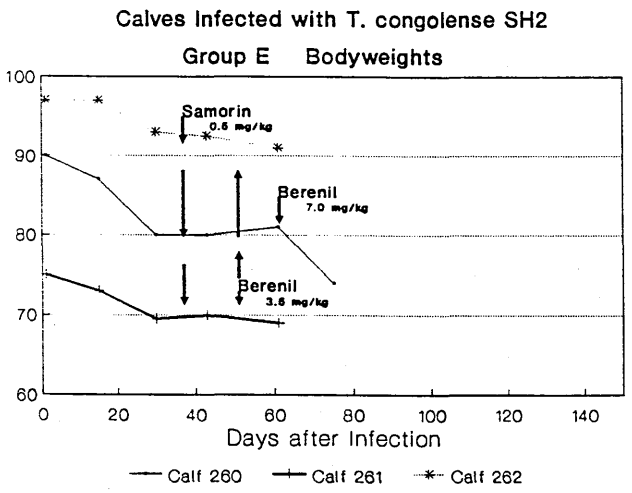


Fig. 19 Bodyweights of calves infected with T. congolense SH2

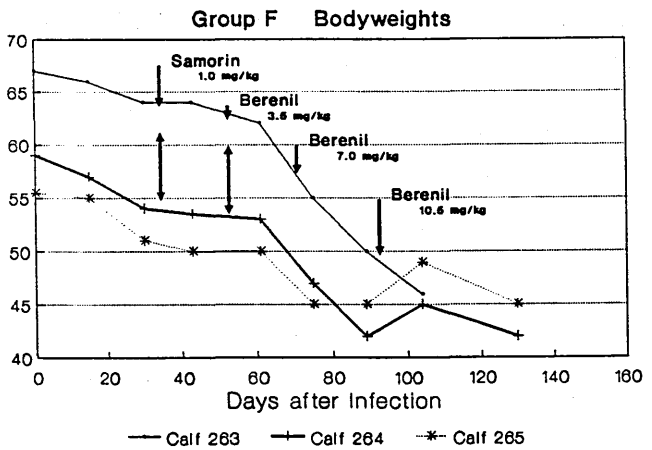


Fig. 20 Bodyweights of calves infected with T. congolense SH2

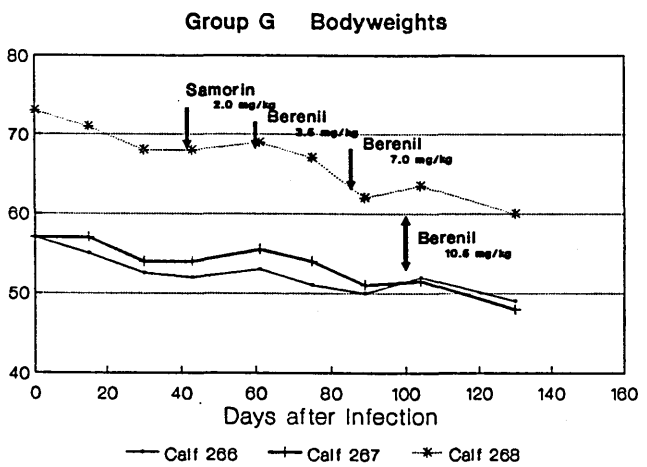


Fig. 21 Bodyweights of calves infected with T. congolense SH2

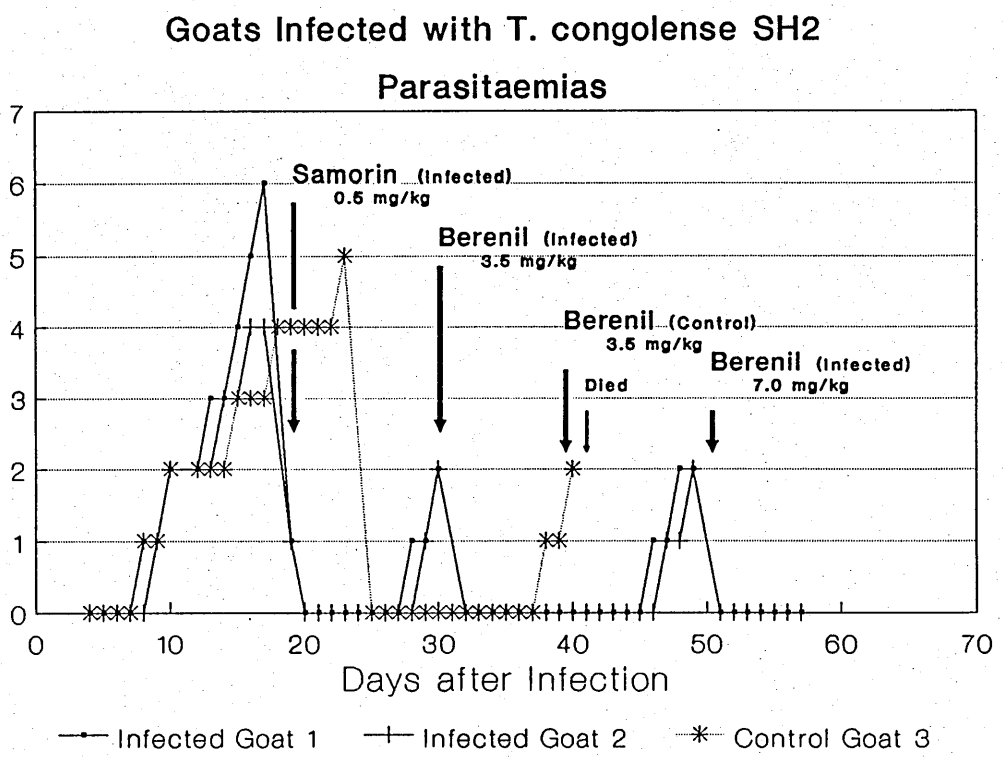


Fig. 22. Parasitaemias of goats infected with T. congolense SH2

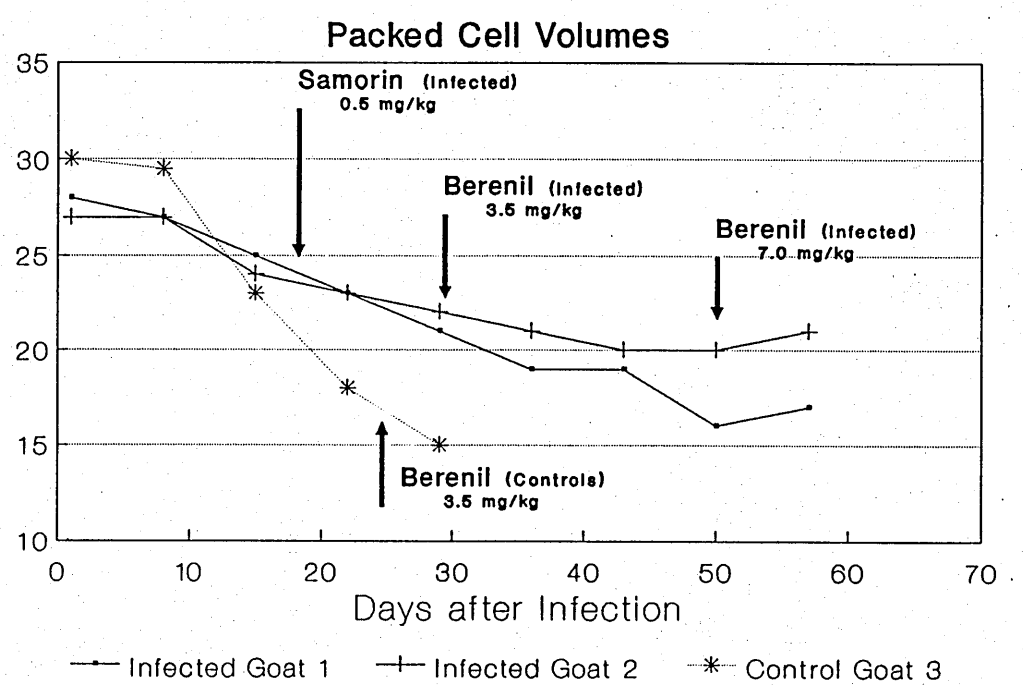


Fig. 23 Packed cell volumes of goats infected with T. congolense SH2

group (N) remained as untreated controls.

The mice were monitored regularly following treatment. Groups L and M remained parasitaemic whilst the mice in the other treated groups became aparasitaemic before developing relapse infections (Table 20). There was a correlation between the time to and incidence of relapse infection with drug dosage and in Group H treated with 20mg/kg only three of five mice developed relapse infections and these occurred between 50-55 days after treatment.

Table 20

The development of relapse infections in mice infected with *T. congolense* isolate SH2 and treated with various doses of isometamidium

Group	Treatment	Days to relapse	No. relapse/ No. treated	Comments
H	20 mg/kg Samorin	55, 50, 55	3/5	
I	10 mg/kg Samorin	27, 29, 42	3/5	1 mouse died before treatment
J	5 mg/kg Samorin	17, 27, 20, 31	4/5	1 mouse died before treatment
K	2 mg/kg Samorin	18, 9, 14, 14, 9	5/5	
L	1 mg/kg Samorin		5/5	All 5 mice remained positive after treatment
M	0.5 mg/kg Samorin		5/5	All 5 mice remained positive after treatment
N	No treatment	-	-	

DISCUSSION

Two isolates of T. congolense have been obtained from the Lower Shabelle region of Southern Somalia which displayed high levels of resistance to therapy with Samorin (isometamidium chloride) and Berenil (diminazine aceturate) in experimental cattle, goats and mice.

Drug resistance by trypanosomes has been reported from several areas of Africa but the strains isolated in the present experiment displayed a remarkably high level of resistance to the two most commonly available trypanocides. Furthermore these two compounds are normally regarded as a 'santative pair' since cross-resistance between them has rarely been reported at least by T. congolense, although isolates of T. vivax from the Tana River area of Kenya have been shown to possess multiple resistance (Rottcher and Schillinger, 1985). Other examples of high levels of drug-resistance by T. congolense have been reported by Gitaha (1981) from Kenya and Pinder and Authie (1984) in West Africa.

In the present study unsophisticated and relatively low cost methods were used to isolate and examine drug resistant strains of T. congolense. This was because of logistical and financial restraints in Somalia. Nevertheless the methods used were shown to be highly effective and clearly demonstrated the existence of drug-resistant strains in the sampled village cattle. For reasons of eonomy multiple inoculations into a single recipient calf were conducted and for that reason it is not possible to know how many of the donor cattle were infected with drug-resistant strains of T. congolense. All that can be stated is

that in both sampling exercises at least one of the donor animals was infected with a drug-resistant strain. The subsequent treatment and expansion of the relapse infection into naive cattle, goats and mice was a novel development and is a valuable demonstration of a procedure which could be adopted in many field situations in Africa, with the proviso that the experimental cattle are not exposeed to tsetse challenge.

Other methods for the detection of drug-resistant trypanosomes have often relied on direct subinoculation into mice or more recently by adaptation of the trypanosomes to laboratory culture and tests in vitro. Both of these techniques have a number of disadvantages (Sones, Njogu and Holmes, 1988). In the case of the former, not all strains of T. congolense are infective to mice and the results from mice may only give a general indication of drug resistance. In the case of in vitro culture it is frequently difficult and time-consuming to adapt 'field' strains to culture and requires relatively expensive sophisticated laboratory equipment.

In the present study it was possible to make direct comparison between cattle and mice and to a limited extent from goats also. In the case of both isolates the minimum curative dose in cattle was above 2mg/kg for isometamidium and above 7.5mg/kg and 10.5mg/kg for Berenil for isolates AF1 and SH2 respectively. In mice the minimum curative dose was less than 20mg/kg isometamidium for isolate AF1 and more than 20mg/kg for isolate SH2. These results support the general view that there is an approximate ten-fold relationship for many strains of

T. congolense between the minimum curative dose for trypanocidal drugs in mice and cattle (e.g. Pinder and Authie, 1984).

A different approach to the quantitation of drug-resistance was suggested by Williamson and Stephen (1960) who developed a method using sheep, to assess the sensitivity of strains of T. vivax to homidium, based on the interval from subcurative treatment to subsequent relapse. The interval from subcurative treatment to relapse was taken as an index of the degree of homidium resistance : the shorter the interval, the more resistant the strain. A good indication of the relative sensitivities of the two isolates of T. congolense used in the present study could be obtained by a similar approach. Thus isolate SH2 which was found to be more resistant to isometamidium in mice than isolate AF1, consistently had a shorter time to relapse in cattle following treatment with isometamidium and Berenil. However in goats the distinction was less clear, with SH2 relapsing at 10 and 11 days post-treatment whilst AF1 relapsed on days 11 and 12 in the two treated goats.

A further important observation in the present study was that both isolates were highly pathogenic and this was especially the case with the more resistant isolate, SH2, in which most of the cattle died before termination of the experiment. Previously it has been suggested that drug-resistant strains of trypanosomes may be less pathogenic then drug-sensitive strains (Stephen, 1962).

The high levels of drug-resistance and pathogenicity observed in the two isolates raises important practical problems over the control of such infections in the field. Fortunately it

would appear from field observations and anecdotal evidence that such infections are relatively rare but this can only be determined by much wider use of tests for drug-resistance in the field possibly using similar methods to those used in the present study. In the meantime it is important for veterinarians to develop possible strategies for the control of drug-resistant strains of trypanosomes in the field. One possible approach is examined in the following chapter.

CHAPTER THREE

STUDIES ON DRUG-RESISTANT STRAINS OF *Trypanosoma congolense*

SECTION II

A COMPARISON OF INTRAVASCULAR AND INTRAMUSCULAR ISOMETAMIDIUM ADMINISTRATION FOR THE TREATMENT OF A DRUG-RESISTANT STRAIN OF *T.congolense* IN GOATS

INTRODUCTION

It is apparent from the literature reviewed in the introduction and the studies in Somalia described in Section I of this chapter that drug-resistance can emerge as a serious problem in the control of bovine trypanosomiasis. In situations such as that described in Somalia where resistance to the 'sanative pair' of drugs, namely isometamidium and diminazine aceturate, exists, the veterinarian is faced with serious difficulties in selecting suitable treatment.

In most cases of drug resistance the resistance is not absolute and is generally dose dependent. Therefore if higher levels of drug concentration in the blood can be achieved it may be possible to successfully treat the infection. A possible way of achieving high blood concentrations was investigated in the present study using isometamidium to treat a strain of T. congolense in goats which had been previously shown to be resistant to treatment with 1.0 mg/kg in cattle (Sones et al, 1988). The treatment consisted of intravenous administration of isometamidium. Administration of isometamidium by the intravenous route has been used by various workers in the past (Toure, 1973; Balis, 1977; Schillinger, Maloo and Rottcher, 1984) and it has recently been advocated as a valuable method of treatment in cattle by field veterinarians working at the Kenyan coast (Dowler, 1987 personal communication; Munsterman, 1988 personal communications). They claim it has two important advantages over the intramuscular route, namely that tissue lesions in muscle at the site of injection are avoided, and it is a more effective treatment in areas where drug-resistant trypanosomes may be a

problem, possibly because higher blood concentrations of drug may be achieved by this route. This method of treatment was compared with the more conventional intramuscular administration of isometamidium in goats.

In this study there was also an opportunity to quantify the concentration of isometamidium in the blood of the treated goats using a recently developed ELISA technique (Holmes, Rowell, Phillips, Sutherland, Gault and Urquhart, 1988). The competition micro ELISA is in the process of development for the detecion of isometamidium of treated ruminants in field laboratories in Africa. The optimal assay conditions have been determined and using spiked samples, standard curves within the range of 0.05 - 500ng/ml have been obtained. The assay is apparently specific for isometamidium and there is insignificant cross reactivity with Ethidium (homidium bromide) or Berenil (diminazine aceturate). Only small amounts (5ul) of serum or plasma are required for the assay.

Experimental design

Groups of two or three goats were infected with a drug-resistant strain of T. congolense and subsequently treated with isometamidium at a dose of 0.5 mg/kg by intravenous (iv) (goats 106,107 and 108) or intramuscular (im) (goats 109 and 115) injection. Following the development of relapse infections the goats were retreated at the higher dose of 1.0 mg/kg by the same method as previously. One goat (114) was maintained as an infected untreated control but subsequently treated by intravenous injection at 0.5mg/kg isometamidium and later by

intramuscular injection at a dose of 1.0mg/kg. The animals were examined for at least 50 days post treatment.

Materials and methods

Experimental goats

British cross-bred goats were obtained from a commercial source. The goats were aged approximately nine months. Prior to infection all goats were treated with 10% suspension of fenbendazole (Panacur, Hoechst).

The goats were housed in a fly-proof isolation unit on a concrete floor and bedded with wood shavings. They were cleaned out daily. The goats were provided with hay ad libitum, supplemented with 1.0 kg/day of concentrate (306 Ewbol store lamb finisher pellets, BOCM Silcock Ltd., England). Water, rock-salt and a mineral lick (super chelated rockies Tythebarn Ltd., England) were always available.

Trypanosomes

An isometamidium-resistant strain of T. congolense GRVPS 56/2 (originally isolated by Pinder and Authie, 1984 and subsequently designated KETRI 2880 by Sones et al, 1988) was used in this experiment. Each goat was infected intravenously, with 1×10^5 trypanosomes.

Trypanosome detection

Jugular blood samples were regularly obtained using heparinised vacutainers. Prior to examination the blood samples were placed on a rotary mixer for at least five minutes to ensure thorough mixing. Duplicate plain capillary tubes were filled with blood, sealed at one end with cristaseal and centrifuged for

five minutes using a microhaematocrit centrifuge. Following centrifugation the buffy coat was subjected to microscopic examination.

Drugs

The trypanocidal drug used in this experiment was isometamidium chloride (Samorin, RMB). It was administered either by intramuscular or intravenous injection of a 0.25% w/v solution. The doses of isometamidium used in this experiment were 0.5 and 1.0 mg/kg.

Packed cell volume

The packed cell volume (PCV) was determined using the jugular blood sample, prior to examination of the buffy coat for trypanosomes. Blood samples were centrifuged in a microhaematocrit centrifuge for five minutes and PCV's read using a microhaematocrit reader (Hawksley, England).

Body temperature

The rectal temperature of the goats was measured thrice weekly at approximately 10.00 a.m.

Body weight

The goats were weighed once per week.

