The Epidemiology of Trypanosomiasis in Village Livestock in an Endemic Sleeping Sickness Area of Western Kenya

by

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Abstract

A longitudinal epidemiological study of trypanosome infections in domestic livestock was carried out over thirteen months in Busia District, an endemic area of Rhodesian sleeping sickness. It was demonstrated that chemoprophylaxis of domestic livestock with Samorin ® (Rhône Poulenc, France) could virtually eliminate the reservoir of potentially human infective *T. brucei* spp. infections in domestic animals. In addition to being a potentially useful control measure during outbreaks of sleeping sickness, chemoprophylaxis showed a net economic benefit in the productivity of local cattle. The duration of chemoprophylaxis of domestic livestock with Ethidium ® (Laprovet, France) was much less, as was the economic benefit to livestock production, however the level of challenge was much less in this trial.

From the relative preference of tsetse flies for each species of domestic animal and the prevalence and incidence of trypanosome infections, it was concluded that cattle and to a lesser extent pigs were the most important species of domestic livestock in the animal reservoir of Rhodesian sleeping sickness in Busia District. Of the risk factors investigated for cattle acquiring trypanosome infections, the most important was an existing trypanosome infection. The effect on the epidemiology of sleeping sickness of a greater than expected interaction between species of trypanosome infecting cattle offers a possible mechanism for the natural control of the animal reservoir of Rhodesian sleeping sickness.

Various parasitological diagnostic techniques for the detection of trypanosome infections in cattle in the field were assessed. Maximum sensitivity of diagnosis and minimum bias in the relative importance of each species of trypanosome was achieved by selecting a suitable combination of diagnostic techniques.

The pathological effects of trypanosome infections in local East African breeds of domestic livestock under conditions of natural tsetse challenge and village husbandry practices were investigated. Although *T. brucei* spp. infections in cattle were shown to cause a mild anaemia, there were no apparent detrimental effects on liveweight gain or reproductive performance. While infections of *T. vivax* or *T. congolense* in cattle tended to cause anaemia and significant weight loss, some infected cattle were able to maintain both their PCV and liveweight status. By definition East

African zebu cattle exhibit a degree of trypanotolerance which increases their capacity to act as an animal reservoir of Rhodesian sleeping sickness.

East African zebu cattle naturally infected with *T. brucei* spp. were found to rarely develop CNS infections and never to develop clinical disturbance of the CNS associated with infection with *T. brucei* spp. infection within their natural lifespan. In contrast all pigs infected with *T. brucei* spp. rapidly developed a CNS infection, which proved fatal in most cases during the period of study. CNS infections of East African zebu cattle had no significant effect on their ability to act as animal reservoirs of Rhodesian sleeping sickness, whereas CNS infections in pigs acted to limit the duration of potentially human infective *T. brucei* spp. infections.

From RFLP analysis of trypanosome DNA it was possible to differentiate *T. b. rhodesiense* from *T. b. brucei* and using this technology it was found that the same stock of *T. b. rhodesiense* which was found in human patients during the last sleeping sickness epidemic in 1989 was found to be circulating in the cattle population in 1994. Since the same trypanosome stocks were present during both the epidemic phase of sleeping sickness in 1989 and the endemic phase in 1994, the likely origins of Rhodesian sleeping sickness epidemics lie in disturbances of the ecological balance of the disease.

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Declaration

I hereby declare that the work presented in this thesis is original and was conducted solely by the author except where the contribution of others has been acknowledged.

I also hereby certify that no part of this thesis has been submitted previously in any form to any university. It has in part been published in the following scientific publication:

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Field records, laboratory records and computer data files related to the work described in this thesis are deposited in the Department of Veterinary Physiology, the University of Glasgow.

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List of abbreviations

BC	Buffy coat technique
°C	Degrees Celsius
CATT	Card latex agglutination test
CFT	Compliment fixation test
CNS	Central nervous system
CSF	Cerebro spinal fluid
df	Degrees of freedom
EATRO	East African Trypanosomiasis Research Organisation
ECF	East coast fever
ELISA	Enzyme linked immunosorbtion assay
FAO	Food and Agricultural Organisation
ftd	Flies per trap per day
HAT	Human African trypanosomiasis
HCT	Haematocrit centrifugation technique
IFAT	Indirect fluorescent antibody test
ILRAD	International Laboratory for Research on Animal Disease
ILCA	International Livestock Centre for Africa
i.m.	Intramuscular
i.p.	Intraperitoneal
i.v.	Intravenous
KETRI	Kenya Trypanosomiasis Research Institute
kg	Kilogram
mf	Microfilaria
mg	Milligram
ml	Millilitre
mm	Millimetre
OAU	Organisation of African Unity
ODA	Overseas Development Administration
OIE	Office International des Epizootie
PCV	Packed cell volume

RH%	Relative Humidity
RFLP	Restriction fragment length polymorphism
s.d.	Standard deviation
s.e.	Standard error
STDM	Standard trypanosome detection methods
STRC	Scientific Technical and Research Commission
Tc	*T. congolense*
Tb	*T. brucei* spp.
Tt	*T. theileri*
Tv	*T. vivax*
WHO	World Health Organisation
µm	Micrometre

Chapter I

General introduction

1.1 Introduction

The trypanosomiases are a group of diseases which have medical and economic importance throughout large areas of Africa, Asia and South America. Trypanosomiasis in man (sleeping sickness and Chaga's disease) has been responsible for much ill health and social disturbance while trypanosomiasis in domestic livestock (nagana, surra, mal de caderas, murina, derrengadera and dourine) has directly contributed to widespread malnutrition, particularly in Africa by limiting the development of draught animal power in addition to reducing the amount of animal protein and by-products available to man (Kershaw, 1970). Since trypanosomiasis affects both man and domestic animals its impact on public health and the economy is greater than the estimated mortality by the World Health Organisation of 55,100 people in Sub-Saharan Africa from sleeping sickness in 1990 might suggest (Murray and Lopez, 1994). The major economic impact of animal trypanosomiasis is due to the threat of the disease preventing farmers from using productive grazing in the tsetse fly belts which has the effect of concentrating animal production in the more arid areas where tsetse challenge is limited but production levels remain low (Jordan, 1986).

1.2 The organisms

The causal organisms of trypanosomiasis are flagellate protozoa of the genus *Trypanosoma*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora (Honigberg and Balmouth, 1963).
Subphylum	Mastigophora (Diesing, 1866).
Class	Zoomastigophora (Calkins, 1909)
Order	Kinetoplastida (Honigberg, 1963 and Vickerman, 1963).
Suborder	*Trypanosoma*tina (Kent, 1880)
Family	*Trypanosoma*tidae (Doflein, 1901, emend Grobben, 1905)

(Levine *et al.*, 1980)

These unicellular organisms are characteristically leaf-like in shape and vary in size from 10 µm to over 120 µm depending on species. They possess a single flagellum as means of locomotion which emerges from the basal body and passes anteriorly to form an undulating membrane on the

outer margin of the pellicle. Some species have a free flagellum whereas in others the flagellum terminates at the anterior end. In close proximity to the basal body is a disc shaped organelle containing DNA, the kinetoplast. These important morphological features along with characteristics of motility can be used as an aid to the identification of the different species of Trypanosome (Table 1.1).

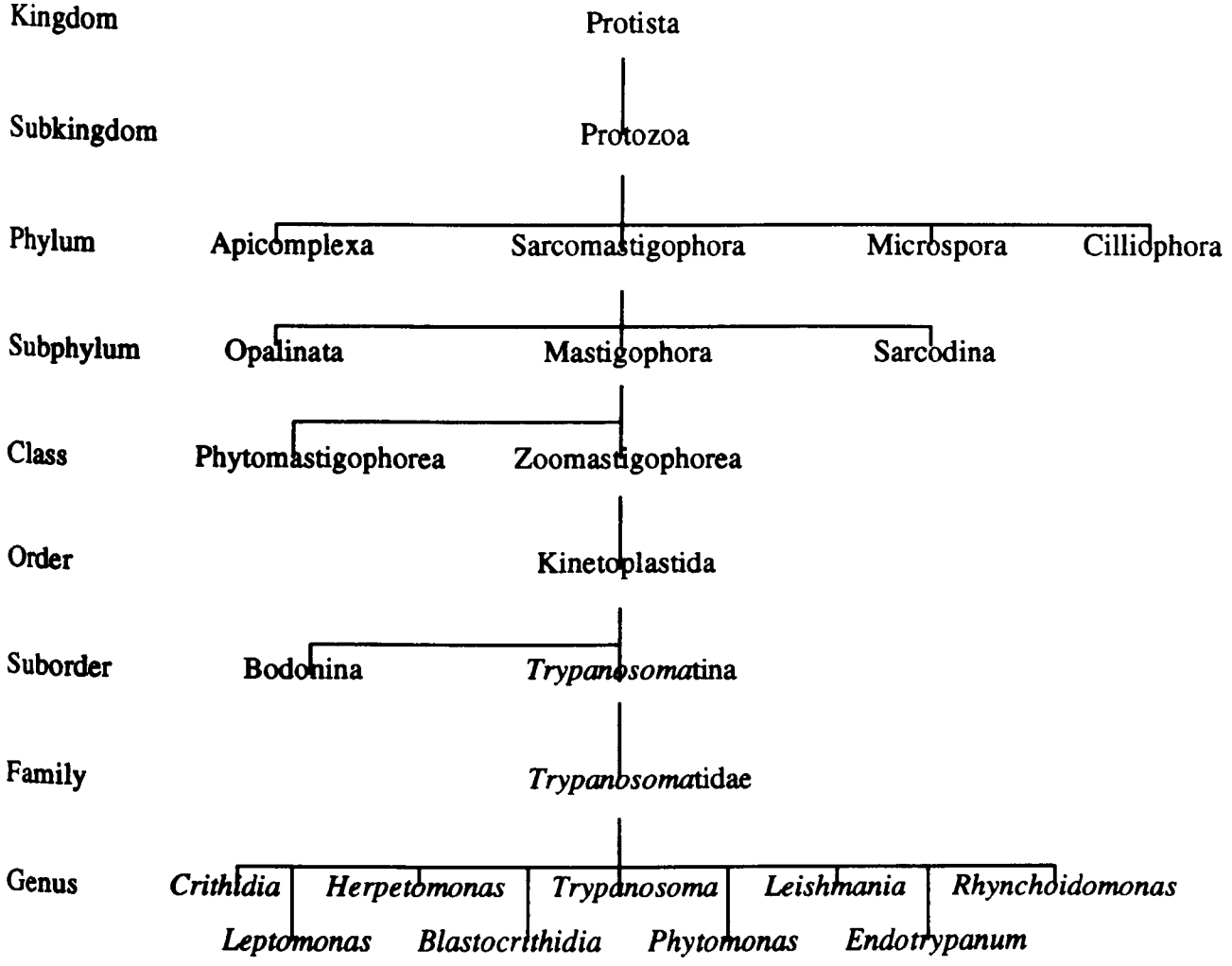
Despite being obligate parasites most species of trypanosome are non-pathogenic. The important pathogenic species of trypanosomes occur in vertebrate hosts principally in blood and tissue fluids although a few may be tissue invasive. Generally trypanosomes, both pathogenic and non-pathogenic, are transmitted by blood sucking arthropods with the exception of *T. equiperdum* which is spread by coitus, *T. evansi* which can be transmitted by vampire bats in South America and carnivores which can be infected by eating the carcass of an infected animal (Moloo *et al.*, 1973). Most trypanosomes undergo cyclical development in the invertebrate host as the life cycle alternates between vertebrate and invertebrate host. Characteristically the trypomastigote form predominates in the vertebrate host whereas it is the promastigote which predominates in the invertebrate. Hoare (1964) divided the genus *Trypanosoma* into two sections Stercoraria (Posterior Station, Group A) and Salivaria (Anterior Station, Group B) (Table 1.2). In the former group development stages within the arthropod vector take place in the hind gut and infection is by faecal contamination of wounds or skin whereas in the latter group the infective stages are to be found in the mouthparts or salivary glands of the vector so that infection takes place when the arthropod takes a blood meal.

Table 1.1 Morphological characteristics of trypanosomes

Species	Site of development in tsetse fly	Free flagellum	Kinetoplast	Undulating membrane	size in μm	Size and motility in wet film and DG
T. vivax	Proboscis	Present	Large, terminal	Not prominent	20-26	Large, extremely active, traverses the whole field very quickly, pausing occasionally.
T. brucei	Midgut salivary glands	Present in all but stumpy forms	Small, sub-terminal, central	Prominent	12-35*	Large, rapid movements in confined areas.
T. congolense	Midgut proboscis	Absent	Medium, sub-terminal, marginal	Not prominent	9-18	Small, sluggish active, adheres to red blood cells by anterior end

*polymorphic - slender, intermediate and stumpy forms (ILCA/ILRAD, 1983)

Table 1.2 Classification of Kinetoplastida



(Molyneux and Ashford, 1983)

1.3 Trypanosomiasis

Any trypanosome can be transmitted mechanically without cyclical changes taking place. Experimentally this can be done by 'syringe passage' and in nature it is accomplished by blood-sucking insects which feed on several different animals before repletion (Soulsby, 1982). There are a few species however where the ability to develop cyclically has been lost and mechanical transmission is the only means available; examples of such species include *T. evansi* and South American strains of *T. vivax*.

Isoenzyme analysis of the trypanozoon group (Gibson *et al.*, 1980) indicates that *T. brucei*,

Isoenzyme analysis of the trypanozoon group (Gibson *et al.*, 1980) indicates that *T. brucei*, *T. rhodesiense*, *T. gambiense* and *T. evansi* may be more correctly classified as subspecies of *T. brucei*, only differentiated by geographical distribution and host range (Godfrey *et al.*, 1987). Despite the similarities between trypanosomes of this group it has been demonstrated by analysis of DNA that these trypanosomes can be uniquely identified (Hide *et al.*, 1990).

1.3.1 Tsetse transmitted trypanosomiasis

Trypanosomiases in domestic livestock caused by the salivarian trypanosomes *T. vivax*, *T. uniforme*, *T. congolense*, *T. brucei brucei*, *T. simiae* and *T. suis* in tropical Africa and transmitted by tsetse flies are often grouped together under the collective term 'nagana'. Similarly human trypanosomiasis caused by the salivarian trypanosomes *T. brucei gambiense* and *T. brucei rhodesiense* are collectively known as 'sleeping sickness'. The distribution of these two groups of diseases (with the exception of mechanically transmitted *T. vivax*) is confined to the tsetse belt of Africa lying between latitudes 14°N and 20°S, extending to 29°S in the Mozambique area, over a total of 10 million square kilometres. These trypanosomes are all cyclically transmitted through tsetse flies (genus *Glossina*) and vary in their infectivity and pathogenicity to different mammalian hosts, summarised in Table 1.3.

Table 1.3 Tsetse transmitted African trypanosomes of medical and veterinary importance

Parasite	Mammalian host	Susceptible laboratory animals	Distribution
anosoma (Duttonella)	Bovines, sheep, goats, equines, dogs, antelopes	Rodents (adapted)	Tropical Africa
.) *uniforme*	Bovines, sheep, goats, equines, antelopes	None	East and Central Africa, Angola, Tropical Africa
anosoma omonas) congolense	Bovines, sheep, goats, equines, pigs, dogs, antelopes	Rodents etc.	Tropical Africa
.) *simiae*	Pigs, warthogs and possibly: bovines, equines, camels	Monkeys rabbits (adapted)	Tropical Africa
anosoma homonas) suis	Pigs, warthogs, bush-pigs	None	Tanzania, Burundi Tropical Africa
anosoma anozoon) brucei	All domestic animals, antelopes	Rodents etc.	Tropical Africa
.) *rhodesiense*	Man, bovines, antelopes	Rodents etc.	East Africa
.) *gambiense*	Man	Rodents etc.	Tropical Africa

(Mulligan, 1970)

1.4 The disease

1.4.1 The pathogenesis and pathology of tsetse transmitted animal trypanosomiasis

The animal trypanosomiases are regarded as a group of diseases equal in number to the species of pathogenic trypanosomes. The disease course and severity depends on the one or more species and strains of trypanosomes that cause the infection, the species of the host (Hornby, 1921) and its breed and prior experience of the disease (Murray *et al.*, 1982) and is summarised in Table 1.4.

Table 1.4 Susceptibility of animals to trypanosomes pathogenic for domestic animals

Trypanosome	Equine	Cattle	Sheep	Goat	Camel	Pig	Dog	Rat	Mice	Rabbit	Guinea pig	Monkey
T. brucei	+++	+	+	++	+++	+/-	++	+++	+++	++	+	+
T. congolense	++	+++	++	++	++	+/-	+/-	+/-	+/-	+	+	+
T. equiperdum	+++	+	+	+	?	+/-	+	+/-	+/-	+/-	+/-	?
T. evansi	++	++	+	+	+++	+/-	++	++	+	+	+	+
T. simiae	-	-	+	++	+++	+++	-	-	-	+/-*	-	+/-
T. suis	-	-	-	-	-	++	-	-	-	-	-	-
T. uniforme	++	++	++	++	?	-	-	-	-	-	-	-
T. vivax	+++/++	+++	++	++	+	-	-	-	-	-	-	-

*only splenectomised rabbits (Mulligan, 1970)

In reviewing the pathology of animal trypanosomiasis Losos and Ikede (1972) divided the pathogenic trypanosomes transmitted by tsetse into two groups. The haematic group that are confined to the blood and lymphatic vessels, namely *T. vivax* and *T. congolense* and the humoral group that invade intercellular space cavities in addition to being found in blood and lymphatic vessels including *T. brucei* spp. This classification is not absolutely valid as *T. vivax* is capable of development outside the circulatory system (Whitelaw *et al.*, 1988). However this general classification is useful for the purpose of this study as (i) it separates trypanosomes of the subgenus *Trypanozoon* which in addition to causing cachexia and anaemia also cause significant tissue damage and (ii) that this difference in the distribution of trypanosomes in the host's body leads to varying susceptibilities to the different trypanocidal drugs (Williamson, 1970).

1.4.1.1 The haematic group

Two species of trypanosome, *T. congolense* and *T. vivax* cause by far the greatest economic loss in cattle (Williamson, 1970). Infection with *T. vivax* produces a wide range of clinical syndromes ranging from a peracute haemorrhagic syndrome characterised by pyrexia, sustained parasitaemia, massive haemorrhages throughout the body, and death within 3 weeks of infection (Hudson, 1944) to a chronic low grade infection. *T. congolense* infections tend to exhibit a similar range of virulence,

ranging from an acute syndrome with pyrexia, petechial haemorrhages, severe anaemia and death within 2 - 4 weeks to the more usual chronic course. Neither infection with *T. vivax* nor *T. congolense* produces pathognomonic gross lesions at post mortem (Losos and Ikede, 1972). The more common course of infection with these two trypanosomes is a chronic condition characterised by progressive emaciation, intermittent periods of pyrexia coinciding with an intermittent and often scanty parasitaemia, and anaemia (Morrison *et al.*, 1981).

At least experimentally initial infection through the bite of a tsetse is followed by the development of a local skin reaction known as a chancre, which appears 7-11 days after infection and 4-12 days before the onset of a detectable parasitaemia. This lesion is a firm swelling 10-20mm in diameter which increases over the next 3-4 days to 15-30mm in diameter and becomes softer in consistency. The swelling often disappears by the 21st day but occasionally it forms a diffuse plaque before resolving (Roberts *et al.*, 1969). Initial parasite multiplication takes place in the dermal collagen and local lymphatic vessels 7-15 days after infection inducing an acute inflammatory response with an infiltration of mononuclear cells mainly consisting of monocytes, lymphocytes and plasma cells (Gray and Luckins, 1980).

Following development of a chancre there is a progressive enlargement of the local lymph nodes prior to the onset of pyrexia and parasitaemia 8 - 16 days later. There then follows a series of crises of fluctuating parasitaemias and fever approximately every 12 days, each lasting 2 - 3 days. If the animal dies during the acute phase, post mortem examination reveals an anaemic carcass with enlarged spleen and lymph nodes, swollen, congested liver, multiple petechial haemorrhages on mucous and serous surfaces and sometimes increased fluid in the pericardium, thorax and abdomen. With *T. congolense* infections there are often ischaemic lesions in the brain due to the affinity of this parasite for the endothelium of capillaries (focal polioencaphalomalacia) (Losos and Ikede, 1972; Banks, 1979). The acute phase lasts about one month, after which the animal may recover, die or more likely go on to develop a chronic infection. During the second phase of the disease, which may last several months, pyrexia and parasitaemias become lower and more sporadic and the anaemia which started during the acute phase becomes progressively more severe. Infected cattle continue to lose condition and the spleen and lymph nodes diminish in size.

Animals progressing to the third phase of the disease remain anaemic but become apparently aparasitaemic. During this phase a small proportion of animals may spontaneously recover, however the majority die 6 to 12 months after initial infection, while a few remain stunted, emaciated or infertile with a persistent low grade parasitaemia. During phases two and three of the chronic disease

acute relapses have been reported (Edwards *et al.*, 1956) which may be related to stress e.g. draught work, pregnancy, malnutrition, vaccination or other concurrent disease (Fiennes, 1970). Under field conditions it is likely that many of these 'acute relapses' may be due to re-infection. Death during the chronic disease is caused by congestive cardiac failure brought about by a combination of anaemia, circulatory disturbance associated within increased vascular permeability and myocardial damage (Murray, 1974). Infections in cattle do not invariably cause disease and infected animals which show no visible ill effects have been associated with low parasitaemias. Development of a chancre and a clear cut distinction between the three phases of the disease may not be apparent in cattle undergoing natural infection (Losos and Ikede, 1972).

The main and most consistent pathological features of animal trypanosomiasis are emaciation and anaemia. Although the precise mechanism causing emaciation is unknown, since the disease is not reported to cause anorexia (Goodwin, 1970), experimental infections of trypanosomes have been demonstrated to cause disruption of lipid metabolism (Wellde *et al.*, 1989b and Katunguka-Rwakishaya *et al.*, 1995) and carbohydrate metabolism (Wellde *et al.*, 1989b) in ruminants which may help explain emaciation occurring in the absence of anorexia. There is evidence both from the field (Agymang *et al.*, 1992) and experimentally (Katunguka-Rwakishaya *et al.*, 1995) that trypanosome infections impose a metabolic burden on ruminants and that the physiological production status and the level of nutrition are critical factors in an animal's ability to tolerate trypanosome infections.

The anaemia occurs in two distinct phases (Morrison *et al.*, 1981). During the first phase the onset and severity of anaemia are associated with the first wave of parasitaemia (Murray and Dexter, 1988). Initially anaemia is caused by extravascular haemolysis which occurs in an expanded mononuclear phagocytic system (Jennings *et al.*, 1974; Mamo and Holmes, 1975). Aetiology of the anaemia in trypanosomiasis is likely to be multifactorial involving trypanosome haemolysis, complement activation and immune complexes (Murray and Dexter, 1988). At this stage in the disease erythropoesis is usually elevated (Holmes, 1976) but not sufficiently to fully compensate for the anaemia (Dargie *et al* 1979; Katunguka-Rwakishaya *et al.*, 1992). The anaemia is usually normocytic and normochromic with the role of haemodilution caused by an expanding plasma volume being uncertain (Mamo and Holmes, 1975; Dargie *et al.*, 1979). Self cure or treatment with a suitable trypanocidal drug at this stage of infection generally leads to rapid resumption of normal haematological parameters. During the second phase of the disease which appears to be less dependent on the presence of the trypanosome, the high rate of destruction of red blood cells

continues in the apparent absence of the parasite (Murray and Dexter, 1988) and the bone marrow shows dyshaemopoiesis (Dargie *et al.*, 1979) caused by a defect in iron metabolism (Murray and Dexter, 1988). Treatment with trypanocidal drugs at this phase in the disease most often gives a very poor response and self cure is much less likely.

The immune response of animals to trypanosomiasis is complicated by the ability of the organisms to alter their antigenic characteristics (phenotype) by changing the variable surface glycoprotein (VSG) expressed on their coat (Williams *et al.*, 1979). The successive variant antigenic types (VAT) stimulate the formation of a series of variant-specific antibodies. The phenomenon of antigenic variation is responsible for the relapsing parasitaemias seen in the clinical disease (Gray, 1967). A large proportion of the immunoglobulins produced during the course of an infection are not specific for the VATs expressed at any one point in time, thereby imposing a burden on the host's immune system (Houba *et al.*, 1969). These antibodies may even contribute to the pathology of trypanosomiasis by causing the deposition of vasoactive immune complexes (Boreham, 1979). The leucopaenia (Losos *et al.*, 1974) and thrombocytopaenia (Davis *et al.*, 1974) which develop concurrently with the anaemia may also contribute to the development of the disease.

Different species of domestic livestock which show varying susceptibilities to the pathogenic effects of *T. congolense* and *T. vivax* are summarised in Table 1.4.

1.4.1.2 The humoral group

1.4.1.2.1 Experimental infections of cattle with *T. brucei* spp.

When Wellde *et al* (1989b) infected a number of cattle (*Bos taurus*) (39 cattle infected with 13 uninfected controls) with various strains of *Trypanosoma brucei rhodesiense* (by injection) they developed a CNS infection resulting in a fatal disease characterised by severe clinical disturbance of the CNS, comparable to sleeping sickness in man in half of the infected cattle (20 cattle), with a duration of between 85 and 1613 days. The remaining 19 cattle developed a mild or inapparent infection, not involving the CNS, which apparently resolved spontaneously. Three to five days after inoculation a low level parasitaemia developed which fluctuated over the following 3 to 5 months when it was no longer possible to detect parasites in the blood even by sub-inoculation of laboratory rodents. Parasites however remained detectable in the lymph nodes in many cases throughout the course of the disease. Usually a mild to moderate anaemia developed soon after the onset of patent parasitaemia which became more severe in animals with clinical CNS disturbances and usually

persisted. Initially a leucocytosis developed, concurrent with the time of detectable parasitaemia followed by a leucopaenia and thrombocytopaenia which persisted throughout the first month of infection. Later in the course of the disease a leucocytosis was evident again. Total serum protein levels in infected animals were raised due to a large increase in the level of gamma-globulins, particularly IgG and IgM. Infected animals also had a lower level of albumin and alpha-globulin which gave the combined effect of reducing the albumin/globulin and albumin/gamma-globulin ratios. Blood glucose levels were significantly lower in infected cattle as were the values of total serum lipid and cholesterol when compared to the control animals. However there was no apparent difference in serum triglyceride levels between either group.

Trypanosomes could always be detected in the cerebro-spinal fluid (CSF) in animals with acute clinical signs of CNS disturbance, whereas in many chronic cases they were only detectable when the animal was approaching the terminal stages of the disease. These experimental infections of cattle produced a markedly variable course of disease. The early acute cases, where trypanosomes could be found in the CSF soon after infection, resembled Rhodesian sleeping sickness in man, whereas the chronic CNS cases, where infection persisted for years and diagnosis was difficult, more closely resembled Gambian sleeping sickness (Losos and Ikede, 1972; Wellde *et al.*, 1989b).

The findings of Wellde *et al.* (1989b) were supported by Morrison *et al.* (1983), who demonstrated that in eleven cattle experimentally infected with three different stocks of *T. b. brucei* (the remainder being apparently resistant) all developed a low-grade fluctuating parasitaemia over a period of 2 - 5 months with a moderate anaemia (minimum PCV 25%) but unlike Wellde *et al* (1989b) these workers found that some stocks of the parasite, *T. b. brucei*, can produce a marked anaemia and occasional mortality during the acute phase of the disease. However *T. b. brucei* is essentially a connective tissue parasite causing most of it pathogenic effect by tissue invasion and the associated inflammatory reaction (Losos and Ikede, 1972). Four of the infected cattle died less than seven months after infection, all showing clinical and histopathological signs of CNS infection. The remaining seven animals were treated with diminazine aceturate and four of them also subsequently developed signs of CNS infection (Morrison *et al.*, 1983).

During the early stages of infection with *T. brucei* spp. the spleen and lymph glands are enlarged (up to three times larger) with oedematous skin plaques and a serous effusions into the peritoneum and pericardial cavities (Ikede and Losos, 1972). The most common organs affected by tissue invasion are the heart and brain (Morrison *et al.*, 1983, Wellde *et al.*, 1989b), although other organs may be involved such as the spinal cord, eye, testes, skin and skeletal muscle (Ikede and

Losos, 1972). Long standing cases are usually emaciated and sometimes exhibit a transient parasitaemia just prior to death. In cases where myocarditis develops the heart is generally enlarged due to ventricular hypertrophy with areas of haemorrhage and fibrous scarring. Histologically lesions are found throughout the myocardium with small numbers of trypanosomes and an extensive cellular infiltration of lymphocytes, plasma cells and macrophages being observed. In cases with severe cardiac lesions an enlarged congested liver was a consistent finding (Morrison *et al* 1983).

These studies by Wellde *et al.* (1989b) and Morrison *et al.* (1983) using predominantly European breeds of cattle experimentally infected with *T. brucei* spp. clearly demonstrated that a significant proportion of the animals developed a clinical disease syndrome and pathological lesions in the CNS which were similar to those described in humans infected with *T. b. rhodesiense* or *T. b. gambiense* (Apted, 1970 ; Losos and Ikede, 1972 and Poltera, 1985). The clinical signs of neurological disturbance reported in cattle were: depression, weakness, akinesia, ataxia, head pressing, salivation, hyperaesthesia, tremor, blindness, circling and in recumbent animals opistthotonus and paddling prior to death (Wellde *et al.*, 1989b and Morrison *et al.*, 1983). All cattle in both studies which developed clinical signs of CNS disturbance were found to have a diffuse meningoencephalitis with a marked cellular infiltration of the perivascular area, meninges and choroid plexus of mainly lymphocytes and plasma cells and in the most severe cases vacuolar change of the parenchyma and astrocyte proliferation could be found, indicating ischaemic degeneration of the CNS (Morrison *et al.*, 1983; Wellde *et al.*, 1989b).

1.4.1.2.2 Natural infections of cattle with *T. brucei* spp.

Natural infections with *T. brucei* spp. are common in cattle and prevalences of 20 - 30% have been reported in West Africa (Godfrey and Killick-Kendrick, 1961) and up to 53% in Kenya (Wellde *et al.*, 1989c). These infections are usually considered of low pathogenicity because they only produce a low parasitaemia, a very moderate anaemia and do not appear to cause appreciable disease (Hornby, 1921; Richardson, 1928; Killick -Kendrick, 1971). However during an outbreak of human trypanosomiasis in the Lambwe Valley, Kenya in 1980 the mortality of domestic cattle was reported to be 38%, over a period of 7 months (Wellde *et al.*, 1989c). Although no post mortem examinations were carried out, it was known that the most prevalent infection of cattle in this area was *T. brucei* spp. Due to the nature of the lesions, the protracted course of the disease and the relative difficulty of detecting the parasites in blood it is likely that infections and disease caused by *T. brucei* spp. are often not diagnosed (Losos and Ikede, 1972 and Willet, 1972). Infections are often mixed with *T.*

congolense and *T. vivax* which are present in much larger numbers in blood samples than *T. brucei* spp. which can often only be detected by rodent inoculation (Godfrey and Killick-Kendrick, 1971). There is evidence that natural infections under certain conditions are pathogenic for cattle causing anaemia, chronic wasting, lacrimation, corneal opacity, nasal discharge, signs of central nervous disturbance and sometimes death often over a period of years. The disease is initially associated with a fluctuating parasitaemia which is relatively frequent during the first 2 months, thereafter becoming scarce (Losos and Ikede, 1972). This disease syndrome is consistent with that found after experimental infection of *Bos taurus* cattle with the *T. brucei* group (Ikede and Losos, 1972).

Following the experimental results (Wellde *et al.*, 1989b), a second survey was carried out in the Lambwe Valley of the cattle known to have been infected with *T. brucei* spp. two years previously (Wellde *et al.*, 1989b). Subsequent observation of these cattle over a prolonged period (5 years) showed that native zebu cattle under natural conditions of challenge could develop cerebral trypanosomiasis with very similar clinical signs and pathology to the experimental infections with *T. b. rhodesiense* described by Wellde *et al.* (1989b). Although a limited number of cattle (14) were examined in this study, it indicated that the prevalence of CNS infections in native cattle (6) was of similar magnitude to that found in the experimental infections carried out by Wellde *et al.* (1989b), and that apparent self-cure also occurs in native cattle. However it is difficult to draw conclusions on the clinical severity of the disease because most of the cattle observed were either euthanased or treated with a sub-curative dose of trypanocide. As was the case in experimentally infected cattle, both *T. b. rhodesiense* and *T. b. brucei* were shown to produce clinical disease in native cattle. Many more native zebu cattle, which harboured *T. brucei* spp. in CSF, were negative for trypanosomes in both blood samples and lymph node aspirate, when compared to the predominantly European breed cattle used in the experimental infections. The natural disease was of markedly variable duration, similar to the findings in experimentally infected animals (Wellde *et al.*, 1989b and Wellde *et al.*, 1989c)

1.4.1.2.3 Experimental infections of sheep and goats with *T. brucei* spp.

Using a stock of *T. brucei* spp. isolated from a naturally infected goat in the Lambwe Valley, Kenya, Wellde *et al.* (1989d) infected a group of native goats (16) from a tsetse-free area. All of the goats in this study developed cerebral trypanosomiasis and if left untreated died 19 to 68 days after infection. While clinical signs of CNS disturbance were found in all infected, untreated goats, histopathological lesions in the CNS were absent or extremely mild in goats dying within 30 days of infection. Goats

surviving more than 30 days had histopathological lesions in the CNS similar to those described in cattle by Wellde *et al.* (1989d). Similarly central nervous system involvement in goats undergoing primary infections with *T. b. brucei* and relapse infections following treatment with diminazine aceturate have been reported (Whitelaw *et al.*, 1985).

Experimental infections in sheep with *T. b. brucei* (Bouteille *et al.*, 1988) resulted in anaemia, emaciation, clinical signs of CNS disturbance and histopathological lesions in the CNS similar to that found in human trypanosomiasis. Similar findings were reported in sheep experimentally infected with *T. b. rhodesiense* (Angus *et al.*, unpublished).

1.4.1.2.4 Natural infections of sheep and goats with *T. brucei* spp.

The prevalence of natural infection and disease attributed to *T. brucei* spp. in goats and sheep is usually considered to be low (Losos and Ikede, 1972; Okuna and Mayende, 1981), giving rise to a chronic disease with moderate parasitaemia which often resolves in self cure (Fiennes, 1970). However Corson (1934) reported that sheep and antelopes experimentally infected with *T. b. rhodesiense* developed abnormal CNS signs, including inco-ordination, circling and convulsions. In another study experimental infection of goats with *T. brucei* spp. showed characteristic mononuclear cell infiltrations in brain and heart tissues after infections ranging from 45 to 201 days in duration (Büngener and Mehlitz, 1976).

The prevalence of *T. brucei* spp. in sheep and goats during a time of decline in prevalence in human trypanosomiasis in the Lambwe Valley, Kenya was reported to be 1.2% for sheep and 3.9% for goats (Robson and Ashkar, 1972). In the same area 12 years later, during an outbreak of sleeping sickness, Wellde *et al* (1989c) reported 55% mortality in sheep and 47% in goats, however the cause of these deaths was never investigated.

It appears from these limited studies that most attempts to infect sheep or goats with *T. brucei* spp. produce pathogenic effects, although under normal circumstances sheep and goats are not preferred hosts of the tsetse fly (Weitz, 1963). The normally low prevalence of *T. brucei* spp. infections in sheep and goats may be more associated with the distaste of the tsetse for these two species rather an innate resistance to infection.

1.4.1.2.5 Experimental infections of pigs with *T. brucei* spp.

Experimental infection of large white pigs with a West African strain of *T. brucei brucei* resulted in an acute fulminating disease syndrome characterised by a high parasitaemia, severe anaemia, which

later developed into severe clinical disturbance of the CNS. At post mortem anaemia and emaciation were the most evident signs with a meningoencephalitis being observed on histopathological examination (Otesile *et al.*, 1991). While experimental infection of mini-pigs with a West African strain of *T. b. brucei* produced similar lesions of myocarditis and meningo-encephalitis infection with *T. b. gambiense* resulted in no clinical or histopathological abnormalities being detected (Büngener and Mehlitz, 1984).

Attempts experimentally to infect wild pigs by Ashcroft (1959) gave a very low success rate leading only to a very brief parasitaemia.

1.4.1.2.6 Natural infections of pigs with *T. brucei* spp.

Natural infections of *T. b. brucei* in pigs are usually considered of low pathogenicity causing a mild, chronic disease (Stephen, 1970). However an acutely fatal outbreak of disease in pigs caused by *T. brucei brucei* has been described in Nigeria by Agu and Bajeh (1986). The disease was characterised by anorexia, pyrexia, emaciation and severe anaemia. Another survey of trypanosome infections in pigs in Nigeria found the prevalence of *T. brucei* spp. infections to be 24% and while one third of exotic pigs (107) which were infected were clinically ill with pyrexia and anaemia none of the local long snouted West African breed of pig (43) were found to be ill (Onah, 1991).

A parasitological survey of pigs in Upper Volta and Ivory Coast revealed an incidence of *Trypanozoon* infection in domestic pigs of 55% (Mehlitz *et al.*, 1983), the majority of isolates being human serum sensitive *T. b. brucei* and the minority of human serum sensitive *T. b. gambiense* (Mehlitz *et al.*, 1984). This evidence suggests that while certain strains of *T. brucei* spp. can produce an acutely fatal disease in pigs the majority of infections are probably mild, chronic or inapparent.

1.4.1.2.7 Experimental infections of dogs and cats with *T. brucei* spp.

Dogs and cats are highly susceptible to infection with *T. brucei* spp. and experimental inoculation of *T. brucei* spp. consistently produces an acutely fatal disease similar to that seen following natural infection (Losos and Ikede, 1972). Trypanosomes could be detected in experimental infections 5 days after infection with the parasitaemia rising to high levels on days 6 and 7. This was followed by a drop in parasitaemia to undetectable levels on day 9 and 10 and a second rise to equal the previous level 9 to 12 days later where it remained until death. Associated with the parasitaemia a severe anaemia progressively developed until the PCV reaches approximately 50% of the pre-infection level (Morrison *et al.*, 1981). The major pathogenic effects of the infection resulted from invasion of

tissues by trypanosomes and involved a wide range of organs of which the heart and CNS were always severely affected (Morrison *et al.*, 1983). Pyrexia developed in close parallel to the parasitaemia with general enlargement of all the lymph glands (up to 5 times normal size) and spleen. Ocular lesions could be seen about 8 days post-infection which developed into keratitis with corneal opacity. Clinical signs of congestive cardiac failure and neurological changes became evident after the first week and subcutaneous oedema and inflammation of the testes and skeletal muscle were commonly found after the second week.. Death within 3 - 4 weeks was the usual outcome of canine infection with *T. brucei* spp. (Morrison *et al.*, 1981).

The most notable lesions at post mortem examination was oedema, congested liver and other fluid associated with congestive cardiac failure including wasting of cardiac muscle, lesions in the eye and brain, a general muscle wasting in addition to enlarged lymph glands and spleen; (Losos and Ikede, 1972). Grossly the heart was dilated and globular with a mottled pale haemorrhagic appearance. Histologically there was a severe diffuse myocarditis with many extravascular trypanosomes and a cellular infiltrate initially mainly composed of lymphocytes and plasma cells but latterly macrophages and neutrophils were present. These changes were accompanied by degeneration and necrosis of myocardial fibres and interstitial oedema. In areas of extensive myocardial damage, this tissue damage was thought to be initiated by an excessive immunological response by the host to this highly antigenic parasite (Ndung'u, 1990). Within the eye there was oedema, haemorrhage and fibrinous exudate with a cellular infiltrate associated with extravascular trypanosomes. Lesions in the brain were most severe in the choroid plexus where there were numerous trypanosomes and a cellular infiltrate similar to that found in the heart. Small numbers of trypanosomes and inflammatory cells could be found in the meninges and CSF with only occasional foci of perivascular infiltration being seen (Morrison *et al.*, 1981, Morrison *et al.*, 1983).

Comparing the disease syndrome caused by *T. brucei* spp. in dogs with that in cattle it appears that dogs undergo an acute uncontrolled infection with the rapid development of a high parasitaemia with many extravascular trypanosomes whereas in cattle the disease develops much more slowly, and although the parasitaemia is readily controlled by the host there is a persistence of parasites within the tissues (Morrison *et al.*, 1983). Dogs infected with *T. brucei* spp. showed significantly raised levels of total lipids, triglycerides and cholesterol, unlike *T. brucei* spp. infections in cattle which showed a lowering of total lipids and cholesterol with triglyceride levels remaining the same. Intense deposition of lipid in the myocardium, leading to myocardial damage is a feature of canine infections (Ndung'u, 1990), but not in cattle (Morrison *et al.*, 1983 and Wellde *et al.*, 1989b).

These pathological differences may help to explain why an acute clinical syndrome, mainly involving the heart, was not seen in cattle.

1.4.1.2.8 Natural infections of dogs and cats with *T. brucei* spp.

Losos and Ikede (1972) described the clinical course and pathology of *T. b. brucei* in the dog being likened to sleeping sickness in man. In general infection with *T. brucei brucei* and *T. brucei rhodesiense* produced a more acute disease while disease caused by *T. brucei gambiense* was more chronic. Supporting this view, a survey during a sleeping sickness epidemic in Busoga, Uganda found that 15.4% of dogs (75% of parasitaemic dogs) were infected with *T. brucei* spp. (Okuna and Mayende, 1981). This implies that a more chronic disease syndrome may be more common than the literature on experimental infection suggests because of the relatively high prevalence of infection and dogs from an endemic tsetse area may have some innate resistance to the disease. However experimental infection of 3 local breed dogs in South East Uganda with a locally derived serodeme of *T. brucei* spp. produced an acute, fatal disease in all 3 cases (Mwambu, 1977). Although this experiment is only based on 3 dogs, it suggests that any immunity in local dogs may be an acquired immunity, initiated by exposure to the disease early in life, and is only confined to a very localised serodeme of *T. brucei* spp.

1.4.1.2.9 Infection of other domestic animals with *T. brucei* spp.

Natural infections with *T. b. brucei* are known to be highly pathogenic for horses, donkeys, dogs and camels but less so for cattle, goats, sheep and pigs (Fiennes, 1970). In horses and donkeys the disease may follow an acute, sub-acute or chronic course which is almost invariably fatal. Pathological signs are typical of *T. brucei* spp. infections in other species but with the addition of icterus and a marked subcutaneous oedema (Losos and Ikede, 1972).

The clinical manifestations of *T. brucei* infections in camels are the same as those seen in cases of acute surra (*T. evansi*) (Fiennes, 1970) although it is likely that a chronic form of the disease exists.

1.4.1.2.10 Infection of wild animals with *T. brucei* spp.

Many wild animals are known to be hosts to *T. brucei* subgroup infections, in particular; lions, hyenas, warthog, waterbuck and hartebeests, although they rarely show signs of clinical disease (Geigy *et al.*, 1971). *T. brucei* spp. has been shown to invade the tissues of some of these species

and cause pathological lesions, however the mild nature of these histopathological lesions suggests that these infections were probably asymptomatic (Losos and Gwamba, 1973). Experimental infection of Thomsons's gazelle (*Gazella thomsoni*), steinbok (*Rhaphicerus campestris)* and dikdik (*Rhynchotragus kiriku*) with *T. brucei brucei* or *T. brucei rhodesiense* proved fatal while other species of wild game exhibited a transient parasitaemia of varying duration without apparent ill effect (Ashcroft, 1959).

1.4.1.2.11 Mixed infections of more than one species of trypanosome

Mixed infections of more than one species of trypanosome (*T. brucei* spp., *T. congolense* or *T. vivax*), which occur in all combinations, are reported to be very common in cattle (Losos and Ikede, 1972, Willet, 1972). The pathology caused by mixed infections including *T. brucei* spp. may easily go undetected because of the low *T. brucei* spp. parasitaemia making its diagnosis difficult by examination of blood alone for trypanosomes (Godfrey and Killick-Kendrick, 1961; Losos and Gwamba, 1973; Geigy *et al.*, 1973, Wellde *et al.*, 1989b; Losos and Ikede, 1972; Ikede and Losos, 1972).

Concurrent infections of *T. congolense* and *T. b. brucei* spp. in experimentally infected cattle have been reported to exhibit a greater virulence by causing a higher incidence of cerebral trypanosomiasis and allowing *T. congolense* to cross the blood brain barrier (Masake *et al.*, 1984). However considering the ease of which CSF samples become contaminated by blood, this finding requires confirmation with the parasitaemia being cleared by treatment with a trypanocide prior to sampling the CSF.

A field survey of bovine trypanosomiasis carried out in the Lambwe Valley, Kenya during 1967-68 showed that infections with *T. brucei* spp. were more frequently diagnosed in multiple infections than would be expected by chance (Willet, 1972). It was proposed that during an infection with only *T. brucei* spp. the parasitaemias were often so low as to escape detection, but when another trypanosome infection was present the resulting immunosuppression allows the *T. brucei* spp. parasitaemias to rise to detectable levels (Robson and Ashkar, 1972).

1.4.2 The pathogenesis of human African trypanosomiasis (HAT)

The two forms of human trypanosomiasis, Gambian sleeping sickness and Rhodesian sleeping sickness, are caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*

respectively. They are differentiated by their host range and geographical distribution in addition to the differing disease syndromes they cause in man (see section on diagnosis). Classically *T. b. rhodesiense* is considered to cause a more acute form of the disease in which a patient may rapidly succumb within a few weeks or months if untreated while *T. b. gambiense* tends to cause a more chronic syndrome in which a patient may spend many months or years in a progressively emaciating stupor, again most commonly culminating in death if not treated. Typical forms of the disease are by no means universal with acute forms of *T. b. gambiense* (Scott, 1970) and chronic forms of *T. b. rhodesiense* (Apted, 1970) being reported within West and East Africa respectively.

The first sign of infection is often the development of a swelling or chancre at the site of the tsetse bite within 7 - 10 days. Typically it develops into a hard, red, painful raised swelling about 3 cm in diameter surrounded by a 'waxen zone' of minute vesicles 3 - 4 cm in width which in turn is surrounded by a further swollen red area. Within this oedematous lesion initial multiplication of trypanosomes and a mononuclear cell infiltration with tissue damage takes place. After reaching its full development the chancre subsides over the following two weeks leaving an area of desquamated skin. Appearance of the chancre can be variable, being more commonly reported in Europeans, and almost never seen in Africans in an endemic sleeping sickness area (Apted, 1970).

Enlargement of local lymph glands draining the site of an infected bite then develops especially if there has been a chancre. Within the enlarged glands an increased number of macrophages can be found along with the development of germinal centres of the lymphocyte-plasma cell series. One to three weeks (typically about 10 days) following the infective bite an attack of fever is experienced in response to invasion of the trypanosome into the bloodstream which may occur directly by the local dermal venules or via the thoracic duct (Asenyonga and Adam, 1975). There then follow repeated waves of parasitaemia and pyrexia associated with the serial production of antigenic variants (VAT) within the trypanosome population (Barry and Emberg, 1984). Anaemia is a common finding in human trypanosomiasis although it is much less pronounced than in some animal infections (notably *T. congolense* and *T. vivax* infections in cattle and *T. brucei* infections in dogs). The anaemia which is probably caused by extravascular haemolysis appears to be multi-factorial in origin involving immune complexes, haemolysin and complement (Poltera, 1985).

Initially a leucocytosis occurs with a relative lymphocytosis, the most evident proliferation of cells being found in the B-lymphoid system, followed in the later stages of the disease by a leucopaenia (Poltera, 1985). In addition to generalised immunosuppression caused by leucopaenia, experimentally a parasite-specific immunosuppression has been described which initially is transient but becomes complete in the chronic disease (Dempsey and Mansfield, 1983).

A thrombocytopaenia develops which is more severe in *T. b. rhodesiense* than in *T. b. gambiense* infections when platelets show a reduced life span and splenic pooling (Robins-Brown *et al.*, 1975).

As serum IgM levels increase this leads to an increase in circulating immune complexes, which themselves lead to the release of pharmacologically active substances by the kallikrein-kinin system. These substances in turn cause an increase in vascular permeability, therefore contributing to oedema, inflammation and hypotension (Boreham, 1979).

The trypanosomes circulating in the bloodstream if not lysed or phagocytosed reach the interstitium as a result of increased permeability of blood vessels (Goodwin and Hook, 1968). The presence of trypanosomes in the interstitial space of various organs causes cellular damage by their physical presence or by the production of cytotoxins (Murray, 1974). Parasitic invasion has been reported sporadically in many tissues, including skeletal muscle, serous membranes, adrenal glands, thyroid, gut, joints and gonads. More commonly parasites are found in the liver, spleen, heart and central nervous system (Poltera, 1985), the latter two sites of invasion accounting for most of the clinical signs seen. Poltera *et al* (1976) described the histological lesions of a pancarditis involving all heart components including the valves and conducting fibres. These pathological findings were associated with a clinical presentation of cardiac insufficiency with electro-cardiograph disturbances which are commonly found in human trypanosomiasis.

A generalised lymphadenopathy is commonly found during the acute phase of sleeping sickness and particularly with *T. b. gambiense* infections enlarged cervical glands are easily palpable (Winterbottom's sign). Laterally as the disease becomes chronic lymph glands become progressively smaller and more firm in consistency.

Splenic enlargement associated with an increased mononuclear phagocytic system occurs (Woodruff *et al.*, 1973). The spleen is reported to enlarge in proportion to the anaemia (Ormerod, 1970) but is of little diagnostic value as it is a common sign in other tropical

diseases, especially malaria. As the disease progresses to the chronic phase the spleen becomes fibrosed and reduced in size.

Commonly the liver is palpably enlarged in the early stages of the disease with some biochemical evidence of malfunction (Jenkins and Robertson, 1959), however jaundice is rare.

Oedema involving the eyelids and face is sometimes found during the initial stages of infection, possibly due to increased vascular permeability, and a peripheral oedema, especially of the legs, associated with anaemia can be seen latterly.

The second stage of the disease is usually considered to begin when trypanosomes can be detected in the cerebro-spinal fluid which occurs after about three to four weeks with *T. b. rhodesiense* infections and after some months with *T. b. gambiense* infections. Clinical evidence suggests that the brain is invaded at a much earlier stage than this, perhaps as early as eight to ten days post infection (Ormerod, 1970). The principal pathological features in the CNS are a diffuse meningoencephalitis mainly affecting the base of the brain with adhesions of the meninges in later cases these lesions are not suppurative in cases where there is no secondary infection. Within the CNS there is also a vasculitis with a marked perivascular cuffing of inflammatory cells including lymphocytes, macrophages and plasma cells. Invasion of the spinal cord has occasionally been reported but involvement of the eye is a much more common finding causing keratitis, iridocyclitis and retinitis (Apted, 1970).

Death is caused by meningeoencephalitis with subsequent cardiac failure or less commonly, in acute cases of Rhodesian sleeping sickness, by acute cardiac failure due to extensive myocardial damage (Poltera, 1985).

1.5 The diagnosis of trypanosome infections in animals

When considering the detection of trypanosome infections in domestic animals attention should first be given to history (e.g. presence or contact with tsetse flies) and clinical signs (e.g. ailing, unthrifty animal) although the clinical syndrome alone is not pathonomonic for trypanosomiasis.

The certain diagnosis of current infection with trypanosomiasis in livestock is dependent on demonstrating the parasite in blood or other tissue fluids. The severity of the disease is not necessarily related to the number of parasites seen and particularly in chronic relapsing infections found in the field, parasitaemias are often low and sporadic (Killick-

Kendrick, 1968; Paris *et al.*, 1982). There are a number of serological tests available for the detection of trypanosomes or their immunological effects. However with the possible exception of the newly developed ELISA tests they are more applicable to experimental and survey work rather than routine field diagnosis. Recently it has been possible to identify trypanosome subspecies and strains by isoenzyme or DNA analysis which is of benefit in the study of the epidemiology of trypanosomiasis.

1.5.1 Parasitological diagnostic techniques

1.5.1.2 Standard trypanosome detection techniques (STDM)

Wilson (1969) defined the standard trypanosomiasis detection methods (STDM) namely: wet, thin and thick blood films and mouse inoculation. Wet films have the advantage of being simple and quick and may give an indication of species by demonstrating the parasite's characteristics of motility particularly in early infections with *T. vivax* or *T. simiae* (Table 1.1). However low grade infections may be missed and precise species identification is often not possible. Thin fixed stained films are best used for species identification because the morphology of trypanosomes is well preserved and it is possible to examine slides using oil immersion lenses with a magnification of up to 100x. However searching for trypanosomes in a low-grade parasitaemia using thin fixed stained slides is very inefficient on account of the small volume of blood used to make thin films. Because of the greater quantity of blood examined on a thick stained blood film it is a more efficient method of detecting infections than either wet or thin blood films. However some trypanosomes may be deformed making species identification less precise than thin films (Killick-Kendrick, 1968). Mouse inoculation is a useful technique where trypanosomes are so scarce in the peripheral blood or other body fluids that exhaustive searches of stained smears fail to reveal a single parasite. It is possible to infect a susceptible host by the injection of a single viable trypanosome. The technique of animal inoculation has two main problems; firstly the varying susceptibility of different species of animal to common trypanosomes (Table 1.4), e.g. inoculation of blood into rodents is a good method for detecting infections of *T. brucei* spp., certain strains of *T. congolense* but poor for *T. vivax; T. simiae; T. suis* and *T. uniforme* (Mulligan, 1970) and secondly the logistical problem of handling large numbers of laboratory animals and blood samples making the widespread use of this technique difficult in the field (Knowles, 1927).

1.5.1.2 Concentration techniques

There are a number of techniques which concentrate the trypanosome fraction of a blood sample in order to increase the sensitivity of the test, namely the haematocrit centrifugation technique (HCT) (Woo, 1970); the capillary concentration technique (CCT) (Walker, 1972); the miniature anion-exchange/centrifugation technique (m-AEC) (Lumsden *et al.*, 1979) and the dark ground phase contrast buffy coat technique (DG) (Murray *et al.*, 1977). Species identification is most easily achieved using the DG technique but is possible with experience using the other three techniques (Paris *et al.*, 1982). Another advantage of HCT, CCT and DG techniques is that it is possible to read simultaneously the PCV as a measure of anaemia. A summary of the comparative sensitivities of the STDM and concentration techniques is given in Tables 1.5 and 1.6.

Another concentration technique is the hypotonic lysis method (Leeflang *et al.*, 1974) which is as sensitive as HCT but less easy to perform.

Table 1.5 Sensitivity of different parasitological techniques in detecting *T. congolense, T. vivax* and *T. brucei* spp.

trypanosomes per ml	DG*			HCT			Wet film			Thick film			Thin film			Mouse
	Tc	*Tv*	*Tb*	*Tc*	*Tv*	*Tb*	*Tc*	*Tv*	*Tb*	*Tc*	*Tv*	*Tb*	*Tc*	*Tv*	*Tb*	*Tb*
5.0×10^4	4+	3+	4+	+	+	+	+	+	+	+	+	+	+	+	+	ND
2.5×10^4	3+	2+	3+	+	+	+	+	+	+	+	+	+	+	-	+	ND
1.25×10^4	2+	2+	2+	+	+	+	+	-	+	+	+	+	-	-	-	ND
8.3×10^3	2+	2+	2+	+	+	+	-	+	+	+	+	+	-	-	+	ND
6.25×10^3	2+	2+	1+	+	+	+	-	-	-	-	+	+	-	-	-	+
5.0×10^3	2+	1+	1+	-	+	+	-	-	-	-	-	+	-	-	-	+
2.5×10^3	2+	1+	-	-	+	+	-	-	-	-	-	-	-	-	-	+
1.25×10^3	1+	1+	-	-	+	+	-	-	-	-	-	-	-	-	-	+
8.3×10^2	1+	1+	-	-	-	+	-	-	-	-	-	-	-	-	-	+
6.25×10^2	1+	1+	-	-	-	+	-	-	-	-	-	-	-	-	-	+
5.0×10^2	1+	1+	-	-	-	+	-	-	-	-	-	-	-	-	-	+
2.5×10^2	1+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
1.25×10^2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8.3 x 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Scoring system only applied to DG

ND = Not Done
DG = Darkground/phase contrast buffy coat technique
HCT = Haematocrit centrifugation technique (Woo)
Tc = *T. congolense*
Tv = *T. vivax*
Tb = *T. brucei spp.*

(Paris *et al.*, 1982)

Table 1.6 Sensitivity of different concentration techniques in detecting *T. congolense* and *T. vivax* in bovine blood

Trypanosomes per ml	DG*		HCT		CCT		m-AEC	
	Tc	*Tv*	*Tc*	*Tv*	*Tc*	*Tv*	*Tc*	*Tv*
5.0×10^3	2+	2+	+	+	+	+	+	+
2.5×10^3	2+	2+	+	+	+	+	+	+
1.25×10^3	2+	2+	-	+	+	+	-	-
6.0×10^2	1+	1+	-	-	+	+	-	-
3.0×10^2	1+	1+	-	-	-	-	-	-
1.5×10^2	1+	1+	-	-	-	-	-	-
7.5 x 10	-	-	-	-	-	-	-	-

* Scoring cystem only applied to DG

DG = Darkground/phase contrast buffy coat technique
HCT = Haematocrit centrifugation technique (Woo)
CTT = Capillary concentration technique
m-AEC = Miniature anion-exchange/centrifugation technique
Tc = *T. congolense*
Tv = T. vivax

(Paris *et al.*, 1982)

1.5.1.3 Examination of other body fluids

Lymph gland samples may be more sensitive for detecting trypanosomes in cattle infected with *T. brucei* spp. (Wellde *et al.*, 1989b), or early infections of cattle with *T. congolense* (Hornby, 1921) or *T. vivax* (Adams, 1936), however along with examination of cerebro-spinal fluid (CSF) in *T. brucei* spp. infections, practical problems of sample collection may outweigh their benefits (Mulligan, 1970).

1.5.1.4 *In vitro* cultivation techniques

Although successful *in vitro* culture techniques for salivarian trypanosomes have been developed (Hirumi *et al.*, 1984) field isolates from vertebrates do not readily grow under laboratory conditions. The sophisticated technical input required for these techniques makes them impractical for the routine diagnosis of animal trypanosomiasis, particularly under field conditions encountered in Africa.

1.5.2 Non-specific diagnostic tests

Mercuric Chloride Test and the formal gel test have been used for the diagnosis of *T. evansi* infection in camels. These tests are largely empirical, relying on the detection of an overall increase in immunoglobulin levels, particularly IgM, and are therefore not specific but may be of use as a rapid screening method of diagnosis (Killick-Kendrick, 1968).

1.5.3 Serological diagnostic techniques

Several serological tests have been developed for the diagnosis of animal trypanosomiasis to overcome the problem of insufficient sensitivity of the parasitological techniques, particularly when investigating chronic infections where parasitaemias may be low and intermittent. With the exception of the recently developed antigen-detection ELISA all other serological tests rely on the detection of antibodies to trypanosomes in the serum of an animal. These tests may give cross reactions to other protozoal diseases or in cases of mixed trypanosome infections produce mixed or varied responses accounting for the lack of test specificity found when using many serological tests (Luckins, 1977). The demonstration of antibodies in a single animal is often inconclusive and in a group of animals it only indicates that this group of animals has been exposed to trypanosomiasis some time in the past (Molyneux, 1975).

1.5.3.1 Complement fixation test (CFT)

The complement fixation test is a useful means of diagnosing dourine, the venereal trypanosomiasis of equines caused by *T. equiperdum* (Killick-Kendrick, 1968). Experimentally in cattle the complement fixation test was possible to differentiate, by degree of reaction, *T. congolense* infections from other trypanosome infections but not with *T. vivax* or *T. brucei* infections and gave no false positive results (Mehlitz and Diendle, 1972). However it has been reported that the test is unreliable in the field for the diagnosis of bovine trypanosomiasis and that it is difficult to perform in practice in the tropics (Killick-Kendrick, 1968).

1.5.3.2 Indirect fluorescent antibody test (IFAT)

The indirect fluorescent antibody test (IFAT) has been the most widely used immunodiagnostic test for animal trypanosomiasis (Molyneux, 1975). The IFAT test is more sensitive than any STDM parasitological methods (Wilson, 1969; Ashkar and Ochilo, 1972) with a reported sensitivity of 70 - 89% compared with STDM sensitivity in cattle of 15% (experimental) (Zwart *et al.*, 1973). The IFAT test is also more sensitive than CFT for the detection of bovine trypanosomiasis, however IFAT produces non-specific cross reactions, possibly to *T. theileri,* whereas the CFT does not (Mehlitz and Diendle, 1972). Mehlitz and Diendle (1972) reported that using the IFAT, under experimental conditions, serological reactions with homologous antigens was always stronger than the reaction with heterologous antigens but the heterologous reaction was always present. This contrasts with experience in the field, where infection with multiple species is common, the IFAT was found to be of limited use in species differentiation (Wilson, 1969; Zwart, 1973). The IFAT test can be measured quantitatively to indicate the stage of infection (Wilson, 1969) however early infection and self cure may be missed (Zwart, 1973) with a positive reaction being recorded as long as 42 days post treatment (Wilson, 1969). The sensitivity and specificity of the IFAT makes this test more applicable to surveys and herd diagnosis rather than diagnosis of the individual (Ashkar and Ochilo, 1972; Zwart *et al.*, 1973).

1.5.3.3 Card latex agglutination tests (CATT)

Card agglutination tests (CATT) have been widely used in West Africa for the diagnosis of *T. b. gambiense* infections in man (Magnus and Vervoort van Miervenne , 1978). More recently card agglutination tests have been undergoing evaluation for the diagnosis of *T. b. rhodesiense* in man (CIATT ® , Brentec Diagnostic Ltd. Nairobi, Kenya) (Olahu-Mukani *et al.*, 1993) and T. evansi in camels (Suratex ® , Brentec Diagnostic Ltd. Nairobi, Kenya) (Olahu-Mukani and Nyang'ao, 1993). These tests use either serum or whole blood and can be carried out in the field at the time of sampling with minimal equipment. However, at present a card agglutination test for the diagnosis of *T. brucei* spp. in animals has not been developed. In the future card agglutination tests may prove of value in the diagnosis of trypanosome infections in animals but such a system would have to be able to diagnose *T. vivax*, *T. congolense* and *T. brucei* spp. infections and be able to differentiate infections of each species of trypanosome.

1.5.3.4 Enzyme linked immunosorbent assay (ELISA)

There are two possible approaches to the immunodiagnosis of trypanosomiasis; the detection of antibodies to *Trypanosoma* spp. and the detection of *Trypanosoma* spp. antigens.

1.5.3.4.1 Antibody detection ELISA

Voller, Bidwell and Bartlett (1975) demonstrated that their micro-scale modification of the ELISA developed by Engvall and Perlman (1972) using *T. brucei* antigen is of use in the detection of antibodies to *T. b. rhodesiense* in man although cross reactions occur with *Leishmania donovani* and *T. cruzi*. Similarly a micro-plate ELISA has been evaluated for the diagnosis of bovine trypanosomiasis by Luckins (1977) which proved very sensitive (84 - 92%) compared to the IFAT (70 - 89%) and had no cross reactions with other haemoprotozoa often found where tsetse transmitted trypanosomiasis is found, such as *T. theileri, Theileria parva, Th. mutans, Th. annulata, Babesia divergens* and *Anaplasma marginale*. However the test did not distinguish between infections with *T. brucei*, *T. vivax* and *T. congolense*. *Trypanosoma*l antibodies could be detected within 7 - 14 days post infection and persisted for up to 83 days after treatment with diminazine aceturate (Berenil®, Hoechst, Germany).

1.5.3.4.2 Antigen-detection ELISA

Species-specific monoclonal antibodies against *T. vivax, T. congolense* and *T. brucei* have been used to develop an antigen captive enzyme linked immunosorbent assay (antigen-ELISA) for the diagnosis of bovine trypanosomiasis (Nantulya and Lindqvist, 1989). Experimentally this test was able to detect species-specific antigens at between 8 and 14 days post infection which coincided with the first detection of parasitaemia and was reported to be more sensitive than the buffy coat method (DG). Following treatment with diminazene aceturate, antigens of *T. vivax* and *T. congolense* were no longer detectable after 2 weeks but this period was longer and more variable with *T. brucei* infections.

The observations reported by Nantulya and Lindqvist in 1989 indicate the possibility of a sensitive, specific, simple and practical antigen detection test for the use of diagnosis of bovine trypanosomiasis. The test would be useful in not only epidemiological studies but also possibly as a tool for testing drug efficacy post treatment. Unlike all the other immunodiagnostic tests the antigen ELISA has the advantage of detecting current infection.

1.5.3.5 Other serological tests

Two tests used in the diagnosis of human sleeping sickness have been evaluated for the diagnosis of animal trypanosomiasis; the capillary agglutination test (Clarkson *et al.*, 1973) and raised immunoglobulin levels (Luckins, 1977). Both techniques were found not to be consistent or reliable enough for the diagnosis of bovine trypanosomiasis. There are a number of other immunodiagnostic tests for trypanosomiasis such as the indirect haemagglutination test (Gill, 1964), micro-counter immuno-electrophoresis test (Taylor and Smith, 1983) and a skin test (Tizard and Soltyz, 1971) but these remain at present only experimental techniques.

1.5.3.6 Future serodiagnostic tests

The ideal serodiagnostic techniques should:

(I) indicate the presence or absence of infection

(ii) differentiate between infections with different species of trypanosome

(iii) be specific for the parasite concerned, i.e. no cross reactions with other organisms.

The test should also be simple and quick to perform; produce reliable results; use standard reagents and be economic to use on a large number of samples. These criteria are hard to

satisfy because of the special problems encountered with trypanosome infections, i.e. multiple infections, non-pathogenic trypanosome infections, repeated exposure of animals to infection and occasional drug treatments.

1.5.4 Identification of *Trypanosoma brucei* subspecies

Identification of the *Trypanosoma brucei* subspecies *T. brucei brucei, T. brucei rhodesiense* and *T. brucei gambiense* which are morphologically identical has classically been based on host range, geographical distribution and the disease syndrome they cause.

(1) Host range. *T. b. brucei* infects only wild and domestic animals while *T. b. rhodesiense* and *T. b. gambiense* also infect man.

(2) Disease in man. *T. b. rhodesiense* causes acute disease in man while *T. b. gambiense* causes chronic disease.

(3) Infectivity to laboratory rodents. Both *T. b. brucei* and *T. b. rhodesiense* are highly virulent in laboratory rodents while *T. b. gambiense* is less so.

(4) Geographical distribution. *T. b. rhodesiense* is found in East Africa while *T. b. gambiense* is found in West and Central Africa and *T. b. brucei* is found throughout the tsetse region of Africa (Hoare, 1972).

The only true test of whether a *T. brucei* spp. strain is capable of producing sleeping sickness in man is to infect a human and observe the clinical manifestations. The results of experimental human infection are inconclusive since rapid treatment on detection of a parasitaemia only proves infectivity and not the ability to cause sleeping sickness (Gibson *et al.*, 1985). As human experimentation is now considered unethical the blood incubation infectivity test (BIIT) was developed to differentiate between *T. b. brucei* and *T. b. rhodesiense*. This attempts to provide a simple safe method to identify the *T. brucei* spp. subspecies from fly or animals without recourse to experiments on human volunteers (Rickman and Robson, 1970a, 1970b, 1971). The BIIT involves incubating a trypanosome isolate in human serum at 37^0 C for 5 hours before inoculating the sample into a rodent. Human infective isolates (i.e. human serum resistant *T. b. rhodesiense)* will infect a rodent whereas non-human infective isolates (i.e. human serum sensitive *T. b. brucei*) will not. There are however reports of trypanosome stocks whose resistance to human serum was of variable quality that disappeared and reappeared with passage (e.g. Fairbairn, 1933; Joshoea *et al.*,

1978; Jennings and Urquhart, 1985). An *in vitro* human serum sensitivity test (HSRT) has been developed using metacyclic trypanosomes from tsetse salivary glands cultured in medium containing serum and fibroblast cells (Brun and Jenni, 1987). This test is reported to be more reliable than the BIIT, however it can take 2 weeks to complete and is technically complex. *T. b. gambiense* can be differentiated from either *T. b. brucei* or *T. b. rhodesiense* using isoenzyme electrophoresis (the latter two being indistinguishable) which lead to the conclusion that they were subspecies (Gibson *et al.*, 1985; Gibson and Wellde, 1985). More recently using restriction endonuclease fragment length polymorphism (RFLP) analysis of repetitive DNA sequences all three trypanosome types are able to be uniquely identified as determined by cluster analysis of DNA patterns (Hide *et al.*, 1990; Hide, 1996). These techniques are very useful for investigating the epidemiology and taxonomy of trypanosomiasis but due to their technical complexity and cost are unsuitable for mass sample analysis.

1.5.5 Conclusions

Despite much progress in recent years there still exists a need for a rapid accurate test to supplement clinical diagnosis. The ideal diagnostic test would be a parasitological technique which is rapid, simple, sensitive and specific for the etiologic agent and able to be performed in the field (Molyneux, 1975). Since no single technique meets all the requirements for the certain diagnosis of trypanosomiasis a combination of techniques should be employed (Paris *et al.*, 1982). The combination of techniques should be selected to suit the situation and purpose as regards sensitivity and specificity of the diagnosis, taking into consideration limitations of time, manpower and other resources available (Molyneux, 1975).

1.6 The epidemiology of trypanosomiasis in East Africa

1.6.1 Human African trypanosomiasis (HAT)

Man is affected by only two subspecies of *T. brucei* spp. which in West Africa produces a typically chronic disease (*T. b. gambiense*) and in East Africa a more acute form resulting in death after only a few months (*T. b. rhodesiense*). The two diseases, often referred to as

'Gambian sleeping sickness' and 'Rhodesian sleeping sickness', respectively, differ in their epidemiology.

1.6.1.1 The epidemiology of Rhodesian sleeping sickness

The area affected by Rhodesian sleeping sickness extends from Central and South Nyanza provinces of Kenya through Southern Uganda, Rwanda and Burundi to Tanzania, Mozambique, Zambia, Malawi, Zimbabwe, Northern Botswana and Eastern Angola, an area of 1.7 million square miles inhabited by over 50 million people. The number of new cases of Rhodesian sleeping sickness reported each year rarely exceeds 2,000 (Apted, 1970) which does not convey the fear with which this disease is regarded in East Africa because of its epidemic nature. During an epidemic of Rhodesian sleeping sickness between 1980 and 1984 a human prevalence of 5.4% was recorded in one area of the Lambwe Valley in Kenya (Wellde *et al.*, 1989a). Similarly the number of cases of human African trypanosomiasis in Busoga district, Southern Uganda during 1980 was 8465 (1.2% of the population) (Abaru, 1985).

The epidemiology of human infections with *T. b. rhodesiense* is essentially that of a zoonosis, an infection or disease primarily of animals which may be contracted by man if he exposes himself to risk by interrupting the natural animal-fly-animal cycle. In addition man-fly-man transmission of the disease is possible but in practice this is much less important because of the acute course of the disease in humans making them a poor reservoir of infection, and probably only occurs during an epidemic condition (Apted, 1962). However chronic and relapsing cases of sleeping sickness in people fleeing infected areas have been implicated in the spread of the disease from one area to another (Abaru, 1985). Host preference of the important vector tsetse species does not favour man except under conditions where the choice of host is severely limited. The most widespread vectors are members of the *morsitans* group *G. morsitans, G. pallidipes* and *G. swynnertoni* except in a restricted area of Tanzania, Kenya and Uganda surrounding Lake Victoria extending into the Busoga region of South East Uganda where the vector is *G. fuscipes fuscipes* (Willett, 1965) and an area in south west Ethiopia where *G. tachinoides* was found to be the vector (Hutchison, 1971).

Classic Rhodesian sleeping sickness occurs sporadically in sparsely inhabited woodland which contains game; *G. morsitans, G. pallidipes* (which requires thicket), or *G. swynnertoni*, in a confined area. This endemic state of low prevalence in humans tends to be maintained

unless conditions occur which either break the cycle of infection or create those necessary for an epidemic (Nash, 1978).

Although suspected for many years, an animal reservoir for *T. b. rhodesiense* was only conclusively proved by the Corson experiment initiated at Tinde, Tanzania in 1936 where an isolate of *T. b. rhodesiense* was fly-passaged between sheep and antelopes for years and was subsequently shown to be cyclically transmissible to human volunteers (Fairbairn and Burtt, 1946). Proof of a wild animal reservoir was demonstrated by Heisch *et al.* (1958) when a human volunteer was infected with *T. b. rhodesiense* from a bushbuck (*Tragelaphus scriptus*) shot in Kenya. For an animal to provide a good reservoir of *T. b. rhodesiense* infection it must be:

(a) tolerant to the infection and have blood-positive periods of considerable duration.
(b) attractive to the tsetse fly
(c) have circumscribed habitats (Nash, 1978).

Although Ashcroft *et al* (1959) listed seven species which are tolerant of infection and maintain long periods of parasitaemia, of these seven only the bushbuck is also a favourite host of the tsetse and is a sedentary species (Nash, 1960; Allsopp *et al.*, 1972).

Woodland containing thickets, near water, is an essential habitat for both *G. pallidipes* and bushbuck. Since bushbuck is a favoured host of *G. pallidipes* (Weitz (1963) found 49% of blood meals from this species), this could give rise to a persistent focus of *T. b. rhodesiense* infection with only an intimate bushbuck-fly cycle. Man may intrude into this cycle, occasionally if for example his hunting activities take him into close contact with the tsetse, or continuously if the thicket is close to permanent habitation giving rise to an animal-fly-man zoonosis. Prevalence of *T. b. rhodesiense* in man typically remains low under conditions where plenty of game exists, particularly bovids, since ordinarily man is not a favoured host of *G. pallidipes* (Weitz (1963) found only 2.7% of blood meals came from man). However in a sleeping sickness epidemic around Lugala in South West Uganda, where game is relatively scarce, the percentage of blood meals taken by *G. pallidipes* from man rose to 4.5% as the proportion of bushbuck blood meals fell to 27.1% (Southon, 1963). *G. morsitans* is unlikely to play a major role in a bushbuck-fly cycle since only 2.5% of blood meals came from this host but during epidemic conditions. *G. morsitans* may be more important than *G. pallidipes* in any man-fly-man cycle as it takes 9.1% of its meals from man (Weitz, 1963). However the proportion of tsetse blood meals from differing hosts may vary with the geographical location

and time of sampling reflecting changes in the population of each host species (Okoth and Kapaata, 1986; Okoth and Kapaata, 1988).

MacKichan (1944) believed that Rhodesian sleeping sickness spread from Uganda to Kenya in 1942, aided by a contiguous *G. pallidipes* fly belt. However more recent evidence suggests that Rhodesian sleeping sickness has existed in both countries since before 1900 (Koerner *et al.*, 1995). Similarly it has been shown that *G. pallidipes* alone is capable of maintaining an endemic focus of infection as well as being the sole vector during an epidemic in the Lambwe Valley in 1964 (England and Baldry, 1972). However the Lambwe Valley epidemic was not epidemiologically typical of classic Rhodesian sleeping sickness since a significant reservoir of infection may have existed in local domestic zebu cattle (up to 53% prevalence of *T. brucei* spp. infections in cattle) (Wellde *et al.*, 1989c). *G. swynnertoni* has been implicated in human infections in the Maasai Mara area of Kenya (Heisch, 1948) and it may be more important along with *G. morsitans* in the spread of sleeping sickness over greater distances infecting migratory species of game animal.

During an epidemic of sleeping sickness in 1940 in the Busoga District of Uganda *G. pallidipes* was found to be a vector (MacKichan, 1944) but later *G. f. fuscipes* was implicated (Southon and Robertson, 1961). From a three mile radius around an infected village within the endemic area these two species of tsetse were caught and their blood meals identified (Table 1.7).

Table 1.7 Percentage of blood meals from different hosts in South East Uganda in 1963

	G. fuscipes	*G. pallidipes*
Man	28.7	4.3
Bushbuck	18.2	27.1
Bushpig	6.6	45.3
Reptiles	34.9	0.8

(Southon, 1963)

It can be seen that *G. fuscipes* alone is capable of either maintaining an endemic zoonosis or by virtue of its frequent feeding on man contribute to an epidemic man-fly-man cycle. Both of these species of tsetse have a favoured host species which acts to reduce the

probability of infection spreading to man by being a poor reservoir for *T. b. rhodesiense*. In the case of *G. fuscipes* reptiles are the favoured hosts (Duke, 1921), and for *G. pallidipes* bushpigs (Ashcroft *et al.*, 1959). If these host species are reduced in number this could lead to a situation of higher human infection. In an epidemic starting in 1964 at Alego, 32 Km inland from Lake Victoria in Kenya, a heavily populated area where game was scarce, *G. fuscipes* was shown to be the only vector. Conditions were reported to have become favourable for *G. fuscipes* after there was a succession of rains and the introduction of the shrub lantana (*Lantana camara*) to build field demarcating hedges. These helped to create favourable conditions of the encroachment of secondary bush and high relative humidity for the riverine tsetse to move out from areas surrounding water courses to occupy and breed in the high hedges surrounding villages (Okoth, 1986). This new-found peri-domestic habitat allowed intimate cattle-fly-man contact at both the watering point and in the villages where the cattle were kept at night. Of 201 blood meals from *G. fuscipes* at Alego: 44% were from primates; 40% from cattle and 15% from lizards (Boreham, 1973). Conclusive proof of a cattle reservoir was found when *T. b. rhodesiense* was isolated from a cow and identified by infecting a human volunteer (Onyango *et al.*, 1966). Cattle make an ideal reservoir for *T. b. rhodesiense* because after infection even these which eventually succumb to the disease remain intermittently parasitaemic for up to 5 years (Wellde *et al.*, 1989c). The degree of cattle-fly-man contact in Alego made the existence of endemic or even epidemic sleeping sickness a high probability.

G. tachinoides, another riverine species of tsetse, has also shown itself capable of causing a sleeping sickness epidemic in South-West Ethiopia. In an area with abundant game and ubiquitous *G. morsitans* the prevalence of sleeping sickness was low except at Pinybago, a village of four to five hundred people, situated in a dense thicket. The thicket was shared by bushbuck and *G. tachinoides* which regularly fed on people in the village. Over a period of 15 months there were 62 cases (30 male, 32 female) of sleeping sickness in Pinybago (Hutchison, 1971).

The evidence for the roles played by the different species of tsetse indicate that *G. pallidipes* is very efficient at maintaining inter-animal infection but is much less so with zoonotic infection because of its relative distaste for man (Southon, 1963). While *G. fuscipes* and *G. tachinoides* with their more catholic tastes, have a greater potential to transmit *T. b.. rhodesiense* locally to both man and animals (Nash, 1978).

In addition to the factors which influence the degree of contact between reservoir host, fly and man; and the prevalence of infection in reservoir hosts; the prevalence of infection within the population of tsetse flies is important in the epidemiology of Rhodesian sleeping sickness. A number of factors (which may vary by species) influence tsetse infection rates:

(a) temperature of pupal incubation
(b) species of tsetse
(c) timing of first feed
(d) tsetse immunity
(e) serodeme of the trypanosome
(f) sex of tsetse fly
(g) infective dose of trypanosomes

(a) Incubating pupae at 22°C (3°C lower than normal) has been shown to significantly reduce infection rates of *T. congolense* in *G. morsitans* but not to affect the maturation rates (Welburn and Maudlin, 1990). However despite reports in the literature that raising pupal incubation rates increases fly infection rates (Burtt, 1946; Buxton, 1955; Ndegwa *et al.*, 1992) this result could not be repeated in an experiment involving more than 1000 flies (Maudlin, unpublished observations).

(b) The relatively low vectorial capacity of *palpalis* group of flies for *T. congolense* has been known for some time (Duke, 1933; Harley and Wilson, 1968; Moloo and Kutuza, 1984) however this has been shown not to be the case with the *trypanozoon* group. Harley (1971) discovered that the infection rates with *T. b. rhodesiense* were much greater in *G. pallidipes* and *G. fuscipes* than *G. morsitans*, using newly emerged flies in the laboratory.

(c) Using *G. fuscipes* (Harley, 1971) and *G. morsitans* (Wijers, 1958) it has been found that infection rates with *T. b. rhodesiense* and *T. b. gambiense* are highest if the first (infected) feed is taken within 24 hours of emergence and reduces to less than half this at between 24 and 48 hours and although it is possible to infect older flies this is not a common occurrence.

(d) Tsetse immunity to the establishment of midgut infections of *T. b. rhodesiense* appears to depend on the level of lectin present in this site at the time of infection (Welburn and Maudlin,

1989). Lectins are a group of naturally occurring carbohydrate binding glycoproteins of non-immune origin which have specific receptors for certain carbohydrate configurations and can selectively agglutinate cells and/or precipitate complex carbohydrates (Ibrahim *et al.*, 1984). These compounds are secreted by the fly gut in response to bloodmeal serum . Consequently the midgut of teneral flies have been found to have little lectin activity (Maudlin and Welburn, 1988). The levels of lectins within the midgut of the fly are associated with the presence of an infection of ricketsia-like organisms (RLO) which have been shown to produce the enzyme chitinase (Maudlin and Welburn, 1988). Chitinase hydrolyses the chitin which is found in the gut of teneral flies, as a remnant of metamorphosis, to D-glucosamine (Tracey, 1955) which specifically inhibits the secretion of lectins from the midgut of a tsetse fly (Ibrahim *et al.*, 1984). The effect of RLO's is only seen in teneral flies as the chitin remnants are defecated after the first blood meal.

The susceptibility of tsetse flies to develop midgut infections of *T. brucei* or *T. congolense* infections is heritable, following the maternal line with RLO infections being transmitted between generations within the cytoplasm of the ova (Maudlin, 1985).

A field survey in Liberia of populations of *G. p. palpalis* and *G. nigrofusca* showed strong association between rate of RLO infections and the rate of trypanosome infections (Maudlin *et al.*, 1990a).

Lectins are also important in the maturation of midgut infections to the salivary glands for *T. brucei* and the mouthparts for *T. congolense.* They appear to provide a signal to the trypanosome to undergo this process of maturation which is necessary if the tsetse is to be able to infect subsequent hosts (Maudlin and Welburn, 1988).

(e) The effect of lectins on differing stocks of trypanosome vary, suggesting a genetic difference within the trypanosomes related to the number of lectin binding sites (Maudlin and Welburn, 1988).

(f) The rate of mature salivary gland infections of *T. b.. rhodesiense* is greater in male tsetse flies compared to female flies and is under genetic control. However the epidemiological significance of this phenomena is partially negated by the difference in life span of the two sexes (Maudlin, 1990b).

(g) The number of trypanosomes ingested by a tsetse fly during an infective feed was considered by Molyneux (1977) to influence the infectability of the fly. Whereas Wijers and Willet (1960) concluded that the infection rate of *T. b. gambiense* was dependent on the absolute number of short stumpy forms in the infective feed rather than the total number of trypanosomes in the host blood. It has now been demonstrated that a single trypanosome is sufficient to infect a tsetse fly, provided that the organism is capable of infection, and that the fly is susceptible to trypanosome infection (Maudlin and Welburn, 1989). However it is reasonable to conclude that the probability of a susceptible fly ingesting an infective trypanosome would be greater when the host had a high parasitaemia.

This relationship between vector and parasite may explain the often very low tsetse infections found in the field. Jordan (1974) found that in 9000 dissections of *G. morsitans* the overall infection rate was only 10%, comprising of 7% *T. vivax*, 2.6% *T. congolense* and only 0.3% mature salivary gland infections with *T. brucei* spp. Similar fly infection rates were obtained in the laboratory by Harley (1966a) and, Harley and Wilson (1968) using *G. pallidipes* and *G. fuscipes* infected with *T. congolense*. The infection rate of *T. vivax* correlates with the proportion of feeds from infected hosts whereas the infection rates of *T congolense* and *T. brucei* do not. Since *T. vivax* undergoes its development entirely within the tsetse fly's mouthparts, a site free of lectin activity, this may offer a possible explanation (Maudlin, 1985).

1.6.1.1.1 Mechanical transmission

Infection rates of tsetse flies with *T. brucei* subspecies is very low, even under epidemic conditions (Buxton, 1955; Roberts *et al.*, 1989). During a sleeping sickness epidemic in the Lambwe Valley in 1980 only 0.8% of 2011 *G. pallidipes* dissected had mature *T. brucei* infections despite the fact that up to 53% of cattle were infected in certain areas (Wellde *et al.*, 1989c). Mechanical transmission of *T. brucei* spp. offers a possible explanation for the high incidence of infection in cattle and the low fly infection rate observed. It has been demonstrated in the laboratory that tsetse, stomoxys, tabanids and other biting flies are capable of mechanically transmitting *T. brucei* spp infections if the period between blood meals is sufficiently short (Taylor, 1930, 30 minutes; Dixon *et al.*, 1971, 30 minutes; and Roberts *et al.*, 1989, 160 minutes). These experiments relied on the use of rodents which tend to have a much higher parasitaemia than either wild bovidae, domestic cattle or man. Under

experimental conditions, Roberts *et a.,l* (1989) failed to transmit *T. brucei rhodesiense* between cattle using artificially reared *G. morsitans morsitans*, despite some of the infected cattle having a relatively high parasitaemias (up to 4.8 x 10^4 trypanosomes per ml). A more likely explanation for the high prevalence of *T. brucei* spp. infections in cattle in certain areas of the Lambwe Valley is that tsetse flies interrupted during feeding would result in the infection of more than one host during each feeding cycle (Corson, 1932; Harley *et al.*, 1966; Jenni *et al.*, 1980).

1.6.1.1.2 Alternative routes of infection

Congenital infection of *T. b. gambiense* has been reported in human cases of sleeping sickness, but it remains a very rare event. Werner (1954; 1955) concluded that after attempting experimentally to infect over 900 foetuses by infecting pregnant animals of various species (rats, mice, guinea pigs and hamsters), that transplacental transmission without placental damage is unlikely.

Trypanosome infections of carnivores can occur through minor oral abrasions when eating infected prey (Sachs *et al.*, 1971). Infection by this route would be possible in the Maasai tribe and other pastoral groups who drink fresh ox blood (Soltys, 1971).

1.6.1.1.3 Climatic factors

Hot dry weather reduces the range of tsetse flies, which in turn reduces the choice of host upon which the fly is able to feed. This may lead to an increased chance of contact between man and the tsetse fly, and a higher incidence of sleeping sickness during the hotter and drier seasons of the year (Nash, 1944). This pattern was seen in the Lambwe Valley where *G. pallidipes* was the vector, where there was an inversely proportional relationship between the average monthly rainfall and number of new cases of sleeping sickness per month (Wellde *et al.*, 1989a). However an alternative explanation of this observation is that there is a lag phase between the rainy season and a build up of fly numbers.

1.6.1.1.4 Human susceptibility and prevalence

In order to explain the apparent range of susceptibility to trypanosomiasis seen in man (Fairbairn and Burtt, 1946) it has been suggested that groups of people with a history of uninterrupted contact with tsetse, and presumably with man-infective trypanosomes over

thousands of years, by a process of natural selection, have developed some form of innate resistance. Whether this resistance to trypanosomiasis is acquired as opposed to genetic is unknown (Murray *et al.*, 1982).

The risk of an individual contracting Rhodesian sleeping sickness is directly related to the probability of contact with the tsetse fly and there is no inherent susceptibility or resistance to the disease based on either sex or age. However the male/female ratio of infected people can be as high as 6:1 when the thicket harbouring the fly is distant from the village and be 1:1 when the village is close to thicket. The male/female ratio in disease prevalence also tends to increase with age with the maximum prevalence tending to be found in the middle age groups of people with relatively small numbers of children under 10 years of age or people over 50 years of age found to be infected. This difference in prevalence reflects occupational risk, with tsetse at a distant site men hunting would be at higher risk of contact whereas with tsetse in a peri-domestic site the entire village would be at equal risk. The duration of residence within an infected area also affects the prevalence of infection (Wellde *et al.*, 1989a).

1.6.1.1.5 Strain of trypanosome

The virulence of the strain of *T. b. rhodesiense* may affect the epidemiology of the disease. From clinical evidence, the disease tends to be relatively mild in Southern Africa increasing in severity towards East Africa, to Northern Uganda where sleeping sickness is usually acute and severe (Ormerod, 1960). Accompanying these clinical observations Ormerod (1960) observed morphological differences between strains of *T. b. rhodesiense* from the north and south, in isolates from the south there were greater numbers of dense inclusion bodies. These differences are borne out by isoenzyme analysis of several different strains of *T. b. rhodesiense* from different geographical locations (Gibson *et al.*, 1980). Recently analysis of repetitive DNA sequences by restrictive endonuclease fragment length polymorphism (RFLP) has shown more accurately the differences between strains from other locations, however strains found within a location during an epidemic are remarkably uniform indicating clonal development (Hide *et al.*, 1990; Hide *et al.*, 1991; Stevens and Welburn, 1993; Hide *et al.*, 1994).

Willet (1965) considered that transmission by different species of tsetse fly vectors could influence the virulence of trypanosomes in the host. The discovery of genetic exchange within a population of naturally transmitted trypanosomes (Tait, 1980; Jenni *et al.*, 1986) is a

possible explanation for the evolution of a hybrid trypanosome with increased virulence which may be the mechanism by which new strains emerge from a population (Stevens and Welburn, 1993).

1.6.1.1 The animal reservoir of Rhodesian sleeping sickness

Numerous species of wild animal have been shown to have the ability to be infected experimentally with *T. b. rhodesiense*, summarised in Table 1.8. These infections are of varying severity and duration as is their prevalence.

Table 1.8 The effect on various species of wild animal of infection with *T. b. brucei* and *T. b. rhodesiense*

GROUP 1. Animals usually killed by *T. b. brucei* and *T. b. rhodesiense*	
Thomson's gazelle	(*Gazella* sp.)
Dikdik	(*Madoqua* sp.)
Blue forest duiker	(*Cephalophus* sp.)
Jackal	(*Canis* sp.)
Fox	(*Vulpes* sp.)
Ant-bear	(*Orycteropus* sp.)
Hyrax	(*Hyracoidea* sp.)
Serval cat	(*Felis* sp.)
Monkey	(*Cebidae* family)
Group 2. Animals usually tolerant or resistant to *T. b. brucei* and *T. b. rhodesiense*	
(a) All infectable; blood positive period of considerable length	
Common duiker	(*Sylvicapra* sp.)
Eland	(*Taurotragus* sp.)
Bohor reedbuck	(*Redunca* sp.)
Spotted hyena	(*Crocuta* sp.)
Oribi	(*Ourebia* sp.)
Bushbuck	(*Tragecephalus* sp.)
Impala	(*Aepyceros* sp.)
(b) Usually infectable; trypanosomes very scanty in blood	
Warthog	(*Phacochoerus* sp.)
Bush pig	(*Potamochoerus* sp.)
Porcupine	(*Hystricidae* family)
(c) Not infectable	
Baboon	(*Papio* spp.)

(Ashcroft *et al.*, 1959)

Under certain circumstances domestic cattle make an ideal reservoir of infection of *T. b.. rhodesiense* (Onyango *et al.*, 1966). Cattle in Africa are often kept in a kraal close to a village, confined for their protection at night and by day they are trekked to a grazing area and watering points accompanied by local herdsmen. This pattern of activity places the cattle at maximum risk of infection should the appropriate tsetse fly be present, particularly *G. fuscipes* but also *G. pallidipes*. Cattle, when confined in close proximity to a village, would provide an ideal reservoir of infection for humans requiring only the presence of a vector, especially if the timing of confinement coincides with the period of maximum activity of the fly. Cattle are good reservoirs of *T. brucei* spp. infection because of the nature of the disease caused which is most often inapparent or protracted over a long period of time.

Movement of cattle out of a sleeping sickness area whether for their own protection or for trade to another area which contains the appropriate tsetse vector has been known to cause the spread of sleeping sickness. Marked cattle, known to be infected with *T. b. rhodesiense* (Zymodeme 25) were moved from the Lambwe Valley to the shore of Lake Victoria where 11 cases of sleeping sickness occurred (all zymodeme 25). The shores of Lake Victoria are devoid of *G. pallidipes* but they do harbour a low density of *G. f. fuscipes* (Wellde *et al.*, 1989c).

T. b. rhodesiense has been shown experimentally to infect sheep (Corson, 1934; Angus *et al.*, unpublished) and serum resistant *T. brucei* spp. were found in sheep from the Lambwe Valley, Kenya (Robson *et al.*, 1972). Sheep from Busoga, Uganda (Okuna and Mayende, 1981) and Busia, Kenya (Chapter V); goats from the Lambwe Valley (Robson and Ashkar, 1972; Wellde *et al.*, 1989d), Busia (Chapter V) and Busoga (Okuna and Mayende, 1981); and pigs from Busia (Chapter V) and Busoga (Okuna, 1993); and dogs from Busia (Chapter V) Busoga (Gibson and Gashumba, 1983) were shown to have *T. brucei* spp. infections in endemic sleeping sickness areas. The relative distaste of tsetse flies for sheep, goats and dogs (Weitz, 1963) may limit the importance of these species as reservoirs for Rhodesian sleeping sickness. However in an endemic sleeping sickness area of Uganda near Lake Victoria, where there were few cattle, pigs were found to be important hosts of *Glossina f. fuscipes* accounting for 20% of blood meals (Okoth and Kapaata, 1988).

Under experimental conditions domestic chickens have been shown to be capable of maintaining an infection of *T. b. rhodesiense* for more than a year (Joshua *et al.*, 1978). However birds generally provide less than 2% of blood meals for the species of tsetse

important in the transmission of Rhodesian sleeping sickness (Weitz, 1963; Okoth and Kaapata, 1988).

1.6.1.2 The epidemiology of Gambian sleeping sickness

The distribution of Gambian sleeping sickness is discontinuous in both geographical location and time. The endemic foci, which tend to be isolated, are located from the Ivory Coast through West and Central Africa extending to Southern Chad, Central African Republic, Zaire and Northern Uganda. From these endemic foci there is a periodic tendency for the disease to break out in epidemic form. In an endemic period when sleeping sickness is confined to small circumscribed areas the prevalence among people in these areas may only be 2% or less (Scott, 1970) whereas during an epidemic period local prevalence of up to 70% has been reported (Nash, 1944).

The classic epidemiology of Gambian sleeping sickness involves essentially a man to man cycle transmitted by the bite of an infected tsetse fly. After a fly has fed on an infected parasitaemic person the parasite undergoes cyclical development within the tsetse. The tsetse then becomes capable of transmitting the disease 20 - 30 days after this blood meal and remains infected for life.

Although experimentally almost any species of tsetse fly can transmit *T. b. gambiense* the natural vectors are members of the palpalis group; *G. palpalis, G. fuscipes* and *G. tachinoides*. These species are often found in close contact with man, along river courses and sometimes in peri-domestic sites. The incidence of the disease is not entirely dependent on *Glossina* population density as epidemics can occur in areas where tsetse are relatively scarce. This can be explained by the nature of man - fly contact which can be referred to as impersonal or personal (Nash, 1948; Hutchinson, 1953). Impersonal man - fly contact may occur in a widely roaming population which have little dependence on human blood meals. A tsetse fly infected with *T. b. gambiense* which feeds on man only once in its life is incapable of transmitting the disease. Whereas personal man-fly contact refers to the situation where a population of tsetse, often confined at the limit of their range, repeatedly feed on man, e.g. at a water collection or ferry point. In these circumstances the chances of man to man transmission are greatly increased. The prevalence of the disease is known to increase during the hotter dry season when the tsetse can only survive in restricted habitats such as shady water courses, thus increasing the chances of personal man-fly contact (Nash, 1944).

Infection rate in the flies is very dependent on the age of the fly at its first infected feed and relies on the fly being infected as a teneral to produce a high infection rate in flies (Wijers, 1958). Most importantly the first host which the fly feeds upon must be an infected human in the early stages of a *T. b. gambiense* infection when transmissible trypanosomes are present in the peripheral blood. Thus when personal man - fly contact increases it will greatly increase the infection rate in tsetse and as a consequence will similarly increase the prevalence of *T. b. gambiense* infection in a human population. As the local prevalence in the human population can rise to 70% (Nash, 1944) it can be understood how epidemic conditions can develop.

Classic Gambian sleeping sickness in man runs a typically chronic clinical course over a period of months and years which may even in the early stages be inapparent to the sufferer. This in itself provides an ideal reservoir for further human infection even during periods of low endemic prevalence of the disease. However *T. b. gambiense* has been isolated from a number of wild (kob, hartebeest; Mehlitz and Zillman, 1983) and domestic animals (dog; Gibson, *et al.*, 1978) (chickens; Küpper *et al.*, 1981) (pigs; Mehlitz, 1977) and even peri-domestic rodents (Mulligan, 1970). However their importance in the epidemiology of classic Gambian sleeping sickness is uncertain as their role in the infection of tsetse flies is unknown. In a low prevalence endemic area human cases are always found, with epidemic spread always being associated with human movement (Ford, 1970). This also provides evidence that the spread of this disease most likely involves man to man transmission with the occasional infection of livestock, although some livestock may play a role in the maintenance of endemic foci.

It is known that the vector species *G. palpalis* and *G. tachinoides* have a wide range of acceptable hosts (Jordan *et al.*, 1962); if man destroys the game, these tsetse will survive on reptiles, man and his domestic animals, and if man subsequently destroys the reptiles the conditions then favour an epidemic situation. Human population density is an important factor in the diversion of the fly's attentions to man (Scott, 1970).

Experimentally it is possible to transmit *T. brucei* spp. mechanically with tsetse and other biting flies (Wells, 1972) which may be of some importance during epidemics of Gambian sleeping sickness. However the geographical distribution of the disease, being confined to the areas of riverine tsetse infestation, strongly indicate that the role of mechanical transmission by biting flies other than tsetse is of little epidemiological importance.

1.6.2 The epidemiology of animal trypanosomiasis in East Africa

Tsetse transmitted animal trypanosomiasis is essentially a group of diseases which affect a number of species of African fauna which often cause negligible pathological effects to them. This sylvatic cycle between wild mammals and tsetse flies continues uninterrupted until domestic animals make their unnatural intrusion.

The role of various species of wild animals as reservoirs of salivarian trypanosomes has been reviewed by Ashcroft, Burtt and Fairbairn (1959) and Lumsden (1962). Most of this work is based on the collection and examination of blood smears which because of their lack of sensitivity in detecting low parasitaemias, would tend to underestimate the level of infection, particularly of *T. brucei* spp. in the wild fauna. Rodent inoculation was also used in some cases, which lends its own bias, being a sensitive technique for *T. brucei* spp., less so for *T. congolense* and very poor for the detection of *T. vivax*. A summary is given in Table 1.9.

Table 1.9 The prevalence of trypanosome infections in wild animals

Animal species	No. examined	% infected	*G. morsitans* No. meals	*G. morsitans* % of total meals	*G. pallidipes* No. meals	*G. pallidipes* % of total	Index of * importance as reservoir
Suidae							
Warthog	154	10	1416	45.0	430	20.5	327
Bushpig	26	12	155	4.9	301	14.4	116
Bovidae							
Hartebeest	144	10	0	0	1	0	0
Duiker	41	15	40	1.3	2	0	10
Oribi	50	16	0	0	0	0	0
Dikdik	34	3	0	0	0	0	0
Kob	70	4	0	0	0	0	0
Waterbuck	110	15	15	0.5	1	0	13
Reedbuck	39	43	96	3.1	10	0.5	77
Impala	151	11	11	0.3	0	0	1
Gazelle	20	10	1	0	1	0	03
Roan	62	15	38	1.2	18	0.9	15
Kudu	40	45	552	17.5	9	0.4	420
Eland	63	29	123	3.9	4	0.2	59
Bushbuck	55	31	112	3.6	1099	52.5	869
Buffalo	87	7	357	11.3	180	8.6	69
Primates							
Baboon	not examined		31	1.0	0	0	0
Monkey	not examined		8	0.3	0	0	0
Others							
Hippopotamus	655	0.5	41	1.3	14	0.7	1
Giraffe	68	37	61	1.9	10	0.5	44
Rhinoceros	8	0	89	2.8	13	0.6	0
Zebra	109	6	0	0	0	0	0

* The index of importance of each species of aminal as a reservoir of trypanosomes for these two species of *Glossina* are given by the product of the prevalence of infection and the mean percentage of bloodmeals taken from that species. Although not statistically acceptable it gives a useful indicator as to the importance of each host species.

(adapted from Jordan, 1986)

It is interesting that all species of animals examined are capable of supporting a trypanosome infection, with the exception of rhinoceros (*Diceros bicornus*) of which only eight were sampled. The prevalence of infection varies greatly, from less than 1% in

hippopotamus (*Hippopotmus amphibis*) to over 50% in waterbuck (*Koleus spp*), but does not vary directly with the tsetse fly's preference of species for its blood meals. A species of wild animals may be an important source of food for tsetse flies but may be a less important carrier of trypanosomiasis if it has some resistance to infection. Similarly certain highly susceptible species may be less important than might be supposed because they are seldom fed on by tsetse flies in the wild (Ashcroft *et al* 1959; Fairbairn and Burtt, 1946).

Carnivores are commonly found to be infected with trypanosomes, e.g. lion (*Felix leo*) and hyena (*Crocuta crocuta*), probably being infected through oral abrasions while eating freshly killed infected prey (Geigy *et al.*, 1971). Because these species are rarely fed upon by tsetse flies their importance in the epidemiology of trypanosomiasis is limited.

The ideal reservoir for trypanosome infections is a species of wild animal which is tolerant to infection and has parasitaemias of reasonable duration such as occurs in certain wild bovidae e.g. buffalo, roan antelope and bushbuck. Wild equidae and suidae provide a poor reservoir of infection as they tend to be highly resistant to trypanosomiasis and only produce a very transient scanty parasitaemia when infected e.g. zebra, warthog or bush pig. Similarly domestic animals which are highly susceptible to the disease and often rapidly succumb to infection are poor reservoirs of the disease e.g. domestic cattle infected with either *T. congolense* or *T. vivax* , except in the case of chronic infections. The ideal reservoir for trypanosomiasis should be attractive to tsetse flies and be non-migratory to achieve maximum infection rates in that species e.g. bushbuck, whereas migratory species of wild fauna may provide the vehicle for more widespread dissemination of the disease e.g. buffalo and roan antelope (Table 1.9).

The pattern of disease in animals is closely linked to the species, density and infection rate of the tsetse fly vector. The savannah species (*morsitans* group) and the forest species (fusca group) exhibit higher rates of infection than the riverine species (palpalis group) (Harley, 1971). Tsetse distribution is dynamic, advancing when conditions such as climate, vegetation and suitable hosts to feed on are favourable and retreating when conditions become less favourable such as a dry season, bush clearing or a change in local fauna.

The role played by mechanical vectors was reviewed by Wells (1972) who concluded that *tabanus*, *stomoxys* and other haematophagous diptera in addition to tsetse flies have been shown to be capable of transmitting *T. congolense, T. vivax* and *T. brucei* spp. mechanically if the time between blood meals is sufficiently short and that these biting flies may be of some

significance in the local spread of the disease but, with the exception of *T. vivax*, it has never been shown that infection within an area can spread or even be maintained in the absence of the tsetse fly (Ford, 1964).

1.7 The control of trypanosomiasis

With present technology the only way to eradicate trypanosomiasis is to eradicate the vector (Jordan, 1986). In many cases the cost of this would far outweigh any benefits and a more realistic target is often disease control rather than eradication.

There are five basic approaches to the problem of Trypanosomiasis control:

1. Passive control
2. Control measures directed against the parasite
3. Control measures directed against the vector
4. Use of livestock adapted to survive trypanosome infections
5. A combination of above (i.e. an integrated approach to control)

1.7.1 Passive control

The simplest approach to the control of trypanosomiasis is to avoid taking susceptible hosts (man or animals) into tsetse infested areas. This strategy can clearly be seen when cattle distribution in Africa south of the Sahara is compared to the distribution of the tsetse fly. Where there is heavy fly challenge cattle tend not to be present. Similarly during the human sleeping sickness epidemic in Southern Uganda between 1901 and 1908 areas of high risk around Lake Victoria were evacuated. Although this undoubtedly is a very effective method of avoiding trypanosomiasis it is extremely wasteful of existing land resources. Since the tsetse fly inhabits an area of 10 million square kilometres, an area of similar size to the USA, much of which is potentially productive savannah land, the idea that this vast area of Africa will remain largely devoid of livestock or human rural development is inconceivable, especially at a time when many countries are facing an ever increasing pressure on land from a rapidly rising population (Hursey, 1993). A frequently used tactic of pastoralists is to use tsetse infested areas for grazing at times of the year when tsetse numbers are naturally low during the dry season (Roderick, 1995).

1.7.2 Control measures taken against the parasite

1.7.2.1 Chemotherapy

Despite a significant investment of time and resources a vaccine against trypanosomiasis is not an immediate prospect. This leaves trypanocidal drugs as the only effective control measure, directly against the parasite available at present. The range of drugs available is very limited with only one new drug coming on to the market since isometamidium in 1961. Mel-Cy (Cymelarsan®, Rhône Mérieux, France) which was introduced in 1990 is licensed solely for use in camels and is only active against the *trypanozoon* group. This situation is unlikely to improve in the near future as high research and licensing costs appear to outweigh expected commercial returns by international pharmaceutical companies. There are broadly two types of trypanocidal drug available, ones which bring about a sterile cure but have little or no residual activity (chemotherapy) and those which retain residual trypanocidal activity in the body for a period of time (chemoprophylaxis). The currently available trypanocidal drugs are summarised in Table 1.10. However not all the trypanocides listed are being currently manufactured.

