SOME ASPECTS OF THE EPIDEMIOLOGY AND CHEMOTHERAPY OF AFRICAN TRYPANOSOMIASIS

A thesis submitted for the Degree of Doctor of Philosophy in the Faculty of Veterinary Medicine of the University of Glasgow

by

HIMAPONDO GEORGE BUSIKU CHIZYUKA D.V.M.

Department of Veterinary Parasitology,

University of Glasgow.

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CONTENTS

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SUMMARY

Trypanosomiasis affects man and his domestic livestock in 10 million km^2 of tropical Africa. It is a major constraint to the economic development of animal production in Zambia in one third of which mixed farming is rendered impracticable.

In this study an attempt has been made to investigate the epidemiology of animal trypanosomiasis in Zambia and to evaluate the efficiency of the currently used drug regime for the control of the disease. Further, the prevalence of infected domestic livestock in selected areas of the country and the efficacy of two anti-trypanosomal drugs Berenil and Samorin were investigated.

It was found that the incidence of bovine trypanosomiasis is high. Trypanosoma vivax infections in one of the areas surveyed became more prevalent as distance from the tsetse-belt increased. There was an apparent increase in the incidence of T. brucei infections in cattle and five clinical cases of such infections are described.

Treatment of calves with either Berenil (diminazene aceturate) or Samorin (isometamidium chloride) either 24 hours or 21 days after infection with T. vivax or T. congolense subsequently resulted in a relapsing parasitaemia. This is the first time that relapsing T. congolense and T. vivax infections following Berenil or Samorin treatment have been described in Zambia.

Further investigation of the effects of Berenil on T. brucei infections in mice demonstrated that the source of relapsing infection was the brain. This finding has relevance to trypanosomiasis of

domestic animals in which Berenil is a commonly used drug and also to human trypanosomiasis which, in the late stages of the disease, affects the central nervous system.

The study indicated that the efficacy of current chemotherapeutic regime in Zambia should be critically examined and that more information on the incidence of animal trypanosomiasis is required.

CHAPTER 1

GENERAL INTRODUCTION:

ANIMAL TRYPANOSOMIASIS IN ZAMBIA

General Introduction

The literature review presented in this introduction on animal trypanosomiasis in Zambia is divided into two sections. The first section (1.1 to 1.7) covers the geographical, historical and economic background of Zambia, and the incidence of trypanosomiasis in domestic animals and game in the early days of the country's history up to about 1912. The author fe@ls it necessary to give a brief history of Zambia and also of the prehistoric background of the region and its people in order to assist the reader to follow some of the arguments which are advanced later in this study. Aspects of pathogenicity exhibited by some trypanosomes may be as a result of evolutionary genetic reponses by the domestic animal and the human hosts. Planning of control measures and their popularity within official government circles largely depend on the economic structure of the country. Therefore, the economic background of Zambia will be mentioned. Mention of the incidence of trypanosomiasis as it affects the human population is made at the end of the first section.

The second section (1.8 to 1.11) describes the incidence of animal trypanosomiasis from about 1914 to 1978 and deals with chemotherapeutic and chemoprophylactic control measures of the animal disease. Other measures taken to control trypanosomiasis are also briefly discussed.

The subject of this thesis is concerned with studies of some aspects of the epidemiology and chemotherapy of African trypanosomiasis.

Trypanosomiasis (Greek: trypan = borer, soma = body) is a general term for a group of diseases occurring in man and his domestic animals. These diseases are caused by members of the genus Trypanosoma, flagellated parasitic protozoa infecting the blood stream (Ford, 1962; Hoare, 1966). Trypanosomes are transmitted from man to man, animal to

animal and between animals and man by the bites of various flies. The most important vectors in Africa are the tsetse flies, members of the genus Glossina (Bruce, 1895; Ford, 1962; Hoare, 1966). The trypanosome undergoes cyclical development within the fly which becomes infected for the duration of its life (Ford, 1962). Flies of the families Stomoxydae and Tabanidae are primarily responsible for the transmission of Trypanosoma evansi in India and Africa (Rüchel, 1975). In Africa, these biting flies may be responsible for mechanical transmission by the passive transfer of infective blood from one host to another (Montgomery and Kinghorn, 1908; Hornby, 1921; Ann.Rep., 1935, Sheehy, 1956; Shaw, 1960; Ford 1962; Ormerod, 1979).

The enormity of trypanosomiasis problem in Africa can be gauged from the fact that approximately ten million square kilometres (about 37% of the continent), wholly within the tropics, except for small areas on the south-east coast, are infested with tsetse (Buxton, 1955; Ford, 1975). Within almost the whole of that area productive cattle cannot be kept because of the threat of trypanosomiasis. This threat, and that of human trypanosomiasis, has had a devastating effect on the health, economic development and history of Africa (Willett, 1970; Apted, 1970a and 1976; Ford, 1971). Animal trypanosomiasis, as a cause of protein malnutrition, is now probably a greater problem, even on purely medical grounds, than human trypanosomiasis (Willett, 1970).

Trypanosomiasis caused by Trypanosoma congolense and T. vivax occur widely in all parts of Africa except the arid areas of the North and the extreme South, affecting cattle, sheep, goats, horses and dogs (Ford, 1962; Willett, 1970; Griffin and Allonby 1979a). T. brucei causes a severe and often fatal disease in horses, camels, dogs, sheep and goats, but has low pathogenicity for cattle (Ford, 1962). T. simiae causes a

hyperacute disease in pigs, a mild one in sheep and goats whilst horses and cattle are not known to be infected (Ford, 1962; Stephen, 1966).

The disease known as "Surra" occurs in India in cattle and horses as a result of infection by T. evansi. This parasite has spread to Mauritius, Malagasy, Sri Lanka, Pakistan, China and Indonesia. In the Sudan, Somalia and Northern Kenya camels are mainly infected.

In South America horses suffer from "Mal de Caderas" as a result of infection with T. equinum transmitted by biting flies from the reservoir of infection, Capybara, a wild rodent. Dourine is a venereal disease of the horse caused by T. equiperdum and is found in India, Asia, parts of Africa and South America (Ford, 1962).

1.1 Geographical features

Zambia lies between the latitudes 8^{o} and 18^{o} South and longitudes 22^{o} and 34^{o} East and has a total area of 750,000 square kilometres (Fig.1). With a human population of approximately five million, the overall density is six per square kilometre, while the rural population density is 3.6 per square kilometre (UNDP Report, 1976).

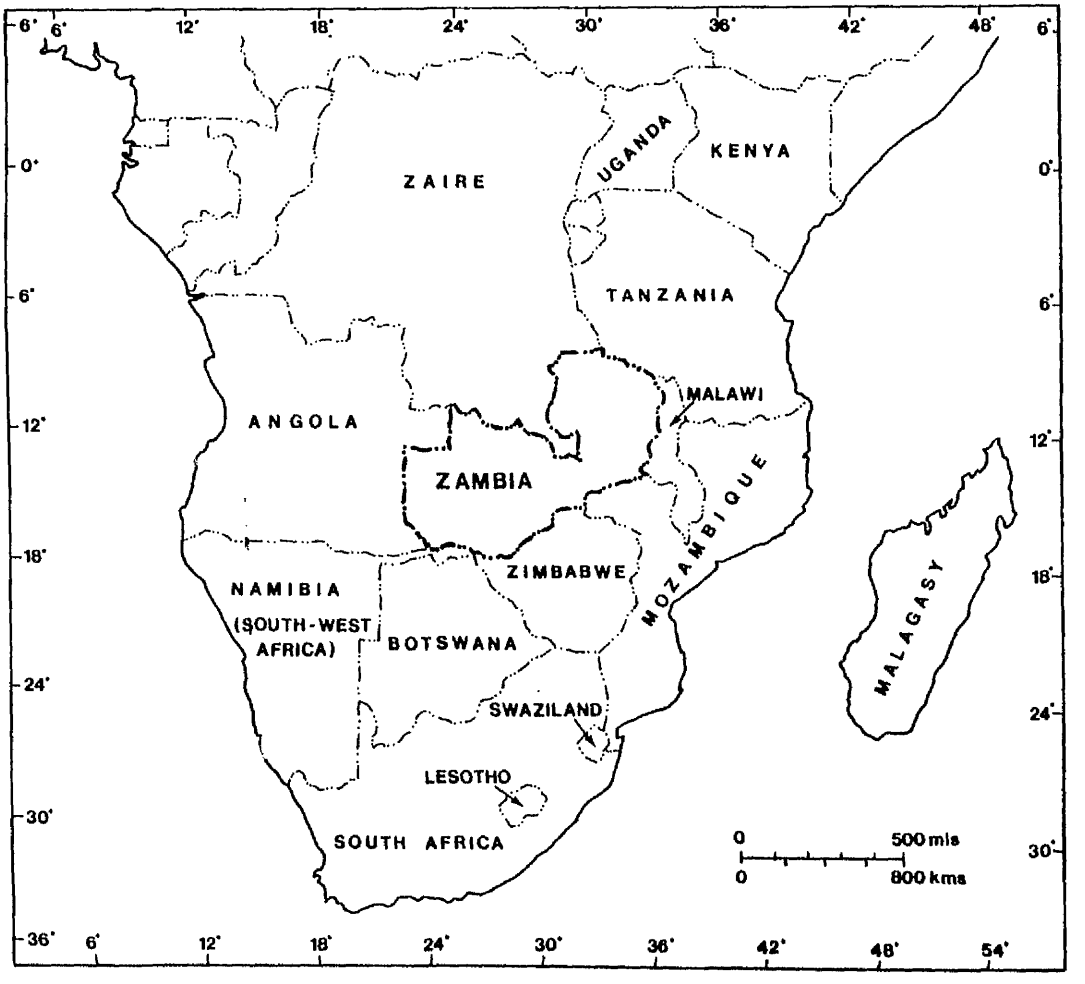
The tropical heat is moderated by altitude, since the country forms part of the central African plateau, the height of which varies between 1200 and 1500 metres above sea level.

Zambia's rainfall is concentrated in one rainly season, from November to March or April. Rainfall is heaviest in the north (100-150 cm) and lowest in the south with as little as 64 cm per annum in the Zambezi Valley.

Temperatures vary with rainfall. Towards the end of the dry season, during September and October, mean temperatures rise to between 29^{o}C and

Figure 1

ZAMBIA AND HER NEIGHBOURS



32°C in the north and north-east, and to as much as 35°C over most of western Zambia. Night-time temperatures during this hot season are 10° to 16° lower. In June and July daytime temperatures average less than 13°C in the north and east, and less than 10°C in the west; to the south and west, ground frost often occurs at night.

Most parts of Zambia are fairly well watered. There are several lakes, major rivers and flood plains, all of which are fed by a multitude of tributaries. Smaller rivers dry up towards the end of the dry season, but meantime water has collected in long shallow depressions, known as "dambos".

In the north-western and north-eastern regions where annual rainfall is more than 100 cm, the typical vegetation is deciduous miombo woodland intersected by grassy dambos.

The best soils, which are relatively rich in base metals and ferro-magnesian minerals, are found in stretches of undulating country where the rainfall is less than 100 cm in Central, Southern and Eastern Provinces. The vegetation here is a mixture of acacia, combretum and afromosia trees known as "munga" woodland, standing in tall grasses suitable for grazing cattle.

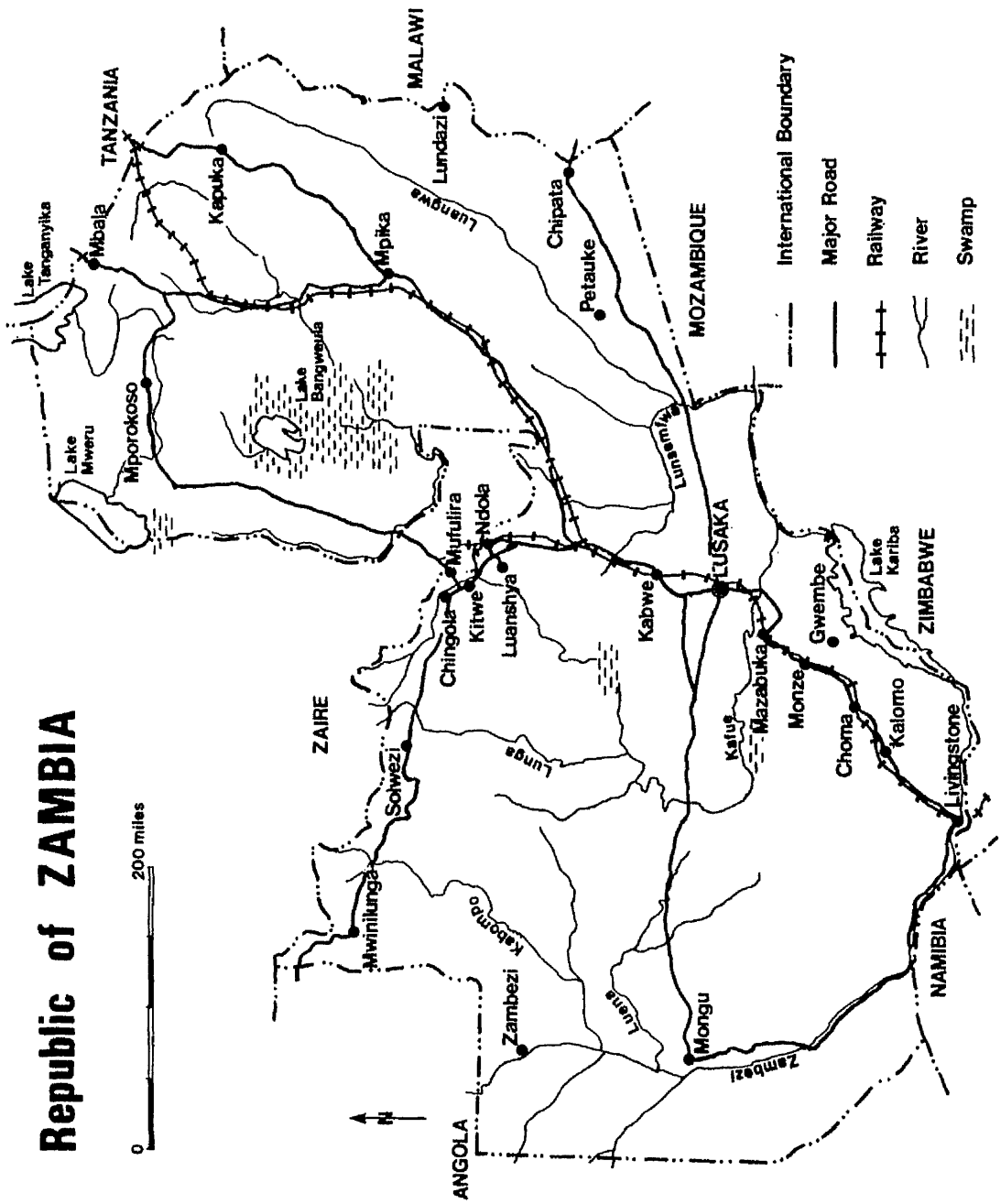
The Kafue Flats and the upper Zambezi flood plains, which are enriched by alluvium deposited by the two major rivers, provide excellent grazing for cattle.

The main geographical features, towns and lines of communication are shown on Figure 2. The administrative districts of Zambia which are frequently referred to below are shown on Figure 3.

1.2 Historical perspective

Zambia (then known as Northern Rhodesia) was created, like many

Figure 2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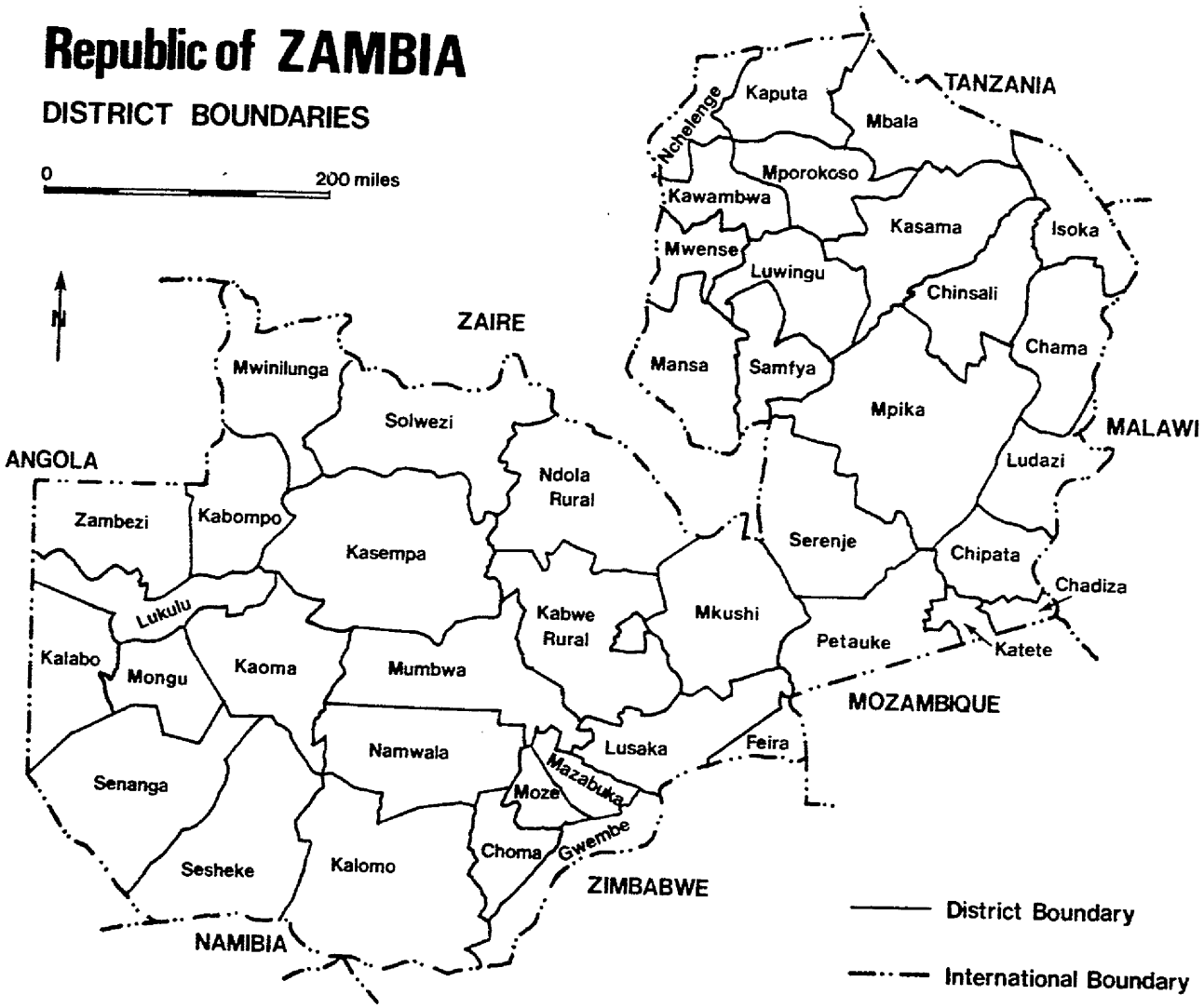
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Kalabo
Mongu
Kaoma
Mumbwa
Namwala
Lusaka
Feira
Mazabuka
Moze
Senanga
Choma
Gwembe
Sesheke
Kalomo
District Boundary
International Boundary

Figure 3

other African countries, by the partition of Africa in the late nineteenth century (Roberts, 1976). British interest in the territory north of the Zambezi was first aroused by the publication of David Livingstone's "Missionary Travels and Researches in South Africa" (Livingstone, 1857). In 1889 Cecil Rhodes, a millionaire businessman, persuaded the British Government to grant a charter to his newly formed company - the British South Africa Company. By this charter, Rhodes was able to use the authority of the British Government in staking out claims to African territory at the expense of other European powers (Roberts, 1976). By 1895, Rhodes had acquired treaties with African Kings and chiefs in the Northern territories, which from 1897 were officially called Northern Rhodesia. For administrative purposes the Company divided the region in 1899 into two: North-Eastern Rhodesia with Headquarters at Fort Jameson (= Chipata), and North-Western Rhodesia with Headquarters at Kalomo. In 1911 the territory was reunited to form Northern Rhodesia with Headquarters at Livingstone. By 1915 the Company had become anxious to reduce its expenses in Northern Rhodesia, especially as it had to bear the cost of defending the country against German troops from East Africa. In 1924 the Colonial Office took over Northern Rhodesia from the British South Africa Company.