Drug assay

Preparation of Immunogen and Coating Conjugate

Isometamidium chloride (Samorin) was conjugated with bovine serum albumin (BSA), Keyhole limpet haemacyanin (KLH) or porcine thyroglobulin (PTG) and used to immunise sheep.

ELISA Method - Indirect Heterogenous 96 Well Competition Micro ELISA

The wells of microtitre plates were coated by passive adsorption with isometamidium-protein conjugate in coating buffer. After overnight incubation at 4°C, the plates were washed twice with distilled water, dried at 37°C and stored at 20°C in the dark. The plates were washed four times with phosphate buffered saline/Tween (PBST) pH 7.2 immediately prior to assay.

Test serum samples (5ul) or isometamidium standards diluted in pooled normal serum were added to the wells followed by sheep anti-isometamidium antisera (100ul of 1 : 100 dilution in PBST). Assays were performed in duplicate and standards in triplicate.

The plate was incubated overnight in the dark at room temperature. The wells were emptied, then washed five times in distilled water. Alkaline phosphatase - anti-sheep IgG (H + L) conjugate solution (100 ul of a 1 : 1000 dilution in PBST) was added to each well. After incubation for a further hour the plate was again washed with water x 5 and 100ul of enzyme substrate solution (2mg/ml^{-1}, Sigma 104) added. Plates were read in a Multiscan MCC at 405nm after 30 minutes.

RESULTS

Parasitaemia

The results from individual goats are presented in Figures 24-29 and summarised in Table 21. Trypanosomes were first detected in the blood between days 14 and 17 post-infection and treatment with 0.5 mg/kg of isometamidium was given on day 23. In the case of the control (goat 114) treatment was

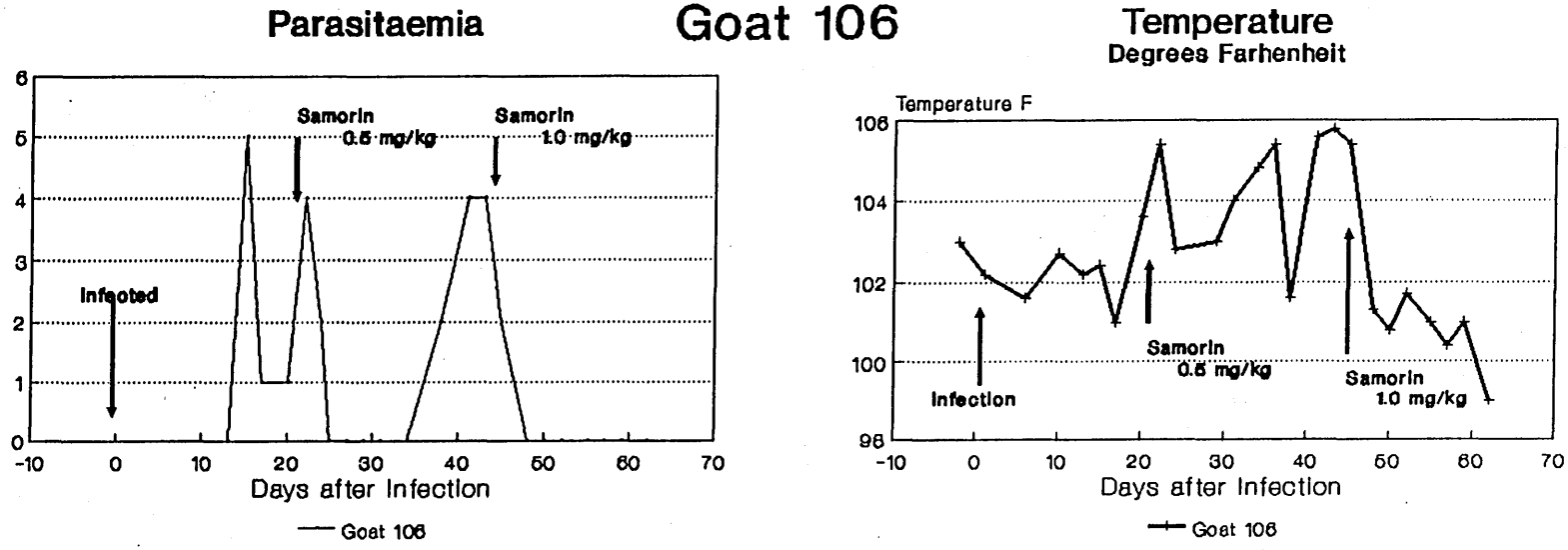
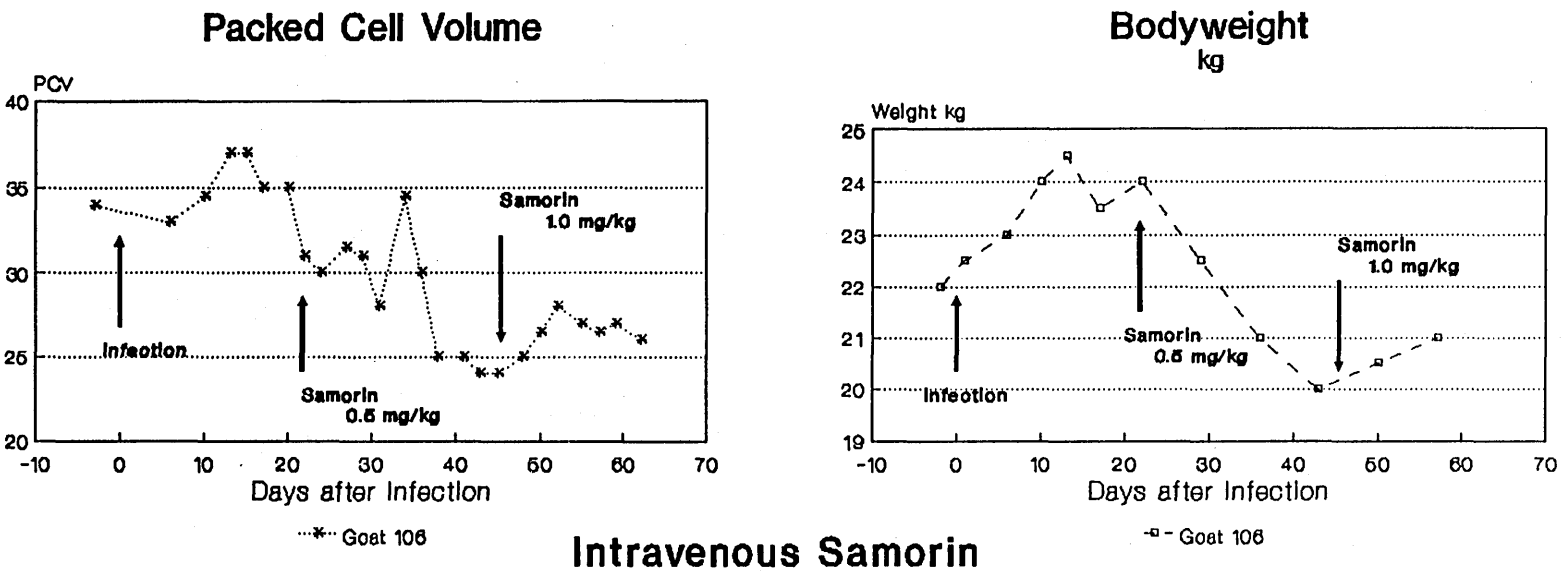


Fig. 24 Goat infected with an isometamidium-resistant *T. congolense*



Intravenous Samorin

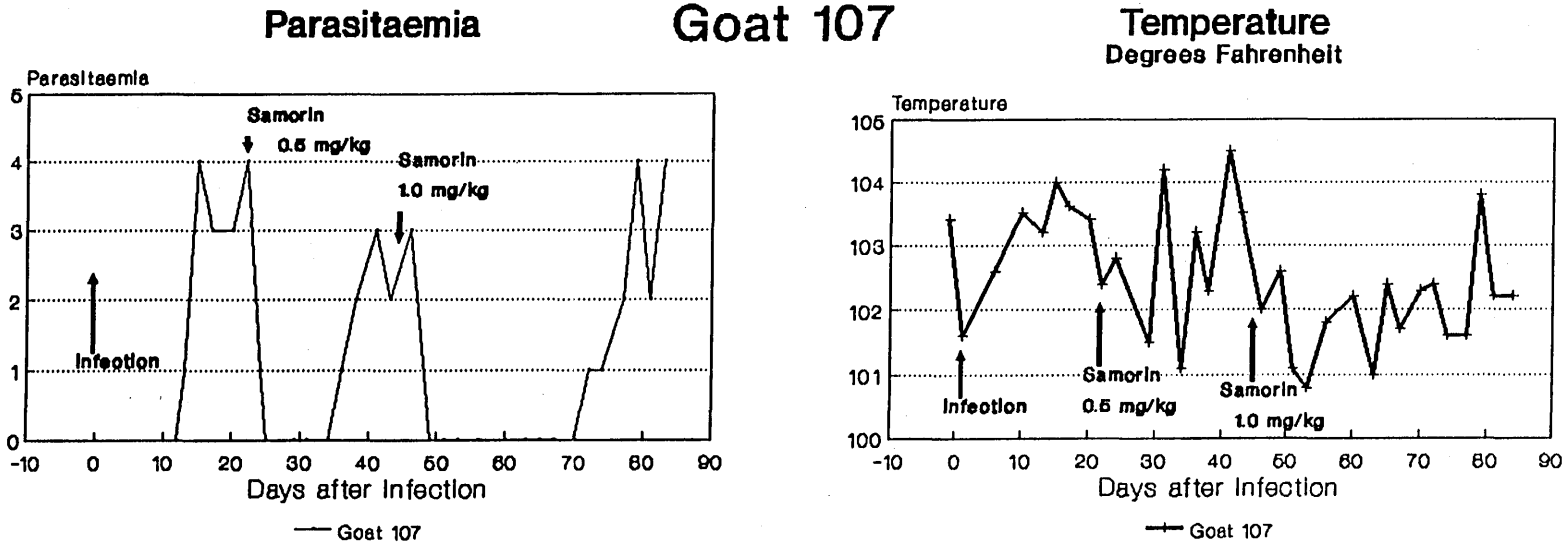
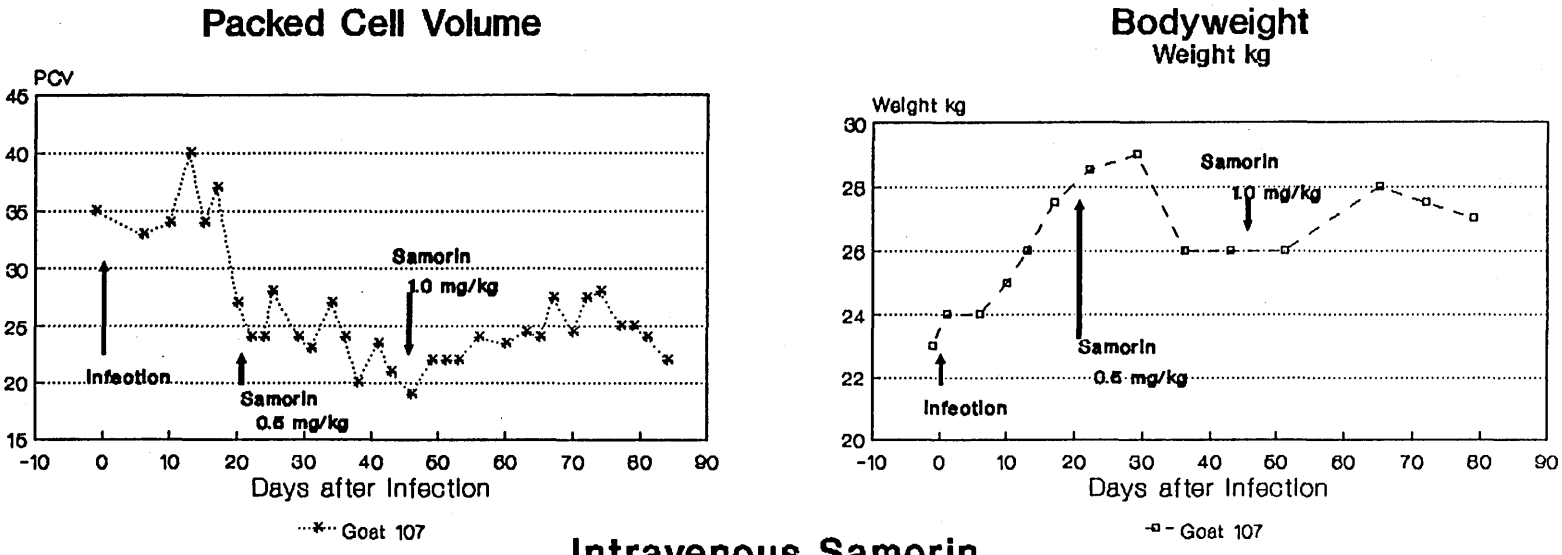


Fig. 25 **Goat infected with an isometamidium-resistant T. congolense**



Intravenous Samorin

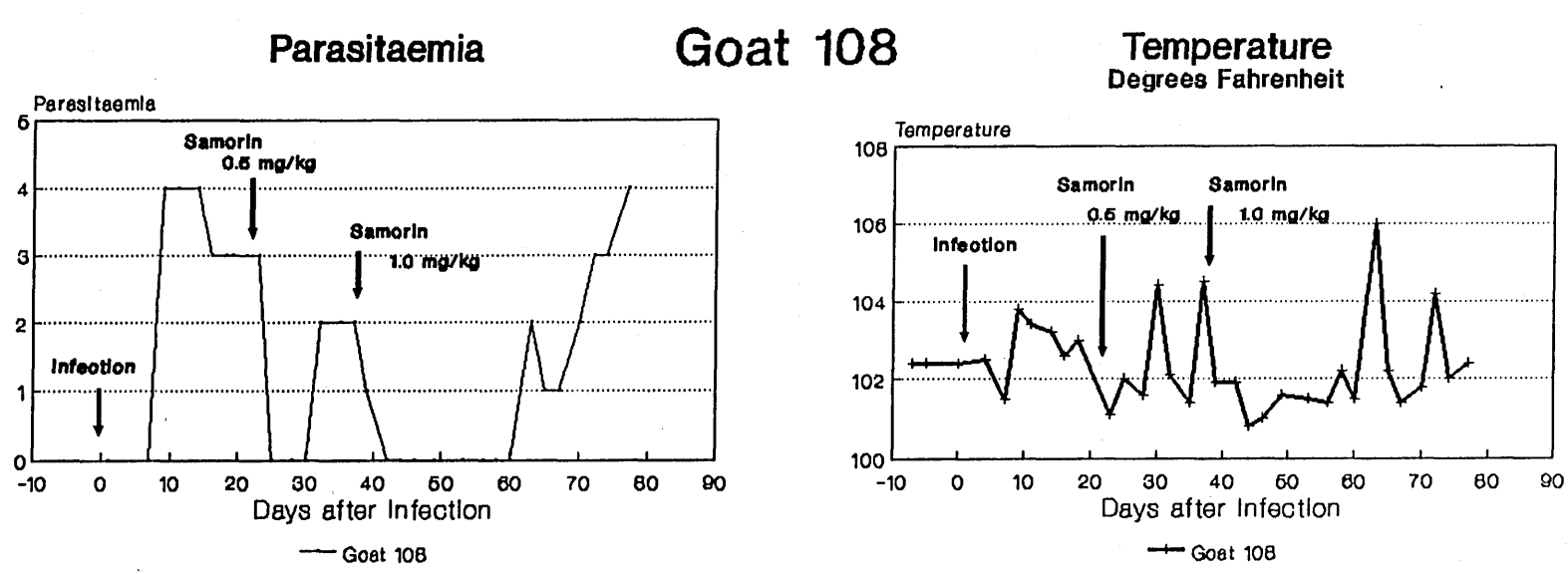
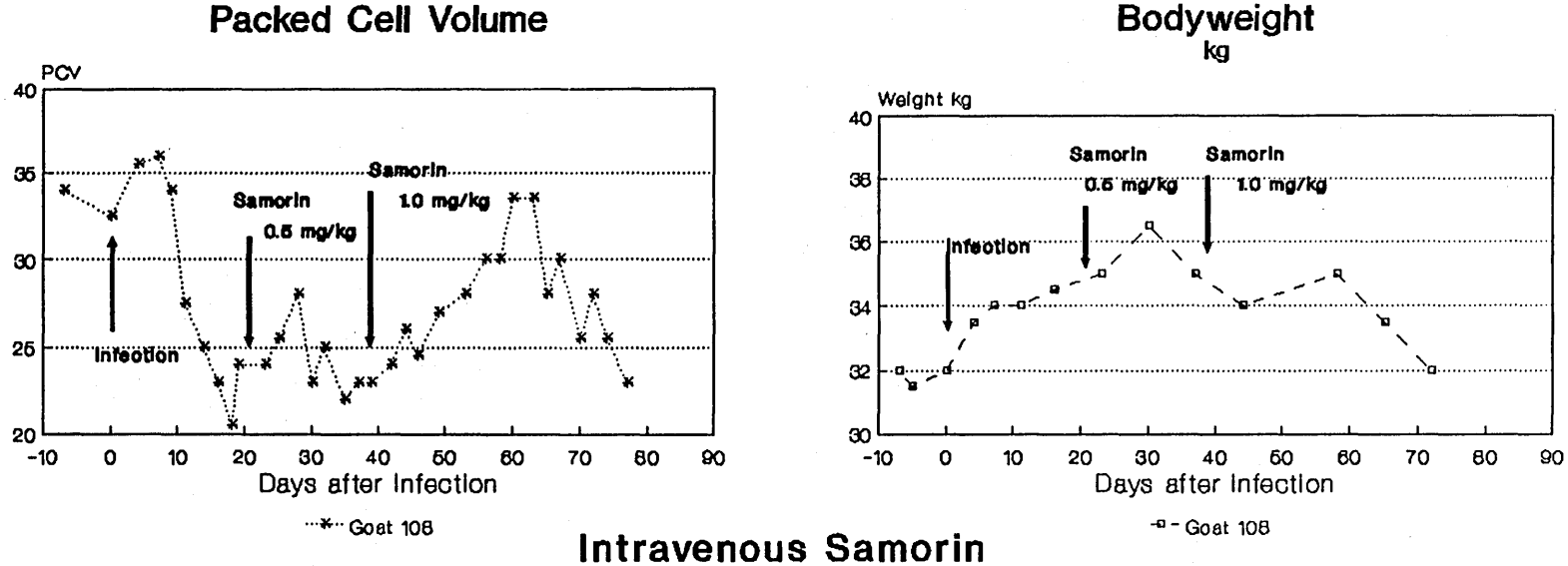


