VARIATION IN SUSCEPTIBILITY TO TSETSE-BORNE TRYPANOSOMIASIS AMONG THREE *BOS INDICUS* CATTLE BREEDS IN DIFFERENT TSETSE ENDEMIC LOCALITIES IN KENYA

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DECLARATION

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

Eric Karanja Mwangi,

January 1993.

DEDICATION

I dedicate this thesis to my parents, family and all friends who gave me constant encouragement.

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Ab-ELISA	Antibody ELISA
Ag-ELISA	Antigen ELISA
ABTS	2,2 - azino bis (3-ethyl)-benzatnine-6-sulphonic acid
A.D.	Apparent Density
CATT	Card agglutination test
CBPP	Contagious bovine pleuropneumonia
DG	Darkground-phase contrast/buffy coat technique.
df	Degrees of freedom
DFMO	Difluoromethylornithine
DNA	Deoxyribonucleic acid
e.p.g.	Eggs per gramme
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-linked immunoabsorbent assay
ECF	East Coast Fever
f	Frequency
FAO	Food Agricultural Organization
F	Fat for body condition score
F1	Filia one (First generation offspring)
IgM	Immunoglobulin M
ICIPE	International Centre of Insect Physiology and Ecology
IBAR	Interafrican Bureau for Animal Resources
ILCA	International Livestock Centre for Africa
ILRAD	International Laboratory for Research on Animal Diseases
kg	Kilogram (s)

KETRI	Kenya Trypanosomiasis Research Institute
KARI	Kenya Agricultural Research Institute
kDa	KiloDalton
km	Kilometre (s)
L	Lean for body condition score
ml	Millilitre (s)
M	Medium for body condition score or 1 Molar solution in case of Chemical reagents
mg	Milligram (s)
m	Metre (s)
mm	Millimetre
m-AEC	Miniature anion-exchange/centrifugation technique
ms	Mean square
not sig.	Not statistically significant
NPE	No previous exposure
nm	Nanometer (s)
Nacl	Sodium chloride
O.D.	Optical density
^{o}C	Degrees Celsius
p	Probability
PCV	Packed cell volume
PE	Previous exposure
PBS	Phosphate buffered saline
r^2	Correlation coefficient
SD	Standard deviation
SIT	Sterile Insect Technique

sig.	Statistically significant
SAT1	South African type 1
ug	Microgramme (s)
ul	Microlitre (s)
ULV	Ultra Low Volume
VAT	Variant antigen type

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SUMMARY

Tsetse-transmitted Afican bovine trypanosomiasis is a major disease limiting livestock production in large areas of Africa. The current methods of disease control are aimed at vector (tsetse) control or the use of drugs to treat or prevention of infection. Tsetse control operations are expensive to implement and maintain, while there are few drugs currently available. In addition, the parasites frequently develop resistance to the drugs following prolonged use.

Currently the utilization of cattle breeds that possess a degree of resistance to the disease has been viewed as an additional tool for the control of trypanosomiasis. This approach has been studied extensively in West African cattle but there has been little attention in East African livestock.

This thesis was therefore designed to investigate the variation in susceptibility among three breeds of *Bos indicus* cattle breeds, the Maasai Zebu, Orma Boran and Galana Boran kept under varying levels of tsetse challenge in different parts of Kenya.

The first chapter reviews the disease epidemiology and current control methods with emphasis on genetic resistance. Chapter 2 summarizes the previous epidemiological work on breed variation carried out at the Kenya Trypanosomiasis Research Institute (KETRI) which led to this investigation. In Chapter 3, the study areas are described together with the livestock production systems and the constraints to production. In addition, a brief review of the history of *Bos indicus* cattle in East Africa with emphasis on the breeds studied and the epidemiological data collected is given.

The field experiments are reported in Chapter 4. Section 4.1 presents the work carried out between September 1989 and September 1990 in high and low tsetse challenge areas at the Nguruman escarpment, South Western Kenya,

involving Maasai Zebu cattle from Nguruman, Orma Boran and Galana Boran cattle transferred from the Galana Ranch on the Kenya coast. In the high challenge area, it was observed that, both the Maasai Zebu and Orma Boran were less susceptible to trypanosome infections as judged by the disease incidence, degree of anaemia, drug requirements and the body weight gains. In the low challenge area, where the tsetse fly population was controlled using odour-baited traps, only the Maasai Zebu and Orma Boran were compared. The results indicated that, there were no significant differences in the disease incidence, degree of anaemia and the growth rates between the two breeds.

The cattle that survived from the high tsetse challenge area at Nguruman were transferred to the Galana Ranch, where they were compared with new groups of Maasai Zebu and Orma and Galana Boran with no previous exposure, for a period of nine months from May 1991 to February 1992. The Maasai Zebu cattle were purchased from farmers at the Nguruman escarpment and transferred to the Galana Ranch, while the Orma and Galana Boran cattle were purchased locally at the Galana Ranch. The results, presented in section 4.2, showed no significant differences in susceptibility among the cattle with previous exposure. On the other hand, among the cattle with no previous exposure, the Maasai Zebu and Orma Boran were significantly less susceptible to trypanosomiasis than the Galana Boran as indicated by the disease incidence, degree of anaemia, drug treatments and the growth rates.

This study has therefore indicated that the Maasai Zebu and Orma Boran possess a superior resistance to trypanosomiasis than the Galana Boran and that, the resistance is not restricted to one locality. They also suggest that previous exposure does influence the susceptibility.

The last aspect of this study (Chapter 5) involved the comparison of the parasitological (darkground/phase contrast buffy coat technique and mouse inoculation) and immunological techniques (antigen and antibody enzyme-linked immunoassays) for the diagnosis of trypanosome infections in cattle exposed to natural tsetse challenge. It was observed that for complete epidemiological information, a combination of more than one technique preferably the darkground phase/buffy coat contrast and the antigen ELISA would be ideal.

Chapter 6 presents the general discussion, conclusions and recommendations.

CHAPTER 1

INTRODUCTION

1.1 AFRICAN TRYPANOSOMIASIS

The African trypanosomiases form a group of diseases of man and domestic livestock caused by trypanosomes, which are flagellate haemoprotozoan parasites.

1.1.1 THE PARASITE

Trypanosomes are protozoan organisms of the Genus: *Trypanosoma*, Family: *Trypanosomatidae*, Order; *Kinetoplastidae* Class: *Zoomastigophora* and Phylum: *Protozoa* (Hoare, 1970, 1972; Molyneux and Ashford, 1983).

They inhabit the plasma, body fluids and tissues of a wide range of animal hosts world-wide (Hoare, 1970). Generally, the bloodstream forms are motile unicellular organisms usually elongate spindle shaped, flattened and have a flagellum for locomotion. They possess a kinetoplast which is located near the basal body of the flagellum. Identification of the various species is based on these morphological features, i.e., the size and shape of the body, position of the kinetoplast, the length and form of the undulating membrane and the flagellum.

Depending on the site of development and mode of transmission of infection, trypanosomes have been divided into two groups, the stercoraria and the salivarian. The stercoraria develop in the alimentary tract of the vector with the production of metacyclics occurring in the hind gut and bring about infection either by contamination of the skin or oral ingestion by the host. *Trypanosoma cruzi*, the causative agent of Chagas' disease in South and Central America, is the most important in this group. On the other hand, the salivarian group complete their development in the anterior station, i.e., the salivary glands and the proboscis and so, their transmission is

inoculative. All parasites of veterinary and medical importance in tropical Africa fall into this group. The salivarian group trypanosomes are divided into four subgenera according to their morphological and biological characteristics (Table 1.1).

1.1.2 TRANSMISSION

The parasites are transmitted from one host to another by intermediate invertebrate hosts which are generally haematophagus arthropods. Trypanosomes were first described in 1894 by Bruce in Natal through microscopic examination of blood, and he also identified the tsetse flies as the disease carriers (Bruce, 1895, 1897). Tsetse flies belong to the genus *Glossina.* There are 36 different species and subspecies of tsetse flies which are capable of transmitting trypanosomes (Ford, 1970a). These species fall into three groups which are adapted to various ecological zones within the African continent; the *morsitans* group mainly found in the savannah, *palpalis* inhabiting the riverine zone and the *fuscipes* in the forests.

Mechanical transmission by biting flies such as *Tabanidae* or *Stomoxys* is known to occur (reviewed by Wells, 1972, 1984), and is important in the disease caused by *Trypanosoma evansi.* This disease is significant among Africa's 12 million camels which make up 80% of the world's total (Mohamoud and Gray, 1980). Non-tsetse transmitted trypanosomiasis due to *Trypanosoma vivax* and *T. evansi* has been shown to occur in South America and Asia where tsetse flies do not exist (Wells, Bentacourt, and Raminez, 1982; Mohamoud and Gray, 1980). *Trypanosoma vivax* also occurs in the absence of tsetse flies in Mauritius where it is thought to be mechanically transmitted (Jordan, 1986). *Trypanosoma equiperdum* which causes a venereal disease in

Table 1.1

Classification of the trypanosomes pathogenic to domestic animals and man in Africa

Subgenus	Species	Subspecies	Major hosts
Duttonella	*T. vivax*		Cattle, sheep, goats
Nannomonas	*T. congolense*		Cattle, sheep, goats
	T. simiae		Pigs
Pycnomonas	*T. suis*		Pigs
Trypanozoon	*T. equiperdum*		Horses, donkeys
	T. evansi		Camels, horses, donkeys
	T. brucei	*T. b. brucei*	Cattle, sheep, goats, dogs
		T. b. rhodensiense	Man
		T. b. gambiense	Man

horses and donkeys is transmitted by coitus and therefore no intermediate vector is involved. The vampire bat (*Desmondus rotundus*) has been thought to transmit *T. evansi* in South and Central America. Transmission by blood transfusion is of significant importance in *T. cruzi*, while transplacental transmission of *T. vivax* has also been reported (Wells, 1972; Ogwu, Njoku, and Osori, 1985).

The normal vertebrate hosts of tsetse-transmitted trypanosomes are the wild large mammals of Africa. They suffer no obvious pathogenic effects unless stressed and therefore are important as reservoir hosts. These wild animals have been shown to play a major role in the epidemiology of trypanosomiasis in East and Central Africa (Ashcroft, 1959; Karstard, Grootenhuis, and Mushi, 1978). The disease becomes important when man and the domestic animals are involved since they are both susceptible to the pathogenic effects of the parasite.

Once infected, the tsetse fly remains infected for its lifetime and becomes a reservoir. The parasite undergoes developmental stages in the invertebrate host. Bloodstream forms or trypomastigotes are ingested by the vector, develop into epimastigotes, and then transform into the infective metacyclic forms. For *T. congolense*, this process occurs in the proboscis and midgut, while that of *T. brucei* occurs in the salivary glands and midgut. The initial cyclical development of *T. vivax* starts in the oesophageal region; the parasites then migrate to the food canal of the proboscis and mature to infective metacyclics in the hypopharynx (Moloo and Gray, 1989). Trypanosomes have a wide range of hosts which include man, domestic livestock, wild animals and rodents (reviewed by Ford, 1970b).

1.1.3 THE DISEASE IN CATTLE

Bovine trypanosomiasis also referred to as 'nagana' is caused mainly by two species of trypanosomes, *T. vivax* and *T. congolense* and to a lesser extent *T. brucei* (Fiennes, 1950, 1970; Morrison, Murray and McIntyre, 1981a). Mixed infections are commonly observed in the field (Stephen, 1970). Identification and differentiation of the three species in the field is based on the morphological differences in stained blood smears and characteristic motility behaviour when observed on a fresh blood film (Hoare, 1972). The clinical disease produced in cattle shows considerable variation in severity and duration. The severity depends on several factors including, the degree of tsetse challenge, the cattle breed, age, intercurrent disease, nutritional status and the virulence of the strain (Murray, Morrison and Whitelaw, 1982).

The clinical signs shown by the three species varies from an acute to a chronic syndrome. The acute form of the disease is generally characterized by high temperature following the infection, with high persistent parasitaemia and death may occur within two weeks (normally two to six weeks). Following infection with some strains of *T. vivax*, death may occur within two weeks, while virulent strains of *T. congolense* may cause death six to ten weeks after infection (Morrison *et al.*, 1981a). Animals infected for longer than three months are normally regarded as having the chronic disease.

An extremely acute disease caused by some strains of *T. vivax* characterized by pyrexia, persistently high parasitaemia, blood stained diarrhoea and on gross pathology, ecchymotic and petechial haemorrhages on all visceral organs and in the gastro-intestinal tract has been reported in Kenya (Hudson, 1944; Mwongela, Kovach and Fazil, 1981; Wellde, Chumo, Adoyo, Kovach, Mwongela and Opiyo, 1983), Ethiopia (Roeder, Scott and Pegram,

1984), and Somalia (Dirie, Wallbanks, Molyneux and Borstein, 1988).

The common clinical syndrome for all the three species is a chronic condition associated with waves of parasitaemia, intermittent pyrexia, enlargement of superficial lymph nodes, partial anorexia, loss of condition, lethargy (Morrison *et al.*, 1981a; Murray, 1979), progressive anaemia (Hornby, 1921; Murray, 1974; Murray and Dexter, 1988), weakness and a terminal recumbency.

There is an initial fall in the packed red cell volume (PCV) associated with the first wave of parasitaemia. During the infection with both *T. congolense* and *T. vivax*, periods of pyrexia have been associated with high parasitaemia (Hudson, 1944; Van den Ingh, Zwart, Vanmiet, and Schotman, 1976). In animals inoculated with bloodstream forms, the first wave can occur within few days, while in cattle subjected to tsetse-transmitted infection, the parasites are detected from the second or third week of infection (Morrison *et al.*, 1981a).

Following single needle or fly challenge, PCV drops progressively to by about 40 to 60% or even lower within the first four to six weeks. At this stage, the PCV may continue to fall until the animal dies, while in others, it is maintained at these low levels for a variable length of time, which may be up to several months (Morrison *et al.*, 1981a; Murray and Dexter, 1988). Animals treated with trypanocidal drugs at this stage normally recover and the PCV rises back to normal haematological levels (Holmes and Jennings, 1976). The rate of recovery depends on when the treatment is administered; animals treated early show a rapid and dramatic response (Murray and Dexter, 1988).

Animals surviving this stage without treatment progress to the chronic phase of the disease. In the chronic stage, some animals recover

spontaneously without treatment, while others survive with a constantly low PCV but eventually die. The chronic stage of the disease can persist for months or years. Animals that survive are uneconomical to keep since they become anaemic, emaciated, lethargic and even recumbent. The chronic stage is associated with progressively decreasing waves of parasitaemia and in most cases the trypanosomes cannot be detected by parasitological techniques. This stage has been associated with infertility, anoestrus and abortions in females (Ogwu *et al.*, 1985) and reduced sperm quality in males (Losos and Ikede, 1972; Grundler, 1985).

Exposure of animals in the chronic stage to any form of stress such as trekking, use for draught, inadequate feeding or any intercurrent disease may result in the recurrence of the acute form of the disease. The animals are also very susceptible to other diseases due to the poor general body condition. Animals in this stage respond poorly to drug treatment. The chronic stage is often fatal, death being caused by congestive heart failure brought by combination of anaemia and circulatory disturbance associated with vascular permeability (Murray, 1974). The tissue lesions seen at necropsy vary due to the difference in the distribution of the parasites within the host, except for the hyperacute *T. vivax* infections. *Trypanosoma brucei* and *T. vivax* are found intra- and extra- vascularly, while apart from the initial site of inoculation, *T. congolense* is restricted to the bloodstream. Organ and tissue damage has been reported in the skeletal muscles and heart following *T. vivax* infections (Van den Ingh, Zwart, Vanmiert, and Schotman, 1976; Van den Ingh, and De Neij-Bakker, 1979), and in the central nervous system (Morrison, Murray, Whitelaw and Sayer, 1983), reproductive system and endocrine organs (Ikede and Losos, 1975; Morrison, Murray, Sayer and Preston, 1981b) in *T. brucei* infections.

Severe tissue damage including myocarditis and meningoencephalitis has been reported to be a common feature of *T. brucei* infections in cattle (Morrison *et al.,* 1983).

1.2 IMPORTANCE OF TRYPANOSOMIASIS

Animal trypanosomiasis in Africa represents one of the major veterinary problems and, while most of the other animal diseases have been controlled, trypanosomiasis continues to present a major threat to animal production in the Sub Saharan Africa (Holmes and Torr, 1988). Most of the best watered land is infested with tsetse flies, while large areas of good grazing in the sub humid zone could be available for pastoralism if trypanosomiasis was controlled (MacLennan, 1980).

In 1982, it was estimated that, the world population was 4.2 billion expanding at a rate of 2%, i.e., about 70 to 80 million people per year and about 30 million tonnes of staple food were needed annually to cope with this growth (Gavora, 1982). There is therefore the need to increase the food production of the continent to cope with this population growth. One of the major solutions is to increase livestock production by reduction of livestock losses due to disease. It has been estimated that if a 2% reduction in livestock death due to disease would be achieved, it would provide food for an additional 80 million people (Gavora, 1982).

Seventy five percent of the world's population lives in developing countries. Sixty to 70% of the world's livestock is found in the developing countries but accounts for only 30 % of the worlds meat output. The estimated animal protein production per 1000 hectares in 1975 was 542 kg for Africa, 4113 kg for Latin America, and 38,083 kg for Europe (FAO, 1975). These

figures show that Africa's farming produces less animal protein than any other continent. There are still large areas of Africa which have not been developed and one of the most significant factors restricting the extension of cattle producing areas of Africa is the tsetse fly (FAO, 1961; Jordan, 1986).

Trypanosomiasis is a threat to both man and animals. Human trypanosomiasis (sleeping sickness) caused by *T. b. rhodesiense* and *T. b. gambiense* is a major problem among the human population and it is estimated that 50 million people in 36 countries in Africa are at risk with an estimated increase of 20,000 newly reported cases per year (Molyneux, 1986). Sleeping sickness is a major factor in the depopulation of large areas of Africa and is therefore a constraint on rural development. Tsetse flies infest about 11 million km^2 of the continent and affect 37% of the continent in 40 countries (FAO/WHO/OIE, 1982). If tsetse flies were controlled, 7 million km^2 of this land would be suitable for mixed agriculture and livestock development without stress to the environment (MacLennan, 1980).

Of the 173 million cattle in Africa, only about 44 million are in the tsetse-infested area (IBAR, 1989) and, while there is no information on sheep, goats, pigs, horses, donkeys and camels, the situation is probably similar. In 1963, the annual loss due to tsetse in meat production was estimated to be $5 billion (Murray and Gray 1984), excluding the value of milk and hides. If trypanosomiasis control was successful, it is estimated that, the development of livestock and agriculture in the tsetse-infested areas in Africa could generate further $50 billion annually (Murray, Stear, Trail, d'Iteren, Agyemang and Dwinger, 1991).

The Central and the West African regions are severely affected by disease. About 26% of Africa's population is in this region (from Senegal to

Zaire) but the area holds only 9% of Africa's livestock population (ILCA, 1979). The livestock biomass per inhabitant is only 26 kg per capita compared to 136 kg for the remainder of Africa south of the Sahara and 79 kg for the whole of Africa (ILCA, 1979). If tsetse control was achieved in this region, its estimated that the carrying capacity could be 20 cattle km^{-2} compared to the present 3.4 cattle km^{-2}, and the sheep and goat population would increased five times (FAO, 1974). In Eastern and Central Africa, about 70% of some countries such as Tanzania are infested with tsetse (FAO, 1974).

Beside losses in meat production, further losses occur in milk production and mixed agriculture. Animals provide 80% of the traction power in Africa and it has been estimated that, the possession of a single oxen can increase the food output of a family six times (McDowell, 1977). The manure produced by livestock is used to improve food crop production and is a potential source of energy in form of biogas. Beside the losses in mortality due to the disease, the non-fatal acute or chronic syndrome causes severe losses in production due to poor growth, severe weight loss, low milk production, reduced capacity to work, infertility and abortions (FAO/WHO/OIE, 1963; McDowell, 1977).

Tsetse reduction has taken place due to activities such as increased agricultural cultivation associated with the expansion of human population. In other places, tsetse control programmes have been successful, e.g., MacLennan (1980) planned one of the greatest tsetse control operations by ground application of residual insecticide which by 1977, had reclaimed 200,000 km^2 of land infested with *G. morsitans* in Nigeria. Insecticides have also been used successfully for tsetse control in Zimbabwe, Botswana and Zambia (MacLennan, 1980; 1981). However at the continental level, territorial expansion of

tsetse in savannah areas has been continuing, e.g., *G. morsitans* has invaded 26,000 km^2 in Nigeria since 1952, 21,000 km^2 in central Cameroon since 1950 and 11,700 km^2 in Zambia since 1953 (MacLennan, 1980). The full extent of tsetse spread is not known but significant advances have been observed in Botswana, Zimbabwe, Malawi, Uganda, Tanzania, Sudan, Ethiopia, Senegal and Mali, while recently, the most active advance has been taking place in Angola (FAO, 1979; MacLennan, 1980; Jordan 1986). Tsetse expansion is increasing production pressure on the remaining tsetse free areas resulting to pasture degradation, fall in agricultural output, and increase production costs (Jordan, 1986). In order to meet the needs of the increasing African population, and to decrease the pressure on the tsetse free regions, the tsetse-infested areas must be utilized, and therefore, there is an urgent need to control the tsetse fly using all practicable methods available (FAO, 1979; Murray and Gray, 1984).

However note should be taken that some workers take the contrary view that the development of the tsetse-infested land for grazing is environmentally detrimental. Omerold and Rickman (1988) consider trypanosomiasis control as one of the worst initiators of land degradation. They suggest that land cleared of tsetse and rapidly overstocked is vulnerable to overgrazing which can easily lead to an ecological imbalance. This argument is unrealistic since it is improper to ignore the control of such an economically significant disease, especially when it poses a threat to human life. Far from increasing overgrazing, planned tsetse control would lead to opening up of new areas for agriculture and livestock development and would greatly reduce the pressure on the arid and semi-arid areas (Jordan, 1986).

1.3 TSETSE CHALLENGE OR TRYPANOSOMIASIS RISK

Human beings and livestock living in the tsetse-infested areas are at the risk of contracting trypanosomiasis as a result of the successful feed of an infected fly. Trypanosomiasis risk is the probability of a host becoming infected per unit time. The terms tsetse risk and tsetse challenge have been used by various workers, but considering the mechanical transmission of *T. vivax* and *T. evansi* by biting flies, the term trypanosomiasis risk is possibly more appropriate.

At present, no precise definition of tsetse challenge or trypanosomiasis risk for use in the field has been formulated. From an economic aspect, what requires to be known is quantification of the trypanosomiasis risk and the likelihood of animals becoming infected in a tsetse-infested area. The risk is greater in some areas than others and is influenced by many variables such as the tsetse density, tsetse species, tsetse infection rates, tsetse transmission rates, infection rates in the hosts, the numbers and species of the hosts and the climatic and ecological conditions (Rogers, 1983). Such correlates of risk are generally incorporated in the term 'challenge' and it has been assumed that by assessing the challenge in any area, the consequent risk can be predicted.

Factors which interact with each other to determine the level of trypanosomiasis risk have been classified into three categories (Whiteside, 1960a); vector related, non-vector related and accessory factors (host related). The vector related consist of; tsetse species, infection rates, tsetse density, disposition to feed on cattle and mechanical transmission. The non-vector related factors include; the trypanosome species, pathogenicity of the trypanosomes, susceptibility to drugs and liability to become drug resistant. The accessory factors comprised of the breed, sex, place of origin of the host, previous history and factors that modify susceptibility such as general condition, pregnancy,

lactation, intercurrent, latent or, chronic disease, grazing, watering and the climatic conditions. These factors have a complex interaction which makes the definition of the challenge or risk difficult.

The concept of trypanosome challenge originated from Eric Whiteside of the Kenya Veterinary Department and since then, many workers have used various definitions in attempts to describe the tsetse challenge or trypanosomiasis risk in their studies. The term Apparent Density (A.D.) was used to refer to the number of non-teneral flies caught in a hand net per 10,000 yards by a man walking through a path made in a tsetse-infested area (fly round) (Glasgow, 1970). Whiteside (1955) found a correlation between the A.D. of *G. pallidipes* and the incidence of animal trypanosomiasis at two different sites, Makueni and Simba in Kenya. Since the degree of correlation in the two areas was different, he suggested that there were other additional factors involved.

The term index of challenge obtained as a product of the A.D. and the mean fly infection rate (Smith and Rennison, 1960; Whiteside, 1962a) was later used in several field studies in Zimbabwe (Boyt, Lovemore, Pilson and Smith, 1962), Tanzania (Cawdery, 1958) and Uganda (Cawdery and Simmons, 1965). Smith and Rennison (1960) defined trypanosome challenge as the number of infective bites from a tsetse which a host receives in a unit of time.

Later, Whiteside (1962b) used the term 'trypanosomiasis incidence' which he defined as, the average number of infections per head per annum recorded from cattle maintained in a tsetse-infested area, treated only with diminazene aceturate (Berenil^{R}, Hoechst) once infected. He referred to the number of treatments per animal as the Berenil Index. According to this method, he regarded 12.5, 6.5, three and one treatments per annum to indicate very high, high, medium and low trypanosomiasis challenge, respectively. This definition

has been criticized as it does not take into account fly densities or infection rates in the determination of trypanosomiasis incidence and therefore only quantifies risk (Rogers, 1983). Boyt *et al.,* (1962) used the Berenil Index (treatments per head per annum) as a measure of what they called 'trypanosomiasis risk', while Cawdery and Simmons (1965), used the term 'challenge index' obtained as product of A.D. and the infection rate.

Progress in research from the mid-1960's revealed shortcomings in the methods previously use for the estimation of the of challenge. The A.D. figures were regarded as poor and biased indicators of fly bites on cattle as experiments in Zimbabwe showed that the presence of humans decreased the fly catches at a cattle bait (Vale 1974). The vectorial capacity was shown to be species dependent and therefore, fly infection rates were not a direct measure of the probability of infection (Harley and Wilson, 1968). The emergence of drug resistant strains (Braber, 1968; Mwambu and Mayende, 1971b) and a slight but significant prophylactic effect of diminazene aceturate (Berenil^R, Hoechst) (van Hoeve, Cunninghham and Graenge, 1964; Gitatha and Maudlin, 1968) were noticed to influence the Berenil Index. The method of Llyod and Johnson (1924) for detecting infection in tsetse is not sensitive enough especially for *T. brucei*, and by this technique, infection rates in wild flies have usually been found to be very low (Jordan, 1974).

Wilson *et al.,* (1972) introduced the concept of rate of disease transmission when they used the term 'transmission index' which they defined as the proportion of infected inocula each from a single infected fly that gives rise to an infection in a susceptible vertebrate host.

Rogers (1983, 1985), suggested that the prophylactic period for diminazene aceturate (Berenil^R, Hoechst) is about 22 days, a figure close to that

suggested by van Hoeve *et al.*, (1964), which when he added to a minimum prepatent period of seven days gave a Berenil interval of 29 days. He recommended that this should be used as a correcting factor when calculating the Berenil Index. Wilson *et al.*, (1983, 1986) used the term parasite attack rate to describe the Berenil Index which he obtained as the mean number of infections per annum calculated on a monthly time interval. Njogu *et al.*, (1985b) described a modified calculation of the Berenil Index in which they allowed a prophylactic period of only seven days for diminazene aceturate.

Recently, ILCA (1986a) modified the measurement of challenge to include, tsetse density, trypanosome fly infection rates and the proportion of feed taken on the domestic animals. The tsetse density was expressed as the relative density (flies /trap/day) using biconical traps, while trypanosome infection rates were determined by the method of Llyod and Johnson, (1924). Blood meal analysis was carried out on smears of undigested blood from the guts of recently fed tsetse using the enzyme linked immunosorbent assay (ELISA). The tsetse challenge was then obtained as the product of the relative density, infection rates and the percentage of blood meals from livestock.

By obtaining the apparent density as a product of tsetse density and mean fly infection rates as measure of the tsetse challenge, and the Berenil Index as a measure of trypanosomiasis incidence in cattle, (Rogers, 1985) demonstrated that the logarithm of challenge was linearly related to the Berenil Index. After analysing data from experiments in which tsetse control and chemotherapy have been used for the control of animal trypanosomiasis, he concluded that the challenge and the risk of infection are related and one can be used as an indicator of the other (Rogers, 1985).

With the current research on the improvement of the detection of infections in flies by *in vitro* culture techniques (Mehlitz,1988) and DNA probes (Kukla, Majiwa, Young, Moloo and ole-MoiYoi, 1987), diagnosis of infection in cattle using antigen detection immunoassays (Nantulya and Lindqvist, 1989) and the development of analytical disease models (Milligan and Baker, 1988; Rogers, 1988; Rawlings, 1991), a precise definition of the trypanosomiasis risk may be feasible.

In order to develop appropriate management and preventive medicine schemes in tsetse-infested areas, and to determine the likely cost benefits of tsetse and trypanosomiasis control, it is essential that accurate estimation of tsetse challenge and trypanosomiasis risk can be predicted.

1.4 DIAGNOSIS OF TRYPANOSOMIASIS IN CATTLE

Diagnosis of trypanosomiasis still presents major problems. This subject has been reviewed by several workers (Molyneux, 1975; Voller 1977; Nantulya, 1990). An accurate epidemiological assessment of animal trypanosomiasis is important in the application of effective disease control programmes. The rapidity and accuracy of any one technique or a combination is vital if suitable treatment of any individuals is to be effective or for chemoprophylactic control in livestock kept in endemic areas.

In the acute phase of the disease, there is fluctuating parasitaemia arising from antigenic variation (Doyle, 1977), while chronic infections usually have low or no parasitaemia (Murray and Dexter, 1988). The demonstration of the parasites can also vary with the sample, time, technique and the microscopist (Greig, 1979).

In terms of the overall disease control strategy, more sensitive methods are necessary. The diagnosis of a limited number of cases in a herd will indicate exposure to disease and depending on the level of trypanosomiasis challenge the type of treatment can be decided (Molyneux, 1975).

The clinical signs of animal trypanosomiasis are not pathognomonic and therefore, the specific diagnosis of the disease still depends on the demonstration of the parasite in blood or tissue fluids by light microscopy or by immunological techniques.

1.4.1 PARASITOLOGICAL DIAGNOSTIC TECHNIQUES

These consist of the thick, thin and wet blood films, inoculation of blood into susceptible mice, and the trypanosome concentration methods consisting of the haematocrit centrifugation (Woo, 1970), the darkground/phase contrast technique (Murray, Murray, and McIntyre, 1977) and the miniature anion exchange techniques (m-AEC) (Lumsden, Kimber, and Strange, 1977; Lumsden, Kimber, Evans and Doig, 1979).

Trypanosomes in the peripheral blood can be detected by microscopic examination of the wet blood film or Giemsa stained thin and thick blood smears. Direct microscopy though cheap and good for screening large herds of cattle, may miss about 50% of the infections (Barnett, 1947).

Inoculation of laboratory animals is more sensitive than direct microscopy (Paris, Murray and McOdimba, 1982). This method is more sensitive for *T. brucei* but other trypanosome strains, mainly the East African *T. vivax*, and to a lesser extent *T. congolense*, do not infect laboratory rodents (Paris *et al.*, 1982; Nantulya, 1990). Diagnosis by this method is not immediate since the mice have to be examined for a minimum of 30 days before they are declared not to be

parasitaemic.

The sensitivity of the direct microscopic methods is improved through concentration of the parasites by centrifugation. Centrifugation of unclotted blood concentrates the parasites on the buffy coat making it more sensitive than the blood films (Woo, 1969; Murray, Murray and McIntyre, 1977). In addition, the darkground/phase contrast buffy coat technique (DG) (Murray *et al.,* 1977) enables the estimation of the intensity of parasitaemia.

Further improvement in the sensitivity of the concentration methods by the separation of the hosts red blood cells prior to the spinning has been achieved by the miniature anion exchange/centrifugation technique (Lumsden *et al.*, 1977). This method is however expensive and time consuming. Although the concentration techniques are more sensitive they require a source of electricity which limits their use in the field.

1.4.2 IMMUNOLOGICAL TECHNIQUES

An ideal immunodiagnostic technique should detect all infected cases and be negative in all other conditions, permit identification of the species of infecting trypanosomes and indicate the efficacy of treatment, i.e., should become negative after parasite clearance and cure (Gray, 1974). However, none of the tests used satisfy these criteria since the development of more sensitive immunological techniques is hindered by the occurrence of antigenic variation.

In spite of these shortcomings, immunodiagnosis can supplement traditional methods for individual diagnosis and epidemiological purposes. The suitability of a test for large scale use, such as its cheapness and ease of use in the field, can outweigh deficiencies in the sensitivity and specificity so long as these are recognized (Voller, 1977). The immunological tests are based on

demonstration of either antibodies or antigens.

a) Antibody detection

At present, the two most commonly used immunological methods for the detection of antibody responses in cattle are the indirect immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA).

i) Indirect immunofluorescent antibody test (IFAT)

This has been the most widely used test for serodiagnosis of trypanosomiasis and several workers demonstrated its superiority over the parasitological methods in the proportion of infected animals detected (Wilson, 1969; Zwart, 1973; Weisenhutter, 1973; Wery, van Wettere, Wery-Paskoff, van Meirvenne and Mesatewa, 1970). However, antibodies do not reach detectable levels for some days (Zwart, 1973), therefore early infections are missed and also, the presence of positive IFAT reactions in treated animals still remains a problem. Weisenhutter (1969) found that animals treated with the trypanocidal drug diminazene aceturate (Berenil^{R}, Hoechst) remained positive 40 days post treatment, while Wilson (1969) reported antibody titres for as long as 112 days.

The antigens used are prepared by making smears of parasitized blood using a variety of fixatives (Zwart, Perie, Keppler and Goedbloed, 1973). The main disadvantages of the method are; the standardization of the antigen preparation is difficult, the slides require ultra-low temperature for storage and transportation, and, the antigens so prepared provide substantial nonspecific reactions. However, the technique for antigen preparation has recently been greatly improved by fixing the antigens in a mixture of acetone and formaldehyde suspension so that the test can give results which are specific enough, to

differentiate to a limited extent between *T. vivax T. congolense* and *T. brucei* infections cattle (Katende, Musoke, Nantulya and Goddeeris, 1987).

Although IFAT has been used extensively recently and the method has considerable practical merit, it requires skilled operators, expensive equipment which cannot be used in the field and also, the interpretation of the results is subjective.

ii) Enzyme-linked immunosorbent assay (ELISA)

The search for new tests as sensitive as the IFAT but cheap to perform and less subjective in interpretation led to the development of enzyme immunoassays. This technology is sensitive, requires simple equipment, assessment of the results is easy and eliminates subjective bias in the interpretation of results and can be used for large scale screening of samples. ELISA was first used for the human disease (Voller, Bidwell, Bartlett, 1975). Luckins *et al.*, (1978, 1979) used ELISA for the diagnosis of *T. evansi* and the results were comparable to those of IFAT with regard to sensitivity and specificity.

The major limitation of its use as a diagnostic test was that the antigens used were crude lysates and their quantity was ill defined thus making the standardization with respect to sensitivity and specificity difficult. Further improvement on the test making it specific enough to distinguish between infections with different trypanosome species through the use of purified antigens has been reported (Ijagbone, Staak and Reinhard, 1989).

iii) Card agglutination test (CATT)

The main disadvantage of trypanosomiasis serodiagnosis is the lack of well defined standardized antigen. The development of the card agglutination test

(CATT) has attempted to overcome this problem for *T. b. gambiense* (Magnus, Vervoort and van Meirvenne, 1978) since it detects antibodies to the surface coat antigens of a commonly occurring variable antigen type (VAT). This is achieved by fixing and stabilizing the antigens on the parasite using formaldehyde, so that the whole trypanosome is used in a direct agglutination test. The *T. b. gambiense* CATT has shown some success in the laboratory for *T. evansi* (Zweygarth, Sabwa and Rottcher, 1984) and this is because the VAT used in the test is found in the repertoire of VAT's expressed by *T. b. brucei, T. b. gambiense*, and *T. evansi* (van Meirvenne, Magnus and Vervoort, 1977). Recent epidemiological studies in Benin have shown that the *T. b. gambiense* CATT can be used to detect infections caused by *T. congolense*, *T. vivax* and *T. brucei* in cattle indicating that the parasite strain (LiTat 1.3) used in the CATT has somatic and variable surface glycoprotein antigens common to other species of salivarian trypanosomes (Doko, Guedegbe, Baelmans, Demey, N'Diaye, Pandey and Verhulst, 1991).

Serological methods based on the antibody detection are sometimes difficult to interpret due to occurrence of mixed infections, the persistence of antibodies in treated animals (Luckins, Boid, Rae, Mahmoud, Elmalik, and Gray, 1979), or in animals with spontaneous recovery (Nantulya, Musoke, Rurangirwa and Moloo, 1984). Presence of antibodies only provides information on previous exposure but cannot be used to assess the extent of active infections or the success of treatment.

b) Antigen detection

Earlier attempts to detect trypanosome antigens in infected animals showed very low sensitivity and specificity (Araujo, 1982; Rae and Luckins, 1984). Recently, trypanosome species specific monoclonal antibodies which are able to

demonstrate trypanosomal antigens in infected hosts have been developed (Nantulya, 1981, 1989; Nantulya, Musoke, Rurwangirwa, Saigar and Minja, 1987; Richardson, Jenni, Beecroft and Pearson, 1986). Using *T. brucei* group-specific monoclonal antibodies, a sandwich enzyme immunoassay (ELISA) was developed for the diagnosis of *T. b. brucei* infections in cattle and *T. b. rhodesiense* and *T. b. gambiense* in man (Nantulya, 1989). When the same test was used for *T. evansi* in camels and buffaloes, antigens were detected as early as six days following experimental infection (Nantulya, Hammers and Bajyana Songa, 1989). Species specific monoclonal antibodies against *T. congolense* and *T. vivax* have also been developed leading to an antigen capture sandwich ELISA for these species in cattle (Nantulya and Lindqvist, 1989).

Results of field studies in camels (Nantulya, Lindqvist, Diall, and Olaho-Mukani, 1989) and cattle (Nantulya, Lindqvist, Stevenson and Mwangi, 1992) from endemic areas, show that the antigen ELISA detected infection in 92% and 96% of the parasitologically positive cases and 55% and 52.6% of the parasitologically negative animals, respectively. Recent laboratory studies (Masake and Nantulya, 1990) indicated that the antigen ELISA was four times more sensitive than the darkground/phase contrast buffy coat technique (Murray *et al.*, 1977), in monitoring *T. congolense* infections in cattle and goats. However, the test misses a proportion of the early parasitologically positive infections and shows positive reactions for some period post-treatment.

The outstanding advantages of antigen ELISA are; it is easy to perform, allows the analysis of large numbers of serum samples, can be used for individual diagnosis, the results can be read visually and it is more sensitive than the current techniques for parasitological diagnosis (Nantulya, 1990). The major disadvantage is that it requires access to specialized reagents and gives a small

number of false positive results.

For epidemiological purposes, the diagnostic strategy for the future is likely to be a combination of one of the more sensitive parasitological methods with antigen trapping, while antibody detection will remain an important tool for the assessing exposure to the disease.

1.5 TRYPANOSOMIASIS CONTROL METHODS

Trypanosomiasis control is normally aimed at applying all practical methods that will reduce the prevalence of the disease in man and livestock to minimal manageable levels and possibly lead to eventual eradication. As with other diseases, trypanosomiasis control is a continuous process and requires constant vector and disease surveillance and countermeasures as appropriate (reviewed by MacLennan, 1981; Jordan, 1986). Control methods are either direct or indirect. The direct control methods are those aimed at the parasite, while the indirect methods are those aimed at the vector.

1.5.1 TSETSE CONTROL

Tsetse control methods are normally expensive because the affected areas are not isolated and are therefore prone to reinvasion unless the control operations are constantly monitored. A careful assessment taking into account technical, economic and social factors must be made before embarking on a long term tsetse control programme. Several important factors like the habitat, tsetse fly species, trypanosome species and their sensitivity to chemotherapy, must be considered (Jordan, 1978, 1986; MacLennan, 1978, 1981). However, economic considerations may be ignored in emergencies such as epidemics occurring in areas where sleeping sickness and animal trypanosomiasis are already endemic,

or in areas where the activities of man have resulted in formation of woody habitats, thereby, increasing the man-fly or animal-fly contact (FAO,1979).

Vector control methods are generally classified into two groups, non-chemical and chemical. The non-chemical methods include, vegetation clearing, destruction or elimination of the reservoir hosts, use of traps, screens and targets, genetic, biological and physiological control. The chemical methods involve the control by application of insecticides (Jordan, 1986).

a) Vegetation clearing

The principal behind vegetation clearing is the destruction of the tsetse habitat. As mentioned earlier there are 36 species and subspecies of *Glossina* with a wide range of climatic and vegetation requirements. Successful and economical or cost effective vector control depends on thorough knowledge of tsetse ecology and population dynamics in a geographical area (FAO, 1979).

Before the introduction of modern insecticides, clearing operations against riverine tsetse were the major methods used for the suppression of epidemics of sleeping sickness, caused by *G. palpalis* and *G. tachinoides*, in Nigeria, in the 1930's (Ford, Nash and Welch, 1970; McLennan, 1981). Vegetation clearing in Western Kenya controlled sleeping sickness due to *G. f. fuscipes* between 1950 and 1973 (Maina, 1977).

Bush clearing may be indiscriminate or partial. Indiscriminate or sheer clearing removes all the tsetse habitat, i.e., bushland, scrubland and forests. Partial clearance is aimed at clearing the specific habitat or part of habitat of the particular tsetse species (Jordan, 1986). The major drawbacks of the former are that it is labour intensive when performed manually, expensive when using machinery, and it has to be repeated regularly (Jordan, 1986; MacLennan, 1981).

Sheer clearing of vegetation is mainly considered important in creation of barrier zones or blocks for tsetse eradication by insecticidal spraying or in intensive livestock production systems, where the cleared land is used for fodder production or pasture improvement (MacLennan, 1981). Selective methods are difficult to apply in humid environments since there is no clear-cut relationship between the fly and the habitat but nevertheless, they have been shown to be successful in some areas of Sudan and Northern Guinea (MacLennan, 1967; MacLennan and N'aisa, 1971).

b) Destruction or the elimination of the reservoir hosts

The principle behind this method is to reduce or remove the food source of the tsetse and normally involves intensive hunting and destruction of the game animals. This method has been used in several countries, including, Zimbabwe, Botswana and Mozambique (Ford, 1970c). From 1946 to 1966 *G. m. submorsitans* and *G. pallidipes* were eliminated from more than 2000 km^2 in Uganda following intensive hunting campaign in which large numbers of wild animals were shot (Wooff, 1968). In Zambia and Zimbabwe, fences have been used to separate game areas from livestock with an animal free buffer zone where any wild animal entering is shot (Jordan, 1986). With the current world-wide wildlife conservation efforts, this method is considered unacceptable and is widely unpopular.

The rapidly expanding human population is resulting in massive clearing of the vegetation for settlement and agriculture. This is having the practical effects of destroying the tsetse habitat and also reducing the number of hosts since bush clearing results in the migration of the game.

c) Hand catching

Direct hand catching was first tried on a large scale in 1913 against *G. palpalis* in the Principe' Island off the West African coast, following a sleeping sickness epidemic. It was also used by the Germans against *G. fuscipes* in the Riamugasire Islands on Lake Victoria (Glasgow and Potts, 1970). This method is not applicable to the savanna species due to their vast and diffuse distribution, as opposed to the linear distribution of the riverine species (Vale, Bursell and Hargrove, 1985).

d) Traps

The first successful use of traps was that reported by Harris when he attempted to eliminate *G. pallidipes* from Zululand between 1931 and 1937. In 1949, Morris and Morris designed the 'animal trap' which had the shape of a sheep or goat. They used this type of trap against the riverine species, *G. palpalis* and *G. tachinoides*, in West Africa, and they recommended its use in combination with other methods of tsetse control (Glasgow and Potts, 1970).

Work on the design of more efficient traps led to the development of the biconical trap (Challier and Laveissiere, 1973), which produces a visual stimuli by contrasting the white, blue and black surfaces. This trap has proved to be effective for a wide range of tsetse species and has been used extensively in Africa for control and ecological studies (Allsop, Hall and Jones, 1985; Vale *et al.*, 1985; Jordan, 1986). Recent research has led to trap designs which have shown marked improvements in fly control for the various species in different ecological zones (Vale, 1982c; Brightwell, Dransfield, Kyorku, Golder, Tarimo and Mungai, 1987).

e) Combination of traps, screens and targets and odour

Experiments done by Laveissiere *et al.*, (1981) showed that, biconical traps impregnated with insecticides had a potential for the control of linear infestations of the *palpalis* group in Burkina Faso. This method has been used successfully in Nigeria and Burkina Faso, to suppress fly populations before the release of sterile males in the sterile insect technique (Allsop, 1984).

In the non-linear savanna habitat occupied mainly by the *morsitans* group, the efficiency of traps has been shown to increase if natural host odour, carbon dioxide and acetone are included in the setting of the trap (Vale, 1974a; Vale and Hargrove, 1979; Vale, 1980). Octenol, a component of the cattle odour has shown to be potent olfactory attractant for some *Glossina* species (Bursell, 1984; Hall, Beevor, Cork, Nesbitt and Vale, 1984) and this has led to the manufacture of synthetic odours, and at the moment acetone and phenols are in use.

The concept of combining traps with attractants is currently being widely applied in tsetse control programmes. Odour-baited visually attractive insecticide impregnated targets were used to completely eradicate *G. m. morsitans* and *G. pallidipes* from an island in Lake Kariba (Vale and Hall, 1985a,b), while odour-baited traps have been used to reduce the fly population (dominantly *G. pallidipes*) by over 90% at Nguruman, Kenya (Dransfield, Williams, Brightwell, 1991). The use of targets and screens on the 600 km^2 Rifa triangle in Zimbabwe reduced the population of *G. m. morsitans* and *G. pallidipes* drastically (Vale, Flint and Hall, 1986).

Recent approaches have been made towards techniques which not only involve the capturing, but also sterilizing and releasing the captured flies instead of killing them, basing on the principle that, the female only mates once (House, 1982; Vale, 1982c). The costs of such complex methods in comparison to simple

traps and targets need critical evaluation before they are adopted into tsetse control programmes.

f) Biological control

Biological control involves the use of predators parasites or pathogens for the control of *Glossina* species (reviewed by Nash, 1970; Laird and Simmonds, 1977). The method is specific and non contaminating. Most research in this aspect has been carried out on the insect predators. The genus *Nesolynx* has been used in attempts to control *Glossina* species in Malawi in 1925, Nigeria, and Tanzania. The largest release was in Tanzania in 1933 in which 13.75 million *Nesolynx* were released with poor results (Jordan, 1986). Further research into both fly predators and pathogens with attention to mass production and commercialization with hopes of incorporating them into the integrated tsetse control approach has been suggested (Laird and Simmonds, 1977), but again, the cost of such technology must be considered as it may be prohibitive.

g) Pheromones

In the tsetse fly, the pheromones (sex hormones) are non volatile and species specific. The principle behind their use is that the odour stimuli attracts the fly a point where they are visually attracted to the decoys placed on the targets and in an attempt to copulate with them, they receive a 'dose' of the chemosterilant.

This method may provide a highly specific way of autosterilization for particular *Glossina* species (Langley, Trewern and Carlson, 1982).

h) Genetic control

The aim of this method is to control *Glossina* populations by suppressing or decreasing significantly the population of the fertile females in the wild. Large numbers of males are reared and sterilized using a chemosterilant or irradiation, and then released to mate with the wild females. Sterilization involves the induction of dominant lethality in the male gametes. The sperms appear normal but suffer chromosomal damage that arrests cell division at some early stage in the life cycle. As mentioned earlier, the female mates only once (Dame, 1970) and, therefore, when it mates with a sterile male it becomes infertile for the rest of its life.

The success of the sterile insect technique (SIT) depends on, the number of the males released, their ability to compete with the wild males and to disperse in a given habitat (reviewed by Knippling, 1982; Oludunmade, Takken, Dengart, Tenabe, Feldman and Hamman, 1977). The release patterns in any programme are dictated by the density of local populations and the behavioural characteristics of the target species as the method is species specific. The population density of the target species should be reduced with some other method (traps, targets or residual insecticide spray) prior to the release of the sterile males. Experimental work in Nigeria showed that the method was most effective when the ratio of the sterile to the natural flies was 10:1 (Oludunmade, Takken, Dengart, Tenabe, Feldman and Hamman, 1977). The advantage of this method is that the control effort becomes more effective as the programme proceeds due to decrease in the natural population following an increase in the ratio of the sterile to wild males with time (Dame, 1970).

Field trials performed in Burkina Faso on the riverine species (Politzar and Cuisance, 1982) and in Tanzania on the savanna species (Williamson, Dame,

Gates, Cobb, Bakur and Warner, 1983), showed that, in combination with other methods, the sterile insect technique was able to achieve local eradication in linear habitats. In Burkina Faso, the study was reinforced with residual insecticide and biconical traps, while in Tanzania, two non-residual applications of endosulfan were performed before the release of the males. In both trials, the experimental areas were isolated by cleared barriers. Localized eradication was achieved in Burkina Faso, while in Tanzania, the barriers proved inadequate resulting to reinvasion.

i) Insecticide application

The principal method of tsetse control has been the use of insecticides (reviewed in FAO, 1977; MacLennan, 1981). For effective control the habitat must be made lethal for periods longer than the pupal duration (Jordan, 1986). The insecticides used fall into two categories, residual and non-residual.

Residual insecticides are applied by ground spraying and to a less extent aerial spraying. The insecticides that have been used are DDT and more recently dieldrin. Residual insecticides have persistent effects and thus need only single applications. Hand-operated spraying machines are employed to apply the chemical to the tsetse habitat. Ground spraying is cheap, has less environmental contamination but requires thorough knowledge of the tsetse ecology (Burnett, 1970; MacLennan, 1967; Davies, 1971).

Non-residual insecticides are applied by aerial spraying. They are not persistent and, therefore, require repeated applications (FAO, 1977; MacLennan, 1981). Currently endosulfan is the most commonly used insecticide and is applied using Ultra-Low-Volume techniques (ULV). Recent advances in the technology of aerial spraying have resulted in better use of insecticides by using fixed wing

aircraft for the savanna species and helicopters for tsetse inhabiting galleries (Lee, Parker, Baldry and Molyneux, 1978; MacLennan, 1981). The choice of the method of application, equipment and material used depend on the nature of the habitat and the topography (FAO, 1977). The major disadvantages of aerial spraying are that it is expensive and has environmental effects on the fauna and flora. However, these environmental effects appear to be transient and do not last for more than a year (Jordan, 1986).

Although no resistance to the organochlorides has been reported (FAO, 1979), efforts are being made in search of alternative compounds with less persistence in the environment and lower accumulation in animal tissues. More recent trials with the synthetic pyrethroids, permethrin, cypermethrin and deltamethrin have shown that they may replace the organochlorides (Spierberger *et al.*, 1979). In one of these experiments, aerial application of deltamethrin in 0.75% ULV eradicated *G. p. palpalis* and *G. tachinoides* from 360 km^2 of riverine vegetation in Nigeria. Deltamethrin trials for control of *G. tachinoides* have also achieved acceptable levels in Burkina Faso (Baldry *et al.*, 1981). The use of the synthetic pyrethoids for aerial application may be at the moment hampered by their high cost.

The various insecticides used for tsetse control can have some adverse environmental effects. Destruction of birds and wildlife have been noticed after the use of organochlorides in tsetse control operations (Koeman and Hadden, 1968; Langridge and Mgutu, 1968). Residual spraying of a single heavier dose of insecticide to a selected part of the vegetation is more likely to have a greater impact on the non target organisms than repeated non-residual aerial sprays (FAO, 1977). Endosulfan is toxic to fish, while deltamethrin is toxic to fresh water crustacea, a factor that should be considered when planning control

operations near rivers and lakes. However, the above effects appear to be transient and they do not usually last for more than one year (Jordan, 1986).

Dipping of cattle in insecticides has been mainly practised for tick control. However, some workers in Zambia noticed that, the use of deltamethrin in dips reduced the incidence of trypanosomiasis in cattle from 40% to 5%, in addition to the reduction in tick challenge (Chizyuka and Luguru, 1986). Application of pour-on formulations of these synthetic pyrethroids (flumethrin and deltamethrin) on cattle to assess their efficiency as mobile targets, is being tested in various parts of Africa. Marked decrease in the disease incidence and higher body weight gains have been achieved using flumethrin in high tsetse challenge areas in Kenya (Lohr, Omukuba, Njogu, Maloo, Gishemba, Okedi and Mwongela, 1991) and Burkina Faso (Bauer, Kabore, Liebish, Meyer and Petrich-Bauer, 1992) and under low tsetse challenge in Zanzibar (Schoenfield, 1988). Similar results with deltamethrin have been reported in several parts of Southern Africa with varying tsetse challenge (Thompson, 1991) and under high challenge in Kenya (Stevenson, Munga, Makumi, Baylis and Alushula, *in press*).

In conclusion, many different methods have been attempted for tsetse control, but the recent use of insecticides applied to traps and targets may provide a simple and environmentally safe method for some fly species, while the treatment of cattle with the insecticides either as dipwash or a pour-on formulation may effectively reduce the disease incidence in cattle.

1.5.2 CHEMOTHERAPY

At present, the development of a vaccine against trypanosomiasis is hindered by the phenomenon of antigenic variation (Murray and Urqurhart, 1977). Vector control methods are expensive to initiate and difficult to sustain. Only 10.7

million cattle representing 24% of the total population in the tsetse-infested areas are regarded as trypanotolerant (Hoste, Chalon, d'Iteren and Trail, 1989), and they are mainly confined to West and Central Africa (ILCA, 1979). Therefore, the use of trypanocidal drugs still plays a major role in the control of the disease.

The first use of drugs was described by Plimmer and Thompson (1908) who showed that, tartar emetic (potassium antimonyl tartrate) cured mice infected with *T. evansi* or *T. brucei.* This observation led to the first application of chemotherapy to bovine trypanosomiasis. Intravenous administration of the drug in Tanzania, Rhodesia and Zululand demonstrated the efficacy of the drug against *T. congolense* and *T. vivax* (Bevan, 1928). In 1938, Browning and his colleagues showed that phenanthridines also had marked activity against *T. congolense.* Further research led to the release of quinapyramine (Davey 1950), homidium (Watkins and Woolfe, 1952) and later diminazene (Jensch, 1955).

Until the recent release of melarsenoxyde cysteamine (MelCy, Cymelarsan^{R}, Rhone Merieux) for the treatment of *T. evansi* in camels (Raynaud, Sones, Friedheim, 1989), no new trypanocidal drug had been released for the last 30 years mainly due to the high costs of drug development and limited trypanocidal market (Murray *et al.,* 1991). By 1980, it was estimated that it would cost about $20 to $30 million to make a new trypanocidal drug (Williamson, 1980). The market for trypanocidal drug is potentially large but is made up of countries with unreliable economies and political stability (Williamson, 1976).

At present, the use of drugs is faced with two problems, namely, the lack of new trypanocides and the development of resistance by the trypanosomes to the existing ones. If effective use of the current drugs is to continue, well co-ordinated programmes on surveillance and testing or screening for drug

resistance need to be implemented (Leach and Roberts, 1981; FAO, 1979). Tacher (1982) estimated that 25 million doses of trypanocides are administered annually despite the fact that, there are about 50 million cattle, 40 million goats and 30 million sheep exposed to the disease. Even if the animals were to be treated only twice per year, about 240 million doses would be required or even more considering that there are also 12 million camels exposed to the disease (Murray *et al.*, 1991).

There is a big gap between the number of treatments required and number of treatments actually performed due to logistic and financial problems such as the high drug costs, lack of funds to implement control programmes, lack of well trained personnel, lack of the infrastructure required for drug administration and erratic supplies of drugs (MacLennan, 1981; Holmes and Scott, 1982; Murray and Gray, 1984).

Chemotherapy and chemoprophylaxis can be effective and economical if managed and used in an organized system (Leach and Roberts, 1981). In areas of high tsetse challenge, drug use should be accompanied by vector suppression (Finelle, 1976). The choice of practical and effective therapy or prophylaxis depends on the type of animal husbandry. Mass curative treatment and strategically timed prophylaxis have been advocated (MacLennan, 1981). This should be accompanied with regular surveillance for infection in the entire group. The frequency of treatment depends on the level of challenge to which the animals are exposed.

A chemotherapeutic strategy is indicated for animals in an area of light tsetse challenge or in cattle no longer exposed to the disease risk following complete withdrawal from an endemic area (MacLennan, 1970). Where seasonal transhumance is practised, prophylactic treatment should be given to cattle

before they move into the tsetse-infested area, while curative treatment should be performed as soon as the cattle are back to the tsetse free area (FAO, 1979). Such curative treatment is important in preventing mechanical transmission of infection (FAO, 1979; Gray, 1983).

For cattle maintained in endemic areas in farms and ranches with good veterinary supervision, chemoprophylaxis has proved to be successful through regular monitoring of all animals and a proper drug regime which incorporates a curative drug at regular intervals (FAO, 1979; Finelle, 1976; Holmes and Scott, 1982; Trail, Sones, Jibbo, Durkin, Light and Murray, 1985). Chemoprophylaxis is also indicated for animals trekking to market through tsetse-infested stock routes. In nomadic areas, prophylaxis is difficult since it requires trained staff, reliable transport and easy access to the animals (Jordan, 1986). Trypanosomiasis control using prophylactic drugs must be accompanied by a constant surveillance for drug resistance.

Successful maintenance of herds of cattle economically in tsetse-infested areas of using strategic treatment has been reported in Kiburine Ranch (Wilson, Le Roux, Paris, Davison and Gray, 1975b), Kilifi Plantations (Wissocq, Trail, Wilson and Murray, 1983) and the Galana Ranch (Wilson, Njogu, Gatuta, Mgutu, and Alushula, 1983; Njogu, Dolan, Sayer, Wilson and Alushula, 1985a) in Kenya, Mkwaja Ranch in Tanzania (Trail *et al.*, 1985), Mali (Logan, Goodwin, Tembely, and Craig, 1984) and under village husbandry in Muhaka, Kenya coast (ILCA, 1986a,b).

The strategic chemoprophylactic trials in areas of low to medium tsetse challenge on the Galana Ranch in Kenya between 1982 and 1983, showed a 60% reduction in drug costs (Njogu *et al.*, 1985a). Similarly the studies on cattle under village husbandry system at Muhaka on the Kenyan coast, indicated that,

strategic use of drugs reduced the disease incidence by 39%, increased the productivity of the breeding cattle by 20%, and had significant effect on post weaners daily liveweight gains (Maloo *et al.,* 1987; 1988). Bourn and Scott (1978) reported successful maintenance of 450 draught oxen in the Angar-Gutin settlement scheme, an area of high tsetse challenge, by the use of trypanocidal drugs and good veterinary supervision. The strategic use of isometamidium (Samorin^{R}, RMB) and diminazene (Berenil^{R}, Hoechst) has enabled the maintenance of Boran cattle in Mkwaja Ranch an area of high challenge to attain levels of productivity close to those of Boran cattle reared in tsetse-free areas (Trail *et al.*, 1985).

In areas of low tsetse challenge and with good organization which enables examination of individual animals, strategic use of therapeutic trypanocidal drugs has been successful. Therapeutic strategic treatment with diminazene aceturate (Berenil^{R}, Hoechst) has been used to maintain a dairy herd of over 800 cows in Kilifi Plantation for over 20 years. The animals were bled regularly and treatment was given to those with a PCV of less than 30% (Wissocq *et al.*, 1983). Before the adoption of this system, the cattle had previously experienced constant severe abortion storms associated with trypanosomiasis.

a) Trypanocidal drugs available currently

The drugs in current use for the treatment of animal trypanosomiasis are shown Table 1.2. Drugs vary in effectiveness to different species of trypanosomes and it is important to identify the most prevalent trypanosome species in an area before the selection of the drug is made. In the case where mass treatment is to be done, random examination of samples from a representative group of animals preferably by the DG technique (Murray *et al.,* 1977) which enables species

Table 1.2

Drugs used for the treatment of trypanosomiasis in domestic animals.

Compound	Generic name	Action	Susceptible trypanosomes
Diamidines	Diminazene aceturate[1]	Curative	T. vivax, T. congolense (T. brucei, T. evansi).
Phenanthridiums	Homidium bromide[2]	Curative	T. vivax, T. congolense.
	Homidium chloride[3]	Curative	T. vivax, T. congolense.
	Isometamidium chloride[4]	Prophylactic	T. vivax, T. congolense (T. brucei).
	*Pyrithridium[5]	Curative Prophylactic	T. vivax, T. congolense
Quinoline Pyrimidine group	Quinapyramine Sulphate[6,7]	Curative	T. evansi, T.equiperdum, T. brucei (T. vivax, T. congolense).
	Quinapyramine[8] Sulphate:Chloride (3:2 w/v)	Prophylactic	As for the sulphate
Napthalidines	Suramin[9]	Curative	T. evansi, T. brucei, T. equiperdum.
Arsenicals	Melarsenoxyde[10] cysteamine (MelCy)	Curative	T. evansi, T. equiperdum T. brucei.

Parentheses indicate species less susceptible to the drug.

[1]Berenil[R], Hoechst, W.Germany
[2]Ethidium[R], CAMCO, U.K.
[3]Novidium[R], RMB, U.K.
[4]Samorin[R], RMB, U.K.
[5]Prothridium[R], May and Baker.
*Not currently in market.
[6]Trypacide[R], RMB, U.K.
[7]Trypamidium[R], Rhone Merieux, France
[8]Trypacide Prosalt[R], RMB, U.K.
[9]Naganol[R], RMB, U.K.
[10]Cymelarsan[R], Rhone Merieux, France

identification, may be necessary. The use of homidium bromide in a *T. congolense* area in Nigeria resulted in the development of resistance (Killick-Kendrick and Godfrey, 1963). This is because at normal curative doses homidium bromide is more effective against *T. vivax* , a factor which had been overlooked. Scott and Pegram (1974) made similar observations with homidium bromide in Ethiopia. Thus, where mass treatment has to be given for a long period, it is important to determine the prevailing infection rate and sensitivity of the parasites to the drug in use.

b) Drug resistance

Drug resistance has been referred to as the ability of formerly exposed trypanosome strains to survive despite the administration of a drug at doses equal to or higher than the recommended dose (Schillinger, 1984). It has been associated with several factors such as, the incorrect dosing following poor body weight estimation or miscalculation of the dosage, irregular treatments with prophylactics and high incidence of the disease (Whiteside, 1962a).

Resistance to trypanocidal drugs has been encountered in various parts of Africa. Diminazene resistant *T. vivax* strains have been reported in East Africa (Mwambu *et al.,* 1971a,b; Mbwambo *et al.,* 1988; Njau *et al.,* 1983) as well as in West Africa strains (Jones-Davies, 1967a,b; Graber, 1968, Gray and Roberts, 1971; MacLennan and N'Isa, 1970; MacLennan, 1971, 1972). Resistant *T. congolense* strains have been reported in East Africa in Kenya (Gitatha, 1979) and West Africa (Jones-Davies, 1968; Clausen, Sidibe, Kabore, Bauer, 1992).

Homidium resistant *T. congolense* strains have been reported in Kenya (Gitatha, 1979), Ethiopia (Scott and Pegram, 1974) and Sudan (Gadir, Tahir, Razing and Osman, 1972). A few cases of isometamidium resistance have been

reported in Kenya (Gitatha, 1981; Dolan, Stevenson, Alushula and Okech, 1992), Ethiopia (Bourn and Scott, 1978), Ivory Coast (Kupper and Wolters, 1983) and Burkina Faso (Pinder and Authie, 1984; Clausen, Sidibe, Kabore, Bauer, 1992).

Drug resistant trypanosome strains can be transmitted cyclically by the tsetse fly and retain their drug resistance and pathogenicity. They can persist in an area after cessation of drug treatment and even after removal of all the domestic livestock (Gray and Roberts, 1968, 1971). In the field, if cattle are withdrawn from an area and use of drug in question is stopped, drug resistant strains of *T. congolense* and *T. vivax* tend to disappear from the tsetse within six to nine months (Whiteside, 1960b).

Occurrence of drug resistance sometimes can be controlled by designing a programme involving the alternate use of trypanocidal drugs, for example, quinapyramine/diminazene/isometamidium/diminazene. The alternate use of diminazene and isometamidium has been recommended in the field (Finelle, 1974).

c) Cross resistance

Cross resistance refers to the situation where one strain of trypanosome resistant to one trypanocide is also resistant to another trypanocide even though it might not have been exposed to the latter.

Extensive cross resistance studies were done in Kenya by Whiteside in the 1950's using strains of *T. vivax* and *T. congolense* (Whiteside, 1960b). He showed cross resistance was not related to chemical structure, e.g., there was cross resistance between homidium and quinapyramine. He also demonstrated that trypanosomes resistant to quinapyramine were resistant to diminazene aceturate. He used the term 'sanative' pairs to refer to pairs of drugs that do not induce

cross resistance to each other. The two sanative pairs are homidium/diminazene and isometamidium/diminazene. Currently, cross resistance has been avoided by the use of sanative pairs of drugs. However, recently, some *T. congolense* strains have been reported to show cross resistance between isometamidium and diminazene (Pinder and Authie, 1984) and between isometamidium, homidium and diminazene in West Africa (Clausen *et al.*, 1992)

d) Drug stimulated immunity

It has been observed that animals exposed to an infection-treatment regime are cured of the infection and also develop a substantial degree of acquired immunity. Bevan (1928) was the first to describe this type of immunity after treatment of cattle with tartar emetic and called it 'tolerance'. Field studies by Soltys (1955) in East Africa, demonstrated acquired immunity to *T. congolense* following a prophylactic regime of quinapyramine.

Further field studies by Whiteside confirmed this observation (Whiteside, 1962). Cunningham (1968) demonstrated that under experimental conditions cattle acquired an immunity to a single strain of *T. b. brucei* following infection and a single curative dose of diminazene aceturate. Wilson *et al.*, (1975a,b,; 1976) conducted a series of experiments to examine the development of immunity in the field using different trypanocidal regimes. It was observed that the group treated with diminazene on individual basis had partial immunity after two years, while the group treated on a herd basis developed no immunity. The group under isometamidium treatment developed a higher degree of immunity and had better performance than the others. This work showed that provided a carefully planned drug regime was used, development of immunity was not a prerequisite

for the successful maintenance of cattle in a tsetse-infested area. They also observed that on withdrawal of the drug the incidence of the disease became more frequent suggesting that the immunity was short-lived.

Laboratory experiments on cattle primed by cyclically-transmitted fly infection with clones of *T. congolense* and treated with diminazene aceturate (Berenil^{R}, Hoechst) after three or four weeks showed that the animals became immune to cyclically-transmitted challenge with homologous clones three to five weeks later as, judged by the lack of development of chancre reactions and parasitaemia (Akol and Murray, 1983).

e) Recent approaches to chemotherapy

As mentioned above the use of drugs is faced with the problem of drug resistance and cross resistance. Development of new drugs belonging to other chemical groups and acting in different ways is urgently required. Such new compounds should be highly active, have low toxicity and should not have any structural relationship with the existing drugs (FAO, 1979). The development of new trypanocides requires detailed knowledge of comparative biochemistry of the host and parasite and mode of action of the existing drugs.