Northern Rhodesia became the independent state of Zambia on 24th October, 1964.

1.3 Economic background

The economic development of Zambia has been based largely on the exploitation of one of the world's richest deposits of copper. The First World War stimulated the market for all base metals. However, the main watershed in the copper industry came in the early 1930s and by 1940

production had increased 40 times and a hundred fold by 1960. This rapid expansion in economic activity led to a rapid and substantial number of European farmers settling along the line of the railway, which linked the copperbelt and seaports in South Africa and which, for the most part, runs through tsetse-free areas of the plateau. Agricultural policies were thus designed to encourage these farmers to grow cash crops to feed the mining community. Little of the economic structure has changed since Independence.

1.4 Prehistoric background

Most of the indigenous Zambian population are Bantu-speaking. Bantu-speaking people are a cultural group who, today, number some 70 million and occupy most of Africa south of the Equator (Oliver, 1970). Although the keeping of goats, sheep, pigs and cattle in Africa started about five thousand B.C. (Clark, 1970), it is postulated that the earliest Zambian farmer may have come in the country as a result of the expansion of the Bantu-speaking peoples from their nuclear dispersal area in the Congo (Fagan, 1970; Clark, 1970; Oliver, 1970; Buyst, 1974, 1977b). According to Fagan (1970), there is evidence from Gundu, Kalomo district, and Ing'ombe Ilede, Gwembe Valley in the Southern Province to suggest that Early Iron Age inhabitants kept cattle and small livestock.

1.5 Incidence of animal trypanosomiasis (1857 - 1912)

The destructive effect of trypanosomiasis on the rearing of livestock has long been recognised by the local people in areas where this disease occurs. During his second Missionary journey across central Africa, David Livingstone (1857) noted of "tsetse-fly disease" in Zambia that "many tribes on the Zambezi can keep no domestic animals

except the goat, in consequence of the scourge existing in their country".

Trypanosomiasis has always been known to local people under a variety of names. Some of these denote symptoms - e.g. Kaodzera (Ngoni language) = to nod; Ndulu (Chewa) = to nod (Bruce, Harvey, Hamerton, Davey and Bruce, 1912a; Shircore, 1912), while others are names of the fly itself - e.g. Luuka (Tonga), tushembe (Bemba), Kamdzembe (Nsenga), Zeze (Lozi) (personal observations).

Montgomery and Kinghorn (1908) recorded such synomyms of trypanosomiasis as 'fly disease', 'fly struck', or simply 'fly'.

Nagana (from a zulu word 'anagana' = powerless, frail, useless (Hoare, 1972)) nowadays denotes those African parasitoses which are caused by trypanosomes, are pathogenic for domestic animals and are transmitted by biting flies (Rüchel, 1975).

The earliest recorded observations on trypanosomiasis of domesticated livestock in Africa was made in Zambia by David Livingstone (1857).

Whereas the discovery of the nagana parasite by David Bruce in 1894 was a result of extensive examination of blood slides (Bruce, 1915), the relationship between 'nagana' and the 'tsetse' was first described by Livingstone. In his account on tsetse-fly disease in the South West of Zambia, Livingstone (1857) noted that, "the symptoms seem to indicate what is probably the case, a poison in the blood. The poison-germ, contained in a bulb at the root of the proboscis, seems capable, although very minute in quantity, of reproducing itself". Livingstone had also suspected poisonous plants to be the cause of tsetse-fly disease. He gave up this theory when "Major Vardon of the Madras Army rode a horse up to a hill infested by the insect without allowing him time to graze, and, though he only remained long enough to take a view

of the country and catch some specimens of tsetse on the animal, in ten days afterwards the horse was dead". About forty years later, Bruce (1915) conducted a similar experiment using dogs and oxen which he kept in a tsetse-infested area in Zululand and for the first time demonstrated that the nagana parasites were transmitted by tsetse flies.

The problem of animal trypanosomiasis in Zambia was really first appreciated during the period 1890-1910 when European settlement and development in the country started (Shaw, 1960), and it has since remained the biggest single problem confronting the Department of Veterinary and Tsetse Control Services (Ann.Rep., 1934-39; 1957, 1958; Sheehy, 1963; MacLennan, 1975).

With the opening of mines, cattle primarily intended for agricultural work had to be used for transport, and this exposed them to greater risks of infection by bringing them into contact with tsetse (Montgomery and Kinghorn, 1908). At about this time human porterage as a means of transport was fast being replaced by cattle and draught animals.

The first investigation into the occurrence of trypanosomiasis in Zambia was carried out by the Liverpool School of Tropical Medicine Sleeping Sickness Expedition to the Zambezi in 1907-1909 headed by Kinghorn and Montgomery - the latter paying special attention to animal trypanosomiasis (Montgomery and Kinghorn, 1908; Kinghorn and Montgomery, 1908). The Expedition established a camp and laboratory at Broken Hill (= Kabwe) which was then the terminus of the "Cape to Cairo Railway" and an important communications centre to the copper mines in the north and German East Africa in the north-east. There were also a few European farmers around this important centre. During their three months' work around Kabwe, Montgomery and Kinghorn detected 36 cases of bovine trypanosomiasis, three cases of T. brucei infection

in dogs and three T. dimorphon (= T. congolense, Godfrey, 1960) infection in sheep. Details of the results of the investigation are shown in Table 1.

Experimentally (by inoculation of infected blood) these workers showed that T. dimorphon (= T. congolense) was fatal to cattle, goats, dogs, rabbits, guinea-pigs and rats, but would not infect donkeys. Also T. vivax was fatal to cattle in five out of six cases in which it was observed. Sheep and goats became infected with T. vivax and of eight animals infected six recovered. Dogs, rabbits, guinea-pigs and rats were refractory to T. vivax.

In a later paper on the nomenclature of the mammalian trypanosomes observed in North-Western Rhodesia (= Zambia), Montgomery and Kinghorn (1909) classified the trypanosomes they isolated according to their increased or decreased virulence to laboratory animals and on morphological grounds into three groups:-

1. T. evansi group, which included T. brucei and T. sudanense
2. T. dimorphon group, including T. congolense and T. pecaudi
3. Group including T. nanum, T. vivax and T. cazalboui

These groups were later renamed after exhaustive comparative studies into what presently are known as subgenus Trypanozoon or the Trypanosoma (Trypanozoon) brucei subgroup (Hoare, 1972). T. dimorphon and T. nanum are synonymous to T. congolense (Godfrey, 1960; Fairbairn, 1962; Hoare, 1972) and T. cazalboui to T. uniforme (Hoare, 1972). T. pecaudi is a synonym of T.(T.) brucei brucei (Hoare, 1972).

From Kabwe the Expedition toured Luampula Province, Lake Bangweulu, Northern Province and in June, 1908 reached Abercon (= Mbala) in North-Eastern Rhodesia (Montgomery and Kinghorn, 1909). They established a laboratory at Kambole Mission Station, some fifty miles west of Mbala

TABLE 1

Incidence of animal trypanosomiasis, Kabwe, 1907 (Montgomery and Kinghorn, 1908)

Species of Animal	No. of cases diagnosed	Species of trypanosome
Cattle	36	29 *T. dimorphon* (= *T. congolense*)
		7 *T. vivax*
Sheep	3	*T. dimorphon* (= *T. congolense*)
Dogs	3	*T. brucei*

(Fig. 2 and 3). At this time there were between 8,000 and 9,000 head of cattle in North-Eastern Rhodesia (= Northern, Eastern and parts of Luapula Provinces) localised around Mbala and Chipata. Horses, donkeys, mules, dogs and a few cattle were kept at various Boma (district) offices. Sheep and goats were scattered over the territory, but more extensively bred around Lake Bangweulu and in the Luangwa Valley.

Montgomery and Kinghorn (1909) also reported that trypanosomiasis in cattle appeared to vary considerably in intensity. The severe outbreaks at Kabwe and Chipata contrasted markedly with the infection at Chinsali, which they though was old having been in the herd probably since 1905. Results of the investigation in the West Luangwa area are shown in Table 2.

It has not been possible to obtain literature on the identity of the Chinsali "Scotdale" trypanosome on which more work, presumably, was carried out once specimens had arrived at Cape Town, South Africa. However, the trypanosome most probably was T. congolense as the authors describe 'tadpole forms' very similar to T. nanum.

One of the Kambole trypanosomes from a cow was designated by Montgomery to their dimorphon group, but later Laveran (Montgomery and Kinghorn, 1909) proposed to call this strain T. montgomeryi. Controversy about the validity of this species existed until Stephen (1963, 1966) finally settled the issue when he showed that T. montgomeryi is "an atypical form found in members of the T. congolense group (= Nannomonas). Sometimes it occurs in apparently pure form in individual animals, but it has not maintained its morphology through successive subinoculations to susceptible animals." T. montgomeryi thus becomes a synonym of both T. congolense and T. simiae (Hoare, 1972).

TABLE 2

Incidence of animal trypanosomiasis, West Luangwa valley area

(Montgomery and Kinghorn, 1909)

Location	No. of animals examined	Total number positive for trypanosomiasis	Species of trypanosome
CHINSALI (Scotdale Farm)	5 cattle	4	T. nanum (= T.congolense
	11 sheep	4	(?)* "
	4 goats	1	(?)* "
CHINSALI BOMA	9 cattle	2	T. nanum
KASAMA (Lake Bangweulu)	1 sheep	1	T. nanum
CHUNGA (Mbala)	3 dogs	3	2 T. congolense 1 (?)*
KAMBOLE (Mbala)	1 pig	1	T. congolense (= T. simiae)
KAMBOLE	2 cows	2	1 T. congolense (?)* T. dimorphon (= T. montgomeryi = T. congolense)

(?)* = Authors were not certain of the diagnosis

The Kambole trypanosome which Montgomery and Kinghorn saw in a pig was, in fact, a new species which we now know as T. simiae. They were the first to describe the characteristic acute nature of the disease caused by this trypanosome in pigs. In their case the six months old male pig suddenly developed severe symptoms of the disease. The animal was recumbent, semi-comatose, breathing heavily, had oedema of the prepuce and a temperature of 102°F. Trypanosomes were swarming in the blood and the pig died six hours later. No details of movement of the parasites in fresh blood film preparations could be observed as the trypanosomes were so numerous and, more especially, because they were agglutinating. Kinghorn and Yorke (1912a), while working at Nawalia, South Luangwa Valley, described a similar trypanosome which they isolated by feeding Glossina morsitans on monkeys and called it T. ignotum. At about this time, Bruce, Harvey, Hamerton, Davey and Bruce (1912b) described a trypanosome causing a hyperacute disease in monkeys in Nyasaland (= Malawi) and called it T. simiae. Like Montgomery et al., (1909), these workers also showed that the new trypanosome caused a chronic disease in goats, but did not infect rats, guinea-pigs, dogs, oxen and baboons. It also showed the characteristic autoagglutination, which Bruce et al., (1912b) described thus:- "it would seem as if multiplication took place so rapidly that the individual trypanosomes had no time to disengage themselves".

Most of the early work on trypanosomiasis of domestic livestock in the East Luangwa Valley area (= Eastern Province) was done by Kinghorn and Yorke, members of the Luangwa Sleeping Sickness Commission of the British South Africa Company. Kinghorn and Yorke (1912b) noticed that, from their camp at Nawalia (= Petauke District), in several villages on the main road to Fort Jameson (= Chipata) goats were found between August, 1911 and beginning of April, 1912, but at the end of that month

TABLE 3

Incidence of trypanosomiasis in domestic stock, Nawalia, South Luangwa Valley. (Kinghorn and Yorke, 1912b)

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Cow	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum* (= *T. congolense*)
Cow	*T. pecorum* or *T. nanum*	Result not available	*T. pecorum* or *T. nanum* (= *T. congolense*)
Goat No.39	*T. vivax*	Negative	*T. vivax*
Goat No.94	*T. vivax* and *T. nanum* or *T. pecorum*	Negative	*T. vivax* and *T. nanum*
Goat No.202	*T. pecorum* or *T. nanum*	Negative	*T. nanum*
Goat No.258	*T. vivax*	Negative	*T. vivax*
Dog	*T. rhodesiense*	*T. rhodesiense*	*T. rhodesiense* (= *T. brucei*)
Dog	*T. pecorum*	*T. pecorum*	*T. pecorum*
Dog	*T. pecorum*	No inoculation	*T. pecorum*
Dog	*T. sp.(montgomeryi)*	Negative	*T. sp. (montgomeryi)*

TABLE 4

Incidence of trypanosomiasis in domestic stock, Ngoa, North Luangwa Valley. (Kinghorn and Yorke, 1912c)

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Goat 369	*T. nanum* or *T. pecorum*	No inoculation	*T. nanum* or *T. pecorum* (= *T. congolense*)
Goat 375	*T. vivax* and *T. nanum* or *T. pecorum*	No inoculation	*T. vivax* and *T. nanum* or *T. pecorum*
Goat 378	*T. vivax* and *T. nanum* or *T. pecorum*	*T. pecorum*	*T. vivax* and *T. pecorum* (= *T. congolense*)

not a single animal was alive. All the animals were presumed dead from trypanosomiasis.

In April, 1912 the Commission moved from the south of Luangwa to the north and established a camp at Ngoa on the Congo - Zambezi watershed (Kinghorn and Yorke, 1912c).

Results of the investigations are given in Tables 3 and 4.

1.6 Trypanosomiasis of game animals

While investigating the incidence of trypanosomiasis in domestic livestock, Montgomery and Kinghorn (1909), wherever possible, also examined game animals. In most cases they examined wet films of blood taken from the ear or heart, but later gland juice was included and, sometimes, juices from other organs. Out of the 158 game examined, only three showed flagellates, two of which were trypanosomes. One of these was identified as T. congolense, isolated from a bushbuck Tragelaphus scriptus; the other was never identified as it was not seen in dry films (Montgomery and Kinghorn, 1909).

A list of the game animals examined is given in Table 5. Animals were obtained in both clean and tsetse-infested areas.

An extensive survey of trypanosome infections in wild animals in the Luangwa Valley was carried out in 1911-1912 by Kinghorn and Yorke (1912a, b, 1913). These workers found out that trypanosomes were more readily detected in an animal's blood by the examination of thin stained smears, than by that of fresh preparations (Kinghorn et al., 1912a). Owing to non-availability of goats and sheep, Kinghorn et al (1912a, b) used monkey and rat inoculations to isolate trypanosomes from game animals.

TABLE 5

List of game animals examined for trypanosomiasis, Northern Rhodesia (Montgomery and Kinghorn, 1909)

Name of Game Animal		No. Examined
Elephant	*Elephus africanus*	2
Hippopotamus	*H. amphibius*	1
Buffalo	*Bos caffer*	1
Eland	*Tragelaphus spekei*	2
Sable Antelope	*Hippotragus niger*	1
Roan Antelope	*Hippotragus equinus*	13
Zebra	*Equus burcbelli*	11
Hartebeest	*Bubalis lichstensteini*	18
Sessaby	*Damaliscus lunatus*	4
Waterbuck	*Cobus ellipsirymnus* and *C. defasa*	9
Puku	*Cobus vardoni*	34
Lechwe	*Cobus licbi*	2
Impala	*Aepiceros melampus*	3
Reedbuck	*Cervicapra arundinum*	30
Bushbuck	*Tragelaphus scriptus*	4
Oribi	*Oribia scoparia*	8
Warthog	*Phacocboerus aethiopicus*	2
Bush pig	*Potomocboerus chaeropotomus*	2
Duikder	*Cephalopus grimmi*	1
Lion	*Felis leo*	1

TABLE 6

Results of examination of game for trypanosomes at Nawalia, South Luangwa, (Kinghorn and Yorke, 1912a)

Animal	Number examined	Number in which trypanosomes were found in animal's blood	Number inoculations made	Number positive inoculations in which parasites were seen in animal's blood	Number positive inoculations in which no parasites were seen in animal's blood	Total number positive by examination and inoculations
Elephant	7	0	1	0	0	0
Rhinoceros	7	0	1	0	0	0
Hippopotamus	7	0	0	0	0	0
Zebra	5	0	3	0	0	0
Roan	8	1	2	0	0	1
Wildebeest	2	0	1	0	0	0
Puku	10	1	6	0	0	1
Impala	29	1	13	1	1	2
Bushbuck	9	4	6	1	2	6
Bushpig	4	0	1	0	0	0
Warthog	9	0	3	0	1	1
Lion	2	0	0	0	0	0
Hunting dog	1	0	1	0	0	0
Giant rat	1	0	0	0	0	0
Genet	2	0	0	0	0	0
Squirrel	1	0	0	0	0	0
Kudu	7	3	3	1	1	4
Hartebeest	6	0	1	0	1	1
Waterbuck	28	16	14	5	1	17
19	127	26	56	8	7	33

TABLE 7

Results of examination of game for trypanosomes at Ngoa, North Luangwa, (Kinghorn and Yorke, 1912b)

Animal	Number examined	Number in which trypanosomes were found in animal's blood	Number inoculations made	Number positive inoculations in which parasites were seen in animal's blood	Number positive inoculations in which no parasites were seen in animal's blood	Total number positive by examination and inoculations
Rhinoceros	6	0	3	0	0	0
Zebra	17	0	5	0	0	0
Buffalo	6	0	3	0	0	0
Eland	15	0	12	0	4	4
Roan	5	0	3	0	1	1
Hartebeest	8	0	4	0	0	0
Waterbuck	27	12	15	3	0	12
Puku	8	1	6	0	0	1
Sitatunga	2	1	0	0	0	1
Duiker	9	2	4	0	0	2
Klipspringer	2	0	1	0	0	0
Warthog	12	0	3	0	0	0
Hyaena	2	0	1	0	0	0
Caracal	2	0	0	0	0	0
Galago	1	0	0	0	0	0
Reedbuck	2	0	0	0	0	0
	124	16	60	3	5	21

A total of 127 head of game was examined at Nawalia, and trypanosomes were found by direct examination (dry stained thin blood film), by inoculation or by both methods, in 33 animals (25.9%). At Ngoa, 124 game animals were examined and 21 (16.9%) were harbouring trypanosomes. Details of the results are shown in Tables 6 and 7.

During their investigations at Nawalia, Kinghorn et al. (1912c) carried out some experiments with the object of determining what species of trypanosomes were transmitted by Glossina morsitans. Flies, freshly caught, were brought to the laboratory and fed on clean monkeys, which were the only animals available for this purpose.

By this method, Kinghorn et al. (1912c) were able to isolate three species of trypanosomes - T. rhodesiense (= T. brucei), T. pecorum (= T. congolense) and a new trypanosome, for which they proposed the name T. ignotum (= T. simiae).

1.7 Human trypanosomiasis

Rüchel (1975) traces the zoonotic nature of trypanosomiasis to the discovery by Dutton in 1902 of trypanosomes causing sleeping sickness (human trypanosomiasis). Since sleeping sickness is caused by T. brucei subgroup trypanosomes whose reservoir hosts are game (Kinghorn et al., 1912c; Keymer, 1969; Dillmann and Awan, 1972) and domestic animals ((Onyango, Van Hoeve and de Raadt, 1966; Mwambu, 1973) the studies of human and animal trypanosomiasis are closely related.

The first recorded case of sleeping sickness in Zambia was diagnosed in 1908 (Ormerod, 1974; Buyst, 1976) when parasites were seen in the blood of a mineral prospector travelling through the Luangwa Valley and from whom the species Trypanosoma rhodesiense was first described by Stephens and Fantham (1910).

In Zambia, the Gambian form of sleeping sickness which is transmitted by Glossina palpalis appeared on the southern shores of Lake Tanganyika in 1936 reaching a maximum of 20 cases in 1941, but no further cases have occurred since 1962 (Ormerod, 1974). In the rest of the country the disease is that of the acute and severe Rhodesian form transmitted by G. morsitans (Sheppard, 1946; Ormerod, 1974; Buyst, 1976, 1977a).

Some workers trace the origin of Rhodesian sleeping sickness in Zambia (and elsewhere) to pre-existing foci of Gambian sleeping sickness (Willett, 1965) such as those which appeared on the Zaire side of the Luapula river, on the shores of Lake Tanganyika and at Hargreaves (= Kakumbi) in the Luangwa Valley (Sheppard, 1946; Ormerod, 1974). Ormerod (1974) suggests that Rhodesian sleeping sickness arose from Trypanosoma brucei in game animals which survived the rinderpest panzootic of 1896 in residual fly areas in the Zambezi basin. These fly areas expanded as the game population recovered (Neave, 1911; Ford, 1965; Buyst, 1977b). From these beginnings Rhodesian sleeping sickness spread northwards reaching greater virulence and epidemic proportions in Tanzania in the 1930s and Uganda in the 1940s but the original focus remained in the Zambezi basin (Ford, 1971; Ormerod, 1974).