Fig. 26 Goat infected with an isometamidium-resistant T. congolense



Intravenous Samorin

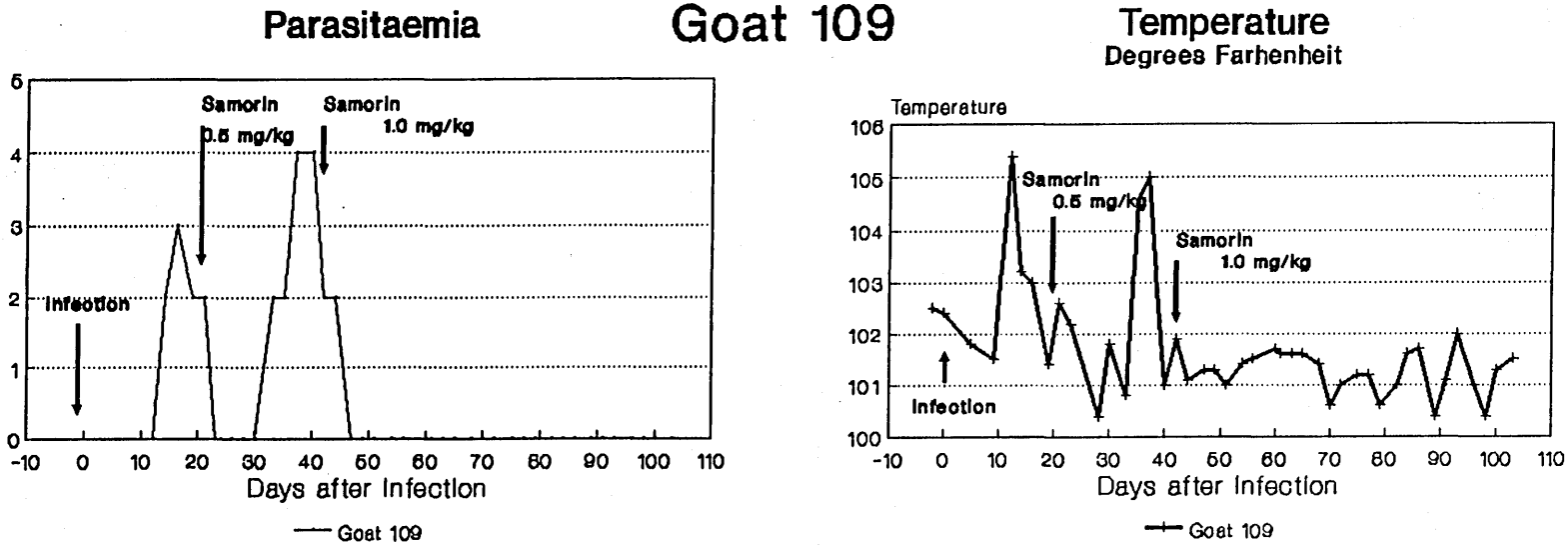
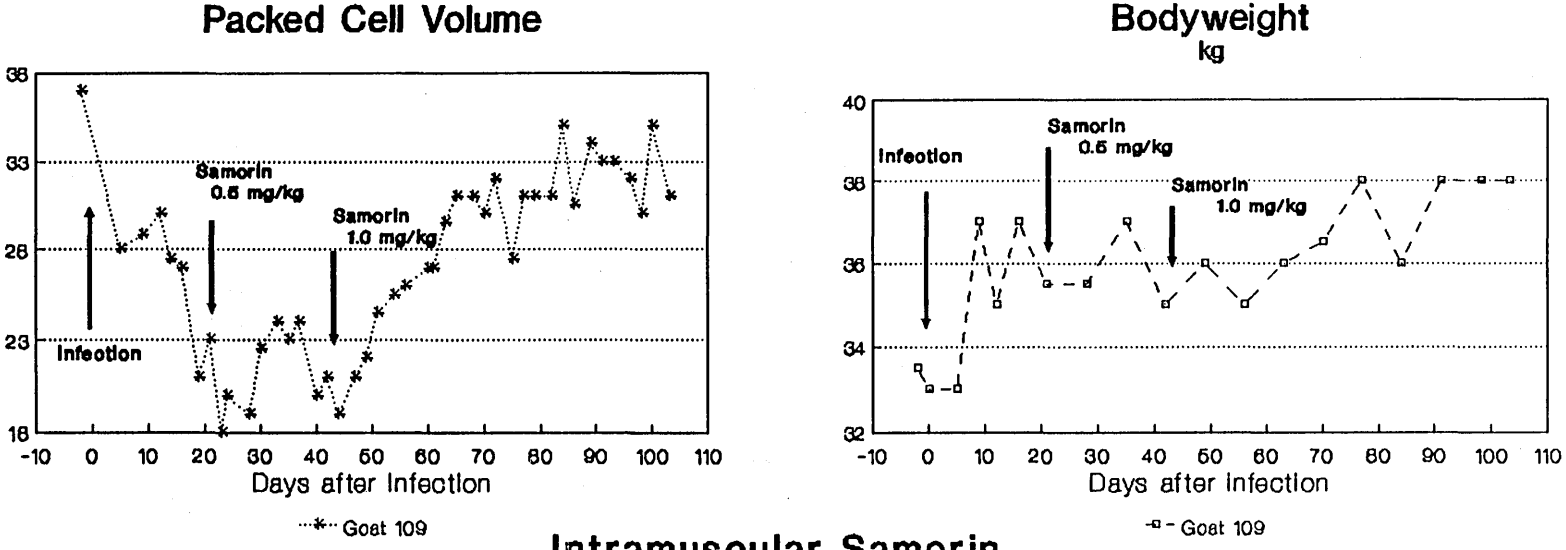


Fig. 27 Goat infected with an isometamidium-resistant T. congolense



Intramuscular Samorin

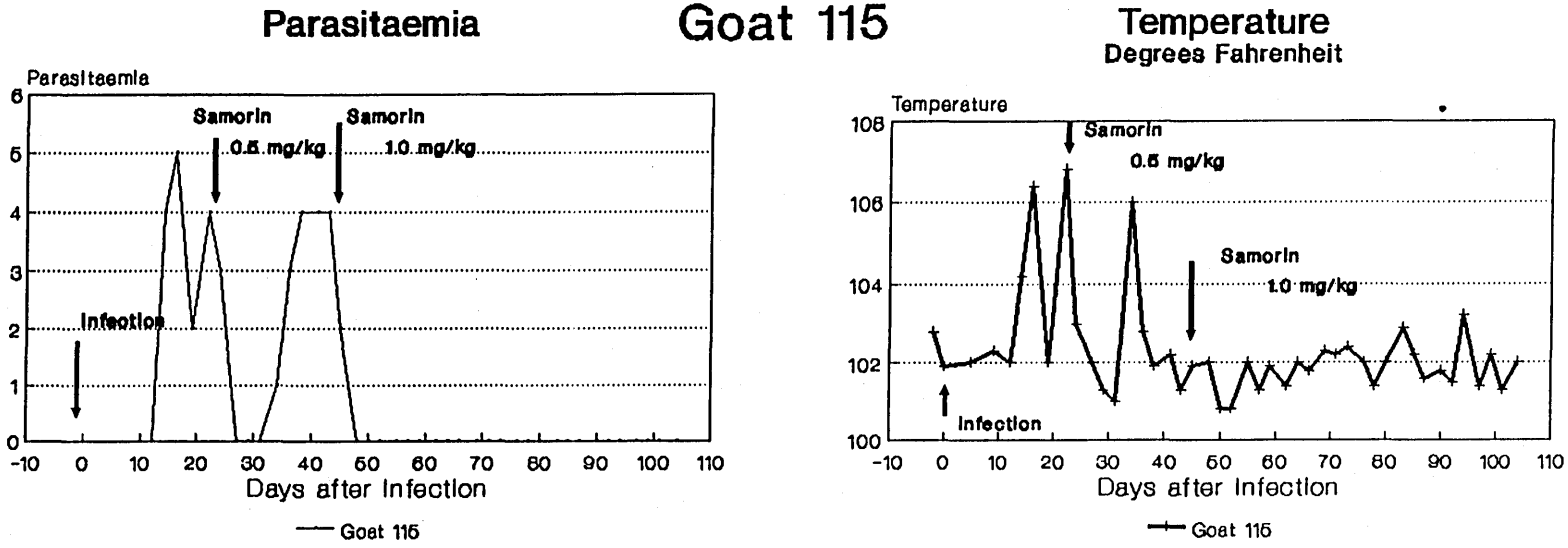
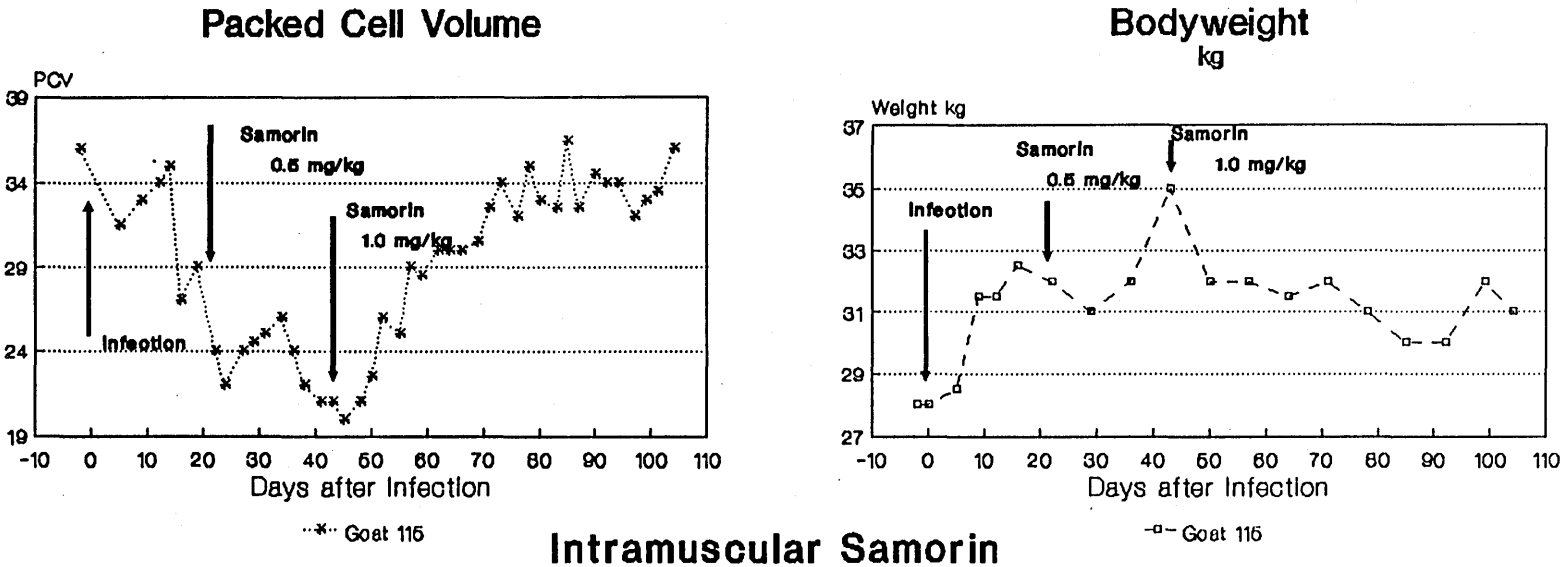


Fig. 28 Goat infected with an isometamidium-resistant T. congolense



Intramuscular Samorin

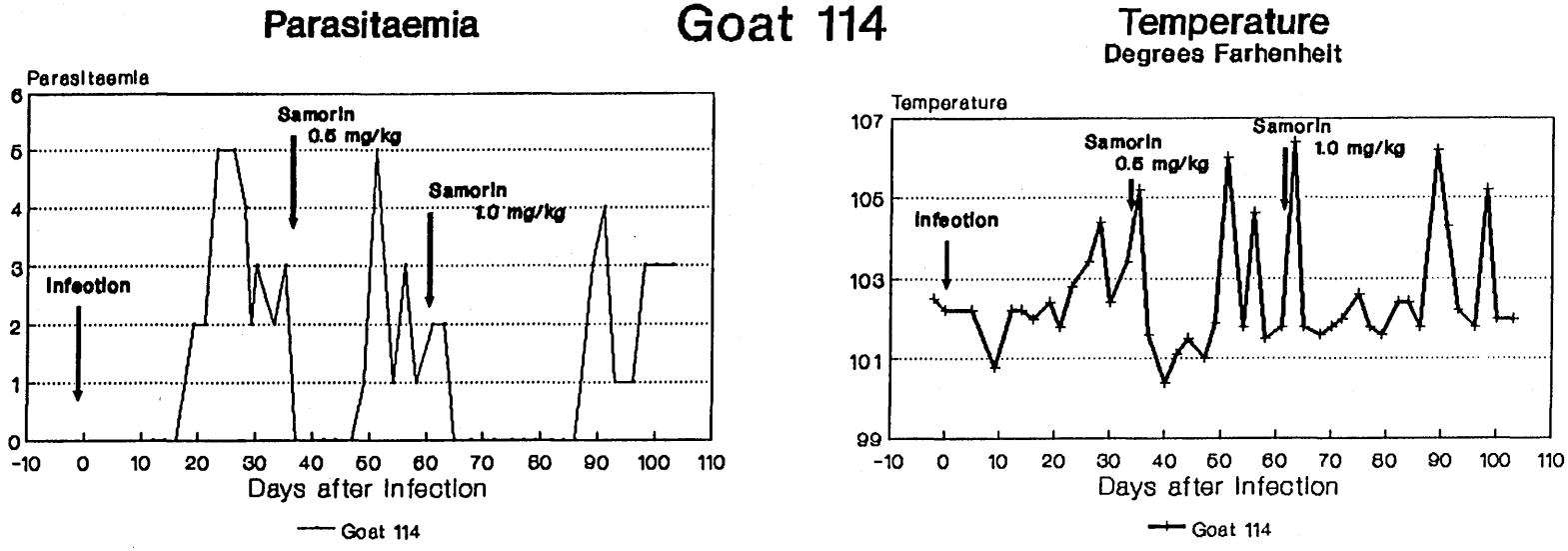
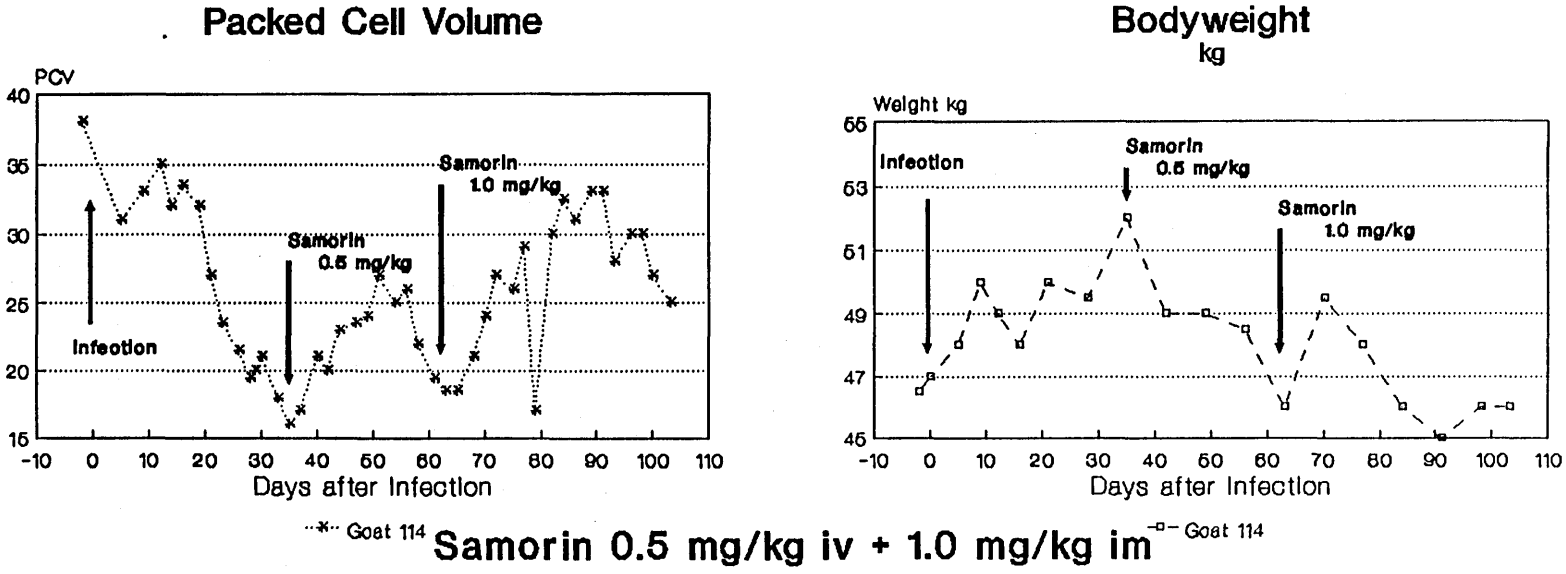


Fig. 29 Goat infected with an isometamidium-resistant T. congolense



Samorin 0.5 mg/kg iv + 1.0 mg/kg im

Table 21

The response to treatment with isometamidium by the intravenous or intramuscular route to goats infected with *T. congolense* GRVPS 56/2

Goat No.	1st Treatment (0.5mg/kg)			2nd Treatment (1.0mg/kg)			Termination
	Route	DA1*	Days to Relapse	Route	DA1	Days to Relapse	DA1
106	IV	23	14	IV	45	No relapse	62
107	IV	23	14	IV	45	27	83
108	IV	23	16	IV	45	25	83
109	IM	23	12	IM	45	No relapse	104
115	IM	23	12	IM	45	No relapse	104
114	IV	37	14	IM	63	28	104

DA1* = Days after infection

delayed until day 37.

In the goats given the intravenous injection (goats 106, 107 and 108) parasites rapidly disappeared from the blood and became undetectable. However within 14-16 days parasites reappeared in all three goats. On day 45 after infection the goats were retreated, this time with 1 mg/kg. One goat (106) remained aparasitaemic until its premature removal from of the experiment whilst the other two goats in this group developed relapse parasitaemias at 25 and 27 days after treatment respectively.