Table 1.10 Drugs for the treatment of African trypanosomiasis in man and domestic animals

Drug	Trade name(s)	Action	Species mainly affected	Used especially in	Remarks
melarsoprol	Mel-B, Arsobal	C	*T. b. gambiense* *T. b. rhodesiense*	man	Most commonly used drug in late stage sleeping sickness
nitrofurazone		C	*T. b. gambiense* *T. b. rhodesiense*	man	Can be used in melarsoprol resistant cases, but little used because toxic
pentamidine	Pentamidine, Lomodine	C, P	*T. b. gambiense* *T. b. rhodesiense*	man	Only effective prior to nervous system involvement
eflornithine	DFMO	C	*T. b. gambiense* *T. b. rhodesiense*	man	Requires large doses administered over several days
diminazene	Berenil Veriben	C	*T. congolense* *T. vivax* *T. brucei spp.*	cattle, sheep and goats	Rapidly excreted, widely used
homidium	Ethidium Novidium	C, P	*T. vivax* *T. congolense* *T. brucei spp.*	cattle, sheep and goats	Shorter duration of prophylaxis than isometamidium
isometamidium	Samorin Trypamidium	C, P	*T. vivax* *T. congolense* *T. brucei spp.*	cattle, sheep, goats, and horses	Most widely used prophylactic
quinapyramine	Antrycide Triquin	C, P	*T. vivax* *T. congolense* *T. brucei spp.*	cattle, sheep, goats and camels	Widespread resistance reported and problems with cross resistance
suramin	Antrypol Naganol	C, P	*T. b. gambiense* *T. b. rhodesiense* *T. brucei spp.* *T. evansi*	man, camels and horses	Most commonly used drug in early stage sleeping sickness
mel-cy	Cymelarsan	C	*T. evansi*	camels	Very rapidly excreted, and only effective against *Trypaozoom* group

C curative
P prophylactic

Human trypanosomiasis is routinely treated with suramin in the acute phase of the disease and melarsoprol in the chronic phase when parasites can be found in the CNS. Suramin (Bayer, Germany) is strongly anionic and readily binds to plasma proteins (albumin) which explains why this drug does not cross the blood brain barrier but has a prophylactic effect (Leach and Roberts, 1981).

Melarsoprol (Mel-B®) is used in late stage cases because of its unique ability among the commercially available trypanocidal drugs for humans of being able to cross the blood-brain barrier into the CNS. Treatment with melarsoprol is associated with a number of serious side effects, including reactive encephalopathies (Robertson, 1963) making early diagnosis and treatment or prevention of human sleeping sickness infinitely preferable to its eventual treatment. In the last 10 years eflornithine (DFMO) has been used to treat late stage cases. It was found to be very effective against Gambian sleeping sickness but less so for Rhodesian sleeping sickness. It has the disadvantage of having to be administered daily for 14 days in very high doses and is unfortunately no longer commercially produced (de Raadt, 1993).

For the treatment of trypanosomiasis in cattle three drugs have gained the widest acceptance, diminazene (Berenil®, Höechst, Germany and Veriben®, Sanofi Santé animale, Canada), homidium (Novidium®, Rhône Mérieux, France and Ethidium®, Laprovet, France) and isometamidium (Samorin®, Trypamidium®, Rhône Mérieux, France). All of these drugs when used correctly can be highly effective. Suramin (Bayer, Germany) is also used in the treatment and prophylaxis of animal trypanosomiasis, usually in horses and camels, and the more recently introduced Mel-Cy (Cymelarsan®, Rhône Mérieux, France) for curative treatment in camels against species of the *trypanozoon* subgenus as it is ineffective against *T. congolense* and *T. vivax* (Stephen, 1966).

1.7.2.2 Management, financial and logistical problems of chemoprophylaxis

The effective use of trypanocidal drugs to control trypanosomiasis relies on the level of management operating a cattle enterprise. The management must be capable of regularly and accurately injecting the cattle with a prophylactic or curative drug and be able to monitor the level of trypanosomiasis within the herd. Regular monitoring is essential to determine the optimum dose and timing of drug treatment made necessary by the variable duration of prophylaxis and the varying susceptibilities of differing species and strains of trypanosome (Whiteside, 1962b). It has been demonstrated at Mkwaja Ranch, Tanzania that under a good

ranch management system a high level of production could be maintained over a 27 year period using chemoprophylaxis and chemotherapy in an area where cattle would otherwise die of trypanosomiasis (Trail *et al.*, 1985). Under this system a herd of about 12,000 Grade Boran cattle were maintained using an average of 4.6 prophylactic treatments of Samorin® and 0.7 curative treatments of Berenil® per year. Despite this area of Northern Tanzania having a high tsetse challenge the level of productivity achieved in this ranch herd was about 80% of the level of the best Boran herds kept in a tsetse free area of Kenya and 35% more productive than trypanotolerant N'Dama cattle in medium to high trypanosomiasis risk ranching situations in West and Central Africa. At Mkwaja in areas where an integrated trypanosomiasis control approach was practised with the addition of bush clearing, spraying and tsetse trapping, levels of herd productivity equalled the average of that on Kenyan ranches (Trail *et al.*, 1985). However more recently an increase in drug resistance has been observed at Mkwaja Ranch, with the dose of Samorin ® increasing from 0.5 mg/kg every 3 months in the 1960's to 1.0 mg/kg every 5 weeks in 1989. At this point dipping cattle in deltamethrin was started, resulting in a rapid reduction in the tsetse population and a marked improvement in herd health and productivity (Fox *et al.*, 1993).

In the case of smallholder sedentary farmers it has been shown that in a project involving more than 450 draught oxen in a tsetse infested area of Ethiopia that the use of chemoprophylaxis (Samorin®, 1.0 mg/kg) could maintain these cattle in a sufficiently good state of health for productive work. However initial problems over the duration and regularity of the prophylactic dose combined with suspected drug resistance in *T. congolense* infections stressed the need for effective veterinary supervision (Bourn and Scott, 1978).

In a truly nomadic pastoral society such as the Karamojong in Northern Uganda it would not be an easy task to control trypanosomiasis in cattle using chemotherapy, as these societies may often not have access to the necessary drugs even if they had the necessary cash readily available to pay for them. By the very nature of their nomadism it would be difficult in a developing country with an inadequate veterinary service to provide the necessary supervision. Trials have however shown that chemoprophylaxis (Samorin® 0.5 mg/kg) can be effective in limiting losses when trekking pastoralists' cattle 450 miles from the Sahel to the richer markets of Southern Nigeria, despite high tsetse challenge en route (Jones-Davies, 1967). The development of efficient rail and road links to rapidly transport cattle to market

and slaughter within the incubation period of trypanosomiasis was however shown to be more effective than chemoprophylaxis (Kilgour and Godfrey, 1978).

It is difficult to analyse the cost effectiveness of the use of trypanocidal drugs even where some animal production data exists as in the case of Mkwaja Ranch. Because the project could not exist without some form of trypanosomiasis control it is only realistic to compare chemoprophylaxis with the alternative of tsetse control. Chemoprophylaxis can be shown in some cases to be a more economically beneficial control strategy than tsetse control, despite its recurrent cost, with the difference becoming greater as the cattle carrying capacity of the land decreases (Jahnke, 1974).

A four year study in the Muhaka area of Kenya has shown that local East African zebu cattle, kept under a traditional village husbandry system, in an area of moderate tsetse challenge, can improve their productivity by 20% under a program of chemotherapy using isometamidium chloride (Samorin®) and diminazene aceturate (Berenil®) (Maloo *et al.*, 1987). This demonstrated that with good organisation and infrastructure it is possible to control trypanosomiasis, in local cattle, by means of chemoprophylaxis in a cost effective manner, at village level (Itty *et al.*, 1987).

Of the 37 countries (FAO, 1982) infested with tsetse it is unfortunate that, in general, they are also the countries with the lowest gross national product and the poorest ratio of veterinarians per livestock unit (Braend, 1979). The true cost of the use of trypanocidal drugs must include adequate veterinary supervision for diagnosis and monitoring of trypanosomiasis, other skilled personnel, cattle handling facilities, transport (including vehicle, fuel and adequate roads) in addition to diagnostic equipment, needles, syringes and imported drugs.

1.7.2.3 Duration of prophylaxis

Although the recommended dose for Samorin® is 0.5 mg/kg, and 1.0 mg/kg under heavy challenge, to be repeated at intervals of 10 - 12 weeks or more, depending on the level of fly challenge (Samorin® data sheet), the timing and dose rate are often selected empirically rather than based on sound scientific knowledge. Reports of the duration of prophylaxis from the field vary between less than 12 weeks (Pinder and Authie, 1984) to 36 weeks (Robson, 1962). This variation is most likely to result from varying dose rates of drug and the levels of tsetse challenge. This view is supported by observations made by Whiteside (1962a) in Kenya where he equated the level of tsetse challenge to the number of Berenil® treatments required per year

giving rise to the concept of the 'Berenil index' as a measure of trypanosomiasis challenge. Another possibility is that the apparent duration of chemoprophylaxis in cattle is influenced by the development of a degree of immunity while under the protection of a trypanocidal drug. In a field experiment in Northern Kenya cattle treated with either Berenil® or Samorin® were reported to have developed a degree of immunity to trypanosomiasis demonstrated by the presence of antibodies and longer durations of prophylaxis (Wilson *et al.*, 1976). However more recently under controlled experimental conditions, cattle which were repeatedly challenged with metacyclic *T. congolense* under the chemotherapeutic protection of isometamidium chloride (Samorin®) did not develop detectable skin reactions nor did they produce trypanosome specific antibodies. It was concluded that drug residues effectively limited trypanosome multiplication at the site of inoculation, thus preventing the development of a parasitaemia or priming of the host immune system (Whitelaw *et al.*, 1986).

There is experimental evidence that the duration of prophylaxis against *T. vivax* infections (less than one month with one strain) is less than that against *T. congolense* despite the *T. vivax* strains used having been shown to be sensitive to the chemotherapeutic action of Samorin® (Peregrine *et al.*, 1987). This finding is supported by field observations in Kenya, that cattle under a chemoprophylactic regime showed a higher prevalence of *T. vivax* infections when compared to non-treated animals in the same area (Njogu *et al.*, 1985).

There is some evidence that homidium, in addition to having a curative action against trypanosome infections, also confers a degree of prophylaxis. The prophylactic action of a single dose of homidium bromide (Ethidium®) in cattle at 1mg/kg and 5mg/kg was tested at 14 day intervals by syringe transmitted *T. congolense* and *T. vivax*. It was concluded that homidium afforded some protection against *T. congolense* for 4 weeks at 1 mg/kg and for 6 weeks at 5 mg/kg. With *T. vivax* there was a prophylactic period of up to 6 weeks (Kirby, 1964). Kirby (1964) also compared homidium bromide at 1 mg/kg with diminazine aceturate at 3.5 mg/kg in cattle under identical conditions of high tsetse risk. Animals receiving homidium were re-infected predominantly with *T. congolense*, between 31 and 41 days post treatment and those given diminazine predominantly with *T. vivax* between 21 and 25 days. A more recent trial involving Boran steers treated with homidium bromide (Ethidium®) at 1 mg/kg in Kenya reported a period of up to 17 weeks prophylaxis, however the tsetse challenge was very low and these results have never been repeated (Dolan *et al.*, 1990).

Diminazene (Berenil®) is reported to have a prophylactic effect in cattle for 16 - 21 days by van Hoeve *et al.* (1964) and 21 days by Wellde *et al.* (1983).

Suramin being highly anionic binds readily to plasma protein (albumin) and traces may be found in the blood up to three months after intravenous injection, thus accounting for its prophylactic activity (Dewey and Wormal, 1946).

1.7.2.3 Drug resistance

With the very limited number of trypanocidal drugs available, almost all of which have been in use for more than 30 years, it comes as no surprise that there is growing evidence that drug resistant trypanosomes are widespread in many areas of Africa (Holmes and Scott, 1982). The occurrence of drug resistance has been reported to all the currently available trypanocides and cross resistance is common, even between chemically unrelated drugs (summarised in Table 1.11).

Table 1.11 Cross resistance of drugs used in bovine trypanosomiasis

	Response of strain resistant to:			
Drug	Diminazine	homidium	Isometamidium	Quinapyramine
Diminazine	-	0	0	+
homidium	0	-	+	+
Isometamidium	0	+	-	+
Quinapyramine	+	+	+	-

\+ cross resistance

0 no cross resistance

(adapted from Mulligan 1970)

Problems of drug resistance in the field can be reduced by several factors. Cross resistance between the two most widely used trypanocides, Samorin® and Berenil®, are rare and alternating their use as a 'sanative pair' (Whiteside, 1962b) will reduce the chance of developing a resistant strain of trypanosome to either individual drugs. The development of drug resistance in the field is generally considered to result from the prolonged use of sub-curative doses which is supported by experimental evidence from the laboratory (Whiteside, 1962b). Therefore it is more likely that resistance will develop to drugs with prophylactic

activity than those which are only curative. Diminazine (Berenil®), unlike isometamidium (Samorin®), only has cross resistance with one other trypanocidal drug (see Table 1.10). These facts offer some explanation of the comparative rarity of Berenil® resistance (Holmes and Torr, 1988). Although resistance to isometamidium (Samorin®) has been reported (Pinder and Authie, 1984), Mkwaja Ranch used Samorin® as a prophylactic on over 12,000 cattle between 1954 and 1983 with no evidence of the development of drug resistance (Trail *et al.*, 1985). However drug resistance to isometamidium (Samorin®) at Mkwaja Ranch has been observed from 1988, but this only occurred after 34 years of continuous use of the drug (Fox *et al.*, 1993).

Drug resistance must be defined in terms of resistance to a particular dose rate of a trypanocidal drug. Because of the toxic nature of the drugs involved they have a very narrow therapeutic index which limits the ability to simply raise the dose rate to combat any drug resistant strains of trypanosome.

It has been reported that drug resistant field strains of trypanosomes may often be of comparatively low pathogenicity (Goble *et al.*, 1959; Stephen, 1962; Sones *et al.*, 1989).

1.7.2.5 Tests for drug resistance

At present the use of *in vivo* drug assays provides the only definitive method for the determination of the level of sensitivity or resistance of a trypanosome isolate (Sones *et al.*, 1988). These tests involve the infection of either cattle or mice and their subsequent treatment with a range of doses of a trypanocide after the development of a patent parasitaemia. Ideally cattle should be used but because of expense and the problem of finding and maintaining trypanosome-naive cattle in Africa a murine model is most often used. Mice are reliable laboratory hosts but pose two major problems; *T. vivax* and some strains of *T. congolense* fail to infect mice; and, while the correlation of drug dose rates between the bovine and murine host is often taken to be 1:10 (Pinder and Authie, 1984) the murine model is only able to predict the broad sensitivity of a strain of trypanosomes and not the curative dose for cattle (Sones *et al.*, 1988).

With the advent of successful *in vitro* culture techniques for trypanosomes, several methods for *in vitro* drug assay have been developed (Zweygarth and Kaminsky, 1991). One such method by Borowy *et al* (1985) relies on the growth inhibition of trypanosomes on a culture medium of bovine fibroblast feeder layer cells. Problems associated with these *in vitro*

tests include difficulty in adapting field isolates of trypanosomes to culture; a high technological input which may not be appropriate for Africa; and a need to correlate results with sensitivity tests carried out in cattle.

1.7.2.6 Systemic and local reactions to trypanocides

Isometamidium (Samorin®) has a low therapeutic index. When it is injected intramuscularly into cattle at a dose rate of 4 mg/kg it has a severe systemic effect causing unease, incoordination, recumbency and death in about 50% of animals but at the lower dose of 2.0 mg/kg the systemic reaction causing unease soon passes. A more chronic systemic toxicity has been reported in cattle given isometamidium (Samorin® , dose rate of 1 mg/kg) at monthly intervals over a period of time, particularly if diminazene (Berenil ®) was also used, and the cattle were in poor condition under nutritional stress. These cattle had reduced weight gains and there were some deaths with signs of liver damage (Stevenson *et al.*, 1993). The drug is very irritant causing a hot painful swelling at the site of injection which regresses over a period of 2 - 3 months when given at a dose rate of 2 mg/kg (Robson, 1962). Repeated injection of isometamidium (1 mg/kg) has been shown to cause a crippling fibrosis in the neck muscles of cattle (Boyt, 1971). However reports from Mkwaja Ranch, Tanzania, have revealed no deleterious effects in beef cattle regularly treated with isometamidium (on average 4.6 times per year at a dose rate of 0.5mg/kg) throughout their life (Trail *et al.*, 1985), presumably because the site of injection was varied. In the case of draught oxen it is important to minimise local reaction at the injection site to avoid any lameness or impairment of ability to work (Holmes and Scott, 1982).

Suramin has also been shown to cause a systemic reaction with lysosymal deposits being found in the kidneys resulting in nephritis and a delayed toxicity. If severe local reactions to suramin are to be avoided the drug must be administered intravenously (Leach and Roberts, 1981).

A slight transient swelling at the site of injection with homidium has been reported (Ethidium® data sheet)

1.7.2.7 Treatment of *Trypanosoma brucei* spp. infections in animals

A dose rate of 3.5 mg/kg Berenil® will eliminate infections with *T. congolense* and *T. vivax* in cattle, however a dose rate of 7 mg/kg is recommended for infections with *T. brucei* spp. by the manufacturers (Höechst, Germany). Berenil® is well tolerated in cattle at doses up to 21 mg/kg when injected subcutaneously (Fairclough, 1963).

Relapse of *T. brucei* spp. infections following treatment with Berenil® have been reported in mice (Jennings *et al.*, 1977), dogs (Chukwu *et al.*, 1990), goats (Whitelaw *et al.*, 1985) and cattle (Morrison *et al.*, 1983). Similarly relapse of *T. b. brucei* infections in dogs treated with isometamidium chloride (1 mg/kg) (Kaggwa *et al.*, 1988) and relapse of CNS disease in goats experimentally infected with *T. brucei* spp. treated with suramin (50 mg/kg) has been reported (Wellde *et al.*, 1989d). Because of the propensity of *T. brucei* spp. for invading connective tissues and body cavities, particularly the CNS (Losos and Ikede, 1972) the location of the organisms within the tissues makes them less susceptible to drug action. Neither diminazine (Berenil®), isometamidium (Samorin®) or suramin, because of their molecular size and structure, can cross the blood brain barrier in effective quantities. Despite these drugs being able to eliminate the bloodstream forms of *T. brucei* spp. a reservoir of infection remains within the brain. Even repeated doses of Berenil® fail to clear the infection in the CNS (Chukwu *et al.*, 1990). Jennings *et al.* (1977) reported a distinct relationship between the duration of infection with *T. brucei* spp. and the efficacy of chemotherapy, using a murine model. Treatment with Berenil® at 3 and 7 days post infection elicited a permanent cure whereas if treatment was delayed later than 14 days after infection then all the mice relapsed, usually between 20 and 50 days post treatment.

The use of melarsoprol (Mel-B®) and tryparsamide, two organoarsenical compounds capable of crossing the blood-brain barrier, have been used in natural cases of clinical cerebral trypanosomiasis in cattle. Although a temporary improvement was reported the four animals eventually died. Failure of the treatment was attributed to the advanced stage of the disease (Wellde *et al.*, 1989c).

Experimentally melarsoprol (Mel-B®) (four daily doses) and high doses of suramin (50 mg/kg) have been shown to cure goats infected with *T. brucei* spp. showing clinical cerebral trypanosomiasis. However in the same experiment some of the goats treated with suramin alone relapsed and were subsequently treated successfully with melarsoprol (Mel-B®) (Wellde *et al.*, 1989d).

1.7.3 Control measures against the vector

1.7.3.1 Destruction of habitat

Prior to the widespread availability of insecticides after the Second World War, the most widely used method of tsetse control was removal of vegetation which forms the local tsetse habitat (Jordan, 1986). Total or sheer clearing is rarely practised today except in narrow strips to create barrier zones. It has however been shown in South East Uganda that a 5 km bush cleared barrier along a river course failed to prevent re-infestation of *G. f. fuscipes* (Glasgow and Duffy, 1951). Clearing by bulldozer is expensive (Jahnke, 1974) and by hand it is very labour intensive, although there is evidence of improved grazing after clearing (Ford *et al.*, 1970). Clearing bush also has the disadvantages of incurring a recurrent cost to prevent re-growth and may also be ecologically damaging. However urban development caused by an increasing human population has a very similar effect in removing or altering tsetse habitats. Partial 'selective' or 'discriminative' clearing of particular species of plant or tree thereby altering the micro-climate has been shown to be effective in reducing circumscribed tsetse populations (Nash, 1940), but is probably only the method of choice to combat tsetse presence when insecticides are not readily available (Jordan, 1986).

1.7.3.2 Destruction or exclusion of hosts

Removal of the food supply and wild animal reservoirs of trypanosomes by mass destruction of wild game has proved in the past to lead to the disappearance of tsetse flies and trypanosomiasis from certain areas. This occurred naturally during the rinderpest pandemic in the 19th century (Ford, 1971) and by planned hunting in Uganda (Wooff, 1968). Hunting must be ruthless in order to be effective as some species of tsetse can survive on warthog alone, which are much less easily shot than the larger bovids (Bushrod and Evanson, 1983). This may not be effective against members of the palpalis group which can survive in the absence of man and game (Jordan *et al.*, 1962). Today such extreme measures are considered environmentally unsound and aesthetically unacceptable, however large scale extension of human habitation has a similar long term effect. The only modern application of this method is between 'game proof' fences in a buffer zone as practised in Zambia and Zimbabwe (Jordan, 1986).

Selective elimination of one or two of the most favoured hosts of *Glossina m. morsitans* in demarcated areas of the Chirisa Game Reserve, Zimbabwe, has been shown to cause stress and a change in diet for the flies, but not an overall drop in the tsetse population (Vale and Cumming, 1976). However simultaneous hunting of all four favoured hosts of *G. m. morsitans* in the Nagupande Region, Zimbabwe, resulted in a spectacular decline in the tsetse catches in the area (Cockerbill, 1971). A significant reduction in the tsetse population in a given area may therefore be achieved by selective elimination of favoured hosts only with local knowledge of preferred tsetse hosts and game populations.

1.7.3.3 The use of insecticides

Because of the unusual lifecycle of the tsetse fly it is only vulnerable to insecticides as an adult fly. The adult female is larviparous depositing a third larval instar on the ground in a moist shady area about 18 days after its own emergence. When the larva is deposited on the ground it immediately burrows into the soil and forms a puparium, from which it emerges around 30 days later. Pupal development is temperature dependent and may take considerably longer than this at temperatures less than the optimal 25°C. Female tsetse flies only mate once in life and from this can produce a larva every 9 - 10 days under ideal conditions (Glasgow, 1963). While female flies can live for about 90 days, and males a little less, with this low rate of reproduction it is essential that the female survives long enough to produce two viable offspring in order that the species survives.

There are two basic strategies for the application of insecticides. The use of residual insecticide applied to the vegetation which is the favoured resting site of the tsetse fly and the use of non-residual insecticides which are applied every few weeks during the maximum pupation period. Residual insecticides are usually applied by either ground spraying, commonly in the past with DDT or dieldrin or by helicopter using the modern insecticides endosulphan or deltamethrin. Ground spraying remains the only proven successful method of tsetse eradication, particularly in West Africa where 194,500 km^2 of Nigeria were cleared of the fly using residual insecticide (Jordan, 1978). However this method requires the maintenance of a large skilled spray team and is only of use in terrain where man and vehicles can pass. Similarly the application of residual insecticides is not suitable for wet climates where the spray deposits may be rapidly washed off and in most areas ground spraying must be confined to the dry season. The problem of applying residual insecticide over difficult

terrain, particularly river courses, can be overcome by using helicopters, however this method is very expensive, relies on suitable weather conditions and has a high technical input (Jordan, 1986). The use of residual insecticides, particularly DDT and dieldrin, is becoming increasingly frowned upon because of their damaging environmental effect and it can be expected that their use will become less common in the future (Holmes and Torr, 1988).

Non-residual insecticides are usually applied by fixed wing aircraft using ultra low dose endosulphan or deltamethrin. Blanket covering over an area must be repeated every 10 - 20 days during the pupation period (usually 5 - 6 applications) to ensure that no viable pupae remain in the soil. This method is of importance because of its lower environmental impact and a reduced need for a large skilled labour force compared to ground spraying. However the method is not without its problems as it is expensive, it has a high technical input and is very dependent on weather conditions. A summary of the three techniques is given in Table 1.12.

Table 1.12 Comparative features of the three main chemical methods of tsetse control

	Ground spray: knapsack	Aerial spray: helicopter	Aerial spray: fixed wing
Main insecticides	DDT: dieldrin	Dieldrin: endosulfan	Endosulfan
Application principal	Discriminative	Discriminative	Blanket
Spray type	Residual	Residual	Non-residual aerosol
Dry season meteorological conditions	Any	Temperature inversion	Temperature inversion
Applications	1	1	5 - 6
Application rate (g/ha)	150 - 600 *	800 - 1000 *	30 - 100 (Total for 5 applications)
Optimum droplet size (μm)	200 - 500	150 - 160	20 - 40
Labour requirements	Highest	Medium	Lowest
Cost (re: foreign exchange)	Lowest	Highest	Medium
Environmental side effects	Medium	Highest	Lowest

* over area actually sprayed. If 10% discrimination is possible the application rate in relation to the total area reclaimed would be one-tenth of these quantities.

(Jordan, 1986)

More recently as an alternative to applying insecticide to the environment, persistent insecticide of low mammalian toxicity has been applied to livestock to control tsetse flies. Most commonly deltamethrin has been applied to cattle, either as a dip (Fox *et al.*, 1993) or as

a pour-on preparation (Mangwiro and Wilson, 1993). This method of tsetse control has been shown to last for up to six weeks per application and cause an apparent reduction in the tsetse population of 90% as measured by tsetse trapping (Fox *et al.*, 1993).

Selection of technique of insecticide application must take into consideration economics, climate, vegetation and topography and a combination of techniques may be appropriate. Regardless of the technique employed it is essential that there is an effective monitoring team surveying the area before and after spraying (Jordan, 1986).

1.7.3.4 Tsetse fly trapping

Tsetse trapping has been used extensively for sampling tsetse populations to study the ecology of the fly but only in the last 20 years has it become the subject of more intense research for its application as a means of tsetse control. Early use of traps for the control of tsetse tended to be of empirical design resembling the shape of the mammalian host (Harris, 1938). These traps proved particularly effective against the riverine species of *Glossina* (Morris and Morris, 1949), especially when impregnated with DDT (Morris, 1950). However after some early success they proved much less effective against the *morsitans* groups (Harris, 1938). With the development of the more efficient biconical trap (Challier and Laveissière, 1973) there was renewed interest in trapping tsetse for their control because of the increasing problems associated with the widespread use of insecticides. The use of biconical traps impregnated with deltamethrin (340 mg active ingredient per trap) have proved very successful in controlling the riverine species *G. tachinoides* and *G. p. gambiensis* in West Africa with a reduction in numbers of more than 98%. This reduction was achieved after 2 weeks and was shown to be sustainable (Laveissière *et al.*, 1981). A similar experiment was repeated the following year with simple screens impregnated with deltamethrin (100 mg active ingredient per screen) which proved almost as effective (Laveissière and Couret, 1981). Both these methods are extremely economical and require little in the way of technical equipment or skilled manpower and have enormous potential in the control of human trypanosomiasis associated with linearly distributed populations of *Glossina* spp. However the technique of trapping flies in these experiments was carried out during the dry season and it has been shown that siting the traps on the river bank and maintaining adequate levels of insecticide during the wet season is a problem (Politzer and Cuisance, 1984).

Control of the *morsitans* group, which are more widely dispersed, is more difficult to achieve using traps which rely on attracting flies by visual stimulus alone. This would require a large number of traps per unit area as it is estimated that the range of visual attraction of a biconical trap is only about 20 metres (Dransfield, 1984). In order to improve the efficiency of this technique the addition of host animal odour greatly improves the catch because most tsetse are attracted to stationary hosts mainly by their olfactory sense (Vale, 1974). Since this discovery a number of the constituent attractants have been identified and synthesised artificially, notably acetone, carbon dioxide (Vale, 1980), 1 octen-3-ol p-cresol and 3-m-propylphenol (Vale *et al.*, 1985). Some of these compounds can be packaged in slow release containers for prolonged action in the field and have been shown to increase the fly catch by up to 20 fold.

If odour is not used the visual attractiveness must be optimised as this is the initial attraction for tsetse in the vicinity. Size, shape and movement have been conclusively shown to affect the efficiency of a trap (Vale, 1974) as has the colour of targets or traps with blue (460 nm) and black being the most attractive (Green, 1994).

When considering the population dynamics of the tsetse fly a reduction in female flies of only 2 - 3% per day would reduce the population by 95% in one year (Langley and Weidhaas, 1986). Theoretically with a population of *Glossina* spp which naturally has a low rate of reproduction and in which female flies only mate once, it would be better to trap sterilise and release both male and female flies rather than kill them with insecticides. This technique is undergoing field trials using synthetic juvenile hormone (Langley and Pimley, 1986).

Trapping tsetse with odour baited traps is likely to become a more widespread method of control, especially as the technology improves and because of its specific targeting and low environmental impact. It must however be noted that the technology is not universally applicable as trap design and attractants effective in one area or against one species may not be effective elsewhere or against another species of *Glossina* (Vale *et al.*, 1988).

When considering the merits of 'environmentally friendly' tsetse trapping techniques their relative economic benefits must be evaluated. In a two year campaign to control the riverine species *G. palpalis gambiense* and *G. tachinoides* in Northern Ivory Coast there was a 99.8% and a 100% reduction respectively in the two species of fly and an 88% reduction in the incidence of trypanosomiasis in sentinel cattle herds. The cost of this operation was

calculated at between US$ 0.7 and US$ 1.1 per head (Kupper and Douati, 1984) which compares very favourably with chemotherapy. It is impossible to evaluate the benefit of reducing the prevalence of sleeping sickness on the human population in the area, but when this method is compared to other forms of human trypanosomiasis control the cost per head of cattle may be as low as US$0.3 in subsequent years due to the re-use of equipment.

1.7.3.5 Biological control

While a number of biological control techniques such as the use of predators, parasites and pathogens have been the subjects of research the only method (now considered to be genetic control) used in the field to date has been the release of sterile male flies (Dame and Schmidt, 1970). This technique involves the release of male flies after giving a sub-lethal dose of radiation or chemo-sterilant in such numbers that they exceed the natural male population and is based on the principle that these flies will out-compete the natural flies by virtue of their greater numbers. Since the female fly only mates once, if it mates with a sterile male either non viable or no offspring are produced. Sterile male release is most efficient at low tsetse density, for example after initial insecticide spraying. Although the technique has no environmentally damaging effect it is expensive and requires a sophisticated technical input to maintain the artificial colony of flies and is further complicated if more than one species of tsetse is involved.

The ability of gamma-irradiated sterile male tsetse to act as vectors for pathogenic species of trypanosome is not impaired by the process of sterilisation (Moloo and Kutuza, 1984). However the risk of these flies acting as disease vectors can be significantly reduced if they are given a blood meal before their release because of the much greater probability of establishing *T. b. gambiense* or *T. b. rhodesiense* infection if a fly is infected at its first feed (Wijers, 1958).

1.7.4 The use of trypanotolerant livestock

It is well known that certain breeds of cattle, sheep and goats can exist and thrive in tsetse infested areas of Africa when the more common zebu cattle cannot (Stewart, 1951), although some strains of Kenya Boran have been found to exhibit a degree of disease resistance (Dolan *et al.*, 1985). This reduced susceptibility to trypanosomiasis has been most closely studied in

the N'dama breed of cattle from West Africa where it has been found that although the cattle become parasitaemic and anaemic after infection, these are rapidly brought under control (Murray *et al.*, 1979). This resistance to the disease is innate and heritable, not requiring previous exposure to trypanosomes (Roberts and Gray, 1973). In the past trypanotolerant breeds of cattle were considered to be of low productivity, possibly due to their small size, however studies have shown them to be capable of similar performance to zebu cattle (ILCA, 1979). Although the use of trypanotolerant cattle may contribute to a disease control strategy, they are not a universal panacea for the control of trypanosomiasis as they constitute only 8% of Africa's cattle population (ILCA, 1979). Their trypanotolerance can also be overcome by high levels of challenge (Stewart, 1937), concurrent disease, poor nutrition, parturition, lactation, or the stress of heavy work (MacLennan, 1970), and at these times of stress may require chemotherapeutic support (Hill, 1956). The most important factor is likely to be the nutritional state of the host. In arid conditions when fodder is in short supply, animals may have to treck long distances to find sufficient feed, and under these circumstances infected cattle, with anaemia and possible myocardial damage will be less likely to survive and that their poor nutritional status will tend to exacerbate the disease (Murray *et al.*, 1982).

Many species of wildlife are also known to be resistant to trypanosomiasis (Ashcroft *et al.*, 1959) and it has been argued that since these animals are well adapted to the African environment, they would be preferable to cattle for meat production. Game cropping (Roth, 1973) and game ranching (Skinner, 1970) have had some success, but have their own associated problems such as processing, transport, marketing and disease. Although the use of indigenous African wildlife can and will play a part in meat production, unless there is a social revolution, they will not replace cattle, especially in pastoral societies which value their cattle for their role in traditional tribal law and customs as well as a source of animal protein and by products (Jordan, 1986). It is also difficult to envisage game animals replacing domestic cattle for draught power and milk production.

The replacement of domestic livestock with trypanotolerant breeds or African game species would not have any significant effect on the control of zoonotic *T. b. rhodesiense* infections in man, because although these animals have a greater resistance to disease caused by trypanosomes, many become infected and remain parasitaemic over long periods of time, thus providing an ideal reservoir for human infection (Ashcroft *et al.*, 1959).

1.7.5 Integrated control

The simultaneous and co-ordinated use of more than one method of trypanosomiasis control may offer solutions which are more economic and run less inherent risk than relying too heavily on a single method of control. In Ethiopia, where over-dependence on chemotherapy and chemoprophylaxis has led to the development of widespread drug resistance, simultaneous tsetse trapping has been introduced to reduce the fly challenge and hence the frequency of drug therapy (ILRAD report, April 1990). Similarly at Mkwaja ranch, Tanzania where tsetse control, by applying deltamethrin to cattle, has been used in conjunction with mass chemotherapy, thereby reducing the frequency of prophylactic injections, it has been shown to improve cattle productivity over groups on chemoprophylaxis alone (Trail *et al.*, 1985; Fox *et al.*, 1993).

The combination of a trapping system using insecticide impregnated screens and biconical traps followed by the release of sterile males has been shown to control *G. palpalis gambiense* and *G. tachinoides* in Burkina Faso. Along 650 km of rivers bordered by gallery forests, 6,500 impregnated screens were deployed during the dry season, which reduced the population by 94%. This enabled the release of 55 sterile males per kilometre of river, a ratio of 7 sterile males per wild male. The combination of these two complementary non-polluting techniques is claimed to be of comparable cost to other methods of tsetse control (Politzar and Cuisance, 1984).

Integrating the control of trypanosomiasis with tick control and the control of other cattle ectoparasites such as biting diptera has been demonstrated using deltamethrin dips and sprays (Thomson, 1987), and flumethrin pour-ons (Bauer *et al.*, 1989). These methods do not protect individual animals from trypanosomiasis transmission, as pyrethroids are not repellent to tsetse flies (Thomson, 1987). However the reduction in the tsetse population tends to reduce the level of challenge experienced by the cattle over a period of time. Disadvantages of using pyrethroids on cattle to control trypanosomiasis include; their need for frequent application, often every two weeks (Bauer *et al.*, 1989), and in the case of modern pour-on preparations, their high cost. Deltamethrin impregnated ear tags, used in the control of biting flies and the cattle ear tick (*Rhipicephalus apendiculatus*), are largely ineffective at controlling tsetse flies because of insufficient overall cover of the cattle by the insecticide (Thomson, 1987). When calculating the cost/benefit ratio of controlling tsetse flies using these pyrethroid

preparations consideration must also be given to the benefits accrued by the control of ticks, tick-borne disease, and other cattle ectoparasites.

1.8 Introduction to the present study

Busia District, in the Western Province of Kenya, comprises mainly of low lying swamp land draining into Lake Victoria and forms part of a contiguous tsetse fly belt with bordering South East Uganda. Both regions are areas of endemic Rhodesian sleeping sickness and have suffered recent sleeping sickness epidemics; South East Uganda from 1976 ending in 1990 (Mbulamberi, 1990) and Busia District in 1989 and 1990 (Chapter III). An epidemiological study was carried out over 13 months in Busia District with the broad aims of investigating: chemoprophylaxis of domestic livestock as a potential control measure for Rhodesian sleeping sickness, and the role played by domestic animals in the epidemiology of the disease.

1.8.1 The use of chemoprophylaxis for the control of Rhodesian sleeping sickness

From field observation (Maudlin *et al.*, 1990a) and theoretical calculations (Rogers, 1988) the incidence of human cases during a sleeping sickness epidemic would be more sensitive to a reduction in the prevalence of *T. b. rhodesiense* infections in the animal reservoir than to a reduction in the prevalence of infection within the human population. To achieve this chemoprophylaxis of the domestic animal reservoir of potentially human infective *T. brucei* spp. was carried out using two different drugs. The practical and theoretical considerations of this technique as a control measure for Rhodesian sleeping sickness are discussed in chapter IV.

1.8.2 The epidemiology of trypanosome infections of domestic animals in an area of endemic Rhodesian sleeping sickness

Although there have been some studies of trypanosome infections of domestic animals in sleeping sickness areas (Onyango *et al.*, 1966; Willet *et al.*, 1972; Okuna *et al.*, 1983; Gitatha *et al.*, 1987; Wellde *et al.*, 1989c; Okech *et al.*, 1990), they have all taken the form of purely descriptive epidemiological studies, reporting the distribution of trypanosome infections in

some species of domestic animals at one discrete point in time as a point prevalence. The present study aimed to investigate trypanosome infections in the same group of domestic animals in an endemic sleeping sickness area over a prolonged period of time as part of a longitudinal (cohort) epidemiological study. Monthly prevalence and incidence or trypanosome infections in domestic animals in four different villages of the district were monitored and all infections were followed over time to discover their likely outcome. Various risk factors associated with trypanosome infections were investigated as were the possible interactions between infections with trypanosomes of differing species in domestic livestock. The observations and implications of these epidemiological findings are discussed in chapter V.

1.8.3 Comparison of field diagnostic techniques for the diagnosis of trypanosome infections in cattle

Point prevalence of trypanosome infections in past field epidemiological studies are difficult to compare with each other because of the differing diagnostic test used. This study compares the sensitivity of various diagnostic techniques under field conditions with the aim of being able to more accurately predict the true prevalence of trypanosome infections in previous studies thereby allowing more reliable comparison between them.

1.8.4 The pathological effects of trypanosome infections in domestic livestock in an area of endemic Rhodesian sleeping sickness

The pathogenesis of *T. brucei* spp. infections in East African zebu cattle in the literature is equivocal. On one hand some workers report slight or inapparent disease (Hornby, 1921; Richardson, 1928; Killick-Kendrick, 1971; Losos and Ikede, 1972), however others report that all infected cattle eventually succumb to a fatal CNS infection of *T. brucei* spp. (Wellde *et al.*, 1989c). Recent research also indicates that the level of nutrition on infected animals has an important role in the pathogenesis of the disease (Otesile *et al.*, 1991; Katunguka-Rwakishaya *et al.*, 1995). During this study the pathogenesis of trypanosome infections were studies in local breeds of domestic livestock, particularly East African zebu cattle, in conditions of natural tsetse challenge, kept under traditional local village husbandry systems.

Particular attention was paid to the role of infection of the CNS of animals on the epidemiology of the disease. The findings and their implications as to the epidemiology of Rhodesian sleeping sickness are discussed in chapters VII and VIII.

1.8.5 Characterisation of *T. brucei* spp. samples from domestic livestock in an area of endemic Rhodesian sleeping sickness

T. brucei spp. samples from domestic livestock (1993 to 1994) were characterised using restriction endonuclease fragment length polymorphism (RFLP) analysis of repetitive DNA sequences (Hide, 1996) and these were compared to human stocks from Busia District collected during the most recent sleeping sickness epidemic in 1989 and 1990 and to stocks of animal and human origin from the sleeping sickness epidemic in South East Uganda during the 1980's and (Hide *et al.*, 1994). Comparison was made between samples from an endemic state of sleeping sickness and samples taken during a sleeping sickness epidemic. The epidemiological implications of theses finding are discussed in chapter IX.

1.8.6 Conclusions

Finally the findings of all the chapters are pooled together and discussed to give a broader insight into the role of domestic animals in the epidemiology of Rhodesian sleeping sickness, and the practicalities and limitations of chemoprophylaxis of domestic animals in the control of the disease.

Chapter II

Study Area: Busia District, Western Kenya

2.1 Summary

Busia District lies on the Uganda border in the westernmost province of Kenya. The District consists mainly of swampland with the many rivers draining into Lake Victoria and a few granite hills. Annual rainfall is between 1000mm and 2000mm with the wettest months being April and May and the mean monthly temperature is 22° C. There are few areas of natural forest remaining except along the river courses, it having been cleared for agriculture, however over 50% of the land area is covered with poorly managed pasture land with extensive encroachment of secondary bush, mainly *Tithonia diversofolia* and *Lantana camara*. Subsistence agriculture is the main activity with maize, cassava, millet, sorghum, beans, groundnuts and bananas being grown mainly for local consumption with any excess being sold as cash crops along with tobacco and sugar cane. Livestock farming forms an integral and increasing part in local agriculture with East African zebu cattle being the most important but including sheep, goats, poultry, pigs and a small but increasing dairy industry. Busia District is a densely populated area with up to 391 people per km^2 and an increasing total population of 297,840 at the last census.

There have been 165 cases of sleeping sickness in Busia District between 1977 and 1992 with the majority of cases occurring during the epidemic years of 1989 and 1992.

Over 80% of Busia District is tsetse infested with the most numerous species being *Glossina f. fuscipes* but *Glossina pallidipes* is also found in hilly areas. Since 1990 when regular tsetse trapping started using deltamethrin impregnated pyramidal traps (Gouteux and Lancien, 1986) there has been an apparent reduction in tsetse fly numbers in some areas by up to 99%.

2.1 Introduction

Information was gathered on the geography, climate, natural flora and fauna, agriculture, human population, the history of sleeping sickness and tsetse control measures in Busia District from various departments of the Government of Kenya. This was compiled to form a description of the geography and history of the study area particularly as it relates to the ecology of Rhodesian sleeping sickness in the District.

2.2 Materials and methods

Data collection was from the following departments of the Government of Kenya:
Physical geography (District Survey Office, Busia)
Climate (Meteorological Department)
Natural vegetation and wildlife (Kenya Rangelands Ecological Report, Ministry of Planning and National Development, Technical Report No. 118, Land Use in Busia District and Kenya Wildlife Services)
Soil type and crops (District Agriculture Office, Busia)
Human population (Central Bureau of statistics)
Animal population (District Livestock Production Office, Busia)
History of human sleeping sickness in Busia District 1977-1992 (KETRI Sleeping Sickness Hospital, Alupe)
Tsetse fly control 1977-1994 (Tsetse Control Section, Veterinary Department (Busia), Department of Agriculture, Livestock and Marketing)

2.3 Description and history of the study area

2.3.1 Physical geography

Busia is one of the four districts comprising the Western Province of Kenya and is situated at the western extremity of the country. The district is bordered by Bungoma District to the north, Kakamega District to the east, and Siaya District in Nyanza Province to the south east and Uganda to the West. It lies between latitude 0° and 0° 45' north and longitude 34° 25' east (fig.2.1). The district has an area of 1819^2 km with a permanent water surface area of 137^2 km which increases dramatically during the wet season when the low lying swampland along the river courses floods (fig. 2.3).

Most parts of Busia District lie within the Lake Victoria basin rising from 1130m above sea level on the shores of Lake Victoria to a maximum of about 1500m in the Samia and North Teso hills. The central part of the district is a peneplain marked by low flat divides of approximately uniform height, often capped by laterite and a shallowly incised drainage system. The northern part of the central region features large granite out-crops,

originating from Mount Elgon, which are undergoing wind erosion and forms large granite hills or tors such as at Amukura, where the villages of Apatit and Katelenyang can be found. The Southernmost part of the district is covered by the Yala swamp which is lowland associated with the formation of Lake Victoria. The area forms a colony of papyrus growth and is broken by regular water channels and occasional small lakes with grassy islands and is covered with lacustrine and alluvial deposits of recent and Pleistocene times. To the west of these low lying plains in the south of the district are the Samia and Funyula hills which run south easterly to Port Victoria on the lake shore.

The river drainage system for the central region of Busia district runs into the river Malaba which originates in the north of this region and forms the border with Uganda north of Busia town. In Nambale district and to the south in Funyula the numerous rivers drain into the river Sio, which also forms part of the Uganda border but to the south of the town. All river courses in central and Southern Busia district form part of the lake basin drainage system. The villages of Ngelechom and Rukada lie along the low lying land adjoining the rivers Malaba and Sio respectively (fig. 2.2 and 2.3).

2.3.2 Climate

Most parts of Busia District receive between 1270mm and 1790mm mean annual rainfall. However parts of northern Amagoro Division and western parts of Bunyala and Budalangi Division and Samia (including Rukada village) in Funyula Division only receive 1015mm to 1270mm mean annual rainfall. The driest part of the District is found marginally around the lake shore and receives from 760mm to 1015mm per year. The northern and central parts of the District (including the villages of Katelenyang, Apatit and Ngelechom) are the wettest with a mean annual rainfall of between 1400mm and 2000mm, which has no definite seasonal pattern although the maximum monthly rainfall is expected in April and May. Throughout the remainder of the District about 50% of the annual rainfall falls in the long rains, which run from late March to late May. A further 25% of the rains fall during the short rains between August and October. Rainfall reliability in most parts of the District is more than 66% during the long and short rains. Dry spells are experienced from December through to February, particularly in the south (Rukada village in Samia). The mean annual rainfall generally decreases from north to south.

The rainfall distribution and reliability have a significant effect on the major agricultural and livestock activities practised in the District. In central division, where the villages of Katelenyang, Apatit and Ngelechom are situated, where the rainfall is more reliable, the area forms a very rich agricultural zone producing subsistence crops of maize, millet, sorghum with sugar cane, cotton and tobacco as the main cash crops. Further south in Samia, where the village of Rukada is situated, and down towards the lake shore where the annual rainfall is lower, more drought resistant crops such as cassava, sorghum and some maize tend to be grown with surplus food crops and cotton as cash crops. Livestock is kept throughout the District for both economic and social reasons.

Temperatures throughout Busia District are almost uniform with annual mean maximum temperatures ranging from 26° C to 30° C and mean minimum temperatures between 14° C and 18° C. The potential evaporation throughout the District lies between 1800mm and 2000mm per year due to its proximity to Lake Victoria and its drainage systems rendering the humidity of the air to be relatively high (fig. 2.4 and 2.5).

2.3.3 Natural vegetation and wildlife

There are very few areas of natural forest remaining in Busia District as most of it has been cleared for agricultural purposes or to harvest timber and fuel wood (there are only 578.8 hectares of gazetted forest in Busia District). Most of the small area of trees in this district are plantation forests of evergreen cassarina or individual mango trees, usually found on private land. The demand for timber for construction and firewood, and farmland outstrips supply making the destruction of natural vegetation an increasing problem. This is particularly evident on the hilltops where the topsoil is thinner and natural floral ecosystem more delicate. There is much secondary bush encroachment, notably *Tithonia diversofolia* and *Lantana camara*, which is mainly to be found on fallow land, as hedges between fields or along water courses.

About 90% of Busia's land area is suitable for vegetation growth including agricultural crops. However the nature of the soils limits perennial crop production in most parts of the district. This together with traditional and cultural reasons has left a large area (approximately 51%) of the total arable land in the District under mismanaged pasture and

secondary bush. Much of the land adjacent to river courses is subject to regular flooding and is covered by bamboo, papyrus and other reeds.

Busia District has no game reserves or national parks. The rare incursion of any large African wildlife species into this area is met with a response designed to protect the local population and to prevent the destruction of crops. Wild birds are also rare in Busia District with the exception of the environs of Lake Victoria. The lake and, to a much lesser extent, its feeder river systems provide an ideal environment for hippopotamus, crocodiles and monitor lizards which can be found there. Small rodents, lizards and snakes are abundant in this District.

2.3.4 Soil type and crops

In the uplands of North Teso, Amukura, and the Samia hills the soil consists of thin to moderately deep sandy red clays of reasonable natural fertility with many rocks and stones. On the higher areas of the lowland plain the soils tend to be deeper brownish sandy clay soils of high natural fertility. The lowest lying lands are composed mainly of deep firm fine textured clays with a fairly high organic content. Despite being fertile these areas of land are frequently flooded. Most of the land in the central region of Busia falls within the high potential agro-ecological zone with the Southern region, nearest Lake Victoria being graded as of medium potential, graded on soil type and rainfall.

The main staple foods grown in Busia District are: maize (26.5% of all cultivated land), cassava, millet, sorghum, beans, groundnut, sweet potatoes, cow-peas, sesame and various varieties of bananas. These crops are consumed at subsistence level but are sometimes sold, thereby serving a dual purpose of food and cash crop. The main cash crops in this area are cotton and in the hilly northern areas tobacco with only a small areas given over to sugar cane as there is no nearby factory.

2.3.5 Human population

The ethnic origin of the people north of Busia town are mainly Teso (nilotic) and to the south of Busia town and to the east of Teso land the predominant tribe is Luhya (bantu) but there are also a number of Luo (nilotic) people. Intermarriage between the three main tribes of the

District is common. In Busia town representatives of most of the ethnic groups of Kenya and some of Uganda can be found.

Busia District's population was 200,486 in the 1969 census but by the next census in 1979 it had risen to 297,841 people (fig. 2.6) and from this base point it has been projected that the population would be 481,959 in 1993 and 498,871 in 1994, assuming an annual growth rate of 3.5%.

2.3.6 Animal population

In Busia District there has been a marked increase in the population of local East African Zebu cattle from 154,000 in 1987 to 177,000 in 1993 (Table 2.1). Although these cattle are officially classified as beef cattle by the Kenyan Department of Agriculture they are multifunctional performing in many roles e.g. draught power, local milk production and providing dung for fertilizer and building material. The reported offtake is 9.5%, which is probably an artificially high estimate due to importation of slaughter stock into the district, estimated to account for half of this figure. Natural pasture, estimated at 50,000 ha, provides most of the feed for these cattle with the addition of a few crop by-products.

The dairy sector utilising exotic cattle (Friesian) kept under zero grazing systems also recorded a steady growth in both numbers and output between 1987 and 1990 as did sheep, goats and poultry (Tables 2.1 and 2.2). Poultry are almost entirely free range and are ubiquitous throughout Busia District.

Pig production is carried out on a small scale mainly for the production of weaners which are sold to commercial units outwith the District. Back-yard keeping of rabbits and turkeys are also reasonably common.

Table 2.1 Livestock population in Busia District

Year	Dairy Cattle (exotic breeds)	Sheep	Poultry	Beef Cattle (indigenous breeds)
1987	1,930	22,500	366,440	154,000
1988	2,120	20,210	401,210	153,130
1989	2,120	24,100	413,790	166,160
1990	2,210	25,480	433,580	171,340

Source: District Livestock Office, Busia, Annual Report 1991

Table 2.2 Livestock Production per annum in Busia District (kg)

Year	Milk	Beef	Sheep	Poultry	Eggs
1987	7,437,750	1,815,440	193,750	72,000	12,840,000
1989	8,284,350	2,011,360	208,900	80,078	15,760,480

Source: District Livestock Office, Busia, Annual Report 1991

2.3.7 History of human sleeping sickness in Busia District between 1977 and 1992

Until the break up of the East African community in the mid 1970's, Kenya, Uganda and Tanzania shared a trypanosomiasis research institute, the East African Trypanosomiasis Research Organisation (EATRO)which was based near Tororo in Uganda where all sleeping sickness patients from this district were treated. Since the end of 1977 all sleeping sickness cases in Busia District were admitted to the Kenya Trypanosomiasis Research Institute (KETRI) sleeping sickness hospital at Alupe, 5 km north of Busia town. The incidence of human trypanosomiasis occurring in Busia District from 1977 to 1992 ranged from 0 to 88 cases per year (fig. 2.7). Over 75% of sleeping sickness cases occurred in the epidemic years of 1989 and 1990 with only sporadic cases in other years (fig. 2.7). Most cases came from South Teso, which includes the administrative divisions of Amogoro, Amukura and Nambale, the majority of the remainder coming from Funyula Division with relatively few cases from Butula Division in the east and Budalangi Division on the lake shore (figs. 2.8 and 2.2). There was no significant difference in the incidence of sleeping sickness between male and female cases ($p = 0.252$, Appendix II, Table II.1) however there was a significantly greater incidence in patients between the ages of 30 and 60 years than would be expected by chance and a significantly lower incidence in the under 10 year olds ($p < 0.001$, Appendix II, Table II.2) (fig. 2.9), probably reflecting differences in fly-contact between these groups.

2.3.8 Tsetse fly control in Busia District between 1977 and 1994

Over 80% of Busia District is tsetse infested ranging from the islands and lake shore along the river systems to the hilly hinterland. Two species of tsetse fly are found, *Glossina fuscipes fuscipes* and *Glossina pallidipes*. The former is the predominant species and is widely distributed in lacustrine and riverine habitats and has adapted to peri-domestic habitats in some areas while *G. pallidipes* is confined to the hilly hinterland mainly in the south of the District.

Prior to 1990, when regular systematic fly trapping was introduced, tsetse control was carried out on an ad hoc basis by bush clearance and ground spraying in villages and their surrounding areas where cases of sleeping sickness had occurred. Initially DDT (25%) and dieldrin (1.8%) were used but these were later replaced by cypermethrin (0.3%)

after the use of DDT and deildrin was banned by an Act of Parliament in 1983. The policy of bush clearing and ground spraying in and around any village where there has been a case of sleeping sickness was continuing in 1994.

Until 1984 sampling of the tsetse population was by a tsetse patrol with hand nets using a moving black target between two men wearing blue overalls as an attractant. Thereafter biconical traps (Challier and Lavissière, 1973) have been routinely used to sample the tsetse population and estimate the level of challenge.

Following the 1989-1990 sleeping sickness epidemic centred on Apatit and Katelenyang villages, systematic tsetse fly trapping has been carried out in many areas of the District using pyramidal Lancien traps (Gouteux and Lancien, 1986) impregnated with 0.1% deltamethrin (Glossinex®, ROUSSEL UCLAF) every 3 months. The traps are re-located along the river courses of the District every few weeks. Systematic fly trapping has dramatically reduced the tsetse population in the District by up to 99% (Table 2.3).

Regular meetings have been taking place between the Kenyan and the Ugandan authorities since 1992 to co-ordinate tsetse and trypanosomiasis control along the border region.

Table 2.3 Tsetse fly traps and targets deployed in various areas of Busia District from 1990 to 1994

Division	No sites	No traps*	No targets+	1990 ftd	1994 ftd
Amagoro	3	710	0	1.9	0.5
Amukura	4	1240	0	13.5	0.1**
Nambale	3	570	0	13.5	0.1
Funyula	4	750	350	6.0	0.3
Budalangi	3	425	0	10.5	1.4
Total	17	3695	350	8.0	2.3

* Lancien traps impregnated with deltamethrin
+ Black targets impregnated with deltamethrin and baited with cow urine, acetone and octenol deployed where there are thought to be *G. pallidipes*.
ftd number of flies caught per trap per day
** an apparent reduction in tsetse population by over 99%
Sourse: Tsetse control Section, Veterinary Department (Busia)

2.4 Discussion

Since Busia District is geographically similar to South East Uganda and forms part of a contiguous tsetse fly belt of *Glossina f. fuscipes*, it is not surprising that it is also an area endemic for Rhodesian sleeping sickness which shows occasional epidemics. The climate is always favourable for tsetse flies and the District's topography of low lying swampland forms an ideal habitat for *Glossina f. fuscipes* while the drier hilly areas may offer a habitat for smaller populations of *Glossina pallidipes*. Encroachment of secondary bush on land previously cleared for agriculture may offer sufficient vegetation cover to create a suitable peri-domestic habitat for tsetse flies. An increasing human and livestock population may lead to changes in land use practices as more land comes under cultivation. The resulting change in the vegetation pattern of the District may in turn affect the ecology of the tsetse population. Although it has been shown that regular tsetse fly trapping with insecticide impregnated traps could significantly reduce the apparent tsetse population, it has equally been shown that this reduction in population does not lead to eradication. This implies that the effects of trapping are likely to be temporary and the tsetse population would be expected to recover should trapping cease

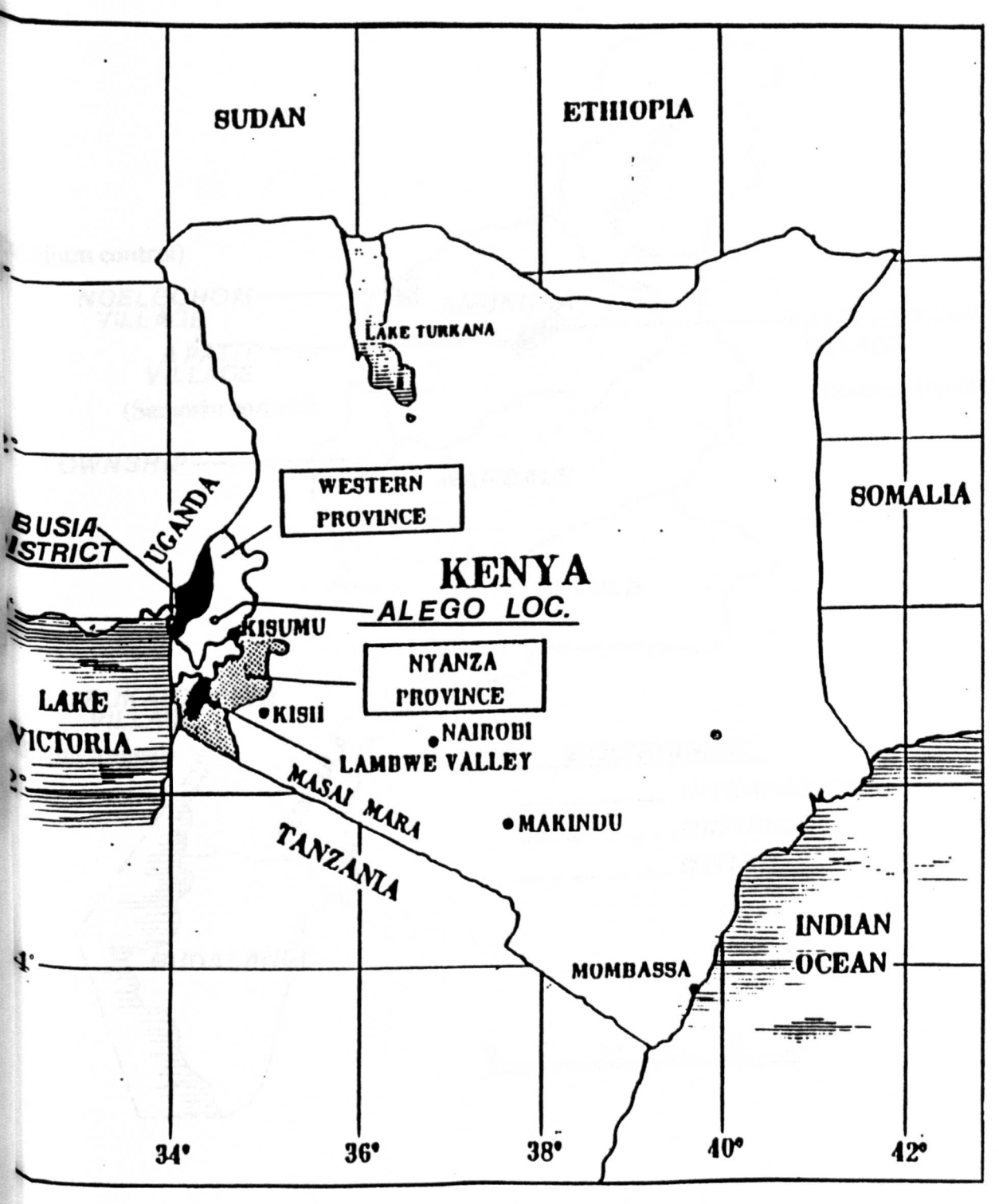


fig. 2.1 Location of sleeping sickness areas of Kenya

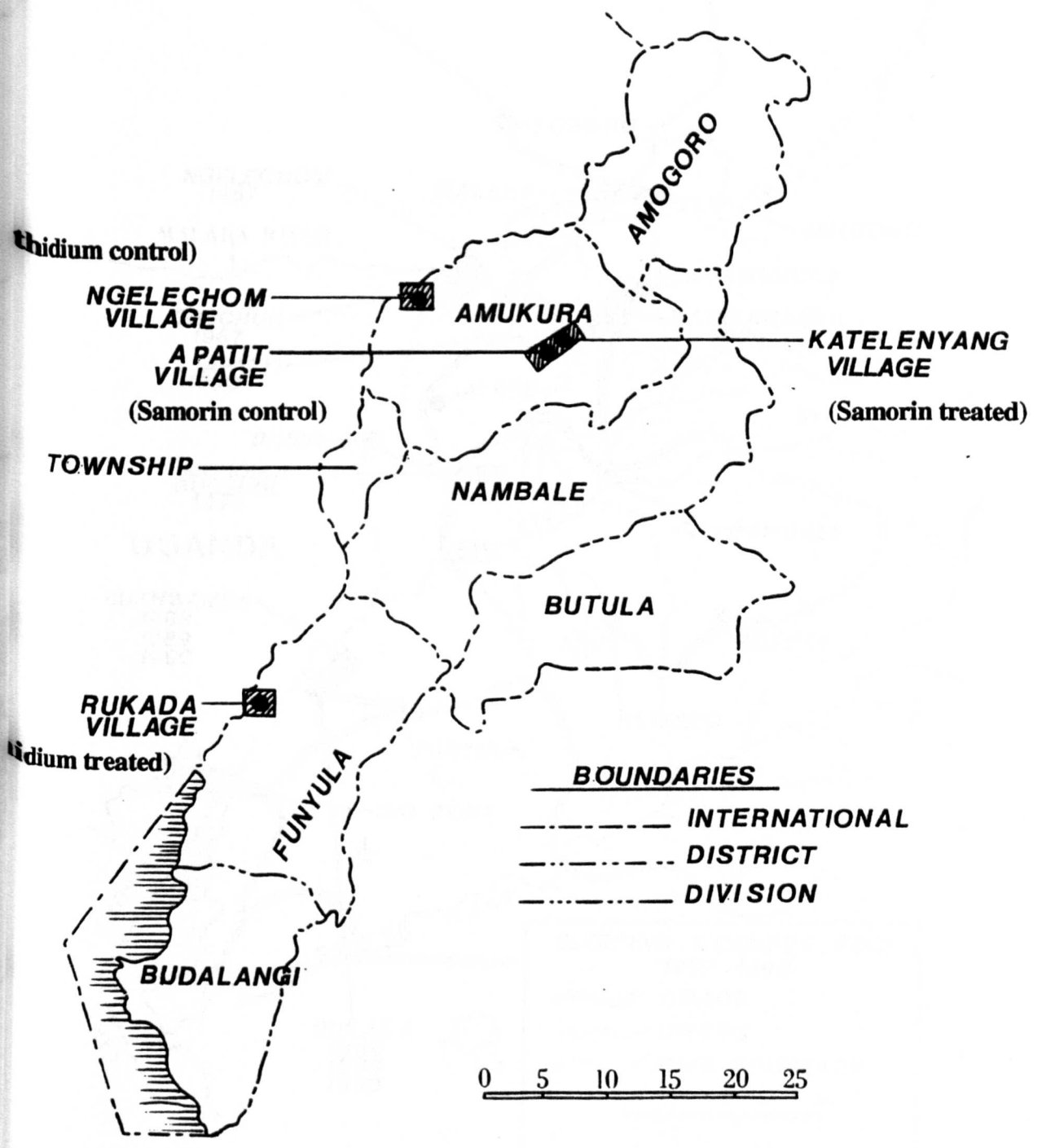


fig.2.2 Busia District administrative boundaries

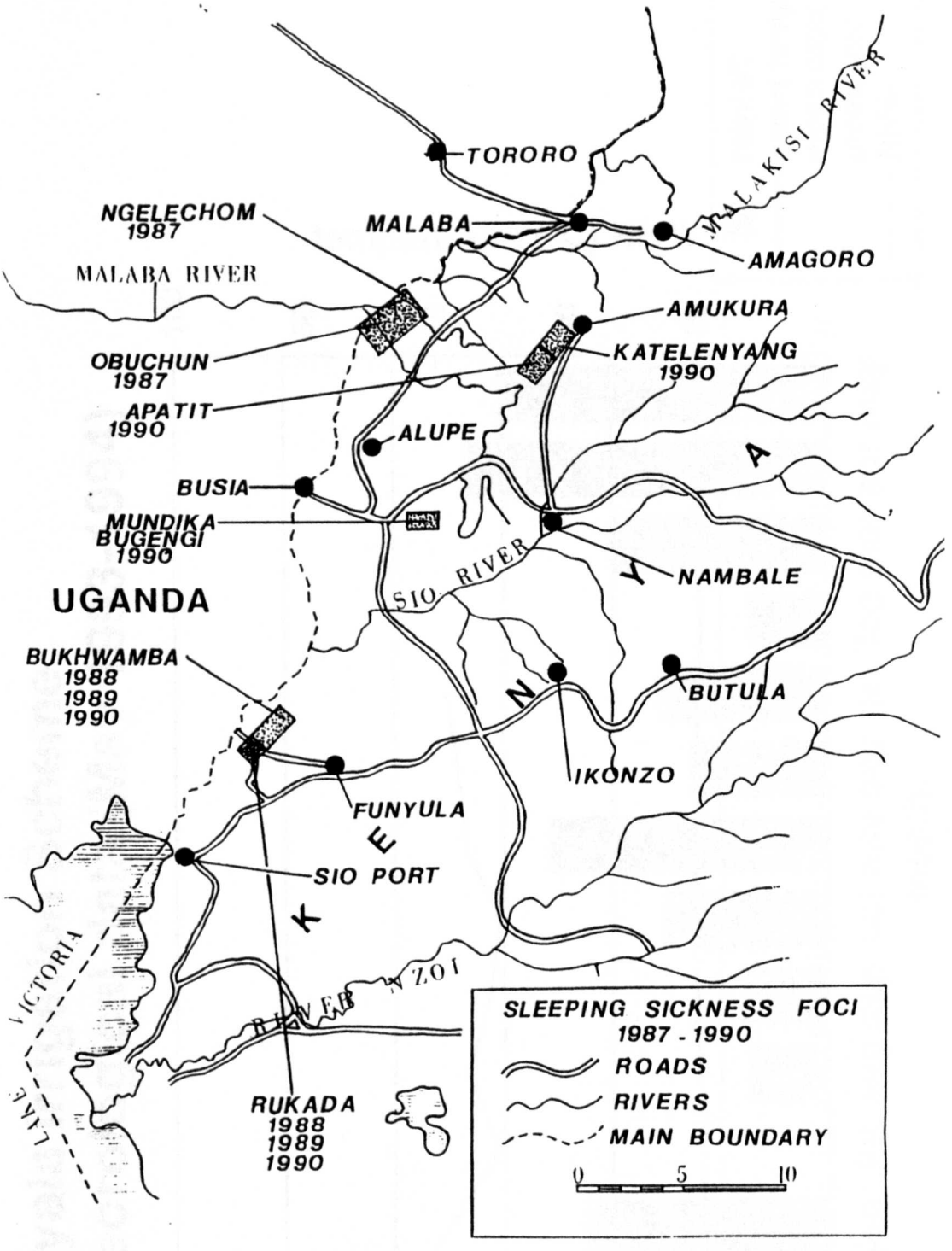


fig. 2.3 Sleeping sickness foci (1987-1990) Busia District

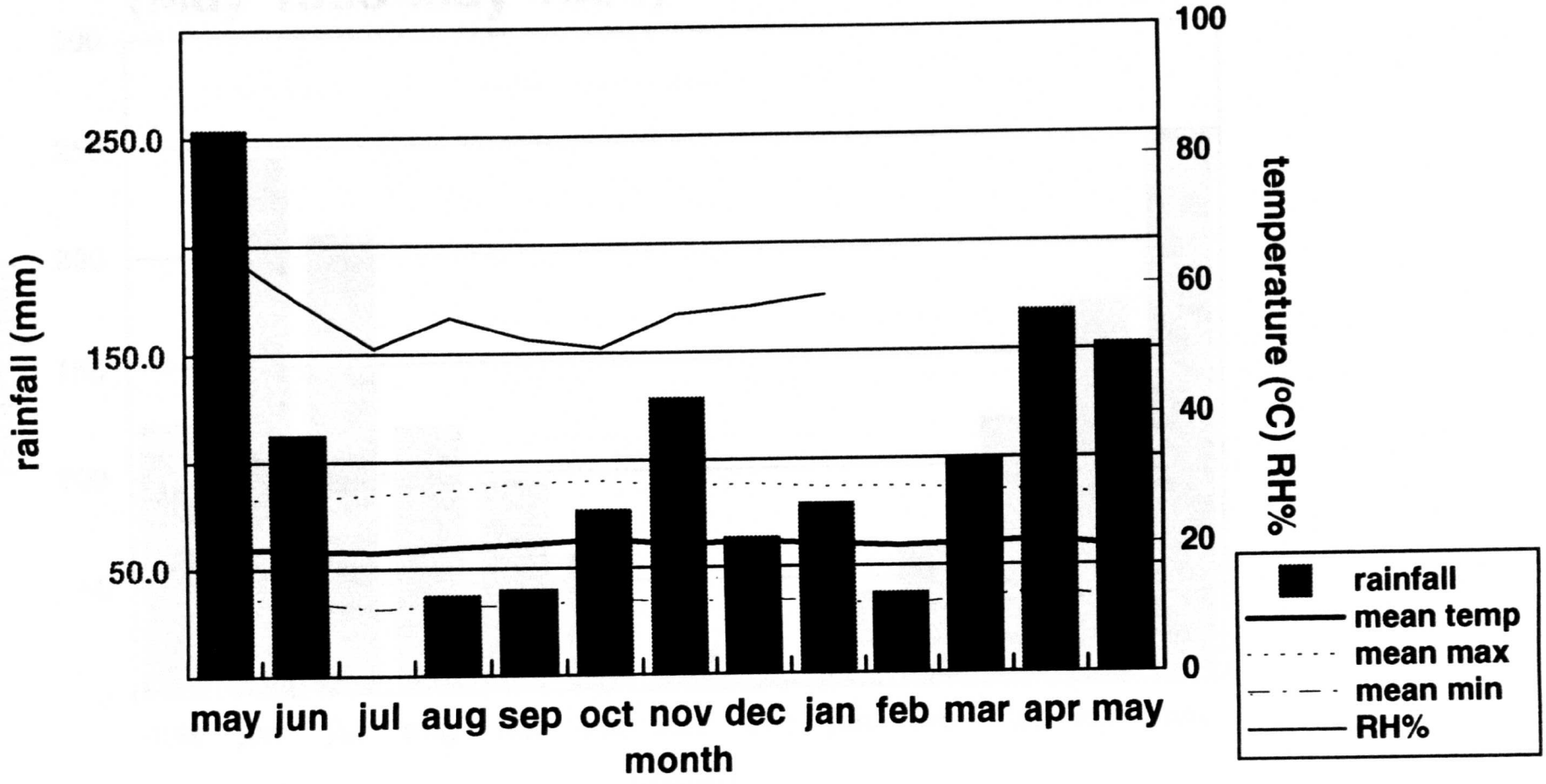

Bunyala Irrigation Scheme
meteorological data (May 1993-1994)
rainfall (mm)
250.0
150.0
50.0
100
80
60
40
20
0
temperature (°C) RH%
may jun jul aug sep oct nov dec jan feb mar apr may
month
rainfall
mean temp
mean max
mean min
RH%

fig. 2.4

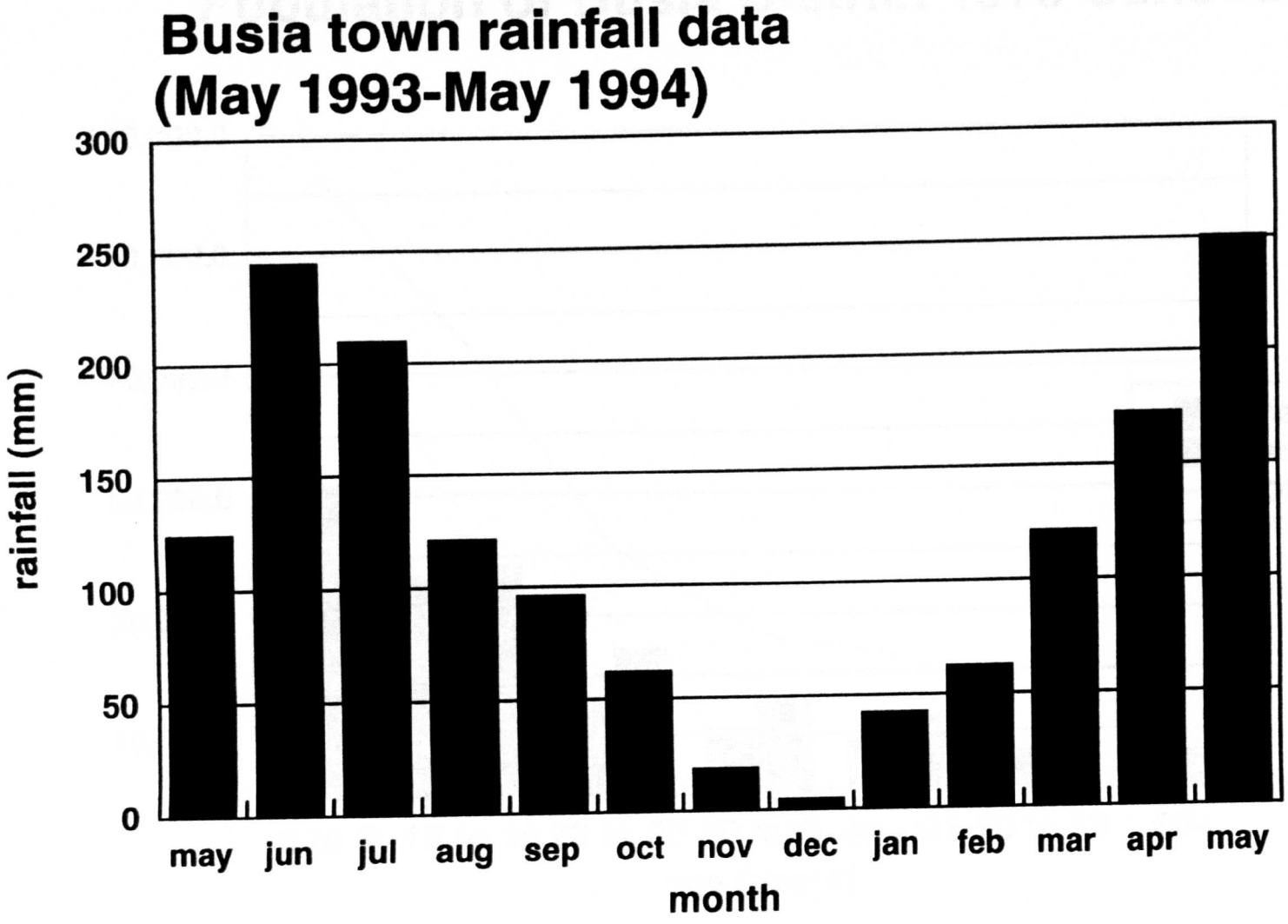

Busia town rainfall data
(May 1993-May 1994)
rainfall (mm)
300
250
200
150
100
50
0
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 2.5

Population of Busia District 1979 Census

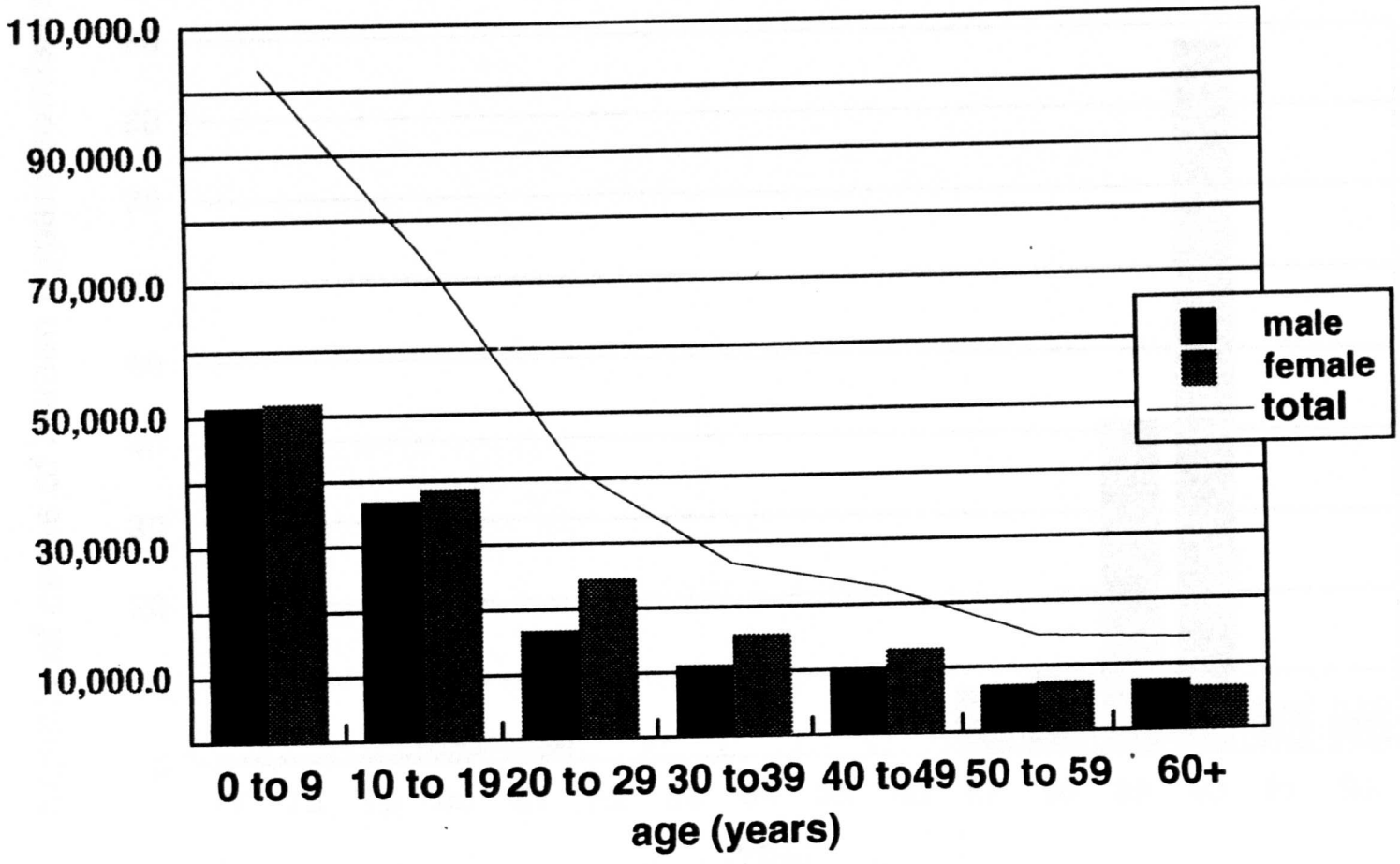


fig. 2.6

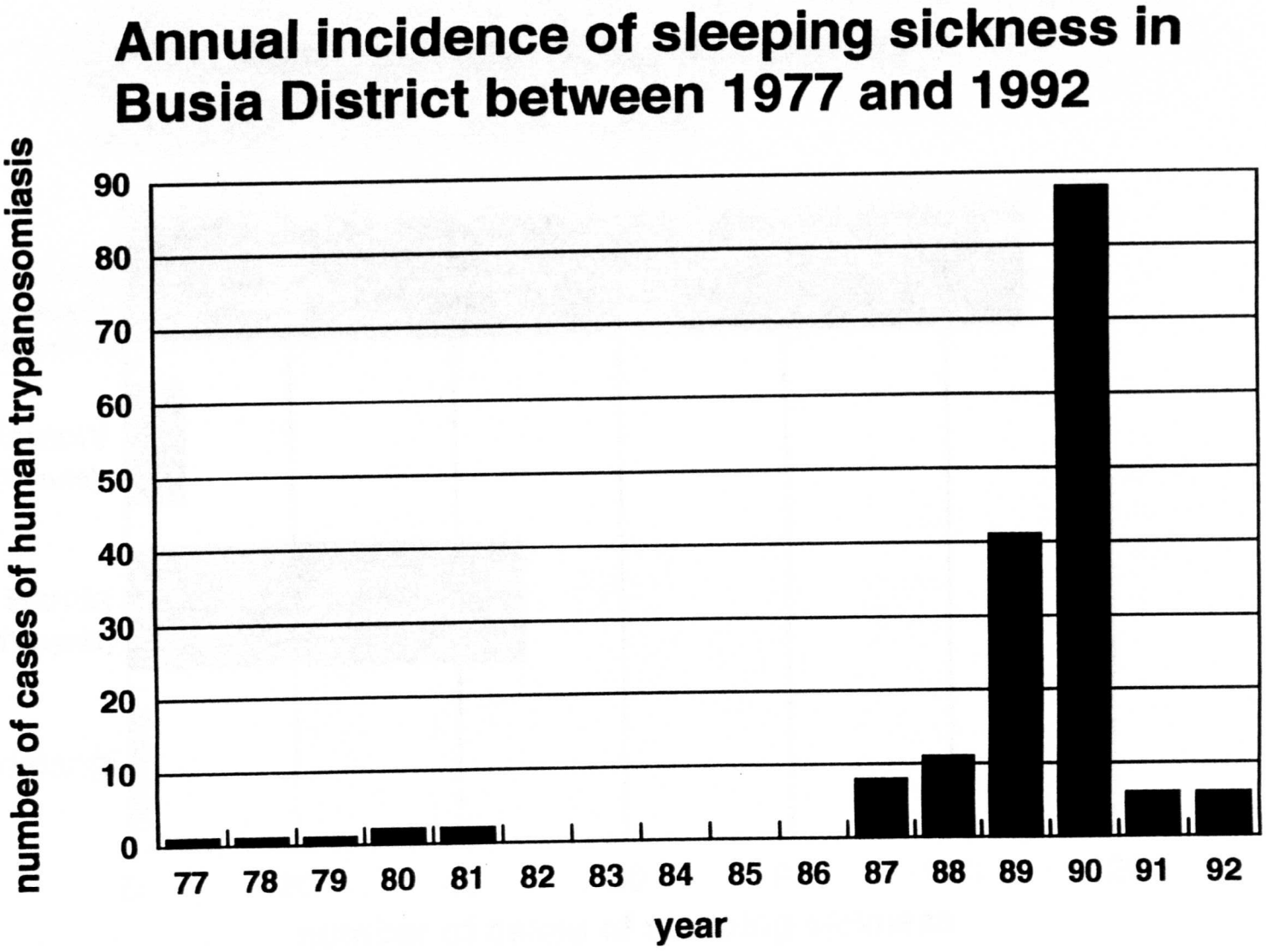

Annual incidence of sleeping sickness in Busia District between 1977 and 1992
number of cases of human trypanosomiasis
90
80
70
60
50
40
30
20
10
0
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
year

fig. 2.7

Number of new cases of sleeping sickness from each area of Busia District between 1977 and 1992

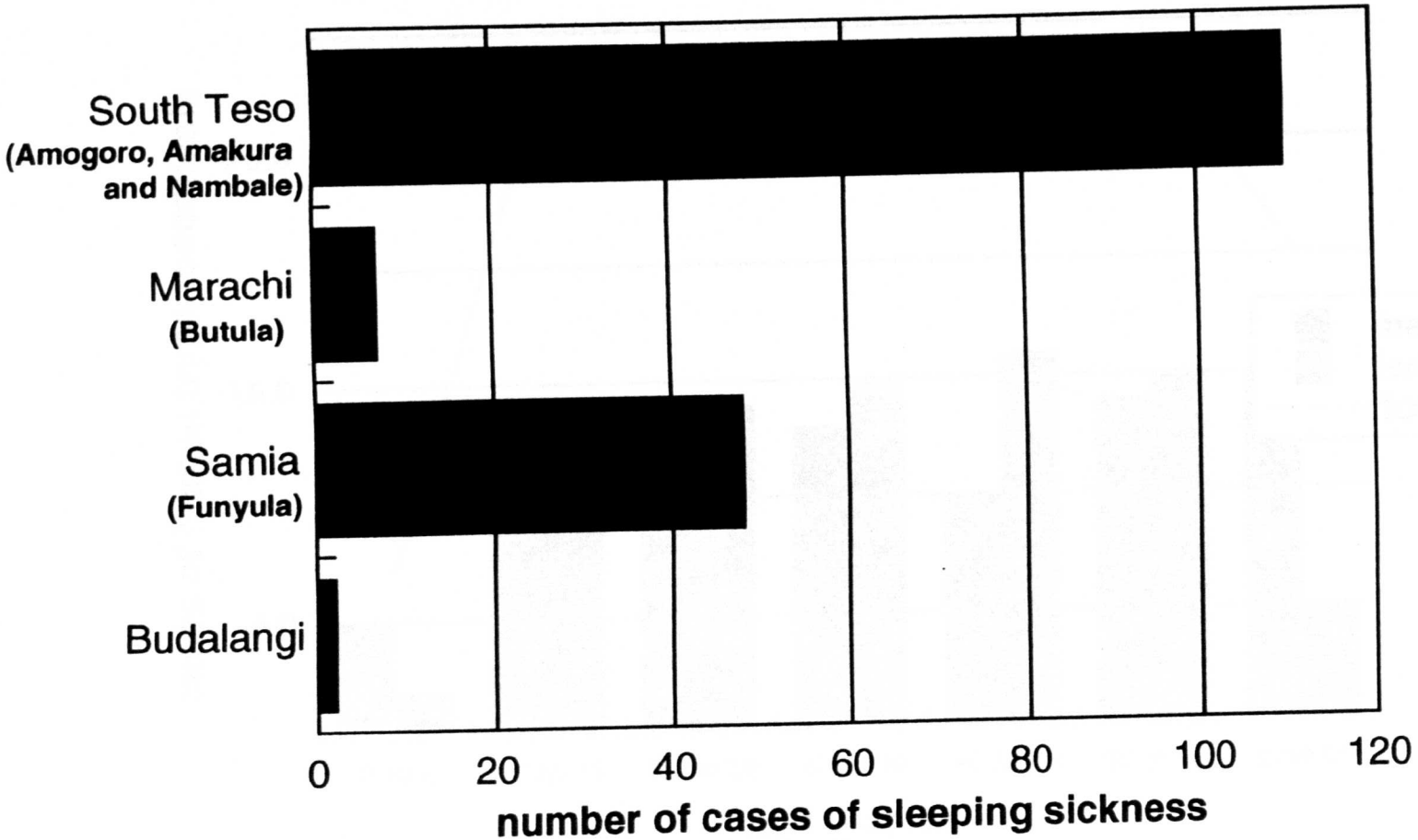


fig. 2.8

The distribution of sleeping sickness cases by age and sex in Busia District between 1977 and 1992

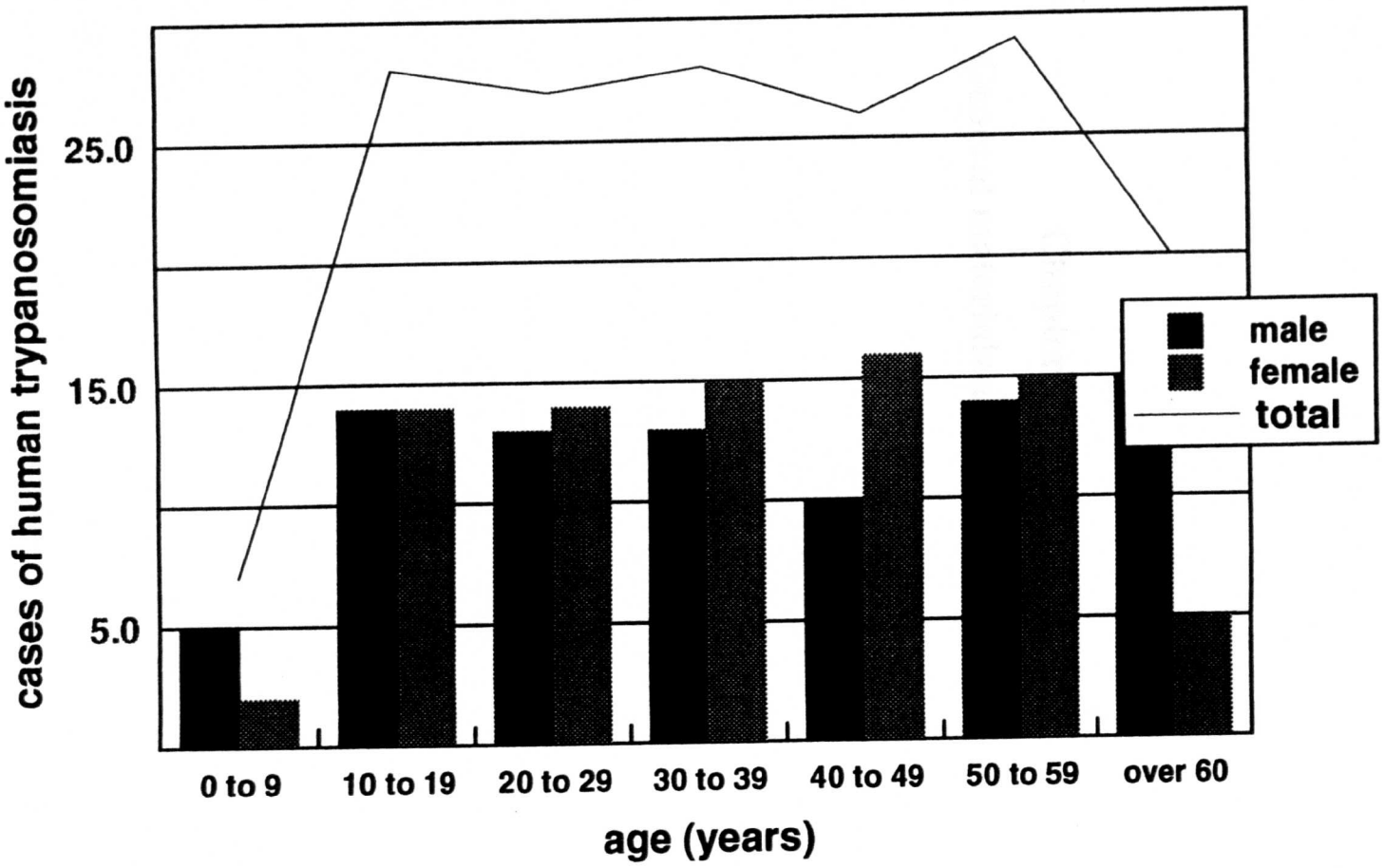


fig. 2.9

Chapter III

General materials and methods

3.1 Introduction

This chapter provides a broad description of all the standard materials and methods employed during the study. The chapter is divided into materials and methods used in the field, the laboratory, for epidemiological analysis and the analysis of data. Any adaptations of these standard methods are given in separate materials and methods sections at the beginning of each chapter.

3.2 Field methods

3.2.1 Village animals used

All animals sampled in the four villages were indigenous East African breeds of cattle, sheep and goats with the exception of one Ayrshire bull (N19) in Ngelechom village and a very small number of cross-bred cattle found in Rukada village (8 cattle) and Ngelechom (1 heifer). These animals were all kept by local smallholder farmers under traditional village husbandry practices. The pigs were cross- bred local types of pig with varying degrees of large white or landrace in their lineage.

3.2.2 Sample selection and animal identification

From an initial selection of 11 villages based on the occurrence of recent cases of human trypanosomiasis, four villages were subsequently selected on the basis of a high prevalence of *T. brucei* spp. in village livestock (survey January 1993).

Within each village a census of domestic livestock was carried out, and from this a random sample (using random number tables (Cannon and Roe, 1982)) of cattle, sheep, goats and pigs were selected. Each animal was given a plastic ear tag and individually numbered, including a letter code to indicate the village where it was sampled.

For each ear-tagged animal an individual record was taken of the species, age, sex, colour, name, sub-location within a village and owner.

The sample of livestock in each village was supplemented each month by recruiting a random sample of animals entering the village herd through purchases, gifts, loans or dowry

payments. The data from all recruited livestock were analysed from the second month onwards because the origin of any trypanosome infection diagnosed on first sampling would have been uncertain. In addition all new births to tagged cows were also tagged and monitored from the first month of sampling as these cattle had never been out of their village.

The sample size of cattle which were ear tagged for the epidemiological study was enumerated in March 1994 and is given in Table 3.1. This ranged from 94% in Katelenyang village (Samorin treated) to 44% in Ngelechom village (Ethidium control) with the other villages of Apatit (Samorin control) and Rukada (Ethidium treated) villages at 75% and 62% respectively.

Table 3.1 Number of cattle ear tagged for the epidemiological study

Village	Total number of village cattle	Number of cattle with ear tags	% cattle with ear tags
Katelenyang	300	282	94%
Apatit	310	232	75%
Rukada	393	244	62%
Ngelechom	672	296	44%
Total	1675	1054	63%

3.2.2 Blood sampling

All ear tagged animals were be bled at monthly intervals. Blood for diagnosis in the field was taken from the animal's ear vein (using a sterile lancet) in heparinised capillary tubes and sealed with Cristaseal ®. The capillary tube was then spun in a microhaematocrit centrifuge (Hawksley ®) at

12,000 rpm for 5 minutes. The packed cell volume (PCV), which is a measure of the volume of packed red blood cells expressed as a percentage of the total blood volume, was monitored using a microhaematocrit reader (Vickers, UK).

Blood for inoculation into mice, thin blood smears and making stabilates was collected from the jugular vein in 7 ml heparinised tubes (vacutainer ®, Becton and Dickinson, USA)

3.2.3 Lymph sampling

Lymph node aspirates were obtained by injecting sterile normal saline into a prescapular lymph node and subsequently aspirating the fluid (Chapter VI).

3.2.4 CSF sampling

Cattle, goats and sheep were sedated prior to sampling and all samples were taken aseptically. CSF samples from cattle were collected from the cisterna magna via the atlanto-occipital junction and samples from pigs, goats and sheep were collected from the lumbo-sacral junction.. The CSF was then allowed to drip into a sterile bottle under its own pressure (Chapter VIII).

3.2.5 Diagnosis of trypanosome infections in the field

Diagnosis of trypanosomiasis from blood samples in the field was carried out using the Haematocrit centrifugation technique (HCT) (Woo *et al.*, 1970); the buffy coat technique (BC), a modification of the dark ground buffy coat technique (DG) (Murray *et al.*, 1977) and by mouse inoculation.

The HCT used the same capillary tube following centrifugation and reading of the PCV, which was then placed on a glass slide which was prepared as follows: two rectangular pieces of glass 25 x 10 x 1.2 mm thick from a microscope slide were fixed 1.5 mm apart on a microscope slide. The capillary tube was placed in the groove formed by the rectangular glass pieces under a coverslip and the space between the slide and coverslip was flooded with water. By slowly rotating the tube, the buffy coat-plasma junction was examined using a Leitz field microscope (Leitz Wetzlar, Germany) and a 40x long working distance objective with the condenser diaphragm partially closed to reduce transmitted light. The use of the long working distance (6.7 mm) allowed

considerable depth of focus through the capillary, unlike a standard objective where the average working distance is approximately 0.5 mm.

After examination of the microhaematocrit tube by HCT the BC technique was used. The capillary tube was cut with a diamond pen 1 mm below the buffy coat to incorporate the uppermost layer of erythrocytes, and 1 cm above to include some plasma. By partially removing a thumb from the end of the capillary tube the contents of the tube were gently allowed to flow onto a clean slide, mixed and covered with a 22 x 22 mm coverslip. The preparation was examined using the same microscope arrangement as for HCT except for the 40x objective lens which was of standard focal length. A minimum of 20 microscope fields were examined.

Species identification for both the HCT and BC techniques was carried out using morphological and motility characteristics as described in Chapter I.

Buffy coat and thin blood smears were taken from all positive cases diagnosed in the field for confirmation of species identification in the laboratory.

Fresh heparinised blood (0.2 ml) from all animals with trypanosome infections diagnosed by the HCT or the BC techniques was inoculated intraperitoneally (i.p.) into immunosuppressed mice in the field. Similarly blood was taken and inoculated into mice from all animals with a PCV of 20 or less and animals which were positive for *T. brucei* spp. the previous month. One mouse was used for each sample.

For diagnosis of cerebral trypanosome infections 0.2 ml of CSF was inoculated intraperitoneally (i.p.) into immunosuppressed mice. Three mice were used fore each sample.

During one month in the village of Apatit, lymph node aspirate was inoculated intraperitoneally (i.p.) into immunosuppressed mice. One mouse was used for each sample.

3.2.6 Veterinary treatment

Whenever a pathogenic trypanosome infection (*T. brucei* spp., *T. vivax* or *T. congolense*) was detected in a domestic animal, which also had a PCV of 20 or less, it was treated with diminazine aceturate (Veriben ®, Sanofi Santé Animale, Canada inc.) at a dose rate of 7 mg/kg. Infected animals with a PCV greater than 20 were given a placebo injection of a diluted multivitamin solution (Parentrovite ®, Beecham Animal Health, UK). All animals with no detectable trypanosome infection and a PCV of 20 or less were treated with a single injection of

oxytetracycline (Adamycine ® 10%, Teva Pharmaceutical Industries Ltd, Israel.) at a dose rate of 10 mg/kg.

3.2.7 Block drug treatment

Block treatment of all domestic livestock (cattle, goats, sheep and pigs) using isometamidium chloride (Samorin®, Rhône Mérieux) at a dose rate of 1 mg/kg was carried out in Katelenyang village in October 1993 and repeated in January 1994. All imported livestock were treated as soon as was practical after their arrival in Katelenyang. The adjacent village of Apatit, with a similar prevalence of trypanosomiasis in domestic livestock, was used as a control.

Block treatment of all domestic livestock using homidium bromide (Ethidium®, Laprovet, France) at a dose rate of 1 mg/kg was carried out in Rukada village in November 1993 and repeated in January 1994. All imported livestock was treated as soon as was practical after their arrival in Rukada. The geographically similar village of Ngelechom, with a similar prevalence of trypanosomiasis in domestic livestock, was used as a control.

All block treated animals had their ear tags notched in such a manner as to indicate the month of treatment.

3.2.8 Animal production data

The weight and body score of each animal was recorded monthly at the time of blood sampling. Mortality and reproductive performance was also recorded at this time.
A second census of all livestock in each of the four villages was carried out in March 1994.

3.2.8.1 Liveweight

A transportable electronic weighing balance was used to weigh all animals in the study every month. A wooden base was placed on two weighing bars. These were attached to a digital recorder capable of weighing to the accuracy of 1 kg. This was powered by a 12V DC source supplied by a Landrover battery. The entire weighing apparatus was transported on each weighing session to cattle handling facilities which had been constructed at each of the four villages in the study to accommodate the weighing apparatus.

3.2.8.2 Body condition scoring

Scoring of body condition was carried out at the same time as weighing in order to investigate changes in body condition relating to trypanosome infections, anaemia and environmental factors e.g. season. The scoring method used was that described by Nicholson and Butterworth (1986), and was based on three categories of body condition (lean, medium and fat), which were further subdivided into three divisions producing a scale of nine possible scores. In order to maintain consistency under such a system, which inevitably involves some degree of subjective assessment, the same member of the KETRI field technical staff carried out the task of body condition scoring each month.

3.2.8.3 Economic data

Records were kept for all sales and purchases of livestock from each of the four villages during the 13 months of the study. This was cross referenced to price, village, date, sex, age, weight, and body score.

3.2.9 Fly trapping

Regular monthly monitoring of tsetse and biting flies was carried out in the four villages using 20 biconical traps (Challier and Laveissière, 1973) deployed along the river courses at 100m intervals for a period of 24 hours. The traps were checked regularly throughout this time and the species and number of each fly caught was recorded. All tsetse flies were classified by species, sex and whether they were teneral or not and then dissected to identify any trypanosome infections.

3.3 Laboratory methods

3.3.1 Identification of trypanosome species

Confirmation of trypanosome species was carried out in the laboratory using the thin blood films made in the field from all positive cases of trypanosome infections and all cattle with a PCV of 20% or less. In addition thin films were made from the buffy coats of all samples which were found with

trypanosome infections by the BC technique. These slides were fixed using methyl alcohol and stained with Giemsa stain before being examined by light microscopy using 40x and 100x oil immersion objective lenses (ILCA/ILRAD, 1983). Trypanosome species were identified by their morphology as described in Chapter I.

3.3.2 Diagnosis of trypanosome infections in the CNS

Samples were placed in a heat-sealed Pasteur pipette, spun in a centrifuge at 2000 rpm for 5 minutes. The pipette was then fixed to a glass slide with Cristaseal ® and any residue at the sealed end of the pipette examined microscopically for the presence of trypanosomes as for blood samples by HCT.

3.3.3 Subinoculation of mice

The mice used for diagnosis of trypanosomiasis in the field were inbred Swiss white albino BALB/C from ILRAD, Nairobi which had been immunosuppressed with 300 mg/kg cyclophosphamide (Endoxana®, Asta Medica, Cambridge) administered intraperitoneally 24 hours previously. One mouse was used for each sample.

For passage of trypanosome samples outbred Swiss albino mice from KETRI, Alupe, Busia, similarly immunosuppressed, were used.

During the experiment comparing different diagnostic techniques the two breeds of mice were used both immunosuppressed and non-immunosuppressed.

All mice were bled three times weekly from the tail and fresh wet blood films were examined for trypanosomes by light microscopy until 42 days after inoculation.

3.3.4 Cryopreservation

The mice were euthanased using chloroform and the trypanosomes were harvested by cardiac puncture using a heparinised syringe with a 25G needle. A 20% solution of glycerol in phosphate buffer was then added to the trypanosomes drop by drop to give a final concentration of glycerol of 10%. The glycerolated blood was then put into plain capillary tubes sealed with Cristaseal ® and put into plastic test tubes which had been pierced to allow the ingression of liquid nitrogen. These tubes were later transferred in an insulated container to the vapour phase of a liquid nitrogen container for 2 hours before being immersed in liquid nitrogen (ILCA/ILRAD, 1983).

3.3.5 Human trypanosome samples

The samples of trypanosomes from human patients were cryopreserved in an identical method to that described for the samples of animal origin. They were obtained from the KETRI trypanosome banks (Alupe and Muguga, Kenya) and were recent examples of trypanosomes from human patients from the four villages and closely surrounding areas where the animals were sampled.

3.3.6 Passage of trypanosomes in mice

The *T. brucei* spp. stocks collected throughout the study were passaged twice in mice in Kenya as a requirement of the Ministry of Agriculture Fisheries and Food, United Kingdom for importation into the UK. An immunosuppressed BALB/C mouse was used for the first passage followed by an immunosuppressed outbred Swiss white albino mouse (KETRI, Alupe). The imported stabilates were then passaged in immunosuppressed BALB/C mice in the United Kingdom to ensure their viability before feeding them to tsetse flies (*Glossina morsitans morsitans*) at the Tsetse Research Laboratory, Langford, Bristol).

3.3.7 Passage of trypanosomes in tsetse flies

The passaged stabilate (0.4 ml) was added to 1 ml of defibrinated horse blood. To this a 1.5 molar solution of D-glucosamine in normal saline was added to give a final concentration of D-glucosamine of 0.03 molar. This mixture was then fed to *Glossina morsitans morsitans* (5 Flies

per sample, FX9 colony flies, Tsetse Research Laboratory, University of Bristol) using an artificial membrane. The flies were dissected 10 days later when the midguts were removed from infected flies.

3.3.8 Culture of trypanosomes

The midgut from infected tsetse flies were then put in 100 ml of culture medium containing Cunnningham's medium (Cunningham, 1977), 10% foetal calf serum and 120μg gentamycin. When the procyclic trypanosomes started to divide, the culture medium was removed by pipette and replaced with culture medium containing 20μg gentamycin. The procyclic culture was incubated at 25°C throughout this period until the trypanosome count reached 10^8 trypanosomes per ml.

3.3.9 Preparation of DNA

The procyclic culture was centrifuged at 1500 g-force for 10 minutes, washed by re-suspending in PBS and spun down again (repeating twice). The trypanosomes were then lysed and their DNA extracted using the chloroform / isoamyl alcohol extraction process and dialysis as described by Hide (1996).

3.3.10 Southern blotting and cluster analysis

Analysis of DNA was carried out using restriction length polymorphism (RFLP) in repetitive DNA sequences of trypanosome genes using λ104, λ109 and pBE2 probes. These RFLP's are observed as ladders of bands on an autoradiograph. The banding patterns generated by each individual trypanosome stock can be used as unique "molecular fingerprints" to identify individual stocks. Cluster analysis using similarity coefficients derived from pairwise comparisons of banding patterns from trypanosome stocks was used to produce a dendogram illustrating the relatedness of trypanosome stocks (Hide, 1996).