For a long time sleeping sickness in Zambia was regarded as a minor problem (Buyst, 1976) and an endemic disease characterised by sporadic cases, "health carriers" and with few instances of self-cure (Sheppard, 1946; Foulkes, 1970; Buyst, 1970; Ford, 1971; Ormerod, 1974). However, the situation is now different in the Luangwa Valley where outbreaks of epidemic proportions have been reported (Rickman, 1974; Buyst, 1974; 1977 a, b). Buyst (1977b) suggests these epidemics could be considered as the late results of the 1896 rinderpest panzootic. In the North Luangwa Valley, Buyst (1977a) diagnosed 241 cases in just under

TABLE 8

Notification of cases of sleeping sickness, (Sheppard, 1946 and Ormerod, 1974).

YEARS	NO. OF CASES
1925-1934* (-1929)	82
1935-1944	626
1945-1954 (?1953)	608
1955-1964 (?1958)	652
1965-1973	1604

*-1929 = no cases reported

?1953, ?1958 = no records available

three years. In other parts of the country the disease is endemic except in the Western part where, normally, a chronic or subacute course is observed in local people, but an acute and severe disease manifests itself in tourists and newcomers to the area (Foulkes, 1970).

Table 8 shows the number of recorded cases of sleeping sickness in Zambia from 1925 to 1973.

1.8 Animal Trypanosomiasis: 1914 - 1978

During the period 1857-1913 investigators in Zambia had identified animal and human pathogenic trypanosomes, i.e. T. congolense, T. vivax, T. simiae, T. brucei and T. rhodesiense. With increased economic development and cultural change (Shaw, 1960; Buyst, 1977b) which led to improved communications and inter-district trading and travelling, animal trypanosomiasis became a problem not only in areas marginal to tsetse fly-belts, but also in tsetse-free areas.

Shaw (1960) quotes Owen who reported a loss of 1,000 cattle in 1914 from "Sesheke Sickness" (= trypanosomiasis) caused by T. dimorphon (=T. congolense) in the Western Province. Hornby (1921) studied a number of herds of cattle on the Tanganyika plateau in the Northern Province. In one herd of 30 cattle he recorded 16 cases of trypanosomiasis of which 10 were caused by T. congolense and 6 by T. vivax. He also observed spontaneous recovery in two of four calves infected with T. vivax while none of the calves infected with T. congolense survived for more than three months after diagnosis.

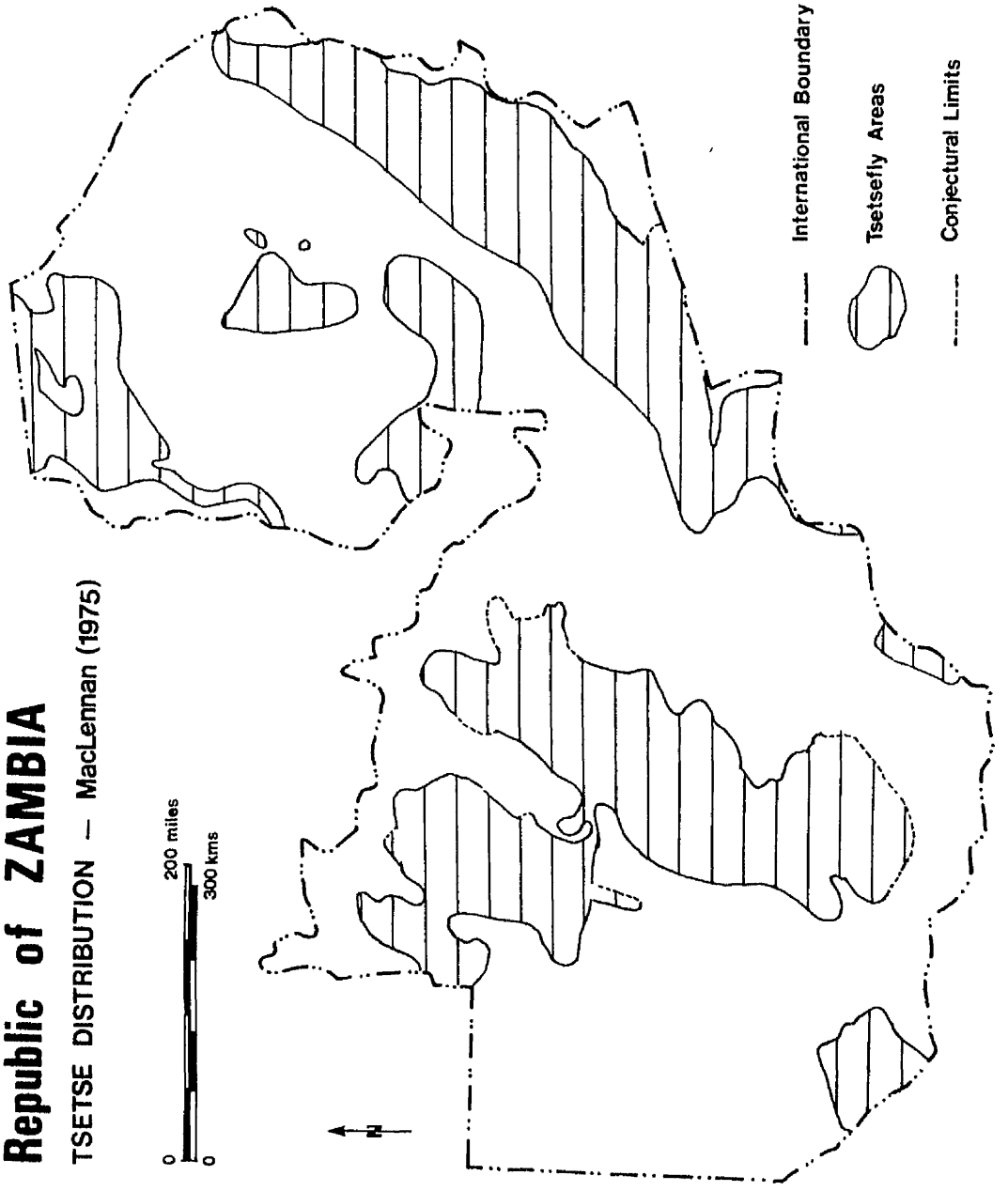
The ubiquity and high pathogenicity of T. congolense in cattle in Zambia and the rest of south-eastern Africa is in contrast to T. vivax which causes fewer and less virulent infections (Shaw, 1960; Ford, 1964; Willett, 1970; Hoare, 1972).

By the 1930s it was generally believed within the Department of Veterinary Services that approximately five-eighths of Zambia was infested with tsetse fly and trypanosomiasis was enzootic in these areas. Vehicular traffic aided dissemination of Glossina. Field observations (Ann.Rep. 1935) appeared to indicate that mechanical transmission by other biting flies played a very considerable part in the spread of infection - an occasional tsetse fly infects one animal, and the subsequent spread of infection in the herd is effected by Stomoxydae, Tabanidae, Hippoboscidae, etc. This may account for certain outbreaks occurring far from known tsetse fly belts (Ann. Reps. 1934-37; Shaw, 1960).

In 1938 the first outbreak of cattle trypanosomiasis in North-Western Province was reported on the Lealui-Balovale (= Lukulu - Zambezi) border along the Zambezi plains in an area where tsetse fly did not exist. Blood-sucking flies other than tsetse were held responsible for the spread of infection which originated in oxen which had been in a fly belt. Around the 1940s, the most severely affected parts of the country were the economically important areas in Eastern, Southern and Central Provinces. This is also true at the present time (Fig. 4). In 1940 (Ann.Rep., 1940) a serious outbreak of cattle trypanosomiasis was reported in Namwala district (Southern Province) along the Kafue flats and five cases of trypanosomiasis in donkeys and several in horses were reported in Gwembe (Southern Province) and Ndola (Copperbelt Province) districts respectively.

In 1956 Sheehy (1956) reported a growing problem of mechanical transmission of bovine trypanosomiasis, particularly where there had been large-scale seasonal movements of stock (to and from Kafue flats and Zambezi plains) during which contact is made between uninfected herds and infected cattle moving from marginal fly areas to common dry season

Figure 4


Republic of ZAMBIA
TSETSE DISTRIBUTION – MacLennan (1975)
0
200 miles
300 kms
0
N
International Boundary
Tsetsefly Areas
Conjectural Limits

grazing grounds.

T. brucei was, for the first time, diagnosed in cattle in Lusaka area in 1956 and two outbreaks of T. simiae infection in pigs were reported in the same year at Feira and Chisamba both in the Central Province. A minor outbreak of T. brucei infection in sheep at Kawambwa in Luapula Province was also reported (Sheehy, 1956).

Since around 1950 onwards, the best available indicator of the magnitude of cattle trypanosomiasis has been the quantity of trypanocides used, the number of cattle which have required treatment (Hobday, 1954; Sheehy, 1963; MacLennan, 1975) and the number of trypanosome positive blood smears during routine laboratory examinations (Ann.Rep., 1958; Awan, 1976).

Table 9 shows the number of blood smears from domestic livestock (predominantly cattle) positive for trypanosomiasis and the trypanosome species diagnosed at the Central Veterinary Research Station (CVRS), Mazabuka during the period 1949-1959.

It is noteworthy that an outbreak of T. congolense infection, which caused high mortality, occurred in 1953 on the North bank of the Kafue river in Lusaka and Mumbwa districts. Since 1960 animal trypanosomiasis, particularly of cattle, has continued to increase. In 1960 tsetse flies were reported to have invaded Western Province along the Mashi river spreading the infection from there to Kalabo (Swan, 1961). By 1967 trypanosomiasis had become a very serious problem in the following areas (in decreasing order of magnitude): Eastern Province, Southern Province (Zambezi Valley, Choma West and Namwala), Central Province (Mumbwa, Lusaka East, Chisamba), Western Province (Sesheke, Senanga West).

Today the tsetse infested area of Zambia amounts to 32% of the

TABLE 9

Number of blood smears positive for animal trypanosomiasis and trypanosome species causing them; 1949 - 1959, (Annual Reports, 1950-1960, CVRS, Mazabuka).

SPECIES	YEAR											TOTAL
	49	50	51	52	53	54	55	56	57	58	59	
T. congolense	79	107	85	39	110	92	153	110	197	227	364	1578
T. vivax	-	15	8	5	1	16	37	4	60	135	180	461
T. brucei	-	-	4	13	4	4	1	5	4	3	5	43
T. simiae	-	-	-	3	-	-	8	6	5	25	1	48
T. theileri	-	-	-	-	-	1	12	2	1	5	4	25
T. ingens	-	-	-	-	-	-	1	-	-	-	4	5
T. lewisi	-	-	-	-	-	-	1	-	-	-	-	1
TOTAL	79	122	97	60	115	113	213	127	267	395	558	

TABLE 10

Number of trypanosomiasis cases diagnosed between 1970-1978, (Annual Reports, 1970-1978, CVRS, Mazabuka).

	YEARS								
	70	71	72	73	74	75	76	77	78
CASES	1437	1021	632	345	227	231	251	324	188

total (MacLennan, 1975) and this puts at risk about 60% of the cattle population (Clarkson, 1977; Awan, Chizyuka and Akafekwa, 1978).

Figures shown in Table 10 are the total number of positive cases of trypanosomiasis diagnosed at the Central Veterinary Research Station from 1970 to 1978. The decline in numbers from 1973 is largely due to the establishment of regional laboratories which between them now handle about three-fifths of routine blood smear examinations and not due to a decline in the incidence of the disease.

1.9 Control of Animal trypanosomiasis in Zambia

In Zambia the primary tsetse infested area amounts to 32% of the total (see Fig.4), with further extensive areas adjacent to the primary tsetse belts where tsetse may occasionally be found (MacLennan, 1975). Demand for the expansion and development of the national herd exists and therefore control of trypanosomiasis has been and will continue to be an increasing national problem.

The control of trypanosomiasis in Zambia has been carried out by use of chemotherapeutic and chemoprophylactic drugs in conjunction with vector control.

1.10 Control by drugs

Chemotherapy of trypanosomiasis has a long history which started in 1905 when Thomas demonstrated the trypanocidal effect of atoxyl on *T. gambiense* in mice. The first decade of this century saw the use of substances such as trypan blue, trypan red and trypasafrol (Ford, 1962). The successful treatment of a French Medical Officer, who had contracted sleeping sickness in Congo (= Zaire), with subcutaneous injections of

atoxyl and intravenous injections of tartar emetic (Bevan, 1928), led early workers to try these drugs on animals with trypanosomiasis. In 1907 Montgomery and Kinghorn (1908), working at Kabwe, were able to cure syringe-transmitted bovine trypanosomiasis with 5.0 g of atoxyl and recommended further trials of this drug under natural conditions. However, the work of Bevan (1928, 1936) and his success in the treatment of bovine trypanosomiasis with Potassium antimonyl tartrate (Tartar emetic) in neighbouring Zimbabwe greatly influenced workers in Zambia and other South-eastern African countries to adopt chemotherapy as a method of control.

Since 1908 the following drugs have been used for the control of trypanosomiasis in Zambia.

Tartar emetic (Potassium antimonyl tartrate)

Between 1908 and 1920, Tartar emetic and atoxyl were the two drugs in general use (Shaw, 1960). Atoxyl was, due to its toxicity, withdrawn from use in 1912. Tartar emetic was administered intravenously as a 4% solution at a dose of 25.00 cc and was effective against T. congolense and T. vivax infections but not against T. brucei. Tartar emetic had two main disadvantages. First, a course of four or five weekly infections was required to avoid relapses (Shaw, 1960) and this sometimes led to symptoms of acute shock. Second, it required skilled operators to administer the drug by the intravenous route necessary to avoid abscesses or necrosis of tissue. The drug was withdrawn from field use and replaced with phenanthridines and quinaldines in 1945 (Ann.Rep., 1949; Shaw, 1960).

Antimosan and Naganol

Antimosan, a more soluble and less irritant derivative of Potassium antimony tartrate (Bevan, 1928) and Naganol, a quinaldine, were

introduced into Zambia in 1932. These drugs were tried in experimental cases and found to be more effective than Tartar emetic and their administration by subcutaneous injection was simpler. However, the high cost of these drugs precluded their routine field use (Shaw, 1960).

Phenidium (Phenanthridinium) Chloride

This became available in 1944 as a ½% solution administered intravenously at a dose rate of 0.6 mg/kg bodyweight. A single injection was sufficient to cure T. congolense and T. vivax infections (Shaw, 1960). However, phenidium chloride was less soluble, had a low therapeutic index (Williamson, 1970) and swellings, necrosis and fibrous changes invariably occurred in the muscles at the site of inoculation. Also it was shown to be ineffective against T. brucei infection in a horse (Shaw, 1960).

Dimidium (Phenanthridinium) bromide

This drug was effective against T. vivax, T. congolense and T. simiae. Dimidium bromide replaced phenidium chloride in 1946 as a drug for general field use at a dose rate of 1.25 mg/kg intravenously as a 3% solution. It was used as a curative as well as a prophylactic drug and was particularly useful for treating animals travelling through tsetse belts for short periods as the drug had an effective prophylactic period of one week. Shaw (1960) regarded the use of Dimidium bromide as a great success in the control of trypanosomiasis. For example in the Namwala District (Southern Province) where Dimidium bromide was used from 1946-1955 there was a 70% increase in the cattle population. In 1955 the national cattle herd for the first time exceeded one million, which was an increase of over 300,000 since 1945 (Swan, 1956). This increase was attributed partly to reduced mortality of cattle in major trypanosomiasis areas (Sheehy, 1956). In 1952 and 1954 extensive

outbreaks of photosensitisation following the use of Dimidium bromide were reported in most parts of the country north of the Kafue river (Swan, 1956; Shaw, 1960). Due to its toxicity, Dimidium bromide was withdrawn from general use in 1957.

Ethidium (Homidium) bromide

This was introduced in Zambia as a field chemotherapeutic agent in 1957. The drug was given intramuscularly at a dosage of 2.0 mg/kg. It was effective against T. congolense, T. vivax and T. brucei infections, but failed to cure T. simiae infections at 1.0 mg/kg and 2.0 mg/kg at Feira and Chisamba respectively (Sheehy, 1956).

Antrycide methyl-sulphate

This quinoline derivative became available for general field use in 1957 (Ann.Rep., 1957, 1958) after results from field trials had indicated the drug had a prophylactic effect of up to two months against T. vivax and three months against T.congolense (Shaw, 1960). Antrycide methyl-sulphate was used as a prophylactic drug, but because of its high cost and its delayed toxicity, it was replaced in 1958 with prothidium.

Antrycide pro-salt

This drug, though very effective against most of the animal trypanosomes, had the serious disadvantage of leaving large permanent swellings at the site of inoculation. These swellings were intolerable to the traditional stock owner and because of this Antrycide pro-salt was precluded from general field use (Swan, 1959; Shaw, 1960).

Novidium (Homidium) chloride

This curative drug was used to treat clinical cases in cattle from 1958 to 1971.

Prothidium

A curative and prophylactic drug effective against T. congolense and T. vivax infections (Rüchel, 1975), prothidium was introduced in Zambia in 1958. Unfortunately prothidium was so widely used that by 1962 widespread resistance had developed particularly in the Eastern Province where cattle were under constant tsetse challenge (Sheehy, 1962). T. congolense and T. vivax strains resistant to prothidium were reported in Eastern and Southern Provinces and were subsequently confirmed at the Central Veterinary Research Station, Mazabuka (Ann.Rep., 1966; 1968). Prothidium was withdrawn from general use in 1972.

Berenil (Diamidine - diazoaminobenzene aceturate)

Berenil is a curative drug which became available for field use in 1958 at a dose rate of 3.5 mg/kg for T. congolense and T. vivax and 7.0 mg/kg for T. brucei infections in bovines. Berenil was also used for sanative treatments of prothidium and, in rare cases, Antrycide - resistant trypanosomes (Sheehy, 1962; Ann.Rep. 1966).

Samorin (Isometamidium chloride)

Samorin has both curative and prophylactic properties. In 1967 Samorin, at a dose rate of 0.5 mg/kg, replaced Berenil as a general therapeutic drug (Ann.Rep. 1967) and an apparent low incidence of cattle trypanosomiasis that year was attributed to this change of drug.

From 1972 up to the present day, only two anti-trypanosomal drugs - Berenil and Samorin - have been used to control animal trypanosomiasis.

Berenil at a dosage of 3.5 mg/kg is used in areas remote from the tsetse belts and where the incidence of the disease is low.

Samorin at a dosage of 0.5 mg/kg (in low challenge) and 1.0 mg/kg

(high challenge) is used in areas near to or within the tsetse belts where the disease is endemic. Samorin is normally applied at two, three or four monthly intervals depending on the intensity of the challenge. It is advocated that Berenil be used twice yearly in such areas.

To avoid toxicity an interval of at least six weeks is allowed between administration of the two drugs.

1.11 Vector control

Immediately following the 1896 rinderpest panzootic, game and consequently tsetse were reduced to isolated areas in the Zambezi and Luangwa river valleys (see Fig.2), and in a small area at the headwaters of Nanzhila river in Namwala district (Shaw, 1960; Ormerod, 1974). From these areas game and tsetse gradually re-established themselves (Shaw, 1960) and from 1928 to 1957 it was generally accepted that five-eighths of the area of Zambia was occupied by tsetse (Ann.Rep., 1935, 1949; Shaw, 1960).

In these early days, tsetse control measures were limited to avoidance of contact between livestock and tsetse. Planned human re-settlement worked successfully in Petauke district in 1909 when game and tsetse were driven out of the area due to human pressures of wood-cutting and cultivation. Subsequently cattle were introduced and the population rose from zero in 1909 to 19,000 in 1949 (Shaw, 1960).

Game fences (Holding lines) were intended to prevent entry of game and the attendant fly into cattle rearing areas and the first of these was erected in Chipata district in 1919. By 1963 a total of over 1,449 kilometres of game fence had been constructed along the fly belts flanking the livestock - rearing areas of the country. Although it was

claimed that holding lines had been reasonably successful (Swan, 1960), MacLennan (1975) doubted their effectiveness as a tsetse control measure. Game fences are now being phased out due to ineffectiveness and high maintenance costs.