In the two goats (goats 109 and 115) given intramuscular doses of isometamidium treatment with 0.5 mg/kg produced only a brief period of aparasitaemia (12 days) before relapse infections developed. However a subsequent treatment with 1.0 mg/kg on day 45 achieved cures in both animals.

Finally a single goat (114) was kept as a control but on clinical grounds had to be treated. The first treatment given on day 37 was 0.5 mg/kg of isometamidium by intravenous injection to which the infection relapsed in 14 days (Figure 29). Subsequently the animal was treated with 1.0 mg/kg isometamidium on day 63 by intramuscular injection and following an aparasitaemic period of 28 days a relapse infection developed.

Haematocrit

The haematocrit values showed that infection with this strain of T. congolense was pathogenic in goats and caused the animals to become anaemic (Figures 24-29). By day 20 post infection the mean haematocrit had fallen to 25% and in the control goat (114) in which treatment was delayed the haematocrit had reached 16% by day 35 post infection.

Treatment at 0.5 mg/kg was associated with a rise in haematocrit in all the goats, with a subsequent fall as the relapse infections developed. Following the second treatment a rise in haematocrit was again observed and this improvement continued until preinfection levels were reached in all the goats in which the treatment at 1.0 mg/kg isometamidium achieved a cure, namely goats 109, 112, 115 and 117. In the goats which were not cured by the second treatment the haematocrits remained low and subsequently declined.

Body temperature

All the goats showed intermittent periods of pyrexia and these were closely correlated with the peaks in parasitaemia both before and after treatment (Figures 24-29). In the four goats which were cured by the second treatment body temperatures remained in the normal range until termination of the study.

Body weight

All the goats showed modest weight gains for approximately the first three weeks of the study (Figures 24-29). However later the weights did not increase and in some animals there was a fall in body weight. Successful cures following the second treatment were in general not associated with marked improvements in weight gain.

Drug assay

The plasma concentrations of isometamidium as measured by an ELISA assay are presented in Figures 30-33.

At ten minutes after the intravenous injection of 0.5mg/kg of isometamidium very high blood concentrations (mean 270ng/ml)

Plasma Samorin Levels

Intravenous Injection

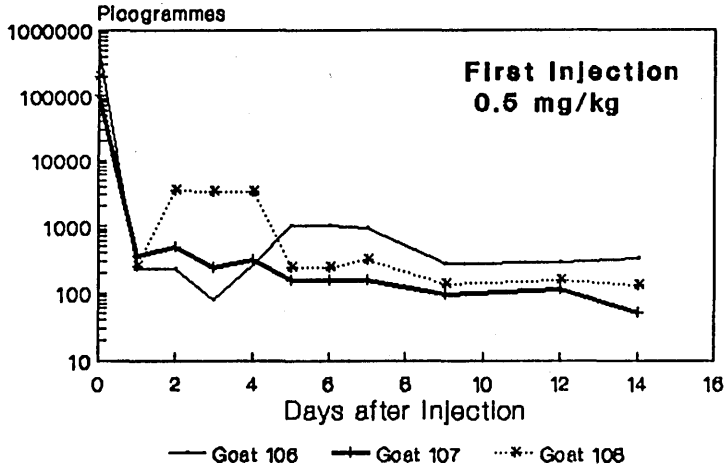


Fig. 30

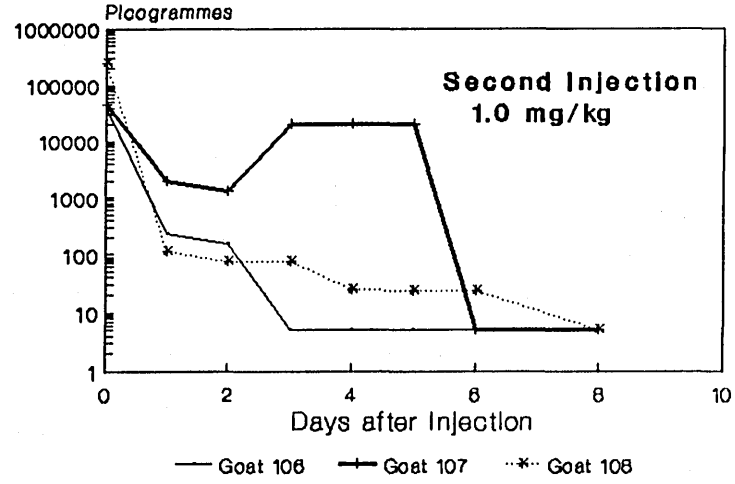


Fig. 31

Plasma isometamidium levels in goats 106, 107 and 108 after intravenous injection of Samorin.

Plasma Samorin Levels

Intramuscular Injection

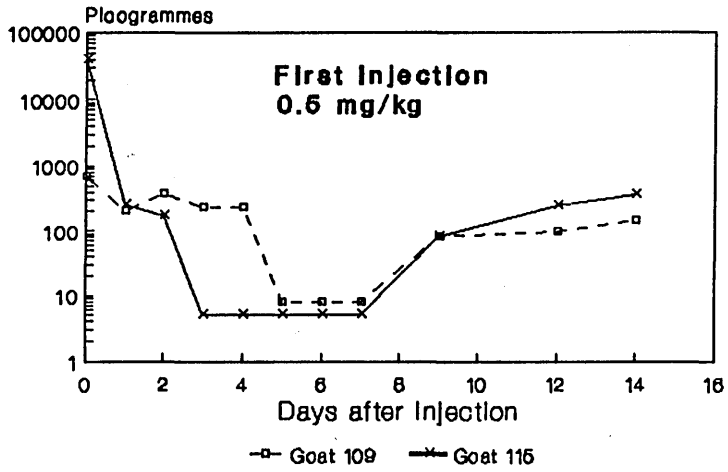


Fig. 32

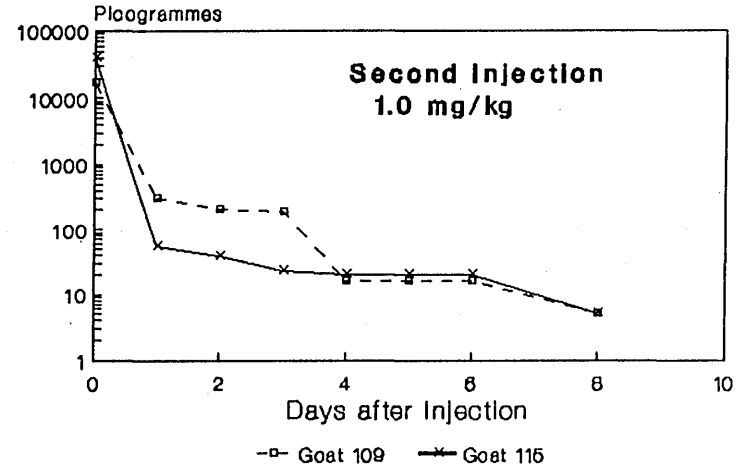


Fig. 33

Plasma isometamidium levels in goats 109 and 115 after intramuscular injection of Samorin.

were detected. By four hours after injection goats receiving the intravenous injection had on all occasions higher blood levels than goats which received the same dose of drug by intramuscular injection. However by 24 hours and beyond there were wide differences between individual goats and from day to day but no obvious effect of the route of administration of the drug.

Mean values from Day 1 to Day 8 of the blood concentration of isometamidium are presented in Table 22 and confirm the lack of obvious differences between treatment groups.

Table 22

Mean plasma concentration of isometamidium over 8 days following injection, as measured by ELISA

Group	Treatment	Goat no.	Mean isometamidium plasma concencentration (ng/ml) Following 1st infection (0.5 mg/kg)	Following 2nd infection (1.0 mg/kg)
A	Isometamidium iv	106	0.53	0.07
		107	0.37	9.15
		108	1.60	0.04
B	Isometamidium im	109	0.16	0.09
		115	0.08	0.03

DISCUSSION

Two routes of administration, namely intravenous and intramuscular, of isometamidium were used to treat goats infected with a drug-resistant strain of T. congolense. The results indicate that following treatment at 0.5mg/kg both treatments were equally ineffective in that relapse infections developed 12-16 days post treatment. However following retreatment with a higher dose of drug there were clear indications that intravenous administration was less efficacious than intramuscular treatment as relapses developed in two goats on days 25 and 27 after intravenous treatment (goats 107 and 108). Unfortunately the third goat (106) had to be removed from the experiment on day 17 after treatment due to a persistent urinary infection.

In contrast neither of the two goats (109 and 115) treated with 1.0mg/kg by the intramuscular route developed relapses during the 59 days of observation after treatment. However the parasitaemic profile of the control goat (114) which was initially treated with 0.5mg/kg intravenously and later with 1.0mg/kg intramuscularly suggests that the efficacy of the 1.0mg/kg intramuscular treatment in goats 109 and 115 may in part be due to the residual effect of the first intramuscular treatment. In the control goat a relapse infection developed 28 days after treatment with 1.0mg/kg im at a similar time to the relapses which developed in the IV treated group.

It must be recognised that the number of animals used in this experiment was very small, due to limited facilities in Glasgow for maintaining trypanosome-infected ruminants, and therefore caution should be exercised in evaluating the results.

Nevertheless they do strongly suggest that the intravenous administration of isometamidium confers no obvious benefits over intramuscular administration when treating drug-resistant strains of T. congolense, and may possibly be less efficacious.

The strain of T. congolense used in this study had previously been demonstrated to be resistant to isometamidium at a dose of 1.0mg/kg im by the workers who originally isolated this strain (Pinder and Authie, 1984) and later by Sones et al (1988). The latter workers demonstrated a minimum curative dose of 2mg/kg for this strain in cattle. In the present study the response to treatment at 1mg/kg has most probably been influenced by the early treatment 22 days previously and the results suggest that there was a greater residual effect when the earlier treatment was given by the intramuscular route rather than intravenously. The timing between treatments in the present experiment was largely dictated by the clinical condition of the goats as many of the goats became severely anaemic following the development of relapse infections after the first treatment at 0.5mg/kg. Likewise the severe anaemia of the control goat (114) required that treatment be given to this animal by day 37 post infection.

The advocates of the use of intravenous isometamidium (e.g. Dowler and Munstermann) claim that in addition to being an improved treatment for drug-resistant strains of T. congolense it also avoids tissue damage at the site of injection, which can in certain circumstances lead to lameness or neck deformities particularly if short and/or unclean needles are used. Certainly intravenous administration would avoid these potential adverse

reactions. However the intravenous administration of isometamidium requires considerable care for several reasons. First, rapid injection of concentrated solutions can lead to collapse and even death (Schillinger et al, 1985), and secondly perivascular or subcutaneously leakage of drug can lead to sloughing. For these reasons alone it is difficult to recommend intravenous administration by unskilled operators. Thirdly the prophylactic effects of the drug are considerably reduced following iv injection (Toure, 1973).

The development of an ELISA assay for isometamidium holds considerable promise for the quantitation of the drug in cattle in the field. Furthermore it offers greater sensitivity than any available technique including HPLC. The intention is that the test could not only be used to quantify drug levels in treated cattle, but also indirectly could indicate the presence of drug-resistant trypanosomes when parasitaemias are observed in cattle with drug levels normally considered to be trypanocidal.

In the present experiment an unrefined form of the ELISA test was used and the test requires further development. Nevertheless the results do indicate perhaps not surprisingly that by four hours post injection intravenous administration does result in higher blood concentrations of drug than intramuscular administration. However by 24 hours and beyond this difference was no longer apparent. The tentative suggestion from this result is that to be effective against drug-resistant strains of T. congolense high drug levels must be attained for longer than four hours. Possibly new formulations of isometamidium may achieve this in the future for the treatment of drug-resistant

strains.

In conclusion, the results of the present study, although involving a relatively small number of animals, indicate that the intravenous administration of isometamidium offers no clear advantage over intramuscular administration in the treatment of drug-resistant strains of *T. congolense* and may possibly be less efficacious than the conventional route of treatment. Further studies using larger numbers of animals, preferably Zebu cattle, are required to confirm these preliminary observations.

CHAPTER FOUR

STUDIES ON THE CHEMOTHERAPY OF *T. evansi* INFECTIONS

SECTION I

STUDIES IN MICE

INTRODUCTION

The first trypanocidal drugs used in the treatment of T. evansi infections were various compounds of arsenic and antimony (Lingard, 1899; Salmon and Stiles, 1902). The authors used these drugs in Surra-infected equines, bovines and canines. Later, suramin which is a member of the naphthalene compounds was found to be effective against naturally occurring T. evansi and T. equiperdum infections (Knowles, 1925).

The general pattern of chemotherapy and chemoprophylaxis has been reviewed by many workers and Williamson (1980) commented that "reviews of this subject tend to be reviews of reviews because of the almost complete absence of new drugs since the 1950's". Leach and Roberts (1981) also mentioned that between 1950 and the early 1960's was the period when most of the current trypanocidal drugs were introduced and when they were tested in the laboratory and in the field.

When trypanocidal drugs were first introduced the system was effective mainly because the treatments were professionally supervised and controlled. Although they were expensive in terms of drugs, when both chemotherapy and chemoprophylaxis were used together, they allowed the animals to be reared in tsetse endemic areas. The efficacy of the trypanocidal drugs were usually assessed by their action against bloodstream forms which occur in the mammalian host, however resistance has been detected with all the compounds which are currently in commercial use. In addition, toxicity, especially in certain species of animals, has been observed.

However, the effectiveness of quinapyramine sulphate against Trypanosoma evansi infections in all species had been recognised by many workers including Pellegrini and Bonelli (1951). Moreover, Arshadi and Farhangear (1971), stated that quinapyramine sulphate provides protection of three to four months in camels. The drug disappeared from the market in early 1970 (Holmes and Scott, 1982) but it is now available again from a number of different companies under various commercial names such as Trypacide (RMB) and Noroquin (both as sulphate and prosalt by Norbrook Laboratories).

Compounds such as diminazene aceturate (Berenil) and isometamidium (Samorin), which are not normally used in camels, due to their toxicity, have also been tried against T. evansi, using lower than normal doses, on many occasions in the field.

Deaths have been recorded after 7.0mg/kg of diminazene was given to camels (Leach, 1961) and Raisinghani and Lodha (1980), suggested that the best curative dose of diminazene for camels, experimentally infected with T. evansi was 2.5mg/kg intramuscularly. A few years earlier, Petrovski and Khamiev (1977) found that 5mg/kg of diminazene aceturate was effective in clearing T. ninae lohlyakimovae (T. evansi) from the blood of 25 Bacterian camels without any adverse effects.

In India, isometamidium chloride (Samorin) was found not to be effective against T. evansi in dogs (Srivastava and Malhotra, 1967). Likewise Schillinger, Maloo and Rottcher (1982) reported isometamidium to have low trypanocidal action against T. evansi in East African camels. These authors also observed local toxicity, high doses producing abscesses at the injection sites,

and these local reactions were similar to those caused by the drug in cattle. To avoid the local reaction which the drug produces at the injection site, these workers administered the drug intravenously and camels tolerated 1.0mg/kg intravenously. Temporary paralysis and severe shock reactions were reported with doses higher than 1.0mg/kg (Schillinger et al, 1982; Balis and Richard, 1977).

Karebe, Bottger, McCann, Sjoerdsma and Freitas (1982) found that difluoromethylornithine (DFMO) has the ability to arrest the multiplication of tumour cells in mice and prevent replication in different protozoa and is effective in treating early infections of trypanosomes.

In the late-stage infections the trypanosomes pass into the central nervous system (CNS). Here the immune mechanisms cannot be expressed and so long as the blood-brain barrier remains reasonably intact, the trypanosomes in the CNS can survive.

However, in his recent work, Jennings (1988) has found that both CNS and blood circulating infections of T. b. brucei can be cured permanently by the combination of DFMO and reduced quantities of melarsoprol injections. The author also stated that the use of such small quantities of melarsoprol might reduce the incidence of encephalopathies encountered in the treatment of human trypanosomiasis.

Melarsoprol (Mel B) and Trimelarsan (Mel W) which were known for their effectiveness in the treatment of Trypanosoma gambiense and Trypanosoma rhodiense infections, were screened against animal trypanosomes and shown to be effective against Surra

(Gill, 1961a; 1961b; 1977; Gill and Sen, 1971).

In this thesis, all of the above mentioned trypanocidal drugs have been used against experimental infections with several T. evansi strains. In addition, the thesis records experiments carried out to investigate the efficacy of Mel Cy, a new compound which is a melaminylthioarsenate, for its chemotherapeutic activity against both late and early infections of T. evansi. The prophylactic effect of this compound was also tested. Since this compound (Mel Cy) is in the early development stages, there is no published information on its use in animal trypanosomiasis. As trypanosome strains and stabilates can be genetically heterogenous, clones were prepared at the beginning of these experiments in order to obtain populations grown up from single organisms.

MATERIALS AND METHODS

Mice

Swiss white mice (CD-1) were purchased from the animal suppliers (Charles River Ltd., Margate, England). All the mice were female, weighed between 25-30 g and were 6-8 weeks of age at the start of each experiment.

Feeding and Housing

All the mice were housed in metal/plastic cages with sawdust bedding which was changed twice weekly. The cages were kept on the shelves of metal stands in an animal house where the temperature was kept at approximately normal room temperature (21^{o}C). The animals were fed ad libitum on pellets (Angus Milling Company, Kirriemuir, Perth, Scotland). Drinking water was constantly available.

Trypanosomes

The trypanosomes used in these experiments were kindly supplied by Dr. F.W. Jennings from the Department of Parasitology, Veterinary School, Glasgow. The stabilates were derivatives of TREU 1412, TREU 1444 and TREU 1445 originally obtained from the Centre of Tropical Veterinary Medicine, Royal (Dick) School of Veterinary Medicine, Edinburgh.

Infection

Each mouse was inoculated intraperitoneally with 1×10^5 trypanosomes in phosphate buffered saline pH 8.0 prepared directly from the frozen stabilates, which were maintained in liquid nitrogen. All mice were checked for circulating trypanosomes, before drug treatment and at least two times per week after treatment. Fresh blood smears from the tail of each mouse were examined for at least 20 microscope fields at x 400 magnification. Mice which developed relapse infections were removed from the experiment and any deaths were noted.