3.4 Epidemiological methods

3.4.1 Prevalence

The prevalence of a condition is the proportion of the total population with that condition at a particular stated time. The prevalence of a condition is related to both its incidence and duration and is a measure of the total burden of a condition to a population (Wayne Martin *et al.*, 1987). During this study monthly point prevalence was used except in chapter VIII where an annual prevalence was stated.

3.4.2 Incidence

The incidence of a condition is the number of new cases that occur within a stated period as a proportion of the number of individuals at risk. During this study a monthly incidence was used and, except where discussed, it was assumed that the total animal population was at risk.

3.4.3 Diagnostic tests

Several useful criteria were used to evaluate differing diagnostic tests (Table 3.2).

Table 3.2 Parameters for the evaluation of a diagnostic test

test \ true result	infection present	no infection	total
test positive	a	b	a + b
test negative	c	d	c + d
total	a + c	b + d	a + b + c + d

Sensitivity

test sensitivity = a / (a + c)

The sensitivity of a diagnostic test is the proportion of truly infected individuals that is recognised as such by a particular diagnostic test. It is a measure of how good the test is at recognising infected animals.

Specificity

Test specificity = d / (b + d)

The specificity of a diagnostic test is the proportion of truly infection free individuals that is recognised by a particular diagnostic test. It is a measure of how good the test is at recognising uninfected animals.

True prevalence

True prevalence of infection = (a + c) / (a + b + c + d)

The true prevalence of infection is the actual prevalence of infection that exists.

Apparent prevalence

Apparent prevalence of infection = (a + b) / (a + b + c + d)

The apparent prevalence of infection is the prevalence of infection indicated by a particular diagnostic test.

Kappa value

The kappa value is used to compare diagnostic tests and is defined as the observed agreement between diagnostic tests minus the chance level of agreement divided by the maximum possible agreement beyond chance level. No agreement beyond chance gives a kappa value of 0 , and a kappa value of 1 indicates perfect agreement. A kappa value of at least 0.4 - 0.5 indicates a moderate level of agreement between diagnostic tests (Wayne Martin *et al.*, 1987).

3.5 Data analysis

Data analysis was carried out using standard statistical methods as described by Draper and Smith (1980); Mead and Curnow (1983) and Bailey (1985). The epidemiological application of statistical methods are discussed by Putt *et al.* (1987); Wayne Martin *et al.* (1987) and ILCA (1990). The standard methodologies are discussed with particular reference to problems associated with the analysis of field data from a large scale longitudinal (cohort) epidemiological study.

3.5.1 Computer software

Field records were digitally coded and entered directly from the animal record books onto an Excel ® (Microsoft, USA) spreadsheet. Most of the analysis was carried out using GLIM ® computer software program (Royal Statistical Society, London) including tabulation, regression analysis, and 2-tailed-t-tests; Chi^2 analysis was carried out using Minitab ® (Minitab Corporation, USA); Fisher's exact test was carried out using Epi Info 6 ® (CDC, USA) and the Graphics were produced using Stanford Graphics ® (Stanford Corporation, USA).

3.5.2 Contingency Tables

This method of analysis was used when comparing frequencies of events between two or more groups or time spans. The principle is based on a measure of the deviation of an observed rate from an expected rate based on an aggregate of comparable rates with the null hypothesis that there is no difference between group rates. This involves the calculation of an overall rate from the total of all group samples and events and the derivation of an expected rate from this for each group. A Chi^2 value was estimated as a measure of the difference between groups with n-1 degrees of freedom. This test statistic is represented by the equation:

$$X^2 = \Sigma (O - E)^2 / E$$

O = observed

E = expected

A Chi^2 test was used provided that the expected values were greater than 5. When expected values were less than 5 a Fisher's exact test was used to predict the probability (p) of an event occurring by chance (Bailey, 1985).

3.5.3 Analysis of covariance

PCV and live weight gain were analysed by Analysis of Covariance (GLIM statistical package, Numerical Algorithms Group, Oxford). The periods before and after chemoprophylaxis was administered were distinguished by one factor (referred to as 'period of prophylaxis'), and the main

effect of this factor, the main effect of village, and the interaction of the two factors, were investigated by fitting them in regression models of increasing complexity. The drop in residual variance at each step (measured using an 'F' ratio, whose denominator was the residual variance of the complete model) was used to judge the effect of each parameter added to the model. The drop in residual deviance, as a percentage of overall deviance, was calculated for each step of model fitting. When investigating the importance of factors other than sex, age, month of sampling and village, affecting the monthly weight change of cattle the residual deviance after accounting for these factors was used. This removes the effects of sex, age, season and village on the liveweight change of cattle from subsequent analyses.

Considering the size of the data base many factors proved statistically significant despite explaining significantly less than one per cent of the residual deviation. While these factors cannot be ignored, only factors explaining more than one per cent of the residual deviation were considered of major importance.

3.5.4 Comparison of means

Comparison of the mean PCV and monthly liveweight change of cattle was carried out in order to estimate the magnitude of the effect caused by any factor found to have a significant effect on these variables by analysis of covariance e.g. infection with differing trypanosome species. The significant of any difference in the mean PCV and monthly liveweight change in any two groups of cattle was tested using a 2- tailed-t -test (Bailey, 1985).

Chapter IV

The prevalence and incidence of trypanosome infections in cattle and their effect on productivity in four villages in Busia District before and after the use of prophylactic drugs to control the potential human reservoir of *T. brucei* spp. infections

4.1 Summary

A longitudinal epidemiological study was carried out in Busia District in the Western Province of Kenya, an endemic area of Rhodesian sleeping sickness, to examine the importance of domestic livestock as a potential reservoir for human infection. The study was carried out in 4 villages over a 13 month period. After collecting 6 months base line data the villages were paired, with one village in each pair acting as a control, for comparative drug trials using the chemoprophylactic trypanocidal drugs, isometamidium chloride (Samorin ®) and homidium bromide (Ethidium ®). Block treatment of all livestock (cattle, sheep, goats and pigs) was carried out in each of the trial villages at a dose rate of 1 mg/kg and all untreated livestock entering these villages during the trial period were treated with the same drug. In the control villages no prophylactic drugs were given although clinical cases of trypanosomiasis were treated with diminazene aceturate.

In the village treated with Samorin it took up to 7 days to clear animals of pathogenic trypanosome infections and *T. theileri* and microfilaria remained present during this period. However re-sampling these animals 7 to 8 weeks later revealed no trypanosome infections, only microfilaria. Samorin was 98% effective as a prophylactic in cattle against all trypanosomes at 10 weeks post treatment, 94% at 12 weeks, 81% at 14 weeks and 51% at 17 weeks. A significant increase in the mean PCV and liveweight gain of cattle was seen during the period of prophylaxis.

Ethidium was effective at curing infections with *T. vivax* and *T. congolense* and *T. brucei* but was much less effective than Samorin as a prophylactic. Despite very low tsetse challenge, breakthrough infections in the Ethidium group were seen at 3 and 4 weeks post treatment and *T. theileri* and microfilaria infections were seen throughout the period of prophylaxis. Because of the very low level of challenge it was only possible to estimate the degree of Ethidium prophylaxis. This was estimated to be 85% at 3 to 4 weeks and at a negligible level at 8 weeks post treatment. Ethidium prophylaxis did not significantly affect either mean PCV or liveweight gain of cattle compared to the control village.

4.2 Introduction

A longitudinal epidemiological study was carried out in Busia District in the Western Province of Kenya, an area where Rhodesian sleeping sickness is endemic, to examine the importance of domestic livestock as a potential reservoir for human infection. The study was carried out in 4 villages over a 13 month period from May 1993 to May 1994, with the same random sample of livestock being blood sampled each month. After collecting 6 months base line data the villages were paired, with one village in each group acting as a control, for comparative drug trials using the chemoprophylactic trypanocidal drugs, isometamidium chloride (Samorin®) and homidium bromide (Ethidium®). While it is well known that these drugs have a prophylactic effect in cattle against infection by *T. vivax* and *T. congolense*, prophylaxis against *T. brucei* spp. infection has usually been considered to be of lesser importance (Dolan *et al.*, 1990) being seen as only a minor pathogen of cattle (Losos and Ikede, 1972). Block treatment of all livestock (cattle, sheep, goats and pigs) was carried out in each of the trial villages at a dose rate of 1 mg/kg and all untreated livestock entering these villages during the trial period were treated with the same drug.

Control of Rhodesian sleeping sickness in the recent past has relied on treatment of human patients and vector control. Methods to control the vector have included the application of insecticides either by ground or aerial spraying, the use of traps and targets with or without odour baits or impregnation with insecticide and sterile insect release (Brandl, 1988). For vector control to be successful the reduction in the tsetse fly population has to be in excess of 90% before the transmission rate of the disease is reduced to a level which significantly affects the incidence of trypanosomiasis (Okoth *et al.*, 1991; Laveissière and Meda, 1992). During a sleeping sickness epidemic, which often occurs as a result of great social upheaval (Koerner *et al.*, 1995), it may not be possible for logistical reasons for this level of control to be achieved.

It has been known that domestic cattle can act as a reservoir for *T. b. rhodesiense* for some time (Onyango *et al.*, 1966), however only recently has it been possible to investigate the importance of this reservoir more fully with the advent of a molecular technique (restriction fragment length polymerisation, RFLP) which can reliably differentiate *T b. brucei* from *T. b. rhodesiense* (Hide *et al.*, 1990). Blood meal analysis carried out towards the end of the most recent epidemic of sleeping sickness in the Busoga District of Uganda

showed that the major tsetse hosts were monitor lizards (59%) and domestic cattle (23%) while humans provided only 7% of blood meals (Welburn *et al.*, 1995). At the same time the incidence of sleeping sickness in the human population was 0.6% and the prevalence of *T. brucei* spp. in domestic cattle was 4.9% over the entire District and up to 51% in some isolated villages (Maudlin *et al.*, 1990c). However only one in four of the *T. brucei* spp. infections in cattle were found to be human infective *T. b. rhodesiense*, the remainder being human serum sensitive *T. b. brucei* (Hide *et al.*, 1991). From this information it can be estimated that the likelihood of a human becoming infected by a tsetse fly which has itself been infected by feeding on an infected cow is up to 50 times greater than the fly being infected from an infected human. Similarly mathematical modelling of sleeping sickness has predicted that the low value of the basic reproductive rate of the disease in the human population means that infection levels in humans are determined almost entirely by characteristics in the non-human reservoir and that a greater reduction in human disease may be achieved by treating the animal reservoir rather than human beings (Rogers, 1988). Although flies remain infected for life their mean lifespan is estimated to be between 29 days (Phelps and Vale, 1978) and 76 days (Glasgow, 1963) and if emergent flies feed on uninfected hosts during their lifetime then the transmission cycle of the disease would be broken. Furthermore if infected tsetse flies feeding on cattle with sufficiently high levels of Samorin in their blood stream this will kill trypanosomes developing in the vector, and this helps to further reduce the infection rate in tsetse flies and so reduce the transmission of the disease (Agu, 1984 ; Jeffries and Jenni 1987).

The level of tsetse challenge during the study was measured by tsetse fly trapping and the 'Berenil index'. The 'Berenil index' is an indirect measure of tsetse and trypanosome challenge as measured by the mean annual number of treatments with a curative trypanoside in a herd of cattle (Whiteside, 1962a) (Chapter III).

The differences in the productivity of cattle in the four villages was estimated by comparing the monthly weight change and PCV of cattle in the treated and untreated control villages.

4.3 Materials and methods

For detailed materials and methods see Chapter III.

4.3.1 Experimental design

In the village of Katelenyang block treatment of all cattle, goats, sheep and pigs using isometamidium chloride (Samorin®, Rhône Mérieux, France) at a dose rate of 1 mg/kg was carried out in October 1993 and repeated 14 weeks later in January 1994. Thereafter the village was monitored monthly until 17 weeks after the second treatment. All imported livestock were treated as soon as was practical after their arrival in the village. The adjacent village of Apatit, with a similar prevalence of trypanosomiasis in domestic livestock, was used as a control and treatment was limited to clinical cases of trypanosomiasis using diminazene aceturate (7 mg/kg).

In the village of Rukada block treatment of all cattle, goats, sheep and pigs using homidium bromide (Ethidium®, Laprovet, France) at a dose rate of 1 mg/kg was carried out in November 1993 and repeated 8 weeks later in January 1994. Thereafter the village was monitored until 17 weeks after the second treatment. All imported livestock was treated as soon as was practical after their arrival in the village. The geographically similar village of Ngelechom, with a similar prevalence of trypanosomiasis in domestic livestock, was used as a control village where treatment with diminazene aceturate (7 mg/kg) was restricted to clinical cases as in the other control village (Apatit).

4.3.2 The prevalence and incidence of trypanosome infections in cattle

All cattle were blood sampled monthly and these samples were examined in the field using the haematocrit centrifugation technique (HCT) and the buffy coat technique (BC). All blood samples containing trypanosomes and those from cattle with a PCV of 20 or less were subinoculated into immunosuppressed mice, also in the field. Thin blood films were taken from all samples inoculated into mice and taken to the laboratory where they were fixed and stained with Geimsa stain and used to aid species identification of trypanosomes. The prevalence of trypanosome infections in cattle was calculated on a monthly basis as the number of cattle with trypanosome infections as a percentage of the number of cattle sampled. The incidence of trypanosome infections in cattle was similarly calculated on a monthly basis but as the number of cattle with a new trypanosome infection as a percentage of cattle sampled (Chapter III).

4.3.3 Analysis of the PCV of cattle

The packed cell volume (PCV) of cattle blood sampled was measured (as described in Chapter III) and the effect of chemoprophylaxis on cattle PCV was estimated by calculating the mean PCV of each village herd of cattle before and after treatment. The mean PCV of the village cattle herds in the treated villages of Katelenyang (Samorin) and Rukada (Ethidium) were compared to their respective control villages of Apatit and Ngelechom. Statistical analysis was carried out using a two tailed t-test and stepwise fitting of regression models as described in Chapter III.

4.3.4 Analysis of the monthly liveweight change of cattle

All the village cattle from which blood samples were obtained were also weighed each month (as described in Chapter III) and the effect of chemoprophylaxis on cattle weight was estimated by calculating the mean monthly liveweight gain of each village herd of cattle before and after treatment. The mean monthly liveweight gain of the village cattle herds in the treated villages of Katelenyang (Samorin) and Rukada (Ethidium) were compared to their respective control villages of Apatit and Ngelechom. Statistical analysis was carried out using a two tailed t-test as described in Chapter III. These analyses rely on the assumption that the composition of each sample of village cattle was comparable between the respective treated and control villages (see sample selection Chapter III).

4.4 Results

4.4.1 Prevalence of trypanosome infections of cattle in Katelenyang and Apatit villages before and after treatment with Samorin

The prevalence of trypanosome infections in cattle in Katelenyang (Samorin treated) and Apatit (Samorin control) villages varied throughout the year, tending to be higher following the long rains from March to June and lower in the drier months of November and December (see Chapter II). Before the treatment with Samorin in Katelenyang, the prevalence of trypanosomiasis in the cattle was always higher than in the control village Apatit with *T. vivax* being the most common infection found followed by *T. brucei* spp.. *T. congolense* was much

less common than the other two species of trypanosomes for most of the year. Following treatment with Samorin the prevalence dropped to zero for a number of months with no breakthrough infections of *T. brucei* spp. within 12 weeks of treatment, while the prevalence of trypanosomiasis in Apatit (control) was never less than 15% (figs. 4.1 and 4.2).

The prevalence of mixed infections (concurrent infections with two or more species of trypanosome) mirrored the total prevalence of trypanosome infections (figs. 4.1 and 4.2). However, all mixed infections occurred at a much greater frequency than would be expected by chance (see Chapter V).

4.4.2 Incidence of trypanosome infections of cattle in Katelenyang and Apatit villages before and after treatment with Samorin

The incidence of trypanosome infections in Katelenyang (Samorin treated) and Apatit (Samorin control) villages closely followed the same pattern of monthly change as the prevalence of trypanosome infections but at a lower level. *T. vivax* was the most common new infection in all but one month, with *T. brucei* spp. being the next most common species in every month except October and November when the incidence of *T. congolense* was at its highest (figs. 4.5 and 4.6).

The incidence of mixed infections (concurrent new infections with two or more species of trypanosome) mirrored the total incidence of trypanosome infections (figs. 4.5 and 4.6) and occurred at a much greater frequency than would be expected by chance (see Chapter V).

4.3.3 The relative prevalence of infections in cattle with the three species of pathogenic trypanosomes in Katelenyang and Apatit villages before and after the time of Samorin prophylaxis

The proportion of all trypanosome infections in cattle which involved a *T. brucei* spp. infection in Katelenyang and Apatit villages was not significantly different before the prophylactic treatment of all cattle in Katelenyang village with Samorin ($X^2 = 0.30$, df = 1, $p = 0.58$) (Appendix IV, Table IV.1). However following Samorin prophylaxis the proportion of all trypanosome infections which involved a *T. brucei* spp. infection was significantly less in

Katelenyang village (Samorin treated) than Apatit village (Samorin control) ($X^2 = 3.84$, df = 1, p = 0.05) (Appendix IV, Table IV.2).

The proportion of all trypanosome infections in cattle which involved a *T. vivax* infection before Samorin prophylaxis was significantly higher in Katelenyang village (Samorin treated) than Apatit village (Samorin control) ($X^2 = 9.99$, df = 1, p = 0.002) (Appendix IV, Table IV.3). However there was no significant difference in the proportion of all trypanosome infections which involved a *T. vivax* infection between the two villages during the time of Samorin prophylaxis ($X^2 = 2.41$, df = 1, p = 0.12) (Appendix IV, Table IV.4).

There was no significant difference in the proportion of all trypanosome infections which involved a *T. congolense* infection between Katelenyang village (Samorin treated) and Apatit village (Samorin control) either before ($X^2 = 1.61$, df = 1, p = 0.21) (Appendix IV, Table IV.5) or after the time of Samorin prophylaxis ($X^2 = 2.42$, df = 1, p = 0.12) (Appendix IV, Table IV.6).

4.4.4 Prevalence of trypanosome infections of cattle in Rukada and Ngelechom villages before and after treatment with Ethidium

The prevalence of trypanosome infections of cattle in Rukada (Ethidium treated) and Ngelechom (Ethidium control) villages varied throughout the year, tending to be higher following the long rains from March to June and lower in the drier months of November and December (see Chapter II). Before the treatment with Ethidium in Rukada the prevalence of trypanosomiasis in cattle was similar to the control village Ngelechom (fig. 4.3 and 4.4). In Ngelechom (Ethidium control) the most common infection was always *T. vivax*, followed by *T. brucei* spp. and *T. congolense* was the least prevalent. In contrast the prevalence of *T. brucei* spp. in Rukada village (Ethidium treated) was very similar to that of *T. vivax*. Following treatment with Ethidium the prevalence of trypanosome infections dropped significantly for a number of weeks although the first breakthrough infections of *T. brucei* spp. were observed within four weeks of treatment (fig 4.3). During this period the prevalence of trypanosome infections in cattle in Ngelechom (Ethidium control) started to rise from February onwards (fig. 4.4) after an early start to the long rains (see Chapter II), whereas there was a very late start to the long rains in Rukada village (Ethidium treated).

The prevalence of mixed infections (concurrent infections with two or more species of trypanosome) mirrored the total prevalence of trypanosome infections (figs. 4.3 and 4.4). However, all mixed infections occurred at a much greater frequency than would be expected by chance (see Chapter V).

4.4.5 Incidence of trypanosome infections of cattle in Rukada and Ngelechom villages before and after treatment with Ethidium

The incidence of trypanosome infections in Rukada (Ethidium treated) and Ngelechom (Ethidium control) villages closely followed the same pattern of monthly change as the prevalence of trypanosome infections but at a lower level. *T. vivax* was the most common new infection in all but one month, with *T. brucei* spp. being the next most common species and *T. congolense* being the least common. The incidence of *T. vivax* in Rukada following the second treatment with Ethidium was zero for three months with no new *T. vivax* infections being detected until four months post treatment (figs. 4.7 and 4.8).

The incidence of mixed infections (concurrent new infections with two or more species of trypanosome) mirrored the total incidence of trypanosome infections (figs. 4.7 and 4.8) and occurred at a much greater frequency than would be expected by chance (see Chapter V).

4.4.6 The relative prevalence of infections in cattle with the three species of pathogenic trypanosomes in Rukada and Ngelechom villages before and after the time of Ethidium prophylaxis

The proportion of all trypanosome infections in cattle which involved a *T. brucei* spp. infection in Rukada village (Ethidium treated) was significantly greater than that of Ngelechom village (Ethidium control) both before ($X^2 = 20.50$, df = 1, $p < 0.001$) (Appendix IV, Table IV.7) and after ($X^2 = 4.69$, df = 1, $p = 0.03$) (Appendix IV, Table IV.8) the time of prophylactic treatment.

The proportion of all trypanosome infections in cattle which involved a *T. vivax* infection in Rukada village was significantly less than that of Ngelechom village both before

(X^2 = 19.90, df = 1, $p < 0.001$) (Appendix IV, Table IV.9) and after (X^2 = 45.17, df = 1, $p < 0.001$) (Appendix IV, Table IV.10) the time of prophylactic treatment.

There was no significant difference in the proportion of all trypanosome infections which involved a *T. congolense* infection between Rukada village and Ngelechom village before the time of Ethidium prophylaxis (X^2 = 2.09, df = 1, p = 0.148) (Appendix IV, Table IV.11). However during the time of prophylaxis the proportion of all trypanosome infections which involved a *T. congolense* infection was significantly greater in Rukada village (Ethidium treated) compared to Ngelechom village (Ethidium control) (X^2 = 18.22, df = 1, $p < 0.001$) (Appendix IV, Table IV.11).

4.4.7 Therapeutic efficacy of Samorin and Ethidium treatment in cattle

Both Samorin and Ethidium were effective at curing infections of *T. brucei* spp. , *T. vivax* and *T. congolense* in cattle but only Samorin cleared infections with *T. theileri.* In the village treated with Samorin (Katelenyang), it took up to 10 days to clear animals of pathogenic trypanosomes. *T. theileri* and microfilaria remained present during this period, however re-sampling these animals 7 to 8 weeks later revealed no trypanosomes, only microfilaria.

4.4.8 Prophylactic efficacy of Samorin and Ethidium treatment in cattle

Samorin gave over 90% prophylactic protection up to 12 to 14 weeks post treatment (Table 4.1, figs. 4.1 and 4.5). Continued monthly monitoring of the effect of prophylaxis up to 17 weeks after the second treatment showed that there was a very significant reduction in the number of cases of trypanosomiasis which had to be treated compared to the period before prophylaxis (Table 4.2; Appendix IV, Tables IV.14 to IV.17 and fig. 4.9 and 4.10).

Table 4.1 Incidence of trypanosomiasis (%) during Samorin prophylaxis

village	Katelenyang	Apatit	apparent prophylaxis [1]
weeks post treatment	Samorin	control [3]	
10	0.4 (n=197)	19.3 (n=181)	97.9%
12	0.8 (n=244)	12.6 (n=197)	93.7%
14	5.1 (n=226)	26.3 (n=205)	80.6% [2]
17	9.4 (n=197)	19.3 (n=181)	51.3%

[1]The apparent prophylaxis is the observed incidence (in Katelenyang) as a proportion of the predicted incidence (based on the control village).

[2] Samorin gives a significant level of prophylaxis up to 14 weeks post treatment.

[3] Control village not given any prophylactic drug.

Table 4.2 Comparison within each village of the number of curative trypanocide treatments per number of animals sampled before and after prophylaxis ('Berenil index')

village	treatment	Chi^2	d.f.	p
Katelenyang	Samorin	90.5	1	< 0.001 ***
Apatit	control [3]	5.4	1	0.020 *
Rukada	Ethidium	0.133	1	0.715
Ngelechom	control [3]	0.079	1	0.779

*** There is a highly significant reduction in the number of doses of trypanocide (Veriben®, Samorin® or Ethidium®, see materials and methods) used to treat trypanosome infections in cattle in Katelenyang village during the period of Samorin prophylaxis which is not seen in any other village over this period of time.

* significant

[3] Control villages not given any prophylactic drug.

Because of the very low tsetse challenge (as measured by the 'Berenil index') in the village where Ethidium prophylaxis was being carried out (Rukada) (fig. 4.11) it was only possible to estimate the degree of prophylaxis at approximately 85% at three to four weeks and at a negligible level eight weeks post treatment (based on a comparison between the incidence of trypanosome infections in cattle in Rukada village before and after treatment with Ethidium). Three to four weeks after both the first and second treatments with Ethidium there were breakthrough infections when it could be estimated that there should have been seven, based on the previous months' incidence, and by eight weeks post treatment there were the expected four new infections. The level of tsetse challenge in the control village for the Ethidium trial (Ngelechom) remained significantly higher than that of Rukada (figs. 4.11 and 4.12 and see Chapter V, figs. 5.5 and 5.6) making a direct comparison between disease incidence of little value. Monitoring the effect of prophylaxis up to 17 weeks after the second treatment, there was no significant reduction in the number of cases of trypanosomiasis which had to be treated compared to the period before prophylaxis (Table 4.2).

4.4.9 The 'Berenil index'

The level of tsetse challenge, as measured by the number of curative doses of trypanocide used per hundred cattle sampled, varied from month to month and by village (figs. 4.9 to 4.12). While Katelenyang (Samorin treated) and Apatit (Samorin control) villages had similar levels of challenge before prophylaxis Rukada (Ethidium treated) and Ngelechom (Ethidium control) villages did not. Ngelechom had significantly higher levels of challenge as measured by the 'Berenil index' than Rukada throughout the time of the study.

4.4.10 The prevalence of anaemia in cattle

The prevalence of anaemia as defined by a packed cell volume (PCV) of less than or equal to 20% closely reflected the prevalence of trypanosome infections in all four villages (figs. 4.1 to 4.4 and 4.13 to 4.16). The prevalence of anaemia in Katelenyang village (Samorin treated) was very low between the months of November and April when the prevalence of trypanosome infections was very low or zero (see figs. 4.1 and 4.13). Although the prevalence of anaemia in Rukada village (Ethidium treated) also was reduced following

treatment, the effect was less than that of Katelenyang village (Samorin treated) (fig. 4.15). The control villages of Apatit (Samorin control) and Ngelechom (Ethidium control) consistently had higher prevalences of anaemia in cattle than their respective treated villages during the period of prophylaxis.

4.4.11 The incidence of anaemia in cattle

The incidence of anaemia as defined by a new case of an animal having a packed cell volume (PCV) of less than or equal to 20% closely reflected the incidence of trypanosome infections in all four villages (figs. 4.17 to 4.20 and 4.13 to 4.16). The incidence of anaemia in Katelenyang village (Samorin treated) was very low or zero between the months of November and April when the incidence of trypanosome infections was very low or zero (see figs. 4.5 and 4.17). The incidence of anaemia in Rukada village (Ethidium treated) was reduced in each of the months following treatment when the incidence of trypanosome infections was low (figs. 4.7 and 4.19). The control villages of Apatit (Samorin control) and Ngelechom (Ethidium control) consistently had higher incidence of anaemia in cattle than their respective treated villages during the period of prophylaxis.

4.4.12 The effect of Samorin prophylaxis on the PCV of village cattle

The mean PCV of all infected and uninfected cattle sampled in Katelenyang village (Samorin treated) (24.17%) was less than that of the cattle of Apatit village (Samorin control) (24.83%) before the period of Samorin prophylaxis (i.e. during the 17 weeks following Samorin treatment), which was statistically significant ($p = 0.0360$). Following Samorin prophylaxis this was reversed when the mean PCV of all cattle in Katelenyang village (28.62%) was significantly greater than that of the cattle of Apatit village (26.09%) ($p < 0.0001$). However between the periods before and during prophylaxis there was a significant increase in the mean PCV of all cattle in both villages ($p < 0.0001$) (Appendix IV, Table IV.19). A comparison of the mean PCV of uninfected cattle between the two villages (26.10% and 26.70%) before prophylaxis showed no significant difference ($p = 0.1227$). However during prophylaxis the mean PCV of uninfected cattle in Katelenyang village (28.95%) was greater than those in Apatit village (27.75%), which was statistically significant ($p < 0.0001$). A small but

statistically significant increase in the mean PCV of uninfected cattle was seen in both villages between the periods before and during prophylaxis ($p < 0.0001$ and $p = 0.0001$) (Appendix IV, Table IV.20).

Comparing the mean PCV of cattle with a trypanosome infection from Katelenyang (20.98%) and Apatit (20.05%) villages, there was no significant difference before the period of prophylaxis ($p = 0.8480$). However during the period of prophylaxis the mean PCV of cattle with a trypanosome infection from Katelenyang village (23.70%) was greater than those of Apatit village (21.41%), which was statistically significant ($p = 0.0272$). A significant increase was also seen in the mean PCV of infected cattle from both villages between the periods before and during prophylaxis ($p = 0.0067$ and $p = 0.0060$) (Appendix IV, Table IV.21).

The analysis of covariance (Appendix IV, Table IV.18) confirmed these results which showed that the period of prophylaxis had a significant overall effect on PCV in the two pairs of villages, indicating a change over time unconnected with prophylaxis itself (as this was only administered in one village of each pair); there were also differences between the villages themselves. The prophylactic effect of the drug treatment given by the interaction of these two factors was only significant for Samorin, explaining nearly 2% of overall deviance ($p < 0.0001$).

4.4.14 The effect of Ethidium prophylaxis on the PCV of village cattle

The mean PCV of all infected and uninfected cattle sampled in Rukada village (Ethidium treated) (27.28%) before the period of Ethidium prophylaxis was greater that of cattle in Ngelechom village (Ethidium control) (26.73%), which was statistically significant ($p = 0.0010$) and this remained the case during the period of Ethidium prophylaxis when the mean PCV of all sampled cattle was 28.43% and 27.35% respectively ($p < 0.0001$) (Appendix IV, Table IV.25). However between the periods before and during prophylaxis there was an increase in the mean PCV of all cattle in both villages, which was statistically significant($p < 0.0001$ and $p = 0.0002$) (Appendix IV, Table IV.25).

There was no significant difference in the mean PCV of uninfected cattle between the two villages either before or during the period of prophylaxis (i.e. during the 14 weeks following Ethidium treatment) (Appendix IV, Table IV.26). However as with all

sampled cattle there was a small, but statistically significant, increase in the mean PCV of uninfected cattle was seen in both villages between the periods before and during prophylaxis ($p < 0.0001$ and $p = 0.0001$) (Appendix IV, Table IV.26).

The mean PCV of cattle with a trypanosome infection in Rukada village (Ethidium treated) (24.80%) before the period of Ethidium prophylaxis was significantly greater that of cattle in Ngelechom village (Ethidium control) (21.70%) ($p < 0.0001$) and this remained the case during the period of prophylaxis when the mean PCV of cattle with a trypanosome infection was 26.57% and 21.84% respectively ($p = 0.0001$) (Appendix IV, Table IV.27). Unlike the case of uninfected cattle the mean PCV of cattle with a trypanosome infection was not significantly different between the periods before and during prophylaxis in either the Ethidium treated village (Rukada) ($p = 0.0869$) or its control village (Ngelechom) ($p = 0.8465$) (Appendix IV, Table IV.27).

The analysis of covariance(Appendix IV, Table IV.18) confirmed these results which showed that the period of Ethidium prophylaxis had a significant overall effect on PCV in the two pairs of villages, indicating a change over time unconnected with prophylaxis itself (as this was only administered in one village of each pair); there were also differences between the villages themselves. However the prophylactic effect of drug treatment given by the interaction of these two factors was not significant, explaining only 0.02% of overall deviance ($p = 0.2748$).

4.4.13 The effect of Samorin prophylaxis on the monthly liveweight change of village cattle

Prior to the period of prophylaxis there was no significant difference between the mean monthly liveweight gain of all infected and uninfected cattle sampled in Katelenyang (2.35 kg) and Apatit (2.37) villages ($p = 0.9702$). During the period of prophylaxis (i.e. during the 17 weeks following treatment with Samorin) there was a difference of more than one kilogram in the mean monthly liveweight gain of all cattle sampled between the Samorin treated village (Katelenyang) (5.01 kg) and the untreated control village (Apatit) (3.89 kg) ($p = 0.0004$). However there was a significant increase in the monthly liveweight gain of all tested cattle in both villages from the period before prophylaxis to the period during prophylaxis ($p < 0.0001$ and $p = 0.0022$) (Appendix IV, Table IV.22).

There was no significant difference in the mean monthly liveweight gain of uninfected cattle from Katelenyang village (Samorin treated) or Apatit village (Samorin control) either before ($p = 0.2352$) or during prophylaxis ($p = 0.7540$) (Appendix IV, Table IV.23). However the mean monthly liveweight gain of uninfected cattle improved slightly during the period of prophylaxis in Apatit village (control), which was statistically significant ($p = 0.0068$) whereas this was not significant in Katelenyang village (Samorin treated) ($p = 0.1836$) (Appendix IV, Table IV.23).

Prior to the period of prophylaxis there was no significant difference in the mean monthly liveweight gain of cattle with trypanosome infections between the two villages ($p = 0.9678$). However during the period of prophylaxis the mean monthly liveweight gain of cattle with a trypanosome infection in Katelenyang village (4.75 kg) was very significantly greater than that of trypanosome infected cattle in Apatit village (0.04 kg) ($p < 0.0001$) (Appendix IV, Table IV.24).

The overall difference in the mean monthly liveweight gain of all infected and uninfected cattle between the two villages of Katelenyang (Samorin treated) and Apatit (Samorin control) during the period of Samorin prophylaxis (Appendix IV, Table IV.23) arises because the cattle with breakthrough infections of trypanosomes under Samorin prophylaxis were very few in number (30 cattle months) and had weight gains similar to uninfected cattle whereas the many more cattle infected during this period in the untreated control village (308 cattle months) on average did not gain weight (Appendix IV, Table IV.24).

4.4.15 The effect of Ethidium prophylaxis on the monthly liveweight change of village cattle

There was no significant difference in the mean monthly liveweight gain of all infected and uninfected cattle sampled between the two villages either before ($p = 0.6053$) or during ($p = 0.8508$) the period of Ethidium prophylaxis (i.e. during the 14 weeks following Ethidium treatment) (Appendix IV, Table IV.28). Similarly there was no significant change in the mean monthly liveweight gain of all cattle sampled between the periods before and during prophylaxis in either village (Appendix IV, Table IV.28).

Prior to the period of prophylaxis there was no significant difference in the mean monthly liveweight gain of uninfected cattle between Rukada village (Ethidium treated)

(3.08 kg) and Ngelechom village (Ethidium control) (3.74 kg) (p = 0.1267) (Appendix IV, Table IV.29). However during the period of prophylaxis the mean monthly liveweight gain of uninfected cattle in the control village (Ngelechom) (4.55 kg) was significantly greater than that of uninfected cattle in the treated village (Rukada) (3.76 kg) (p = 0.0317) (Appendix IV, Table IV.29). Similarly while a significant increase in the mean monthly liveweight gain was seen in uninfected cattle between the periods before and during prophylaxis in the control village (Ngelechom) (0.81 kg) (p = 0.0170), this was not the case with uninfected cattle from the Ethidium treated village (Rukada) (0.68 kg) (p = 0.1343) (Appendix IV, Table IV.29).

The mean monthly liveweight gain of cattle with a trypanosome infection in Rukada village (Ethidium treated) was significantly greater than that of cattle with a trypanosome infection in Ngelechom village (Ethidium control) both before (6.02 kg, p = 0.0004) and during the period of prophylaxis (6.77 kg, p = 0.0063) (Appendix IV, Table IV.30).

4.4.16 The prevalence and incidence of *T. theileri* in village cattle

The prevalence of *T. theileri* in Katelenyang (Samorin treated) and Apatit (Samorin control) villages were comparable except during the period of Samorin prophylaxis when there was a complete absence of *T. theileri* (fig. 4.21). Similarly the prevalence of *T. theileri* in Rukada (Ethidium treated) and Ngelechom (Ethidium control) villages were comparable (fig. 4.22). However there was a significant difference between the prevalence of *T. theileri* in the two villages on the Samorin trial compared to the two villages on the Ethidium trial (figs. 4.21 and 4.22). The prevalence of *T. theileri* appears to be unaffected by the Ethidium treatment (fig. 4.22). The incidence of *T. theileri* infections in cattle in all four villages displayed a similar pattern to the prevalence (figs. 4.23 and 4.24).

4.4.1.7 The prevalence and incidence of microfilaria in village cattle

The prevalence of microfilaria in Katelenyang (Samorin treated) and Apatit (Samorin control) villages was similar even during the period of Samorin prophylaxis (fig. 4.25). The prevalence of microfilaria in Rukada (Ethidium treated) and Ngelechom (Ethidium control) villages was also comparable (fig. 4.26). However there was a significant difference between the

prevalence of microfilaria in the two villages on the Samorin trial compared to the two villages on the Ethidium trial although microfilaria appeared unaffected by either drug (fig. 25). The incidence of microfilaria infections in cattle in all four villages displayed a similar pattern to the prevalence (fig. 26).

4.5 Discussion

It has been demonstrated that two block treatments with Samorin at 12 week intervals can virtually eliminate the potential reservoir of *T. brucei* spp. including *T. b. rhodesiense* (Chapter IX) in cattle for up to 6 months. This proved that chemoprophylaxis of domestic livestock would be a very useful control method in the advent of an outbreak of Rhodesian sleeping sickness and since all trypanosome infections were eliminated within ten days of initial treatment it would provide means for the rapid removal of this potentially human infective reservoir, thereby greatly reducing the chances of zoonotic spread of the disease.

Furthermore because the mean life span of tsetse flies in the wild is considerably less than six months (77 days, Glasgow, 1963; 29 days, Phelps and Vale, 1978) most newly emergent teneral flies would be expected to receive their first blood meal from an uninfected animal and further infections would be rare. However it is important to take into account of the possible encroachment of infected flies from surrounding areas, alternative animal reservoirs of infection or the importation of infected cattle into the study area. *Glossina f. fuscipes* is the predominant tsetse vector in Busia District and this species has a well circumscribed habitat (Mwangelwa *et al.*, 1990) and where this is the sole tsetse species the effect of recruitment to the fly population may not be great. Small numbers of *Glossina pallidipes* have been previously been identified in the hills surrounding Funyula in the south of the district and recently a small population of *Glossina pallidipes* has been discovered in the hilly area around Amukura, where the villages of Katelenyang and Apatit are situated (KETRI 1994). There may be an increase in the effect of recruitment in hilly areas of the district where *Glossina pallidipes* is found together with *Glossina f. fuscipes*. However evidence which suggests that *Glossina f. fuscipes* is a more important vector of trypanosomiasis than *G. pallidipes* in this region comes from the relative lack of *T. congolense* infections in infected livestock in the study villages. As *G. pallidipes* is a relatively good vector of *T. congolense* and *G. f. fuscipes* a relatively poor vector (Harley, 1966b) it would be expected that the

incidence of *T. congolense* would be greater in Katelenyang and Apatit villages where *G. pallidipes* exists, than in the villages of Rukada and Ngelechom which are situated in the low lying swampland on the Kenya Uganda border and where only *G. f. fuscipes* is present. The data presented here show that the *T. congolense* proportion of total trypanosome infections was not significantly different in the four villages ($p = 0.153$) (Appendix IV, Table IV.10). From blood meal analysis the major tsetse hosts in this region are monitor lizards (59%) and domestic cattle (23%) while humans only provide 7% of bloodmeals (Welburn *et al.*, 1995). From the bloodmeal data and the data shown here on the prevalence of trypanosome infections in domestic livestock (see Chapter V), it is clear that cattle are the most important domestic animal reservoir of *T. brucei* spp. in this area and any effect from alternative animal reservoirs would not be great.

The characteristic of Rhodesian sleeping sickness is a series of endemic foci of infection from which epidemics develop when condition become favourable (Koerner *et al.*, 1995). In order for an epidemic state of Rhodesian sleeping sickness to exist the transmission rate of the disease must exceed that when the disease is in an endemic state (Rogers, 1988). Chemoprophylaxis of domestic livestock may result in the transmission rate of the disease being reduced below this critical level and as a result the disease would tend to return to a low endemic state.

The apparent prolonged prophylactic effect of Samorin past 12 weeks may be due in part to a reduction in the infection rates of the local tsetse population which itself may been caused by chemoprophylaxis with Samorin. Because cattle are frequently traded or exchanged in this area, in order to maintain a cordon sanitaire by drug prophylaxis of domestic cattle around an area experiencing an outbreak of sleeping sickness, any cattle moving into the area would have to be treated on arrival.

The one early breakthrough (*T. vivax*) infection in the Samorin prophylaxis of village cattle at 10 weeks post treatment was an 18 months old heifer which had increased in weight from 69 kg to 87 kg during this period, an increase of some 26% in body weight. Although 69 mg of Samorin represents 1 mg/kg for a 69 kg animal, it is less than 0.8 mg/kg for one of 87 kg. If the exponential decay of the drug (Eisler *et al.*, 1994) is considered then this effect would have been even greater. This phenomenon of cattle 'outgrowing' their dose of prophylactic drug may contribute to infections breaking through sooner than would be

expected particularly when the drug is being given to cattle with trypanosomiasis which often show a dramatic increase in weight after treatment (see Chapter VI).

Analysis of the proportion of total trypanosome infections which involved *T. brucei* spp. infection following Samorin prophylaxis in Katelenyang village compared to its control Apatit village indicates that Samorin was an equally effective prophylactic for *T. brucei* spp. as it was for *T. vivax* and *T. congolense*.

Ethidium proved very effective at treating existing infections of *T. brucei* spp., *T. vivax* and *T. congolense* in the village cattle population and gave some degree of prophylaxis of between 4 and 8 weeks duration. Because of the low level of tsetse challenge in the Ethidium treated village compared to the control village (figs. 4.11, 4.12 and Chapter V figs. 5.5 and 5.6) it was only possible to make an estimation of the level and duration of prophylaxis. It is uncertain whether this level or duration of prophylaxis would be sufficient to reduce the transmission rate of sleeping sickness to below the critical level required to maintain an epidemic state.

In the past there have been reports of drug resistance in *T. brucei* spp. infections in cattle to Ethidium treatment (EATRO, 1974, 1975, 1976). However analysis of the proportion of total trypanosome infections which involved *T. brucei* spp. infection following Ethidium prophylaxis in Rukada village compared to its control Ngelechom village indicates that Ethidium was an equally effective prophylactic for *T. brucei* spp. infections as it was for *T. vivax* and *T. congolense*. These results do not support previous observations of Ethidium drug resistance and possibly reflects the relatively infrequent use of trypanocides over the last twenty years.

The prolonged time taken by Samorin to clear the host's bloodstream of pathogenic trypanosomes (*T. brucei* spp., *T. vivax* and *T. congolense*) after treatment (up to 10 days) may lead to false reporting of drug resistance. Mistaken identification of *T. theileri* as a pathogenic trypanosome may also result in false reports of drug resistance since in animals treated with Samorin it takes between ten days and six weeks to clear such an infection and in the case of Ethidium the infection is never cleared.

The mean PCV of all infected and uninfected cattle sampled in Katelenyang village (Samorin treated) showed a significant rise to equal that of the mean of uninfected cattle (see Chapter VI) following treatment with Samorin. While the mean PCV of all infected and uninfected cattle sampled in Apatit village (Samorin control) also showed a significant rise

between the period before and the same period as that of prophylaxis in Katelenyang village, however the rise in PCV was significantly less than that in the Samorin treated village. This may have been due to the treatment of clinical cases of trypanosomiasis in the control village.

The highly significant rise in the mean PCV of uninfected cattle in Katelenyang village (Samorin treated) compared to Apatit village (Samorin control) between the periods before and after treatment may have been due to the effect of Samorin preventing the advent of prepatent, sub clinical and undiagnosed trypanosome infections.

The difference in the mean PCV of cattle with trypanosome infections between the two villages on the Samorin trial during the period of prophylaxis may only be a reflection in the duration of the infections.

The mean PCV of cattle with trypanosome infections was significantly less in Ngelechom village (Ethidium control) compared to Rukada village (Ethidium treated) both before and after treatment. This may be due to the strains of trypanosomes in Ngelechom village being more pathogenic.

Since the incidence of anaemia in cattle during Samorin or Ethidium prophylaxis was almost zero, as was the incidence of trypanosome infections, it must be concluded that either trypanosomiasis is the only significant cause of anaemia in East African zebu cattle of this District or that Samorin and Ethidium also have an effect on other diseases which cause anaemia e.g. babesiosis or anaplasmosis, which were known to be present in Busia District (Gov. of Kenya, 1993).

The significant effect of Samorin increasing overall village cattle herd liveweight gain was caused by a reduction in the prevalence of trypanosome infections. There was no evidence that Samorin given to uninfected cattle at a dose rate of 1 mg/kg was either beneficial or detrimental to the overall performance of the village cattle herd.

In addition to the obvious benefit to a human population at risk of contracting sleeping sickness, Samorin prophylaxis was beneficial to cattle production by controlling *T. vivax*, *T. congolense* and *T. brucei*. During each month of prophylaxis the mean monthly weight gain was 1.12 kg greater in the Samorin treated group than the control, which represents 3.36 kg per Samorin treatment. From observations made at local markets during the time of the study 1 kg liveweight was valued at 30/= (Kenya shillings), which makes the estimated benefit per animal per Samorin treatment of 100/=. At the same time the cost for Samorin treatment for cattle of mean weight (155 kg) was 60/=, making block treatment of

the village cattle herd cost effective on the basis of liveweight production alone without taking account of an improvement in cattle mortality or reproduction. It may be expected that the other livestock production outputs would be similarly increased e.g. draught power, milk, dung, as a means of storing wealth and other social reasons such as dowry payment. This would have had the effect of further improving the positive economic benefit of block treatment of village cattle herds with Samorin.

The overall village cattle herd liveweight gain in Ngelechom village (Ethidium control) was significantly greater than that of Rukada village (Ethidium treated) following treatment with Ethidium. This probably reflects the level of nutrition in the two villages during the period of prophylaxis when there was no rain in Rukada village, rather than any detrimental effect on cattle liveweight gain caused by Ethidium treatment.

The mean monthly liveweight gain of cattle with trypanosome infections was significantly less in Ngelechom village (Ethidium control) compared to Rukada village (Ethidium treated) both before and after treatment. This appears to be due to the strains of trypanosomes in Ngelechom village being more pathogenic.

The prevalence and incidence of the stercorarian trypanosome *T. theileri* was much greater in Rukada village (Ethidium treated) and Ngelechom village (Ethidium control) than in Katelenyang village (Samorin treated) and Apatit village (Samorin control) (figs. 4.21 to 4.24). Interestingly enough this trend was exactly the opposite pattern to that found with the salivarian trypanosomes (figs. 4.1 to 4.8). This leads to the conclusion that the major vector of *T. theileri* in this area may not have been tsetse flies and that tabanids and other biting flies, which were more numerous in Rukada and Ngelechom villages than in Katelenyang or Apatit villages (Chapter V, figs. 5.7 to 5.10), or ticks, which have been shown to be the vector of *T. theileri* in other regions (Burgdorfer *et al.*, 1973) may have been more important vectors.

Similarly the prevalence and incidence of microfilaria was much greater in Rukada village (Ethidium treated) and Ngelechom village (Ethidium control) than in Katelenyang village (Samorin treated) and Apatit village (Samorin control) which followed the pattern of *T. theileri* infections (figs. 4.25 and 4.26).

It is envisaged that the use of prophylactic drugs to control the reservoir of *T. brucei* spp. in cattle would occur in conjunction with treatment of human cases and appropriate control of the tsetse vector in a fully integrated approach to the control of sleeping

sickness. While vector control would reduce the tsetse population and thereby reduce the transmission of sleeping sickness in the short term, it may often be difficult or impossible to achieve or maintain a sufficient reduction in the tsetse population to significantly affect the incidence of human infection. Creating a cordon sanitaire by regularly treating all cattle within an area afflicted by Rhodesian sleeping sickness with Samorin would augment the more immediate effects of vector control and would be expected to help reduce the incidence of sleeping sickness in the human population over a period of a few months. Three monthly treatment of all cattle with Samorin in a village could be extended past the six month period of chemoprophylaxis in this trial since no detrimental effects were observed apart from a local reaction at the site of injection. Indeed block drug treatment of village cattle with Samorin proved of net economic benefit in terms of animal production in addition to its potential contribution to a reduction in the incidence of human cases in an outbreak of Rhodesian sleeping sickness.

The prevalence of trypanosome infections in cattle Katelenyang village (Samorin treated)

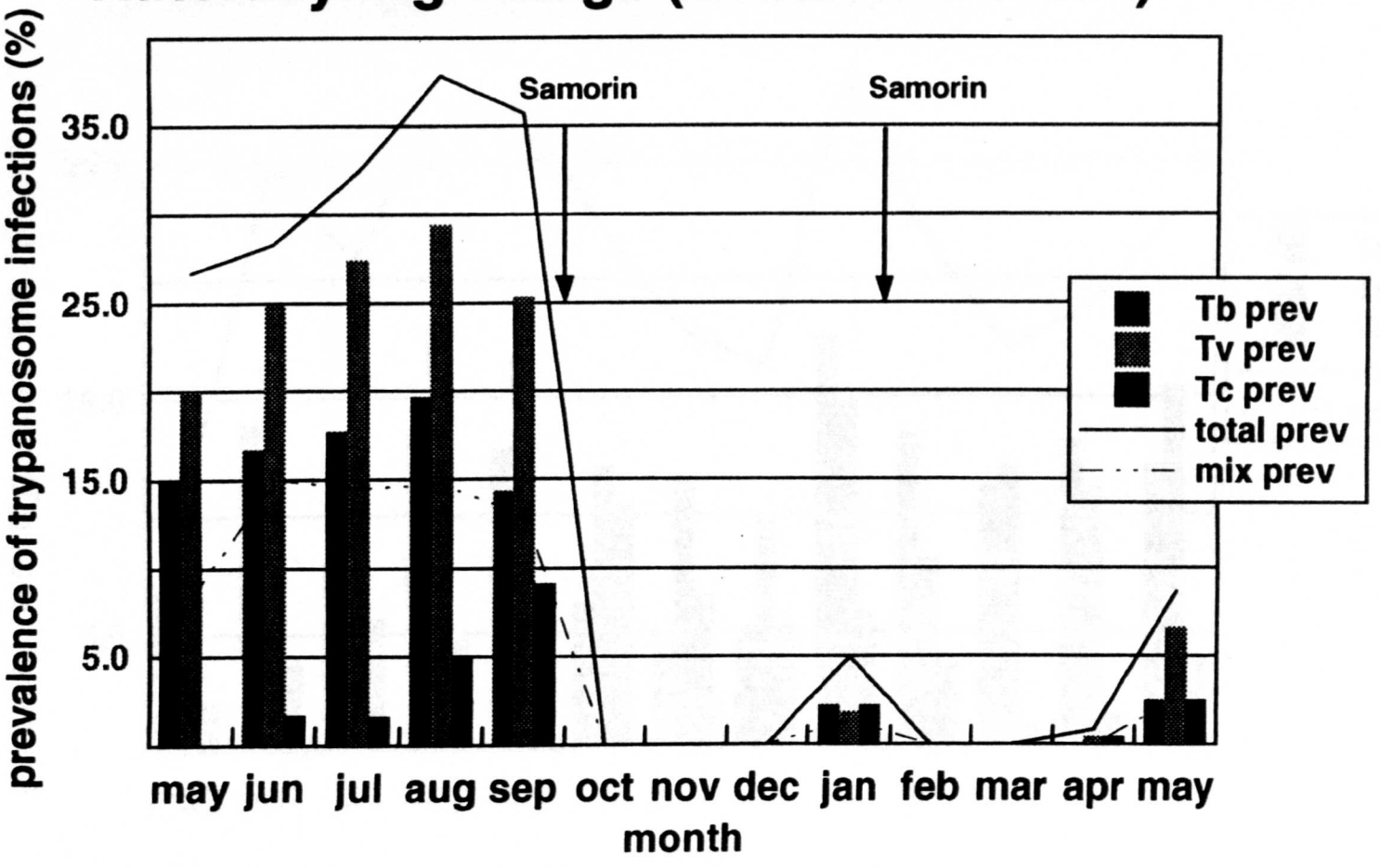


fig. 4.1

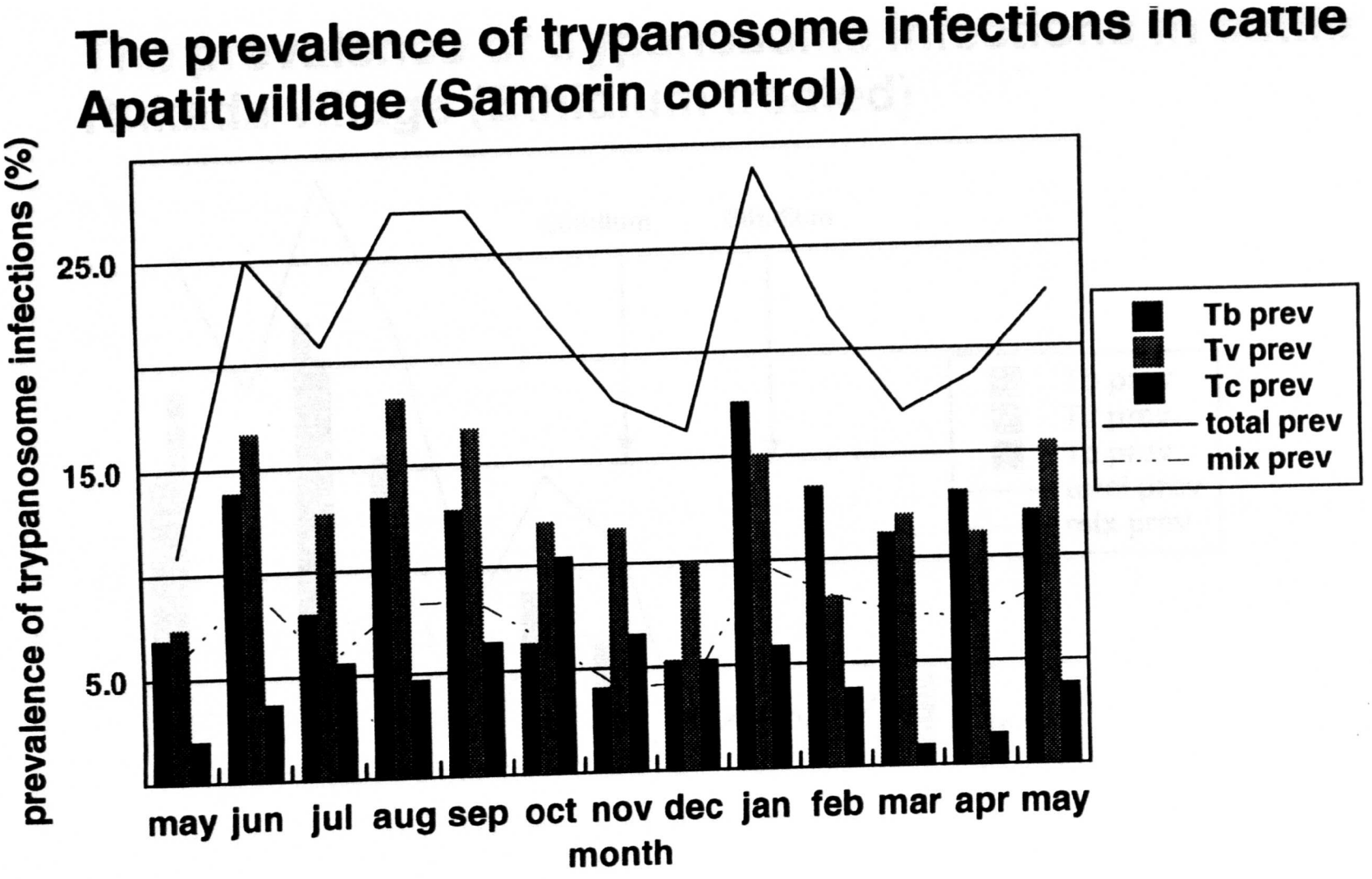

The prevalence of trypanosome infections in cattle
Apatit village (Samorin control)
prevalence of trypanosome infections (%)
25.0
15.0
5.0
Tb prev
Tv prev
Tc prev
total prev
mix prev
may jun jul aug sep oct nov dec jan feb mar apr may
month

fig. 4.2

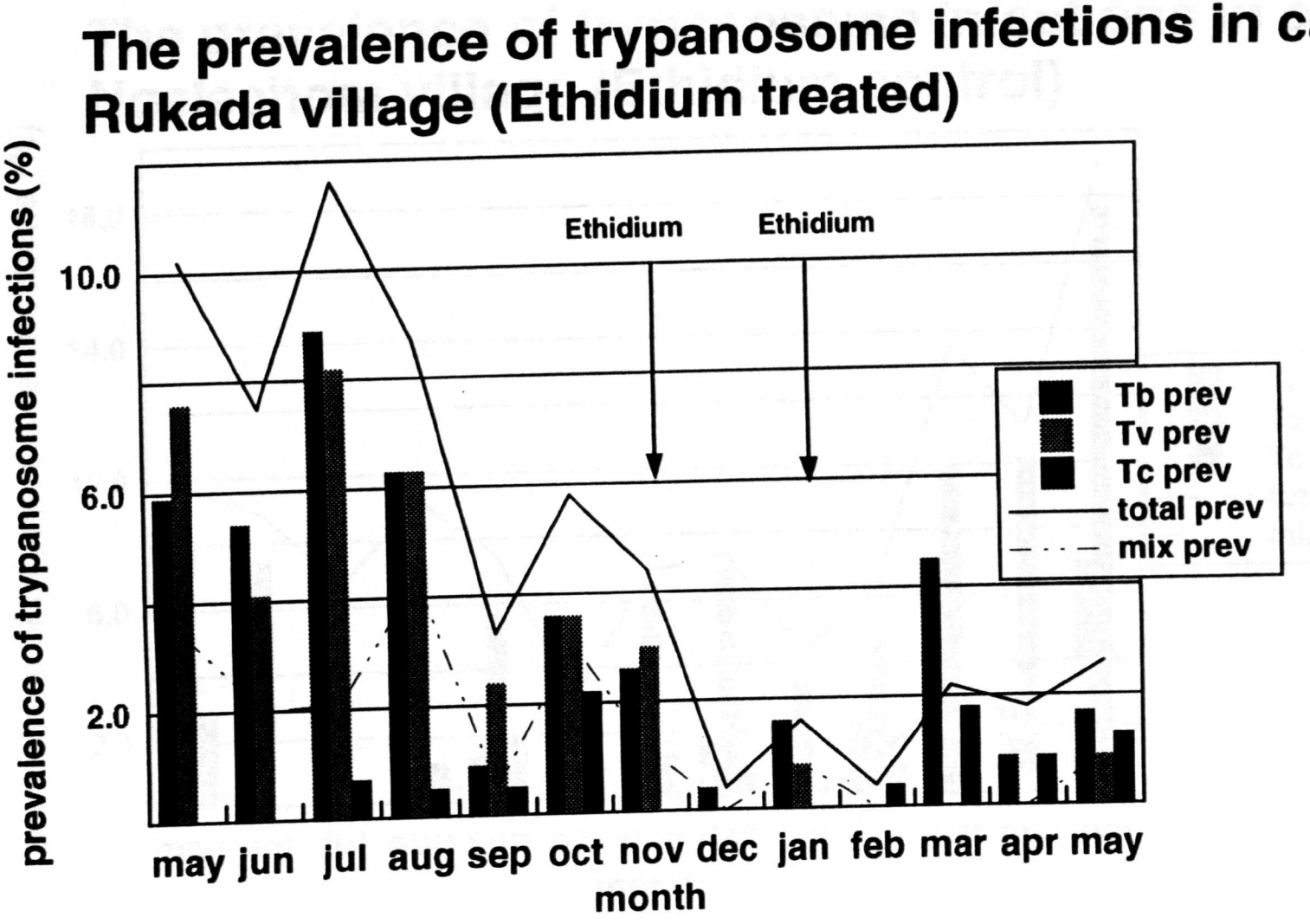

The prevalence of trypanosome infections in cattle
Rukada village (Ethidium treated)
prevalence of trypanosome infections (%)
10.0
6.0
2.0
Ethidium
Ethidium
Tb prev
Tv prev
Tc prev
total prev
mix prev
may jun jul aug sep oct nov dec jan feb mar apr may
month

fig. 4.3

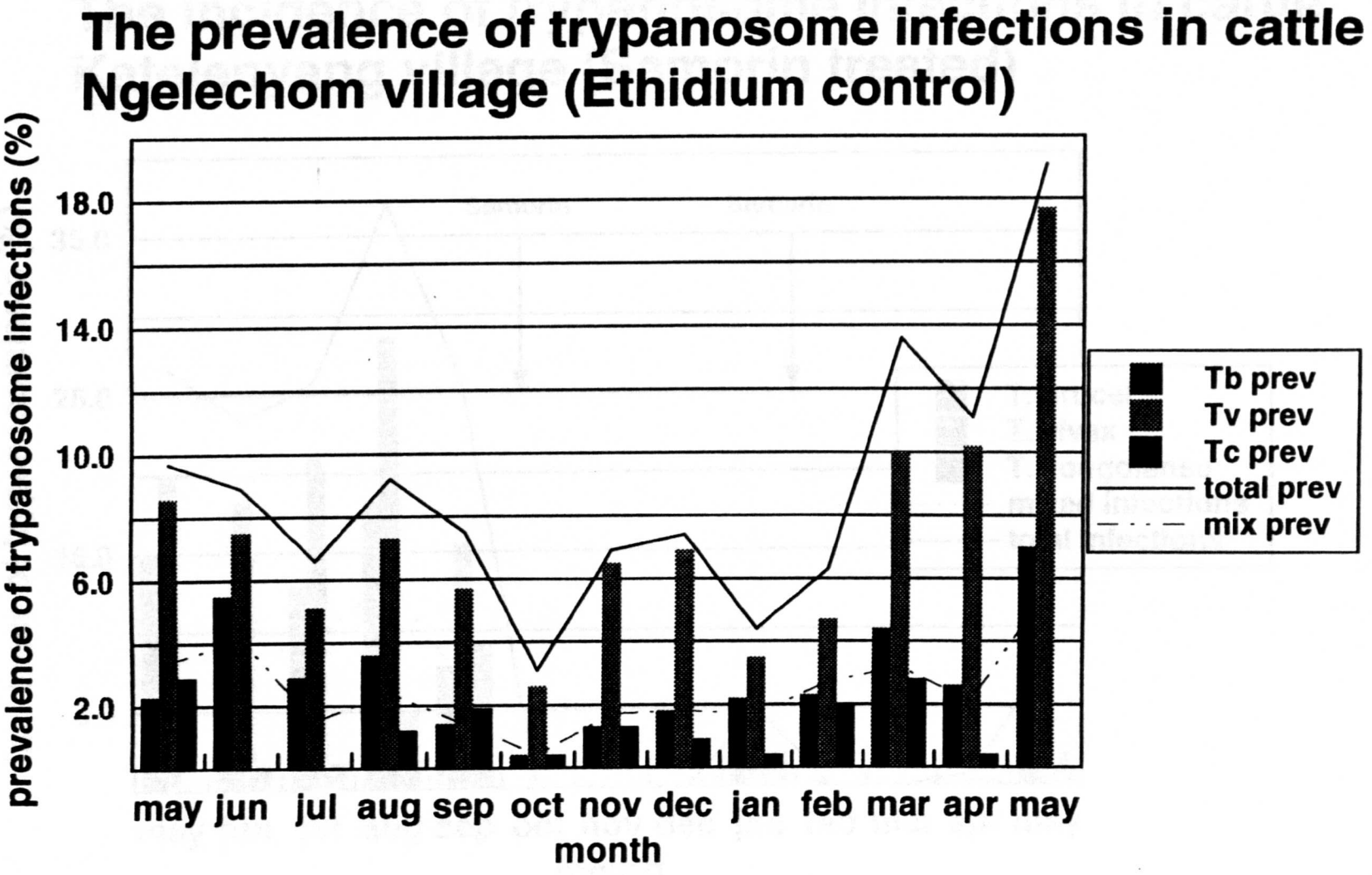

The prevalence of trypanosome infections in cattle
Ngelechom village (Ethidium control)
prevalence of trypanosome infections (%)
18.0
14.0
10.0
6.0
2.0
may jun jul aug sep oct nov dec jan feb mar apr may
month
Tb prev
Tv prev
Tc prev
total prev
mix prev

fig. 4.4

The incidence of trypanosome infections in cattle Katelenyang village (Samorin treated)

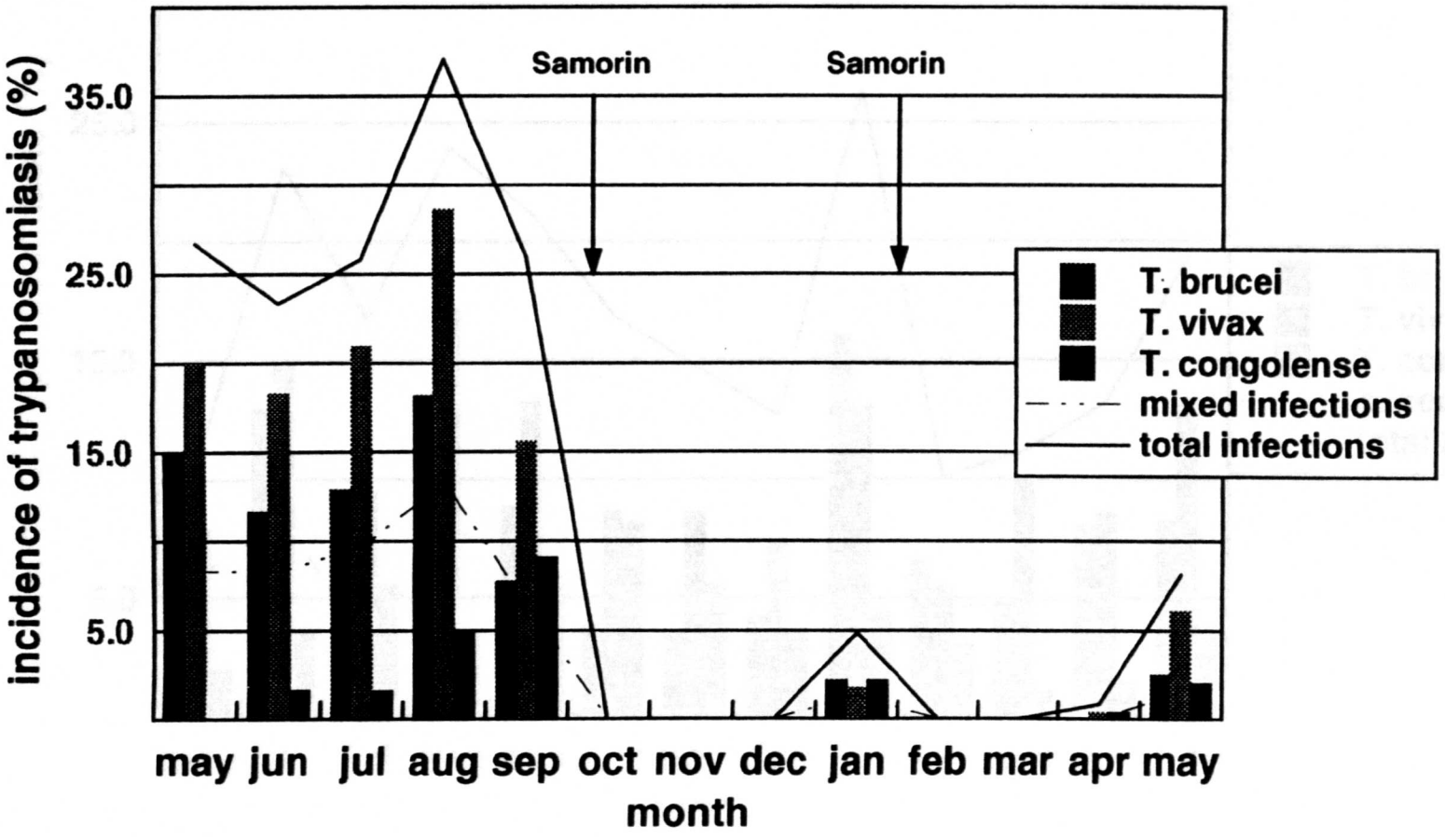


fig. 4.5

The incidence of trypanosome infections in cattle Apatit village (Samorin control)

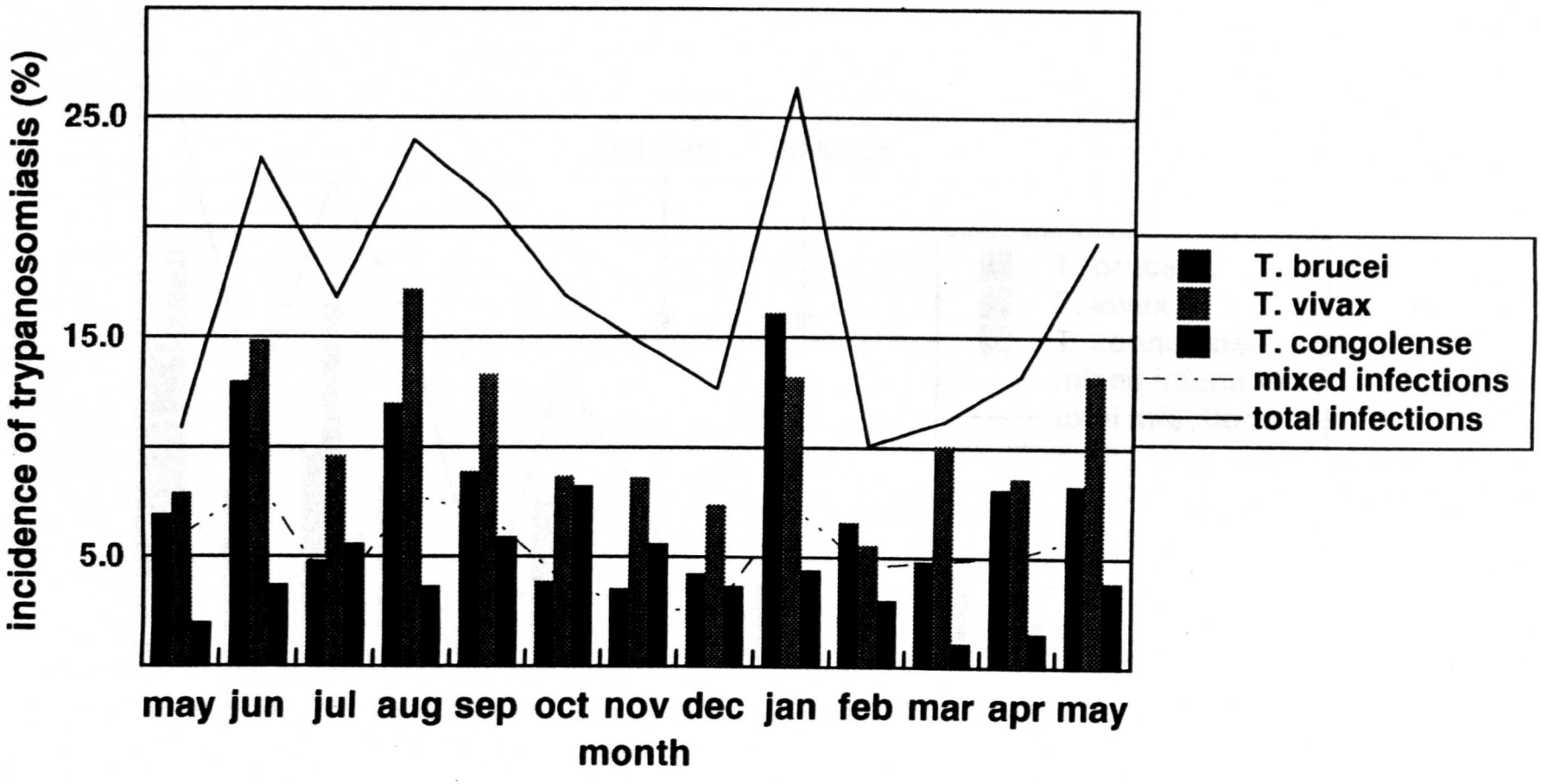


fig. 4.6

The incidence of trypanosome infections in cattle Rukada village (Ethidium treated)

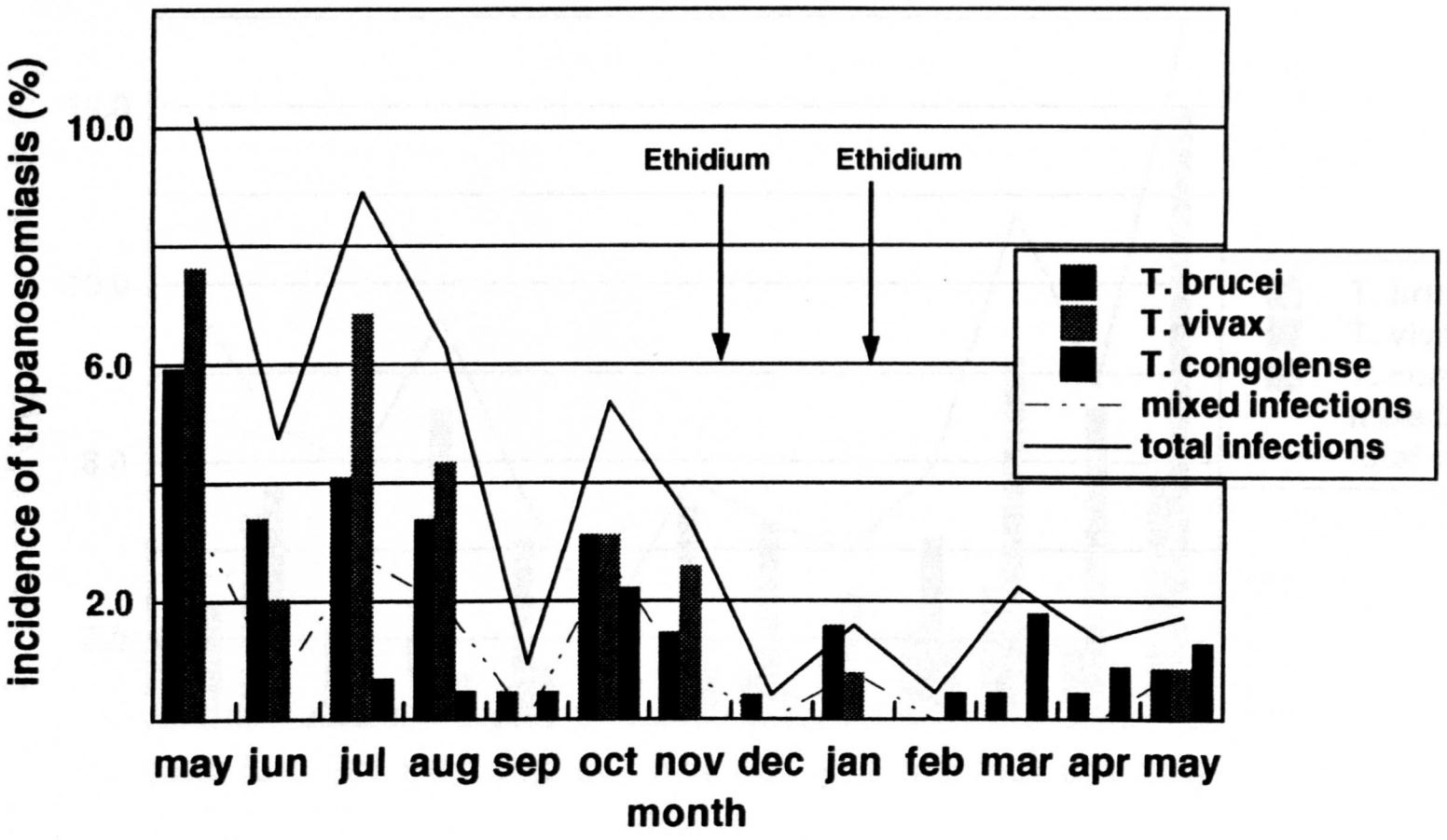


fig. 4.7

The incidence of trypanosome infections in cattle Ngelechom village (Ethidium control)

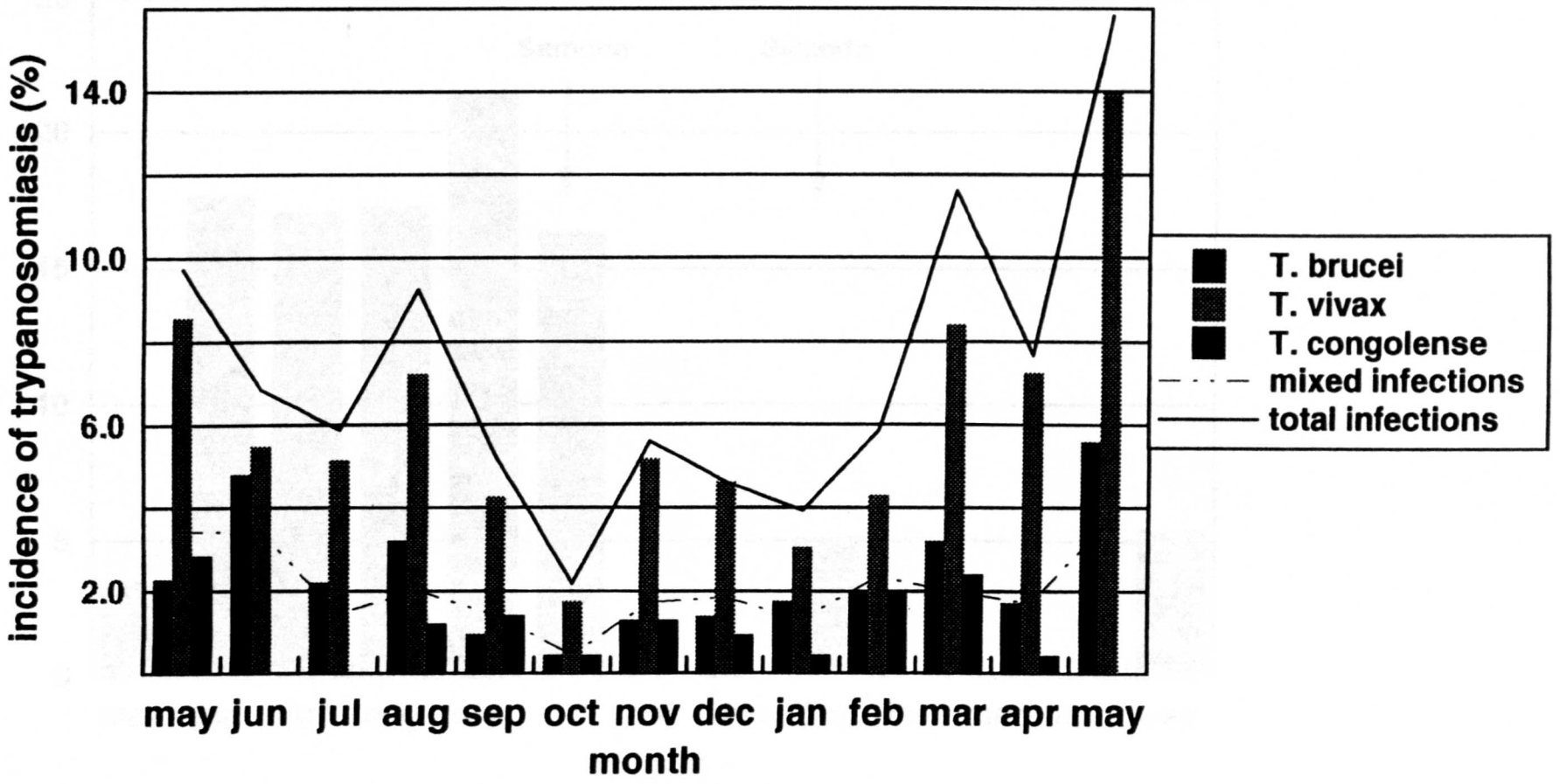


fig. 4.8

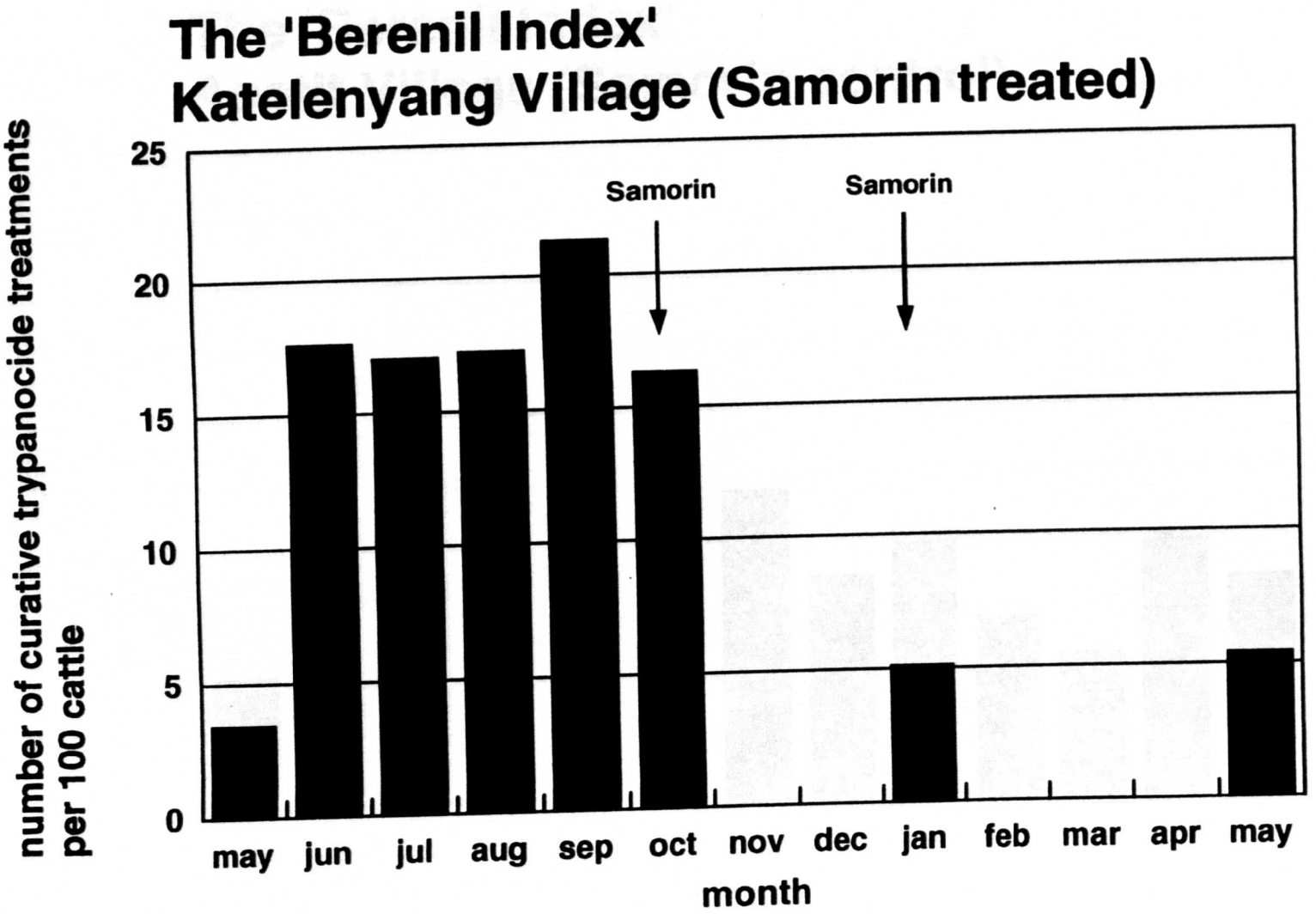

The 'Berenil Index'
Katelenyang Village (Samorin treated)
number of curative trypanocide treatments per 100 cattle
25
20
15
10
5
0
Samorin
Samorin
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 4.9

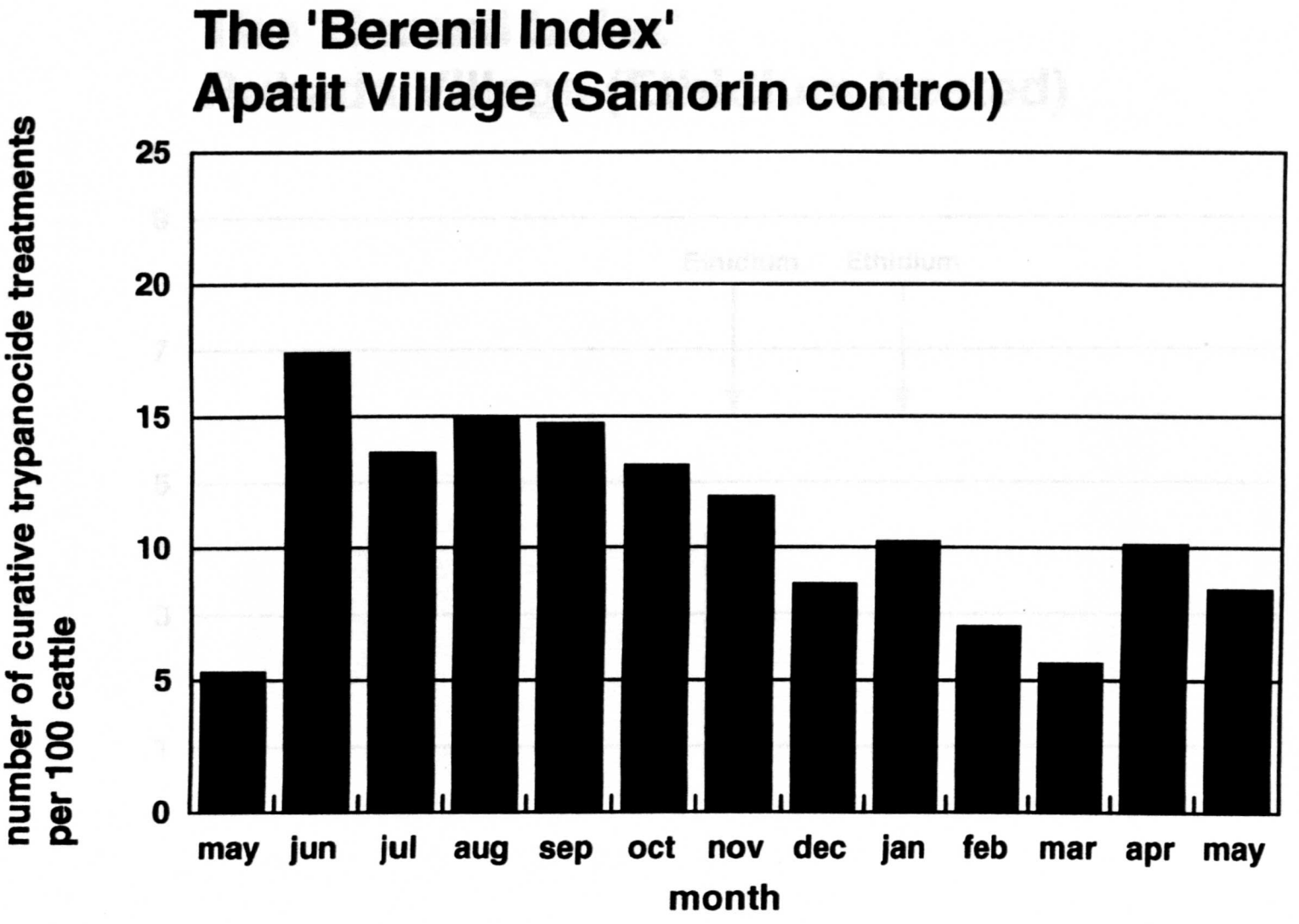

The 'Berenil Index'
Apatit Village (Samorin control)
number of curative trypanocide treatments per 100 cattle
25
20
15
10
5
0
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 4.10

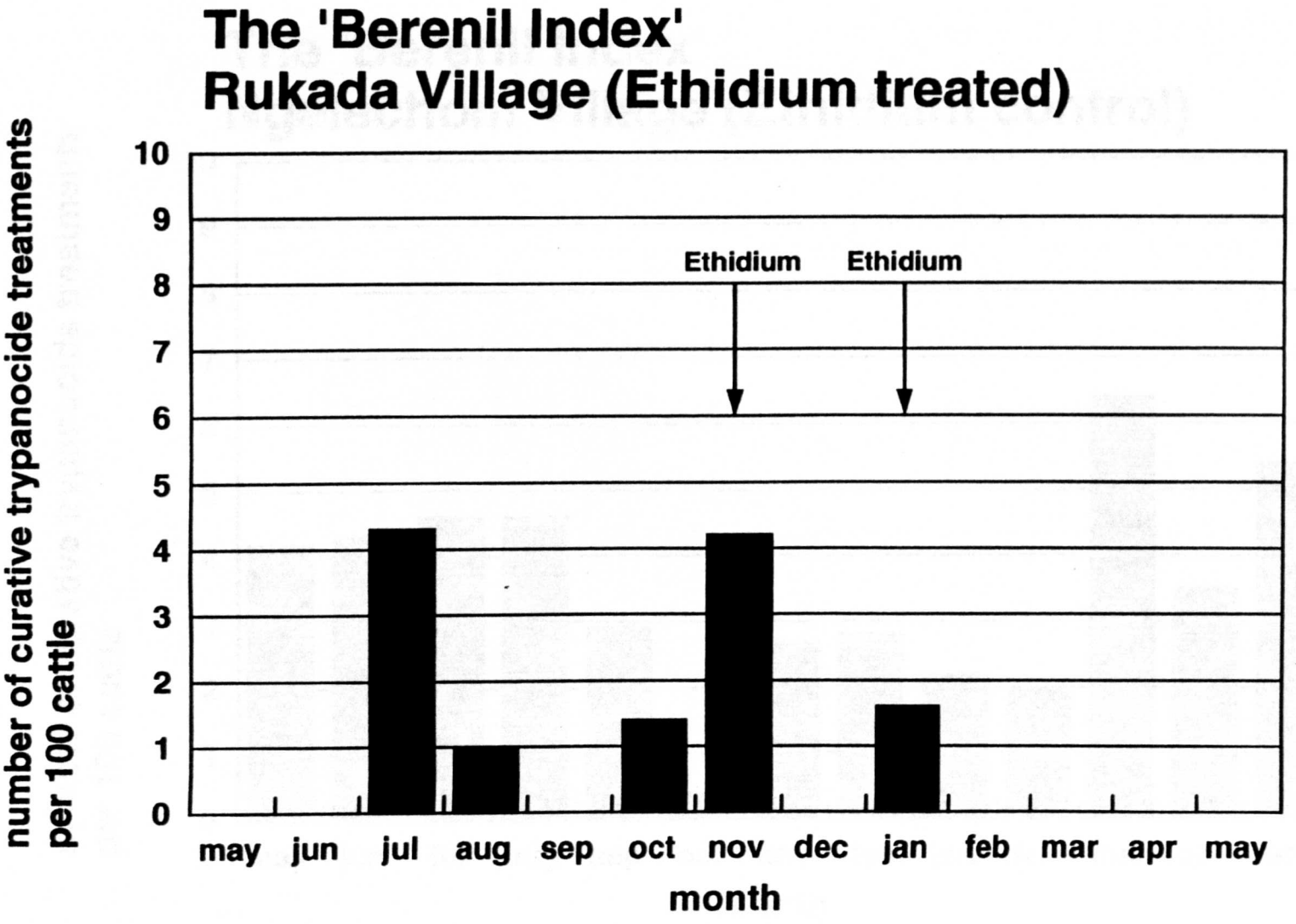

The 'Berenil Index'
Rukada Village (Ethidium treated)
number of curative trypanocide treatments per 100 cattle
10
9
8
7
6
5
4
3
2
1
0
Ethidium
Ethidium
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 4.11

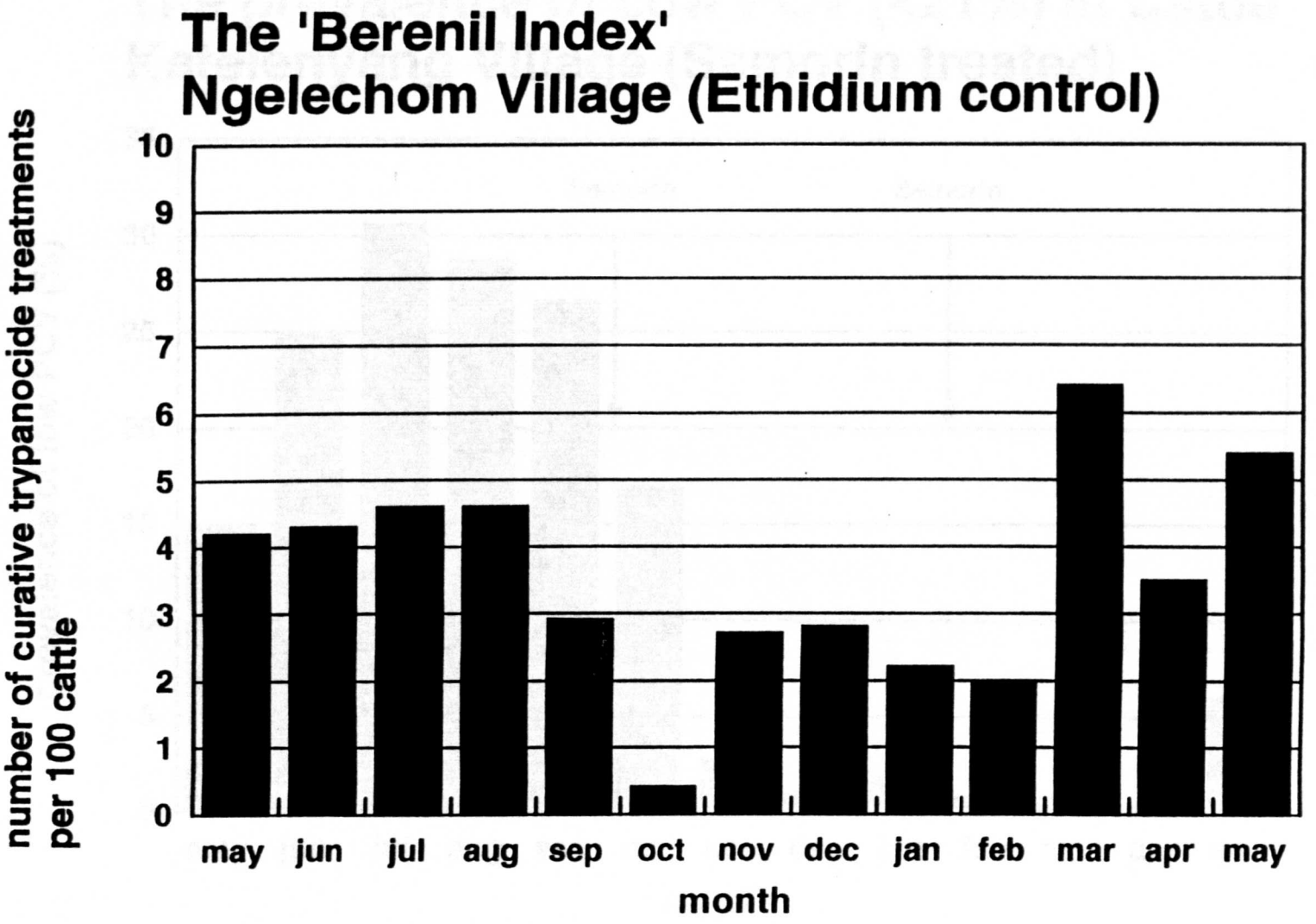

The 'Berenil Index'
Ngelechom Village (Ethidium control)
number of curative trypanocide treatments per 100 cattle
0
1
2
3
4
5
6
7
8
9
10
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 4.12

The prevalence of Low PCV (<21%) in Cattle Katelenyang Village (Samorin treated)

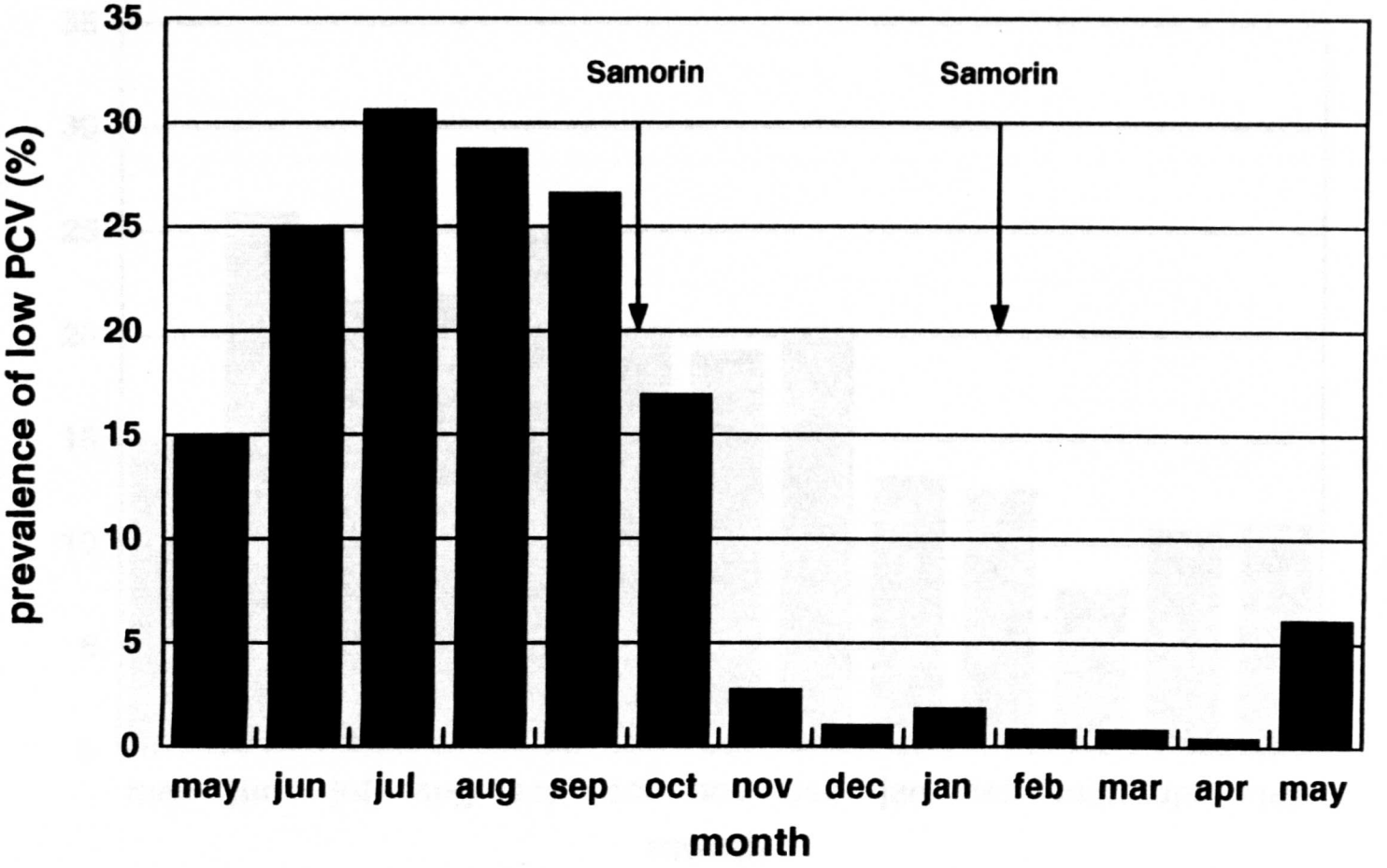


fig. 4.13

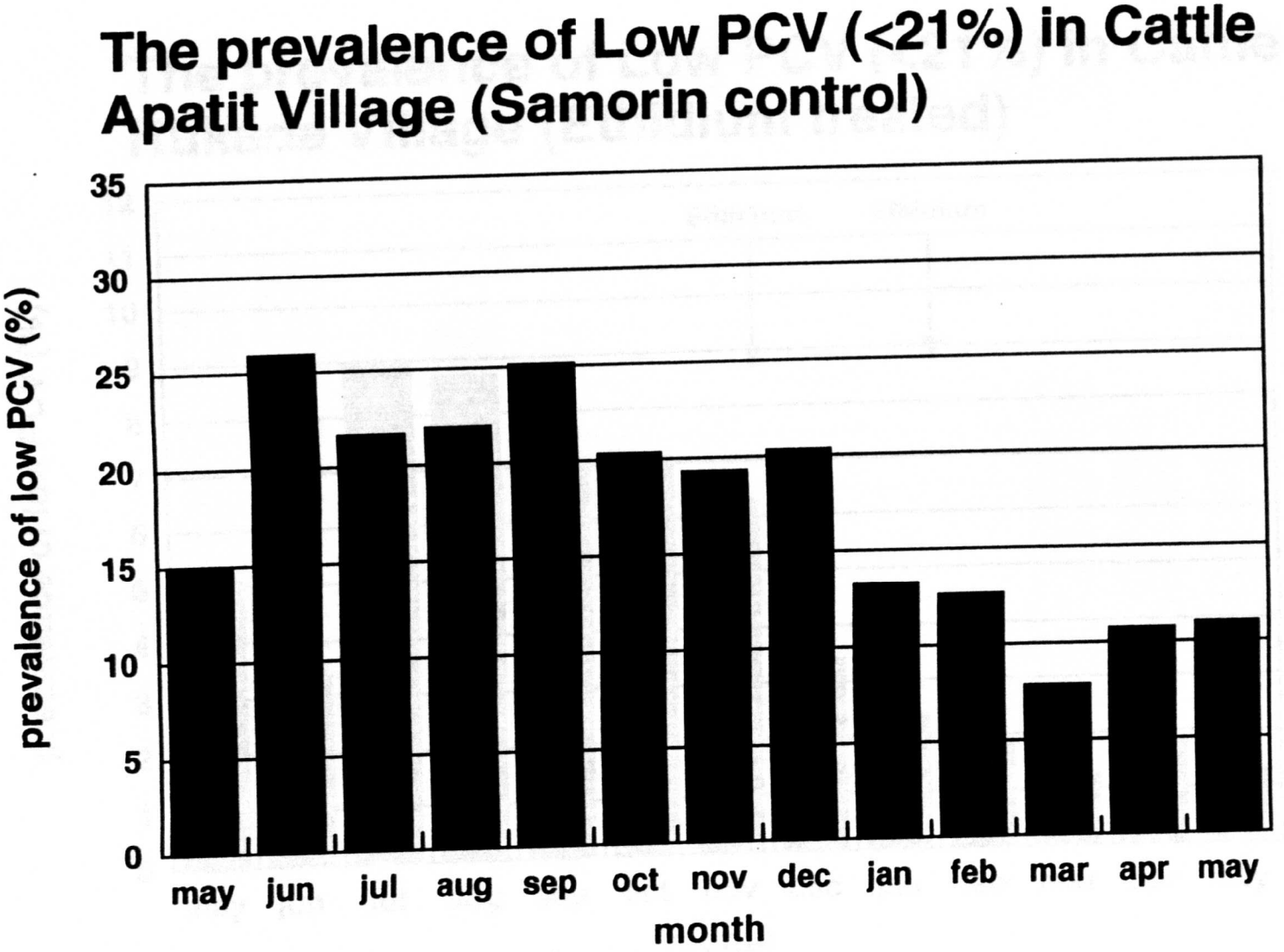

The prevalence of Low PCV (<21%) in Cattle Apatit Village (Samorin control)
prevalence of low PCV (%)
35
30
25
20
15
10
5
0
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 4.14

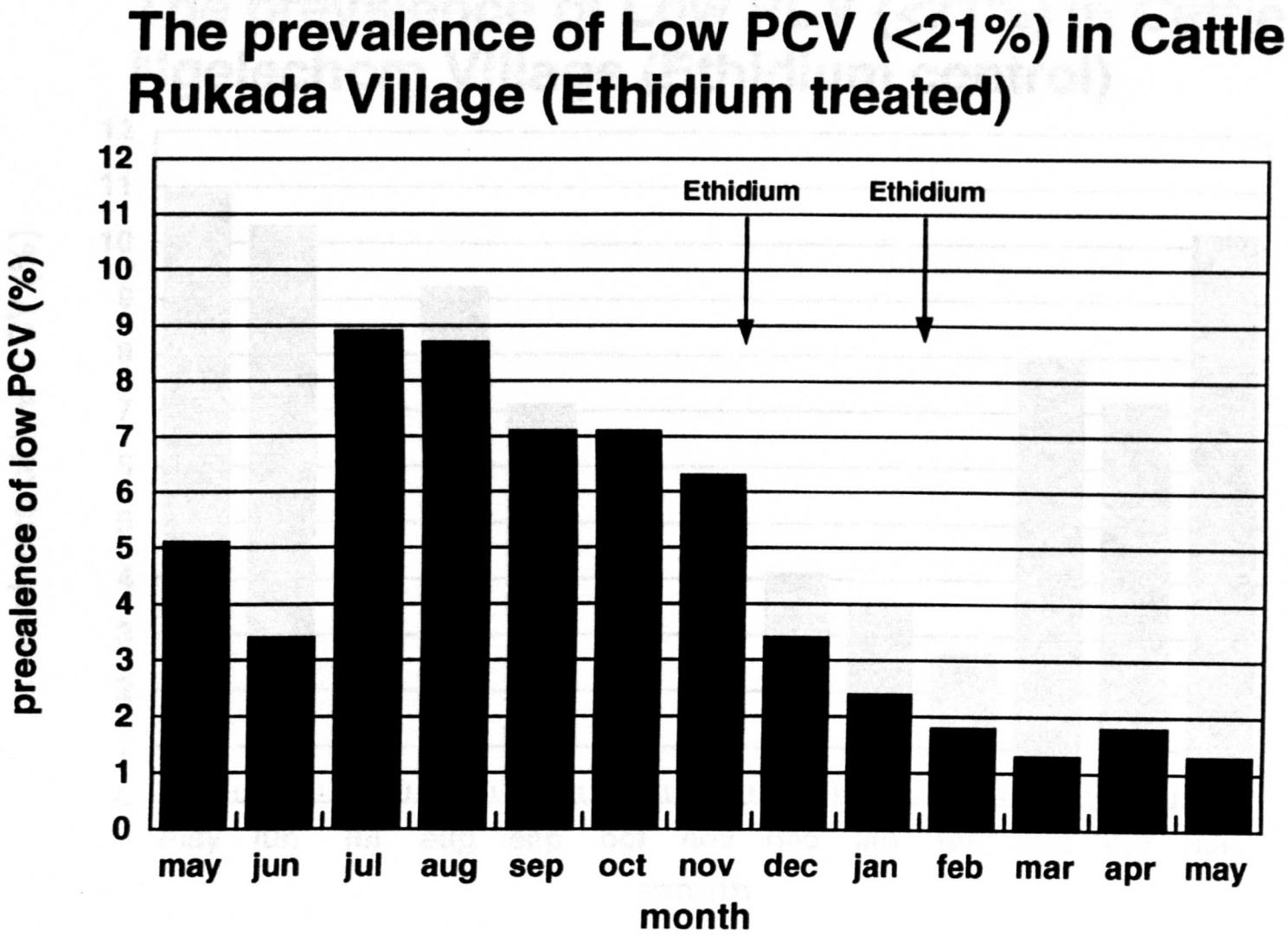

The prevalence of Low PCV (<21%) in Cattle
Rukada Village (Ethidium treated)
precalence of low PCV (%)
12
11
10
9
8
7
6
5
4
3
2
1
0
Ethidium
Ethidium
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 4.15