Smudge houses (fly chambers) and fly pickets were instituted in 1936 to check dispersal of fly by vehicles and bicycles on all major roads passing through tsetse belts (Ann.Rep., 1939) by use of insecticides and fly catching nets. Smudge houses were less efficient than fly pickets (Shaw, 1960) and were abandoned in the 1960's. Fly pickets are still widespread in the country (MacLennan, 1975).

Fire exclusion and discriminative bush clearing; this method was tried out in Mbala from 1935 to 1945 to eliminate fly from an area of 280 square miles which was being prepared for cattle rearing. Glover et al (1955) were not able to detect any flies when they surveyed the area ten years later and the cattle which had been introduced in 1948 into the area remained in good health up to 1953 (Hobday, 1953). Despite its apparent effectiveness, this method was not extensively used due to bush fire risks.

Game elimination, barrier and discriminative clearing. In 1949 barrier clearing of about 910 metres wide along the edge of known tsetse belts was introduced. Game found crossing from the fly side of the barrier were shot. Later barriers were reinforced by discriminative clearing on either side, the erection of game fences (Shaw, 1960) and selective hunting of game between the fences (Clarke, 1964). Game elimination as a tsetse control method was phased out in the 1970's.

Use of insecticides. The introduction of knapsack sprayers along with discriminative bush clearing (Swan, 1961) led to a gradual switch from game destruction to selective spraying of residual insecticides.

Today, ground and aerial application of insecticides are the main methods of tsetse control.

Ground spraying is effected by use of knapsack and unimog (tractor) sprayers selectively spraying 10% of the vegetation (MacLennan, 1975).

Air spraying, a technique of aerial application of insecticide by aircraft supplemented by other equipment (MacLennan, 1975), was first used in 1948 in the Zululand campaign against G. pallidipes (Du Toit, 1954) which lead to the extermination of the species. Cockbill, Lovemore and Phelps (1963) showed that aerial spraying sufficiently reduced G. morsitans, in a relatively isolated population in the Zambezi valley, to permit settlement of people and their livestock. In 1968 the first air spray operation was carried out in south-west Zambia using a fixed wing aircraft (Park et al., 1968). These authors claimed extermination was achieved although reinvasion has since occurred.

Sequential dispensation - i.e. repeated treatment at intervals of three to four weeks - of aerosols from fixed-wing aircraft is the method currently being practised in Zambia.

CHAPTER 2

MATERIALS AND METHODS

2.1 Experimental animals

Mice

Mice used in all the experiments in Chapters 3 and 4 were females of the Swiss White strain aged 4-6 weeks. They weighed between 20 and 25 g and were obtained locally from the breeding colony of the Central Veterinary Research Station (CVRS), Mazabuka, Zambia. The mice were kept in plastic cages with sawdust as bedding and were cleaned out twice weekly. The cages were kept on metal shelves in flyproofed accommodation.

The animals were fed ad libitum on pellets (National Milling Company, Lusaka, Zambia).

Female CFLP mice (Anglia Laboratory, Alconbury, Huntingdon, England) were used in the experiments described in Chapter 5. These mice were 5-7 weeks old and weighed 25-30 g. The mice were kept in plastic cages with sawdust bedding which was changed twice weekly. The cages were kept on shelves of metal stands in an animal house whose temperature was kept at about 21.6°C.

The mice were fed ad libitum on diet 41 (Angus Milling Company, Kirriemir, Perth, Scotland).

Drinking water was available to all mice ad libitum from plastic water bottles.

Sheep

Blackhead-Persian adult castrated male sheep were used in some experiments in Chapter 4. They were part of the flock of sheep bred and reared by the Animal Husbandry Research Branch at the CVRS, Mazabuka.

After selection the sheep were dosed with Thiabendazole (Thibenzole: Merck, Sharp and Dohme Limited) and dipped prior to experimentation. After inoculation with infective material they were housed in concrete pens and bedded on wood shavings which were cleaned out twice weekly. The animal houses were fly-proofed. The sheep were fed on a supportive diet of hay and maize bran and water was available ad libitum. A gutter along the outer perimeter of the pens inside the animal house was filled with "Coopertox" dip wash (Cooper Zambia Ltd.) to prevent tick contamination.

Calves

The calves used in the cattle experiments were 7-9 months old Angoni-Boran castrated male crosses. They were reared at two Agricultural Institutions. One batch of calves was obtained from Mochipapa Agriculture Research Station near Choma and the other from Palabana Farm Training Centre near Lusaka. Both areas are tsetse-free and have had no history of animal trypanosomiasis. At these institutions the calves were reared under good management. With effective tick and anthelmintic control regimes. During experiments the calves were kept in groups of between 4 and 8 in concrete pens in screened and fly-proofed accommodation. They were sprayed weekly with "Coopertox" dipwash. A gutter surrounding the pen was permanently full of similar dipwash to prevent any tick contamination of the pen. The calves were fed on a supportive diet of hay and maize bran and drinking water was available ad libitum.

Weighing procedures

Calves were weighed using Avery cattle scales before the start of experiments. During the course of experiments, bodyweight was determined by the use of weigh bands.

Sheep were weighed on a Salter balance scale.

Transportation

Calves and sheep were transported from their places of origin to Mazabuka in cattle trucks. These trucks were also used to carry the calves and sheep from Mazabuka to and from crushpens in the field.

Infection procedures

In the initial experiments on drug sensitivity (Chapter 4), blood was collected from the experimental herds and sub-inoculated into recipient cattle and sheep. The technique used was a modification of that described by Jones-Davies (1966) as follows: 20 ml of blood were collected from the jugular vein of each donor animal. Some of this blood was put into two glass tubes; one for the preparation of serum and the other for the preparation of blood films and the measurement of packed red cell volume (P.C.V.). 0.5 - 1.0 ml of the remainder of the blood sample was inoculated intraperitoneally (i.p.) into each of a group of 3 mice and 2-3 ml from the rest of the blood sample was injected subcutaneously into each experimental calf or sheep. The calves and sheep thus received numerous injections in different sites over the body.

In subsequent experiments calves were inoculated with infected blood which was either freshly collected from infected cattle or collected deep-frozen and then thawed prior to inoculation (see 2.2). Counts of trypanosomes were made using haemacytometer (Lumsden, Herbert and McNeillage, 1973). Infected blood was diluted in phosphate buffered glucose saline, pH 8.0 (di-Sodium hydrogen orthophosphate anhydrous 13.48 g, Sodium dihydrogen orthophosphate 0.78 g, Sodium chloride 4.25 g, glucose 15.0 g and 1.0 l. distilled water).

2.2 Trypanosomes

Origin of stocks and stabilates

Stocks of trypanosomes used in the experiments described in Chapter 4 were isolated in calves from cattle in Kalomo district in the Southern Province of Zambia. *T. congolense* was isolated in an untreated calf number 202. Hence this isolate was designated *T. congolense* 202 and cryopreserved as stabilate CVRS *T. congolense* 202. In another untreated control calf number 224 *T. vivax* was isolated from cattle in the same district but from a different area. This organism was designated CVRS *T. vivax* 224.

Trypanosoma brucei stabilates used in the experiments described in Chapter 5 were obtained originally from different sources. Stabilate TREU 667 was obtained from Dr. A.R. Gray, Edinburgh. Stabilates LUMP 571 and LUMP 1001 came from Professor W.H.R. Lumsden of the London School of Hygiene and Tropical Medicine, London.

Cryopreservation

The derivative stabilates of TREU 667 and LUMP 571 and 1001 were passaged in mice irradiated with 500 rad the day before infection. The mice were exsanguinated by cardiac puncture under deep anaesthesia at peak parasitaemia using heparin as anticoagulant. This blood was cryopreserved in polythene tubing in liquid nitrogen (Taylor, 1972) after addition of 10% glycerol by volume.

Stabilates of *T. congolense* and *T. vivax* were prepared from infected cattle blood at peak parasitaemia and preserved at CVRS, Mazabuka. 1.0 ml of infected cattle blood obtained by jugular vein puncture was mixed with 5 ml Alsevers' solution (glucose 4.66 g, sodium citrate 2.0 g, sodium chloride 1.05 g, distilled water 200 ml)

containing 0.4 ml glycerol. Ten to fifteen ampoules each filled with 1.0 ml of this mixture were suspended in liquid nitrogen vapour for 45 minutes or until the contents were frozen. The ampoules were then lowered and stored in liquid nitrogen.

2.3 Diagnostic methods

Parasitological

For detection of live trypanosomes, the wet blood film examination technique was used. Tail blood from mice and ear vein blood from cattle was examined by this method.

The thick blood film examination technique was used in most cattle experiments. The thick blood smears were prepared and stained according to the technique described by MacLennan (1957). Thin blood films stained with Giemsa's stain were also used for detection and identification of trypanosomes.

Serological

Enzyme-linked immunosorbent assay (ELISA)

The technique used was essentially the 'micro ELISA' described by Luckins (1977) and Luckins, Boid, Rae, Mahmoud, El Malik and Gray (1979). 0.3 ml of antigens diluted with 0.05M carbonate/bicarbonate buffer, pH 9.6 were coated on to wells of disposable flat bottom polystyrene plates (Dynatech Cat. No. M29A) by incubating at 37^{o}C for 1 hour and then leaving overnight at 4^{o}C. The plates were washed 3x with PBS/Tween-20 (washing buffer), five minutes each wash and shaken dry. 0.3 ml of serum diluted 1:200 with PBS/Tween-20 was added to each well after which the plates were incubated for two hours at room temperature. The plates were then washed 3x with washing buffer, five minutes each wash and

dried. 0.3 ml of conjugated immunoglobulin + enzyme was then added to each well and left at room temperature for a further two hours. The plates were washed 3x with washing buffer, five minutes each wash and dried. 0.3 ml of substrate was added to each well and left at room temperature for 15 minutes. In order to stop the binding reaction between the substrate and the enzyme conjugated rabbit anti-bovine IgG, 0.05 ml of 3M sodium hydroxide were added to each well and the absorbance of the solution in each well was determined visually.

The dilution of the antigen used was 1:200 for T. brucei and 1:100 for T. vivax.

Haematological values

Packed red cell volume (P.C.V.)

The packed red cell volume percentage of blood from experimental animals was determined by the microhaematocrit method, using a micro-haematocrit centrifuge and reader (Hawksley & Son Ltd., London, England).

2.4 Chemotherapy

Berenil

Berenil (Diminazene aceturate, Fabwerke Hoechst) was administered to cattle as a freshly prepared 7% aqueous solution. The calculated dose was given by deep intramuscular injection (i.m.) in the thigh or neck region.

In mice a dose (40 mg/kg) was selected to give maximum trypanocidal effects without toxicity to the host; each mouse was weighed and the requisite dose administered intraperitoneally (i.p.) in a volume of 0.05 ml/5 g bodyweight.

Samorin

Samorin (Isometamidium chloride, May & Baker) was freshly prepared as a 1% w/v aqueous solution. The drug was administered by deep intramuscular injection in the neck region or thigh.

CHAPTER 3

FIELD INVESTIGATIONS

3.1. General Introduction

In this chapter the incidence of animal (mainly cattle) trypanosomiasis in the Eastern Province and in the areas of Kalomo and Mazabuka districts of the Southern Province is reported. The Southern and Eastern Provinces have the most fertile soils in Zambia (UNDP Rep., 1976) suited for both crop and livestock farming.

Whereas the share of agriculture in the nation's Gross Domestic Product (GDP) fell from 13.7% in 1964 to 11.5% in 1974 (UNDP Rep., 1976), approximately 65% of this (cattle, maize and cotton) represented the contribution of the traditional sector i.e. the traditional hoe and plough systems as practised in the Southern and Eastern Provinces where animal husbandry plays a more significant role (Siddle, 1971). Transition from these traditional systems to commercial agriculture and stock rearing is relatively easy to achieve. In 1974 1.5 million out of a total of about 1.7 million cattle in Zambia were held in the traditional sector, the majority of which were in the Southern Province.

The Eastern Province area (Fig. 7) extending from Chipata to Petauke and the belt from Kalomo (Fig. 2) northward to the Kafue river are among some areas in Zambia with denser rural population (9.6 per sq. km.) and advanced agricultural systems. Ironically, these areas are also among those which are affected by trypanosomiasis thus limiting their agricultural exploitation (McGlashan, 1971). In these areas there is an increasing trend towards permanent land occupancy which is being encouraged by Government farming schemes.

Cattle in these areas are at constant risk of trypanosomiasis (see Fig.4) and are being maintained under chemotherapeutic and chemoprophylactic regimes (Shaw, 1960; Ann.Rep., 1972; Awan *et al.*, 1978).

Weisenhütter, Turner and Kristensen (1968) and Wilson, Paris and Dar (1975) have reported keeping beef cattle successfully in tsetse-infested areas of East Africa using anti-trypanosomal drugs. However, Bourn and Scott (1978) have pointed out that dependence on regular drug treatment for trypanosomiasis control leads to a major risk of drug resistance developing particularly where a high standard of veterinary supervision is not practised. Since 1972 Berenil and Samorin have been the only two anti-trypanosomal drugs in use in the country.

It was against this background that investigations into the incidence of trypanosomiasis and the relative occurrence of trypanosome species in domestic livestock were carried out in these areas. 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.

3.2 Trypanosomiasis survey: Kalomo West

Introduction

Kalomo district (Fig.5) is one of the six administrative districts that make up Southern Province. It lies on the Batoka Plateau which is part of the central African plateau. Kalomo district is a major farming area with both commercial and traditional systems of agriculture. In 1974 Kalomo had 107,591 head of cattle (Ann.Rep., 1974).

Kalomo, together with the neighbouring districts of Choma and Namwala, accounted for 84% of the trypanosomiasis outbreaks in the Southern Province (MacLennan, 1975). Thus, in 1974 4,507 curative and 16,831 prophylactic drug treatments were given.

It can be seen from Figure 5 that a tongue of tsetse infection extends from the central *G. morsitans* belt (see Fig.4) into Kalomo district and this is possibly the cause of infection to be described.

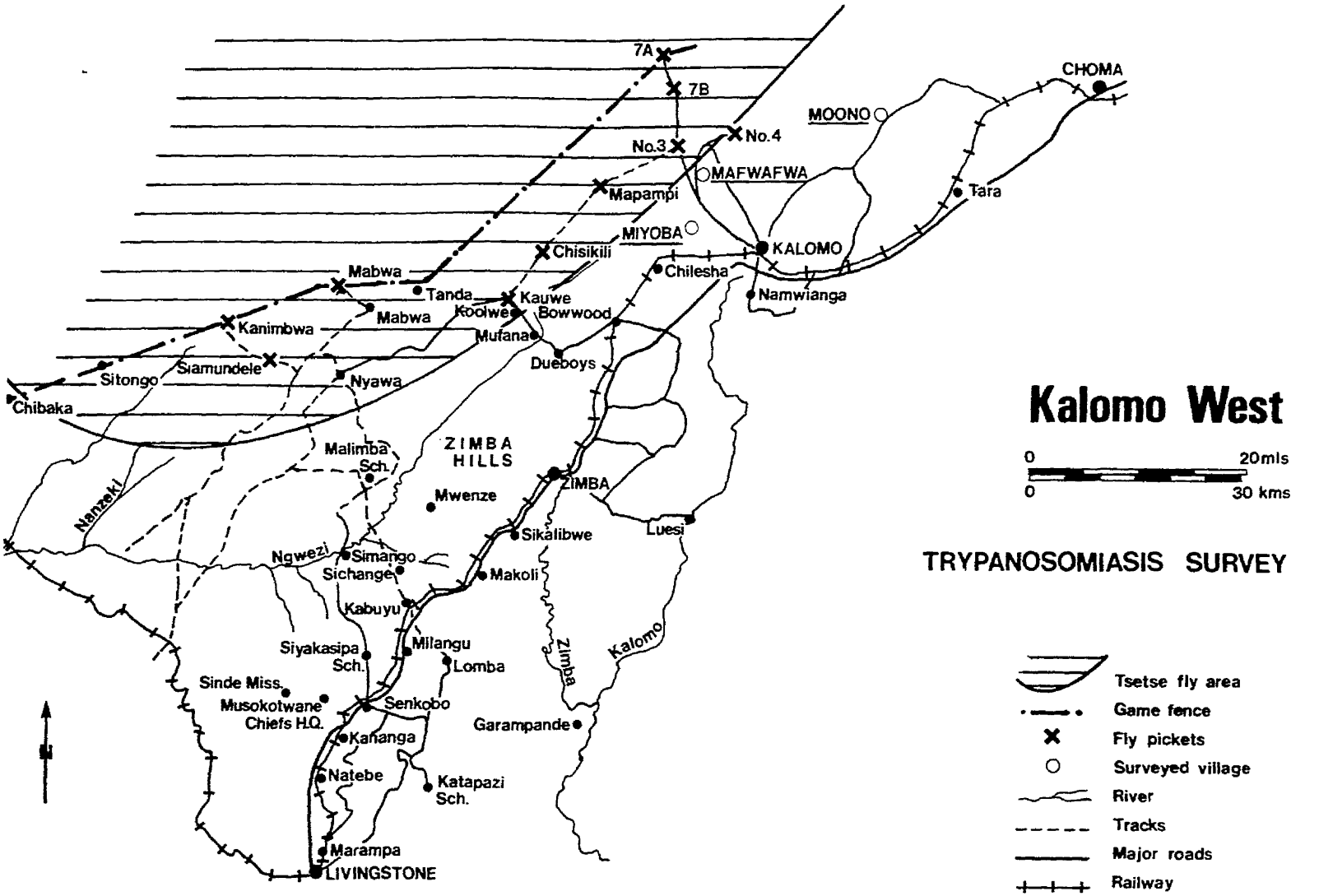

7A
7B
No.3
No.4
Mapampi
MIYOBA
Chisikili
Mabwa
Tanda
Kauwe
Koolwe
Bowwood
Mabwa
Kanimbwa
Mufana
Siamundele
Dueboys
Sitongo
Nyawa
Chibaka
ZIMBA HILLS
Malimba Sch.
Nanzeki
Mwenze
ZIMBA
Sikalibwe
Luesi
Ngwezi
Simango
Sichange
Makoli
Kabuyu
Siyakasipa Sch.
Milangu
Lomba
Zimba
Kalomo
Sinde Miss.
Musokotwane Chiefs H.Q.
Senkobo
Garampande
Kananga
Natebe
Katapazi Sch.
Marampa
LIVINGSTONE
CHOMA
MOONO
MAFWAFWA
Tara
KALOMO
Chilesha
Namwianga
Kalomo West
0
20mls
0
30 kms
TRYPANOSOMIASIS SURVEY
Tsetse fly area
Game fence
Fly pickets
Surveyed village
River
Tracks
Major roads
Railway

Figure 5

Kalomo West has excellent land resources and there has been considerable traditional and planned agricultural development. Cattle in this area are used as draught animals and as a source of cash income. In 1978 the Kalomo district Veterinary office received frequent reports of deaths of cattle, pigs and even dogs from villages and settlements in Kalomo West. Trypanosomiasis was suspected and was later, in some cases, confirmed at the Central Laboratories, Mazabuka. Cattle in this and other marginal areas are kept under a chemoprophylactic regime using Samorin. In a situation suggestive of an epizootic, it was decided to investigate the prevalence of trypanosomes in domestic animals of the area.

Investigations protocol

Routine diagnosis of animal trypanosomiasis in Zambia is carried out by examination of stained thin blood slides. These are submitted to the Central Veterinary Research Station or Regional Laboratories (in Eastern, Northern, Western and Copperbelt Provinces) as dried preparations from the field by Veterinary Assistants (VAs). Veterinary Assistants are grassroot personnel trained in basic Veterinary Medicine and Practice. They are based in the rural areas (Veterinary camps) amongst villages.

In the present survey, the assessment of the incidence of trypanosomiasis in domestic livestock in Kalomo West was made by examination of stained thin blood films. Thin blood smears were collected, labelled and dispatched to the author at Central Veterinary Research Station, Mazabuka for staining and microscopic examination.

Results

Blood slides were taken from 953 different domestic animals from

TABLE 11

Prevalence of animal trypanosomiasis in Kalomo West, 1978.

Animal	Total number of blood smears exd.	Number positive	Species of trypanosome
Cattle	601	11	T. congolense (4) T. brucei (4) T. vivax (3)
Goats	125	Nil	Nil
Pigs	115	Nil	Nil
Dogs	112	4	T. congolense

41 villages and settlements (Table 11). Of the 601 cattle blood slides examined, 11 (18%) were found to contain trypanosomes. Thin blood preparations were taken from 112 dogs, from which 4 (3.6%) were shown to be positive for trypanosomes. Blood smears from goats and pigs showed no trypanosomes.