Drugs

The drugs used in these experiments were Samorin (isometamidium) RMB, Dagenham, England; Moranyl (suramin) Specia Ltd., Paris; Berenil (diminazene aceturate) Hoechst, Frankfurt, West Germany; Difluoromethylornithine (DFMO) supplied by Merell, Dow Research Institute, Strasburg, France; Arsobal (melarsoprol) diluted in rapeseed oil, supplied by Specia Ltd., Paris; Trypacide and Trypacide Pro-salt (quinapyramine) RMB, Dagenham, England; melarsonyl potassium (Trimelarsan, Specia, Paris) and Mel Cy which has no generic name as it is under

experimental study (Rhone Merieux, Toulouse, France). The drugs were all prepared according to the manufacturers recommendations so that the requisite amount (mg/kg) could be administered at a dose of 0.05ml per/5g bodyweight, and the drugs were administered either orally, intraperitoneally or subcutaneously. The mice were injected either at the early stage or chronic stage of infection.

Preparation of Clones

This experiment was carried out to prepare clones of T. evansi, from the stabilates GVR 72, GVR 74 and GVR 75 and two drug-relapsed stabilates of GVR 74 named GVR 87 and GVR 91 (see the history of the trypanosomes) (Figures 34, 35 and 36).

Groups of five mice were irradiated with 600 rads (cobalt-60 source), the mice were infected with at least 1×10^5 trypanosomes of each stabilate to be cloned. The parasitaemias were high within a few days. Initially, a mouse with a high parasitaemia was bled into heparin so that the blood did not clot and the blood was kept on ice, and was diluted in PBSG.

For each stabilate one 96 well "NUNC plate" with lid was used. The cloning operation was carried out by filling the two outside rows with water (approximately 200ul) so that the atmosphere in the plate would remain saturated and delay evaporation of the very small drops containing the trypanosomes. The first four wells of each row were used and down the side of each well 30ul of PBSG, was carefully introduced so that it formed a bead. A pointed needle was taken and dipped into the diluted trypanosomes solution and a microdroplet was transferred into the first four wells, so that it was beside but not into,

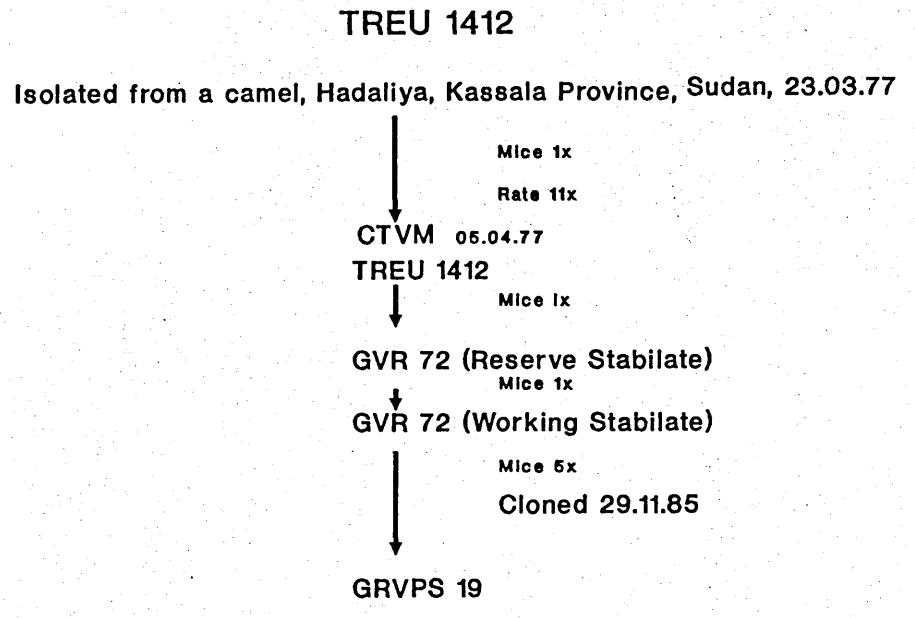


Fig. 34 History of T. evansi TREU 1412 and clone GVRPS 19

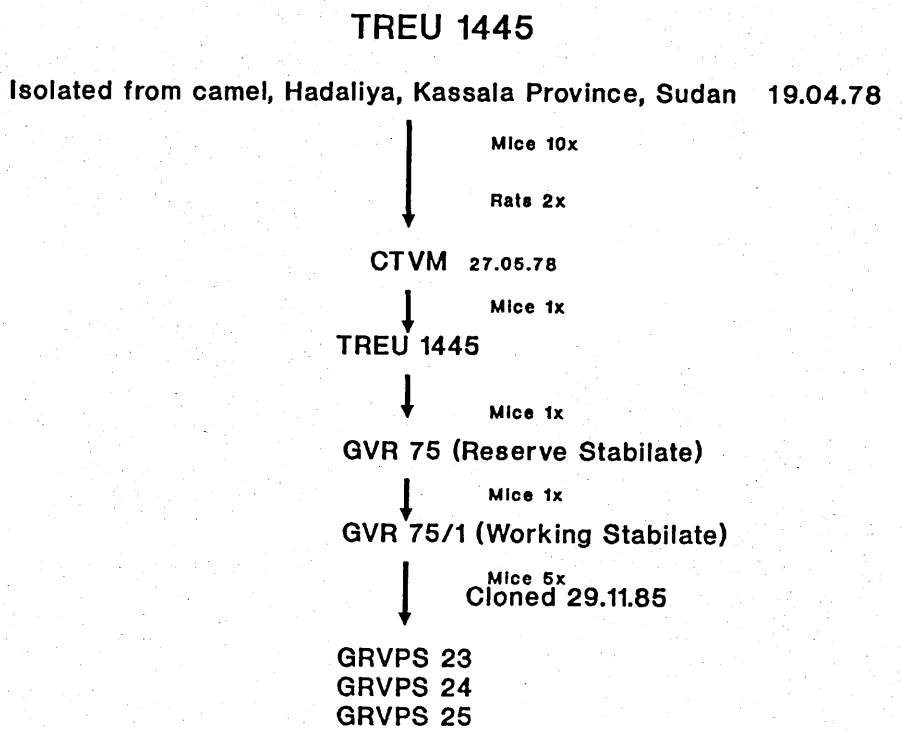
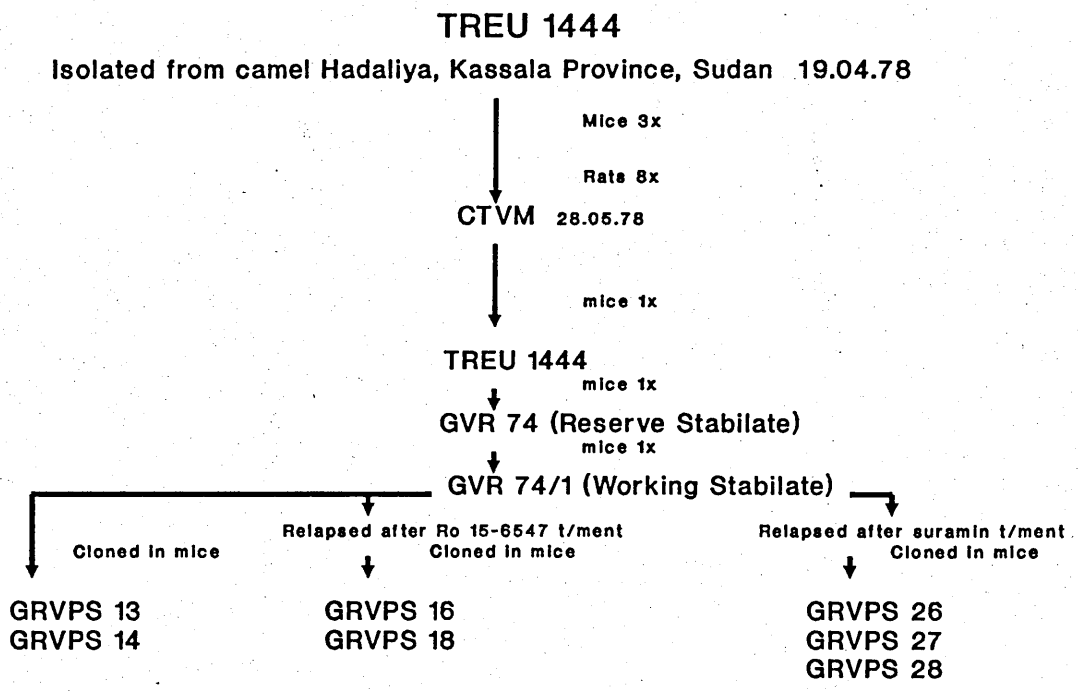


Fig. 35 History of T. evansi TREU 1445 and clones GRVPS 23, 24, 25

Fig. 36 History of T. evansi TREU 1444 and seven cloned isolates.



the bead of PBSG. These droplets were examined using an inverted microscope. Each droplet was searched thoroughly and those wells containing only one trypanosome in the droplet were marked whilst the plate was still in position under the microscope. A second observer was invited to check the drops and if a single trypanosome was confirmed, the droplet and the bead of PBSG were mixed. Then a further 100ul of PBSG were added and the total volume was taken up into a 1.0ml syringe. The liquid containing the single trypanosome was injected intravenously into an irradiated mouse, ensuring that the complete contents of the syringe was inoculated. The used plate was discarded and a fresh plate was taken for each isolation.

Examination of the infected mice was carried out by wet-films of the tail blood from day 2 onwards. Blind passage was done every three days by taking the maximum amount of blood (approx. 1.0ml) and transferring it by injection into another irradiated mouse until a population of trypanosomes adequate for cryopreservation, was obtained i.e. until circulating parasitaemia became high (> log 8.0).

The mice were killed at this peak parasitaemia by cervical dislocation. The chest was cut open and the main arteries and veins to the heart were cut. Also several cuts were made in the heart. Blood collected was pooled and kept on ice. The amount of blood was measured with a pipette and glycerol, to a final concentration of 12.5% as cryopreservative was added. The blood and glycerol were mixed well, and left for a few minutes. The blood-glycerol mixture was taken up into capillaries and both ends were sealed in a hot flame by forming a terminal bead.

Capillaries were put into plastic tubes, with four holes and a square of gauze in the bottom. The plastic tubes were suspended in the vapour phase of liquid nitrogen and slowly frozen. They were stored finally, in the vapour phase of liquid nitrogen, following the proposals of Dar, Lighthart and Wilson (1972) and Dar, Wilson and Goedbloed (1973).

The clones were each named under the Veterinary Physiology trypanosome research code, GRVPS (see Table 23).

Table 23

Relation between various stabilates of T.evansi and GRVPS clones

Original stabilates	Clones made
GVR 74	GRVPS 13
[TREU 1444]	GRVPS 14
	GRVPS 15
GVR 87	GRVPS 16
[TREU 1444]	GRVPS 17
Relapse after RO 15-6547	GRVPS 18
GVR 91	GRVPS 26
[TREU 1444]	GRVPS 27
Relapse after suramin	GRVPS 28
GVR 72	GRVPS 19
[TREU 1412]	
GVR 75	GRVPS 23
[TREU 1445]	GRVPS 24
	GRVPS 25

Infectivity Test of the Stabilates

Occasionally, some difficulty was encountered in the infectivity of cryopreserved stabilates, because some of the organisms were either killed by shock during the process of cryopreservation or they revealed very slow activity during the suspension of the trypanosome i.e. before they were inoculated into normal recipient mice.

Infectivity tests on all prepared stabilates were carried out and for each stabilate groups of 15 mice were infected. The infecting dose was 1×10^4 trypanosome in 0.2ml of PBSG injected ip, and the prepatent period and the behaviour of each stabilate was studied. There was a total of 13 stabilates used and none gave a good infection at the first attempt.

In most of the stabilates, death of the organism was observed, immediately after suspension in PBSG. Therefore a new solution of PBSG (15% glucose) was prepared which contained an addition of 20% foetal calf serum (20%) and Minimum Essential Media (MEM). This PBSG preparation improved the condition of some of the stabilates while others were still unsatisfactory after thawing from the frozen stabilate giving low numbers of motile trypanosomes with lots of dead trypanosomes. The results also showed that in some of the stabilates the numbers of trypanosomes rapidly declined after being prepared in PBGS and MEM while others were stable in the solution of PBSG and foetal calf serum.

Five stabilates proved to be in good condition after employing the later preparations (solution of PBSG and 20% of foetal calf serum and MEM) and these stabilates were used for the

experiments recorded in this section of the thesis.

I. Sensitivity of five cloned T. evansi stabilates to quinapyramine (Trypacide sulphate)

The objective of this experiment was to examine the sensitivity of the five cloned T. evansi stabilates (GRVPS 13, GRVPS 18, GRVPS 19, GRVPS 23 and GRVPS 27) to quinapyramine at three different dose levels of 0.05, 0.5 and 5mg/kg administered on day 7 of the infections.

All non-treated infected controls were removed from the experiment after making Giemsa stain smears weekly for four weeks.

A summary of the results on the use of quinapyramine with the five cloned stabilates is given in Table 24. Treating seven day-old infections of T. evansi GRVPS 13 with 0.05mg/kg dose level of quinapyramine showed an initial removal of the parasites from the circulation but relapses occurred in all mice between day 23-40 after treatment. Similar relapses occurred when quinapyramine at 0.5mg/kg was administered. However, no relapses occurred when the mice were treated with 5mg/kg.

In the case of T. evansi GRVPS 18 infections all the mice relapsed after being treated with 0.05mg/kg but when 0.5kg of the drug was given only three out of five mice relapsed, while in the 5mg/kg treatment group, four out of five mice were permanently cured.

In fact it would appear that the "suramin-relapse GRVPS 27" was slightly more resistant to quinapyramine than the original stabilate, while the "nitroimidazole-relapse GRVPS 18" was

Table 24

Sensitivity of murine infections with five stabilates of *T. evansi* to quinapyramine sulphate when administered seven days after infection

Group	*T.evansi* stabilates	Dose rate mg/kg	Cured/ Treated	Day of relapse
1	GRVPS 13	0.05	0/5	23,25,32,35,40
		0.5	0/5	35,47,47,54,75
		5	5/5	-
2	GRVPS 18	0.05	0/5	28,28,30,30,54
		0.5	2/5	40,61,75
		5	4/5	47
3	GRVPS 27	0.05	0/5	All remained +ve
		0.5	0/5	23,23,28,35,35
		5	5/5	-
4	GRVPS 19	0.05	0/5	All remained +ve
		0.5	2/5	47,54,61
		5	4/5	47
5	GRVPS 23	0.05	0/5	All remained +ve
		0.5	2/5	49, 49, 100
		5	5/5	-

perhaps slightly more sensitive.

T. evansi GRVPS 27 was totally insensitive at 0.05mg/kg Trypacide. All mice relapsed at 0.5mg/kg, after initial disappearance of the parasite. 5mg/kg was high enough to clear blood parasites immediately and to give permanent cures.

T. evansi GRVPS 19 was completely insensitive when treated on day 7 with 0.05mg/kg of the drug and the mice remained positive. However, after an initial clearance of the parasites, three out of five mice relapsed following treatment with 0.5mg/kg dose level whilst four out of five mice were cured (80%) at the 5mg/kg treatment level.

In the case of T. evansi GRVPS 23 infections 0.05mg/kg failed to clear the parasite from the circulation. However, using 0.5mg/kg of the drug, after an initial clearance of infection, three out of five mice relapsed. All mice were cured using 5mg/kg Trypacide sulphate, thus proving that this stabilate was also sensitive to quinapyramine.

II. Sensitivity of two stabilates of T. evansi to six trypanocidal drugs

(a) Quinapyramine (Trypacide)

In this experiment two stabilates (GRVPS 13, GRVPS 19) which showed the greatest differences in sensitivity to quinapyramine were treated on day 7 and day 21 after infection with six different trypanocidal drugs at various dose levels. Treatment with Trypacide sulphate of T. evansi GRVPS 13 at day 7 and day 21 after infection produced permanent cures at the dose rate of 5mg/kg and this confirmed the sensitivity of this stabilate to

the drug quinapyramine.

When the same dose rate of 5mg/kg of Trypacide sulphate was used to treat the T. evansi GRVPS 19 the treatment on day 7 cured four out of five mice. However, when the treatment was delayed until day 21 after infection, all five mice were permanently cured (see Table 25).

Chemotherapy of both T. evansi GRVPS 13 and 19 infections with Trypacide Prosalt demonstrated that if treatment was initiated seven days after infection then Trypacide Prosalt at 5mg/kg could clear the parasites and give permanent cures. However, when the treatment was delayed until day 21 after infection a few relapses occurred with both stabilates.

(b) Diminazene Aceturate (Berenil)

Permanent cures were produced when 10mg/kg of diminazene aceturate (Berenil) was used to treat T. evansi GRVPS 13 infections on 7 and 21 days after infection. However, when T. evansi GRVPS 19 infections were treated with diminazene aceturate at 10mg/kg, on day 7 and day 21 after infection, one out of five mice relapsed from the day 7 and three out five mice from the day 21 treatments (see Table 25).

(c) Isometamidium (Samorin)

Treatment with isometamidium (Samorin) of T. evansi GRVPS 13 infections indicated permanent cures when the treatment was started on day 7 after infection at 1mg/kg, but when the treatment was delayed until day 21 after infection, two out of the five mice relapsed. On the other hand when isometamidium was used to treat T. evansi GRVPS 19 infections at the same dose rate (1mg/kg) two out of five mice relapsed after treatment on day 7

Table 25

Sensitivity of two stabilates of *T. evansi* to various trypanocidal drugs

Drug	Dose mg/kg	Stabilate GRVPS 13 Day 7*	GRVPS 13 Day 21	GRVPS 19 Day 7	GRVPS 19 Day 21
Quinapyramine (Trypacide Sulphate)	5 mg/kg	5/5**	5/5	4/5	5/5
Quinapyramine (Trypacide Prosalt)	5 mg/kg	5/5	3/5	5/5	3/5
Diminazine aceturate (Berenil)	10 mg/kg	5/5	5/5	4/5	2/5
Isometamidium (Samorin)	1 mg/kg	5/5	3/5	3/5	1/5
Melarsoprol (Arsobol)	3.6 mg x 4 days	5/5	5/5	5/5	5/5
Difluoromethyl-ornithine (DFMO)	2% soln. (5-19 days)	5/5	-	5/5	-
Suramin	20 mg/kg	0/6	0/5	5/5	0/5
	40 mg/kg	0/6	0/6	5/5	2/5
	80 mg/kg	1/6	0/6	-	-
	160 mg/kg	6/6	0/5	-	-

* Day of treatment

** Cured/treated

and four out of five mice relapsed after treatment was given on day 21. This result confirms the difference in sensitivity of the two stabilates to this drug.