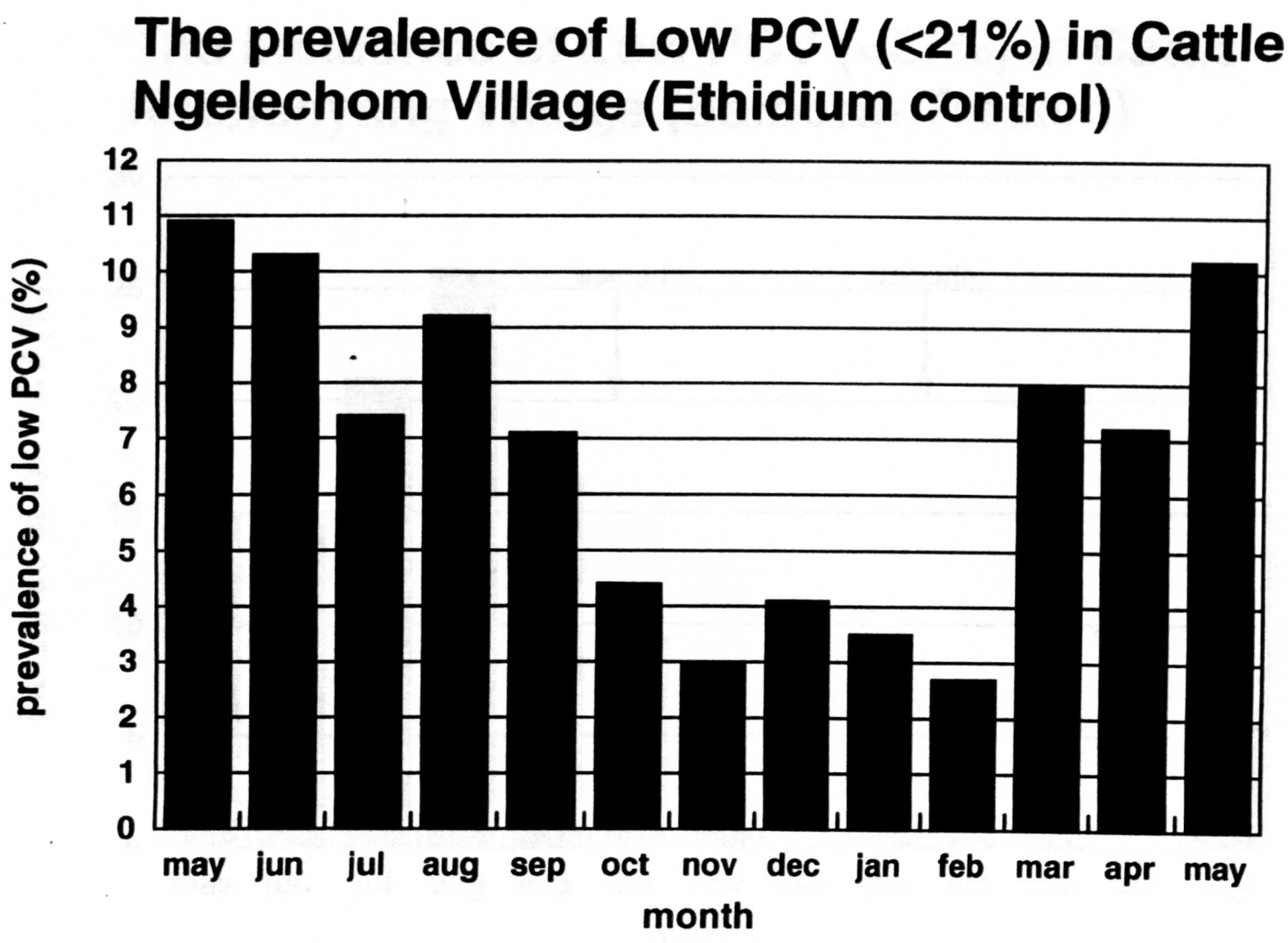

The prevalence of Low PCV (<21%) in Cattle
Ngelechom Village (Ethidium control)
prevalence of low PCV (%)
0
1
2
3
4
5
6
7
8
9
10
11
12
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 4.16

The incidence of Low PCV (<21%) in Cattle Katelenyang Village (Samorin Treated)

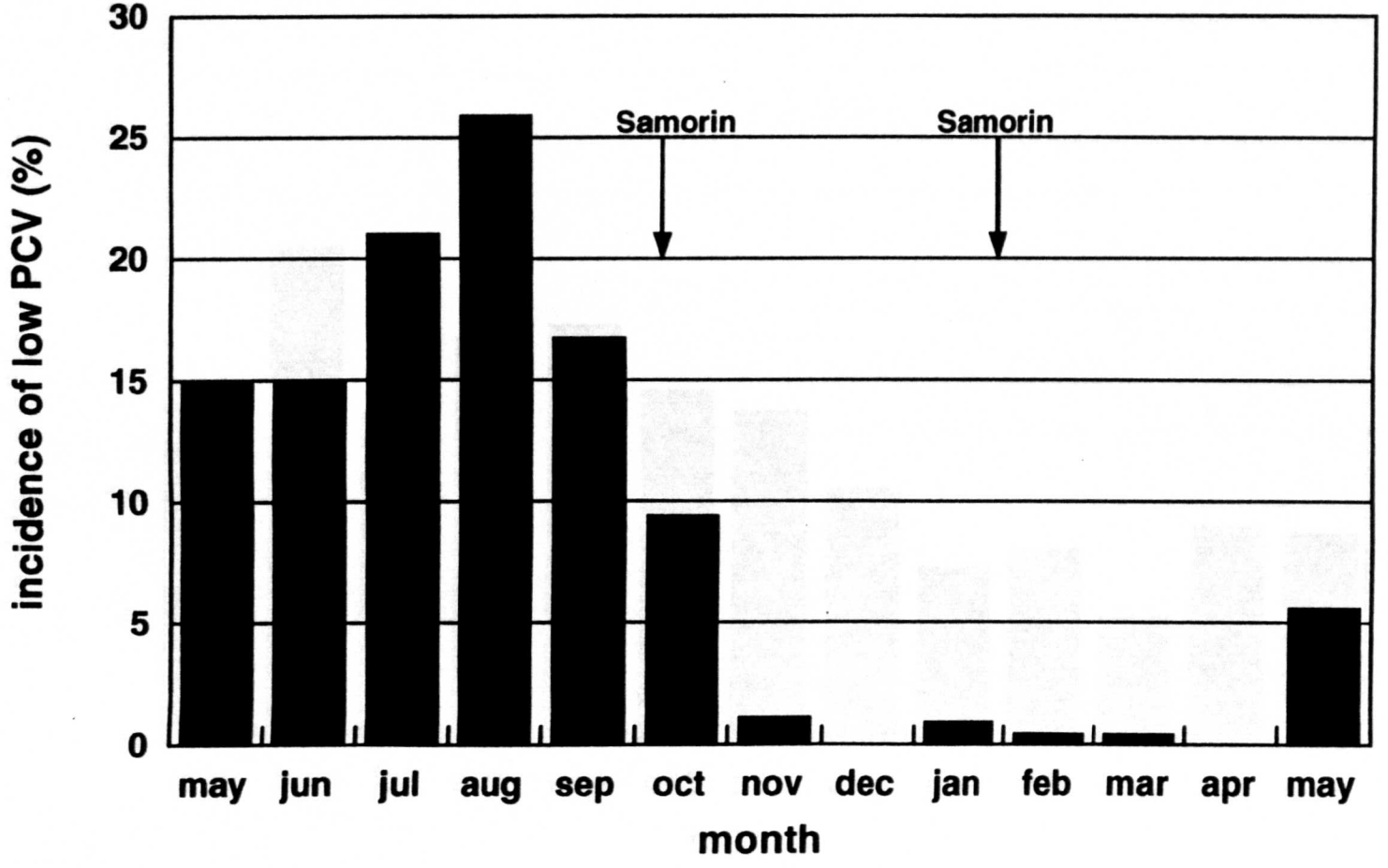


fig. 4.17

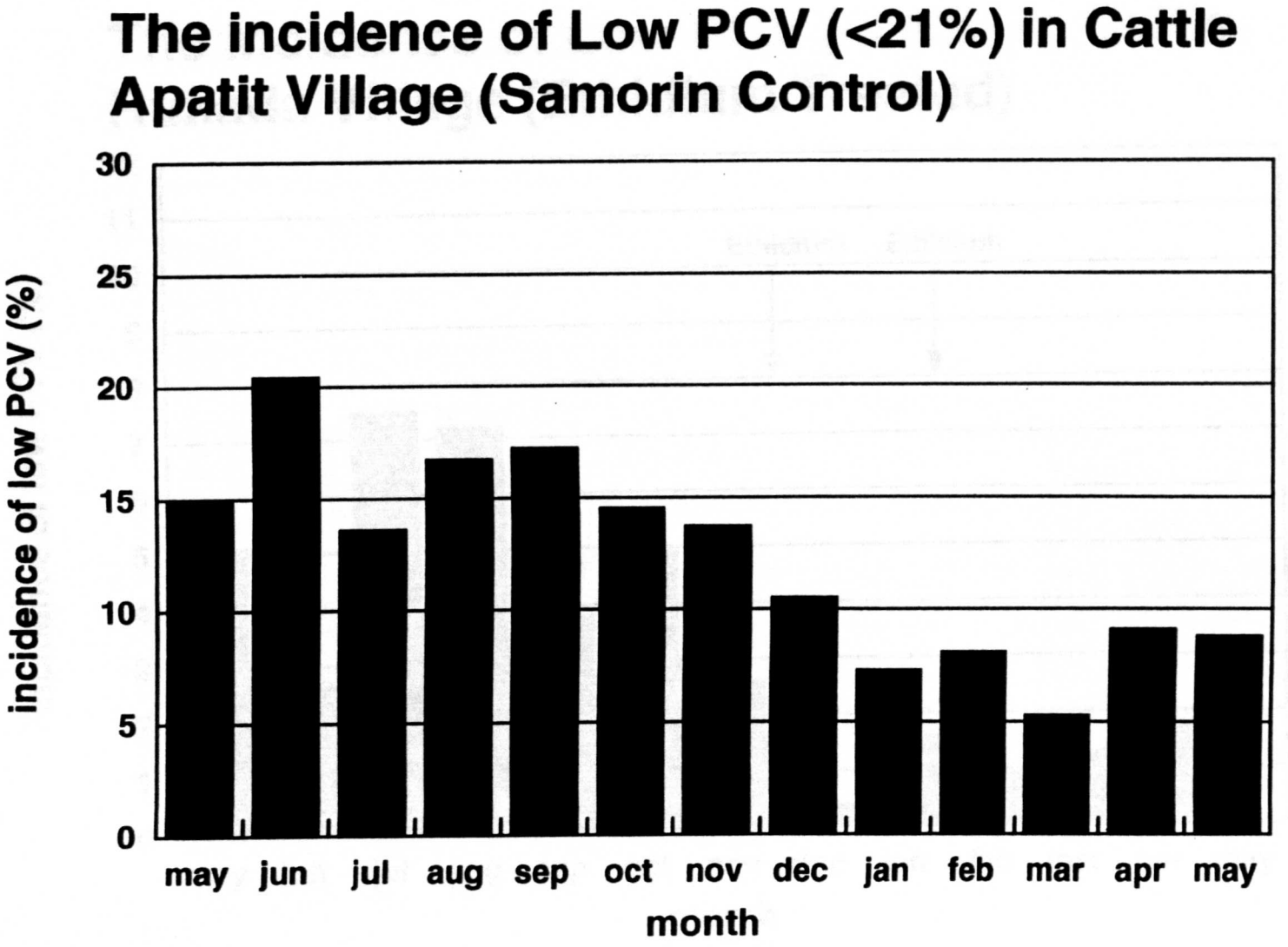

The incidence of Low PCV (<21%) in Cattle Apatit Village (Samorin Control)
incidence of low PCV (%)
30
25
20
15
10
5
0
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 4.18

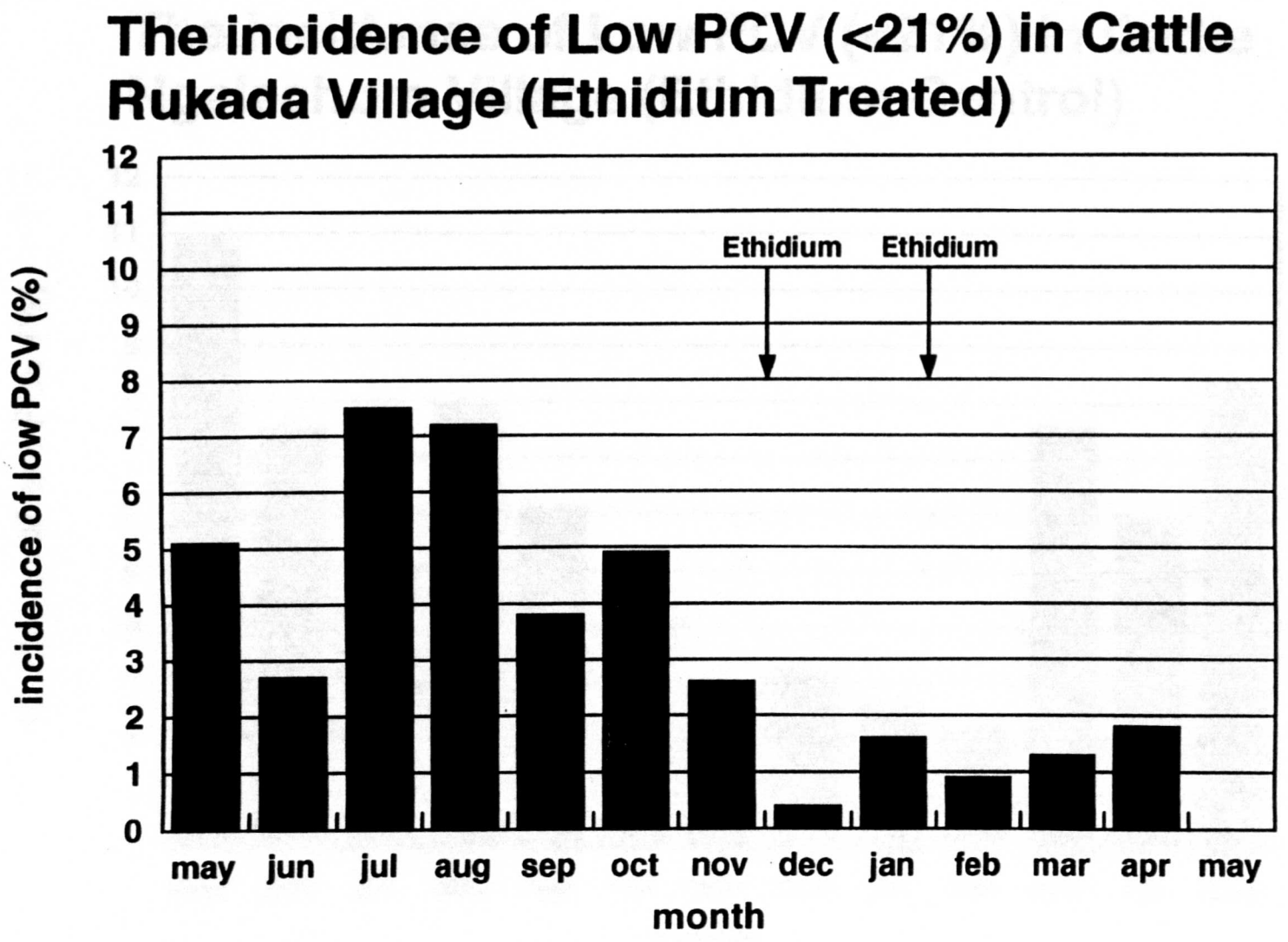

The incidence of Low PCV (<21%) in Cattle
Rukada Village (Ethidium Treated)
incidence of low PCV (%)
12
11
10
9
8
7
6
5
4
3
2
1
0
Ethidium
Ethidium
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 4.19

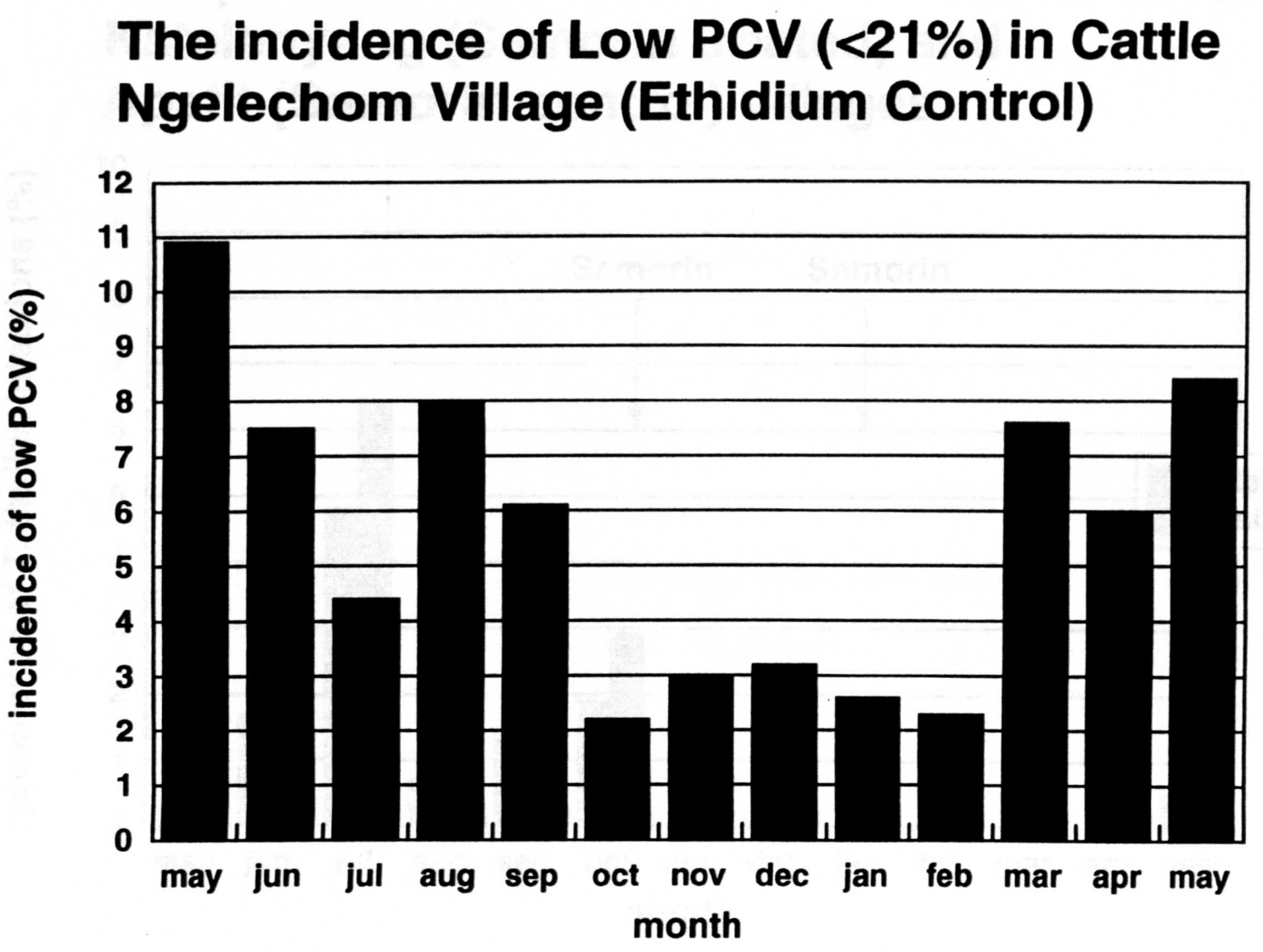

The incidence of Low PCV (<21%) in Cattle
Ngelechom Village (Ethidium Control)
incidence of low PCV (%)
0
1
2
3
4
5
6
7
8
9
10
11
12
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 4.20

The prevalence of *T. theileri* infections in cattle in Katelenyang (Samorin treated) and Apatit (Samorin control) villages

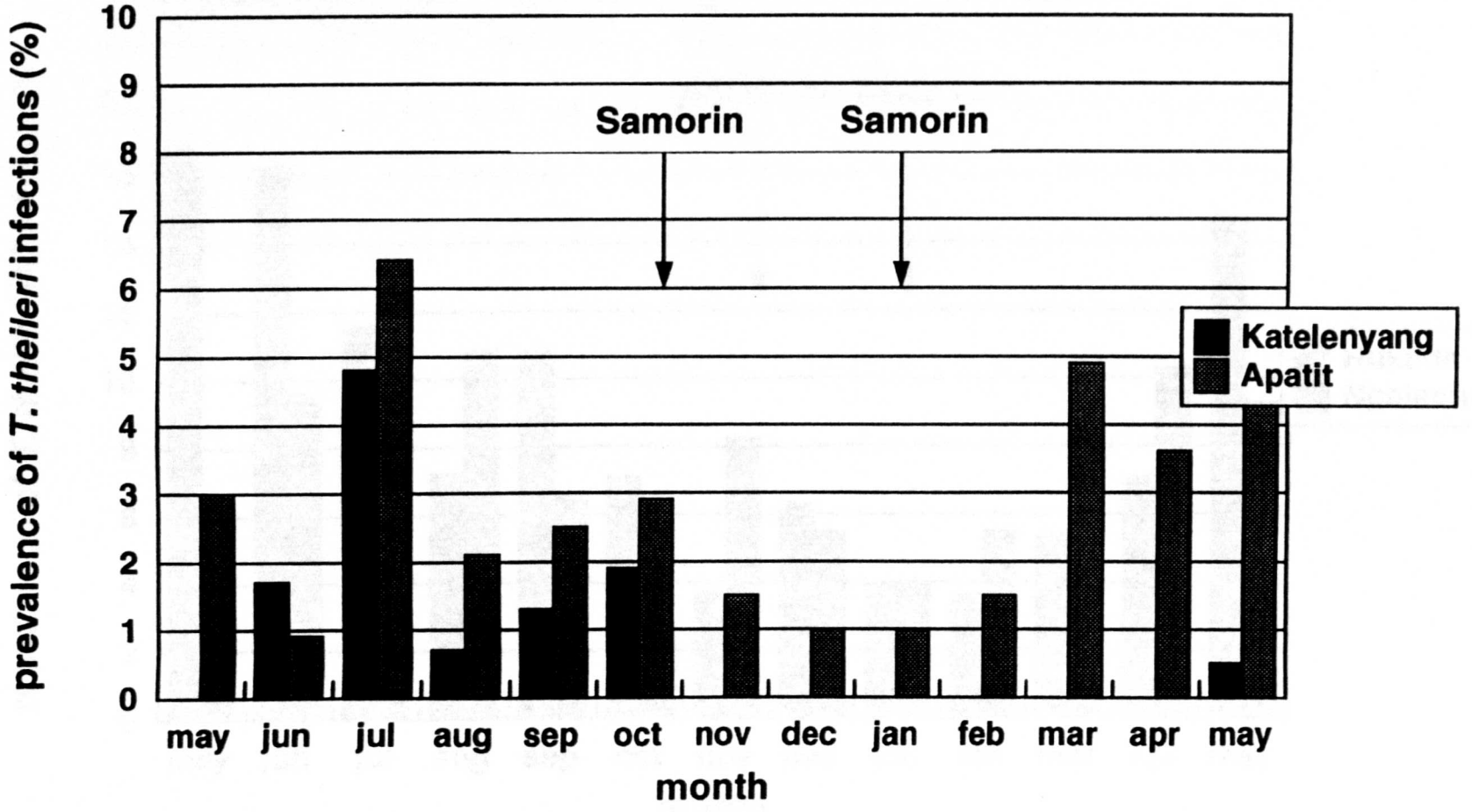


fig. 4.21

The prevalence of *T. theileri* infections in cattle in Rukada (Ethidium treated) and Ngelechom (Ethidium control) villages

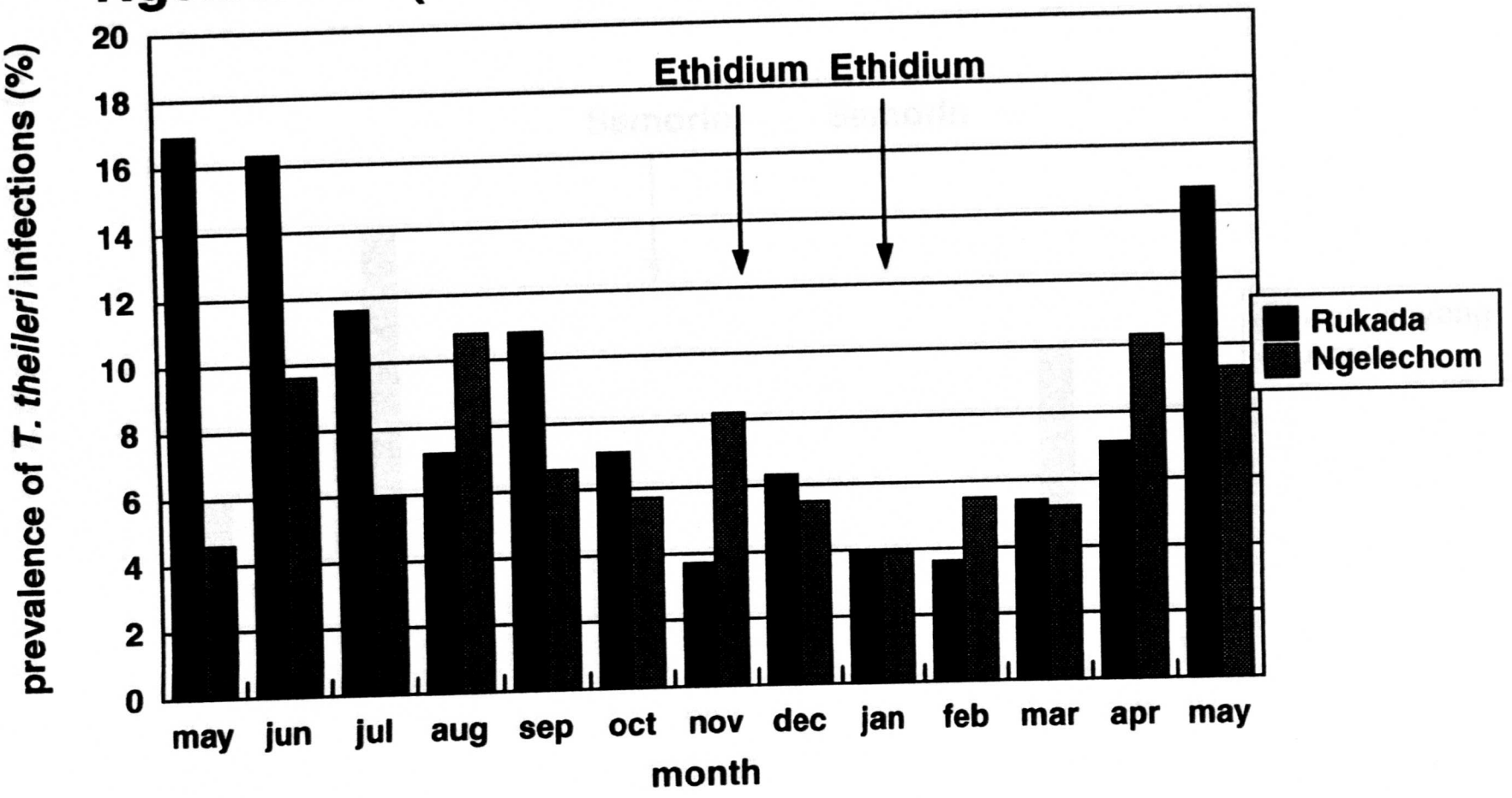


fig. 4.22

The incidence of *T. theileri* infections in cattle in Katelenyang (Samorin treated) and Apatit (Samorin control) villages

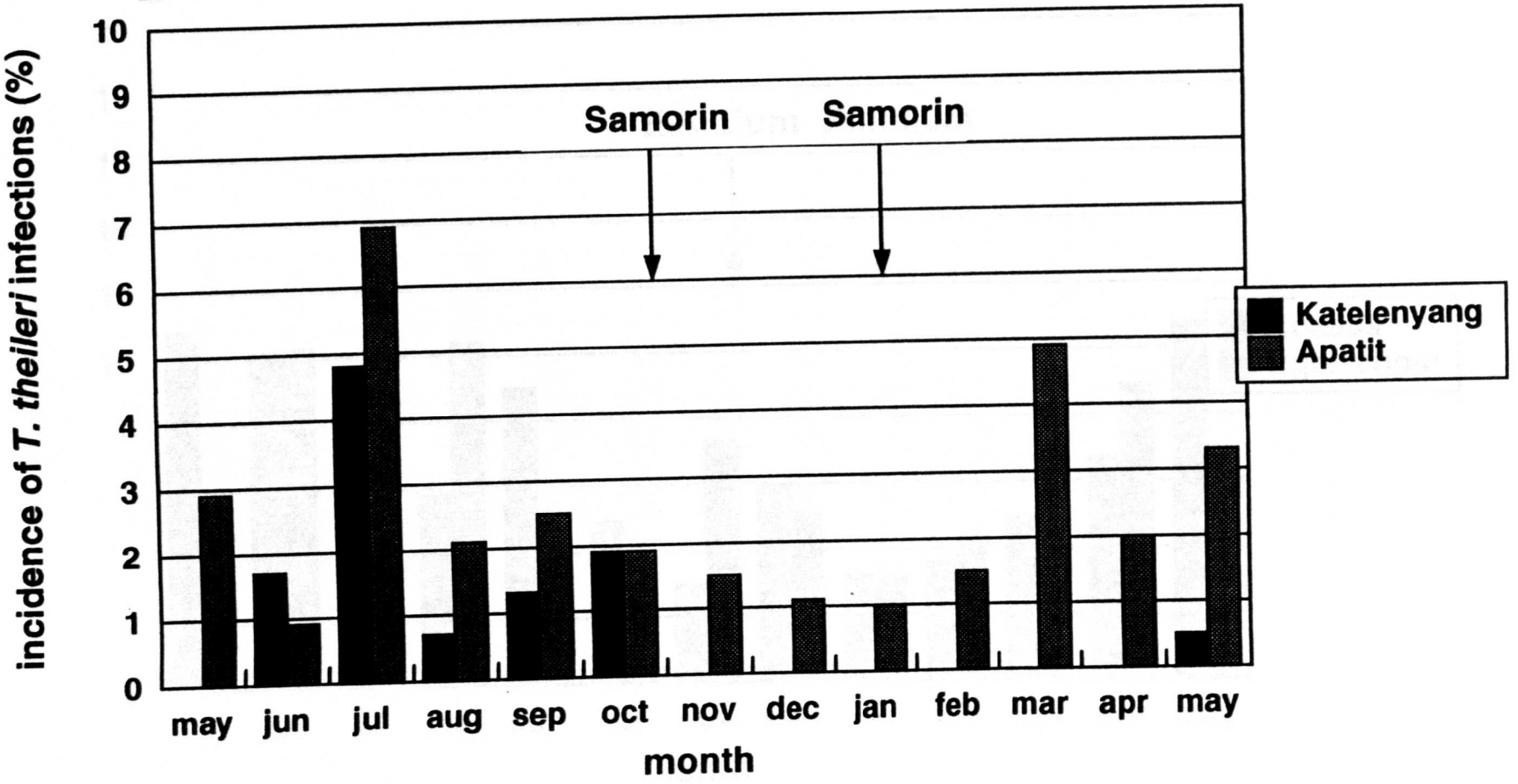


fig. 4.23

The incidence of *T. theileri* infections in cattle in Rukada (Ethidium treated) and Ngelechom (Ethidium control)

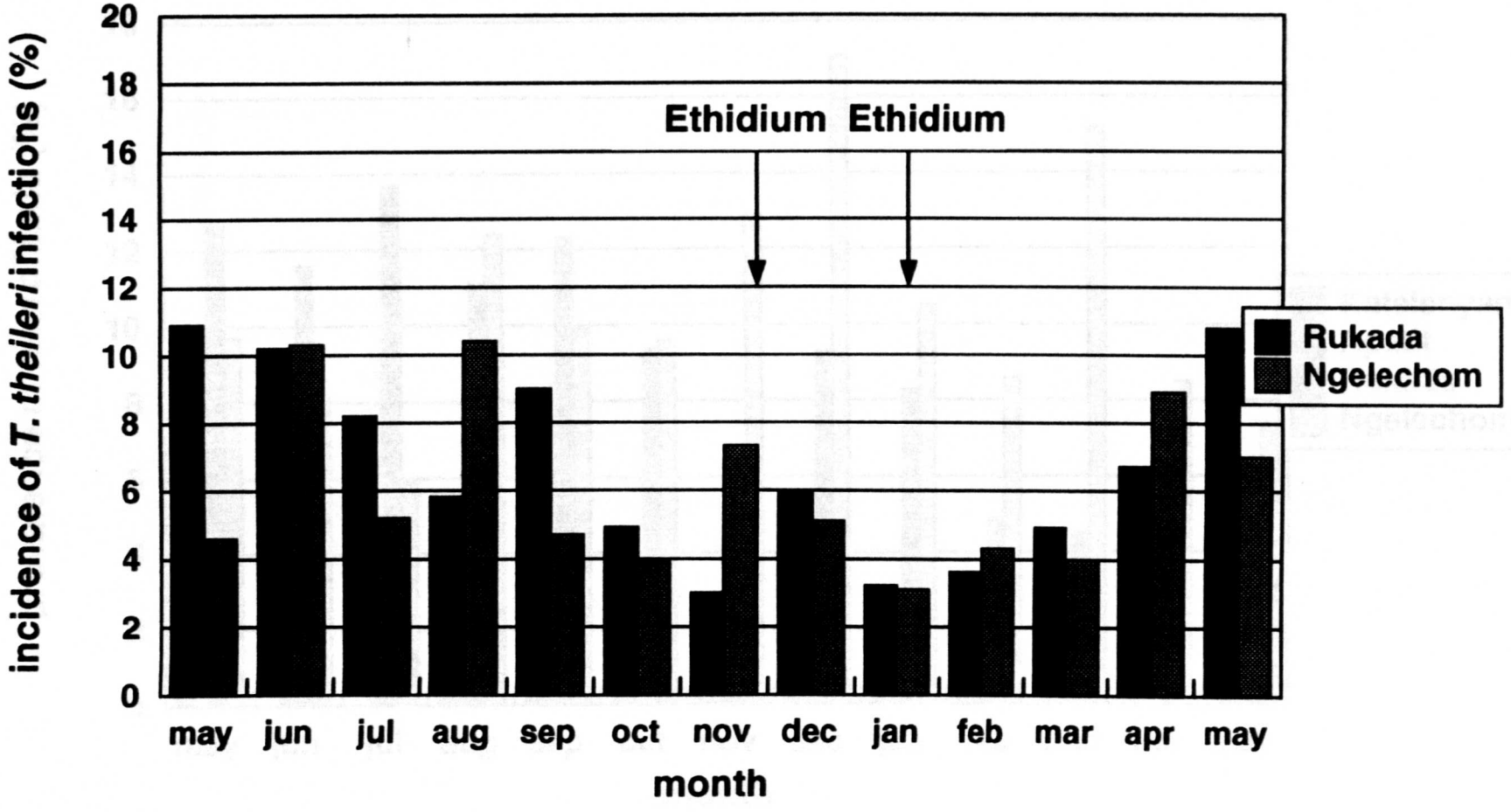


fig. 4.24

The prevalence of microfilaria in all 4 villages

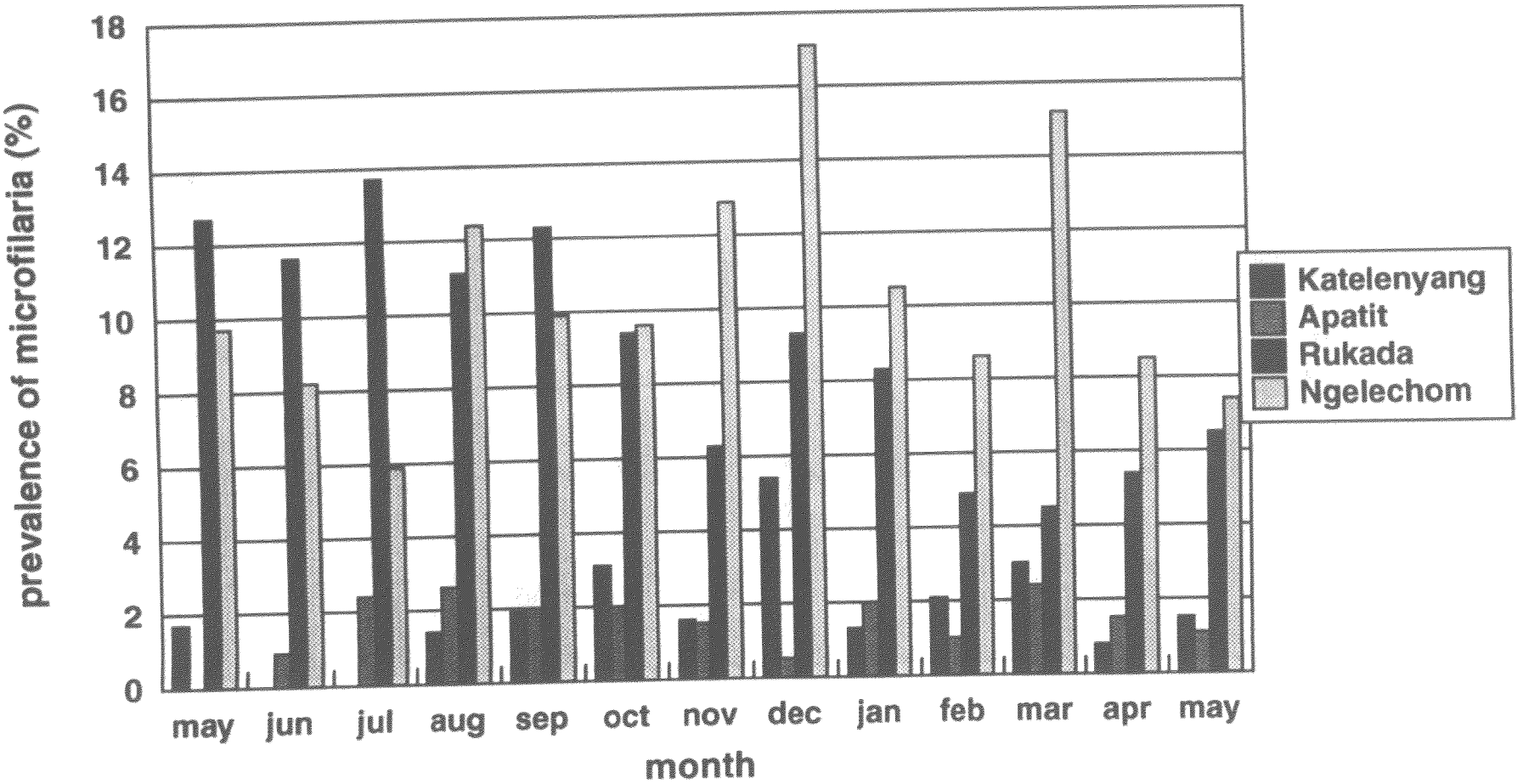


fig. 4.25

The incidence of microfilaria in all 4 villages

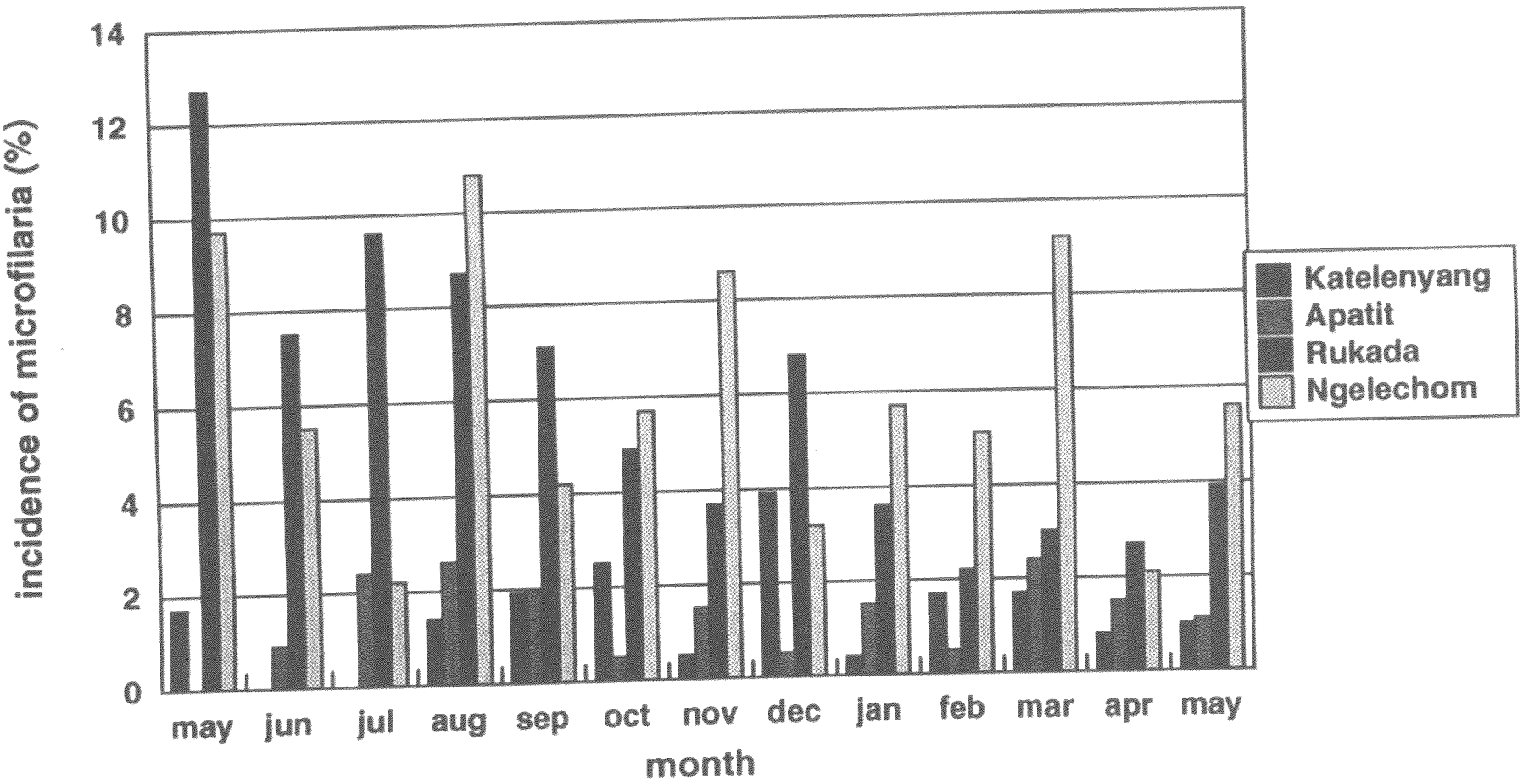


fig. 4.26

Chapter V

The epidemiology of trypanosomiasis in domestic livestock with particular reference to the potential animal reservoir of human infective *T. b. rhodesiense*

5.1 Summary

During an epidemiological study to investigate the potentially human infective *T. brucei* spp. reservoir of domestic livestock in Busia District in the Western Province of Kenya, an area where Rhodesian sleeping sickness is endemic, more than 12,000 blood samples were examined for evidence of trypanosome infections. Cattle were found to be the most important species in the potentially human infective reservoir of *T. brucei* spp. in domestic livestock, with a prevalence of up to 15%. Cattle were also one of the favoured hosts of *Glossina f. fuscipes* and were the most numerous species of domestic livestock in this area. However pigs may play a role in the animal reservoir of Rhodesian sleeping sickness under certain circumstances. The prevalence and incidence of mixed infections of more than one species of trypanosome in cattle was found to be much greater than would be expected by chance. This increase in mixed infections was much greater in mixed infections involving a *T. brucei* spp. infection rather than a mixed infection of *T. vivax* and *T. congolense*.

Tsetse fly trapping revealed that despite there being a 99% reduction in the number of *Glossina f. fuscipes* being caught in the four villages the incidence of trypanosome infections in cattle remained high (up to 35%). Biting flies could not explain this discrepancy.

There was a positive correlation between rainfall and the incidence of trypanosome infections in cattle in Busia District.

The implications of a greater than expected interaction between and within species of trypanosome for the epidemiology of sleeping sickness are discussed. A mechanism for the natural control of the animal reservoir in Rhodesian sleeping sickness is suggested.

5.2 Introduction

A longitudinal epidemiological study was carried out in Busia District in the Western Province of Kenya, an area where Rhodesian sleeping sickness is endemic, to examine the importance of domestic livestock as a potential reservoir for human infection, since the district is largely devoid of game. The study was carried out in four villages over a thirteen month period from May 1993 to May 1994, with the same random sample of livestock being blood sampled each month. During this study more than 12,000 blood samples were collected from domestic animals of which 9,973 were from cattle, 1,029 from goats, 965 from sheep and 284 from pigs

to examine the extent and the nature of the potentially human infective *T. brucei* spp. reservoir in domestic animals. Although it has been known for some time that domestic animals could act as a potential reservoir for *T. b. rhodesiense*, the role of differing species and the extent and nature of this domestic animal reservoir remains the subject of speculation (Corson, 1934; Onyango *et al.*, 1966; Mehlitz, 1987; Wellde *et al.*, 1989c). Within each species of domestic animal it is important to know what the risk factors for trypanosome infections are in order to devise appropriate strategies for the avoidance and control of these trypanosome infections. Under experimental conditions interaction between differing species of trypanosome (Masake *et al.*, 1984) and between differing strains of the same species of trypanosome (Morrison *et al.*, 1982; Dwinger *et al.*, 1986; Sones *et al.*, 1989; Turner *et al.*, 1996) has been observed, however neither the causes nor the effects of such interactions have been investigated in the field.

Meteorological data were collected throughout the period of the study from weather stations nearest the four villages and from the District reference weather station at Bunyala Irrigation Scheme to examine any association between climate and the incidence of trypanosome infections in domestic livestock.

Similarly tsetse and biting flies were trapped on a monthly basis in order to try to relate the number of flies to the incidence of trypanosome infections.

It has long been known that sleeping sickness has an endemic level and from this it rises to epidemic levels and then falls back to endemic levels again. In the past it has been speculated that sleeping sickness epidemics are the result of the emergence of new strains of trypanosome, virulent to humans, from the animal population of *T. b. brucei* (Gibson and Wellde, 1985), however more recent evidence suggests that this is not what happens. It is now known that the same strains of *T. b. rhodesiense* have remained circulating very locally over periods of at least 30 years (Hide *et al.*, 1994). This being the case the natural mechanism for the emergence of an epidemic of sleeping sickness and its equally rapid demise remains speculative.

5.3 Materials and methods

For general materials and methods see Chapter III.

5.3.1 Blood sampling of livestock and the diagnosis of trypanosome infections

Following a random selection of village livestock all animals were uniquely identified with ear tags and were blood sampled monthly over a period of thirteen months. Diagnosis of trypanosome infections were carried out by haematocrit centrifugation technique (HCT), buffy coat technique (BC), subinoculation of immunosuppressed mice and thin blood films fixed and stained with Geimsa (Chapter III).

5.3.2 Meteorological data

Meteorological data was collected by the field stations of the Meteorological Office, Government of Kenya at Bunyula, Amukura and Funyula in Busia District.

5.3.3 Tsetse and biting fly trapping

Twenty biconical traps (Challier and Laveissière, 1973) were deployed at a spacing of 100 meters in each of the four villages of Katelenyang, Apatit, Rukada and Ngelechom. Trapping was carried out over period of 48 hours and all trapped flies were counted and identified at intervals of 12 hours.

5.3.4 Statistical analysis

Statistical analysis was carried out using a Chi^2 square test where possible and a Fisher's exact test when expected values were less than five.

5.4 Results

5.4.1 The monthly prevalence and incidence of trypanosome infections in domestic cattle

The prevalence of trypanosome infections in cattle was highest in Katelenyang village (Samorin treated) at 42.6% for all trypanosome infections with the prevalence of *T. brucei*

spp. infections being 26.2% before treatment with Samorin. At the same time Katelenyang village had the highest incidence of all trypanosome infections at 36.2% with the incidence of *T. brucei* spp. infections being 16.5%. The lowest prevalence of trypanosome infections was observed in Rukada village (Ethidium treated) at 3.7% for all trypanosome infections, with all infected cattle having a *T. brucei* spp. infection, before treatment with Ethidium. At the same time Rukada village had the lowest incidence of all trypanosome infections at 1% (See Chapter IV sections 4.3.1, 4.3.2, 4.3.4 and 4.3.5).

5.4.2 The monthly prevalence and incidence of trypanosome infections in domestic species other than cattle

The incidence and prevalence of trypanosome infections in domestic species of livestock other than cattle was very low (Table 12). Pigs in the village of Katelenyang, where cattle had the highest prevalence of trypanosome infections (at times over 42% (26% *T. brucei* spp.)) (fig. 4.1), the prevalence of trypanosome infections in pigs was only 5.2% at its highest; however all the infections were *T. brucei* spp. (Table 5.1). These figures were much lower in other villages except Apatit village where the prevalence of *T. brucei* spp. infections in pigs (12.5%) was similar to that in cattle (fig. 4.2 and Tables 5.1 to 5.4). The prevalence of trypanosome infections in goats was 4.3% in Apatit, the village with the highest prevalence, compared to a prevalence in cattle of around 20% (fig. 4.2 and Table 5.2). Again these figures tended to be lower in other villages with only two goats in the entire study being found to be infected with *T. brucei* spp. (five samples positive for *T. brucei* spp. from 729 samples taken from goats). Sheep had an even lower prevalence (3.5%) and incidence (1.2%) of trypanosome infections again in Apatit, the village with the highest prevalence of trypanosome infections in sheep. Three sheep were found to be infected with *T. brucei* spp. (10 samples positive for *T. brucei* spp. from 956 samples taken from sheep) (Tables 5.1 to 5.2). One dog was found to be infected with *T. brucei* spp. from the 72 dogs which were sampled.

Table 5.1 The number of trypanosome infections in domestic livestock other than cattle in Katelenyang village (Samorin treated) during the complete study

species	number of animals	number of samples	*T. brucei* spp.	*T. vivax*	*T. congolense*	mixed infections	total
goats	43	169	0	0	0	0	0
sheep	36	121	0	0	0	0	0
pigs	24	115	6 (5)	0	0	0	6 (5)

( ) number of positive animals

Table 5.2 The number of trypanosome infections in domestic livestock other than cattle in Apatit village (Samorin control) during the complete study

species	number of animals	number of samples	*T. brucei* spp.	*T. vivax*	*T. congolense*	mixed infections	total
goats	56	208	3 (1)	6 (2)	0	0	9 (3)
sheep	61	265	8 (2)	0	1 (1)	0	9 (3)
pigs	31	56	7 (2)	0	0	0	7 (2)

( ) number of positive animals

Table 5.3 The number of trypanosome infections in domestic livestock other than cattle in Rukada village (Ethidium treated) during the complete study

species	number of animals	number of samples	*T. brucei* spp.	*T. vivax*	*T. congolense*	mixed infections	total
goats	82	469	2 (1)	6 (2)	0	0	8 (3)
sheep	84	356	2 (1)	0	0	0	2 (1)
pigs	23	111	0	0	0	0	0

( ) number of positive animals

Table 5.4 The number of trypanosome infections in domestic livestock other than cattle in Ngelechom (Ethidium control) during the complete study

species	number of animals	number of samples	*T. brucei* spp.	*T. vivax*	*T. congolense*	mixed infections	total
goats	57	183	0	0	0	0	0
sheep	52	214	0	0	0	0	0
pigs	2	2	0	0	0	0	0

( ) number of positive animals

5.4.3 Bloodmeal analysis of *Glossina f. fuscipes* from Tororo District, Uganda during a sleeping sickness epidemic 1988 to 1990

Bloodmeal analysis of *Glossina f. fuscipes* from Tororo District, Uganda (an area immediately adjacent to Busia District on the opposite bank of the river Malaba) taken towards the end of the sleeping sickness epidemic from 1988 to 1990 reveals that the most favoured hosts of the tsetse fly were monitor lizards (59.4%) and domestic cattle, with man only providing 7.3% of blood meals (Table 5.5). These three hosts combined account for 90% of all tsetse feeds in this area (data Maudlin unpublished).

Table 5.5 Bloodmeal analysis of *Glossina f. fuscipes* from Tororo District, Uganda during a sleeping sickness epidemic 1988 to 1990

species	number	%
monitor lizard	82	59.4
domestic cattle	32	23.2
MAN	10	7.3
wild ruminant	4	2.9
bushbuck	3	2.2
dog	2	1.5
hippopotamus	2	1.5
crocodile	1	0.7
chicken	1	0.7
rodent	1	0.7
total	138	100

(Maudlin unpublished data)

5.4.4 The prevalence of mixed infections in domestic cattle

The prevalence of mixed infections mirrored the total prevalence of trypanosome infections (figs. 4.1 to 4.4).

However, all mixed infections occurred at a much greater frequency than would be expected by chance. Mixed infections of *T. brucei* spp. and *T. vivax* were up to 22 times more common; mixed infections of *T. brucei* spp. and *T. congolense* were up to 18 times more common while mixed infections with *T. vivax* and *T. congolense* up to 5 times more common than would be expected by chance. This phenomenon was repeated in all 4 villages regardless of the overall prevalence of trypanosomiasis (Table 5.6 and Appendix V Tables V.1 to V.15)

Table 5.6 The factor of increase in observed occurrence of the prevalence of mixed infections over that expected by chance

village	tsetse challenge[1]	*T. brucei* spp. / *T. vivax*	*T. brucei* spp. / *T. congolense*	*T. vivax* / *T. congolense*
Katelenyang	high	10.1 ***	8.5 ***	2.7 ***
Apatit	high	3.5 ***	17.6 ***	1.7 *
Rukada	low	22.0 ***	10.9 ***	4.7 **
Ngelechom	medium	9.4 ***	11.6 ***	6.3 ns
all 4 villages		7.6 ***	6.8 ***	3.4 ***

[1] based on 'Berenil index' (Chapter IV, 4.3.10)

ns not significant

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

5.4.5 The incidence of mixed infections in domestic cattle

The incidence of mixed infections (simultaneous new infections with 2 or more species of trypanosome) mirrored the total incidence of trypanosome infections (figs. 4.5 to 4.8) and occurred at a much greater frequency than would be expected by chance. The incidence of mixed infections of *T. brucei* spp. and *T. vivax* were up to 28 times more common, mixed infections of *T. brucei* spp. and *T. congolense* were up to 13 times more common while mixed infections with *T. vivax* and *T. congolense* were up to 8 times more common than would be expected by chance. This observation was repeated in all 4 villages regardless of the overall prevalence of trypanosomiasis (Table 5.7 and Appendix V Tables V.16 to V.30).

Table 5.7 The factor of increase in observed occurrence of the incidence of mixed infections over that expected by chance

village	tsetse challenge	*T. brucei* spp. / *T. vivax*	*T. brucei* spp. / *T. congolense*	*T. vivax* / *T. congolense*
Katelenyang	high	3.4 ***	9.1 ***	1.9 ***
Apatit	high	4.5 ***	3.5 ***	2.0 ***
Rukada	low	27.7 ***	13.3 ***	6.3 **
Ngelechom	medium	11.3 ***	11.6 ***	7.7 *
all 4 villages		9.2 ***	7.5 ***	4.1 ***

[1] based on 'Berenil index' (Chapter IV, 4.3.10)

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

5.4.6 The risk factors for trypanosome infections in cattle

5.3.6.1 Sex of cattle as a risk factor for infection with a trypanosome

Sex was only found to be a significant risk factor in Rukada village (Ethidium treated) where male animals were twice as likely to become infected with a pathogenic trypanosome than female animals (Tables 5.8 to 5.11). When the data from all 4 villages were combined there appeared to be a significant difference in the risk factor for all trypanosome infections. However this was caused by large difference in the sex ratios between Rukada and the other 3 villages (Appendix V Tables V.61 to V.75).

Table 5.8 Sex as a risk factor for *T. brucei* spp. infections

	male / female ratio	significance (p)
Katelenyang	1.2	0.464
Apatit	0.97	0.839
Rukada	1.7	0.082
Ngelechom	1.0	0.900
total	1.3	0.025

Table 5.9 Sex as a risk factor for *T. vivax* infections

	male / female ratio	significance (p)
Katelenyang	1.1	0.491
Apatit	1.0	0.850
Rukada	1.6	0.142
Ngelechom	1.2	0.259
total	1.4	0.000

Table 5.10 Sex as a risk factor for *T. congolense* infections

	male / female ratio	significance (p)
Katelenyang	0.8	0.560
Apatit	1.4	0.099
Rukada	3.2	0.010
Ngelechom	0.8	0.501
total	1.5	0.010

Table 5.11 Sex as a risk factor for infection with any pathogenic trypanosome

	male / female ratio of infected cattle	significance (p)
Katelenyang	1.0	0.946
Apatit	1.1	0.272
Rukada	2.0	0.004
Ngelechom	1.2	0.193
total	1.4	0.000

5.4.6.2 Age of cattle as a risk factor for infection with a trypanosome

There is a significant reduction in the actual incidence compared to the expected incidence of infections of *T. brucei* spp. ($p < 0.001$), *T. vivax* ($p < 0.001$), *T. congolense* ($p < 0.001$) or an infection with any one of these pathogenic trypanosomes ($p < 0.001$) in cattle less than 1 year old. After this age there was no increase or decrease in incidence of trypanosome infections compared to their expected values (Appendix V Tables V.76 to V.79).

5.4.6.3 An existing trypanosome infection as a risk factor for infection with another species of trypanosome

The probability of an individual animal becoming infected with a new trypanosome infection was much greater for an animal already with a trypanosome infection than for an animal which was uninfected when one of the infections is *T. brucei* spp. (Tables 5.12 to 5.14 and Appendix V Tables 31 to V.60). This effect of interaction between species of trypanosome was much greater between *T. brucei* spp. and *T. vivax* and was most significant in animals with an existing infection of *T. brucei* spp. which subsequently become infected with *T. vivax* (Table 5.12 and Appendix V Tables V.31 and V.32).

Table 5.12 The factor of increase in observed occurrence of the incidence of infection of one species of trypanosome in an animal already infected with another species of trypanosome over that expected by chance *T. brucei* spp. and *T. vivax*

new trypanosome infection		*T. vivax*	*T. brucei* spp.
established trypanosome infection		*T. brucei* spp.	*T. vivax*
village	challenge [1]		
Katelenyang	high	7.0 ***	5.0 **
Apatit	high	1.3 ns	1.8 ns
Rukada	low	12.5 ***	6.7 ns
Ngelechom	medium	5.6 **	1.1 ns
all 4 villages		3.6 ***	3.1 ***

[1] based on 'Berenil index' (Chapter IV, 4.3.10)

ns not significant

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 5.13 The factor of increase in observed occurrence of the incidence of infection of one species of trypanosome in an animal already infected with another species of trypanosome over that expected by chance *T. brucei* spp. and *T. congolense*

new trypanosome infection		*T. congolense*	*T. brucei* spp.
established trypanosome infection		*T. brucei* spp.	*T. congolense*
village	challenge [1]		
Katelenyang	high	3.3 ns	33.3 *
Apatit	high	2.3 *	3.1 *
Rukada	low	5.0 ns	n/a
Ngelechom	medium	n/a	n/a
all 4 villages		3.9 ***	7.1 ***

[1] based on 'Berenil index' (Chapter IV, 4.3.10)

n/a not able to calculate because of zero values

ns not significant

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 5.14 The factor of increase in observed occurrence of the incidence of infection of one species of trypanosome in an animal already infected with another species of trypanosome over that expected by chance *T. vivax* and *T. congolense*

new trypanosome infection		*T. vivax*	*T. congolense*
established trypanosome infection		*T. congolense*	*T. vivax*
village	challenge [1]		
Katelenyang	high	n/a	n/a
Apatit	high	0.8 ns	1.1 ns
Rukada	low	n/a	n/a
Ngelechom	medium	n/a	10 ns
all 4 villages		0.8 ns	2.5 ns

[1] based on 'Berenil index' (Chapter IV, 4.3.10)

n/a not able to calculate because of zero values

ns not significant

5.4.7 Removal of trypanosome infections from the epidemiological cycle

Removal of trypanosome infections from the epidemiological cycle occurs by four main methods: death of the animal; slaughter for reason of ill health; sold for reason of ill health or treatment with a trypanocidal drug. The probability of an infected animal being removed from the epidemiological cycle varied with the species of trypanosome or trypanosomes involved.

There was a significant reduction in the probability (by a factor of 4) of the removal of an infection of a single infection of *T. brucei* spp. from the epidemiological cycle compared to either a single infection of *T. vivax* ($p < 0.001$) or a single infection of *T. congolense* ($p < 0.001$). However there was no significant difference in the chances of removal between single infections of *T. vivax* and *T. congolense* ($p = 0.442$). When comparing the probability of the removal single infections of *T. brucei* spp. with that of mixed infections of either *T. brucei* spp. and *T. vivax* ($p < 0.001$) or *T. brucei* spp. and *T. congolense* ($p < 0.001$) there was a very significant increase in the probability of both mixed infections being removed from the epidemiological cycle. However there was no significant

difference between a single infection of *T. vivax* and a mixed infection with *T. brucei* spp. and *T. vivax* ($p = 0.139$) or a single infection of *T. congolense* compared to a mixed infection of *T. brucei* spp. and *T. congolense* ($p = 0.117$). There was a significant increase in the probability of removal from the epidemiological cycle between a mixed infection of *T. vivax* and *T. congolense* when compared to either a single infection of *T. vivax* ($p = 0.001$) or a single infection of *T. congolense* ($p = 0.001$). Numerically the most important factor in this effect was the greatly increased likelihood of a mixed infection of *T. brucei* spp. and *T. vivax* being removed from the epidemiological cycle compared to a single infection of *T. brucei* spp. (Table 5.15 and Appendix V Tables V.80 to V.88).

Table 5.15 The number of trypanosome infections removed from the epidemiological cycle by type of infection

fate type of infection	remains in the epidemiological cycle	removed from the epidemiological cycle	percentage (%) removed
T. brucei spp. only	153	21	12.1 **
T. vivax only	218	199	47.7
T. congolense only	56	43	43.4
T. brucei spp. and *T. vivax*	146	105	41.8
T. brucei spp. and *T. congolense*	21	28	57.1
T. vivax and *T. congolense*	7	25	78.1 *
T. brucei spp., *T. vivax* and *T. congolense*	5	10	66.7

** highly significant $p < 0.001$

* significant $p < 0.01$

5.4.8 Human trypanosomiasis in Busia District

There were three admissions to Alupe sleeping sickness hospital in 1993, two of which originated in Uganda and were people seeking medical treatment in Kenya. The third was a 17 year old girl from Ikonzo village in Marachi location, Busia District. The previous year there had also been a single case from this village. Human and animal disease surveys were carried out by KETRI staff at the same time a few days after the admission of the sleeping sickness patient (Tables 5.16 and 5.17). All trypanosome infections in the cattle of this village were *T. brucei* spp.

Table 5.16 Human disease survey, Ikonzo village 1993

site	number	trypanosomiasis	malaria
village	143	1	47
market	67	0	19
total	209	0	66

Table 5.17 Cattle disease survey, Ikonzo village 1993

site	number	*T. brucei* spp.	*T. vivax*	*T. congolense*	*T. theileri*	microfilaria
village	35	3	0	0	1	1
market	15	1	0	0	0	0
total	50	4	0	0	1	1

5.4.9 Meteorological data

The rainfall, temperature and relative humidity at Bunyala Irrigation Scheme showed the general meteorological trends of Busia District between May 1993 and May 1994 (fig. 2.4). Because of the patterns of convection rainfall in the district very local variations in rainfall were common. While the rainfall data from Amukura (fig. 5.1), the nearest meteorological station, accurately depicts the conditions in Katelenyang (Samorin treated) and Apatit (Samorin control) villages during the period of the study this was not always the case in either Rukada (Ethidium treated) (Funyula meteorological station) or Ngelechom (Ethidium control) (Amukura meteorological station) villages where the short rains failed in both villages in October and November. However the long rains started towards the end of February in Ngelechom (Ethidium control) but did not start until May in Rukada (Ethidium prophylaxis). While the rainfall data from Funyula (fig. 5.2) was similar to that in Rukada village (Ethidium treated) from May to October, during the period of Ethidium prophylaxis from November onwards there was a complete drought with no rainfall until May 1994.

5.4.10 Trapping tsetse flies

The number of tsetse flies caught in the two villages used for the Samorin drug trial throughout the study were very similar as were the number of flies caught in the two villages used in the Ethidium trial. However the number of tsetse flies caught in both the hilly villages of Katelenyang (Samorin treated) and Apatit (Samorin control) were very low compared to the lower lying villages of Rukada (Ethidium treated) and Ngelechom (Ethidium control) (figs. 5.2 to 5.6). No seasonal pattern could be found in the tsetse catch in either Katelenyang or Apatit villages because of the very low catch (figs. 5.3 and 5.4). However in the villages of Rukada and Ngelechom more flies tended to be caught in the wetter months from April to July and less flies in the drier months from August to February (figs. 5.5 and 5.6). All tsetse flies caught were *Glossina f. fuscipes.*

5.4.11 Trapping biting flies

Similar numbers of biting flies (*Stomoxys, Tabanidae* and *Haematopota*) were caught in the three villages of Apatit (Samorin control), Rukada (Ethidium treated) and Ngelechom (Ethidium control) (figs. 5.8 to 5.10) while many fewer flies were caught in Katelenyang village (Samorin treated) (fig. 5.7). More biting flies were caught in the wetter months between April and October in all three villages.

5.5 Discussion

In terms of the prevalence and incidence of *T. brucei* spp. infections in cattle, goats, sheep and pigs it can be seen that cattle are the most important species of domestic animal acting as a potential reservoir of human infective *T. b. rhodesiense* in this area. Furthermore from the blood meal analysis (Table 5.5) it can be seen that domestic cattle are one of the favoured hosts of tsetse flies, unlike the other species of domestic animals, which made domestic cattle the most important source of *T. brucei* spp. infections for tsetse flies in this area. In one village (Apatit) the prevalence of *T. brucei* spp. infections in pigs (12.5%) was of a similar magnitude to that found in the cattle there, however there were numerically fewer pigs (88) than cattle (310) in Apatit village. Under other circumstances it has been demonstrated that

pigs may account for up to 20% of blood meals of *Glossina f. fuscipes* in Busoga district, Uganda (Okoth and Kapaata, 1988) and should the population of pigs be sufficiently great then pigs may play a significant role in the animal reservoir of *T. b. rhodesiense*. Although it has been shown experimentally that goats (Whitelaw *et al.*, 1985 ;Wellde *et al.*, 1989d) and sheep (Ikede and Losos 1972; Bouteille *et al.*, 1988) can readily be infected with *T. brucei* spp. the prevalence of *T. brucei* spp. infections in goats and sheep under local village conditions was very low. The relative distaste of the tsetse fly for goats and sheep may more readily explain the low prevalence of *T. brucei* spp. infections in these two species rather than any innate resistance to infection (Weitz, 1963). The role of goats and sheep in the animal reservoir of *T. b. rhodesiense* must therefore be limited. The prevalence and incidence of *T. vivax* and *T. congolense* infections followed the same trends as that of *T. brucei* spp. infections in cattle, goats and sheep, however in pigs it was notable that the only species of trypanosome found was *T. brucei* spp.

Mixed infections of more than one species of trypanosome in cattle occur in a vastly increased frequency (up to 22 times greater) than would be expected by chance (Table 5.6). However the magnitude of this effect is twice as great with mixed infections including *T. brucei* spp. compared to mixed infections of *T. vivax* and *T. congolense* (Table 5.6). The risk factor of an existing *T. brucei* spp. infection being superinfected with either *T. vivax* or *T. congolense* is greater than an existing infection of *T. vivax* or *T. congolense* being superinfected with *T. brucei* spp. although both do occur (Table 5.6). Because of the very high prevalence of mixed infections there must be many tsetse flies feeding on hosts infected with more than one species of trypanosome, which should in return result in tsetse flies with mixed infections. This is supported by the very high (up to 27 times than expected by chance) incidence of mixed trypanosome infections where an animal becomes newly infected with more than one species of trypanosome simultaneously (Table 5.7). However to examine this in the wild population of tsetse flies in Busia District would be difficult because of the typically low daily catch of *Glossina f. fuscipes* (figs. 5.3 to 5.7) and the very low mature *T. brucei* spp. infection rate in this area, which is of the order of 1 in 750 (Maudlin *et al.*, 1990c). Recent experimental evidence suggests that tsetse flies already infected with one species of trypanosome are more easily infected with another species of trypanosome which would greatly increase the chances of flies developing mixed infections (Maudlin and Rego unpublished observations, 1995). The epidemiological significance of this is that the

interaction between different species of trypanosomes seen in mixed infections in the mammalian host (Appendix V, Tables V.1 to V.30) (Masake *et al.*, 1984) may be repeated in the tsetse fly.

There are several ways in which mixed trypanosome infections can come about in cattle, however some of these events are more likely to occur than others. Cattle with mixed trypanosome infections of *T. brucei* spp. and *T. vivax* exist up to 22 times more frequently than would be expected by chance and mixed trypanosome infections of *T. brucei* spp. and *T. congolense* in cattle are up to 18 times more frequent (Table 5.6). However the interaction between *T. brucei* spp. and *T. vivax* is numerically much more important than the interaction between *T. brucei* spp. and *T. congolense* (Table 5.7). The first mechanism for acquiring a mixed infection is if cattle are infected by a tsetse fly which itself has a mixed infection. This occurs up to 28 times greater with mixed infections of *T. brucei* spp. and *T. vivax*, and up to 13 times greater than would be expected by chance in the case of mixed infections with *T. brucei* spp. and *T. congolense* (Table 5.7). Secondly cattle with an existing infection of *T. brucei* spp. were up to 12 times more likely than an uninfected animal to become newly infected with a *T. vivax* and up to 4 times more likely to be newly infected with *T. congolense* (Tables 5.12 and 5.13). Thirdly the chances of a new infection of *T. brucei* spp. occurring in an animal already infected with *T. vivax* was up to 7 times more likely and an animal already infected with *T. congolense* up to 7 more likely than for an uninfected animal (Tables 5.12 and 5.13). The most common means of cattle acquiring a mixed trypanosome infection is to be infected with *T. brucei* spp. and *T. vivax* or to a lesser extent *T. brucei* spp. and *T. congolense* simultaneously (Table 5.7). This is followed by superinfection by *T. vivax* of an already existing *T. brucei* spp. infection (Table 5.14).

In a survey of cattle carried out towards the end of an epidemic of sleeping sickness in the Lambwe valley in 1969, where *Glossina pallidipes* was the vector, a two fold increase in the point prevalence of mixed infections of *T. brucei* spp. with *T. vivax* or *T. brucei* spp. with *T. congolense* but not *T. vivax* with *T. congolense* was observed (Willet, 1972). It was concluded that infection with *T. brucei* spp. predisposed cattle to superinfection with *T. vivax* or *T. congolense* or that infection with either of these two species of trypanosome predispose an animal to infection with *T. brucei* spp. Although the present study, which used more modern methods of diagnosis, showed that cattle with an existing trypanosome infection were more likely to become infected with another trypanosome species,

this observation would also occur if only a percentage of the cattle population within a village was at risk of trypanosome infections by being exposed to tsetse flies. In Apatit village (Samorin control), where trypanosomiasis challenge was high and there was no drug prophylaxis, if only between a quarter and an eighth (95% confidence level) of the population of village cattle were exposed to tsetse flies, or were susceptible to infection with trypanosomes, then the number of mixed infections observed in cattle would be no greater than expected by chance. However the increase in observed prevalence of mixed infections of *T. vivax* with *T. congolense* over that expected in cattle was much less than in cattle with mixed infections involving a *T. brucei* spp. infection. This indicates that the idea of only a sub-population within a village being exposed to infection cannot fully explain the increased observation of mixed infections in cattle from that which would be expected by chance. Another explanation for the higher than expected prevalence of mixed infections in cattle would be that the immunosuppressive effect of a *T. vivax* or a *T. congolense* infection would cause a rise in the parasitaemia of a concurrent *T. brucei* spp. infection allowing easier diagnosis of a *T. brucei* spp. infection and perhaps its more frequent observation. This explanation relies on the assumption of under-diagnosing the true prevalence of single *T. brucei* spp. infections in cattle. If this was the case in Apatit village (Samorin control) then the true prevalence of *T. brucei* spp. infections in cattle would be at least 33% (95% confidence level), three times the observed prevalence of *T. brucei* spp. in that village. Alternatively the various trypanosome infections in cattle are not independent events as would be the case if there was a greater than expected proportion of tsetse flies which themselves had mixed trypanosome infections.

By deduction, with many infected tsetse flies having mixed trypanosome infections, cattle with existing single infections of *T. brucei* spp. would be exposed to other *T. brucei* spp. infections in addition to the detected superinfections of either *T. vivax* or *T. congolense* (Table 5.12 and 5.13). Therefore the risk factor of an existing *T. brucei* spp. infection being superinfected with a different subspecies of *T. brucei* spp. would be greater than would be expected by chance. It was not possible to measure this effect directly with field diagnostic techniques. Although the subspecies *T. b. gambiense* can adequately be identified from either *T. b. rhodesiense* or *T. b. brucei* by isoenzyme electrophoresis (Gibson *et al.*, 1980) all three subspecies can only be reliably identified by resorting to DNA analysis (RFLP) (Hide *et al.*, 1990). The epidemiological significance of this is that in addition to the

interaction between different species of trypanosome seen in mixed infections occurring at a far greater frequency than would be expected by chance, the possibility of interaction between *T. b. brucei brucei* and *T. b. rhodesiense* are similarly increased. It has already been demonstrated that trypanosomes of the same species interact within the mammalian host (Morrison *et al.*, 1982; Dwinger *et al.*, 1986; Turner *et al.*, 1996) and tsetse fly (Tait, 1981; Jenni *et al.*, 1986). It may be possible for one subspecies of *T. brucei* spp. to become dominant by overgrowing and replacing an existing infection of another subspecies of *T. brucei* spp. which would have significant epidemiological consequences. This has already been suggested as an explanation for a drug resistant stock of *T. congolense* to overgrow drug sensitive stock when superinfected with a drug sensitive stock (Sones *et al.*, 1989).

Only in Rukada village was there a significant bias in the sex ratio of infected cattle with bulls being infected twice as often as cows (Tables 5.9 and 5.10). This may be due to the relatively small number of bulls found in this village which were used mainly for breeding and tended to be more mature than in the other three villages. These larger bulls were more frequently trekked across the swamp (where tsetse were most common) into Uganda for grazing which was more plentiful on that side of the border and this may explain the increase in risk of infection.

Age was only a significant risk factor for cattle in their first year of life when they were at a lower risk of being infected with trypanosomes than any other age group. This may have been due to previously exposed dams conferring some protection to their calves via colostrum (Murray *et al.*, 1982) or because of the husbandry practice of tying young calves to a peg in the homestead while the rest of the herd were taken for water or grazing. This is done until weaning at between 6 months and one year of age when the calf can keep up with rest of the herd so that the cow can be milked for human consumption. It might be expected that as animals increase in size they present an increasingly large target to a tsetse fly (Hargrove and Vale, 1978). However there was no significant difference between the incidence of trypanosome infections in cattle in their second, third, fourth or subsequent years. Because the habitat of *Glossina f. fuscipes* is swamp and secondary bush a fly may not at any one time see the whole of the hosts body and cattle movement rather than size may be a more important attractant.

Cattle with trypanosome infections are removed from the epidemiological cycle by death, slaughter for reasons of ill health, selling for reasons of ill health or treatment with a

trypanocidal drug. Because of the experimental protocol whereby all infected cattle with a PCV of 20 or less (i.e. assumed to have clinical trypanosomiasis) were treated with diminazene aceturate there is the introduction of a bias towards treatment because the drug was available to the farmers and at no cost. Prior to the time period of this study cattle critically ill with trypanosomiasis were usually slaughtered to salvage the meat before their death. However from an epidemiological point of view the bias towards treatment rather than death, slaughter or sale is largely irrelevant. Cattle with mixed infections of *T. vivax* and *T. congolense* had an almost a 80% probability of being removed from the epidemiological cycle which was significantly higher than either cattle with single infections of *T. vivax* ($p = 0.001$) or *T. congolense* ($p = 0.001$). This suggests some form of synergy between the two species of trypanosome causing an enhanced pathogenicity. The probability of cattle with a single infection of *T. brucei* spp. being removed from the epidemiological cycle is approximately 12% whereas the probability for cattle with single infections of either *T. vivax* or *T. congolense* is almost 50% ($p < 0.001$, $p < 0.001$). The probability of cattle with mixed infections of either *T. brucei* spp. and *T. vivax* or *T. brucei* spp. and *T. congolense* being removed from the epidemiological cycle are similar to that of single infections of either *T. vivax* or *T. congolense* at 50% ($p = 0.139$, $p = 0.117$) and significantly different from single infections of *T. brucei* spp. ($p < 0.001$, $p < 0.001$). Since mixed infections of *T. brucei* spp. with *T. vivax*, and *T. brucei* spp. with *T. congolense* are removed from the epidemiological cycle four times more frequently than single infections of *T. brucei* spp. (Table 5.15) the effect on the epidemiology of the animal reservoir of *T. brucei* spp. would be significant. The combination of a high probability of removal from the epidemiological cycle by slaughter, death or treatment of mixed infections of *T. brucei* spp. and *T. vivax*, and *T. brucei* spp. and *T. congolense* compared to single infections of *T. brucei* spp. and a very much increased occurrence over that expected by chance of mixed infections containing *T. brucei* spp. would have the effect of removing *T. brucei* spp. infections from the epidemiological cycle. Therefore *T. vivax* and to a lesser extent *T. congolense* acted as a check, limiting the build up of the reservoir of *T. brucei* spp. in cattle. Thus the presence of a sufficient prevalence of *T. vivax* and *T. congolense* may act as a controlling factor for the animal reservoir of *T. brucei* spp. in cattle, and by so doing may act as a natural control for epidemic Rhodesian sleeping sickness. Cattle sampled in Ikonzo village, where human infections had recently occurred, were the only herd of village cattle of 14 different villages sampled over a period of 18 months in Busia

District where *T. brucei* spp. infections were found in the absence of *T. vivax* and *T. congolense* (Tables 5.16 and 5.17)).

The numbers of tsetse flies caught appeared to bear some correlation with the incidence and prevalence (figs. 5.6 and 5.7) of trypanosome infections in Rukada (Ethidium treated) and Ngelechom (Ethidium control) villages. However in Katelenyang (Samorin treated) and Apatit (Samorin control) villages where the incidence (figs. 4.5 to 4..8) and prevalence (figs. 4.1 to 4.4) of trypanosome infections was much greater, the tsetse fly catch was very much lower (figs. 5.3 and 5.5). This difference cannot be explained by biting flies acting as an alternative vector as Katelenyang village (Samorin treated) with the highest monthly incidence and prevalence of trypanosome infections, had by far the lowest catch of biting flies. The efficiency of blue and black biconical tsetse traps at catching biting flies may be questioned but assuming the efficiency or inefficiency remains the same in each village this allowed a comparison between villages if not a true measure of the overall numbers of biting flies in each of the four villages. The difference in success of tsetse trapping may be attributable to the differing terrain in the villages. In the low lying flat swampy area of Ngelechom at the beginning of an epidemic of sleeping sickness in June 1987 the number of flies per trap per day (ftd) was 26.7 prior to ground spraying with cypermethrin whereas in the hilly village of Apatit, where the 1990 sleeping sickness epidemic was centred, the maximum tsetse catch was only 4.23 ftd prior to ground spraying (Busia District Veterinary Department Annual Reports, Chapter II). The 'Berenil index' gives a much more accurate measure of tsetse challenge than fly trapping as reflected in the closer correlation with incidence and prevalence of trypanosome infections (figs. 4.9 to 4.12). Whereas the 'Berenil index' in the villages of Katelenyang (Samorin treated) and Apatit (Samorin control) are of the same magnitude, with Katelenyang village (Samorin treated) having slightly higher challenge prior to the period of Samorin prophylaxis (figs. 4.9 and 4.10), the 'Berenil index' in Rukada (Ethidium treated) and Ngelechom (Ethidium control) were not so alike as Ngelechom (Ethidium control) has a higher level of challenge prior to the period of prophylaxis with Ethidium in Rukada village (figs. 4.11 and 4.12). The data used for the incidence of trypanosome infections however was similar to that used for the 'Berenil Index' in that the incidence was the number of new trypanosome infections in cattle per month and the 'Berenil Index' was the number of cattle with trypanosome infections, new and old, requiring treatment on the grounds of clinical trypanosomiasis per month.

Tsetse fly trapping revealed that despite there being an apparent 99% reduction in the number of *Glossina f. fuscipes* being caught in the four villages (Chapter II) the incidence of trypanosome infections in cattle remained high (up to 36%). Biting flies could not explain this discrepancy. The lack of correlation between the numbers of tsetse flies (all *Glossina f. fuscipes*) which were caught and the incidence of trypanosome infection in cattle in Katelenyang and Apatit villages may be partially explained by the recent discovery of a small population of *Glossina pallidipes* on this hilly location (Chapter IV), which is a species of tsetse fly not readily caught in biconical traps (Douati *et al.*, 1986).

The climate in Busia District is never too cold and never too dry to support a tsetse fly population (figs. 5.1 to 5.3). There is a reasonable correlation between the rainfall at Amukura and the incidence of trypanosome infections in Ngelechom village (Ethidium control) two months later. Similarly with Apatit village (Samorin control) with the exception of the unusually high incidence of trypanosome infections in January. The rainfall pattern in Funyula (about 10 km from Rukada village (Ethidium treated)) does not reflect the rainfall at Rukada village after October because the rains failed completely in this village during the period of Ethidium prophylaxis from November to April. Prior to this time there was reasonable correlation in Rukada village (Ethidium treated) between rainfall and the incidence of trypanosome infections in cattle the following month (fig. 4.7 and 5.3). There was only a one month shift from rainfall influencing incidence compared to the two months seen in other villages because this village was always the last to be tested each month. The unusually high prevalence of trypanosome infections in cattle in Apatit village (Samorin control) in the month of January may be due to the changing pattern of agriculture in this village at this time of year when tobacco seedlings are grown next to the stream (because they need irrigation) making it necessary to water the cattle elsewhere.

This epidemiological study has demonstrated the existence of the opportunity under natural conditions for interactions between and within species of trypanosome to occur at a very much increased frequency than was previously thought (Willet, 1972). Indeed the interaction of *T. brucei* spp. with *T. vivax* or *T. congolense* may serve as the natural control of the animal reservoir of *T. brucei* spp. infections by limiting the time span of any *T. brucei* spp. infections in cattle (Table 5.15). As *T. b. rhodesiense* was shown to be present in the cattle population of Busia District during the study it can be projected that the same mechanism of natural control of the of the animal reservoir of *T. b. rhodesiense* may exist. Since it has been

suggested both theoretically (Rogers, 1988) and from field data (Maudlin *et al.*, 1990c) that the transmission of Rhodesian sleeping sickness is much more sensitive to alterations in the prevalence of *T. b. rhodesiense* in the animal reservoir rather than the prevalence within the human population, the interaction of *T. vivax* or *T. congolense* with *T. b. rhodesiense* within the animal reservoir may act as a natural regulator for sleeping sickness. This may be a mechanism involved in the sudden appearance and equally rapid disappearance of sleeping sickness epidemics. Following the sleeping sickness epidemic in Katelenyang and Apatit villages in 1990 it was reported that most of the village cattle died. As it has been shown (Chapter IV) that the major lethal disease of cattle in that area was trypanosomiasis it can be assumed that this was the main cause of the cattle deaths. Since *T. vivax* and *T. congolense* infections in cattle are often fatal (unlike infections with *T. brucei* spp.) infections, it may be interpreted that following human infections in 1989 and 1990 the cattle reservoir of *T. b. rhodesiense* was drastically reduced by the emergence of *T. vivax* and to a lesser extent *T. congolense* infections in cattle.

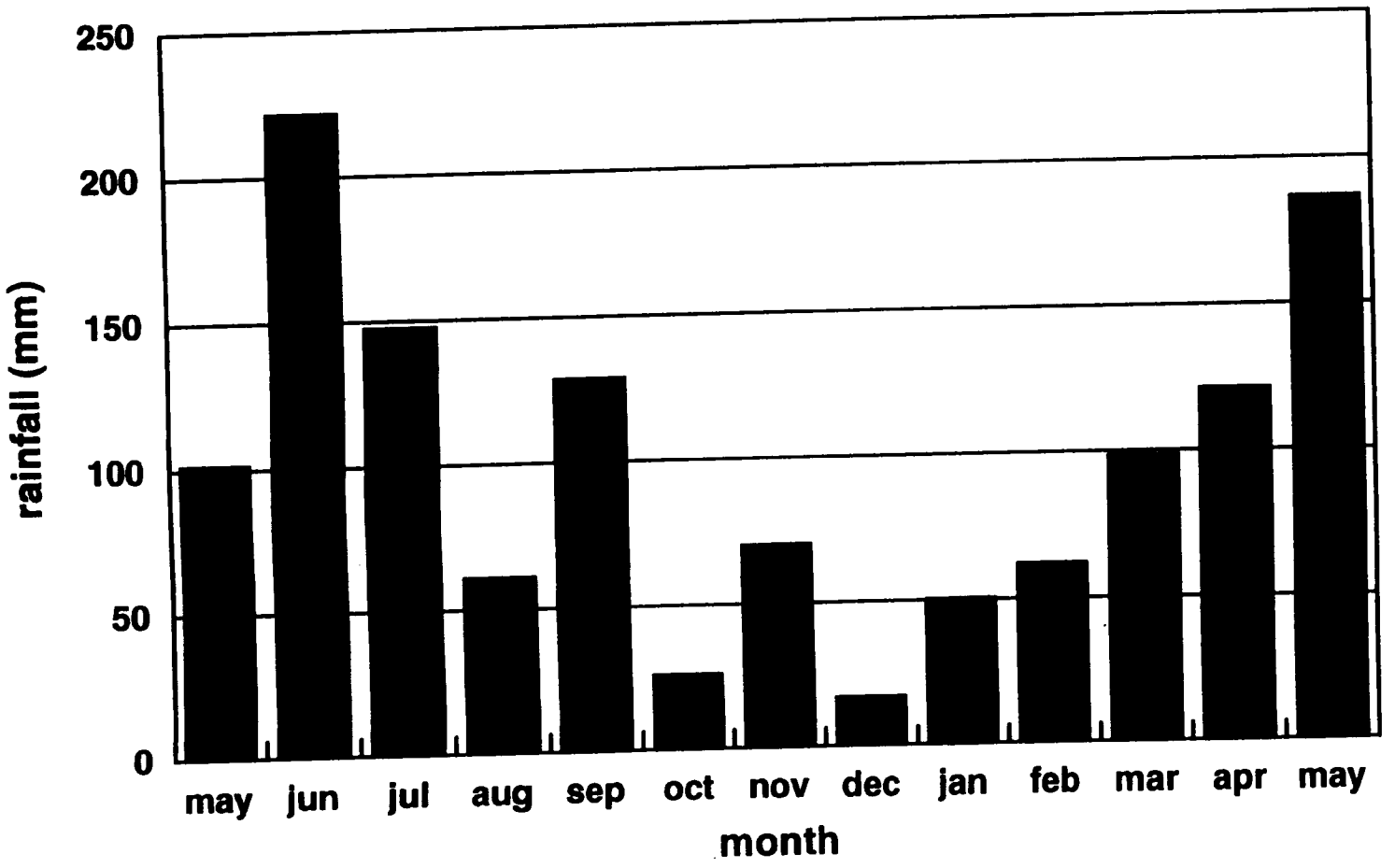

Amakura rainfall data (May 1993-May1994)
250
200
150
100
50
0
rainfall (mm)
may
jun
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month

fig. 5.1

Funyula rainfall data (May 1993-May 1994)

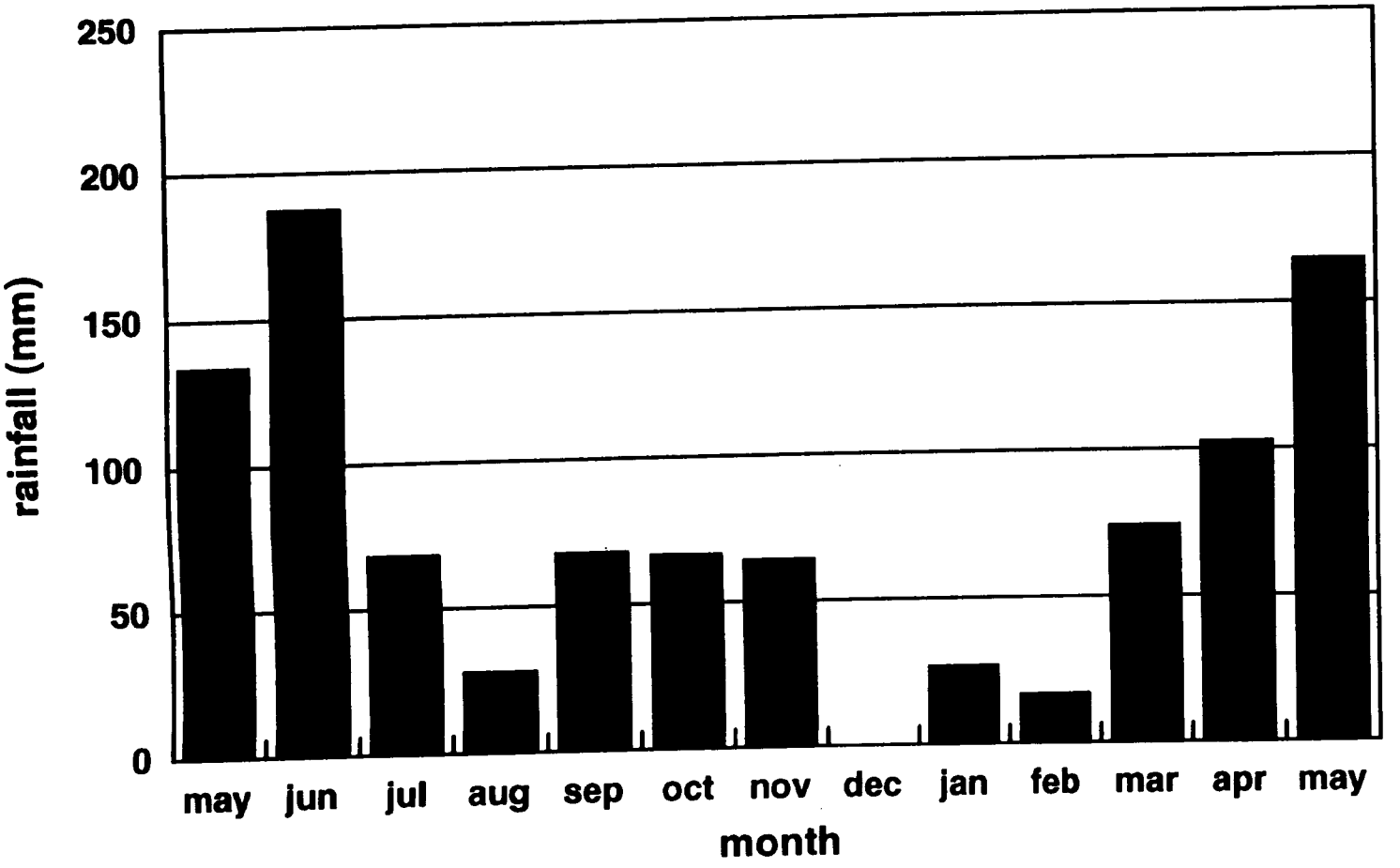


fig. 5.2

Tsetse fly trapping in Katelenyang village (May 1993-April 1994)

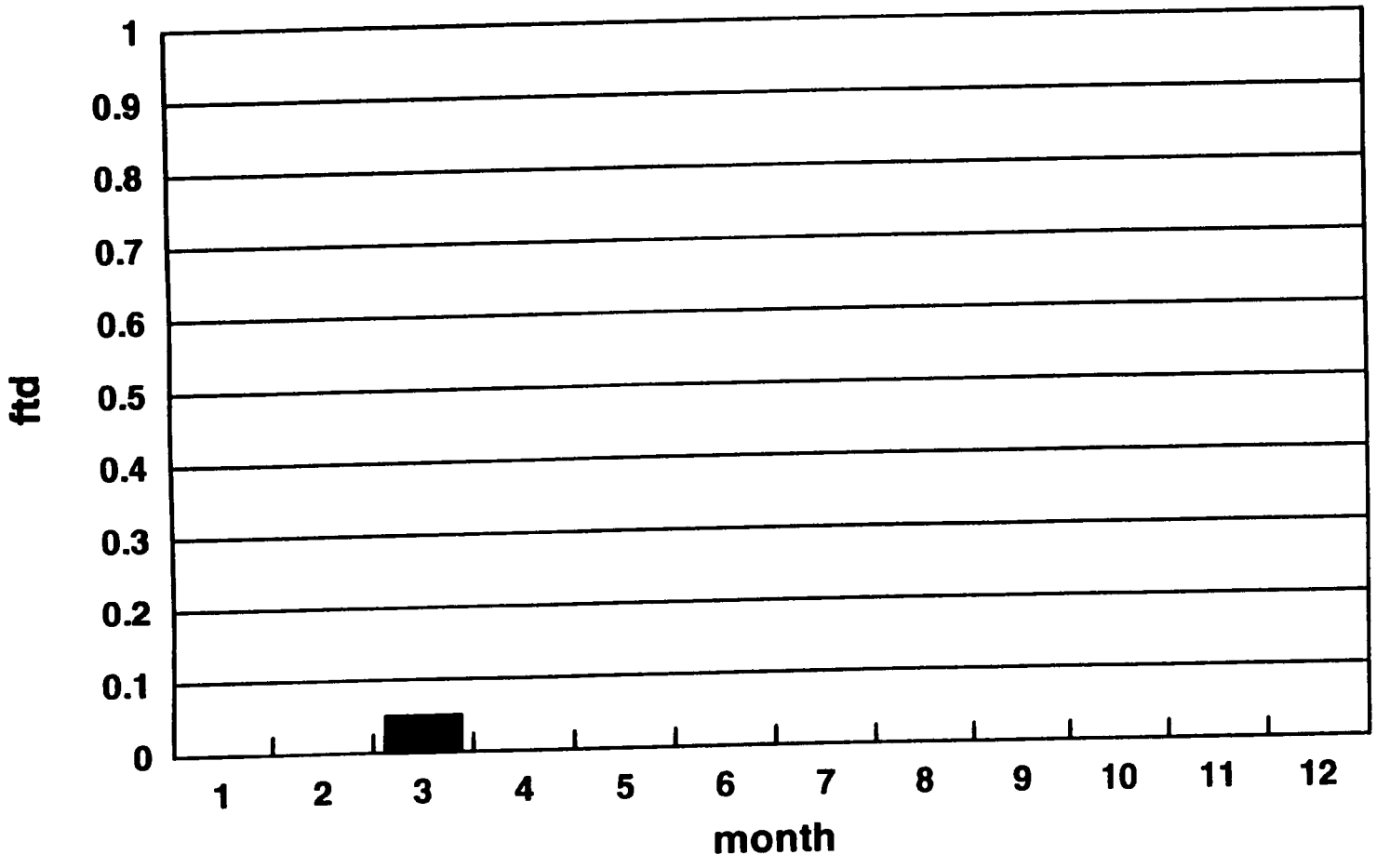


fig. 5.3

Tsetse fly trapping in Apatit village (May 1993-April 1994)

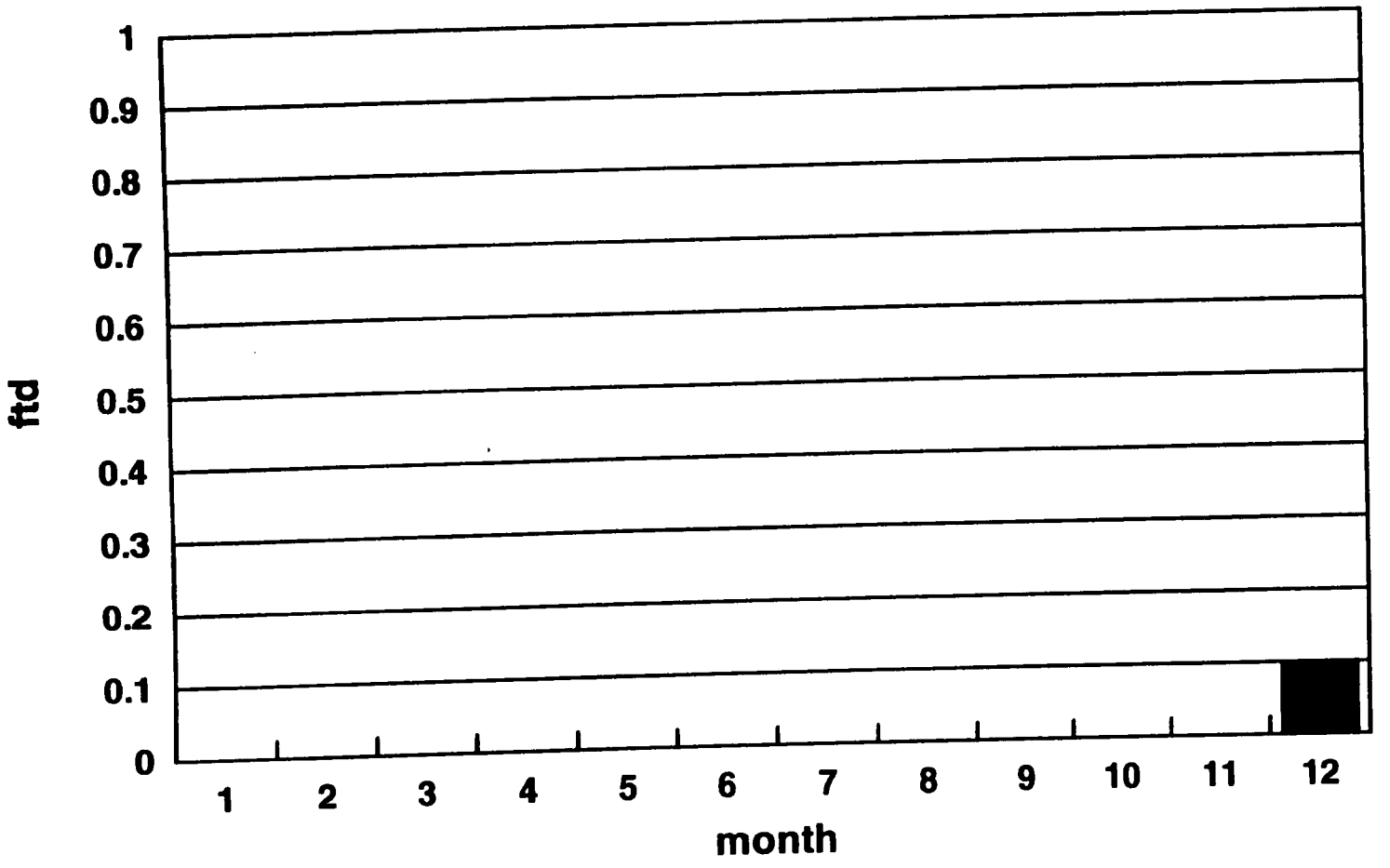


fig. 5.4

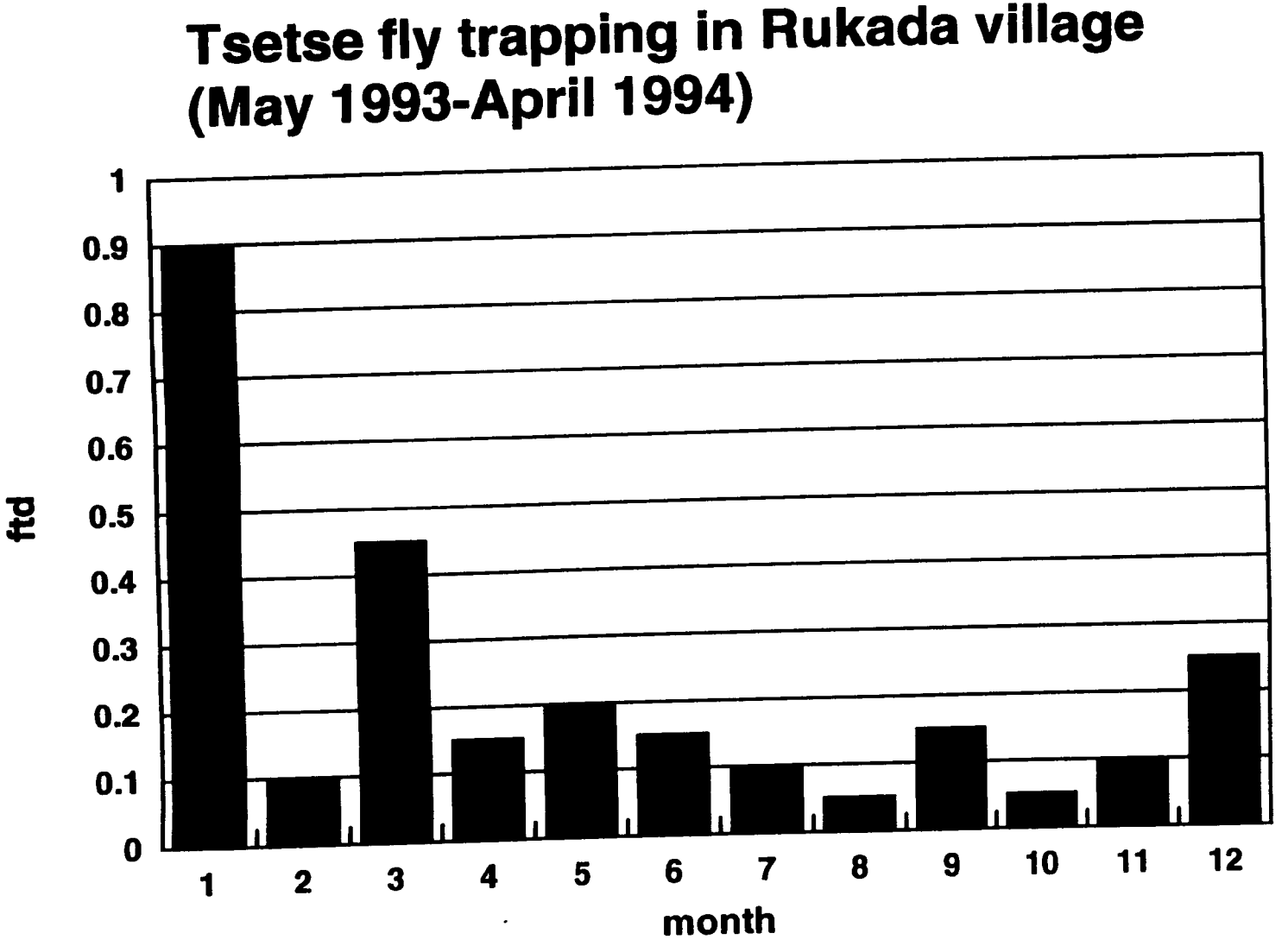

Tsetse fly trapping in Rukada village
(May 1993-April 1994)
ftd
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
10
11
12
month

fig. 5.5

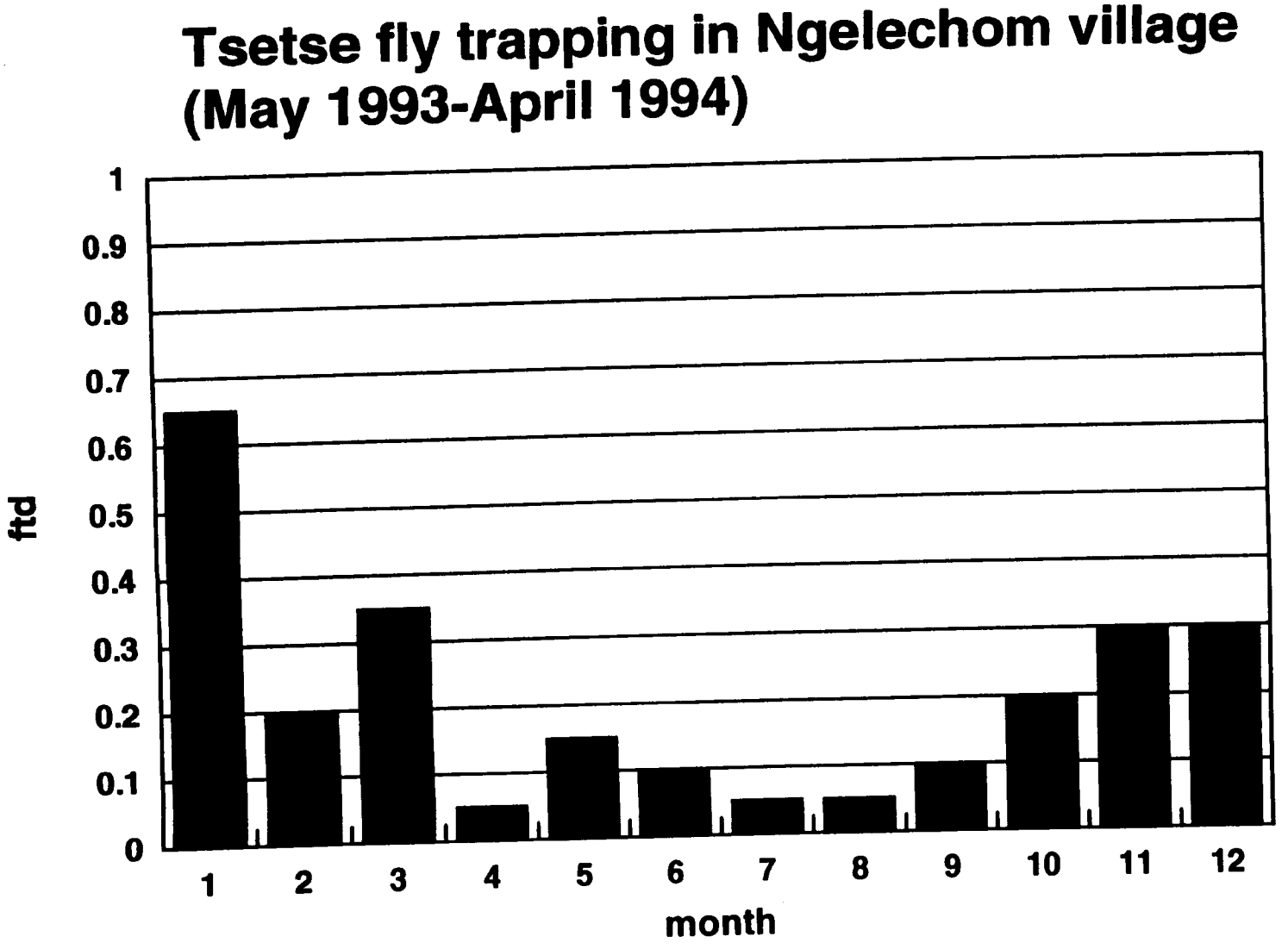

Tsetse fly trapping in Ngelechom village
(May 1993-April 1994)
ftd
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
10
11
12
month

fig. 5.6

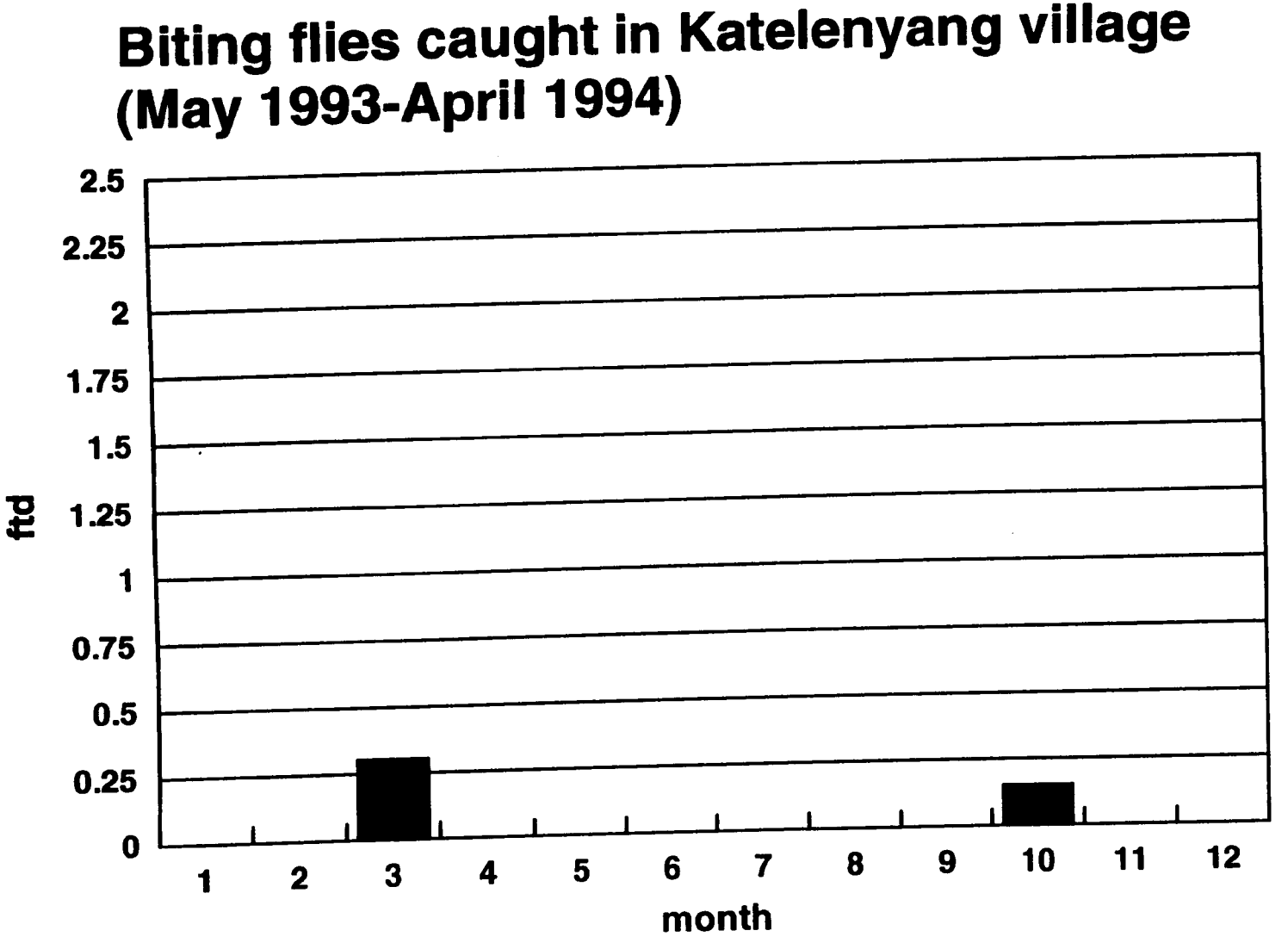

Biting flies caught in Katelenyang village
(May 1993-April 1994)
ftd
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
1
2
3
4
5
6
7
8
9
10
11
12
month

fig. 5.7

Biting flies caught in Apatit village (May 1993-April 1994)