Discussion

Trypanosomiasis has been a persistent problem in this part of Kalomo district and there is evidence that the tsetse population has been increasing since 1958 (MacLennan, 1975). Since 1966 up to the present date, a variety of reclamation activities such as planned and traditional settlement, bush clearing, arboricide treatments, air and ground spraying have had little success (Ann.Rep., 1972; MacLennan, 1975).

In an area where cattle are routinely given the prophylactic drug Samorin (every three or four months), 11 slides showing trypanosomes out of a total of 601 perhaps represent a tolerable incidence of the disease in cattle.

As far as the author is aware, Kalomo West has had no history of goat trypanosomiasis, but severe outbreaks of T. simiae infection in pigs have been reported (Ann.Rep., 1955, 1958; Stephen, 1966). The rapidity with which T. simiae wipes out herds of pigs minimises the chances of diagnosing the infection in isolated remote cases.

3.3 Trypanosomiasis Investigations: Mazabuka East

General Introduction

Mazabuka (Fig.6) is a small district in the Southern Province characterised by a dense rural population (9.6 per sq. km). It has a

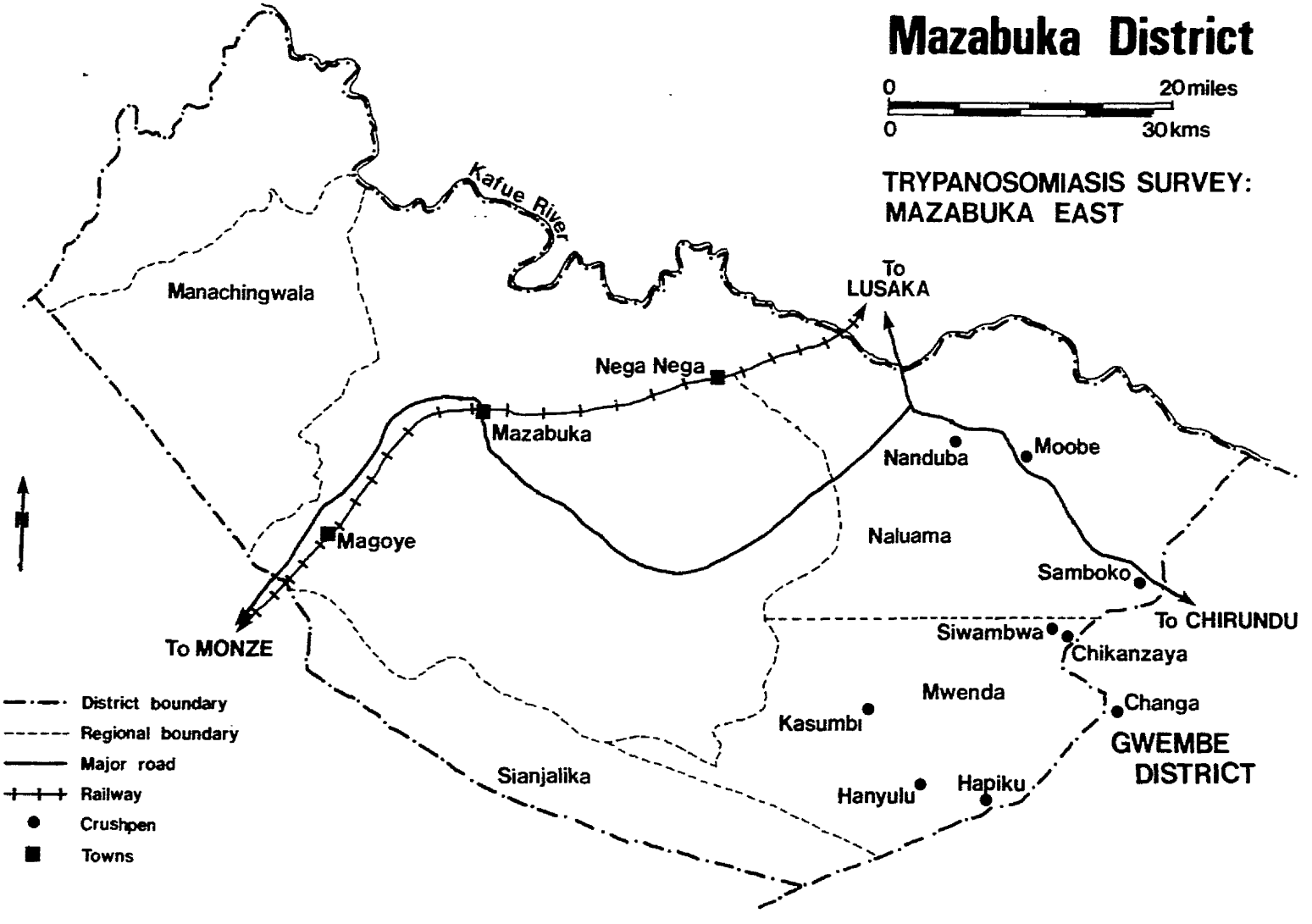

Mazabuka District
0 20 miles
0 30 kms
TRYPANOSOMIASIS SURVEY:
MAZABUKA EAST
Kafue River
Manachingwala
To LUSAKA
Nega Nega
Mazabuka
Nanduba
Moobe
Magoye
Naluama
Samboko
To CHIRUNDU
To MONZE
Siwambwa
Chikanzaya
Mwenda
Changa
Kasumbi
GWEMBE DISTRICT
Sianjalika
Hanyulu
Hapiku
District boundary
Regional boundary
Major road
Railway
Crushpen
Towns

Figure 6

comparatively broad-based economy with both prosperous commercial and traditional crop and livestock farming.

Mazabuka is classified as a tsetse-free area (Clarke, 1966) and does not share borders with a defined tsetse zone. However, Mazabuka has a common eastern border with Gwembe district (Fig.3) which is wholly situated in the Zambezi valley. The Zambezi valley has been known to be infested with tsetse fly (Livingston, 1857; Neave, 1911; Ford, 1971; Ormerod, 1974) of the G. morsitans morsitans species or by this fly in combination with G. pallidipes (MacLennan, 1975).

These infestations have been dealt with effectively by ground spraying and selective bush clearing. Tsetse was believed to have been eliminated, except on two islands in Lake Kariba (Ann.Rep., 1972). After the creation of Lake Kariba, people and their livestock within a 50 or more mile radius had to be evacuated and resettled elsewhere. In Gwembe district, people and their livestock from the flooding areas near Lake Kariba were resettled in the Lusitu from which tsetse-fly had been eliminated (Fig.2). A narrow buffer zone running parallel to the Zambezi river had been successfully maintained between 1966 and 1972 by twice annual applications of persisting insecticide by ground spray (Ann.Rep., 1970).

Border security requirements have precluded anti-tsetse activity which ceased following the insecticide application in 1972 (MacLennan, 1975). By 1975 reinvasion had progressed to a distance of 25 km north of the Zambezi (see Fig.4).

It is this tsetse invasion from the Gwembe (Zambezi) valley which is presumed to have reached the edges of the valley and through into bordering villages in the east of Mazabuka district.

Background information

Investigations into the incidence of cattle trypanosomiasis were carried out in chief Mwenda's area, locally known as Mapangazya and in chief Naluama's area (see Fig.6). These areas had never experienced severe outbreaks of trypanosomiasis until April 1977 when several head of cattle died from trypanosomiasis. This outbreak was put down by blanket treatment of the whole area with Samorin at 0.5 mg/kg. In February - March 1978 a much more severe outbreak occurred and some villagers told of having lost whole herds. During our tour of the areas, it was also alleged that mortality decreased further away from the border with Gwembe district. Big game (elephants and buffaloes) from Lusitu in Gwembe valley had been observed since two years previously, but more were seen during the rainy season of 1977-78.

Investigations protocol

A mobile investigations team comprising the author and a number of laboratory and field assistants established a temporary base at chief Mwenda's palace while working in Mapangazya area. The survey carried out in chief Naluama's area (Fig. 6) was conducted from the Central Veterinary Research Station at Mazabuka.

Wet, thin and thick blood film examination techniques were used. Also 0.5 ml of blood from each sample was inoculated into three mice while later in the survey blood was also inoculated into sheep and calves in an attempt to diagnose T. vivax infections.

Because it rained for most of the survey period, it was not possible to examine wet blood preparations at every crushpen. Therefore, results obtained by this method have not been included.

TABLE 12

Incidence of cattle trypanosomiasis in areas of chiefs Mwenda and Naluama, Mazabuka district, 1978.

Crushpen	Total number of blood smears exd.	Total number positive	Species of trypanosome
*Chaanga	141	12	T. vivax (6) T. brucei (5) T. vivax and T. brucei (1)
Hapiku	52	1	T. vivax
Hanyulu	49	5	T. brucei
Kasimbi	220	8	T. vivax (6) T. brucei (1) T. brucei and T. bigemina and T. ingens (1)
Moobe and Nanduba	215	3	T. vivax (2) T. brucei (1)
Samboko	31	3	T. brucei

* = Chaanga is in Gwembe district. This crushpen was included in the survey because cattle from Chaanga do mix with those of Mazabuka district and is situated on the edge of the Gwembe valley.

Results

Figure 6 shows the relative positions of various crushpens from the Zambezi valley (Gwembe district). During the survey thick and thin blood films from 708 cattle (Table 12) were examined. 32 (4.5%) of these were found positive for trypanosomes which were identified on morphological grounds as T. vivax or T. brucei. Attempts to isolate trypanosomes by mouse, sheep and calf inoculations failed.

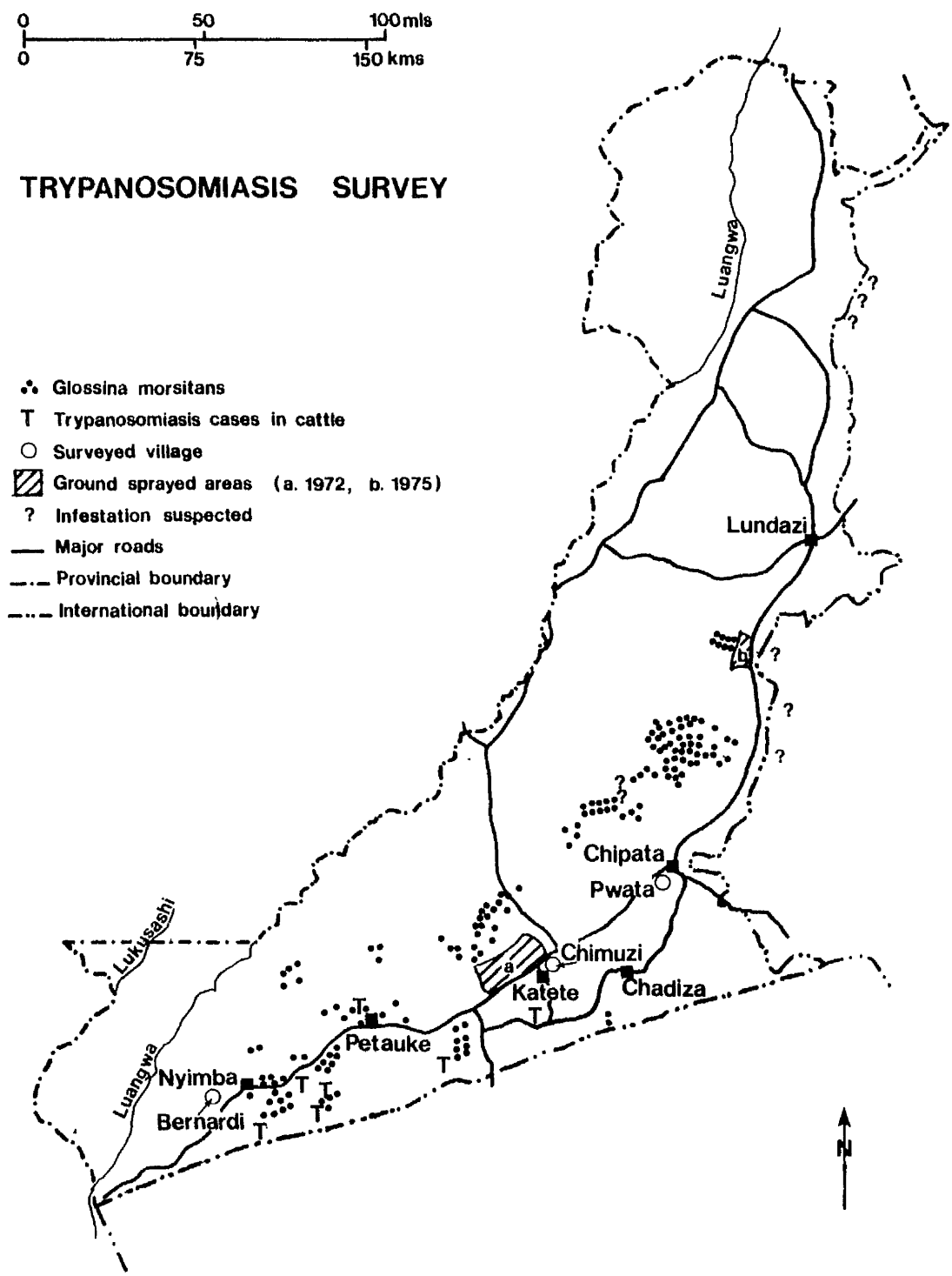
Discussion

In April 1977 the District Veterinary Officer of Mazabuka conducted a similar survey at Moobe, Nanduba, Samboko, Simwaaba and Chikanzaya crushpens prior to giving blanket treatment. Of 220 blood slides examined, 22 (10%) were positive for trypanosomes, 16 of which were showing T. vivax organisms and six T. brucei. Our results suggest a 1:1 ratio of T. vivax to T. brucei infection rates in cattle while the District Veterinary Officer recorded a 7:3 ratio in the 1977 survey. In agreement with the results of the local Veterinary Office, we did not record any T. congolense organisms.

Further, our results seem to indicate that the proportion of T. vivax infections increases with the distance from the detectable tsetse belt. Ford (1964), while working in the Zambezi valley on the south bank (Zimbabwe), observed that T. congolense infections in cattle predominated in areas of high tsetse challenge, but as distance increased from such a centre, so the proportion of smears showing T. vivax increased. From our investigations it would appear that T. brucei infections in cattle are increasing compared to the figures of 1977 survey. The fact that the proportion of T. brucei infections appears to increase as one nears the tsetse belt may be indicative of tsetse encroachment towards the plateau from the valley.

Figure 7

EASTERN PROVINCE, Zambia.



3.4 Trypanosomiasis survey: Eastern Province

General Introduction

Eastern Province (Fig.7) covers an area of 69,100 square kilometres with a human population of 509,515 (Jackman, 1971) and a cattle population of 213,830 (Briault, 1978). Much of the human population and nearly all the livestock (including cattle, pigs, sheep, goats and dogs) are highly concentrated on the plateau (MacLennan, 1975).

Most of the plateau area is tsetse-free, but is bounded to the west by the heavily tsetse infested Luangwa Valley. The infestation here is by *G. morsitans morsitans* but *G. pallidipes* and *G. brevipalpis* are also present. Tsetse in Chadiza and Katete districts (Fig.7) are sparsely distributed, but a substantial part of Chipata and most of Lundazi and Chama districts are infested. In Petauke tsetse infestations have built up in the centre and south of the district (Fig.7) and these have serious repercussions on the rural economy.

Cattle trypanosomiasis is a very serious problem in the Eastern Province. As a result, Chama district has only 202 head of cattle (Briault, 1978) and was not included in the survey. Also Chadiza district was not included because previously it had been shown to have the lowest incidence of trypanosomiasis (Geysen, 1978).

Investigations protocol

The collection of some of the blood samples and preparation of thin films was done, on our request, by the local district veterinary staff. Therefore results summarised in Table 13 are based on examination of stained thin blood films.

At about the same time of our visit to Eastern Province, *T. rhodesiense* sleeping sickness was diagnosed in one man at Bernardi

TABLE 13

Incidence of Animal Trypanosomiasis in 4 districts of Eastern Province, 1978.

District	Animal	Number of slided exd.	Total positive for trypanosomes	Species of trypanosome
Chipata	Cattle	92	4	T. congolense
	Goats	27	-	-
	Dogs	23	-	-
	Sheep	23	-	-
Lundazi	Cattle	154	12	T. congolense
	Goats	46	-	-
	Dogs	12	-	-
	Sheep	7	-	-
Katete	Cattle	20	3	T. congolense
	Goats	27	-	-
	Dogs	4	-	-
	Sheep	11	-	-
Petauke	Cattle	240	34	T. congolense (32) T. vivax (1) T. brucei (1)
	Goats	85	-	-
	Dogs	51	-	-
	Sheep	6	-	-

TABLE 14

Isolation of trypanosomes from a sleeping sickness village, Nyimba, Petauke District, Eastern Province.

Animal and number exd.	Method used					Results
	Mouse Inoc.	Rat Inoc.	Thick Smear	Gland puncture	Wet film	
Cattle 18	57	-	36	18	18	2 cases of *T. congolense* from mouse inoc.
Dogs 10	17	4	30	-	10	Negative
Donkeys 5	15	-	10	-	15	Negative
Goats 14	12	-	28	10	14	Negative
Pigs 12	16	14	24	-	12	Negative

Village, Nyimba area in Petauke district. Since the village is not very close to Luangwa National Park (Fig. 7) we were interested to investigate whether or not domestic animals in and around the village were carrying T. (T.) brucei subgroup organisms. Details of the investigation design are shown in Table 14.

Results

From four districts of Eastern Province, we examined thin blood smears from 506 cattle, 185 goats, 90 dogs and 37 sheep. Fifty-three (10.5%) slides out of 605 taken from cattle showed trypanosomes, but no trypanosomes were seen in blood slides from goats, sheep and dogs (Table 13).

At Nyimba (Table 14) on only two occasions was T. congolense isolated by mouse inoculation from 18 cattle. The rest of the samples were negative for trypanosomes.

Discussion

It is noteworthy that 53 (10.47%) slides out of 506 taken from cattle in the four districts surveyed showed trypanosomes, but no trypanosomes were found in small ruminants and dogs. Briault (1978) reported 225 cases of animal trypanosomiasis in his annual report for Eastern Province. No cases of trypanosomiasis were reported in sheep and goats whose population stood at 3,866 sheep and 90,885 goats in the Province. It is possible that the apparent low or unrecorded incidence of trypanosomiasis in goats and sheep is a result of little effort exerted in the field to look for the disease in these animals. Rickman (1974) found 13 (39.4%) goats out of 33 infected with T. congolense (11), T. brucei (1) or T. vivax (1) at Luembe village in Petauke district. Another possible explanation could be one noted by Griffin and Allonby (1979a) in Kenya that indigenous sheep were less susceptible to

trypanosomiasis than exotic breeds.

3.5 Discussion of Chapter 3

Although the number of samples examined is small, the results found during these investigations in the three areas have practical implications. Further, the use of more sensitive methods - for example, the Haematocrit Centrifuge Technique (HCT) (Woo, 1970; Robson and Rickman, 1972) or the Buffy Coat Darkground Illumination Technique (Murray et al, 1977), might have resulted in the detection of more cases.

Until recently the proportion of T. congolense infections in Zambian cattle has been approximately 80%, T. vivax 15% and T. brucei about 5% (Ann.Rep., 1974, 1975). The results found from the survey of parts of Eastern Province indicate an infection rate in cattle of 92.15% T. congolense, T. vivax 5.8% and 1.96% T. brucei. This is roughly in agreement with the results of previous surveys of cattle trypanosomiasis in other areas of Zambia (Ann.Rep. 1976; Awan et al, 1978) and in neighbouring countries (Ford, 1964), but is at variance with some reports from Eastern Province.

MacLennan (1975) noted from results of a random survey in Petauke district that 46% of all the positive blood smears (which was 3.3% of the total number examined) contained T. brucei. Geysen (1978) reported an approximate ratio of 6:3:1 T. congolense to T. brucei to T. vivax in cattle during 1976 and 1977. Most of his cases were diagnosed during routine laboratory diagnostic and some investigation work. In his annual report for Eastern Province, Briault (1978) reported 45 (20%) T. brucei infections out of 225 cases of cattle trypanosomiasis.