Various approaches have been made towards the development of new compounds. Research is being carried out on parasite biochemistry with the hopes of identifying trypanosome specific metabolic pathways not found in the host which may be vulnerable to attack by enzyme inhibitors, such as diflouromethylornithine (DFMO) and salicyl hydroxamic acid (Croft, 1986; Jennings, 1988). Recent studies on trypanothione metabolism in trypanosomatids indicate that the enzyme trypanothione reductase may be a target for the design of new trypanocidal drugs (Henderson and Fairlamb, 1987).

The complexing of the current drugs with dextran has also been attempted. Aliu and Sannusi (1979), reported prolonged prophylaxis and reduced skin reaction with isometamidium-dextran complex. Liposomal delivery systems of drugs have shown success in the treatment of leishmaniasis (Croft, 1988). This approach has been suggested for trypanocidal drugs. A twelve months study on liposomal formulations of diminazene aceturate, homidium chloride and isometamidium chloride, showed that this approach may provide new means of improving the activity of the few drugs currently available (Fluck and Hopkins, 1987). The slow release technology aimed at making slow release formulations of both the prophylactic and curative drugs has been suggested as an area for future research.

1.5.3 GENETIC RESISTANCE

The current methods for control of animal trypanosomiasis methods by chemotherapy and vector control are faced with several limitations which include the high cost of implementation and lack of trained man power. Vector eradication has been successful where small areas are involved but at the continental level it cannot be extended to the whole of tsetse-infested Africa (Jordan, 1985). Chemotherapy and chemoprophylaxis are applicable in low and to an extent in medium tsetse challenge areas but under high challenge, the high frequency of treatments may lead to drug resistance (Holmes and Scott, 1982; Kupper and Wolters, 1983; Pinder and Authie, 1984). Attempts to produce a vaccine are not promising due to the ability of the parasite to undergo antigenic variation (Gray and Luckins, 1976; Cross, 1977; Vickerman, 1984). There is therefore the need to develop other methods which may enable more efficient utilization of the vast tsetse-infested areas of Africa. This has led to the need for

investigation into the possibility of exploiting cattle breeds that appear naturally resistant to trypanosomiasis.

Genetic resistance to African trypanosomiasis occurs in certain breeds of livestock and many species of wildlife (reviewed by Murray *et al.,* 1982; Murray, Trail and Grootenhuis, 1984). There is no standard definition of trypanotolerance. Pagot (1974) defined trypanotolerance as the racial aptitude of cattle allowing them to maintain themselves in good condition and reproduce without showing signs of the disease. Trypanotolerance has also been defined as the ability of certain breeds of cattle, sheep, goats as well as some species of wildlife to survive and reproduce in endemic fly infested areas without the aid of chemotherapy, while other breeds cannot (Murray *et al.*, 1982). Trypanotolerance is not absolute since in some cases, infections in animals considered trypanotolerant may cause severe disease (Murray *et al.*, 1982).

a) Evidence of genetic resistance in cattle in West Africa

Pierre (1906) observed that certain breeds of West African livestock could survive in tsetse-infested areas. Further laboratory and field experiments by other workers confirmed this observation (Stewart, 1951; Chandler, 1958; Desowitz, 1959). Since then there have been several breed comparison studies in which the superior resistance of the N'Dama and the West African Shorthorn over the Zebu have been reported (Murray *et al.,* 1982; Roelants 1986).

Experimental fly challenge with wild caught tsetse flies to the N'Dama, Muturu and Zebu which had never been previously exposed, showed that all cattle had constant parasitaemia early in the infection but as the infection progressed, most taurines had slightly lower levels than the Zebu (Stephen, 1966; Roberts and Gray, 1973). Based on the weight loss, degree of anaemia, and

survival, the N'Dama and Muturu showed a superior resistance than the Zebu. Similar observations were made in later experiments under natural tsetse challenge (Toure, Gueye, Ba and Mane, 1978; Murray, Clifford, Gettinby, Snow and McIntyre, 1981a; Roelants, Fumoux, Pinder, Quival, Bassinga, and Authie, 1987) and by syringe needle inoculation of trypanosomes (Murray, Murray, Wallace, Morrison, and McIntyre, 1979a; Saror *et al.,* 1981).

Using natural field challenge, Murray *et al.,* (1981a) exposed two groups each of ten young adult female animals of the N'Dama and Zebu breeds which had no previous exposure. All the Zebu died within eight months, while only three N'Dama, which were lactating and suckling calves died between eleven and fourteen months after the initial exposure. Less severe anaemia, lower intensity and a shorter duration of parasitaemia were observed in the N'Dama. There were no abortions in the N'Dama as compared to the Zebu where abortions occurred in early and late pregnancy.

Previously, trypanotolerant cattle breeds have been regarded as non-productive due to their small size (Stephen, 1966). It was believed that their trypanotolerance was restricted to local trypanosome populations and that they would become susceptible if moved to distant tsetse-infested areas and exposed to different trypanosome strains. However, recent surveys have been done on trypanotolerant livestock in 18 countries in West and Central Africa in which indices of productivity were examined using all the basic production data from different regions with differing management systems and different levels of challenge. The results indicate that in areas of no or low tsetse challenge the productivity of the N'Dama and the West African shorthorn was equal to that of the Zebu (ILCA, 1979). There were no comparative data from the high tsetse challenge areas since only the trypanotolerant cattle breeds could survive.

Field studies in The Gambia on the milk production potential of the N'Dama have shown that, a N'Dama cow with an average weight of 225 kg, and constantly exposed to trypanosomiasis risk and difficult nutritional conditions, can produce a calf every 18 to 20 months, raise the calf to 60 kg in 10 months and yet produce over 320 kg milk with 5% fat (Agyemang, Jeanin, Grieve, and Dwinger, 1987), a level of production which was higher than previously thought.

Evidence that trypanotolerance is not due resistance acquired to local trypanosome populations has been provided by the successful establishment of trypanotolerant breeds like the N'Dama and Lagune from West Africa in distant tsetse-infested areas of Central Africa (Mortelmans and Kageruka, 1976; ILCA, 1979; FAO, 1987).

b) Evidence of genetic resistance in cattle in East Africa

Differences in susceptibility to trypanosomiasis among breeds in East African cattle have not been studied to the same extent as in the *Bos taurus* in West Africa. The first reports of East African cattle resistant to trypanosomiasis were made by Balfour (1913) in Sudan and later, similar observations in the same area were reported by Archibald (1927) who described the ability of some *Bos indicus* cattle to survive and reproduce under trypanosomiasis challenge without drug treatment. Later, studies by Cunningham (1966) on Zebu cattle around Lake Victoria demonstrated a 30% prevalence rate of trypanosomiasis and neutralizing antibodies in 90% of the Zebu cattle examined and, he also noted that the parasitaemic animals were in good body condition, suggesting a degree of reduced susceptibility.

A comparative study on *Bos indicus* cattle involving two groups of Zebu cattle from tsetse endemic areas of Kenya with Boran and Ayrshire cattle which

had no previous exposure, using needle challenge with *T. congolense,* showed that the Ayrshire developed a rapid and severe anaemia and were all dead within eight weeks after infection (Monirei, Murray, Whitelaw, Trail, Wissocq, and Chema, 1982). On the other hand, the development of anaemia in the other breeds was gradual and of a severe degree. There were no differences in terms of the degree of anaemia, level of parasitaemia or in survival between the two groups of the East African Zebu and the Boran. These results indicated that the Ayrshires were more susceptible than the other breeds.

Between 1972 and 1978, a six year epidemiological study was conducted at the Kilifi Plantations (Kenya coast) an area of low tsetse challenge, on a dairy herd of 800 breeding animals consisting of two genotypes, 1/3 Ayrshire x 2/3 Sahiwal and 2/3 Ayrshire x 1/3 Sahiwal (Wissocq *et al.,* 1983). The entire herd was bled four to five time per year and animals with a PCV of less than 30% were treated with diminazene (Berenil^{R}, Hoechst) or homidium chloride (Novidium^{R}, RMB) irrespective of the detection of parasitaemia. The results showed that, the 1/3 Ayrshire x 2/3 Sahiwal required less than half the number of trypanocidal drugs treatments needed by the other group during one calving interval of thirteen months. The increased resistance was not associated with decreased productivity, assessed in terms of milk production and calf viability. This work demonstrated that even a small degree of resistance may be exploited in areas of low tsetse challenge.

Field studies done under medium to high natural tsetse challenge on the Galana Ranch in Kenya for over ten years have shown that, the Galana Boran is more susceptible to trypanosome infections than the Orma Boran (Wilson, Njogu, Gatuta, Mgutu, and Alushula, 1983; Njogu, Dolan, Wilson, and Sayer, 1985a,b; Dolan, Njogu, Sayer, Wilson, and Alushula, 1985). It was found that the

Orma Boran had a longer prepatent period, lower disease incidence and required fewer trypanocidal drug treatments. Laboratory studies using needle and fly challenge with both *T. vivax* and *T. congolense,* showed that, basing on severity of clinical responses, intensity of parasitaemia, severity of anaemia, ability to gain weight and number of trypanocidal drug treatments required, the Orma Boran appeared to be more resistant to trypanosomiasis than the Galana Boran (Ishmael, Njogu, Gettinby and Murray, 1985; Ishmael, 1988).

The above observations provide evidence of variation in susceptibility to trypanosomiasis among *Bos indicus* cattle breeds in East Africa.

c) Resistance in sheep and goats

The epidemiology of trypanosomiasis in sheep and goats is similar to cattle with the dwarf indigenous breeds being more resistant than the larger imported breeds. In West Africa, Djallonke sheep were found to be more resistant than the Fulani sheep to syringe passaged *T. congolense* infections (Toure, Seye, Dieye and Mbengue, 1983). In East Africa, the indigenous sheep (Red Maasai and the Blackhead Persian) and goats (Galla and East African) have been shown to be more resistant than the imported breeds (Merino sheep and Saanen goats) to syringe passaged *T. congolense* and also under field challenge (Griffin and Allonby, 1979a,b). No significant differences in susceptibility were demonstrated among the East African Galla goats and their crosses with either Nubian or Toggenburg, following fly and needle challenge *T. congolense* (Whitelaw, Kaaya, Moulton, Moloo and Murray, 1985). This was attributed to the high virulence of the parasite strain used.

Comparative studies on indigenous goat breeds from three different tsetse endemic localities in East Africa (Morogoro, Arusha and Lambwe Valley) and

one tsetse free area (Imbo, Central Nyanza, Kenya) to needle challenge with *T. congolense* indicated that the Morogoro goat was most resistant, the Arusha and Lambwe Valley intermediate, while the Imbo was most susceptible (Mutayoba, Gombe, Waindi and Kaaya, 1989).

d) Resistance in wildlife

Wild animals are highly resistant to trypanosome infections and in some cases completely refractory. This is shown by their ability to survive and reproduce in areas infested with tsetse flies and subsequent to experimental infection (Ashcroft, 1959; Murray, Grootenhuis, Akol, Emery, Shapiro, Moloo, Dar, Bovell and Paris, 1981b; Murray *et al.,* 1982; Murray and Njogu, 1989). Several parasitological surveys in wild animals in East Africa showed infection rates of 19.5%, with the highest being in the waterbuck and the lowest in the zebra (Ashcroft, 1959). Following the results of needle challenge experiments (Ashcroft, Burt, and Fairburn, 1959), wild animals were classified into two groups, those that usually died of the infection and the less susceptible ones. The latter group could further be divided into three groups, those that had a parasitaemia for a considerable period, those with scanty parasitaemias and those that were refractory to infection.

Using needle challenge and experimental infection with *T. congolense, T. vivax* and *T. brucei* on the African buffalo, oryx, eland and waterbuck which had not been previously infected with trypanosomes, it was found out that, all the four species showed marked degree of resistance to trypanosomiasis as reflected by the low transient parasitaemias and only minor temporary reductions in the PCV (Grootenhuis *et al.,* 1982; 1990). Information on the epidemiological role of game is still scanty. Their characteristic of being less susceptible or refractory to

infection makes them an important model for the study of the mechanisms of trypanotolerance.

e) Mechanisms of trypanotolerance and its criteria

Current evidence shows that resistance to trypanosomiasis depends on the ability of the host to control and reduce parasitaemia, develop a superior immune response and to resist anaemia (Murray *et al.*, 1982; Black, Sedashonga, Lalor, Whitelaw, Jack, Morrison and Murray, 1985; Roelants, 1986).

Following an infected tsetse bite a chancre develops and this is where the parasite replicates before disseminating into the blood stream. The reaction has been shown to be smaller or less in certain resistant animals than the susceptible ones (Dwinger, Grootenhuis, Murray, Moloo, and Gettinby, 1986). Comparative studies on the N'Dama, N'Dama/Baoule, Baoule and the Zebu, showed that the local skin reactions in the Zebu were large and severe, while those that occurred in other breeds were smaller, less severe or mild (Akol, Authie, Pinder, Moloo, Roelants, and Murray, 1986). However, no differences were observed between the N'Dama and Boran in the number of chancres or in the kinetics of the reaction observed clinically (Paling, Moloo, Scott, Gettinby, McOdimba and Murray, 1991). The wild species have also been shown to exhibit a reduced number and size of skin reactions in addition to transient reduction in PCV and reduced parasitaemia (Grootenhuis, Varma, Black, Moloo, Akol, Emery, and Murray, 1982). It is possible that the factors that regulate the parasite growth and differentiation could be operative at the skin level and are important in determining the susceptibility of the host.

Trypanotolerance has also been associated with the ability of the host to mount a more superior immune response. Desowitz (1959) demonstrated that

previously exposed N'Dama cattle were able to eliminate trypanosomes more rapidly and efficiently than the Zebu following a repeat challenge. Experiments on mice have shown that resistant strains produce a more persistent antibody response to *T. congolense* than the susceptible strains (Murray, Morrison, Clifford, Murray, and McIntyre, 1981c). In experimental infections with *T. brucei* in the N'Dama and the Zebu, the ability to control anaemia was found to have a correlation with the ability of the host to recognize at least one of the three common trypanosome antigens of molecular weights 110, 150 and 300 kDa All the N'Dama recognized the three antigens, while none of the Zebu did, thus demonstrating the superior immune response of the former (Shapiro and Murray, 1982). Recent studies show that, all N'Dama resistant to trypanosomiasis recognize and produce antibodies against a protein of molecular weight 33 kDa which the Boran do not (ILRAD, 1991). Characterization of this protein shows that it is a cysteine protease which may be associated with the activation of the host immune system to release large quantities of tumour necrosis factor and interleukins which are capable of producing the clinical signs of trypanosomiasis.

In comparative studies between N'Dama, N'Dama/Baoule, Baoule and the Zebu to *T. congolense* infection, there were no significant differences in the time to detectable parasitaemia, but the taurine breeds showed a better capacity to control and reduce subsequent waves of parasitaemia (Akol *et al.*, 1986). This was attributed to a superior antibody response to the first peak of parasitaemia of the taurine breeds. It has also been shown that during challenge with captured flies infected with *T. congolense,* cattle which recover spontaneously show a more rapid antibody response and a higher antibody titre to trypanosome variants present in the first wave of parasitaemia (Pinder, Fumoux, Van Melick and Roelants, 1987).

Another explanation to trypanotolerance is that the resistant animals are less susceptible to immunodepression. A relationship between the immunodepression and susceptibility has been reported (Selkirk and Sacks, 1980). It was shown that strains of mice of different susceptibilities exhibited different responses to heterologous antigens. The resistant mice were able to mount an early IgM response, while the IgM was more rapidly depressed in more susceptible strains. They concluded that the extent and rapidity of onset of suppression of IgM responses determined the course of infection.

Infected trypanotolerant animals have been shown to develop less severe anaemia than susceptible animals. Studies on the kinetics of anaemia in the N'Dama and Zebu cattle infected with *T. congolense* and *T. brucei* showed that the degree of anaemia depended on the number of parasites in the blood (Dargie, Murray, Murray, Grimshaw and McIntyre, 1979; Dargie, 1980). It was therefore thought that the capacity to control anaemia was due to the ability to control parasitaemia and not due to innate erythropoietic responses (Dargie, 1980).

Comparative studies on the relationship between anaemia and parasitaemia in the N'Dama and the Boran following tsetse-transmitted *T. congolense* showed that the N'Dama had a better ability to control anaemia which improved significantly with consequent challenges (Paling *et al.*, 1991). In this study the ability to control anaemia was not directly related to the intensity of parasitaemia.

Trypanotolerance has been suggested to be associated with a reduced susceptibility to the effects of infection due to some of the physiological factors possessed by trypanotolerant breeds which aid survival (Murray *et al.*, 1982). These factors include, a superior adaptation to food utilization, heat tolerance

and water conservation but so far, there are no experimental data on these parameters.

Analysis of over 22,600 samples from various parts of Africa, showed that tsetse flies have host preferences which are affected by many environmental factors (Weitz, 1963). The colour, size and odour of the host are thought to be associated with the host preferences. Definite tsetse host preference was demonstrated in experiments between cattle and oryx (Roberts, Bhoghal and Karstad, 1980) where it was observed that, five times as many tsetse were attracted to cattle than to oryx. Whether attractiveness differs between cattle breeds is not known.

Another possible explanation of trypanotolerance is that such animals are bitten less by the tsetse probably because they are less attractive as has been shown for some African wild animals (Murray *et al.*, 1982). This is supported by the observation that resistant cattle develop parasitaemia when infected tsetse flies are forced to feed on them under experimental conditions. It has been thought that resistant animals may be more efficient at preventing the flies from feeding on them by tail flicking or neuromuscular twitching (skin rippling movements). This mechanism may be reduced in emaciated cattle or those debilitated by disease. This may explain the loss of trypanotolerance in such animals (Murray *et al.*, 1982).

However, from most of the studies carried out among breeds under natural field challenge and in the laboratory, it has been confirmed the trypanotolerance of the N'Dama and the West African shorthorn is due to their ability to resist the effects of infection, i.e., to gain weight and reproduce when infected with *T. vivax, T. congolense* or *T. brucei* (Murray *et al.*, 1982; Murray and Dexter, 1988). At the same time, anaemia which the most important effect of

trypanosome infection in cattle (Hornby, 1921; Murray, 1979) has been shown to be less severe in the trypanotolerant breeds (Dargie, *et al.,* 1979). It was concluded that, trypanotolerance is probably an innate trait associated with the capacity to control parasitaemia and resist anaemia and that the ability to resist anaemia would appear to be related at least in part to the ability to control parasitaemia.

Most of the above conclusions have been criticized for several reasons. The previous history of trypanosome infections in the animals used in most of the studies was unknown (Stewart, 1951; Desowitz, 1959; Toure *et al.,* 1978; Roelants, 1983; Akol *et al.,* 1986). In cases where the animals had no previous exposure, a uniform challenge among the different animals was not guaranteed (Stephen, 1966; Roberts and Gray 1973; Murray *et al.,* 1981a). In other studies, cattle were infected by syringe inoculation with bloodstream forms by-passing the skin where the tsetse deposits the infective organisms, as it has been suggested that, the initial skin reaction following the tsetse bite is important in determining host susceptibility (Akol *et al.,* 1986). In majority of the studies, the trypanosomes used for the challenge had not been characterized in terms of antigenicity and virulence and probably consisted of a mixture of serodemes.

Recently, in a study where all above variables were eliminated, the susceptibility of the N'Dama cattle to four sequential fly challenges with different clones of *T. congolense* was compared with that of Boran cattle, both reared in tsetse-free environment under similar management and nutrition (Paling *et al.,* 1991). The N'Dama were obtained as embryos from donors in The Gambia and implanted into surrogate Boran mothers in Kenya (Jordt, Mahon, Toure, Ngulo, Morrison, Rawle and Murray, 1986). The N'Dama gained weight at the same rate as uninfected control animals, did not develop a severe anaemia to require

treatment, and the PCV recovered to normal values within two to four months. Furthermore, the infected females showed normal oestrous cycle activity (Lorenzini, Scott, Paling and Jordt, 1988). In contrast, all the Boran needed treatment during the course of the four infections and had markedly reduced body weight gains. This investigation provided the most complete evidence so far, on the superior resistance of the N'Dama compared to the Boran.

Trypanotolerance is not a stable character and the degree varies among breeds and individuals and can be affected by a variety of environmental factors which include, the degree of tsetse challenge, previous exposure, intercurrent disease, age, poor nutrition, and any stress such as work, pregnancy, parturition, lactation and suckling, and poor management (Murray *et al.,* 1981a, 1982).

The most important factors appear to be the nutrition status and the trypanosomiasis risk. It has been shown that the lack of adequate nutrition which is prevalent under field conditions can result in severe anaemia and weight loss in N'Dama with trypanosomiasis (Agyemang *et al.,* 1990). Results on cattle exposed to natural tsetse challenge show that as the level of the trypanosomiasis risk increases, the productivity the trypanotolerant cattle falls and they can suffer severely from the disease and display the typical signs of wasting, abortion and even death (ILCA, 1979).

There are several reports indicating that cattle with previous exposure to trypanosomiasis with or without chemotherapy are subsequently more resistant to reinfection. This has been confirmed in West Africa in the N'Dama (Chandler, 1958; Desowitz, 1959; Toure *et al.,* 1978 and Saror *et al.,* 1981) and among *Bos indicus* breeds in East Africa (Bevan, 1928; Whiteside, 1962a; Wilson *et al.,* 1976; Bourn and Scott, 1978). Paling *et al.,* (1991) observed that over the course of four infection periods in N'Dama cattle infected with *T. congolense,* the overall

severity of anaemia produced became progressively and significantly less and therefore concluded that the N'Dama cattle possessed an innate ability to acquire resistance to the disease as assessed by increasing resistance to anaemia.

There have been few studies on the heritability of trypanotolerance. Stewart (1951) reported that crossbreeding the N'Dama, Zebu and Ghanian Shorthorn produced a larger more productive animal that retained its resistance to trypanosomiasis. Similarly, N'Dama/Zebu crossbreeds retained a significant degree of trypanotolerance when exposed to natural challenge (Chandler, 1952). In breeding experiments in the Ivory Coast involving large numbers of N'Dama and Jersey, Letteneur (1978) found that the F1 crosses produced an excellent animal as regards growth and milk production and they retained their tolerance although the level of tsetse challenge or trypanosome prevalence was not reported.

Heritability of trypanosomiasis in cattle is not well defined and there have been no established estimates (Dolan, 1987). It has been shown that the ability to control parasitaemia and resist anaemia are two factors associated with this trait. Accumulated evidence also shows that, the severity of anaemia as assessed by the PCV in infected cattle is correlated with production traits such as reproductive performance (Trail, Colardelle, d'Iteren, Itty, Jeannin, Maehl, Nagda, Ordner, Paling, Rarieya, Thorpe and Yangari, 1988). This suggests that the PCV and parasitaemia during the course of a trypanosome infection might serve as a selection criteria for trypanotolerance. Recent studies on this aspect in N'Dama cattle exposed to medium to high tsetse challenge in Zaire and Gabon (Trail, d'Iteren and Murray, 1991c) have shown that the ability to resist anaemia and control parasitaemia was associated with performance in both adults and calves. When all the environmental and parasitaemia information was taken into

account, the heritability of growth and of average PCV following infection reached 0.39 and 0.64, respectively, with a genetic correlation of 0.7 between the two. The results of this work have indicated that the ability to control the development of anaemia may serve as a reliable criterion of selection for trypanotolerant individual animals.

In addition to the trypanotolerant trait, the N'Dama has been shown have a degree of resistance to dermatophilosis (Stewart, 1937; Coleman, 1967), heartwater, anaplasmosis, babesiosis (Epstein, 1971) and helminthiasis (Kaufmann, Dwinger, Hallebeek, van Dijk and Pfister, 1992).

Trypanotolerant breeds, however, represent only 6% (10.7 out of 173 million of the total cattle population in the 40 countries where tsetse occur (IBAR, 1989; Hoste *et al.*, 1989). It has been estimated that 2 million km^2 in West Africa is suitable for trypanotolerant cattle without any additional control measures (FAO, 1976). This area can attain a carrying capacity of 20 cattle per km^2 as compared to the present 3.4 cattle per km^2. Similar increases would also be possible for sheep and goats. This means there would be approximately five fold increase in the total livestock population in this area. It is also believed that a similar approach might be possible in East Africa a consideration that is the basis of this thesis.

The use of trypanotolerant breeds is still one of the best approaches to the control of bovine trypanosomiasis. A complete understanding of the mechanisms of trypanotolerance and the environmental factors affecting it could lead to identification of genetic markers for selective breeding of trypanotolerant livestock or might enable methods to be devised for enhancing resistance to trypanosomiasis in the more susceptible breeds.

CHAPTER 2

OBJECTIVES OF THE THESIS

2.1 INTRODUCTION

2.1.1 BACKGROUND OF THE EPIDEMIOLOGICAL STUDIES AT THE NGURUMAN ESCARPMENT

In 1983, the International Centre for Insect Physiology and Ecology (ICIPE) entomology team initiated studies to investigate means of reducing trypanosomiasis challenge at Nguruman, an area in Southwestern Kenya that is heavily infested with tsetse flies. Consequently, the team has been carrying out studies on tsetse population dynamics and looking at methods to develop and design cheap technology to allow the local Maasai community to control tsetse flies using odour-baited traps. As a result of their work, precise information is available concerning the tsetse density and infection rates in the area (Dransfield, Chaundhury, Tarimo, Golder and Brightwell, 1985; Tarimo, Golder, Dransfield, Chaundhury and Brightwell, 1985)

In 1987, the Kenya Trypanosomiasis Research Institute (KETRI) initiated studies on the epidemiology of animal trypanosomiasis in the area, aimed at improving the disease control. This has mainly consisted of regular monitoring of livestock belonging to the farmers to determine the disease incidence. At the same time, an assessment of the use of chemotherapy for the control of the tsetse-transmitted trypanosomiasis in Maasai livestock and surveys for drug resistance were made.

Following these preliminary studies, it is now possible to identify with a degree of certainty the areas with high or low trypanosomiasis risk during different times of the year.

2.1.2 BACKGROUND OF TRYPANOTOLERANCE STUDIES CARRIED OUT BY KETRI

Epidemiological studies done by KETRI on the Galana Ranch over a period of about 10 years (from 1980 to 1990), have indicated that the Orma Boran cattle are more resistant to trypanosomiasis than the Galana Boran under natural field challenge. The Orma Boran cattle were observed to have lower incidence of trypanosomiasis thus requiring fewer trypanocidal treatments, and had higher body weight gains (Wilson *et al.*, 1983; Njogu *et al.*, 1985a,b; Dolan *et al.*, 1985).

Later, laboratory studies using needle and experimental fly challenge with pathogenic strains of *T. vivax* and *T. congolense*, confirmed the superior resistance of the Orma Boran compared with the Galana Boran, in terms of clinical responses (temperature, heart rate and respiratory rate), intensity of parasitaemia , severity of anaemia, ability to gain weight and the number of trypanocidal treatments required (Ishmael *et al.*, 1985).

2.2 AIMS OF THE STUDY

It is of importance to confirm the possible trypanotolerance of the Orma Boran in other tsetse-infested areas and to compare it with other local breeds. Such studies were undertaken at the Nguruman escarpment and the Galana Ranch with the aims;

1) to compare, under different levels of trypanosomiasis challenge at Nguruman, the epidemiology of the disease in the Orma Boran with local Maasai Zebu and Galana Boran and,

2) to compare the epidemiology of the disease in the Maasai Zebu, Orma Boran and Galana Boran with and without previous exposure at the Galana Ranch. The results would;

a) indicate whether the resistance of the Orma Boran was localized to the Galana Ranch or was more generalized,

b) establish if the farmers at Nguruman would benefit from the introduction of the Orma Boran in the areas with high disease risk and,

c) gauge the susceptibility of the Maasai Zebu to trypanosome infections in comparison to the Orma Boran and Galana Boran under different environments at the Nguruman escarpment and on the Galana Ranch.

2.3 JUSTIFICATION OF THE STUDY

Kenya's population growth rate is approximately 3.4% and in 1986, it was estimated that, it will be 35 million by the year 2000 (Anon., 1986). The beef requirements will be an estimated 540,000 tonnes, but with the present production systems and the area of land currently utilized, it is projected that, the country will be able to produce only 420,000 tonnes by then (Anon., 1986). Similarly, the milk requirements will be more than double the 1986 annual consumption of 1.6 billion litres.

The arid and semi-arid lands occupy 81% of the country, and are not suitable for arable farming (KETRI, 1991). About 60% of the semi-arid area consists of rangelands which form the most suitable area for livestock production. However, over 50% of these rangelands are infested with tsetse flies with the consequent risk of trypanosomiasis, a major factor which limits the expansion of livestock production (Anon., 1986; KETRI, 1991). To meet the above targeted beef requirements, there is the need to look for methods of either reducing the tsetse infestation or appropriate trypanosomiasis control to improve livestock production in these rangelands. One possibility is the utilization of breeds of livestock that are genetically resistant to trypanosomiasis, in combination with

established control measures. There is therefore the need to identify local cattle breeds that may possess this trait and compare their productivity under different levels of trypanosomiasis challenge in different environmental situations.

CHAPTER 3

MATERIALS AND METHODS

3.1 THE STUDY AREAS

3.1.1 THE NGURUMAN ESCARPMENT

a) General description of the area

The Nguruman escarpment and its environs are located in Kajiado District, Southwestern Kenya (Figure 3.1). The altitude is about 900 m, while the escarpment rises up to 1500 m. The Olkirimatian Group Ranch where the studies were conducted, is situated at the foot of the escarpment and has an approximate area of 300 km^2. The general features and the specific study sites are shown on Figure 3.2. This ranch is one of the many group ranches set up by the government in the late 1960's, as a part of a new approach to transform pastoral development from nomadic subsistence to a commercially oriented system.

It lies in a semi-arid area falling under the agroecozone V of the Kenyan ecological zones (Sombroek, Braun and van der Pouw, 1982). There are a variety of soils but the main ones include alluvial deposits and broken rocky terrain.

b) Vegetation

The main vegetation types in the part of the ranch between the Ewaso Ngiro River and the escarpment have been described by Dransfield *et al.,* (1985). Briefly, acacia woodland is found along the Ewaso Ngiro River. On either side of the river there are open plains consisting of scattered bushes and grassland. To the west of the river, these are followed by open acacia woodland and then dense woodland near the base of the escarpment. Open woodland dominates the slopes of the escarpment except for the narrow belts of dense woodland along several streams from the escarpment.

The northern region has areas of dense woodland forests stretching from the river to the base of the escarpment. Several streams from the escarpment

Figure 3.1 The location of study areas at the Nguruman escarpment and the Galana Ranch.

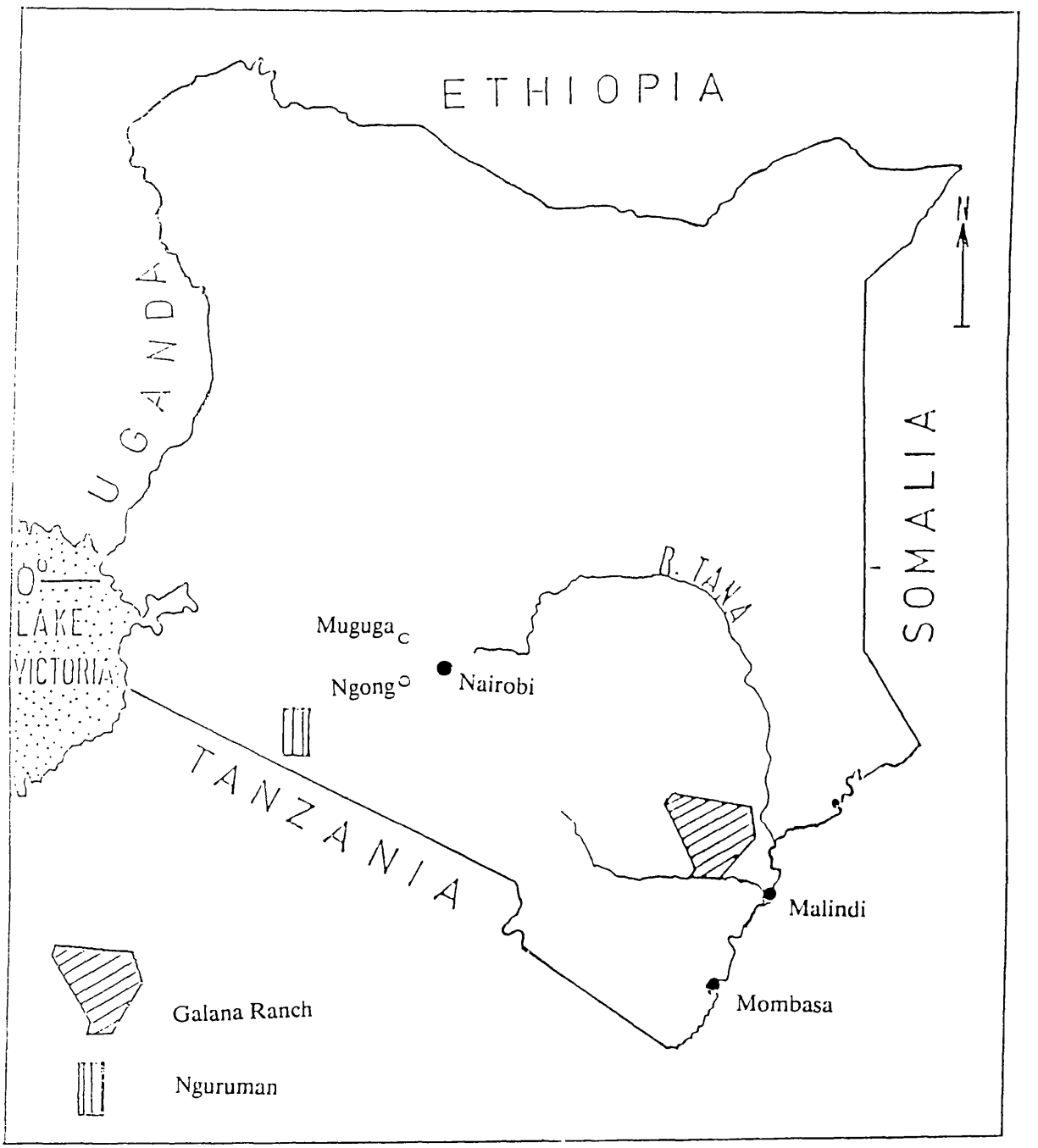

ETHIOPIA
UGANDA
SOMALIA
R. TANA
0°
LAKE VICTORIA
Muguga
Ngong
Nairobi
TANZANIA
Malindi
Mombasa
Galana Ranch
Nguruman

Figure 3.2 Nguruman; the Olkirimatian Group Ranch showing the low and high tsetse challenge areas, the KETRI camp and the experimental cattle bomas. (Original maps courtesy of R. Dransfield and R. Brightwell, OSCDP)

K - KETRI camp

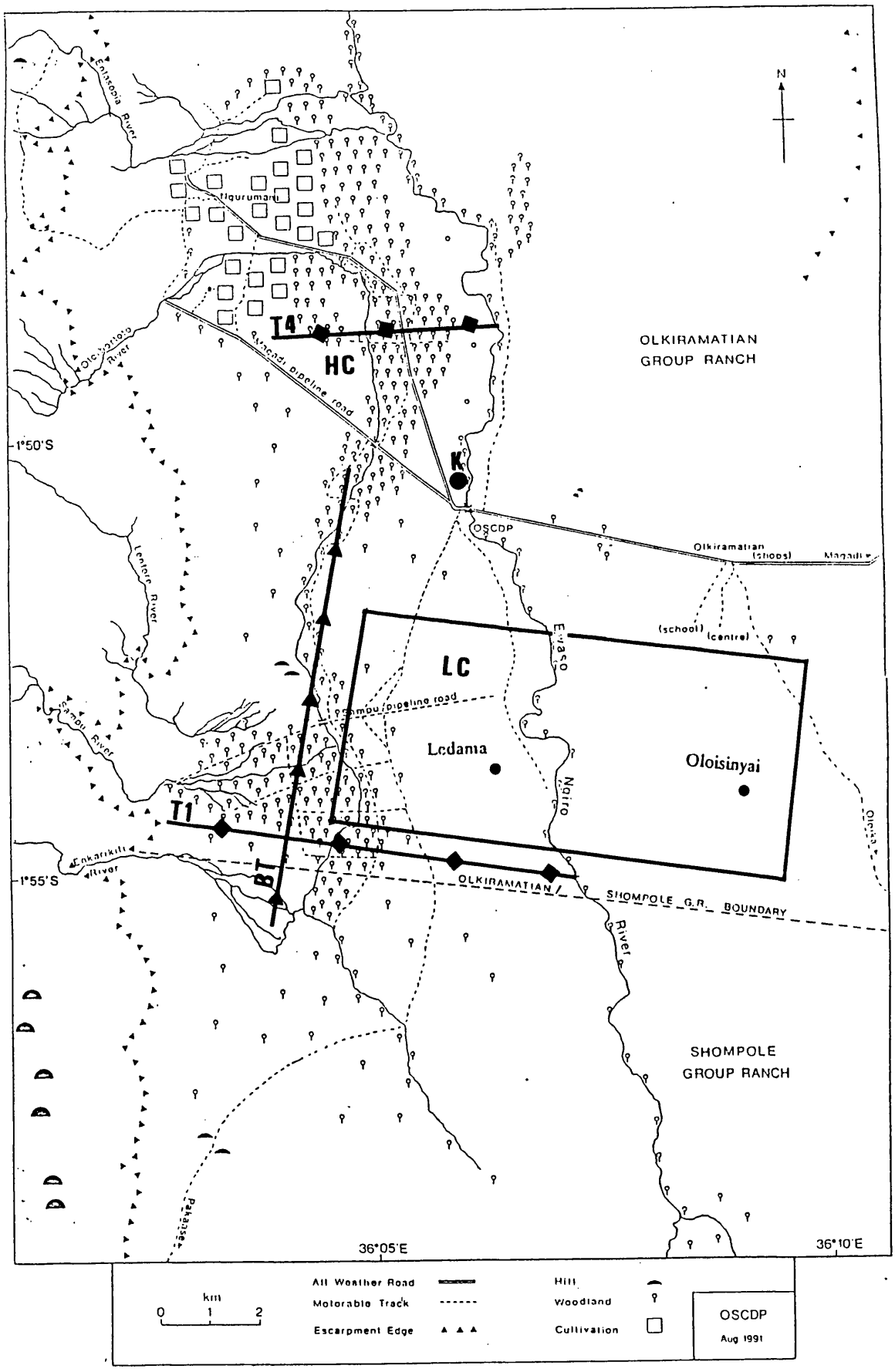
HC - High tsetse challenge area

LC - Low tsetse challenge area (includes zone enclosed by the rectangle on either sides of the river)

T1 - ICIPE fly Transect I (approximate location)

T4 - ICIPE fly Transect IV (approximate location)

BT - ICIPE barrier traps


N
Enasopia River
Ngurumani
T4
HC
Amboseli pipeline road
Olchorro River
OLKIRAMATIAN
GROUP RANCH
1°50'S
K
OSCDP
Olkiramatian (shops)
Magadi
Lenkisi River
(school)
(centre)
Engaso Nyiro
LC
Ngombu pipeline road
Ngombu River
Ledama
Oloisinyai
T1
BT
Enkarikili River
1°55'S
OLKIRAMATIAN / SHOMPOLE G.R. BOUNDARY
Nyiro River
SHOMPOLE
GROUP RANCH
Pastoral
36°05'E
36°10'E
km
0 1 2
All Weather Road
Motorable Track
Escarpment Edge
Hill
Woodland
Cultivation
OSCDP
Aug 1991

drain into the area making it suitable for agriculture, and as a result, there has been some human settlement with small scale agricultural activities being practised. Pastures are available for a greater part of the year. However, the area has a high tsetse density and except for several flocks of sheep and goats, very few cattle have been kept in this part.

c) Climate

The average monthly maximum temperature varies between 32 - 37°C, and a mean annual rainfall of 500 - 700 mm. The rainfall is bimodal with the long rains from March to May and the short rains from late October or early November to December (Dransfield *et al.*, 1985).

d) Livestock management

Basically, a group ranch normally consists of an area of tribal land allocated to a number of pastoralist householders who have been its traditional occupants. The individual family retains its livestock ownership and management, but the grazing and pasture management policy together with the development of the ranch, is under the guidance of an elected committee.

For this ranch, the grazing policy rotates very much around the availability of pastures and the presence of tsetse flies. The strategy is normally is to graze the cattle where pastures are available avoiding contact with the tsetse flies as much as possible and preserving other pastures for the dry season. As a result, the Maasai people in the area practice a seasonal transhumance.

During the rainy season the animals are grazed on the plains to the east of the river where there is grass and a relatively low trypanosomiasis challenge, which is confined mainly to the river belt. However, at certain times of the year

especially after the rains, flies can spread out several kilometres east of the river. As the dry season advances, the cattle move to the open plains on the west of the river. In the dry season there is no grass on the open plains (Figure 3.3) and the animals move to the wooded plains, an area with moderate disease risk.

In severe drought, the cattle are moved into the dense woodlands and the slopes of the escarpment, areas with high fly density (Figure 3.4). The animals have to walk for long distances in search for pastures and are often watered only once every two days. The exposure to the high trypanosomiasis risk accompanied by malnutrition and the stress of long distance trekking can sometimes result in heavy mortality. In the 1984 drought, at the end of several years of very dry weather, the combination of drought stress and trypanosomiasis was considered to have led to the deaths of about three quarters of the cattle in the area (Dransfield, Williams and Brightwell, 1991).

The study area has an approximate population of 10,000 cattle, 18,000 sheep, 19,000 goats and 500 donkeys (Anon., 1988). The cattle are predominantly of the East African Zebu breed, although recently, farmers have started importing bulls of exotic breeds especially Sahiwal, Boran and their crosses from commercial beef farms and there is a local livestock development project to upgrade stock for beef production. In addition, the livestock owners have a preference for animals with a large stocky conformation. The sheep consist of the Red Maasai, the Black head Somali and their crosses, while the goats are of mainly what has been generally referred to by several workers as the local East African breed (Mason and Maule, 1960; Griffin and Allonby, 1979; Kanyari, 1982; Waiyaki 1985), although many Galla goats are now being introduced.

Cattle are the main source of milk for domestic consumption, while goats (though also milked) and sheep are the main sources of meat for domestic

Figure 3.3 Nguruman; a view of the Olkirimatian group Ranch between the Ewaso Ngiro River and the escarpment, showing plains (PL), dense woodland forests (DW) at the base and the open woodlands (OW) on the slopes. In the foreground are Maasai Zebu cattle grazing on the almost bare ground in the dry season.

PL - Plains

DW - Dense woodlands

OW - Open woodlands

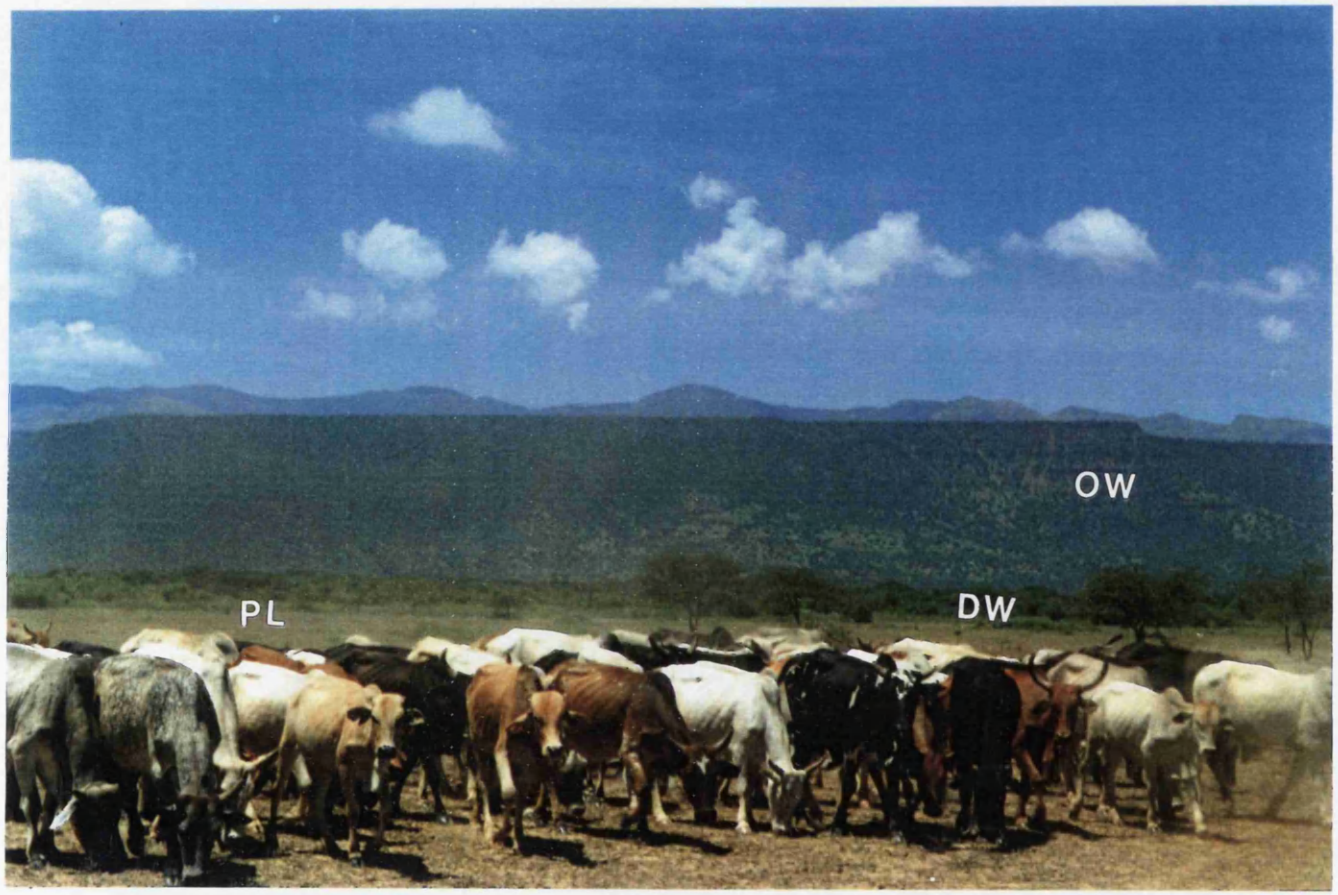

OW
DW
PL

Figure 3.4 Nguruman; Maasai Zebu cattle grazing in the tsetse-infested woodland forests at the base of the escarpment.

consumption. Livestock from this area are mainly marketed in Nairobi and its suburbs.

Donkeys are the only source of animal power and are used for fetching water, firewood and transportation of household goods during the seasonal transhumance.

e) Constraints to livestock production

The main constraints to livestock production are believed to be trypanosomiasis, seasonal lack of pasture and water, inadequate veterinary services and to a less extent predators (mainly lions on cattle and baboons killing kids and lambs). About 30% of the cattle were reported to have trypanosomes in their blood (Tarimo *et al.*, 1985) but more recent detailed epidemiological work by KETRI indicates that there is great seasonal variation in the different parts of the ranch (unpublished results).

The tsetse fly species that have been identified here are *Glossina pallidipes* and *G. longipennis* with the former being predominant. Preliminary studies have indicated that the apparent density varies from 19.4 flies/trap/day in the dense woodlands at the base of the escarpment to 0.16 near the river. In the same study, the infection rates were found to be 4.6% for *G. pallidipes* and 1.6% for *G. longipennis* with infections due to *T. vivax* being higher than *T. congolense* (Tarimo *et al.*, 1985).

There are many species of game which act as hosts for tsetse in the area, including giraffe, impala, buffaloes, Grant's gazelle, warthog, zebra, dik dik and waterbuck. In studies on the feeding pattern of *G. pallidipes*, analysis of 703 bloodmeal samples showed a high percentage of feeds from the suids (34.9 %), waterbuck (12.7 %) and buffalo (11.1 %) (Tarimo *et al.*, 1985).

f) Selection of study areas with differing tsetse challenge

Prominent physical features were used to identify two areas, which from previous work by the ICIPE entomology team, were known to have either high or low tsetse fly density.

i) Low tsetse challenge area

This part, represented by the rectangular block on Figure 3.2, was roughly regarded as the area between the KETRI boma at Oloisinyai to the west and the ICIPE barrier traps to the east. The northern limit was an imaginary line parallel to the Olkirimatian-KETRI camp road, but lying about 5 km to the south of this road, and extending on both sides of the Ewaso Ngiro River. The southern limit was the ICIPE sampling transect I and its arbitrary extension to the east of the river.

The vegetation in this area consists mainly of a narrow belt of woodland along the river, open plains and open acacia woodland on either sides of the river. There is more dense woodland towards the ICIPE barrier traps but in the course of the experiment, grazing into this part was avoided.

The part of this block lying to the west of the Ewaso Ngiro River was within the tsetse suppression zone, where since 1987, the fly population has been controlled using odour-baited NG2B traps (Figure 3.5). These traps were developed at Nguruman (Brightwell *et al.*, 1987) and within eight months following their installation, the fly population was reduced by 99% relative to catches outside the control zone. In addition, this control programme has been able to maintain the fly population always at about 10% of the fly density outside the suppression area (Dransfield *et al.*, 1991). As described earlier, the fly density to the east of the river is normally very low and confined to the riverine belt.

Figure 3.5 Nguruman; the NG2B trap used for tsetse fly control in the low challenge area. The combination of the blue and black colours on the cloth acts as visual attractants, while the cow urine and phenol provide odour stimuli. The the acetone (a) is dispensed in the plastic jar with a white top and the cow urine (u) in the blue topped one. Trapped tsetse flies accumulate in the white plastic paper bag (b) where they eventually die.

a - Acetone dispenser

b - Plastic paper bag

u - Urine dispenser


b
a u

ii) High tsetse challenge area

This was designated as the area bound by the Ewaso Ngiro River to the east, the ICIPE sampling Transect IV to the north, and the Magadi pipeline road to the southwest (Figure 3.2). The actual areas to which grazing was restricted are shown Figures 3.6, 3.7 and 3.8. The area has dense woodlands which are heavily infested with tsetse and although pastures are available for most of the year, livestock owners avoid grazing their animals in this zone. The area has a high population of wildlife particularly buffaloes. At the time of this study, there was no tsetse control programme in this part of the ranch.

3.1.2 THE GALANA RANCH

General description of the ranch and the livestock production system

This ranch has been described by several workers (King, Heath and Hill, 1977; Njogu *et al.,* 1985b; Wilson *et al.,* 1986). Briefly, the Galana Ranch, one of the largest ranches in Africa, is located in the coastal hinterland of Kenya (Figure 3.1 and 3.9) and occupies 667, 500 hectares lying between 2° and 3°S and 39° and 40°E. The mean altitude is 270 m above sea level and the mean annual rainfall increases from 250 mm in the west to 625 mm near the coastal area. The rainfall is bimodal with the most of the precipitation occurring between March and April and the rest between October and November. The western part of the ranch has an arid climate with a vegetation dominated by shrubs and acacia woodland, while nearer the coast it changes to semi-arid with a mainly thick coastal bush.

Commercial beef cattle production is the major livestock enterprise. There are about 20,000 cattle which consist of 16,000 Galana Boran and 4,000 Orma Boran. The Galana Boran are bred on the ranch, while, the Orma Boran are purchased from the Orma tribe in the Tana River basin at 12 to 18 months of

Figure 3.6 Nguruman; details of high challenge area showing the main block where the grazing was confined to.

K - KETRI camp

B - Cattle boma for the experimental animals

T4 - ICIPE Transect IV (approximate location)

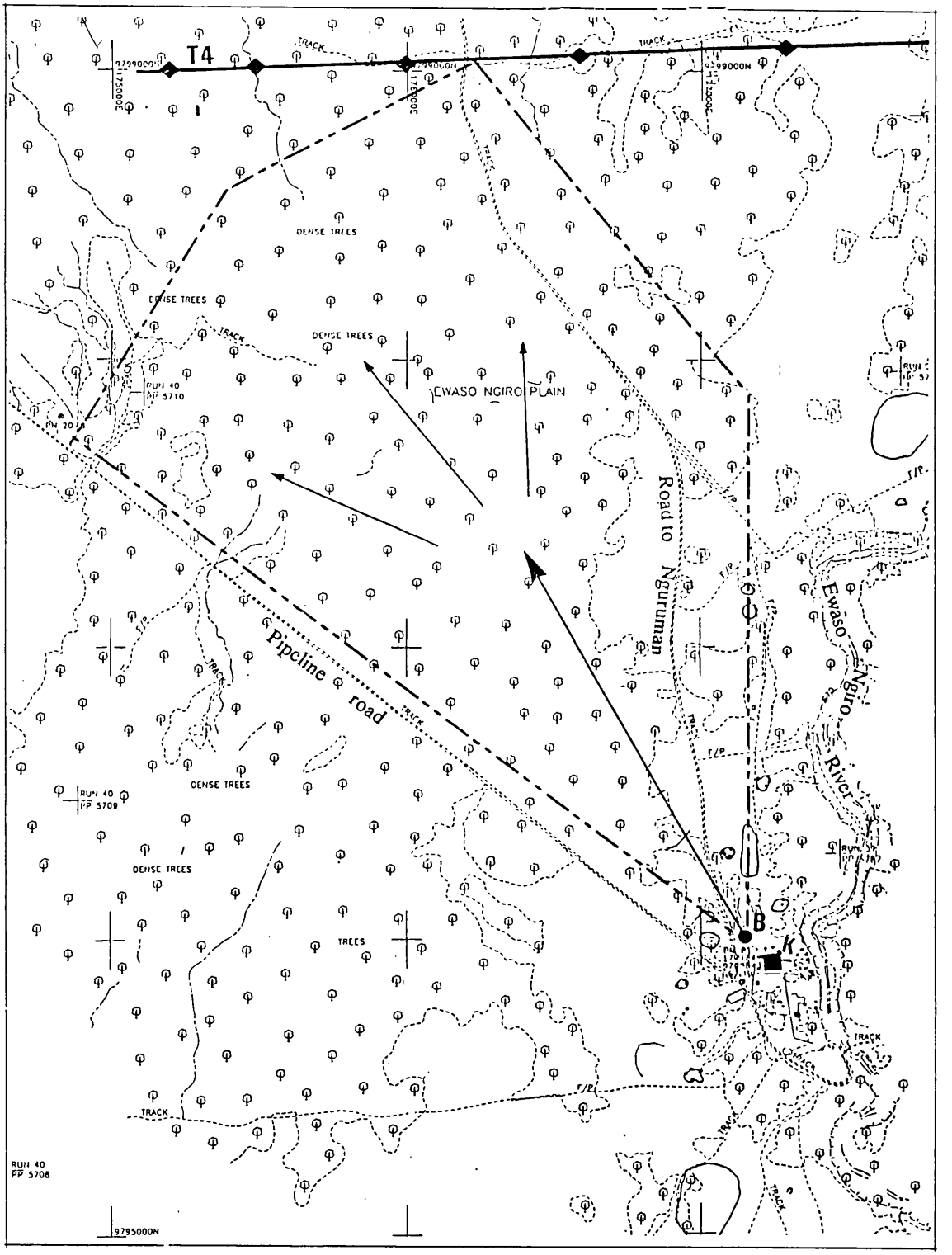

T4
TRACK
DENSE TREES
EWASO NGIRO PLAIN
Road to Ngurumman
Ewaso Ngiro River
Pipeline road
B
K
RUN 40
PP 5710
RUN 40
PP 5709
RUN 40
PP 5708
9795000N

Figure 3.7 Nguruman; topographical view of the high challenge area showing the distribution of the densely forested tsetse habitat.

K - KETRI camp

B - Cattle boma for the experimental animals

T4 - ICIPE Transect IV (approximate location)

DF - Dense forests

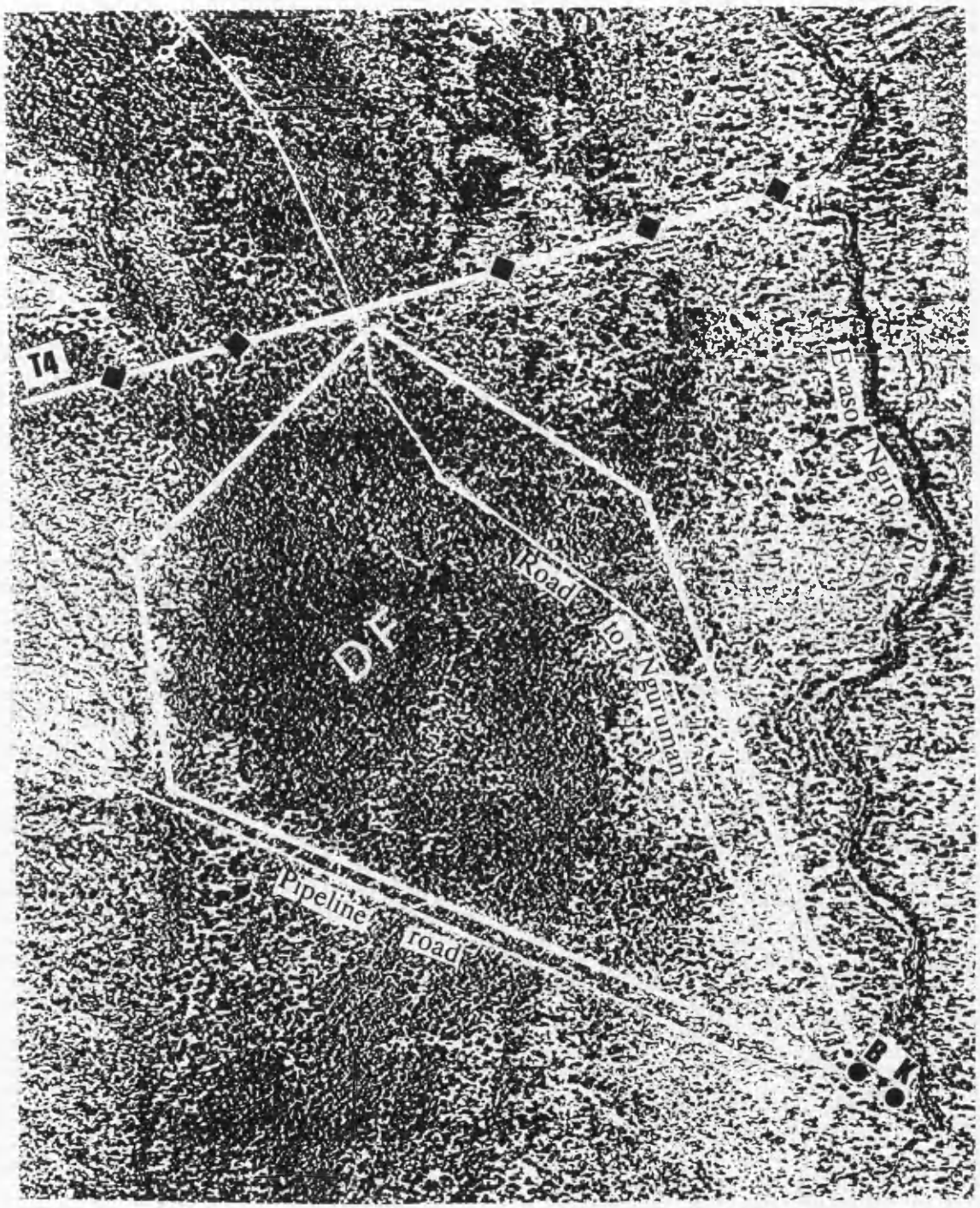

T4
DF
Road to Nguruman
Ewaso Ngiro River
Pipeline road
B K

Figure 3.8 Nguruman; aerial photograph of the northern part of the grazing block in the high challenge area showing the distribution of the densely forested tsetse habitat.

1 - Pipeline road

2 - Road to Nguruman shopping centre

3 - Ewaso Ngiro River

T4 - ICIPE Transect IV (approximate location)

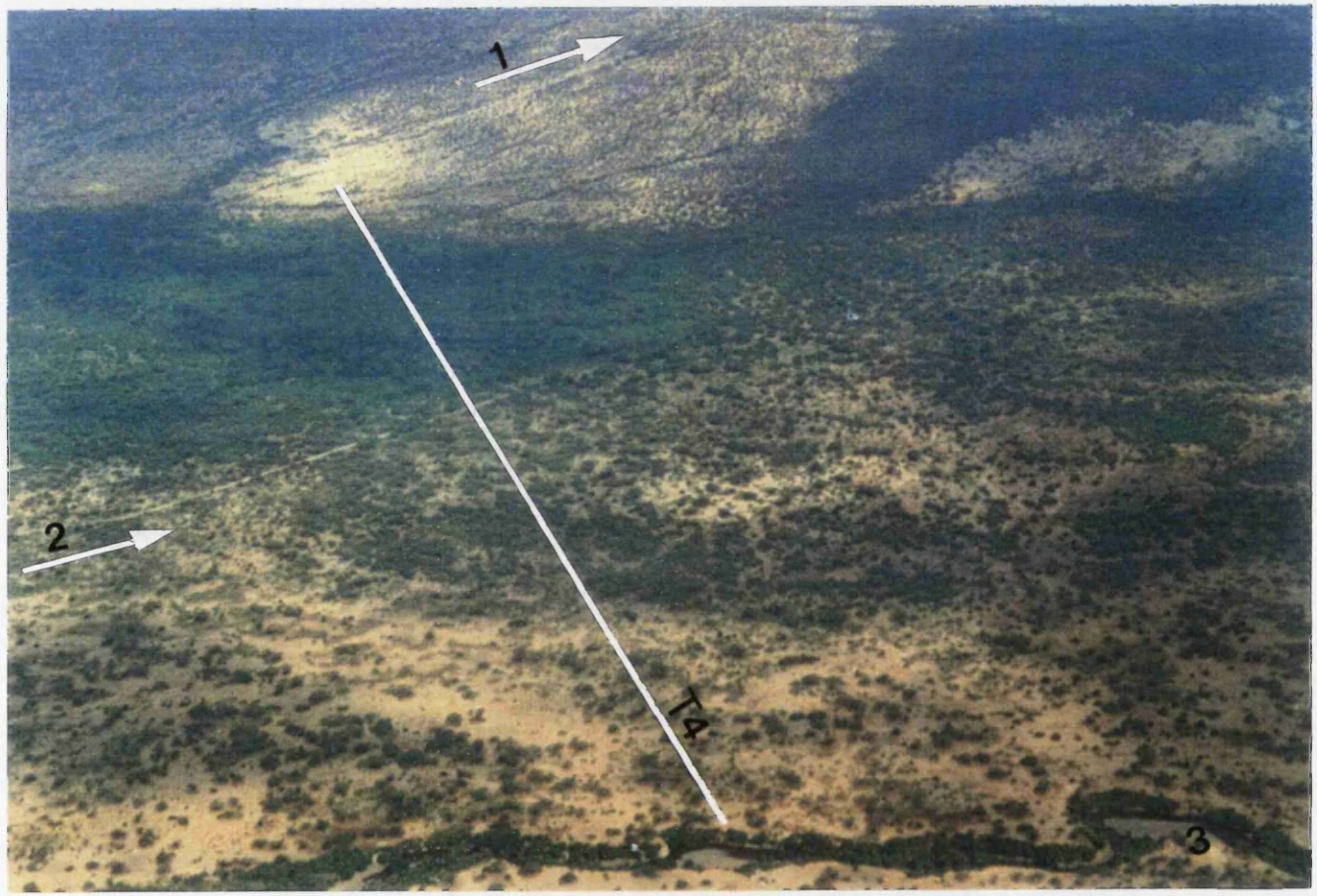
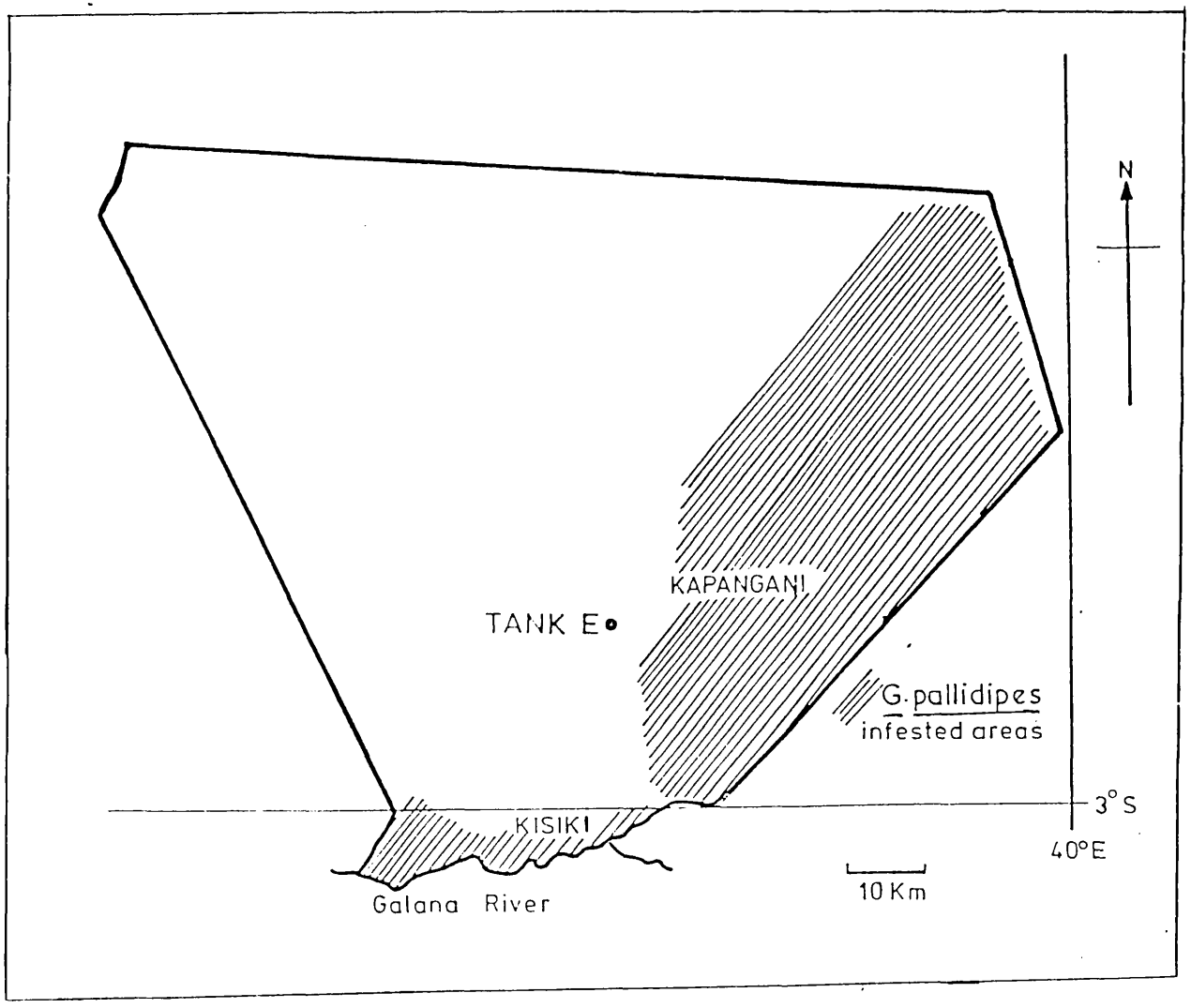

1
2
T4
3

Figure 3.9 The Galana Ranch


N
KAPANGANI
TANK E
G. pallidipes
infested areas
3°S
KISIKI
40°E
10 Km
Galana River

age and kept on the ranch, where they reach a market weight of 300 - 350 kg at around four years of age.