(d) Melarsoprol (Arsobal, Mel B)

Melarsoprol (Arsobal) (diluted with rapeseed oil) was used against an early infection of T. evansi GRVP 13. The treatment was begun five days after infection and drugs were given daily for four consecutive days i.e. day 5,6,7 and 8. The result indicated permanent cures. When the treatment was delayed until day 21 the same result of permanent cures was obtained. This is in contrast with the findings with 21 day-old T. brucei infections, were 4 x 3.6mg/kg failed to permanently cure any of the mice (Jennings, 1988). Moreover, a collateral experiment carried out using T. evansi GRVPS 19 stabilates infections with similar dose rates of melarsoprol and the same days of treatment also demonstrated permanent cure of the mice. This result showed that this drug is effective against experimental murine Surra.

(e) Difluoromethylornithine (DFMO)

The effect of oral difluoromethylornithine (DFMO) administration for 14 days was studied. The administration of a 2% solution of DFMO in the drinking water was begun on day 5 after infection in both T. evansi stabilates, GRVPS 13 and GRVPS 19. All mice receiving 2% DFMO in drinking water showed a rapid clearance of circulating trypanosomes and were permanently cured during a period of observation of 114 days for both stabilates.

(f) Suramin (Moranyl)

As it was already known the GRVPS 13 was suramin-resistant, different dose rate levels of suramin (40, 80, 160mg/kg) were used to treat infections of T. evansi GRVPS 13, and the mice were monitored for relapse infections. It was found that mice infected with T. evansi GRVPS 13 and treated with 40mg/kg of suramin at day 7 and day 21, failed to clear the circulating parasitaemias. It was also found at higher doses of suramin up to 80mg/kg and 160mg/kg were still unable to produce permanent cures of T. evansi GRVPS 13. (Although 160mg/kg is approaching the maximum tolerated dose).

Two different dose levels of 20 and 40mg/kg of suramin were given to mice infected with T. evansi GRVPS 19 stabilate, on day 7 and day 21. Both dose levels on day 7 treatment, demonstrated 100% cures, however, when the treatment was delayed until day 21, three out of five mice relapsed at 40mg/kg and all the mice relapsed in 20mg/kg. This illustrates that early infections of T. evansi are more readily treated than are the more established infections.

III. Studies of chemotherapeutic activity of Mel Cy and Mel W against both early and late infections of T. evansi

The results of groups of mice infected with T. evansi GRVPS 13 and treated with various dose ranges of Mel Cy and Mel W (Trimelarsan) are shown in Table 26.

The mice treated with dose rates of 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/kg of Mel Cy on day 7 and day 9 after infections were permanently cured.

Table 26

The sensitivity of the arsenicals, Mel Cy and Trimelarasan against early (7-9 DAI) and late (21 DAI) infections of T. evansi GRVPS 13

Group	Dosage mg/kg	Treated DAI	Number Cured/Total Treated Mel Cy	Trimelarsan
A	20	9	5/5	5/5
B	10	9	5/5	5/5
C	5	9	5/5	5/5
D	2.5	9	5/5	5/5
E	1.25	7	4/4	5/5
F	0.625	7	5/5	5/5
G	0.3125	7	5/5	3/5*
H	40	21	4/4	5/5
I	20	21	5/5	5/5
J	10	21	5/5	5/5
K	5	21	5/5	5/5
L	2.5	21	4/4	3/4**

* relapsed 49 & 49 days after treatment

** relapsed 73 days after treatment

Observed for 60 days (Groups A to G) and 100 days (Groups H to L) after treatment

DAI - Days after infection

The lowest dose used was effective against the stabilate used in this experimental work. However, it is reasonable to assume that the dose range of the drug could be further reduced.

The response of the other groups of mice infected with the same stabilate of T. evansi GRVPS 13 and then treated with the corresponding dose ranges of another compound, Trimelarsan, was also interesting. The first six groups of mice covering the range of dosages of 20mg/kg to 2.5mg/kg were permanently cured, and no breakthrough was observed up to the termination of the experiment. However, two out of five mice treated with 0.3125 mg/kg (see Table 26) both showed circulating trypanosomes only on day 49 after treatment. However, 60% of the mice treated with this dose were cured.

Another experiment was carried out to test the efficacy of the two compounds, Mel Cy and Trimelarsan, against more chronic T. evansi infections in mice.

Mice, infected with T. evansi GRVPS 13 were treated with various dose ranges of Mel Cy (40, 20, 10, 5 and 2.5 mg/kg) after 21 days of infection. The parasites disappeared from the blood 1-2 days after treatment and the blood remained free of trypanosomes until the end of the experiment, which was 100 days of observation (see Table 26). It was interesting to note the absence of toxic reactions even at the 40 mg/kg dose level of the drug in mice, which is a higher dose rate than can be administered to larger animals. One mouse died before treatment from group L and another mouse died the day after treatment.

Following the same procedure, mice infected with T. evansi GRVPS 13 were given Trimelarsan. The dose rates and the day of

treatment were the same as Mel Cy treatment. This compound exhibited a high therapeutic activity against the stabilates of T. evansi. All doses removed the parasites from the blood of the infected mice. Monitoring the infection by the diagnostic methods used in this thesis, the blood remained negative until the end of the experiment (14 weeks). Only one mouse relapsed on day 73 after treatment with 2.5 mg/kg of Trimelarsan. Another mouse died at treatment (see Group L).

IV. Period of prophylaxis given by Trypacide Prosalt against T. evansi infections

This experiment was designed to study the period of prophylaxis which a single dose of Trypacide Prosalt could give against an experimental infection with T. evansi.

So that all the groups of mice could be infected on the same day, the injections of the Trypacide Prosalt was staggered at two weekly intervals. Two groups of five mice were each injected with 2.5mg/kg or 5.0mg/kg Trypacide Prosalt on 0, 2, 4 and 6 weeks and then all the groups of mice were challenged with T. evansi at week 8; corresponding to 2, 4, 6 and 8 weeks after treatment.

Two stabilates, T. evansi GRVPS 18 and GRVPS 19, were used to challenge the mice.

The results are shown in Table 27 and show that using 2.5mg/kg Trypacide Prosalt with the stabilate T. evansi GRVPS 18 one mouse out of five became infected at two weeks and at four weeks and it was only at six weeks that all 5/5 mice were infected.

Table 27

The prophylactic period conferred by quinapyramine (Trypacide Prosalt) against *T. evansi* GRVPS 13 challenge

Weeks after treatment	Drug dose (mg/kg)	Number of mice infected after challenge with GRVPS 18	GRVPS 19
0	0	5/5	5/5
2	2.5	1/5	1/5
	5.0	0/5	0/5
4	2.5	1/5	3/5
	5.0	0/5	2/5
6	2.5	5/5	5/5
	5.0	1/5	2/5
8	2.5	5/5	5/5
	5.0	5/5	5/5

In the case of T. evansi GRVPS 19 using 2.5mg/kg Trypacide Prosalt, 1/5 mice became infected at two weeks, 3/5 at four weeks and all 5/5 at six and eight weeks. Therefore it would appear that 2.5mg/kg cannot give prophylaxis for even a period of two weeks and certainly not beyond four weeks. The results also show that T. evansi GRVPS 18 is more sensitive to Trypacide than GRVPS 19.

Using a dose level of 5.0mg/kg the prophylactic period against T. evansi GRVPS 18 was at least six weeks when only 1/5 mice became infected. In the case of T. evansi GRVPS 19 the prophylactic period does not extend beyond two weeks, although some mice failed to become infected at both four and six weeks.

V. Studies on chemoprophylactic activity of Mel Cy against T. evansi infections in mice

This experiment was designed to determine the protection period which Mel Cy could confer against a challenge with a T. evansi stabilate. The results of groups of mice treated with 30 mg/kg of Mel Cy and then challenged with T. evansi GRVPS 13 at approximately weekly intervals after administration of the drug are summarised in Table 28.

As can be seen from the table all the mice challenged on week 4, 3 and 2 after treatment became infected and at one week four out of the five became parasitaemic. Those challenged three days after treatment failed to become infected indicating a prophylactic period for this dose rate of only three days.

The parasitaemia of the trypanosome-positive mice from the week one challenge, remained scanty for about 48 days (i.e. one

Table 28

The prophylactic period conferred by Mel Cy against challenge with *T. evansi* GRVPS 13

Time after Mel Cy treatment	Mel Cy dose mg/kg	No. of mice infected after challenge with GRVP 13
3 days	30	0/5
1 week	30	4/5
2 weeks	30	5/5
3 weeks	30	5/5
4 weeks	30	5/5

trypanosome per ten fields). However when blood was taken from one of these mice and inoculated intraperitoneally into five naive recipient mice, all these mice developed high parasitaemias nine days after infection. These infections caused mortality in the mice by 23-27 days which was very similar to the pathogenicity of the original stabilates. Some of the mice showed debility after two weeks of infection. However, this experiment demonstrated the inability of Mel Cy to protect the mice against T. evansi for any significant period of practical value when administered as a water-soluble injection of 30 mg/kg.

DISCUSSION

From its inception, quinapyramine consistently yielded good results against natural infections of Surra (Pellegrini and Bonelli, 1951; Ray, Short, Shivnani and Hawkins, 1953; Novinska, 1961; Auguadra, 1963).

The activity of the soluble quinapyramine dimethesulphate against most of the trypanosomes pathogenic to livestock in Africa was demonstrated experimentally by Curd and Davey (1949; 1950), and in field trials by Davey (1950).

Investigating the sensitivity of five cloned T. evansi stabilates to quinapyramine at different dose levels has produced some interesting results (Experiment I). Three out of the five stabilates showed 100% sensitivity while the remaining two showed 80% sensitivity at 5mg/kg of quinapyramine. It was noted that 0.05mg/kg was not effective against these stabilates and moreover, similar failures occurred at the 0.5mg/kg dose level, which although it cleared the circulation of trypanosomes immediately after treatment, allowed many of the mice to relapse.

The results of this experiment clearly demonstrated that the highest dose level (5mg/kg) of quinapyramine can eliminate the parasitaemias caused by the five cloned stabilates and effect permanent cures.

This experiment also indicated that, although all the stabilates tested were cured by 5mg/kg of quinapyramine, there were minor variations in sensitivity between the various stabilates. It was also encouraging to see that GRVPS 13 which was a derivative of TREU 1444 was sensitive to quinapyramine as this stabilate is highly resistant to suramin (see history of the clones) (Abebe et al., 1983).

A significant problem in the chemotherapy of animal trypanosomiasis is the failure of some drugs to eliminate the parasitaemia from infected animals if treatment is delayed. However, Jennings, Whitelaw and Urquhart (1977a) showed that treatment, if given late in T. brucei infections (21 days) may fail to remove a cryptic focus of trypanosomes residing in the brain, which subsequently gives rise to a relapse parasitaemia once the drug concentration has fallen below a critical level.

The results of experimental studies, with six different trypanocidal drugs against mouse infections of T. evansi GRVPS 13 and T. evansi GRVPS 19 (Experiment II) showed varying trypanocidal activities when treated on day 7 compared with day 21.

Melarsoprol (Mel B) diluted for administration to mice by mixing with rapeseed oil and difluoromethylorinithine (DFMO) used chemotherapeutically against early infections (day 7) and delayed

infections (day 21) of both T. evansi GRVPS 13 and T. evansi GRVPS 19 stabilates illustrated their effectiveness against these parasite stabilates. This is in contrast with the finding with the pleomorphic T. brucei infections and might possibly indicate that monomorphic T. evansi do not penetrate into the central nervous system (CNS) at least during the first 21 days of the infection.

This aspect was investigated in a later experiment (Section II) in which groups of black mice (C57 Bl) were infected with T. evansi and treated with 40mg/kg of diminazine aceturate given 60 days after infection.

It has been shown that DFMO is remarkably effective in curing acute trypanosomiasis. This is true not only in the case of infections with the veterinary trypanosomes. T. brucei and T. congolense, (Schillinger and Gorton, 1984) but also those which mainly affect humans, T. rhodesiense and T. gambiense (Sjoerdsma and Schechter, 1984).

The quinapyramine (Trypacide sulphate) was effective against T. evansi GRVPS 13 and T. evansi GRVPS 19 at a dose rate of 5mg/kg after administration or either 7 or 21 days after infection. Trypacide sulphate, which again became available recently, is the only available alternative in suramin-resistant T. evansi infections in camels. Trypacide Prosalt at 5mg/kg also provided an effective cure against T. evansi GRVPS 13 and GRVPS 19 after treatment on day 7 and day 21.

Both quinapyramine sulphate and prosalt are used against T. brucei and T. evansi infections, but resistance to this drug is widespread, and this has been confirmed by many workers

including Gill (1971). In equines, quinapyramine can be administered by giving 4.4mg/kg in 10% solution, divided into two subcutaneous doses, the second half at a different site five or six hours after the first (Leach and Roberts, 1981).

Diminazene aceturate (Berenil), which is more active against T. congolense and T. vivax, was used against the two strains of T. evansi (GRVPS 13 and GRVPS 19). All doses given to T. evansi GRVPS 13 produced permanent cures, while relapses were observed in both early and delayed treatments of T. evansi GRVPS 19 infections. There was a difference of sensitivity of the two stabilates, T. evansi GRVPS 13 being resistant to suramin and sensitive to diminazene aceturate while T. evansi GRVPS 19 is sensitive to suramin and relatively resistant to diminazene aceturate.

Isometamidium (Samorin) which was once described as "an irritant but valuable drug against animal trypanosomes" (Boyt, 1971) was used successfully at 5 mg/kg against T. evansi GRVPS 13 and T. evansi GRVPS 19 infections. Only a few reports have indicated high activity of the drug against T. evansi in dogs (Srivastava and Malhotra, 1967). However, the results obtained from the present tests showed its remarkable effectiveness in curing early infections of T. evansi GRVPS 13. In spite of this, the delayed treatment of T. evansi GRVPS 13 and, both early and delayed treatment of T. evansi GRVPS 19 have shown a slightly reduced effectiveness of the drug. Balis and Richard (1977) carried out trials in guinea pigs and dromedaries in Ethiopia with isometamidium chloride and they found that it was curative

in guinea pigs at a dose rate ranging from 0.25mg/kg body weight. Their experiments in dromedaries were extremely disappointing and from their results one, could conclude that isometamidium was only moderately active against T. evansi infections.

The results of early treatments against T. evansi GRVPS 19 infections indicated its sensitivity to suramin, but in contrast, the delayed treatments of both T. evansi GRVPS 19 and T. evansi GRVPS 13, and the early treatments of T. evansi GRVPS 13 infections demonstrated their resistance to suramin.

However, the results obtained from Experiment III indicated that Trimelarsan and Mel Cy are highly effective and can easily cure both early and chronic infections of T. evansi and so could be added to the available compounds useful in treating Surra, and especially suramin-resistant strains.

Many authors including Williamson (1980) have recommended the employment of the arsenicals against T. evansi strains. Later, Schillinger and Rottcher (1986) obtained remarkable results from camels infected with T. evansi and treated with a melaminyl arsenical compound (melarsoprol). The results obtained with the two melaminyl compounds (Trimelarsan and Mel Cy) in this thesis and those of Schillinger and Rottcher (1986) indicate that the arsenical compounds could be used as a reliable therapeutic agent against T. evansi infections. One possible explanation for the arsenical compounds high activity against T. evansi infections, is that these compounds were never used extensively in the field.

Leese (1927) has described adverse side effects of the arsenical compounds, but both compounds (Mel Cy and Trimelarsan)

used in these experiments showed neither side effects nor toxicity in all dose ranges given to the infected mice.

The minimum effective dose level of Mel Cy has still to be determined in the mouse model as the lowest doses used against the stabilate in this study were effective.

The results of these experiments suggest that further studies should be done especially in camels infected with suramin-resistant strains of T. evansi. Also it would be wise to study the side effects and toxicity of the drugs in camels under field conditions.

There has been little published on the prophylactic activity of quinapyramine prosalt (Trypacide Prosalt) against infections of T. evansi (Surra) in both experimental or naturally infected animals. The main reason for this could be due to the withdrawal of this drug (Antrycide) from the market for a considerable period and its only recent reintroduction.

Wilson (1984) reported that Antrycide only provided a relatively short period of prophylaxis. Other workers however, claim that Antrycide Prosalt could give cattle protection against trypanosome infection for up to twelve months (Lodha and Singh, 1963; Fernandez et al., 1965 and Woo, 1977). The explanation for this conflict of opinion may be possibly due to variations in the risk of infection and the degree of susceptibility of the local strains of trypanosomes.

After many years of field experience with naturally infected animals, MacLennan (1970) recommended that the drugs used as a curative and a prophylactic should be chemically different. If

one recognises this point, the limited number of drugs available for treatment of T. evansi infections, restricts the possibility of utilising a chemoprophylactic approach.

In Experiment IV it would appear that 5.0mg/kg dose of quinapyramine prosalt (Trypacide Prosalt) confers reasonable protection for at least four weeks against a relatively sensitive strain of T. evansi but only two weeks against a more resistant strain.

A dose of 2.5mg/kg could not be recommended as a prophylactic dose in mice, but as the relationship of effective dose between cattle and that in mice has still to be evaluated it is highly probable that the recommended dose of 0.5mg/kg would confer a reasonable period of prophylaxis in cattle.

The study (Experiment V) testing the prophylactic activity of Mel Cy against T. evansi infections in mice unfortunately failed to demonstrate any protection beyond three days.

CHAPTER FOUR

STUDIES ON THE CHEMOTHERAPY OF *T. evansi* INFECTIONS

SECTION II

FURTHER STUDIES IN GOATS AND MICE

INTRODUCTION

The control of African trypanosomiasis in domestic animals largely depends on the effective use of chemotherapy and chemoprophylaxis (Holmes and Torr, 1988). This is particularly so in the case of T. evansi infections because the control of the principal vectors is impractical (Nieschulz, 1929; Sergent and Donatien, 1922). However, as no new drugs have been marketed for the last 30 years and because of the small range of drugs suitable for use in T. evansi infections there is a major problem in the control of the disease. Furthermore there are reports of drug resistance, as judged by the failure of trypanosomes to disappear from blood or the detection of parasitaemia at comparatively short intervals of time after treatment (Trail et al., 1985).