The results from the Kalomo survey may not be statistically significant, but they too point to an epidemiological pattern in which

T. brucei in cattle would appear to be as common as or even more than T. vivax.

The situation in Mazabuka East is different. Here, our findings are in agreement with previous work in that no T. congolense organisms were diagnosed using various methods. The infection rates of T. vivax and T. brucei in cattle are almost at a 50:50 ratio. We have seen that trypanosomiasis in these areas was severe during the 1977 and 1978 outbreaks. If we were to go by the results of this survey and those of previous workers (Dist.Vet.Office report, 1977), then we would have a disease situation, at least in Mazabuka East and probably elsewhere in the country, where the causative agents are mainly T. vivax and T. brucei. Cases described in the Appendix suggest that T. brucei organisms are not only present in the blood of some cattle, but that they may cause clinical disease.

In Zambia, the first clinical T. brucei infection in cattle was probably that reported in 1956 in a settlement near Lusaka (Sheehy, 1956). It is interesting and relevant (see Chapter 5) to note that in 1963 another outbreak of T. brucei infection characterised by chronic syndrome and cerebral complications was reported. This is the first description of cerebral trypanosomiasis in cattle.

Losos and Ikede (1972) demonstrated the pathogenicity of T. brucei in cattle under experimental infection with one animal dying from the disease 54 days after infection. Further, Mettam (Losos and Ikede, 1972) stressed the need to recognise that T. brucei under certain conditions was pathogenic for cattle. The cases described in the Appendix demonstrate that T. brucei subgroup organisms, in Zambia, can cause cattle trypanosomiasis clinically similar to the diseases caused by T. vivax and/or T. congolense. Robson and Ashkar (1972) noted that when another infection such as Babesiosis or Anaplasmosis is superimposed

on the T. brucei infection the animal's resistance is lowered, and the T. brucei subgroup parasites reach a level at which they can be diagnosed by simple parasitological techniques. Of our five cases (see Appendix) one might be placed in this category having concurrent Babesiosis at the time of examination. Further, Losos and Ikede (1972) quote Mettam to have observed that T. brucei organisms obtained from a "G. morsitans" belt were distinctly more pathogenic than those isolated from a "G. palpalis" belt. In Zambia, G. morsitans is responsible for the spread of animal trypanosomiasis.

In conclusion, it would appear that T. brucei might be a more serious cattle pathogen in Zambia than has been recognised. The failure to have detected more T. brucei organisms in blood slides from the Eastern Province might have been due to parasitaemias too low to be diagnosed by thin film examination. Also it could be that samples were taken from areas where, whatever is the cause, infections of T. vivax and T. congolense predominate.

Our own observation which confirms that of Ford (1964) that the proportion of T. vivax increased with the distance from tsetse-belts perhaps merits entomological investigation direct towards noncyclical transmission by biting flies.

CHAPTER 4

RELAPSING PARASITAEMIAS OF *T. CONGOLENSE* AND *T. VIVAX* IN CATTLE TREATED WITH VARIOUS DOSES OF BERENIL OR SAMORIN

4.1 Introduction

Control of cattle trypanosomiasis by the use of anti-trypanosomal drugs has been widely used with considerable success in Zambia (Hobday, 1953; Shaw, 1960; Ann.Rep., 1976) and elsewhere in Africa (Bevan, 1928; Smith 1959; Scott and Pegram, 1974; Bourn and Scott, 1978).

This practice, however, leads to several practical problems one of which is the development of drug resistance (Wilson et al., 1975; Bourn and Scott, 1978). Widespread drug resistance was reported in the 1960s in areas of Zambia where trypanosomiasis is endemic (Shaw, 1960; Sheehy, 1962; Ann.Rep., 1966; 1967; 1971). Because of this difficulty, the only drugs available for field use since 1972 have been Berenil (Diminazene aceturate) and Samorin (Isometamidium chloride) which are used as therapeutic and prophylactic agents respectively.

The common cause of drug resistance in the field is underdosage or irregular treatments (Mwambu, Mayende and Masinde, 1969). However Fussgänger and Bauer (1960) and Bauer (1962) concluded that Berenil did not induce the development of resistant T. congolense strains even after continuous administration of subcurative doses. Similarly, Folkers (1966) reported failure to produce resistance by a series of 0.25 mg/kg Samorin injections of cattle infected with T. congolense or T. vivax. In contrast, Hawking (1963), Whiteside (1963), de Raadt, van Hoeve, Hart and Grainge (1965) showed that resistance to Berenil can be induced by the administration of inadequate doses of the drug. Further, Scott and Pegram (1974) reported that after Samorin had been administered prophylactically for 6 months at 8-weekly intervals, thick and thin blood films from cattle 5 weeks after the third treatment revealed a 66% infection rate. Also, resistance to Berenil can arise in trypanosomes

made resistant to stilbamidine (Bauer, 1962; Gill, 1971), to antrycide (Fussgänger and Bauer, 1960; Whiteside, 1963 and Williamson, 1976) and to ethidium (MacLennan and Jones-Davies, 1967; Jones-Davies, 1967a, 1968b).

Since 1967 it has become apparent that certain strains from T. congolense and T. vivax naturally relapse after therapeutic doses of Berenil (Jones-Davies, 1967a, b; MacLennan, 1967; 1972; Jones-Davies, 1968a and Mwambu, 1973). Fenelle (1972), however, suggested that strains which are naturally resistant to Berenil were still sensitive to Homidium and Samorin.

In view of the large-scale use of Berenil and Samorin, and the consequent risk of the development of resistant strains, we decided to investigate drug sensitivity of cattle trypanosomes in Zambia. The experiments described below are the first in a longterm programme to monitor drug susceptibility of cattle trypanosomes to customary curative doses of Berenil and Samorin.

4.2 Isolation of trypanosomes from MAFWAFWA and MOONO crushpens and treatment with Berenil or Samorin

In order to make field observations two crushes in Kalomo district were chosen for the study.

Experimental design

MAFWAFWA CRUSHPEN

Five clean (previously uninfected) 8-months old Angoni-Boran weaner male calves were used as recipients of blood from the cattle being examined. These calves were driven in a cattle truck to Mafwafwa crushpen (Fig.5). A herd of about 50 cattle from the local villages had

been gathered at the crushpen on the day of the inspection. From this herd, 24 cattle in rather poor condition were selected and sampled. 20 ml of blood was taken from the jugular vein of each of these animals. Thick and thin films were prepared, wet films examined and packed red cell volume measured using a portable haematocrit centrifuge; blood was put into test tubes for the preparation of serum. Three mice were inoculated intraperitoneally with 0.5 ml blood from each of the samples. Further, about 3 ml of blood from each of the 24 cattle was inoculated subcutaneously (according to the method of Jones-Davies and Folkers, 1966) into each of the five recipient calves. The calves were then transported back to the Central Veterinary Research Station at Mazabuka. At the Research Station, the calves were kept in screened, flyproof accommodation and examined every other day for parasitaemia.

One calf (272) was treated with 0.5 mg/kg Samorin twenty-four hours after inoculation with blood and one other (265) at the same time with 3.5 mg/kg Berenil. Two further calves (260 and 269) were treated with the same doses of each drug when the infection became patent, 22 days after inoculation. Calf 224 was not treated.

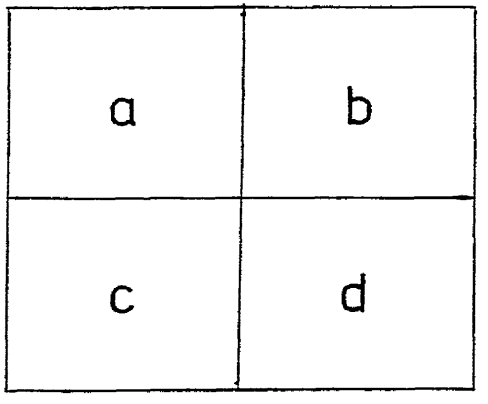
MOONO CRUSHPEN

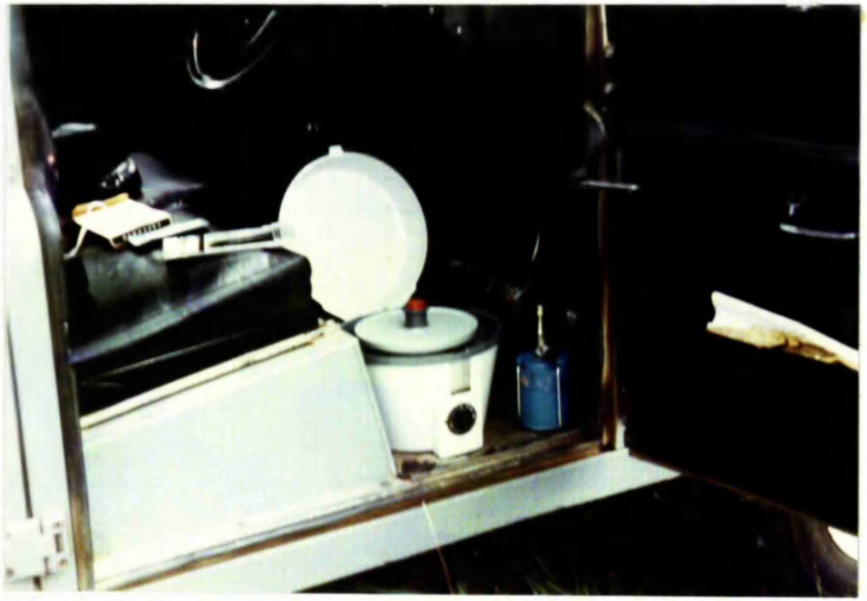
Four calves and five sheep, all trypanosome-free and whose serum contained no anti-trypanosomal antibodies (as determined by the ELISA test - see Chapter 2) were taken to Moono crushpen in another area of Kalomo district (Fig.5). In general the same procedures as described above for Mafwafwa were repeated at Moono crushpen. At Moono 56 cattle from three small herds were examined and blood from 32 (one of which was positive on blood smear examination) of these animals was injected into four recipient calves. The five sheep were injected with blood from the smallest herd of 15 animals. Since there were only four calves, it was

Figure 8

Photographs taken at Mafwafwa and Moono crushpens which illustrate (a) general terrain and cattle truck carting recipient cattle, (b) crushpen and bleeding of donor cattle, (c) temporary laboratory where wet film examinations, preparation of thick blood films and serum and mouse inoculation were carried out and (d) portable microhaematocrit centrifuge for determination of P.C.V.'s.

Photographs:





decided to treat one calf (282) with Samorin twenty-four hours after inoculation and two calves one with Berenil (237) and another with Samorin (205) at patent parasitaemia. Calf 202 was not treated.

Photographs of the terrain and procedure are shown in Figure 8.

Results

Incidence of trypanosomiasis in donor cattle

MAFWAFWA CRUSHPEN

At Mafwafwa cattle had been treated with Berenil 42 days before they were examined. A trypanosome was identified in a thick blood smear from one of the 24 cattle. Its species could not be identified. No trypanosomes were seen in either wet or thin blood films. Serum from 13 out of 24 cattle contained antibody as determined by the ELISA test at a serum dilution of 1:200 using *T. vivax* antigen. Mice inoculated with blood did not become infected. Recipient calves became infected with *T. vivax* 18-20 days after inoculation with blood from the 24 cattle. *Babesia bigemina* was identified in thick blood smears from four out of the five calves 42, 42, 44 and 90 days post blood inoculation respectively.

MOONO CRUSHPEN

A total of 56 cattle were examined. These cattle were in three herds of 24, 17 and 15 head and each group belonged to different individuals resident in the same area.

The 24 cattle in the first group had been treated with 0.5 mg/kg Samorin six weeks previously. Examination of thick films showed that three out of the 24 cattle were infected with trypanosomes. One of these was identified as *T. congolense*, another as *T. vivax* while the third could not be identified due to distortion of the shape of the

trypanosome. Because these cattle had been treated with Samorin only six weeks previously, it was thought they were unlikely to be infected and therefore their blood was not inoculated into recipient calves.

Examination of thick blood smears from the second herd of 17 cattle revealed no trypanosomes. This herd of cattle had been treated with Samorin six months previously.

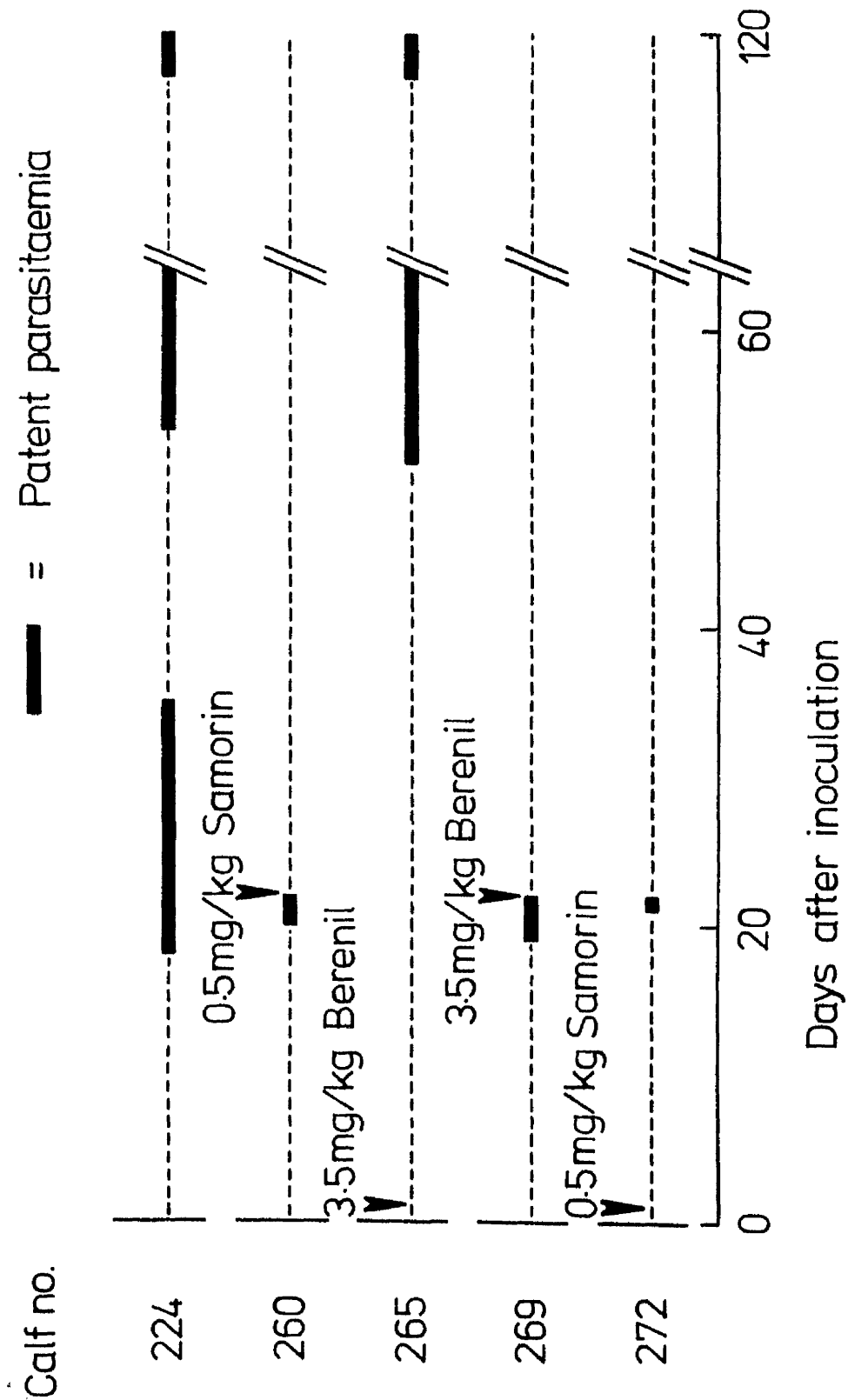
One animal was found positive for T. congolense in the third herd of 15 head of cattle on thick blood smear examination. This herd had been treated with 0.5 mg/kg Samorin four months before the cattle were examined.

The calves which received blood from the 32 cattle in the second and third herds subsequently developed a T. congolense parasitaemia 18 days later.

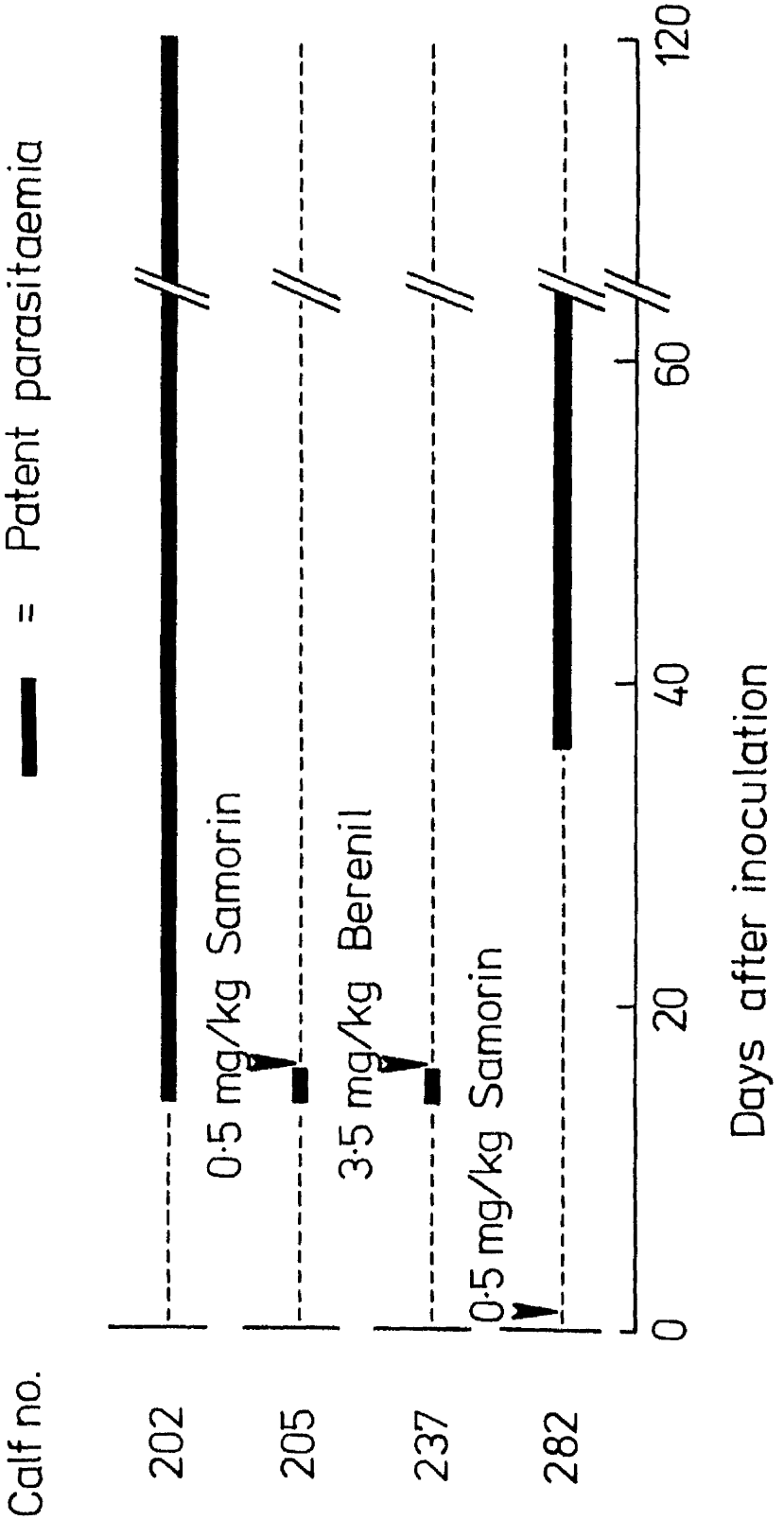
Babesia bigemina was identified in the thick blood smears from calf 202 on day 69 after inoculation. The five sheep which were inoculated with blood from the third herd of 15 cattle did not develop any trypanosome infection. Mice inoculated with blood from the 32 cattle (second and third herds) did not subsequently become infected.

Drug susceptibility of isolated trypanosomes

Calves inoculated with blood from the cattle at Mafwafwa crushpen became infected with T. vivax. Results of the treatment of this infection with 3.5 mg/kg Berenil or 0.5 mg/kg Samorin are shown in Figure 9. Both calves treated 24 hours after blood inoculation became parasitaemic. However, the two calves treated with the same doses of either drug at patent parasitaemia remained aparasitaemic during the 120 days period of observation.



Parasitaemias in five calves following inoculation of blood from 24 cattle at MAFWAFWA crush-pen. Four calves were treated with either Berenil or Samorin. Calf 224 was not treated. The trypanosome was identified as T. vivax.