The main constraints to production are lack of water, trypanosomiasis and the presence of predators. Tsetse flies occupy about 35% of the ranch and an estimated 8000 cattle are at a risk from trypanosomiasis (Wilson, Gatuta, Mgutu and Alushula, 1986). In 1981, the estimated potential loss in the total population at trypanosomiasis risk was approximately, Kshs. 5 million (about US$ 700 000) (Wilson *et al.*, 1986). The predominant fly species is *G. pallidipes*, but *G. longipennis, G. brevipalpis* and *G. austeni* are also present (Njogu *et al.*, 1985b). The Galana Ranch has a large wildlife population including oryx, buffalo, gazelle, giraffe, eland, and zebra (King, Heath and Hill, 1977) which provide reservoir host for tsetse.

The present experiments were set up at Kapangani, an area of the ranch heavily infested with tsetse flies, the main species being *G. pallidipes* (Njogu *et al.*, 1985b). This part of the ranch has acted as a study site for KETRI field experiments on chemoprophylaxis and trypanotolerance for over 10 years and represents a typical high challenge area where ranch cattle are grazed.

3.2 HISTORICAL BACKGROUND OF THE *BOS INDICUS* CATTLE BREEDS OF EAST AFRICA

The name 'Zebu' (derived from Portuguese 'Giba' or Tibetan 'Zeba') means humped. Zebu cattle probably originated from Eastern Iran (Reed, 1984). They were moved by sea through the Persian Gulf around 2000 B.C., to the Somali coast and then to Egypt (Epstein and Mason, 1984). Around 400 A.D. more Zebu, camel and horses moved to Somalia and Ethiopia. However, the main influx of Zebu cattle from Asia coincided with the Arab invasions around 700

A.D. and these shorthorned Zebu are now the dominant breed in East Africa (Payne, 1990).

3.2.1 SANGA

The first group of Zebu cattle that arrived from Asia interbred with the Hamitic Longhorn cattle in the horn of Africa, to form the longhorned small humped Sanga cattle. From their origin in Ethiopia, the Sanga then spread southwards into Central and Southern Africa (Reed, 1984; Mason 1990). At present, most of the Sanga have been replaced by the Zebu since the latter appear to be more resistant to rinderpest and therefore survived the pandemic of the 1890's (Payne, 1964). In addition, the Zebu appear to be well adapted to living in arid and semi-arid areas (Payne, 1964; Epstein, 1971).

The term Sanga has been used for the small cervico-thoracic humped cattle, while the large humped 'true' Zebu are simply referred to as Zebu. From their distribution, the Sanga in the hinterland and the East African Zebu on the coastal area, and the similarity of the latter with the Zebu of India and Southwestern Asia, it appears that the Sanga were the first to arrive and the Zebu arrived more recently from the east (Mason, 1960).

Along the boundaries of the areas of distribution of the two types there is considerable intermixture and in some places, more or less fixed types referred to as intermediate, which have moderate size humps varying between thoracic and cervico-thoracic position, have emerged.

3.2.2 BORAN

The indigenous Boran cattle are found in the Borana province of southern Ethiopia on the Liban plateau. Due to constant tribal movements and the

nomadic habits of these tribes, they spread throughout southern Ethiopia, northern and central Kenya. The Ethiopian Boran is a fairly large, long legged animal with good body conformation. It is light coloured varying from white to light fawn and sometimes red.

a) Tanaland (Orma) Boran

This term refers to the Galla cattle found to the south and west of the Tana River in Kenya and, to distinguish them from other Galla cattle the name Tanaland is used (Mason and Maule, 1960; Maule, 1990). The Tanaland Boran now forms a uniform type differing slightly in conformation from the Ethiopian Boran. In this study the name Orma Boran is used (Figure 3.10).

The Orma are the remnants of the Ormo people of southern Somalia (Kenya Official Handbook, 1983). They came from central Arabia through the Red sea and settled in Somalia around 2000 B.C. They later moved southwards with their animals and settled on the banks of Tana River from Garissa to Garsen in Kenya about 500 years ago (Feeders and Salvadori, 1984; Amin and Moll, 1980; Njogu, Ishmael, Dolan, Okech, Sayer and Opiyo, 1988). The Tana River basin where the Orma tribe have traditionally selected their animals for milk yield for several centuries is heavily infested with tsetse flies (Ford and Katondo, 1973).

b) Kenya Boran

These originated from large numbers of Boran type cattle brought to the northern Kenyan by Somali traders. Over the last 100 years, as a result of selection, careful breeding and greatly improved management on beef ranches in the Rift Valley and Central provinces, they have developed into large robust

Figure 3.10 Orma Boran steers approximately two and a half years old.

animals with excellent conformation. The 'improved' Boran has been bred and selected mainly as a beef animal (Mason and Maule, 1960; Njogu *et al.*, 1988).

The Kenyan Boran were first introduced into the Galana Ranch in 1978 from the Laikipia District of Kenya which is a tsetse-free area. The selection process was continued at the Galana Ranch, where these cattle reach 350 - 400 kg in three and a half years (Njogu *et al.*, 1985b). These type of animals have been referred to as the Galana Boran in the study (Figure 3.11).

3.2.3 SMALL EAST AFRICAN ZEBU

These are the predominant type of cattle found in north and eastern Uganda, south and western Kenya and most of Tanzania. Because the typical East African Zebu of this type is small framed and in order to avoid confusion with other East African cattle types, the name 'Small' East African Zebu has been used (Mason,1960). There are several types of which the one in this study is the Maasai Zebu.

Maasai Zebu

The Maasai Zebu shows considerable variation in size, colour, conformation and horn development. They are black, white, grey fawn and various combinations of these colours (Figure 3.12). Maasai cattle tend to be larger than other Small East African Zebu cattle (Mason, 1990) and at Nguruman, the bulls reach an average adult liveweight of about 300 kg.

The present Maasai territory where this breed is predominant, extends from the south of Lake Naivasha in Kenya to central Tanzania. In this study, this breed has been referred to as the Maasai Zebu.

Figure 3.11 Galana Boran steers approximately two and a half years old.

Figure 3.12 Maasai Zebu steers approximately two and a half years old.

3.3 VARIABLES MONITORED IN THE STUDY

3.3.1 TRYPANOSOMIASIS RISK

The trypanosomiasis risk was assessed by measuring the apparent fly density and the disease incidence in sentinel cattle herds as described later in the respective study areas.

3.3.2 TRYPANOSOMIASIS IN CATTLE

a) Parasitaemia and anaemia

The parasitaemia and anaemia in the cattle were monitored weekly. The animals were bled by pricking the superficial ear veins with sterile lancets and the blood was drawn directly into a pair of heparinized capillary tubes. The tubes were sealed on one end with Cristaseal^{R}(Hawksley and Sons Ltd. U.K.) and spun at 12,000 g for 5 minutes on a microhaematocrit centrifuge. The percentage of the packed red blood cells (PCV) was measured on a haematocrit reader. The buffy coat was then examined for trypanosomes using the darkground/phase contrast technique (DG) as described by Murray *et al.*, (1977). Briefly, after the centrifugation of the microhaematocrit capillary tube, it was cut with a diamond tipped pencil at 1 mm below the buffy coat, to include the upper most layer of the red blood cells and 1 cm above to include plasma. The contents were expressed onto a clean glass slide, mixed, and a 22 x 22 mm cover slip placed to spread the drop. The preparation was examined using a phase contrast which was created by a normal bright field microscope system with the diaphragm closed to achieve critical illumination. A minimum of 100 microscope fields was examined and the trypanosome species identified by their size and motility characteristic as described in Table 3.1. Thick and thin smears were also prepared from positive samples, and stained with 10% Giemsa for laboratory morphological

Table 3.1

Morphological characteristics used for the identification of trypanosomes species

Species	Free fagellum	Kinetoplast	Undulating membrane	Size and motility in wet film and DG
T. vivax	Present	Large, terminal	Not prominent	Large, extremely active, transverse the whole field very quickly, pausing occasionally.
T. brucei	Present in all except stumpy forms	Small, sub-terminal, central	Prominent	Large with slender, intermediate and stumpy forms, rapid movement in confined areas.
T. congolense	Absent	Medium, sub-terminal, marginal	Not prominent	Small, sluggish active, adheres to red blood cells by anterior end.

confirmation of the trypanosome species.

b) Parasitaemia score

The parasitaemia was estimated using the scoring system described by Paris *et al.*, (1982), as shown in Table 3.2.

c) Wet, thick and thin smears

For the wet films, 5 ul of blood (one drop) on a coverslip, was placed on a clean glass slide and examined using phase contrast microscopy. For the thick and thin smears, 5 ul of blood was spread onto a slide and then dried by rapid waving in the air. In case of the thick smears, they were placed in distilled water for five minutes to dehaemoglobinise, while the thin films were fixed for three minutes in methanol. Both were then stained with 10% Giemsa for 30 minutes and examined under oil immersion.

d) Trypanocidal drug treatments

In this study parasitaemic animals with were treated with diminazene aceturate (Berenil^{R}, Hoechst) at 7 mg kg^{-1} body weight only when the PCV dropped to 17% or less. This value was choosen because from previous observations (personal experience), cattle infected with trypanosomes at Nguruman had been noticed to survive with such low PCV values, while at risk from dying.

e) Inoculation of blood into susceptible mice

This was done by inoculating fresh whole jugular blood from every animal collected into an EDTA treated vacutainer, into a pair of adult male mice (Swiss), each with 0.5 ml intraperitoneally. After five days, the mice

Table 3.2

Estimation of parasitaemia by the darkground/phase contrast buffy coat (DG) method

Trypanosomes/field*	Score	Estimated parasitaemia (trypanosomes/ml)
1 per preparation	1+	10^2 - 10^3
1 - 10 per preparation	2+	10^3 - 10^4
1 per field - 1 per 10 fields	3+	5 x 10^3 - 5 x 10^4
1 -10 per field	4+	10^4 - 5 x 10^5
> 10 per field	5+	> 5 x 10^5
Swarming > 100 per field	6+	> 5 x 10^6

* Magnification x 250

were bled twice weekly from the tail, wet preparations made and examined for trypanosomes by light microscopy. The mice were monitored for a minimum of 30 days before they were declared non parasitaemic.

3.3.3 OTHER HAEMOPARASITIC DISEASES

Thick and thin smears were made from animals with PCV of 20% or less with no trypanosomes detected on the buffy coat, and stained with 10% Giemsa for examination of tick-borne infections mainly, anaplasmosis, babesiosis and theileriosis.

3.3.4 MANAGEMENT

a) Tick control

Tick-borne diseases were controlled by a weekly spraying programme with amitraz (Triatix^R, Cooper Ltd.). On several occasions during the study, ticks were collected from the animals and later submitted to the Kenya Agricultural Research Institute (KARI) laboratory at Muguga for species identification.

b) Helminthiasis

To ensure that there was no anaemia associated with helminths, drenching with 10% albendazole (Valbazen^R, Beecham) at 7.5 mg kg^{-1} body weight was carried out every three months. In addition, faecal egg counts were performed on at least 50% of the steers of each breed every two months as described below.

Two grammes of freshly collected faeces was sieved into a beaker with 30 ml 1% formalin solution. The residue was discarded. The suspension was thoroughly mixed and 10 ml were poured into a conical centrifuge tube and spun at 1,200 g for two minutes. The supernatent was discarded, the deposit

resuspended in 10 ml saturated NaCl solution, mixed thoroughly and using a pipette, dispensed into a McMaster counting chamber, and left to stand for five minutes. Both chambers were counted and an estimate of the number of eggs per gram (e.p.g.) made by multiplying the total count by 50.

3.3.5 PERFORMANCE

a) Body weights

Changes in the body weight were obtained by weighing all the animals fortnightly. This was carried out early in the morning before the animals went for grazing. The growth rate was then obtained by expressing the mean changes in weight as percentages the original body weight using the formula;

$$\text{Weight gain } (\%) = \frac{Bwt_t - Bwt_o}{Bwt_o} \times 100,$$

where, Bwt_o - is the mean herd body weight (kg) at the start of the experiment, and, Bwt_t - is the mean herd body weight (kg) at a particular time.

b) Condition score

The plane of nutrition to which an animal has been exposed over a reasonable length of time is reflected by the extent to which either the fat is stored or the muscle mass has diminished. This can be assessed visually and expressed as a condition score. The main anatomical parts used to determine the score are; the tail-head, brisket and hump, transverse processes of the lumbar vertebrae, hips (trochanter major) and ribs, and, the shape of the muscle mass between the *tuber coxae* and *tuber ischii*. The score of an animal depends on the visibility of these

anatomical parts and the flesh and fat cover at these points (Lowman, Scott and Sommerville, 1976; Nicholson and Butterworth, 1986).

In this study, a nine point scale was used in which the three main conditions, lean (L), medium (M), and fat (F) were subdivided into three categories. The animals were scored early in the morning before any feeding. A description of how the different scores were assessed as described for *Bos indicus* cattle by Nicholson and Butterworth (1986), is summarized in Table 3.3.

3.3.6 METEOROLOGICAL DATA

Meteorological data were obtained from small weather stations set up in the study areas at both Nguruman and the Galana Ranch.

3.4 SEROLOGY

On a monthly basis, 10 ml of jugular blood was collected from each animal into a clean vial and allowed to clot. The serum was separated by centrifugation and stored at -20°C and later analysed for trypanosomal antigens and antibodies.

3.4.1 ANTIGEN ELISA

Antigens were detected in sera by a sandwich enzyme-linked immunoabsorbent assay (ELISA) following the procedure described by Nantulya and Lindqvist (1989). In brief, flat-bottomed micro-titre (micro-ELISA) plates were coated overnight at 4°C with monoclonal antibodies against *T. vivax*, *T. congolense* or *T. brucei*, using 100 ul per well of a 1:100 (1 ug/well) dilution in carbonate buffer pH 9.6 (coating buffer). The excess antibody was drained off the plates and 100 ul washing buffer (0.15M phosphate buffered saline (PBS), pH 7.4 containing 0.05% Tween 80) added to each well. Test and control sera were added to the wells in

Table 3.3

The nine point scale used scale used to condition score *Bos indicus* cattle (after Nicholson and Butterworth, 1986)

Condition	Score	Main features observed
L-	1	Marked emaciation
L	2	Prominent transverse processes and the neural spines
L+	3	Prominent hips, *tuber ischii* and ribs, dorsal spines pointed to the touch
M-	4	Ribs, hips and *tuber ishcii* clearly visible
M	5	Ribs visible, little fat cover, dorsal spines barely visible
M+	6	Animal smooth, well covered, dorsal spines invisible but easily felt
F-	7	Animal smooth, well covered, dorsal spines felt with pressure
F	8	Fat cover, transverse process not seen or felt
F+	9	Heavy fat deposits on tail head, dorsal spines, *tuber coxae*

duplicates, 5 ul for *T. brucei* and 10 ul for both *T. congolense* and *T. vivax*. They were then incubated at room temperature for 15 minutes, flipped empty and rinsed with the washing buffer. 100 ul of the appropriate monoclonal antibody conjugated to horseradish peroxidase diluted 1:100 in washing buffer containing 1% bovine serum albumin were added. The plates were then incubated for 15 minutes at room temperature, emptied, rinsed once then soaked three times at 10 minute intervals with the washing buffer to wash off the excess conjugate. 100 ul of substrate and chromogen in citrate buffer pH 4.0 were added to each well. The substrate and chromogen consisted of 1% hydrogen peroxide and 250 ug/ml of 2,2'-aziono bis (3-ethyl)-benzthiazoline-6-sulphonic acid (ABTS, Sigma, St. Louis, USA), respectively. The plates were incubated at room temperature for 30 minutes. The optical densities (O.D.) of the colour reaction were read at 414 nm wavelength using a Titertek Multiskan MCC/340 MK II (Type 347) micro-ELISA autoreader. The threshold reading for a sample regarded to indicate presence of antigens was 0.050.

3.4.2 ANTIBODY ELISA

The micro-ELISA technique described by Luckins (1977) was used. Briefly, a crude antigen extract was prepared from *T. congolense* IL3000, which is a clone derived from an isolate collected originally in 1966 from a naturally infected cow from Trans-Mara, Kenya (Wellde *et al.*, 1974). Flat-bottomed 96-well micro-ELISA plates were coated with the crude lysate antigen extract diluted at 1:100 with carbonate buffer pH 9.6 (coating buffer) and incubated overnight at 4°C. The working dilution of the antigen was determined by titrating at serial dilutions with positive and negative sera. The antigen dilution which gave the greatest difference between the negative and positive sera was used in the assays.

The plates were then rinsed once with washing buffer (0.15M PBS, pH 7.4, containing 0.05% Tween 80) to remove the excess antigen. The test and control sera were diluted 1:50 in washing buffer containing 1-2% ovine serum albumin. 100 ul were dispensed in duplicates to each well and incubated at 37°C for 30 minutes. The plates were rinsed three times then soaked for 30 minutes and the buffer drained off. 100ul of the conjugate consisting of 1:20,000 goat anti-bovine immunoglobulin diluted in 1% sheep albumin in washing buffer was added to each well and then incubated for 30 minutes at 37°C. The plates were washed and soaked with the washing buffer for 30 minutes and this procedure was repeated twice. The substrate (same as the one described above for the antigen-ELISA) was added, the plates incubated at room temperature for 30 minutes and the optical densities read at 414 nm wavelength using the micro-ELISA autoreader. The serum samples were considered positive if the O.D. reading was more than double the mean of the control negative sera.

CHAPTER 4

VARIATION IN SUSCEPTIBILITY TO TSETSE-BORNE TRYPANOSOMIASIS AMONG THREE *BOS INDICUS* CATTLE BREEDS IN DIFFERENT TSETSE ENDEMIC LOCALITIES IN KENYA

4.1. EPIDEMIOLOGICAL STUDIES AT THE NGURUMAN ESCARPMENT: COMPARISON OF THE MAASAI ZEBU, ORMA BORAN AND GALANA BORAN IN AREAS OF DIFFERING TSETSE CHALLENGE FROM SEPTEMBER 1989 TO SEPTEMBER 1990

4.2. EPIDEMIOLOGICAL STUDIES AT THE GALANA RANCH, KENYA COAST: COMPARISON OF THE MAASAI ZEBU, ORMA BORAN AND GALANA BORAN CATTLE WITH (PE) AND WITHOUT (NPE) PREVIOUS EXPOSURE TO TRYPANOSOMIASIS FROM MAY 1991 TO FEBRUARY 1992.

4.1 EPIDEMIOLOGICAL STUDIES AT THE NGURUMAN ESCARPMENT: COMPARISON OF THE MAASAI ZEBU, ORMA BORAN AND GALANA BORAN IN AREAS OF DIFFERING TSETSE CHALLENGE FROM SEPTEMBER 1989 TO SEPTEMBER 1990.

4.1.1 INTRODUCTION

Studies on the susceptibility of the Orma Boran and Galana Boran under natural tsetse challenge in Kenya have been carried out only at the Galana Ranch (Wilson *et al.*, 1983; Njogu *et al.*, 1985a,b). The work described in this section was aimed at investigating how these two breeds would respond to natural challenge in a different environment at Nguruman. At the same time, information would be obtained on the susceptibility of the local Maasai Zebu in comparison with these breeds. The results would also indicate if there was any potential for the exploitation of breed advantage of the Orma Boran in those parts of Nguruman that are heavily infested with tsetse flies.

4.1.2 MATERIALS AND METHODS

a) Areas with high and low tsetse challenge

The selection of the areas with high and low tsetse challenge has been described in Chapter 3.

b) Selection of experimental cattle

i) Maasai Zebu

Forty four steers aged about one and a half years were purchased locally from the farmers at Nguruman. The age was estimated as described by Carles and Lampkin (1977), where steers with the first pair of incisors fallen or just erupting

were regarded as between one and a half and two years.

The previous history of each animal was obtained from the owner and this included, last trypanocidal drug treatments (Table 4.1), and previous exposure to tsetse challenge. Overall, 19 animals (44%) had no history of trypanocidal drug treatment, while 13 animals (30%) had received treatment within the last three months and seven animals (16.3%) between three and six months. From interviews with the farmers, most of these animals had been in the tsetse challenge area for varying lengths of time during the dry seasons.

In the pre-weaning period, as a tradition of the Maasai livestock owners, the calves are grazed separately from the dam, and are allowed to suckle in the morning and evening only. Milk from the dam is therefore shared between calf rearing and domestic consumption. The calves are generally grazed around the homesteads and are therefore not exposed to high tsetse challenge until they are weaned.

ii) Orma Boran

Forty three steers, approximately one and a half years old, were selected from a herd bought into the Galana Ranch six months earlier, from the Tana River District, an endemic area with high tsetse challenge. The same method of age estimation was used as for the Maasai Zebu .

There was no clear information on the pre- and post-weaning periods, but most likely, these animals were exposed to varying levels of trypanosomiasis challenge from birth and must have received repeated trypanocidal drug treatments from the owners. On purchase by the ranch, the animals were given a prophylactic treatment with isometamidium chloride (Samorin^{R}, RMB, U.K.) at a dosage of 1 mg kg^{-1} body weight.

Table 4.1

Approximate time before the date of purchase, when the Maasai Zebu steers received their last trypanocidal drug treatments

Drug	Last treatment	Number of animals
Diminazene aceturate[1]	1 - 6 months	5
Diminazene aceturate and Homidium chloride[2]	1 - 5 months	2
Homidium chloride	1 - 3 months	13
No treatment		19
No clear history		4

[1]Berenil[R], Hoechst, W. Germany.

[2]Novidium[R], RMB, UK.

The pre-weaning calf management of the Orma Boran is similar to that of the Maasai Zebu, where a portion of the dams milk is also extracted for domestic consumption, and young calves are normally grazed away from areas with high tsetse density.

iii) Galana Boran

Nineteen steers aged about one and a half years were selected from a herd born and bred in the Galana Ranch. The ranch has precise records and it was therefore easy to obtain the exact ages. During the pre-weaning period, the calves are generally allowed to graze with the dam and kept in areas of low tsetse challenge to minimize pre-weaning mortality. They therefore get milk *ad libitum*. They are weaned at eight months and still kept in areas with low tsetse challenge. The steers bought were selected from a group which had been treated with isometamidium chloride at 1 mg kg^{-1} body weight, about six months earlier.

c) Pre-experimental procedures

i) Vaccinations

Vaccinations were carried out against foot-and-mouth disease (types A, O, SAT 1 and 2), rinderpest, contagious bovine pleuropneumonia (CBPP), blackquarter and anthrax.

ii) Treatments

All animals were treated with diminazene aceturate (Berenil^{R}, Hoechst) at 7 mg kg^{-1} body weight to clear any trypanosome infections and drenched with an anthelmintic, 10% albendazole (Valbazen^{R}, Beecham) at 7.5 mg kg^{-1} body weight, in preparation for the transportation to the Nguruman escarpment.

Anaplasmosis is common on the Galana Ranch; therefore, a long acting tetracycline (Terramycin/LA^R, Pfizer), at 20 mg kg^{-1} body weight was administered to control any incubating cases or other stress related infections that were likely to flare up during the transportation. In addition, the animals were also sprayed with an acaricide (Triatix^R, Cooper Ltd.) three days before the journey.

The Orma and Galana Boran steers were then moved by lorry to the Nguruman escarpment, a distance of about 500 km. On arrival, a physical examination was performed, and any animals that were looking weak and stressed were kept on multivitamins (Catasol^R, Bayer) for three days. Following the transportation, a few animals had lacerations and traumatic superficial skin wounds which were treated with topical antibiotics. The whole herd was allowed to rest and grazed in an area with no tsetse challenge for a period of one week. Any weak looking animals were examined daily until they fully recovered from the transportation stress.

Baseline data on anaemia (PCV), parasitaemia and body weight were collected. Blood and lymph node smears made from all the animals were stained with 10 % Giemsa and examined for haemoparasites, specifically anaplasma, babesia and theileria. Serum was collected and stored at -20^{o}C for later screening for pre-experimental trypanosomal antibodies and antigens.

d) Experimental design

The cattle of the three breeds were grouped and introduced into the study areas as shown in Table 4.2. There were no Galana Boran in the low challenge area primarily because the group ranch raised concern about the introduction of a large number of cattle from the Galana Ranch. The groups were herded

Table 4.2

Experimental design at Nguruman; - number of animals of each cattle breed introduced into the high and low tsetse challenge areas

Breed	High challenge		Low challenge	
	N	W(kg)	N	W(Kg)
Maasai Zebu*	22	140	21	138.8
Orma Boran	22	157	21	155.5
Galana Boran**	19	196.8	-	-

N - Number of animals.

W(kg) - Mean body weight (kg) at the start of the experiment.

* One Maasai Zebu steer was dropped to make the groups in the low challenge area even.

** No Galana Boran steers were introduced into the low tsetse challenge area.

together for a period of one year, from September 1989 to September 1990, in the high and low tsetse challenge areas. In March 1990, the animals in the low challenge area were moved to the east of the Ewaso Ngiro River in accordance with the pasture management practice of the group ranch.

e) Parameters monitored

i) Trypanosomiasis risk

The trypanosomiasis risk was assessed by measuring the apparent fly density and the disease incidence in sentinel Maasai Zebu cattle in the respective areas.

Tsetse challenge

Data on the tsetse situation were provided by the ICIPE entomology team based at Nguruman. Briefly, odour-baited biconical traps (Challier and Laveissiere, 1973), were set at 1 km apart in both study blocks and operated for six days per month. The fly density was then estimated from counts on 48 hourly trap catches. When the cattle in the low challenge area were moved to the east of the river, the KETRI team set up biconical traps opposite Transect I; these were managed in the same way as the others.

Disease incidence in sentinel cattle

In the low challenge area, the disease incidence was calculated from a sentinel herd of 40 Maasai Zebu cattle grazing together with the experimental groups. There were no sentinel animals in the high challenge area for the first five months. However, in February 1990, a group of 21 Maasai Zebu steers aged two and a half years, which had previously been used as sentinel animals in the tsetse suppression zone, was treated with diminazene aceturate (Berenil^{R}, Hoechst) at

7 mg kg^{-1} body weight to clear any infections which could be incubating, were introduced into the high area where they served as the sentinel herd. They were monitored weekly and any trypanosome infections detected were treated immediately with diminazene aceturate (Berenil^R, Hoechst) at 7 mg kg^{-1} body weight and the results used to calculate the disease incidence, expressed as the proportion of the animals infected weekly.

ii) Trypanosomiasis in cattle

The disease monitoring in the experimental cattle and the assessment of the growth rate were performed as described in section 3.3.

iii) Meteorological data

Data on daily minimum and maximum temperature, rainfall, and relative humidity, were obtained from a small weather station set at the camp site at Nguruman.

f) Data analysis

Comparison for differences between breeds and breed x time interactions in the in the two areas with differing tsetse challenge was done using the Generalized Linear Interactive Modelling system (GLIM, release 3.77, Royal Statistical Society, 1987). Differences between groups were investigated using the one way analysis of variance, two way analysis of variance for repeated measures, while the multigroup mean comparisons were done by the Newman-Keul's range test (multiple range test) using the Minitab release 7 statistical package (Ryan, Joiner and Ryan, 1985). The analyses took into account animals that died in the course of the study. Unless otherwise stated, the level of significance was $p < 0.05$.

4.1.3 RESULTS

4.1.3.1 Comparison of the Maasai Zebu, Orma Boran and Galana Boran in the high tsetse challenge area.

a) Weather

The mean monthly rainfall distribution and the variation in the mean daily maximum and minimum temperatures from August 1989 to October 1990 are shown in Figure 4.1. There was a total rainfall of 875 mm during the study period with peaks of 186 mm in December 1989 and 252 mm in March 1990, and a period of no rain between June and August 1990.

There were broadly three distinct periods; the first four months (August to November 1989) with light rainfall, followed by six months when most of the rain fell (December 1989 to May 1990), and a five months dry season (June to September 1990). The proportion of the total precipitation in each of these periods was 12%, 84% and 2%, respectively. The mean maximum temperatures ranged from 30.7 to 34.7^{o}C, while the minimum temperature from 20.8 to 23.9^{o}C.

b) Trypanosomiasis risk

The trypanosomiasis risk, assessed by the mean monthly apparent fly density and the disease incidence in the sentinel herd of Maasai Zebu cattle grazing alongside the experimental animals, is illustrated in Figure 4.2. The mean monthly disease incidence was obtained by calculating the average of the weekly incidence. As stated earlier, data collection on the disease incidence in the sentinel animals started in mid-February 1990.

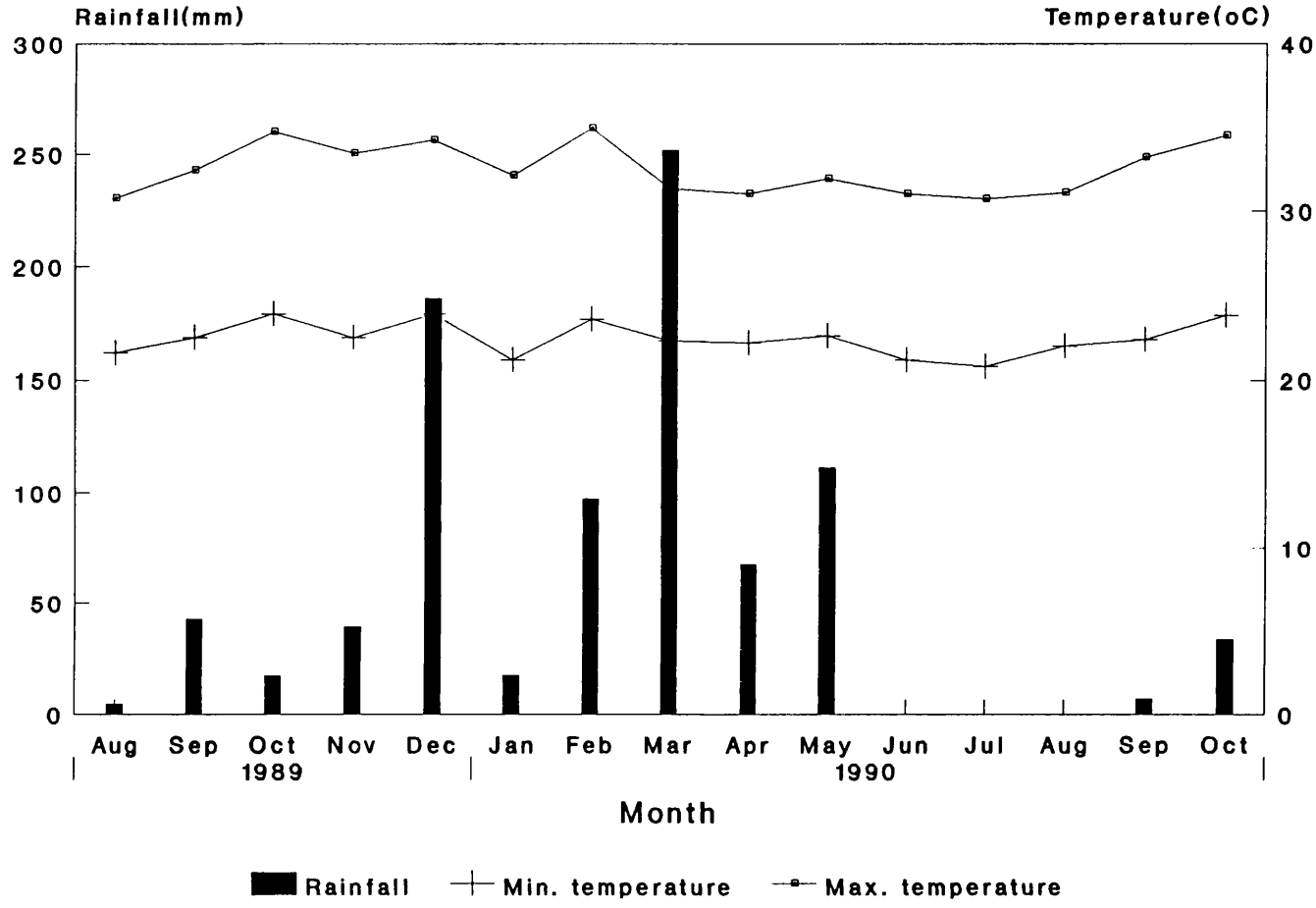


Figure 4.1. The mean maximum and minimum daily temparature (oC) and the monthly rainfall (mm) at Nguruman.

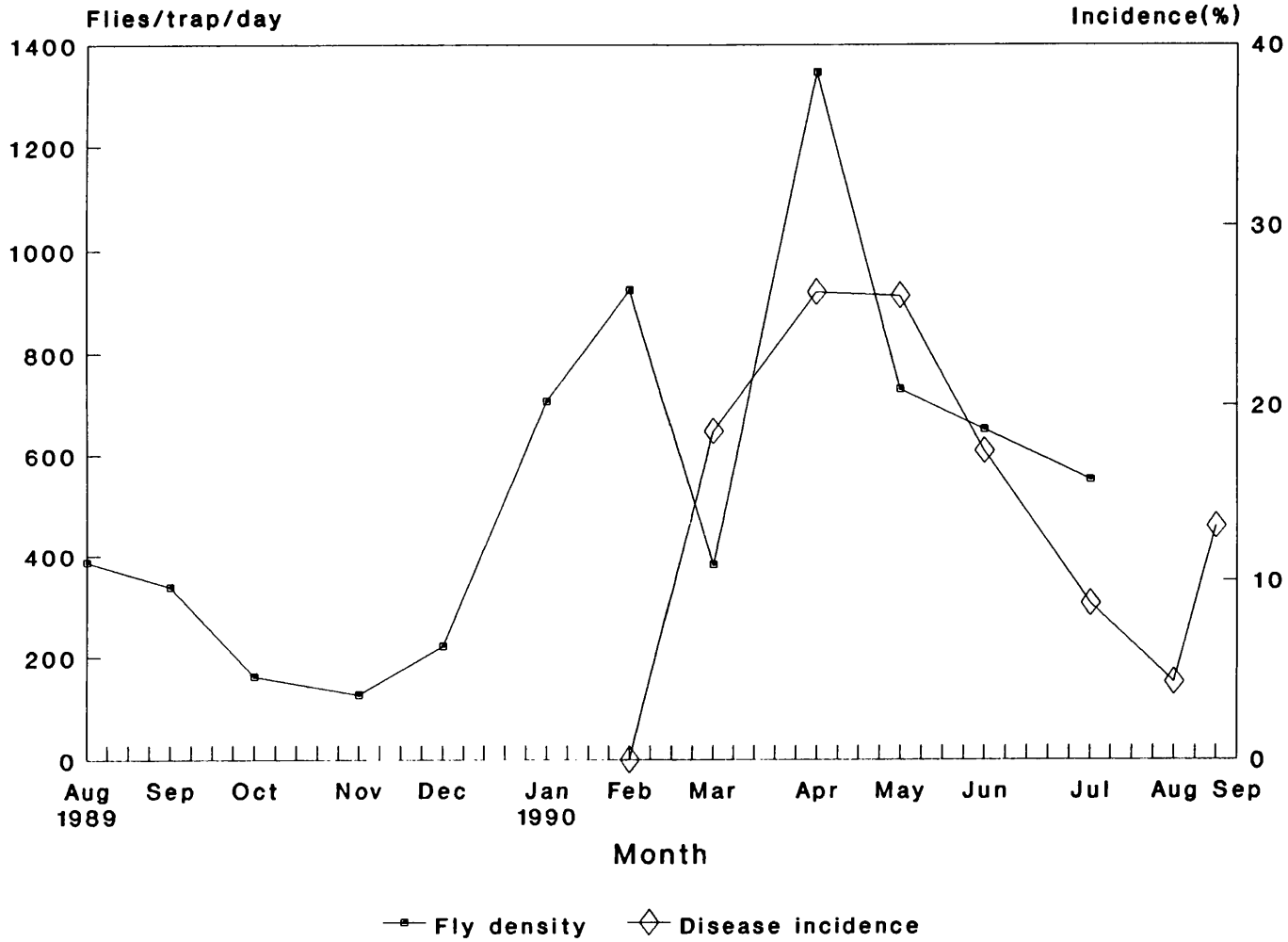


Figure 4.2. The mean monthly fly density and the incidence of trypanosomiasis in sentinel Maasai Zebu cattle in the high tsetse challenge area. The mean monthly incidence was obtained as an average of the weekly incidence.

The fly density was high with a mean of 558 flies/trap/day, a minimum of 161 in October 1989 and a maximum of 1345 in April 1990. The fly population, however, showed marked monthly fluctuation. The dominant fly species was *G. pallidipes* accounting for 99.3% of the total fly catches over the entire study period, while only 0.7% was due to *G. longipennis*.

The mean monthly disease incidence in the sentinel herd was 16.6%, with the highest levels (>30%) during the rainy season (April to June 1990) followed by a decline two months, after the rains in July 1990. Overall, the disease incidence was high during the rainy season and decreased with the onset of the dry season reaching minimum levels two months after the end of rains.

c) Pre-experimental samples

Blood samples collected from all the cattle at the beginning of the experiment (before they were split into the high and low challenge areas) were examined for parasites using the DG technique, while the serum was analysed for the antigens and antibodies by ELISA techniques as described in section 3.3. No parasites were detected on the buffy coat, while similar number of cases with antibodies were demonstrated in all the breeds (Table 4.3). Antigens were detected in fourteen Maasai Zebu, eight Orma Boran and four Galana Boran, with *T. congolense* being the dominant species in all the breeds. There were no differences among breeds in the buffy coat and antibody results, while it appears that, there was a higher proportion of the Maasai Zebu and Orma Boran with antigens than the Galana Boran.

Table 4.3

Number of positive cases detected after screening the pre-experimental blood and serum samples at Nguruman using the darkground/phase contrast buffy coat (DG), antibody and antigen ELISA techniques

Diagnostic technique	Trypanosome species identified	Maasai Zebu *N=44*	Orma Boran *N=43*	Galana Boran *N=19*
DG		-	-	-
Ab-ELISA		2	4	3
Ag-ELISA				
	Tc	8	4	3
	Tv	3	-	1
	Tb	1	-	-
	Tc/Tv	1	2	-
	Tc/Tb	1	2	-
	Tv/Tb	-	-	-
	Tv/Tc/Tb	-	-	-

N - Number animals examined

Tc - *T. congolense*

Tv - *T. vivax*

Tb - *T. brucei*

- = Negative sample

d) Trypanosomiasis

i) Disease incidence

The incidence was calculated as the proportion of the animals with new trypanosome infections detected by the darkground/phase contrast technique (DG) per week and for every month, an average for all weeks was obtained. The seasonal variation in the disease incidence is shown in Figure 4.3. The disease incidence was high during the first five months (September 1989 to January 1990); while it increased rapidly in all the breeds, it was higher in the Galana Boran, where over 20% of the animals were infected within the first month, and remained so. persistently higher than the other breeds. During this period, except in the month of November, the incidence in the Galana and Orma Borans was three and two times, respectively, that of the Maasai Zebu.

In the next five months (February to May 1990), a season characterized by heavy rainfall and an increase in the fly population, the incidence increased proportionally and was above 10% in all breeds except in Galana Boran for the month of March 1990. Similar maximum levels of 19%, 22%, and 23% in the Maasai Zebu, Orma and Galana Borans, respectively, were attained between April and May 1990.

With the onset of the dry season (June to September 1990) there was a decline in the incidence in all the breeds, which was lower in the Maasai Zebu than the Galana Boran for most of the time, while it remained intermediate in the Orma Boran.

The Galana Boran had higher disease incidence than the Maasai Zebu for the entire period (except in March 1990), while that of the Orma Boran was higher than the Maasai Zebu for a total of eight months. In the season with low

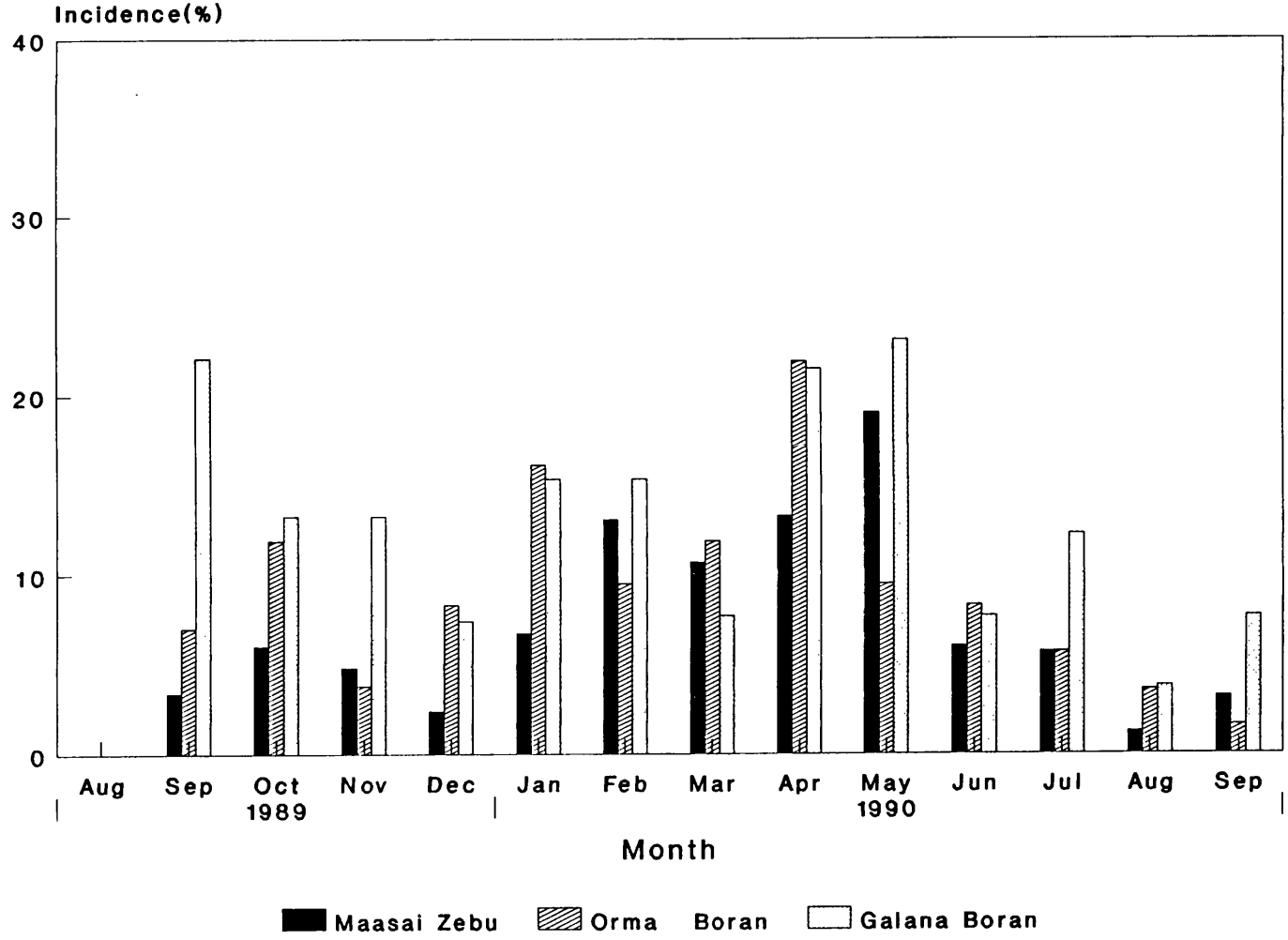


Figure 4.3. The mean monthly disease incidence in the three cattle breeds in the high tsetse challenge area at Nguruman.

to medium challenge, the incidence in Maasai Zebu and Orma Boran was lower than in the Galana Boran but during the high challenge, it increased and was similar in all breeds. Results of analysis of variance on the weekly disease incidence indicate that for the one year period, the Galana Boran had significantly higher disease incidence than the other breeds, while there was no significant difference between the Maasai Zebu and the Orma Boran (Table 4.4).

ii) Infections in individual animals

Table 4.5 shows the frequency of infections in the animals that survived up to the end of the study. During the one year period, all animals became infected. A total of 19 Maasai Zebu steers were infected less than five times compared to ten Orma and only two Galana Borans. There was a maximum of seven infections in two Maasai animals, nine in two Orma steers, while in the Galana Boran ten infections were recorded in two animals, and a maximum of fourteen in one steer.

The analysis of variance on the number of infections in the animals that survived up to the end of the study period in the three breeds are presented in Table 4.6. The infections per animal per year were significantly higher in the Galana Boran than the other breeds, being nearly twice the number in the Maasai Zebu.

iii) Intervals between infections

The intervals between subsequent infections in the three breeds are summarized in Table 4.7. On introduction into the high tsetse challenge, the mean duration

Table 4.4

The mean weekly disease incidence (% ± SD) of the Maasai Zebu, Orma Boran and the Galana Boran in the high tsetse challenge area at Nguruman

	Maasai Zebu	Orma Boran	Galana Boran
Observations	54	54	54
Mean	7.6 ± 8.1	9.6 ± 8.9	13.7 ± 10.6 *

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	520	7.7	sig.
Time	53	121.4	1.8	sig.
Error	106	67.9		

* Significantly higher than the Maasai Zebu and Orma Boran

Table 4.5

Frequency distribution of infections in animals that survived up to the end of the experiment

Number of infections	Frequency		
	Maasai Zebu	Orma Boran	Galana Boran
	N = 21	N = 21	N = 13
0	0	0	0
1	1	0	0
2	2	2	0
3	6	2	1
4	3	3	1
5	7	3	0
6	0	8	4
7	2	1	0
8	0	0	4
9	0	2	0
10	0	0	2
14	0	0	1

N - Number of animals that survived.

Table 4.6

The mean number of infections per animal per year (±SD), recorded in the animals that survived in the high challenge area at Nguruman

	Maasai Zebu	Orma Boran	Galana Boran
Observations	21	21	13
Mean	4.0 ± 1.5	5.2 ± 1.9	7.5 ±2.8 *

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	48.2	11.5	sig.
Error	52	4.2		

* Significantly higher than the Maasai Zebu and Orma Boran

Table 4.7

Mean interval in days (±SD) between infections

	Maasai Zebu		Orma Boran		Galana Boran	
	N	Mean	N	Mean	N	Mean
Duration to** first infection	21	103.3±63*	21	56.6±42.9*	19	21.8±15.4*
Intervals between other infections						
1 and 2	20	48.3±31.9	21	43.8±26.9	15	38.2±23.2
2 and 3	18	30.8±12.5	19	37.7±19.7*	13	24.1±6.5*
3 and 4	12	29.5±12.9	17	29.2±9.8	12	29.2±10.6
4 and 5	9	43.6±23.9	14	26.6±9.7m	11	27.6±7.2m
5 and 6	-	-	11	35.3±12.9	11	33.9±19.7
6 and 7	-	-	-	-	7	28±7.1
7 and 8	-	-	-	-	7	42.9±16.6

N - Number of observations

* Means within the row significantly different from each other.

** - One steer from each of the Maasai Zebu and Orma Boran groups died before they got trypanosome infections.

m - Means along the row significantly lower than in the Maasai Zebu.

- = Observations too few for analyses.

to first parasitologically detectable infection was significantly different among the three breeds, with the Maasai Zebu having longest (103 days), Orma Boran intermediate (57 days) and Galana Boran the shortest (22 days). The interval between the second and third infections was significantly longer in the Orma Boran than the Galana Boran, while that between the fourth and fifth infection was significantly longer in the Maasai Zebu than the other breeds. There were no differences in the intervals between any of the infections within the breeds.

iv) Duration of parasitaemia

Table 4.8 shows the mean duration in days that the cattle of each breed remained infected before the drop of PCV to ≤ 17% (thus the need for drug treatment) after the parasite detection on the buffy coat for each of the infections. Analysis was done up to the fifth infection in Maasai Zebu and seventh in Orma Boran as there were very few cases above these levels in these two breeds.

There were no significant differences in the duration animals remained infected in the first four infections among the three breeds, although it appeared longer in the Maasai Zebu, and to an extent in the Orma Boran. During the fifth infection, the Maasai Zebu were parasitaemic for longer period than both the Orma and the Galana Borans. No differences within the breeds were observed in the duration it took for the PCV to fall to ≤ 17% in all the infections.

v) Trypanosome species prevalence

The weekly trypanosome prevalence was obtained as the proportion of animals detected parasitaemic on the weekly sampling. The monthly prevalence was then obtained by calculating a mean of the weekly observations.

Table 4.8

Mean duration (days ± SD) after the detection of infection that the animals stayed before the PCV dropped to ≤ 17%, hence the need for drug treatment

	Maasai Zebu		Orma Boran		Galana Boran	
Infection	N	Mean	N	Mean	N	Mean
1	21	42 ± 62.2	21	36 ± 55.4	19	26 ± 44.7
2	20	29.7 ± 30.8	21	21.2 ± 32.4	15	29.2 ± 36.3
3	18	32.9 ± 30.9	19	28.9 ± 31.8	13	13.2 ± 10.8
4	12	26.3 ± 33.8	17	12 ± 13.3	12	14.6 ± 12.7
5	9	31.8 ± 31.6	14	14.1 ± 11.1[m]	11	11.1 ± 14.2[m]
6	-	-	11	21.2 ± 21.7	11	10.4 ± 11.6
7	-	-	-	-	7	6 ± 4.4
8	-	-	-	-	7	6.1 ± 8.2

N - Number of animals infected.

[m] Duration significantly shorter than in the Maasai Zebu along the row.

- = Observations too few for analyses.

Maasai Zebu

Figure 4.4 shows the seasonal variation in the prevalence of trypanosome species. The first two months had only *T. vivax* infections, with its peaks occurring later during the rainy season between February and March 1990, affecting over 20% of the herd. The *T. vivax* infections persisted up to June but fell for the rest of the year.

The prevalence of other species was low for the first six months, reaching only 3.8% for both *T. congolense* and mixed infections, and 2.9% for *T. brucei*, but increased in the rainy season between March and April 1990, to a maximum of 13% for *T. congolense*, 6% for *T. brucei* and 13% for mixed infections. Thereafter, the *T. congolense* infections remained persistently high for the rest of the study, while *T. brucei* and mixed infections dropped sharply after the rains. The prevalence of *T. congolense* exceeded that of *T. vivax* for a total of four months only in the entire period. The *T. brucei* and mixed infections were observed mainly during the rainy season with the peaks coinciding with the maximum rainfall in March.

Of the 199 infections detected, 89 (45%) were due to *T. vivax*, 54 (27%) *T. congolense* and the remainder due to *T. brucei* and mixed infections (Figure 4.5). *Trypanosoma vivax* was the predominant species in the Maasai Zebu, with a *vivax:congolense* ratio of 1.7.

The analysis of variance of the mean weekly prevalence of the various trypanosome species and a breakdown of the mixed infections in the three cattle breeds are summarized in Table 4.9. The prevalence of *T. vivax* was significantly higher than all the other species, while that of *T. congolense* was higher than *T. brucei* but similar to mixed infections. There were no differences between *T. brucei* and mixed infections. The *T. vivax/T. brucei* infections in the Maasai

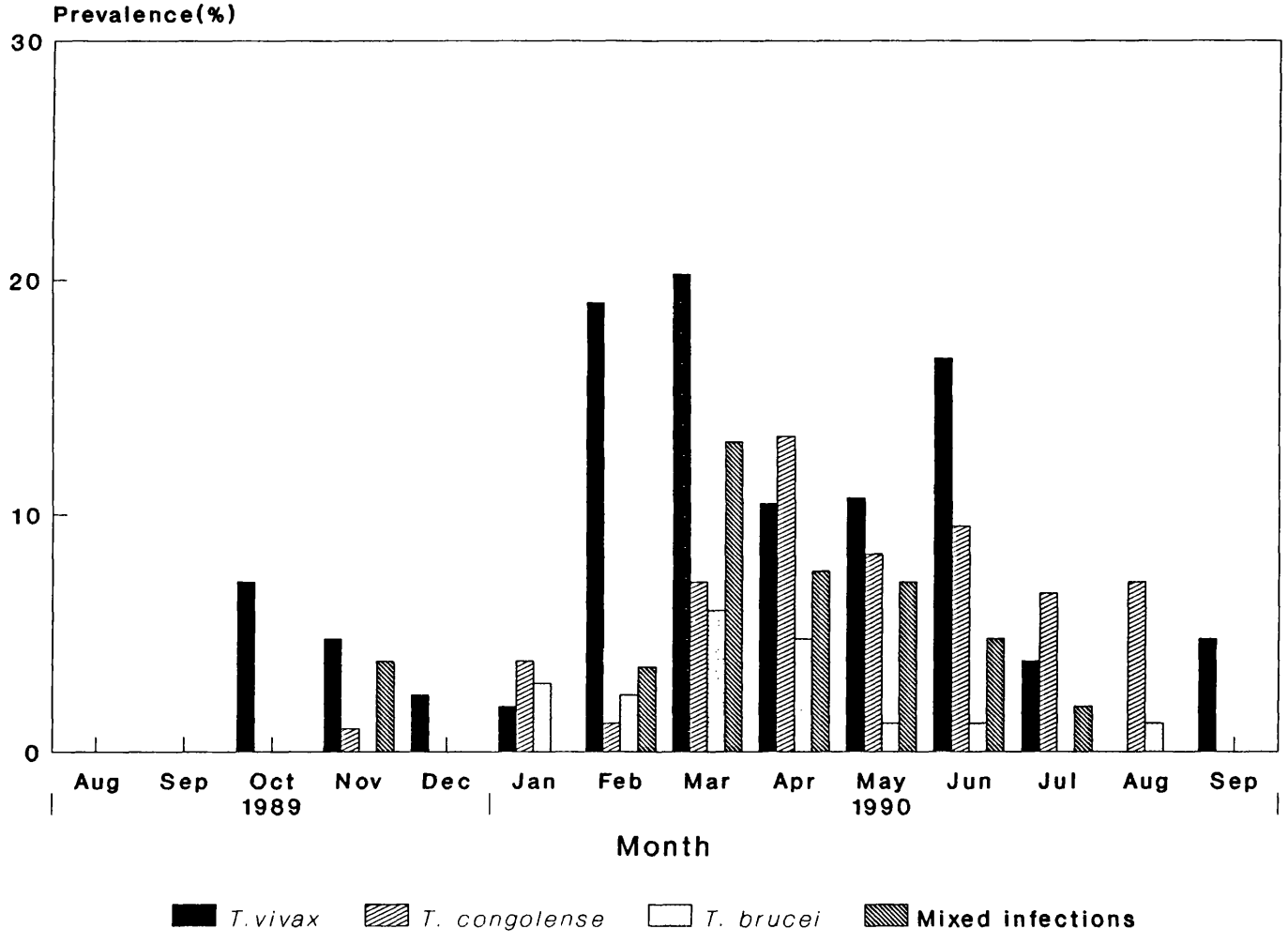


Figure 4.4. The mean monthly trypanosome prevalence (obtained as an average of the weekly prevalence) in the Maasai Zebu Steers.

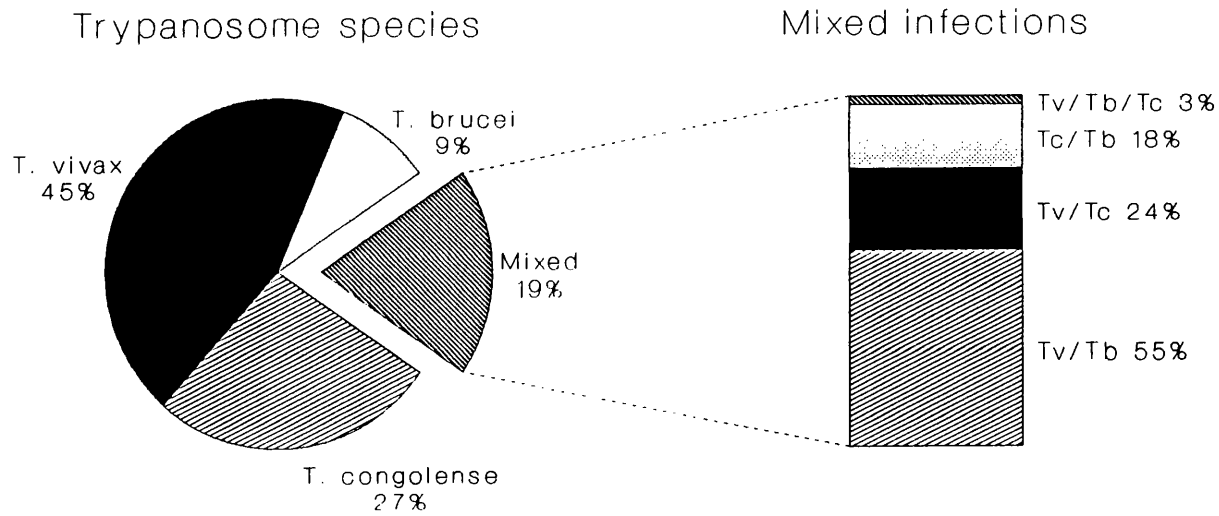


Figure 4.5. The proportion of infections due to the three trypanosome species in the Maasai Zebu

Table 4.9

Mean weekly prevalence (%) of the trypanosome species and mixed infections in the three breeds in the high challenge area at Nguruman

Trypanosome species	Breed		
	Maasai Zebu	Orma Boran	Galana Boran
T. vivax	7.7	8.9	9.6
T. congolense	4.7[v]	4.9[v]	7.8[o,m]
T. brucei	1.6[v,c]	1.1[v,c]	1.4[v,c]
Mixed	3.3[v]	2.2[v,c]	3.1[v,c]
Mixed Infections			
T. vivax/T. congolense	0.8	0.3	0.4
T. vivax/T. brucei	1.8*	0.9[m]	1.5
T. congolense/T. brucei	0.6	1.0	1.2

[v] Significantly different from *T. vivax* in the column.

[c] Significantly different from *T. congolense* in the column.

* Significantly higher than the other mixed infections in the column.

[o] Significantly different from the Orma Boran along the row.

[m] Significantly different from the Maasai Zebu along the row.

Zebu group were significantly higher than the *T. vivax/T. congolense* and *T. congolense/T. brucei*.

Orma Boran

The onset of trypanosome prevalence in the Orma Boran occurred earlier and reached higher levels than in the Maasai Zebu (Figure 4.6) and within the first month, over 7% of the herd became parasitaemic with *T. vivax*. In the first six months (September 1989 to February 1990), *T. vivax* infections were predominant reaching over 10%, while all other species were below 5%. The *T. vivax* infections continued to be high attaining a peak of 23% in May. There was a low prevalence of *T. congolense* for the first six months increasing later after the rains, to reach similar levels as *T. vivax* between March and June 1990, with a peak of 15%, and became the dominant species in the last three months. The prevalence of *T. brucei* was periodic with two peaks occurring during the short rains (December 1989 to January 1990) and another peak in the middle of the heavy rains in April 1990. Mixed infections occurred in low numbers during the first five months but increased during the rainy season, and were higher than the other species in April 1990. Both *T. brucei* and mixed infections declined rapidly after the rains. Figure 4.7 shows the proportion of infections due to the various species. A total of 197 infections were detected, consisting of 103 (52%) *T. vivax*, 56 (28%) *T. congolense*, 13 (7%) *T. brucei* and 25 (13%) mixed infections. The number of *T. vivax* infections was almost double that of *T. congolense*, with a *vivax:congolense* ratio of 1.9.

The prevalence of *T. vivax* was significantly higher than all the other species (Table 4.9). *T. congolense* was higher than *T. brucei* and mixed infections, while there were no differences between the latter. There were no differences

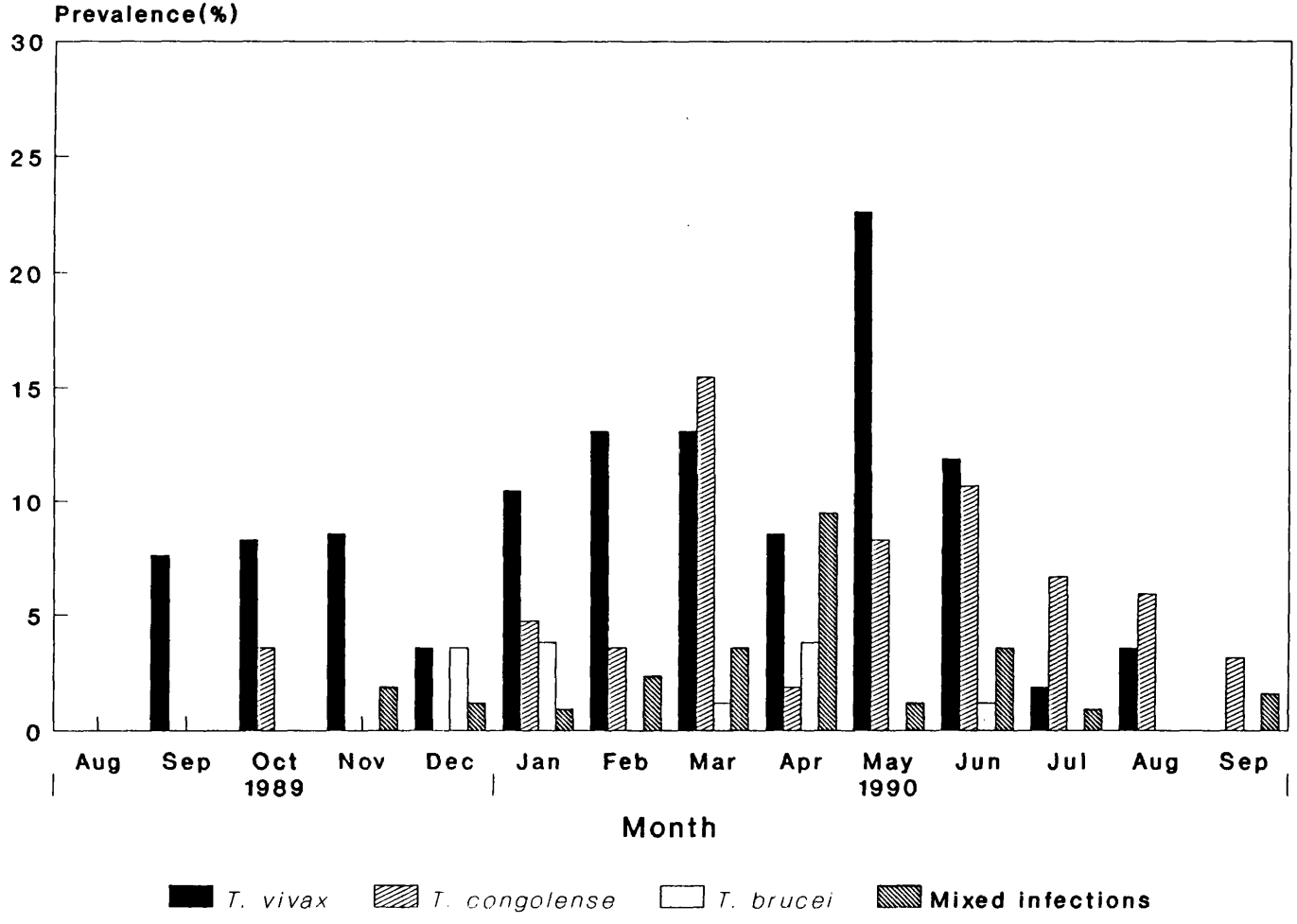


Figure 4.6. The mean monthly trypanosome prevalence in the Orma Boran.

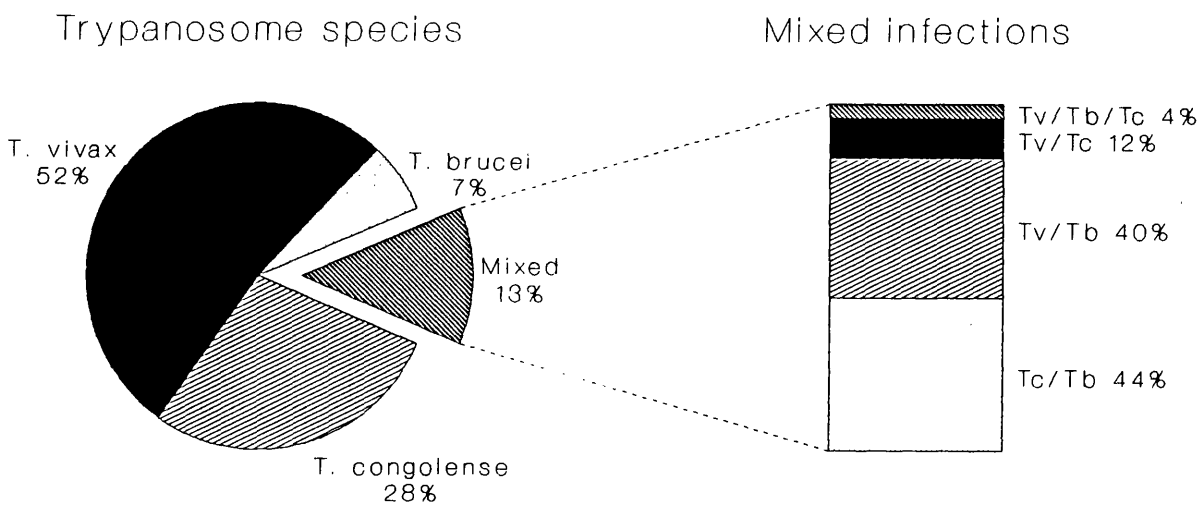


Figure 4.7. The proportion of infections in the Orma Boran due to the three trypanosome species.

among the three types of mixed infections.

Galana Boran

In the Galana Boran, the trypanosome prevalence occurred earlier, with infections being detected as early as the second week, and reaching higher levels than the other breeds (Figure 4.8). This group experienced a heavy *T. vivax* challenge for the first six months from September 1989 to February 1990. By the end of the first month, over 11% of the herd had *T. vivax* infections and this progressed to reach its highest level of 22% in October 1989. The prevalence of *T. congolense* was low in the first four months but increased progressively after the rains and remained persistently high for the rest of the time becoming the dominant species in the last four months, rising to a maximum level of 21% at the end of the experiment in September 1990. Mixed infections and *T. brucei* were more prevalent in the rainy season and reached their maximum levels of 14% in December 1989 and 5% in January 1990, respectively.

Unlike in the other breeds, there were three clear phases in the Galana Boran; the first phase from September to November 1989, which was a period of mainly *T. vivax* challenge, followed by second phase from December 1989 to May 1990 during the rainy season, when there was a similar prevalence of both *T. vivax* and *T. congolense*, and a high number of both *T. brucei* and mixed infections, and a third phase from June to September 1990, when the animals were predominantly under *T. congolense* challenge.