The need for improved diagnostic techniques, the development of new drugs and/or the improved use of trypanocidal drugs presently available has been recommended on many occasions. A further series of experiments were conducted on T. evansi infections. The development of an isolation unit at the University of Glasgow Veterinary School licenced for work with trypanosome infected ruminants provided an opportunity to conduct some of these studies in goats, as well as mice. Following the success of the new arsenical compound Mel Cy in mice (Section I) an experiment was conducted in goats to test its therapeutic activity against T. evansi in a ruminant host (Experiment VI).

It is a common feature in both animal and human trypanosomiasis for the parasite to be temporarily absent from the blood circulation for a period, only to be followed by

recrudescence of the parasitaemias. A similar behaviour can also be attributed to drug-resistance, which appears to be acquired by exposure of the trypanosomes to inadequate trypanocidal dosages (Whiteside, 1962a). Sometimes the relapse infections retain equal sensitivity to the drug as the first infections.

This latter relapse infection was reported by Jennings et al. (1977a, b) in T. brucei infected mice. The brain was found to be the site where the trypanosomes, which escaped the action of the drug, finally emerged and re-established the circulating parasitaemia (Jennings et al., 1979).

It is well established that T.b.brucei is a pathogenic trypanosome which penetrates the brain of animals in chronic infections. However, in the closely related species, T. evansi, incoordination in a dog infected by the parasites has been reported by Hutyara and Marek (1938). Later Innes and Saunders (1962), found T. evansi in the grey and white matter, and in the CSF of dogs. The latter two workers also mentioned that the CSF from infected horses caused infections when inoculated into rats.

However, little attention has been paid to T. evansi infections so that less is known about the possible role of CNS involvement. The second experiment described here (Experiment VII) was designed to study whether or not during infections of T. evansi the parasite passes to the brain like T. brucei.

From its introduction up to the present time, isometamidium chloride has been shown to be efficacious against most of the pathogenic trypanosomes of cattle (Trail et al, 1985).

In their extensive work on camel trypanosomiasis (T. evansi), Schillinger et al (1982) found a low trypanocidal action for isometamidium chloride against T. evansi infections in East African camels. These authors also emphasised the development of local toxicity reactions at the injection sites, temporary paralysis and shock reactions in the treated camels.

The effectiveness of Samorin in treating T. evansi infections has also been described by Petrovskii (1974). However, the prospect of using isometamidium chloride against T. evansi infections in camels appears to be hampered by the severity of the toxic reactions. In contrast there is a growing awareness of the importance of T. evansi in cattle and buffaloes, particularly in Asia and this drug may be used there in the future.

To date, there is little evidence in the field indicating the existence of Samorin-resistant strains of T. evansi but it is possible this may emerge as a problem. An experiment was conducted in mice to determine whether it was possible to build up Samorin-resistance in two strains of T. evansi.

One of the strains T. evansi GRVPS 13 was highly resistant to suramin (Abebe et al, 1983) and the other strain T. evansi GRVPS 19 was initially slightly resistant to Samorin.

MATERIALS AND METHODS

Mice

Details of the materials and methods used in the mice studies have been described in Section I of this chapter.

Goats

Details of the goats, their housing and maintenance and the measurements of parasitaemia, haematocrit, body temperature and

body weight have been described in Chapter Three (Section II).

Trypanosomes

The origins of the trypanosomes used in these studies have been described in Section I of this chapter.

VI. The therapeutic activity of Mel Cy in goats infected with T. evansi GRVPS 13/3

The aim of this study was to determine the efficacy of Mel Cy to treat goats infected with T. evansi.

Three goats only could be used because of the limitations of accommodation in the isolation unit. Each goat was infected with T. evansi GRVPS 13/3 by the intravenous injection of 1×10^5 trypanosomes. After sufficient time had elapsed to allow establishment of the infection the goats were treated with Mel Cy kindly provided by RMB Animal Health at a dose of 0.25mg/kg. The drug was dissolved in saline and administered by subcutaneous injection as a 0.25% (w/v) solution. At weekly intervals throughout the latter part of the study sublethally irradiated mice were subinoculated with blood (0.5ml) by intraperitoneal injection in order to identify infections undetectable by standard microscopy.

The results of the three goats (179, 180 and 189) are presented in Figures 37, 38 and 39.

The prepatent period of goat 180 was 35 days whilst in goats 179 and 189 it was delayed until 44 and 42 days respectively. However, in the latter two goats subinoculation of their blood into irradiated mice on day 28 revealed they had circulating parasitaemias. When the goats had had patent

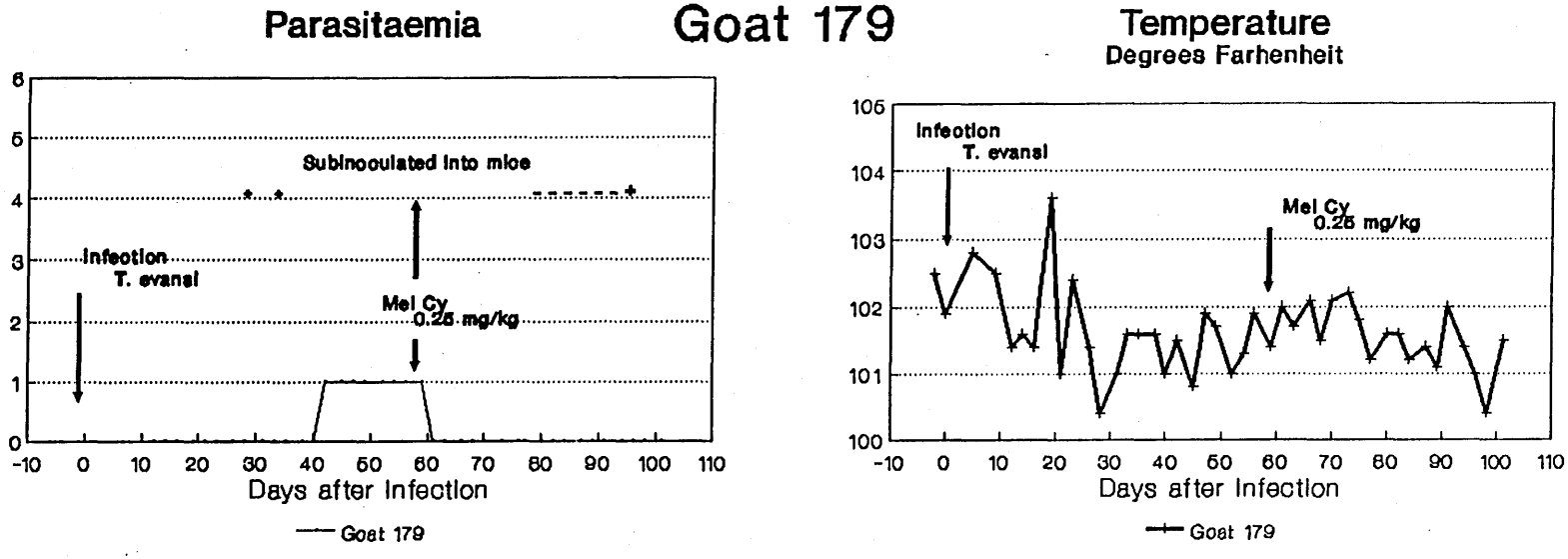
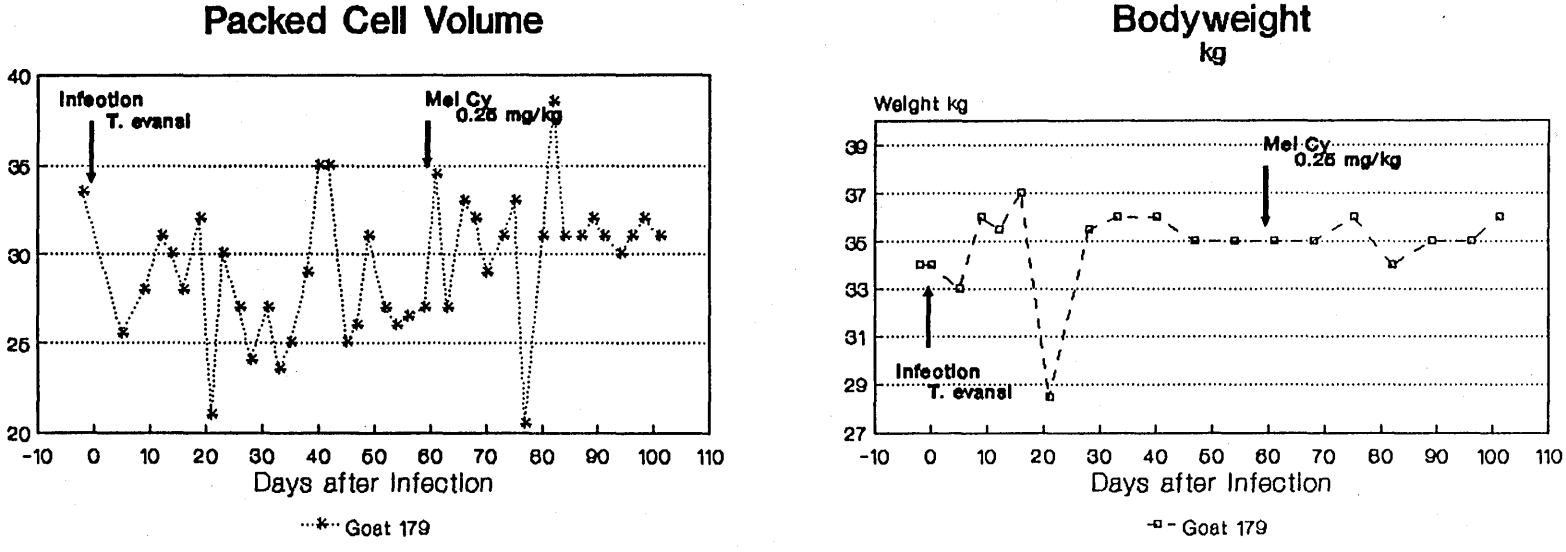


Fig. 37 Goat 179 infected with T. evansi and treated with Mel Cy (s.c.)



Subcutaneous Mel Cy (0.25 mg/kg)

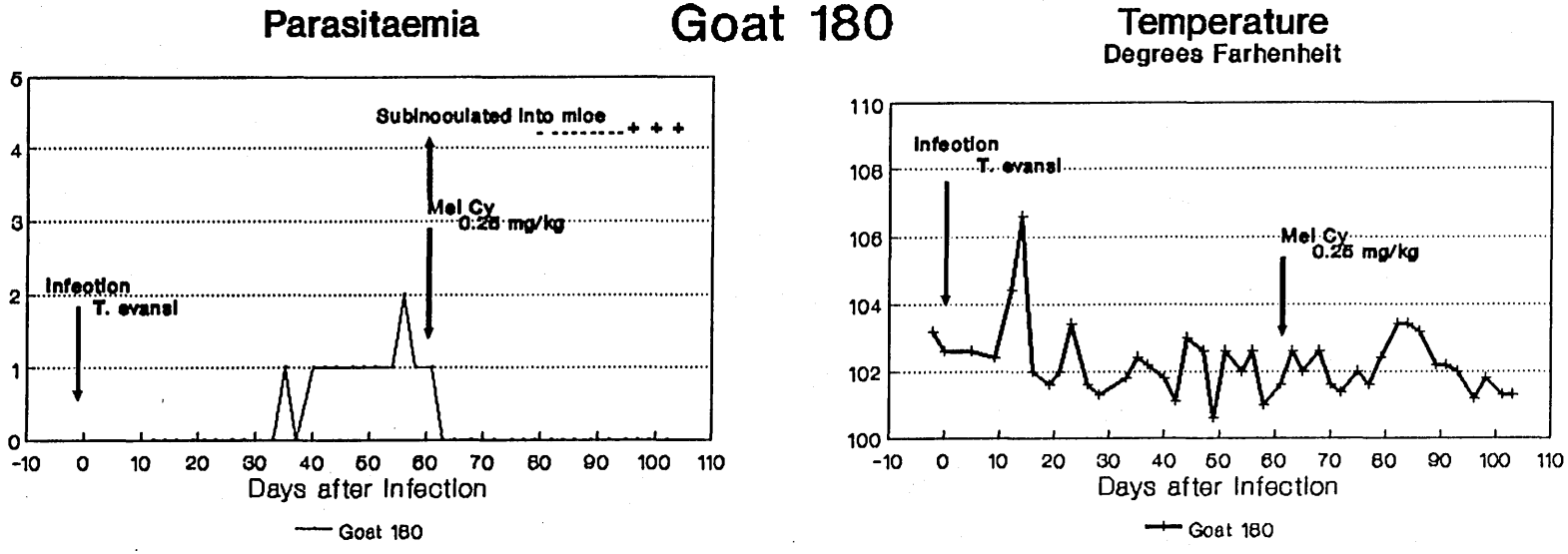
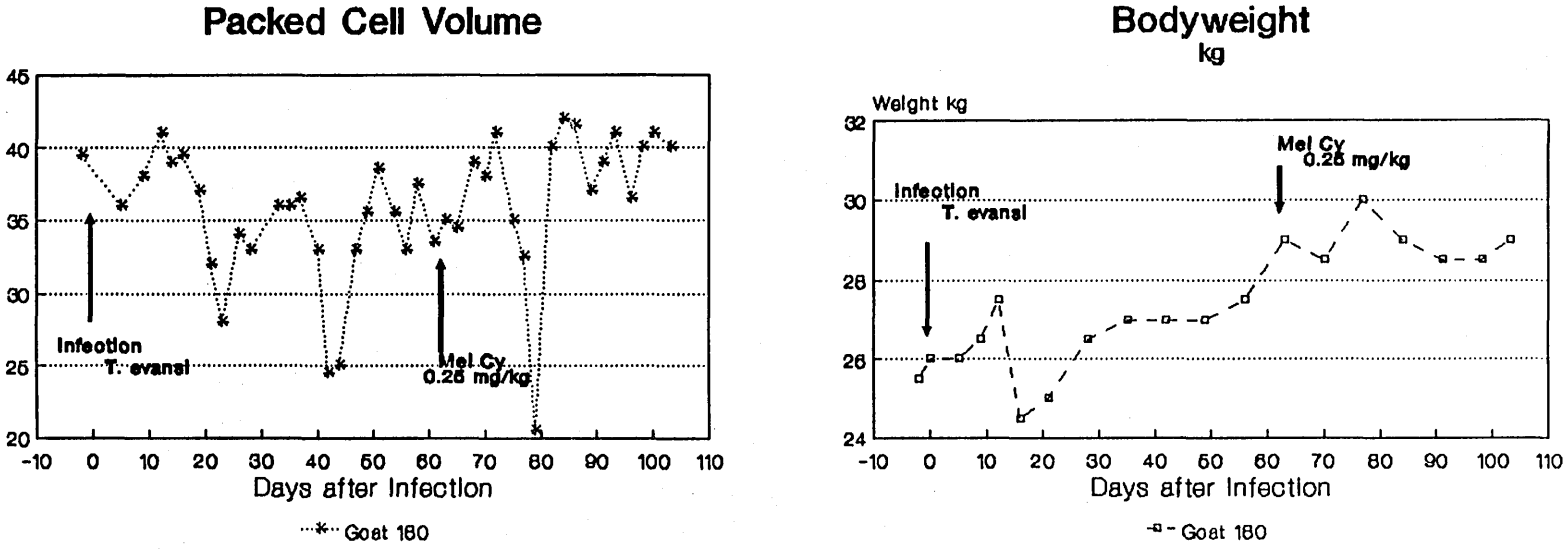


Fig. 38 Goat 180 infected with T. evansi and treated with Mel Cy (s.c.).



Subcutaneous Mel Cy (0.25 mg/kg)

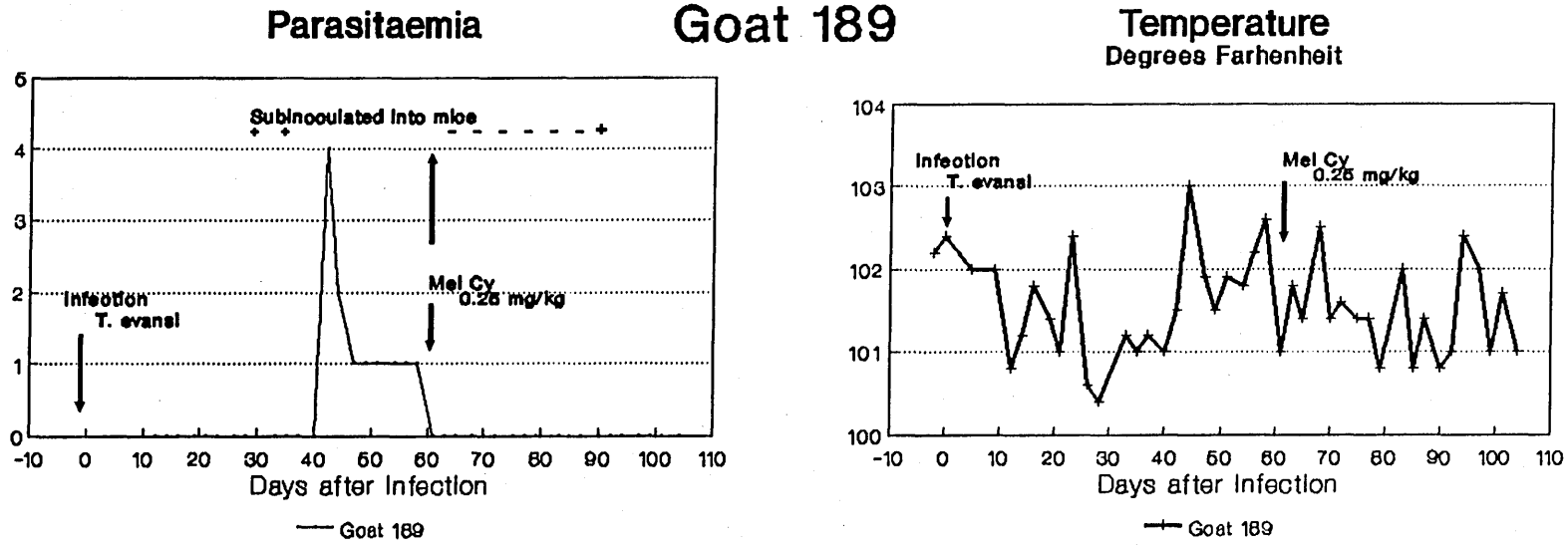
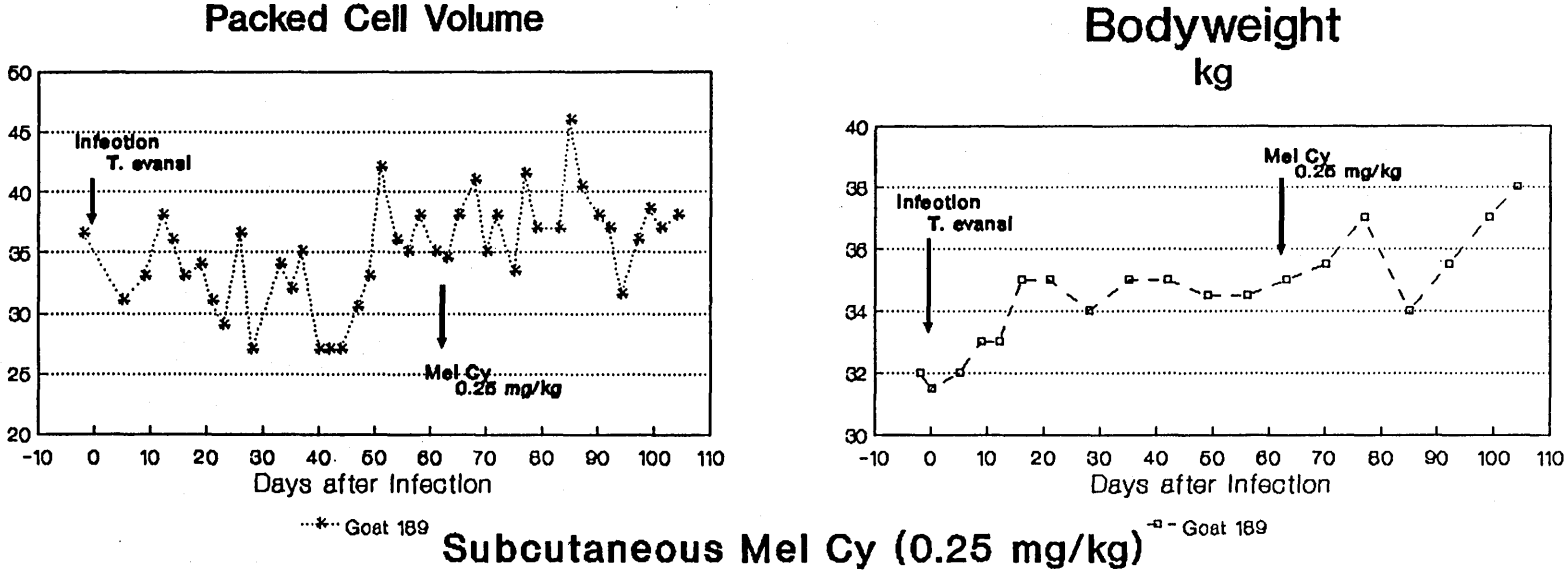