Parasitaemias in four calves following inoculation of blood from 32 cattle at MOONO crush-pen. Three calves were treated with either Berenil or Samorin. Calf 202 was not treated. The trypanosomes was identified as T. congolense.

The one calf (Fig.10) treated with 0.5 mg/kg Samorin 24 hours after inoculation with cattle blood at Moono became parasitaemic on day 36. The two calves treated with either Berenil or Samorin two days after the infection became patent remained aparasitaemic during the observation period of 120 days.

The results from the two experiments above suggested that infections caused by these isolates of T. vivax or T. congolense became patent despite treatment with Berenil or Samorin 24 hours after infection. On the other hand infection with either isolate appeared to have been cured if treatment was made immediately after the infection became patent.

It is curious that the T. vivax infection in calf 272 (Fig.9) was observed on one day only. T. vivax in cattle is known to display aparasitaemic phases (Fiennes, 1950) and this occurred in the untreated control calf (224). In a later experiment (unpublished observations) when this isolate of T. vivax was put into sheep, some of the animals showed an intermittent parasitaemia as determined by thick blood film examinations. Others died without showing parasites in their blood, but with a packed red cell volume as low as 10.0%, suggesting that such animals might have died from a low level T. vivax infection.

4.3 Relapsing T. congolense infections following treatment with 3.5 mg/kg Berenil

Introduction

The results from the two experiments above suggested that calves were permanently cured if they were treated with 0.5 mg/kg Samorin or 3.5 mg/kg Berenil after the trypanosome infection had become patent. Similar treatments immediately (24 hours) after infection resulted in relapses. In view of these observations it was decided to further

investigate the effect of these drugs on the parasites at a late stage of the infection.

Experimental design

Isolates of T. vivax and T. congolense from untreated control calves were used in this and subsequent experiments. Trypanosoma congolense was isolated from calf No. 202 (T. congolense 202) and T. vivax from calf No.224 (T. vivax 224).

Three calves were each injected subcutaneously with about 1×10^5 T. congolense 202 and another three with a similar number of T. vivax 224. All the calves were treated with 3.5 mg/kg Berenil on day 21 after infection.

Results

The significance of the results of the effect of Berenil on T. vivax in calves is limited since one calf died 19 days after infection, having shown parasitaemia from day 7. The remaining two calves which also became parasitaemic around this time and were treated on day 21 died 32 and 80 days later respectively having shown no signs of relapse.

The results of treating calves infected with T. congolense 202 with 3.5 mg/kg Berenil on day 21 after infection are shown in Table 15. Relapses were observed within 10 and 21 days after treatment.

There were no secondary relapses when the calves were treated with 7.0 mg/kg Berenil 24 days after the initial treatment. The periods between relapse and second treatment are shown in Table 15. Thick blood smears from the calves were examined every other day for 60 days after the second treatment.

These results indicated that treatment with 3.5 mg/kg Berenil, 9 days

TABLE 15

The effect of Berenil on primary and relapse syringe transmitted T. congolense 202 infections in cattle.

Calf number	Primary infection treated with 315 mg/kg Berenil on day 21		Relpase infection treated with 7.0 mg/kg Berenil	
	Duration of patent infection before treatment	Result of treatment	Period between relapse and treatment (days)	Result of treatment
113	9	Relapse 21 days after treatment	4	cure
117	9	Relapse 12 days after treatment	13	cure
320	9	Relapse 10 days after treatment	15	cure

after a T. congolense 202 infection had become patent, resulted in a relapsing parasitaemia. The results from 4.2 above suggested that a similar treatment immediately after the infection had become patent resulted in cure while treatment 9 days after the infection had become patent resulted in relapse. Thus it was suggested that there is a critical period during which T. congolense 202 may or may not be susceptible to 3.5 mg/kg Berenil. This is further investigated in the following experiment.

4.4 The effect of various doses of Samorin or Berenil on T. congolense 202 in cattle

Experimental design

A group of 25 calves was infected with T. congolense 202. Each calf was inoculated subcutaneously with about 1×10^4 organisms of T. congolense 202 on day 0. On day 1 - i.e. 24 hours after infection, 12 of the calves were divided into four groups of three animals. Two of these groups were treated with Berenil, one at a dosage of 3.5 mg/kg and another at 7.0 mg/kg. The other two groups were treated with Samorin, one at a dosage of 0.5 mg/kg and another at 2.0 mg/kg.

On day 21 after infection 12 out of the remaining 13 calves were divided into the same number of groups and treated with the same doses of either drug as above.

A further calf served as an untreated control.

Results

Two calves (372 and 19) died 27 days after treatment due to accidental poisoning.

TABLE 16

The effect of the treatment of T. congolense 202 in cattle with Berenil or Samorin, 24 hours after infection.

Calf No.	Drug and doage in mg/kg		Duration of aparasitaemic period after treatment (days)	Remarks
	BERENIL	SAMORIN		
116	-	-	14	Untreated control
115	3.5		*80	
112	3.5		39	
372	3.5		-	Died 24 days after treatment
1994	7.0		*80	
2210	7.0		*80	
2470	7.0		*80	
9		0.5	39	
19		0.5	-	Died 24 days after treatment
309		0.5	41	
120		2.0	*80	
122		2.0	*80	
363		2.0	*80	

* = Duration of experiment

TABLE 17

The effect of the treatment of T. congolense 202 in cattle with Berenil or Samorin 21 days after infection.

Calf Number	Drug and dosage in mg/kg		Duration of prepatent period (days after infection)	Duration of aparasitaemic period (days after treatment)	Remarks
	BERENIL	SAMORIN			
01	3.5		16	18	
21	3.5		14	*80	
127	3.5		10	20	
M13	7.0		12	*80	
M15	7.0		12	18	
111	7.0		12	-	Died day 57 after treatment
00		0.5	12	7	
102		0.5	12	5	
386		0.5	12	7	
1931		2.0	10	13	
2403		2.0	14	11	
2437		2.0	12	11	

* = Duration of experiment

It is clear from the results shown in Table 16 that T. congolense 202 relapsed following treatment with 3.5 mg/kg Berenil and 0.5 mg/kg Samorin 24 hours after infection. No relapsing parasitaemias were observed in cattle treated with either 7.0 mg/kg Berenil or 2.0 mg/kg Samorin.

If, however, treatment was delayed until day 21 after infection (Table 17) all calves treated with 0.5 and 2.0 mg/kg Samorin relapsed as early as 5 days after treatment. Two out of three calves treated with 3.5 mg/kg Berenil relapsed and one out of three treated with 7.0 mg/kg also relapsed. It is significant that in all the groups the time between treatment and relapse is much shorter after treatment on day 21 than at 24 hours after infection.

4.5 Discussion of Chapter 4

Relapsing strains of T. vivax and T. congolense following curative doses of Berenil have been reported in Nigeria by Jones-Davies (1967a, b; 1968a, b), MacLennan (1967, 1970) and in East Africa by Whiteside (1963) and Mwambu (1973). These naturally occurring strains have been shown to retain their relapsing property after transmission through tsetse fly and game (Van Hoeve and Grainge, 1966; Gray and Roberts, 1971a, b) and by syringe passage (Whiteside, 1963). In all these cases relapsing infections have been ascribed to drug resistance naturally inherent in certain isolates of T. vivax and T. congolense.

Little has been documented in the literature about trypanosomes naturally resistant to Samorin - i.e. in areas where the drug has not been used before. Scott and Pegram (1974) suspected that T. congolense was resistant to Samorin in Ethiopia, while Lewis and Thomson (1974) in Zimbabwe reported T. congolense resistance to Samorin.

In Zambia this is the first time that cattle trypanosomes relapsing after treatment with doses of either Berenil or Samorin which have previously been shown to be curative have been described.

Two out of 24 cattle treated with 0.5 mg/kg Samorin six weeks previously were found infected with T. vivax and T. congolense at Moono crushpen. This strongly suggests the presence of strains resistant to that dose of the drug or that the tsetse challenge is too high for 0.5 mg/kg Samorin to give prophylaxis longer than six weeks. Alternatively, the animals were underdosed. The fact that T. congolense 202, isolated from a different herd of cattle but within the same area, relapsed after treatment with Berenil or Samorin seems to confirm that relapsing strains occur in the area. Further, T. vivax was isolated from the same district and ecological zone. Preliminary results of the effect of the drugs on this strain also suggested that it relapsed after treatment with normally curative doses.

The results from our experiments indicated that relapse occurred following treatment with 3.5 mg/kg Berenil and 0.5 mg/kg Samorin 24 hours after infection. If, however, the dose for Berenil was doubled and that for Samorin was increased four times, relapses did not occur (at least with T. congolense 202).

On the other hand, 3.5 mg/kg Berenil and 0.5 mg/kg Samorin appeared to give permanent cure for both T. congolense 202 and T. vivax 224 if treatment was carried out immediately after the parasitaemias become patent (Fig.9 and 10), but failed to do so in the case of T. congolense 202 (Table 17) when treatment was delayed until 7-11 days post parasitaemia i.e. 21 days after infection. At this stage of infection all three calves treated with 2.0 mg/kg Samorin and one out of three treated with 7.0 mg/kg Berenil relapsed.

We seem to be dealing with probably two phenomena of relapsing trypanosome infections.

First the possibility that the inoculum is sequestered from the drug in the local lesion for at least one or two days. Fiennes (1950) reported that T. congolense acted as a tissue parasite in the adrenal cortex and the anterior pituitary glands and he suggested that such tissue foci must be the source of the secondary stage of the disease in cattle. Roberts, Gray and Gray (1969) reported that T. congolense can develop in connective tissue of the dermis in local reactions at the site of the tsetse fly bite. Recently it has been shown that T. congolense develops and persists (Luckins and Gray, 1978, 1979; Gray and Luckins, 1979) in the connective tissue at the sites of the bites of tsetse-flies in rabbits, calves and sheep. Gray and Luckins (1979) showed that trypanosomes could be detected from these local reactions in the collagen at least until day 30 after infection in calves. Our inoculations were subcutaneous in which case a lesion at the site of injection could have developed just as in the case of a tsetse-fly bite. The drugs were administered intramuscularly. Samorin owes its prophylactic effect to its ability to adhere to protein and thus forms a depot at the site of injection (Hill and McFadzean, 1963) from which it is slowly released into the circulating blood to act on the parasites. Although Berenil is known to display rapid action on trypanosomes and is quickly excreted from the blood stream (Hawking, 1958; Fussgänger and Bauer, 1960; Fairclough, 1963), recent work has shown that anti-trypanosomal activity against T. brucei persists in tissue fluids and plasma up to two weeks (Goodwin and Tierney, 1977). Further, Raether, Hajdu, Seidenath and Damm (1974) obtained a prophylactic period of approximately 14 and 21 days following intramuscular injections of 10 and 20 mg/kg Berenil into rhesus monkeys against needle challenge of T. rhodesiense.

The prolonged prepatent period following treatment with both drugs 24 hours after infection suggested that there must have been chemotherapeutic action exerted on a proportion of the trypanosomes as they entered the blood stream. We suggest that when blood and tissue fluid concentrations of the drugs from the depots became too low to have any antitrypanosomal effect, the parasites, on the other hand, continued to multiply at the site of injection in the dermis from which they infiltrated the blood system in increasing numbers reaching a detectable level in the peripheral blood many days after the untreated control had shown patent parasitaemia. Gray and Luckins (1979) have shown that numbers of the trypanosomes decrease with time at the site of the tsetse-fly bite. Probably this may explain the apparent cure when calves were treated immediately (2-4 days) after the infection had become patent.

Secondly, the possibility of relapse infections similar to those with Trypanosoma brucei in mice described by Jennings et al. (1977). These workers noted that infections resulting from inoculations of mice with six different stabilates of T. brucei and treated with 40, 7.5 and 10 mg/kg of Berenil, Ethidium and Prothidium respectively at either 3 or 7 days after infection elicited a permanent cure. If, however, they delayed treatment later than 14 days after infection, then all the mice relapsed, generally between 20 and 50 days after treatment. MacLennan and Na'lsa (1970) described a similar relapsing infection of T. vivax in Zebu cattle in Nigeria following treatment with Berenil at normal and increased doses. The relapses occurred within 10 to 25 days after treatment and were uncorrelated with the dose rate. Further, MacLennan (1971) showed that if treatment of T. vivax infections at varying dose rates of 3.5, 7.0, 10.5 and 14.0 mg/kg Berenil was administered when parasitaemia was well established all animals relapsed between 14 and 31 days after treatment. In our hands, T. congolense 202 infections in cattle relapsed between 5 and 21 days (Tables 16 and 17) after treatment

with 3.5 or 7.0 and 0.5 or 2.0 mg/kg Berenil or Samorin respectively.

It is rather difficult to postulate the cause of relapses in T. vivax and T. congolense infections observed after treatment 21 days post-infection. In Trypanosoma brucei relapsing infections, Jennings et al. (1979 and also see Chapter 5) showed that the source of the relapses was the brain. It may be that T. congolense, as well as T. vivax, at a certain stage of the disease, invade tissues of various organs as suggested by Fiennes (1950) in which either they become inaccessible or acquire forms insusceptible to the drugs.

Finelle (1972) suggested that Berenil-resistant trypanosomes are sensitive to Samorin. The isolates of T. congolense and T. vivax described here appeared to be less susceptible to normally curative doses of both drugs. If this observation from the results of working with two strains is representative of the general situation in Zambia, then the implications could be serious since Berenil and Samorin are the only two drugs currently in use in Zambia. For practical purposes in the field it is resistance to a normally curative dose of a drug that is important (Hawking, 1963).

Judging by these preliminary results and by the evidence from the field that despite the massive use of Samorin and Berenil the incidence of cattle trypanosomiasis is still high, it may be possible that there has been selection of less susceptible trypanosome populations following the use of drugs.

CHAPTER 5

THE BRAIN AS A SOURCE OF RELAPSING *TRYPANOSOMA BRUCEI* INFECTION IN MICE AFTER CHEMOTHERAPY

5.1 Introduction

Relapsing infections following drug treatment of trypanosome infections in the field have been largely associated with drug resistance (Hawking, 1958; Whiteside, 1963; Jones-Davies and Folkers, 1966; Gray and Roberts, 1971a) although MacLennan and Na'lsa (1970) have described a relapse phenomenon of T. vivax in Zebu cattle which they ascribed neither to previous experience of the organism to the drug diminazene nor to the dose rate.

Browning and Calver (1943) were probably the first to show a relationship between the stage of infection and chemotherapeutic response in trypanosome infection. These workers noted that if they delayed treatment of their otherwise sensitive T. congolense strain in mice until infection became chronic, cure was more difficult to obtain. Treated animals invariably relapsed.

Jennings, Whitelaw and Urquhart (1977) subsequently described relapsing T. brucei infection in mice after treatment with Berenil, which could be ascribed neither to drug resistance nor under-dosage. Treatment of infections during the first 7 days invariably produced permanent cure. If, however, treatment was delayed later than 14 days after infection, relapses almost always occurred, normally between 20 and 50 days after treatment, but occasionally up to 120 days after. The morphology and site of the parasite in the host during the aparasitaemic phase was unknown.

Soltys and Woo (1970) suggested that tissue forms of T. brucei which they found in the liver and spleen may give rise to blood forms when inoculated into recipient animals. Ormerod and Venkatesan (1971) also postulated that if infection is prolonged by drugs, parasitaemic

relapses, which may have their origin in a tissue or 'occult' phase, may occur.

In this chapter, using the system described above by Jennings et al (1977), we describe investigations of the 'occult' phase of trypanosomiasis which followed delayed chemotherapy, the results demonstrate the persistence of infection only in the brain tissue.

5.2 Experimental design

Donor mice were each inoculated intraperitoneally (i.p.) with approximately 1×10^4 trypanosomes, prepared from the frozen stabilates, in phosphate glucose saline buffer, pH 8.0. Tail blood from the mice was examined for trypanosomes by the wet film technique prior to chemotherapy to ensure that all were infected, and every second day after chemotherapy to confirm the absence of trypanosomes. In later experiments, the absence of circulating trypanosomes on the day of tissue transfer was further substantiated by the miniature anion-exchanger/centrifugation technique (Lumsden, Kimber and Strange, 1977). Wet films from mice retained to monitor for recrudescence of parasitaemia after chemotherapy were checked twice per week. Recipients of transferred tissue were similarly examined for parasites every second day for 10 days after transfer and thereafter twice per week.

5.3 Preparation of tissue homogenates

Donor mice were exsanguinated by cardiac puncture under deep trichloroethylene anaesthesia (Trilene, I.C.I., England) during the aparasitaemic period after chemotherapy, and immediately dissected. In the initial experiment, the various organs were mechanically homogenised for a few seconds (Silverson, London, England) but in subsequent

experiments they were gently teased apart through a wire gauze into sterile saline, using a rubber-tipped spatula. Each cell suspension derived from an entire organ was then injected i.p. into one recipient; the volume of blood transferred was 1 ml.

5.4 Results

Experiments with T. brucei TREU 667

Eight mice which had been infected with T. brucei TREU 667 for 21 days were treated with 40 mg/kg Berenil and killed 14 days later during the aparasitaemic phase. Brain, liver, kidney and spleen cell suspensions were prepared from individual mice, together with a blood sample, and these were each injected into recipient mice. Parasitaemia occurred 22-26 days later in 3 of 8 recipients, all of which had received brain homogenate (Table 18). A further 6 mice which had not been killed after Berenil treatment all relapsed between 28 and 47 days after chemotherapy.

A second experiment identical in design was performed except that lung and heart tissue were also included, and the teasing method of tissue disruption was used. As before, infection was transmitted only by brain tissue, from 3 of 5 donors (Table 18) the mice becoming parasitaemic 10-14 days after transfer of brain homogenate. Mice which were not killed developed parasitaemia 59-68 days after chemotherapy.

In a third experiment, a higher incidence of transmission from brain was achieved from mice which had been infected with T. brucei TREU 667 for 7 weeks and killed one week after treatment (Table 18): brain homogenate from 6 of 8 mice produced parasitaemias between 7 and 27 days after inoculation into recipient mice. Spleen, liver, heart, lymph node, kidney and lung tissue failed to transmit infection. Donor

TABLE 18

Transmission of infection with tissues from aparasitaemic* mice 1-2 weeks after chemotherapy with Berenil (40 mg/kg). Each organ was inoculated into one recipient.

Group	*T. brucei* Stabilate	Duration of infection at chemotherapy (weeks)	Tissue transfer after chemotherapy (weeks)	No. of donors	Number of recipients becoming parasitaemic when tissues were transferred from Berenil-treated donors							
					Brain	Blood	Liver	Spleen	Kidneys	Lung	Heart	Lymph nodes
1	TREU 667	3	2	8	3	0	0	0	0	ND	ND	ND
2	TREU 667	3	2	5	3	0	ND	0	0	0	0	ND
3	TREU 667	7	1	8	6	0	0	0	0	0	0	0
4	LUMP 1001	3	1	12	12	0	ND	ND	ND	ND	ND	ND
5	LUMP 571	3	1	6	6	0	ND	ND	ND	ND	ND	ND

ND = Not done

* Unsacrificed mice subsequently relapsed after chemotherapy as follows:

Group	No. of mice	Day of relapse
1	6	28, 30, 30, 40, 40, 47
2	3	59, 59, 68
3	4	25, 32, 37, 41
4	3	24, 25, 27, 30
5	3	40, 48, 54

blood also failed to transmit infection; this confirmed the failure to detect parasites on the day of transfer by both wet films and DEAE-cellulose chromatography.

Experiments with T. brucei LUMP 1001 and LUMP 571

These two stabilates were examined to find if infection similarly resided in the brain tissue during the aparasitaemic period after Berenil treatment. With T. brucei LUMP 1001 blood transfer from mice killed 1 week after treatment of a 3 week infection was negative, thus confirming the absence of circulating trypanosomes as indicated by wet film and DEAE-cellulose chromatography examinations. All 12 recipient mice which received brain homogenate from the 12 donors developed parasitaemias (Table 18). All recipients were parasitaemic by day 24 except for one which became patent after 43 days. Controls relapsed 24-30 days after Berenil treatment.

Similarly, in the case of T. brucei LUMP 571 the brains of 6 donor mice sacrificed 1 week after treatment of a 3 week infection transmitted infection to recipients while the blood from the same donors was non-infective.