Over the study period, the prevalence of *T. vivax* was not significantly greater than *T. congolense*, while both were greater than *T. brucei* and mixed infections (Table 4.9). There were no differences between *T. brucei* and mixed infections. The Galana Boran experienced similar levels of *T. vivax* and

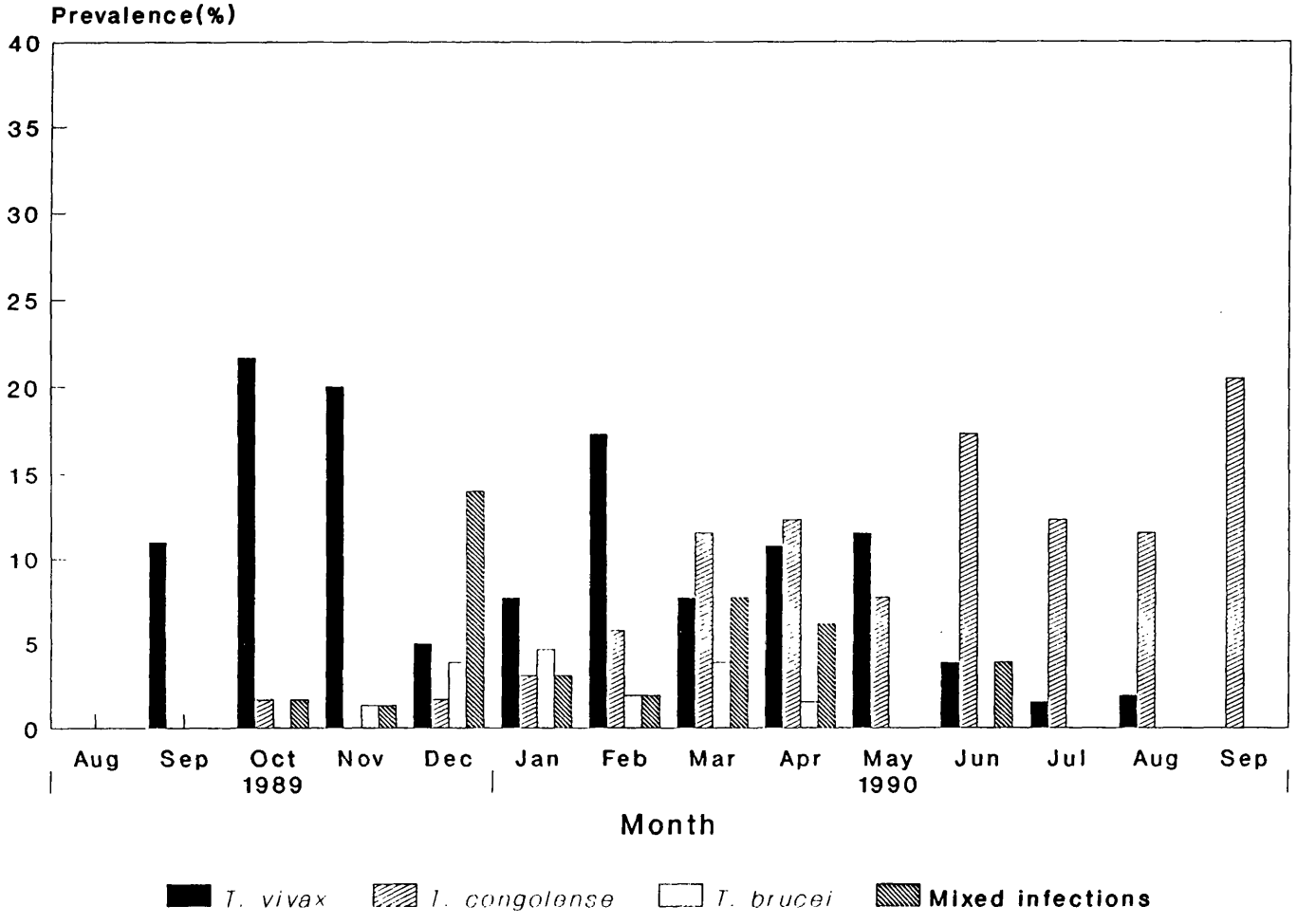


Figure 4.8. The mean monthly trypanosome prevalence in the Galana Boran.

T. congolense challenge but at different times of the year. There were no significant differences in the prevalence of various types of mixed infections encountered.

The relative frequencies infections due to the various species are presented in Figure 4.9. Of 164 infections detected 75 (46%) were *T. vivax*, 56 (34%) *T. congolense*, 10 (6%) *T. brucei* and 23 (14%) mixed infections. There were more *T. vivax* than *T. congolense*, with *vivax:congolense* ratio of 1.3.

Trypanosome species prevalence among the three breeds

There was no difference in the prevalence of *T. vivax* and *T. brucei* among the breeds (Table 4.9). *Trypanosoma congolense* had a significantly higher prevalence in the Galana Boran than the Orma Boran and Maasai Zebu, but was similar in the latter two breeds. There were no differences in the overall prevalence of mixed infections consisting of either *T. vivax/T. congolense*, or *T. congolense/T. brucei* among the breeds but, *T. vivax/T. brucei* infections were significantly more in the Maasai Zebu than Orma Boran.

vi) Intensity of parasitaemia

As described earlier, parasitaemias resulting from the trypanosome infections were identified by the causal trypanosome species and categorized for intensity of infection using a parasitaemia score, ranging from 1+ for the lowest to 6+ for the highest. The percentages from each breed falling within the low (1-2), medium (3-4) and high (4-6) parasitaemia score classes are shown in Table 4.10.

There were very similar proportions of *T. congolense* and mixed infections in the low and medium score class in the three breeds. In addition, there were more infections in the medium score for *T. congolense* and mixed infections than

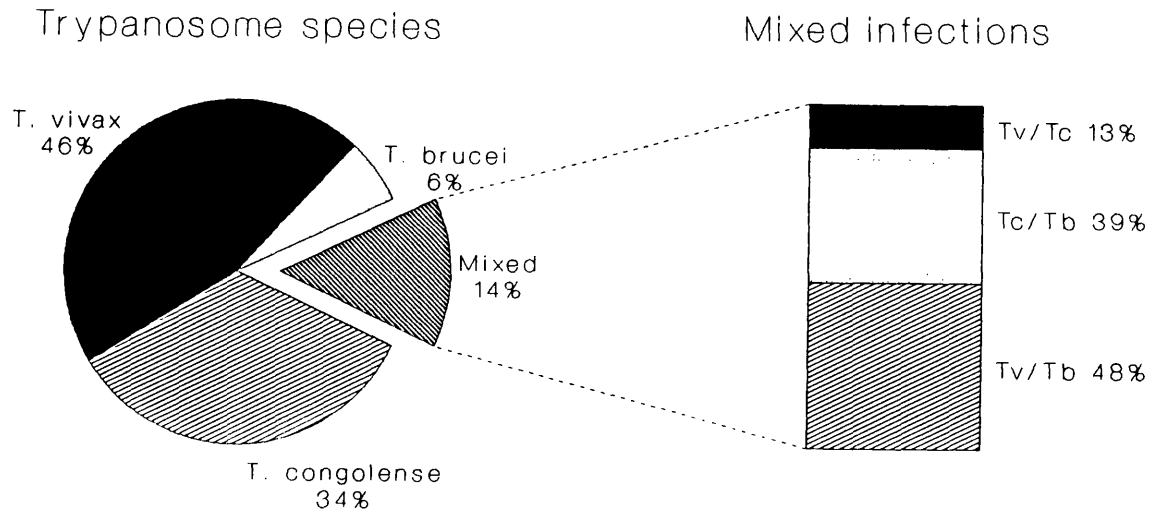


Figure 4.9. The proportion of infections in the Galana Boran due to three trypanosome species.

Table 4.10

Relative frequencies (%) of parasitaemia scores of three trypanosome species in the three breeds of cattle in the high challenge area

Trypanosome species	Breed	No. of infections	Parasitaemia score class		
			1-2	3-4	5-6
T. vivax	Maasai Zebu	103	71	27	2
	Orma Boran	101	70	26	4
	Galana Boran	73	78	19	3
T. congolense	Maasai Zebu	52	50	50	0
	Orma Boran	55	42	53	5
	Galana Boran	53	55	43	2
T. brucei	Maasai Zebu	16	88	13	0
	Orma Boran	12	100	0	0
	Galana Boran	8	75	25	0
Mixed infections	Maasai Zebu	28	43	54	4
	Orma Boran	28	43	57	0
	Galana Boran	18	50	50	0

for *T. vivax* and *T. brucei* in all the breeds. Majority of the *T. vivax* infections were in the low parasitaemia score class. All *T. brucei* infections had either low or medium scores, while there were none in the high score class. Similarly, there were no *T. congolense* with high score parasitaemia in the Maasai Zebu. There was no evidence of important breed effects in the intensity of parasitaemia in any of the trypanosome species.

vii) Treatment requirements

Trypanocidal drug treatment was only administered to parasitaemic animals after the PCV dropped to the critical value of 17% or less. This allowed for time to monitor the ability of the parasitaemic animals to withstand trypanosome infections. The monthly herd treatment requirements displayed a similar seasonal pattern to the disease incidence (Figure 4.10).

No treatments were needed in the Maasai Zebu during the first month compared to 2.4% and 11% in the Orma and Galana Boran herds, respectively. In the next six months (October 1989 to March 1990), treatments increased in the order Maasai Zebu, Orma and Galana Borans. For most of this period the treatments needed in the Galana Boran herd were more than twice those of the Maasai Zebu. Maximum treatments were required in March 1990 in the Galana Boran (23%) and Orma Boran (19%), and a month later (April 1990) in the Maasai Zebu (17%) and this coincided with high disease incidence season. After April, there was a steady decline in the treatments in all the herds but again the requirement was clearly lower in the Maasai Zebu than the Galana Boran.

Results of the analysis on the weekly treatments for the entire period indicated that, the Galana Boran herd needed significantly more treatments than the other breeds (Table 4.11). In comparison with the Maasai Zebu, more than

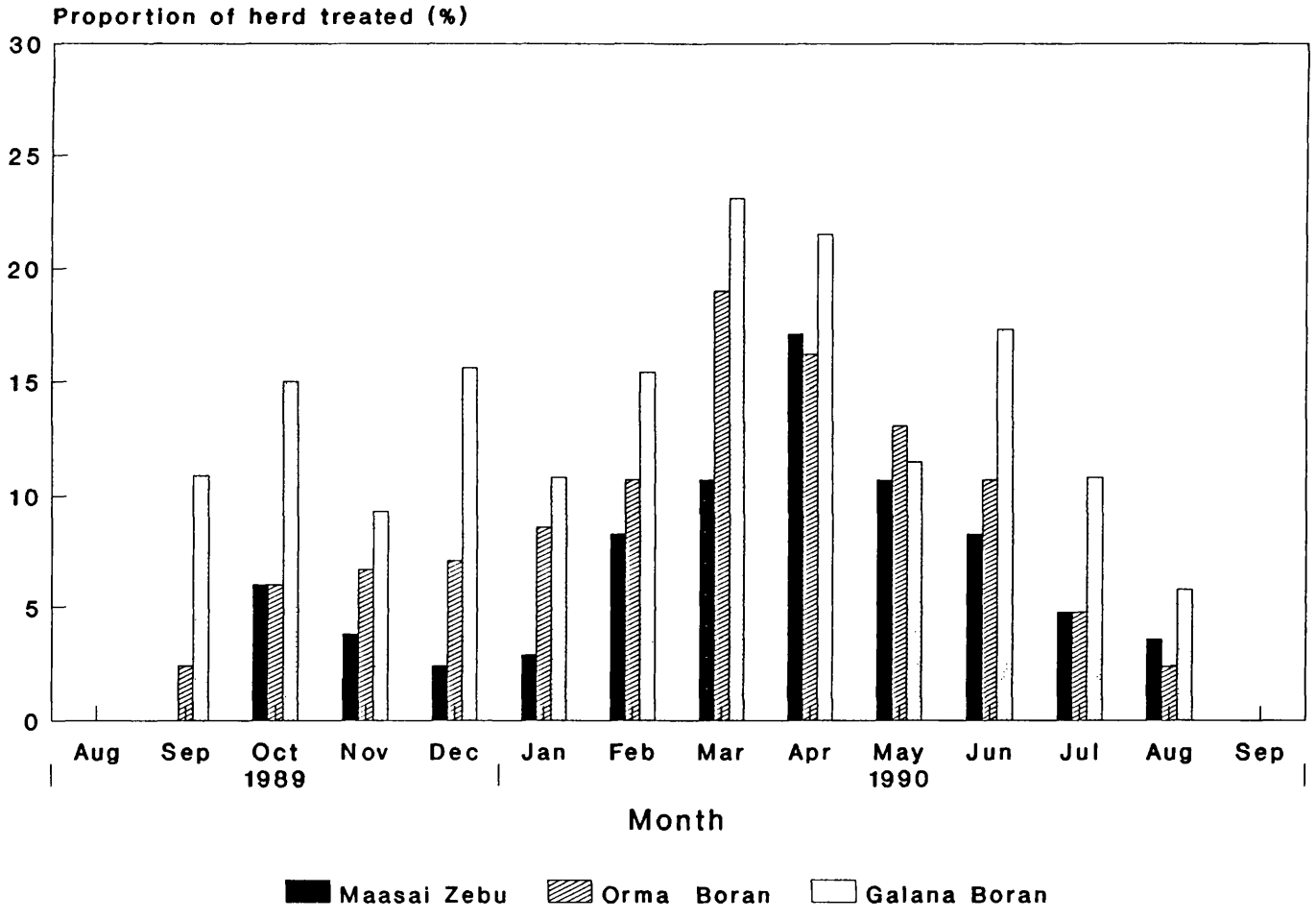


Figure 4.10. The mean monthly herd treatments in the three cattle breeds in the high tsetse challenge area.

Table 4.11

The mean weekly herd drug treatments (% ± SD) in the Maasai Zebu, Orma Boran and the Galana Boran in the high tsetse challenge area at Nguruman

	Maasai Zebu	Orma Boran	Galana Boran
Observations	54	54	54
Mean	6.4 ± 6.7	8.6 ± 7.9	13.3 ± 10.9*

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	686	14.9	sig.
Time	53	132.0	2.9	sig.
Error	106	46.1		

* Significantly higher treatments than the Maasai Zebu and Orma Boran

twice the number of treatments were required for nine months (75% of the time) in the Galana Boran, and for three months (25% of the time) in the Orma Boran (Figure 4.10).

Treatments in animals that survived up to end of the study

One Maasai Zebu steer required no treatment during the period of study, while eighteen steers (86%) needed less than five treatments (Table 4.12). In the Orma Boran, 13 (62%) animals had less than five treatments with a maximum of nine treatments in one case. In the Galana Boran, eleven of the animals (84.6%) needed more than five treatments with a maximum of fourteen in one case, while only two (15.5%) animals needed less than five treatments.

Table 4.13 shows mean number diminazene aceturate (Berenil^{R}, Hoechst) treatments in the animals that survived up to the end of the study period. The Galana Boran required 7.2 treatments/animal/year compared to 4.9 and 3.4 in the Orma Boran and Maasai Zebu, respectively. The treatments in the Galana Boran were significantly higher than in the Orma Boran and Maasai Zebu, while there were no differences between the latter. The Galana Boran required more than twice the number of treatments needed by the Maasai Zebu, while those of the Orma Boran were intermediate.

Self cure

In all the breeds, a self cure phenomenon was observed. This referred to cases where animals were detected parasitaemic but consequently, the parasites were seen for only a brief period or rarely, developed no obvious clinical signs, and the PCV never dropped to the critical value of ≤17%, hence needed no treatment. Such cases were considered to have undergone spontaneous recovery.

Table 4.12

Frequency distribution of treatments in animals that survived up to the end of the experiment

Number of treatments	Frequency		
	Maasai Zebu	Orma Boran	Galana Boran
	N = 21	N = 21	N = 13
0	1	0	0
1	1	1	0
2	4	3	0
3	5	0	1
4	6	3	1
5	2	6	0
6	0	6	4
7	2	0	2
8	0	1	2
9	0	1	1
10	0	0	1
14	0	0	1

N - Number of animals that survived.

Table 4.13

The mean number of drug treatments per animal per year (±SD) required by the animals that survived in the high challenge area at Nguruman

	Maasai Zebu	Orma Boran	Galana Boran
Observations	21	21	13
Mean	3.4 ± 1.7	4.9 ± 2.0	7.2 ± 2.8*

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	58.0	13.2	sig.
Error	52	4.4		

* Significantly more treatments than the Maasai Zebu and Orma Boran.

Self cure occurred in 11 (13%), 5 (4.5%) and 2 (2.1%) of the total infections in the Maasai Zebu, Orma and Galana Borans, respectively (Table 4.14). Most of the infections which self cured in the Maasai Zebu and Orma Boran were *T. vivax*. The cases of self cure in the Maasai Zebu were significantly higher than in the other breeds.

viii) Anaemia

The seasonal variation of the mean weekly PCV of the three cattle breeds is shown in Figure 4.11, and it can be classified into four phases. The first phase covering the first three months (September to November 1989) was characterized by an initial drop in the mean PCV in all the breeds to values ≤25% in the Galana and Orma Boran, but not below 26.2% in the Maasai Zebu. In the second phase, from December 1989 to February 1990, the PCV in the Galana and Orma Borans stabilized around 25%, exceeding this value for only one and four weeks (10% and 40% of the time), respectively, while in the Maasai Zebu, there were some weekly fluctuations but it remained above 26%. The third phase, lasting for three and a half months (March to May 1990), showed a marked PCV drop in all breeds to ≤25%. Minimum mean values of 20.1%, 21.4%, and 21.6% in the Galana Boran, Orma Boran and Maasai Zebu, respectively, were attained in this period. The fourth phase (from June 1990 to the end) was characterized by recovery of the PCV to ≥25% in the three breeds. This recovery was rapid in the Maasai Zebu taking one week only, while it took over a month longer (five weeks) in the Orma Boran. In the Galana Boran, it was transiently above 25% for one week only in July 1990 but never exceeded this value again.

Table 4.14

The number of cases with the self cure phenomenon in the three cattle breeds in the high challenge area

	Maasai Zebu	Orma Boran	Galana Boran
Total number of infections detected	84	110	97
Self cure			
T. vivax	9	4	1
T. congolense	-	1	-
T. brucei	-	-	1
Mixed infections	2	-	-
Total	11(13%)*	5(4.5%)	2(2.1%)

- = No cases of self cure

* Significantly more than the other breeds.

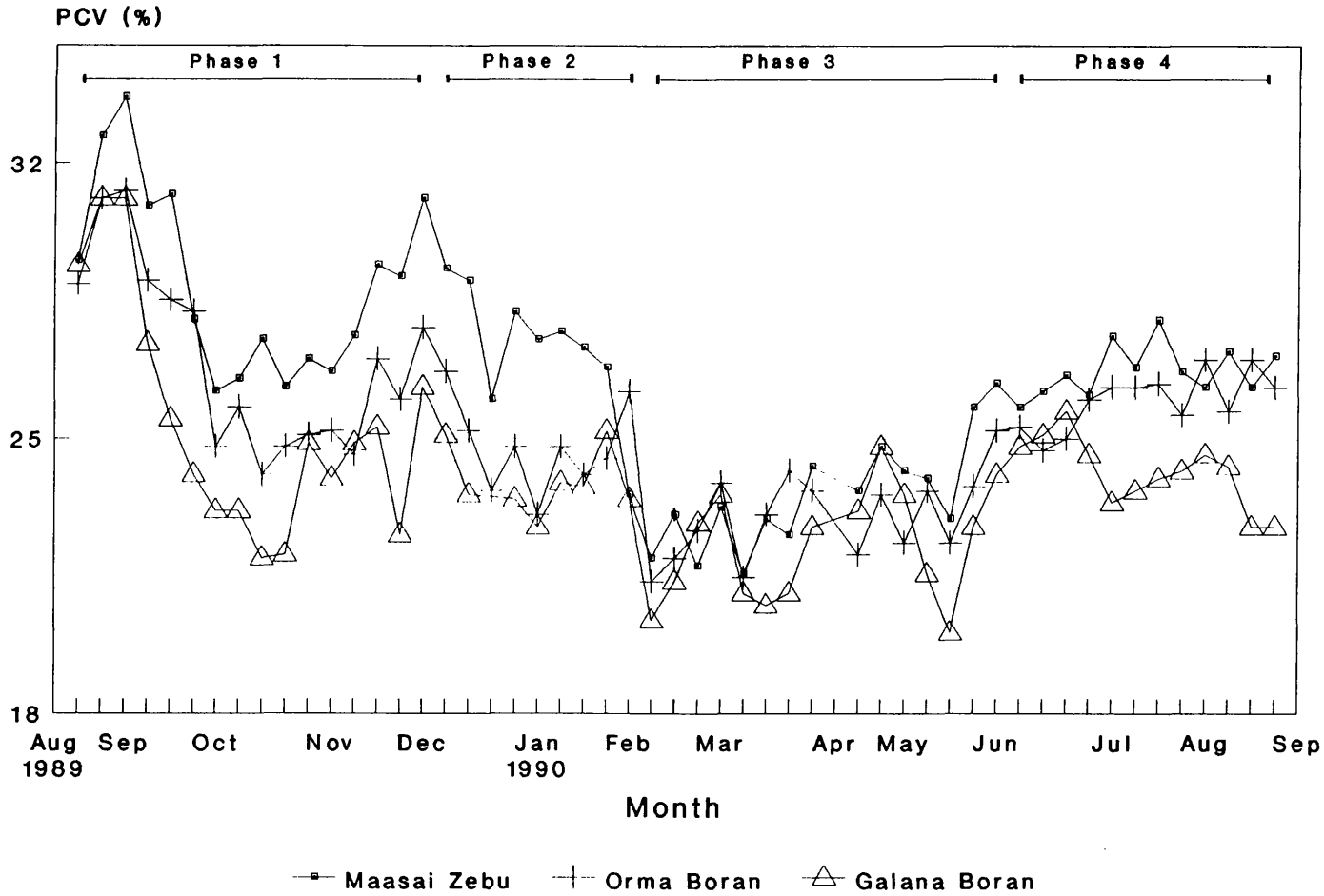


Figure 4.11. The mean weekly packed cell volume (PCV) of the three cattle breeds in the high tsetse challenge area.

In the entire observation period, the PCV was ≥25% for 41, 21 and 9 weeks (76%, 39% and 17% of the time) in the Maasai Zebu, Orma and Galana Borans, respectively. Analysis of the mean weekly PCV, presented in Table 4.15, indicated that the breeds were significantly different from each other, with the Maasai Zebu having the highest, the Orma Boran intermediate, while the Galana Boran had the lowest.

e) Performance

i) Growth rates

The steers from the three cattle breeds did not have the same average weight at the start of the experiment. Therefore, the growth rate was obtained by expressing the changes in weight as percentages of the original body weight as described in section 3.3.

The body weight changes occurred in two phases (Figure 4.12). During the first phase lasting five and a half months (September 1989 to mid-February 1990), there were periods of weight loss in the three breeds which appeared longer and more severe in the Galana Boran. By the end of this period, the changes in weight gains appeared to be insignificant. In the second phase beginning from mid-March 1990, all breeds had progressive increase in the weight gains, reaching 21.5% in Galana Boran, 32.4% in Orma Boran, and 33.6% in the Maasai Zebu by the end of the study period.

Significant differences in weight gains occurred between the Maasai Zebu and Galana Boran, while the Orma Boran was intermediate and not significantly different from the others (Table 4.16). The growth rate in the Maasai Zebu was more than twice that of the Galana Boran.

Table 4.15

The mean weekly Packed Cell Volume (% ± SD) of the Maasai Zebu, Orma Boran and the Galana Boran in the high tsetse challenge area at Nguruman

	Maasai Zebu	Orma Boran	Galana Boran
Observations	1095	1090	698
Mean	26.6 ± 2.8*	25.2 ± 2.2*	24.0 ± 2.2*

Analyses of variance

Source	df	ms	f	p < 0.05
Group	2	1689.5	9.9	sig.
Error	60	171.1		
Time	51	271.1	22.1	sig.
Time x group	101	19.5	1.6	sig.
Error	2668	12.3		

* Means significantly different from each other.

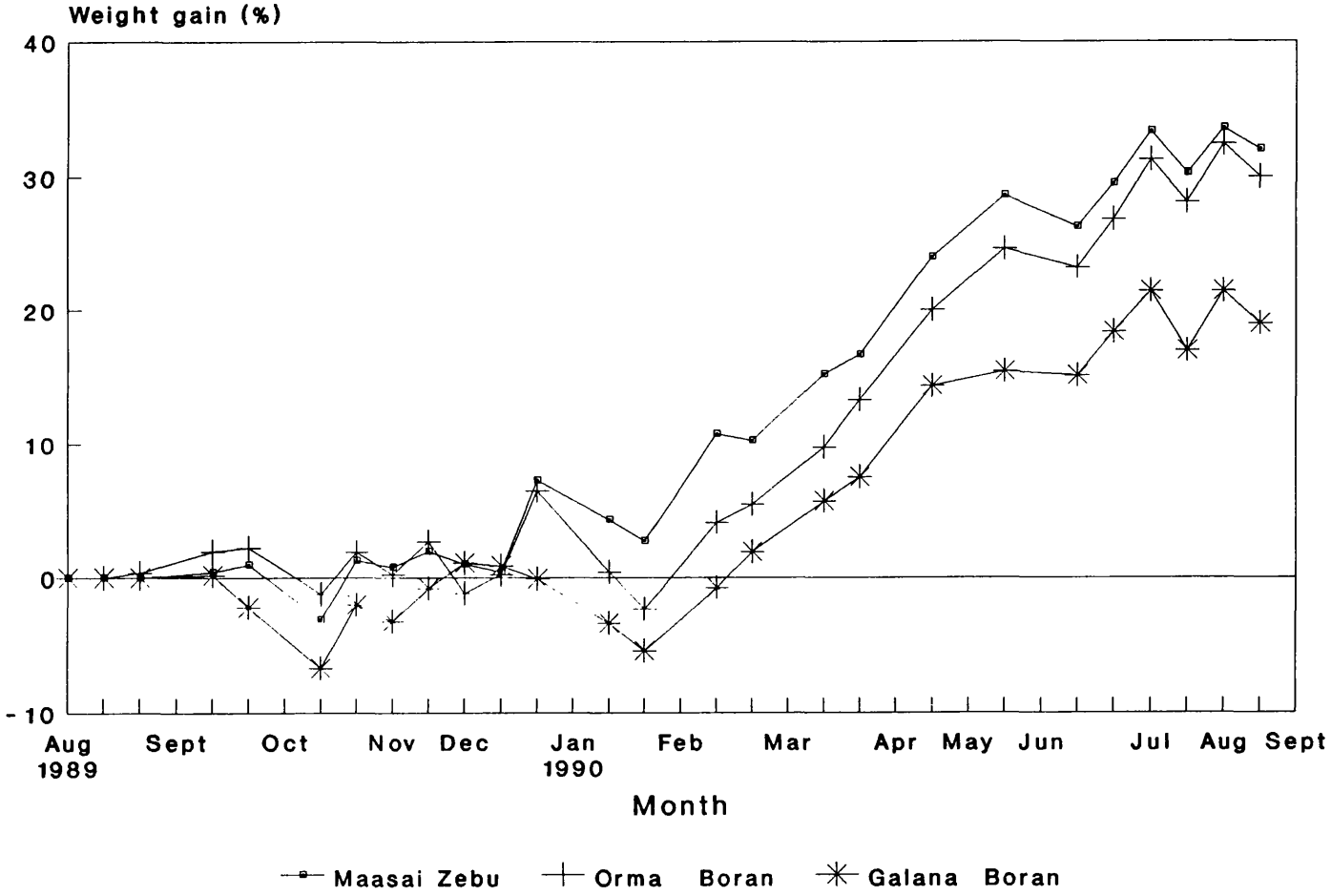


Figure 4.12. The mean fortnightly body weight gains in the three cattle breeds in the high challenge area.

Table 4.16

The mean percentage monthly body weight gains (% ± SD)) of the Maasai Zebu, Orma Boran and the Galana Boran in the high tsetse challenge area at Nguruman

	Maasai Zebu	Orma Boran	Galana Boran
Observations	478	478	294
Mean	11.7 ± 13.2	9.7 ± 12.4	4.2 ± 9.3*

Analyses of variance

Source	df	ms	f	p < 0.05
Group	2	5074.5	3.3	sig.
Error	55	1549.2		
Time	22	7884.6	251.8	sig.
Time x group	43	101.1	3.2	sig.
Error	1127	31.3		

* Weight gain significantly lower than the Maasai Zebu.

ii) Body condition score

The seasonal variations in the body condition scores are illustrated in Figure 4.13. The Orma and Galana Borans showed a loss in the body condition by the second month, while in the Maasai Zebu, the loss was noticeable by the third month. In all the breeds, the body condition had become worse by the end of the rainy season (June) but improved towards the end of study (August to September 1990) when they recovered to the pre-experimental values. There were no significant differences in the mean monthly body condition scores among the three breeds (Table 4.17).

4.1.3.2 Comparison of the Maasai Zebu and Orma Boran in the low tsetse challenge area.

a) Weather

The weather changes seen in this study area were as described in section 4.1.3.1 and illustrated in Figure 4.1.

b) Trypanosomiasis risk

Figure 4.14 shows the mean monthly apparent fly density and the disease incidence in a sentinel herd of Maasai Zebu cattle kept in the low challenge area. The mean fly density was 9 flies/trap/day with a minimum of 1 in September 1989, and a maximum of 30 flies/trap/day in April 1990. The mean monthly disease incidence was 5.7% with a peak of 18.7% in February 1990 and minimum of 0% in September 1989 and August 1990. For the whole period, the disease incidence was less than 5% for eight months, indicating that, the challenge in this

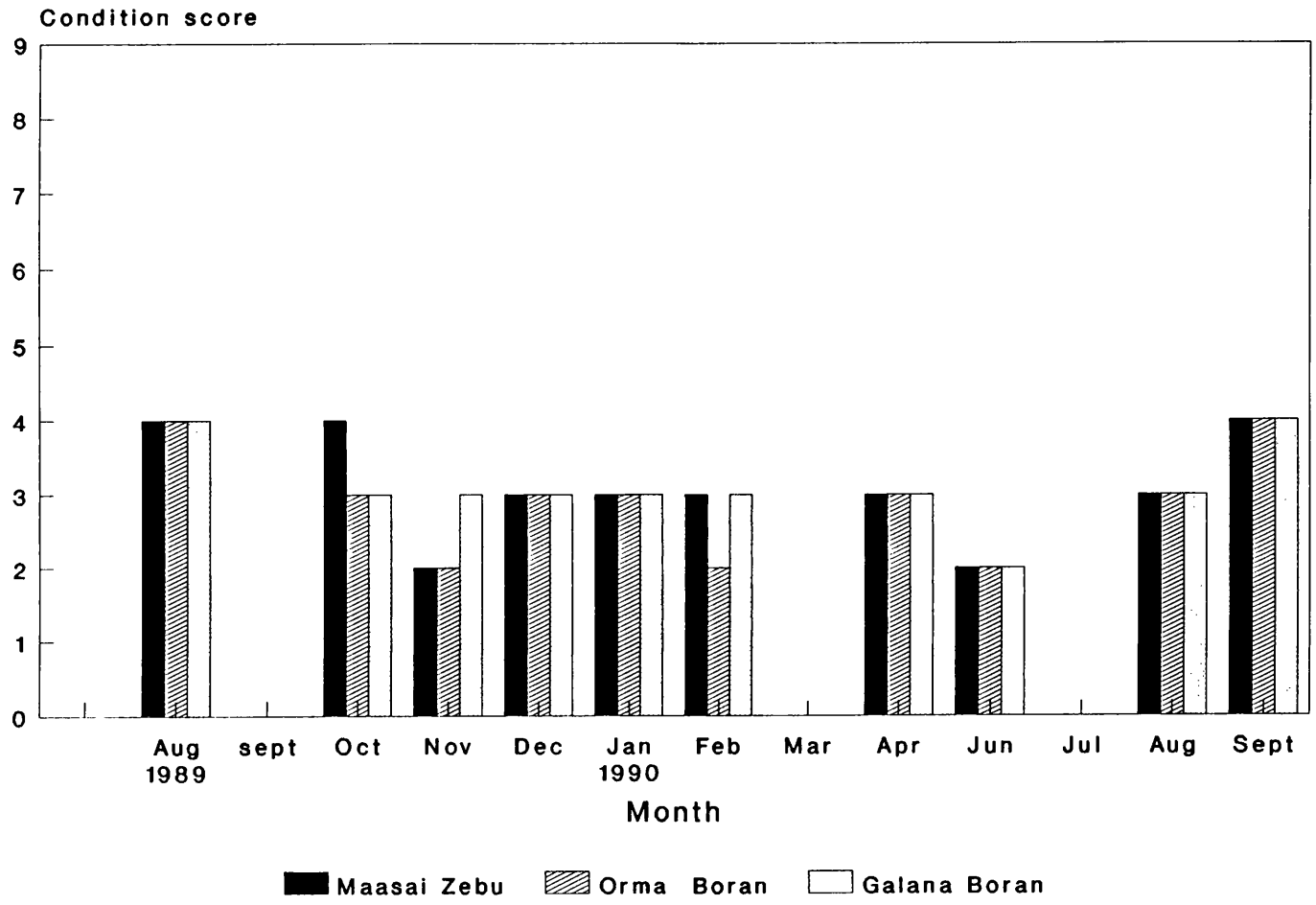


Figure 4.13. Changes in the mean body condition score of three cattle breeds in the high tsetse challenge area.

Table 4.17

The mean monthly body condition scores of the Maasai Zebu, Orma Boran and the Galana Boran in the high tsetse challenge area at Nguruman

	Maasai Zebu	Orma Boran	Galana Boran
Observations	185	161	108
Mean*	3	3	3

Analyses of variance

Source	df	ms	f	p < 0.05
Group	2	1.5	0.5	not sig.
Error	54	3.1		
Time	9	13.4	34.8	sig.
Time x group	15	0.5	1.3	not sig.
Error	399	0.4		

* No significant differences among the breeds.

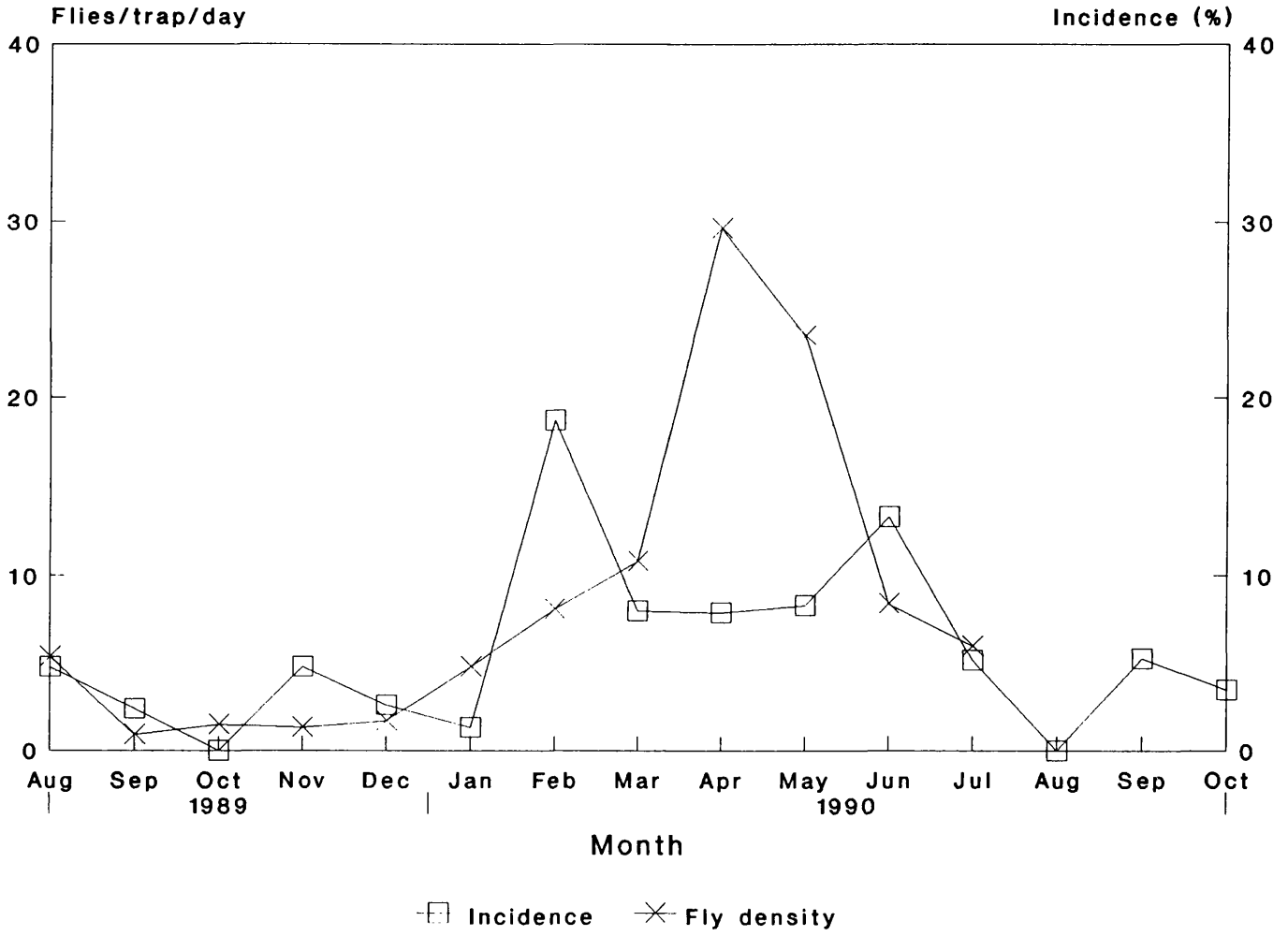


Figure 4.14. The mean monthly fly density and disease incidence in sentinel Maasai Zebu cattle in the low tsetse challenge area at Nguruman.

area was relatively low compared to that in the high challenge area described in the previous section.

c) Trypanosomiasis

The results on the pre-experimental screening of the animals has already been discussed in section 4.1.3.1

i) Disease incidence

Ten Maasai Zebu and five Orma Boran were infected. Both groups exhibited long mean periods of over four months prior to the detection of the first infection (Table 4.18). Though the period appeared longer in the Maasai Zebu, there was a big standard deviation in the Orma Boran and hence the differences were not significant.

Maasai Zebu

A total of eleven infections were recorded in this group (Table 4.18). The first infection occurred within the first month, while the rest appeared after four months (Figure 4.15). One steer was infected twice with an interval of nine months between the two infections.

Orma Boran

Five infections were detected (Table. 4.18) two of which occurred in the first four months, while the other three were recorded in the remaining period (Figure 4.15). In both the groups majority of cases were recorded following the high rainfall peaks in the period from December 1989 to May 1990, which also coincided with an increase in fly population. Once infected, there were no

Table 4.18

The mean duration in days (±SD) to the first infection and that infected animals stayed before PCV dropped to ≤ 17% in the low challenge area

	Maasai Zebu	Orma Boran
	N = 21	N = 21
Number of animals infected	10	5
Total infections detected	11*	5
Mean duration to the first infection	200.6 ± 81.4	142.2 ± 109.1
Duration infected animals stayed before PCV dropped to ≤ 17%	26.2 ± 15.5	87.5 ± 116.9

N - Total number of animals.

* One Maasai Zebu steer was infected twice.

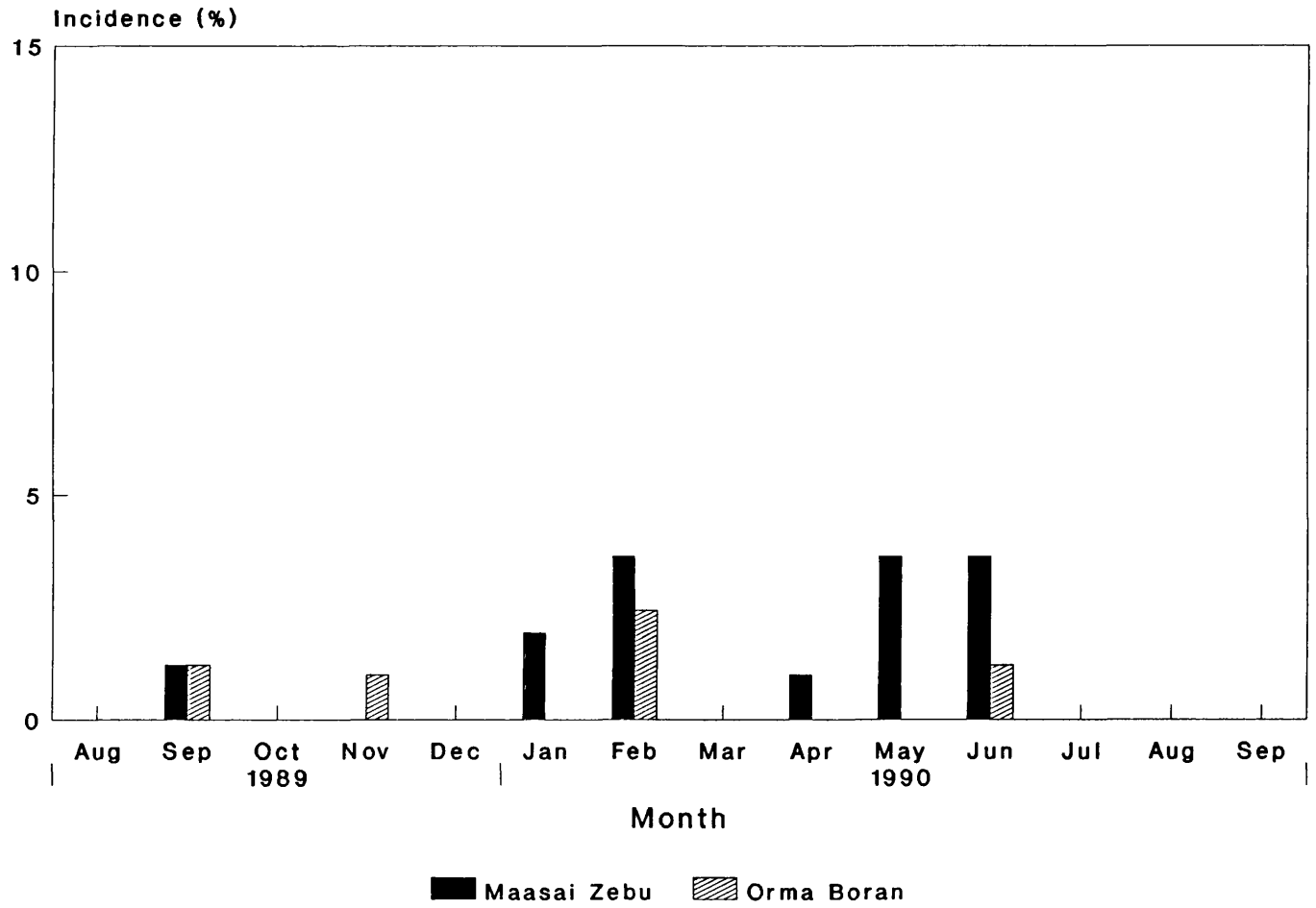


Figure 4.15. The mean monthly disease incidence in the Maasai Zebu and Orma Boran in the low tsetse challenge area.

significant differences between the two breeds in the mean duration that the animals remained parasitaemic before the PCV dropped to below the critical level of 17% (Table 4.18). The duration appears longer in the Orma Boran, but again, due to the a big standard deviation, there were no significant differences.

As shown on Table 4.19, there were no differences in the disease incidence between the two breeds.

ii) Trypanosome species prevalence

In the Maasai Zebu, the eleven infections detected consisted of six *T. vivax*, four *T. congolense* and one mixed infection with *T. congolense/T. brucei*, while in the Orma Boran, the five infections consisted of three *T. vivax* and two *T. congolense* (Table 4.20).

iii) Treatment requirements

Only one Maasai Zebu steer required treatment during the first five months, while the rest of the treatments occurred between February and July 1990 (Figure 4.16). In one of the *T. congolense* and three *T. vivax* infections in the Maasai Zebu, animals were parasitaemic for over four months and eventually, the parasitaemia disappeared without the PCV dropping to the critical value for treatment and therefore self cured. In the Orma Boran, all the infected animals except one with a *T. congolense* infection, needed drug treatment (Table 4.20).

In total, seven out of the eleven infections (58.3%) in the Maasai Zebu needed treatment compared to four out of the five (80%) in the Orma Boran. There were no significant differences in the overall herd weekly drug requirements between the two breeds in the entire study period (Table 4.21).

Table 4.19

The mean weekly disease incidence (% ± SD) of the Maasai Zebu and Orma Boran in the low tsetse challenge area at Nguruman

	Maasai Zebu	Orma Boran
Observations	54	54
Mean*	1.2 ± 2.4	0.4 ± 1.4

Analysis of variance

Source	df	ms	f	p < 0.05
Group	1	13.4	3.7	not sig.
Time	53	4.3	1.2	not sig.
Error	53	3.6		

* No significant differences between the two breeds.

Table 4.20

Number of infections, trypanosome species, drug treatments and self cures in the Maasai Zebu and Orma Boran in the low tsetse challenge area at Nguruman

Trypanosome species	Breed	No. of infections	Treatments	Self cure
T. vivax	Maasai Zebu	6	3	3
	Orma Boran	3	3	-
T. congolense	Maasai Zebu	4	4	-
	Orma Boran	2	1	1
Mixed*	Maasai Zebu	1	-	1
	Orma Boran	-	-	-

- = No cases

* Only one mixed infection of *T. congolense/T. brucei* was encountered in the low tsetse challenge area.

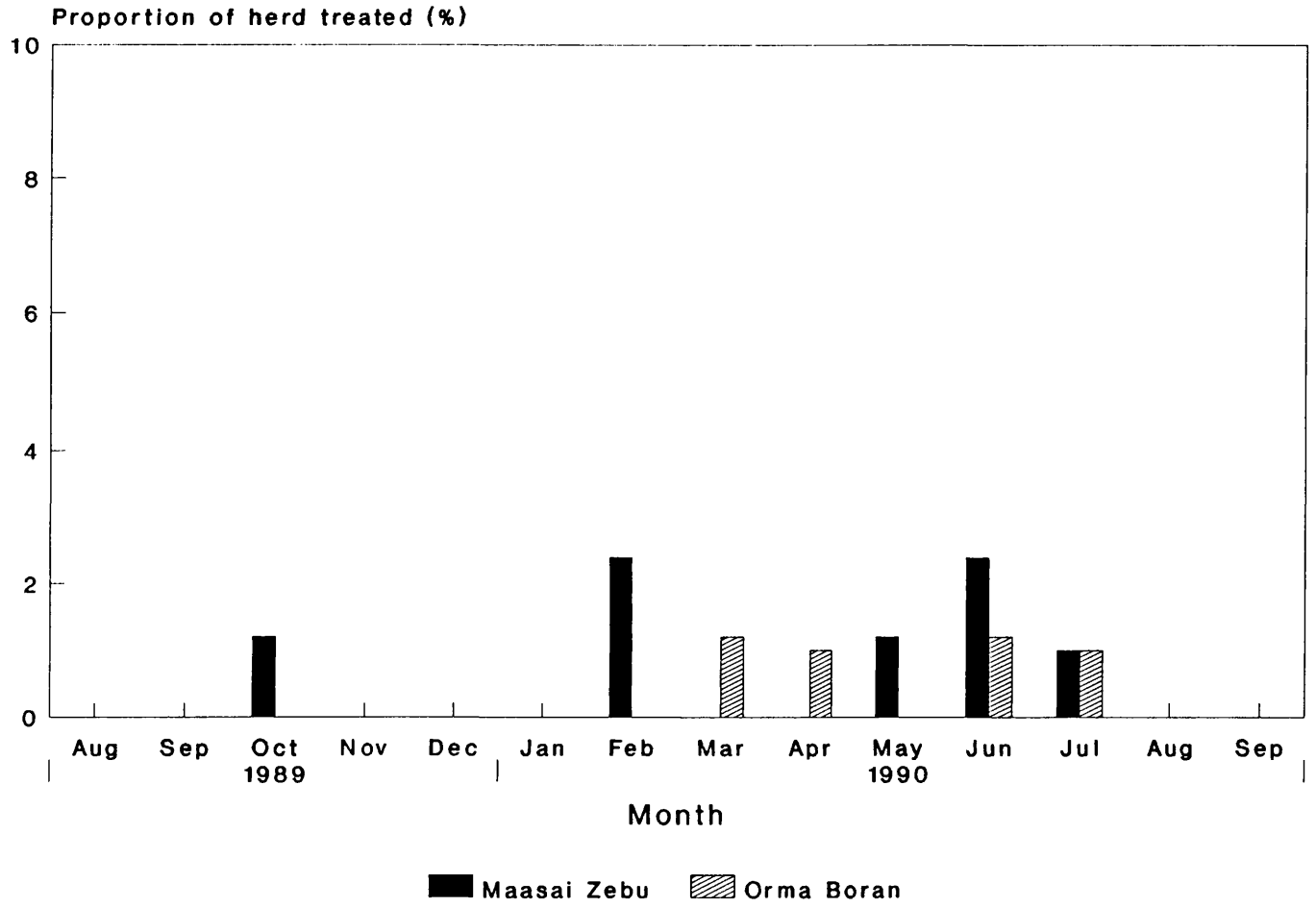


Figure 4.16. The mean monthly herd treatments in the Maasai Zebu and Orma Boran in the low tsetse challenge.

Table 4.21

The mean weekly drug treatments (% ± SD) in the Maasai Zebu and Orma Boran herds in the low tsetse challenge area at Nguruman

	Maasai Zebu	Orma Boran
Observations	54	54
Mean*	0.6 ± 2.1	0.4 ± 1.3

Analysis of variance

Source	df	ms	f	p < 0.05
Group	1	1.9	0.6	not sig.
Time	53	2.7	0.9	sig.
Error	53	3.2		sig.

* No significant differences between the breeds.

iv) Anaemia

The seasonal variation in the mean weekly PCV in the two cattle breeds is illustrated in Figure 4.17. The changes in the PCV may be divided into three phases. During the first phase from September to mid-November, the PCV was similar in both breeds and above 28%. In the second phase from mid-November to May, the two groups showed some fluctuations in PCV. For most periods during this phase, PCV of Orma Boran was $\leq 28\%$, while that of the Maasai Zebu was mainly $\geq 28\%$, though it was transiently less than 28% for a period of three weeks in April 1990. During the third phase from May 1990 to the end of experiment, both breeds maintained PCV values higher than 28%.

Although the mean PCV of the Maasai Zebu was higher than the Orma Boran for most of the time, the differences were not significant (Table 4.22).

d) Performance

i) Growth rate

Figure 4.18 shows the mean fortnightly percentage herd body weight gains. There was an initial four months lag with no changes in body weight (September to December 1989), but a steady increment took place in both breeds from January 1990 to the end of the study. There was marked improvement in the growth rate after the rains in December 1989.

By the end of the study, net weight increases of 54% and 60% had occurred in the Maasai Zebu and Orma Boran, respectively and as illustrated in Table 4.23, the difference was not significant.

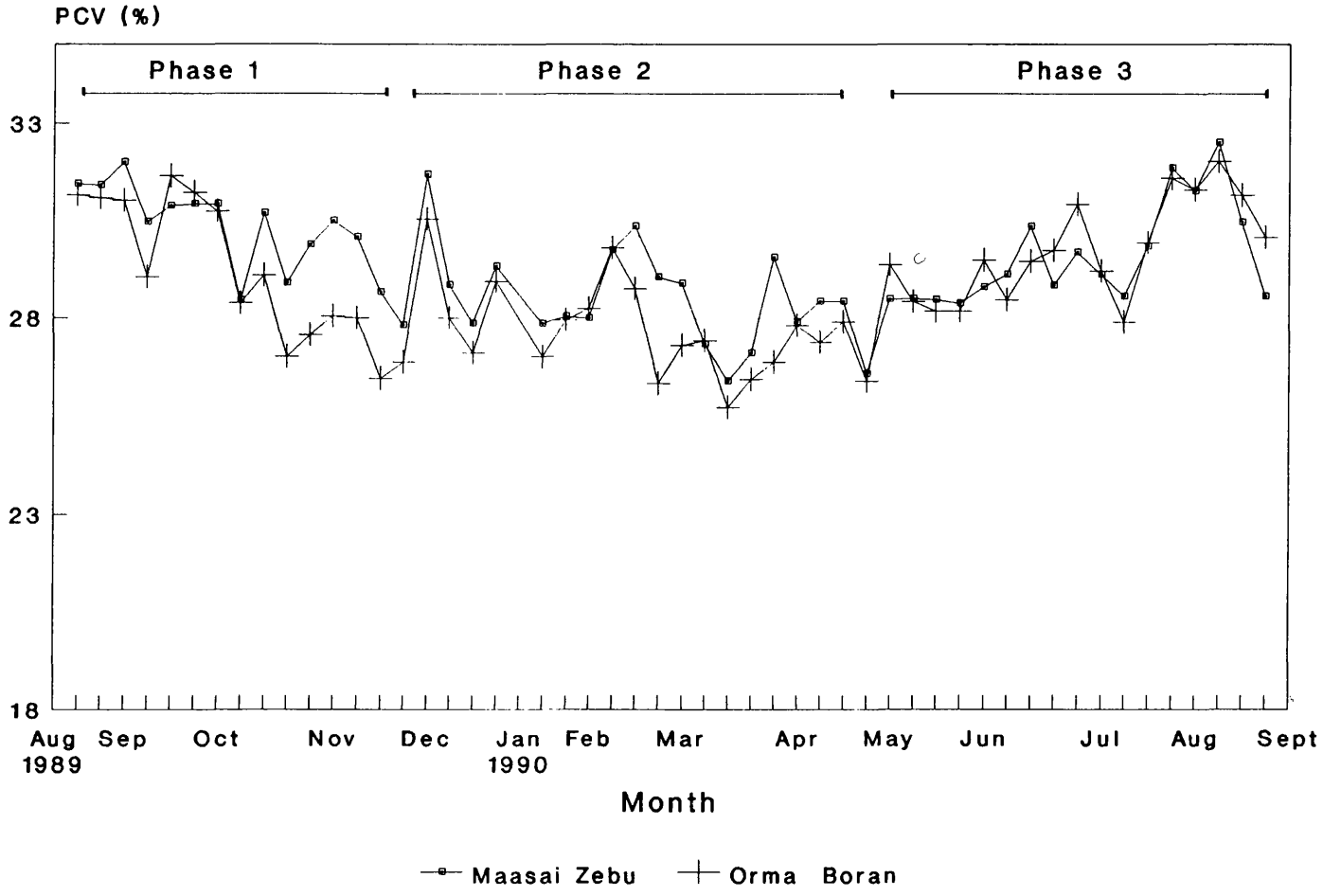


Figure 4.17. The mean weekly packed cell volume (PCV) of the Maasai Zebu and Orma Boran in the low tsetse challenge area.

Table 4.22

The mean weekly Packed Cell Volume (% ± SD) of the Maasai Zebu and Orma Boran in the low tsetse challenge area at Nguruman

	Maasai Zebu	Orma Boran
Observations	1046	1046
Mean*	29.4 ± 1.5	28.8 ± 1.7

Analysis of variance

Source	df	ms	f	p < 0.05
Group	1	170.4	0.9	not sig.
Error	40	196.8		
Time	49	94.7	11.9	sig.
Time x group	49	10.5	1.3	sig.
Error	1952	7.9		

* No significant differences between the breeds.

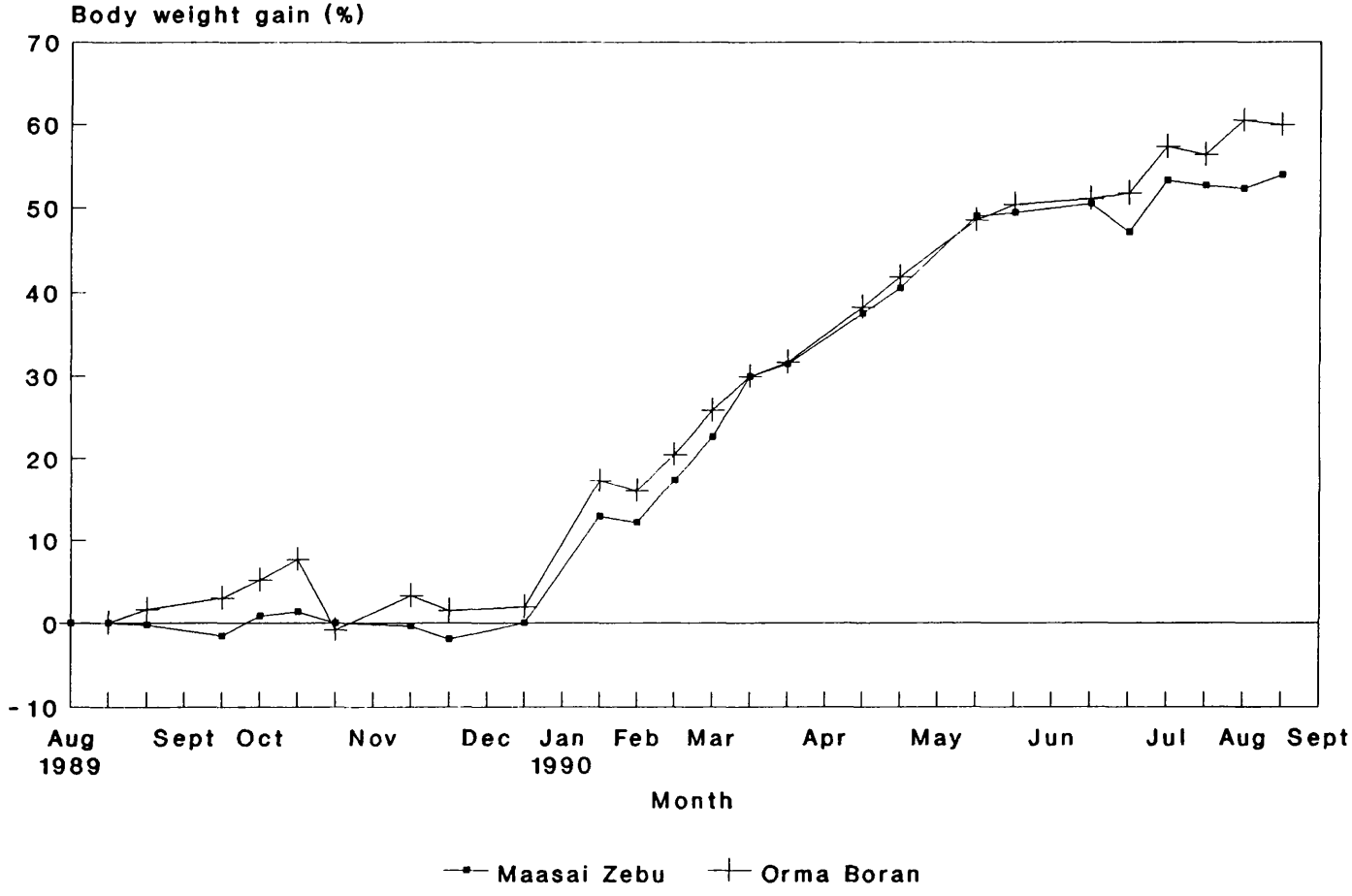


Figure 4.18. The mean fortnightly body weight gain in the Maasai Zebu and Orma Boran in the low tsetse challenge area.

Table 4.23

The mean monthly percentage body weight gains (±SD) of the Maasai Zebu and Orma Boran in the low tsetse challenge area at Nguruman

	Maasai Zebu	Orma Boran
Observations	504	479
Mean*	23.2±21.5	26.3±21.6

Analysis of variance

Source	df	ms	f	p < 0.05
Group	1	1790	1.3	not sig.
Error	40	1336.1		
Time	23	18361.5	545.5	sig.
Time x group	22	22.5	0.7	not sig.
Error	896	33.7		

* No significant differences between the breeds.

ii) Body condition

The mean monthly body condition scores are illustrated in Figure 4.19. For the first four months, there was no change in the body condition of the Orma Boran, while the Maasai Zebu had a transient decline in the third month. By the end of February, there had been an improvement in both groups. This was followed by a drop in the next two months which was more marked in the Maasai Zebu than the Orma Boran. By the end of the study, animals from both breeds had a net improvement in the body condition. The Orma Boran had significantly higher mean monthly body condition scores than the Maasai Zebu (Table 4.24).

4.1.3.3 Other diseases encountered in the two areas at Nguruman

a) Tick-borne diseases

As described earlier, blood smears were made from animals reported sick or with PCV < 20%. These were then stained in the laboratory with 10% Giemsa and examined for blood parasites as described in section 3.3. A total of 283 blood smears were examined (Table 4.25).

i) Theileriosis

Following the introduction of the cattle to the high tsetse challenge area 19 cases of theileriosis were detected (Table 4.25). The disease was found to occur following the grazing of the cattle in some pockets of very thick bush known to harbour high population of buffaloes.

The main clinical signs observed in the affected animals were, pyrexia, anorexia, dyspnoea, pulmonary oedema, enlarged lymph nodes, lacrimation, corneal opacity, petechial haemorrhages on the visible mucous membranes, bloody diarrhoea, and a terminal recumbency followed by death. The signs

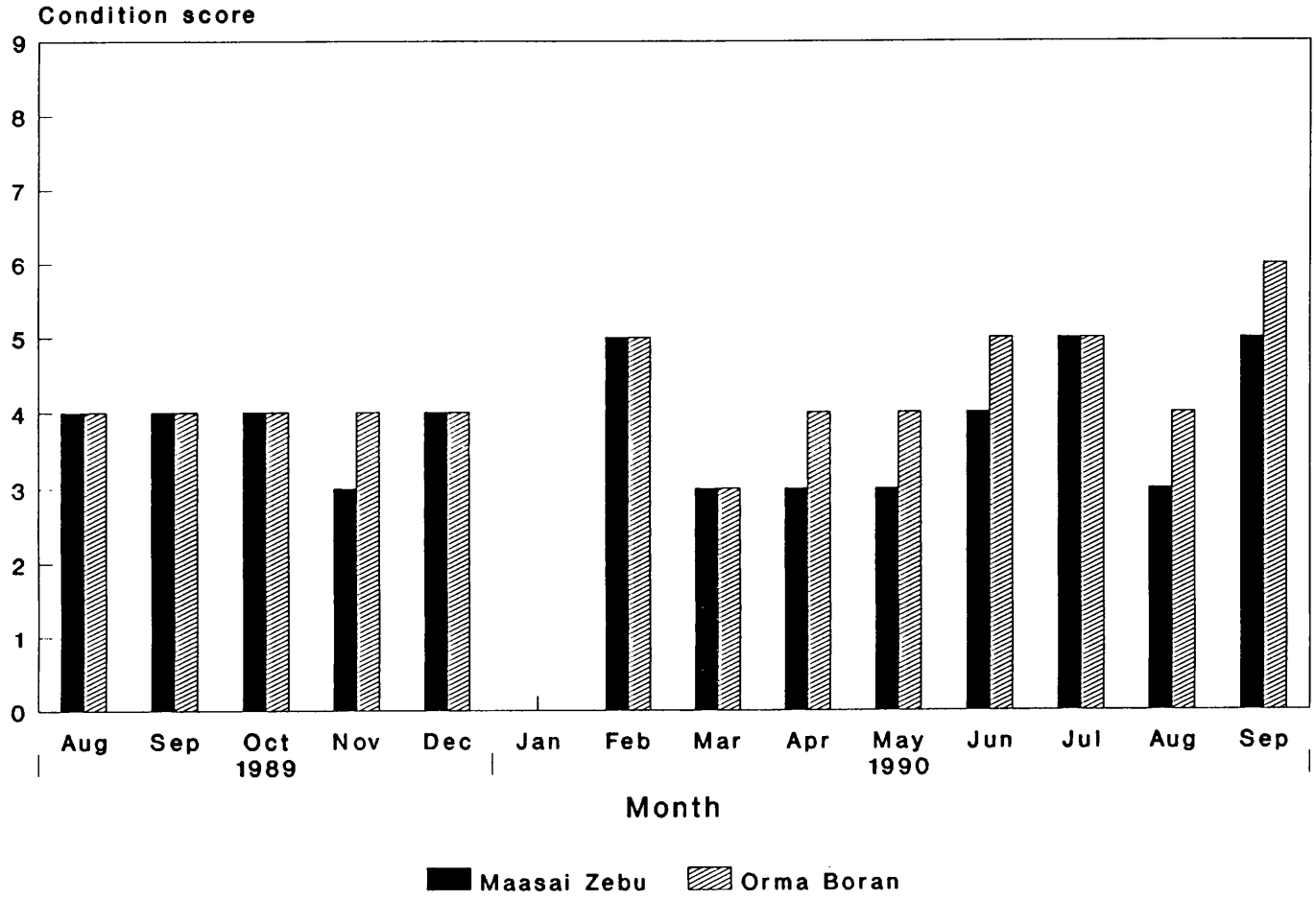


Figure 4.19. The mean monthly body condition scores of the Maasai Zebu and Orma Boran in the low challenge area.

Table 4.24

The mean monthly body condition scores (± SD) of the Maasai Zebu and Orma Boran in the low tsetse challenge area at Nguruman

	Maasai Zebu	Orma Boran
Observations	228	231
Mean	3.9 ± 0.8	5.0 ± 0.8*

Analysis of variance

Source	df	ms	f	p < 0.05
Group	1	11.9	5.0	sig.
Error	40	2.4		
Time	10	31.2	76.2	sig.
Time x group	10	0.5	1.1	not sig.
Error	397	0.4		

* Significantly higher score.

Table 4.25

Results of the blood and lymph node smears examined from animals reported sick and those with PCV < 20%

Sample	Total examined	Results					
		Diagnosis	High challenge			Low challenge	
			M	O	G	M	O
Blood smears	283	Anaplasma	4	3	2	4	2
		Theileria	1	2	4*	-	-
		Anaplasma and Theileria	2	1	1	-	-
		Anaplasma and Trypanosomiasis	1	3	2	-	1
		Theileria and Trypanosomiasis	3	2	3	-	-
Lymph node smears	30	Theileria	1	1	4*	-	-

M - Maasai Zebu

O - Orma Boran

G - Galana Boran

- = No cases detected

* Samples from the same animals

were severe, and six of the 19 affected animals died within one week after the onset of clinical signs, while the rest were treated successfully.

Lymph node biopsy smears and thin blood smears prepared from the affected animals were stained with 10% Giemsa. Schizonts of *Theileria parva* were demonstrated in the lymphocytes, while rod shaped piroplasms were seen in the thin blood smears. Of the 19 theileria infections detected, 4(21%) were mixed with anaplasma, while 8 (42%) were mixed with trypanosomes. Of the 30 lymph node smears, schizonts were seen in all the six animals that died from theileriosis. No cases of theileriosis occurred in the low tsetse challenge area. Due to the close association of the buffalo habitat and the occurrence of the disease, it was concluded to be the buffalo-derived form of theileriosis.

To avoid further losses from tick-borne diseases, constant monitoring was maintained where, any animal reported sick had lymph node biopsy smears and thin blood smears examined for theileriosis and anaplasmosis, in addition to the routine examination for trypanosomiasis. It must be stressed that theileriosis occurred because there was an initial delay in the implementation of the tick control, and as soon as the application of an acaricide (Triatix^{R}, Cooper Ltd.) started, the incidence was reduced remarkably. In addition, the herdsmen were advised to keep the cattle away from the areas heavily populated with buffaloes.

ii) Anaplasmosis

The only anaplasma species encountered was *Anaplasma marginale.* Of the 26 anaplasma infections, 4 (15%) were mixed with theileria, while 6 (23%) were mixed with trypanosomiasis (Table 4.25).

There were no differences among the breeds in the incidence of anaplasmosis and theileriosis, but the mortality associated with theileriosis in the

Galana Boran was higher than in the other two breeds.