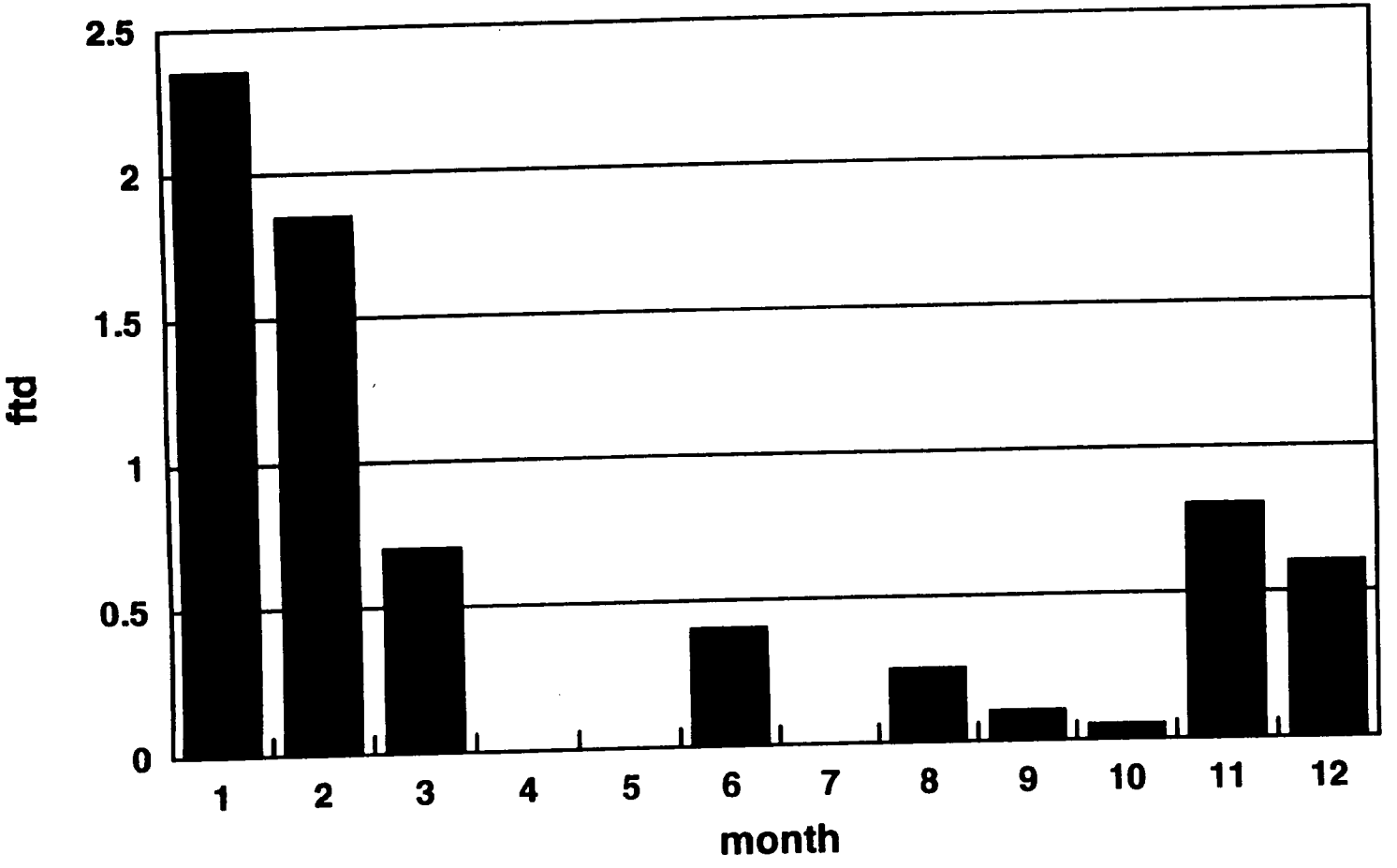


fig. 5.8

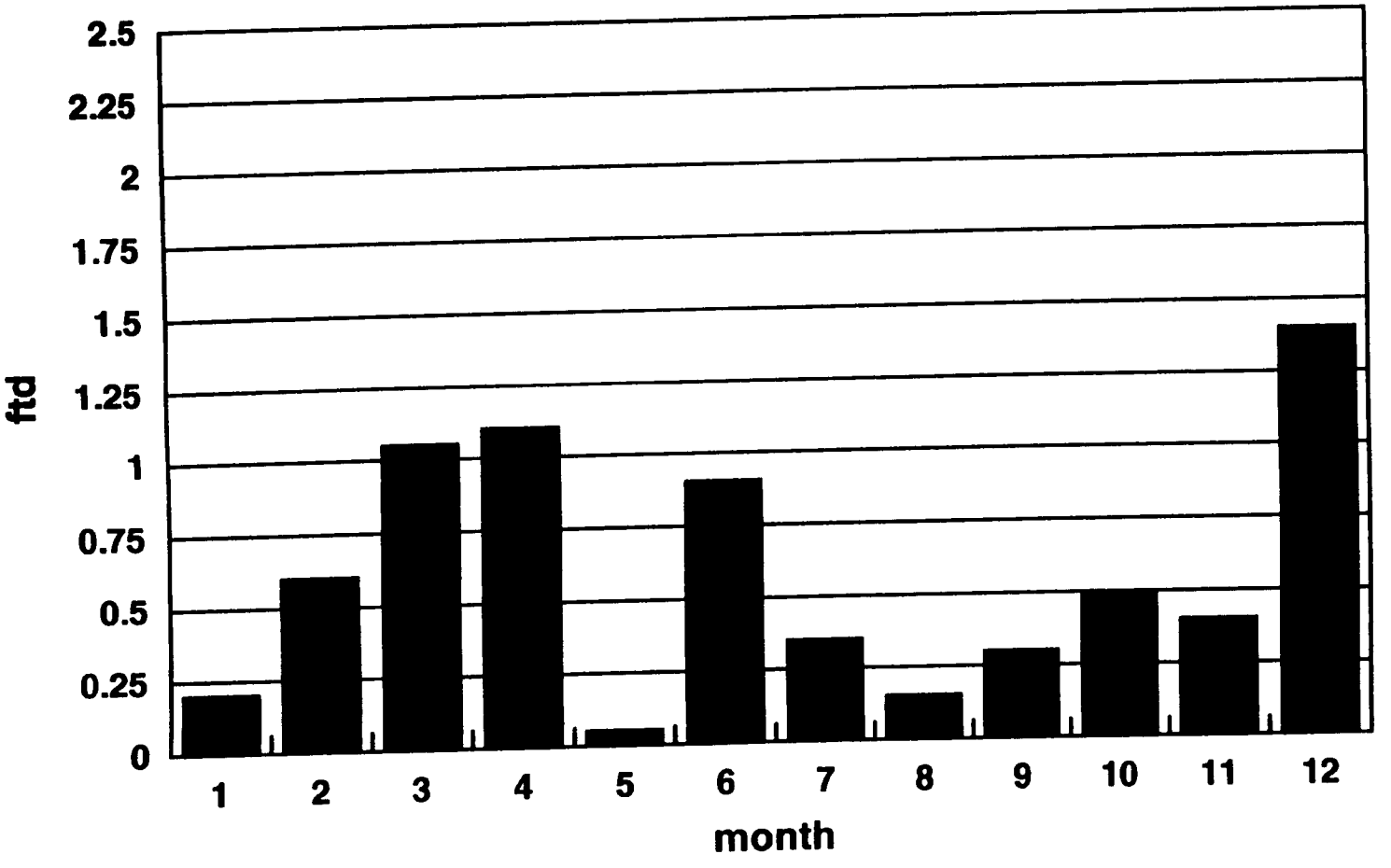

Biting Flies Caught in Rukada Village
May 1993-April 1994
ftd
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
1
2
3
4
5
6
7
8
9
10
11
12
month

fig. 5.9

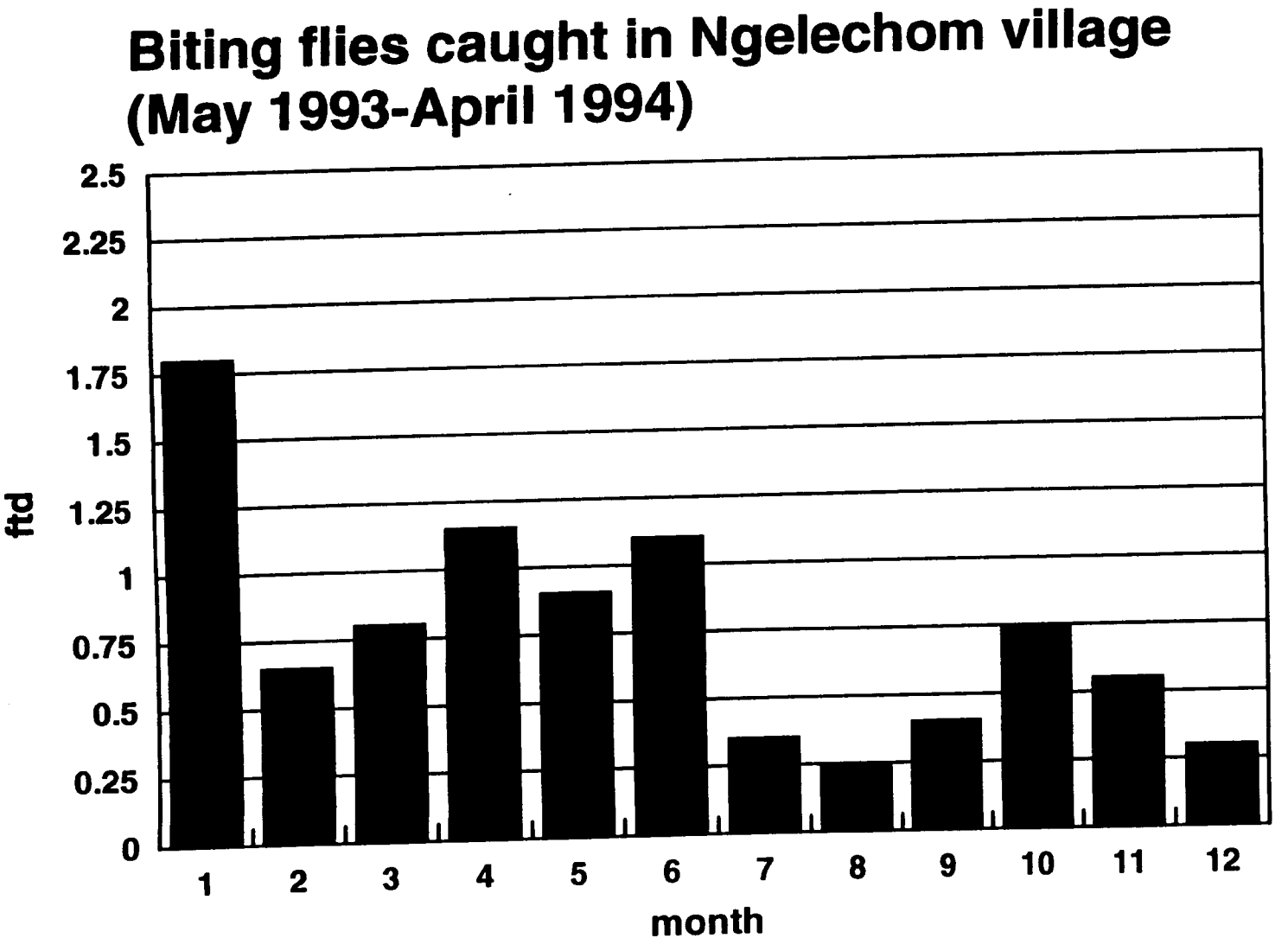

Biting flies caught in Ngelechom village
(May 1993-April 1994)
ftd
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
1
2
3
4
5
6
7
8
9
10
11
12
month

fig. 5.10

Chapter VI

Comparative evaluation of diagnostic techniques for trypanosome and microfilaria infections in cattle under field conditions

6.1 Summary

During an epidemiological survey of cattle in Busia District, Western Kenya, an area of endemic Rhodesian sleeping sickness, over a period of thirteen months more than 11,000 blood samples were examined for the presence of trypanosomes using the haematocrit centrifugation technique (HCT), buffy coat technique (BC) and mouse inoculation.

For the diagnosis of *T. brucei* spp. infection in cattle the HCT technique and inoculation into immunosuppressed mice were equally sensitive but the BC technique was significantly less so. The HCT and BC techniques proved equally sensitive for the diagnosis of *T. vivax* infections in cattle, while *T. vivax* failed to infect mice. For the diagnosis of *T. congolense* infections in cattle the BC technique was significantly more sensitive than the HCT technique, however mouse inoculation was very poor at detecting *T. congolense* infection in the cattle of Busia District. Maximum sensitivity was achieved for the diagnosis of *T. brucei* spp., *T. vivax* and *T. congolense* by using a combination of the HCT and BC techniques which proved significantly more sensitive than either technique alone. The HCT technique was the only sensitive technique for the diagnosis of *T. theileri* or microfilaria infections in cattle.

Immunosuppression of mice prior to the inoculation of bovine blood was shown to significantly increase the sensitivity (by up to 100%) of diagnosing *T. brucei* spp. infections in cattle. However there was no difference in sensitivity between the use of inbred and outbred mice.

No advantage was found to the inoculation of mice with lymph rather than blood for the routine diagnosis of *T. brucei* spp. infections in cattle.

The PCV of cattle was found to be an accurate predictor of the prevalence of trypanosome infections in a population of cattle but it was more accurate at predicting all trypanosome infections and *T. vivax* infections than infections of *T. brucei* spp.

6.2 Introduction

Certain diagnosis of a current trypanosome infection in livestock can only be made at present by demonstrating the parasite in blood or other tissues of an infected animal. The reliability of such parasitological techniques to detect the often low and intermittent parasitaemias commonly encountered in natural infections is often questioned (Murray *et al.*, 1977) .

Serological techniques which rely on detecting trypanosome antibodies by their very nature do not necessarily detect a current infection regardless of their sensitivity or possible cross reactions with other antibodies. The recent development of an ELISA test (Nantulya and Lindqvist, 1989) which detects the presence of trypanosome antigens has proved difficult to standardise to produce reliable and repeatable results. Although the PCR (Majiwa *et al.*, 1993) is a very sensitive test for trypanosomiasis, in common with the ELISA test it requires specialised laboratory equipment.

This study used diagnostic techniques applicable to the field which use a minimum of specialised equipment, only requiring a centrifuge and a microscope. The techniques used were the Haematocrit centrifugation technique (HCT) (Woo, 1970), a modification of the dark ground / phase contrast buffy coat technique (BC) (Murray *et al.*, 1977) and inoculation of susceptible mice and compares the various techniques for the diagnoses of *T. brucei*, *T. vivax*, *T. congolense*, *T. theileri* and microfilaria. Two breeds of mice were compared, both with and without immunosuppression and the comparative value of inoculation of lymph gland aspirate was compared with blood for the diagnosis of *T. brucei*. The use of PCV as a predictor of trypanosomiasis was also investigated. Confirmation of trypanosome species was done using thin blood and buffy coat smears stained with Giemsa stain. In addition to apparent sensitivities being stated kappa values are given as a measure of agreement of diagnostic techniques.

The sensitivity, which is a measure of the ability of a test to identify infected animals, was measured for each diagnostic technique. The specificity of a diagnostic test is the ability of a diagnostic technique not to identify uninfected animals as infected but since only parasitological techniques were used whereby all trypanosome samples were identified by morphology, motility and the ability to infect mice (ILRAD/ILCA, 1983) this was assumed to be 100% (Murray *et al.*, 1977). During the early stages of a disease control programme it may be advantageous to maximise the sensitivity of the diagnostic test used in order to prevent a failure to identify infected animals, whereas in the latter stages of a disease control program it may be more advantageous to maximise the specificity of a diagnostic test in order not to falsely identify diseased animals. (Wayne Martin *et al*, 1987)

6.3 Materials and methods

For general materials and methods on diagnostic and epidemiological techniques see chapter III.

6.3 1 Blood sampling of cattle

Blood for diagnosis in the field was taken from ear veins (using a sterile lancet) in heparinised capillary tubes and spun in a microhaematocrit centrifuge. The packed cell volume (PCV) was monitored using a microhaematocrit reader (Vickers).

Blood for inoculation into mice, thin blood smears and making stabilates was collected from the jugular vein in 7 ml heparinised tubes (vacutainer®, Becton and Dickinson).

6.3.2 Lymph sampling of cattle

Lymph node aspirates were obtained by injecting 0.5 ml sterile normal saline into a prescapular lymph node with a 20G needle and subsequently aspirating the fluid, which was then inoculated into immunosuppressed BALB/C mice as described by Wellde *et al.* (1989b).

6.3.3 Diagnosis of trypanosome infections in cattle

Diagnosis of trypanosome infections in the field was carried out using the Haematocrit centrifugation technique (HCT) where the top of the buffy coat was examined through the capillary tube used for the measurement of the PCV (Woo *et al.*, 1970) and a modification of the dark ground buffy coat technique (BC) where the capillary tube was cut below the buffy coat and aspirate examined under a cover slip (Murray *et al.*, 1977) (Chapter III).

Buffy coat and thin blood smears were taken from all positive cases diagnosed in the field and stained with Giemsa for species identification in the laboratory.

Fresh heparinised blood (0.2 ml) from all cases of trypanosomiasis diagnosed by the HCT or the BC techniques was inoculated intraperitoneally (i.p.) into immunosuppressed mice in the field. Similarly blood was taken and inoculated into mice from all animals with a PCV of 20 or less and animals which were positive for *T. brucei* spp. the previous month.

6.3.4 Identification of trypanosomes

Identification of trypanosomes was carried out by observation of trypanosome morphology, motility and the ability to infect mice (ILCA/ILRAD, 1983) using all the possible diagnostic techniques (6.2.3).

6.3.5 Mice

The mice used for diagnosis of trypanosome infections in the field were inbred Swiss white albino BALB/C from *ILRAD*, Nairobi which had been immunosuppressed with 300 mg/kg cyclophosphamide (Endoxana®, Asta Medica, Cambridge) administered intraperitoneally 24 hours previously unless otherwise stated. One mouse was used for each sample.
Outbred Swiss white mice from *KETRI*, Alupe were used in the experiment comparing the effects of mouse breed on the sensitivity of diagnosis of *T. brucei* spp. infections in cattle (6.3.8).
The experiment comparing the effect of mouse breed and immunosuppression on the sensitivity of diagnosis was carried out over a period of three months and the experiment comparing the sensitivity of blood inoculation with lymph inoculation for the diagnosis of trypanosome infections in cattle was carried out over a period of one month.

6.3.6 Epidemiological parameters used to compare diagnostic tests

Sensitivity is the ability of a diagnostic test to detect diseased animals and is defined as the proportion of diseased animals which test positive.
Specificity is the ability of a diagnostic test to detect non diseased animals and is defined as the proportion of non diseased animals which test negative.
The kappa value is used to compare diagnostic tests and is defined as observed agreement between diagnostic tests minus the chance level of agreement divided by the maximum possible agreement beyond chance level. No agreement beyond chance gives a kappa value of 0 , and a kappa value of 1 indicates perfect agreement. A kappa value of at least 0.4 - 0.5 indicates a moderate level of agreement between diagnostic tests.
True prevalence is the actual prevalence of a condition within a population.

Apparent prevalence is the prevalence indicated by a particular diagnostic test (Wayne Martin *et al.*, 1987)

6.3.7 Statistical analysis

Comparison of the sensitivity of diagnostic tests was carried out using a Chi square test.

6.4 Results

6.4.1 The sensitivity of field diagnostic techniques for the diagnosis of different species of trypanosome and microfilaria

The comparative sensitivities of the HCT, BC and mouse inoculation techniques for the diagnosis of *T. brucei* spp., *T. vivax*, *T. congolense*, *T. theileri* and microfilaria ranged from zero to 98.8% (Table 6.1).

Table 6.1 The sensitivity of field diagnostic techniques for different species of trypanosome and microfilaria

	Tb		Tv		Tc		Tt		mf	
	No	%	No	%	No	%	No	%	No	%
HCT	302	47.5	751	76.2	121	36.6	1029	98.8	1255	97.8
BC	212	33.3	775	78.6	229	69.2	180	17.3	20	1.6
HCT+BC	359	56.4	900	91.3	266	80.4	1032	99.5	1274	99.3
MOUSE^{+}	308	48.4	0	0	2	0.6	0	0	0	0
TOTAL *	636	100	986	100	331	100	1037	100	1283	100

Total number of cattle blood samples taken 11903
$^{+}$immunosuppressed BALB/C Swiss white mice
* includes infections diagnosed on examination of Giemsa stained thin blood smears and buffy coat smears.
Tb *T. brucei*
Tv *T. vivax*
Tc *T. congolense*
Tt *T. theileri*
mf microfilaria
HCT haematocrit centrifugation technique
BC dark ground / phase contrast buffy coat technique
MOUSE mouse inoculation with blood

6.4.2 A comparison of the sensitivity of field diagnostic techniques for the diagnosis of *T. brucei* spp. infections in cattle

The HCT technique was significantly more sensitive than the BC technique ($X^2 = 26.45$, df = 1, $p < 0.001$) for the diagnosis of *T. brucei* spp. infections in cattle. However the HCT and BC techniques combined were more sensitive than either HCT ($X^2 = 10.23$, df = 1, $p = 0.001$) or BC ($X^2 = 29.98$, df = 1, $p < 0.001$) technique alone (Table 6.1).

Mouse inoculation was significantly more sensitive than the BC technique ($X^2 = 29.98$, df = 1, $p < 0.001$) for the diagnosis of *T. brucei* spp. infections in cattle, but there was no significant difference between the sensitivity of the HCT technique and mouse inoculation ($X^2 = 0.11$, df = 1, $p = 0.736$) (Table 6.1).

There was good correlation between the BC technique and mouse inoculation (kappa value = 0.59) for the diagnosis of *T. brucei* spp. infections in cattle, however the correlation between the HCT technique and mouse inoculation was poor (kappa value = 0.27) (Table 6.2).

Table 6.2 Kappa values for the diagnosis of *T. brucei* spp. infections in cattle

	HCT	BC	MOUSE
HCT	1	0.59	0.27
BC	0.59	1	0.2
MOUSE	0.27	0.2	1

6.4.3 A comparison of the sensitivity of field diagnostic techniques for the diagnosis of *T. vivax* infections in cattle

There was no significant difference between the sensitivity of the HCT and BC techniques (X^2 = 1.67, df = 1, p = 0.197) for the diagnosis of *T. vivax* infections in cattle. However the HCT and BC techniques combined were more sensitive than either HCT (X^2 = 82.61, df = 1, p < 0.001) or BC (X^2 = 61.94, df = 1, p < 0.001) techniques alone (Table 6.1).
Mouse inoculation with bovine blood failed to infect a single mouse with *T. vivax* (Table 6.1).
There was very good correlation between the HCT and BC techniques for the diagnosis of *T. vivax* infections in cattle (kappa value = 0.81) (Table 6.3).

Table 6.3 Kappa values for the diagnosis of *T. vivax* infections in cattle

	HCT	BC	MOUSE
HCT	1	0.81	0
BC	0.81	1	0
MOUSE	0	0	1

6.4.4 A comparison of the sensitivity of field diagnostic techniques for the diagnosis of *T. congolense* infections in cattle

The BC technique was significantly more sensitive than the HCT technique ($X^2 = 70.71$, df = 1, $p < 0.001$) for the diagnosis of *T. congolense* infections in cattle. However the HCT and BC techniques combined were more sensitive than either HCT ($X^2 = 130.78$, df = 1, $p = 0.001$) or BC ($X^2 = 10.96$, df = 1, $p < 0.001$) techniques alone (Table 6.1).

Mouse inoculation was a very poor technique for the diagnosis of *T. congolense* infections in cattle in this District (Table 6.1).

While there was reasonable correlation between the HCT and BC techniques (kappa value = 0.47), the correlation between these two techniques and mouse inoculation was very poor (kappa value < 0.01) (Table 6.4).

Table 6.4 Kappa values for the diagnosis of *T. congolense* infections in cattle

	HCT	BC	MOUSE
HCT	1	0.47	<0.01
BC	0.47	1	<0.01
MOUSE	<0.01	<0.01	1

6.4.5 A comparison of the sensitivity of field diagnostic techniques for the diagnosis of *T. theileri* infections in cattle

The HCT technique was significantly more sensitive than the BC technique ($X^2 = 1429.49$, df = 1, $p < 0.001$) for the diagnosis of *T. theileri* infections in cattle and combining the two techniques did not significantly improve the sensitivity of the diagnosis over the use of the HCT technique alone ($X^2 = 0.70$, df = 1, $p = 0.404$) (Table 6.1).

Mouse inoculation with bovine blood failed to infect a single mouse with *T. theileri* (Table 6.1).

Table 6.5 Kappa values for the diagnosis of *T. theileri* infections in cattle

	HCT	BC	MOUSE
HCT	1	0.007	0
BC	0.007	1	0
MOUSE	0	0	1

6.4.6 A comparison of the sensitivity of field diagnostic techniques for the diagnosis of *microfilaria* infections in cattle

The HCT technique was significantly more sensitive than the BC technique ($X^2 = 2377.68$, df = 1, $p < 0.001$) for the diagnosis of microfilaria in cattle and combining the two techniques did not significantly improve the sensitivity of the diagnosis over the use of the HCT technique alone ($X^2 = 0.99$, df = 1, $p = 0.352$) (Table 6.1).

Mouse inoculation with bovine blood failed to infect a single mouse with microfilaria (Table 6.1).

There was poor correlation between the HCT and BC techniques for the diagnosis of microfilaria in cattle (kappa value = 0.11) (Table 6.6).

Table 6.6 Kappa values for the diagnosis of microfilaria infections in cattle

	HCT	BC	MOUSE
HCT	1	0.11	0
BC	0.11	1	0
MOUSE	0	0	1

6.4.7 A comparison of the sensitivity of mouse inoculation with bovine blood or lymph for the diagnosis of *T. brucei* spp. infections in cattle

During the experiment to examine the sensitivity of mouse inoculation with bovine blood or lymph, where a relatively small number of samples were used (n = 229), there was no significant difference in sensitivity between the HCT, BC, mouse inoculation with blood and mouse inoculation with lymph ($X^2 = 3.00$, df = 3, p = 0.393) as diagnostic techniques for the detection of *T. brucei* spp. infections in cattle (Table 6.7). Similarly there was no significant difference in the sensitivity of the combined direct examination techniques of HCT and BC compared to the sensitivity of mouse inoculation with blood and mouse inoculation with lymph combined ($X^2 = 2.98$, df =1, p = 0.085) (Table 6.7).

Correlation between the HCT and the BC techniques was good (kappa value = 0.62), but correlation between mouse inoculation with blood and mouse inoculation with lymph was relatively poor (kappa value = 0.35) (Table 6.8). However correlation between the direct examination techniques (HCT and BC) and mouse inoculation with either blood or lymph was very poor with kappa values less than 0.1 (Table 6.8).

Table 6.7 A comparison of the sensitivity of different diagnostic techniques including blood and lymph inoculation into immunosuppressed mice for the diagnosis of *T. brucei* spp. infections in cattle

	No	%
HCT	14	33.3
BC	11	26.2
HCT+ BC	17	40.5
MOUSE (blood)	20	47.6
MOUSE (lymph)	15	35.7
MOUSE (blood + lymph)	28	66.7
Total number of positive *T. brucei* spp. samples	42	100

Total number of cattle sampled = 229

Table 6.8 Kappa values for the diagnosis of *T. brucei* spp. infections in cattle by mouse inoculation of blood or lymph

	HCT	BC	Blood	Lymph
HCT	1	0.62	0.05	0.01
BC	0.62	1	0.07	0.02
Blood	0.05	0.07	1	0.35
lymph	0.01	0.02	0.35	1

6.4.8 A comparison of the sensitivity of two breeds of mice both with and without immunosuppression for the diagnosis of *T. brucei* spp. infections in cattle

There was a significant difference between the sensitivity of mouse inoculation using immunosuppressed and non-immunosuppressed *KETRI* outbred mice ($X^2 = 10.14$, df = 1, p = 0.001) for the diagnosis of *T. brucei* spp. infections in cattle (Table 6.9).

There was also a significant difference between the sensitivity of mouse inoculation using immunosuppressed and non-immunosuppressed BALB/C inbred mice ($X^2 = 7.49$, df = 1, p = 0.006) for the diagnosis of *T. brucei* spp. infections in cattle (Table 6.9).

However there was no significant difference between the sensitivity of mouse inoculation using immunosuppressed *KETRI* outbred mice and immunosuppressed ILRAD BALB/C inbred mice ($X^2 = 0.011$, df = 1, p = 0.915) for the diagnosis of *T. brucei* spp. infections in cattle (Table 6.9). Similarly there is no significant difference between the non-immunosuppressed *KETRI* outbred mice and the non-immunosuppressed ILRAD BALB/C inbred mice ($X^2 = 0.33$, df = 1, p = 0.565) for the diagnosis of *T. brucei* spp. infections in cattle (Table 6.9).

In the experiment to examine the effects of immunosuppression and breed of mouse, where the number of samples (n = 375) was greater than those used in the blood and lymph experiment (6.3.7), the HCT technique was significantly more sensitive than the BC technique ($X^2 = 7.44$, df = 1, p = 0.007) for the diagnosis of *T. brucei* spp. infections in cattle (Table 6.9). The HCT technique proved as sensitive as mouse inoculation with either immunosuppressed *KETRI*

outbred mice or immunosuppressed ILRAD BALB/C inbred mice (X^2 = 0.202, df = 1, p = 0.904) and the BC technique proved of similar sensitivity to mouse inoculation with either non-immunosuppressed *KETRI* outbred mice or non-immunosuppressed ILRAD BALB/C inbred mice (X^2 = 0.362, df = 1, p = 0.834) (Table 6.9).

The correlation between mouse inoculation with differing breeds of mice either immunosuppressed or non-immunosuppressed was generally good (kappa value > 0.4), except for the correlation between immunosuppressed and non- immunosuppressed *KETRI* outbred mice (kappa value = 0.31) which had the lowest number of infections detected (Table 6.10). Correlation was very good between mouse inoculation with both immunosuppressed breeds of mice (kappa value = 0.63) (Table 6.10).

The sensitivity of diagnosis of *T. brucei* spp. infections in cattle using both the direct examination techniques (HCT and BC) combined improved significantly only when immunosuppressed mice of either breed (*KETRI* outbred or ILRAD BALB/C inbred) were also used (X^2 = 6.90, df = 2, p = 0.032) and not when non-suppressed mice were used (X^2 = 2.73, df = 2, p = 0.256) (Table 6.9 and Appendix VI, Table VI.1 and VI.2).

Table 6.9 A comparison between two breeds of mice with and without immunosuppression for the diagnosis of *T. brucei* spp.

	No.	%
*KETRI** immunosuppressed	50	50.5
*KETRI** non- immunosuppressed	24	24.2
BALB/C+ immunosuppressed	51	51.5
BALB/C+ non-immunosuppressed	28	28.3
HCT	47	47.5
BC	25	25.3
HCT + BC	52	52.5
Total number of positive *T. brucei* spp. samples	99	100

Total number of cattle sampled = 375

* outbred Swiss white mice bred at *KETRI*, Alupe

+ inbred BALB/C Swiss white mice bred at ILRAD, Nairobi

Table 6.10 Kappa values for the diagnosis of *T. brucei* spp. by mouse inoculation using inbred and outbred strains of mice both immunosuppressed and non-suppressed

mouse type	outbred (S)	outbred (N)	inbred (S)	inbred (N)
outbred (S)	1	0.31	0.65	0.55
outbred (N)	0.31	1	0.46	0.63
inbred (S)	0.65	0.46	1	0.46
inbred (N)	0.46	0.63	0.46	1

(S) immunosuppressed

(N) non immunosuppressed

6.4.9 The use PCV as a diagnostic technique for detecting trypanosome infections in cattle

Using the PCV of cattle as an indirect predictor of trypanosome infections varied in sensitivity and specificity depending on the PCV value selected as an indicator of trypanosome infections (figs. 6.1 to 6.3). The higher the value of the PCV selected as an indicator for trypanosome infections the more sensitive the test (i.e. the less false negatives which were detected) and at the same time the less specific the test was (i.e. the more false positives which were detected). Similarly the lower the value of the PCV selected as an indicator for trypanosome infections the specificity of the test increased (i.e. the less false positives which were detected) at the expense of sensitivity, which decreased (i.e. the more false negatives which were detected) (figs. 6.1 to 6.3). Where the apparent prevalence as predicted by PCV equates to the true prevalence of trypanosome infections within a population of cattle (this lies between 19 and 21) depended on the species of trypanosome being detected (figs. 6.1 and 6.3). At this point on the graphs for infections with all trypanosomes (fig. 6.1) and infections with *T. vivax* (fig. 6.3) the kappa value is at its maximum which indicated that at this PCV value there was maximum degree of agreement between the use of PCV as a diagnostic test for the presence of a trypanosome infection and the true prevalence of trypanosome infections in cattle as indicated by the combination of all the parasitological techniques used (HCT, BC, thin blood film and mouse inoculation). However this was not the case using the PCV of cattle as an indirect diagnostic test for *T. brucei* spp. (fig. 6.2). The PCV of cattle was most accurate at predicting an infection with *T. vivax* (kappa value = 0.34 at PCV = 19) or an infection with any trypanosome (kappa value = 0.43 at PCV of 21), which included mixed infections, and was least accurate at predicting infections of *T. brucei* spp. only (kappa value = 0.15 at PCV = 20) (figs. 6.1 to 6.3). There were too few infections of only *T. congolense* to graph.

6.5 Discussion

Inoculation of immunosuppressed mice and HCT proved consistently to be the most sensitive methods for the detection of *T. brucei* spp., however there is a poor degree of agreement between the tests (kappa value = 0.27). Of the 522 positive *T. brucei* spp. samples detected by either test only 88 were detected by both with 220 being only identified in mice and 214 in

HCT only. The BC technique was significantly less sensitive then these two tests but had a moderate level of agreement with HCT (kappa value = 0.62) and a low level with mouse inoculation (kappa value = 0.07). Since HCT and BC techniques rely on the level of parasitaemia to be sufficient for detection (Paris *et al*, 1982) and correlation between these tests and mouse inoculation is low, infection of mice may rely more on the strain and form of trypanosome rather than the infective dose. A possible explanation for only a moderate level of agreement between HCT and BC lies the pleomorphic nature of *T. brucei* spp. which has the ability to exist in long slender forms as well as short stumpy forms and various intermediates. Since both HCT and BC rely on the differing sizes and specific gravities (Murray *et al.*, 1977) of the trypanosomes, some smaller intermediate forms may preferentially be found under the buffy coat rather than on top where most of the long slender forms are found accounting for the higher level of agreement between the BC technique and mouse inoculation (kappa value = 0.59) (Table 6.2).

For the detection of *T. brucei* spp. infections in cattle it is necessary to use a range of diagnostic tests simultaneously to maximise the sensitivity of diagnosis. Using the HCT and BC techniques together significantly increases the sensitivity of diagnosis of *T. brucei* spp. infections in cattle over either technique alone (6.3.2). If mouse inoculation is used in addition to the HCT and BC techniques then the sensitivity of diagnosis of *T. brucei* spp. infections in cattle is significantly increased again (6.3.2) but only if the mice are immunosuppressed (6.3.8). However non-immunosuppressed mice may improve the overall sensitivity of diagnosis of *T. brucei* spp. in cattle over that of the HCT and BC techniques if more than one mouse is used per blood sample.

It has been claimed that inoculation of lymph mode aspirate into mice is a more sensitive diagnostic technique than the inoculation of blood (Wellde *et al.*, 1989b). However the samples used in that trial from the Lambwe Valley were mostly from cattle chronically infected with *T. brucei* spp. for a number of years. In the present experiment which compared inoculation of blood and lymph node aspirate into mice there was no advantage in sensitivity of using lymph node aspirate rather than blood to inoculate mice. Only half of the samples positive for lymph inoculation were also positive for blood which suggests that samples from blood and lymph may be separate sub-populations. During the present experiment there was a very poor correlation between the two mouse inoculations and direct examination by either HCT or BC techniques (Table 6.2) which may be related to the lower than average tsetse

challenge in the month of this experiment causing fewer new infections which tend to have higher parasitaemias. Given the technical difficulty of lymph node sampling it is probably not a useful technique for routine cattle surveys; however for detection of *T. brucei* spp. in specific chronically infected animals it would be of value.

In a study which compared parasitological techniques in cattle Paris *et al* (1982) observed that *T. vivax* could be detected at parasitaemias as low as 2.5×10^2 trypanosomes per ml using the BC technique whereas the lowest detectable parasitaemia using the HCT technique was 1.25×10^3 trypanosomes per ml. Despite this obvious difference in sensitivity seen in the laboratory, under field conditions in the present study there was no significant difference in the sensitivity of the two tests. Natural infection of *T. vivax* in cattle tends to produce a high parasitaemia which may explain why the increased sensitivity of the BC seen in the laboratory is not repeated in the field. In order to get a more accurate estimate of the prevalence of *T. vivax* infections in cattle when surveying in the field both the HCT and the BC techniques should be used concurrently to maximise the sensitivity of the diagnosis of *T. vivax* in cattle.

The BC technique was repeatedly up to twice as sensitive as the HCT technique for the diagnosis of *T. congolense* in the field which is consistent with the findings of Paris *et al.* (1982) in the laboratory. Since *T. congolense* infections tend to produce lower parasitaemias than *T. vivax* in cattle this may explain the agreement between findings from the field and those from the laboratory. Correlation between the HCT and the BC techniques for the detection of *T. congolense* infections in cattle was relatively good (kappa value = 0.47) which may be due to a failure to differentiate between *T. congolense* and short stumpy forms of *T. brucei* spp. on the basis of morphology (ILCA/ILRAD, 1983).

The HCT proved to be the only worthwhile diagnostic test used in this study for the diagnosis either *T. theileri* or microfilaria infections in cattle.

When selecting suitable susceptible mice for inoculation no advantage in the sensitivity of diagnosis of *T. brucei* spp. infections in cattle was found by using expensive inbred ILRAD BALB/C mice rather than *KETRI* outbred mice. However since only one mouse was used per sample and the sample size was only 375 the increase in susceptibility of inbred mice to infection with *T. brucei* spp. may not have been apparent. Immunosuppression with cyclophosphamide prior to inoculation with bovine blood can increase the sensitivity of the technique by a magnitude of up to 100%. There was moderate to good levels of

agreement between the groups of mice (kappa value > 0.4) except between the immunosuppressed and the non-suppressed outbred *KETRI* mice (kappa value = 0.31) which had the lowest number of infections of all the groups of mice. Higher mortality within the immunosuppressed groups may account for this difference.

Using the PCV of cattle as a predictor of trypanosome infections in cattle is a potentially useful tool as it requires less equipment and skilled manpower to carry out than the HCT, the BC technique or mouse inoculation. Depending on the choice of PCV value the sensitivity and specificity of the test can be raised or lowered but necessarily the lower the PCV value chosen as an indicator of trypanosomiasis specificity increases and sensitivity decreases. At a PCV of 20%, there is a 95% accurate prediction of the true prevalence of trypanosome infections in cattle, with approximately 50% of actual cases of trypanosomiasis being detected using only PCV as an indicator of infection. PCV is a more accurate predictor of *T. vivax* and any trypanosome infection compared to the more tissue invasive *T. brucei* spp. (Morrison *et al.*, 1983). As anaemia is one of the most consistent clinical signs of trypanosomiasis (Murray and Dexter, 1988) and the PCV of cattle is simple and quick to measure using only a centrifuge and a PCV reader, with little skill required, it may be a useful guide in an epidemiological study to estimate the prevalence of trypanosome infections within a population of cattle. In this study if a PCV of 21% was chosen then the apparent prevalence of trypanosome infections would equal the true prevalence which is of value in an epidemiological study but of less value in diagnosing individual cases (fig. 6.1).

Surveys of the prevalence of trypanosome infections in cattle are notoriously difficult to compare with each other because of the differing diagnostic tests used. However using the information in this study, covering the diagnosis of bovine trypanosome infections under field conditions using over 11,000 samples, it is possible to calculate a more accurate estimation of the true prevalence of cattle infections from the prevalence reported in past studies which used only a selection of the diagnostic techniques evaluated here.

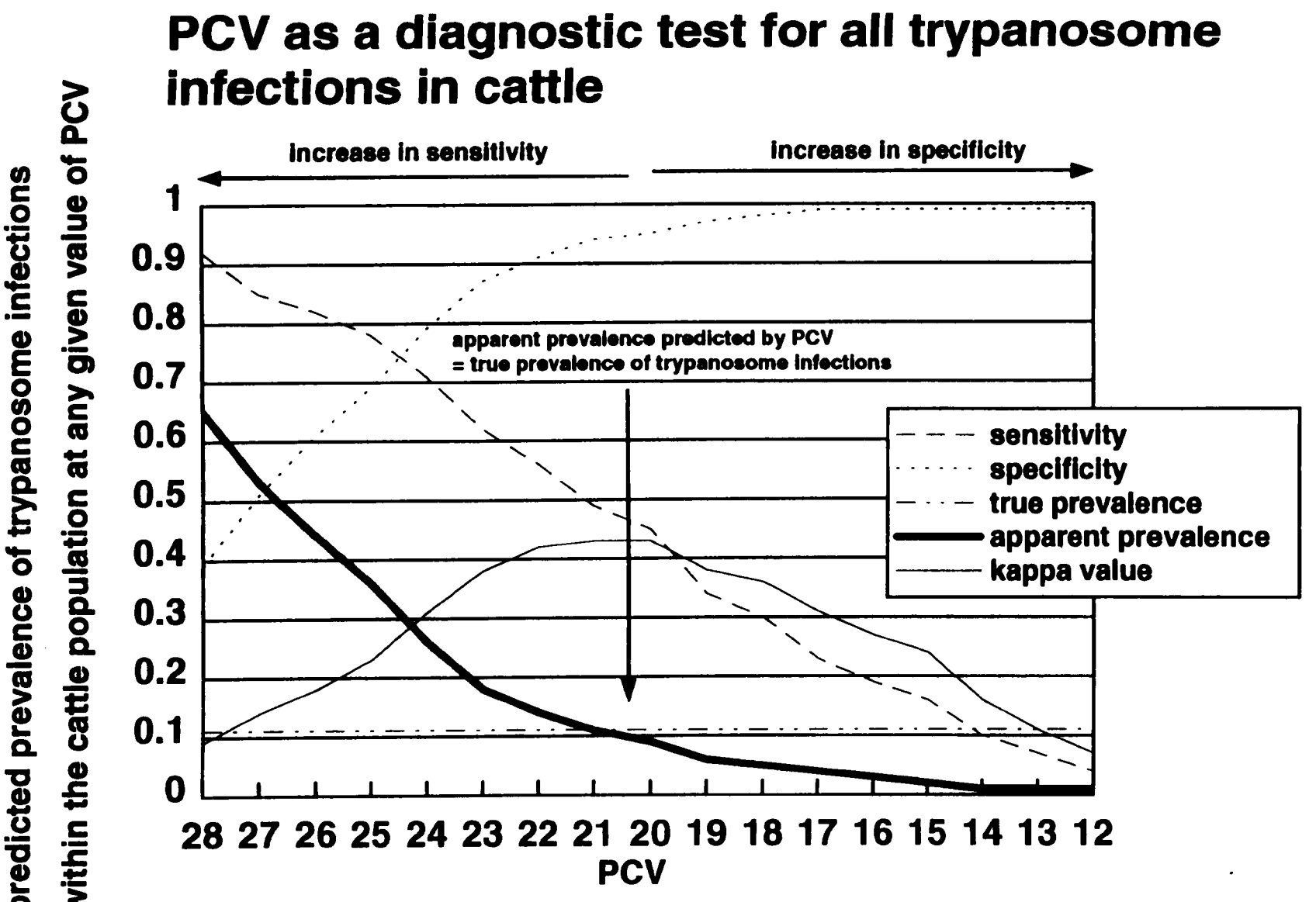

PCV as a diagnostic test for all trypanosome infections in cattle
predicted prevalence of trypanosome infections within the cattle population at any given value of PCV
increase in sensitivity
increase in specificity
apparent prevalence predicted by PCV
= true prevalence of trypanosome infections
sensitivity
specificity
true prevalence
apparent prevalence
kappa value
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
28 27 26 25 24 23 22 21 20 19 18 17 16 15 14 13 12
PCV

fig. 6.1

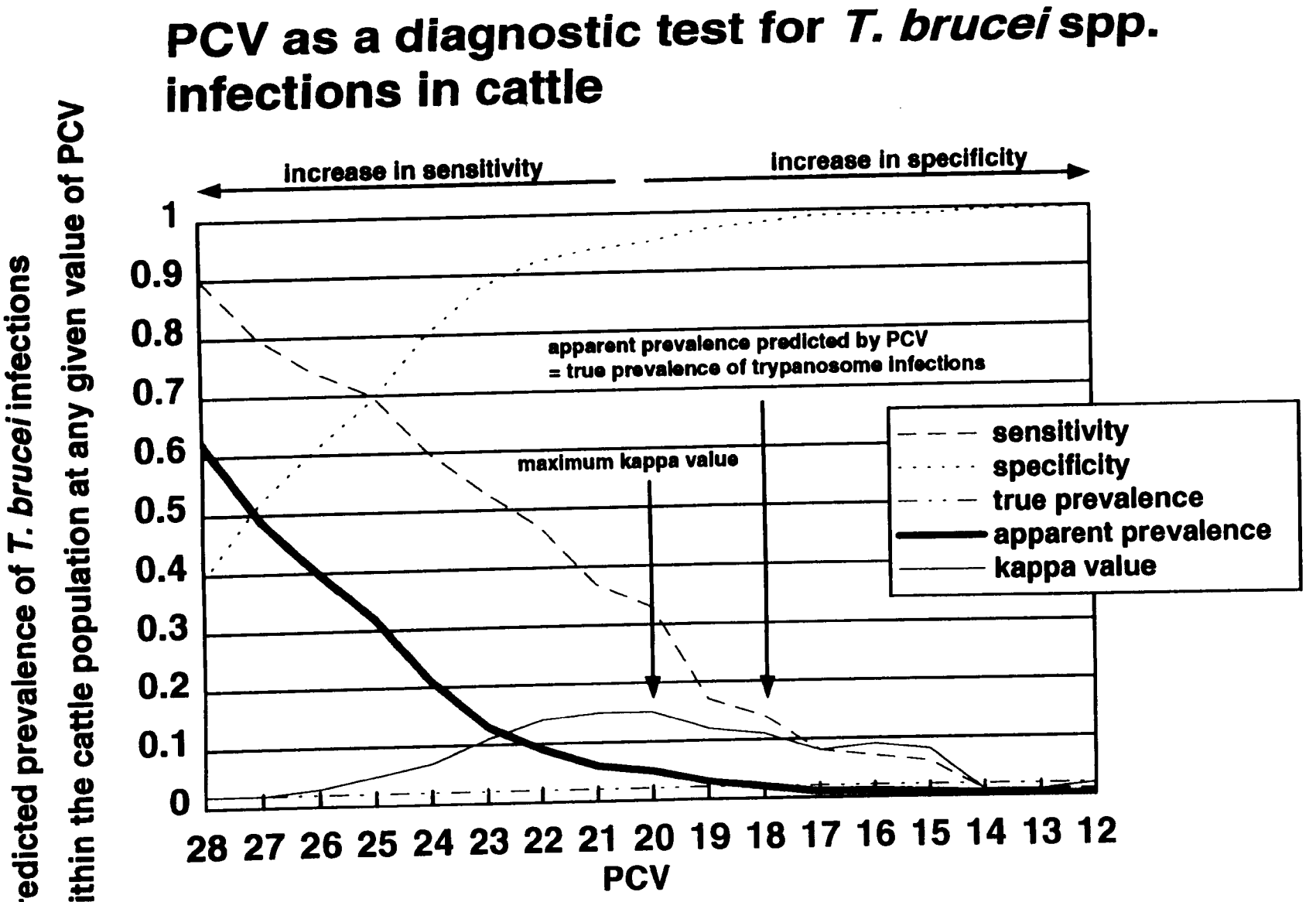

PCV as a diagnostic test for T. brucei spp. infections in cattle
predicted prevalence of T. brucei infections within the cattle population at any given value of PCV
increase in sensitivity
increase in specificity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
apparent prevalence predicted by PCV
= true prevalence of trypanosome infections
maximum kappa value
sensitivity
specificity
true prevalence
apparent prevalence
kappa value
28 27 26 25 24 23 22 21 20 19 18 17 16 15 14 13 12
PCV

fig. 6.2

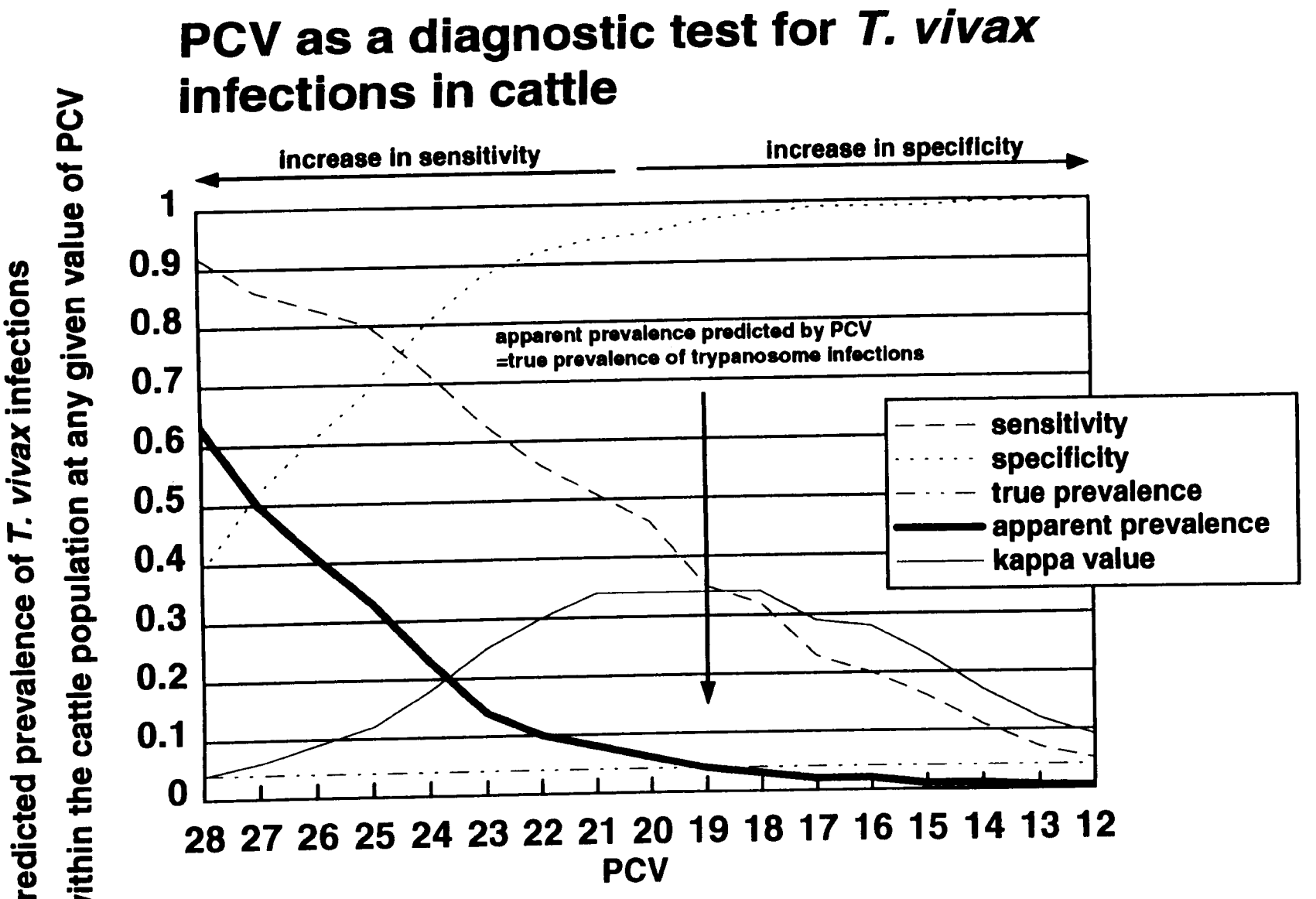

PCV as a diagnostic test for T. vivax infections in cattle
increase in sensitivity
increase in specificity
predicted prevalence of T. vivax infections within the cattle population at any given value of PCV
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
apparent prevalence predicted by PCV
=true prevalence of trypanosome infections
sensitivity
specificity
true prevalence
apparent prevalence
kappa value
28 27 26 25 24 23 22 21 20 19 18 17 16 15 14 13 12
PCV

fig. 6.3

Chapter VII

The pathogenesis of natural trypanosome infections in East African zebu cattle

7.1 Summary

The pathogenesis of *T. brucei* spp., *T. vivax*, *T. congolense*, *T. theileri* and microfilaria were studied in monthly samples of more than one thousand East African zebu cattle, kept under local village husbandry conditions, in Busia District, Western Kenya over a period of thirteen months. Although it is known that trypanosome infections in cattle can cause anaemia, weight loss and abortion and increased mortality it was unknown what degree these events occurred under natural tsetse challenge and local husbandry practices. Infections of *T. brucei* spp. alone were of relatively low pathogenicity to East African zebu cattle whereas cattle infected with *T. vivax* or *T. congolense* had a more varied outcome. While some cattle were able to tolerate infections of either *T. vivax* or *T. congolense* for some time, others became anaemic, lost weight and were clinically ill. Cattle with mixed infections of *T. brucei* spp. and *T. vivax* or *T. brucei* spp. and *T. congolense* showed similar pathology to cattle with single infections of *T. vivax* or *T. congolense* respectively. There was some evidence of synergy between species of trypanosome in mixed infection in the pathogenesis of anaemia. Mixed infections of *T. vivax* and *T. congolense* were more pathogenic for East African zebu cattle than either single infections of *T. vivax* alone or *T. congolense* alone.

Infection of pregnant cattle with trypanosomes rarely resulted in abortion and congenital infection was never detected.

T. theileri and microfilaria infections in East African zebu cattle were not considered pathogenic.

The effects of the pathogenesis of the differing species of trypanosome on the epidemiology of the animal reservoir of Rhodesian sleeping sickness are discussed.

7.2 Introduction

It has long been regarded that animal trypanosomiasis is a group of diseases equal in number to the species of pathogenic trypanosomes and that the disease process depends on the one or more species of trypanosome causing infection, the strain within each species of trypanosome and on the species of the infected host (Hornby, 1921). However this fundamental definition has not found broad support, and animal trypanosomiasis appears usually to be considered as a group of closely similar diseases (Losos and Ikede, 1972). Advances in the knowledge of the

pathogenesis of trypanosomiasis over the past 30 years have largely been the results of experiments using European breeds of humpless (*Bos taurus*) cattle (Morrison *et al.*, 1983; Wellde *et al.*, 1989b), improved *Bos indicus* Boran cattle (Dolan *et al.*, 1985; Dolan *et al.*, 1990; Kamango-Sollo *et al.*, 1991) or small ruminants (Whitelaw *et al.*, 1985; Bouteille *et al.*, 1988; Katunguka-Rwakishaya *et al.*, 1992). Although the pathogenesis of single trypanosome infections in experimental animals has been extensively studied the pathogenesis of mixed trypanosome infections is less well understood (Losos and Ikede, 1972; Masake *et al.*, 1984).

The main pathogenic effects of trypanosomiasis are anaemia, weight loss, abortion, infertility and increased mortality (Fiennes, 1970). The development of anaemia is a well recognised consequence of trypanosome infections in domestic animals in general and cattle in particular (Murray and Dexter, 1988) and it has been established that the measurement of anaemia gives a reliable indication of the disease status (Murray *et al.*, 1979) and productive performance of trypanosome infected cattle (ILCA/ILRAD, 1986). The severity of the anaemia which follows infection is affected by many factors, including the differences in virulence of the differing species of trypanosome and among the large number of strains within each species (Murray and Dexter, 1988). In the pathogenesis of anaemia host factors such as age, nutritional status and breed are also important (Murray *et al.*, 1982). Recent research indicates that there is direct interaction between nutrition status and the pathogenesis of the trypanosomiasis (Katunguka-Rwakishaya *et al.*, 1992). The pathogenesis of trypanosomiasis in local East African zebu (*Bos indicus*) is much less well understood except by inference to similarities with the pathogenesis in experimental animals.

In order to investigate the pathogenesis of trypanosome infections in cattle in Busia, native East African zebu cattle were studied under conditions of natural tsetse challenge and local husbandry practices during a period of eighteen months. The effects of trypanosome infections on the monthly change in the PCV and weights of cattle were investigated by analysis of covariance and an estimate of the magnitude of these effects was made by comparing the means. The effects of trypanosome infections on body condition, clinical condition, reproduction and mortality were also examined.

7.3 Material and methods

For general materials and methods see chapter III.

7.3.1 Measuring the effects of trypanosome infections on the PCV of cattle

The packed cell volume (PCV) of all ear-tagged cattle was measured monthly as described in chapter III. Cattle were assumed to be uninfected when they had no trypanosome infection on the month of recording, the month prior to recording and the month after recording. Cattle undergoing chemoprophylaxis with either Samorin or Ethidium were excluded from analysis. The effects of trypanosome infections on the PCV of cattle were measured over time with the time of infection represented by sample event 0, the sample events preceding and post infection were represented by -1, +1 etc.

7.3.2 Measuring the effects of trypanosome infections on monthly liveweight change of cattle

All ear-tagged cattle were weighed monthly as described in chapter III. Cattle were assumed to be uninfected when they had no trypanosome infection on the month of recording, the month prior to recording and the month after recording. Cattle undergoing chemoprophylaxis with either Samorin or Ethidium were excluded from analysis, as were pregnant cows, calving cows and cows in early lactation. The effects of trypanosome infections on the monthly liveweight change were measured over time with the time of infection represented by sample event 0, the sample events preceding and post infection were represented by -1, +1 etc.

7.3.3 Measuring the effects of trypanosome infections on the body condition score of cattle

The body condition score of all ear-tagged cattle was recorded monthly as described by Nicholson and Butterworth (1986).

7.3.4 Measuring the effects of trypanosome infections on the clinical condition of cattle

All ear-tagged cattle with a trypanosome infection or a PCV of 20% or less were clinically examined and were assigned a clinical score from 1 to 4 as described in Table 7.24.

7.3.5 Measuring the effects of trypanosome infections on cattle reproduction

All calvings and abortions of ear-tagged cattle during the previous month were recorded at the subsequent time of blood sampling and weighing and calved and aborted cows and their calves were clinically examined as soon after the event as was practical. Pregnancy and lactation were calculated from calving dates.

7.3.6 Measuring the effects of trypanosome infections on cattle mortality

Deaths of ear-tagged cattle during the previous month were recorded at the subsequent time of blood sampling and weighing.

7.3.7 Statistical analysis

The significance of the effects of trypanosome infections on the PCV and monthly liveweight change of cattle was tested by analysis of covariance and an estimation of the magnitude of these effects was carried out by comparing the mean values using a two tailed t-test. Analysis of the categories of body condition score and clinical score were carried out using a Chi square test. For a full description of statistical methods see chapter III.

7.4 Results

7.4.1 The effects of trypanosome infections on the PCV of cattle.

7.4.1.1 The PCV of cattle with no trypanosome infection

The mean PCV of all uninfected cattle was 27.58% (Table 7.1). Calves up to the age of six months had a mean PCV 2.42% higher than cattle over six months of age ($t = 6.75$, $p < 0.0001$) (Table 7.1). Analysis of covariance of the effect of age on PCV also showed this to be highly significant for cattle less than six months of age ($p < 0.0001$, Appendix VI, Table VII.1). The distribution of the PCV of uninfected cattle is given in Fig. 7.1.

Table 7.1 The PCV of cattle with no trypanosome infection

	mean %	s.e.	number of samples
cattle over 6 months age	27.58	0.07726	2833
cattle under 6 months age	30.00	0.35106	225
all cattle	27.76	0.07691	3058

7.4.1.2 The PCV of cattle in which only infections with *T. brucei* spp. were observed

The mean PCV of cattle in which only infections of *T. brucei* spp. were observed was 23.18%, 4.75% less than the mean PCV of uninfected cattle (27.76%, $p < 0.0001$) (Table 7.2). This difference was highly significant explaining more than one per cent of residual deviation ($p < 0.0001$, Appendix VII, Table VII.2 and VII.3). The mean PCV of cattle with an infection of *T. brucei* spp. only (23.18%) was significantly greater than those in which only infections of *T. vivax* (21.33%, $p < 0.0001$), mixed infections of *T. brucei* spp. and *T. vivax* only (21.91%, $p = 0.0075$) or mixed infections of *T. brucei* spp. and *T. congolense* only (20.29%, $p = 0.0005$)

were observed but not significantly different from the PCV of cattle with only a *T. congolense* infection (22.02%, p = 0.10) or mixed infections of *T. brucei* spp., *T. vivax* and *T. congolense* together (21.00%, p = 0.12) (Table 7.3). The distribution of the PCV of cattle infected with *T. brucei* spp. alone is given in Fig. 7.2.

The mean PCV of cattle with an infection of *T. brucei* spp. only of less than one month's duration (22.70%) was significantly lower than cattle with *T. brucei* spp. infections of longer standing than one month (24.12%, p = 0.037) (Table 7.4) and a comparison between the mean PCV of cattle from the first month of diagnosis of an infection of *T. brucei* spp. alone to that of one month later with the *T. brucei* spp. infection still present there is a small (1.43%) but significant rise in the PCV (Table 7.5 and fig. 7.3). There was only one infection of *T. brucei* spp. only in a cow which had a PCV of less than 20% at the time of first diagnosis and required treatment.

Table 7.2 The PCV (%) of cattle with trypanosome infections

infections	mean	s.e.	difference from uninfected cattle	number of samples
T. brucei spp. only	23.18%	0.342	-4.75% (t = 13.07) (p < 0.0001)	174
T. vivax only	21.33%	0.259	-6.43% (t = 23.74) (p < 0.0001)	417
T. congolense only	22.02%	0.616	-5.74% (t = 9.25) (p <0.0001)	99
T. brucei spp. with *T. vivax* only	21.91%	0.327	-5.85% (t = 17.43) (p < 0.0001)	251
T. brucei spp. with *T. congolense* only	20.29%	0.721	-7.47% (t = 11.81) (p < 0.0001)	49
T. vivax with *T. congolense* only	17.41%	0.873	-7.47% (t = 11.81) (p < 0.0001)	32
T. brucei spp. with *T. vivax* and *T. congolense*	21.00%	1.28	-6.76% (t = 5.27) (p = 0.0001)	15
T. theileri only	28.06%	0.210	0.30% (t = 1.36) (p = 0.17)	400
microfilaria only	26.58%	0.178	-1.18% (t = 6.09) (p <0.0001)	491

Table 7.3 Comparison between the PCV (%) of cattle with differing trypanosome infections

infection	*T. brucei* spp. only compared to	*T. vivax* only compared to	*T. congolense* only compared to
T. brucei spp. only (n = 174)	-	-1.85% ** (t = 1.64) (p < 0.0001)	-1.16% ns (t = 1.64) (p = 0.10)
T. vivax only (n = 417)	1.85% ** (t = 1.64) (p < 0.0001)	-	0.69% ns (t = 1.03) (p = 0.31)
T. congolense only (n = 99)	1.16% ns (t = 1.64) (p = 0.10)	-0.69% ns (t = 1.03) (p = 0.31)	-
T. brucei spp. and *T. vivax* (n = 251)	1.27% ** (t = 2.69) (p = 0.0075)	-0.58% ns (t = 1.38) (p = 0.17)	ND
T. brucei spp. and *T. congolense* (n = 49)	2.89% ** (t = 3.63) (p = 0.0005)	ND	1.73% ns (t = 1.83) (p 0.070)
T. vivax and *T. congolense* (n = 32)	ND	3.92% ** (t = 4.31) (p = 0.0001)	4.61% ** (t = 4.32) (p = 0.0001)
T. brucei spp., *T. vivax* and *T. congolense* (n = 15)	2.18% ns (t = 1.64) (p = 0.12)	0.33% ns (t = 0.26) (p = 0.80)	1.02% ns (t = 0.72) (p = 0.48)

ND not done
** highly significant
ns not significant

Table 7.4 Comparison of the mean PCV (%) of cattle with newly diagnosed trypanosome infections with those of cattle with trypanosome infections of longer duration

infection	*T. brucei* spp. only	*T. vivax* only	*T. congolense* only
new infections	22.70% (s.e. 0.44) (n = 115)	21.54% (s.e. 0.29) (n = 330)	22.21% (s.e. 0.64) (n = 89)
older infections	24.12% (s.e. 0.51) (n = 59)	20.54% (s.e. 0.58) (n = 87)	20.30% (s.e. 2.1) (n = 10)
all infections	23.18% (s.e. 0.34) (n = 174)	21.33% (s.e. 0.26) (n = 417)	22.02% (s.e. 0.62) (n = 99)
difference in duration of infection	1.42% (t = 2.10) (p = 0.037)	-1.00% (t = 1.54) (p = 0.13)	-1.91% (t = 0.86) (p = 0.41)

new infections - a trypanosome infection in cattle of less than one month's duration
older infections - a trypanosome infection in cattle of more than one month's duration

Table 7.5 Change in the mean PCV (%) of cattle before and after infection with *T. brucei* spp. alone without treatment

months before and after infection	change in mean PCV	2-sample t	p value
-1 to 0	-0.97%	1.60	0.1201 ns
0 to +1	1.43%	2.24	0.0332 *
-1 to +1	0.47%	0.52	0.6107 ns

n = 30
* significant
ns not significant
infection detected at month 0

7.4.1.3 The PCV of cattle in which only infections of *T. vivax* were observed

The mean PCV of cattle in which only infections of *T. vivax* were observed was 21.29%, 6.49% less than the mean PCV of uninfected cattle (27.76%, $p < 0.0001$) (Table 7.2). This difference was highly significant explaining more than seven per cent of residual deviation ($p < 0.0001$, Appendix VII, Table VII.2 and VII.3). The mean PCV of cattle with an infection of

T. vivax only (21.33%) was significantly less than that of cattle with only a *T. brucei* spp. infection (23.18%, $p < 0.0001$) and was significantly greater than that of cattle with a mixed infection of *T. vivax* and *T. congolense* only (17.41%, $p < 0.001$), but was not significantly different from cattle with an infection of *T. congolense* alone (22.02%, $p = 0.13$) or mixed infections with either *T. brucei* spp. and *T. vivax* only (21.91%, $p = 0.17$) or *T. brucei* spp. *T. vivax* and *T. congolense* together (21.00%, $p = 0.80$). The distribution of the PCV's of cattle infected with *T. vivax* alone is given in Fig. 7.4.

The mean PCV of cattle with an infection of less than one month's duration of *T. vivax* only (21.54%) was higher than that of cattle with *T. vivax* infections alone of longer standing than one month (20.54%), however this difference was not statistically significant ($p = 0.13$) (Table 7.4).

Although there was a small but significant drop in the PCV (-1.57%) of cattle infected with *T. vivax* only not requiring treatment from the month before infection to the month when infection is first diagnosed there was no significant drop in PCV over the subsequent two months (Table 7.6, Fig. 7.5).

Table 7.6 Change in the mean PCV (%) of cattle before and after infection with *T. vivax* alone without treatment

months before and after infection	change in mean PCV	2-sample t	p value
-1 to 0	-1.57%	2.07	0.0458 *
0 to +1	-1.35%	1.66	0.1059 ns
+1 to +2	0.49%	0.70	0.4870 ns
-1 to +1	-2.92%	3.39	0.0017 **
-1 to +2	-2.43%	3.09	0.0038 **

n = 37
** highly significant
* significant
ns not significant
infection detected at month 0

The mean PCV of cattle with an infection of *T. vivax* only which required treatment dropped sharply (-8.20%) between the month before diagnosis of *T. vivax* infection and the month of diagnosis and treatment (Table 7.7). Following treatment there was a similar

sharp increase in mean PCV which continued over the following two months (Table 7.7, Fig. 7.6).

Table 7.7 Change in the mean PCV (%) of cattle before and after infection with *T. vivax* alone and treatment with diminazene aceturate

months before and after infection	change in mean PCV	2-sample t	p value
-1 to 0	-7.66%	8.67	< 0.0001 **
0 to +1	7.70%	11.76	< 0.0001 **
+1 to +2	1.80%	2.92	0.0058 **
-1 to +1	-0.50%	0.61	0.5480 ns
-1 to +2	1.30%	1.57	0.1253 ns

n = 40
** highly significant
* significant
ns not significant
infection detected and treated at month 0

7.4.1.4 The PCV of cattle in which only infections with *T. congolense* were observed

The mean PCV of cattle with an infection of *T. congolense* only was 21.18% 6.58% less than the mean PCV of uninfected cattle (27.76%, $p < 0.001$) (Table 7.2). This difference was highly significant explaining more than one per cent of residual deviation ($p < 0.0001$, Appendix VII, Table VII.2 and VII.3). The distribution of the PCV's of cattle infected with *T. congolense* alone is given in fig. 7.7.

The mean PCV of cattle with an infection of less than one month's duration of *T. congolense* (22.21%) was higher than that of cattle with *T. congolense* infections of longer standing than one month (20.30%), however this difference was not statistically significant ($p = 0.41$, Table 7.4).

The mean PCV of cattle with an infection of *T. congolense* alone did not require treatment did not significantly alter before or after infection (Table 7.8, fig. 7.8). Whereas the mean PCV of cattle with a *T. congolense* infection only which did require treatment dropped sharply (-8.37%) on infection and treatment and had a similar sharp increase (8.58%) following treatment (Table 7.9, fig. 7.9).

Table 7.8 Change in the mean PCV (%) of cattle before and after infection with *T. congolense* alone without treatment

month before and after infection	change in mean PCV	2-sample t	p value
-1 to 0	-0.94%	0.81	0.4318 ns
0 to +1	0.11%	0.13	0.8981 ns
-1 to +1	-0.83%	0.60	0.5545 ns

n = 18
ns not significant
infection detected at month 0

Table 7.9 Change in the mean PCV (%) of cattle before and after infection with *T. congolense* alone and treatment with diminazene aceturate

months before and after infection	change in mean PCV	2-sample t	p value
-1 to 0	-8.37%	5.43	< 0.0001 **
0 to +1	8.58%	8.67	< 0.0001 **
-1 to +1	0.21%	0.11	0.9108 ns

n = 19
** highly significant
* significant
ns not significant
infection detected and treated at month 0

7.4.1.5 The PCV of cattle in which only mixed infections of *T. brucei* spp. and *T. vivax* were observed

The mean PCV of cattle with a mixed infection of *T. brucei* spp. and *T. vivax* only was 21.80%, 5.96% less than the mean PCV of uninfected cattle (27.76%, $p < 0.0001$) (Table 7.2). This difference was highly significant explaining more than four per cent of residual deviation ($p < 0.0001$, Appendix VII, Table VII.2). The mean PCV of cattle with a mixed infection of *T. brucei* spp. and *T. vivax* only (21.80%) was significantly less than an infection with *T. brucei* spp. only (23.18%, $p = 0.0075$), but not significantly different from an infection with *T. vivax* alone (21.33%, $p = 0.17$). The interactive effect of between *T. brucei* spp. and

T. vivax on PCV after accounting for the effects of each infection individually, was significant explaining more than one per cent more residual deviation ($p < 0.0001$, Appendix VII, Table VII.3). The distribution of the PCV's of cattle with a mixed infection of *T. brucei* spp. and *T. vivax* alone is given in fig. 7.10.

The effect on the mean PCV of cattle with a mixed infection of *T. brucei* spp. and *T. vivax* alone which required treatment was a sharp drop in PCV between the month before diagnosis and the month of diagnosis and treatment (-8.2%, Table 7.10). Following treatment there was a similar sharp increase in mean PCV which continues over the following two months (7.7% and 1.8%, Table 7.10 and fig.7.11). There were too few infections of cattle of *T. brucei* spp. and *T. vivax* alone with a duration of three months which did not require treatment to allow statistical analysis (n = 8).

Table 7.10 Change in the mean PCV (%) of cattle infected with *T. brucei* spp. and *T. vivax* alone in the same month and treated with diminazene aceturate

months before and after infection	change in mean PCV	2-sample t	p value
-1 to 0	-8.20%	8.67	< 0.0001 **
0 to +1	7.70%	11.76	< 0.0001 **
+1 to +2	1.80%	2.92	0.0058 **
-1 to +1	-0.50%	0.61	0.5480 ns
-1 to +2	1.30%	1.57	0.1253 ns

n = 28
** highly significant
* significant
ns not significant
infection detected and treated at month 0

7.4.1.6 The PCV of cattle in which only mixed infections of *T. brucei* and *T. congolense* were observed

The mean PCV of cattle with a mixed infections of *T. brucei* spp. and *T. congolense* only was 20.18%, 7.58% less than the mean PCV of uninfected cattle (27.76%, $p < 0.0001$) (Table 7.2). This difference was highly significant explaining more than one per cent of residual deviation ($p < 0.0001$, Appendix VII, Table VII.2). The mean PCV of cattle with mixed infections of *T. brucei* spp. and *T. congolense* alone (20.29%) was significantly less than the

PCV of cattle with infections of *T. brucei* spp. alone (23.18%, p = 0.0005), but not significantly different from the PCV of cattle with infections of *T. congolense* alone (22.02%, p = 0.07) (Table 7.3). The interactive effect between *T. brucei* and *T. congolense*, after accounting for the effects of each infection individually was significant (p < 0.0001), but explained only 0.19 per cent of residual deviation (Appendix VII, Table VII.3). The distribution of PCV's of cattle with mixed infections of *T. brucei* spp. and *T. congolense* only is given in fig. 7.12.

7.4.1.7 The PCV of cattle in which only mixed infections of *T. vivax* and *T. congolense* were observed

The mean PCV of cattle with a mixed infection of *T. vivax* and *T. congolense* only was 20.38%, 7.38% less than the mean PCV of uninfected cattle (27.76%, p < 0.0001) (Table 7.2). This difference was highly significant explaining more than one per cent of residual deviation (p < 0.0001, Appendix VII, Table VII.2). The mean PCV of cattle with a mixed infection of *T. vivax* and *T. congolense* only (17.41%) was significantly less than the PCV of cattle with a single infection of *T. vivax* only (21.33%, p = 0.0001), but not significantly different from that of cattle with a single infection of *T. congolense* alone (22.02%, p = 0.07). The interactive effect between *T. vivax* and *T. congolense*, after accounting for the effects of each infection individually was significant (p=0.0124), but explained less than 0.1 per cent of residual deviation (Appendix VII, Table VII.3). The distribution of PCV's of cattle with a mixed infection of *T. vivax* and *T. congolense* only is given in fig. 7.13.

7.4.1.8 The PCV of cattle in which mixed infections of *T. brucei* spp., *T. vivax* and *T. congolense* together were observed

The mean PCV of cattle with a mixed infection of *T. brucei* spp. , *T. vivax* and. *T. congolense* together was 21.00%, 6.76% less than the mean PCV of uninfected cattle (27.76%, p = 0.0001) (Table 7.2). This difference was significant (p < 0.0001) but explained less than one per cent of the residual deviation (Appendix VII, Tables VII.2). The mean PCV of cattle with mixed infections of *T. brucei* spp., *T. vivax* and *T. congolense* together (21.00%) was not significantly different from the PCV of cattle with single infections of *T. brucei* spp. (23.18%, p = 0.12), *T. vivax* (21.33%, p = 0.0.80) or *T. congolense* (22.02%, p = 0.48) (Table 7.3). The interactive effect on the PCV of cattle between *T. brucei* spp., *T. vivax* and *T. congolense*

accounting for the effects of each infection individually and as mixed infections was not significant (p = 0.6445, Appendix VII, Table VII.3). The distribution of PCV's of cattle with mixed infections of *T. brucei* spp., *T. vivax* and *T. congolense* together is given in fig. 7.14.

7.4.1.9 The PCV of cattle in which infections of *T. theileri* only were observed

The mean PCV of cattle with an infection of *T. theileri* only was 28.06%, 0.3% greater than the mean PVC of uninfected cattle (27.76%) (Table 7.2). However this difference was not statistically significant (p= 0.17, Table 7.2). The interactive effects of *T. theileri* with *T. brucei* spp., *T. vivax* and *T. congolense* on PCV of cattle were examined and found not to be significant (Appendix VII, Table VII.4).

7.4.1.10 The PCV of cattle in which infections of microfilaria only were observed

The mean PCV of cattle with an infection of microfilaria only was 26.58%, 1.18% less than the mean PCV of uninfected cattle (27.76%, p < 0.0001, Table 7.2). However when the effect of infections of microfilaria only on the PCV of all cattle was examined this was found not to be significant (Appendix VII, Table VII.5).

7.4.1.11 Factors other than trypanosome infections affecting PCV of cattle

Village (1.1% deviation, p < 0.0001), body score (5.48% deviation, p < 0.0001) (fig. 7.), age (2.28% deviation, p < 0.0001) were found to have very significant effects on PCV of cattle (Appendix VII, Table VII.6). Month of sampling (0.99% deviation, p = 0.0005), the interaction between age and village (0.9% deviation, p< 0.0001) (Appendix VII, Table VII.6), east coast fever (0.60% deviation, p< 0.001) death or disposal (0.28% deviation, p= 0.0140), weight category (0.23% deviation, p= 0.0195) (Appendix VII, Table VII.7), were all statistically significant but explain less than 1% of residual deviation. Pregnancy and calving was found not to have a significant effect on the PCV of cattle (p= 0.9696) (Appendix VII, Table VII.7).

7.4.2 The effects of trypanosome infections on the liveweight of cattle

7.4.2.1 The monthly weight change of uninfected cattle

Analysis of covariance of weight change (kg) in cattle with no prophylactic trypanocide treatment and excluding pregnant, calving, aborting cows and cows in early lactation showed that the sex of cattle was not a significant factor in the deviance of monthly weight change of cattle and although age was a statistically significant factor it explains much less than one per cent of residual deviance (Appendix VII, Table VII.9). The month of sampling was a highly significant factor in the deviance of monthly weight change of cattle, explaining more than six per cent of residual deviation and the differences between villages, which although statistically significant, explained less than a quarter of one per cent of residual deviation (Appendix VII, Table VII.9).

The mean monthly weight change in male cattle does not change significantly from adjacent age groups in Table 7.12 ($p = 0.29$, $p = 0.69$, $p = 0.84$, $p = 0.16$). However for female cattle (excluding pregnancy, calving and early lactation), while there was no significant difference between adjacent age groups ($p = 0.89$, $p = 0.88$, $p = 0.46$) up to the age of four years, there was a significant reduction in monthly liveweight gain after this age ($t = 2.42$, $p = 0.016$) (Table 7.11).

The mean monthly weight gain of male cattle in their first year of life was significantly greater than that of female cattle of the same age. ($t = 10.49$, $p < 0.001$) (Table 7.12). Similarly in cattle over 4 years of age there was a significant difference between male and female ($t = 2.07$, $p = 0.0387$). However there were no significant differences between the sexes in the other age groups (Table 7.11).

Table 7.11 The mean monthly liveweight change (kg) of cattle with no trypanosome infection (not given any prophylactic trypanocidal drug and excludes pregnant, calving and lactating cows)

age group /sex	0 - 1 year	1 - 2 years	2 - 3 years	3 - 4 years	over 4 years	all cattle
male	5.35 s.e. 0.300 n = 172	4.79 s.e. 0.448 n = 229	4.54 s.e. 0.446 n = 322	4.68 s.e. 0.538 n = 288	3.76 s.e. 0.365 n = 601	4.39 s.e. 0.202 n = 1612
female	4.56 s.e. 0.245 n = 203	4.61 s.e. 0.321 n = 416	4.55 s.e. 0.318 n = 441	4.13 s.e. 0.470 n = 330	2.66 s.e. 0.385 n = 714	3.85 s.e. 0.178 n = 2104
total	4.93 s.e. 0.192 n = 375	4.68 s.e. 0.261 n = 645	4.54 s.e. 0.263 n = 763	4.38 s.e. 0.354 n = 618	3.16 s.e. 0.268 n = 1315	4.09 s.e. 0.134 n = 3716

7.4.2.2 The monthly weight change of cattle with trypanosome infections

Analysis of covariance of residual deviation in weight change of cattle (with no prophylactic trypanocide treatment and excluding pregnant, calving, aborting cows and cows in early lactation) after accounting for differences in sex, age, month and village (Appendix VII, Table VII.9) showed that infections with either *T. vivax* and *T. congolense* have a highly significant effect on the monthly weight change of cattle whereas infection with *T. brucei* spp. does not (Appendix VII, Table VII.10).

The mean monthly weight gains of cattle infected with *T. vivax* only, *T. congolense* only, mixed infections of *T. brucei* spp. and *T. vivax* only, *T. brucei* spp. and *T. congolense* only, *T. vivax* and *T. congolense* only and infection with *T. brucei* spp., *T. vivax* and *T. congolense* together are all significantly less than uninfected cattle (Table 7.13). However the mean monthly weight gain for cattle infected with *T. brucei* spp. only was not significantly different from that of uninfected cattle (Table 7.12).

Table 7.12 The mean monthly weight change of cattle with trypanosome infections compared to uninfected cattle

infection	mean weight change (kg)	mean weight change (%)	compared to mean weight change in uninfected cattle (kg)
T. brucei spp. only	3.64 (s.e. 0.757) (n = 142)	2.79 (s.e. 0.508) (n = 142)	-0.45 ns (t = 0.58) (p = 0.56)
T. vivax only	-0.36 (s.e. 0.535) (n = 301)	0.21 (s.e. 0.384) (n = 301)	-4.45** (t = 8.07) (p <0.0001)
T. congolense only	-1.38 (s.e. 1.19) (n = 82)	0.37 (s.e. 0.994) (n = 82)	-5.47** (t = 4.56) (p < 0.0001)
T. brucei spp. and *T. vivax*	-0.45 (s.e. 0.720) (n = 202)	0.49 (s.e. 0.448) (n = 202)	-3.60** (t = 7.70) (p < 0.0001)
T. brucei spp. and *T. congolense*	0.21 (s.e. 1.41) (n = 39)	0.65 (s.e. 0.908) (n = 39)	-3.88** (t = 2.74) (p = 0.0094)
T. vivax and *T. congolense*	-5.25 (s.e. 2.23) (n = 26)	-3.03 (s.e. 1.31) (n = 26)	-9.39** (t = 4.18) (p = 0.0003)
T. brucei spp., *T. vivax* and *T. congolense*	-3.40 (s.e. 2.82) (n = 10)	-2.46 (s.e. 2.41) (n = 10)	-7.49* (t = 2.65) (p = 0.026)

** highly significant
* significant
ns not significant

Comparison between the monthly liveweight change of cattle with differing trypanosome infections showed that the monthly liveweight change of cattle with infections involving *T. vivax* or *T. congolense* in all combinations were significantly less than cattle with infections of *T. brucei* spp. alone (Table 7.13). Cattle with mixed infections of *T. vivax* and *T. congolense* only had a mean weight loss significantly greater than those with infections of *T. vivax* alone (Table 7.13).

Table 7.13 Comparison between the mean monthly weight change (kg) of cattle with differing trypanosome infections

mean monthly weight change (kg) in cattle infected with	cattle infected with *T. brucei* spp. only compared to	cattle infected with *T. vivax* only compared to	cattle infected with *T. congolense* only compared to
T. brucei spp. only (n = 142)	-	-4.00 ** (t = 4.32) (p < 0.0001)	-5.04 ** (t = 3.55) (p = 0.0005)
T. vivax only (n = 301)	4.00 ** (t = 4.32) (p < 0.0001)	-	-1.04 (t = 0.78) (p = 0.44)
T. congolense only (n = 82)	5.04 ** (t = 3.55) (p = 0.0005)	1.04 ns (t = 0.78) (p = 0.44)	-
T. brucei spp. and *T. vivax* (n = 202)	3.68 ** (t = 3.91) (p = 0.0001)	-0.32 ns (t = 0.10) (p = 0.92)	ND
T. brucei spp. and *T. congolense* (n = 39)	3.43 * (t = 2.14) (p = 0.036)	ND	-1.61 ns (t = 0.86) (p = 0.39)
T. vivax and *T. congolense* (n = 26)	ND	4.94 * (t = 2.13) (p = 0.042)	3.9 ns (t = 1.53) (p = 0.13)

ND not done
** highly significant
* significant
ns not significant

7.4.2.3 The effect of infection with *T. brucei* spp. alone on the liveweight of cattle over time

There was no significant change in the mean monthly weight change of cattle infected with *T. brucei* spp. only before and after infection (Table 7.14 and fig. 7.15).

Table 7.14 The mean monthly weight change (kg) of cattle before and after infection with *T. brucei* spp. alone without treatment

months before and after infection	change in mean monthly weight gain (kg)	2-sample t	p value
-1 to 0	-2.2 kg	0.81	0.4273 ns
0 to +1	1.7 kg	0.69	0.4978 ns
-1 to +1	-0.5 kg	0.20	0.8458 ns

n = 21
ns not significant
infection detected at month 0

7.4.2.4 The effect of infection with *T. vivax* alone on the liveweight of cattle over time

The mean monthly liveweight of cattle infected with *T. vivax* alone dropped during the first month of infection but this was only significant in cattle which failed to maintain their PCV (and required treatment) (Table 7.15 and 7.16, fig. 7.16 and 7.17). However by the second month of infection in untreated cattle there was a significant drop in liveweight gain from the month pre-infection (Table 7.16 and fig. 7.16). While the cattle infected with *T. vivax* alone which were able to maintain their PCV (see Table 5.15) were also able to maintain some monthly liveweight gain (Table 7.15 and fig. 7.16), this was not the case with those unable to maintain their PCV (Table 7.16 and fig. 7.17). Cattle infected with *T. vivax* alone which were treated at the time the infection was first diagnosed showed a highly significant increase in liveweight gain over the following month (Table 7.16 and fig. 7.17).

Table 7.15 The mean monthly weight change (kg) of cattle before and after infection with *T. vivax* alone without treatment

months before and after infection	change in mean monthly weight gain (kg)	2-sample t	p value
-1 to 0	-3.77 kg	1.87	0.0749 ns
0 to +1	-0.65 kg	0.2519	0.8033 ns
+1 to +2	1.96 kg	0.81	0.4292 ns
-1 to +1	-4.42 kg	2.09	0.0479 *
-1 to +2	-2.46 kg	1.33	0.1971 ns

n = 24
* significant
ns not significant
infection detected at sample 0

Table 7.16 The mean monthly weight change (kg) of cattle before and after infection with *T. vivax* alone and treatment with diminazine aceturate

months before and after infection	change in mean monthly weight gain (kg)	2-sample t	p value
-1 to 0	-5.2 kg	2.39	0.0237 *
0 to +1	8.1 kg	3.35	0.0023 *
+1 to +2	-2.7 kg	0.97	0.3384 ns
-1 to +1	2.9 kg	1.39	0.1752 ns
-1 to +2	0.2 kg	0.08	0.9352 ns

n = 29
* significant
ns not significant
infection detected and treated at sample 0

7.4.2.5 The effect of infection with *T. congolense* alone on the liveweight of cattle over time

Cattle infected with *T. congolense* alone which managed to maintain their PCV (not requiring treatment) also had no significant change in mean monthly liveweight gain and managed to maintain their weight, before and after infection (Table 7.17 and fig. 7.18), while those which failed to maintain their PCV (required treatment) also failed to maintain their live weight (Tables 7.18 and fig. 7.19). There is a very significant increase in liveweight gain in cattle

infected with *T. congolense* alone over the month following treatment (Table 7.18 and fig. 7.19).

Table 7.17 The mean monthly weight change (kg) of cattle infected with *T. congolense* alone without treatment

months before and after infection	change in mean monthly weight gain (kg)	2-sample t	p value
-1 to 0	3.5 kg	1.36	0.1945 ns
0 to +1	-1.2 kg	0.37	0.7163 ns
-1 to +1	2.3 kg	0.71	0.4875 ns

n = 16
ns not significant
infection detected at sample 0

Table 7.18 The mean monthly weight change (kg) of cattle infected with *T. congolense* alone and treated with diminazene aceturate

months before and after infection	change in mean monthly weight gain (kg)	2-sample t	p value
-1 to 0	-6.3 kg	2.06	0.0573 ns
0 to +1	13.6 kg	3.76	0.0019 **
-1 to +1	7.3 kg	1.93	0.0722 ns

n = 16
** highly significant
* significant
ns not significant
infection detected and treated at sample 0

7.4.2.6 The effect of a mixed infection with *T. brucei* spp. and *T. vivax* only on the liveweight of cattle over time

Cattle infected with a mixed infection of *T. brucei* spp. and *T. vivax* which failed to maintain their PCV (required treatment) showed a significant drop in their mean liveweight gain, however the variance was large (Table 7.19 and fig. 7.20). There were too few cattle infected with a mixed infection of *T. brucei* spp. and *T. vivax* which maintained their PCV (no treatment required) for statistical analysis (n = 8).

Table 7.19 The effect of a mixed infection with *T. brucei* spp. and *T. vivax* on liveweight change (kg) and treated with diminazene aceturate

months before and after infection	change in weight gain (kg)	2-sample t	p value
-1 to 0	-0.86 kg	2.52	0.0215 *
0 to +1	8.3 kg	1.48	0.1552 ns
+1 to +2	3.3 kg	0.74	0.4693 ns
-1 to +1	-0.5 kg	0.14	0.8936 ns
-1 to +2	3.1 kg	1.32	0.2024 ns

n = 20
* significant
ns not significant
infection detected and treated at sample 0

7.4.2.7 The monthly weight change of cattle with infections of *T. theileri* or microfilaria alone

Analysis of covariance of residual deviation in weight change of cattle (with no prophylactic trypanocide treatment and excluding pregnant, calving, aborting cows and cows in early lactation) after accounting for differences in sex, age, month and village (Appendix VII, Table VII.9) showed that infections with either *T. theileri* or microfilaria had no significant effect on the monthly weight change of cattle (Appendix VII, Table VII.11).

There is no significant difference in the mean monthly liveweight gain between cattle infected with either *T. theileri* or microfilaria alone and uninfected cattle (Table 7.20).

Table 7.20 The mean monthly weight change (kg) of cattle with infections of *T. theileri* or microfilaria

infection	mean monthly weight change (kg)	difference from uninfected cattle (kg)
***T. theileri* only**	**4.12** **(s.e. 0.480)** **(n = 340)**	**-0.16 ns** **(t = 0.350)** **(p = 0.7262)**
microfilaria only	**3.75** **(s.e. 0.436)** **(n = 414)**	**-0.53 ns** **(t = 1.254)** **(p = 0.2099)**

ns not significant

7.4.3 The effect of trypanosome infections on the body condition score of cattle

Trypanosome infections in cattle significantly affect their body condition score. There were more cattle with a body score of four or less and less cattle with a body score of six which had a trypanosome infection than cattle which were uninfected ($p < 0.001$) (Table 7.21).

Table 7.21 The effect of a trypanosome infection on the body condition score of cattle

body score	1 to 3	4	5	6	7 to 9
infected cattle	80 (38.8)	191 (163.2)	329 (320.0)	421 (503.5)	17 (11.7)
uninfected cattle	293 (334.2)	1374 (1405.9)	2743 (2757.1)	4415 (4338.5)	95 (100.4)
total	373	1569	3077	4842	112

( ) expected values

($X^2 = 70.26$, df = 4, $p < 0.001$)

There was no apparent difference in the effect of infection with differing species of trypanosome on the body score of cattle ($p = 0.80$, Table 7.22). There was a strong correlation between PCV and body score (Appendix VII, Table VII. and fig. 7.20).

Table 7.22 The effect of differing trypanosome infections and body condition score of cattle

infection / body score	*Tb*	*Tv*	*Tc*	*Tb+Tv*	*Tb+Tc*	*Tv+Tc*	*Tb+Tv +Tc*	total
1-3	13	32	9	18	3	3	2	80
4	28	75	17	55	7	7	2	191
5	58	141	26	73	14	12	5	329
6	74	162	46	100	24	9	6	421
7-9	1	7	1	5	1	1	1	17
total	174	417	99	251	49	32	16	1038

(Chi^2 test on body scores of *T. brucei* spp., *T. vivax* , *T. congolense* and mixed infections with *T. brucei* spp. and *T. vivax* $X^2 = 7.8$, df = 12, p = 0.80)

7.4.4 The effect of trypanosome infections on the clinical condition of cattle

Cattle infected with *T. brucei* spp. only had a clinical score of 1 much more frequently than would be expected by chance, and a clinical score of 3 much less frequently than would be expected by chance if all trypanosome infections were equally pathogenic (Table 7.23). There were no cattle infected with a single infection of *T. brucei* spp. which had a clinical score of 4. Cattle with mixed infections of either *T. brucei* spp. and *T. vivax* only or *T. brucei* spp. and *T. congolense* had similar clinical scores cattle with single infections of either *T. vivax* or *T. congolense* alone.

Table 7.23 The effect of trypanosome infections on the clinical score of cattle

infection / clinical score *	*Tb*	*Tv*	*Tc*	*Tb+Tv*	*Tb+Tc*	*Tv+Tc*	*Tb+Tv +Tc*	total
1	91 (50.8)	103 (121.8)	37 (28.9)	58 (73.3)	8 (14.3)	4 (9.4)	2 (4.4)	303
2	68 (76.7)	180 (183.8)	38 (43.6)	124 (110.6)	27 (21.6)	12 (14.1)	8 (6.6)	457
3-4	15 (46.5)	13 (111.4)	24 (26.4)	69 (67.1)	14 (13.1)	16 (8.6)	5 (4.0)	277
total	174	417	99	251	49	32	15	1037

($X^2 = 85.6$, d.f. = 12, $p < 0.001$)
( ) expected count
* clinical score
1 no abnormalities detected
2 lymph nodes enlarged, pale mucous membranes, otherwise as 1
3 clinically ill, dull, partial anorexia, otherwise as 2
4 severely ill, very dull, anorexic, petaechial haemorrhages on mucous membranes and in some cases a full heamorrhagic syndrome, otherwise as 2

7.4.5 The effect of trypanosome infections on cattle reproduction

Only three abortions were observed in ear tagged cattle during the study (Table 7.24). More than 10% of ear tagged cattle which calved normally did so with a current trypanosome infection which is similar to the overall prevalence of trypanosome infection in cattle of 11% (Table 7.25). The varying species of trypanosome infections of calving cows are given in Table 7.26.

Table 7.24 Observed abortions in ear tagged cattle

village	PCV	trypanosome infection	clinical signs	treatment	outcome
Apatit village (Samorin control)	29%	none	trauma and no other abnormalities detected	antibiotic	1
Ngelechom village (Ethidium control)	10%	*T. vivax*	haemorrhagic syndrome *	diminazene aceturate	2
Ngelechom village (Ethidium control)	16%	*T. vivax*	haemorrhagic syndrome *	diminazene aceturate	3

* cattle with petaechial haemorrhages on mucous membranes and haemorrhaging through the gut

1. uneventful recovery
2. dam recovered after treatment; calf born one month premature, weak but alive
3. despite treatment when the PCV of the cow was 16 the PCV continued to fall to PCV 4, the cow then became recumbent, aborted and died

Table 7.25 Normal calving reported in ear tagged cattle

village	Katelenyang village (Samorin treated)	Apatit village (Samorin control)	Rukada village (Ethidium treated)	Ngelechom village (Ethidium control)	total
cows calving with a trypanosome infection	3	4	1	2	10
cows calving without a trypanosome infection	6	9	48	18	81
total	9	13	49	20	91

Table 7.26 Trypanosome infections of cows calving normally

trypanosome infection	number of calvings
T. brucei spp. only	3
T. vivax only	5
T. congolense only	1
T. brucei spp. and *T. vivax*	1
T. brucei spp. and *T. congolense*	0
T. vivax and *T. congolense*	0
T. brucei spp., *T. vivax* and *T. congolense*	0
total	10

More then 20% of cattle which were known to be pregnant had a trypanosome infection at some time during pregnancy (Table 7.27). The varying species of trypanosome are given in Table 7.28.

Table 7.27 Trypanosome infections of pregnant ear tagged cattle

village	Katelenyang village (Samorin treated)	Apatit village (Samorin control)	Rukada village (Ethidium treated)	Ngelechom village (Ethidium control)	total
number of pregnant cattle with trypanosome infections	6	8	7	4	25
number of positive samples from pregnant cattle	7	16	12	8	43
number of pregnant uninfected cattle	7	14	48	22	91
number of samples from uninfected pregnant cattle	37	45	240	110	432
total number of pregnant cattle sampled	13	22	55	26	116
total number of samples from pregnant cattle	44	61	252	118	475

Table 7.28 Trypanosome infection in pregnant ear tagged cattle

trypanosome infection	number of samples
T. brucei spp. only	8
T. vivax only	17
T. congolense only	2
T. brucei spp. and *T. vivax*	10
T. brucei spp. and *T. congolense*	4
T. vivax and *T. congolense*	2
T. brucei spp., *T. vivax* and *T. congolense*	0
total	43

Some cattle with trypanosome infections which calved normally were known to have carried the infections for extended periods during pregnancy and on to lactation (Table 7.29). One cow (R32) was known to have been infected with *T. brucei* spp. prior to conception, carried the infection throughout its pregnancy, gave birth to a normal calf and still had the infection at post mortem some five months after calving.

Table 7.29 Individual histories of cows with long standing trypanosome infections which calved normally

cow number	village	month of conception	month of calving	duration of infection at calving (months)			treatment
				Tb	*Tv*	*Tc*	
K158	Katelenyang	Nov 92	Aug 93	0	2	0	none
K180	Katelenyang	Dec 92	Sep 93	3	3	0	Sept 93
K234	Katelenyang	Oct 92	Jul 93	2	0	0	none
K485	Katelenyang	Dec 92	Sep 93	2	2	0	none
A71	Apatit	Jan 93	Oct 93	0	2	0	none
A254	Apatit	Sep 93	May 94	0	8	8	none
A271	Apatit	Feb 93	Nov 93	7	2	2	none
A337	Apatit	Jun 93	Feb 94	2	2	0	none
A358	Apatit	Dec 92	Sept 93	1	0	0	none
A426	Apatit	Feb 93	Nov 93	0	4	0	none
A570	Apatit	Jan 93	Oct 93	0	1	0	Oct 93
R32	Rukada	Jul 93	Mar 94	11	11	0	none
R33	Rukada	Jan 93	Oct 93	5	6	0	none
R459	Rukada	Aug 93	Apr 94	2	0	0	none
N141	Ngelechom	Mar 93	Dec 93	0	3	0	none
N320	Ngelechom	Aug 93	Apr 94	0	6	0	none
N378	Ngelechom	Aug 93	Apr 94	1	1	0	Apr 94
N464	Ngelechom	Aug 93	Apr 94	0	1	0	Apr 94

7.4.6 Trypanosome infections of new born calves

Clinical examination and blood sampling of all new born calves revealed no trypanosome infections.