Fig. 39 Goat 189 infected with T. evansi and treated with Mel Cy (s.c.)



parasitaemias for approximately three weeks they were treated with Mel Cy on day 61 post infection. Following this treatment the parasitaemias became undetectable by microscopy and subinoculation for at least 30 days. However before termination of the experiment all three goats had developed relapse infections which were detectable by mouse subinoculation. These were first detected on days 33, 33 and 29 post treatment in goats 179, 180 and 189 respectively.

Measurement of body temperature revealed a brief period of pyrexia at approximately 20 days post infection. Intermittent pyrexia was later detected in goat 189 but not in the other two animals. Similarly a fall in haematocrit was found at approximately 20 days post infection and two goats (179 and 180) had two other brief periods of anaemia. However in general the infection was of low pathogenicity and body weights were not adversely affected with the exception of a brief period of weight loss in goats 179 and 180 at about 20 days post infection (see Figures 37, 38 and 39.

VII. The treatment of chronic T. evansi infections in mice

Twenty C57 Bl mice were infected intraperitoneally with 1×10^5 T. evansi GRVPS 13, prepared from the frozen stabilate. Tail-blood from the mice was examined for trypanosomes by the wet film technique for about 60 days prior to chemotherapy. At 60 days after infection, 40 mg/kg of diminazene aceturate (Berenil) which is known not to penetrate through the blood-brain barrier was given to the mice.

Tail blood was examined every second day for 120 days after

chemotherapy to determine whether relapse infections developed.

The results are presented in Table 29. The prepatent period of the 20 infected C57 Bl mice was between 13-17 days.

Following the onset of patent parasitaemia there was a remission of the infection and the number of parasites in the blood circulation was low. This was followed by a long aparasitaemia period (28 days) during which no trypanosomes in the circulating blood were observed. Later, the reappearance of the parasites in the circulating blood was confirmed by tail-blood examination. It was after this that some of the mice had their maximum parasitaemias while others remained at low levels.

Ten highly parasitaemic mice died between 45-60 days after the initial onset of the infection. The remaining ten mice which were treated with 40 mg/kg of diminazene aceturate, were all permanently cured and no relapse infections were encountered through the whole period of observation of 120 days.

Table 29

C57 Bl mice infected with T. evansi GRVPS 13 and treated with Berenil

No. mice infected	Treated DAI	Dosage mg/kg	No. mice died before treatment	No. cured/ total treated	Observation days after treatment
20	60	40	10	10/10	120

DAI - Days after infection

VIII. An attempt to decrease the drug sensitivity of T. evansi GRVPS 13 and GRVPS 19 by repeated subcurative treatments with isometamidium

Groups of normal and irradiated CD-1 mice were infected intraperitoneally with T. evansi GRVPS 19 and T. evansi GRVPS 13, prepared from frozen stabilates.

Ten passages and eight passages through normal and irradiated mice respectively were conducted and at each stage either similar or increased doses of isometamidium chloride were administered. Stabilates were prepared and stored in liquid nitrogen after each passage.

The Samorin dosages given to T. evansi GRVPS 19 infected mice ranged between 0.001 - 5 mg/kg while the dose ranges given to T. evansi GRVPS 13 infected mice ranged between 0.001 - 1.5 mg/kg of samorin. The exact experimental regimens are given in Figures 40 and 41.

The two stabilates GRVPS 13 and GRVPS 19 were tested at the beginning for their sensitivity to Samorin and it was found that both relapsed after treatment with 0.1 mg/kg. This dosage was taken as the starting point in producing a Samorin-resistant stabilate. At each relapse the trypanosomes were taken and inoculated usually into both irradiated and normal mice and the resulting infections subjected to increasing dosages of Samorin. The exact regimen varied between the two strains but the details are set out in Figures 40 and 41. It was found that the sensitivity of GRVPS 13 increased from 0.1 mg/kg at the beginning to 1.5 mg/kg while GRVPS 19 increased from 0.1 mg/kg to

Fig. 40 Production of Samorin-resistant T. evansi GRVPS 19

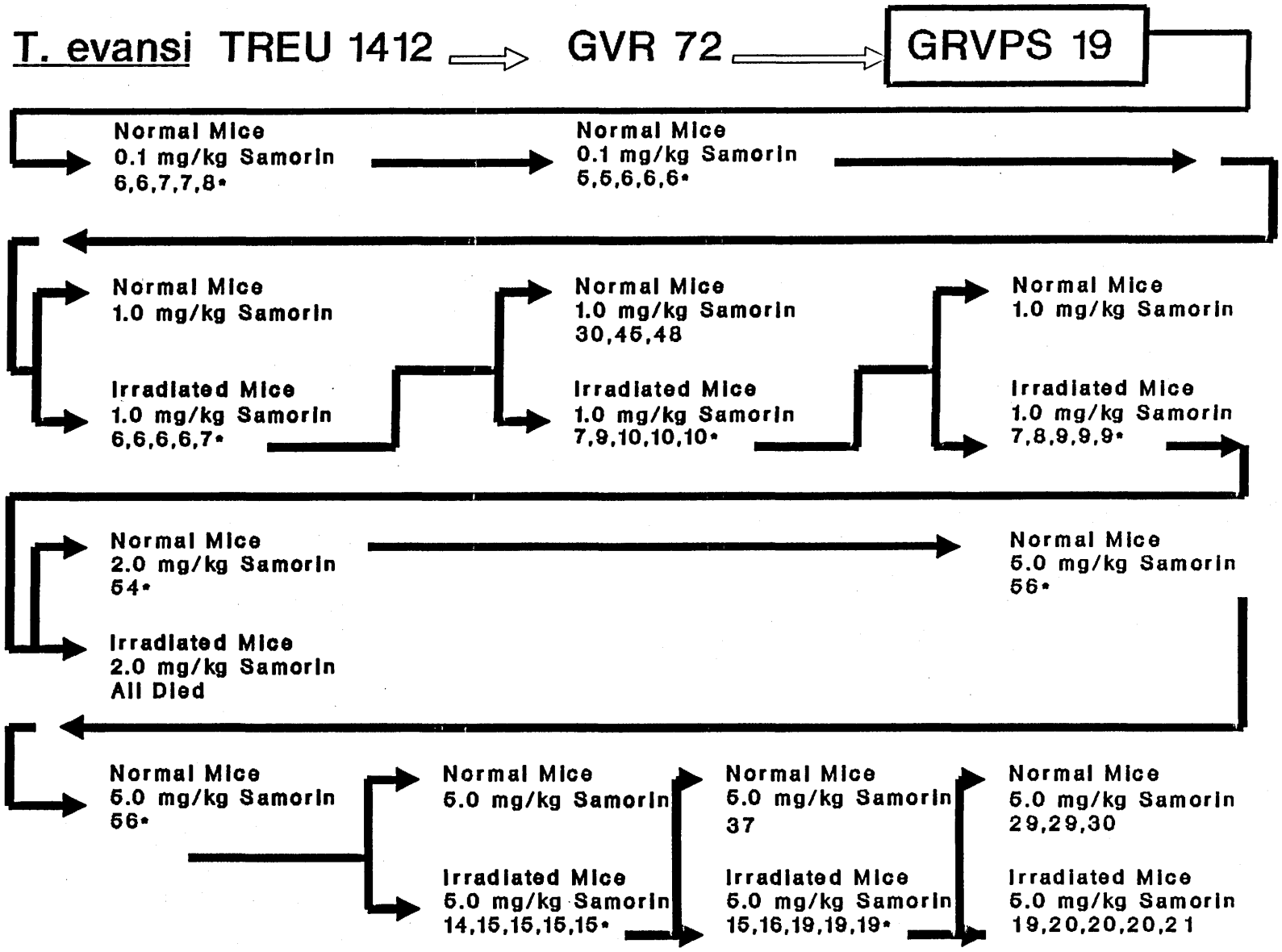
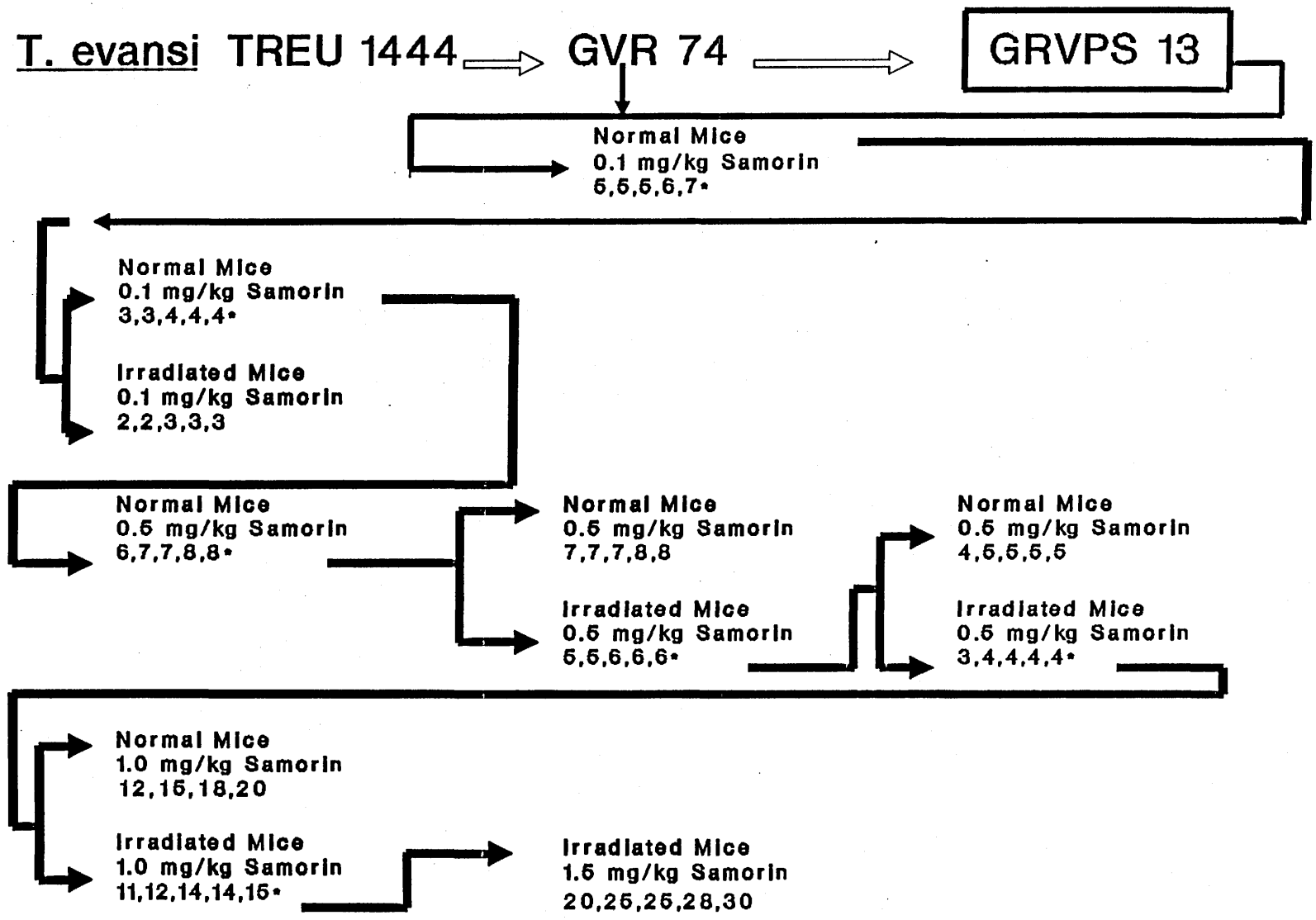


Fig. 41 Production of Samorin-resistant T. evansi GRVPS 13



5.0 mg/kg.

DISCUSSION

The course of Trypanosoma evansi infections in goats demonstrated that there were long pre-patent periods. However, the finding that blood subinoculated from the three infected goats, apparently aparasitaemic in the prepatent period, resulted in establishment of parasitaemias in irradiated recipient mice indicated that the parasites were present at a low level despite being microscopically undetectable. This finding substantiates the reports of Cross (1921a) and Kuppaswamy (1941) who described the course of T. evansi infections in goats as a chronic form in which the parasites are normally scanty in the circulating blood.

The temperature rise in the early stage of infection about day 20, when no trypanosomes were detected in the blood, and then the low but intermittent rise of temperature when the trypanosomes appeared in the peripheral blood, indicated that the pyrexia was not correlated with the number of trypanosomes.

It is perhaps possible that the high fever recorded during the prepatent period was, due to the tissue invasion of the parasites, as T. evansi is known for its extracellular invasions.

Similarly the PCV values of the goats dropped during the prepatent period, but were later correlated with the parasitaemia to some degree.

All goats gained weight during the experiment. This weight gain was similar in all the goats. The therapeutic activity demonstrated by Mel Cy at a dose rate of 0.25mg/kg was reflected in the negative microscopical blood examinations for circulating trypanosomes. However, blood collected 30 days after treatment

from all three treated goats and subinoculated into irradiated mice resulted in patent infections. Previously mice infected with a suramin-resistant strain of T. evansi and treated with the 0.5mg/kg of Mel Cy recorded permanent cure. However, as the drug is still under experimental study, it may be possible that this dose rate 0.25mg/kg is not the optimum dose level to use for T. evansi infected ruminants.

The results of experiment VII indicated that infections of T. evansi are different from the closely allied T. brucei infections observed by Jennings et al (1977a; b). This work had shown that treatment with diminazene aceturate failed to permanently cure chronic infection of T. brucei in mice. However, with this T. evansi GRVPS 13 infection, permanent cures were obtained to chronic infections which indicates that no focus of infection such as in the brain remained in the mice which could re-establish parasitaemia. This does not correlate with those reports that record CNS involvement in T. evansi infections (Laveran and Mensil, 1912; Leese, 1927; Wilson, 1984) However, it is possible that 60 days does not allow sufficient time in the mouse for the establishment of a CNS infection.

It does, however, correlate with the work of Evans and Brightman (1980) who were able to clear a fairly chronic 21-day old infection of T. evansi with SHAM-glycerol, which also suggested that there was no CNS involvement.

The development of resistant Trypanosoma strains is of great importance in view of the wide application of trypanocidal drugs. The results of experiment VIII indicate that in the laboratory

the development of Samorin-resistance could be readily promoted by using irradiated mice infected with T. evansi rather than the normal mice infected with the same strain.

After T. evansi GRVPS 19 was passaged ten times and T. evansi GRPS 13 was passaged eight times through normal or irradiated groups of mice, receiving increasing dosages of Samorin, the two strains built up a high degree of Samorin-resistance, 5 mg/kg for T. evansi GRVPS 19 and 1.5 mg/kg for T. evansi GRVPS 13. Such rapid acquisition of Samorin-resistance was mainly observed in irradiated groups of mice. Furthermore the irradiated mice demonstrated the shortest intervals between treatment and relapse infection and in some cases no aparasitaemic interval was observed and the mice died shortly after treatment. The interval period of the normal mice was longer than the irradiated mice and some normal mice did not show any relapse infection.

It would appear that the irradiated-infected groups of mice have lost their humoral immune responses to the disease and the treatment alone was not able to prevent rapid re-establishment in the irradiated mice while the combination of immune response and trypanocidal effects were able to slow up the re-establishment of the parasites in non-irradiated animals.

Similarly, Von Jansco and Von Jansco (1935) and Schnitzer, Laferty and Buck (1946) have suggested that the rapid-passage method of producing resistant strains in the laboratory may have an advantage because the rapidly emerging trypanosomes may avoid the effect of the host's immune response.

The present experiment suggests that under laboratory studies, building up of Samorin-resistance may be promoted more readily in immunosuppressed mice than in normal mice.

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