Treatments

Cases of anaplasmosis were treated with imidocarb dipropionate (Imizol^R, Wellcome) at 1 mg kg^{-1} body weight or oxytetracycline (Terramycin/LA^R, Pfizer), at 20 mg kg^{-1}, while for theileriosis, either parvaquone (Clexon^R, Wellcome) at 10 mg kg^{-1} body weight or halofuginone (Terit^R, Hoechst) at 1 mg kg^{-1} body weight was used. Other veterinary clinical conditions encountered were treated as diagnosed.

Ticks

On several occasions, ticks were collected from the animals and submitted to the Kenya Agricultural Research Institute (KARI) laboratory at Muguga, for identification. *Rhipicephalus* spp. were predominant in the high challenge area with *R. evertsi* constituting 52% of the total collection, while *Hyalomma truncatum* was the major species in the low challenge area making up 43% (Table 4.26).

b) Helminthiasis

Figures 4.20 and 4.21 show the faecal egg counts in the three cattle breeds in the high and low challenge areas. As mentioned earlier, regular drenching at three month intervals was carried out. The level of helminthiasis never reached clinically significant levels (300 - 600 e.p.g.) and therefore the anaemia observed was unlikely to be associated with this condition.

Table 4.26

Ticks species identified from the cattle at Nguruman

Tick species	High challenge area N=473		Low challenge area N=594	
	X	(%)	X	(%)
Rhipicephalus appendiculatus	62	13	33	6
Rhipicephalus evertsi	248	52	205	35
Rhipicephalus pulchellus	119	25	94	16
Amblyomma gemma	19	4	9	2
Hyalomma truncatum	25	5	253	43

N - Number of ticks collected for identification

X - Number identified.

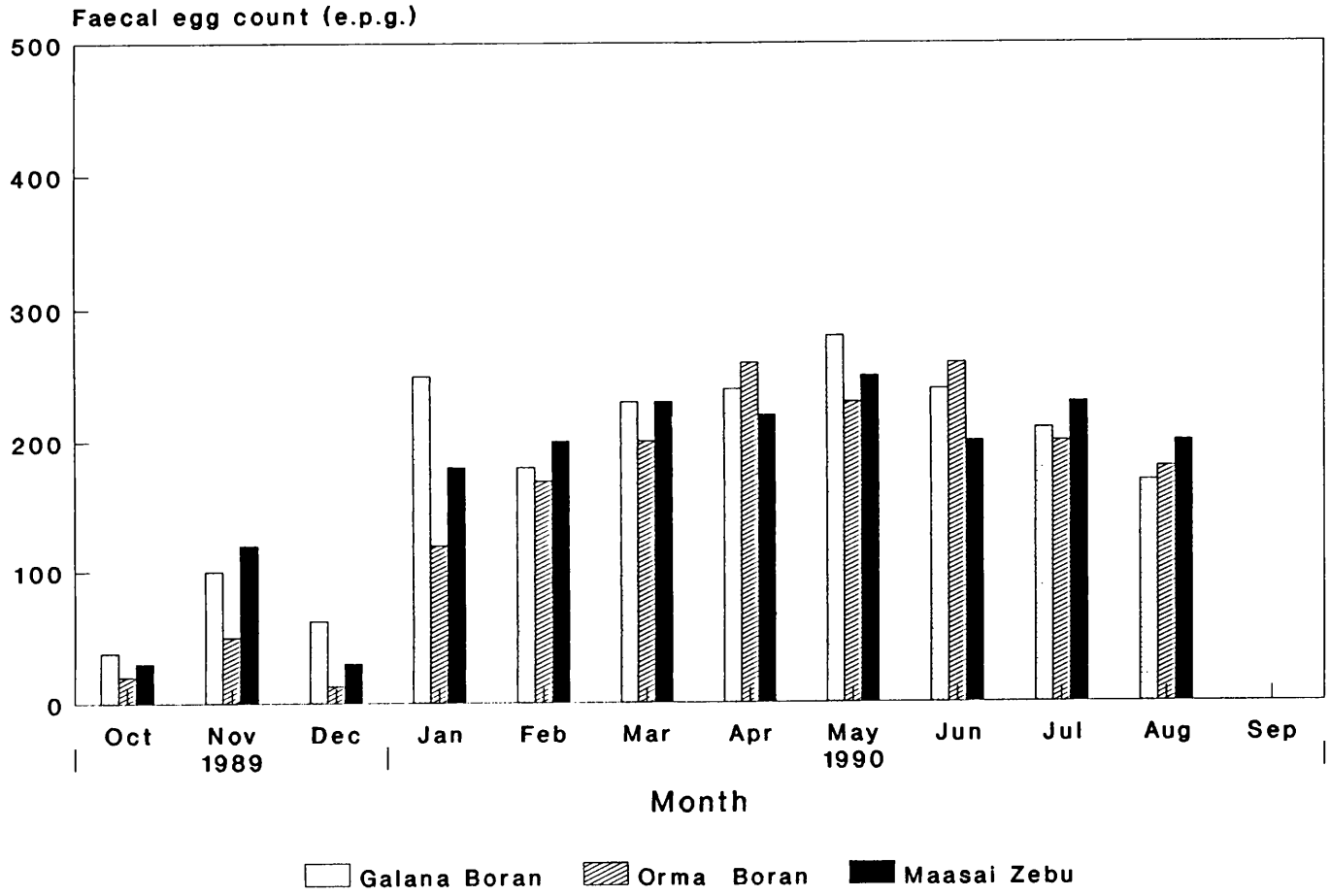


Figure 4.20. Mean monthly faecal egg counts (e.p.g.) in the three cattle breeds in the high challenge area.

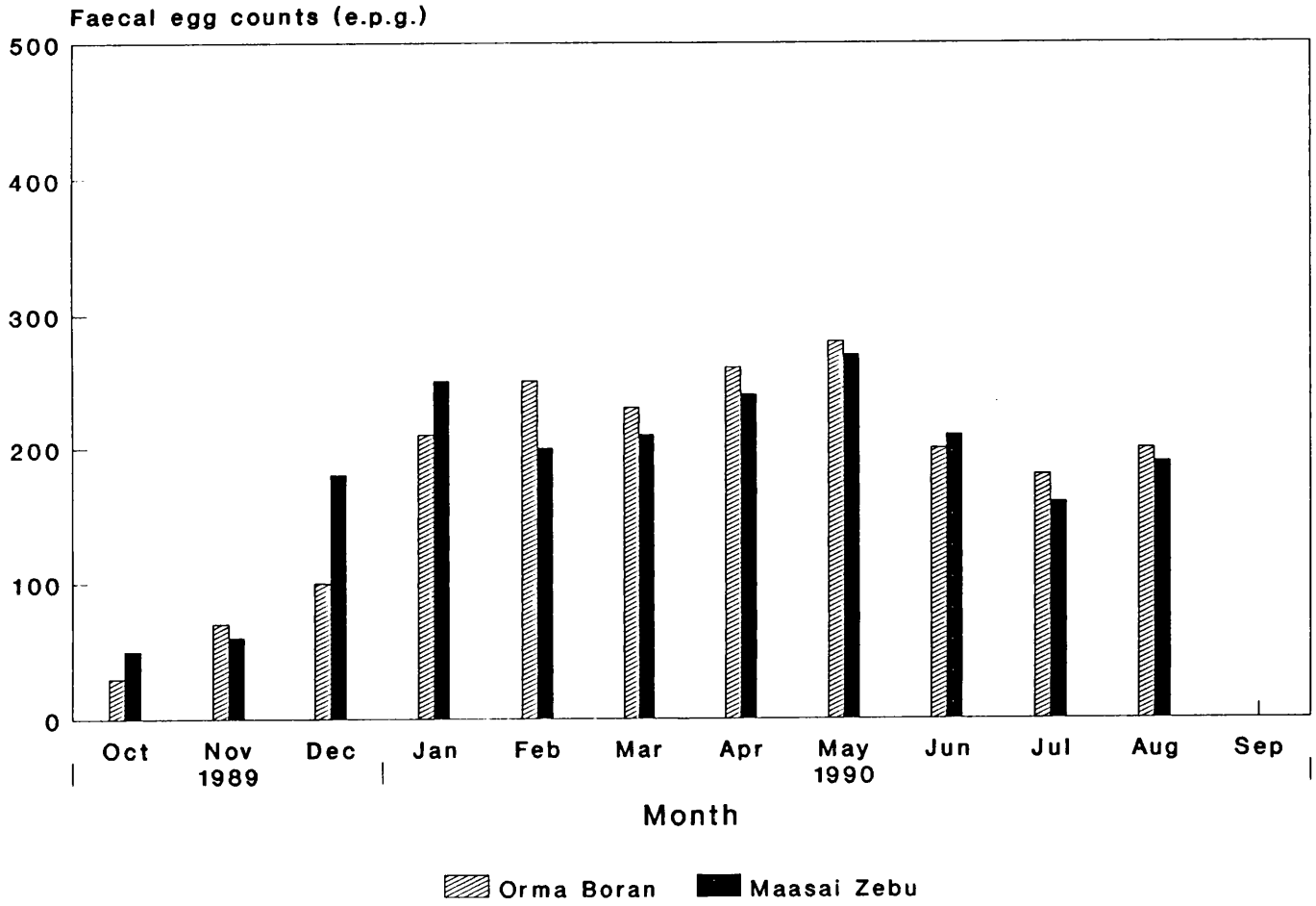


Figure 4.21. Mean monthly faecal egg counts (e.p.g.) of the cattle breeds in the low challenge area.

c) Other clinical conditions

Other clinical conditions encountered beside tick-borne diseases, are summarized in Table 4.27 and these were all recorded in the high challenge area, probably due to the thick vegetation harbouring a variety of disease vectors and hosts (wildlife).

d) Mortality

Following the introduction of cattle in the high tsetse challenge area, one Maasai Zebu, one Orma and two Galana Boran steers died of theileriosis within the first month (Table 4.28). Two more Galana Boran died from mixed infections of theileriosis and trypanosomiasis within the same period, while another two died from trypanosomiasis alone. There was no mortality in the Maasai Zebu and Orma Boran due to trypanosomiasis. No mortality occurred in the low tsetse challenge area.

The main post-mortem lesions observed in the cases of theileriosis were, corneal opacity, frothing from nostrils, pulmonary oedema, subcutaneous oedema, enlarged superficial lymph nodes and spleen, ecchymotic and petechial haemorrhages on the muscles, gastrointestinal tract, serosal surfaces of the kidneys and the heart, multifocal white foci on the liver and congestion on mucous membranes of abomasum. The main features seen in the carcasses from the animals that died of trypanosomiasis were emaciation, anaemia and slight lymph node enlargement.

Table 4.27

Cases of other diseases and conditions encountered in the high challenge area at Nguruman*

Disease	Number of cases		
	Galana Boran	Orma Boran	Maasai Zebu
Eye infections (infectious keratoconjuctivitis)	6	6	3
Lameness			
Laminitis	1	1	-
Sprain	-	-	1
Broken hoof (due to trauma)	-	-	1
Ephemeral fever (Three days sickness)	3	2	1
Upper respiratory tract infection	1	1	-

* Excluding anaplasmosis and theileriosis.

- = No cases.

Table 4.28

The number of animals that died in the high tsetse challenge area at Nguruman

Breed	Disease			
	Trypanosomiasis	Theileriosis	Mixed trypanosoma and theileria infections	Total
Maasai Zebu	-	1	-	1
Orma Boran	-	1	-	1
Galana Boran	2	2	2	6

- = No deaths.

4.1.3.4 Comparison of the Maasai Zebu and Orma Boran groups in the high and low tsetse challenge areas

Since the cattle groups of the same breed introduced into the low and high challenge areas had similar pre-experimental treatments, and the disease monitoring during the experiment was performed at the same points in time, it was possible to compare their performance in the two areas and therefore determine the effects attributable to the differences in the tsetse challenge.

A comparison of the trypanosomiasis risk in the low and high challenge areas is presented in Table 4.29. The disease incidence in the sentinel Maasai Zebu cattle in the high challenge area was almost three times higher than in the low challenge, while the fly density was nearly 90-fold.

The various disease parameters monitored and the performance in the two areas are summarized in Table 4.30. The groups in the low challenge area had lower disease incidence and prevalence and needed fewer drug treatments. In addition, both the Maasai Zebu and Orma Boran herds in the low challenge area had significantly higher PCV values than their counterparts in the high challenge area. The differences appeared higher between the Orma Boran than in Maasai Zebu groups (Figures 4.22 and 4.23). Similarly, the groups in the low challenge area attained higher body weight gains than their counterparts in the high challenge area (Figures 4.24 and 4.25); Maasai Zebu gained twice and Orma Boran nearly three times as much (Table 4.30). In addition, animals in the low challenge area maintained better body condition than their counterparts.

There was no mortality in the low challenge area, while one Maasai Zebu and one Orma Boran died of theileriosis in the high challenge area.

A summary of the results which have been discussed from the high and low challenge areas at Nguruman is presented in Table 4.31.

Table 4.29

Comparison of the trypanosomiasis risk in the low and high tsetse challenge areas at Nguruman. The mean values of the fly density and disease incidence for the whole study period were obtained by calculating the average of the monthly observations

	High challenge	Low challenge	Ratio
Fly density (Flies/trap/day)	358	4	89.5
Disease incidence(%) in sentinel cattle	16.6	5.7	2.9

Table 4.30

Comparison of the mean values on disease incidence, infections, drug treatments, PCV(%), body weight gains and mortality between the Maasai Zebu and Orma Boran cattle in the high and low challenge areas at Nguruman

	Maasai Zebu		Orma Boran	
	High challenge	Low* challenge	High challenge	Low* challenge
Mean weekly disease incidence(%)	7.6	1.2	9.6	0.4
Infections/ animal/year	4	0.6	5.2	0.2
Treatments/ animal/year	3.4	0.3	4.9	0.2
PCV(%)	26.7	29.4	25.2	28.8
Monthly body weight gain (%)	11.7	23.2	9.7	26.3
Condition score	3	3.9	3	5.0
Mortality	1(4.5%)	0	1(4.5%)	0

* All the parameters significantly different from those obtained from the high tsetse challenge area.

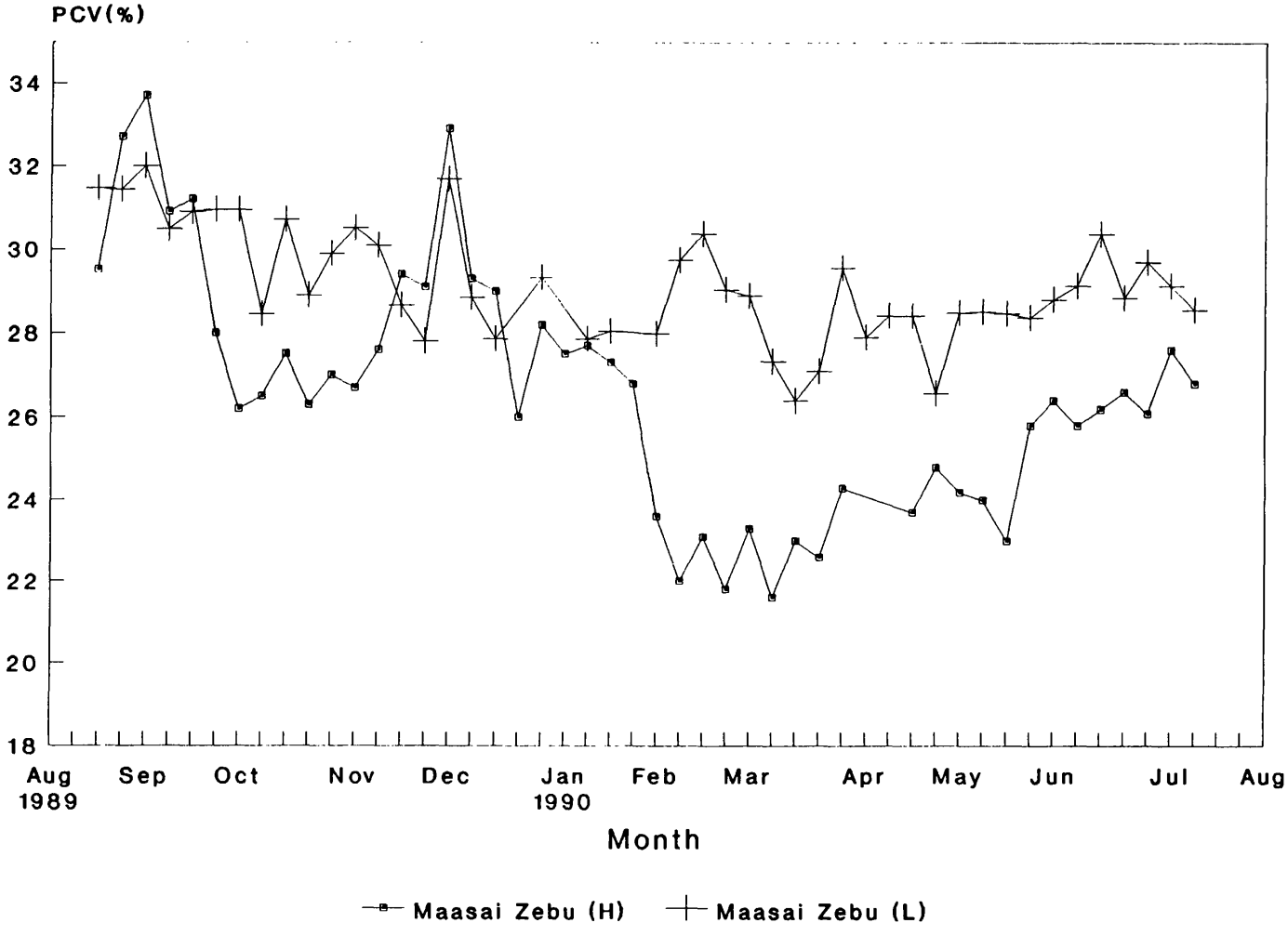


Figure 4.22. Comparison of the mean weekly packed cell volume (PCV) in the Maasai Zebu groups in the high (H) and low (L) tsetse challenge areas at Nguruman.

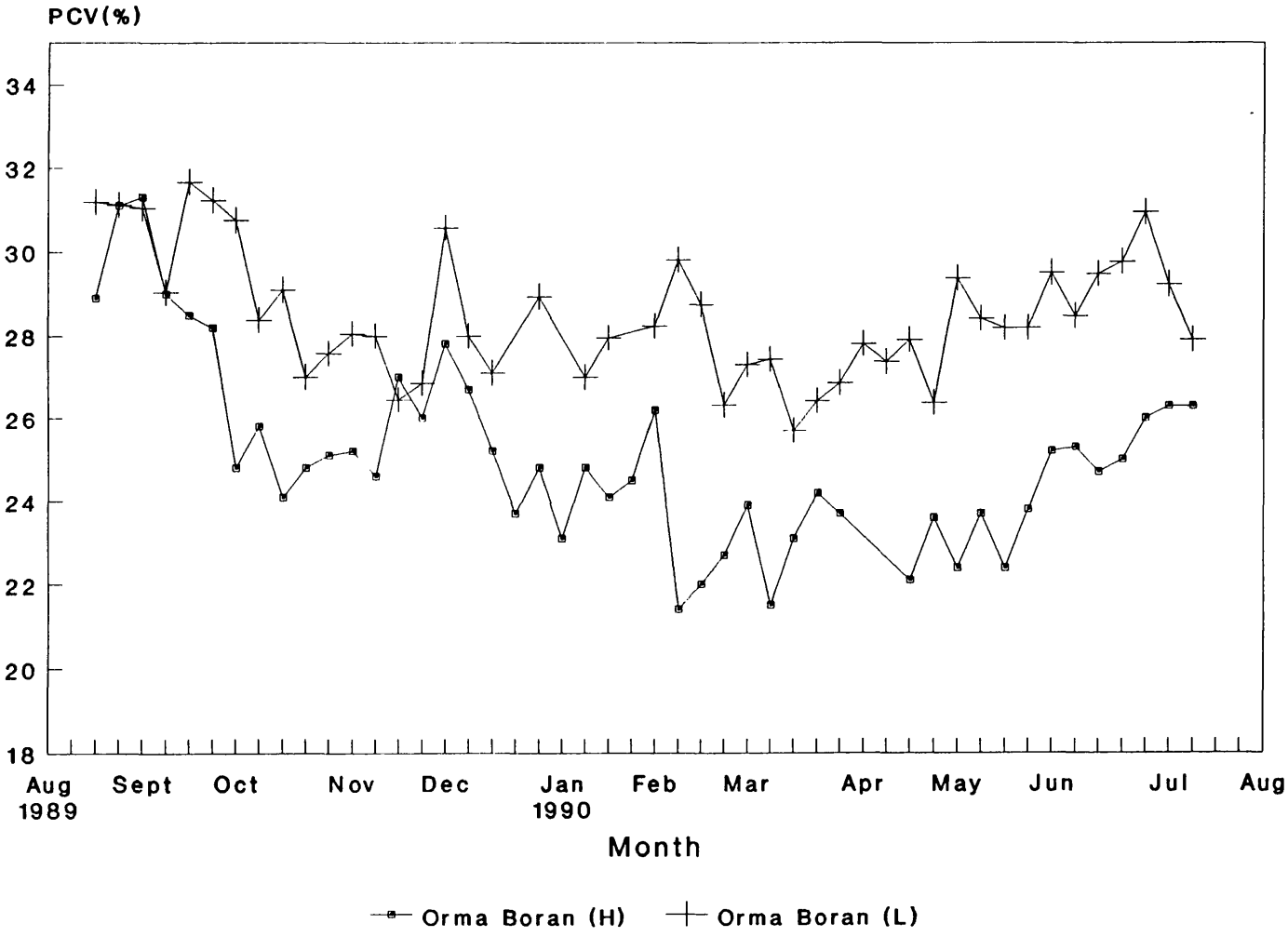


Figure 4.23. Comparison of the mean weekly packed cell volume in the Orma Boran groups in the high (H) and low (L) tsetse challenge areas at Nguruman.

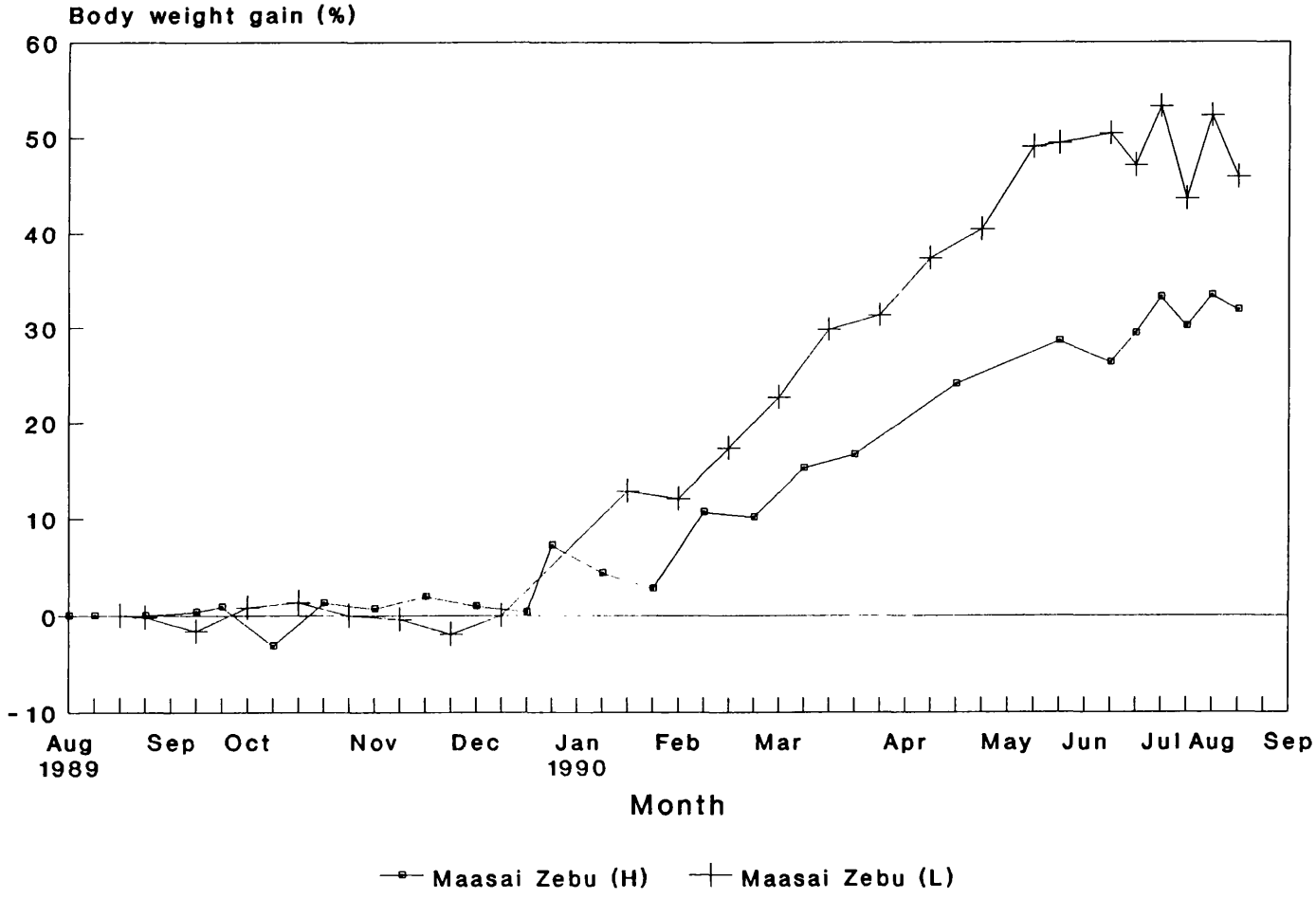


Figure 4.24. Comparison of body weight changes in Maasai Zebu groups in the high (H) and low (L) challenge areas.

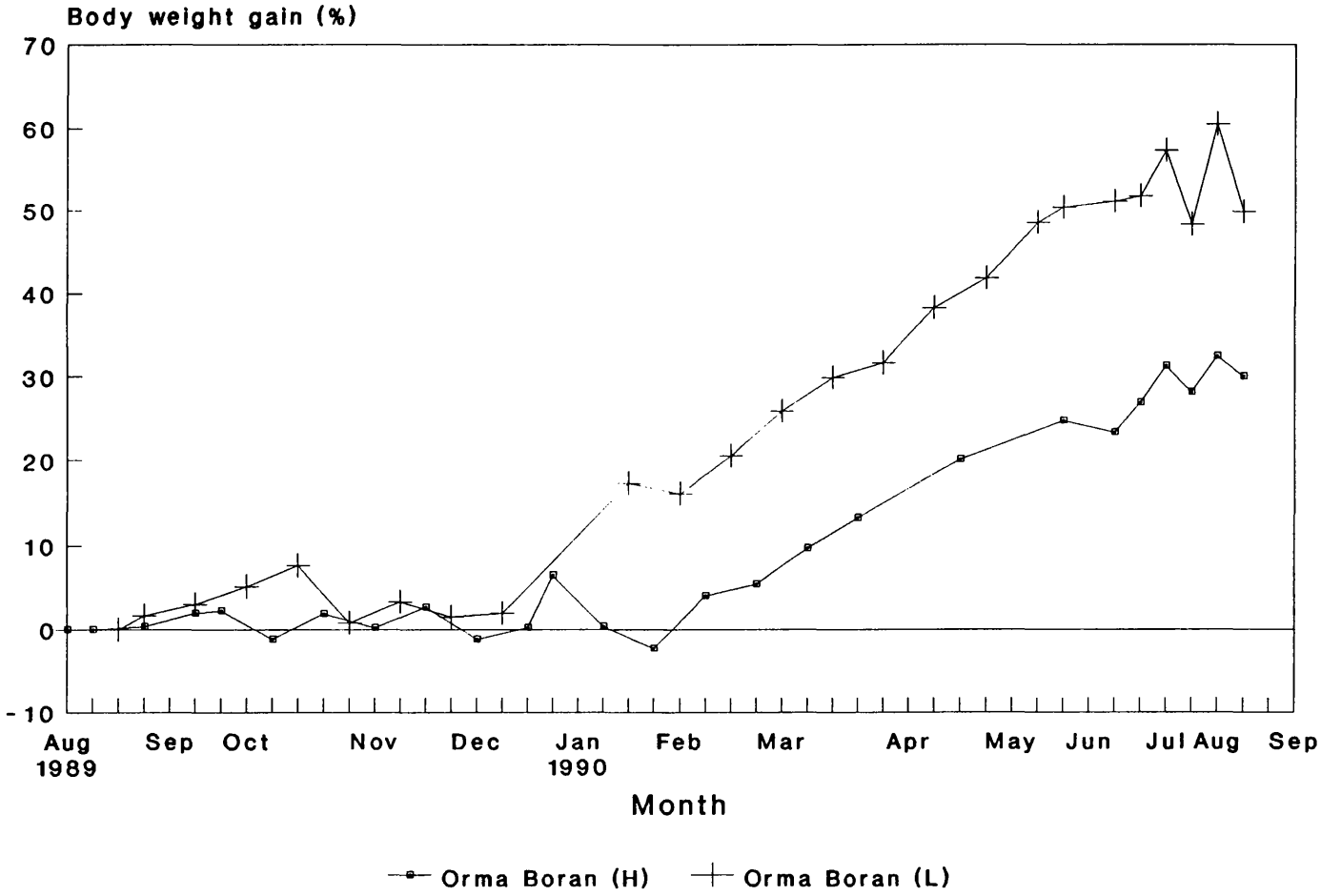


Figure 4.25. Comparison of the body weight gain in the Orma Boran groups in high (H) and low (L) challenge areas.

Table 4.31

Summary of observations on the groups in the high and low tsetse challenge areas at Nguruman

	High challenge			Low challenge*, G	
	Maasai Zebu *a*	Orma Boran *b*	Galana Boran *c*	Maasai Zebu	Orma Boran
Prepatent period	103.3	56.6	21.8	200	142.2
Disease incidence	7.6	9.6	13.7[a,b]	1.2	0.4
Infections/animal/year	4	5.2	7.5[a,b]	0.5	0.2
Treatments/animal/year	3.4	4.9	7.2[a,b]	0.3	0.2
Herd treatment (%)	6.4	8.6	13.3[a,b]	0.6	0.4
Prevalence of *T. vivax*	7.7	8.9	9.6	2.6	1.3
Prevalence of *T. congolense*	4.6	4.9	7.8[a,b]	2.7	0.9
Self cure	11 (13)	5 (4.5)	2 (2.1)	4 (36.3)	1 (20)
PCV**	26.6	25.2	24.0	29.4	28.8
Growth rate	11.7	9.7	4.2[a]	23.2	26.3
Condition score***	3	3	3	3.9	5.0
Mortality					
Trypanosomiasis related	-	-	4	-	-
Theileriosis	1	1	2	-	-

* All observations on the Maasai Zebu and Orma Boran in the high tsetse challenge area significantly different from their counterparts in the low challenge area.
a,b,c - Refer to the Maasai Zebu, Orma and Galana Borans respectively in the high challenge and the superscripts indicate the groups with significantly different results.
** - All groups in the high challenge area significantly different from each other.
*** - No significant differences among the three groups in the high challenge area.
Numbers in parenthesis are percentages of the total infections that self cured.
G - No Galana Boran steers kept in the low challenge area.

4.2 EPIDEMIOLOGICAL STUDIES AT THE GALANA RANCH, KENYA COAST: COMPARISON OF THE MAASAI ZEBU, ORMA BORAN AND GALANA BORAN CATTLE WITH AND WITHOUT PREVIOUS EXPOSURE TO TRYPANOSOMIASIS FROM MAY 1991 TO FEBRUARY 1992.

4.2.1 INTRODUCTION

The study at Galana Ranch consisted of two groups of the three cattle breeds with different background histories of exposure to trypanosomiasis. The first group consisted of herds of the Maasai Zebu, Orma and Galana Borans, which survived from the high tsetse challenge area at Nguruman, and had therefore been subjected to constant exposure to tsetse challenge for a period of one year.

In this study these groups are referred to as the ones with previous exposure (PE). The second group consisted of a freshly purchased group of the Maasai Zebu from Nguruman and fresh groups of the Orma and Galana Boran from the Galana Ranch. All the newly purchased groups had variable backgrounds, but certainly had not been under constant previous exposure. They have been referred to as the cattle groups with no previous exposure (NPE).

4.2.2 MATERIALS AND METHODS

a) Cattle used in the study and the pre-experimental procedures

After the end of the observation period at Nguruman, all cattle were treated with diminazene aceturate (Berenil^{R}, Hoechst) at 7 mg kg^{-1} body weight, long acting oxytetracycline (Terramycin/LA^{R}, Pfizer), at 20 mg kg^{-1} body weight, and sprayed with an acaricide (Triatix^{R}, Cooper Ltd.). The three groups of the Maasai Zebu, Orma Boran and Galana Boran cattle from both the high and low

challenge areas at Nguruman were then moved 100 km away and kept in a tsetse-free area at Kisames, Ngong, near Nairobi, for a period of five months from October 1990 to February 1991. They were monitored monthly for parasitaemia and PCV to confirm complete recovery from trypanosomiasis. In addition, serum was collected monthly for the analysis of the trypanosomal antigens and antibodies.

During this period there was severe drought in the area and the cattle were watered once every two days and supplemented with hay. Most animals lost weight and generally were in poor body condition. As a result, the animals became very susceptible to the bovine-derived form of theileriosis (East Coast Fever) and anaplasmosis which were prevalent at Kisames. A total of seven animals, four Galana Boran, one Orma Boran and two Maasai Zebu, died from a combination of either ECF or anaplasmosis, and starvation stress. Post-mortem examinations were not carried out since the death reports were not received in time.

In February 1991, a group of 25 Maasai Zebu steers aged approximately two and a half years were purchased from the farmers at Nguruman. Their age estimation was performed as described previously in section 4.1.2. Previous treatment history on the Maasai Zebu as obtained from the farmers at purchase indicated that, 16 of the animals had no trypanocidal treatment within the last one year (Table 4.32). Three animals had received either homidium chloride (Novidium^{R}, RMB, UK) or diminazene aceturate (Berenil^{R}, Hoechst). Only one animal had received a treatment within less than six months.

At the same time, 25 Galana Boran and 20 Orma Boran aged approximately two and a half years were purchased from the Galana Ranch. These steers were selected from ranch cattle herds which had received

Table 4.32

Treatment history of the 25 Maasai Zebu steers newly purchased at Nguruman in February 1991, and transferred to the Galana Ranch

Drug	Last treatment administered	Number of animals
Diminazene aceturate[1]	> 6 months	1 (4%)
Diminazene aceturate and homidium chloride[2]	< 6 months	1 (4%)
Homidium chloride	< 6 months	1 (4%)
No treatment		16 (64%)
No clear history		6 (24%)

[1] Berenil^{R}, Hoechst, W. Germany.

[2] Novidium^{R}, RMB, UK.

prophylactic cover six months previously with isometamidium (Samorin^{R}, RMB, UK) at 1 mg kg^{-1} body weight, and were grazed in areas with minimal tsetse challenge by the ranch management. All the newly selected animals may have been exposed to varying levels of trypanosomiasis challenge for uncertain periods of time.

i) Pre-experimental treatments of the cattle at Nguruman, Ngong and the Galana Ranch

At the beginning of March 1991, the newly purchased cattle from Nguruman and Galana, and the herds from the previous Nguruman experiment kept at Kisames, Ngong, were vaccinated against foot-and-mouth disease, rinderpest, contagious bovine pleuropneumonia (CBPP), blackquarter and anthrax. They were also treated with diminazene aceturate (Berenil^{R}, Hoechst) at 7 mg kg^{-1} body weight, long acting oxytetracycline (Terramycin/LA^{R}, Pfizer), at 20 mg kg body weight, and sprayed with an acaricide (Triatix^{R}, Cooper Ltd.).

The newly purchased Maasai Zebu herd (NPE) at Nguruman and the surviving PE Maasai Zebu, Orma and Galana Boran herds from the high challenge area at Nguruman (kept at Kisames) were then transported by lorry to the Galana Ranch. The Maasai Zebu and Orma Boran from the low challenge area were transported to the KETRI farm at Muguga, where monthly monitoring for trypanosome parasitaemia and serum collection continued for three months (and the results are presented in Chapter 5).

ii) Pre-experimental management at the Galana Ranch

At the Galana Ranch, the cattle moved from Nguruman were grazed in a tsetse-free area, together with the newly purchased groups of NPE Orma and

Galana Boran steers from the ranch, for a period of two months. This gave the transferred animals time to adjust to the Galana Ranch environment and to recover from the nutritional stress they had suffered at Kisames, Ngong. Ten odour-baited biconical traps were set at 500 m apart within the area where the cattle were grazing and monitored weekly. During the two months period only biting flies were caught in the traps, confirming that, the animals were under minimal trypanosomiasis risk.

b) Experimental design

In May 1991, all the steers at were given another treatment with diminazene aceturate (Berenil^{R}, Hoechst) at 7 mg kg^{-1} body weight, to ensure that, they were not incubating trypanosome infections prior to the experiment. They were then moved to Kapangani, an area of the ranch which has high tsetse challenge for most of the year, and where it is known that, cattle cannot survive without treatment (Njogu *et al.*, 1985a,b). Two Maasai Zebu steers were lost to predators (lions) before the start of the experiment, and therefore this group was reduced to 23 animals.

The animals were grouped as shown on Table 4.33 and monitored for a period of nine and a half months, from mid-May 1991 to February 1992. The trypanosomiasis risk, disease monitoring in cattle, drug treatments and the assessment of the growth rate were performed as described in section 3.3.

c) Management

i) Tick control

The tick challenge in the Galana Ranch is high and therefore, a weekly acaricide spraying programme (Triatix^{R}, Cooper Ltd.) was implemented.

Table 4.33

Experimental design at the Galana Ranch;- the number of animals of the three breeds with and without previous exposure introduced into high natural tsetse challenge at the Galana Ranch in April 1991

	Group 1 Previously exposed (Survivors from the Nguruman experiment)		Group 2 No previous exposure (New animals)	
	N	W(kg)	N	W(kg)
Maasai Zebu	20	180.8	23*	152.8
Orma Boran	19	195.8	20	236
Galana Boran	9	231.4	25	306

N - Number of animals.

W(kg) - Mean body weight (kg) at the start of the experiment.

* Two Maasai Zebu steers killed by lions before start of the experiment.

ii) Helminthiasis

Regular drenching with albendazole (Valbazen^{R}, Beecham) at 7.5 mg kg^{-1} body weight was performed every three months. In addition, constant surveillance was maintained by carrying out faecal egg counts on at least 25% of the herd every two months using the method described in section 3.3.

d) Data analysis

Comparison for differences and interaction in the individual animals within and between breeds, in the herds with and without previous exposure to trypanosomiasis at the Galana Ranch, was done using the Generalized Linear Interactive Modelling system (GLIM, release 3.77, Royal Statistical Society, 1987). Differences between groups were compared as described in section 4.1.2.

4.2.3 RESULTS

4.2.3.1 Observations on the Maasai Zebu, Orma Boran and Galana Boran with previous exposure to trypanosomiasis.

a) Weather

Figure 4.26 shows the mean monthly maximum and minimum temperatures and rainfall at the Galana Ranch during the study period. The mean temperature ranged from 31 - 35^{o}C. There was a total of 370 mm rainfall, 79% (292 mm) which fell in the first five months, while the rainfall was scanty for the rest of the period, except in November 1991.

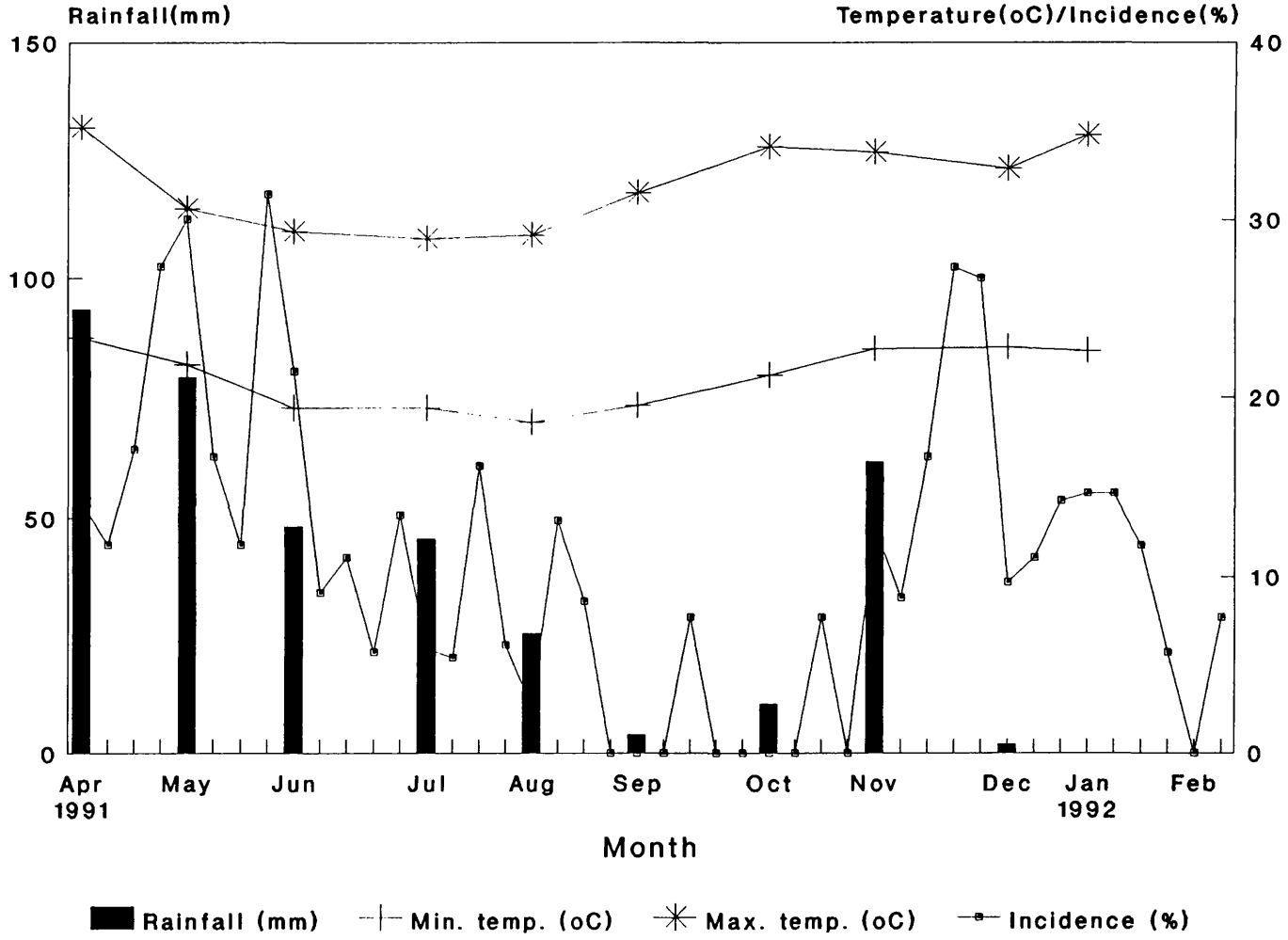


Figure 4.26. The mean monthly rainfall, maximum and minimum daily temperature (oC) and weekly disease incidence in a sentinel herd of Orma Boran steers at the Galana Ranch between April 1991 and February 1992.

b) Trypanosomiasis risk

The mean weekly disease incidence was obtained from a sentinel herd of 30 Orma Boran steers aged two and a half years which were grazed together with the experimental cattle. The disease incidence was high in the rainy season and exceeded 25% for two periods in May and June, and again in November 1991 (Figure 4.26). The incidence declined markedly in September and October 1991.

c) Trypanosomiasis

i) Pre-experimental serum samples.

No parasites were detected by the buffy coat in the three breeds, while there were more cases of antigens in the Maasai Zebu than other breeds (Table 4.34). On the other hand, the Orma Boran had the highest number of cases with antibodies.

ii) Disease incidence

Figure 4.27 illustrates the mean monthly disease incidence. Except in June 1991 when the incidence exceeded 10% in all the breeds, and in December 1991 in the Galana Boran, it was relatively low for the rest of the study period. Most infections occurred in the first four months and the last three months with a spell of low disease incidence in between. There were no significant differences in the mean weekly disease incidence among the three cattle breeds (Table 4.35).

iii) Infections in individual animals

Table 4.36 shows the frequency of infections in animals that survived to the end of the study. All animals became infected. Seventeen of Maasai Zebu (85%), 15 (79%) of Orma Boran and six (67%) of the Galana Boran were infected three

Table 4.34

Number of positive cases detected on the pre-experimental samples from the PE animals using the darkground/phase contrast buffy coat (DG), antibody and antigen ELISA techniques

Diagnostic technique	Trypanosome species identified	Maasai Zebu *N=20*	Orma Boran *N=19*	Galana Boran *N=9*
DG		-	-	-
Ab-ELISA		4	7	3
Ag-ELISA				
	Tc	2	4	2
	Tv	2	-	1
	Tb	-	-	-
	Tc/Tv	2	-	1
	Tc/Tb	1	1	-
	Tv/Tb	-	1	-
	Tv/Tc/Tb	2	-	-

N - Number of cattle
Tv - *T. vivax*
Tc - *T. congolense*
Tb - *T. brucei*
- = Negative

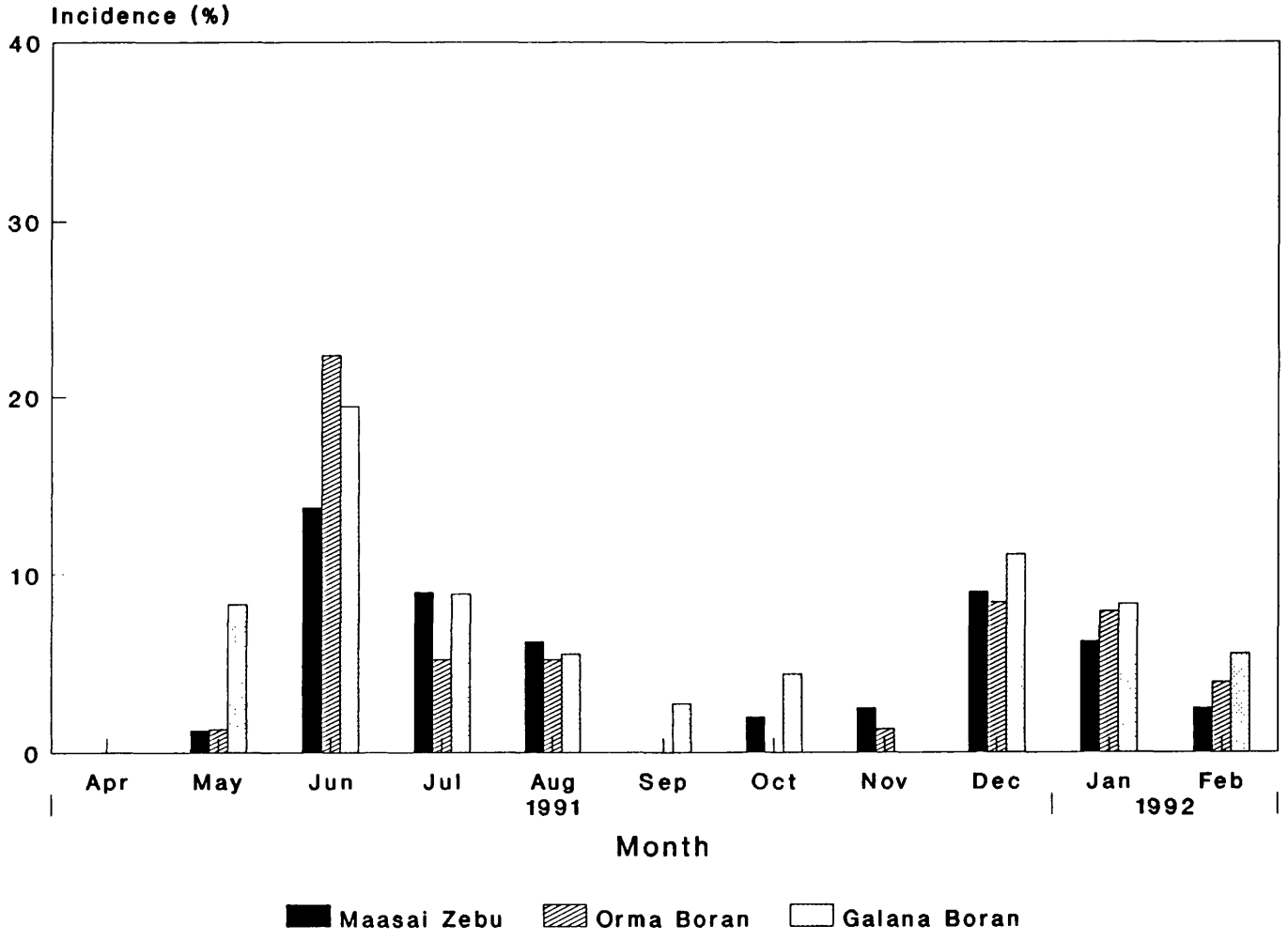


Figure 4.27. The mean monthly disease incidence (obtained as an average of the weekly incidence) in the Maasai Zebu, Orma Boran and Galana Boran with previous exposure.

Table 4.35

The mean weekly disease incidence (±SD) of the PE Maasai Zebu, Orma Boran and the Galana Boran in the high tsetse challenge area at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Observations	42	42	42
Mean*	5.5 ± 6.2	5.6 ± 7.8	7.7 ± 10.3

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	62.8	1.6	not sig.
Time	41	128.7	3.4	not sig.
Error	82	38.4		

* No significant differences among the breeds.

Table 4.36

Frequency distribution of infections in the PE cattle of the three breeds that survived up to the end of the experiment

Number of infections	Frequency		
	Maasai Zebu N=20	Orma Boran N=19	Galana Boran N=9
0	0	0	0
1	5	6	0
2	7	7	3
3	5	2	3
4	2	1	3
5	1	2	0
6	0	1	0

N - Number of animals

times or less. There was a maximum of five infections in one Maasai Zebu, six in one Orma Boran and four in three Galana Boran.

The mean number of infections per animal during the nine months are presented in Table 4.37. There were no significant differences in the mean number of infections among the three cattle breeds.

iv) Intervals between infections and the duration of parasitaemia

Following the introduction into the tsetse challenge area, the Maasai Zebu appeared to have longer mean duration to first infection (Table 4.38); however, this was not significantly different from the other breeds. Similarly, there were no differences among or within the breeds in the intervals between subsequent infections following treatments.

Once infected, the mean duration that the animals were parasitaemic before the PCV dropped to 17% or less is summarized in Table 4.39. There were no significant differences in the duration of the parasitaemia among the infections within the breeds.

v) Trypanosome species prevalence

Maasai Zebu

Figure 4.28 shows the seasonal occurrence of the various trypanosome species in the Maasai Zebu. There were two periods of high challenge from May to August 1991, and December 1991 to February 1992, and in both, *T. congolense* was more commonly identified than other species, and reached a maximum of 16% in August 1991. Mixed infections and *T. brucei* occurred mainly during the rains (June and July 1991). The prevalence of *T. brucei* was very low with a peak of only 1.3% in June after the rainy season. The proportion of infections due to

Table 4.37

The mean total number of infections (±SD) per animal recorded in the PE Maasai Zebu, Orma Boran and Galana Boran in the high tsetse challenge area at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Observations	20	19	9
Mean	2.4 ± 1.1	2.4 ± 1.5	3.0 ± 0.9

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	1.4	0.9	not sig.
Error	45	1.6		

* No significant differences among the breeds.

Table 4.38

Mean duration in days (±SD) between subsequent infections in the PE cattle

Boran	Maasai Zebu		Orma Boran		Galana	
	N	Mean	N	Mean	N	Mean
Duration to first infection	20	53.9±56.5	19	34.8±9.5	9	28.0±11.6
Intervals between other infections						
1 and 2	15	107.4±66.3	13	110.2±81.7	9	56.4±50.4
2 and 3	8	94.8±45.7	6	102.2±57.4	6	81.3±54.2
3 and 4	-	-	4	39.5±28.1	3	75.0±30.8

N - Number of observations.

- = Observations too few for analyses.

Table 4.39

Mean duration in days (±SD) that the PE animals remained parasitaemic before the need for drug treatment

	Maasai Zebu		Orma Boran		Galana Boran	
Infection	N	Mean	N	Mean	N	Mean
1	17	18.7±41.2	17	31.2±68.1	9	26.9±51.7
2	13	24.1±52.7	10	29.6±47.9	9	41.4±71.4
3	6	17.5±15.4	6	3.3±4.3	5	6.2±4.5
4	3	5.3±9.2	4	13.0±11.3	2	10.5±12.0

N - Number of observations.

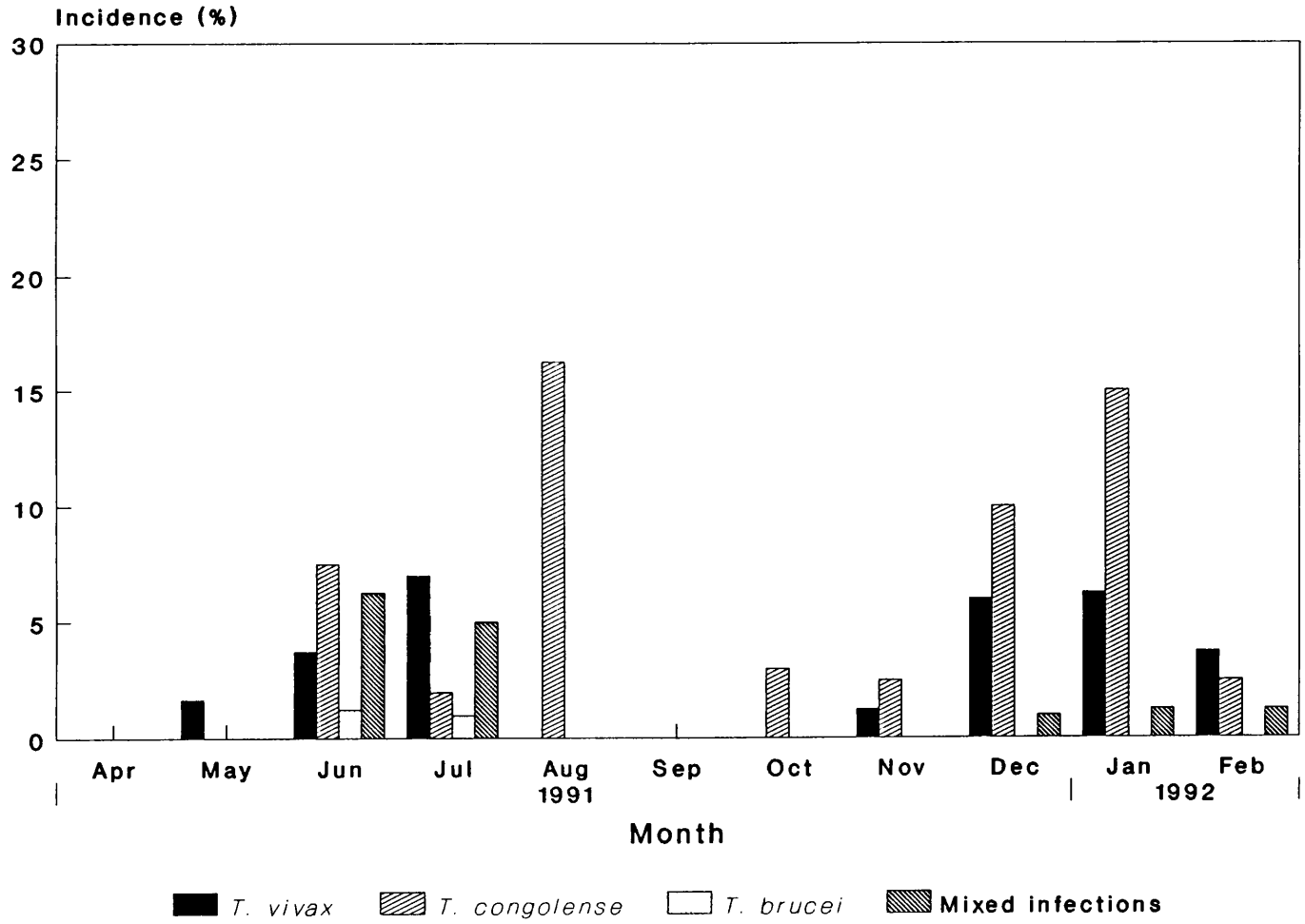


Figure 4.28. Mean monthly trypanosome prevalence in PE the Maasai Zebu.

the various trypanosome species is given in Figure 4.29. A total of 92 infections were diagnosed on buffy coat and the main species was *T. congolense*, making up 54%, while 28%, 15% and 2% were due to T. vivax, mixed infections and T. brucei, respectively.

The results of analysis of variance of the weekly prevalence of trypanosome species in the three breeds are summarized in Table 4.40. The incidence of *T. congolense* was significantly greater than all the other species, while that of *T. vivax* was greater than *T. brucei*. There was no difference in the prevalence of *T. vivax, T. brucei* and the mixed infections.

Orma Boran

In this breed, the seasonal variation in the prevalence of trypanosomiasis occurred in three phases (Figure 4.30). In the first phase from May to August 1991, *T. vivax* infections dominated with the exception of the month of June, when the prevalence of *T. congolense* was more than twice any of the other species. Most of the *T. brucei* and mixed infections were observed in this period and they both attained peak values of 4% and 6.6%, respectively. Infections with the species decreased markedly in the second phase between September and November 1991. In the third phase from December 1991 to February 1992, there was a high number of *T. congolense* infections in the first two months which later decreased, while the *T. vivax* challenge was fairly constant.

Figure 4.31 shows the proportion of infections due to the various species. Of 91 infections detected on the weekly examination on the buffy coat, 53% were due to *T. congolense* as in the Maasai Zebu. The incidence of *T. congolense* was significantly higher than all the others, while *T. vivax* was greater than the *T. brucei* and mixed infections, with no difference between the latter (Table 4.40).

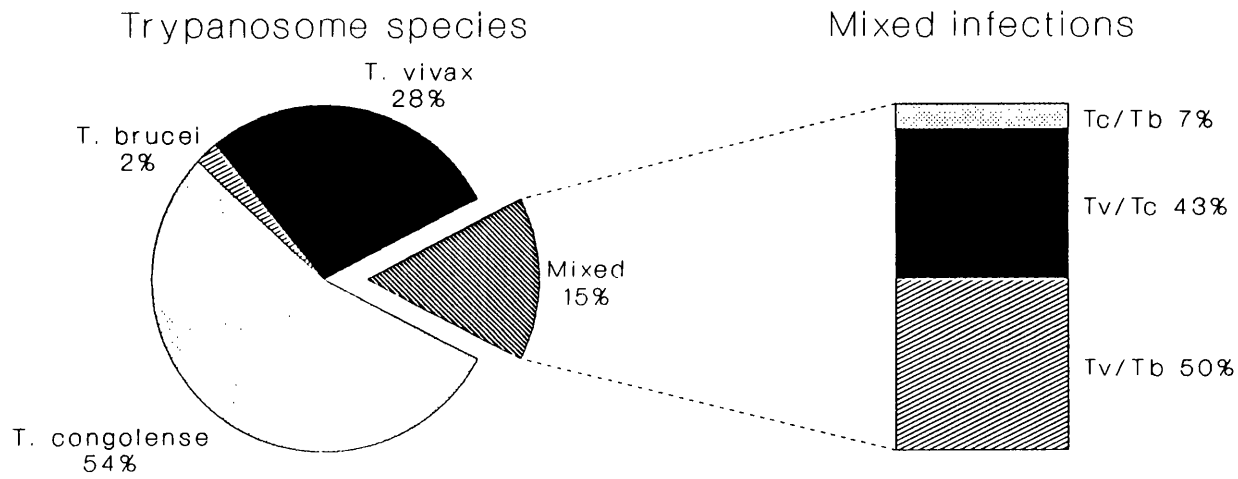


Figure 4.29. The proportion of infections due to the various trypanosome species in the Maasai Zebu with previous exposure.

Table 4.40

Trypanosome species prevalence in the PE groups of three cattle breeds at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
T. vivax	3.2[c]	4.0[c]	4.3[c]
T. congolense	6.1	6.2	8.6
T. brucei	0.2[c,v]	0.5[c,v]	1.1[c]
Mixed	1.6[c]	1.0[c,v]	0.8[c]
Mixed infections			
T. vivax/T. congolense	0.7	0.3	0.3
T. vivax/T. brucei	0.9	0.3	0.5
T. congolense/T. brucei	0.1	0.4	0

[c] Significantly different from *T. congolense* in the column

[v] Significantly different from *T. vivax* in the column

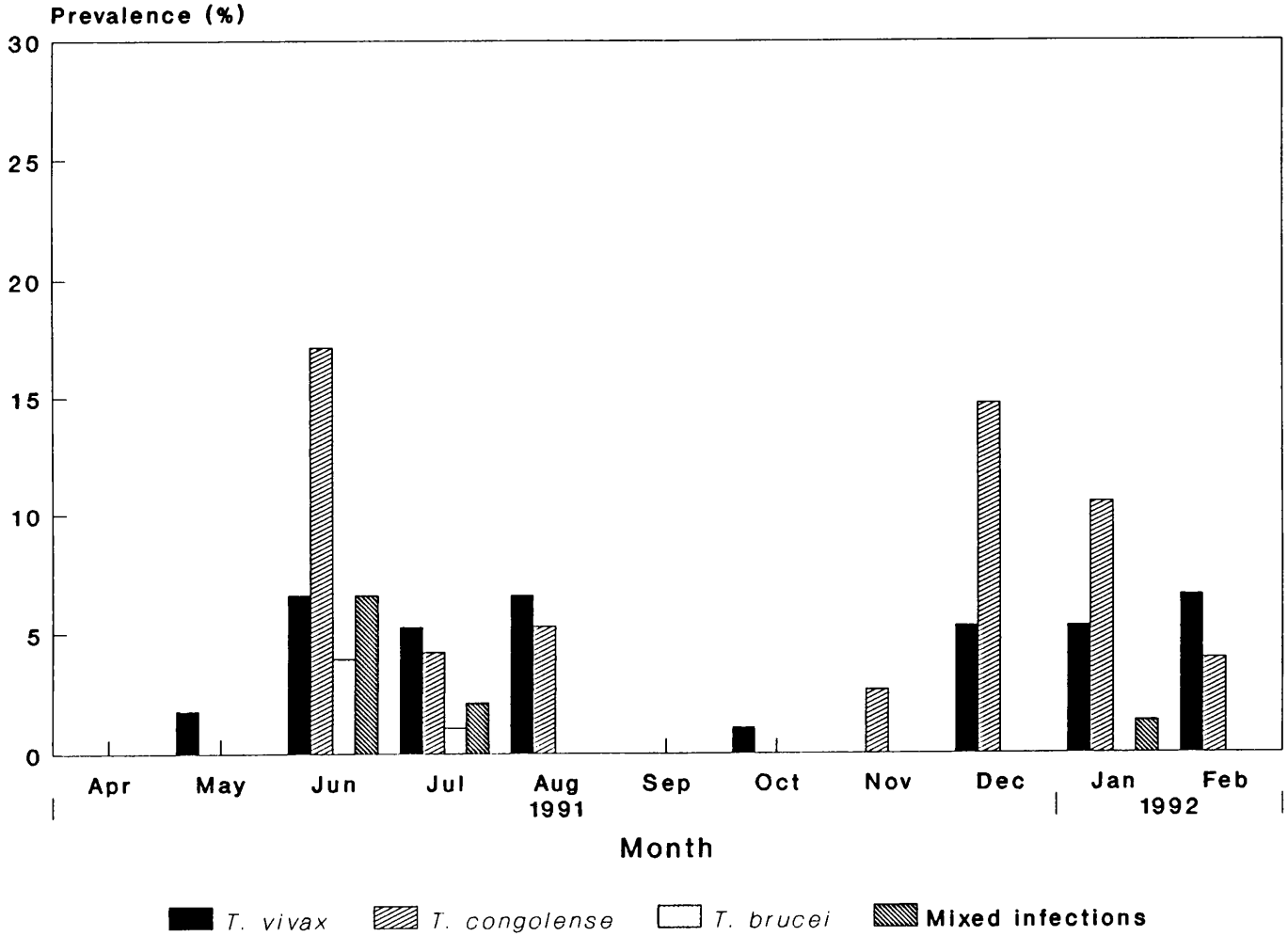


Figure 4.30. The mean monthly trypanosome prevalence (obtained as an average of the weekly prevalence) in the Orma Boran with previous exposure.

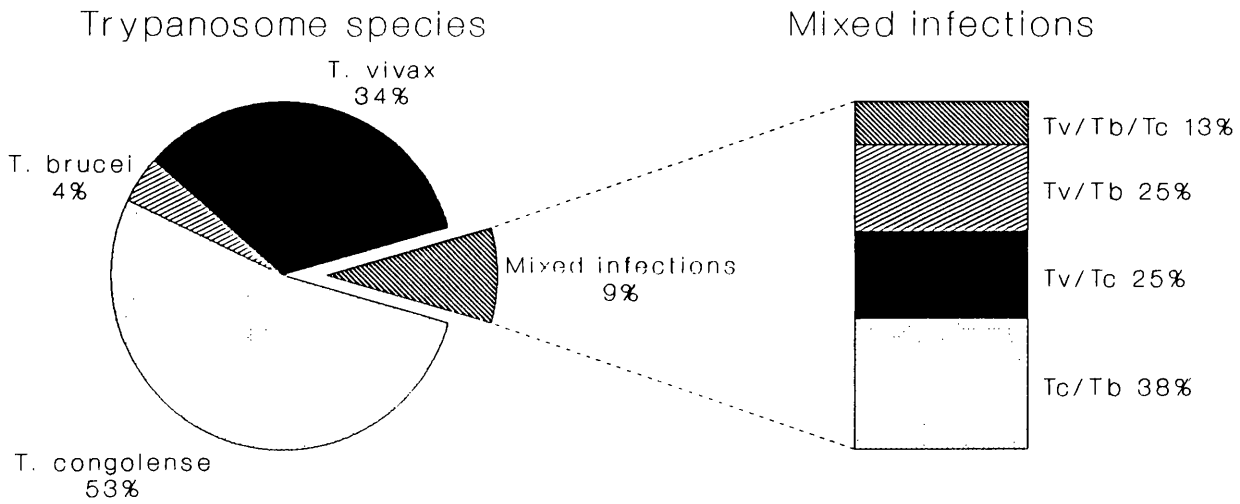


Figure 4.31. Proportion of infections due to the various trypanosome species in the Orma Boran with previous exposure.

Galana Boran

This breed had two periods with high *T. vivax* infections in the first and last three months of the study period (Figure 4.32). Except for the months of September and November 1991, *T. congolense* challenge was constantly high reaching a peak of 27% in December 1991, but declined in January and February 1992. Mixed infections and *T. brucei* occurred mainly during the rainy period from May to July 1991 and both reached maximum values of 5%. A few mixed infections also occurred in December 1991.

Of the 55 infections detected, *T. congolense* was the major species making up 58% (Figure 4.33). The incidence of *T. congolense* was significantly higher than the other species, while that of *T. vivax* was higher than *T. brucei* and mixed infections (Table 4.40). There were no differences between the prevalence of *T. brucei* and mixed infections.