7.4.7 The effect of trypanosome infections on cattle mortality

Of 72 reported deaths of ear tagged cattle, 24 had a trypanosome infection diagnosed on the month prior to reporting death (Table 7.30). Seventeen of the 24 cattle dying with trypanosome infections having been diagnosed in the month prior to death had a *T. vivax* infection and six had a *T. congolense* infection and only three cattle apparently had a single infection of *T. brucei* spp. at death (Table 7.31).

Table 7.30 Deaths reported in ear tagged cattle by village

village	Katelenyang village (Samorin treated)	Apatit village (Samorin control)	Rukada village (Ethidium treated)	Ngelechom village (Ethidium control)	total
trypanosome infection (month before reported death)	5	10	1	8	24
no trypanosome infection (month before reported death)	5	21	19	3	48
total	10	31	20	11	72

Table 7.3.1 Deaths reported in ear tagged cattle known to have a trypanosome infection

trypanosome infection	number of deaths
T. brucei spp. only	3
T. vivax only	10
T. congolense only	3
T. brucei spp. and *T. vivax*	5
T. brucei spp. and *T. congolense*	1
T. vivax and *T. congolense*	2
T. brucei spp., *T. vivax* and *T. congolense*	0
total	24

7.5 Discussion

When examining domestic cattle in Busia district as a potential reservoir of human infective *T. b. rhodesiense* it is essential to consider the pathogenesis of all trypanosome infections since they share a common vector (*Glossinidae*). Similar consideration must be given to the particular breed of East African zebu since this has been shown to have a significant effect on the pathogenesis of trypanosomiasis (Cunningham, 1966; Dolan *et al.*, 1985) and to the system of husbandry and level of nutrition under which they are kept (Murray *et al.*, 1982; Otesile *et al.*, 1991; Katunguka-Rwakishaya *et al.*, 1992). In Busia District, East African zebu

cattle infected with *T. brucei* spp. alone tend to have a mild but significant anaemia but do not have a reduction in liveweight gain. The anaemia was most severe within the first month of infection, but thereafter it tended to partially recover and most infected cattle managed to maintain both PCV and liveweight status, whether gaining weight or maintaining liveweight as adult cattle. It must be concluded that infections of the strains of *T. brucei* spp. found in Western Kenya alone were of very low pathogenicity in East African zebu cattle. Cattle infected with *T. vivax* or *T. congolense* alone on average had a more severe anaemia and had a significant reduction in liveweight gain or a weight loss. However within a group of cattle infected with either of these two species of trypanosome there were significant numbers of cattle which managed to both maintain their PCV and their liveweight status while others suffered anaemia and weight loss and required to be treated. In cattle with mixed infections of *T. brucei spp.* and *T. vivax, T. brucei* spp. and *T. congolense,* and *T. vivax* and *T. congolense,* there appears to be some interactive effect between the two species of trypanosomes beyond the effect of each species alone in the development of anaemia. However this interaction may be the effect of a greater duration of infection or that cattle which failed to control their parasitaemia, and are therefore more likely to be diagnosed by parasitological techniques were the same cattle which failed to maintain their PCV. The course of mixed infections in cattle with *T. brucei* spp. and *T. vivax* or *T. brucei* spp. and *T. congolense* was similar to that of infections with either *T. vivax* or *T. congolense* alone respectively but significantly more pathogenic in either case than an infection with *T. brucei* spp. alone. The course of mixed infections of *T. brucei* spp. and *T. vivax* only in cattle were similar to those infected with *T. vivax* alone, while some cattle failed to maintain their PCV or liveweight status others appeared much less severely affected. There is evidence that mixed infections of *T. vivax* and *T. congolense* only in cattle are more pathogenic, as measured by PCV and liveweight change and clinical condition, than infections of *T. vivax* alone.

Although there was a significant difference in body condition score (Nicholson and Butterworth, 1986) between uninfected cattle and those with trypanosome infections there is no such difference between cattle infected with differing species of trypanosome. This may be an effect of intervention with treatment during the period of study which prevented the development of long standing chronic cases of trypanosomiasis.

There was a significant difference in the clinical condition of cattle with differing trypanosome infections which reflected the greater pathogenicity of infections including *T. vivax* or *T. congolense* compared to infections of *T. brucei* spp. alone. Over 20% of pregnant cattle were found to have a trypanosome infection at some time during their pregnancy and 10% of cattle calving normally were similarly infected which was comparable the overall prevalence of trypanosome infections in cattle of 11%. Few abortions were observed, which were all in the third trimester of pregnancy and were associated with severely ill cattle with a haemorrhagic *T. vivax* syndrome or trauma. It is clear that most trypanosome infections during pregnancy do not result in abortion and by definition these cattle were exhibiting a degree trypanotolerance. Congenital trypanosome infection has been reported in cattle (Sekoni, 1994), but despite examining all new born calves throughout the duration of the study no cases of congenital infection were ever found.

Mortality figures in ear tagged cattle were of limited value since most sick cattle were not allowed to die naturally, rather the carcass was salvaged by slaughtering the animal prior to death. Also the period of time between diagnosis and reported death was usually one month but in some cases exceeded this. However most cattle known to have a trypanosome infection at death had infections of either *T. vivax* or *T. congolense* and much less so *T. brucei* spp., emphasising the greater pathogenicity of these two species of trypanosome.

Despite reports that *T. theileri* can be pathogenic for cattle under certain circumstances (Docherty, 1993) it must be concluded from the PCV and liveweight gain that *T. theileri* infections in East African zebu cattle are neither pathogenic on their own nor do they contribute to the pathology of other trypanosome infections in a mixed infection. Although cattle infected with microfilaria had a slightly lower PCV than uninfected cattle, there was no change to liveweight status, and if considered as a pathogen to East African zebu cattle, its effects are very mild.

Trypanotolerance has been defined by Pagot (1974) as a genetic capability of cattle to "maintain themselves in good condition and to reproduce while harbouring trypanosomes without showing clinical signs of the disease." However this definition can be misleading because infection of animals considered to be trypanotolerant can, in some circumstances, cause severe clinical disease and thus such breeds are not truly trypanotolerant but may be more correctly described as exhibiting a greater degree of resistance to the disease

(Murray *et al.*, 1982). By this definition East African zebu cattle in Busia district must be considered to show a degree of trypanotolerance. However it is clear that their ability to tolerate infections of differing species of trypanosome was neither uniform nor universal. Cattle have a far greater ability to tolerate infections of *T. brucei* spp. alone than infections of either *T. vivax* or *T. congolense* alone. Mixed infections of either *T. brucei* spp. and *T. vivax* only or *T. brucei* spp. and *T. congolense* only were tolerated to a similar degree as infections of *T. vivax* alone or *T. congolense* alone respectively. This has important implications for the epidemiology of trypanosomiasis in these cattle and in particular their ability to act as a reservoir for human infective *T. b. rhodesiense*. Infections of *T. brucei* spp. alone in the East African zebu cattle of this region are of very low pathogenicity causing only a slight drop in PCV with no significant change in liveweight gain. This is an ideal situation for cattle to act as a reservoir for human infective *T. b. rhodesiense* whereby cattle can maintain infections for a long period of time without any apparent detrimental effects. Some cattle with mixed infections of *T. brucei* spp. and *T. vivax* only or *T. brucei* spp. and *T. congolense* only were also able to maintain some level of production for a period of time but this was less common and most such infections required treatment within a few months. Measuring PCV in cattle was found to be a reliable indicator of trypanotolerance, since the cattle which manage to maintain their PCV had the least disruption to their liveweight status which concurs with the conclusions of Murray *et al.* (1982).

The frequency distribution of the PCV of cattle with no current trypanosome infection and not been treated with any trypanocidal drug

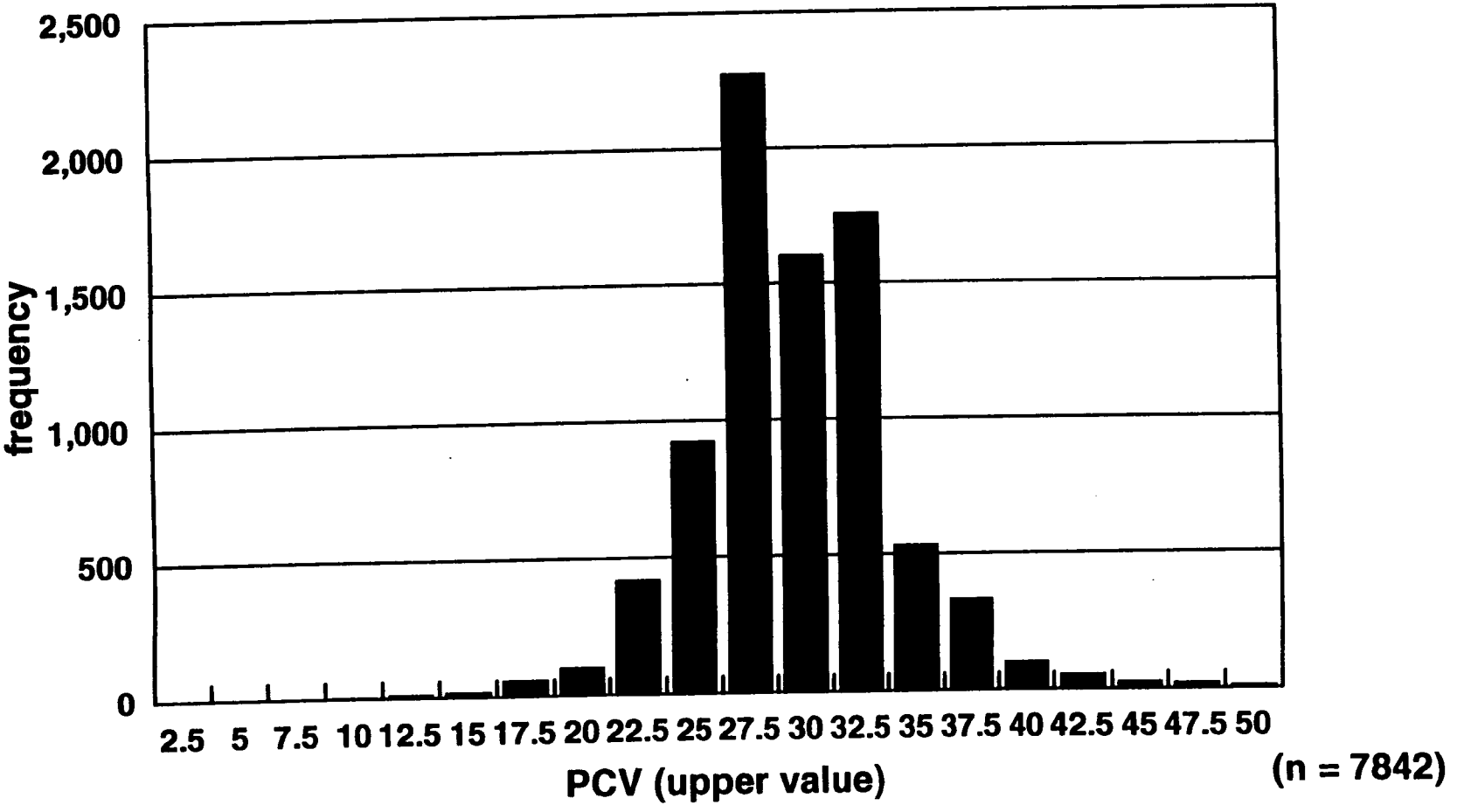


fig. 7.1

The frequency distribution of the PCV of cattle infected with *T. brucei* spp. alone

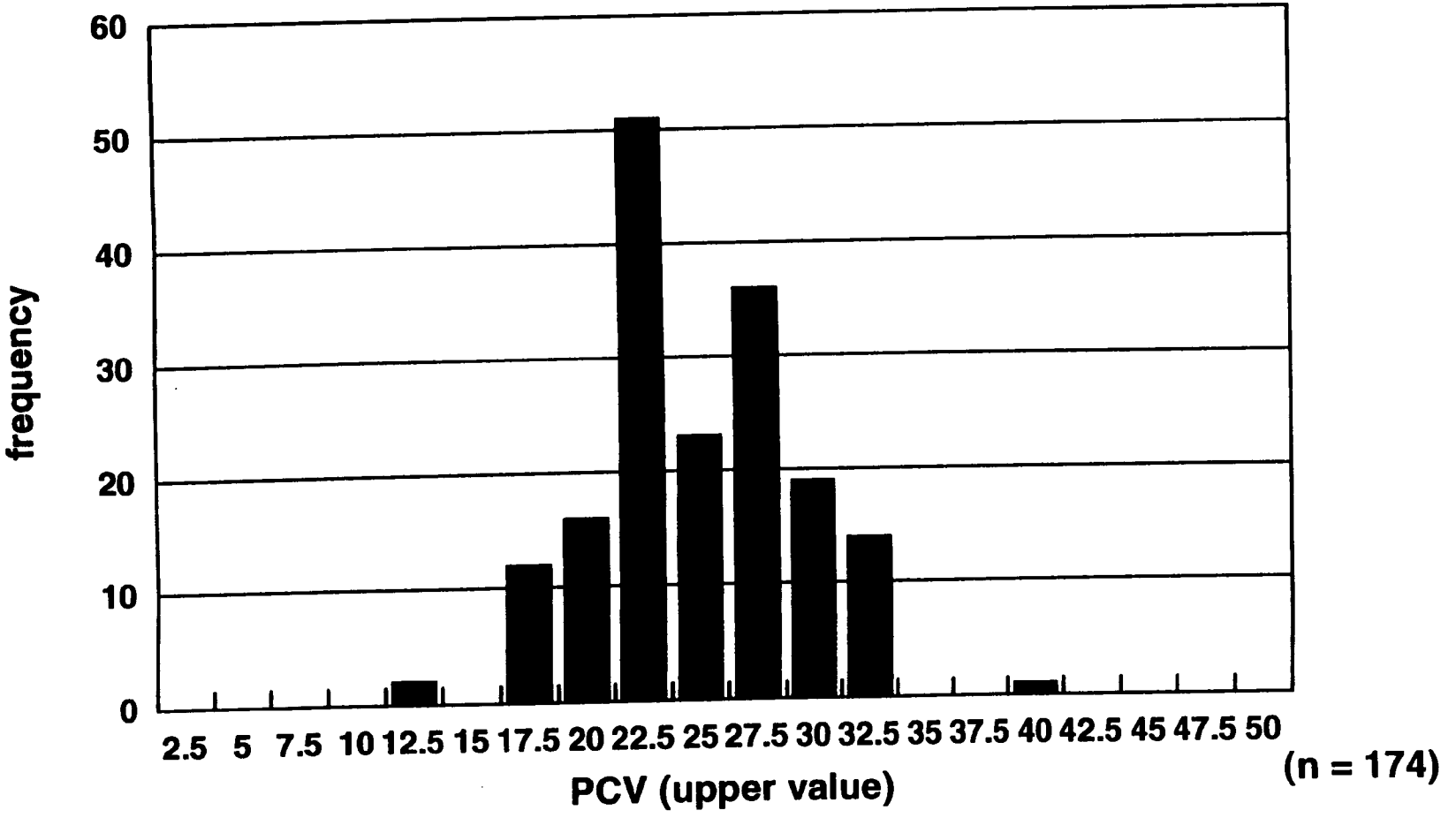
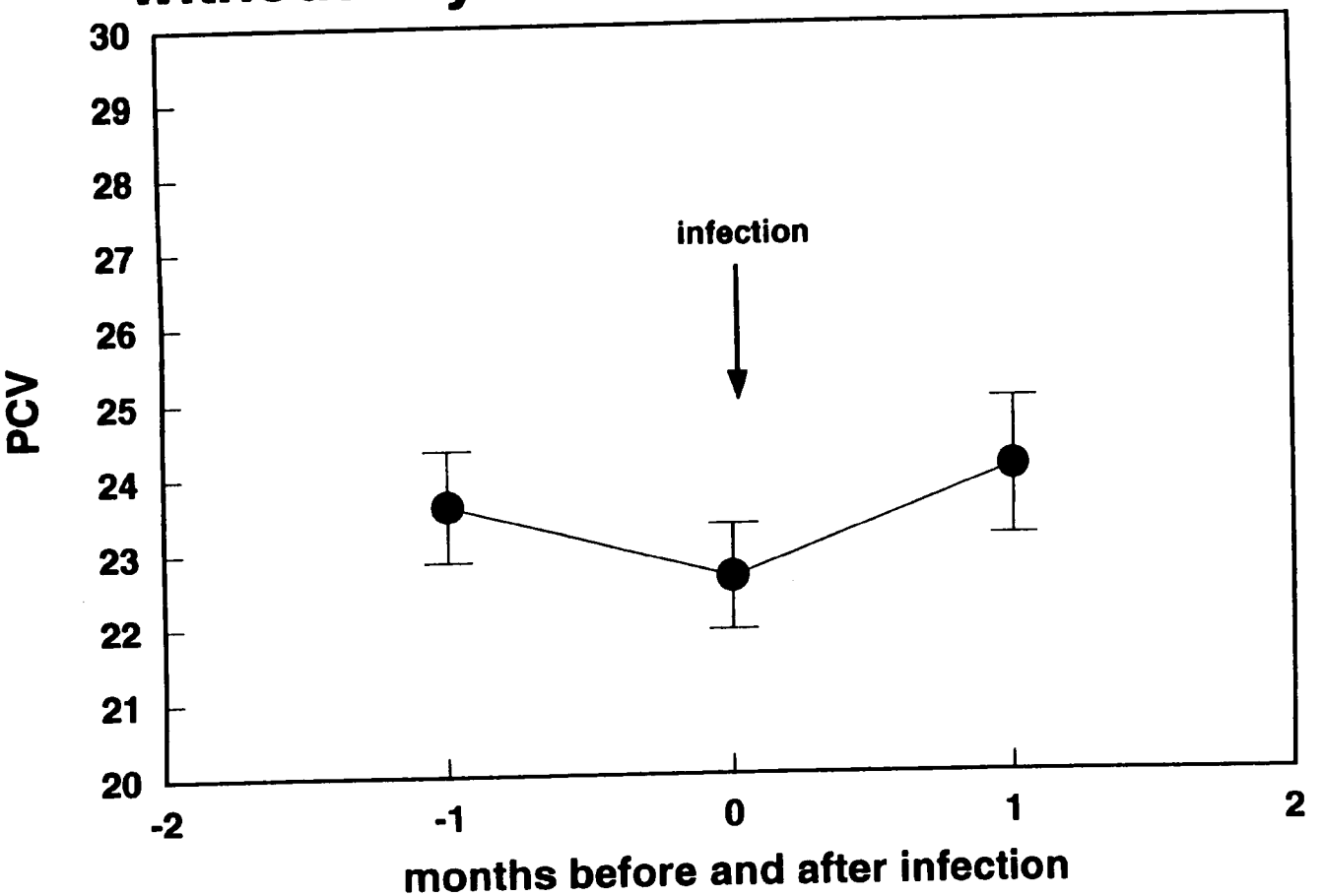


fig. 7.2

The change in the mean PCV of cattle before and after infection with *T. brucei* spp. alone without any treatment



(n = 30)

fig. 7.3

The frequency distribution of the PCV of cattle infected with *T. vivax* alone

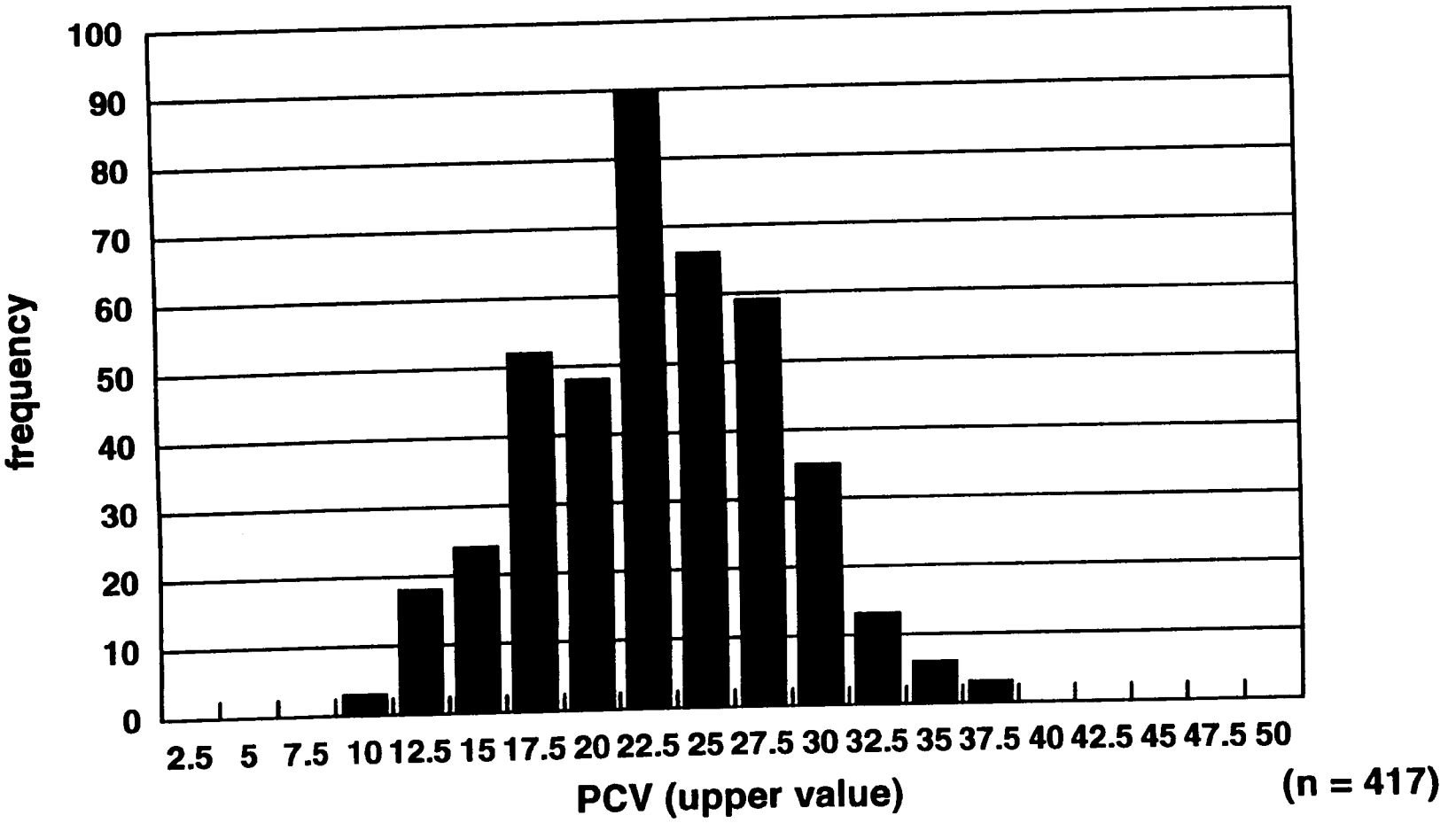
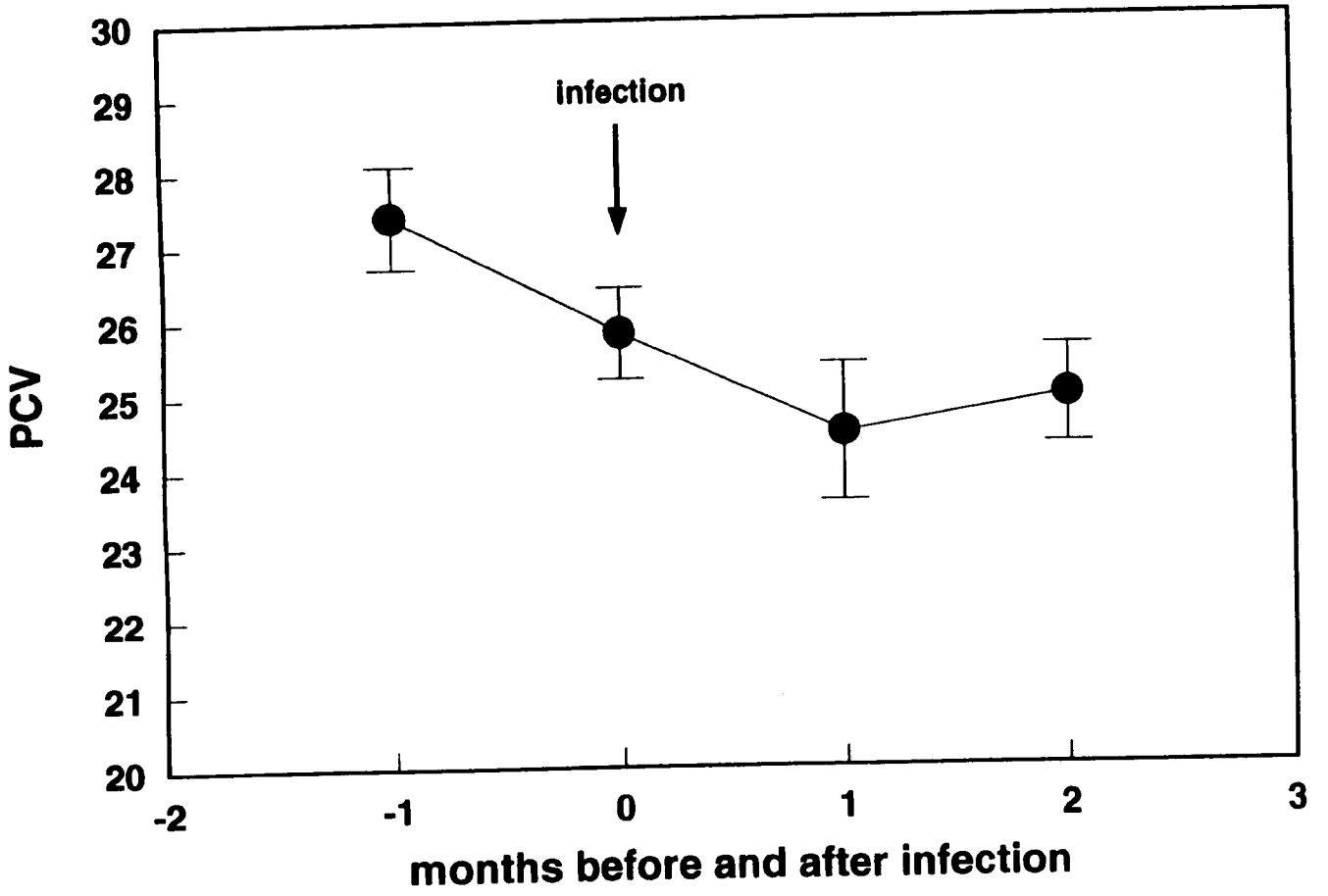


fig. 7.4

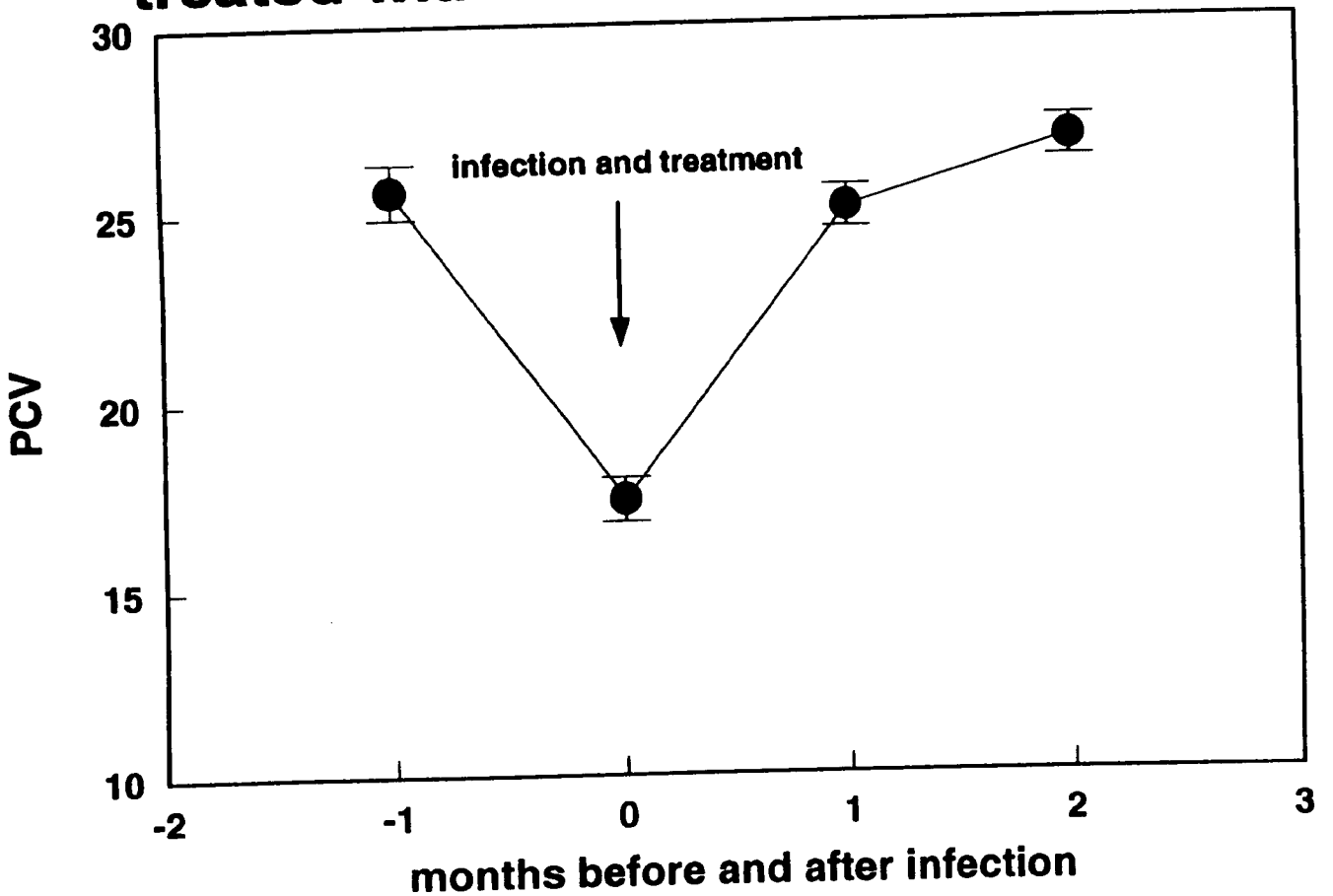
The change in the mean PCV of cattle infected with *T. vivax* alone without any treatment



(n = 37)

fig. 7.5

Change in the mean PCV of cattle before and after infection with *T. vivax* only and treated with diminazine aceturate



(n = 39)

fig. 7.6

The frequency distribution of the PCV of cattle infected with *T. congolense* alone

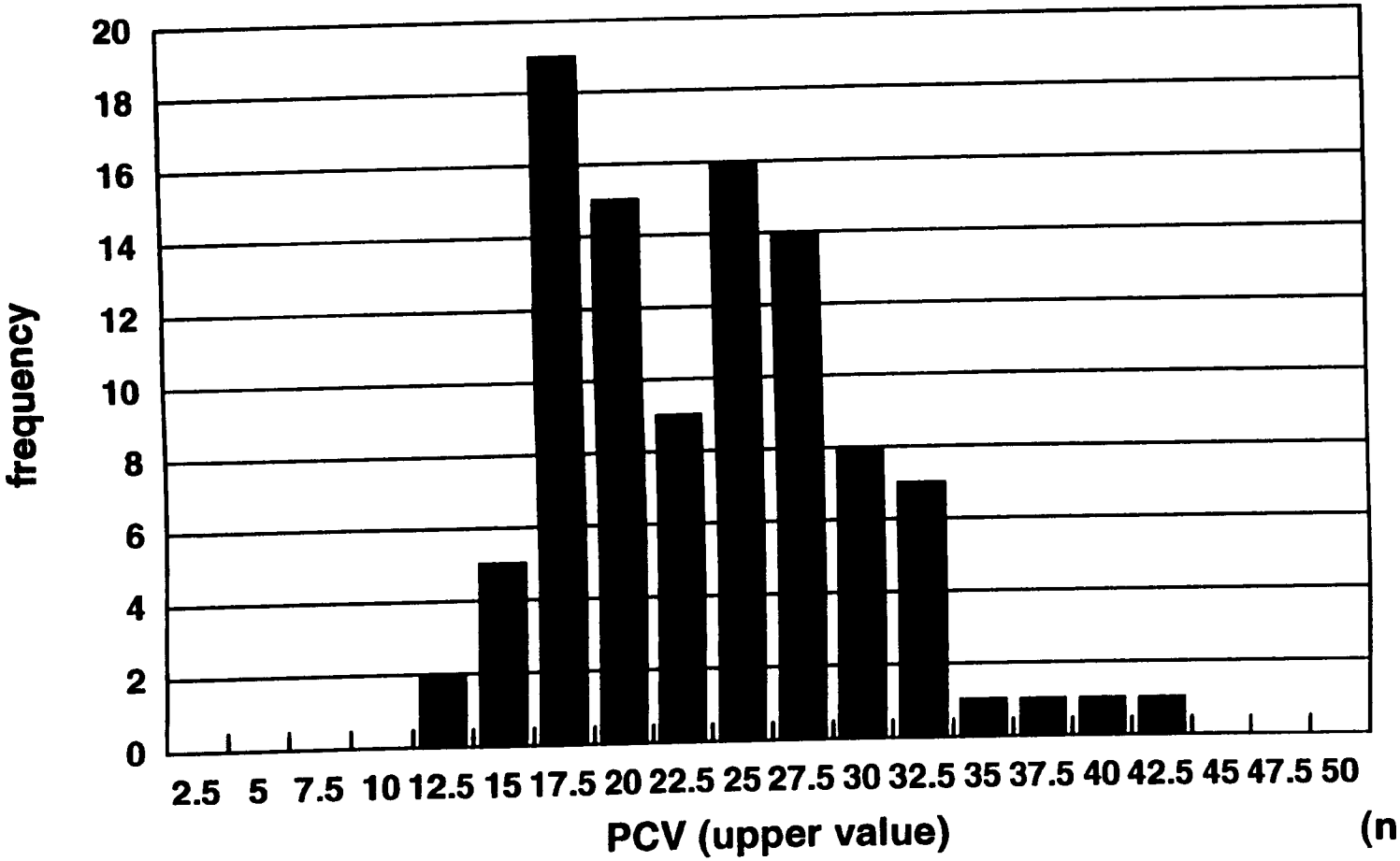
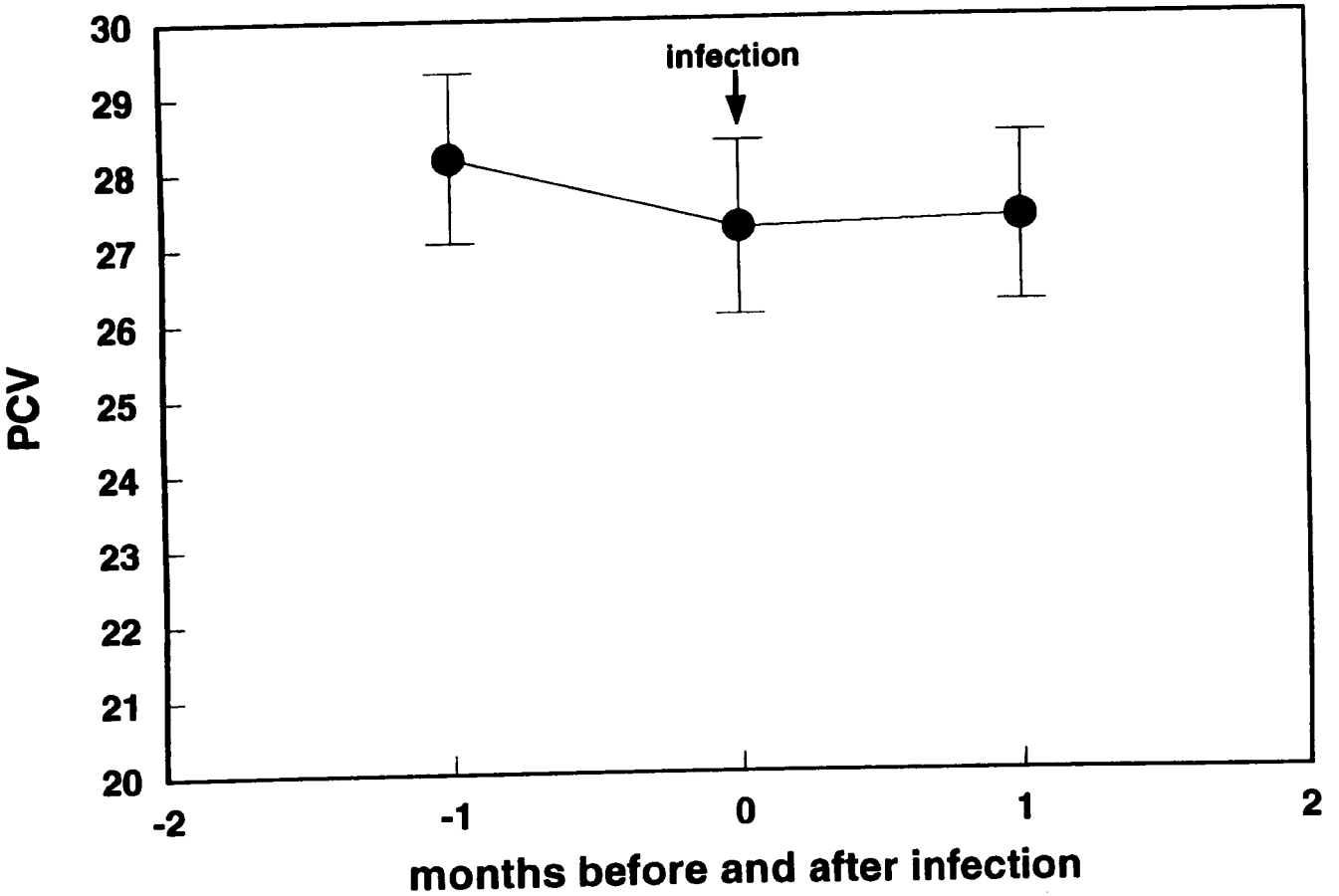


fig. 7.7

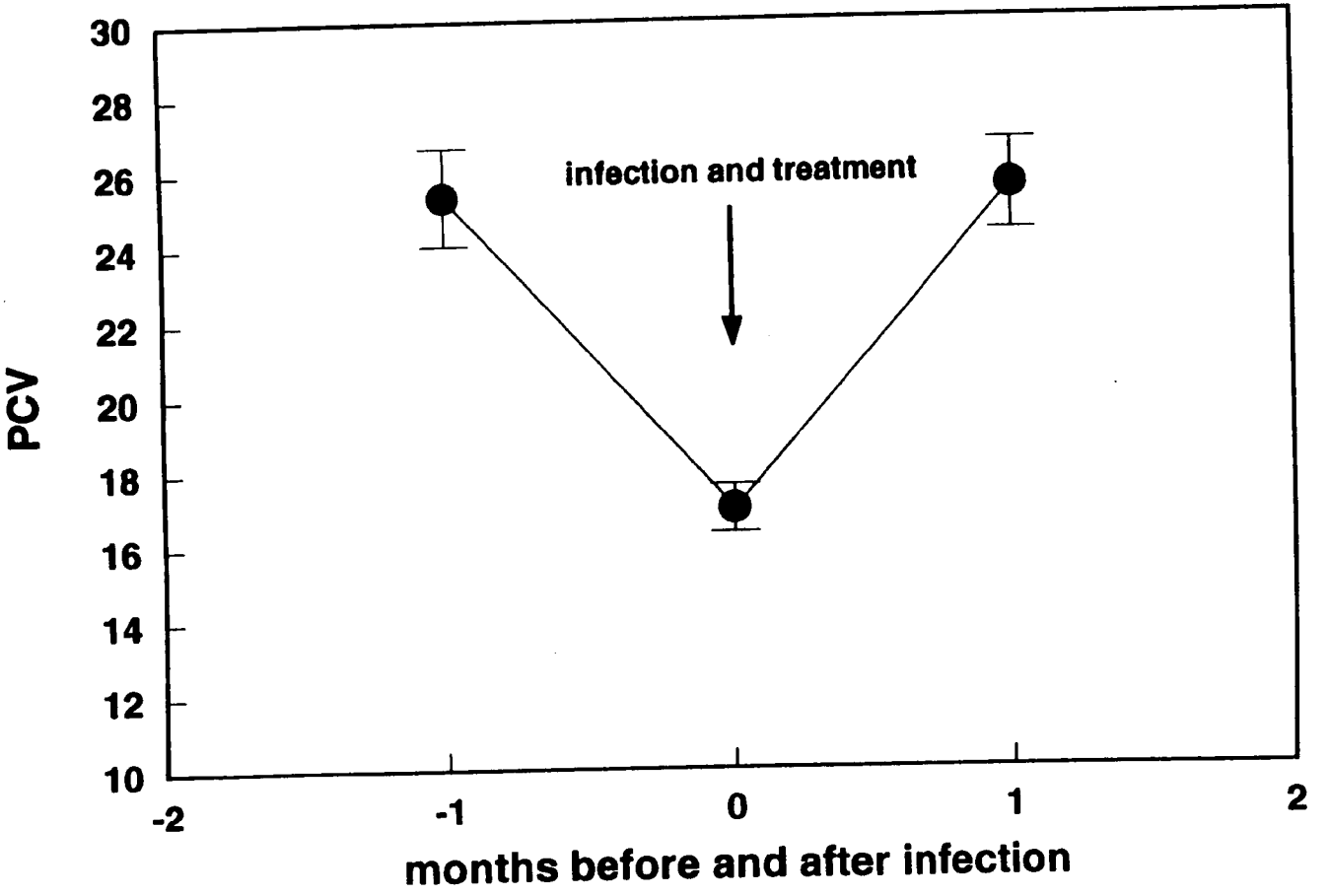
The change in the mean PCV of cattle before and after infection with T. congolense alone without any treatment



(n = 18)

fig. 7.8

The change in the mean PCV of cattle before and after infection with *T. congolense* alone and treatment with diminazene aceturate



(n = 19)

fig. 7.9

The frequency distribution of the PCV of cattle infected with *T. brucei* spp. and *T. vivax* only

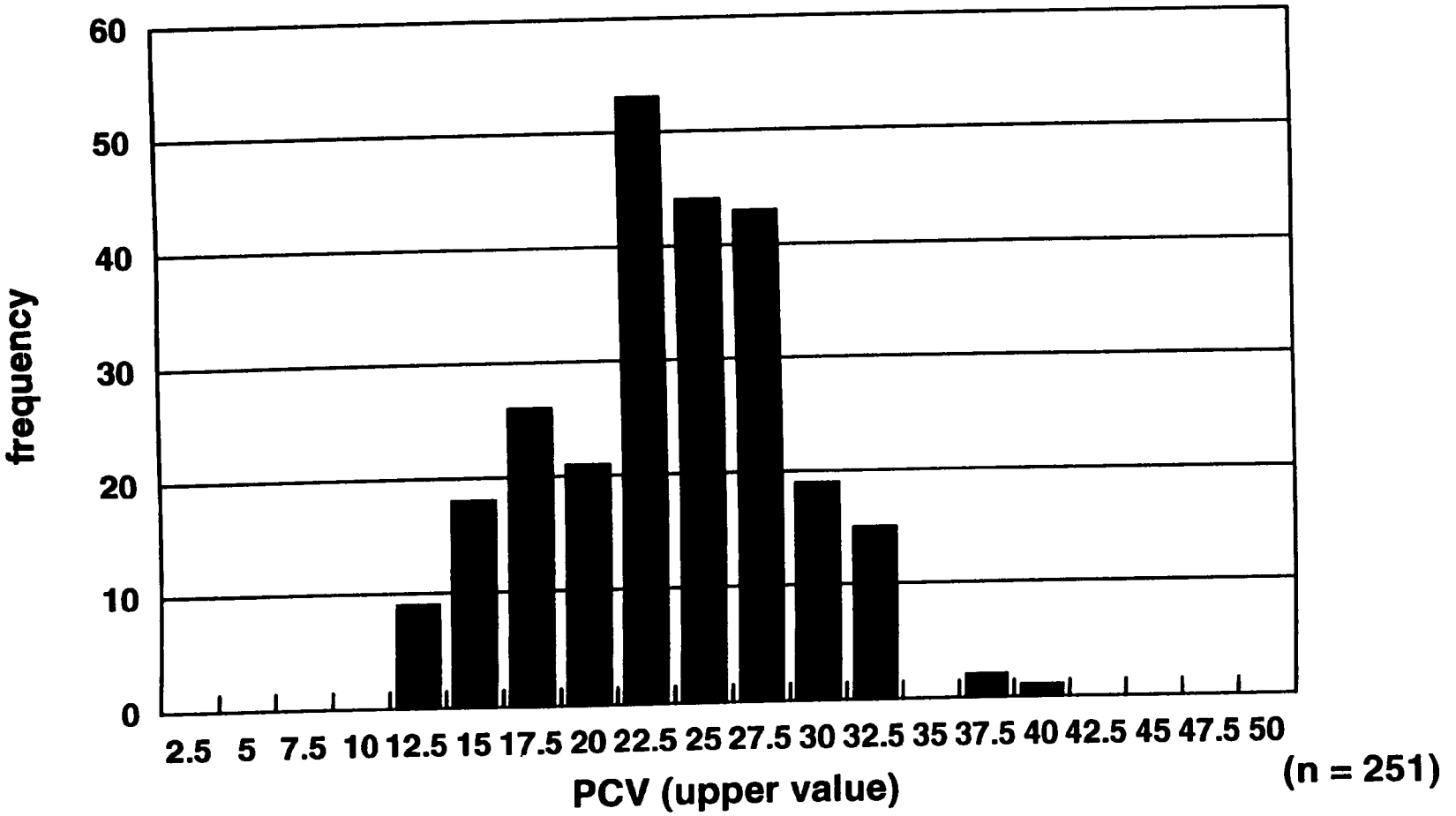


fig. 7.10

The change in mean PCV before and after a mixed infection with T. brucei spp. and T. vivax only treated with diminazene aceturate

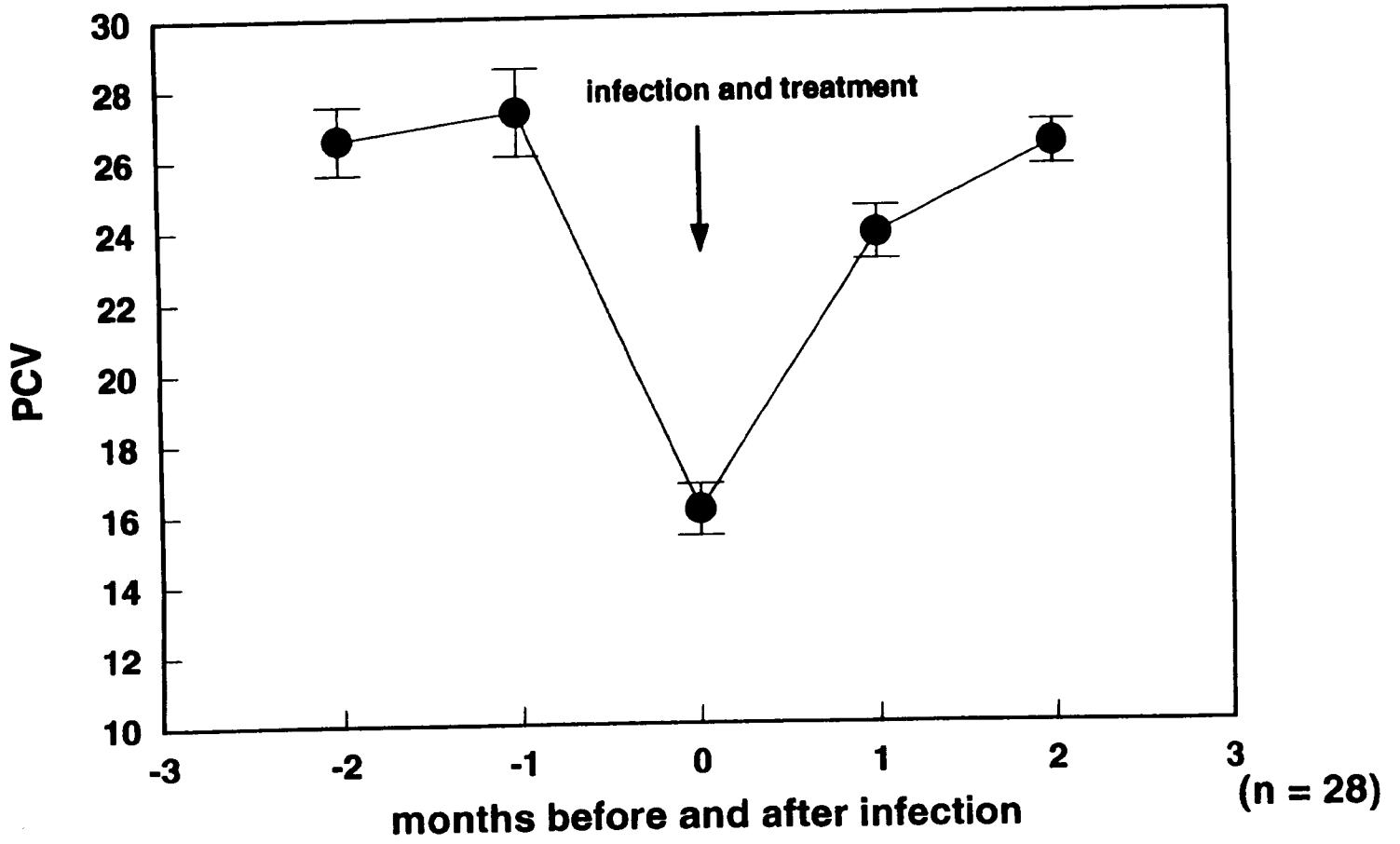


fig. 7.11

The frequency distribution of the PCV of cattle infected with *T. brucei* spp. and *T. congolense* only

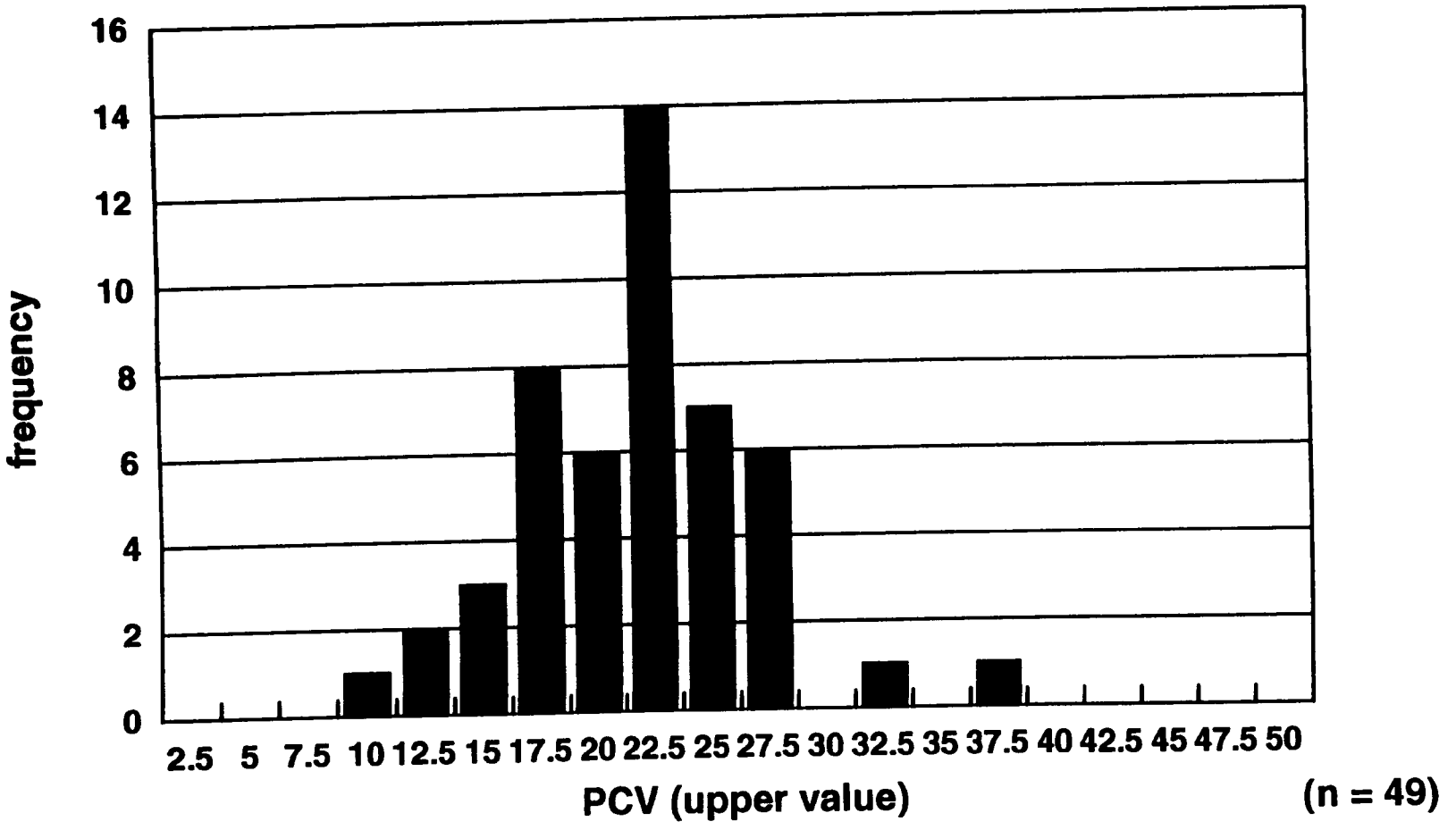


fig. 7.12

The frequency distribution of the PCV of cattle infected with *T. vivax* and *T. congolense* only

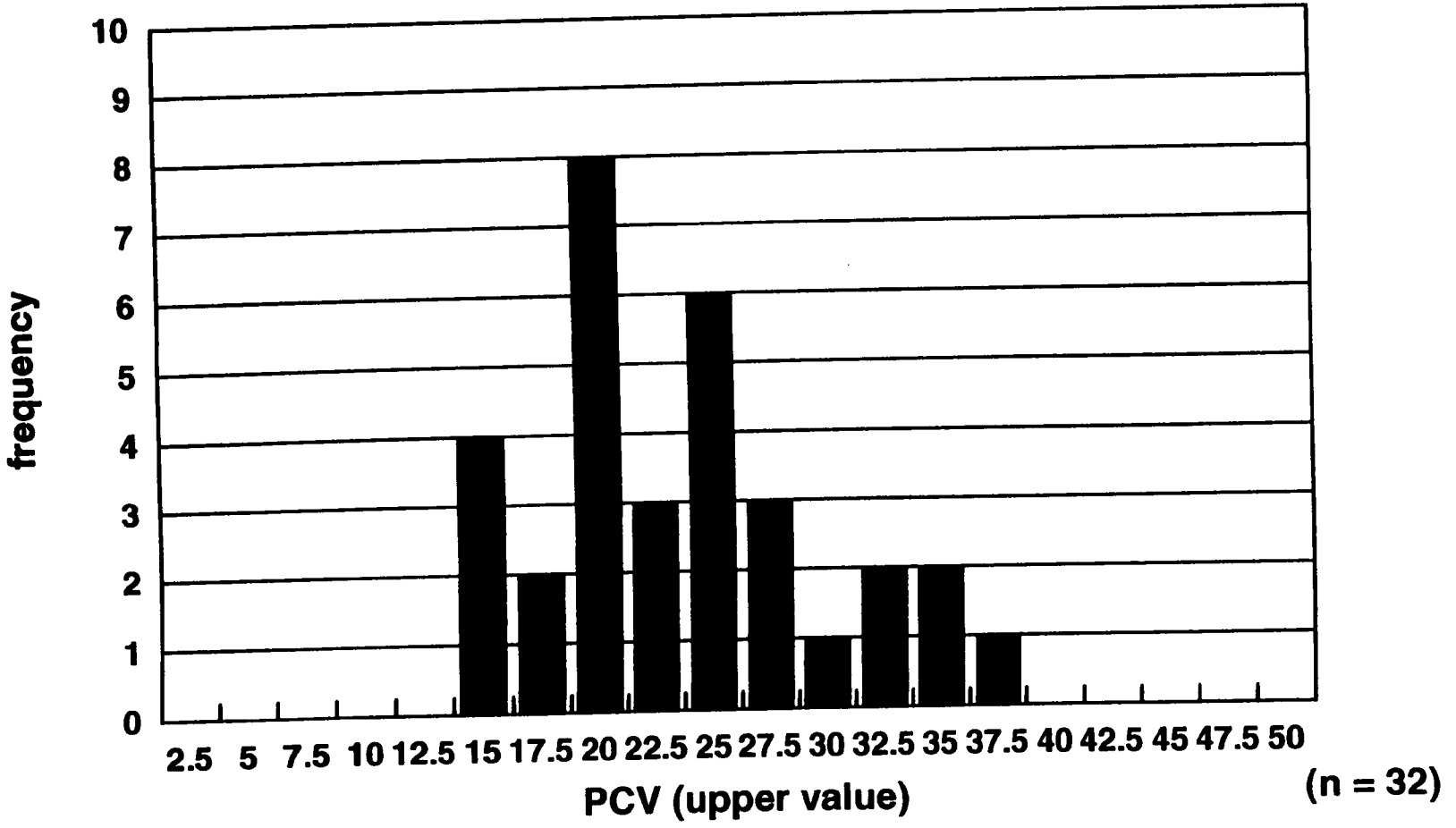


fig. 7.13

The frequency distribution of the PCV of cattle infected with *T. brucei* spp., *T. vivax* and *T. congolense* together

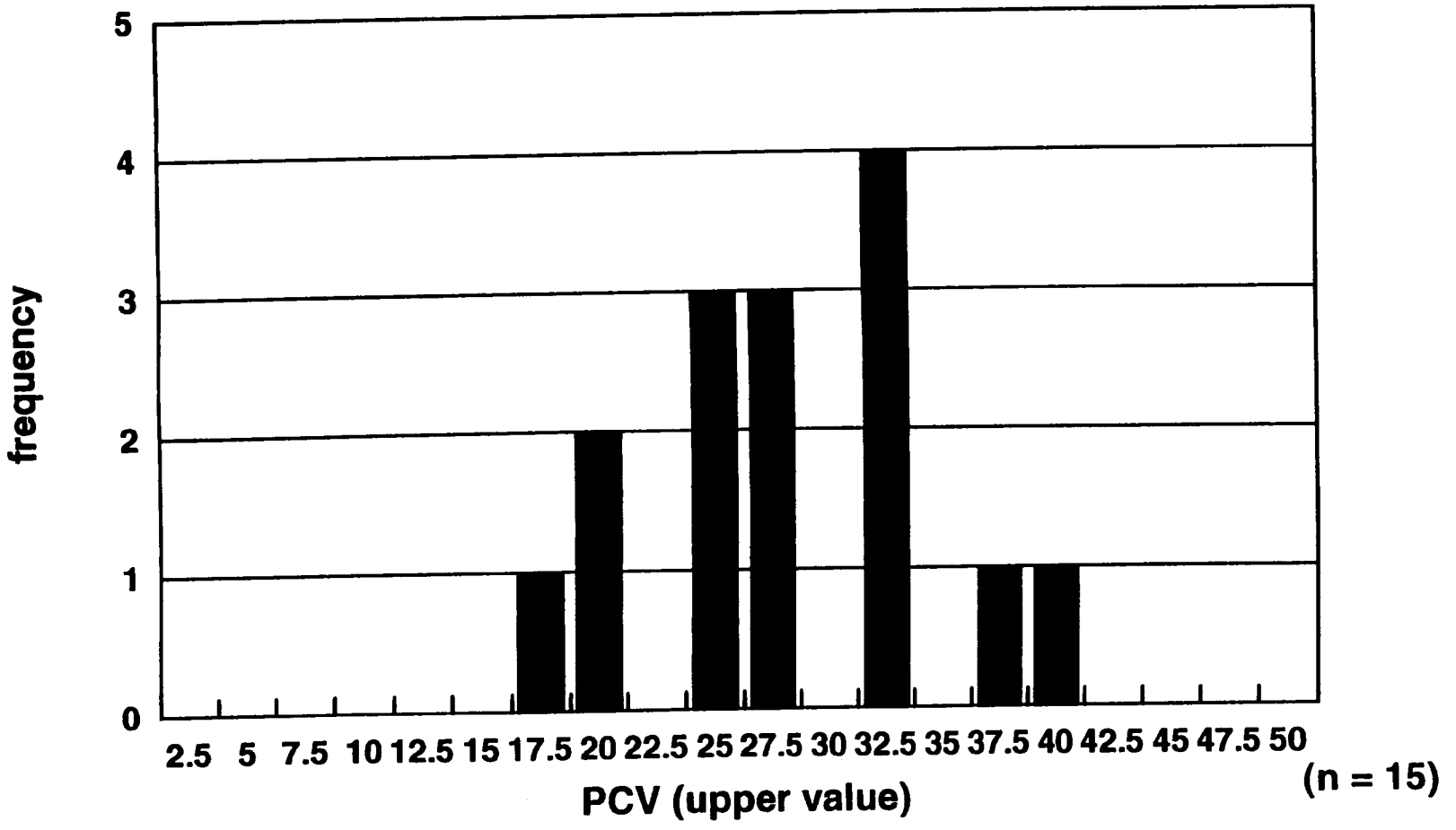
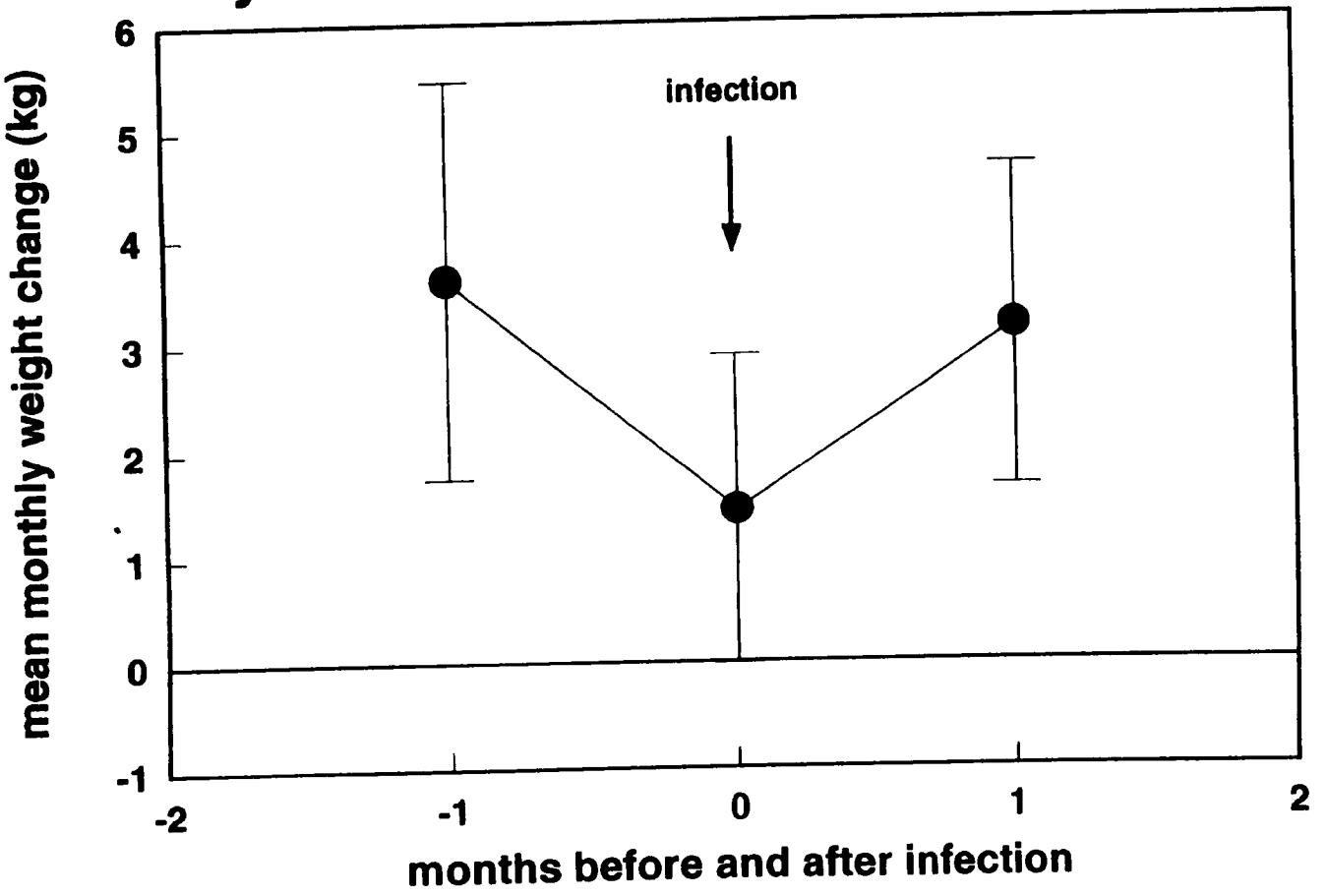


fig. 7.14

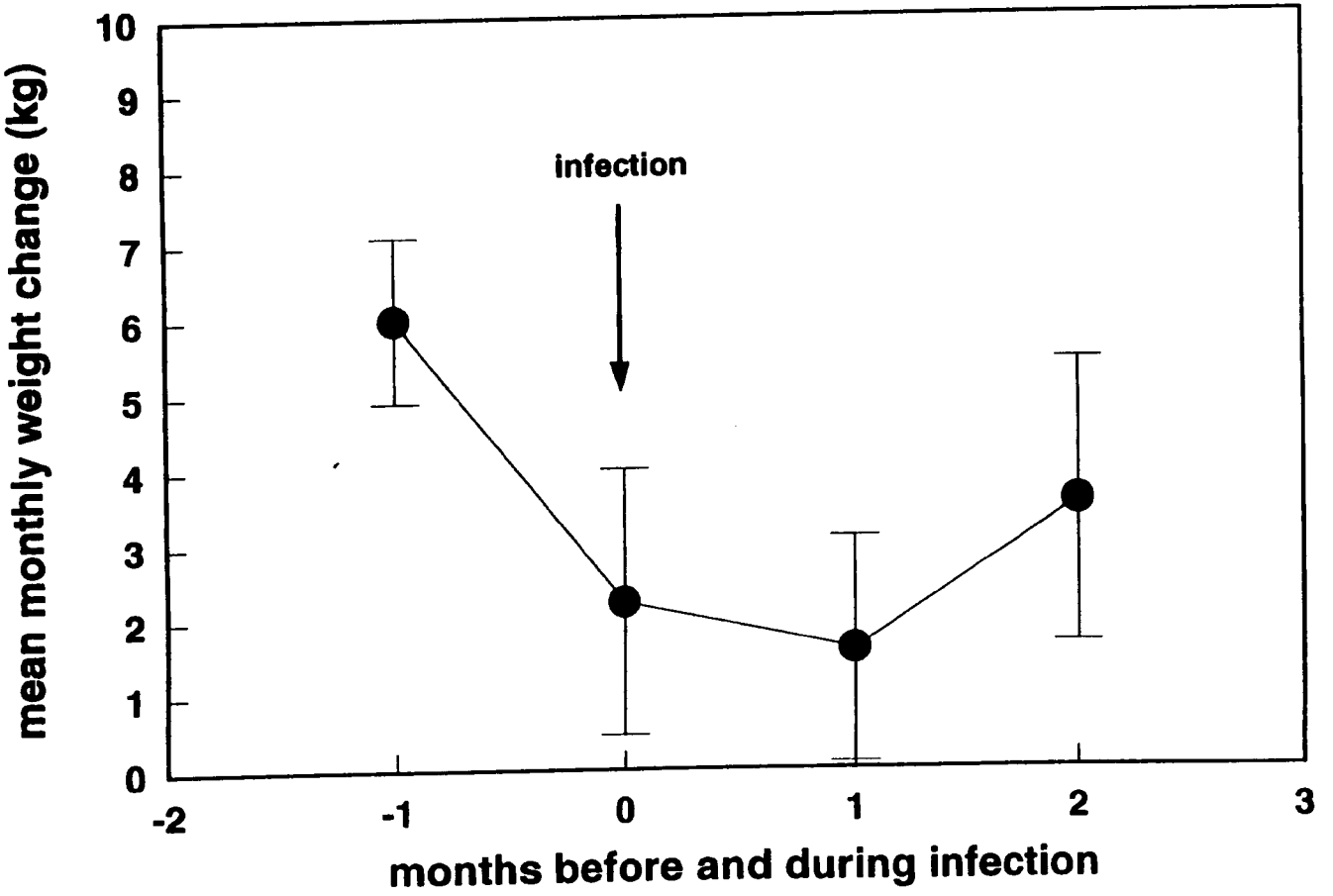
The mean monthly weight change (kg) of cattle before and after infection with *T. brucei* spp. only without any treatment



(n = 21)

fig. 7.15

The mean monthly weight change (kg) of cattle before and after infection with *T. vivax* alone without any treatment



(n = 24)

fig. 7.16

The mean monthly weight change (kg) of cattle before and after infection with *T. vivax* alone and treatment with diminazene aceturate

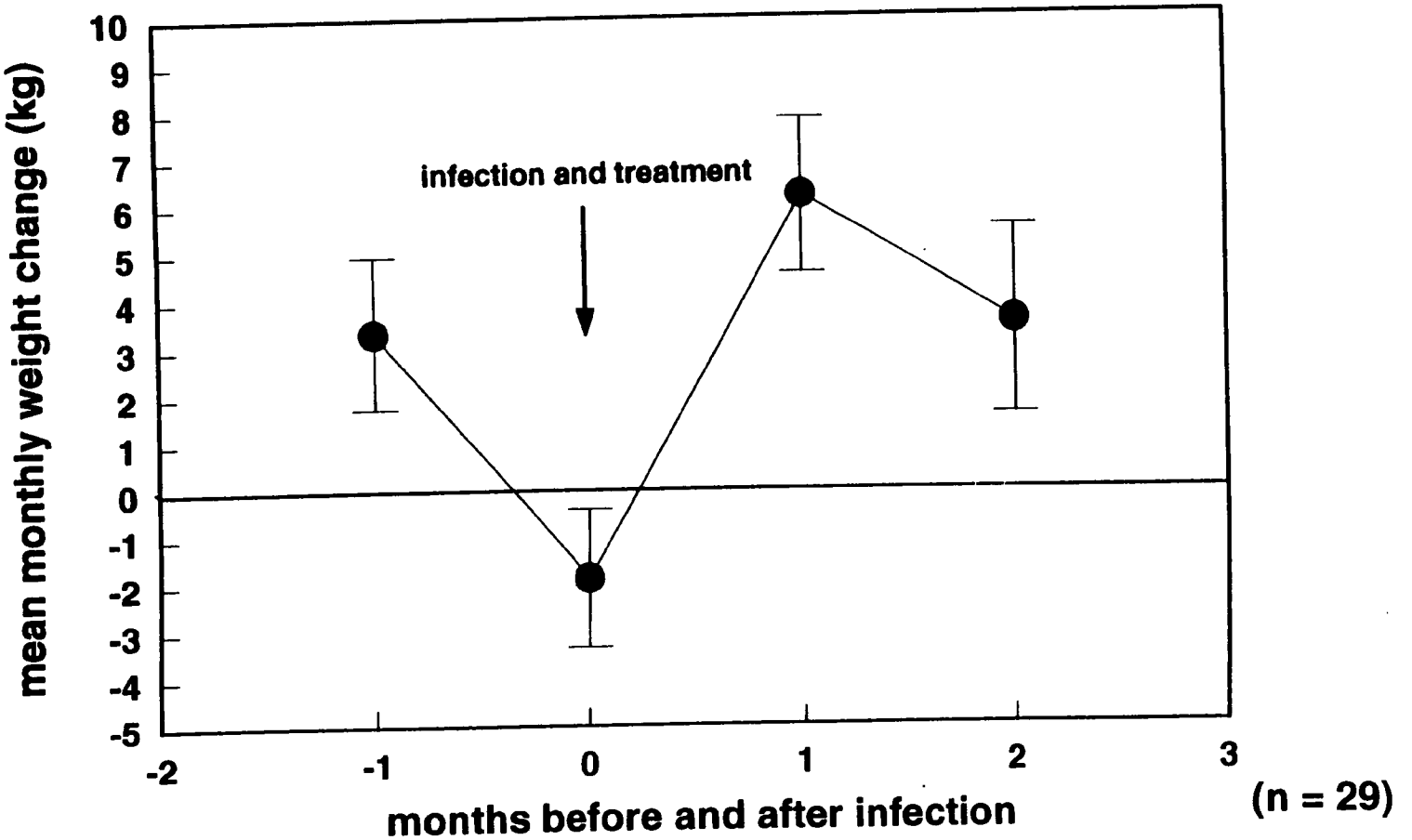


fig. 7.17

The mean monthly weight change (kg) of cattle before and after infection with *T. congolense* alone without any treatment

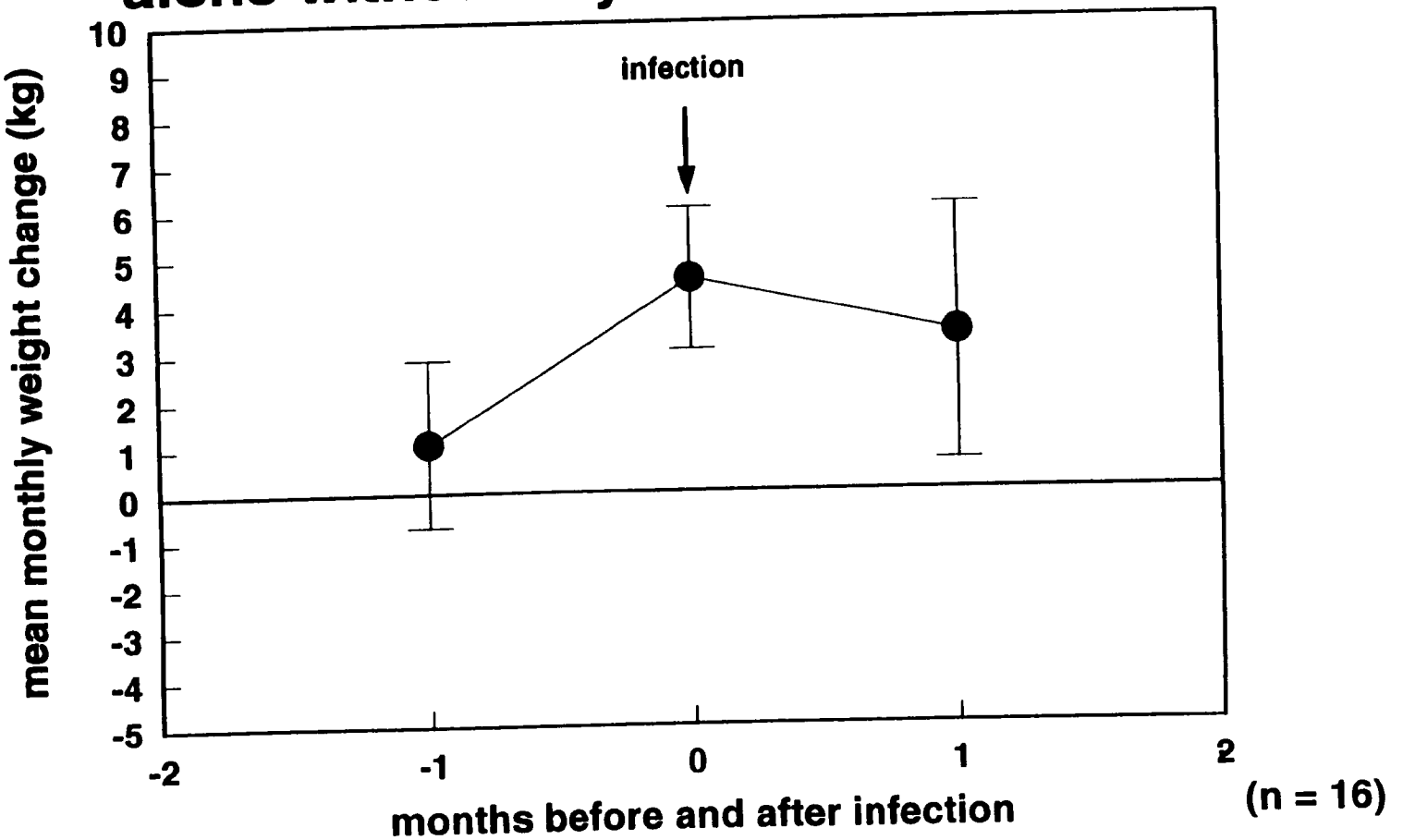
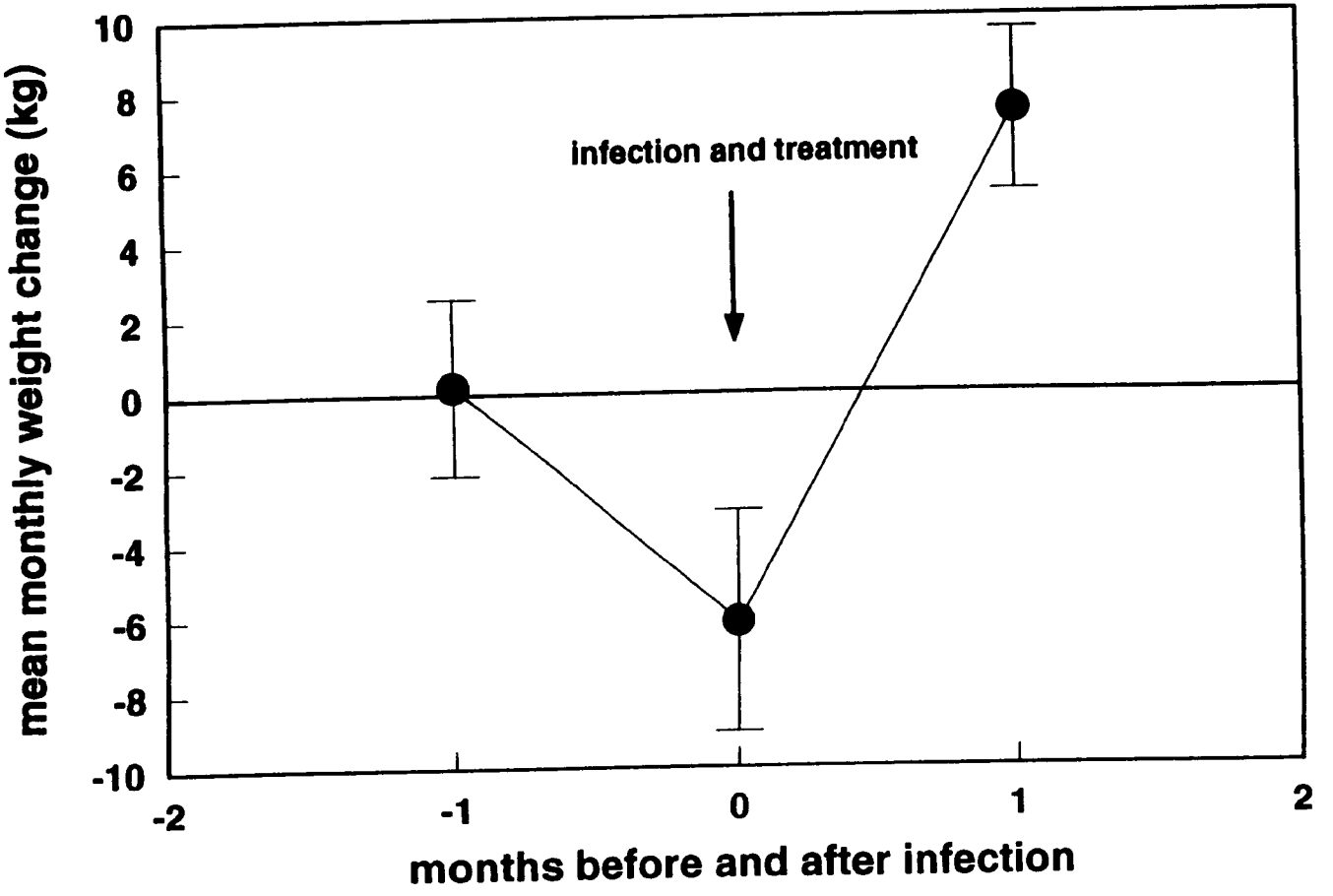


fig. 7.18

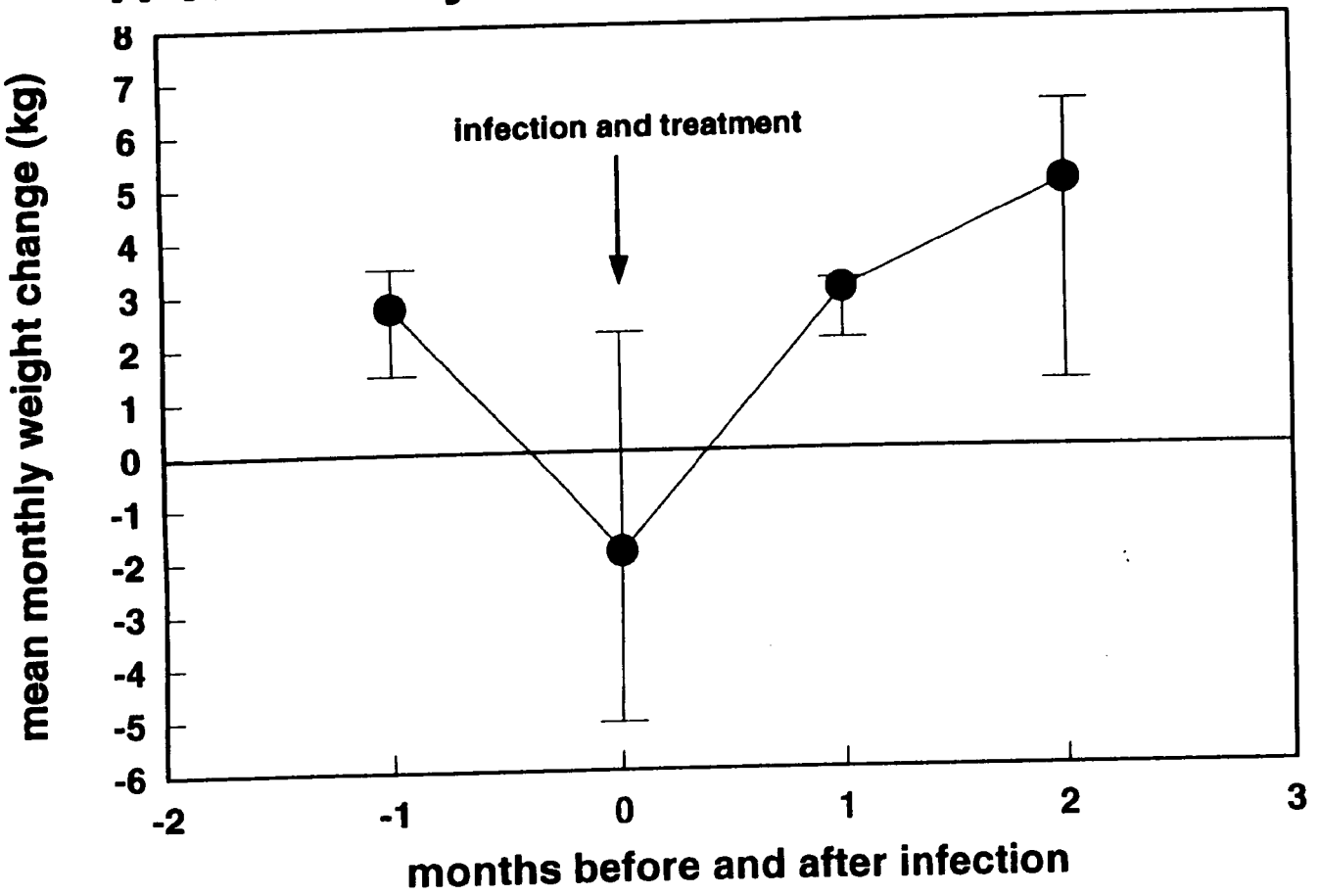
The mean monthly weight change (kg) of cattle before and after infection with *T. congolense* alone and treatment with diminazene aceturate



(n = 16)

fig. 7.19

The mean monthly weight change (kg) of cattle before and after a mixed infection with *T. brucei* spp. and *T. vivax* only and treatment with diminazene aceturate



(n = 20)

fig. 7.20

Chapter VIII

CNS involvement in natural *T. brucei* spp. infections of domestic livestock

8.1 Summary

During an epidemiological study of trypanosome infections in domestic livestock carried out in four villages in Busia District, Kenya over a period of 18 months, the annual prevalence of *T. brucei* spp. infections was 10.8% in cattle, 1.1% in sheep, 1.4% in goats and 12.5% in pigs in Apatit village, where the prevalence of trypanosome infections was the highest. All cattle, sheep and goats with *T. brucei* spp. infections of more than 60 day's duration and all pigs with *T. brucei* spp. infections had their CSF sampled monthly to investigate the role of cerebral trypanosomiasis in the domestic livestock of this district. Of 256 CSF samples taken from cattle nine samples were positive for *T. brucei* spp. from six different cattle. No cases of clinical CNS disease were observed in local East African zebu cattle (*Bos indicus*) with long standing *T. brucei* spp. infections, their CSF total protein tended to remain within the normal range and histological changes in their CNS were mild or absent. In contrast the one exotic bull (*Bos taurus*) with a *T. brucei* spp. infection developed fatal cerebral trypanosomiasis, its CSF total protein was elevated and histological lesions of the CNS were severe. One sheep with a *T. brucei* spp. infection developed cerebral trypanosomiasis and died after being infected for more than seven months. No goats were found to have CNS infections of *T. brucei* spp. Of seven pigs found to be infected with *T. brucei* spp., six developed CNS infections, five of which were fatal within the time of the study. Histological lesions of the CNS of infected pigs were more severe than in local East African zebu cattle.

The implications of these findings on the epidemiology of the domestic animal reservoir of potentially human infective *T. brucei* spp. are discussed.

8.2 Introduction

While *T. brucei* spp. infections in cattle have been found to be prevalent in both East and West African (Godfrey *et al.*, 1964; Onyango *et al.*, 1966; Gray, 1970; Wellde *et al.*, 1983, 1989c) it has generally been reported that natural infections of cattle are relatively non-pathogenic (Hornby, 1921; Buxton, 1955; Stephen, 1970; Killick-Kendrick, 1971 and Losos and Ikede, 1972). However, experimental infections of *T. brucei* spp. have been shown on occasion to cause severe pathological disease in cattle (Peruzzi, 1928; Gray, 1964; Ikede and Losos, 1972; Wellde *et al.*, 1973; Mwambu and Ochola, 1975 and Morrison *et al.*, 1983). Following experimental infections of

mainly European breeds of cattle by *T. b. rhodesiense*, they have been shown to develop a fatal CNS disease comparable to sleeping sickness in man. with a duration of between 85 and 1613 days post infection (Wellde *et al.*, 1989b). Initial field surveys by Wellde *et al.*(1989c) in the Lambwe Valley, Kenya in 1973 and 1979 did not confirm the existence of cerebral trypanosomiasis in native cattle. However a subsequent survey of cattle by the same group in the same area revealed the presence of some native East African zebu cattle with clinical disturbances of the CNS, which were attributed to cerebral trypanosomiasis (Wellde *et al.*, 1989c). Further observation of a group of East African zebu cattle from the Lambwe Valley with long standing natural *T. brucei* spp. infections revealed that some of them developed clinical CNS disease and it was concluded that all cattle would eventually succumb in time (Wellde *et al.*, 1989c).

Experimentally, CNS infections with *T. brucei* spp. have also been demonstrated in sheep (Corson, 1938; Ikede and Losos, 1972 and Bouteille *et al.*, 1988), goats (Whitelaw *et al.*, 1985; Wellde *et al.*, 1989d), pigs (Otesile *et al.*, 1991; Bungener and Mehlitz, 1984) and dogs (Chukwu *et al.*, 1990; Ndung'u *et al.*, 1994).

The present study was undertaken to examine the role of *T. brucei* spp. infections of the CNS of cattle and other domestic species in the epidemiology of the potential reservoir for human sleeping sickness.

8.3 Materials and methods

For general materials and methods see chapter III.

8.3.1 Selection of animals for CSF sampling

Monthly CSF samples were taken from animals which had been infected with *T. brucei spp.* for at least 60 days on the basis of blood sampling, except the pigs which were infected for an unknown period of time. Animals then had their CSF sampled monthly until the end of the study unless they had to be treated, in which case they were only sampled until one month after treatment.

8.3.2 CSF Sampling

Prior to sampling the CSF, all cattle, goats, sheep and pigs were sedated. CSF samples from cattle were collected from the cisterna magna via the atlanto-occipital junction and samples from goats, sheep and pigs were collected from the lumbo-sacral junction (Scott, 1993). The CSF was then allowed to drip into a sterile bottle under its own pressure (Chapter III).

8.3.3 Direct examination of CSF for trypanosomes

Samples were placed in a heat sealed Pasteur pipette, spun in a centrifuge at 2000 rpm for 5 minutes and then examined as for blood samples by HCT (Woo *et al.*, 1970).

8.3.4 Subinoculation of CSF into mice for trypanosome detection

Samples of CSF were injected (i.p.) into inbred Swiss white albino BALB/C mice which had been immunosuppressed . Three mice were used for each sample.

8.3.5 Protein assay for CSF

Total protein assay for CSF samples were carried out using a colorimeter and standard reagents (Biorad ®, USA).

8.3.6 Histopathology of the CNS of domestic livestock

Tissues taken at post mortem were fixed in buffered neutral formalin and embedded in wax before sectioning. Sections were stained with haematoxylin and eosin and examined microscopically at x100 and x400 magnification. Observations of lesions were graded as per Wellde *et al.* (1989b) in order to correlate clinical and laboratory findings with pathological changes in the CNS.

8.4 Results

8.4.1 Natural CNS infections of *T. brucei* spp. in cattle

8.4.1.1 A survey of the prevalence of *T. brucei* spp. infections in cattle

The annual prevalence of *T. brucei* spp. infections in domestic cattle in each village ranged from 2.6% in Rukada village (Ethidium treated) to 10.8% in Apatit village (Samorin control) (Table 8.1). The overall annual prevalence of *T. brucei* spp. infection in cattle was significantly greater than the annual prevalence in sheep ($p < 0.001$), goats ($p < 0.001$) but not significantly different to that in pigs ($p = 0.802$) (Appendix VIII, Tables VIII.1 to VIII.4).

Table 8.1 The annual prevalence of *T. brucei* spp. infections in cattle

village	no. of cattle	no. of samples	no. of positive cattle	no of positive samples	annual prevalence
Katelenyang village (Samorin treated)	343	2167	67	90	4.2%
Apatit village (Samorin control)	359	2310	138	250	10.8%
Rukada village (Ethidium treated)	359	2715	38	70	2.6%
Ngelechom village (Ethidium control)	408	2781	57	79	2.8%
Total of 4 villages	1469	9973	300	489	4.9%

8.4.1.2 Central nervous system involvement in natural *T. brucei* spp. infections in cattle

The number of monthly CSF samples analysed from each species is given in Table 8.2. From a total of 256 CSF samples analysed from domestic cattle over a period of eleven months only nine samples from six different cows were positive for the presence of trypanosome, all of which were *T. brucei* spp. (Table 8.3).

Table 8.2 The number of CSF samples analysed

month	cattle	goats	sheep	pigs	total
July	25	0	1	0	26
August	14	0	1	1	16
September	22	0	1	0	23
October	23	0	1	0	24
November	21	0	1	0	22
December	22	0	1	2	25
January	20	0	0	0	20
February	20	1	0	1	22
March	26	1	0	1	28
April	31	1	0	2	34
May	32	1	1	2	36
total	256	4	7	9	276

Table 8.3 Positive CSF samples from cattle

animal number	age (years / months)	month of sampling	direct examination of CSF	mouse inoculation of CSF	duration of infection
N19	7 / 11	Dec	+	+	40-70 days
R250	5 / 9	Dec	-	+	180-210 days
A68	1 / 8	Dec	-	+	40-70 days
A399	3 / 7	Mar	-	+	60-90 days
A68	2 / 0	Apr	+	-	160-190 days
A84	5 / 0	Apr	+	-	60-90 days
A279	3 / 11	Apr	+	+	150-180 days
R250	6 / 1	Apr	-	+	300-330 days
A279	4 / 0	May	+	+	180-210 days

all positive CSF samples from cattle were *T. brucei* spp.

8.4.1.3 CSF total protein of cattle

There is no significant difference in CSF total protein levels between uninfected cattle and cattle infected with *T. brucei* spp. for more than 60 days ($t = 0.87$, $p = 0.40$); uninfected cattle and cattle with *T. brucei* spp. isolated from their CSF ($t = 0.22$, $p = 0.83$) or cattle infected with *T. brucei* spp. for more than 60 days and cattle with *T. brucei* spp. isolated from their CSF ($t = 0.87$, $p = 0.41$) (Table 8.4). The mean CNS total protein of cattle infected with *T. brucei* spp. for more than two consecutive months, which did not have any trypanosomes isolated from their CSF is given in fig. 8.1 and the CSF total protein over a period of months of individual cattle which had trypanosomes isolated from their CNS is given in fig. 8.2. Only one Ayrshire bull (*Bos taurus*) (number N19) showed a significantly greater CSF total protein level than uninfected cattle (fig. 8.2).

Table 8.4 The total protein concentration (g/dl) of CSF from uninfected cattle and cattle infected with *T. brucei* spp.

sample	n	mean	s.d.
uninfected cattle	17	46.47	21.87
cattle infected with *T. brucei* spp. for more than 60 days	159	47.70	25.38
cattle samples where *T. brucei* spp. was isolated from the CSF	10	54.78	25.06

8.4.1.4 The clinical history of cattle with trypanosome infections in the CNS

No clinical signs of any neurological disturbance were seen in any native East African zebu cattle (*Bos indicus*) over a period of 18 months study. One Ayrshire bull (*Bos taurus*) (number N19) showed clinical signs of severe neurological disturbance prior to death (Appendix VIII, VIII.8).

8.4.1.5 Histopathology of the CNS of cattle infected with *T. brucei* spp.

Histopathological changes in the CNS were recorded and graded as to their severity using the same criteria as described by Wellde *et al.* (1989b) (Table 8.5). The principal histological features of the CNS disease in the two cattle graded 2+ or higher were the same and only differed in severity. The most prominent feature was generalised perivascular infiltration with mononuclear inflammatory cells, mainly lymphocytes and plasma cells. There was a gliocytosis with an increased number astrocytes being associated with perivascular cuffs and distributed in the parenchyma. In the most severe clinical case (number N19) there was evidence of ischaemia and necrosis with large numbers of swollen astrocytes and a rarefaction of the parenchyma with vacuolar changes giving a spongy appearance, disrupting the normal architecture and a meningitis with an infiltration of mononuclear leukocytes.

Table 8.5 Histological changes in the CNS and clinical signs of cattle with long standing infections of *T. brucei* spp.

number	age (years / months)	breed *	trypanosome found in CNS	duration of *T. brucei* spp. infection	clinical signs of CNS disturbance	histological grade **
A68	2 / 1	EAZ	+	8 - 9 months	none	1+
A279	4 / 2	EAZ	+	8 - 9 months	none	1+
R32	6 / 2	EAZ	-	> 16 months	none	1+
R56	1 / 11	EAZ	-	11 - 12 months	none	1+
R63	4 / 2	EAZ	-	> 16 months	none	1+
R111	3 / 2	EAZ	-	> 16 months	none	1+
R180	2 / 2	EAZ	-	> 16 months	none	1+
R250	6 / 3	EAZ	+	13 - 14 months	none	2+
R494	0 / 7	EAZ	-	0	none	1+
N19	7 / 11	AYR	+	40-70 days	severe	4+

* breed
EAZ East African zebu (*Bos indicus*)
AYR Ayrshire (*Bos taurus*)
** as per Wellde *et al.* (1989b)

8.4.1.6 The age of village cattle

The mean age of all ear tagged cattle ranged from 37 ± 40 months in Apatit village (Samorin control) where the prevalence of trypanosomiasis in cattle was the highest of the four villages to 49 ± 72 months in Rukada village (Ethidium treated) where the prevalence of trypanosomiasis in cattle was the lowest. The age of ear tagged cattle in all four villages in Busia District was 43 ± 54 months (95% confidence level) (Table 8.6) i.e. where the prevalence of trypanosome infections was high only 5% of village cattle were more than six years of age.

Table 8.6 The mean age of ear tagged cattle in all four villages

village	mean age (months)	s.d.	n
Katelenyang village (Samorin treated)	40.28	20.41	2167
Apatit village (Samorin control)	37.27	19.68	2310
Rukada village (ethidium treated)	48.51	36.24	2715
Ngelechom village (Ethidium control)	43.91	21.56	2781
all four villages	42.84	26.17	9973

8.4.2 Natural CNS infections of *T. brucei* spp. in domestic species other than cattle

8.4.2.1 A survey of the prevalence of *T. brucei* spp. infections in domestic species other than cattle

The overall annual prevalence of *T. brucei* spp. infection in sheep was very low, ranging from 0% in Katelenyang village (Samorin treated) and Ngelechom village (Ethidium control) to 1.1% in Apatit village (Samorin control) (Table 8.7). This was significantly lower than the annual prevalence of *T. brucei* spp. in cattle ($p < 0.001$) or pigs ($p < 0.001$) but not significantly different from the annual prevalence in goats ($p = 0.572$) (Appendix VIII, Tables VII.5 to VIII.7).

Table 8.7 The annual prevalence of *T. brucei* spp. infections in sheep

village	no. of sheep	no. of samples	no. of positive sheep	no of positive samples	annual prevalence
Katelenyang village (Samorin treated)	36	121	0	0	0%
Apatit village (Samorin control)	61	265	1	3	1.1%
Rukada village (Ethidium treated)	84	365	1	2	0.5%
Ngelechom village (Ethidium control)	52	214	0	0	0%
Total of 4 villages	233	965	2	5	0.5%

The overall annual prevalence of *T. brucei* spp. infection in goats was very low, ranging from 0% in Katelenyang village (Samorin treated) and Ngelechom village (Ethidium control) to 1.4% in Apatit village (Samorin control) (Table 8.8). This was significantly lower than the annual prevalence of *T. brucei* spp. in cattle ($p < 0.001$) or pigs ($p < 0.001$) but not significantly different from the annual prevalence in goats ($p = 0.572$).

Table 8.8 The annual prevalence of *T. brucei* spp. infections in goats

village	no. of goats	no. of samples	no. of positive goats	no of positive samples	annual prevalence
Katelenyang village (Samorin treated)	43	169	0	0	0%
Apatit village (Samorin control)	56	208	1	3	1.4%
Rukada village (Ethidium treated)	82	469	1	2	0.4%
Ngelechom village (Ethidium control)	57	183	0	0	0%
Total of 4 villages	238	1029	2	5	0.5%

The overall annual prevalence of *T. brucei* spp. infection in pigs ranged between 0% in Rukada village (Ethidium treated) and Ngelechom village (Ethidium control) to 12.5% in Apatit village (Samorin control) (Table 8.9). This was significantly greater than the annual prevalence of *T. brucei* spp. in either sheep ($p < 0.001$) or goats ($p < 0.001$) but not significantly different from the annual prevalence of *T. brucei* spp. in cattle ($p = 0.802$).

Table 8.9 The annual prevalence of *T. brucei* spp. infections in pigs

village	no. of pigs	no. of samples	no. of positive pigs	no of positive samples	annual prevalence
Katelenyang village (Samorin treated)	24	115	5	6	5.2%
Apatit village (Samorin control)	31	56	2	7	12.5%
Rukada village (Ethidium treated)	23	111	0	0	0%
Ngelechom village (Ethidium control)	2	2	0	0	0%
Total of 4 villages	80	284	7	13	4.6%

8.4.2.2 Central nervous system involvement in natural *T. brucei* spp. infections in domestic species other than cattle

The number of monthly CSF samples analysed from each species is given in Table 8.2. From a total of seven CSF samples analysed from sheep over a period of eleven months only two samples from the same sheep were positive for the presence of trypanosomes, both of which were *T. brucei* spp. (Table 8.10). While none of the four CSF samples analysed from a goat with a long standing infection of *T. brucei* spp. were positive for the presence of trypanosomes (Table 8.10). However of nine CSF samples analysed four samples from three different pigs were positive for the presence of trypanosomes, all of which were *T. brucei* spp. (Table 8.10).

Table 8.10 Positive CSF samples from species other than cattle

animal number	age (months)	date sampled	species	direct exam of CSF	mouse inoc. of CSF	duration of infection
A294	25	Nov	ovine	-	+	> 6 months
A294	26	Dec	ovine	-	+	> 7 months
A775	24	Dec	porcine	-	+	unknown
A850	10	Feb	porcine	-	+	unknown
A850	11	Mar	porcine	+	+	unknown
A802	7	May	porcine	+	+	> 1 month

All CNS infections were *T. brucei* spp.

8.4.2.3 The clinical history of domestic animals other than cattle with trypanosome infections in the CNS

Of domestic animals other than cattle one local East African fat tailed hair sheep and five pigs showed severe clinical CNS disturbance prior to death (Appendix VIII). No clinical CNS disorders were observed in local goats during the period of study.

8.4.2.4 Histopathology of the CNS of domestic species of animal other than cattle

Histopathological changes in the CNS were recorded and graded as to their severity using the same criteria as described by Wellde *et al.* (1989b) (Table 8.11). The principal histological features of the CNS disease in animals graded 2+ or higher were the same as was found in cattle and only differed in severity except for pigs treated with diminazene aceturate (numbers A 617 and A850) which had a severe meningoencephalopathy with a massive infiltration of mononuclear leukocytes in the meninges.

Table 8.11 Histology and clinical signs of domestic species other than cattle with long standing infections of *T. brucei* spp.

number	age (months)	species	trypanosome found in CNS	duration of *T. brucei* spp. infection	clinical signs of CNS disturbance	histological grade **
A63	17	sheep	-	90-120 days	none	1+
A77	24	goat	-	120-150 days	none	1+
A516	15	pig	-	> 300 days	none	3+
A617	11	pig	-	60-90 days	severe	4+
A802	9	pig	+	90-120 days	none	2+
A850	11	pig	+	unknown	severe	4+

** as per Wellde *et al.* (1989b)

8.4 Discussion

This study shows that village cattle with long term *T. brucei* spp. infections have, prolonged periods when trypanosomes are absent from CSF, very mild or absent histopathological changes in the CNS and no clinical signs of CNS disease. Indeed some East African zebu cattle infected with *T. brucei* spp. over a prolonged period may never develop CNS disease. It is therefore concluded that East African zebu cattle infected with *T. brucei* spp. rarely develop a fatal CNS disease within their natural lifetime.

There is evidence that the *Trypanosomal* drugs, diminazene aceturate, isometamidium chloride and homidium bromide do not efficiently cross the blood brain barrier (Gutteridge and Coombs, 1977), and it has been shown that treatment of mice infected with *T. brucei* spp. for 14 or more days, once the trypanosomes have invaded the CNS, does not clear parasites from the brain despite elimination of the parasitaemia (Jennings *et al.*, 1977). These mice all relapsed with a more severe meningoencephalitis than mice which died of a primary infection of *T. brucei* spp. without treatment (Jennings and Gray, 1983). Severe fatal relapse infections of *T. brucei* spp. following treatment have been described in cattle (Morrison *et al.*, 1983), goats (Whitelaw *et al.*, 1985) and dogs (Chukwu *et al.*, 1990). Such relapse infections were never observed in *Bos indicus* East African zebu cattle during the present study, despite them being infected with *T. brucei* spp. for prolonged periods prior to treatment. However relapse infections of *T. brucei* spp. were observed in one *Bos taurus* Ayrshire bull (N19) and one pig (A850). This

evidence supports the conclusion that some East African zebu cattle infected with *T. brucei* spp. over prolonged periods may never develop CNS infections.

Two cases of clinical CNS disturbance were seen in East African zebu cattle (*Bos indicus*) during the 18 month period of study which presented themselves as cattle which were dull, uncoordinated and with signs of hyperaesthesia, both of which had a haemorrhagic *T. vivax* infection and no infection with *T. brucei* spp. It has been demonstrated that certain strains of *T. vivax* can cause extravascular haemorrhages throughout the carcass (Wellde *et al.*, 1989e), which may include the CNS. However these two East African zebu cattle made a full and uneventful recovery after treatment with diminazene aceturate. In addition to these two zebu cattle with *T. vivax* infections showing clinical CNS disturbances one Ayrshire bull (*Bos taurus*) had a severe clinically evident CNS infection of *T. brucei* spp., and died within four months of infection despite treatment with diminazene aceturate. The course of this *T. brucei* spp. infection of a *Bos taurus* bull was consistent with the findings during experimental infection of exotic breeds (*Bos taurus*) cattle by Morrison *et al.* (1983) and Wellde *et al* (1989b).