5.5 Discussion of Chapter 5

The results indicated that Berenil treatment (40 mg/kg) of T. brucei infections at 3 weeks or more was followed by an aparasitaemic phase of several weeks, after which parasites reappeared in the circulation. Tissue transmission experiments during this period implicated the brain as the sole source of subsequent relapse and provide further evidence that this type of relapse after the chemotherapy of T. brucei infections was caused by the re-entry into the systemic circulation of trypanosomes from

the brain and was not attributable to drug resistance or under-dosage (Jennings et al., 1977). The morphology of these parasites, their location in the brain and the stimulus for their re-emergence into the systemic circulation is still unknown.

With regard to location, they may be in the brain tissue itself, the cerebrospinal fluid (CSF) or the choroid plexus. Of these, the most probable are the brain tissue or the CSF, since trypomastigote forms in the choroid plexus would presumably be susceptible to chemotherapy. This of course may not be the case if the trypanosomes survive in a capillary closed by a shunt mechanism (Ormerod and Venkatesan, 1971a) or in a form insusceptible to drug action. The latter, however, is inconsistent with the findings that infective forms less than 0.8 μm in diameter were present in the liver and spleen as early as 72 hours after infection (Soltys, Wood and Gillick, 1969; Soltys and Wood, 1970; Ormerod and Venkatesan, 1971a, b). Obviously, these forms must be drug sensitive since treatment at 3 days would not otherwise give a permanent cure, nor would transfer of brain tissue after Berenil treatment at 3 days fail to establish an infection in recipient mice (unpublished observation). In addition successful transmission of infection after treatment at 3 weeks should also still have been possible with tissue other than brain.

Whatever the location of the parasite in the brain, it may occur in trypomastigote or amastigote form; the latter has been described by Ormerod and Venkatesan (1971b) in the choroid plexus of rats infected with T. brucei. After penetration into the central nervous system, presumably by trypomastigotes, the parasites are beyond the blood-brain barrier (Angevine, 1975). This barrier prevents Berenil from reaching sufficient concentration in the CSF to be effective. It is also possible that once in the CNS they then change to forms (which may or may not be drug-resistant) which revert to trypomastigotes at intervals

and so re-establish parasitaemia. It seems likely that infection may persist in the CNS for prolonged periods, since a proportion of mice infected with T. brucei for 3 weeks and then treated at 21-day intervals with Berenil (40 mg/kg) for 6 months (during which time blood examination was persistently negative) eventually became parasitaemic 7-8 weeks after treatment had ceased (unpublished observations).

Treatment of human infections caused by T.rhodesiense and T.gambiense is not uncommonly followed by relapse up to 3-4 years later (Apted, 1970b), with trypomastigotes reappearing in the CSF or blood. These relapses could be similar to those described here in mice, in which case the mouse model may be of value in the search for improved chemotherapy in man. Some work on the successful chemotherapy of such infections using Berenil-nitroimidazole combinations has been described recently by Jennings, Urquhart, Murray and Miller, (1980).

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

In this chapter an attempt is made to discuss broadly the results of the present study and their probable impact on the socio-economic structure of rural Zambia. It was stated in Chapter 1 that the economy of Zambia has been sustained principally by the copper mining and refining industry. However, the current world recession in demand for copper has adversely affected the national economy. This, therefore, has given added impetus to the Government to diversify the economic base of the country through rural development (MacLennan, 1975). This drive to develop agriculture is emphasised in the Third National Development Plan (TNDP) (1978-82) whose theme is rural development.

Up against this drive are the problems presented by trypanosomiasis which affect almost every aspect of human activity. Trypanosomiasis, both of humans and of livestock, is one of the most important factors restricting economic development in Africa today (Wilson, Morris, Lewis and Krog, 1963). In Zambia, the effects on agriculture are chiefly seen through the failure to establish a system of mixed farming in provinces where trypanosomiasis makes livestock farming uneconomic.

Thus the economic and public health problems caused by trypanosomiasis are great. In some areas of the upper Luangwa Valley, dogs and chickens are the only domestic animals seen in villages (Ormerod, 1974). Also some whole villages were either wiped out or abandoned in the Nyimba area of the south Luangwa Valley as a consequence of trypanosomiasis (Rickman, 1974). Buyst (1976, 1977b) and Rickman (1974) have reported outbreaks of sleeping sickness of epidemic proportions the epidemiology of which have not been fully investigated. Ormerod (1974) suggested that the reasons why this epidemic situation has come about are mainly historical, pointing out that endemic foci of Rhodesian sleeping sickness have existed in Zambia since its discovery in 1908. In such endemic areas rearing of domestic livestock is also almost impossible

e.g. there are only 203 cattle in Chama district of the Eastern Province (Briault, 1978).

In Zambia, and it is true for all tropical Africa, tsetse infestation has altered the distribution of cattle. Apart from acting as vectors of a widespread debilitating disease, tsetse flies have created local over-stocking problems in some tsetse-free grazing areas such as in Kalomo and Namwala districts of the Southern Province.

The results from our field investigations (Chapter 3) suggest that the incidence of animal trypanosomiasis is high in these areas, but not as high as reported by other workers e.g. Geysen (1978) and Briault (1978) in the Eastern Province. This might have been because relatively small numbers were examined or due to some parasitaemias being too scanty to be detected by the diagnostic techniques used. Paris, Murray and Agure (1980) have pointed out that this is especially a problem in cattle where parasitaemias in naturally-occurring field cases are generally low and sporadic. Further a prophylactic regime with Samorin is maintained in most of the areas from which samples were taken. Smith (1959) and Lewis and Thomson (1974) have reported that in animals which have received a prophylactic drug the parasitaemia is low and intermittent.

Another interesting point from the results of the epidemiological survey is that no cases of trypanosomiasis were recorded in sheep and goats. Naturally acquired trypanosomiasis in goats in the Eastern Province was suspected by Kinghorn and Yorke (1912b) and Rickman (1974) diagnosed the disease in 13 out of 33 goats in Nyimba area of Petauke district. Griffin and Allonby (1979b) reported considerable economic losses resultant from the effects of trypanosomiasis in sheep and goats under range conditions. In another study, despite such losses, these workers (Griffin and Allonby, 1979c) concluded that because of the

possible trypanotolerance of the indigenous breeds, sheep and goats should be considered in small stock improvement programmes in trypanosomiasis-endemic areas of Kenya. In Zambia small ruminants (especially goats) are extensively reared in the Zambezi (Gwembe district) and the Luangwa valleys where cattle keeping is almost impossible. On the basis of the Kenyan experience of Griffin and Allonby (1979c), further detailed investigations on the epidemiology of trypanosomiasis and trypanotolerance in goats and sheep in these areas would be of value and could lead to improved productivity of these species.

If the incidence of animal tryapnosomiasis has been portrayed as moderate, the results in the present study have revealed a possible change in the epidemiological pattern which might have important practical implications. Other workers have reported an apparent increase in numbers of blood smears from cattle positive for T. brucei (MacLennan, 1975; Geysen, 1978; Briault, 1978). We have reported clinically apparent T. brucei infections in five cattle and of a situation in Mazabuka where the causative agents of bovine trypanosomiasis were predominantly T. brucei and T. vivax. This suggests a major shift in the epidemiological situation whose pattern was generally, in a descending order of importance, T. congolense, T. vivax and T. brucei. A situation like that observed in Mazabuka district is important because it presents logistical problems in the event of a severe outbreak such as that which occurred in the same district in 1977 (Dist.Vet.Office report, 1977). Trypanosoma brucei does not respond to treatment with 3.5 mg/kg Berenil which normally would be used as a sanative in mass treatment in an area distant from a known tsetse-belt. Infection with T. vivax could lead to a further spread of the disease by mechanical transmission in communal grazing areas (Shaw, 1960; Sheehy, 1956).

The results from studies on chemotherapy have been discussed at

length in the relevant chapters (4 and 5). It is, however, desirable to carry out more investigations in order to determine conclusively the source of relapsing T. congolense and T. vivax infections in cattle following chemotherapy as has been done in the case of T. brucei infections in mice (Jennings et al, 1979). This is necessary because the question of relapsing trypanosome infections, whatever their cause and/or source, has far reaching implications in field control of trypanosomiasis. More information on this phenomenon will greatly assist in the planning and execution of chemotherapeutic and chemoprophylactic regimes designed to give better results in the field. It was against this background that we carried out investigations in large animals. Unfortunately, the large sums of money required for the purchase and maintenance of such animals under laboratory conditions restricted the numbers of cattle that we could use in the experiments.

Whatever the deficiencies may be, the study presented in this thesis has shown that animal trypanosomiasis in Zambia is still a major problem and that its epidemiological pattern may be changing from what has been generally known. Therefore, there is need for more detailed trypanosomiasis surveys in other parts of the country. Preliminary results from the studies on drug sensitivity have suggested the presence of relapsing T. congolense and T. vivax post treatment with normally curative doses of Samorin and Berenil. This is the first time that apparent resistance to these drugs has been reported in the country. Further this appears to be the first time cross-resistance between Samorin and Berenil has been reported in naturally occurring T. congolense and T. vivax infections in cattle.

APPENDIX

CLINICALLY APPARENT *T. brucei* INFECTIONS IN CATTLE IN ZAMBIA

INTRODUCTION

Trypanosoma (Trypanozoon) brucei has for a long time been regarded at most as being mildly pathogenic to cattle. Thus Stephen (1970) concludes "cattle in all regions of tropical Africa exhibit a high degree of resistance to T. brucei".

In Zambia, however, clinical cases of T. brucei infection in cattle are not uncommon. The account presented below is a description of five clinical cases of T. brucei sub-group natural infections observed in indigenous cattle of Zambia. Four cases were diagnosed in Southern Province and one in the Eastern Province during the course of trypanosomiasis surveys from 1977-78.

The parasites were identified as T. brucei on morphological grounds by examination of stained blood films. In three cases, Swiss white mice were inoculated intraperitoneally with 0.5 ml fresh blood taken from the jugular vein of the sick cattle. However, in no case did a parasitaemia develop during the subsequent 40 days period of examination.

CLINICAL OBSERVATIONS

Case 1

Location

This case was observed in a heifer from Miyoba village, Kalomo district (Fig.5) in the Southern Province.

Clinical symptoms

The heifer was in a fair condition, alert and rather excited. The superficial lymph nodes were very enlarged. Their sizes were as follows:-

prescapular 8 x 5 cm; prefemoral 10 x 6 cm and sub-mandibular 5 x 3 cm. Visible mucous membranes appeared normal in colour. Despite the apparent fair physical condition of the heifer, jugular vein pulsation was visible when the animal was not restless. Anaemia was indicated by a low packed red cell volume (25%).

Diagnosis

Trypanosomiasis was diagnosed on wet film examination during which one trypanosome was seen in about every 20 fields. On examination of thick and thin blood films, the organisms seen were morphologically indistinguishable from T. brucei.

Three Swiss white mice each inoculated intraperitoneally with 0.5 ml of blood did not subsequently become infected.

History of the herd

The village owned about 200 head of cattle of which 50 were presented at the crushpen for inspection. Blood samples were taken from 24 out of 50 cattle.

Examination of stained blood films revealed three more positive slides all of which showed only T. congolense organisms. Therefore, four (16.6%) out of 24 cattle sampled were infected with trypanosomes - T. brucei (1) and T. congolense (3).

The entire village herd had been inoculated with Samorin at a dosage of 0.5 mg/kg ten weeks previously.

The sick cow was treated with 7.0 mg/kg Berenil. No further information about the fate of the animal was available thereafter.

Case 2

Location

During one of our survey visits to the Eastern Province, we inspected a herd of about 60 cattle at Chimwa crushpen very near to Katete Boma. Ten animals were examined, two of which showed infection with T. brucei by thick film but none had shown infection by wet film examination. After examining the herd, one of the herd boys told us that a sick animal had been left in the village (Chimuzi, Fig.7) because it was too weak to walk.

Clinical symptoms

We found the animal, which was an adult cow of about five years, lying flat in the muddy Kraal. The cow looked dejected with drooping ears and twitching muscles. It was in a very poor condition with a harsh hair coat. Despite the apparent stage of collapse, the cow stood up and charged at the owner as he approached her. The cow was very emaciated, the mucous membrane of the gums was very pale and the packed red cell volume was 13.5%. It had lymphadenitis and slight oedema of the sternum and forelimbs.

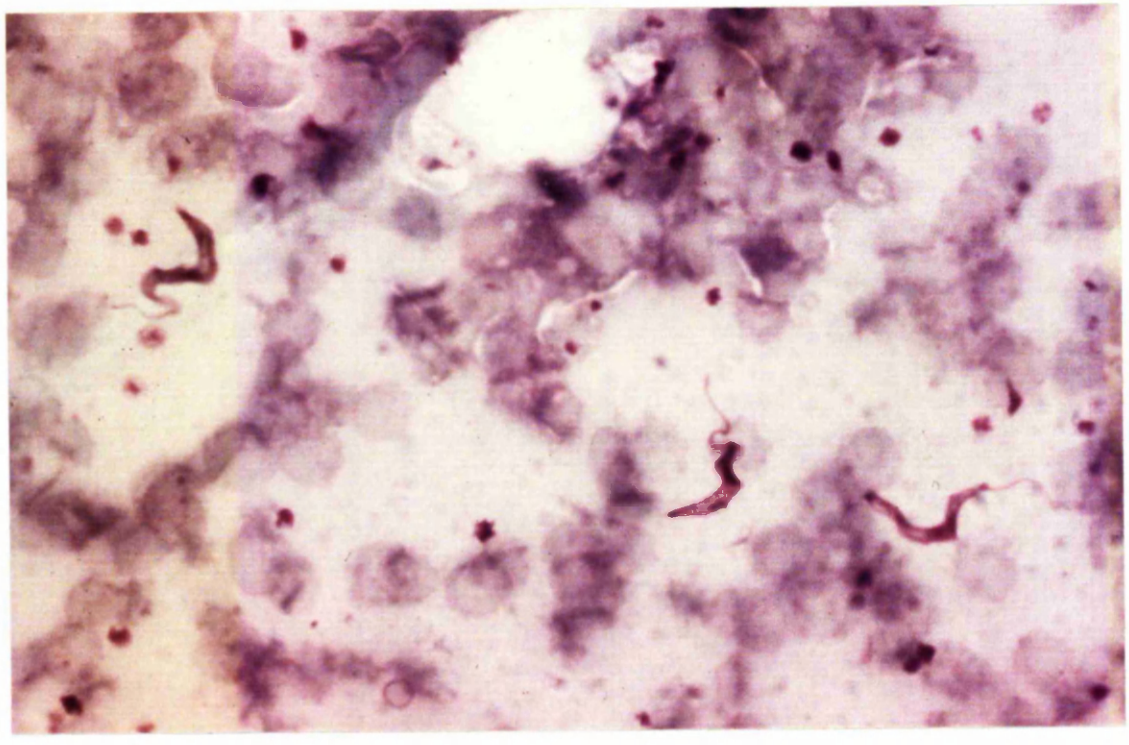
Diagnosis

7

Wet film examination showed a heavy infection with T. brucei. 0.05 ml of the blood was inoculated into each of three mice which, however, did not become subsequently infected. Later examination of stained films showed that the cow was heavily infected also with Babesia bigemina.

The cow was treated with 7.0 mg/kg Berenil, but did not respond to treatment and died three days later.

Figure 11 Photograph of thick blood smear from a cow at Samboko. The cow showed clinical signs of trypanosomiasis - see case three in text.



History of the herd

The Veterinary Assistant in charge of the area informed us that cases of trypanosomiasis were regularly diagnosed in cattle. Despite this, he said that to his knowledge tsetse flies were not seen in the area.

Case 3

Location

This case was seen at Samboko village (Fig.6) in Mazabuka district of the Southern Province.

Clinical symptoms

This animal, a cow of about six years, showed such classical clinical signs of chronic trypanosomiasis that it was picked for examination from the other animals as they were entering the crushpen. The animal was very emaciated, trailing slowly behind the main herd. It had enlarged superficial lymph node glands, visibile jugular vein pulsation, very rough hair coat and slight lacrimation. The cow walked with its head low and kept turning towards the left. Visible mucous membranes were pale.

Diagnosis

The cow was positive for trypanosomiasis on wet film examination. T. brucei organisms were confirmed on morphology by examination of stained thin blood films (Fig.11). Blood samples for thick and thin blood films as well as for mice inoculations were taken from a further 29 cattle. One of these slides showed T. brucei organisms. Mice inoculated with blood from all the 30 cattle did not subsequently become infected.

History of the herd

The village had a total herd of about 150 cattle. Cattle trypanosomiasis is known to occur in this area and a severe outbreak in and around the area was recorded in 1977. Cattle in the village had been treated with Samorin at a dosage of 0.5 mg/kg one year previously.

Cases 4 and 5

Location

Two cases with T. brucei infection were seen at Chaanga crushpen, Gwembe district (Fig.6). Both cases concerned two adult cows.

Clinical symptoms

Cow No.80

This cow was in a very poor condition - i.e. emaciated, dull and utterly weak. The cow could not cope with long distances (10-15 km) that the main herd covered in search of fresh grass and water. Consequently this cow grazed around the village in a small herd of calves. It had enlarged superficial lymph node glands and pale mucous membrane of the gum. The packed red cell volume was 18.0%. The cow was very old (about 9-10 years) and had lost most of its teeth. This might have contributed towards its pitiful physical condition.

Cow No.7

This animal of about 6-7 years old looked thin, rough coat, lacrimation of the right eye and had enlarged superficial lymph node glands. She had little appetite, most of the time stood staring with drooped ears and head while others vigorously grazed. Packed red cell volume was 25.0%.

Diagnosis

Trypanosoma brucei was diagnosed in both animals by examination of thick and thin stained blood films. No mice inoculations were done. Parasitaemia was heavy in cow No.80 and moderate (2-5 per microscope field) in cow No.7.

Treatment

Both animals were treated with 7.0 mg/kg Berenil. Cow No.80 was never seen despite an intensive search two days after treatment. It was presumed dead. Cow No.7 was clinically re-examined one week after treatment. No significant clinical changes were noticed except for absence of lacrimation. Thick blood smears were also made on this occasion, but no trypanosomes were seen in the smears.

History of the herd

The cattle of Chaanga area graze widely. Consequently these animals come into contact and share grazing with game animals from Lake Kariba which is a tsetse-infested area. In the dry season cattle most probably wander into tsetse-infested areas near the lake in search of grass and water. At the same time tsetse infestation around Chaanga cannot be ruled out since there is no evidence to the contrary.

Discussion and Conclusion

In recent years T. brucei infections in cattle in Zambia have tended to be on the increase (MacLennan, 1975; Geysen, 1978; and Briault, 1978) particularly in the Eastern Province. The encounter of the five clinical cases described above cannot, therefore, be treated as a coincidence. There is, however, very little published on the incidence of T. brucei infections in cattle. One such publication was

in Kenya by Robson and Ashker (1972) who reported up to 100 (2.7%) T. brucei subgroup infection out of 3,695 cattle they examined. Mwambu et al. (1973) recommended a change of drugs from Ethidium to Berenil and Samorin in the treatment of cattle trypanosomiasis in Alego location, Kenya in order to get rid of chronic T. brucei subgroup infections. Presumably there must have been a considerable number of chronically sick cattle.

More recently, in an experimental study of T. brucei infections in The Gambia, Murray et al. (1977) conclude "while it is generally believed that T. brucei is not a significant pathogen in cattle, the present results show that it produces anaemia in N'dama and Zebu and is capable of killing Zebu".

Although the trypanosomes were morphologically indistinguishable from T. brucei subgroup trypanosomes, in three out of the five cases described above blood failed to infect inoculated mice. This anomaly has been observed by other workers. Dillmann and Awan (1972) inoculated 225 Swiss white mice with separate blood samples from 75 hippopotami. Only four out of 225 mice became positive for T. brucei subgroup. These workers attributed this low infectivity of T. brucei to low parasitaemias and also to the chronicity of these infections in hippopotami. Rickman (1974) while investigating Rhodesian sleeping sickness in Luangwa valley infected 56 Swiss white mice with venous blood from four people who had positive thick blood films. Five out of 56 mice subsequently became parasitaemic. Later he infected mice with blood from a goat positive for T. brucei on thick film examination but this failed to infect the mice.

The reasons for this refractory tendency to some strains of T. brucei by the Swiss white mouse are not clear. It may be that some mutation has taken place in the breed rendering it more tolerant to

T. brucei organisms. Another possibility might be reduced infectivity or adaptation of chronic strains of the *T. brucei* subgroup in the susceptible hosts.

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