Trypanosome species prevalence among the three breeds

There were no differences in the incidence of any of the trypanosome species or the mixed infections among the breeds (Table 4.40).

vi) Intensity of parasitaemia

A summary of the weekly prevalence of the trypanosome species is presented in Table 4.41. Most of the *T. vivax* infections in the three breeds were in the low parasitemia score class, while majority of the *T. congolense* were in the medium score class. In the Maasai Zebu, *T. brucei* infections were all in the low parasitaemia score class. There were higher parastaemias of *T. brucei* in the Orma and Galana Boran. The majority of mixed infections in the Maasai Zebu and Orma Boran were in the middle parasitemia score class, while in the Galana

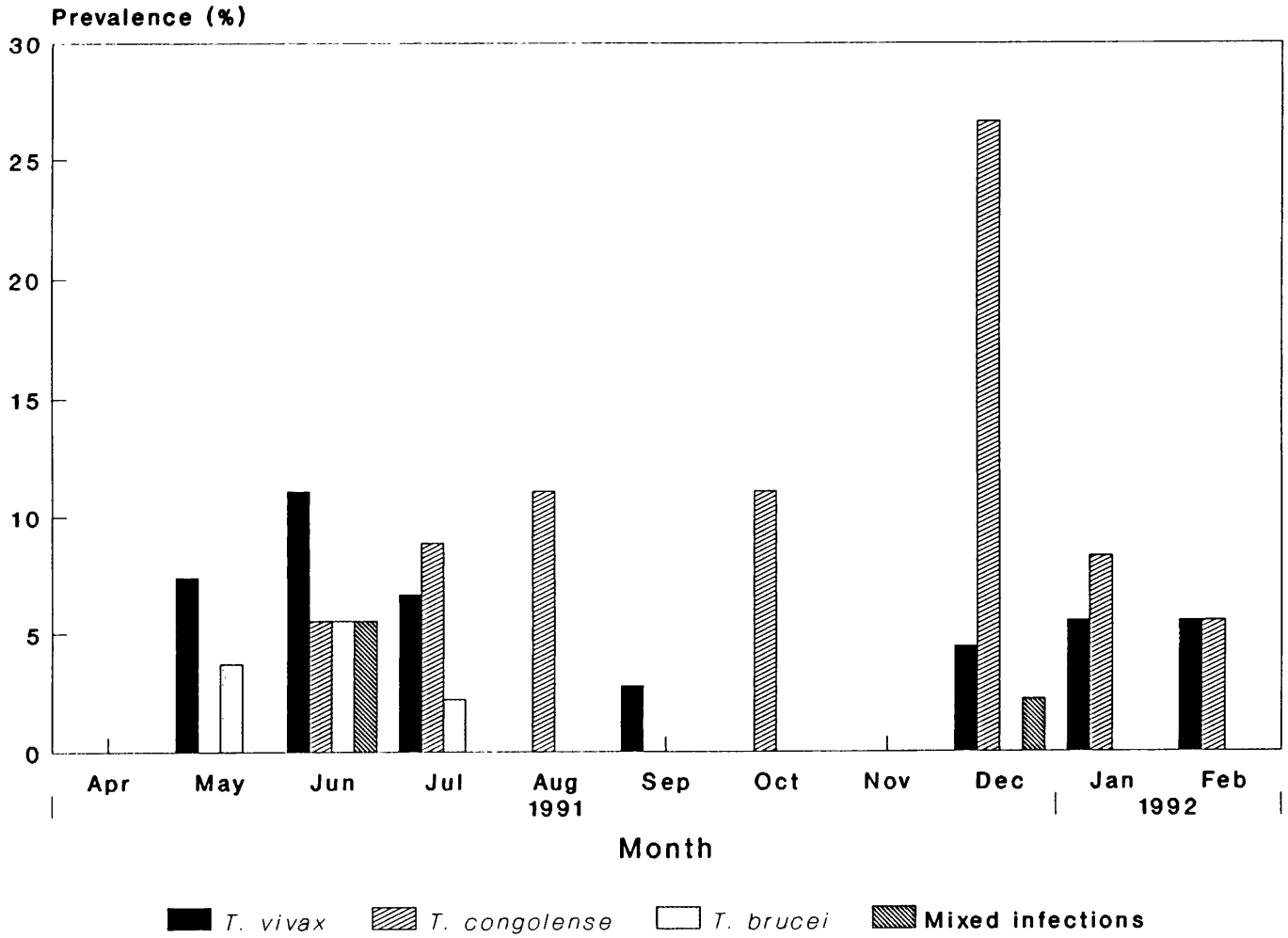


Figure 4.32. The mean monthly trypanosome prevalence (obtained as an average of the weekly prevalence) in the Galana Boran with previous exposure.

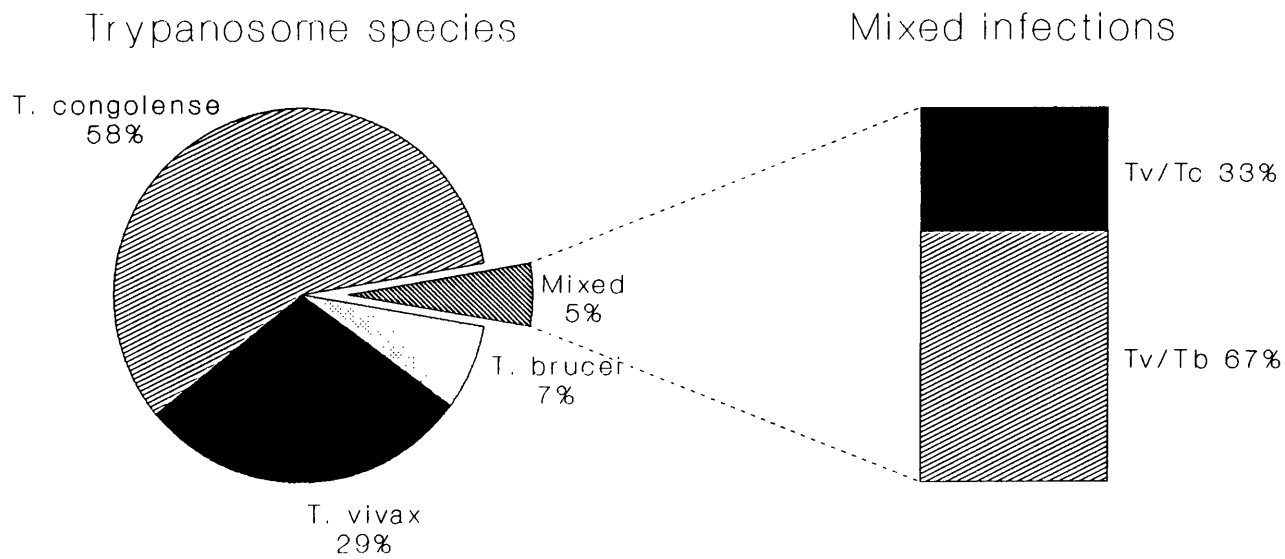


Figure 4.33. Proportion of the total infections due to the various trypanosome species in the Galana Boran with previous exposure.

Table 4.41

Relative frequencies (%) of parasitemia scores of three trypanosome species in the PE Maasai Zebu, Orma Boran and Galana Boran cattle

Trypanosome species	Breed	Total infections	Parasitaemia score class		
			1-2	3-4	5-6
T. vivax	Maasai Zebu	30	60	37	3
	Orma Boran	28	64	36	0
	Galana Boran	16	75	19	6
T. congolense	Maasai Zebu	47	30	66	4
	Orma Boran	46	35	63	2
	Galana Boran	31	29	68	3
T. brucei	Maasai Zebu	2	100	0	0
	Orma Boran	5	40	60	0
	Galana Boran	3	67	33	0
Mixed infections	Maasai Zebu	10	10	90	0
	Orma Boran	4	0	100	0
	Galana Boran	2	50	50	0

Boran there were equal proportions in the middle and low score class. However, the observations on the *T. brucei* and mixed infections were too few for reasonable conclusions to be made.

vii) Treatment requirements

There was a similar pattern to the disease incidence with more treatments occurring in the seasons of high challenge (Figure 4.34). The herd treatment requirements in all the breeds was less than 10% for over 60% of the time. For most of the months more treatments were required in the Galana Boran herd than the other breeds, while the Orma Boran and Maasai Zebu needed a similar number of treatments, but overall, there were no significant differences among the three breeds (Table 4.42).

Frequency of treatments in individual animals

Three Maasai Zebu and two Orma Boran needed no treatment. Fourteen Maasai Zebu (70%), 13 Orma Boran (68%) and seven Galana Boran (78%) required three treatments or less (Table 4.43). The mean number of treatments per animal in the nine months are shown in Table 4.44. There were no significant differences among the three breeds.

Self cure

Seven infections in the Maasai Zebu, five in the Orma Boran and two in the Galana Boran recovered without treatment (Table 4.45). Although the cases of self cure were more in the Maasai Zebu and Orma Boran than Galana Boran, there were no significant differences.

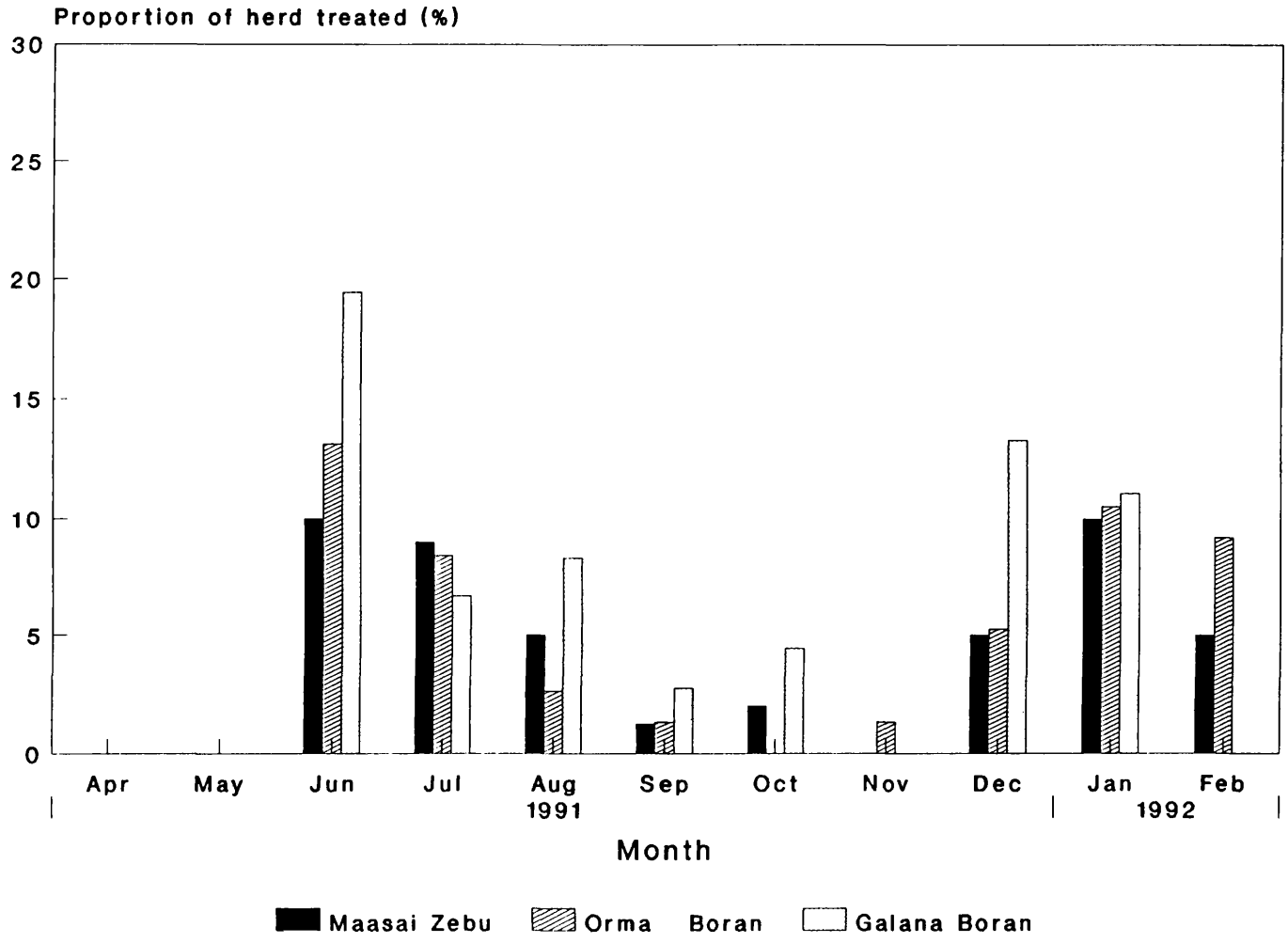


Figure 4.34. The mean monthly herd treatment (obtained as an average of the weekly observations) in the three cattle breeds with previous exposure.

Table 4.42

The mean weekly herd drug treatments (% ± SD) of the PE Maasai Zebu, Orma Boran and the Galana Boran in the high tsetse challenge area at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Observations	42	42	42
Mean*	4.9 ± 6.2	5.3 ± 8.1	6.9 ± 9.2

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	47.2	1.2	not sig.
Time	41	106.7	2.6	not sig.
Error	82	40.9		

* No significant differences among the breeds.

Table 4.43

Frequency distribution of drug treatments in the PE cattle from the three cattle breeds that survived up to the end of the experiment

Number of treatments	Frequency		
	Maasai Zebu N=20	Orma Boran N=19	Galana Boran N=9
0	3	2	0
1	4	7	0
2	7	4	4
3	3	2	3
4	2	1	2
5	1	2	0
6	0	1	0

N - Number of animals

Table 4.44

The mean total number of drug treatments (±SD) per animal required by the PE Maasai Zebu, Orma Boran and Galana Boran the high tsetse challenge area at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Observations	20	19	9
Mean*	2.0 ± 1.4	2.2 ± 1.7	2.8 ± 0.8

Analysis of variance

Source	df	ms	f	$p < 0.05$
Group	2	1.9	0.9	not sig.
Error	45	2.1		

* No significant differences among the breeds.

Table 4.45

Number of infections that self cured in the PE Maasai Zebu, Orma Boran and Galana Boran cattle at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Total number of infections	44	46	27
Number of infections* that recovered spontaneously	7(15.9%)	5(10.9%)	2(7.4%)

* No significant differences among the breeds.

viii) Anaemia

The changes in the weekly PCV observed in the three cattle breeds may be broadly classified into three phases (Figure 4.35). During the first nine weeks (May to mid-July 1991), there was a steady decline in PCV to values ≤23%. For the next five and a half months (mid-July to the end of November 1991), the PCV in the Maasai Zebu and Orma Boran increased to ≥23%, while that of the Galana Boran remained below 23% for a further five weeks before any indication of recovery. From mid-November 1991 to the end of the experiment, the PCV of all the breeds dropped to values ≤23%.

The PCV of the Galana Boran was ≤23% for longer periods than the other breeds. Except for two weeks in June 1991 and one week in January 1992, when PCV of the Orma Boran was less than that of Galana Boran, and the last two weeks in January 1992, when the PCV of the Maasai Zebu was lower than Galana Boran, the latter maintained lower PCV values than the other breeds. Despite the variations with time, there were no significant differences between the three groups for the overall study period (Table 4.46). The PCV of the Galana Boran was higher than that of the Orma Boran for a total of four weeks (two weeks in June, one week in November 1991, and one week of January 1992), equivalent to a total of 11% of the entire period. The PCV of the Galana Boran was higher than the Maasai Zebu for only two weeks at the beginning and two weeks at the end of the observation period (a total of 11% of the time).

d) Performance

Figure 4.36 shows the changes in body weight in the three cattle breeds. There were no weight gains during the first month but thereafter, changes occurred in all the breeds. By the end of the experiment, there were increments of 24.3%,

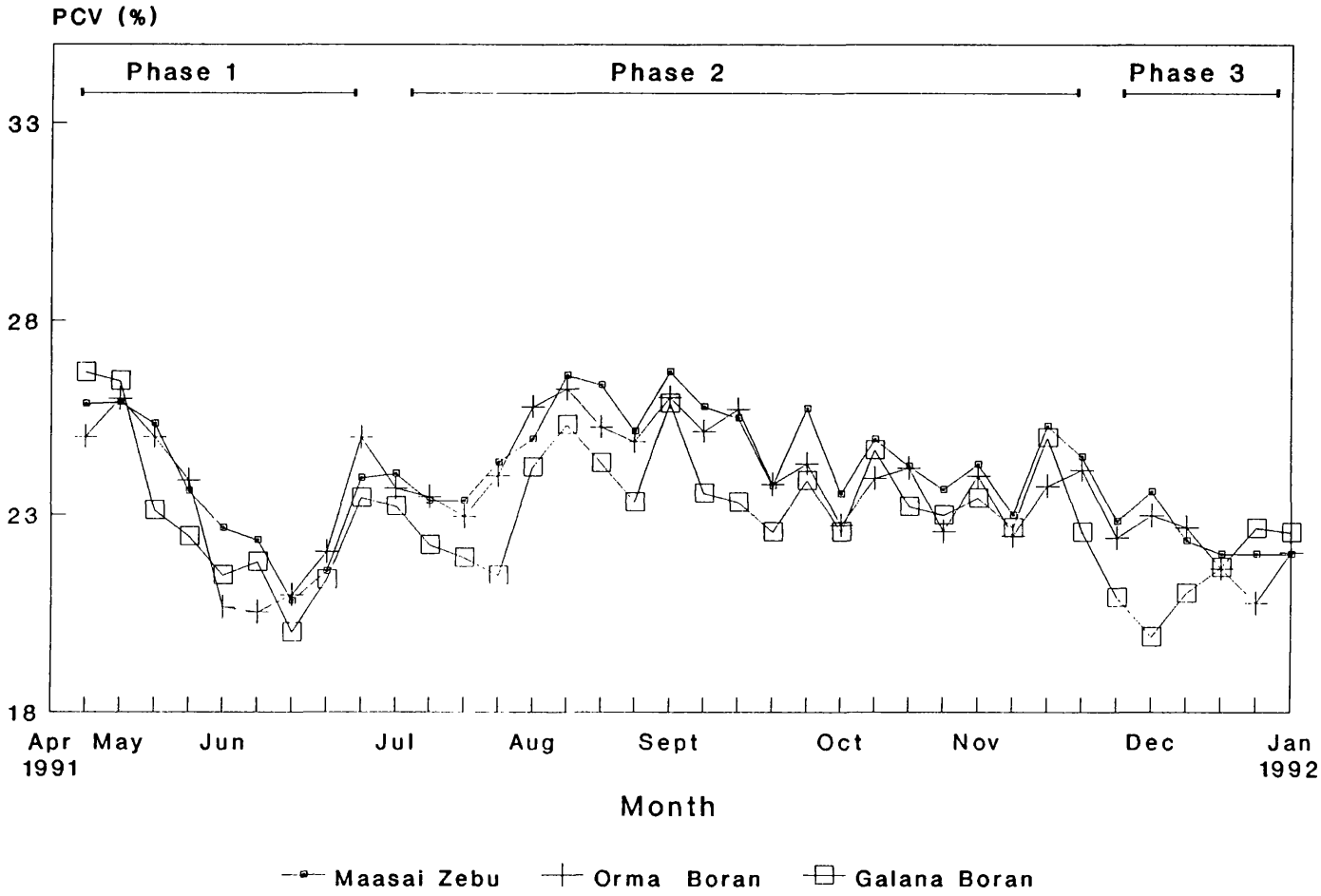


Figure 4.35. The mean weekly packed cell volume (PCV) of the three cattle breeds with previous exposure.

Table 4.46

The mean weekly Packed Cell Volume (PCV) (% ± SD) of the PE Maasai Zebu, Orma Boran and Galana Boran cattle under high tsetse challenge at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Observations	720	684	324
Mean*	24.1 ± 1.5	23.6 ± 1.6	23.0 ± 1.6

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	128.2	1.4	not sig.
Error	45	92.8		
Time	35	107.1	14.1	sig.
Time x group	70	6.4	0.8	not sig.
Error	1575	7.6		

* No significant differences among the breeds.

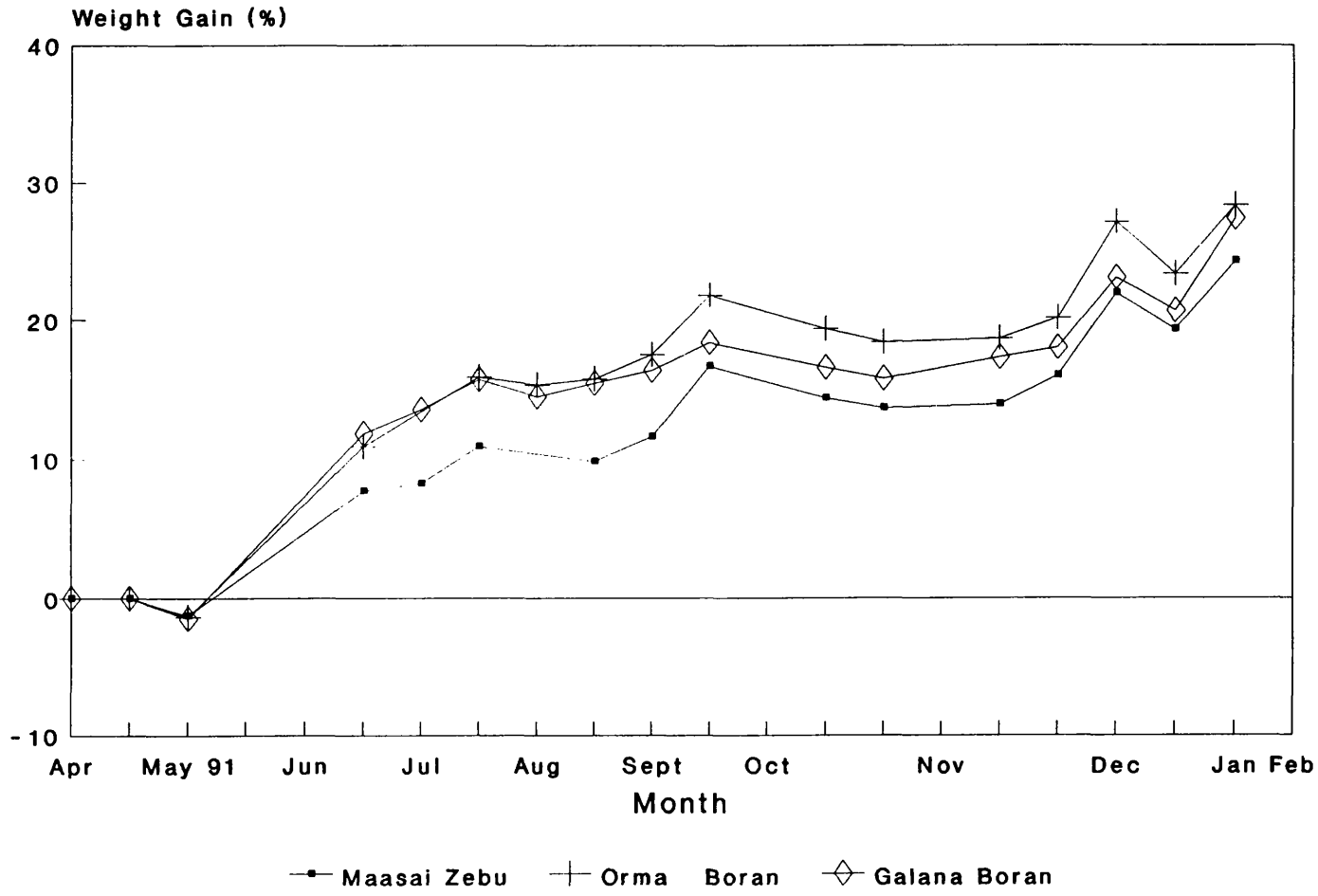


Figure 4.36. The mean fortnightly body weight gains in the three cattle breeds with previous exposure.

28.4% and 27.5% in the Maasai Zebu, Orma and Galana Borans, respectively. The Orma Boran had a significantly higher growth rate than the other breeds (Table 4.47).

4.2.3.2 Observations on newly purchased groups of the Maasai Zebu, Orma Boran and Galana Boran cattle with no history of previous constant exposure to trypanosomiasis

This part of the study was aimed at comparing the susceptibility of the three cattle breeds with no history of previous constant exposure, to high tsetse challenge at the Galana Ranch. In addition, the response of the Maasai Zebu to trypanosomiasis challenge outside the Nguruman area was investigated. The results were compared to those of the previous year (September 1989 to September 1990) at Nguruman, to determine if the lower susceptibility to the trypanosomiasis observed in the Maasai Zebu was generalized or only restricted to the local trypanosome strains at Nguruman.

The Orma and Galana Boran steers used, were purchased locally at the Galana Ranch, while the Maasai Zebu were purchased from Nguruman and transferred to Galana, a distance of over 500 km, as in section 4.2.2. The experimental design has been discussed in section 4.2.2 (Table 4.32), while the other procedures for assessing the trypanosomiasis risk, disease monitoring, drug treatments and the measurement of the growth rate in the cattle, were carried out as in section 3.3. The effect of other disease was also monitored as described previously.

Table 4.47

The mean monthly percentage body weight gains (±SD) of the PE Maasai Zebu, Orma Boran and the Galana Boran in the high tsetse challenge area at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Observations	300	304	143
Mean	12.4 ± 8.6	17.6 ± 10.2*	13.8 ± 9.9

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	2119.0	9.0	sig.
Error	45	234.5		
Time	16	2546.8	190.4	sig.
Time x group	29	31.3	2.3	sig.
Error	654	13.4		

* Significantly higher than the other breeds.

a) The weather and trypanosomiasis risk

These have already been described in section 4.2.3.1

b) Trypanosomiasis

i) Pre-experimental serum samples

Table 4.48 presents the results on the samples screened for trypanosome parasites, antigens and antibodies. No parasites were detected by the buffy coat (DG) technique in any of the breeds. There were three Maasai Zebu, five Orma Boran and two Galana Boran with antibodies. There did not appear to be any major difference among the breeds in the number of cases with antigens.

ii) Disease incidence

Two periods of high challenge occurred from May to August 1991 and November to February 1992 (Figure 4.37). Following the introduction to the high natural tsetse challenge, infections occurred in all breeds within the first two weeks. The disease incidence rose rapidly in the Galana Boran in comparison to the other breeds, and 16% of the herd had been infected by the second week (end of May 1991). In the next three months (June to August 1991), the disease incidence in the Galana Boran remained distinctively higher than in the Maasai Zebu. In the entire study period, the disease incidence was higher in the Galana Boran than other breeds remaining above 10% for a total of six months, compared to two and three months in the Maasai Zebu and Orma Boran, respectively.

While new cases were still encountered in the Galana Boran in the low challenge period (September to November 1991), there were none for a period of one month (November 1991) in the Orma Boran, and two months in the Maasai Zebu (October to November 1991). Over the nine month period of study, the

Table 4.48

Number of positive cases detected on the pre-experimental samples from the NPE cattle using the darkground/phase contrast buffy coat (DG), antibody and antigen ELISA techniques

Diagnostic technique	Trypanosome species detected	Maasai Zebu *N=23*	Orma Boran *N=20*	Galana Boran *N=25*
DG		-	-	-
Ab-ELISA		3	5	2
Ag-ELISA				
	Tc	3	2	2
	Tv	1	1	3
	Tb	-	-	-
	Tc/Tv	1	1	-
	Tc/Tb	-	-	-
	Tv/Tb	-	-	-
	Tv/Tc/Tb	-	-	-

N - Number of cattle examined.
Tv - *T. vivax*
Tc - *T. congolense*
Tb - *T. brucei*
- = Negative

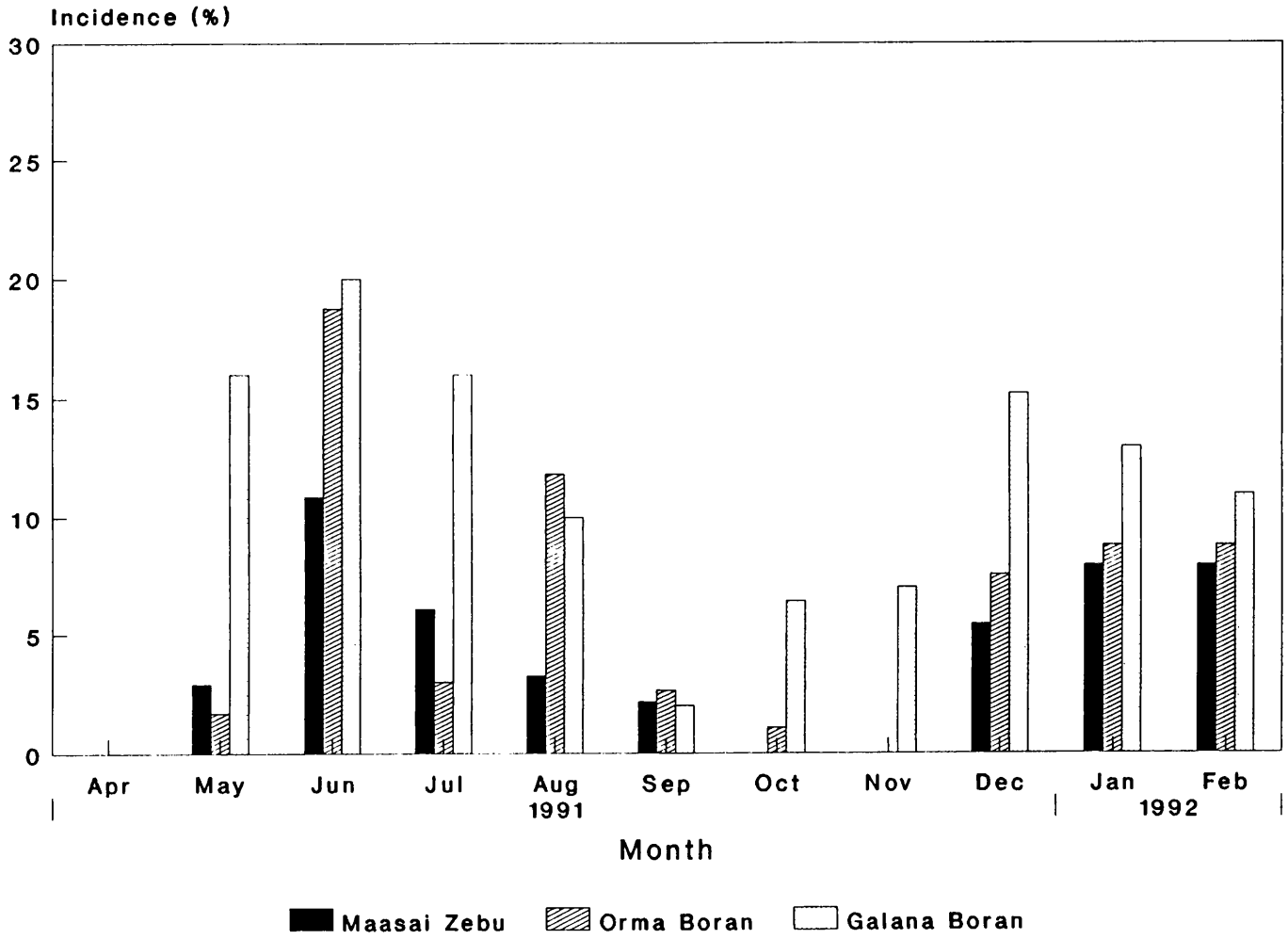


Figure 4.37. The mean monthly disease incidence (obtained as an average of the weekly incidence) in the Maasai Zebu, Orma Boran and Galana Boran with no previous exposure.

incidence in the Galana Boran was more than twice that of the Maasai Zebu and Orma Boran for seven and four months (78% and 44% of the time), respectively. The mean disease incidence in the Galana Boran was significantly higher than both the Maasai Zebu and Orma Boran, while there were no differences between the latter (Table 4.49).

iii) Infections in individual animals.

Table 4.50 shows the frequency of infections in individual animals that survived the entire study period. In total, two animals of the Maasai Zebu and one Orma Boran were never detected parasitaemic. Nineteen Maasai Zebu steers were infected three times or less, compared to 12 Orma Boran and six Galana Boran. A maximum of five, seven and nine infections occurred in two Maasai Zebu, one Orma Boran and one Galana Boran, respectively.

The analysis of variance on the number of the infections per animal in the nine and a half months period (from mid-May 1991 to February 1992) for those that survived, is illustrated in Table 4.51. The Galana Boran had significantly more infections than the other breeds with almost twice that of Orma Boran and two and a half times of the Maasai Zebu. There were no significant differences in the number of infections in the Orma and Maasai Zebu steers.

iv) Intervals between the infections and the duration of parasitaemia

The mean duration to the first infection was 78.9, 49.4 and 20.3 days for the Maasai Zebu, Orma Boran and the Galana Boran, respectively (Table 4.52). The Maasai Zebu had a significantly longer mean duration to first infection than the Galana Boran, while there was no difference between the Orma Boran and either of the other breeds.

Table 4.49

Mean weekly disease incidence (% ± SD) in the NPE Maasai Zebu, Orma Boran and Galana Boran under high tsetse challenge at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Observations	42	42	42
Mean	4.7 ± 6.3	6.4 ± 8.4	11.6 ± 10.1*

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	554.8	12.9	sig.
Time	41	126.8	2.9	sig.
Error	82	43.1		

* Significantly higher than the other breeds.

Table 4.50

Frequency of distribution of the trypanosome infections in the NPE Maasai Zebu, Orma Boran and Galana Boran cattle that survived up to the end of the experiment in the high tsetse challenge at the Galana Ranch

Number of infections	Frequency		
	Maasai Zebu N=22	Orma Boran N=17	Galana Boran N=25
0	2	1	-
1	7	2	-
2	6	7	2
3	4	3	4
4	1	2	4
5	2	1	5
6	-	-	4
7	-	1	3
8	-	-	2
9	-	-	1

N - Number of animals

- = No cases

Table 4.51

Mean number of infections per animal (±SD) in the NPE groups of the three cattle breeds during the entire observation period (between May 1991 and February 1992)

	Maasai Zebu	Orma Boran	Galana Boran
Observations	22	17	25
Mean	2.0 ± 1.4	2.6 ± 1.7	5.1 ± 1.9*

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	60.3	21.3	sig.
Error	61	2.8		

* Significantly higher than the other breeds.

Table 4.52

Mean duration in days (±SD) between infections in the NPE Maasai Zebu Orma Boran and Galana Boran under high tsetse challenge at the Galana Ranch

	Maasai Zebu		Orma Boran		Galana Boran	
	N	Mean	N	Mean	N	Mean
Duration to first infection	21	78.9 ± 83.4	19	49.4 ± 28.8	23	20.3 ± 28.8*
Intervals between other infections						
1 and 2	13	100.2 ± 66.7	17	53.29 ± 48.7*	23	37.4 ± 42.3*
2 and 3	7	111.9 ± 85.8	7	83.3 ± 64.6	21	44.3 ± 32*
3 and 4	-	-	4	24.8 ± 5.5	17	51.4 ± 36

N - Number of observations.

- = Too few observations in the Maasai Zebu for analyses.

* Duration significantly shorter than that of the Maasai Zebu.

The Galana Boran had significantly shorter duration for the intervals between the first to third infections than the Maasai Zebu (Table 4.52). On the other hand, the Orma Boran had significantly shorter interval between the first and second infections than the Maasai Zebu. The analysis was done up to the fourth infection since only two Maasai Zebu and one Orma Boran steers had been infected five times or more by the end of the experiment. There were no significant differences in the intervals between subsequent infections within the breeds, although they appeared longer in the Orma Boran.

Once infected, the mean duration that the animals stayed before the PCV dropped to ≤ 17% is given in Table 4.53. There were no significant differences in the duration of the parasitaemia among the infections within the breeds.

v) Trypanosome species prevalence

Maasai Zebu

In the period with high disease challenge (May to September 1991), the prevalence of *T. vivax, T. congolense* and mixed infections was high, reaching peaks of 7.8%, 4.3% and 6.6%, respectively (Figure 4.38). This was followed by a period of low challenge from October to November 1991, when there were very few parasitaemic animals. The second period with high incidence (December 1991 to February 1992) was dominated mainly by *T. vivax* and *T. congolense* infections attaining maximum levels of 13.6% and 6.8%, respectively. The prevalence of *T. vivax* was higher than *T. congolense* for four months, while the reverse was true for two months. Mixed infections and *T. brucei* were encountered mainly in the rainy period between May and August 1991, when they reached peak prevalence levels of 1% and 6.5%, respectively.

Table 4.53

Mean duration in days (±SD) that the infected NPE animals stayed before the PCV for dropped to ≤17%, hence the need for treatment

	Maasai Zebu		Orma Boran		Galana Boran	
Infection	N	Mean	N	Mean	N	Mean
1	16	14.6 ± 18.2	19	48.3 ± 68.7	25	12.9 ± 7.1
2	11	15.5 ± 23.2	11	22.3 ± 42.9	24	27.9 ± 43.9
3	6	5.8 ± 5.8	7	23.0 ± 34.5	23	23.1 ± 36.4
4	-	-	3	24.8 ± 11.7	18	10.6 ± 12.8
5	-	-	-	-	15	9.8 ± 10.3
6	-	-	-	-	9	5.8 ± 5.6
7	-	-	-	-	5	6.4 ± 11.7

- = Observations too few for analysis.

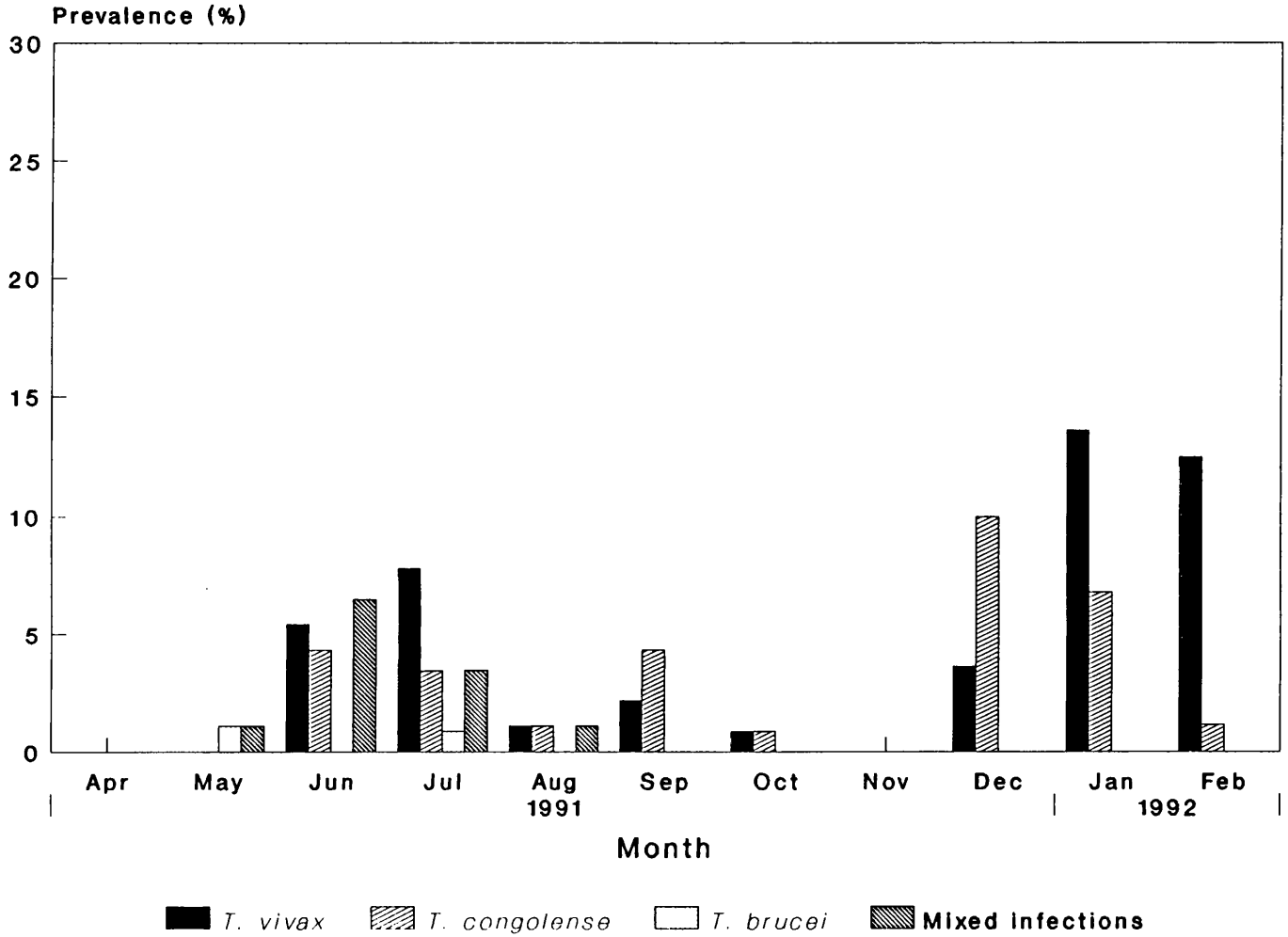


Figure 4.38. The mean monthly trypanosome prevalence (obtained as an average of the weekly prevalence) in the Maasai Zebu with no previous exposure.

A total of 91 infections were recorded and as shown in Figure 4.39, they consisted of 45 (49%) *T. vivax* and 32 (35%) *T. congolense*, while the rest were due to *T. brucei* and mixed species. The major type of mixed infections mainly consisted *T. vivax* and *T. brucei*. There were no differences in the prevalence of *T. vivax* and *T. congolense* but both were significantly higher than either *T. brucei* or mixed infections (Table 4.54).

Orma Boran

The seasonal variation in the prevalence of the trypanosome species in the Orma Boran (Figure 4.40) had a very similar pattern to the Maasai Zebu, but reached higher levels. In the first five months, the prevalence of *T. vivax* reached a maximum of 21% compared to 2.7%, 2.5% and 7.5% for *T. congolense*, *T. brucei* and mixed infections, respectively. The second period with high disease incidence (December 1991 to February 1992) was dominated mainly by *T. vivax* and *T. congolense* both reaching peaks of 16% and 23.5%, respectively.

In the first seven months, this breed appeared to be under mainly *T. vivax* challenge, while during the last three months, there was a high challenge of both *T. vivax* and *T. congolense*. There was no difference in the prevalence of *T. vivax* and *T. congolense* but both were significantly higher than *T. brucei* and mixed infections (Table 4.54). The prevalence of both *T. brucei* and mixed infections was similar.

Of the total 128 infections detected, 63 (49%) were due to *T. vivax*, 44 (34%) to *T. congolense*, while the rest were made up of *T. brucei* and mixed infections (Figure 4.41).

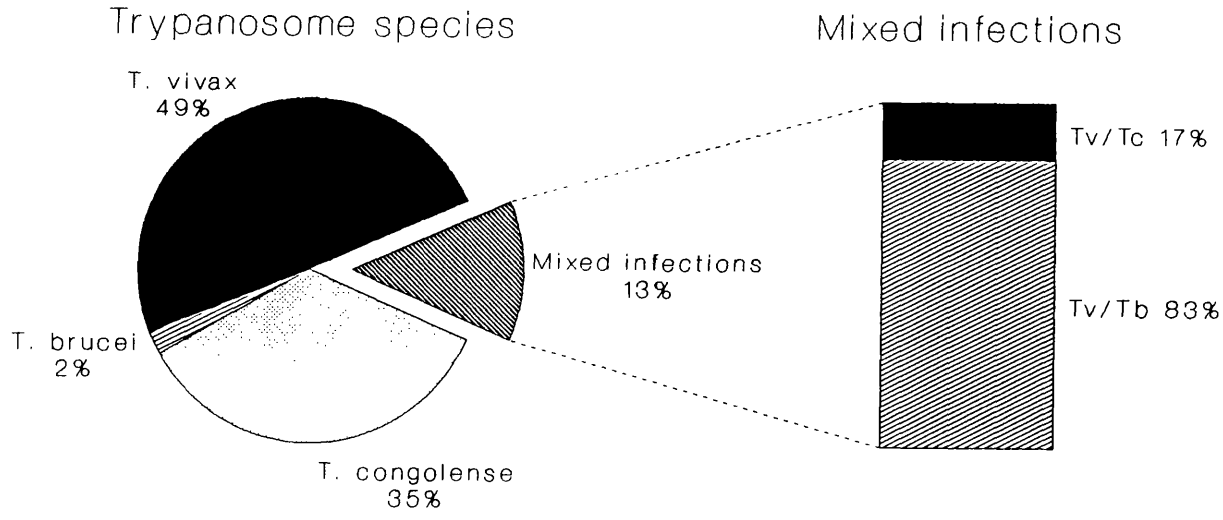


Figure 4.39. Proportion of infections due to the various trypanosome species in the Maasai Zebu with no previous exposure.

Table 4.54

Mean weekly prevalence (%) of the trypanosome species and mixed infections in the NPE groups of the three cattle breeds at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
T. vivax	4.9	8.3[m]	12.2[m]
T. congolense	3.5	5.9[m]	9.4[m]
T. brucei	0.2[v,c]	0.7[v,c]	0.6[v,c]
Mixed	1.3[v,c]	1.9[v,c]	4.3[v,c]
Mixed infections			
T. vivax/T. congolense	0.2	0.5	0.6
T. vivax/T. brucei	1.1	1.2	3.3[m]
T. congolense/T. brucei	0	0	0.1

[m] Significantly lower than the Maasai Zebu along the row.
[v] Significantly lower than the *T. vivax* in the column.
[c] Significantly lower than the *T. congolense* in the column.

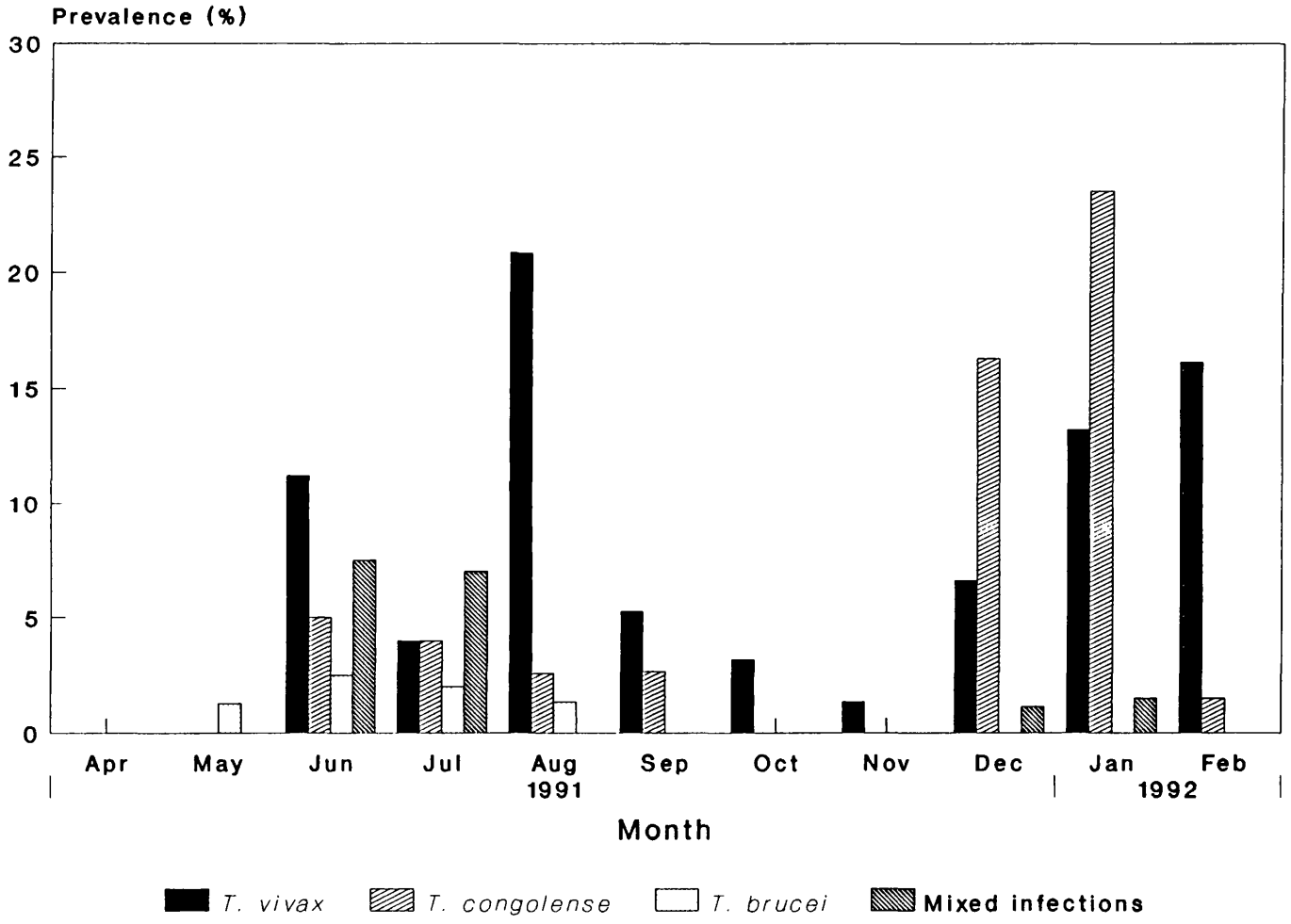


Figure 4.40. Mean monthly trypanosome prevalence (obtained as an average of the weekly prevalence) in the Orma Boran with no previous exposure.

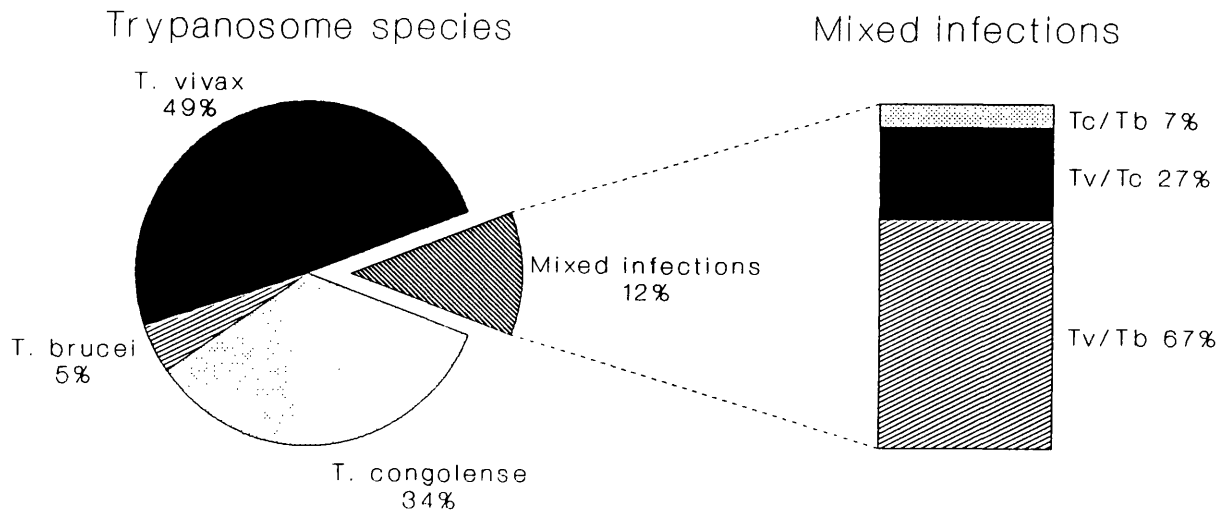


Figure 4.41. Proportion of infections due to the various trypanosome species in the Orma Boran with no previous exposure.

Galana Boran

There were two major phases (Fig 4.42). In the first phase (May to September 1991), this group experienced a high challenge of *T. vivax*, *T. brucei* and mixed infections reaching monthly prevalence peaks of 21%, 6.4% and 26%, respectively. This was followed by a steady increase in the prevalence of *T. congolense* infections and in the second phase (October 1991 to January 1992), this group was mainly under *T. congolense* and *T. vivax* challenge with peaks of 24% and 16%, respectively, in December 1991. *Trypanosoma vivax* dominated in the first phase, while *T. congolense* was dominant in the second phase. In February 1992, the prevalence of *T. vivax* was greater than *T. congolense*. Mixed infections and *T. brucei* were observed to be more prevalent in the rainy season.

Of the 284 infections detected, 125 (44%) were *T. vivax*, and 96 (34%) *T. congolense* and the rest *T. brucei* and mixed infections (Figure 4.43). The predominant combination of the mixed infections was *T. vivax*/*T. brucei* making up 77% of the total mixed. There were no differences in the prevalence of *T. vivax*, and *T. congolense* (Table 4.54).

Trypanosome species prevalence among the three breeds

The prevalence of both *T. congolense* and *T. vivax* in the Maasai Zebu was significantly less than in the other breeds, while there were no significant differences between the Galana and Orma Borans (Table 4.54). There was no difference in prevalence of *T. brucei* among the three breeds. The prevalence of mixed infections in the Galana Boran was significantly higher than in the other breeds, but there were no differences between the Orma Boran and the Maasai Zebu.

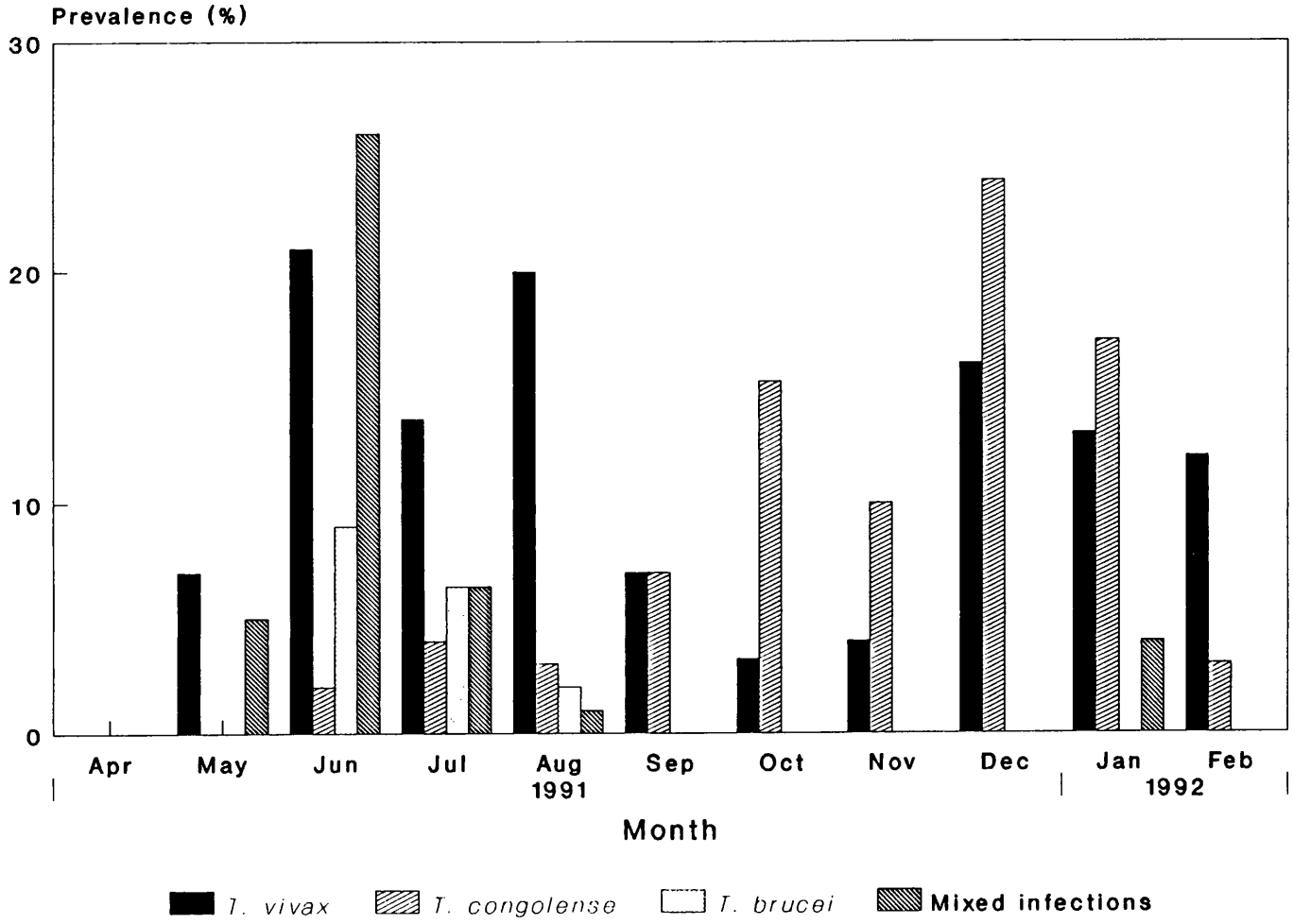


Figure 4.42. The mean monthly trypanosome prevalence (obtained as an average of the weekly prevalence) in the Galana Boran with no previous exposure.

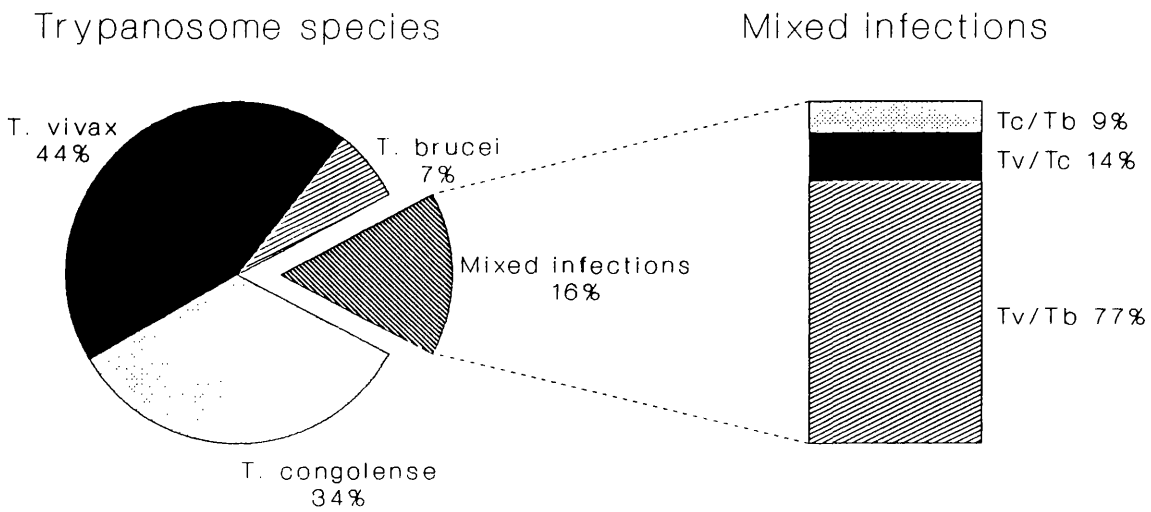


Figure 4.43. Proportion of infections due to the three trypanosome species in the Galana Boran with no previous exposure.

While the prevalence of the *T. vivax/T. brucei* mixed infections was significantly less in the Maasai Zebu than the Galana Boran, there were no differences in the other mixed infections due to *T. vivax/T. congolense* and *T. congolense/T. brucei* among the breeds.

vi) Intensity of parasitaemia

The mean parasitaemia scores of the infections detected on the weekly buffy coat examination are given in Table 4.55. In the Maasai Zebu, the proportion of *T. vivax* infections in the lower and middle score class were similar, while in the Galana and the Orma Boran, there was a higher number with the low parasitaemia score. In all the breeds, majority of *T. congolense* and mixed infections were in the middle score class, while there were similar proportions of *T. brucei* infections with low and medium score classes.

vii) Treatment requirements

The treatment requirements followed a similar pattern to the disease incidence (Fig. 4.44). During the first period of high disease incidence (June to July 1991), there was a high number of treatments in all breeds. No treatments were needed in any of the breeds for the first two weeks. Within the first six weeks (by the end June 1991) 24% of the Galana Boran steers needed treatment compared to 5.4% and 7.5% in the Maasai Zebu and Orma Boran, respectively.

In the next three months (August to November 1991), the drug requirements decreased reaching a minimum in November 1991, when no treatment was required in the Maasai Zebu and Orma Boran. In the last three months (December 1991 to February 1992), there was an increase in drug treatments and, while it remained constantly above 10% in the Galana Boran

Table 4.55

Relative frequencies (%) of parasitaemia scores of three trypanosome species in the NPE cattle groups

Trypanosome species	Breed	Total infections	Parasitaemia score class		
			1-2	3-4	5-6
T. vivax	Maasai Zebu	48	48	52	0
	Orma Boran	53	68	30	2
	Galana Boran	108	61	37	2
T. congolense	Maasai Zebu	31	26	74	0
	Orma Boran	42	29	69	2
	Galana Boran	100	38	61	1
T. brucei	Maasai Zebu	2	50	50	0
	Orma Boran	5	60	40	0
	Galana Boran	24	50	50	0
Mixed infections	Maasai Zebu	7	43	57	0
	Orma Boran	17	0	82	18
	Galana Boran	39	23	77	0

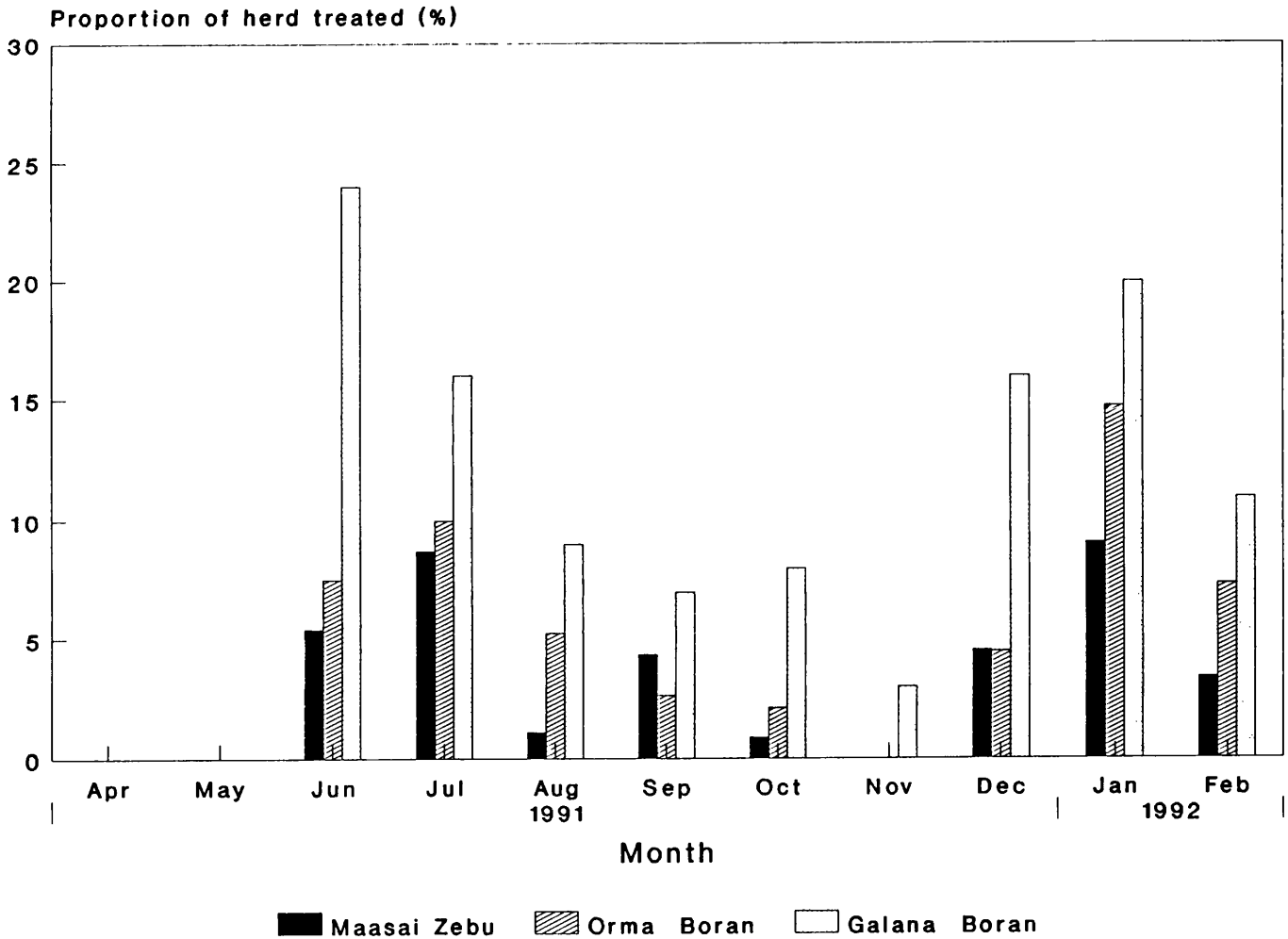


Figure 4.44. Mean monthly herd treatments (obtained as an average of the weekly treatments) in groups of the three cattle breeds with no previous exposure.

and for one month in the Orma Boran, it never exceeded 9% in the Maasai Zebu. For the entire period, the Maasai Zebu herd needed significantly fewer treatments than the other breeds (except in September 1991 when it exceeded that of the Orma Boran), while on the other hand, the Galana Boran required more treatments than the other breeds. The requirements of the Orma Boran were intermediate but closer to the Maasai Zebu. A maximum herd treatment level of 24% in the Galana Boran were reached early in the study, while maxima of 9% and 24% in the Maasai Zebu and Orma Boran, respectively occurred towards the end of the study, in January 1992. Analysis of variance on the weekly herd treatments (Table 4.56) showed that the Galana Boran herd needed significantly more treatments than the Maasai Zebu and Orma Boran.

Treatments in animals that survived up to the end of the study

Five of the seven Maasai Zebu steers infected only once (as previously shown in Table 4.50) in the entire period did not require any treatment (Table 4.57). Eighteen Maasai Zebu needed three treatments or less compared to thirteen Orma and seven Galana Borans. On the other hand, only two Maasai Zebu and three Orma Boran needed more than three treatments compared to eighteen Galana Boran steers. A maximum of eight treatments was administered in three Galana Boran compared to five and seven in one steer of the Maasai Zebu and Orma Boran breeds, respectively.

Table 4.58 shows the mean number of treatments required per animal in those that survived for the nine months. The Galana Boran needed two and three times the number of treatments required by the Orma Boran and Maasai Zebu steers, respectively. The Maasai Zebu needed the least number of treatments, while the Orma Boran was intermediate. The treatment requirements

Table 4.56

Mean weekly herd treatment (% ± SD) in the NPE Maasai Zebu, Orma Boran and Galana Boran cattle kept under high tsetse challenge at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Observations	42	42	42
Mean	3.9 ± 5.4	5.5 ± 6.3	11.8 ± 11.4*

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	730.7	28	sig.
Time	41	98.4	3.8	sig.
Error	82	26.1		

* Significantly higher than the other breeds.

Table 4.57

Frequency distribution of drug treatments in the NPE Maasai Zebu, Orma Boran and Galana Boran cattle kept under high tsetse challenge at the Galana Ranch.

Number of treatments	Frequency		
	Maasai Zebu	Orma Boran	Galana Boran
	N=22*	N=17**	N=25
0	5	-	-
1	4	6	1
2	5	3	1
3	4	4	5
4	1	1	5
5	1	1	4
6	-	-	3
7	-	1	3
8	-	-	3

* Two Maasai Zebu never infected therefore needed no treatment.