Although Wellde *et al.* (1989c) showed that it was possible for East African zebu cattle to develop a fatal CNS infection of *T. brucei* spp., only two of his six observed cattle with natural CNS infections of *T. brucei* spp., of unknown duration, which were observed over a further period of five years, died of the condition. The remaining four cattle had mild or absent clinical signs of CNS disturbance and slight or absent histopathological change in the CNS at post mortem. Similarly in a follow up study in the Lambwe Valley in 1980, the mild and non-specific clinical signs reported in cattle with long standing infections of *T. brucei* spp., by a lay observer, may not have been associated with their *T. brucei* spp. infections (Wellde *et al.*, 1989c).

Wellde *et al.* (1989b) reported that of 39 exotic cattle (*Bos taurus* and *Bos taurus* crossed with improved *Bos indicus* cattle) experimentally infected with *T. b. brucei* or *T. b. rhodesiense*, half went on to develop a fatal CNS infection of between 85 days and four and a half year's duration. Similarly experimental infection of 11 exotic cattle (*Bos taurus*) with *T. b. brucei* by Morrison *et al.* (1983) showed that most cattle succumbed to a fatal CNS disease within a year of infection . From the differences in the morbidity of CNS infection, presentation of clinical signs, severity of histopathological changes in the CNS and duration of the disease between East African zebu cattle (*Bos indicus*) and exotic breeds of cattle (*Bos taurus*) it appears that East African zebu cattle exhibit a significantly greater degree of resistance or tolerance to CNS infection and disease caused by *T. brucei* spp.

The results of this study on cerebral trypanosomiasis do not directly conflict with those of previous workers, rather they present a different overall interpretation. Whereas Wellde *et al.* (1989c) believed that all East African zebu cattle infected with *T. brucei* spp. would eventually succumb to a fatal CNS disease, this was not a common outcome within the mean lifespan of this type of cattle kept under local village husbandry practices in Busia District. Within the normal age range of East African zebu cattle, with very few cattle reaching the age of six years (less than 5%), of the cattle infected with *T. brucei* spp., only some will develop a CNS infection and only a few of these may develop a clinical or fatal CNS disease. With the very slow development of cerebral trypanosomiasis in East African zebu cattle the majority of these cattle would not live long enough to experience its clinical manifestations should they indeed occur at all. The role of cerebral trypanosomiasis in local East African zebu cattle in the epidemiology of cattle as a potential reservoir for human sleeping sickness appears very limited.

It was observed that a *T. brucei* spp. infection of unknown duration in a local sheep caused clinically evident CNS disturbance, which eventually proved fatal. This disease syndrome observed in a local East African sheep was similar to that observed by Bouteille *et al.* (1988) when experimentally infecting European breeds of sheep. The role of cerebral trypanosomiasis in local sheep in the epidemiology of the potential reservoir for human sleeping sickness may be significant because of the relatively rapid removal of infected sheep from the epidemiological cycle through death. However the very low annual prevalence of *T. brucei* spp. infection in sheep would limit the effects of a fatal CNS disease on the epidemiology of Rhodesian sleeping sickness.

Although it has been demonstrated by many workers that goats can succumb to a fatal CNS disease after experimental infection with *T. brucei* spp. (Whitelaw *et al.*, 1985; Wellde *et al.*, 1989b), with trypanosomes being readily isolated from the CNS, no such observation was made in the local village goats of Busia District over 18 months of study. This may be due to the very low prevalence of *T. brucei* spp. in goats making it a rare event in the field.

All of the pigs with a *T. brucei* spp. infection developed cerebral trypanosomiasis within a few weeks or months of infection and most of these developed a clinically evident and fatal CNS disease syndrome within two months of trypanosomes being discovered in the CSF. The annual prevalence of *T. brucei* spp. infections in pigs can be of a similar magnitude to that in cattle but since most pigs rapidly succumb to a fatal CNS infection of *T. brucei* spp. the role of cerebral trypanosomiasis in pigs in the epidemiology of the potential reservoir of domestic livestock for human sleeping sickness must act to limit the importance of this species.

Mean CSF total protein of East African zebu cattle infected with *T. brucei* spp. for more than two months with no detectable trypanosomes in the CNS

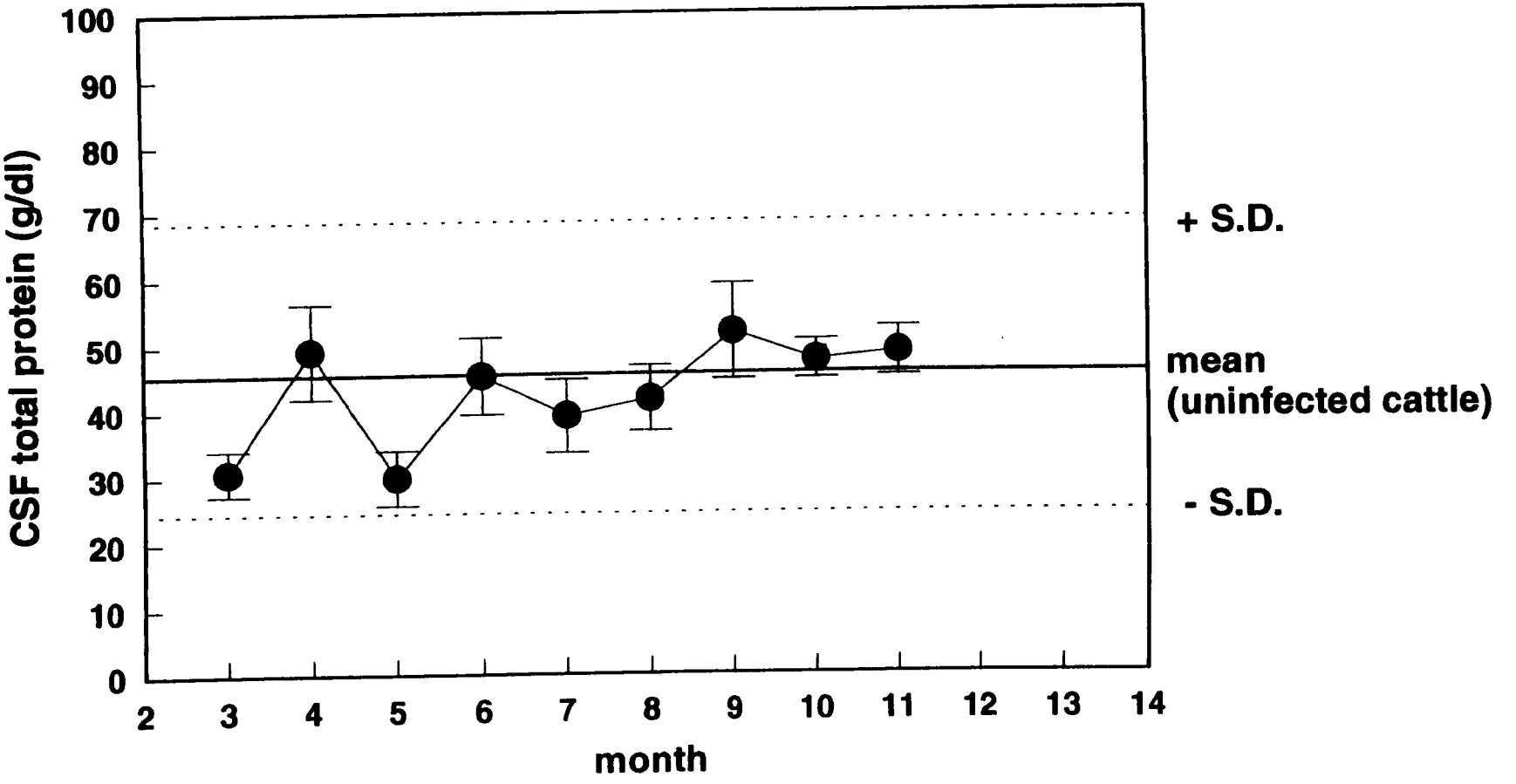


fig. 8.1

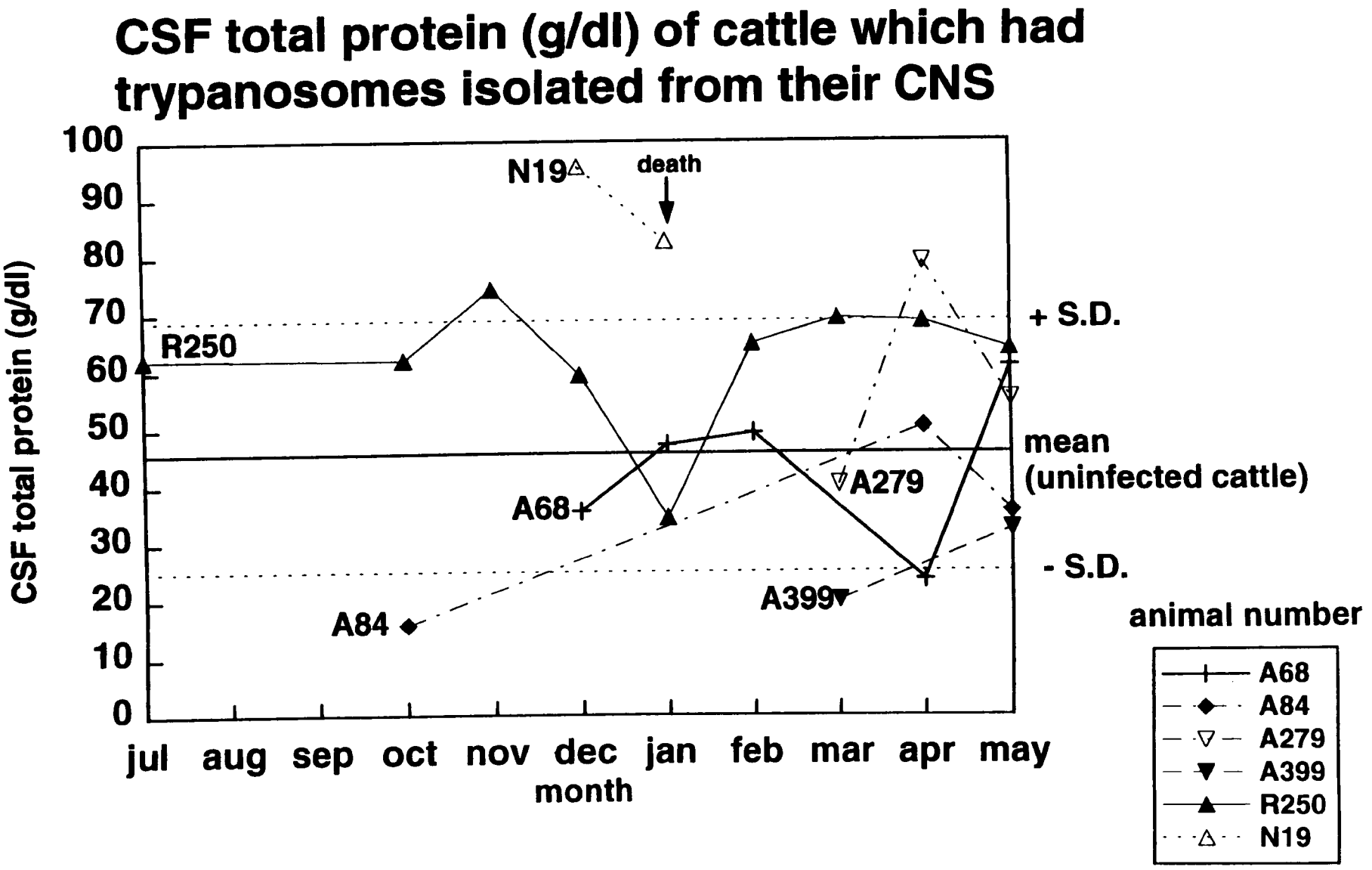

CSF total protein (g/dl) of cattle which had trypanosomes isolated from their CNS
CSF total protein (g/dl)
100
90
80
70
60
50
40
30
20
10
0
jul
aug
sep
oct
nov
dec
jan
feb
mar
apr
may
month
N19
death
R250
A68
A84
A279
A399
+ S.D.
mean
(uninfected cattle)
- S.D.
animal number
A68
A84
A279
A399
R250
N19

fig. 8.2

Chapter IX

Characterisation of *T. brucei* spp. stocks from Busia District using repetitive DNA probes

9.1 Summary

The epidemiological investigation of sleeping sickness foci has always been hindered by the difficulty of identifying the various subspecies of *T. brucei* spp. and the many strains within each subspecies. In this study restriction fragment length polymorphism (RFLP) was used to analyse *T. brucei* spp. stocks collected from domestic livestock in Busia District, Western Kenya in 1993 and 1994 when sleeping sickness was in an endemic phase. These samples were compared to a large data set of *T. brucei* spp. stocks circulating in the population humans and domestic animals in Tororo District, South East Uganda during the last sleeping sickness epidemic from 1988 to 1990 (Hide *et al.*, 1990, 1991, 1994). The *T. brucei* spp. stocks circulating within the human and animal populations between the adjoining areas of Busia District and Tororo District were the same during endemic and epidemic phases of sleeping sickness respectively. This indicates that the strains of trypanosome necessary for a sleeping sickness epidemic may already be present in an endemic area and that ecological and socio-economic factors may be more important in the initiation and maintenance of a sleeping sickness epidemic rather than the introduction to an area of a new more virulent strain of trypanosome. Over a period of up to three months several *T. brucei* spp. strains were found to be stable in infected cattle and a pig, emphasising the clonal nature of infection. Analysis of CSF and blood samples belonging to the same infected animal showed that CNS invasive trypanosomes were not a separate sub-population. The epidemiological implications of these finding are discussed.

9.2 Introduction

The epidemiological investigation of sleeping sickness foci has always been hindered by the difficulty of identifying the various subspecies of *T. brucei* spp. and the many strains within each subspecies. Since all the subspecies of *T. brucei* spp. are morphologically identical, host range, disease in man, infectivity to laboratory rodents and geographical distribution have been used to define these groups. Prior to standardisation of the blood incubation infectivity test (BIIT) (Rickman and Robson, 1970) the only way of differentiating *T. b. rhodesiense* form *T. b. brucei* was the use of human volunteers (Onyango *et al*, 1966). The BIIT relies on the

ability of *T. b. rhodesiense* to resist lysis by human serum (Owen and Gillet, 1992), however this is not an invariably expressed characteristic (Joshua *et al.*, 1978 and Jennings and Urquart, 1985) and leads to inaccuracy. Since then the human serum resistance test (HSRT) has been developed which can more reliably differentiate man infective from non-man infective trypanosomes in East Africa (Brun and Jenni, 1987). To further subdivide these subspecies and attempt to predict groups most likely to be human-infective isoenzyme electrophoresis has been used (Gibson and Wellde, 1985; Enyaru *et al*, 1993), however isoenzyme zymodemes alone cannot differentiate human infective and non-human-infective trypanosomes (Godfrey *et al*, 1990). Using repetitive DNA sequences to identify *T. brucei* subspecies by restriction fragment length polymorphism (RFLP) it is possible to clearly distinguish human-infective and non human infective trypanosomes, trypanosomes from differing regions and to further subdivide each group (Hide *et al*, 1990, 1991, 1994, 1996).

A collection of isolates obtained from humans in 1989 during the last sleeping sickness epidemic in Busia District were compared with isolates from domestic livestock in Busia District over a period of 18 months from January 1993 to July 1994. Characterisation of these isolates was carried out using restriction length polymorphism analysis (RFLP) (Hide, 1996) in order to classify these *T. brucei* spp. isolates into man - infective *T. b. rhodesiense* and non-man-infective *T. b. brucei* in order to assess the potential animal reservoir for human sleeping sickness. A separate data set exists for stocks of *T. brucei* spp. circulating in the population of humans and domestic cattle in Tororo District, South East Uganda during the last sleeping sickness epidemic fr 1988 to 1990 and the *T. brucei* spp. stabilates gathered from Busia District five years late were compared to this. A dendogram was constructed showing the relationships of 101 stocks of *T. brucei* spp. from the 1988-1990 study and three other studies (Hide *et al.*, 1990; 1991 and 1994) as determined by cluster analysis of DNA banding patterns.

During this study a number of domestic animals were blood sampled on several occasions over a period of time to investigate the stability of trypanosome strains within an individual host. In addition samples were analysed in one animal from either side of the blood brain barrier over a time period.

9.3 Materials and methods

For general materials and methods see chapter III and for collection of CSF samples see chapter VIII.

9.3.1 Trypanosome stocks

Blood samples were collected from domestic livestock as described in chapter III and CSF samples as described in chapter VIII. Samples containing *T. brucei* spp. infections were passaged twice through immunosuppressed mice and the resulting mouse blood samples were then cryopreserved as described by ILCA/ILRAD (1983) prior to export from Kenya. On arrival in the United Kingdom the samples were again passaged through immunosuppressed mice. Glucosamine was then added to the mouse blood before feeding it to teneral *Glossina morsitans morsitans* (Langford colony, Bristol). The flies were dissected 14 days later and infected midguts were placed in Cunningham's medium (Cunningham, 1977) and incubated at 26^{0}C until there were sufficient procyclic trypanosomes for DNA extraction.

Trypanosome stocks used in the analysis are listed in Table 9.1. Details of other stocks used in the comparisons are listed elsewhere (Hide *et al.*, 1990, 1991, 1994).

9.3.2 Isolation of DNA, Southern Blotting and Cluster analysis

Isolation of DNA was from a lysed procyclic trypanosome culture using solvent extraction. Analysis of DNA was by restriction length polymorphism (RFLP) in repetitive DNA sequences of trypanosome genes using radio labelled probes which produced a banding pattern on an autoradiograph. The banding patterns were then analysed by cluster analysis using similarity coefficients in order to produce a dendogram illustrating the degree of similarity among trypanosome stocks (Chapter III) (Hide 1996). The samples of *T. brucei* spp. from Busia District were compared to samples from Uganda, Kenya, Zambia and West Africa which were previously analysed (Hide *et al.*, 1994).

Table 9.1 ***Trypanosoma brucei*** **spp. stocks from Busia District**

stock	sample	host	date	location
K221	blood	cattle	16/09/93	South Teso (Katelenyang)
K224	blood	cattle	25/03/93	South Teso (Katelenyang)
K224	blood	cattle	18/06/93	South Teso (Katelenyang)
K235	blood	cattle	14/05/93	South Teso (Katelenyang)
K237	blood	cattle	16/09/93	South Teso (Katelenyang)
A68	blood	cattle	12/11/93	South Teso (Apatit)
A68	blood	cattle	24/01/94	South Teso (Apatit)
A277	blood	cattle	17/01/94	South Teso (Apatit)
A279	blood	cattle	14/02/94	South Teso (Apatit)
A279	blood	cattle	19/05/94	South Teso (Apatit)
A802	blood	pig	20/05/94	South Teso (Apatit)
A802	CSF	pig	20/05/94	South Teso (Apatit)
A802	blood	pig	30/06/94	South Teso (Apatit)
A802	CSF	pig	04/07/94	South Teso (Apatit)
A850	blood	pig	09/02/94	South Teso (Apatit)
R459	blood	cattle	19/05/94	Samia (Rukada)
KETRI 3194	blood	man	1989	Samia
KETRI 3196	blood	man	1989	South Teso
KETRI 3200	blood	man	1989	South Teso
KETRI 3202	blood	man	1989	Samia
KETRI 3203	blood	man	1989	Samia
KETRI 3205	blood	man	1989	Samia
KETRI 3206	blood	man	1989	Samia

9.4 Results

From the dendogram (fig. 9.1) all stocks of *T. brucei* spp. from domestic livestock in Busia District in 1993 and 1994 fell into the same group of East African *T. b. brucei* as those isolated from cattle in Tororo District between 1988 and 1990 except one stock from a cow (R459) which fell in the group of *T. b. rhodesiense* alongside other stocks isolated from humans in Busia and Tororo District some five years previously.

Cow R459 was only three and a half years old at the time of sampling which indicated that there had been transmission of *T. b. rhodesiense* within the cattle population of Busia District since the last sleeping sickness epidemic.

One in eight cattle infected with a *T. brucei* spp. between 1993 and 1994 was found to be carrying a human infective *T. b. rhodesiense* which is not significantly different from the proportion cattle (23%) infected with *T. brucei* spp. found to have human infective

trypanosomes in S. E. Uganda between 1988 and 1990 (Hide *et al.*, 1996) during the last sleeping sickness epidemic ($p = 0.503$) (Appendix IX, Table IX.1).

The trypanosome stocks isolated from human patients in 1989 in South Teso in the north of Busia District represent the same range of *T. b. rhodesiense* stocks isolated from the south of Busia District in Samia. These trypanosome stocks fall into the same group of stocks which have been circulating in the Kenyan and Ugandan sleeping sickness foci since at least the 1960's, and are distinct from *T. b. rhodesiense* stocks isolated in Zambia (fig. 9.1).

T. b. brucei samples from domestic livestock in Busia District formed a more genetically diverse group than the *T. b. rhodesiense* group isolated from man and cow R459. With the exception of one cattle sample (K235) all of the *T. b. brucei* samples taken in Busia District were represented in Tororo District some five years previously. Pigs in Kenya were shown to be infected with the same stocks of *T. b. brucei* as cattle in Kenya and Uganda and these stocks were CNS invasive strains at least in pigs.

To investigate the genomic stability of trypanosome strains in a single host, blood samples were collected from three cattle and one pig over a time period ranging from one to three months. Trypanosomes isolated from two cattle three months apart (K224 and A279), one heifer two months apart (A68) and a pig one month apart (A802) had identical banding patterns showing that the same strain of trypanosome remained present in each of these animals throughout this period (Table 9.1 and fig. 9.1).

To investigate the further possibility that differing strains of trypanosome might be present in different tissues within the same animal, samples were collected two months apart from the cerebrospinal fluid, of a pig, in addition to the sequential sampling of blood (A802). Again, identical banding patterns were observed showing that the same strain of trypanosome was present on both sides of the blood brain barrier in the same animal (Table 9.1 and fig. 9.1).

From the dendogram it can be seen that the *T. brucei* spp. stocks isolated from Busia District between 1993 and 1994 represent some of the same stocks which were circulating in closely neighbouring S. E. Uganda between 1988 and 1990 during the last sleeping sickness epidemic.

9.5 Discussion

The results of this study clearly indicated that cattle remained an important reservoir of human infective *T. b. rhodesiense* during an endemic phase of sleeping sickness (in 1994) as well as during an epidemic phase (Onyango *et al.*, 1966, Hide *et al.*, 1994). Very similar stocks of trypanosomes were found in domestic livestock in Busia District in 1993 and 1994 when the disease was endemic, compared to stocks from man and domestic livestock in Uganda and man in Kenya taken during a sleeping sickness epidemic from 1988 to 1990. Indeed some of the *T. b. rhodesiense* stocks have been circulating within the sleeping sickness foci around the Kenya / Uganda border for more than 30 years and these form a distinct group from *T. b. rhodesiense* isolated in Zambia (Hide *et al*, 1994). These results indicate a very localised circulation of essentially similar trypanosome stocks both during epidemic and endemic phases of sleeping sickness and rather than a new trypanosome stock being introduced from a different geographical region (Ormerod, 1961) or the relatively frequent emergence of virulent human infective strains from the animal population of *T. brucei* spp. (Gibson and Wellde, 1985), the trypanosome stocks required for a sleeping sickness epidemic may already exist within an endemic area. It may be that ecological or socio-economic factors are more important and may result in an increase in the level of infection in tsetse, cattle or humans altering the balance between the endemic and the epidemic states of sleeping sickness (Ford, 1970). It has been observed that past epidemics of sleeping sickness in East Africa in the early 1900's and again in the 1940's and 1980's were all associated with conditions of great social upheaval (Koerner *et al*, 1995).

The discovery of a stock of *T. b. rhodesiense* in 1994 in a cow (R459) which was the same as stocks isolated from human patients in 1989 indicates that the same human infective stock of *T. b. rhodesiense* had remained in circulation in the animal reservoir for at least five years. Since the infected cow was only three and a half years old this indicates that a population of cattle can carry and maintain the same trypanosome stock for very long periods of time. This in turn suggests that domestic livestock form a very serious threat to the human population having the potential to act as extremely long term reservoirs of sleeping sickness. Although individual animals can act as reservoirs of disease (Onyango *et al.*, 1966) it is useful when considering the epidemiology of *T. b. rhodesiense* to consider domestic livestock as a population as well as individuals. Individual cattle have been shown to be able to remain

infected with *T. b. rhodesiense* for periods of up to five years (Wellde *et al.*, 1989b), however it may be more important when considering the long term epidemiology of sleeping sickness in this region to consider the animal reservoir of *T. b. rhodesiense* as a population of trypanosomes circulating within the domestic animal population.

The finding that individual hosts are infected with a single strain of trypanosome which appears to remain stable over time has important epidemiological consequences. Since it is known that mixed infections of more than one species of trypanosome occur in cattle at a much greater frequency then would be expected by chance (Willet, 1972; chapter V) and that an existing trypanosome infection is a risk factor for subsequent infections (chapter V) it is reasonable to assume that cattle with an existing *T. brucei* spp. infection may be naturally challenged with another *T. brucei* spp. stock. This being the case the existing *T. brucei* spp. stock may either prevent superinfection or be replaced by the second stock. This is supported by experimental evidence of a signalling or regulatory mechanism between trypanosomes (Turner *et al*, 1996).

The identification of the same stock of *T. brucei* spp. on both sides of the blood brain barrier on two occasions in one animal indicates that there is not a separate sub-population of CNS invasive trypanosomes within an infection.

Further application of molecular techniques for the identification of trypanosomes needs to be carried out in order to resolve some of these important epidemiological questions of Rhodesian sleeping sickness.

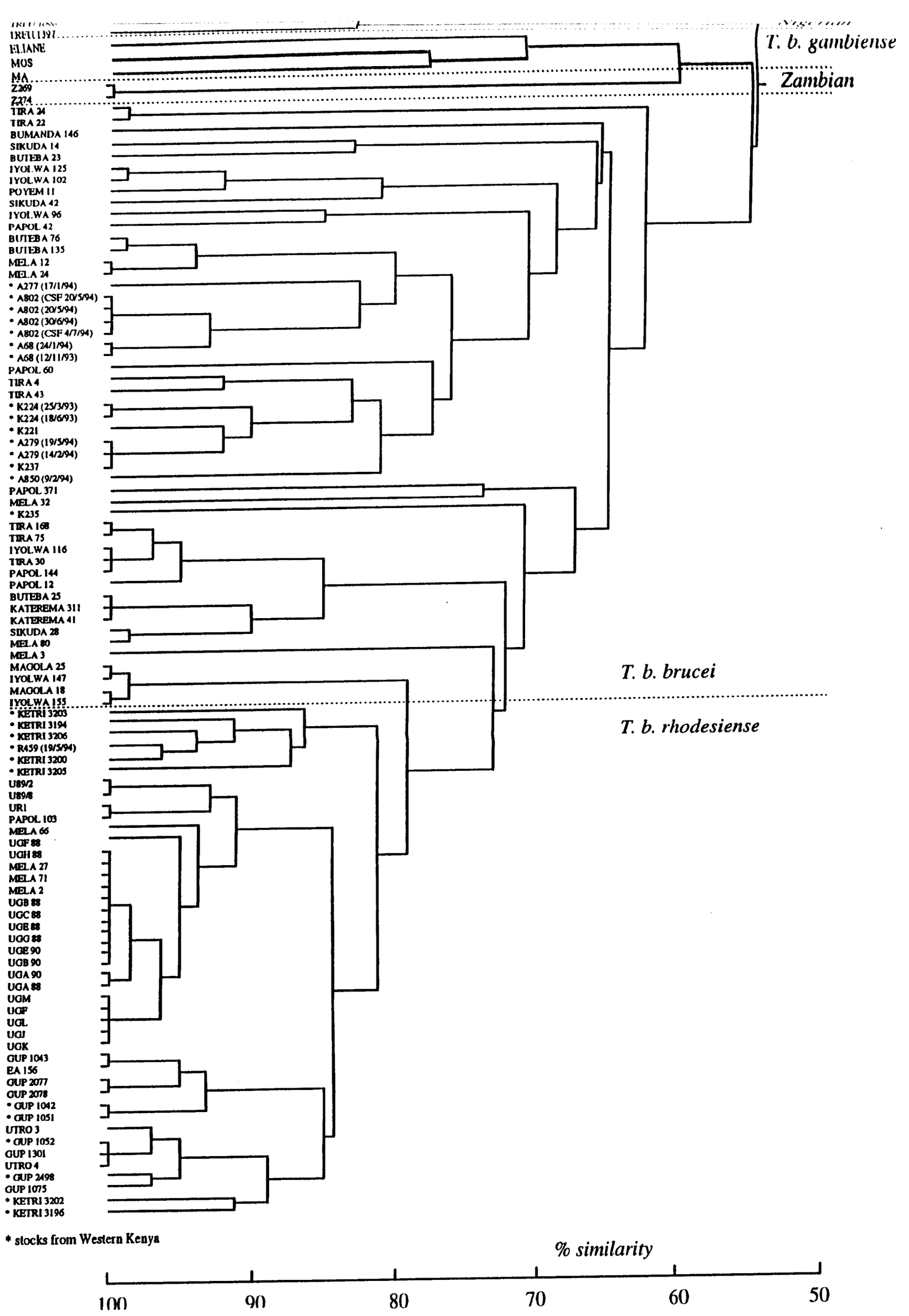


fig. 9.1 A dendogram showing the results of cluster analysis on the *T. brucei* spp. stocks from Western Kenya and South East Uganda

Chapter X

General discussion and conclusions

10.1 Introduction

This chapter will discuss the overall findings of the study as they relate to its broad aims and objectives. The study included; evaluation of chemoprophylaxis of the domestic animal reservoir as a possible control method in the advent of a sleeping sickness epidemic using two different drugs; the epidemiology of trypanosome infections in the local village domestic animal population in an area of endemic Rhodesian sleeping sickness and evaluation of field diagnostic techniques. Throughout the period of study the pathogenesis of natural trypanosome infections in local breeds of livestock kept under local husbandry conditions was recorded and particular note was made of trypanosome infections of the central nervous system. Subspecies and strains of *T. brucei* spp. were characterised using repetitive DNA probes in order to gain further insight into the epidemiology of Rhodesian sleeping sickness. The use and limitation of chemoprophylaxis as a control method for potentially human infective *T. brucei* spp. in domestic livestock are discussed as is the importance of this reservoir and a possible mechanism for its natural control.

Since 1990 the routine control of Rhodesian sleeping sickness in Busia District, Western Kenya has relied on the deployment of insecticide impregnated traps to reduce the tsetse population. This has been augmented by ground spraying with insecticide and the monitoring of the human population in villages where sleeping sickness cases were identified. Although there was an apparent reduction of the tsetse fly population of over 99%, as measured by trapping, at Amukura Location (where the villages of Katelenyang and Apatit are to be found) between the last sleeping sickness epidemic in 1989/1990 and 1994 the prevalence of *T. brucei* spp. infections in domestic cattle remained high (up to 26.2%) (Chapter IV). The continued existence of this potentially human infective reservoir of *T. brucei* spp. infections (Chapter IX) despite continuous fly trapping in this area calls into doubt the effectiveness of tsetse trapping as the sole measure of control for Rhodesian sleeping sickness. There appears to be a need for an alternative approach to the control of the reservoir of potentially human infective trypanosomes found in domestic livestock which may help prevent the necessary conditions occurring for Rhodesian sleeping sickness to become epidemic. Chemoprophylaxis of domestic livestock offers the possibility for the control of this reservoir.

10.2 General discussion

Treatment of domestic livestock with Samorin every three months was shown to virtually eliminate trypanosome infections for this period, including potentially human infective *T. brucei* spp. infections. While Ethidium was also shown to cure all trypanosome infections in cattle the duration of prophylaxis was much less, estimated at six weeks. Demonstrating the practical ability to effectively control the domestic animal reservoir has important implications for the control of Rhodesian sleeping sickness in the future since it is predicted from both theoretical models (Rogers, 1988) and field observations (Maudlin *et al.*, 1990c) that the transmission of the disease is more sensitive to a reduction in the prevalence of infection in the animal reservoir than to a reduction in the prevalence of human infection.

During the period of prophylaxis newly emergent tsetse flies would have their important first feed as a teneral, as well as subsequent blood meals from uninfected hosts and flies already infected before the prophylactic drug was administered to the livestock would tend to die out if the duration of prophylaxis exceed the natural lifespan of infected tsetse flies in the wild. Repeated treatment of domestic livestock with Samorin every three months was shown to achieve this objective, however it was much less certain that treatment with Ethidium, even every six to eight weeks would have the same results. Chemoprophylaxis of domestic livestock with Samorin has also been shown to have an effect on the trypanosome infections of infected flies which feed upon them (Agu, 1984; Jeffries and Jenni, 1987). While this may have an immediate effect on the infection rate within the tsetse population it is uncertain as to the duration of this effect since it is known that blood levels of Samorin diminish over time (Eisler *et al.*, 1994). If the only aim of chemoprophylaxis is to remove the animal reservoir of *T. b. rhodesiense* during a sleeping sickness epidemic then Ethidium may be considered on economic grounds should there be a sufficiently great price difference between the two drugs and allowing for the increased cost of treating animals more frequently,.

Immigration of infected tsetse flies into an area during a disease control program would not be affected by chemoprophylaxis of domestic livestock and the threat of importing disease in this manner would have to be countered by some means of vector control. The effect of migrating tsetse flies may not be great with the riverine species *Glossina f. fuscipes*, which has a well circumscribed habitat (Mwangelwa *et al.*, 1990), however with the

more widely roaming morsitans species *Glossina pallidipes* the problem of immigration of infected flies would have to be addressed (Jordan, 1986). Similarly importing infected livestock threatens the cordon sanitaire created by chemoprophylaxis and in order to counter this threat all domestic animals brought into a control area would have to treated at the point of entry.

From blood meal analysis of tsetse feeding habits (Okoth and Kapaata, 1986, Chapter IV) and the prevalence of *T. brucei* spp. infections in domestic livestock it is clear that cattle, and under certain circumstances pigs, were the only species of domestic animal of significant importance in the reservoir of potentially human infective *T. brucei* spp. infections. In order to remove the threat of this reservoir in domestic livestock it may only be necessary to treat cattle and pigs in order to reduce the transmission of *T. b. rhodesiense* below the critical level for epidemic sleeping sickness. This would have the added advantage of limiting the cost of using chemoprophylaxis as a control measure for Rhodesian sleeping sickness.

Animals with a very rapid weight gain were found to effectively outgrow their dose of Samorin in the three months duration of prophylaxis afforded by each treatment. This was occasionally seen in young cattle but was a far greater problem in pigs, which proportionally grow much more rapidly than cattle. In order to maintain effective chemoprophylaxis within a village with a significant population of pigs the frequency of treatment would have to be greater than that indicated in the drug trials carried out with cattle.

Relying on chemoprophylaxis alone for disease control leaves the system vulnerable to the development of drug resistance. However no evidence of drug resistance was observed during this study and at Mkwaja Ranch, where Samorin was used constantly as the main control measure for bovine trypanosomiasis, it took 30 years for significant resistance to develop (Fox *et al.*, 1993). The development of drug resistance may be further slowed by vector control reducing the level of challenge and the use of two drugs as sanitive pairs (Whiteside, 1962b). The prolonged period taken for Samorin to clear the bloodstream of trypanosomes and the fact that Ethidium never cleared non-pathogenic *T. theileri* infections suggests that reports of resistance to these two drugs may be exaggerated.

Samorin had a significant effect on the productivity of cattle during the period of prophylaxis. The effect on liveweight gain alone was sufficiently great to cover the cost of the drug without considering any beneficial effect on a reduction in mortality, improved reproduction, increased ability to work as draught oxen or an increase in animals products e.g.

milk or dung. The effect of Samorin on cattle productivity was solely due to a reduction in the prevalence of trypanosome infections as no beneficial or detrimental effect to liveweight gain was seen in uninfected animals. No improvement in productivity was seen in the group of cattle treated with Ethidium, however this may have been due to the very low prevalence of trypanosome infections during this trial. In addition to removing the animal reservoir of potentially human infective *T. brucei* spp. infections in cattle (and all other species of domestic livestock) there was a net economic benefit from chemoprophylaxis with Samorin .

When the prevalence of trypanosome infections in cattle was reduced to zero in Katelenyang village during the period of Samorin prophylaxis , the incidence and prevalence of anaemia in cattle was almost zero, which implies that either trypanosome infections were the only significant cause of anaemia in the cattle of this area or that Samorin had an effect on tick-borne diseases, anaplasmosis and babesiosis, known to cause anaemia.

If the control of human trypanosomiasis is the sole aim of chemoprophylaxis of domestic livestock in a village then it would only be necessary to treat *T. brucei* spp. infections, and given that *T. vivax* and *T. congolense* infections in cattle naturally act to limit the duration of *T. brucei* spp. infections (chapter V), in theory it would be advantageous in the control of sleeping sickness if the drug used was only effective against *T. brucei* spp. infections. If suramin, which is effective against *T. brucei* spp. but not the more pathogenic *T. vivax* or *T. congolense* infections, was used as a prophylactic it would be expected that it would give approximately two months prophylaxis against *T. brucei* spp. infections, while not affecting the incidence or prevalence of *T. vivax* or *T. congolense* infections in cattle. However, in practice, local farmer co-operation may not be as good as when Samorin or Ethidium was used because the use of suramin would not be expected to produce the same improvement in village cattle herd productivity. This is because suramin is only effective against *T. brucei* spp. infections which are relatively non-pathogenic in cattle (chapter VII). Furthermore logistical problems exist with suramin as supply of the drug is uncertain and it has to be administered intravenously.

During an outbreak of Rhodesian sleeping sickness chemoprophylaxis of domestic livestock alone would not affect the number of tsetse flies and may not significantly affect the number of tsetse flies already infected. Therefore it would be advantageous to combine chemoprophylaxis with some form of vector control. The vector control would be ideally of such a duration as to exceed the mean lifespan of tsetse in the wild (estimated at up

to 75 days for female flies, Glasgow, 1963; and 20 to 30 days, Phelps and Vale, 1978) to cover the period when flies would be alive which had already been infected by trypanosomes, prior to the treatment of the domestic animal reservoir with a chemoprophylactic. Ground spraying is commonly used in villages of the Kenyan and Ugandan border regions which have outbreaks of Rhodesian sleeping sickness, however the topography of the region (Chapter II) often makes it difficult or impossible to follow the water courses which form the riverine habitat of *Glossina f. fuscipes*. Therefore ground spraying may not be the ideal technique because of failure to access tsetse habitats and the potential risk of polluting rivers. The recent discovery of a population of *Glossina pallidipes* in some inaccessible hilly habitats adjacent to villages which may not be significantly affected by ground spraying along river courses would also cast doubt on the suitability of this vector control technique.

Since it is known from tsetse feeding habits (Table 5.5) and the prevalence of trypanosome infections (chapter IV) that cattle are a favoured host, it is reasonable to assume that insecticide applied to cattle would be an effective method of vector control. Two applications of deltamethrin six weeks apart to all village cattle (Thomson and Wilson, 1992) would be expected to reduce the numbers of tsetse flies, including importantly flies which were already infected, thereby reducing the transmission of sleeping sickness before the effects of chemoprophylaxis of domestic animal reservoir are reflected in a reduction in the trypanosome infection rates within the tsetse population. Since it has been shown in Katelenyang village and Apatit villages that despite an apparent reduction in tsetse fly numbers of over 99% from 1990 to 1993 (chapter II) there was no reflection of this in the prevalence of *T. brucei* spp. infections in domestic livestock, indeed the prevalence was higher in 1993 and 1994 (chapter IV, II) than when measured during the last sleeping sickness epidemic in 1990, (Mukiria *et al.*, 1990). This usefully illustrates the importance of integrated control by chemoprophylaxis and vector reduction by applying insecticide to cattle, which would be complementary control strategies.

The incidence and prevalence of mixed infections of more than one species of trypanosome in cattle was much greater than would be expected by chance in all four villages irrespective of the level of challenge. These observations can be explained by:

(a) flies with mixed infections of more than one species of trypanosome being more common than would be expected because of:

-feeding on animals with mixed infections

-flies already infected may be more susceptible to superinfection than uninfected flies

(b) cattle already infected are more susceptible to superinfection than uninfected animals

(c) only a proportion of the cattle population are either exposed or susceptible to trypanosome infections

(d) the true prevalence of *T. brucei* spp. infections was many times greater than that detected

(e) infections of differing species of trypanosome in cattle are not independent events

Since the effect of mixed infections of more than one species of trypanosome was apparent in both the incidence and the prevalence of infections in cattle this would tend to support explanation (a) and to a lesser extent (b) and (c). However this observed effect was greater in the prevalence compared to the incidence of mixed infections in cattle, which in turn tends to support explanation (b) or (c). The incidence and prevalence of mixed infections of more than one species of trypanosome in cattle which included *T. brucei* spp. (i.e. mixed infections of *T. brucei* spp. or *T. vivax*, and infections of *T. brucei* spp. and *T. congolense*) were more frequently observed at rates greater than expected by chance than mixed infections of *T. vivax* and *T. congolense*. This observation tends to support explanation (d) that single infections of only *T. brucei* spp. in cattle were under-diagnosed by up to a factor of three as it cannot be adequately explained by (a), (b) or (c) alone. The explanations for the observations that the incidence and prevalence of mixed infections of more than one species of trypanosome in cattle occurring at frequencies far greater than would be expected by chance may be a combination of those listed, suggesting that there may be an interaction between infections of differing species of trypanosome, which therefore may not behave as independent events.

The likelihood of infections of differing species of trypanosome in cattle not being independent events has important implications for the epidemiology and pathogenesis of trypanosomiasis. With mixed infections of more than one species of trypanosome there is an increased opportunity for interaction between infections of differing species, subspecies and strains of trypanosome, both in the mammalian host and tsetse flies. The pathogenesis of mixed trypanosome infections in all species of domestic livestock is much less well understood

than single infections of only one species of trypanosome (Losos and Ikede, 1972; Masake *et al.*, 1984)

Three risk factors for cattle acquiring a trypanosome infection were examined, which were age, sex and an existing trypanosome infection. Of these only an existing trypanosome infection, and especially one of *T. brucei* spp., was found to be consistently significant. Since *T. vivax* and *T. congolense* infections in cattle are more pathogenic than those of *T. brucei* spp. alone, cattle infected with *T. vivax* and *T. congolense* infections tend to be removed from the epidemiological cycle by means of death or treatment at a faster rate than those infected with *T. brucei* spp. alone. The chances of cattle with an infection of *T. brucei* spp. alone being removed from the epidemiological cycle was found to be one in eight per month compared to a one in two chance for cattle with infections of either *T. vivax* or *T. congolense* alone, or mixed infections of *T. brucei* spp. with *T. vivax* or *T. congolense*. Looking at the natural duration of trypanosome infections in cattle this gives a half life for a *T. brucei* spp. infection of approximately five months whereas mixed infections of *T. brucei* spp. with *T. vivax* or *T. congolense* have a half life of approximately one month (i.e. within one month 50% of cattle with mixed infections will have been removed from the village either by treatment, slaughter, sale or death, whereas only about 10% of cattle with infections of *T. brucei* spp. alone were removed from the epidemiological cycle in the same period of time). The presence of *T. vivax* and *T. congolense* infections in cattle therefore have the effect of limiting the duration of *T. brucei* spp. infections in cattle and thereby preventing a gradual increase in the prevalence (which is a function of the incidence and duration of infection) of *T. brucei* spp. infections in cattle which forms the reservoir of potentially human infective trypanosomes in the epidemiology of Rhodesian sleeping sickness.

This view is also supported by evidence from re-analysis of data from the last Rhodesian sleeping sickness epidemics in Lambwe Valley and Alego Location, in Kenya. In the Wiga area of the Lambwe Valley, Kenya during 1967 and 1968, when sleeping sickness was in decline, a survey of cattle (1553 cattle) showed an overall prevalence of trypanosome infections to be 11.8% with *T. congolense* accounting for 6.6%; *T. vivax* 4.4% and *T. brucei* spp. only 1.6% (Robson and Ashkar, 1972). Thirteen years later another survey of domestic livestock was carried out using the same diagnostic techniques (STDM) when sleeping sickness was in an epidemic phase. The prevalence of trypanosome infections in the 95 cattle sampled was then 70%; with *T. congolense* accounting for 21%; *T. vivax* 4.2% and *T. brucei*

spp. 53% (10% had mixed infections) (Wellde *et al.*, 1989c). Although there was a 3 fold increase in *T. congolense* infections between these two periods in time; *T. brucei* spp. infections increased by a factor of more than 30, while *T. vivax* infections remained at the same level. Similarly a survey of 152 cattle during an outbreak of sleeping sickness in Alego Location, Central Nyanza, Kenya in 1964 revealed that while the prevalence of *T. brucei* spp. was 20%, the prevalence of *T. congolense* and *T. vivax* infections combined, was less than 10% (Onyango *et al.*, 1966). During the surveys in the Lambwe Valley the vector was *Glossina pallidipes*, while the vector of sleeping sickness in Alego location, Central Nyanza was *Glossina f. fuscipes*.

Where the two species of fly exist there may be an interactive effect on the ecological balance of *T. brucei* spp., *T. vivax* and *T. congolense* infections in domestic livestock because of the differing susceptibilities of these two species of tsetse fly to infection with these three species of trypanosome (Harley, 1971).

T. brucei spp. infections in cattle may be easier to detect in cattle when they exist in mixed infections with either *T. vivax* or *T. congolense*. The immunosuppresive effects of *T. vivax* or *T. congolense* infections in cattle may allow the *T. brucei* spp. parasitaemia to rise to detectable levels (Willet, 1972). If this is the case then the true prevalence of *T. brucei* spp. infections in cattle may be three times greater than that detected by commonly used parasitological diagnostic techniques. The implication of this is that the animal reservoir of potential human infective *T. brucei* spp. may be much greater than was previously suspected.

All the parasitological diagnostic techniques (STDM, HCT and BC, Chapters I and III) have bias towards the detection of one or more species of trypanosome. The use of any single parasitological technique will inevitably bias the result and give a false indication as to the relative importance of each species of trypanosomes (Chapter VI). In the past, when subinoculation of mice has been used for diagnosis in the field they have not usually been immunosuppressed. Immunosuppression of the mice prior to inoculation with bovine blood was shown to double the sensitivity of this method as a diagnostic technique.

It has been known for some time that, at least experimentally, in mainly exotic European breeds of cattle (*Bos taurus*) and improved zebu cattle (*Bos indicus*), infections of either *T. brucei* spp. or *T. congolense* alone are more pathogenic than infections with *T. brucei* spp. at least in the short term (Losos and Ikede, 1972). However the pathological effects of infections with local strains of trypanosomes in the local breed of East African zebu

cattle maintained on traditional village husbandry systems under conditions of natural tsetse challenge were much less certain. While infections of *T. brucei* spp. alone caused a mild anaemia with no apparent effect on the liveweight gain of cattle, infections with either *T. vivax* or *T. congolense* alone caused a more severe anaemia and a significant reduction in liveweight gain. Cattle with mixed infections of *T. brucei* spp. with *T. vivax* or *T. congolense* showed similar pathological effects as single infections of either *T. vivax* or *T. congolense* alone respectively, whereas those with mixed infections of *T. vivax* and *T. congolense* showed more severe effects than infections with either species of trypanosome alone. Mortality in cattle followed a similar pattern with infections including *T. vivax* or *T. congolense* causing significant mortality whereas infections of *T. brucei* spp. alone, accounted for few if any deaths. Abortion was observed in only two cases of *T. vivax* infections in cattle which clinically displayed a haemorrhagic syndrome, whereas the remaining pregnant cattle which became infected with *T. brucei* spp., *T. vivax* or *T. congolense* did not abort. Indeed one cow was observed to have a *T. brucei* spp. infection prior to conception, during pregnancy and on into lactation with no evidence of congenital infection in its calf. The low calving rate in villages with a high prevalence of trypanosome infections may have been caused by infected cattle losing weight, being in poor body condition and failing to conceive, embryonic absorption or early embryonic loss which was not reported as abortion.

East African zebu cattle in the Western Province of Kenya exhibit a degree of trypanotolerance as defined as the ability of some cattle to maintain their PCV and weight gain and are able to reproduce normally despite trypanosome infections. These cattle exhibited a much greater tolerance of *T. brucei* spp. infections compared to infections of either *T. vivax* or *T. congolense*. The ability of cattle to maintain their PCV proved a reliable indicator in individual animals of trypanotolerance and since this trait has been demonstrated to be heritable (Murray *et al.*, 1982), this opens the possibility of selectively breeding for trypanotolerance in East African zebu cattle. However this would have undesirable effects on the epidemiology of Rhodesian sleeping sickness because increasing the degree of trypanotolerance within a population of cattle would increase their ability to act as symptomless reservoirs of potentially human infective *T. brucei* spp. infections.

Previous studies have assumed that *T. brucei* spp. infections in cattle would develop into fatal CNS infections either as an encephalitis associated with prolonged infection with *T. brucei* spp. (Morrison *et al.*, 1983). However this assumed that *T. brucei* spp.

infections in East African zebu cattle (*Bos indicus*) behave in a similar fashion to *T. brucei* spp. infections in humpless European breeds of cattle (*Bos taurus*) (Morrison *et al.*, 1983, Wellde *et al.*, 1989b) or that infected East African zebu cattle would eventually succumb to a fatal CNS *T. brucei* spp. infection (Wellde *et al.*, 1989c). Neither of these assumptions proved to be the normal outcome of *T. brucei* spp. infections in East African zebu cattle kept under traditional village husbandry conditions in Busia District. Clinical CNS disturbance was observed in an Ayrshire bull (*Bos taurus*) associated with invasion of the CNS within a few months of infection, which eventually developed into a fatal menigoencephalits associated with a relapse infection following treatment with diminazene. Whereas CNS infections of *T. brucei* spp. were rarely found in East African zebu cattle (*Bos indicus*) and these were never associated with clinical disease. Similarly no relapse infections were seen in cattle with long standing *T. brucei* spp. infections which were treated with either diminazene, isometamidium or homidium. Wellde *et al.* (1989c) believed that all East African zebu cattle infected with *T. brucei* spp. would eventually succumb to a fatal CNS disease despite this occurring in only two of eight East African zebu cattle of unknown age and duration of *T. brucei* spp. infections which were observed over a period of five years. Since less than five per cent of the cattle population was over six years of age it is concluded that East African zebu cattle rarely, if ever, develop a fatal CNS infection of *T. brucei* spp. within their natural lifespan and as such CNS infections of cattle with *T. brucei* spp. are largely irrelevant to the epidemiology of Rhodesian sleeping sickness.

Local East African zebu breeds of sheep (Chapter VIII) and goats (Wellde *et al.*, 1989d) have been shown to succumb to fatal CNS clinical disturbance associated with CNS infections of *T. brucei* spp. of only a few months duration. However since these species have a limited lifespan and the prevalence of *T. brucei* spp. infections was very low, CNS infections of sheep and goats would only serve to limit this already very limited reservoir of potentially human infective trypanosomes.

All pigs infected with *T. brucei* spp. developed CNS infections within a few weeks or months of infection and in the majority of cases this proved fatal within the period of observation. Both encephalitis associated with primary *T. brucei* spp. infection of the CNS and severe relapse infections following treatment were seen. Central nervous system

infections act to limit the duration of *T. brucei* spp. infections in pigs and consequently serve to limit the prevalence of infection in this species.

The only dog found infected with *T. brucei* spp. throughout the study died within three weeks of infection with a severe disturbance of the CNS, which makes dogs a very poor long term reservoir of infection of potentially human infective *T. brucei* spp.

Characterisation of *T. brucei* spp. stocks form Busia District using repetitive DNA probes (RFLP) (Hide, 1996) showed that the same stock of *T. b. rhodesiense* was circulating in the cattle population in 1994 which was seen during the last sleeping sickness epidemic five years earlier. Similarly a comparison of *T. brucei* spp. stocks isolated from both humans and animals showed that these same stocks have been circulating in the animal and human populations of Western Kenya and South East Uganda for more than 30 years during both epidemic and endemic phases of Rhodesian sleeping sickness. This implies that the origin of epidemic sleeping sickness is more likely to be associated with a disturbance in the ecological balance of the disease rather than the importation or emergence of a new virulent strain of *T. b. rhodesiense*.

Repeated blood and CSF samples from the same animals over a period of time showed that the same trypanosome genotype was consistently found indicating that the population of trypanosomes in a mammalian host is clonal and that the trypanosomes on either side of the blood brain barrier are identical.

10.3 Conclusions

10.3.1 The reservoir of potentially human infective *T. brucei* spp. in domestic livestock and its natural control

From the prevalence of *T. brucei* spp. infections in domestic livestock and the relative importance of each species as hosts for tsetse flies (Weitz, 1963) it is concluded that cattle and pigs are the most important species in the domestic animal reservoir of potentially human infective *T. brucei* spp. infections. In addition to this *T. brucei* spp. infections in the East African zebu cattle of this region were of very low pathogenicity (chapter VII) and appeared

to be able to resist or control the progression of CNS pathology (chapter VIII). The natural control of this reservoir appears to be the presence of the more pathogenic *T. vivax* or *T. congolense* infections which lead to the early elimination of *T. brucei* spp. infections. This is supported by characterisation of *T. brucei* spp. subspecies and strains which indicate that a change in ecology of the trypanosome transmission cycle rather than the appearance of new strains of *T. b. rhodesiense* were responsible for the occurrence of epidemics of sleeping sickness.

Pigs were also shown to have a prevalence of *T. brucei* spp. infections comparable in magnitude to cattle (chapter VIII) and interestingly they consistently showed much higher parasitaemias than cattle. Other studies have also shown that pigs can, under certain circumstances, be favoured hosts of *Glossina f. fuscipes* (Okoth and Kapaata, 1986). However, in the present study, unlike cattle, no *T. vivax* or *T. congolense* infections were ever found in pigs. The natural control of this potentially human infective *T. brucei* spp. reservoir appears to lie in the pathology of *T. brucei* spp. infections in pigs. All pigs rapidly developed a fatal CNS infection within a few months of infection with *T. brucei* spp. which served to limit the duration of the infection. This helped prevent a build up in the prevalence of *T. brucei* spp. infections in pigs, thereby acting as a natural control for this reservoir. Another constraint for pigs acting as a reservoir of infection was their limited lifespan, with most pigs being sold or slaughtered at only a few months of age and only a small number of breeding pigs surviving more than one year. Although it has been shown that it is possible for pigs to act as reservoirs of potentially human infective *T. brucei* spp. infections, the pig population (239) represented only one sixth of the cattle population (1675) in the four villages in this study.

From the low prevalence of *T. brucei* spp. infections (Chapter V) and the relative distaste of tsetse flies for sheep, goats and dogs (Weitz, 1963), it is concluded that these species are of very minor importance in the epidemiology of the potentially human infective *T. brucei* spp. infections in domestic animals. The severe pathological changes caused by *T. brucei* spp. infections in these species serves limit the duration of these infections and thereby further limiting their importance in the epidemiology of Rhodesian sleeping sickness. It is thereforee concluded that in order to control the domestic animal reservoir of potentially human infective *T. brucei* spp. infections drug treatment should be confined to cattle and pigs.

10.3.2 Chemoprophylaxis of the domestic animal reservoir as a means of control of Rhodesian sleeping sickness

Chemoprophylaxis of domestic livestock was shown to be a very effective means of controlling the potentially human infective *T. brucei* spp. reservoir in domestic livestock, provided that it was adequately monitored. In order for chemoprophylaxis to be effective in a village it would probably only necessary to treat cattle and pigs. However during an outbreak of sleeping sickness this method alone may not significantly affect the number of tsetse flies which were already infected and therefore it would be necessary to combine chemoprophylaxis with some form of vector control in order to reduce the incidence of human infection as rapidly as possible.

The drug of choice should ideally produce the maximum duration prophylaxis which would reduce the logistical problems of treating large numbers of animals. It should be a broad spectrum trypanocide which is effective against *T. vivax* and as well as *T. brucei* spp. in order to maximise local farmer co-operation by improving the productivity of village livestock. Of the presently available prophylactic trypanocidal drugs for livestock Samorin most closely fulfils these criteria, however should economic circumstances change Ethidium may be considered. It is further recommended that should the duration of block treatment of all village cattle and pigs in a sleeping sickness focus be prolonged then an annual treatment with diminazene aceturate should be carried out as a sanative to reduce the possibility of the development of drug resistance.

It is envisaged that chemoprophylaxis of domestic cattle and pigs combined with the simultaneous application of persistent insecticide to these animals would be used as the control strategy of choice as part of an integrated disease control program involving monitoring and treatment of the human population for the control of an outbreak of Rhodesian sleeping sickness in this region.

10.3.3 Diagnosis of trypanosome infections

In the absence of a quick, accurate and reliable serological test for the diagnosis of trypanosome infections in domestic livestock which can easily be performed at the time of sampling, parasitological diagnostic techniques will remain the most applicable techniques

used in field surveys of domestic animals. Despite the results of chapter VI broadly agreeing with laboratory investigations as to which individual parasitological diagnostic technique is the most sensitive for each species of trypanosome, the clear conclusion from this large scale field investigation is that for maximum sensitivity in the diagnosis of infections of all three species of pathogenic trypanosomes, *T. brucei* spp., *T. vivax* and *T. congolense*, a suitable combination of diagnostic techniques should be used, namely HCT, BC and subinoculation of blood into immunosuppressed mice with thin fixed smears of positive trypanosome samples being taken for staining in the laboratory for confirmation of species identification. In conclusion, considering the diagnostic techniques used in past field surveys of trypanosome infections in domestic livestock, it is probable that the true prevalence of *T. brucei* spp. infections in particular have been significantly underestimated.

10.3.4 Epidemiological implications of interactions between differing species and differing strains of trypanosome

Since it has been demonstrated that mixed infections of more than one species of trypanosome occur in cattle at a much greater frequency than would be expected by chance (chapter V) it is reasonable to assume that tsetse flies are exposed to blood meals with mixed infections as tenerals more frequently than would be expected. However the interaction between species of trypanosome in tsetse flies cannot currently be evaluated because of the lack of reliable techniques for trypanosome species identification in dissected flies. Cattle with existing trypanosome infections were shown to be at greater risk of being infected with another species of trypanosome than uninfected cattle (chapter V) which implies that superinfection of cattle already infected with a different strain of the same species of trypanosome may also occur at a significant frequency. However molecular techniques for the identification of differing strains of the same species of trypanosome would have to be used to examine the possible epidemiological consequence of this as parasitological technique are unable to differentiate trypanosome strains.

From RFLP analysis of the trypanosome stocks from Busia District, Kenya and Tororo District, Uganda during endemic and epidemic phases of sleeping sickness respectively it appears that the trypanosome stocks necessary for an epidemic remain present during endemic periods. This suggests that the reason for the change from the endemic to the

epidemic phases and vice versa may lie in the complex ecological balance between trypanosome infections, human and animals mammalian hosts and the tsetse fly vector. It is concluded that further investigation of the interaction between trypanosome species, subspecies and strains should be carried out in both domestic animals and tsetse flies using reliable molecular techniques for both a qualitative and quantitative assessment of these interactions and an evaluation of their epidemiological implications.

References

Abaru, D.E. (1985). Sleeping sickness in Busoga, Uganda 1976 - 1983.
Tropical Medicine and Parasitology. **36**:72-76.

Adams, A.R.D. (1936). III The diagnosis and course of untreated *T. vivax* infections in domestic animals.
Annals of Tropical Medicine and Parasitology. **30**:521-531.

Agu, W. E. (1984). The effect of isometamidium chloride on *T. vivax* occurring within the insect vector (*Glossina*)
Zeitschrift für Parasitenkunde. **70**: 431-435.

Agu, W.E. and Bajeh, Z.T. (1986). An outbreak of fatal *Trypanosoma brucei* infection of pigs in Benue State of Nigeria.
Tropical Veterinarian. **4**: 25-28.

Agymang, K., Dwinger, R.H., Little, D.A., Leperre, P. and Grieve, A.S. (1992). Interaction between physiological status in N'Dama cows and trypanosome infections and its effect on health and productivity of cattle in Gambia.
Acta Tropica. **50**: 91-99.

Allsopp, R., Baldry, D.A.T., and Rodrigues, C. (1972). The influence of game animals on the distribution and feeding habits of *Glossina pallidepes* in the Lambwe Valley.
Bulletin of the World Health Organisation. **47**: 795-809.

Angus, S.D., Turner, C.M.R. and Holmes, P.H. (unpublished). The pathogenesis of *T. b. rhodesiense* in experimentally infected Scottish blackface sheep.

Apted, F.I.C. (1962). Sleeping sickness in Tanganyika past, present and future.
Transactions of the Royal Society of Tropical Medicine and Hygiene. **56**: 15-29.

Apted, F.I.C. (1970). The epidemiology of rhodesian sleeping sickness
In: *The African Trypanosomiases* (H. W. Mulligan, ed.).
George Allen and Unwin, London. pp. 645-655.

Ashcroft, M.T. (1959). The importance of African wild animals as reservoirs of trypanosomiasis.
East African Medical Journal. **36**:289-297.

Ashcroft, M.T., Burtt, E., and Fairbairn, H. (1959). The experimental infection of some African wild animals with *Trypanosoma rhodesiense, T. brucei* and *T. congolense.*
Annals of Tropical Medicine and Parasitology. **53**:147-161.

Ashkar, T. and Ochilo, M. (1972). The application of the indirect fluorescent antibody test to samples of sera and dried blood from cattle in the Lambwe Valley south Nyanza Kenya.
Bulletin of the World Health Organisation. **47**: 769-772.

Assenyonga, G.S.C. and Adams, K.M. (1975). The number and morphology of trypanosomes in the blood and lymph of rats infected with *Trypanosoma brucei* and *T. congolense.*
Parasitology. **70**: 255-261.

Bailey, N.T.J. (1985). *Statistical methods in biology.*
Hodder and Stoughton, London.

Banks, K.L. (1979). In vitro binding of *Trypanosoma congolense* to erythrocytes.
Journal of Protozoology. **26**: 103-108.

Barry, J.D. and Emberg, D.L. (1984). Parasite development and host response during the establishment of *Trypanosoma brucei* infection transmitted by the tsetse fly.
Parasitology. **88**: 67-74.

Bauer, B., Meyer, F., and Kabore, I. (1989). Effects of flumethrin pour-on against *Glossina palpalis gambiensis* (Diptera, Glossinidae) during release in a fly proof stable.
Tropical Medical parasitology. **40**: 478-479.

Boreham, P.F.L. (1973). Recent developments in, and possible future applications of the identification of blood meals in vectors of African trypanosomiasis.
Joint FAO/WHO seminar, Kinshasa 1972.

Boreham, P.F.L. (1979). The pathogenesis of African and American trypanosomiasis In: *Biochemistry and physiology of protozoa.*
New York Academic press, USA.

Borowy, N.K., Fink, E., and Hirumi, H. (1985). *Trypanosoma brucei* : Five commonly used trypanocides assayed in vitro with mammalian feeder layer system for cultivation of bloodstream forms.
Experimental parasitology. **60**: 323-330.

Bourn, D. and Scott, M. (1978). The successful use of work oxen in agriculture development of tsetse infested land in Ethiopia.
Tropical animal health and production. **10**: 191-203.

Bouteille, B., Darde, M.L., Dumas, M., Catanzano, G., Pestre-Alexandre, M., Breton, J.C., Nicolas, A. and N'Do, D.C. (1988). The sheep (*Ovis aries*) as an experimental model for African trypanosomiasis I. clinical study.
Annals of Tropical Medicine and Parasitology. **82**: 141-148.

Boyt, W.P. (1971). Trypanosomiasis control in Rhodesia
Bulletin of African Infectious Epizoology. **76**: 310-306.

Braend, M. (1979). World veterinary manpower : comparative aspects.
Veterinary Record. **105**:77-79.

Brandl, F.E. (1988). Costs of different methods to control riverine tsetse in West Africa.
Tropical Animal Health and Production. **20**: 67-77.

Brun, R. and Jenni, L. (1987). Human serum resistance of metacyclic forms of *Trypanosoma brucei brucei, T. brucei rhodesiense* and *T. brucei gambiense.*
Zeitschrift für Prasitenkunde. **73**: 218-223.

Büngener, W. and Mehlitz, D. (1976). Experimentelle trypanosomoninfection bei Kamerun - zwergziegen : histopathologische befunde.
Tropenmedizin und Parasitologie. **27**: 405-410.

Büngener, W. and Mehlitz, D. (1984). Histopathological findings in mini-pigs infected with different strains of *Trypanosoma brucei.*
Tropenmedizin und Parasitologie. **35**: 109-114.

Burgdorfer, W., Schmidt M. L. and Hoogstraal H. (1973). Detection of *T. theileri* in Ethiopian cattle ticks.
Acta Tropica. **30** (4): 340-346.

Burtt, E. (1946). Incubation of tsetse pupae : increased transmission rate of *Trypanosoma rhodesiense in Glossina morsitans.*
Annals of Tropical Medicine and Parasitology. **40**: 18-28.

Bushed, FM and Evison, C. (1981).The value of game exclusion measures in the control of *Glossina morsitans centralise* in Zambia.
Proceeding of the 17th meeting International Science Council for Trypanosomiasis Research and Control (ISCTRC),Arusha, Tanzania. OAU/STRC publication No **112**: 637-642.

Buxton, P.A. (1955). The Natural History of Tsetse Flies.
HK Lewes and co. Ltd, London

Cannon, R. M. and Roe, M. T. (1982). Livestock disease surveys: a field manual for veterinarians.
Australian Bureau of Animal Health, Canberra.

Challier, A. and Laveissière, C. (1973). Un nuveau piège pour la capture des Glossines (*Glossina*: Diptera, Muscidae): description et essais sur le terrain.
Cahiers d'ORSTOM, Série Entomologie Médicale et Parasitologie. **11**: 251-262.

Chukwu, C.C., Anene, B.M., Onuekwusi, K.O., and Anika, S.M. (1990). Relapse infection after chemotherapy in dogs experimentally infected with *Trypanosoma brucei brucei.* *Journal of Small Animal Practice.* **31** (3): 141-144.

Clarkson, M.J., Cottrell, B.A., and Toro, M. (1973). The capillary agglutination test in the diagnosis of animal trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* **67**: 10-11.

Cockerbill, G.F. (1971). The control of tsetse and trypanosomiasis in Rhodesia. *Bulletin of the Office International des Epizootie.* **76**: 347-352.

Corson, J.F. (1932). The results of successive bites of an infected tsetse fly. *Journal of Tropical Medicine and Hygiene.* **35**: 136-137.

Corson, J.F. (1934). *Trypanosoma rhodesiense* in cerebrospinal fluid of sheep and goats. *Annals of Tropical Medicine and Parasitology.* **25**: 145.

Corson, J.F. (1939). The infections produced in sheep and antelopes by a strain of *Trypanosoma rhodesiense.* *Transactions of the Royal Society of Tropical Medicine and Hygiene.* **33**: 37-46.

Cunningham, M.P. (1966). Immunity in bovine trypanosomiasis. *East African Medical Journal.* **43**: 394-397.

Cunningham, I. (1977). New culture medium for culture of tsetse tissues and trypanosomatids. *Journal of Protozoology.* **24**: 325-329.

Dame, D.A. and Schmidt, C.H. (1970). Sterile male technique against tsetse flies: *Glossina* spp. *Bulletin of the Entomological Society of America.* **16**: 24-30.

Dargie, J.D., Murray, P.K., Murray, M., and McIntyre, W.I.M. (1979). The blood volumes and erythrokinetics of N'dama and Zebu cattle experimentally infected with *Trypanosoma brucei.* *Research in Veterinary Science.* **26**: 245-247.

Davis, C.E., Robbins, R.S., and Weller, R.D. (1974.) Thrombocytopaenia in experimental trypanosomiasis. *Journal of Clinical Investigations.* **53**: 1359-1367.

Dempsey, W.L. and Mansfield, J.M. (1983). Lymphocyte function in experimental African trypanosomiasis VI Parasite specific immunosuppression. *Journal of Immunology.* **130**: 2896-2898.

Dewey, H.M. and Wormal, A. (1946). Studies on suramin (Antrypol : Bayer 205) 5. The combination of the drug with the plasma and other proteins. *Biochemistry Journal.* **40**: 119-124.

Dixon, M.A., Cull, R.S., Dunbar, I.F., Greenhill, R.J., Grimshaw, C.G., Hill, M.A., Landeg, F.J., and Miller, W.M. (1971). Non cyclical transmission of trypanosomiasis in Africa. *Veterinary Record.* **89**: 233-235.

Docherty, M.L., Windel, H., Voorkeis, H.P., Larkin, H., Casey, M., Cleary, D. and Murray, M. (1993). Clinical disease associated with *T. theileri* infections in a calf in Ireland. *Veterinary Record.* **132** (26): 653-656.

Dolan, R.B., Njogu, A.R., Sayer, P.D., Wilson, A.J. and Alushula, H. (1985). Trypanotolerance in East African cattle. *19th Meeting International Scientific Council (ISCTRC), Lome, Togo. OAU/ STRC publication No.* **114**: 314-318.

Dolan, R.B., Okech, G., Alushula, H., Mutugi, M., Stevenson, P., Sayer, P.D. and Njogu, A.R.(1990). Homidium bromide as a chemoprophylaxis for cattle trypanosomiasis in Kenya. *Acta Tropica.* **47**: 137-144.

Douati, A., Küpper, W., Kotia, K. and Badou, K. (1986). Contrôle des glossines (*Glossina*: Diptera, Muscidea) à l'aide d'écrans et de pièges (méthodes statistiques): bilan de deux années de lutte à Sirasso dans le nord de la Côte-d'Ivoire. *Révue d'Elevage et de Médicine Vétérinaire des Pays Tropicaux* **39**: 213-219.

Dransfield, R.D. (1984). The range of attraction of the biconical trap for *Glossina pallidepes* and *Glossina brevipalpis.* *Insect Science and its Application.* **5** (5): 363-368.

Draper, N. R. and Smith, H. (1980). Applied regression analysis second edition, John Wiley and sons, USA.

Duke, H.L. (1921). On the zoological status of the polymorphism mammalian trypanosomes of Africa and their relation to man. *Parasitology.* **13**: 352-397.

Duke, H.L. (1933). Studies on the factors that may influence the transmission of the polymorphic trypanosomes by tsetse. III *Glossina morsitans morsitans* versus *Glossina palpalis* as a transmitter of the polymorphic trypanosomes. *Annals of Tropical Medicine and Parasitology.* **27**: 123-132.

Dwinger, R.H., Luckins, A.G., Murray, M., Rae, P. and Moloo, S.K. (1986). Interference between different serodemes of *Trypanosoma congolense* in the establishment of superinfections in goats. *Parasitic Immunology.* **8**: 293-305.

EATRO (1974). East African Trypanosomiasis Research Organisation, Tororo, Uganda. *Annual Report, 1974.*

EATRO (1975).East African Trypanosomiasis Research Organisation, Tororo, Uganda *Annual Report, 1975.*

EATRO (1976). East African Trypanosomiasis Research Organisation, Tororo, Uganda *Annual Report, 1976.*

Edwards, E.E., Judd, J.M., and Squire, F.A. (1956). Observations on trypanosomiasis in domestic animals in West Africa ! : the daily index of infection and weekly haematological values in goats and sheep infected with *T. vivax, T. congolense* and *T. brucei.* *Annals of Tropical Medicine and parasitology.* **50**: 242-251.

Eisler, M.C., Arowolo, R.O.A., Gault, E.A., Moloo, S.K., Holmes, P.H. and Peregrine, A.S. (1994). Isometamidium concentrations in the sera of Boran Cattle: correlation with prophylaxis against tsetse-transmitted *Trypanosoma congolense.* *Acta Tropica.* **56**: 39-50.

England, E.C. and Balry, D.A.T. (1972).The hosts and trypanosome infection rates of *Glossina pallidepes* in the Lambwe and Woo valleys. *Bulletin of World Health Organisation.* **47**: 785-788.

Engvall, E. and Perlmann, P. (1972). Enzyme linked immunosorbant assay ELISA III quantification of specific antibodies by enzyme - labelled ante - immunoglobulin in antigen coated tubes. *Journal of Immunology.* **109**: 129-135.

Enyaru, J.C.K., Stevens, J.R., Odiit, M., Okuna, N.M. and Carasco, J.F. (1993). Isoenzyme comparison of trypanozoon isolates from two sleeping sickness areas of south-eastern Uganda. *Acta Tropica.* **55**: 97-115.

Fairbairn, H. (1933). The action of human serum *in vitro* on sixty four recently isolated strains of *T. rhodesiense.* *Annals of Tropical Medicine and Parasitology.* **27**: 185-205.

Fairbairn, H. and Burtt, E. (1946). The infectivity to man of a strain of *Trypanosoma rhodesiense* transmitted cyclically by *Glossina morsitans* through sheep and antelope : evidence that man requires a minimum infective dose of metacyclic trypanosomes.
Annals of Tropical Medicine and parasitology. **40**: 270-313.

Fairclough, R. (1963). Observations on the use of berenil against trypanosomiasis of cattle in Kenya.
Veterinary Record. **75**: 1107-1112.

FAO (1982). FAO-WHO-OIE Animal Health Year Book 1981.
FAO Animal Health and Production Series No 18.

Fiennes, R.N.T. (1970). Pathogenesis and pathology of animal trypanosomiases
In: *The African Trypanosomiases* (H. W. Mulligan ed.).
George Allen and Unwin, London. pp. 729-750.

Ford, J. (1964). The geographical distribution of trypanosome infections in African cattle populations.
Bulletin of Epizootic Diseases in Africa. **12**: 307-320.

Ford, J. (1970). The geographical distribution of *Glossina*
In: *The African Trypanosomiases* (H. W. Mulligan, ed.).
George Allen and Unwin, London. pp. 274-297.

Ford, J. (1971). *The Role of the Trypanosomiases in African Ecology: A study of the tsetse problem.*
Clarendon, Oxford. 568 pp.

Fox, R.G.R., Mmbando, S.O., Fox, M.S. and Wilson, A. (1993). Effect on herd health and productivity of controlling tsetse and trypanosomiasis by applying deltamethrin to cattle.
Tropical Animal Health and Production. **25**: 203-214.

Geigy, R., Mwambu, P.M., and Kauffmann, M. (1971). Sleeping sickness survey in Musoma district Tanzania IV examination of wild mammals as a potential reservoir of *T. rhodesiense.*
Acta Tropica. **28**: 211-220.

Geigy, R., Kauffmann, M., and Mayende, J.S.P. (1973). Isolation of *Trypanosoma (Trypanozoon) rhodesiense* from game and domestic animals in Nusoma district Tanzania
Acta Tropica. **30**: 49-56.

Gibson, W.C., Mehlitz, D., Lanham, S.M., and Godfrey, D.G. (1978). The identification of *Trypanosoma brucei gambiense* in Liberian pigs and dogs by isoenzymes and by resistance to human plasma.
Tropenmedizin und Parasitolgie. **29**: 335-345.

Gibson, W.C., Marshall, T.F., and Godfrey, D.G. (1980). Numerical analysis of enzyme polymorphism. A new approach to the epidemiology and taxonomy of trypanosomes of the sub genus *Trypanozoon*. *Advances in Parasitology*. **18**: 175-245.

Gibson, W.C. and Gashumba, J.K. (1983). Isoenzyme characterisation of some *trypanozoon* stocks from a recent trypanosomiasis epidemic in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **77**: 113-118.

Gibson, W.C. and Wellde, B.T. (1985). Characterisation of trypanozoon stocks from the South Nyanza sleeping sickness focus in Western Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **79**: 671-676.

Gill, B.S. (1964). A procedure for the indirect haemaglutination test for the study of experimental *Trypanosoma evansi* infections. *Annals of Tropical Medicine and Parasitology*. **58**: 473-480.

Gitatha, S.K., Tengekyon, K.M., Mwabi, D.L.L., Okech, G., Njogu, A.R., Nakuro Leboo, J.L. and Adoyo, R.D.H. (1987). Human and animal trypanosomiasis in South Teso Busia District. *KETRI Internal Report, Kenya Trypanosomiasis Research Institute, Muguga, Kenya.*

Glasgow, J.P. (1963).*The distribution and abundance of tsetse* Oxford, Pergamon Press. pp. 39-48.

Glasgow, J.P. and Duffy, B.J. (1951). Further observations on the extermination of *Glossina fuscipes fuscipes* Newstead by hand catching. *Bulletin of Entomological Research*. **42**: 55-63.

Goble, F.C., Fewell, B., and Steiglitz, A.R. (1959). The virulence and drug susceptibility of certain strains of the *brucei - evansi* group maintained by syringe passage. *Annals of Tropical Medicine and Parasitology*. **53**: 189-202.

Godfrey, D.G. and Killick-Kendrick, R. (1961). Bovine trypanosomiasis in Nigeria I the inoculation of blood into rats as a method of survey in the Donga Valley Benue Province. *Annals of Tropical Medicine and Parasitology*. **55**: 287-297.

Godfrey, D.G., Leach, T.M. and Killick-Kendrick, R. (1964). Bovine trypanosomiasis in Nigeria. III A high incidence in a group of West African humpless cattle *Annals of Tropical Medicine and Parasitology*. **58**: 204-215.

Godfrey, D.G., Scott, C.M., Gibson, W.C., Mehlitz, D. and Zillmann, U. (1987). Enzyme polymorphism and the identity of *Trypanosoma brucei gambiense*. *Parasitology.* **94**: 337-347.

Godfrey, D.G., Baker, R.D., Rickman, L.R. and Mehlitz, D. (1990). The distribution, relationships and identification of enzymic variants within the subgenus *Trypanozoon.* *Advance in Parasitology.* **29**: 1-74.

Goodwin, L.G. and Hook, S.V.M. (1968). Vascular lesions in rabbits infected with *Trypanosoma* (trypanozoon) *brucei.* *British Journal of Pharmacology.* **32**: 505-513.

Goodwin, L.G. (1970). The pathology of African trypanosomiasis . *Transactions of the Royal Society of Tropical Medicine and Hygiene.* **64**: 797-817.

Gouteaux, J.P. and Lancien, J. (1986). Le piège pyramidal à tsétsé (Diptera: Glossinidea) pour la capture et la lutte. Essais comparatifs et description de nouveux systèmes de capture. *Tropical Medicine and Parasitology.* **37**: 61-66.

Gov. of Kenya (1993).Veterinary Department (Busia) Annual Report 1993. Department of Agriculture Ministry of Agriculture, Livestock and Marketing Government of Kenya.

Gray, A.R. (1964). *Annual Report, West African Institute for Trypanosomiasis Research, Nigeria,* pp 28.

Gray, A.R. (1967). Some principles of the immunology of trypanosomiasis. *Bulletin of the World Health Organisation.* **37**: 177-193.

Gray, A.R. (1970). A study of the antigenic relationships of isolates to *Trypanosoma brucei* collected from a herd of cattle kept in one locality for five years *Journal of General Microbiology.* **62**: 301-313.

Gray, A.R. and Luckins, A.G. (1980). The initial stages of infection with cyclically transmitted *Trypanosoma congolense* in rabbits, calves and sheep. *Journal of Comparative Pathology.* **90**: 499-512.

Green, C.H. (1994). Bait methods for tsetse fly control. *Advances in Parasitology.* **34**:229-291.

Gutteridge, W.E. and Coombs, G.H. (1977). *Biochemistry of parasitic protozoa.* MacMillan, London.

Hargrove, J.W. and Vale, G.A.(1978). The effect of host odour concentration on catches of tsetse flies (Glossinidae) and other Diptera in the field. *Bulletin of Entomological Research.* **68**: 607-612.

Harley, J.M.B. (1966) .Studies on age and trypanosome infection rate in females of *Glossina pallidepes* Aust., *G. Palpalis fuscipes* Newst. and *G. Brevipalpis* Newst. in Uganda. *Bulletin of Entomological Research.* **57**: 23-37.

Harley, J.M.B., Cunningham, M.P., and Van Hoeve, K. (1966). The numbers of infective *Trypanosoma rhodesiense* extruded by *Glossina morsitans* during feeding. *Annals of Tropical Medicine and Parasitology.* **60**: 455-460.

Harley, J.M.B. and Wilson,, A.J. (1968). Comparison between *Glosssina morsitans, G. pallidipes* and *G. fuscipes* as vectors of trypanosomes of the *Trypanosoma congolense* group: The proportions infected experimentally and the numbers of infective organisms extruded during feeding. *Annals of Tropical Medicine and Parasitology.* **62**: 178-187.

Harley, J.M.B. (1971). Comparison of the susceptibility to infection with *Trypanosoma rhodesiense* of *Glossina pallidipes, G. morsitans, G. fuscipes, G. brevipalpis.* *Annals of Tropical Medicine and Parasitology.* **65**: 185-189.

Harley, J.M.B. (1971). The influence of the age of the fly at the time of infecting feed on infection of *Glossina fuscipes* with *Trypanosoma rhodesiense.* *Annals of Tropical Medicine and Parasitology.* **65**: 191-196.

Harris, R.H.T.P. (1938). The control and possible extermination of the tsetse by trapping. *Acta Conventus tertii de Tropicis atque Malariae Morbis, Amsterdam.* **1**: 663-677.

Heisch, R.B. and McMahon, J.P. (1958). The isolation of *Trypanosoma rhodesiense* form a bushbuck. *British Medical Journal.* **ii**: 1203-1204.

Hide, G., Cattand, P., Leray, D., Barry, J.D., and Tait, A. (1990). The identification of *Trypanosoma brucei* subspecies using repetitive DNA sequences. *Molecular and Biochemical Parasitology.* **39**: 213-226.

Hide, G. Cattand, P., Le Ray, D. Barry, J.D. and Tait, A. (1990). The identification of *T. brucei* subspecies using repetitive DNA sequences. *Molecular and Biochemical Parasitology.* **39**: 213-226.

Hide, G., Buchanan, N., Welburn, S.C., Maudlin, I., Barry, J.D. and Tait, A. (1991). *Trypanosoma brucei rhodesiense*: Characterisation of stocks from Zambia, Kenya and Uganda using repetitive DNA probes. *Experimental Parasitology.* **72**: 430-439.

Hide, G. Tait, A., Maudlin, I. and Welburn, S.C. (1994). Epidemiological relationships of *Trypanosoma brucei* stock from South East Uganda: Evidence for different population structures in human and non-human trypanosomes. *Parasitology.* **109**: 95-111.

Hide, G. (1996).The molecular identification of trypanosomes. *Methods in Molecular Biology.* **50**: 243-263.

Hill, D.H. (1956). Inter African Bureaux of Epizootic Disease of Africa. *Technical Report 12/55.*

Hirumi, H., Doyle, J.J., and Hirumi, K. (1984). Cultivation of bloodstream *Trypanosoma brucei.* *Bulletin of the World Health Organisation.* **55**: 405-409.

Hoare, C.A. (1964) .Morphological and taxonomic studies on mammalian trypanosomes and revision of the systematics. *Journal of Protozoology.* **11**(2): 200-207.

Hoare, C.A. (1972). *The trypanosomes of mammals.* Blackwell, Oxford.

Holmes, P.H. (1976). Use of radioisotope tracer techniques in the study of the pathogenesis of trypanosomiasis. *Nuclear techniques in Animal Production and Health*: 463-474.

Holmes, P.H. and Scott, J.M. (1982). Chemotherapy against trypanosomiasis. *Perspectives in Trypanosomiasis Research.* 59-69.

Holmes, P.H. and Torr, S.J. (1988). The control of animal trypanosomiasis in Africa : current methods and future trends *Outlook on Agriculture.* **17**: 54-60.

Hornby, H.E. (1921). Trypanosomes and trypanosomiasis of cattle. *Journal of Comparative Pathology.* **34**: 211-240.

Hornby, H.E. (1921). The relative pathogenicity for cattle of *Trypanosoma congolense* and *T. vivax.* *Veterinary Journal.* **77**: 77-78.

Houba, V., Brown, K.N., and Allison, A.C. (1969). Heterophile antibodies, M - antiglobulins and immunoglobulins in experimental trypanosomiasis. *Clinical and Experimental Immunology.* **4**: 113-123.

Hudson, J.R. (1944). Acute and subacute trypanosomiasis in cattle caused by *T. vivax*. *Journal of Comparative Pathology.* **54**: 108-119.

Hursey, B. (1993). FAO presentation
Proceedings of the 22nd meeting International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Kampala, Uganda. OAU/STRC publication No. **117**: 12.

Hutchinson, M.P. (1953). The epidemiology of human trypanosomiasis in British West Africa I and II the Gambia.
Annals of Tropical Medicine and Parasitology. **47**: 153-182.

Hutchison, M.P. (1971). Human trypanosomiasis in south-west Ethiopia (March 1967 March 1970).
Ethiopian Medical Journal. **9**: 3-69.

Ibrahim, E.A.R., Ingram, G.A., and Molyneux, D.H. (1984). Haemaglutanins and parasite agglutanins in haemolymph and gut of *Glossina.*
Tropical Medical Parisitology. **35**: 151-156.

Ikede, B.O. and Losos, G.J. (1972). Pathology of experimental disease in cattle caused by *Trypanosoma brucei.*
Veterinary Pathology. **9**: 272-277.

Ikede, B.O. and Losos, G.J. (1972). Pathology of the disease in sheep produced experimentally by *Trypanosoma brucei.*
Veterinary Pathology. **9**: 278-289.

ILCA (1979). *Trypanotolerant livestock in West and Central Africa'*
International Livestock Centre for Africa, Addis Ababa, Ethiopia.

ILCA/ILRAD (1983). *Livestock productivity and trypanotolerance.*
Network Training Manual (Murray, M., Trail, J.C.M. and Wissocq, Y. Ed.).
International Livestock Centre for Africa, Addis Ababa, Ethiopia.

ILCA/ILRAD (1986). Situation report, December 1985.
Proceeding of a network meeting held at ILCA, Nairobi
International Livestock Centre for Africa, Addis Ababa, Ethiopia.

ILCA (1990). *Livestock Systems Research Manual: Volume 1.*
International Livestock Centre for Africa, Addis Ababa, Ethiopia.

ILRAD (1990). *ILRAD April 1990 report.*
International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

Itty, P., Chema, S., d'Ietere, N., Durkin, J., Leak, S.G.A., Maehl, J.H.H., Maloo, S.H., Mukendi, F., Nagda, S.M., Rarieya, J.M., Thorpe, W., and Trail, J.L.M. (1987). Economic aspects of cattle production and of chemoprophylaxis for control of trypanosomiasis in village East African zebu cattle in Kenya.
The African Trypanotolerant Livestock Network. ILCA/ILRAD Proceedings of Meeting 23 - 27 November 1987, Nairobi, Kenya. pp. 360-376.

Jahnke, H.E. (1974). The economics of controlling tsetse flies and cattle trypanosomiasis in Africa, examined for the case of Uganda.
IFO Forschungsberichte der Africa - studienstelle. **4**: 8.

Jeffries, D. and Jenni, L. (1987). The effect of the trypanocidal drugs berenil and samorin on infections of *Glossina morsitans centralis* by *Trypanosoma congolense.*
Acta Tropica. **44**: 369-370.

Jenkins, A.R. and Robertson, D.H.H. (1959). Hepatic dysfunction in human trypanosomiasis II serum protein in *Trypanosoma rhodesiense* infections and observations on the alterations found after treatment and during convalescence.
Transactions of the Royal Society of Tropical Medicine and Hygiene. **53**: 524.

Jenni, L., Molyneux, D.H., Livesey, J.L., and Galun, R. (1980). Feeding behaviour of tsetse flies infected with trypanosomes.
Nature. **283**: 383-385.

Jenni, L., Marti, S., Schweitzer, J., Betschart, B., Le Page, R.W.F., Neils, J.M., Tait, A., Paindavoine, P., Pays, E., and Stinert, M. (1986). Hybrid formation between African trypanosomes during cyclical transmission.
Nature. **322**: 173-175.

Jennings, F., Murray, P.K., Murray, M., and Urquart, G.M. (1974). Anaemia in trypanosomiasis : studies in rats and mice infected with *T. brucei.*
Research in Veterinary Science. **16**: 70-76.

Jennings, F.W., Whitlaw, D.D., and Urquart, G.M. (1977).The relationship between duration of infection with *Trypanosoma brucei* in mice and the efficacy of chemotherapy.
Parasitology. **75**: 143-153.

Jennings, F.W and Gray, G.D. (1983). Relapsed parasitaemia following chemoprophylaxis of chronic *T. brucei* infections in mice and its relation to cerebral trypanosomes
Contributions to Microbiology and Immunology. 7: 147-154.

Jennings, F.W. and Urquart, G.M. (1985). Induction of human serum sensitive *Trypanosoma brucei* stabilates into human serum resistant "*T. rhodesiense*". *Transactions of the Royal Society for Tropical Medicine and Hygiene.* **79**: 80-85.

Jones-Davies, W.J. (1967). The protection of a small group of trade cattle from trypanosomiasis with samorin. *Bulletin of Epzootic Diseases of Africa.* **15**: 323-335.

Jordan, A.M., Lee-Jones, F., and Weitz, B. (1962). The natural hosts of tsetse flies in Northern Nigeria. *Annals of Tropical Medicine and Parasitology.* **56**: 430-442.

Jordan, A.M. (1974). Recent developments in the ecology and methods of control of tsetse flies (*Glossina* spp.) (Dipt Glossinidae) - a review. *Bulletin of Entomological Research.* **63**: 361-399.

Jordan, A.M. (1978). Principles of the eradication or control of tsetse flies. *Nature.* **273**: 607-609.

Jordan, A.M. (1986). *Trypanosomiasis control and African rural development.* Longman, London & New York.

Joshua, R.A., Herbert, W.J., and White, R.G. (1978). Acquisition by *Trypanosoma brucei* of potential infectivity for man by passage through birds. *Lancet (correspondence).* **1** (8066): 724-725.

Kaggwa, E., Munyya, W.R., and Mugerag, M. (1988). Relapses in dogs experimentally infected with *Trypanosoma brucei* and treated with diminazene aceturate or isometamidium chloride. *Veterinary Parasitology.* **27** (3 - 4): 199-208.

Kamango-Sollo, E.I.P., Musole, A.J., Nantulya, V.M., Rurangirwa, F.R. and Masake, R.A. (1991). Differences between N'Dama and Boran cattle in the ability of their peripheral blood leukocytes to bind antibody-coated trypanosomes. *Acta Tropica.* **49**: 109-117.

Katunguka-Rwakishaya, E., Murray, M. and Holmes, P.H. (1992). Pathophysiology of bovine trypanosomiasis: ferrokinetics and erythrocyte survival studies. *Research in Veterinary Science.* **53**: 80-86.

Katunguka-Rwakishaya, E., Parkins, J.J., Fishwick, G., Murray, M. and Holmes, P.H. (1995). The influence of energy intake on the pathophysiology *of T. congolense* infection in Scottish blackface sheep. *Veterinary Parasitology.* **59**: 207-218.

Kershaw, W.E. (1970). *Foreward.*
In: *The African Trypanosomiases* (H.W. Mulligan, ed.).
George Allen and Unwin, London. pp. vii-ix.

KETRI (1994). Tsetse fly trapping in Busia District May 1994.
Internal report, Kenya Trypanosomiasis Research Institute, Muguga, Kenya.

Kilgour, V. and Godfrey, D.G. (1978). The influence of lorry transport on the *Trypanosoma vivax* infection rate in Nigerian trade cattle.
Tropical Animal Health and Production. **10**: 145-148.

Killick - Kendrick, R. (1968). The diagnosis of Trypanosomiasis of livestock ; A review of current techniques.
Veterinary Bulletin. **38**: 191-197.

Killick - Kendrick, R. (1971). The low pathogenicity of *Trypanosoma brucei* to cattle.
Transactions of the Royal Society of Tropical Medicine and Hygiene. **65**: 104.

Kirby, W.W.(1964). Prophylaxis and therapy under continuous exposure to the risk of natural infection with trypanosomiasis by tsetse flies.
Bulletin of. Epizootic Disease in Africa. **12**: 321-329.

Knowle, S. (1927). Trypanosomiasis of camels in the Anglo - Egyptian Sudan : diagnosis chemotherapy immunity.
Journal of Comparative Pathology. **40**: 59-71.

Koerner, T., de Raadt, P and Maudlin, I. (1995). The 1901 Uganda sleeping sickness epidemic revisited: A case of mistaken identity?
Parasitology Today. **11** (8): 303-306.

Küpper, W., Dräger, N., Mehlitz, D. and Zilmann, U. (1981). On the immobilisation of hartebeest and kob in Upper Volta.
Tropenmedizin und Parasitologie. **32**: 58-60.

Küpper, W. and Douati, A. (1985). The use of insecticide impregnated biconical traps against riverine species of *Glossina* and their impact on animal trypanosomiasis : results of a two year campaign in Northern Ivory Coast.
Proceedings of the 18th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Harare, Zimbabwe. OAU/STRC publication No. **113**: 364-371.

Langley, P.A. and Pimley, R.W. (1986). A role for juvenile hormone and the effects of so called anti-juvenile hormones in *Glossina morsitans.*
Journal of Insect Physiology. **32** (8): 727-734.

Langley, P.A. and Weidhaas, D. (1986). Trapping as a means of controlling tsetse, *Glossina* spp. (Diptera : Glossinidae) : the relative merits of killing and of sterilisation.
Bulletin of Entomological Research. **76**: 89-95.

Laveissière, C. and Couret, D. (1981). Trapping as a means of controlling human trypanosome vectors.
Proceedings of the 17th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Lome, Togo. OAU/STRC publication No. **112**: 609-617.

Laveissière, C., Couret, D., and Kiénon, J.P. (1981). Lutte contre les glossines riveraine à l'aide de piège biconique imprégnés d'insecticide, en zone de savane humide.
Cahiers d'OSTROM Série Entemologie Médicale et Parasitologique. **19**: 49-54.

Laveissièrre, C. and Meda, H.H. (1992). La butte par piègeage contre la maladie du sommeil: pas aussi simple que l'on croit!
Annales de la Société belge de Médicine Tropicale **72** *(suppliment 1)*: 57-68.

Leach, T.M. and Roberts, C.J. (1981). Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the eastern hemisphere.
Pharmacology and Theriogenology. **13**: 91-147.

Leeflang, P., Blotkamp, C., Godfrey, D.G., and Kilgour, V. (1974). A convenient hypotonic lysis method for concentrating trypanosomes from infected blood.
Transactions of the Royal Society of Tropical Medicine and Hygiene. **68**: 412.

Levine, N.D., Corliss, J.O., Cox, F.E.G., Derou, G., Grains, J., and Honigberg, (1980). A newly revised classification of the protozoa.
Journal of Protozoology. **27** (1): 37-58.

Losos, G.J. and Ikede, B.O. (1972). Review of pathology of diseases in domestic and laboratory animals caused by *Trypanosoma congolense, T. vivax, T. brucei, T. rhodesiense* and *T. gambiense.*
Veterinary Pathology (Supplement). **9**: 1-71.

Losos, G.J. and Gwamaka, G.(1973). Histological examination of wild animals naturally infected with pathogenic African trypanosomiasis..
Acta Tropica. **30** (1 - 2): 57-63.

Losos, G.J., Paris, J., and Wilson, A.J. (1974). Pathology of the disease in cattle caused by *T. congolense.*
Bulletin of Epizootic Diseases of Africa. **21**: 239-248.

Luckins, A.G. (1977). Selection of antibodies in trypanosome infected cattle by means of a microplate enzyme -linked immunosorbant assay. *Tropical Animal Health and Production.* **9**: 53-62.

Lumsden, W.H.R. (1962). Trypanosomiasis in African wildlife. *1st International conference on Wildlife Disease, New York*: 68-88.

Lumsden, W.H.R., Kimber, C.D., Evans, D.A., and Doig, S.J. (1979). *Trypanosome brucei* : miniature anion exchange, centrifugation technique for the detection of low parasitaemia : adaptation for field use. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* **73**: 312-317.

Mackichan, I.W. (1944). Rhodesian sleeping sickness in south west Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* **38**: 49-60.

MacLennan, K.J.R. (1970). The epizootiology of trypanosomiasis in livestock in West Africa. In: *The African Trypanosomiases*, (H. W. Mulligan ed.). George Allen and Unwin, London. pp. 751-765.

Magnus, E., Vervoot, T. and van Miervenne, N. (1978). A card agglutination test with stained trypanosomes (CATT) for the serological diagnosis of *T. b. gambiense.* *Annales de la Société belge de Médicine Tropicale.* **58**: 169-176.

Majiwa, P.A.O. (1993). DNA probe- and PCR-based methods for the detection of trypanosomes. *Proceedings of the 22nd Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Kampala, Uganda. OAU/STRC publication No.* **117**: 53-58.

Maloo, S.H., Chema, S., Connor, R., Durkin, J., Kimotho, J., Maehl, J.H.H., Mukendi, F., Murray, M., Rarieya, J.M. and Trail, J.C.M. (1987). The use of chemoprophylaxis in East African zebu village cattle exposed to trypanosomiasis in Muhaka, Kenya. *The African Trypanotolerant Livestock Network. ILCA/ILRAD Proceedings of Meeting 23 - 27 November 1987, Nairobi, Kenya.* pp. 283-288.

Mamo, E. and Holmes, P.A. (1975). The erythrokinetics of zebu cattle chronically infected with *Trypanosoma congolense* *Research in Veterinary Science.* **18**: 105-106.

Mangwiro, C. and Wilson, A. (1993). The control of tsetse and trypanosomiasis by application of insecticides to cattle: An evaluation of deltamethrin pour-ons.
Proceedings of the 22nd Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Kampala, Uganda. OAU/STRC publication No. **117**: 264-266.

Masake, R.A., Nantulya, V.M., Akol, G.W.O., and Musoke, A.J. (1984) Cerebral trypanosomiasis in cattle with mixed *Trypanosoma congolense* and *T. brucei brucei* infections.
Acta Tropica. **41**: 237-246.

Maudlin, I. (1985). Inheritance of susceptibility to trypanosomes in tsetse flies.
Parasitology Today. 1 (2):59-60.

Maudlin, I. and Welburn, S.C. (1988). Tsetse immunity and the transmission of trypanosomiasis.
Parasitology Today. 109-111, 1988.

Maudlin, I. and Welburn, S.C. (1989). A single trypanosome is sufficient to infect a tsetse fly.
Annals of Tropical Medicine and Parasitology. **83** (4): 431-433.

Maudlin, I., Welburn, S.C., and Mehlitz, D. (1990a). The relationship between ricketsia - like - organisms and trypanosome infections in natural populations of tsetse in Liberia.
Tropical Medicine and Parasitology. **41**: 265-267.

Maudlin, I., Welburn, S.C., and Milligan, P. (1990b). Salivary gland infection : a sex-linked recessive character in tsetse?
Acta Tropica. **48**: 9-15.

Maudlin, I. Welburn, S.C., Gashumba, J.K., Okuna, N. and Kalunda, M. (1990c). The role of cattle in the epidemiology of sleeping sickness.
Proceedings of the VII International congress of parasitology, Paris bulletin de la Société Français de Parasitologie. **8** (Supplement 2): 788.

Mbulamberi, D.B. (1990). Recent epidemic outbreaks of Human Trypanosomiasis in Uganda.
Insect Science and its Application. **11** (3): 289-292.

Mead, R and Curnow, R.N. (1983). *Statistical methods in agriculture and experimental biology.*
Chapman and Hall, London.

Mehlitz, D. and Deindl, E. (1972). Serological studies on cattle experimentally infected with African trypanosomes.
Tropenmedizin und Parasitologie. **23**: 411-417.

Mehlitz, D. (1977). The behaviour in the blood incubation infectivity test of four *trypanozoon* strains isolated from pigs in Liberia. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* **71**: 86.

Mehlitz, D. and Zillmann, U. (1983). Recent investigation on the animal reservoir of Gambian sleeping sickness. *Proceedings of the 17th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Arusha, Tanzania. OAU/STRC publication No.* **112**: 157-162.

Mehlitz, D., Zilmann, U. and Sachs, R. (1984). Further evidence of animal reservoir host of gambiense sleeping sickness: mixed populations of *Trypanosoma brucei gambiense* and *T. brucei* spp. in naturally infected pigs in the Ivory Coast. *Proceedings of the 18th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Harare, Zimbabwe. OAU/STRC publication No.* **113**: 128-129.

Mehlitz, D. (1987). Animal reservoir hosts of sleeping sickness, an overview. *Proceedings of the 19th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Lome, Togo. OAU/STRC publication No.* **114**: 213-219.

Molyneux, D.H. (1975). Diagnostic methods in animal trypanosomiasis. *Veterinary Parasitology.* **1**:5-17, 1975.

Molyneux, D.H. (1977). Vector relationships in the Trypanosomatidae. *Advances in Parasitology.* 15:1-82.

Molyneux, D.H. and Ashford, R.W. (1983). *The biology of Trypanosoma and Leishmania, Parasites of man and domestic animals.* Taylor and Francis, London.

Moloo, S.K., Losos, G.J., and Kutusa, S.B. (1973). Transmission of *Trypanosoma brucei* to cats and dogs feeding on infected goats. *Annals of Tropical Medicine and Parasitology.* **67** (3): 331-334.

Moloo, S.K. and Kutuza, S.B. (1984). Vectorial capacity of gamma-irradiated sterile male *Glossina morsitans centralis, G. austeni* and *G. tachynoides* for pathogenic *Trypanosoma* species. *Insect Science and its Application.* **5** (5): 411-414.

Morris, K.R.S. and Morris, M.G. (1949). The use of traps against tsetse in West Africa. *Bulletin of Entomological Research.* **39**: 491-528.

Morris, K.R..S and Morris, M.G. (1949). The persistence of toxicity in DDT impregnated hessian and its use on tsetse traps.
Bulletin of Entomological Research. **41**: 259-288.

Morrison, W.I., Murray, M., and McIntyre, W.M. (1981). Diseases of cattle in the tropics : economic and zoonotic relevance.
Current Topics in Veterinary Medicine and Animal Science. **6**: 469-497.

Morrison, W.I., Murray, M., and Sayer, P.D. (1981). The pathogenesis of experimentally induced *Trypanosoma brucei* in the dog I tissue and organ damage.
American Journal of Pathology. **102**: 168-181.

Morrison, W.I., Wells, P.W., Moloo, S.K., Paris, J. and Murray, M. (1982). Interference in the establishment of superinfections with *Trypanosoma congolense* in cattle.
Journal of Parasitology. **68** (5): 755-764.

Morrison, W.I., Murray, M., Whitelaw, D.D. and Sayer, P.D. (1983). Pathology of infection with *Trypanosoma brucei*: Disease syndromes in dogs and cattle resulting from severe tissue damage.
Contributions to Microbiology and Immunology. **7**: 103-119.

Mukiria, P., Okech, G. and Mwabi, D. (1990). Preliminary tsetse and animal trypanosomiasis survey report. Busia District 17th April- South Teso May 1990.
KETRI Internal Report, Kenya Trypanosomiasis Research Institute, Muguga, Kenya.

Mulligan, H.W. (1970). *The African Trypanosomiases.* (H. W. Mulligan, ed.). George Allen and Unwin, London.

Murray, C.J.L. and Lopez, A.D (1994). Global and regional cause-of-death patterns in 1990.
Bulletin of the World Health Organisation. **72** (3): 447-480.

Murray, M. (1974) .*The pathology of African trypanosomiases.*
In: Progress in immunology II. (L. Brent and J. Holborow, eds.) North-Holland, Amsterdam. vol. 4, pp. 181-192.

Murray, M., Murray, P.K., and McIntyre, W.M. (1977). An improved parasitological technique for the diagnosis of African trypanosomiasis.
Transactions of the Royal Society of Tropical Medicine and Hygiene. **71** (4): 325-326.

Murray, M., Morrison, W.I., and Murray, P.K. (1979). Trypanotolerance : a review.. *World Animal Review.* **31**: 2-12.

Murray, M., Morrison, W.I., and Whitelaw, D.D. (1982). Host susceptibility to African trypanosomiases : trypanotolerance. *Advances in Parasitology.* **21**: 1-68.

Murray, M. and Dexter, T.M. (1988). Anaemia in bovine African trypanosomiasis. *Acta Tropica.* **45**:389-432.

Mwambu, P.M. and Ochola, J.L. (1975). Cases of central nervous system involvement in trypanosomiasis of experimentally infected and treated cattle. *East African Journal of Medical Research.* **2** (special issue) *Trypanosomiasis:* 41.

Mwambu, P.M. (1977). The symptomatology of experimental *Trypanosoma brucei* species infection in dogs as observed at EATRO. *Proceedings of the 15th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Bagui, Central African Republic.* pp. 427-440.

Mwangelwa, M.I., Dransfield, R.D., Otieno, L.H. and Mbata, K.J. (1990). Distribution and diel activity patterns of *Glossina fuscipes fuscipes* Newstead on Rusinga Island and mainland in Mbita, Kenya. *Insect Science and its Application.* **11** (3): 315-321.

Nantulya, V.M. and Lindqvist, K.J. (1989). Antigen detection enzyme immunoassays for the diagnosis of *Trypanosoma vivax, T. congolense* and *T. brucei* infection in cattle. *Tropical Medical Parasitology.* **40**: 267-272.

Nash, T.A.M. (1940). The effect upon *Glossina* of changing the climate in the true habitat by partial clearing of vegetation. *Bulletin of Entomological Research.* **31**:69-84.

Nash, T.A.M. (1944). Low density of tsetse flies associated with a high incidence of sleeping sickness. *Bulletin of Entomological Research.* **35**:51.

Nash, T.A.M. (1948). *Tsetse flies in British West Africa.* HMSO, London.