** One Orma Boran never infected.

- = No cases.

Table 4.58

The mean total number of drug treatments (±SD) per animal in the NPE cattle during the entire study period (May 1991 to February 1992)

	Maasai Zebu	Orma Boran	Galana Boran
Observations	22	17	25
Mean	1.6 ± 1.5	2.4 ± 1.8	4.8 ± 1.9*

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	67.6	22.1	sig.
Error	61	3.1		

* Significantly more treatments than the other breeds.

were significantly higher in the Galana Boran than in both the Orma Boran and Maasai Zebu.

Self cure

In all the three breeds, cases of self cure were observed. The number of cases regarded to have undergone spontaneous recovery are presented in Table 4.59. The results indicate that, the Maasai Zebu had more cases of this nature than the other breeds, as it had 8(21%) compared to 5(3.9%) and 6(2.1%) in the Orma and Galana Boran, respectively. The differences between the Maasai Zebu and Galana Boran were significant.

viii) Anaemia

The PCV changes occurred in three phases (Figure 4.45). In the first phase (May to July 1991), there was gradual a decline in PCV to ≤25% in all the breeds. The next phase was characterized by a rise in the PCV to ≥25% and it lasted for five months (August to December 1991) in the Maasai Zebu and Orma Boran but only one and a half months (mid-August to September 1991) in the Galana Boran. During the third phase there was another drop in PCV to ≤25% which lasted for one month (January 1992) in the Maasai Zebu and Orma Boran, compared to four months (October 1991 to February 1992) in the Galana Boran.

In the Galana Boran, the PCV was above 25% for a total of two months (25% of the time) compared to six and a half months (81% of the time) in the Maasai Zebu and Orma Boran, during the whole study period. The minimum mean PCV values recorded were 22.5%, 23.1% and 20.5% in the Maasai Zebu, Orma Boran and Galana Boran, respectively. The Galana Boran had significantly lower mean weekly PCV values than the Maasai Zebu and the

Table 4.59

Number of infections in the NPE Maasai Zebu, Orma Boran and Galana Boran cattle that recovered without drug treatment

	Maasai Zebu	Orma Boran	Galana Boran
Total number of infections detected	38	44	127
Number of infections that recovered spontaneous	8(21%)*	5(3.9%)	6(2.1%)

* Significantly higher than the Galana Boran.

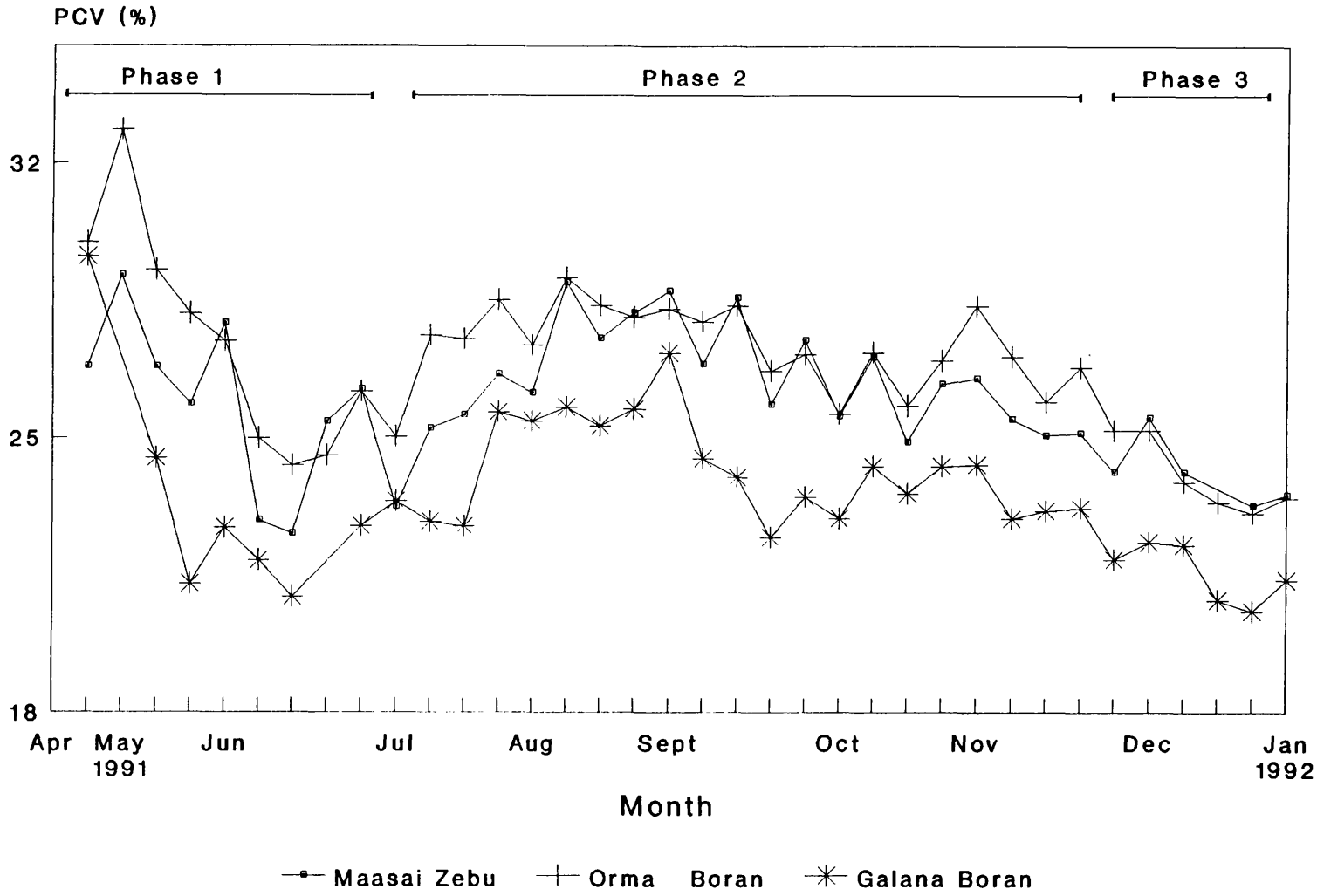


Figure 4.45. The mean weekly packed cell volume (PCV) in groups of the three cattle breeds with no previous exposure.

Orma Boran (Table 4.60). The Orma Boran had a slightly higher mean PCV than the Maasai Zebu but, the difference was not significant.

c) Performance

Body weight changes in the three breeds occurred in two phases (Figure 4.46). In the first seven months (May to November 1991), there were no noticeable changes in body weight. In the last two months, increase in body weights occurred in all the breeds and reached 13.8% in the Maasai Zebu, 6.7% Orma Boran, and 3.2% in the Galana Boran, of the original body weights by the end of the observation period. The mean monthly percentage body weight gain in the Galana Boran was significantly lower than the Orma Boran and Maasai Zebu (Table 4.61).

4.2.3.3 Other diseases

a) Tick-borne diseases

Anaplasmosis

Blood were smears made from animals reported sick by the herdsmen and those with PCV below 20% with no trypanosomes detected, and examined as in section 3.3. Of the 130 smears examined, 15 *Anaplasma marginale* infections were detected, while there were 12 mixed infections of anaplasmosis and trypanosomiasis (Table 4.62). There were no differences among the breeds in the number of cases of anaplasmosis detected.

b) Helminthiasis

Regular surveillance for helminthiasis was maintained by frequent faecal egg counts and, as illustrated on Figures 4.47 and 4.48; the number never reached

Table 4.60

The mean weekly Packed Cell Volume (± SD) of the NPE Maasai Zebu, Orma Boran and the Galana Boran kept under high tsetse challenge at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Observations	794	687	850
Mean	26.0 ± 1.7	26.9 ± 2.2	23.5 ± 1.9*

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	2422.0	14.8	sig.
Error	65	163.8		
Time	35	197.4	15.2	sig.
Time x group	67	20.1	1.6	sig.
Error	2161	13.0		

* Significantly lower PCV than the other breeds.

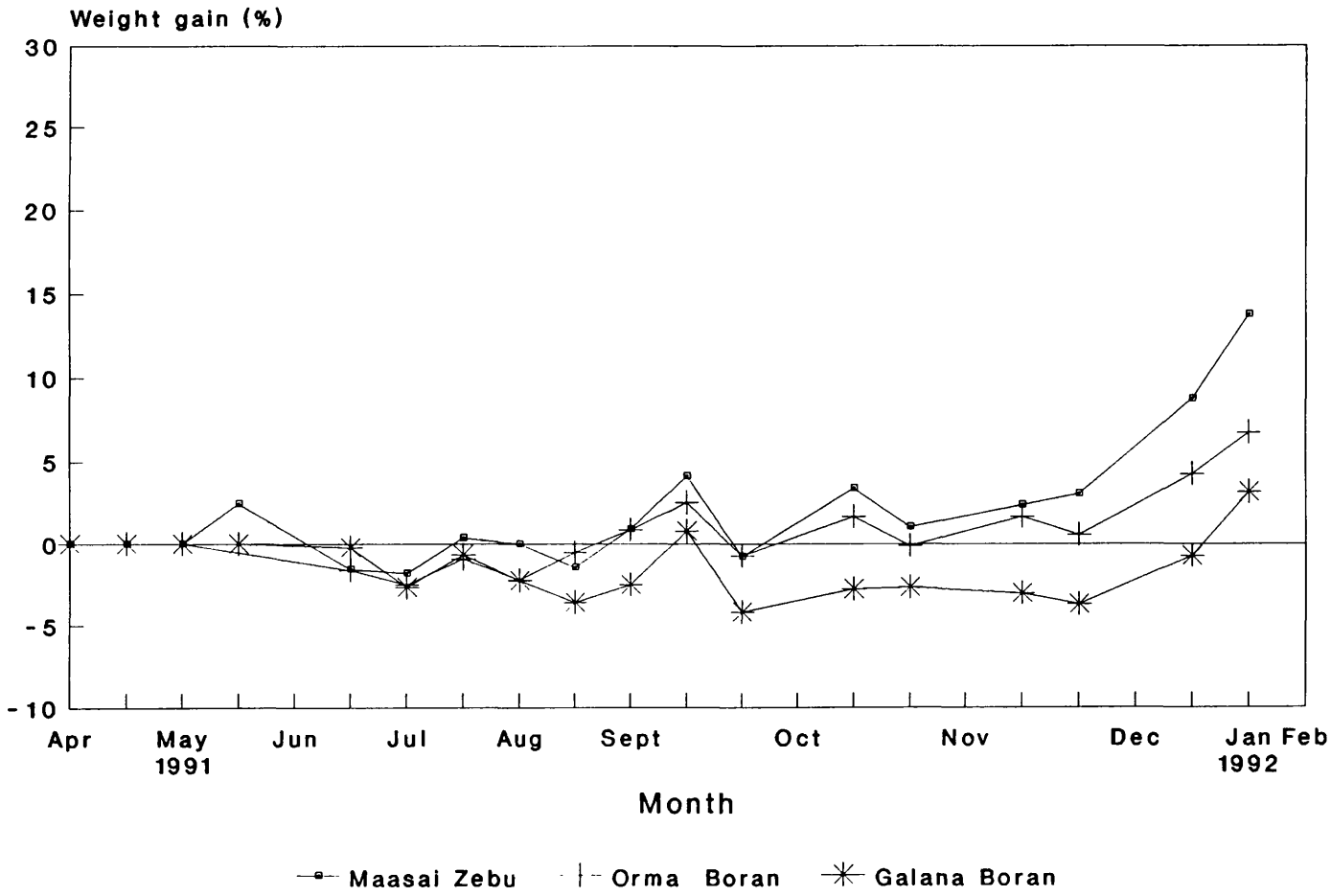


Figure 4.46. The mean fortnightly body weight gains in groups of the three cattle breeds with no previous exposure.

Table 4.61

The mean monthly body weight gains (% ± SD) of the NPE Maasai Zebu, Orma Boran and the Galana Boran in the high tsetse challenge area at the Galana Ranch

	Maasai Zebu	Orma Boran	Galana Boran
Observations	363	268	391
Mean	2.3 ± 3.9	1.3 ± 2.3	-2.6 ± 2.0*

Analysis of variance

Source	df	ms	f	p < 0.05
Group	2	2519.5	7.9	sig.
Error	65	320.1		
Time	16	417.6	34	sig.
Time x group	28	90	7.3	sig.
Error	910	12.3		

* Significantly lower than the other breeds.

Table 4.62

Tick-borne diseases and other conditions encountered in the groups of the three cattle breeds with no previous exposure

Disease	Number of cases		
	Maasai Zebu	Orma Boran	Galana Boran
Anaplasmosis*	4	6	5
Anaplasmosis/* Trypanosomiasis	2	5	5
Eye infections	1	2	-
Skin wound	-	-	1

* A total of 130 blood smears were examined.

- = No cases

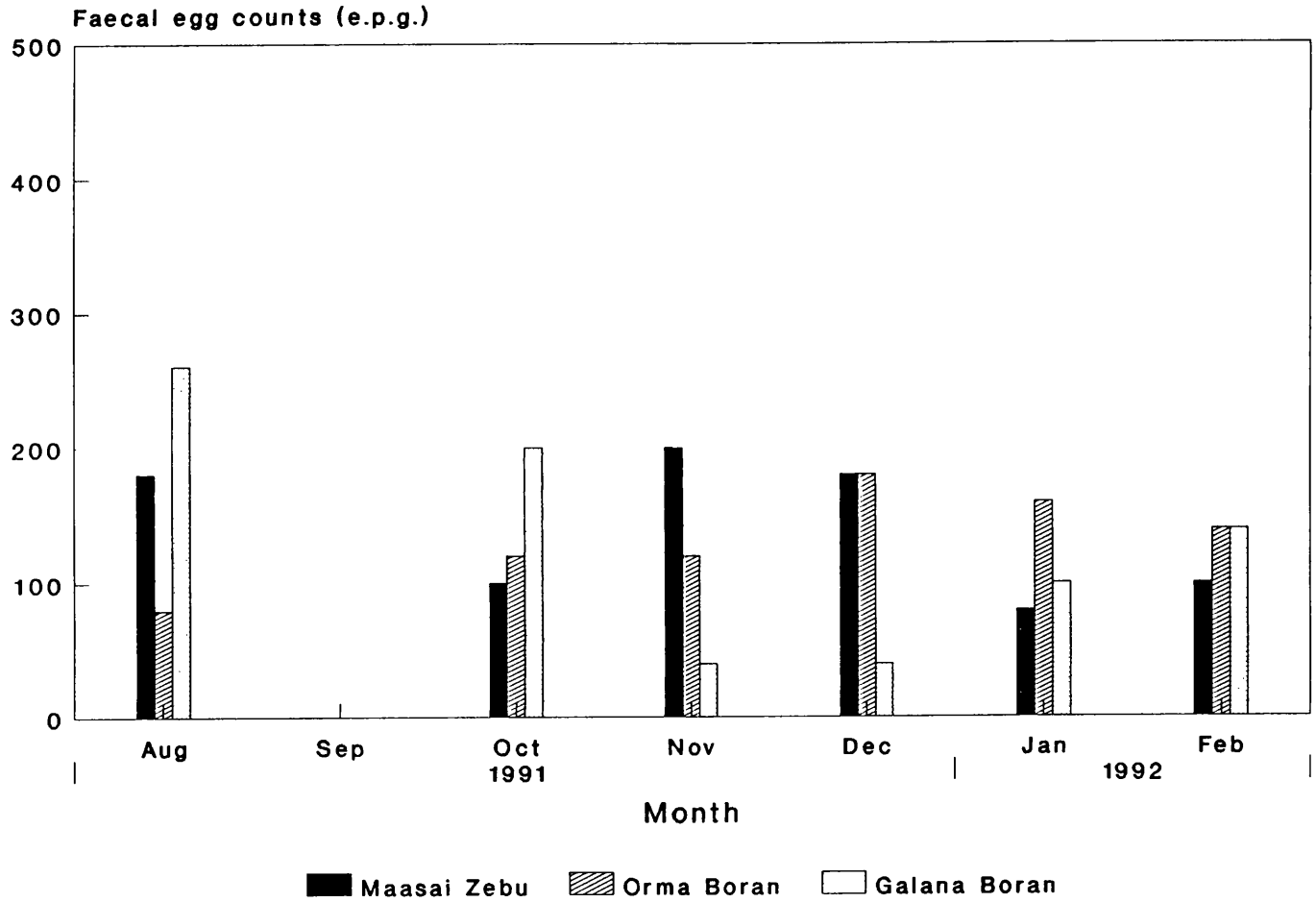


Figure 4.47. The mean monthly faecal egg counts (e.p.g.) in groups of the three cattle breeds with previous exposure.

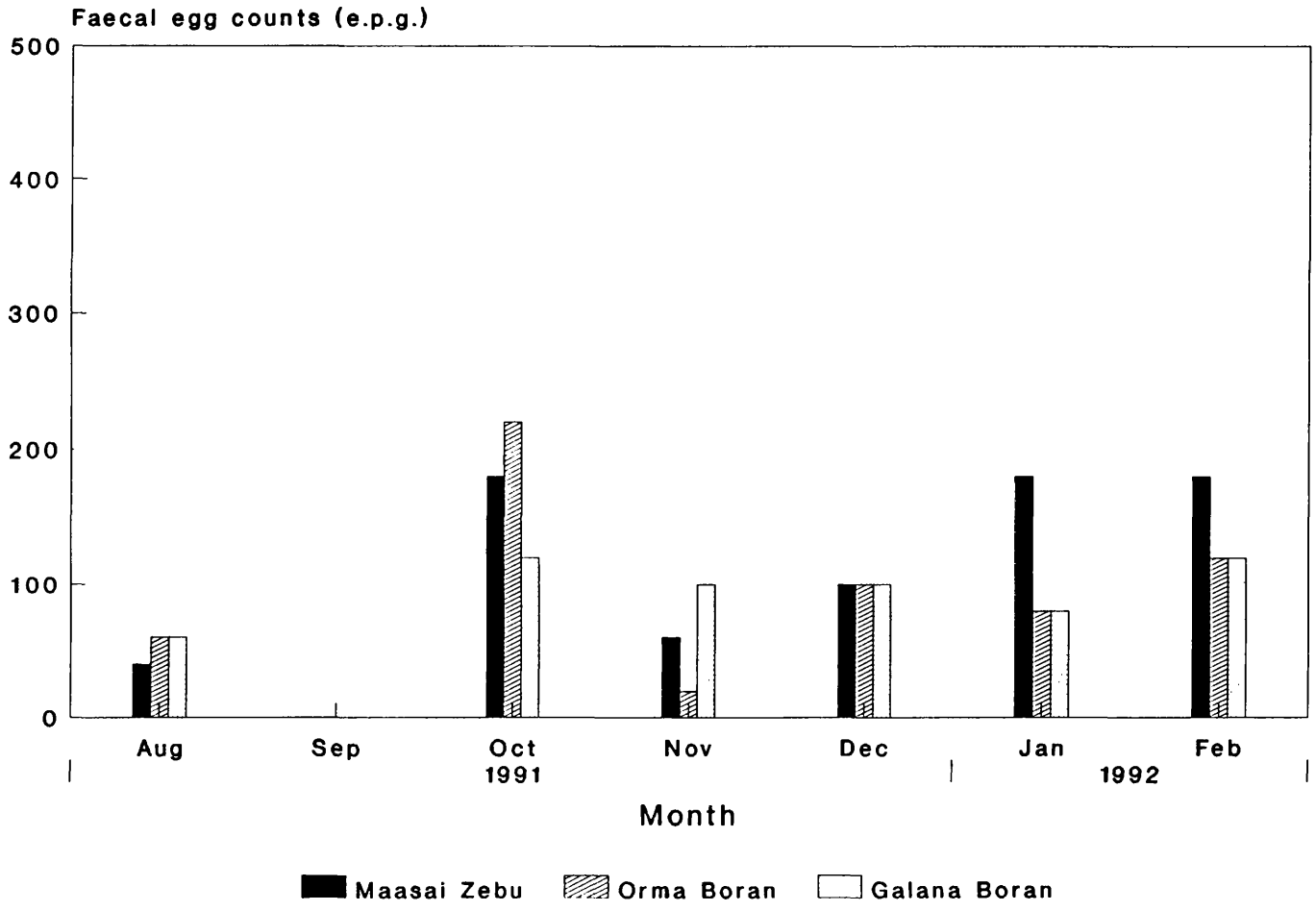


Figure 4.48. The mean monthly faecal egg counts (e.p.g.) of groups from the three cattle breeds with no previous exposure.

levels that could be considered to be of clinical significance.

c) Mortality

There was no mortality due to trypanosomiasis among the animals with previous exposure, while there were four deaths in the groups with no previous exposure (Table 4.63). In the Maasai Zebu, there was no trypanosomiasis related mortality; one steer died following a lion attack.

Two Orma Boran steers died of trypanosomiasis and a third one was killed by lions. In the first case of trypanosomiasis, the animal had a haemorrhagic *T. vivax* infection. It developed acute signs of bleeding from the ears, bloody diarrhoea and died within two days with a PCV of 15%, despite treatment. The second animal initially had a *T. congolense* infection but a week later, mixed infection with *T. vivax* was detected. Two days later, the animal became recumbent and died after three days with a PCV of 23%. The third Orma Boran from the same group died from a lion attack. There were no deaths in the Galana Boran.

4.2.3.4 Comparison of the groups with and without previous exposure at the Galana Ranch

Table 4.64 summarizes the parameters monitored in the animals with previous exposure (PE) and those without (NPE) at the Galana Ranch. There were no differences in the prepatent period among the PE groups, while among the NPE, the Maasai Zebu had significantly longer prepatent period than the Galana Boran. In addition, the NPE Maasai Zebu had a longer prepatent period than the PE Orma and Galana Borans. Similarly, there were no differences in the disease incidence in the PE groups, while the NPE Galana Boran had significantly higher

Table 4.63

Mortality among the NPE Maasai Zebu, Orma Boran and Galana Boran cattle at the Galana Ranch*

	Trypanosomiasis	Predators (lions)
Maasai Zebu	0	1
Orma Boran	2	1
Galana Boran	-	-

* Deaths occurred only in the groups with no previous exposure.

Table 4.64

Summary of the results on groups of the three breeds with (PE) and without (NPE) previous exposure at the Galana Ranch (1991/1992)

	Previous exposure (PE)			No previous exposure (NPE)		
	Maasai Zebu *a*	Orma Boran *b*	Galana Boran *c*	Maasai Zebu *d*	Orma Boran *e*	Galana Boran *f*
Prepatent period	53.9	34.8	28.0	78.9[b,c,f]	49.4	20.3
Disease incidence	5.5	5.6	7.7	4.7	6.4	11.6**
Infections/animal/year	3.2	3.2	4	2.7	3.5	6.8**
Treatments/animal/year	2.7	2.9	3.7	2.1	3.2	6.4**
Herd treatment (%)	4.9	5.3	6.9	3.9	5.5	11.8**
Prevalence of *T. vivax*	3.2	3.9	4.3	4.9	8.3*	12.1*
Prevalence of *T. congolense*	6.1	6.2	8.7	3.5[c,f]	5.9	9.4
Self cure	7 (15.9)	5 (10.9)	2 (7.4)	8 (21)	5 (3.9)	6 (2.1)
PCV***	24.1	23.6	23.0	26.0	26.9	23.5
Growth rate	12.4[b]	17.6*	13.8[b]	2.3[a,b,c]	1.3[a,b,c]	-2.6*
Mortality						
Trypanosomiasis	-	-	-	-	2	-
Predators	-	-	-	2	-	-

a,b,c - Refer to the Maasai Zebu, Orma and Galana Borans with previous exposure, respectively; *d,e,f* - refer to the groups with no previous exposure.

Superscripts indicate groups with significantly different results.

* Significantly different from all the other groups.

** Group *f* - significantly different from all the others, while no differences among the other groups.

*** Groups *d* and *e* significantly different from all other groups.

Numbers in parenthesis represent percentages.

incidence and number of infections in individual animals than all the other groups.

There were no differences in the prevalence of *T. vivax* and *T. congolense* among the PE groups. The NPE Orma and Galana Borans had higher *T. vivax* prevalence than all the other groups, and this was an indication of their higher susceptibility in comparison to the PE Orma and Galana Borans. In the NPE Maasai Zebu, prevalence of *T. vivax* was significantly lower than in NPE Orma and Galana Borans, while that of *T. congolense* was significantly lower than in both PE and NPE Galana Boran. These observations suggest that, the differences observed in the disease response between the PE Galana Boran and NPE Maasai Zebu were largely due to *T. congolense*. Overall, the PE groups were predominantly under *T. congolense* challenge, while the NPE were under *T. vivax* challenge. It was observed that the *T. congolense* and *T. vivax* infections in the NPE groups developed higher parasitaemia than in PE (Tables 4.41 and 4.55).

The NPE Galana Boran needed significantly higher treatments than all the other groups, while there were no differences among the PE groups. In both the PE and NPE groups, the Maasai Zebu had the highest proportion of infections in which the hosts showed the self cure phenomenon.

Both NPE Maasai Zebu and Orma Boran maintained higher PCV than all the other groups. In all breeds, drops in PCV following periods of predominantly *T. vivax* challenge recovered rapidly, while PCV drops associated with high *T. congolense* challenge were chronic in nature and took long for recovery to be noticed (Figures 4.35 and 4.45). It was also noticed that, the PE groups experienced mainly a *T. congolense* challenge, while NPE were mainly under *T. vivax* challenge.

All breeds developed anaemia which became severe under increased disease challenge but later showed a faster recovery in the NPE Maasai Zebu and Orma Boran, while it remained chronically low in the Galana Boran. Among the PE groups, the PCV appeared lower in the Galana Boran throughout but overall, the three groups were fairly close with no significant differences. The PE groups showed a better ability to gain weight than the NPE under the high tsetse challenge.

In conclusion, in the PE, there were no differences among the breeds except that, the Orma Boran had a significantly higher growth rate. In contrast, in the NPE, the Galana Boran was more susceptible to trypanosomiasis than the Orma Boran and Maasai Zebu, as reflected by the shorter prepatent period, higher disease incidence and trypanosome prevalence, development of a higher degree of anaemia, more treatment requirements and a lower growth rate.

4.2.3.5 Comparisons among the three breeds in the high challenge area at Nguruman and the groups without previous exposure at the Galana Ranch

Table 4.65 presents a comparison on the breed pairs on animals kept at high challenge area at Nguruman and those with no previous exposure (NPE) at the Galana Ranch. In the NPE animals, in addition to the differences among the breeds observed in the previous year at Nguruman, the Maasai Zebu had a lower prevalence of *T. vivax* than the Galana Boran and Orma Boran. At the Galana Ranch, the Maasai Zebu exhibited a lower susceptibility to trypanosomiasis than the Galana Boran, similar to that observed at Nguruman. These observations indicate that the higher resistance to trypanosomiasis of the Maasai Zebu than Galana Boran observed at Nguruman was not restricted to the local trypanosome strains at Nguruman.

Table 4.65

Summary of the main observations among the three breeds in the high challenge area at Nguruman (1989/1990) and the groups without previous exposure (NPE) at the Galana Ranch (1991/1992)

	Maasai *vs* Orma Zebu		Maasai *vs* Galana Zebu		Orma *vs* Galana Boran	
	Nguruman	Galana Ranch	Nguruman	Galana Ranch	Nguruman	Galana Ranch
Prepatent period	+	-	+	+	+	-
Disease incidence	-	-	+	+	+	+
Infections	-	-	+	+	+	+
Treatments	-	-	+	+	+	+
Prevalence of *T. vivax*	-	+	-	+	-	+
Prevalence of *T. congolense*	-	-	+	+	+	-
PCV	+	-	+	+	+	+
Growth rate	-	-	+	+	-	+

+ Significant differences between the breeds.

- No significant differences between the breeds.

4.3 DISCUSSION

4.3.1 OBSERVATIONS ON THE THREE CATTLE BREEDS AT THE NGURUMAN ESCARPMENT

During the one year observation period in the high tsetse challenge area at Nguruman, from September 1989 to September 1990, trypanosomiasis was the most common disease found in all the cattle breeds. Most infections occurred in the periods with high rainfall and fly high density.

On introduction into the tsetse challenge, all animals became infected. Significant differences were observed among the Maasai Zebu, Orma Boran and Galana Boran in the prepatent periods, incidence of trypanosomiasis, trypanosome prevalence, degree of anaemia as assessed by the PCV, treatment requirements and growth rates. The prepatent period was significantly different among the three breeds, with that of the Maasai Zebu being the longest, the Galana Boran shortest, while the Orma Boran was intermediate; in fact the prepatent period in the Maasai Zebu and Orma Boran were almost five and three times, respectively, longer than in the Galana Boran. This could suggest that under field conditions, some animals are less liable to tsetse attack (reviewed by Murray *et al.,* 1982). Alternatively, it could imply that under natural challenge, all breeds encountered a similar number of infective bites and, while the Galana Boran succumbed to majority of the trypanosome strains, the Maasai Zebu and the Orma Boran did not develop parasitaemia until they encountered the more virulent strains.

Similar differences in the prepatent period between the Orma and Galana Boran were observed in previous studies under natural tsetse challenge at the Galana Ranch (Dolan *et al.,* 1985). However, when the two breeds were exposed to fly and needle challenge to both *T. congolense* and *T. vivax* in the

laboratory, no significant differences occurred (Ishmael, 1988). In similar studies by other workers involving breeds of varying susceptibility, no differences were observed between the N'Dama and Zebu under natural challenge in The Gambia (Murray *et al.,* 1979b; 1981a) and Senegal (Toure *et al.,* 1978), but it was noticed that most of infections in the Zebu occurred earlier. Paling *et al.,* (1991) found no differences to the duration of detectable parasitaemia between N'Dama and Boran cattle infected with *T. congolense* transmitted experimentally by tsetse fly.

In this study, the incidence of trypanosomiasis was significantly higher in the Galana Boran than the other breeds, while there were no differences between the Maasai Zebu and Orma Boran. These results were consistent with field observations at the Galana Ranch, where in a series of experiments, higher disease incidence in the Galana Boran than Orma Boran was repeatedly observed (Wilson *et al.,* 1983, 1986; Njogu *et al.,* 1985a,b).

On the average, the Galana Boran had 1.9 and 1.4 times the number of infections in the Maasai Zebu and Orma Boran, respectively, in the animals that survived the entire study period. These results are close to the field observations at the Galana Ranch, where the Galana Boran was found to get twice the number of infections in the Orma Boran (Njogu *et al.,* 1985b).

Some but not all intervals between infections showed significant differences among the breeds. The Orma Boran had a longer interval between the second and third infection than the Galana Boran, while the Maasai Zebu had a longer interval between the fourth and fifth infections than the other breeds, with no differences between the other intervals among the three breeds.

The lack of differences in subsequent intervals between infections in the current study, would indicate that repeated infections did not appear to confer

any immunity. These results were similar to those of Njogu *et al.,* (1985b) who observed no differences in intervals between infections with *T. vivax* between the Orma and Galana Boran. In the same way, Roberts and Gray (1973), found no differences among the N'Dama, Muturu and Zebu under natural tsetse challenge. On the other hand, Stephen (1966) reported longer intervals to reinfection following treatments in the N'Dama than in the Zebu. Increase in intervals between infections has been reported in *Bos indicus* breeds maintained under chemotherapy in East Africa (Whiteside, 1962a; Wilson *et al.,* 1976; Trail *et al.,* 1987).

There were significant differences in the prevalence of some but not all trypanosome species among the three breeds. The major trypanosome species in the three breeds was *T. vivax.* There were no differences in the prevalence of *T. vivax* among the breeds, while the prevalence of *T. congolense* was significantly higher in the Galana Boran than Maasai Zebu and Orma Boran. Though there were no differences in the prevalence of *T. vivax,* it was noticed that, the appearance of infections by this species occurred earlier in the Galana Boran, while it was gradual in the Orma Boran and Maasai Zebu. It is possible that, the high prevalence of *T. vivax* seen in all the breeds in the first six months (September 1989 to January 1990), either made the animals, particularly the Galana Boran, more susceptible to *T. congolense* infections, or all the cattle breeds developed some degree of resistance to *T. vivax* after the initial six months of challenge with this species before succumbing to *T. congolense.* Alternatively, there may have been a seasonal variation in fly infections as has been previously reported at Nguruman (Tarimo *et al.,* 1985). These results agree with the observations made by Trail *et al.,* (1991a,b) that under natural trypanosomiasis challenge, N'Dama cattle would appear to acquired immunity to

T. vivax more readily than *T. congolense.*

There were no differences in the intensity of parasitaemia in infections with any of the species among the three breeds. Majority of the *T. congolense* were in the medium score parasitaemia class compared to *T. vivax,* where most were in the low parasitaemia score class. These results were different from other studies where the less susceptible breeds like the N'Dama have been shown to develop lower intensity of parasitaemia than the Zebu infected with *T. congolense*, *T. brucei* (Dargie *et al.,* 1979) and *T. vivax* (Saror *et al.,* 1981). In the same way, Akol *et al.,* (1986) found that the Zebu developed higher parasitaemia than the taurine breeds (N'Dama, N'Dama/Baoule and Baoule). Ishmael (1988) reported a higher prevalence and intensity of parasitaemia in the Galana Boran than Orma Boran following both experimental and needle infection with *T. vivax* and *T. congolense.* Murray *et al.,* (1981a) also noticed significantly higher prevalence of *T. vivax* and *T. brucei* between the N'Dama and Zebu, but not in *T. congolense;* in addition, there was a higher level and longer duration of *T. vivax* parasitaemia in the Zebu. Once the parasitaemia was detected in the Zebu, it persisted till death, while it was more transient in the N'Dama.

Njogu *et al.,* (1985b) reported a higher prevalence of *T. vivax* in the Galana Boran than Orma Boran, and observed that, the Orma Boran appeared to resist the effects of *T. vivax* and showed some resistance to *T. congolense* under low challenge, but succumbed to *T. congolense* when the challenge increased resulting in mortality.

The present study revealed a high proportion of *T. brucei* and mixed infections which mainly occurred during the periods with high rainfall. *Trypanosoma brucei* as a single infection had a prevalence ranging from 6% in the Galana Boran to 9% in the Maasai Zebu, and more important, it caused severe

clinical disease similar to that caused by either *T. congolense* or *T. vivax.*

Some of the animals infected with single species developed a chronic syndrome by the time their PCV dropped to the critical value of ≤17% which combined with other sources of environmental stress, may have favoured the development of mixed trypanosome infections.

These are among the highest levels of both *T. brucei* and mixed infections reported in East African livestock. The prevalence of *T. brucei* was relatively lower than the reported incidence of 20% to 30% in cattle in West Africa (Godfrey and Killick-Kendrick, 1961; Godfrey, Leach and Killick-Kendrick, 1968; Gray 1970). Other workers have also reported natural disease with *T. brucei* associated with anaemia (Godfrey, Leach, Roberts and Killick-Kendrick, 1968), emaciation and death in a few cases (Hornby, 1952), while similar signs were seen in experimental infections (Losos and Ikede, 1972). It has also been noted that mixed infections of *T. brucei* and *T. congolense* caused a more severe disease than *T. congolense* alone (Metam, 1934).

There were significant differences in the treatment requirements among the breeds. Overall, the Galana Boran needed 2.1 and 1.5 more treatments than the Maasai Zebu and Orma Boran, respectively. One Maasai Zebu steer that was infected only once never required treatment during the one year period; no similar cases occurred in the other breeds. Although there were no significant differences between the Maasai Zebu and Orma Boran, there was some indication that the Maasai Zebu required fewer treatments.

Once infected, some animals terminally required treatment, while others remained parasitaemic for some time and eventually the parasites disappeared followed by a recovery of the PCV to normal haematological values, thus exhibiting a self cure phenomenon. The number of cases with such spontaneous

recovery was significantly higher in the Maasai Zebu than the other breeds. Most of the self cures in all the breeds occurred in *T. vivax* infections.

The treatment requirements in the Galana Boran and Orma Boran were similar to those reported from previous field observations at the Galana Ranch (Njogu *et al.,* 1985a), where in a series of experiments on chemoprophylaxis using isometamidium chloride (Samorin^{R}, RMB), the results showed that, under high tsetse challenge, the Galana Boran required five treatments per annum at 1 mg kg^{-1} body weight, compared to three treatments per annum at 0.5 mg kg^{-1} body weight in the Orma Boran. In another part of the ranch with less severe tsetse challenge, they observed that, the Galana Boran required twice the number of treatments as the Orma Boran with the same drug at 0.5 mg kg^{-1} body weight. In a different study involving groups of 30 animals from each breed, 14 Orma Boran never became infected over a twelve months period compared to only two Galana Boran (Njogu *et al.,* 1985b). Similarly, Ishmael *et al.,* (1985) showed that a significantly higher proportion of the Galana Boran required treatments compared to the Orma Boran when experimentally infected by needle and fly challenge with *T. congolense* or *T. vivax* and, over 60% of the Orma Boran recovered spontaneously compared to none in the Galana Boran.

Results of the present study are similar to other cattle breed comparison studies where differences in treatment requirements have been observed to vary among breeds. Murray *et al.,* (1979a) noticed that following needle and fly challenge with *T. congolense* and *T. brucei,* some N'Dama and Zebu cattle were able to eliminate parasites from the circulation accompanied by a gradual return to normal haematological values in some animals. Under natural tsetse challenge, the N'Dama was observed to develop a transient parasitaemia and later became aparasitaemic with the recovery of the PCV to normal levels

(Murray *et al.,* 1981a). Nantulya *et al.,* (1984) reported spontaneous recovery in Zebu x Charollais following needle challenge with *T. congolense* and *T. brucei.* Similarly, Roelants *et al.,* (1987) observed spontaneous recovery from parasitaemia in infected N'Dama/Baoule and resistant animals within the Baoule breed. Paling *et al.,* (1991) found that all the infected N'Dama required no treatment and recovered spontaneously to their normal haematological values within two to four months after infection with tsetse-transmitted *T. congolense,* compared to none in the Boran. In contrast, all these studies most of the Zebu needed treatment (Murray, *et al.,* 1979a; 1981a) or died if untreated (Roelants, 1987).

The mean PCV values for the entire period were significantly different among the three breeds. The Maasai Zebu developed the least degree of anaemia, the Orma Boran was intermediate, while the Galana Boran had the most severe. It was noticed that periods with high disease incidence were accompanied by a marked drop in PCV in all the breeds and the most severe degree of anaemia developed in these periods. Thus, under increased high challenge as seen after the rains, all breeds became equally susceptible and severely affected by the disease.

That trypanotolerance is not absolute and breaks down with increased challenge was demonstrated by Murray *et al.,* (1979a) who showed that in both N'Dama and the Zebu cattle receiving a higher dose of bloodstream forms of *T. brucei* developed more severe clinical signs and anaemia than those receiving a lower dose.

The end of the rains in May 1990 was followed by a decline in the fly challenge and a gradual decrease in the disease incidence. When the challenge decreased, the Maasai Zebu showed faster recovery of the PCV than the Orma

Boran. On the other hand, under increased tsetse challenge, the Galana Boran developed chronically low PCV, which never recovered to normal values even after the decline in tsetse challenge, for a period of eight months (starting from February 1990 to September 1990). During this period, the Galana Boran experienced a predominantly heavy *T. congolense* challenge, which was probably the major cause of the chronic anaemia.

The results of the present study are similar to other breed comparison studies where the degree of anaemia developed following trypanosome infections has been shown to vary significantly. Thus, in experimental infections with *T. congolense* and *T. brucei,* the Zebu was shown to develop a persistently more severe degree of anaemia than the N'Dama (Dargie *et al.,* 1979; Murray *et al.,* 1979a). Similar results were observed when the Zebu was compared with the taurine breeds following tsetse-transmitted *T. congolense* infection (Akol *et al.,* 1986). Under natural challenge, it was shown that, the Zebu developed a more severe anaemia than the N'Dama (Murray *et al.,* 1981a) and N'Dama/Baoule (Roelants *et al.,* 1987). Results from West Africa have confirmed that the resistance of the taurine breeds, namely the N'Dama and the West African Shorthorn, to trypanosome infections is associated with the ability to resist the development of anaemia and control parasitaemia (reviewed by Murray *et al.,* 1982; 1991).

In N'Dama, it was shown that, though the PCV dropped following the initial challenge, and then improved during the aparasitaemic phases (Murray *et al.,* 1981a). In contrast, the development of parasitaemia in the Zebu was accompanied by a progressive development of anaemia till death. Njogu *et al.,* (1985b) observed that, under natural tsetse challenge, the PCV drop in the Galana Boran was more severe, while infected Orma Boran were able to control

their PCV. Paling *et al.,* (1991) found that, the N'Dama maintained significantly higher mean PCV values than the Boran and most infected N'Dama had only transient drops in the PCV which recovered spontaneously to normal haematological values within three weeks.

Differences in performance occurred among the three breeds. The Maasai Zebu had a significantly higher weight gain than the Galana Boran, while there were no differences between the Orma Boran and either of the other breeds. In the first five months (September 1989 to February 1990), there was a growth lag with no body weight gains in any of the breeds. The effects were most severe in the Galana Boran since, in addition to the greater weight loss at this time, the Galana Boran needed most treatments. Following the short rains in December 1989 and heavy rains between February and May 1990, there was an improvement in the pasture quality which was accompanied by progressive weight gains in all the breeds up to the end of the study (September 1990).

Wilson *et al.,* (1983) observed that under natural challenge, in untreated control animals, the Orma Boran performed better than the Galana Boran. Following challenge with *T. vivax* and *T. congolense* in the laboratory, the Orma Boran gained weight, while the Galana Boran lost weight (Ishmael, 1988). The initial loss in body weight and body condition was similar to the observations of Roberts and Gray (1973) who showed more severe effects in the Zebu than N'Dama and Muturu; the loss in the Zebu started immediately after the infection, while it occurred after three to four months in the N'Dama and Muturu. Similarly, *T. congolense* infected N'Dama were shown to gain weight at the same rate as the uninfected N'Dama control animals (Paling *et al.,* 1991).

In this study, differences in the susceptibility within breeds were seen as reflected by the great variation in the number of infections and treatments in the

individual animals that survived the entire period. In each breed the animals fell into three categories; those infected a few times, others intermediate and another group with a high number of infections. A similar pattern was observed in the number of treatment requirements in individual animals.

There was trypanosome related mortality in the Galana Boran, while none occurred in the Orma Boran and Maasai Zebu. The higher mortality due to trypanosomiasis seen in the Galana Boran in this study was similar to that reported in other experiments on the two breeds at the Galana Ranch (Njogu *et al.,* 1985b).

Under laboratory conditions, the Galana Boran was shown to develop more severe clinical signs than the Orma Boran as judged by the lymph node enlargement, increased heart and respiratory rates, and also, the clinical signs occurred in a higher proportion of Galana Boran than the Orma Boran (Ishmael, 1988). Roberts and Gray (1973) reported a less severe clinical disease in the N'Dama and Muturu in comparison to the Zebu, while Murray *et al.,* (1981a) also noted that the N'Dama did not become febrile following natural infection as opposed to the Zebu. Roelants *et al.,* (1987) observed that the Zebu and some susceptible N'Dama/Baoule developed less severe clinical signs and survived. All West African breed comparison studies repeatedly showed that there was higher mortality due to trypanosomiasis in the less susceptible Zebu breed than the N'Dama (Murray *et al.,* 1979a, 1981a; Roelants *et al.,* 1987).

As in the high challenge area at Nguruman, trypanosomiasis was the major disease encountered in the Maasai Zebu and Orma Boran cattle kept in the low tsetse challenge area between September 1989 and September 1990. High peaks of disease incidence were closely related to the increase in the fly density which occurred after the rains. There were no significant differences in

the prepatent period, disease incidence, degree of anaemia, treatment requirements, and the performance between the two breeds.

Majority of the animals from both breeds were never infected, with only five Orma Boran and ten Maasai Zebu steers becoming infected. More infections occurred in the Maasai Zebu than the Orma Boran. There were no differences in the prevalence of the *T. vivax* and *T. congolense* between the two breeds. Only one infection of either mixed infections or *T. brucei* was detected, indicating that both types of infections were not a threat under low tsetse challenge.

There were no significant differences in the degree of anaemia and both breeds had PCV values within the normal range throughout the year. Similarly, there were no differences in the number of required treatments between the two breeds. The Maasai Zebu had a better ability to control *T. vivax* infections as it was observed that, in three of the six infections with this species, the animals were able to control parasitaemia and eventually self cured. In contrast, all except one *T. congolense* infection in the Orma Boran needed treatment.

There were no differences in the growth rates between the two breeds. However, on visual assessment, the Orma Boran had a higher body condition score than the Maasai Zebu. This might be due to the fact that, the Orma Boran has a stockier conformation which was probably associated with a better muscle deposition.

In conclusion, under the low tsetse challenge, there were no differences in the disease incidence, number of infections and treatment requirements, the degree of anaemia or body weight changes between the two breeds. This indicates that, under low disease challenge, both breeds perform equally well. Similarly, previous results from the Galana Ranch showed that under less severe disease challenge, both the Orma and Galana Boran cattle needed similar

number of treatments and gained weight at the same rate (Njogu *et al.,* 1985a).

However, there were significant differences in all the parameters monitored between the Maasai Zebu and Orma Boran in the high challenge area and their counterparts in the low challenge area. The animals in the low challenge area had lower disease incidence, needed fewer treatments, developed only a mild transient anaemia, had higher body weight gains than the matching groups in the high challenge area. No mortality occurred in the low challenge area compared to the high challenge area where deaths due to theileriosis were recorded in both breeds.

Thus, it would appear that the difference in cattle performance in the high and low tsetse challenge areas were attributable to the differences in the levels of trypanosomiasis risk between the two areas, and therefore, the tsetse control in the low challenge area had a significant effect.

4.3.2 OBSERVATIONS ON THE THREE CATTLE BREEDS AT THE GALANA RANCH

Among the animals with previous exposure (PE) at the Galana Ranch, trypanosomiasis was the major disease encountered. There were no differences among the three breeds in the prepatent period, disease incidence, trypanosome prevalence and degree of anaemia. However, significant differences in growth rates were observed.

All animals became infected and although the Maasai Zebu appeared to have a longer prepatent period, there were no significant differences among the three breeds. Once infected, there were no differences in the prevalence of *T. vivax, T. congolense, T. brucei* and mixed infections among the three breeds. Similarly, there were no differences among the breeds in the intensity of

parasitaemia following infections with any of the three trypanosome species. In all breeds, most *T. vivax* were in the low parasitaemia score class, while most *T. congolense* were in the medium score class. There were fewer cases of mixed infections in the Galana Boran in comparison to the other breeds but the differences were not significant.

There were no differences among breeds in the treatment requirements. Among the infected animals, there were three categories in the Maasai Zebu and Orma Boran; animals infected only once and never needed treatment, animals infected repeatedly but in some infections recovered spontaneously and animals that always required treatment once infected. In the Galana Boran, all animals needed treatment and fell into either of the other two categories. The cases of self cure were not significantly different among the breeds, although they appeared fewer in the Galana Boran than the other breeds.

There were no differences in the degree of anaemia among the three breeds though the Galana Boran appeared to maintain persistently lower values than the other breeds. All groups had slightly low PCV values from the start of the study possibly due to the prolonged stress experienced at Kisames, Ngong, where they were exposed to malnutrition due to the severe drought and tick-borne diseases particularly East Coast Fever and anaplasmosis.

Despite the high disease challenge at the Galana Ranch, there were improved growth rates in all the breeds with the Orma Boran having significantly higher weight gains than the other breeds.

No mortality occurred in the PE groups during the nine months observations period.

Among the animals with no previous exposure (NPE), trypanosomiasis was the major disease encountered. There were significant differences in the

prepatent period, disease incidence, degree of anaemia, treatment requirements and growth rates among the three breeds.

Following exposure to natural tsetse challenge, all Galana Boran steers became infected, while two Maasai Zebu and one Orma Boran were never infected. The prepatent periods were significantly longer in the Maasai Zebu than Galana Boran, while that of the Orma Boran was not different from either of the other breeds. High peaks of infections in the three breeds occurred in the periods with high rainfall and tsetse challenge. The disease incidence was significantly higher in the Galana Boran than other breeds and ranged from 2.4 to 1.8 times that of the Maasai Zebu and Orma Boran, respectively. Similarly, in the animals that survived up to the end of the study, significantly more infections occurred in the Galana Boran than in the other breeds.

The prevalence of *T. vivax* was significantly different among the breeds being least in the Maasai Zebu, intermediate in the Orma Boran and highest in the Galana Boran. On the other hand, the prevalence of *T. congolense* was significantly higher in the Galana Boran than Maasai Zebu, while in the Orma Boran, it was not significantly different from other two breeds. These results are similar to the observations made by Njogu *et al.*, (1985b) that, under high natural tsetse challenge, the prevalence of *T. vivax* in the Galana Boran was significantly higher than the Orma Boran, while there were no differences in the prevalence of *T. congolense*.

There were significant differences in the treatment requirements among the breeds with the Galana Boran needing more than twice the number required by the other breeds. In the Maasai Zebu, the animals fell into three categories; those infected only once and never required treatment, others infected more than once but in some infections recovered spontaneously and another group which

was infected frequently and always needed treatment. In the Orma Boran and Galana Boran, all animals needed treatment at some stage. The number of cases with self cure were significantly higher in the Maasai Zebu than Galana Boran, while the Orma Boran was not different from any of the other breeds.

Following trypanosome infections, all animals developed anaemia which was significantly more severe in the Galana Boran than the Maasai Zebu and Orma Boran. Similar seasonal changes in the PCV were observed in all the breeds.

There were significant differences in the body weight gains among the three breeds. The Galana Boran had significantly lower body weight gains than the Orma Boran and Maasai Zebu.

Among the NPE, trypanosomiasis caused the death of two Orma Boran steers, while none occurred in the Maasai Zebu and Galana Boran. These deaths were due to the acute haemorrhagic *T. vivax* which is known to be highly fatal (Mwongela *et al.,* 1981; Wellde *et al.,* 1983). Predators mainly lions were observed to be a major problem as previously reported (Njogu *et al.,* 1985b) and it appeared that the Maasai Zebu were more vulnerable probably due to their smaller size.

In conclusion, at both Nguruman and the Galana Ranch, under high tsetse challenge, the Maasai Zebu and Orma Boran were less susceptible to trypanosomiasis than the Galana Boran. In contrast, in the low tsetse challenge area, no breed differences were observed but the animals kept there performed better than their counterparts in the high challenge area. At the Galana Ranch, previous exposure had a significant effect in that, except for the higher growth rate in the Orma Boran, there were no obvious breed differences.

CHAPTER 5

COMPARISON OF PARASITOLOGICAL TECHNIQUES, ANTIBODY AND ANTIGEN DETECTION ENZYME IMMUNOASSAYS IN THE DIAGNOSIS OF TRYPANOSOME INFECTIONS IN CATTLE

5.1 INTRODUCTION

An essential part of understanding the susceptibility or resistance to infection and consequently the epidemiology of disease is the ability to diagnose the infection specifically and reliably. Parasitological diagnostic techniques usually allow the identification of the species of trypanosomes involved but are not particularly sensitive in detecting parasites, only being able to do so when there are more than 10^2 to 10^4 organisms per ml, depending on the technique used (Paris *et al.*, 1982). The most preferred parasitological method is the phase-contrast darkground illumination buffy coat or DG technique (Murray *et al.*, 1977), it is regarded as the most sensitive, allows species identification even in mixed infections, and provides a semi-quantitative score of the intensity of parasitaemia (Paris *et al.*, 1982).

With the development of immunology, a wide range of techniques have been employed to detect antibodies (Nantulya, 1990). The detection of antibody gives an indication of whether or not an animal has been exposed to infection, but not whether it is actively infected. Therefore, recently major efforts have been made to produce antigen capture tests which are more likely to reflect the presence of an infection. Another major advance in immunological diagnosis has been the development of the enzyme linked immunosorbent assays (ELISA) for the detection of antibody and antigen; this approach is more user friendly, cheaper than other tests, and gives more consistent results, depending on the quality of reagents (reviewed by Nantulya 1990).

The objective of this section was to compare under field conditions the sensitivity of the most sensitive parasitological diagnostic technique, the darkground/phase contrast, (Murray *et al.*, 1977) with the antigen-ELISA (Nantulya and Lindqvist, 1988) and the antibody-ELISA (Luckins, 1977).

5.2 MATERIALS AND METHODS

The collection of serum samples has been discussed in section 4.1.2. On the dates of serum collection the animals were examined for trypanosomes using the buffy coat (DG) technique. On several occasions, in the course of the experiment at Nguruman and the Galana Ranch, blood samples from all the cattle were inoculated into mice as described in section 3.3. In addition, inoculation was also regularly done from any suspected cases of mixed infections and aparasitaemic animals with PCV below 20%. At the same time, the buffy coat was examined and serum collected for the analysis of the circulating trypanosomal antigens and antibodies using the ELISA techniques described in section 3.3.

The samples analysed fell into four groups namely; the pre-experimental samples at Nguruman and the Galana Ranch, the experimental periods in both the high and low tsetse challenge areas at Nguruman, the duration when the animals were moved to a tsetse-free area at Kisames, Ngong, the group of animals that were moved to KETRI, Muguga (as described in section 4.1.2.), and finally the samples collected when the animals under tsetse challenge at the Galana Ranch.

5.3 RESULTS

A breakdown of the samples analysed is presented in Table 5.1. The results on the pre-experimental samples have already been discussed in sections 4.1 and 4.2. Of the total 1378 samples examined when the animals were under challenge at Nguruman and the Galana Ranch, 409 (29.6%) were positive by DG. On the other hand, 693 (50.3%) were positive by antigen ELISA (Ag-ELISA) of which 455 (33%) were mixed infections. The Ag-ELISA test gave positive results in 284 of the samples that were negative by the DG. Of the 689 samples examined for

Table 5.1

Number of trypanosome infections detected by the darkground/phase contrast (DG), antibody and antigen ELISA techniques in cattle under varying levels of trypanosomiasis challenge

	Samples analysed			Positives			
	Buffy coat	Antigen	Antibody	Buffy coat	Antigen		Antibody
					Total*	Mixed	
Nguruman							
Pre-experimental	106	106	106	0	26	7	9
High challenge	582	582	279	282	425	311	218
Low challenge	538	538	152	76	166	84	52
Tsetse free areas							
Kisames (Ngong)	240	240	49	0	88	52	25
KETRI (Muguga)	107	107	97	0	27	7	10
Galana Ranch							
Pre-experimental	116	116	116	0	33	10	24
Experimental	258	258	258	52	102	60	159

* Includes both single and mixed infections.

antibodies over the same period, 429 (62.3%) were positive. In the periods when the cattle were in tsetse-free areas, no infections were detected by the DG in the 347 samples examined, while 115 (33%) had antigens.

5.3.1 SAMPLES COLLECTED FROM NGURUMAN

Samples analysed in this category were collected between September 1989 and September 1990 from animals in both the low and high trypanosomiasis challenge areas. Of the 582 samples analysed from the high challenge area, 282 (48%) were positive by DG and 425 (73%) for antigens (Table 5.1). Of the 279 samples assayed for antibodies, 218 (78%) were positive.

In the high challenge area, the DG results indicated the main species to be *T. vivax* and *T. congolense* both making up 72% (Table 5.2). Single infections were detected in 79% of the samples by DG, while the rest were due to mixed infections. In the samples that were positive by the DG, *T. vivax* occurred in 54%, *T. congolense* in 45%, and *T. brucei* in 24%.

In contrast, the Ag-ELISA results showed that of the 425 positive samples, 311 (73%) had mixed infections (Table 5.1). There was a relatively lower proportion of single species infections than detected by DG, while mixed infections with the three species formed the major proportion occurring 161 (38%) of the samples (Table 5.2). Of the positive serum samples, *T. congolense* was present in 88%, *T. vivax* in 71% and *T. brucei* in 54%.

In the low challenge area, of 538 the samples analysed 166 (31%) had antigens compared to 75 (14%) with parasites seen by DG (Table 5.1). Antibodies were demonstrated in 52 (34%) of the 152 samples (Table 5.1). Using the DG technique, *T. congolense* infections were dominant making up 61%, while *T. vivax* made up 33% and mixed infections were found in only 6% (Table 5.2).

Table 5.2

Trypanosome species identified in the samples detected positive by the darkground/phase contrast (DG) and antigen ELISA techniques, under the different levels of trypanosomiasis challenge at Nguruman

Trypanosome species	High challenge				Low challenge			
	DG		Antigen ELISA		DG		Antigen ELISA	
	N=282	*%*	*N=425*	*%*	*N=75*	*%*	*N=166*	*%*
Tc	90	32	64	15	46	61	43	26
Tv	113	40	25	6	25	33	34	20
Tb	21	7	18	4	0	0	7	4
Tc/Tv	11	4	107	25	0	0	35	21
Tc/Tb	19	7	43	10	0	0	17	10
Tv/Tb	23	8	7	2	4	6	2	1
Tv/Tc/Tb	6	2	161	38	0	0	28	17

N - Number of total positive samples.

Tc - *T. congolense*

Tv - *T. vivax*

Tb - *T. brucei*

In contrast, the Ag-ELISA, detected almost equal numbers for the single species and mixed infections (84 and 82, respectively). Of the samples detected positive by the Ag-ELISA technique, *T. congolense* occurred in 74%, *T. vivax* in 59% and *T. brucei* in 32%.

Table 5.3 presents a comparison of DG and Ag-ELISA in the high and low challenge areas, respectively. Antigens were detected in 152 and 95 of the samples that were negative by DG in the high and low tsetse challenge areas, respectively. On the other hand, the DG detected infections in nine samples from the high challenge area and five in the low challenge from the groups that were negative by Ag-ELISA. The DG also detected infections in 23 and four samples that did not have antibodies in the high and low challenge areas, respectively (Table 5.4).

5.3.2 SAMPLES COLLECTED, WHILE THE CATTLE WERE IN THE TSETSE-FREE AREA

Samples in this category fell into two groups; those collected between October 1990 and February 1991, when all the animals from the Nguruman experiment were kept at Kisames, Ngong, and from March to June 1991, when the cattle kept in the low challenge area at Nguruman in the previous year (September 1989 to September 1990) were moved from Kisames, Ngong to Muguga.

In the 240 samples analysed during the five months period at Ngong (October 1990 to February 1991), no parasites were seen by DG, while by Ag-ELISA, 88 (36.7%) were positive (Table 5.1). *Trypanosoma congolense* was the predominant species (27%), while mixed infections occurred in 52 (59%) (Table 5.5).

Table 5.3

Comparison of the darkground/phase contrast (DG) and antigen ELISA results on samples from the high and low tsetse challenge areas at Nguruman

	darkground/phase contrast (DG)			
	High challenge N = 582		Low challenge N = 538	
	Positive	Negative	Positive	Negative
Antigen ELISA				
Positive	273	152	71	95
Negative	9	148	5	367

N - Number of samples analysed.

Table 5.4

Comparison of the antibody ELISA results with the darkground/phase contrast (DG) in the high and low tsetse challenge areas at Nguruman

	Antibody			
	High challenge N = 279		Low challenge N = 152	
	Positive	Negative	Positive	Negative
DG				
Positive	170	23	13	4
Negative	48	38	39	96

N - Number of samples analysed.

Table 5.5

Trypanosome species identified in the positive samples detected by the antigen ELISA when the cattle were treated and moved to tsetse free areas

Trypanosome species	Kisames*		KETRI*	
	N=88	*%*	*N=27*	*%*
Tc	24	27	11	41
Tv	11	13	5	19
Tb	1	1	4	15
Tc/Tv	18	20	4	15
Tc/Tb	17	19	2	7
Tv/Tb	3	3	0	0
Tv/Tc/Tb	14	16	1	4

N - Number of samples with antigens.

* No parasites detected by the DG

N - Number of positive samples

Tc - *T. congolense*

Tv - *T. vivax*

Tb - *T. brucei*

In the cattle transferred to KETRI, all the 107 samples were negative by DG, while antigens were detected in 27 samples, 7 (26%) of which had mixed infections (Table 5.1). Antibodies were present in 10 samples from the 97 analysed.

5.3.3 SAMPLES FROM THE GALANA RANCH

The Galana Ranch samples consisted of those collected within the first five months following exposure of both groups to natural tsetse challenge (between May and September 1991). From the 258 samples analysed, 52 were positive by DG, compared to 102 with antigens and 159 with antibodies (Tables 5.1 and 5.6). The DG detected infections in seven and 14 samples that were negative by the antigen and antibody-ELISA (Ab-ELISA), respectively (Table 5.6).

At the Galana Ranch, *T. congolense* and *T. vivax* were the major species as single infections by DG as they were detected in 60% and 49% of the positive samples, respectively, while overall, mixed infections were detected in 16% of the same samples (Table 5.7). On the other hand, by the Ag-ELISA technique, *T. vivax* was detected in 67%, *T. congolense* in 58%, and *T. brucei* in 53%, while mixed infections were found in 55% of the positive samples. Mixed infections with all the three species were dominant as detected by the Ag-ELISA, while samples with two species accounted for 32%.

5.3.4 SEASONAL VARIATION OF INFECTIONS AS DETECTED BY THE THREE METHODS

The proportion of infections detected by the three techniques over the entire period in both the high and low tsetse challenge areas is shown in Figure 5.1 and 5.2, respectively. The proportion of infections increased gradually and remained

Table 5.6

Comparison of the antigen and antibody ELISA results with the darkground/phase contrast (DG) in samples collected when the animals were under tsetse challenge at the Galana Ranch

	Antigen ELISA N = 258		Antibody ELISA N = 258	
	Positive	Negative	Positive	Negative
DG				
Positive	45	7	38	14
Negative	57	149	121	85

N - Number of samples analysed.

Table 5.7

Proportion of the three trypanosome species detected in the samples positive for trypanosomes by the darkground/phase contrast (DG) and antigen ELISA techniques during the first five months (May to September 1991) after introduction of cattle into natural tsetse challenge at the Galana Ranch

Trypanosome species	DG		Antigen ELISA	
	N=52	*%*	*N=162*	*%*
Tc	23	44	26	16
Tv	17	33	34	21
Tb	4	8	13	8
Tc/Tv	3	6	17	10
Tc/Tb	2	4	15	9
Tv/Tb	2	4	20	13
Tv/Tc/Tb	1	2	37	23

N - Number of positive samples

Tc - *T. congolense*

Tv - *T. vivax*

Tb - *T. brucei*

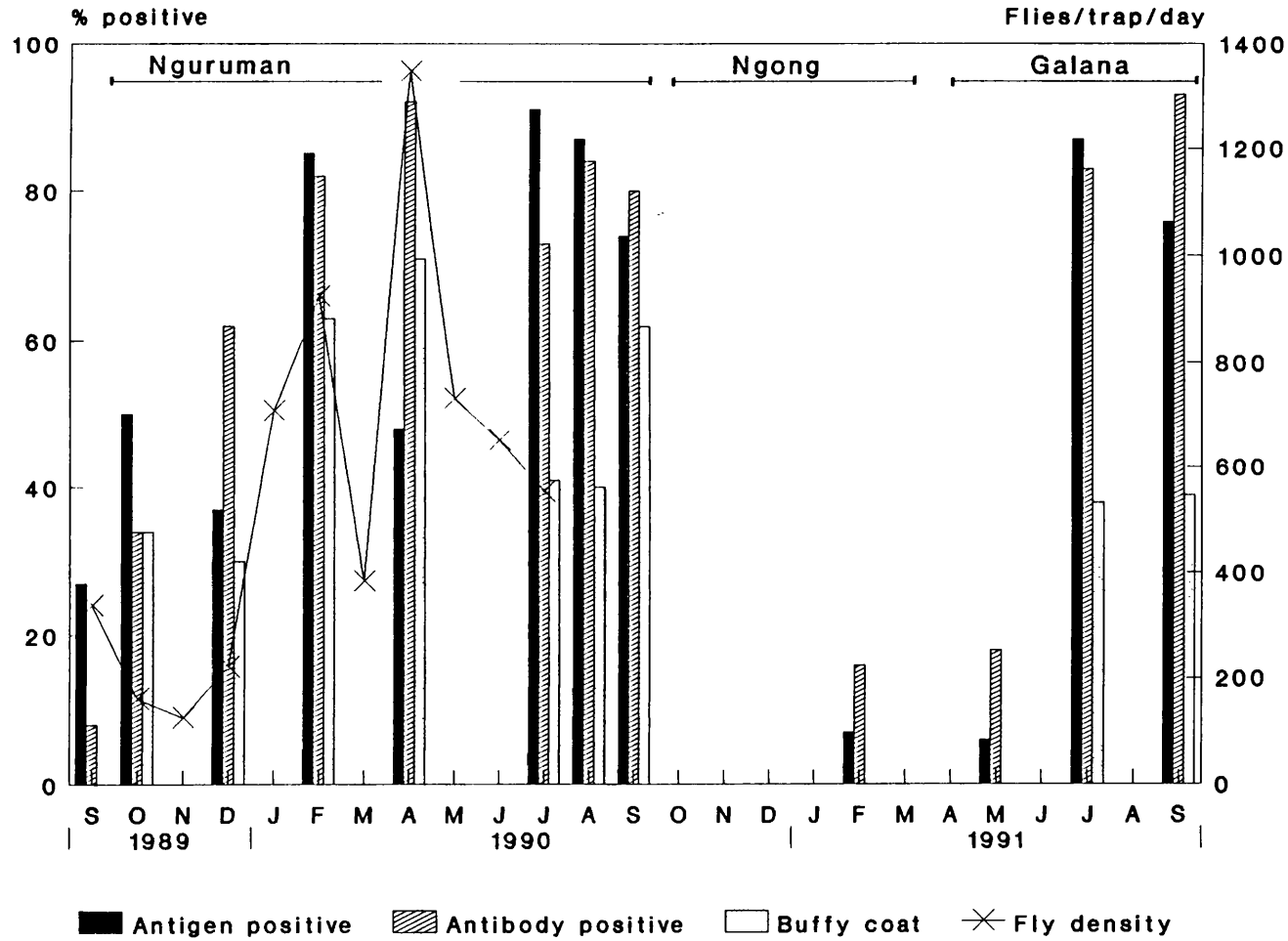


Figure 5.1. The proportion of infections detected positive by each of the three techniques when the cattle were in the high tsetse challenge area at Nguruman (September 1989 to September 1990), in the tsetse free area (October 1990 to May 1991) and after reintroduction to tsetse challenge at the Galana Ranch (July 1991 to September 1991). Fly density presented up to July 1990.