Nash, T.A.M. (1960). A review of the African trypanosomiasis problem. *Tropical Disease Bulletin.* **57**:973-1003.

Nash, T.A.M. (1978). A review of mainly entomological research which has aided the understanding of human trypanosomiasis and its control. *Symposium proceedings. Medical Entomology Centenary. 23-25 November 1977. Royal Society of Tropical Medicine and Hygiene, London.*

Ndegwa, P.N., Irungu, L.W. and Moloo, S.K. (1992). Effect of puparia incubation temperature: Increased infection rate of *Trypanosoma congolense* in *Glossina morsitans centralis, Glossina f. fuscipes* and *G. brevipalpis. Medical and Veterinary Entomology.* **6**: 127-130.

Ndung'u, J.M. (1990). *Clinical and immunopathological aspects of heart damage in dogs infected with Trypanosoma brucei.* PhD thesis, University of Glasgow, Department. of Veterinary Medicine.

Nicholson, M.J. and Butterworth, M.H. (1986). *A guide to condition scoring of Zebu cattle.* ILCA, Addis Ababa, Ethiopia.

Njogu, A.R., Dolan, R.B., Sayer, P.D., Wilson, A.J., and Alashula, H (1985). Strategic chemoprophylaxis for the control of bovine trypanosomiasis. *Proceedings of the 18th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Harare, Zimbabwe. OAU/STRC publication No.* **113**: 199-204.

Okech, G., Mukiria, P.W., Mwabi, D.L.L., Mgutu, S.P., Kwena, M., Kundu, P., Odhiambo, J., Lukhango, J., Nduta, J., Kiboy, M. and Njogu, A.R. (1990). High incidence of trypanozoon in village cattle during an outbreak of Human African Trypanosomiasis (HAT)in Busia District, Kenya. *KETRI Internal Report, Kenya Trypanosomiasis Research Institute, Muguga, Kenya.*

Okoth, J.O. (1986). Peridomestic breeding sites of *Glossina fuscipes fuscipes* Newst. in Busoga Uganda, and epidemiological implications for trypanosomiasis. *Acta Tropica.* **43** (3):283-286.

Okoth, J.O. and Kapaata, R. (1986). Trypanosome infection rates in *Glossina fuscipes fuscipes* Nest. in Busoga sleeping sickness focus Uganda. *Annals of Tropical Medicine and Parasitology.* **80** (4):459-461.

Okoth, J.O. and Kapaata, R. (1988). Hosts of *Glossina fuscipes fuscipes* in Busoga Uganda. *Annals of Tropical Medicine and Parasitology.* **82** (5): 517-518.

Okoth, J. O., Okech, V. and Ogola, A. (1991). Control of tsetse and trypanosomiasis transmission in Uganda by applications of lambda-cyhalothrin. *Medical and Veterinary Entomology.* **5**: 121-128.

Okuna, N.M. and Mayende, J.S.P. (1981). The incidence of trypanosomiasis in domestic animals and their role in the sleeping sickness epidemic in Busoga.
Proceedings of the 17th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Arusha Tanzania. OAU/STRC publication No. **112**: 163-167.

Okuna, N.M., Mayende, J.S.P. and Guloba, A. (1986). *Trypanosoma brucei* infection in domestic pigs in a sleeping sickness epidemic area of Uganda.
Acta Tropica. **43**: 183-184.

Okuna, N.M., Enyaru, J.C.K., Mayende, C.J.P. and Serwada, F.K. (1993). Trypanosomiasis in pigs in south-eastern Uganda, a sleeping sickness endemic area, and its control by isometamidium chloride (Samorin).
In: *OAU/SCTRC* **17** (No. 8321): 184-185.

Olahu-Mukani, W. and Nyang'ao, J.M.N. (1993).Evaluation of Suratex® for the field diagnosis of *Trypanosoma evansi* infections in camels.
Proceedings of the. 22nd Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Kampala, Uganda. OAU/STRC publication No. **117**: 38-41.

Olahu-Mukani, W., Nyang'ao, J.M.N., Tengekyon, K.M. and Omuse, J.K. (1993). Evaluation of the card indirect agglutination test for trypanosomiasis using stored sera from *Trypanosoma brucei rhodesiense* infected patients.
Proceedings of the. 22nd Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Kampala, Uganda. OAU/STRC publication No. **117**: 43-45.

Onyango, R.J., van Hoeve, K., and de Raadt, P. (1966). The epidemiology of *Trypanosoma rhodesiense* sleeping sickness in Alego location, central Nyanza Kenya I evidence that cattle may act as reservoir hosts of trypanosomes infective to man.
Transactions of the Royal Society for Tropical Medicine and Hygiene. **60** (2): 175-182.

Onah, D.N. (1991). Porcine trypanosomiasis in Nigeria: infections in local and exotic pigs in the Nsukka area of Anambra State.
Tropical Animal Health and Production. **23**: 141-146.

Ormerod, W.E. (1960). Cell inclusions and the epidemiology of rhodesian sleeping sickness.
Transactions of the Royal Society of Tropical Medicine and Hygiene. **54**: 299.

Ormerod, W.E. (1970) Pathogenesis and pathology of trypanosomiasis in man.
In: *The African Trypanosomiases,* (H.W. Mulligan, ed.)
George Allen and Unwin, London. pp. 587-601.

Otesile, E.B., Fagbemi, B.O. and Adeyemo, O. (1991).The effect of *Trypanosoma brucei infection* on serum biochemical parameters in boars on different planes of dietary energy.
Veterinary Parasitology. **40**: 207-216.

Owen, J.S. and Gillet, M.P.T. (1992). Cytotoxic effects of human plasma on *Trypanosoma brucei brucei*: insights and confusion from studies in cirrhotic patients, in baboon and in transgenic mice.
Annales de la Société Bèlge de Medicine Tropicale. **72** (Suppl. 1): 94-95.

Pagot, J. (1974.)Les races trypanotolerantes.
In: *Les moyens de lutte contre les trypanosomes et leurs vecteurs.* Actes du Colloque, Paris. pp. 235-248.

Paris, J., Murray, M., and McOdimba, F (1982). A comparative evaluation of the parasitological techniques available for the diagnosis of African trypanosomiasis in cattle.
Acta Tropica. **39**: 3 07-312.

Peregrine, A.S., Moloo, S.K., and Whitelaw, D.D. (1987). Therapeutic and prophylactic activity of isometamidium chloride in Boran cattle against *Trypanosoma vivax* transmitted by *Glossina morsitans centralis.*
Research in Veterinary Science. **43**:268.

Peruzzi, M.R.I. (1928). Pathologico-anatomical and serological observations on trypanosomiasis .
Final Report League of Nations International Committee on Human Trypanosomiasis: 245-328.

Phelps, R.J. and Vale, J.A. (1978). Studies on populations of *Glossina morsitans morsitans* and *Glossina pallidipes* (Diptera: Glossinidae) in Rhodesia.
Journal of Applied Ecology. **15**: 743-760.

Pinder, M. and Authie, E. (1984). The appearance of isometamidium resistant *Trypanosoma congolense* in West Africa.
Acta Tropica. **41**:247-252.

Politzar, H. and Cuissance, D. (1984). An integrated campaign against riverine tsetse, *Glossina palpalis gambiensis and Glossina tachynoides*, by trapping and the release of sterile males.
Proceedings of the 18th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Mombassa, Kenya. OAU/STRC publication No. **113**: 439-443.

Poltera, A.A., Cox, J.N., and Owor, R. (1976) Pancarditis involving the conducting system and all valves in human African trypanosomiasis.
British Heart Journal. **38**: 827-837.

Poltera, A.A. (1985). Pathology of African human trypanosomiasis with reference to experimental African trypanosomiasis and infection of the central nervous system.
British Medical Bulletin. **41** (2):169-174.

Putt, S.N.H., Shaw, A.P.M., Woods, A.J., Tyler, L. and James, A.D. (1987). Veterinary epidemiology and economics in Africa: A manual for use in the design an appraisal of livestock health policy.
ILCA Manual No 3, International Livestock Centre for Africa, Addis Ababa, Ethiopia.

de Raadt, P. (1993). WHO presentation
Proceedings of the *22nd meeting International Science Council for Trypanosomiasis Research and Control (ISCTRC),Kampala, Uganda. OAU/STRC publication No.* **117**: 19.

Richardson, U.F. (1928). Notes in trypanosomiasis of cattle in Uganda.
Transactions of the Royal Society of Tropical Medicine and Hygiene. **22**: 137-146.

Rickman, L.R. and Robson, J. (1970a). The blood incubation infectivity test : a simple test which may serve to distinguish *Trypanosoma brucei* from *T. rhodesiense.*
Bulletin of the World Health Organisation. **42**: 650-651.

Rickman, L.R. and Robson, J. (1970b). The testing of proven *Trypanosoma brucei* and *T. rhodesiense* strains by the blood incubation infectivity test..
Bulletin of the World Health Organisation. **42**: 911-916.

Rickman, L.R. and Robson, J (1971). Some supplementary observations on the blood incubation infectivity test.
Bulletin of the World Health Organisation. **43**:403-404.

Roberts, C.J., Gray, M.A., and Gray, A.R (1969). Local skin reaction in cattle at the site of infection with *Trypanosoma congolense by Glossina morsitans* and *G. tachynoides.*
Transactions of the Royal Society of Tropical Medicine and Hygiene. **63**: 620-624.

Roberts, C.J. and Gray, A.R. (1973). Studies on trypanosome resistant cattle II the effect of trypanosomiasis on N'dama, Mutute and Zebu cattle.
Tropical Animal Health and Production. **5**: 211-219.

Robertson, D.H.H. (1963). The treatment of sleeping sickness (mainly due to *Trypanosoma rhodesiense*) with melarsoprol 1. reactions observed during treatment.
Transactions of the Royal Society for Tropical Medicine and Hygiene. **57**: 122-133.

Robins-Browne, R.M., Schneider, J., and Metz, J. (1975). Thrombocytopaenia in trypanosomiasis.
American Journal of Tropical Medicine and Hygiene. **24**: 226-231.

Robson, J. (1962). Prophylaxis against trypanosomiasis in zebu cattle - iv a field trial of metamidium and isometamidium.
Veterinary Record. **74:** 913-916.

Robson, J. and Ashkar, T.S. (1972). Trypanosomiasis in domestic livestock in the Lambwe valley area and a field evaluation of various diagnostic techniques.
Bulletin of the World Health Organisation. **42**: 727-734.

Roderick, S. (1995). *Pastoral cattle productivity in a tsetse infested area of South Western Kenya.*
PhD thesis, Department of Agriculture, University of Reading.

Rogers, D.J. (1988). A general model for the African trypanosomiasis.
Parasitology. **97**: 193-212.

Roth, H.H. (1973). Game conservation and trypanosomiasis.
British Veterinary Journal. **129**:407-413.

Sachs, R., Schaller, G.B., and Schindler, R. (1971). Untersuchungen uber das vorkommen von trypanosoma bei vildkarnivoren des serengeti national parks in Tanzania.
Acta Tropica. **28**: 323-328.

Scott, D. (1970). The epidemiology of Gambian sleeping sickness.
In: *The African Trypanosomiases,*(H.W. Mulligan, ed.)
George Allen and Unwin, London.

Scott, P.(1993). Collection and interpretation of cerebrospinal fluid in ruminants.
In Practice. **15** (6): 298-300.

Sekoni, V.O. (1994). Reproductive disorders caused by animal trypanosomiasis: a review.
Theriogenology. **42**: 557-570.

Skinner, J.D. (1970). Game ranching in Africa as a source of meat for local consumption and export.
Tropical Animal Health and Production. **2**: 151-157.

Soltys, M.A. (1971). Epidemiology of trypanosomiasis in man.
Tropenmedizin und Parasitologie. **22**: 120-133.

Sones, K.R., Njogu, A.R., and Holmes, P.H. (1988). Assessment of sensitivity of *Trypanosoma congolense* to isometamidium chloride : a comparison of tests using cattle and mice.
Acta Tropica. **45**: 153-164.

Sones, K.R., Holmes, P.H., and Urquart, G.M. (1989). Interference between drug-resistant and drug-sensitive stocks of *Trypanosoma congolense* in goats.
Research in Veterinary Science. **47**: 75-77.

Soulsby, E.J.L. (1982). *Helminths arthropods and protozoa of domesticated animals.*
Baillière and Tindall, London.

Southon, H.A.W. and Robertson, D.H.H. (1961). The isolation of *T. rhodesiense* from *G., palpalis.*
Nature. **189**: 411.

Southon, H.A.W. (1963). The hosts of *Glossina* in South Busoga, Uganda.
EATRO Report. January-December 1961, East African Trypanosomiasis Research Organisation, Tororo, Uganda.

Stephen, L.E. (1962). Some observations on the behaviour of trypanosomes occurring in cattle previously treated with prophylactic drugs.
Annals of Tropical Medicine and Parasitology. **56**: 415-421.

Stephen, L.E. (1966). Pig trypanosomiasis in Africa.
Review series 8 of the Commonwealth Bureaux of Animal Health,
Commonwealth Agriculture Bureaux, London.

Stephen, L.E. (1970). Clinical manifestations of the trypanosomiases in livestock and other domestic animals.
In: *The African Trypanosomiases,* (H.W. Mulligan ed.)
George Allen and Unwin, London.

Stevens, J.R. and Welburn, S.C. (1993). Genetic processes within an epidemic of sleeping sickness in Uganda.
Parasitology Research. **79**: 421-427.

Stevenson, P., Munga, L. and Dolan, R.B. (1993). The detrimental effects of frequent treatment of cattle with trypanocidal drugs.
Proceedings of the 22nd Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Kampala, Uganda. OAU/STRC publication No. **117**: 130-135.

Stewart, J.L. (1937). The cattle of the Gold Coast.
Veterinary Record. **49**: 1289-1297.

Stewart, J.L. (1951). The West African shorthorn cattle : their value to Africa as trypanosomiasis-resistant animals.
Veterinary Record. **63**: 454-457.

Tait, A. (1981). Ploidy and mating in trypanosomes.
Transactions of the Royal Society for Tropical Medicine and Hygiene. **75**: 330.

Taylor, A.W. (1930). Experiments on the mechanical transmission of West African strains of *Trypanosoma brucei* and *T. gambiense* by glossina and other biting flies.
Transactions of the Royal Society for Tropical Medicine and Hygiene. **24**: 289-303.

Taylor, A.E.R. and Smith, V. (1983). Micro - counterimmunoelectrophoresis : a rapid screening technique for trypanosomiasis
Transactions of the Royal Society for Tropical Medicine and Hygiene. **77**: 481-486.

Thomson, J.W., and Wilson, A. (1992.). The control of tsetse flies and trypanosomiasis by the application of deltamethrin to cattle.
Bulletin of Animal Production in Africa. **40**: 5-8.

Thomson, M.C. (1987). The effect on tsetse flies (*Glossina spp*) of deltamethrin applied to cattle either as a spray or incorporated into ear tags.
Tropical Pest Management. **33** (4): 329-335.

Tizard, I.R. and Soltys, M.A. (1971). Cell mediated hypersensitivity in rabbits infected with *Trypanosoma brucei and Trypanosoma rhodesiense*
Infection and Immunity. **4**:674-677.

Tracey, M.V (1955). Chitin.
In: *Modern methods of plant analysis.* (K. Peak and M.V. Tracey eds.) Springer-Verlag, Berlin. pp. 264-274.

Trail, J.C.M., Sones, K., Jibbo, J.M.C., Durkin, J., Light, D.E., and Murray, M. (1985). Productivity of Boran cattle maintained by chemoprophylaxis under trypanosomiasis risk.
ILCA Research Report No. 9 International Livestock centre for Africa, Addis Ababa, Ethiopia.

Turner, C.M.R., Aslam, N. and Angus, S.D. (1996). Inhibition of growth of *Trypanosoma brucei* parasites in chronic infections.
Parasitology Research. **82**: 61-66.

Vale, G.A. (1974). The response of tsetse flies Diptera : Glossinidae to mobile and stationary baits.
Bulletin of Entomological Research. **64**: 545-588.

Vale, G.A. and Cumming, D.H.M. (1976). The effects of selective elimination of hosts on a population of tsetse flies (*Glossina morsitans morsitans* Westood (Diptera, Glossinidae).
Bulletin of Entomological Research. **66**: 713-729.

Vale, G.A. (1980). Field studies on the response of tsetse flies (Glossinidae) and other Diptera to carbon dioxide acetone and other chemicals.
Bulletin of Entomological Research. **70**: 563-570.

Vale, G.A. and Hall, D.R. (1985). The role of 1 - octen3 ol, acetone and carbon dioxide in the attraction of tsetse flies, *Glossina* spp (Diptera : Glossinidae), to ox odour.
Bulletin of Entomological Research. **75**: 209-217.

Vale, G.A., Hall, D.R., and Gouch, A.J.E. (1988). The olfactory responses of tsetse flies, *Glossina* spp (Diptera : Glossinidae) to phenols and urine in the field.
Bulletin of Entomological Research. **78**: 293-300.

van Hoeve, K. and Cunningham, M.P. (1964). Prophylactic activity of berenil against trypanosomes in treated cattle.
Veterinary Record. **76**: 260.

Voller, A., Bidwell, D., and Bartlett, A. (1975). A serological study on human *Trypanosoma rhodesiense* infections using a micro-scale enzyme linked immunosorbent assay.
Tropenmedizin und Parasitologie. **26**: 247-251.

Walker, P.J. (1972). Capillary conc. technique applicable to infections of *T. congolense* in cattle.
Transactions of the Royal Society of Tropical Medicine and Hygiene. **66**: 348.

Wayne Martin, S., Meek, A. H. and Willenberg, P. (1987). *Veterinary Epidemiology Principals and Methods.*
Iowa State University Press, Ames, Iowa, USA.

Weitz, B. (1963). The feeding habits of *Glossina.*
Bulletin of the World Health Organisation. **28**: 711-729.

Welburn, S.C. and Maudlin, I. Lectin (1989). Signalling of maturation of *T. congolense* infections in tsetse.
Medical and Veterinary Entomology. **3**: 141-145.

Welburn, S.C., and Maudlin, I. (1991). Rickettsia-like organisms, puparial temperature and susceptibility to trypanosome infection in *Glossina morsitans.*
Parasitology. **102**: 201-206.

Welburn, S.C., Maudlin, I. and Milligan, P.J.M. (1995). *Trypanozoon*: Infectivity to humans in linked to reduced transmissibility in tsetse
Experimental Parasitology. **81**: 404-408.

Wellde, B.T., Duxbury, R.E., Sadun, E.H., Langbehn, H.R., Lotsch, R., Diendl, G.F. and Warui, G.M. (1973). Experimental infections with African trypanosomes: IV. Immunisation of cattle with gamma-irradiated *Trypanosoma rhodesiense.*
Experimental Parasitology. **34**: 62-68.

Wellde, B.T., Chumo, D.A., Adoyo, M., Kovatch, R.M., Mwongela, G.N and Opiyo, E.A. (1983). Haemorrhagic syndrome in cattle associated with *Trypanosoma vivax* infection.
Tropical Animal Health and Production. **15**: 95-102.

Wellde, B.T., Chumo, D.A., Reardon, M.J., Waema, D., Smith, D.H., Gibson, W.C., Wanyama, L. and Siongok, T.A. (1989a). Epidemiology of Rhodesian sleeping sickness in the Lambwe Valley, Kenya.
Annals of Tropical Medicine and Parasitology. **83**: (Suppl. 1) 43-62.

Wellde, B.T., Reardon, M.J., Kovatch, R.M., Chumo, D.A., Williams, J.S., Boyce, W.L., Hockmeyer, W.T. and Wykoff, D.E. (1989b). Experimental infection of cattle with Trypanosoma brucei rhodesiense.
Annals of Tropical Medicine and Parasitology. **83**: (Suppl. 1) 133-150.

Wellde, B.T., Reardon, M.J., Chumo, D.A., Kovatch, R.M., Waema, D., Wykoff, D.E. Mwangi, J., BoyceW.L. and Williams J.S. (1989c). Cerebral trypanosomiasis in naturally-infected cattle in the Lambwe Valley, South Nyanza, Kenya.
Annals of Tropical Medicine and Parasitology. **83**: (Suppl. 1) 151-160.

Wellde, B.T., Reardon, M.J., Chumo, D.A., Muriithi, R.M., Towett, S. and Mwangi, J. (1989d). Experimental infection of goats with *Trypanosoma brucei* ssp. and effects of treatment with suramin and Mel-B.
Annals of Tropical Medicine and Parasitology. **83**: (Suppl. 1) 161-169.

Wellde, B.T., Chumo, D.A., Onyango, F.K., Reardon, M.J., Roberts, L.M., Njogu, A.G. and Opiyo, E.A. (1989e). *Trypanosoma vivax*: disseminated intravascular coagulation in cattle.
Annals of Tropical Medicine and Parasitology. **83**: (Suppl. 1) 177-183.

Wells, E.A. (1972). The importance of mechanical transmission in the epidemiology of nagana : a review.
Tropical Animal Health and Production. **4**: 74-88.

Werner, R. (1954). Uber die Frage der placentaren trypanosomen infektioner und ubertrangung von trypanosomen und antikorpern durch die milch auf das neugeborene.
Tropenmedizin und Parasitologie. **9**: 17.

Werner, H.(1955). Beobachtungen uber den einflub von trypanosoman infectionen auf die embryonalentwicklung von weiben mausen und goldhamstern. *Zeitschrift für Prasitenkunde.* **6**: 150-158.

Whitelaw, D.D., Moulton, J.E., Morrison, W.I. and Murray, M. (1985). Central nervous system involvement in goats undergoing primary infections with *Trypanosoma brucei* and relapse infections after chemotherapy . *Parasitology.* **90**: 255-268.

Whitelaw, D.D., Bell, I.R., Holmes, P.H., Moloo, S.K., Girmi, H., Urquart, G.M., and Murray, M. (1986). Isometamidium chloride prophylaxis against *Trypanosoma congolense* challenge and the development of immune responses in Boran cattle. *Veterinary Record.* **118**: 722-726.

Whitelaw, D.D., Gardiner, P.R., and Murray, M. (1988). Extravascular foci of *Trypanosoma vivax* in goats : the central nervous system and aqueous humour of the eye as potential sources of relapse infection after chemotherapy. *Parasitology.* **97**: 51-61.

Whiteside, E.F. (1962a). Interactions between drugs, trypanosomes and cattle in the field. *Drugs Parasites and Hosts.* 116-141.

Whiteside, E.F. (1962b). The control of cattle trypanosomiasis with drugs in Kenya : methods and costs. *East African Agricultural Journal.* **28**: 68-73.,

Wijers, D.J.B. (1958). Factors that may influence the infection rate of *Glossina palpalis* with *Trypanosoma gambiense* 1 - the age of the fly at the time of the infected feed. *Annals of Tropical Medicine and Parasitology.* **52**: 385-390.

Wijers, D.J.B. and Willet, K.C. (1960). Factors that may influence the infection rate of *Glossina palpalis* with *Trypanosoma gambiense* II the number and the morphology of the trypanosomes present in the blood of the host at the time of the infected feed. *Annals of Tropical Medicine and Parasitology.* **54**: 431-350.

Willett, K.C. (1965). Some observations on the recent epidemiology of sleeping sickness in Ngaga region and its relation to the general epidemiology of Gambian and rhodesian sleeping sickness in Africa. *Transactions of the Royal Society for Tropical Medicine and Hygiene.* **59**: 374.

Willett, K.C.(1972). An observation on the unexpected frequency of some multiple infections. *Bulletin of the World Health Organisation.* **47**: 747-749.

Williams, A.J., Young, J.R., and Majiwa, P.A.O. (1979). Genomic arrangements correlated with antigenic variation in *Trypanosoma brucei*. *Nature*. **282**: 847-849.

Williamson, J. (1970). Review of chemotherapeutic and chemoprophylaxctic agents. In: *The African Trypanosomiases*. (H.W. Mulligan, ed.). George Allen and Unwin, London. pp. 125-221.

Wilson, A.J. (1969). Value of the indirect fluorescent antibody test as a serological aid to diagnosis of *Glossina* transmitted bovine trypanosomiasis. *Tropical Animal Health and Production*. **1**:89-95.

Wilson, A.J., Paris, J., Luckins, A.G., Dar, F.K., and Gray, A.R. (1976). Observations on a herd of beef cattle maintained in a tsetse area. II assessment of the development of immunity in association with trypanocidal drug treatment. *Tropical Animal Health and Production*. **8** (1):1-11.

Woo, P.T.K. (1970). The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Tropica*. **27**:384-386.

Woodruff, A.W., Ziegler, J.L., Hathaway, A., and Gwata, T.(1973). Anaemia in African trypanosomiasis and 'big spleen' disease in Uganda. *Transactions of the Royal Society for Tropical Medicine and Hygiene*. **67**: 329-337

Woof, W.R. (1968). The eradication of the tsetse *Glossina morsitans* Westw. and *Glossina pallidipes* Aust by hunting. *Proceedings of the 12th Meeting of the International Scientific council for Trypanosomiasis Research and Control (ISCTRC), Bangui, Central African Republic*. pp. 267-286.

Zillman, U. and Mehlitz, D. (1979). Natural occurrence of *Trypanozoon* in domestic chicken in the Ivory Coast. *Tropenmedizin und Parasitologie*. **30**: 244-248.

Zwart, T., Perie, N.M., Keppler, A., and Goedbloed, E. (1973). A comparison of methods for the diagnosis of trypanosomiasis in East African domestic ruminants. *Tropical Animal Health and Production*. **5**: 79-87.

Zweygarth, E. and Kaminsky, R.(1991). Evaluation of DL-alpha-difluoromethylornithine against susceptible and drug-resistant *Trypanosoma brucei. brucei*. *Acta Tropica*. **48**: 223-232.

Appendix II

Appendix II

Table II.1 Sex and age distribution of cases of sleeping sickness in Busia District

sex\age	0-9	10-19	20-29	30-39	40-49	50-59	> 60	total
male	15 (10.2)	14 (14.8)	10 (13.2)	13 (14.3)	13 (14.3)	14 (13.8)	5 (3.6)	84
female	5 (9.8)	15 (14.2)	16 (12.8)	15 (13.8)	14 (13.3)	14 (13.8)	2 (3.4)	81
total	20	29	26	28	27	28	7	165

( ) expected values

($X^2 = 7.83$, df = 6, p = 0.252)

Table II.1 Age distribution of cases of sleeping sickness in Busia District

age	0-9	10-19	20-29	30-39	40-49	50-59	> 60	total
ss *	20 (57.2)	29 (41.8)	26 (22.8)	28 (14.8)	27 (12.5)	28 (8.2)	7 (7.8)	165
pop **	103,249	75,370	41,158	26,614	22,472	14,704	14,108	297,675
total	103,269	75,399	41,184	26,642	22,499	14,732	14,115	297,840

( ) expected values

($X^2 = 105.75$, df = 6, $p < 0.001$)

* ss = cases of human sleeping sickness

** pop = human population of Busia District (1979)

Appendix IV

Appendix IV

Table IV.1 The proportion of the total number of trypanosome infections in cattle involving a *T. brucei* spp. infection before chemoprophylaxis in Katelenyang and Apatit villages (Samorin treated and control)

village period	Katelenyang village (Samorin treated)	Apatit village (Samorin control)
number of *T. brucei* spp. infections before chemoprophylaxis	80 (77.46)	84 (86.54)
number of other trypanosome infections before chemoprophylaxis	82 (84.54)	97 (94.46)

( ) expected values
$X^2 = 0.30$, df = 1, p = 0.58

Table IV.2 The proportion of the total number of trypanosome infections in cattle involving a *T. brucei* spp. infection following treatment in Katelenyang and Apatit villages (Samorin treated and control)

village period	Katelenyang village (Samorin treated)	Apatit village (Samorin control)
number of *T. brucei* spp. infections following treatment	10 (15.13)	166 (160.87)
number of other trypanosome infections following treatment	20 (14.87)	153 (158.13)

( ) expected values
$X^2 = 3.84$, df = 1, p = 0.05

Table IV.3 The proportion of the totalnumber of trypanosome infections in cattle involving a *T. vivax* infection before chemoprophylaxis in Katelenyang and Apatit villages (Samorin treated and control)

village period	Katelenyang village (Samorin treated)	Apatit village (Samorin control)
number of *T. vivax* infections before chemoprophylaxis	125 (111.46)	111 (124.54)
number of other trypanosome infections before time of prophylaxis	37 (50.54)	70 (56.46)

( ) expected values
$X^2 = 9.99$, df = 1, p = 0.002

Table IV.4 The proportion of the total number of trypanosome infections in cattle involving a *T. vivax* infection following treatment in Katelenyang and Apatit villages (Samorin treated and control)

village period	Katelenyang village (Samorin treated)	Apatit village (Samorin control)
number of *T. vivax* infections following treatment	18 (13.90)	190 (194.10)
number of other trypanosome infections following treatment	12 (16.10)	229 (224.9)

( ) expected values
X^2 =2.42, df = 1, p = 0.12

Table IV.5 The proportion of the total number of trypanosome infections in cattle involving a *T. congolense* infection before chemoprophylaxis in Katelenyang and Apatit villages (Samorin treated and control)

village period	Katelenyang village (Samorin treated)	Apatit village (Samorin control)
number of *T. congolense* infections before chemoprophylaxis	23 (27.39)	35 (30.61)
number of other trypanosome infections before chemoprophylaxis	139 (134.61)	146 (150.39)

( ) expected values
X^2 = 1.15, df = 1, p = 0.285

Table IV.6 The proportion of the total number of trypanosome infections in cattle involving a *T. congolense* infection following treatment in Katelenyang and Apatit villages (Samorin treated and control)

village period	Katelenyang village (Samorin treated)	Apatit village (Samorin control)
number of *T. congolense* infections after following treatment	11 (7.48)	76 (79.52
number of other trypanosome infections following treatment	19 (22.52)	243 (239.48)

( ) expected values
X^2 = 2.42, df = 1, p = 0.12

Table IV.7 The proportion of the totalnumber of trypanosome infections in cattle involving a *T. brucei* spp. infection before chemoprophylaxis in Rukada and Ngelechom villages (Ethidium treated and control)

village period	Rukada (Ethidium treated)	Ngelechom (Ethidium control)
number of *T. brucei* spp. infections before chemoprophylaxis	58 (42.41)	32 (47.59)
number of other trypanosome infections before chemoprophylaxis	32 (47.59)	69 (53.41)

( ) expected values
$X^2 = 20.50$, df = 1, $p < 0.001$

Table IV.8 The proportion of the total number of trypanosome infections in cattle involving a *T. brucei* spp. infection following treatment in Rukada and Ngelechom villages (Ethidium treated and control)

village period	Rukada (Ethidium treated)	Ngelechom (Ethidium control)
number of *T. brucei* spp. infections following treatment	12 (7.55)	47 (51.45)
number of other trypanosome infections following treatment	9 (13.45)	96 (91.55)

( ) expected values
$X^2 = 4.69$, df = 1, $p = 0.03$

Table IV.9 The proportion of the total number of trypanosome infections in cattle involving a *T. vivax* infection before chemoprophylaxis in Rukada and Ngelechom villages (Ethidium treated and control)

village period	Rukada (Ethidium treated)	Ngelechom (Ethidium control)
number of *T. vivax* infections before chemoprophylaxis	61 (73.04)	94 (81.96)
number of other trypanosome infections before chemoprophylaxis	29 (16.96)	7 (19.04)

( ) expected values
$X^2 = 19.90$, df = 1, $p < 0.001$

Table IV.10 The proportion of the total number of trypanosome infections in cattle involving a *T. vivax* infection following treatment in Rukada and Ngelechom villages (Ethidium treated and control)

village period	Rukada (Ethidium treated)	Ngelechom (Ethidium control)
number of *T. vivax* infections following treatment	4 (16.13)	122 (109.87)
number of other trypanosome infections following treatment	17 (4.87)	21 (33.13)

( ) expected values
$X^2 = 45.17$, df = 1, $p < 0.001$

Table IV.11 The proportion of the total number of trypanosome infections in cattle involving a *T. congolense* infection before chemoprophylaxis in Rukada and Ngelechom villages (Ethidium treated and control)

village period	Rukada (Ethidium treated)	Ngelechom (Ethidium control)
number of *T. congolense* infections before chemoprophylaxis	8 (11.31)	16 (12.69)
number of other trypanosome infections before chemoprophylaxis	82 (78.69)	85 (88.31)

( ) expected values
$X^2 = 2.09$, df = 1, $p = 0.148$

Table IV.12 The proportion of the total number of trypanosome infections in cattle involving a *T. congolense* infection following treatment in Rukada and Ngelechom villages (Ethidium treated and control)

village period	Rukada (Ethidium treated)	Ngelechom (Ethidium control)
number of *T. congolense* infections following treatment	10 (3.33)	16 (22.67)
number of other trypanosome infections following treatment	11 (17.67)	127 (120.33)

( ) expected values
$X^2 = 18.22$, df = 1, $p < 0.001$

Table IV.13 The proportion of the total number of trypanosome infections in cattle involving a *T. congolense* infection before chemoprophylaxis in all four villages

village period	Katelenyang (Samorin treated)	Apatit (Samorin control)	Rukada (Ethidium treated)	Ngelechom (Ethidium control)
number of *T. congolense* infections before chemoprophylaxis	23 (24.88)	35 (27.79)	8 (13.82)	16 (15.51)
number of other trypanosome infections before chemoprophylaxis	139 (137.12)	146 (153.21)	82 (76.18)	85 (85.49)

( ) expected values
$X^2 = 5.29$, df = 1, p = 0.153

Table IV.14 The 'Berenil index' before and during chemoprophylaxis in Katelenyang village (Samorin treated)

period	before Samorin prophylaxis	during Samorin prophylaxis
number of curative trypanocidal drugs used	108 (32.3)	18 (93.7)
number of cattle sampled in the period	479 (554.7)	1668 (1612.3)

( ) expected values
$X^2 = 253.0$, df = 1, p = 0.000

Table IV.15 The 'Berenil index' before and during the time of prophylaxis in Apatit village (Samorin control)

period	before time of prophylaxis	during time of prophylaxis
number of curative trypanocidal drugs used	87 (71.6)	135 (150.5)
number of cattle sampled in the period	729 (744.5)	1581 (1565.6)

( ) expected values
$X^2 = 5.4$, df = 1, p = 0.020

Table IV.16 The 'Berenil index' before and after prophylaxis in Rukada village (Ethidium treated)

period	before Ethidium prophylaxis	during Ethidium prophylaxis
number of curative trypanocidal drugs used	11 (12.7)	15 (13.3)
number of cattle sampled in the period	1324 (1322.3)	1391 (1392.7)

( ) expected values
$X^2 = 0.43$, df = 1, p = 0.512

Table IV.17 The 'Berenil index' before and after the time of prophylaxis in Ngelechom village (Ethidium control)

period	before time of prophylaxis	during time of prophylaxis
number of curative trypanocidal drugs used	38 (43.5)	50 (44.5)
number of cattle sampled in the period	1379 (1373.5)	1402 (1407.5)

( ) expected values
$X^2 = 1.4$, df = 1, p = 0.237

Table IV.18 Analysis of covariance of the PCV of cattle (stepwise fitting of regression models)

parameter	SAMORIN				ETHIDIUM			
added	% DEV	d.f.	F	p	% DEV	d.f.	F	p
(none)	0	4476			0	5495		
+ period of prophylaxis	5.84	1	291.05	<0.0001	0.88	1	48.92	<0.0001
+ village	2.65	1	132.38	<0.0001	0.81	1	45.09	<0.0001
+ village x period of prophylaxis	1.81	1	90.22	<0.0001 *	0.02	1	1.19	0.2748
error	89.70	4473			98.3	5491		
total	100	4476			100	5495		

* Highly significant

Table IV.19 The effect of Samorin prophylaxis on the PCV of all cattle sampled in Katelenyang village (Samorin treated) and Apatit village (Samorin control)

period \ village	Katelenyang village (Samorin treated)	Apatit village (Samorin control)	difference in mean PCV between villages
before Samorin prophylaxis	24.17 s.e. 0.237 n = 479	24.83 s.e. 0.202 n = 729	-0.66 t = 2.099 p = 0.0360
during Samorin prophylaxis	28.62 s.e. 0.106 n = 1688	26.09 s.e. 0.130 n = 1581	2.54 t = 15.26 p < 0.0001
difference in mean PCV during the time of drug trial	4.45 t = 18.96 p < 0.0001	1.25 t = 5.33 p < 0.0001	-

Table IV.20 The effect of Samorin prophylaxis on the PCV of uninfected cattle in Katelenyang village (Samorin treated) and Apatit village (Samorin control)

period \ village	Katelenyang village (Samorin treated)	Apatit village (Samorin control)	difference in mean PCV between villages
before Samorin prophylaxis	26.10 s.e. 0.311 n = 211	26.70 s.e. 0.229 n = 405	-0.60 t =1.546 p = 0.1227
during Samorin prophylaxis	28.95 s.e. 0.106 n = 1529	27.75 s.e. 0.138 n = 930	1.20 t = 6.870 p < 0.0001
difference in mean PCV during the time of drug trial	2.85 t = 9.217 p < 0.0001	1.05 t = 4.034 p = 0.0001	-

Table IV.21 The effect of Samorin prophylaxis on the PCV of cattle with trypanosome infections in Katelenyang village (Samorin treated) and Apatit village (Samorin control)

period \ village	Katelenyang village (Samorin treated)	Apatit village (Samorin control)	difference in mean PCV between villages
before Samorin prophylaxis	20.98 s.e. 0.386 n = 162	20.05 s.e. 0.372 n = 171	0.93 t = 1.729 p = 0.848
during Samorin prophylaxis	23.70 s.e. 1.030 n = 30	21.41 s.e. 0.302 n = 319	2.29 t = 2.218 p = 0.0272
difference in mean PCV during the time of drug trial	2.72 t = 2.743 p = 0.0067	1.36 t = 2.761 p = 0.0060	-

Table IV.22 The effect of Samorin prophylaxis on the mean monthly liveweight gain of all cattle sampled in Katelenyang village (Samorin treated) and Apatit village (Samorin control)

period \ village	Katelenyang village (Samorin treated)	Apatit village (Samorin control)	difference in mean monthly weight change between villages
before Samorin prophylaxis	2.35 s.e. 0.468 n = 276	2.37 s.e. 0.391 n = 443	-0.02 t = 0.0374 p = 0.9702
during Samorin prophylaxis	5.01 0.204 n = 1620	3.89 s.e. 0.245 n = 1477	1.12 t = 3.538 p = 0.0004
difference in mean monthly weight change during the time of drug trial	2.67 t = 5.03 p < 0.0001	1.52 t = 3.069 p = 0.0022	-

Table IV.23 The effect of Samorin prophylaxis on the mean monthly liveweight gain of uninfected cattle in Katelenyang village (Samorin treated) and Apatit village (Samorin control)

period \ village	Katelenyang village (Samorin treated)	Apatit village (Samorin control)	difference in mean monthly weight change between villages
before Samorin prophylaxis	4.12 s.e. 0.689 n = 113	3.14 s.e. 0.481 n = 220	0.99 t = 1.19 p = 0.2352
during Samorin prophylaxis	5.17 s.e. 0.213 n = 1465	4.90 s.e. 0.305 n = 866	0.27 t = 0.754 p = 0.4507
difference in mean monthly weight change during the time of drug trial	1.05 t = 1.33 p = 0.1836	1.76 t = 2.71 p = 0.0068	-

Table IV.24 The effect of Samorin prophylaxis on the mean monthly liveweight gain of cattle with trypanosome infections in Katelenyang village (Samorin treated) and Apatit village (Samorin control)

period \ village	Katelenyang village (Samorin treated)	Apatit village (Samorin control)	difference in mean monthly weight change between villages
before Samorin prophylaxis	-0.78 s.e. 0.963 n = 87	-0.83 s.e. 0.848 n = 113	0.05 t = 0.040 p = 0.9678
during Samorin prophylaxis	4.75 s.e. 1.254 n =30	0.04 s.e. 0.607 n = 301	4.7 t = 2.40 p = 0.0168
difference in mean monthly weight change during the time of drug trial	5.52 t = 3.10 p = 0.0025	0.87 t = 0.777 p = 0.4376	-

Table IV.25 The effect of Ethidium prophylaxis on the PCV of all cattle sampled in Rukada village (Ethidium treated) and Ngelechom village (Ethidium control)

period \ village	Rukada village (Ethidium treated)	Ngelechom village (Ethidium control)	difference in mean PCV between villages
before Ethidium prophylaxis	27.28 s.e. 0.124 n = 1324	26.73 s.e. 0.109 n = 1379	0.54 t = 3.292 p = 0.0010
during Ethidium prophylaxis	28.43 s.e. 0.117 n = 1391	27.35 s.e. 0.121 n = 1402	1.08 t = 6.448 p < 0.0001
difference in mean PCV during the time of drug trial	1.15 t = 6.795 p < 0.0001	0.62 t = 3.794 p = 0.0002	-

Table IV.26 The effect of Ethidium prophylaxis on the PCV of uninfected cattle in Rukada village (Ethidium treated) and Ngelechom village (Ethidium control)

period \ village	Rukada village (Ethidium treated)	Ngelechom village (Ethidium control)	difference in mean PCV between villages
before Ethidium prophylaxis	27.55 s.e. 0.132 n = 1161	27.27 s.e. 0.103 n = 1164	0.28 t = 1.687 p = 0.0918
during Ethidium prophylaxis	28.57 s.e. 0.122 n = 1430	28.22 s.e. 0.118 n = 1162	0.32 t = 1.902 p = 0.0573
difference in mean PCV during the time of drug trial	0.95 t = 5.398 p < 0.0001	0.92 t = 5.907 p < 0.0001	-

Table IV.27 The effect of Ethidium prophylaxis on the PCV of cattle with trypanosome infections in Rukada village (Ethidium treated) and Ngelechom village (Ethidium control)

period \ village	Rukada village (Ethidium treated)	Ngelechom village (Ethidium control)	difference in mean PCV between villages
before Ethidium prophylaxis	24.80 s.e. 0.460 n = 90	21.70 s.e. 0.556 n = 101	3.10 t = 4.255 p < 0.0001
during Ethidium prophylaxis	26.57 s.e. 0.826 n = 21	21.84 s.e. 0.445 n = 143	4.73 t = 3.948 p = 0.0001
difference in mean PCV during the time of drug trial	1.71 t = 1.727 p = 0.0869	0.14 t = 0.194 p = 0.8465	-

Table IV.28 The effect of Ethidium prophylaxis on the mean monthly liveweight gain of all cattle sampled in Rukada village (Ethidium treated) and Ngelechom village (Ethidium control)

period \ village	Rukada village (Ethidium treated)	Ngelechom village (Ethidium control)	difference in mean monthly weight change between villages
before Ethidium prophylaxis	3.21 s.e. 0.322 n = 807	3.42 s.e. 0.247 n = 944	-0.21 t = 0.5169 p = 0.6053
during Ethidium prophylaxis	3.76 s.e. 0.274 n = 1142	3.82 s.e. 0.231 n = 1307	-0.07 t = 0.1881 p = 0.8508
difference in mean monthly weight change during the time of drug trial	0.55 t = 1.290 p = 0.1971	0.41 t = 1.184 p =0.2363	-

Table IV.29 The effect of Ethidium prophylaxis on the mean monthly liveweight gain of uninfected cattle in Rukada village (Ethidium treated) and Ngelechom village (Ethidium control)

period \ village	Rukada village (Ethidium treated)	Ngelechom village (Ethidium control)	difference in mean monthly weight change between villages
before Ethidium prophylaxis	3.08 s.e. 0.364 n = 695	3.74 s.e. 0.242 n = 792	-0.65 t = 1.528 p = 0.1267
during Ethidium prophylaxis	3.76 s.e. 0.279 n = 1089	4.55 s.e. 0.234 n = 1030	-0.79 t = 2.149 p = 0.0317
difference in mean monthly weight change during the time of drug trial	0.68 t = 1.498 p = 0.1343	0.81 t = 2.388 p = 0.0170	-

Table IV.30 The effect of Ethidium prophylaxis on the mean monthly liveweight gain of all cattle wit trypanosome infections in Rukada village (Ethidium treated) and Ngelechom village (Ethidium control)

period \ village	Rukada village (Ethidium treated)	Ngelechom village (Ethidium control)	difference in mean monthly weight change between villages
before Ethidium prophylaxis	4.96 s.e. 0.855 n = 56	-1.06 s.e. 1.38 n = 60	6.02 t = 3.674 p = 0.0004
during Ethidium prophylaxis	4.87 s.e. 0.914 n = 19	-1.90 s.e. 0.906 n = 136	6.77 t = 2.770 p = 0.0063
difference in mean monthly weight change during the time of drug trial	-0.10 t = 0.0619 p = 0.9508	-0.84 t = 0.516 p = 0.6067	-

Appendix V

Appendix V

Table V.1 The prevalence of mixed infections with *T. brucei* spp. and *T. vivax* in all four study villages

infection	not infected with *T. vivax*	infected with *T. vivax*
not infected with *T. brucei* spp.	9035 (8804.1)	449 (679.9)
infected with *T. brucei* spp.	223 (453.9)	266 (35.1)

( ) expected values
$X^2 = 1723.3$, df = 1, $p < 0.001$

Table V.2 The prevalence of mixed infections with *T. brucei* spp. and *T. vivax* in Katelenyang village (Samorin treated)

infection	not infected with *T. vivax*	infected with *T. vivax*
not infected with *T. brucei* spp.	1994 (1939.9)	83 (137.1)
infected with *T. brucei* spp.	30 (84.1)	60 (5.9)

( ) expected values
$X^2 = 549.7$, df = 1, $p < 0.001$

Table V.3 The prevalence of mixed infections with *T. brucei* spp. and *T. vivax* in Apatit village (Samorin control)

infection	not infected with *T. vivax*	infected with *T. vivax*
not infected with *T. brucei* spp.	1873 (1791.6)	187 (268.4)
infected with *T. brucei* spp.	136 (217.4)	114 (32.6)

( ) expected values
$X^2 = 262.4$, df = 1, $p < 0.001$

Table V.4 The prevalence of mixed infections with *T. brucei* spp. and *T. vivax* in Rukada village (Ethidium treated)

infection	not infected with *T. vivax*	infected with *T. vivax*
not infected with *T. brucei* spp.	2617 (2132.0)	28 (63.0)
infected with *T. brucei* spp.	33 (68.0)	37 (2.0)

( ) expected values
$X^2 = 647.5$, df = 1, $p < 0.001$

Table V.5 The prevalence of mixed infections with *T. brucei* spp. and *T. vivax* in Ngelechom village (Ethidium control)

infection	not infected with *T. vivax*	infected with *T. vivax*
not infected with *T. brucei* spp.	2551 (2501.9)	151 (200.2)
infected with *T. brucei* spp.	24 (73.2)	55 (5.9)

( ) expected values
$X^2 = 458.8$, df = 1, $p < 0.001$

Table V.6 The prevalence of mixed infections with *T. brucei* spp. and *T. congolense* in all four study villages

infection	not infected with *T. congolense*	infected with *T. congolense*
not infected with *T. brucei* spp.	9353 (9297.6)	131 (185.4)
infected with *T. brucei* spp.	425 (479.4)	64 (9.6)

( ) expected value
$X^2 = 332.4$, df = 1, $p < 0.001$

Table V.7 The prevalence of mixed infections with *T. brucei* spp. and *T. congolense* in Katelenyang village (Samorin treated)

infection	not infected with *T. congolense*	infected with *T. congolense*
not infected with *T. brucei* spp.	2055 (2044.4)	22 (32.6)
infected with *T. brucei* spp.	78 (88.6)	12 (1.4)

( ) expected value
$X^2 = 84.1$, df = 1 chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.0010$

Table V.8
Tabulation of prevalence of mixed infections with *T. brucei* spp. and *T. congolense* in Apatit village (Samorin control)

infection	not infected with *T. congolense*	infected with *T. congolense*
not infected with *T. brucei* spp.	1988 (1961.0)	72 (99.0)
infected with *T. brucei* spp.	211 (238.0)	39 (12.0)

( ) expected value
$X^2 = 71.4$, df = 1, $p < 0.001$

Table V.9 The prevalence of mixed infections with *T. brucei* spp. and *T. congolense* in Rukada village (Ethidium treated)

infection	not infected with *T. congolense*	infected with *T. congolense*
not infected with *T. brucei* spp.	2632 (2627.5)	13 (17.5)
infected with *T. brucei* spp.	65 (69.5)	5 (0.5)

( ) expected value
$X^2 = 45.8$, df = 1 chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.0010$

Table V.10 The prevalence of mixed infections with *T. brucei* spp. and *T. congolense* in Ngelechom village (Ethidium control)

infection	not infected with *T. congolense*	infected with *T. congolense*
not infected with *T. brucei* spp.	2678 (2670.9)	24 (31.1)
infected with *T. brucei* spp.	71 (78.1)	8 (0.9)

( ) expected value
$X^2 = 57.6$, df = 1 chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.0010$

Table V.11 The prevalence of mixed infections with *T. vivax* and *T. congolense* in all four study villages

infection	not infected with *T. congolense*	infected with *T. congolense*
not infected with *T. vivax*	9110 (9077.0)	148 (181.0)
infected with *T. vivax*	668 (701.0)	47 (14.0)

( ) expected value
$X^2 = 85.7$, df = 1, $p < 0.001$

Table V.12
Tabulation of prevalence of mixed infections with *T. vivax* and *T. congolense* in Katelenyang village (Samorin treated)

infection	not infected with *T. congolense*	infected with *T. congolense*
not infected with *T. vivax*	1996 (1992.2)	28 (31.8)
infected with *T. vivax*	137 (140.8)	6 (2.2)

( ) expected value
$X^2 = 6.8$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p = 0.021$

Table V.13 The prevalence of mixed infections with *T. vivax* and *T. congolense* in Apatit village (Samorin control)

infection	not infected with *T. congolense*	infected with *T. congolense*
not infected with *T. vivax*	1922 (1912.5)	87 (96.5)
infected with *T. vivax*	277 (286.5)	24 (14.5)

( ) expected value
$X^2 = 7.6$, df = 1, p = 0.006

Table V.14 The prevalence of mixed infections with *T. vivax* and *T. congolense* in Rukada village (Ethidium treated)

infection	not infected with *T. congolense*	infected with *T. congolense*
not infected with *T. vivax*	2634 (2632.4)	16 (17.6)
infected with *T. vivax*	63 (64.6)	2 (0.4)

( ) expected value
$X^2 = 5.9$, df = 1, p = chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.068

Table V.15 The prevalence of mixed infections with *T. vivax* and *T. congolense* in Ngelechom village (Ethidium control)

infection	not infected with *T. congolense*	infected with *T. congolense*
not infected with *T. vivax*	2558 (2545.4)	17 (29.6)
infected with *T. vivax*	191 (203.6)	15 (2.4)

( ) expected value
$X^2 = 73.5$, df = 1, $p < 0.001$

Table V.16 The incidence of mixed infections with *T. brucei* spp. and *T. vivax* in all four study villages

infection	not infected with a new *T. vivax* infection	infected with a new *T. vivax* infection
not infected with a new *T. brucei* spp. infection	9227 (9055.8)	387 (558.2)
infected with a new *T. brucei* spp. infection	167 (338.2)	192 (20.8)

( ) expected values
$X^2 = 1547.9$, df = 1, $p < 0.001$

Table V.17 The incidence of mixed infections with *T. brucei* spp. and *T. vivax* in Katelenyang village (Samorin treated)

infection	not infected with a new *T. vivax* infection	infected with a new *T. vivax* infection
not infected with a new *T. brucei* spp. infection	2019 (1980.9)	151 (200.2)
infected with a new *T. brucei* spp. infection	24 (73.2)	55 (5.9)

( ) expected values
$X^2 = 458.9$, df = 1, $p < 0.001$

Table V.18 The incidence of mixed infections with *T. brucei* spp. and *T. vivax* in Apatit village (Samorin control)

infection	not infected with a new *T. vivax* infection	infected with a new *T. vivax* infection
not infected with a new *T. brucei* spp. infection	1973 (1908.6)	160 (224.4)
infected with a new *T. brucei* spp. infection	94 (158.4)	83 (18.6)

( ) expected values
$X^2 = 269.4$, df = 1, $p < 0.001$

Table V.19 The incidence of mixed infections with *T. brucei* spp. and *T. vivax* in Rukada village (Ethidium treated)

infection	not infected with a new *T. vivax* infection	infected with a new *T. vivax* infection
not infected with a new *T. brucei* spp. infection	2643 (2620.8)	26 (48.2)
infected with a new *T. brucei* spp. infection	23 (45.2)	23 (0.8)

( ) expected values
$X^2 = 613.3$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.001$

Table V.20 The incidence of mixed infections with *T. brucei* spp. and *T. vivax* in Ngelechom village (Ethidium control)

infection	not infected with a new *T. vivax* infection	infected with a new *T. vivax* infection
not infected with a new *T. brucei* spp. infection	2592 (2551.9)	125 (165.1)
infected with a new *T. brucei* spp. infection	20 (60.1)	44 (3.9)

( ) expected values
$X^2 = 450.8$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.0010$

Table V.21 The incidence of mixed infections with *T. brucei* spp. and *T. congolense* in all four study villages

infection	not infected with a new *T. congolense* infection	infected with a new *T. congolense* infection
not infected with a new *T. brucei* spp. infection	9486 (9445.3)	128 (168.7)
infected with a new *T. brucei* spp. infection	312 (352.7)	47 (6.3)

( ) expected value
$X^2 = 277.7$, df = 1, $p < 0.001$

Table V.22 The incidence of mixed infections with *T. brucei* spp. and *T. congolense* in Katelenyang village (Samorin treated)

infection	not infected with a new *T. congolense* infection	infected with a new *T. congolense* infection
not infected with a new *T. brucei* spp. infection	2072 (2063.1)	23 (31.9)
infected with a new *T. brucei* spp. infection	62 (70.9)	10 (1.1)

( ) expected value
$X^2 = 75.9$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.001$

Table V.23 The incidence of mixed infections with *T. brucei* spp. and *T. congolense* in Apatit village (Samorin control)

infection	not infected with a new *T. congolense* infection	infected with a new *T. congolense* infection
not infected with a new *T. brucei* spp. infection	2064 (2046.2)	69 (86.8)
infected with a new *T. brucei* spp. infection	152 (169.8)	25 (7.2)

( ) expected value
$X^2 = 49.6$, df = 1, $p < 0.001$

Table V.24 The incidence of mixed infections with *T. brucei* spp. and *T. congolense* in Rukada village (Ethidium treated)

infection	not infected with a new *T. congolense* infection	infected with a new *T. congolense* infection
not infected with a new *T. brucei* spp. infection	2655 (2651.3)	14 (17.7)
infected with a new *T. brucei* spp. infection	42 (45.7)	4 (0.3)

( ) expected value
$X^2 = 45.8$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.0010$

Table V.25 The incidence of mixed infections with *T. brucei* spp. and *T. congolense* in Ngelechom village (Ethidium control)

infection	not infected with a new *T. congolense* infection	infected with a new *T. congolense* infection
not infected with a new *T. brucei* spp. infection	2695 (2687.7)	22 (29.3)
infected with a new *T. brucei* spp. infection	56 (63.3)	8 (0.7)

( ) expected value
$X^2 = 80.1$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.0010$

Table V.26 The incidence of mixed infections with *T. vivax* and *T. congolense* in all four study villages

infection	not infected with a new *T. congolense* infection	infected with a new *T. congolense* infection
not infected with a new *T. vivax* infection	9261 (9229.2)	133 (164.8)
infected with a new *T. vivax* infection	537 (568.8)	42 (10.2)

( ) expected value
$X^2 = 107.8$, df = 1, $p < 0.001$

Table V.27 The incidence of mixed infections with *T. vivax* and *T. congolense* in Katelenyang village (Samorin treated)

infection	not infected with a new *T. congolense* infection	infected with a new *T. congolense* infection
not infected with a new *T. vivax* infection	2022 (2017.8)	27 (31.2)
infected with a new *T. vivax* infection	112 (116.2)	6 (1.8)

( ) expected value
$X^2 = 10.6$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.0010$

Table V.28 The incidence of mixed infections with *T. vivax* and *T. congolense* in Apatit village (Samorin control)

infection	not infected with a new *T. congolense* infection	infected with a new *T. congolense* infection
not infected with a new *T. vivax* infection	1993 (1982.9)	74 (84.1)
infected with a new *T. vivax* infection	223 (233.1)	20 (9.9)

( ) expected value
$X^2 = 12.0$, df = 1, p = 0.001

Table V.29 The incidence of mixed infections with *T. vivax* and *T. congolense* in Rukada village (Ethidium treated)

infection	not infected with a new *T. congolense* infection	infected with a new *T. congolense* infection
not infected with a new *T. vivax* infection	2650 (2648.3)	16 (17.7)
infected with a new *T. vivax* infection	47 (48.7)	2 (0.3)

( ) expected value
$X^2 = 8.9$, df = 1, p = chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.0406

Table V.30 The incidence of mixed infections with *T. vivax* and *T. congolense* in Ngelechom village (Ethidium control)

infection	not infected with a new *T. congolense* infection	infected with a new *T. congolense* infection
not infected with a new *T. vivax* infection	2596 (2583.8)	16 (28.2)
infected with a new *T. vivax* infection	47 (48.7)	14 (1.8)

( ) expected value
$X^2 = 87.5$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p < 0.0010

Table V.31 The incidence of *T. vivax* in cattle already with established infections of *T. brucei* spp. in all four study villages

infection	no new infection of *T. vivax*	new infection of *T. vivax*
no established *T. brucei* spp. infection	9291 (9271.5)	552 (571.5)
established *T. brucei* spp. infection	103 (122.5)	27 (7.6)

( ) expected values
$X^2 = 53.9$, df = 1, $p < 0.001$

Table V.32 The incidence of *T. vivax* in cattle already with established infections of *T. brucei* spp. in Katelenyang village (Samorin treated)

infection	no new infection of *T. vivax*	new infection of *T. vivax*
no established *T. brucei* spp. infection	2038 (2032.0)	111 (117.0)
established *T. brucei* spp. infection	11 (17.0)	7 (1.0)

( ) expected values
$X^2 = 39.4$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.001023$

Table V.33 The incidence of *T. vivax* in cattle already with established infections of *T. brucei* spp. in Apatit village (Samorin control)

infection	no new infection of *T. vivax*	new infection of *T. vivax*
no established *T. brucei* spp. infection	2004 (2001.7)	233 (235.3)
established *T. brucei* spp. infection	63 (65.3)	10 (7.7)

( ) expected values
$X^2 = 0.809$, df = 1, $p = 0.369$

Table V.34 The incidence of *T. vivax* in cattle already with established infections of *T. brucei* spp. in Rukada village (Ethidium treated)

infection	no new infection of *T. vivax*	new infection of *T. vivax*
no established *T. brucei* spp. infection	2647 (2642.4)	44 (48.6)
established *T. brucei* spp. infection	19 (23.6)	5 (0.4)

( ) expected values
$X^2 = 49.5$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.00105$

Table V.35 The incidence of *T. vivax* in cattle already with established infections of *T. brucei* spp. in Ngelechom village (Ethidium control)

infection	no new infection of *T. vivax*	new infection of *T. vivax*
no established *T. brucei* spp. infection	2602 (2597.9)	164 (168.9)
established *T. brucei* spp. infection	10 (14.1)	5 (0.9)

( ) expected values
$X^2 = 19.6$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p = 0.00142$

Table V.36 The incidence of *T. brucei* spp. in cattle already with established infections of *T. vivax* in all four study villages

infection	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
no established *T. vivax* infection	9493 (9482.9)	344 (354.1)
established *T. vivax* infection	121 (131.1)	15 (4.9)

( ) expected values
$X^2 = 21.9$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.0011$

Table V.37 The incidence of *T. brucei* spp. in cattle already with established infections of. *T vivax* in Katelenyang village (Samorin treated)

infection	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
no established *T. vivax* infection	2074 (2070.8)	68 (71.2)
established *T. vivax* infection	21 (24.2)	4 (0.8)

( ) expected values
X^2 = 12.7, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.008

Table V38 The incidence of *T. brucei* spp. in cattle already with established infections of. *T vivax* in Apatit village (Samorin control)

infection	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
no established *T. vivax* infection	2083 (2079.4)	169 (172.6)
established *T. vivax* infection	50 (53.6)	8 (4.4)

( ) expected values
X^2 = 3.2, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.071

Table V.39 The incidence of *T. brucei* spp. in cattle already with established infections of. *T vivax* in Rukada village (Ethidium treated)

infection	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
no established *T. vivax* infection	2655 (2653.3)	44 (45.7)
established *T. vivax* infection	14 (15.7)	2 (0.3)

( ) expected values
X^2 = 11.3, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.29

Table V.40 The incidence of *T. brucei* spp. in cattle already with established infections of. *T vivax* in Ngelechom village (Ethidium control)

infection	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
no established *T. vivax* infection	2681 (2680.9)	63 (63.2)
established *T. vivax* infection	36 (36.2)	1 (0.9)

( ) expected values
$X^2 = 0.027$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.58

Table V.41 The incidence of *T. congolense* in cattle already with established infections of *T. brucei* spp. in all four study villages

infection	no new infection of *T. congolense*	new infection of *T. congolense*
no established *T. brucei* spp. infection	9677 (9670.3)	166 (172.7)
established *T. brucei* spp. infection	121 (127.7)	9 (2.3)

( ) expected values
$X^2 = 20.4$, df = 1, $p < 0.001$

Table V.42 The incidence of *T. congolense* in cattle already with established infections of. *T. brucei* spp. in Katelenyang village (Samorin treated)

infection	no new infection of *T. congolense*	new infection of *T. congolense*
no established *T. brucei* spp. infection	2117 (2116.3)	32 (32.7)
established *T. brucei* spp. infection	17 (17.7)	1 (0.3)

( ) expected values
$X^2 = 2.0$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.24

Table V.43 The incidence of *T. congolense* in cattle already with established infections of. *T. brucei* spp. in Apatit village (Samorin control)

infection	no new infection of *T. congolense*	new infection of *T. congolense*
no established *T. brucei* spp. infection	2150 (2146.0)	87 (91.0)
established *T. brucei* spp. infection	66 (70.0)	7 (3.0)

( ) expected values
$X^2 = 5.9$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.027

Table V.44 The incidence of *T. congolense* in cattle already with established infections of. *T. brucei* spp. in Rukada village (Ethidium treated)

infection	no new infection of *T. congolense*	new infection of *T. congolense*
no established *T. brucei* spp. infection	2674 ((2673.2)	17 (17.4)
established *T. brucei* spp. infection	23 (23.9)	1 (0.2)

( ) expected values
$X^2 = 4.5$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.15

Table V.45 The incidence of *T. congolense* in cattle already with established infections of. *T. brucei* spp. in Ngelechom village (Ethidium control)

infection	no new infection of *T. congolense*	new infection of *T. congolense*
no established *T. brucei* spp. infection	2736 (2736.2)	30 (29.8)
established *T. brucei* spp. infection	15 (14.8)	0 (0.2)

( ) expected values
$X^2 = 0.16$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.85

Table V.46 The incidence of *T. brucei* spp. in cattle already with established infections of *T. congolense*

infection	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
no established *T. congolense* infection	9599 (9594.7)	354 (358.3)
established *T. congolense* infection	15 (19.3)	5 (0.7)

( ) expected values
$X^2 = 26.4$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p < 0.00159$

Table V.47 The incidence of *T. brucei* spp. in cattle already with established infections of. *T. congolense* in Katelenyang village (Samorin treated)

infection	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
no established *T. congolense* infection	2095 (2094.0)	71 (72.0)
established *T. congolense* infection	0 (1.0)	1 (0.03)

( ) expected values
$X^2 = 29.1$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p = 0.033$

Table V.48 The incidence of *T. brucei* spp. in cattle already with established infections of. *T. congolense* in Apatit village (Samorin control)

infection	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
no established *T. congolense* infection	2120 (2117.3)	173 (175.7)
established *T. congolense* infection	13 (15.7)	4 (1.3)

( ) expected values
$X^2 = 6.1$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test $p = 0.036$

Table V.49 The incidence of *T. brucei* spp. in cattle already with established infections of. *T. congolense* in Rukada village (Ethidium treated)

infection	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
no established *T. congolense* infection	2669 (2699.0)	46 (46)
established *T. congolense* infection	0 (0)	0 (0)

( ) expected values
calculation X^2 not possible

Table V.50 The incidence of *T. brucei* spp. in cattle already with established infections of. *T. congolense* in Ngelechom village (Ethidium control)

infection	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
no established *T. congolense* infection	2175 (2715.1)	64 (64.0)
established *T. congolense* infection	2 (2.0)	0 (0.1)

( ) expected values
$X^2 = 0.047$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.95

Table V.51 The incidence of *T. congolense* in cattle already with established infections of *T. vivax* in all four study villages

infection	no new infection of *T. congolense*	new infection of *T. congolense*
no established *T. vivax* infection	9664 (9664.4)	173 (172.6)
established *T. vivax* infection	134 (133.6)	2 (2.4)

( ) expected values
$X^2 = 0.65$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.57

Table V.52 The incidence of *T. congolense* in cattle already with established infections of *T. vivax* in Katelenyang village (Samorin treated)

infection	no new infection of *T. congolense*	new infection of *T. congolense*
no established *T. vivax* infection	2109 (2109.4)	33 (32.6)
established *T. vivax* infection	25 (24.6)	0 (0.4)

( ) expected values
$X^2 = 0.39$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.68

Table V.53 The incidence of *T. congolense* in cattle already with established infections of *T. vivax* in Apatit village (Samorin control)

infection	no new infection of *T. congolense*	new infection of *T. congolense*
no established *T. vivax* infection	2160 (2160.4)	92 (91.6)
established *T. vivax* infection	56 (55.6)	2 (2.4)

( ) expected values
$X^2 = 0.81$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.577

Table V.54 The incidence of *T. congolense* in cattle already with established infections of *T. vivax* in Rukada village (Ethidium treated)

infection	no new infection of *T. congolense*	new infection of *T. congolense*
no established *T. vivax* infection	2681 (2681.1)	18 (18.0)
established *T. vivax* infection	16 (16.0)	0 (0.1)

( ) expected values
$X^2 = 0.11$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.898

Table V.55 The incidence of *T. congolense* in cattle already with established infections of *T. vivax* in Ngelechom village (Ethidium control)

infection	no new infection of *T. congolense*	new infection of *T. congolense*
no established *T. vivax* infection	2714 (2174.4)	30 (29.6)
established *T. vivax* infection	37 (36.6)	0 (0.4)

( ) expected values
$X^2 = 0.41$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.667

Table V.56 The incidence of *T. vivax* in cattle already with established infections of *T. congolense* in all four study villages

infection	no new infection of *T. vivax*	new infection of *T. vivax*
no established *T. congolense* infection	9377 (9375.2)	576 (577.8)
established *T. congolense* infection	17 (18.8)	3 (1.2)

( ) expected values
$X^2 = 3.1$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.107

Table V.57 The incidence of *T. vivax* in cattle already with established infections of *T. congolense* in Katelenyang village (Samorin treated)

infection	no new infection of *T. vivax*	new infection of *T. vivax*
no established *T. congolense* infection	2048 (2048.1)	118 (118.0)
established *T. congolense* infection	1 (1.0)	0 (0.1)

( ) expected values
$X^2 = 0.058$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.946

Table V.58 The incidence of *T. vivax* in cattle already with established infections of *T. congolense* in Apatit village (Samorin control)

infection	no new infection of *T. vivax*	new infection of *T. vivax*
no established *T. congolense* infection	2052 (2051.8)	241 (241.2)
established *T. congolense* infection	15 (15.2)	2 (1.8)

( ) expected values
$X^2 = 0.028$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.548

Table V.59 The incidence of *T. vivax* in cattle already with established infections of *T. congolense* in Rukada village (Ethidium treated)

infection	no new infection of *T. vivax*	new infection of *T. vivax*
no established *T. congolense* infection	2666 (2666)	49 (49
established *T. congolense* infection	0 (0)	0 (0)

( ) expected values
calculation X^2 not possible

Table V.60 The incidence of *T. vivax* in cattle already with established infections of *T. congolense* in Ngelechom village (Ethidium control)

infection	no new infection of *T. vivax*	new infection of *T. vivax*
no established *T. congolense* infection	2611 (2610.1)	168 (168.9)
established *T. congolense* infection	1 (1.9)	1 (0.1)

( ) expected values
$X^2 = 6.8$, df = 1, chi square probably invalid (expected count less than 5)
Fisher's exact test p = 0.118

Table V.61 Sex as a risk factor for *T. brucei* spp. infections in all four study villages

infection sex	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
male	3695 (3715.3)	159 (138.7)
female	5919 (5898.7)	200 (220.3)

( ) expected values
$X^2 = 5.0$, df = 1, p = 0.025

Table V.62 Sex as a risk factor for *T. brucei* spp. infections in Katelenyang village (Samorin treated)

infection sex	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
male	927 (930.0)	35 (32.0)
female	1168 (1165.0)	37 (40.0)

( ) expected values
$X^2 = 0.54$, df = 1, p = 0.464

Table V.63 Sex as a risk factor for *T. brucei* spp. infections in Apatit village (Samorin control)

infection sex	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
male	981 (979.7)	80 (81.3)
female	1152 (1153.3)	97 (95.7)

( ) expected values
$X^2 = 0.041$, df = 1, p = 0.839

Table V.64 Sex as a risk factor for *T. brucei* spp. infections in Rukada village (Ethidium treated)

infection sex	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
male	535 (539.7)	14 (9.3)
female	2134 (2129.3)	32 (36.7)

( ) expected values
$X^2 = 3.0$, df = 1, p = 0.082

Table V.65 Sex as a risk factor for *T. brucei* spp. infections in Ngelechom village (Ethidium control)

infection sex	no new infection of *T. brucei* spp.	new infection of *T. brucei* spp.
male	1252 (1252.5)	30 (29.5)
female	1465 (1464.5)	34 (34.5)

( ) expected values
$X^2 = 0.016$, df = 1, p = 0.90

Table V.66 Sex as a risk factor for *T. vivax* infections in all four study villages

infection sex	no new infection of *T. vivax*	new infection of *T. vivax*
male	3586 (3630.3)	268 (223.7)
female	5808 (5763.7)	311 (355.3)

( ) expected values
$X^2 = 15.1$, df = 1, p < 0.001

Table V.67 Sex as a risk factor for *T. vivax* infections in Katelenyang village (Samorin treated)

infection sex	no new infection of *T. vivax*	new infection of *T. vivax*
male	906 (909.6)	56 (52.4)
female	1143 (1139.4)	62 (65.6)

( ) expected values
$X^2 = 0.475$, df = 1, p = 0.491

Table V.68 Sex as a risk factor for *T. vivax* infections in Apatit village (Samorin control)

infection sex	no new infection of *T. vivax*	new infection of *T. vivax*
male	948 (949.4)	113 (111.6)
female	1119 (1117.6)	130 (131.4)

( ) expected values
$X^2 = 0.036$, df = 1, p = 0.850

Table V.69 Sex as a risk factor for *T. vivax* infections in Rukada village (Ethidium treated)

infection sex	no new infection of *T. vivax*	new infection of *T. vivax*
male	535 (539.1)	85 (77.9)
female	2131 (2126.9)	35 (39.1)

( ) expected values
$X^2 = 1.3$, df = 1, p = 0.259

Table V.70 Sex as a risk factor for *T. vivax* infections in Ngelechom village (Ethidium control)

infection sex	no new infection of *T. vivax*	new infection of *T. vivax*
male	949 (947.4)	13 (14.6)
female	1185 (1186.6)	20 (18.5)

( ) expected values
$X^2 = 0.339$, df = 1, p = 0.560

Table V.71 Sex as a risk factor for *T. congolense* infections in all four study villages

infection sex	no new infection of *T. congolense*	new infection of *T. congolense*
male	3770 (3786.4)	84 (67.6)
female	6028 (6011.6)	91 (107.4)

( ) expected values
$X^2 = 6.6$, df = 1, p = 0.010

Table V.72 Sex as a risk factor for *T. congolense* infections in Katelenyang village (Samorin treated)

infection sex	no new infection of *T. congolense*	new infection of *T. congolense*
male	949 (947.4)	13 (14.6)
female	1185 (1186.6)	20 (18.4)

( ) expected values
$X^2 = 0.339$, df = 1, p = 0.560

Table V.73 Sex as a risk factor for *T. congolense* infections in Apatit village (Samorin control)

infection sex	no new infection of *T. congolense*	new infection of *T. congolense*
male	1010 (1017.8)	51 (43.2)
female	1206 (1198.2)	43 (50.8)

( ) expected values
$X^2 = 2.73$, df = 1, p = 0.099

Table V.74 Sex as a risk factor for *T. congolense* infections in Rukada village (Ethidium treated)

infection sex	no new infection of *T. congolense*	new infection of *T. congolense*
male	541 (545.4)	8 (3.6)
female	2156 (2151.6)	10 (14.4)

( ) expected values
$X^2 = 6.59$, df = 1 (expected count less than 5)
Fishers exact test p = 0.0164

Table V.75 Sex as a risk factor for *T. congolense* infections in Ngelechom village (Ethidium control)

infection sex	no new infection of *T. congolense*	new infection of *T. congolense*
male	1270 (1268.2)	12 (13.8)
female	1481 (1482.8)	18 (16.2)

( ) expected values
$X^2 = 0.454$, df = 1, p = 0.501

Table V.76 Age as a risk factor for *T. brucei* spp. infections in all four study villages

age (years)	0 - 1	1 - 2	2 - 3	3 - 4	> 4
no new infection of *T. brucei* spp.	934 (919.7)	1525 (1540.5)	1911 (1918.4)	1673 (1683.2)	3562 (3552.4)
new infection of *T. brucei* spp.	11 (34.3)	73 (62.9)	79 (71.6)	73 (62.9)	123 (132.7)

( ) expected values
$X^2 = 24.0$, d.f .= 4, $p < 0.001$

Table V.77 Age as a risk factor for *T. vivax* infections in all four study villages

age (years)	0 - 1	1 - 2	2 - 3	3 - 4	> 4
no new infection of *T. vivax*	936 (898.6)	1488 (1505.2)	1853 (1874.5)	1620 (1644.6)	3497 (3471.1)
new infection of *T. vivax*	18 (55.4)	110 (92.8)	137 (115.5)	126 (101.4)	188 (213.9)

( ) expected values
$X^2 = 44.1$, d.f .= 4, $p < 0.001$

Table V.78 Age as a risk factor for *T. congolense* infections in all four study villages

age (years)	0 - 1	1 - 2	2 - 3	3 - 4	> 4
no new infection of *T. congolense*	946 (937.3)	1564 (1570.0)	1962 (1955.2)	1708 (1715.8)	3618 (3620.3)
new infection of *T. congolense*	8 (16.7)	34 (28.0)	28 (34.9)	38 (30.5)	67 (64.7)

( ) expected values
$X^2 = 9.2$, d.f .= 4, $p = 0.057$

Table V.79
Age as a risk factor for infection with any pathogenic trypanosome all 4 villages

age (years)	0 - 1	1 - 2	2 - 3	3 - 4	> 4
no new trypanosome infection	922 (873.1)	1436 (1462.4)	1801 (1821.2)	1566 (1597.9)	3402 (3372.4)
new trypanosome infection	32 (80.9)	162 (135.6)	189 (168.8)	180 (148.8)	283 (312.6)

( ) expected values
$X^2 = 51.2$, d.f .= 4, $p < 0.001$

Table V.80 The monthly rate of removal from the epidemiological cycle of cattle with a single infection of *T. brucei* spp. compared to a single infection with *T. vivax*

infection/ fate	infected with only *T. brucei* spp.	infected with only *T. vivax*
remains in the epidemiological cycle	153 (109.2)	218 (261.8)
removed from the epidemiological cycle	21 (64.8)	199 (155.2)

( ) expected values
$X^2 = 66.8$, df = 1, $p < 0.001$

Table V.81 The monthly rate of removal from the epidemiological cycle of cattle with a single infection of *T. brucei* spp. compared to a single infection with *T. congolense*

infection/ fate	infected with only *T. brucei* spp.	infected with only *T. congolense*
remains in the epidemiological cycle	153 (133.2)	56 (75.8)
removed from the epidemiological cycle	21 (40.8)	43 (23.2)

( ) expected values
$X^2 = 34.6$, df = 1, $p < 0.001$

Table V.82 The monthly rate of removal from the epidemiological cycle of cattle with a single infection of *T. vivax* compared to a single infection with *T. congolense*

infection/ fate	infected with only *T. vivax*	infected with only *T. congolense*
remains in the epidemiological cycle	218 (221.4)	56 (52.6)
removed from the epidemiological cycle	199 (195.6)	43 (46.4)

( ) expected values
$X^2 = 0.6$, df = 1, $p = 0.442$

Table V.83 The monthly rate of removal from the epidemiological cycle of cattle with a single infection of *T. brucei* spp. compared to a mixed infection with *T. brucei* spp. and *T. vivax*

infection/ fate	infected with only *T. brucei* spp.	infected with *T. brucei* spp. and *T. vivax*
remains in the epidemiological cycle	153 (122.4)	146 (176.6)
removed from the epidemiological cycle	21 (51.6)	105 (74.4)

( ) expected values
$X^2 = 43.6$, df = 1, $p < 0.001$

Table V.84 The monthly rate of removal from the epidemiological cycle of cattle with a single infection of *T. brucei* spp. compared to a mixed infection with *T. brucei* spp. and *T. congolense*

infection/ fate	infected with only *T. brucei* spp.	infected with *T. brucei* spp. and *T. congolense*
remains in the epidemiological cycle	153 (135.8)	21 (38.2)
removed from the epidemiological cycle	21 (38.2)	28 (10.8)

( ) expected values
$X^2 = 34.6$, df = 1, $p < 0.001$

Table V.85 The monthly rate of removal from the epidemiological cycle of cattle with a single infection of *T. vivax* compared to a mixed infection with *T. brucei* spp. and *T. vivax*

infection/ fate	infected with only *T. vivax*	infected with *T. brucei* spp. and *T. vivax*
remains in the epidemiological cycle	218 (227.2)	146 (136.8)
removed from the epidemiological cycle	199 (189.8)	105 (114.2)

( ) expected values
$X^2 = 2.2$, df = 1, $p = 0.139$

Table V.86 The monthly rate of removal from the epidemiological cycle of cattle with a single infection of *T. congolense* compared to a mixed infection with *T. brucei* spp. and *T. congolense*

infection/ fate	infected with only *T. congolense*	infected with *T. brucei* spp. and *T. congolense*
remains in the epidemiological cycle	56 (52.6)	21 (25.5)
removed from the epidemiological cycle	43 (46.4)	28 (23.5)

( ) expected values
$X^2 = 2.5$, df = 1, p = 0.117

Table V.87 The monthly rate of removal from the epidemiological cycle of cattle with a single infection of *T. vivax* compared to a mixed infection with *T. vivax* and *T. congolense*

infection/ fate	infected with only *T. vivax*	infected with *T. vivax* and *T. congolense*
remains in the epidemiological cycle	218 (209.0)	7 (16.0)
removed from the epidemiological cycle	199 (208.0)	25 (25.0)

( ) expected values
$X^2 = 10.989$, df = 1, p = 0.001

Table V.88 The monthly rate of removal from the epidemiological cycle of cattle with a single infection of *T. congolense* compared to a mixed infection with *T. vivax* and *T. congolense*

infection/ fate	infected with only *T. congolense*	infected with *T. vivax* and *T. congolense*
remains in the epidemiological cycle	56 (47.6)	7 (15.4)
removed from the epidemiological cycle	43 (51.4)	25 (16.6)

( ) expected values
$X^2 = 11.658$, df = 1, p = 0.001

Appendix VI

Appendix VI

Table VI.1 Comparison of the improved sensitivity afforded by mouse inoculation using immunosuppressed mice over the combined sensitivity the direct examination techniques (HCT and BC)

diagnostic tests used	HCT + BC	HCT + BC + MOUSE K imm[1]	HCT + BC + MOUSE B imm[2]
number of positive samples	52 (68.0)	76 (68.0)	76 (68.0)
number of negative samples	323 (307)	299 (307)	299 (307)

() expected values

($X^2 = 6.90$, df = 2, p = 0.032)

[1] K imm KETRI outbred immunosuppressed mice

[2] B imm BALB/C inbred immunosuppressed mice

Table VI.2 Comparison of the improved sensitivity afforded by mouse inoculation using non-immunosuppressed mice over the combined sensitivity the direct examination techniques (HCT and BC)

diagnostic tests used	HCT + BC	HCT + BC + MOUSE K non[3]	HCT + BC + MOUSE B non[4]
number of positive samples	52 (61.7)	67 (61.7)	66 (61.7)
number of negative samples	323 (313.3)	308 (313.3)	309 (313.3)

() expected values

($X^2 = 2.73$, df = 2, p = 0.256)

[3] K non KETRI outbred non-immunosuppressed mice

[4] B non BALB/C inbred non-immunosuppressed mice

Appendix VII

Appendix VII

Table VII.1 Analysis of covariance of PCV in cattle for differing age groups

(stepwise fitting of regression models)

parameter	age			
added	% DEV	d.f.	F	p
(none)	0	3057		
+ ACAT 1 *	2.21	1	70.51	0.0000
+ ACAT 2 **	0.07	1	2.32	0.1281
+ ACAT 3 ***	0.63	4	5.00	0.0005
error	95.30	3042		
total	100	3057		

n=9973

Age categories

*	ACAT 1	<6 months, > 6 months
**	ACAT 2	< 6 months, 7-18 months, > 18 months
***	ACAT 3	< 12 months, 1-2 years, 2-3 years, 3-4 years, > 4 years

Table VII.2 Analysis of covariance of the effect on the PCV of cattle of mutually exclusive trypanosome infections

(stepwise fitting of regression models)

parameter		EXCLUSIVE INFECTIONS			
added	n	% DEV	d.f.	F	p
(none)		0	6893		
+ *T. brucei* spp. infection only	174	1.23	1	102.91	0.0000
+ *T. vivax* infection only	417	7.26	1	609.20	0.0000
+ *T. congolense* infection only	99	1.72	1	144.51	0.0000
+ *T. brucei* spp. with *T. vivax* infection	251	4.17	1	350.16	0.0000
+ *T. brucei* spp. with *T. congolense* infection	49	1.33	1	111.73	0.0000
+ *T. vivax* with *T. congolense* infection	32	1.91	1	160.40	0.0000
+ *T. brucei* spp. with *T. vivax* and *T. congolense* infections	15	0.38	1	31.92	0.0000
error		82.01	6886		
total	1037	100	6893		

Table VII.3 Analysis of covariance of the effect on the PCV of cattle of non-exclusive trypanosome infections

(stepwise fitting of regression models)

parameter added	n	NON EXCLUSIVE INFECTIONS % DEV	d.f.	F	p
(none)		0	9972		
+ *T. vivax* infection	715	11.07	1	1302.93	0.0000
+ *T. congolense* infection	195	2.46	1	289.06	0.0000
+ *T. brucei* spp. infection	489	0.65	1	75.92	0.0000
+ *T. vivax* x *T. congolense*	47	0.09	1	10.58	0.0011
+ *T. brucei* spp. x *T. vivax* infection	266	0.94	1	110.72	0.0000
+ *T. brucei* spp. x *T. congolense* infection	64	0.13	1	15.58	0.0001
+ *T. brucei* spp. x *T. vivax* x *T. congolense*	15	0.00	1	0.02	0.8942
error		84.67	9965		
total	1037	100	9972		

Table VII.4 Analysis of covariance of PCV in cattle

(stepwise fitting of regression models)

parameter added	n	interactions of *T. theileri* with other trypanosome infections % DEV	d.f.	F	p
(none)		0	6893		
+ *T. brucei* spp. infections	489	6.02	1	506.09	0.0000
+ *T. vivax* infections	715	7.91	1	664.80	0.0000
+ *T. congolense* infections	195	2.71	1	228.12	0.0000
T. theileri infections	489	0.11	1	9.31	0.0023
T. brucei spp. x *T. theileri* infections	965	0.00	1	0.11	0.7458
T. vivax x *T. theileri* infections	1183	0.03	1	2.52	0.1123
T. congolense x *T. theileri* infections	679	0.00	1	0.15	0.6994
error		81.85	6882		
total		100	6893		

Table VII.5 Analysis of covariance of PCV in cattle

(stepwise fitting of regression models)

parameter		*T. theileri* and microfilaria			
added	n	% DEV	d.f.	F	p
(none)	0	0	6893		
+ *T. brucei* spp. infections	489	6.02	1	506.69	0.0000
+ *T. vivax* infections	715	7.91	1	665.58	0.0000
+ *T. congolense* infections	195	2.71	1	228.39	0.0000
T. theileri infections	489	0.11	1	9.32	0.0023
microfilaria infections	591	0.11	1	8.92	0.0028
error		82.01	6884		
total		100	6893		

Table VII.6 Analysis of covariance of PCV in cattle

(stepwise fitting of regression models)

parameters	village age body score and month			
added	% DEV	d.f.	F	p
(none)	0	3057		
village	1.10	3	12.45	0.0000
ACAT 1 *	2.28	1	77.63	0.0000
village x ACAT 1	0.90	3	10.22	0.0000
body score	5.48	1	186.22	0.0000
body score x ACAT 1	0.01	1	0.34	0.5578
month	0.99	11	3.05	0.0005
month x ACAT 1	0.28	11	0.86	0.5777
error	88.97	3026		
total	100	3057		

n = 9973

* age category ACAT 1 <6 months, > 6 months

Table VII.7 Analysis of covariance of PCV in cattle

(stepwise fitting of regression models)

parameter		calving death selling and east coast fever			
added	n	% DEV	d.f.	F	p
(none)		0	3057		
pregnancy/calving	918	0.02	4	0.13	0.9696
death or disposal	178	0.28	2	4.28	0.0140
east coast fever	27	0.60	1	18.56	0.0000
error		99.1	3050		
total	9973	100	3057		

n = 9973

Table VII.8 Analysis of covariance of PCV in cattle

(stepwise fitting of regression models)

parameter	weight			
added	% DEV	d.f.	F	p
(none)	0	3057		
WTCAT *	0.23	2	3.95	0.0195
ACAT 3	2.91	4	24.95	0.0000
WTCAT x ACAT 3	0.31	7	1.53	0.1520
error		3013		
total	100	3057		

n = 9973

* weight category WTCAT 1- 100 Kg, 101- 200 Kg, 201- 300 kg

** age category ACAT 3 < 12 months, 1-2 years, 2-3 years, 3-4 years, > 4 years

Table VII.9 Analysis of covariance of weight change in cattle with no prophylactic trypanocide treatment and excluding pregnant, calving, aborting cows and cows in early lactation
(stepwise fitting of regression models)

parameter	FACTORS OTHER THAN INFECTION			
added	% DEV	d.f.	F	p
(none)	100	5569		
+ sex	0.05	1	2.87	0.0903
+ age	0.62	4	9.79	0.0000
+ month	6.14	11	35.36	0.0000
+ village	0.21	3	4.34	0.0046
+ sex x age	0.06	4	0.96	0.4263
+ sex x month	0.69	11	3.99	0.0000
+ sex x village	0.04	3	0.89	0.4445
+ age x month	1.56	44	2.25	0.0000
+ age x village	0.09	12	0.49	0.9227
+ month x village	4.45	19	14.85	0.0000
error	86.09	5457		
total	100	5569		

n = 9973

Table VII.10 Analysis of covariance of residual deviation in weight change of cattle with no prophylactic trypanocide treatment and excluding pregnant, calving, aborting cows and cows in early lactation after accounting for differences in sex, age, month and village
(stepwise fitting of regression models)

parameter	TRYPANOSOME INFECTIONS			
added	% DEV	d.f.	F	p
(none)	86.09	5457		
+ *T. vivax*	2.77	1	183.16	0.0000
+ *T. congolense*	0.98	1	64.62	0.0000
+ *T. brucei* spp.	0.01	1	0.67	0.4139
+ *T. vivax* x *T. congolense*	0.02	1	1.20	0.2741
+ *T. vivax* x *T. brucei* spp.	0.00	1	0.16	0.6924
+ *T. congolense* x *T. brucei* spp.	0.02	1	1.12	0.2900
+ *T. vivax* x *T. congolense* x *T. brucei* spp.	0.00	1	0.24	0.6253
error	82.53	5450		
total	86.09	5457		

n = 9973

Table VII.11 Analysis of covariance of PCV of cattle with various trypanosome infections
(stepwise fitting of a regression model)

parameter					
added	n	% DEV	d.f.	F	p
(none)		0	8015		
+ *T. vivax*	715	1.96	1	160.78	0.0000
+ *T. congolense*	195	0.62	1	50.79	0.0000
+ *T. brucei* spp.	489	0.00	1	0.11	0.7448
T. theileri	489	0.00	1	0.00	1.0000
microfilaria	591	0.02	1	1.68	0.1953
error		97.41	8010		
total		100	8015		

Appendix VIII

Appendix VIII

Table VIII.1 Comparison of the annual prevalence of *T. brucei* spp. in cattle, sheep, goats and pigs

species	cattle	sheep	goats	pigs
number of *T. brucei* spp. positive samples	489 (416.8)	5 (40.3)	5 (43.0)	13 (11.9)
number of samples with no *T. brucei* spp. infection	9484 (9556.2)	960 (924.7)	1024 (986.0)	271 (272.1)

() expected values
($X^2 = 80.52$, df = 3, $p < 0.001$)

Table VIII.2 Comparison of the annual prevalence of *T. brucei* spp. in cattle and sheep

species	cattle	sheep
number of *T. brucei* spp. positive samples	489 (450.4)	5 (43.6)
number of samples with no *T. brucei* spp. infection	9484 (9522.6)	960 (921.4)

() expected values
($X^2 = 39.23$, df = 1, $p < 0.001$)

Table VIII.3 Comparison of the annual prevalence of *T. brucei* spp. in cattle and goats

species	cattle	goats
number of *T. brucei* spp. positive samples	489 (447.8)	5 (46.2)
number of samples with no *T. brucei* spp. infection	9484 (9525.2)	1024 (982.8)

() expected values
($X^2 = 42.44$, df = 1, $p < 0.001$)

Table VIII.4 Comparison of the annual prevalence of *T. brucei* spp. in cattle and pigs

species	cattle	pigs
number of *T. brucei* spp. positive samples	489 (488.1)	13 (13.9)
number of samples with no *T. brucei* spp. infection	9484 (9484.9)	271 (270.1)

() expected values
($X^2 = 0.063$, df = 1, p = 0.802)

Table VIII.5 Comparison of the annual prevalence of *T. brucei* spp. in sheep and goats

species	sheep	goats
number of *T. brucei* spp. positive samples	5 (4.8)	5 (5.2)
number of samples with no *T. brucei* spp. infection	960 (960.2)	1024 (1023.8)

() expected values
($X^2 = 0.010$, df = 1, p = 0.919, Fisher's exact test p = 0.572)

Table VIII.6 Comparison of the annual prevalence of *T. brucei* spp. in, sheep and pigs

species	sheep	pigs
number of *T. brucei* spp. positive samples	5 (13.9)	13 (4.1)
number of samples with no *T. brucei* spp. infection	960 (951.1)	271 (279.9)

() expected values
($X^2 = 25.46$, df = 1, p < 0.001, Fisher's exact test p = 0.0000096)

Table VIII.7 Comparison of the annual prevalence of *T. brucei* spp. in goats and pigs

species	goats	pigs
number of *T. brucei* spp. positive samples	5 (14.1)	13 (3.9)
number of samples with no *T. brucei* spp. infection	1024 (1014.9)	271 (280.1)

() expected values
($X^2 = 27.56$, df = 1, $p < 0.001$, Fisher's exact test p = 0.0000053)

VIII.8 Clinical histories and post mortem examinations of animals with cerebral infections of *T. brucei* spp.

Bull N19

N19 was a 7 year old Ayrshire bull introduced to the Ngelechom area two years previously as part of a livestock improvement scheme. It was initially seen in March 1993 in good health with a PCV of 26% and weighing 295 Kg. One month later the bull was seen again with a mixed infection of *T. brucei* spp. and *T. vivax* when it had a PCV of 18% and its weight had dropped to 257 Kg. The animal was very dull, had enlarged superficial lymph glands and a staring coat. At this point it was treated with diminazene aceturate (veriben®) at a dose rate of 7 mg/kg. The bull slowly improved until July when its weight was 326 kg with a PCV of 23%.

In August it again had a mixed infection of *T. brucei* spp. and *T. vivax* with its PCV dropping to 18% and its weight to 289 kg. Clinical signs of anaemia, weight loss, grossly enlarged lymph glands and dullness were seen and the animal was again treated with diminazene aceturate at 7 mg/kg. Two weeks later the same month the animal was examined and found to be suffering from a neurological disorder. The bull was extremely dull, pressing its head against the fence and when forced to walk it circled to the right hand side. No trypanosomes could be detected in the blood and the PCV had risen to 22% but the state of weakness and enlarged lymph glands remained. A tentative diagnosis of

heartwater was made and the animal was treated with 40 ml of 10% oxytetracycline. The bull made an uneventful recovery gaining weight until it weighed 304 kg in September with a PCV of 24%.

No abnormalities were detected in October (weight 297 kg and PCV 25%) however by the 8th November the bull was again found to have a mixed infection of *T. brucei* spp. and *T. vivax*. This was diagnosed as an early infection as the animal only had moderately enlarged superficial lymph glands, showed only a small reduction in weight to 289 kg and the PCV had remained almost constant at 24%. At this time the bull was bright and otherwise clinically normal and was not treated.

On the 7th of December no trypanosomes could be detected but the PCV had dropped to 20 and its weight had reduced to 276 kg. The bull remained bright but was now showing signs of anaemia and nystagmus. Later that month (17/12/93) the PCV had risen to 25 and both blood and CSF samples proved positive for *T. brucei* spp. by mouse inoculation.

Examination on the 11th January 1994 showed a mixed infection of *T. brucei* spp. and *T. vivax* with a PCV of 20% and a reduction in weight to 239 kg. At this time there was a marked deterioration in the animal's clinical condition. The bull was now: blind in the left eye and only had poor vision in the right eye due to uveitis; its gait was exaggerated and staggering with a tendency to circle to the left hand side; its head was held at an angle and it was head pressing; it had a marked mucopurulent nasal discharge; and its lymph glands were markedly enlarged. The animal was also extremely dull and anorexic. At this point bull N19 was again treated with diminazene aceturate at 7 mg/kg. By the 24th January there was a marked improvement in the animal's condition. It was much brighter and was now eating well. Blindness remained evident and although the signs of neurological disturbance were still present they were less obvious. Accompanying this improvement in clinical condition was an increase in PCV to 27%. While both blood and CSF were negative for trypanosomes by direct examination the blood sample was positive for *T. .brucei* spp. by mouse inoculation.

While the bull's weight had risen to 248 kg by the 8th of February, its PCV had dropped to 24% and the animal's clinical condition had deteriorated again. It was very dull, would only walk in a circle to the right hand side and was anorexic again. The

animal's condition became progressively worse until it became recumbent for 24 hours before dying on the 21st February 1994.

At post mortem the carcass was emaciated with enlargement of all lymph nodes. The spleen was grossly enlarged and there was hyperplasia of the white pulp giving it a mottled appearance with petaechial haemorrhages on the surface. The liver was also slightly enlarged with some pale fibrous spots and petaechial haemorrhages on the surface. The heart also had petaechial haemorrhages on the surface of the atria and ventricals, but was otherwise normal in size and appearance. The apical and cardiac lobes of the lungs were congested and there was some interlobular fibrosis. the meninges were congested and the eyes had were clouded over.

Bull R250

R250 was a 5 year old local east African zebu bull which consistently gained weight throughout the period March 1993 to May 1994 except during a period of drought early in 1994. Clinically this animal remained in a state of positive health during this time. Blood samples were positive for *T. brucei* spp. by direct examination only once in October and by mouse inoculation in June, July, August, October, November, December, January and April. Its PCV ranged from 22% to 32% with a mean of 25.6%.

At post mortem the carcass was in very good condition. Apart from a grossly enlarged mottled spleen and a slight enlargement of superficial lymph nodes, no abnormalities were detected.

Heifer A68

A68 was an 11 month old local east African zebu heifer which consistently gained weight throughout the period March 1993 to May 1994. clinically this animal remained in a positive state of health during this time. Blood samples were positive for *T. brucei* spp. by direct examination only once in November and by mouse inoculation in November, January, February and April. Its PCV ranged from 23% to 32% with a mean of 25.7%.

At post mortem the carcass was in good condition. Apart from a grossly enlarged mottled spleen and a slight enlargement of superficial lymph nodes, no abnormalities were detected.

Cow A84

A84 was a 4 year old local east African zebu cow which was first examined in March 1993 when it had a PCV of 30% and weighed 182 kg. By July it had increased its weight to 207 kg and maintained its PCV above 30% during the 5 monthly examinations.

In August a mixed infection of *T. brucei* spp. and *T. vivax* was found, however its PCV was 28%, its weight was 206 kg and the only clinical abnormality was an enlargement of the superficial lymph glands it was only treated with a multivitamin injection.

Blood samples taken in September showed a *T. congolense* infection by direct examination and a *T. brucei* spp. infection by mouse inoculation. By this time the PCV had dropped to 12% and its weight had reduced to 198 kg. Clinical examination revealed enlargement of the superficial lymph glands, very pale mucous membranes, pulsating jugular veins and an incidental thelazia infection in the eyes but otherwise in good condition. The animal was treated with diminazene aceturate at 7 mg/kg.

By October the animal's PCV had risen to 25% and no trypanosomes were detected so this animal was considered cured although its weight remained at 196 kg.

Its PCV continued to rise, reaching 28% in November when it weighed 221 kg.

In December an infection with *T. vivax* was detected, however the animal remained clinically normal and in good condition (PCV 27% and weight 209 kg) and was only treated with multivitamin. No abnormalities were detected in January (PCV 25%, weight 214 kg).

In February a mixed infection of *T. brucei* spp. and *T. vivax* was diagnosed by direct examination of blood and the *T. brucei* spp. infection confirmed by mouse inoculation. Clinically the animal was showing no visible abnormalities and remained in good condition (PCV 24% and weight 221 kg). It was again treated with a multivitamin injection.

The cow continued to gain weight until May when it weighed 244 kg with a PCV of 27%. Blood samples were all negative by direct examination but always positive for *T. brucei* spp. by mouse inoculation.

The cow was in good health and no post mortem was carried out at the end of the study.

Bull A279

A279 was a 3 year old local east African zebu bull which was used as a draught animal throughout the period April 1993 to May 1994. When it was first examined in April 1993 it was in fair condition with a PCV of 30% and weighing 156 kg.

In June a *T. vivax* infection was found, the PCV fell to 18% and its weight dropped to 140 kg. Clinically the animal was dull, not eating well, had enlarged superficial lymph glands, it had pale mucous membranes and had pulsating jugular veins. It was then treated with diminazene aceturate at 7 mg/kg.

Direct examination of blood in July revealed an infection with *T. brucei* spp. with the PCV rising to 26% and the weight to 141 kg. Clinically the animal had greatly improved with the only sign of ill health being slightly enlarged lymph glands. It was treated with multivitamin.

In August a *T. vivax* infection was found by direct examination and a *T. brucei* spp. infection by mouse inoculation. Clinically the animal had enlarged lymph glands and pale mucous membranes but no other abnormalities (PCV 20% and weight 150 kg). It was treated with diminazene aceturate at 7 mg/kg.

By September no trypanosomes could be detected, the PCV risen to 25% and the weight to 152 kg.

In October a mixed infection of *T. brucei* spp. and *T. vivax* was found by direct examination of blood and *T. brucei* spp. by mouse inoculation. The PCV had dropped to 20% while the weight remained similar at 154 kg. Clinically the animal was dull, had enlarged lymph glands, pale mucous membranes and small petaechial haemorrhages under the tongue indicating a mild haemorrhagic strain of *T. vivax*. It was again treated with diminazene aceturate at 7 mg/kg.

Examination in November also showed a mixed infection with *T. brucei* spp. and *T. vivax* by direct examination of blood. However the clinical condition of the animal had improved with enlarged lymph glands as the only abnormality detected, the PCV had risen to 23% and the weight had increased to 168 kg indicating that this was probably a new infection rather than a case of drug resistance. It was treated with multivitamin.

December revealed a mixed infection of *T. brucei* spp. and *T. vivax* by direct examination and a *T. brucei* spp. infection by mouse inoculation. There was no

change in the animal's clinical condition but the PCV went down to 21% and the weight to 164 kg. It was treated with multivitamin.

From this time on the bull maintained its PCV within normal limits and gained weight. *T. brucei* spp. infections were only detected by mouse inoculation between February and May. Despite having a cerebral infection of *T. brucei* spp. this bull remained in positive health an was being regularly used as a draught animal.

At post mortem the carcass was in very good condition. Apart from a grossly enlarged mottled spleen and a slight enlargement of superficial lymph nodes, no abnormalities were detected.

Heifer A399

A399 was a 3 year old local east African zebu heifer which consistently gained weight throughout the period August 1993 to May 1994. Clinically this animal remained in a positive state of health during this time. Blood samples were positive for *T. congolense* by direct examination in January and positive for *T. brucei* spp. by mouse inoculation in January and May. Its PCV ranged from 19% to 37% with a mean of 26.6%.

The heifer was in good health and no post mortem was carried out at the end of the study.

Sheep A294

A294, a local east African fat tailed hair sheep, was found infected with *T. brucei* spp.. in April 1993 when it was 18 months old and weighed 22 Kg. The presenting clinical signs were slight dullness and enlarged lymph glands (prescapular, inguinal, popliteal and submaxillary) without anaemia (PCV 30%). It continued to show the same mild clinical signs until August when it had increased in weight to 31 Kg. From September it began to lose weight until its death in January 1994 when it weighed only 16 Kg (most of the weight loss coming in the last month of life). By December the sheep was in poor condition and started to show signs of neurological disturbance which developed from weakness and inco-ordination to circling, extreme weakness, ataxia and blindness prior to death. Anaemia was not a feature of this disease syndrome (PCV at death was 28% and was never lower than 25%). Blood samples were positive for *T. brucei* spp. by direct examination in April,

May, November and December and by mouse inoculation in April, May, July, August, September, October, November and December. This sheep was never treated.

Since this sheep died naturally and its carcass was eaten by a dog, no post mortem was carried out.

Pig A775

A775 was a 2 year old white sow weighing 55 kg which was first examined on the 11th December when it was reported not to be eating. This examination revealed a *T. brucei* spp. infection by direct examination with a PCV of 26%. The animal was nocturnal and never ate well. It lost weight until its death on the 26th of January when it weighed 35 kg. Two weeks before its death the pig started showing obvious nervous signs of weakness, inco-ordination and ataxia. During the last 5 days of its life it was laterally recumbent, twitching and apparently blind.

At post mortem the carcass was emaciated with a generalised enlargement of all lymph nodes. The spleen was grossly enlarged with hyperplasia of the white pulp giving a mottled appearance. There were petaechial haemorrhages on the surface of the heart and the pericardium contained blood tinged fluid.

Pig A802

A802 was 3 month old white male pig which was first examined on the 13th January when it weighed 5 kg with a PCV of 30%. At this time it was treated with isometamidium chloride at 1 mg/kg.

In April the pig was found to be infected with *T. brucei* spp. by direct examination of blood and by mouse inoculation. At this time the pig weighed 25 kg and had a PCV of 25%.

The pig was similarly positive for *T. brucei* spp. in May when it weighed 32 kg.

At post mortem the carcass was in good condition. There was a generalised enlargement of all lymph nodes of between two and four times normal size. The spleen was grossly enlarged with a mottled appearance. The liver had three fibrous white spots on its surface. No other abnormalities were detected.

Pig A850

A850 was a 10 month old white sow weighing 38 kg which was first examined on the 9th February when it was reported to have aborted on the 4th February and not to be eating well. A *T. brucei* spp. infection was detected by both direct examination of blood and mouse inoculation. Clinically the pig was dull, had a PCV of 24%, was thin, had enlarged lymph glands and a vaginal discharge.

The pig was examined on the 8th March when a *T. brucei* spp. infection could be detected by both direct examination of blood and by mouse inoculation. It was emaciated, in a state of lateral recumbency, it had been anorexic for 3 days, its lymph glands were enlarged and it was showing nervous signs of twitching of the facial muscles, ears and limbs and was apparently blind. It then weighed 28 kg with a PCV of 19% and was treated with diminazene aceturate at 7 mg/kg.

Blood taken on the 11th March was negative for *T. brucei* spp. when the pig weighed 25 kg with a PCV of 24%. The clinical signs had not improved and the animal was euthanased in a moribund state by i.v. pentobarbitone on the 14th March when the blood was again positive for *T. brucei* spp. by mouse inoculation.

At post mortem the carcass was emaciated with a generalised enlargement of all lymph nodes. The spleen was grossly enlarged with hyperplasia of the white pulp. The liver was congested and enlarged. The intestines were congested as were the cardiac and diaphragmatic lobes of the lungs. The heart was enlarged, pale and flabby with petaechial haemorrhages on the surface and the pericardium contained 20 ml of straw coloured fluid. The thymus was enlarged. The meninges were grossly congested and the eyes were clouded over.

Appendix IX

Appendix IX

Table IX.1 The proportion of man-infective and non-man-infective *T. brucei* spp. in Busia District and Tororo District

location	Busia District	Tororo District
man infective *T. brucei* spp.	1 (1.6)	7 (6.4)
non man infective *T. brucei* spp.	8 (7.4)	30 (30.6)

( ) expected values

(Fisher's exact test p = 0.503)