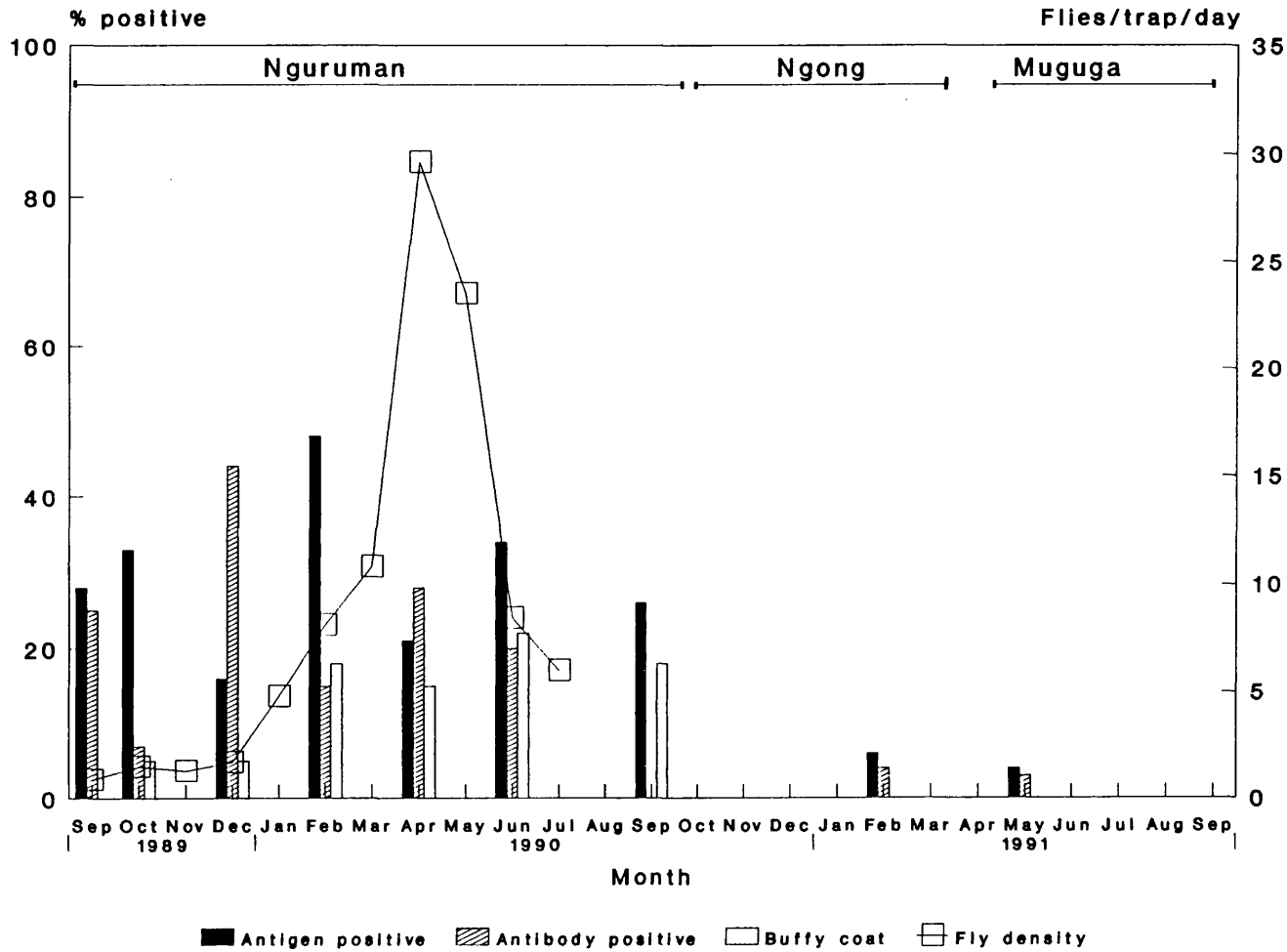


Figure 5.2. Proportion of samples detected positive by the three techniques when the animals were in the low tsetse challenge area at Nguruman (September 1989 to September 1990) and the tsetse free areas at Ngong and Muguga. Fly data presented up to July 1990. No comparisons were done in months without bar charts.

high during the period the animals were under high challenge at Nguruman.

For most of the time, the Ag-ELISA indicated higher disease prevalence than detected by the Ab-ELISA and the DG techniques. After the withdrawal of cattle from the low tsetse area at Nguruman (October 1990), parasites were no longer demonstrated by DG for the periods they were at Kisames, Ngong (Figure 5.3 and 5.4). Similarly, no infections were detected by DG on the group of cattle transferred to KETRI and those moved to the Galana Ranch but kept in tsetse-free area (as described in section 4.2.3). In contrast, antigens and antibodies were frequently detected in both groups even seven months after their transfer from Nguruman (May 1991) (Figure 5.3 and 5.4).

The disease prevalence as detected by all methods increased after the re-introduction into tsetse challenge (Figure 5.1) at the Galana Ranch. It was observed that, by the second month, over 60% of the samples had antigens and antibodies compared to less than 40% detected by the DG (Figure 5.1).

In six of the 11 months (54.5% of the time) that all the three methods were compared, the cases with antigens were more than those either with antibodies or parasites seen by DG, and for a total of five months (45.4% of the time), the samples with antigens were more than twice those with parasites. However, in April 1990, the DG detected more infections than Ag-ELISA, and apparently this was the month with maximum fly challenge at Nguruman. The antibody levels were generally high during the entire period the herds were under tsetse challenge. Similarly, in the low tsetse challenge area, the cases with antigens detected were more than those parasites seen by the DG, except in April 1990.

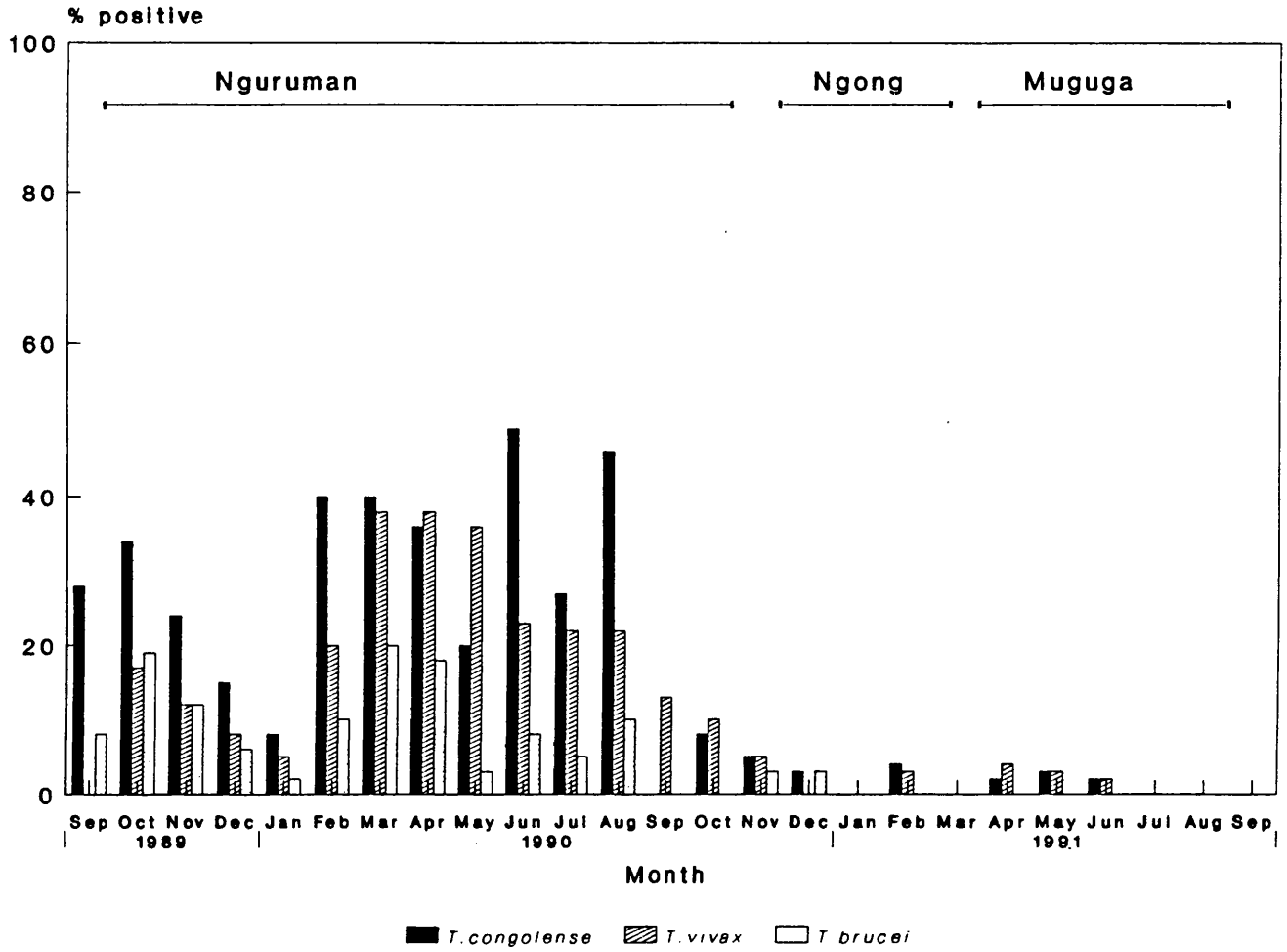


Figure 5.3. Seasonal variation in the prevalence of antigen levels of the three trypanosome species when the cattle were in the low challenge area at Nguruman (September 1989 to September 1990), tsetse free area at Ngong (October 1990 to February 1991), and at Muguga (April to June 1991).

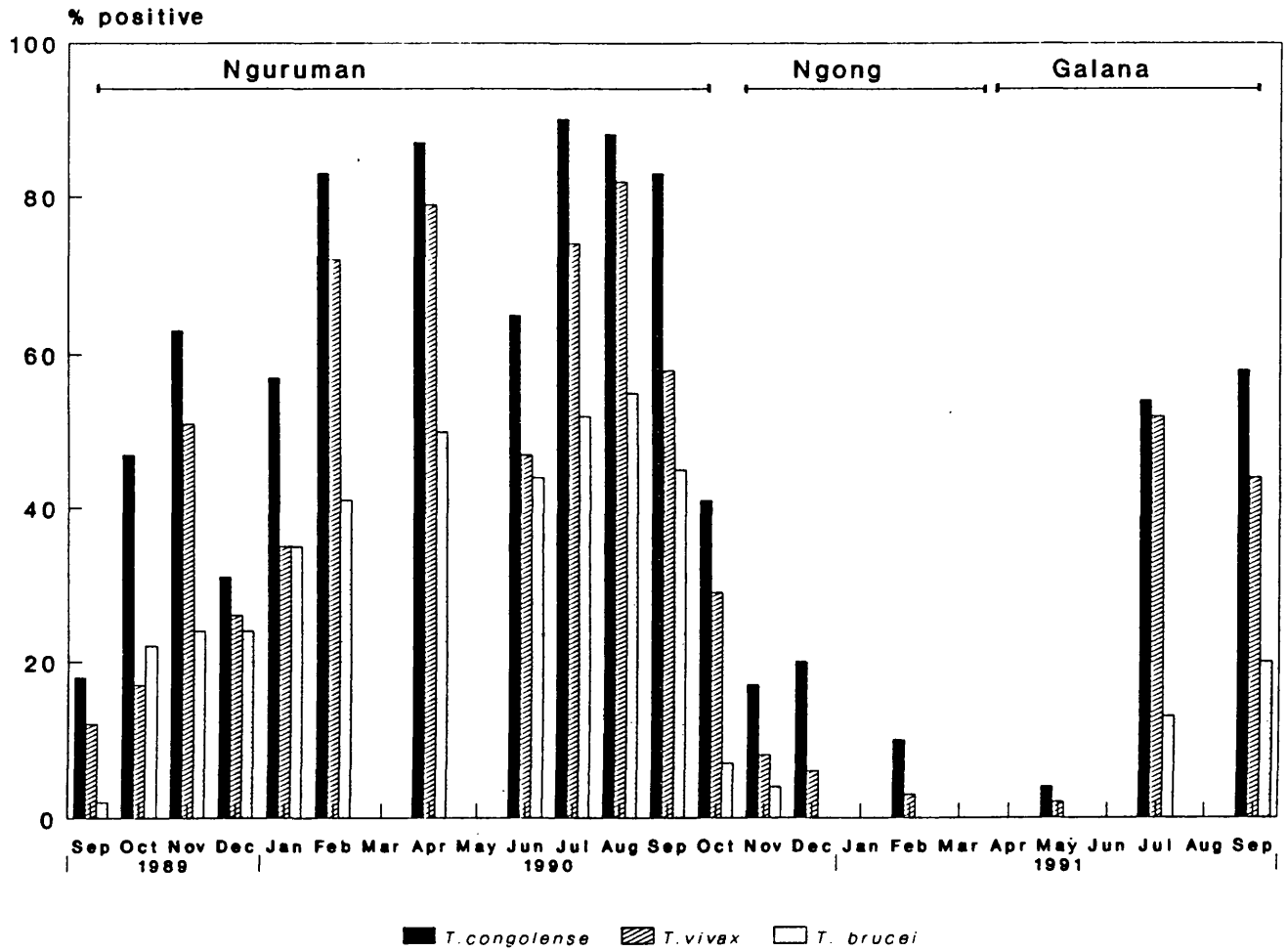


Figure 5.4. Seasonal variationin the prevalence of antigen levels of the three trypanosome species when the cattle were in the high challenge area at Nguruman (September 1989 to September 1990), tsetse free area at at Ngong (October 1990 to February 1991) and after reintroduction to tsetse challenge at the Galana Ranch (July to September 1991).

5.3.5 SPECIES PREVALENCE AS DETECTED BY THE ANTIGEN-ELISA

In both the high and low challenge areas at Nguruman, and at the Galana Ranch, the prevalence of *T. congolense* antigens was higher than the other species (Figure 5.3 and 5.4). The results also indicated a high prevalence of *T. brucei*. In the high challenge area at Nguruman, for a period of six months, between February and September 1990, over 40% of the samples indicated infections by all the three species. The prevalence of all the species, however, decreased gradually following withdrawal from tsetse challenge after October 1990, but increased after the re-introduction of the cattle into tsetse challenge at the Galana Ranch after May 1991 (Figure 5.3).

Under the low tsetse challenge, the number of cases detected by the DG increased after February 1990 following an increase in the fly challenge and remained high up to September 1990. For the entire observation period, antigens were constantly detected in over 15% of the monthly samples in the low challenge area, while it exceeded this value for four and three months on the Ab-ELISA and the DG, respectively.

Antigens of *T. brucei* were no longer detected after December 1991, while those of *T. vivax* and *T. congolense* as well as antibodies persisted up to seven months after the transfer of these animals from the low challenge area (Figure 5.4).

5.3.6 MOUSE INOCULATION

The details of the samples examined in the different areas are shown in Table 5.8. The mouse inoculation detected fewer infections than either the DG or the Ag-ELISA in three cattle groups. The Ag-ELISA detected the highest number, followed by the DG and finally the mouse inoculation with the least. The

Table 5.8

The comparison of the mouse inoculation, darkground/phase contrast (DG) technique and antigen ELISA for the detection of the trypanosome infections

Location	Total samples examined	Positive samples		
		DG	Mouse inoculation	Antigen ELISA
Nguruman				
High challenge	215	59 (27.4)	24 (11.2)	134 (62.3)
Low challenge	91	2 (2.2)	1 (1.1)	37 (40.6)
Galana Ranch	109	12 (11.0)	2 (1.8)	76 (69.7)

Numbers in parenthesis represent percentages.

sensitivity of mouse inoculation at the Galana Ranch was particularly low despite the high disease incidence observed in the cattle.

A breakdown of the trypanosome species in the 27 animals detected by the mouse inoculation, together with the matching results of the DG and Ag-ELISA are presented in Table 5.9. The mouse inoculation detected a high proportion of *T. brucei*, the DG *T. congolense*, while the Ag-ELISA showed a high number of mixed infections. Both the DG and the Ag-ELISA failed to detect infections in six and five of the animals, respectively.

The mouse inoculation detected only *T. congolense* and *T. brucei*, and which was probably one of the reasons for the observed lower sensitivity compared to the other methods. The inoculation method detected a high proportion of *T. brucei* infections missed by the other techniques (Table 5.10). It detected eight *T. brucei* and three mixed infections (*T. congolense/T. brucei*) missed by both the DG and the antigen detection, six *T. brucei* and two *T. congolense* missed by the DG alone, and three *T. brucei* and one *T. congolense* missed by the Ag-ELISA. Mouse inoculation had a poor sensitivity for *T. congolense* in that, it missed seven *T. congolense* detected by the DG and twelve *T. congolense* and one *T. brucei* detected by the antigen-ELISA.

5.4 DISCUSSION

All the methods indicated a gradual increase in infections after the introduction of the animals into tsetse challenge areas. The darkground/phase contrast (DG) results appeared to correlate well with the tsetse challenge in that, infections were only detected when the animals were under fly challenge but as soon as they were treated and withdrawn, infections were no longer detected. At Nguruman,

Table 5.9

A breakdown of the trypanosome species in the 27 samples detected positive by the mouse inoculation and the matching results from the darkground/phase contrast (DG) and the antigen ELISA techniques

	Mouse inoculation	DG	Antigen ELISA
T. congolense	4	11	5
T. vivax	-	3	1
T. brucei	19	2	2
T. congolense/T. vivax	-	-	4
T. congolense/T. brucei	4	3	4
T. brucei/T. vivax	-	2	1
T. congolense/T. vivax/T. brucei	-	-	5
Nothing detected	-	6	5
Total	27	27	27

- No infection detected

Table 5.10

Comparison of the trypanosome species detected or missed by the darkground/phase contrast (DG) and antigen ELISA in the 27 samples that were positive on mouse inoculation

	Mouse inoculation	
	Detected	Missed
DG		
Positive	7 Tb, 2 Tc	7 Tc
Negative	6 Tb, 2Tc	
Antigen ELISA		
Positive	9 Tb, 6Tc	12 Tc, 1 Tb
Negative	3 Tb, 1 Tc	
Samples negative on both the DG and Antigen ELISA	3 (Tc/Tb) 8 Tb	

Tc - *T. congolense*
Tv - *T. vivax*
Tb - *T. brucei*

the DG showed a high positive correlation with the fly density in the high ($r^2 = 0.81$, $p < 0.01$) and low ($r^2 = 0.53$, $p < 0.05$) tsetse challenge areas.

Although the antigen positive cases increased over the season with high tsetse challenge and rainfall, the Ag-ELISA and the fly density had only weak positive correlation in the high challenge area ($r^2 = 0.32$, $p < 0.1$), while it was negative in the low challenge area ($r^2 = -0.17$, $p < 0.1$). In the high challenge area, over 50% of the animals had antigens for most of the period compared to 20% in the low challenge area.

The Ag-ELISA results indicated that most of the infections were mixed and accounted for 73%, and 50.6% of the positive samples in the high and low challenge areas, respectively at Nguruman and, 58.8% in the Galana Ranch. Previous work on samples collected form cattle at Nguruman indicated 74.8% and 15.7% mixed infections in the high and low challenge areas, respectively (Nantulya, Lindqvist, Stevenson and Mwangi, 1992). In a similar study, Trail *et al.*, (1992) reported 14% mixed infections detected by the antigen-ELISA from 283 samples collected from cattle in an area of medium tsetse challenge in Gabon.

In this experiment, trypanosome infections were not treated immediately, hence most animals ran chronic disease course possibly causing enough stress for mixed infections to develop, particularly those of those involving *T. brucei.*

In situations of high trypanosomiasis risk, the high antigen levels may be the result of parasites introduced through repeated infective tsetse bites but do not become established probably due to the ability of the host's immune system to suppress replication, hence the parasitaemia does not reach parasitologically detectable levels. As for the low challenge area, high antigen levels observed are not readily explained particularly in the periods when the fly population was low.

Following treatment with a trypanocidal drug and withdrawal from the tsetse challenge, parasites were no longer demonstrated by DG, while both antigens and antibodies were observed to persist for up to seven months. Although antigens may be expected to last for several weeks (minimum two weeks) (Nantulya and Lindqvist, 1989) following treatment and withdrawal from the tsetse challenge, the reason for the prolonged persistence observed in some cases was unclear. Outside the tsetse challenge, persistent antigens due of *T. brucei* may be expected and to a less extent *T. vivax* since they both invade and may persist in tissues (Losos and Ikede, 1972), while *T. congolense* antigens are not expected to persist long after treatment since the parasite remains in circulation and does not normally invade tissues. Following treatment, Ag-ELISA might be expected to be negative after a short period and if antigens persist longer, this would suggest failure of chemotherapy as has been observed in experimental *T. congolense* infection in cattle (Nantulya and Lindqvist, 1989).

The Ag-ELISA missed a total of 3.2% and 6.5% of the infections detected by the DG in the high and low challenge areas, respectively at Nguruman. These results were close to those obtained from samples collected in the same area which showed that Ag-ELISA failed to detect 3.8% of the cases detected by the DG (Nantulya, *et al.*, 1992). Laboratory observations on cattle infected with *T. congolense* indicated that Ag-ELISA failed to detect 5.7% in goats and 14.2% in cattle (Masake and Nantulya, 1991). False negatives results by the Ag-ELISA in sera from animals in the acute phases of *T. vivax*, *T. congolense* and *T. brucei* infections have been reported (Nantulya and Lindqvist, 1989). Trail, *et al.*, (1992) detected 14 infections by the DG compared to 49 infections by the Ag-ELISA indicating that the DG missed 25 of the infections. They also found that the DG detected parasites in one animal which in which

antigens were never detected over a 13 weeks observations period. It has been suggested that in the early stages of the disease, sufficient parasite destruction has not occurred to produce detectable antigen levels in the circulation (Nantulya and Lindqvist, 1989) and this is possibly why false negative results are observed in the Ag-ELISA technique.

Recent laboratory work on experimental infection in sheep and goats with *T. congolense* suggested that the Ag-ELISA was four times more sensitive than the DG (Masake and Nantulya, 1991), but the results of the present study indicate that, under both high and low natural tsetse challenge, the sensitivity of the Ag-ELISA in the high and low tsetse challenge areas was in the range of 1.6 to 2.5 times better, respectively than the DG technique.

The Ag-ELISA was positive in 95 (20.6%) and 152 (50.7%) of the samples detected aparasitaemic by the DG technique in the low and high challenge areas, respectively. The results from the low challenge area were similar to those of Nantulya *et al.*, (1992), which showed antigens in 50% of the animals without parasitaemia on DG.

In view of the infections missed by the Ag-ELISA and the high proportion of cases detected by the same technique under low tsetse challenge, where a treatment decision has to be made, a combination of the Ag-ELISA and the DG is necessary. Also, constant follow up of cases with antigens and no parasites detected by the DG especially in animals with no clinical disease is necessary, as it may suggest a reservoir status.

The detection of antibodies is an indicator of exposure and cannot be used to differentiate between current and past infections. Antibodies were detected in most of the samples collected three months post exposure. The high number of antibody positive cases detected was possibly due to the fact that animals were

not treated immediately on the detection of parasites. Most of the cases, positive by DG and for antigen, had antibodies in their sera, hence confirming exposure to infection.

The results on the mouse inoculation indicated that, its sensitivity varied with the locality, being lower at the Galana Ranch than Nguruman. It had a high sensitivity for *T. brucei* but poor for *T. congolense*, while no *T. vivax* infections were detected. More important was the observation that, it detected a proportion of the infections missed by both the DG and Ag-ELISA. These observations indicate that mouse inoculation still has a role to play in parasite isolation epidemiological studies.

The results of this work have shown that each of the techniques missed a proportion of infections and, on the other hand, detected infections missed by other methods. For complete assessment of the disease situation in epidemiological studies, a combination of the serological and parasitological methods is necessary. The DG and Ag-ELISA may be recommended as they are both easy to perform in the field and allow the screening of a large number of animals.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

6.1 GENERAL DISCUSSION

At both Nguruman and the Galana Ranch, differences in susceptibility to trypanosomiasis among the breeds were observed. The Maasai Zebu and Orma Boran had fewer infections developed less severe anaemia and performed better than the Galana Boran. Screening of the pre-experimental samples showed presence of antigens and antibodies from some animals in each breed. This suggested that beside the PE groups, some of the animals used in these studies had been exposed for uncertain periods.

In the cattle that were transferred from the Galana Ranch to Nguruman and kept in the high challenge area, the Orma Boran had longer prepatent period, fewer infections and treatments, developed less severe anaemia, had higher body weight gains. Deaths due to trypanosomiasis occurred in the Galana Boran, while none occurred in the Orma Boran.

The results at Nguruman showed that the Orma Boran was less susceptible than the Galana Boran as previously observed at the Galana Ranch (Njogu *et al.,* 1985a,b). The Galana Ranch and Nguruman escarpment are in two extremes locations of Kenya with a distance of over 400 km apart and are separated by a large tsetse free zone. Therefore it is unlikely that any exchange in tsetse population occurs between the two areas. It is therefore possible that the parasite strains to which the Orma Boran and Galana Boran were exposed at Nguruman were entirely different from those they had previously encountered at the Galana Ranch. However, the results indicate that the superiority of the Orma Boran over the Galana Boran to resist trypanosome infections, was not confined to the parasite strains at the Galana Ranch. At Nguruman, the Maasai Zebu similarly had lower susceptibility than the Galana Boran in all the parameters monitored, indicating that, this breed was also less susceptible to trypanosome

infections than the Galana Boran.

In the low tsetse challenge at Nguruman, the Maasai Zebu and Orma Boran had lower disease incidence, fewer treatments maintained higher PCV values and performed better than their counterparts in the high challenge area. This implies that the use of traps to reduce the fly challenge had a significant effect on reducing the disease incidence and improving the performance of both cattle breeds.

As far as the Nguruman escarpment is concerned, the Maasai Zebu and Orma Boran would appear suitable to keep in high disease risk areas. However, when the challenge becomes very high, even these breeds become severely affected and additional disease control measures are necessary. The high disease challenge and the seasonal availability of pasture do not appear suitable for highly selected beef breeds like the Galana Boran.

Whether the observed superiority of the Maasai Zebu was due to previous exposure to the parasite strains at Nguruman was assessed by the transfer of animals of the three breeds with and without previous exposure to the Galana Ranch.

Except for the higher growth rate in the Orma Boran, there were no other differences among the three breeds in the previously exposed animals. In contrast, significant differences in the disease incidence, degree of anaemia and performance occurred in the cattle groups with no previous exposure with the Maasai Zebu and Orma Boran performing better than the Galana Boran.

The PE and NPE groups showed different responses to the trypanosome species; there were more *T. congolense* infections than *T. vivax* in the PE groups, while among the NPE, the reverse was true. A higher proportion of the PE Maasai Zebu developed lower parasitaemia for both *T. congolense* and *T. vivax*

than their NPE counterparts. Similarly, more PE Galana Boran had a lower intensity of parasitaemia following *T. vivax* infections than the NPE Galana Boran, while that of *T. congolense* was similar in the PE and NPE Galana Boran. Both PE and NPE Orma Boran had similar levels of intensity for *T. vivax* and *T. congolense.* These observations suggest that the PE cattle acquired some degree of immunity to trypanosomiasis.

Trypanosome species have an important implication as it has been shown that, animals infected with *T. congolense* on several occasions had significantly lower PCV values (Trail, *et al.,* 1991b; 1992). Results from the same studies indicated that *T. congolense* had significant deleterious effects on growth, while *T. vivax* did not, and that low parasitaemia score was associated with higher growth.

At the beginning of the experiment at the Galana Ranch, the PE groups were in a slightly poorer body condition and had lower PCV values than the NPE groups which was possibly due to the six months nutrition stress the animals experienced at Ngong as a result of drought. The rapid weight gains seen in the PE groups could be a compensatory growth. The growth rates in the PE groups were higher than NPE indicating a better performance of animals with previous exposure under high tsetse challenge. However Trail *et al.,* (1991b) noticed no effect on performance in N'Dama cattle with short term previous exposure.

The Maasai Zebu and Orma Boran at Nguruman and among the NPE groups at the Galana Ranch had higher PCV and growth rates than the Galana Boran. The development of anaemia as assessed by the PCV has been suggested to be the criterion for trypanotolerance closely linked with overall cow productivity, as it was shown that, in N'Dama cattle kept under high natural tsetse challenge, animals maintaining high PCV had significantly shorter calving intervals, heavier calving weight and higher growth rates than those maintaining

low PCV (Trail *et al.,* 1991a).

The study demonstrated that, the disease resistance of the Maasai Zebu was not restricted to the trypanosome strains at Nguruman as this breed also performed better than the Galana Boran when transferred to the Galana Ranch. This suggests that their resistance is innate and does not need previous exposure to particular trypanosome strains. This agrees with findings in the recognized trypanotolerant breeds. In that respect, Roberts and Gray, (1973), showed that following the infection of N'Dama, Muturu and Zebu cattle with no previous exposure with wild caught tsetse, the N'Dama was less susceptible than the Zebu.

The observations on the movement of the Orma Boran and Maasai Zebu in present study agree with other reports which have shown that the susceptibility of breeds regarded as trypanotolerant such as the N'Dama and Lagune was not altered by their transfer to other areas (Mortelmans and Kageruka 1976; ILCA 1979). In the same way, Roelants *et al.,* (1987) also observed that the transfer of the N'Dama/Baoule from one endemic area to another did not alter their susceptibility to trypanosomiasis.

These results on the PE animals from the three breeds are similar to others which suggest that, both susceptible and trypanotolerant cattle with previous exposure with or without chemotherapy, develop an improved resistance to infection. This has been confirmed in several studies carried out on the N'Dama in various parts of West Africa (Chandler, 1952; Desowitz, 1959; Toure *et al.,* 1978). Similarly, N'Dama with previous exposure were shown to have greater ability to limit parasitaemia following *T. vivax* infection (Saror *et al.,* 1981). Similar evidence on *Bos indicus* breeds in East Africa has been obtained in cattle maintained under high tsetse challenge by chemotherapy (Soltys, 1955; Whiteside, 1962a; Bourn and Scott, 1987; Wilson *et al.,* 1976; Trail *et al.,* 1987).

Nantulya *et al.*, (1984) demonstrated that *Bos indicus* cattle (Zebu x Charollais) that recovered spontaneously following needle challenge with bloodstream forms of *T. congolense* and *T. brucei* resisted tsetse-transmitted reinfections with the same strains.

Among the NPE at the Galana Ranch, the differences in susceptibility between the Orma Boran and Galana Boran were similar to all other reported studies at the Galana Ranch (Wilson *et al.*, 1983; Njogu *et al.*, 1985a,b), while those among the three breeds were similar to the observations made at Nguruman during the first year of this study.

The results of studies at Nguruman and the Galana Ranch reported here have indicated three major findings as far as the breed susceptibility is concerned; firstly, they have confirmed the observations which has been repeatedly made in previous studies at the Galana Ranch on the lower susceptibility to trypanosome infections of the Orma Boran than the Galana Boran, secondly, they have shown that the Maasai Zebu is less susceptible to trypanosomiasis than the Galana Boran, and thirdly, indicated that the superior resistance to trypanosomiasis in the Maasai Zebu and Orma Boran is innate and not localized to the parasite strains either at Nguruman or the Galana Ranch.

Tick-borne disease were noticed to be important both at Nguruman and the Galana Ranch. At Nguruman, theileriosis and anaplasmosis were identified as important threats to livestock in the high challenge area, while only anaplasmosis was encountered in the low tsetse challenge area. There were no differences in the incidence of tick-borne diseases among the breeds in both the high and low tsetse challenge areas mainly due to the regular tick control programme. However, in the high challenge area, higher mortality due to theileriosis occurred in the Galana Boran than in the other breeds.

It was noticed that the occurrence of the two parasites, either single or mixed, made the animals weak and more susceptible to trypanosomiasis. In addition, mixed infection of either of these parasites with trypanosomiasis caused a severe syndrome; this resulted in two deaths in the Galana Boran.

This implies that, in the drought season, when there are no optional pastures except in the tsetse-infested areas, relevant tick control measures must emphasized be taken, especially when the cattle are to be grazed in the dense forest pockets heavily populated with wildlife, particularly buffaloes. In the low tsetse challenge area, there were no tick-borne disease problems since the tick challenge was low and the cattle had almost no interaction with wildlife.

At the Galana Ranch, anaplasmosis was the only tick-borne disease encountered and similarly, it occurred either singly or as mixed infection with trypanosomiasis. There were no significant differences in the incidence of anaplasmosis among the breeds because of the rigorous cattle dipping program.

At both Nguruman and Galana Ranch, helminthiasis was controlled by regular drenching and did not contributed to any of the observed effects as it never reached clinically significant levels; no breed differences were observed.

The last part of this study (chapter 5) involved the comparison of the parasitological and serological techniques for the diagnosis of trypanosome infections. The antigen ELISA was able to detect a higher trypanosome prevalence than the other methods and also indicated a high proportion of *T. brucei* and mixed infections both at Nguruman and the Galana Ranch than previously reported.

On comparison of the DG and the antigen ELISA, the samples fell into four categories; those with antigens with or without detectable parasitaemia and those without antigens and with or without parasitaemia, those with antigens and

parasites, those with antigens but no parasites, those with no antigens but with parasites and those with no antigens or parasites. Further work on the category with antigens and no parasites is necessary as this may indicate either the failure of chemotherapy or ability of the host to suppress or control the development of parasitaemia. It has been proposed that presence of antigens in animals not detected parasitaemic by DG reflects the ability to control parasitaemia and therefore, this technique may be used as an additional tool to identify trypanotolerant animals within a breed, as it has been shown that animals with antigens and high PCV had similar growth rates as uninfected animals (Trail *et al.*, 1992).

The results indicate that for the complete epidemiological information, a combination of more than one technique preferably the DG technique and the Ag-ELISA would be ideal. The Ag-ELISA detected a high proportion of infections in the cattle in the low challenge area. In situations where the disease control depends mainly on chemotherapy, a criteria for treatment must be resolved if the judgement to treat is to be based on the antigen-ELISA results as it may be uneconomical to treat all animals with antigens.

For epidemiological purposes and in disease survey, mouse inoculation is still important particularly in the isolation of parasites, as in this study, it was able to detect infections missed on the antigen-ELISA and the DG.

6.2 CONCLUSIONS

The work reported here has shown that, variation in susceptibility to trypanosomiasis exists among the *Bos indicus* cattle in East Africa. The lower susceptibility of the Orma Boran in comparison to the Galana Boran as previously reported in other studies, was confirmed and extended. In addition,

the Maasai Zebu was shown to have a degree of resistance similar to the Orma Boran and significantly higher than the Galana Boran. The results also indicated that the resistance observed in the Maasai Zebu and Orma Boran was not confined to trypanosome strains as similar results were obtained in two distinct locations at Nguruman and the Galana Ranch.

There are still wide gaps in knowledge on the breed variation in *Bos indicus* cattle. Further investigations are therefore required in the following areas;

a) Epidemiological surveys to assess if other *Bos indicus* breeds resistant to trypanosomiasis exist in the tsetse-endemic areas of East Africa and possibly gauge their susceptibility by comparative studies with the Maasai Zebu, Orma Boran and the Galana Boran since information on these breeds is now available.

b) On the already identified resistant breeds like Maasai Zebu and Orma Boran, priorities should be given to;

i) Breed comparison laboratory studies using parasites of known pathogenicity, similar to those conducted by Monirei *et al.,* (1982), Ishmael (1988) and, on animals of known history as by Paling *et al.,* (1991), to provide data for comparison with the field observations.

ii) Field experiments under natural tsetse challenge comparing the susceptibility of the different breeds reared under similar environments. Preliminary work on this aspect for the Orma Boran and Galana Boran is already in progress at the Galana Ranch, where a selection and breeding programme for trypanotolerance and growth rate has been established but needs to be expanded to include the Maasai Zebu and other breeds that may be identified in future.

iii) Assessment of the productivity of the Maasai Zebu, Orma Boran and Galana Boran in different levels of trypanosomiasis challenge under similar

management.

iii) Evaluation of the effects of crossbreeding in areas where it has occurred, on resistance to trypanosomiasis.

c) Once the trypanotolerant breeds have been identified, there will be a need in future to design breed conservation programmes with the aim of studying and understanding the mechanisms of trypanotolerance. This could lead to identification of markers for resistance and the trypanotolerant trait possibly leading to the development of new approaches to chemotherapy and or immunoprophylaxis.

d) Studies on the susceptibility of *Bos indicus* breeds identified as trypanotolerant, to other diseases particularly the tick-borne infections.

After enough experimental evidence is gathered, plans should be made for trials on the introduction of the less susceptible breeds like Maasai zebu and Orma Boran to farmers and ranches in the tsetse-infested areas in addition to other trypanosomiasis control measures.

REFERENCES

Agyemang, K., Jeannin, P., Grieve, A.S. and Dwinger, R.H. (1987). Milk production of the N'Dama cattle kept under village conditions in The Gambia. In: *International Scientific Council for Trypanosomiasis Research and Control*, 19th Meeting, 1987, Lome, Togo, pp. 234-238, (OAU/STRC Publication No. 114).

Agyemang, K., Dwinger, R.H., Toury, B.N., Jeannin, P., Fofana, D. and Grieve, A.S. (1990). Effects of nutrition on degree of anaemia and liveweight changes in N'Dama cattle infected with trypanosomes. *Livestock Production Science*, **26**, 35-51.

Akol, G.W.O. and Murray, M. (1982). Early events following challenge of cattle with tsetse infected with *Trypanosoma congolense*. I. Development of the local skin reaction. *Veterinary Record*, **110**, 293-302.

Akol, G.W.O. and Murray, M. (1983). *Trypanosoma congolense*: Susceptibility of cattle to cyclical challenge. *Experimental Parasitology*, **55**, 386-393.

Akol, G.W.O., Authie, E., Pinder, M., Moloo, S.K., Roelants, G.E. and Murray, M. (1986). Susceptibility and immune response of Zebu and Taurine cattle of West Africa to infection with *Trypanosoma congolense* transmitted by *Glossina morsitans centralis*. *Veterinary Immunology and Immunopathology*, **11**, 361-373.

Aliu, Y.O. and Sannusi, A. (1979). Isometamidium-dextran complex: Therapeutic activity against *Trypanosoma vivax* infection in Zebu cattle. *Journal of Veterinary Pharmacology Therapeutics*, **2**, 265-273.

Allsop, R. (1984). Control of tsetse flies (Diptera: *Glossinidae*) using insecticides: A review of future prospects. *Bulletin of Entomological Research*, **74**, 1-23.

Allsop, R., Hall, D. and Jones, T. (1985). Fatal attraction for the tsetse fly: A review. *New Scientist*, **7**, 40-45.

Amin, M and Moll, P (1980). Portraits of Africa I: Kenya - Description and travel. Harvill press, U.K.

Annon. (1923). *The Annual Report of the Department of Veterinary Science and Animal Husbandry*, 1923. Tanganyika territory, p. **6**, 22.

Annon., 1988. *Livestock census, Kajiado District, Kenya.* ASAL Report, 1988. Ministry of agriculture and livestock development, Kenya.

Araujo, R.G. (1982). Detection of antigen or *Trypanosoma cruzi* by enzyme immunoassay. *Annals of Tropical Medicine and Parasitology,* **76**, 25-36.

Archibald, R.G. (1927). Tsetse fly belt area in the Nuba Mountains Province of the Sudan. *Annals of Tropical Medicine and Parasitology*, **21**, 39-44.

Ashcroft, M.T. (1959). The importance of wild animals as reservoirs of trypanosomiasis. *East African Medical Journal*, **36**, 289-297.

Ashcroft, M.T., Burt, E. and Fairburn, H. (1959). The experimental infection of some African wild animals with *Trypanosoma rhodesiense*, *Trypanosoma brucei* and *Trypanosoma congolense*. *Annals of Tropical Medicine and Parasitology*, **53**, 147-160.

Baldry, D.A.T., Everts, J., Roman, B., Von Onchessee, G.A.B. and Lavessiere, C. (1981). The experimental application of insecticides from a helicopter for the control of riverine populations of *Glossina tachinoides* in West Africa. VIII. The effects of two spray applications of OMS-570 (endosulphan) and OMS-1998 (deltamethrin) on *G. tachinoides* and non-target organisms in Upper Volta. *Tropical Pest Management*, **27**, 83-110.

Balfour, A. (1913). Animal trypanosomiasis in the Lado (Western Mongolla) and

notes on tsetse fly traps and an alleged immune breed of cattle of Southern Kordofan. *Annals of Tropical Medicine and Parasitology*, **13**, 113-124

Barnett S.F. (1947). Bovine trypanosomiasis in Kenya with special reference to its treatment with phenanthridium. *Veterinary Record*, **59**, 459-462.

Bauer, B., Kabore, I., Liebesch, Meyer, F. and Petrich-Bauer (1992). Simultaneous control of ticks and tsetse flies in Satiri, Burkina Faso, by the use of flumethrin pour on for cattle. *Tropical Medicine and Parasitology*, **43**, 41-46.

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

Black, S.J., Sendashonga, C.N., Lalor, P.A., Whitelaw, D.D., Jack, R.M., Morrison, W.I. and Murray, M. (1983). Regulation of growth and differentiation of *Trypanosoma (Trypanozoon) brucei brucei* in resistant (C57BI/6) and susceptible (C3H/HE) mice. *Parasite Immunology*, **5**, 465-478.

Black, S.J., Sendashonga, C.N., O'Brien, C., Borrowy, N.K., Naessens, M., Webster, P. and Murray, M. (1985). Regulation of parasitaemia in mice infected with *Trypanosoma brucei. Current Topics in Microbiology and Immunology*, **117**, 93-118.

Bourn, D. and Scott, M. (1978). The successful use of work oxen in agricultural development of tsetse-infested lands in Ethiopia. *Tropical Animal Health and Production*, **10**, 191-203.

Boyt, W.P., Lovemore, D.F., Pilson, R.D., Smith, I.M. (1962). A preliminary

report on the maintenance of cattle by various drugs in a mixed *Glossina morsitans* and *Glossina pallidipes* fly-belt. In: *International Scientific Council for Trypanosomiasis Research and Control*, 1962, Conakry, pp. 71-79.

Braber, M. (1968). Note sur la resistance au Berenil d'une souche tchadienne de *Trypanosoma vivax. Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*, 21, 463-466.

Brightwell, R., Dransfield. R.D., Kyorku. C., Golder, T.K., Tarimo, S.A. and Mungai, D, (1987). A new trap for *Glossina pallidipes*. *Tropical Pest Management*, **33**(2), 151-159.

Bruce, D. (1895). In: *"Preliminary report on the tsetse fly disease or Nagana in Zululand", Umbombo, Zululand*. Bennett and David, Eds., Durban, pp. 1-28.

Bruce, D. (1897). "Further report on the tsetse fly disease or "Nagana" in Zululand. Harrisson and Sons, Eds., London, pp. 1-69.

Burnett, G.F. (1970). Control by insecticides: Residual deposits, aerial and ground application, pyrethrum aerosols. In: *The African Trypanosomiases*. H.W. Mulligan, Ed., George Allen and Unwin, London, pp. 490-520.

Bursell, E. (1977). Chemosterilization of tsetse flies using pressurized Metepa aerosol. *Transactions of the Rhodesian Scientific Association* **58**, 43-47.

Bursell, E. (1984). Effects of odour on the host behaviour of tsetse. *Insect Science and Application*, **5**, 345-349.

Carles, A.B. and Lampkin, M.K. (1977). Studies on the permanent teeth eruption and body development of the Large East African Zebu (Boran): 1. The ages at first appearance of the incisors, length of the incisor

eruption period and sources of variation. *Journal of Agricultural Science*, Cambridge, **88**, 341-360.

Cawdery, M.J.H. (1958). Estimation of trypanosome challenge. In: *East African Trypanosomiasis Research Organization,* Annual Report, 1958-57, p. 18.

Cawdery, M.J.H. and Robson, J. (1958). Field trial of ethidium bromide (Suramin complex) R.D. 2902, Prothridium and Antrycide Prosalt. In: *East African Trypanosomiasis Research Organization*, Annual Report, 1956-57, pp. 20 - 23.

Cawdrey, M.J.H. and Simmons, D.J.C. (1965). A review of bovine trypanocidal drug trials of the Uganda Veterinary Department. In: *International Scientific Council for Trypanosomiasis Research*, 1964, Kampala, pp. 47-50.

Challier, A. and Laveissiere, C. (1973). A new trap for capturing *Glossina* (*Glossina*; Diptera, Muscide): description et essais sur le terrain, cah. ORSTOM Ser. *Entomologie medicine et Parasitologie*, **11**, 251-262.

Chandler, R.L. (1958). Studies on the tolerance of N'Dama cattle to trypanosomiasis. *Journal of Comparative Pathology*, **68**, 253-260.

Chizyuka, H.G.B. and Luguru, S.M.K. (1986). Dipping cattle to control vectors. *Parasitology Today*, **2**, 123.

Clausen, P.-H., Sidibe, I., Kabore,I. and Bauer, B. (1992). Development of multiple drug resistance of *Trypanosoma congolense* in Zebu cattle under high tsetse fly challenge in the pastoral zone of Samorogouan, Burkina Faso. *Acta Tropica*, **51**, 229-236.

Coleman, C.H. (1967). Cutaneous streptothricosis of cattle in West Africa. *Veterinary Record*, **81**, 251-254.

Croft, S.L. (1986). *In vitro* screens in the experimental chemotherapy of

leishmaniasis and trypanosomiasis. *Parasitology Today*, **2**, 64-69.

Cross, G.A.M. (1977). Antigenic variation in trypanosomes. *American Journal of Tropical Medicine and Hygiene,* **26**, 240-243.

Cuisance, D., Politzar, H., Merot, P., Tomboura, I., Bauer, B., Kabore, I. and Filledier, J. (1985). Integrated campaign against tsetse in the pastoral area of Sideradougou, Burkina Faso. In: *International Scientific Council for Trypanosomiasis Research and Control, 18th Meeting 1985*, Harare, Zimbabwe, pp. 334-343, (OAU/STRC Publication No. 113).

Cunnigham, M.P. (1966). Immunity in bovine trypanosomiasis. *East African Medical Journal*, **43**, 394-397.

Cunningham, M.P. (1968). Vaccination of cattle against trypanosomiasis by infection and treatment. In: *Isotopes and radiation in parasitology*, IAEA, Vienna, pp. 89-91.

Dame, D.A. (1970). The control by sterilization of *Glossina*. In: *The African Trypanosomiases*. H.W. Mulligan, Ed., George Allen and Unwin, London, pp. 533-542.

Dargie, J.D. (1980). Pathophysiology of trypanosomiasis in the bovine. In: *Isotope Radiation Research on Animal diseases and their vectors,* pp. 121-131. IAEA-SM-240/28/Vienna.

Dargie, J.D., Murray, P.K., Murray, M. Grimshaw, W.R.T. and McIntyre, W.I.M. (1979). Bovine trypanosomiasis: The red cell kinetics of N'Dama and Zebu cattle infected with *Trypanosoma congolense*. *Parasitology*, **78**, 271-286.

Davey, D.G. (1950). Experiments with 'Antrycide' in the Sudan and East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **43**,

583-616.

Davey, D.G. (1957). The chemotherapy of animal trypanosomiasis with particular reference to trypanosomal diseases of domestic animals in Africa. *Veterinary Reviews and Annotations*, **3**, 15-36.

Davey, D. G. (1960). The chemotherapy of animal trypanosomiasis with particular reference to trypanosomal diseases of domestic animals in Africa. In: *International Scientific Council for Trypanosomiasis Research and Control,* 1958, Brussels, pp. 47-50.

Davies, H. (1971). Further eradication of tsetse in the Chad and Gongola river systems in Northern-Eastern Nigeria. *Journal of Applied Ecology*, 8, 563-578.

De Raadt, (1975). Review of African trypanosomiasis. In: *International Scientific Council for Trypanosomiasis Research and Control,* 14th Meeting, 1975, Dakar, Senegal, pp. 59-71, (OAU/STRC Publication No. 109).

Desowitz, R.S. (1959). Studies on immunity and host parasite relationship. I. The immunological response of resistant and susceptible breeds of cattle to trypanosomal challenge. *Annals of Tropical Medicine and Parasitology,* **53**, 293-313.

Desowitz, R.S. (1970). African trypanosomes. In: *Immunity to parasitic diseases of animals*, Vol. **2**. G.J. Jackson, R. Herman and I. Singer, Eds., Appleton-Century-Crofts, New York, pp. 551-596.

Dirie, M.F., Wallbanks, K.R., Molyneux, D.H., Bornstein, S. and Omer, H.A. (1988). Hemorrhagic syndrome associated with *Trypanosoma vivax* infections of cattle in Somalia. *Acta Tropica* **45**, 291-292.

Dolan, R.B. (1987). Genetics and trypanotolerance. *Parasitology Today*, **3**, 137-43.

Dolan, R.B., Njogu, A.R., Sayer, P.D. Wilson, A.J. and Alushula, H. (1985). Trypanotolerance in East African cattle. In: *International Scientific Council for Trypanosomiasis Research and Control*, 18th Meeting, Harare, Zimbabwe, pp. 240-246.

Dolan, R.B., Stevenson, P.G.W., Alushula, H. and Okech, G. (1992). Failure of prophylaxis against bovine trypanosomiasis on the Galana Ranch in Kenya. *Acta Tropica,* **51**, 113-121.

Donelson, J.E. (1984). The genetics of antigenic variation in trypanosomes. In: *Molecular Parasitology*. J.T. August, Ed., Academic press, Orlando, 1984, pp. 33-51.

Doyle, J.J. (1977). Antigenic variation in salivarian trypanosomes. In: *Blood-borne parasitic diseases*. J.A. Pino, L.H. Miller and J. Mckelvey, Eds., Plenum press, New York, pp. 31-63.

Doyle, J.J., Moloo, S.K. and Borrowy, N.K. (1984). Development of improved methods for control of animal trypanosomiasis: A review. *Preventive Veterinary Medicine* **2**, 43-52.

Dransfield, R.D., Chaundhury, M.F.B., Tarimo, S.A., Golder, T.K. and Brightwell, R. (1985). Population dynamics of *Glossina pallidipes* under drought conditions at Nguruman, Kenya. In: *International Scientific Council for Trypanosomiasis Research and Control*, 18th Meeting, 1985, Harare, Zimbabwe, pp. 284-292, (OAU/STRC Publication No. 113).

Dransfield, R.D., Williams, B.G. and Brightwell, R. (1991). Control of tsetse flies and trypanosomiasis: Myth or reality?. *Parasitology Today*, **7**, 287-291.

Dwinger, R.H., Grootenhuis, J.G., Murray, M., Moloo, S.K. and Gettinby, G. (1986). Susceptibility of buffaloes, cattle and goats to infection with dif-

ferent stocks of *Trypanosoma vivax* transmitted by *Glossina morsitans centralis*. *Research Veterinary Science*, **41**, 307-315.

Epstein, H. (1971). *The origin of the domestic livestock of Africa.* Volumes 1 and 2. Africana, New York.

Epstein, H. and Mason, I.L. (1984). Cattle. In: *Evolution of domesticated animals.* I.L. Mason, Ed., Longman, London. pp. 6-27.

FAO (1961). *Livestock and meat marketing in Africa.* H.J. Mittendorf and S.G. Wilson, Eds., FAO, Rome, pp. 45-47.

FAO/WHO/OIE (1963). The economic losses caused by animal diseases. In: *Animal Health Year book,* 1962., pp. 284-313. FAO, Rome.

FAO (1974). Report on expert consultation on the programme for the control of animal trypanosomiasis. *AGA/TRYP/74/IE.* FAO, Rome.

FAO (1975). World production of animal protein and needs for a new approach. *AGA/AAAP/75.* FAO, Rome.

FAO (1976). First FAO expert consultation on research on trypanotolerance and breeding of trypanotolerant animals. *AGA-820.* FAO, Rome.

FAO (1977). Insecticides and application equipment for tsetse control. *Animal Production and Health paper*, No. 3. FAO, Rome.

FAO (1979). The African Trypansomiases: Report of joint WHO expert committee and FAO expert consultation, Rome, 8-12th November, 1976.

FAO (1981). Report of FAO panel of experts on the ecological and technical aspects of the programme for the control of African animal trypanosomiasis and related development. 1-5th June, 1981. FAO, Rome.

FAO (1987). Trypanotolerant cattle and livestock development in West and Central Africa. Vol. I. A.P.M. Shaw and C.H. Hoste, Eds., FAO Animal

Production and Health paper 67/1.

FAO/WHO/OIE (1982). *Animal Health Year book.* V. Kouba, Ed., FAO, Rome.

Feeders, A. and Salvadori, C. (1984). Peoples and cultures of Kenya. Transafrica - Nairobi.

Fiennes, R.N.T., Jones, E.R. and Laws, L.G. (1946). The course of pathology of *Trypanosoma congolense* disease in cattle. *Journal of Comparative Pathology*, **56**, 1-27.

Fiennes, R.N.T. (1950). The cattle trypanosomiasis. Some considerations of pathology and immunity. *Annals of Tropical Medicine and Parasitology,* **44**, 42-54.

Fiennes, R.N.T. (1970). Pathogenesis and Pathology of animal trypanosomiasis. In: *The African Trypansomiases.* H.W. Mulligan, Ed., George Allen and Unwin, London, pp. 729-750.

Finelle, P. (1974). Trypanosomiasis: Chemotherapy and chemoprophylaxis. In: *Les moyens de lutte contre les trypanosomes et leur vecteurs,* p. 291. I.E.M.V.T., Maison, Alfort.

Finelle, P. (1976). Chemotherapy and chemoprophylaxis of African trypanosomiasis. *FAO Report AGA/TRYP/WP/76/28.* FAO, Rome.

Finelle, P. (1980). Program for the control of African animal trypanosomiasis and related development. *IAEA*, Vienna, 1980, pp. 3-14.

Fluck, D.J. and Hopkins, J.S. (1987). Chemotherapy and chemoprophylaxis of bovine trypanosomiasis with liposomal trypanocides. In: *International Scientific Council for Trypanosomiasis Research and Control,* 19th Meeting, 1987, Lome, Togo, pp. 303-313, (OAU/STR Publication No. 114).

Ford, J. (1970a). Geographical distribution of *Glossina.* In: *The African*

Trypanosomiases. H.W. Mulligan, Ed., George Allan and Unwin, London, pp. 274-297.

Ford, J. (1970b). The search for food. In: *The African Trypanosomiases*. H.W. Mulligan, Ed., George Allan and Unwin, London, pp. 298-304.

Ford, J, (1970c). Control by destruction of the larger fauna. In: *The African Trypanosomiases*. H.W. Mulligan, Ed., George Allan and Unwin, London, pp. 557-571.

Ford, J., Nash, T.A,M. and Welch, J.R. (1970). Control by clearing the vegetation. In: *The African Trypansomiases*. H.W. Mulligan, Ed., George Allen and Unwin, London, pp. 543-56.

Ford, J. and Katondo, F.M. (1973). The distribution of tsetse flies (*Glossina*) in Africa. OAU/STRC Maps.

Gadir, F.A., Tahir, M.E., Razig, A. and Osman, O.M. (1972). Sensitivity of ethidium bromide resistant *Trypanosoma congolense* strain to Samorin and Berenil. *Sudan Journal of Veterinary Science Animal Husbandry,* **13**, 68-73.

Gardiner, P.R. and Wilson, A.J. (1987). *Trypanosoma (dutonella) vivax. Parasitology Today.*, **3**, (2), 49-52.

Gavora, J.S. (1982). Disease resistance. In: *Second World Congress on Genetics applied to Livestock production*. Vol. V. Madrid, Spain, pp. 143-149.

Geigy, R., Kauffman, M. and Belinger, R. (1967). A survey of wild animals as potential reservoirs of trypanosomiasis in Ulunga District, Tanzania. *Acta Tropica*, **24**, 97-108.

Geigy, R. and Kauffman, M (1973). Sleeping sickness survey in Serengeti area (Tanzania) 1971. I. Examination of large mammals for trypanosomes. *Acta Tropica* **30**, 12-23.

Gitatha, S. K. and Maudlin, I. (1968). Some investigations on Berenil in cattle. In: *International Scientific Council for Trypanosomiasis Research and Control,* 1968, Bangui, pp. 125-129.

Gitatha, S.K. (1979). *Trypanosoma congolense* (Shimba Hills) resistant to various trypanocidal drugs. In: *International Scientific Council for Trypanosomiasis Research and Control*, 16th Meeting, 1979, Younde, Cameroon, pp. 257-263, (OAU/STRC Publication No. 111)

Glasgow, J. P. (1970). Methods for collecting and sampling of *Glossina*. In: *The African Trypanosomiases*. H.W. Mulligan, Ed., George Allen and Unwin Ltd., London, pp. 395-415.

Glasgow, J.P. and Potts, W.H. (1970). Control by hand catching and traps. In: *The African Trypanosomiases*. H.W. Mulligan, Ed., George Allen and Unwin, London, pp. 456-463.

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., Roberts, T.M. and KillicK-Kendrick R. (1968). Plasma protein and haemoglobin levels in Zebu cattle infected with trypanosomiasis in the field. *Bulletin of Epizootic Diseases of Africa*, **16**, 205-212.

Gray, A.R. (1970). A study of the antigenic relationships of isolates of *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 Roberts, C.J. (1968). Experiments on the cyclical transmission of

drug resistant strains of trypanosomes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **62**, 126.

Gray, A.R. and Roberts C.J. (1971). The cyclical transmission of strains of *Trypanosoma congolense* and *Trypanosoma vivax* resistant strains to normal therapeutic doses of trypanocidal drugs. *Parasitology*, **63**, 67-89.

Gray, A.R. (1974). Trypanosomal antigens and the control of trypanosomiasis. In: Control programmes for trypanosomes and their vectors. Report of symposium, Paris 12-15th March 1974. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*. Supplement 1974, pp. 187-191.

Gray, A.R. and Luckins, A.G. (1976). The Antigenic variation in salivarian trypanosomes. In: *Biology of the Kinetoplastida*, I. W.H.R. Lumsden and D.A. Evans, Eds., pp. 493-542.

Gray, A.R. (1983). Chemistry and the control of animal trypanosomiasis and theileriosis. In: *Chemistry and World food supplies: The new frontiers*. (L.W. Shemitt, Ed., Pergamon press, Oxford., pp. 309-317.

Greig, W.A., Murray, M., Murray, P.K. and McIntyre, W.I.M. (1979). Factors affecting blood sampling and parasitaemia in bovine trypanosomiasis. *British Veterinary Journal*, **135**, 130-14.

Griffin, L. and Allonby, L.E.W (1979a). The disease syndromes in sheep and goats naturally infected with *Trypanosoma congolense*. *Journal of Comparative Pathology*, **89**, 457-464.

Griffin, L. and Allonby, E.W. (1979b). Trypanotolerance in breeds of sheep and goats with an experimental *Trypanosoma congolense* infection. *Veterinary Parasitology*, **5**, 97-105.

Grootenhuis, J.G., Varma, Y., Black, S., Moloo, S.K., Akol, G.W.O., Emery, D.L.

and Murray, M. (1982). Host responses of some African wild Bovidae to experimental trypanosome infection. In: *Trypanotolerance and Animal production*. E. Karbe and E.K. Freitas, Eds., GTZ No. 116, pp. 37-42.

Grootenhuis, J.G., Dwinger, R.H., Dolan, R.B., Moloo, S.K. and Murray, M. (1990). Susceptibility of African buffalo and Boran cattle to *Trypanosoma congolense* transmitted by *Glossina morsitans centralis. Veterinary Parasitology*, **35**, 219-231.

Grundler, G. (1985). The influence of trypanosomiasis on sperm quality. In: *International Scientific Council for Trypanosomiasis Research and Control* 18th Meeting, Zimbabwe, 1985, pp. 193-197, Harare, (OAU/STRC Publication No. 113).

Hall, D.R., Beevor, P.S., Cork, A., Nesbitt, B.F and Vale, G.A. (1984). 1-octen-3-ol: A potent olfactory stimulant and attractant for the tsetse, isolated from cattle odours. *Insect Science and Application,* **5**, 335-339.

Hall, M.J.R. (1985). The behaviour of *Glossina morsitans morsitans* towards pheromone baited decoys in the field. In: *International Scientific Council for Trypanosomiasis Research and Control,* 18th Meeting 1985, Harare, Zimbabwe, pp. 300-313, (OAU/STRC Publication No. 113).

Hargrove, J.W. (1977). Towards a new method of controlling tsetse. *Rhodesian Scientific News*, **11**, p. 141.

Harley, J.M.B. and Wilson A.J. (1968). Comparison between *Glossina morsitans, G. pallidipes*, and *G. fuscipes* as vectors of trypanosomes of the *Trypanosoma congolense* group: the proportions infected experimentally and the number of infective organisms extruded during feeding. *Annals of Tropical Medicine and Parasitology* **62**, 178-187.

Henderson, G.B. and Fairlamb, A.H. (1987). Trypanothione metabolism: A chemotherapeutic target in Trypanosomatids. *Parasitology Today*, **3**, 1987.

Hoare, C.A. (1970). Mammalian trypanosomes of Africa. In: *The African Trypanosomiases*. H.W. Mulligan, Ed., George Allan and Unwin, London, pp. 3-23.

Hoare, C.A. (1972). *The trypanosomes of mammals.* Oxford, Blackwell Scientific Publication, 1972.

Holmes, P.H. and Jennings, F.W. (1976). The effect of treatment on anaemia of the African trypanosomes. In: *Pathophysiology of parasitic infections*. E.J.L. Soulsby, Ed., Academic press, New York, pp. 199-210.

Holmes, P.H. (1980). Vaccination against trypanosomes: A review. *Symposium of the British Society for Parasitology,* **18**, 75-105.

Holmes, P.H. and Scott, J.M. (1982). Chemotherapy against animal trypanosomiasis. In: *Perspectives of trypanosomiasis research*. J.R. Baker, Ed., Research studies press, Chichester, pp. 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.

Hudson, J.R. (1944). Acute and subacute trypanosomiasis in cattle caused by *Trypanosoma vivax. Journal of Comparative Pathology,* **54**, 108-119.

House, A.P.R. (1982). Chemosterilization of *Glossina morsitans* West. and *G. pallidipes* Aust. (Diptera: *Glossinidae*) in the field. *Bulletin of Entomological Research*, **72**, 65-70.

Hornby, H.E. (1921). Trypanosomes and trypanosomiasis of cattle. *Journal of Comparative Pathology* **34**, 211-240.

Hoste, C.H., Chalon, E., d'Iteren, G.D.M. and Trail, J.C.M. (1989). Trypanotolerant Livestock in West and Central Afica, vol. III. Situation after a decade. FAO Animal Production and Health Paper 20/3. Food and Agriculture Organization of the United Nations, Rome.

IBAR (1989). Cattle Distribution Maps. Interafrican Bureau for Animal Resources. Organization of African Unity, Nairobi, Kenya.

Ijagbone, I.F., Staak, C. and Reinhard, R. (1989). Fractionation of trypanosome antigens for species-specific sero-diagnosis. *Veterinary Parasitology,* **32**, 293-299.

Ikede, B.O. and Losos, G.J. (1979). Pathogenesis of *Trypanosoma brucei* infections in sheep. III. Hypophyseal and other endocrine lesions. *Journal of Comparative Pathology,* **85**, 37-44.

ILCA (1979). Trypanotolerant livestock network in West and Central Africa. Monograph 2, ILCA, Addis Ababa, Ethiopia.

ILCA (1983). Livestock productivity and trypanotolerance - Network Training Manual. M. Murray, J.C.M. Trail, D.A. Turner and Y. Wissocq, Eds., ILCA, Addis Ababa, Ethiopia.

ILCA (1986a). The ILCA/ILRAD Trypanotolerance network. Situation report, December, 1985: *Proceedings of a network meeting held at ILCA,* 30th November to 4th December 1985, Nairobi.

ILCA (1986b). The African Trypanotolerant Livestock Network. Indications from the results 1983-1985. ILCA, Addis Ababa.

ILCA (1991). Maasai herding. Analysis of the livestock production system of Maasai pastoralists in eastern Kajiado Distict, Kenya. ILCA, Addis Ababa, Ethiopia.

Ilemobade, A.A. and Buys, J. (1970). The isolation of *Trypanosoma vivax* resistant against Novidium from cattle in Northern Nigeria. *Veterinary Record,* **87,** 761-762.

ILRAD (1991). The mechanisms by which cattle acquire immunity to trypanosomiasis. ILRAD Report

Ishmael, A.A. (1988). Studies on the susceptibility of the Orma and Galana Boran cattle to trypanosome infection. *Ph.D. Thesis*, University of Nairobi.

Ishmael, A.A., Njogu, A.R., Gettinby, G. and Murray, M. (1985). Susceptibility of Orma and Galana Boran cattle to infection with blood stream forms of *Trypanosoma congolense* and *Trypanosoma vivax.* In: *International Scientific Council for Trypanosomiasis Research and Control,* 18th Meeting, 1985, Harare, Zimbabwe, pp. 176-181, (OAU/STRC Publication No. 113).

James, D.M. (1978). Prophylactic activity in rodents of trypanocides complexed with dextran. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **72,** 471-476.

Jennings, F.W. (1988). Chemotherapy of trypanosomiasis: The potential of melarsoprol by concurrent difluoromethylornithine (DFMO) treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **82,** 572-573.

Jensch, H. (1955). 4,4'-Diamidino-diazoaminobenzol, ein neues Mittel gegen Trypanosomen-und Babesien-Infektionen. *Arzneimittel-Forschung,* **5,** 634-635.

Jones-Davies, W.J. (1967a). The discovery of Berenil resistant *Trypanosoma vivax* in Northern Nigeria. *Veterinary Record,* **80,** 531-532.

Jones-Davies, W.J. (1967b). A Berenil resistant strain of *Trypanosoma vivax* in

cattle. *Veterinary Record,* **81**, 567-568.

Jones-Davies, W.J. (1968). Berenil resistance in naturally occurring *Trypanosoma congolense. Bulletin of Epizootic Diseases of Africa*, **16**, 213-216.

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. (1985). Tsetse eradication plans for Southern Africa. *Parasitology Today,* **1**, 121-123.

Jordan, A.M. (1986). *Trypanosomiasis control and African rural development.* Longman, London.

Jordt, T., Morgan, G.D., Toury, G.N., Ngulo, W.K., Morrison, W.I., Rawle, J. and Murray, M. (1986). Successful transfer of frozen N'Dama embryos from The Gambia to Kenya. *Tropical Animal Health and Production,* **18**, 65-75.

Kanyari, P.N., Allonby, E.W., Wilson, A.J. and Munyua, W.K. (1983). Some economic effects of trypanosomiasis in goats. *Tropical Animal Health Production*, **15**, 153-160.

Karstard, L., Grootenhuis, J.G. and Mushi, E.Z. (1978). Research on wildlife diseases in Kenya, 1967-1978. *Kenya Veterinarian*, **2**, 29-32.

Katabaazi, B.K. and Matechi, H.T. (1977). Assessment of the residues effectiveness of Gardona against the tsetse flies in the savanna habitats. In: *International Scientific Council for Trypanosomiasis Research and Control*, 15th Meeting 1977, Banjul, Gambia, pp. 541-547.

Katende, J.M., Musoke, A.J., Nantulya V.M. and Goddeeris, B.M. (1987). A new method for fixation and preservation of trypanosomal antigens for use in the indirect immunoflourescence antibody test for diagnosis of bovine

trypanosomiasis. *Tropical Medicine and Parasitology,* **38**, 41-44.

Kaufmann, J., Dwinger, R.H., Hallebeek, A., van Dijk, B. and Pfister, K. (1992). The interaction of *Trypanosoma congolense* and *Haemonchus contortus* infections in trypanotolerant N'Dama cattle. *Veterinary Parasitology,* **43**, 157-170.

Kenya (1983). Kenya Official Handbook. Publisher - Kenya government.

Killick-kendrick, R.R. and Godfrey, D.G. (1963). Bovine trypanosomiasis in Nigeria. II. The incidence among some migrating cattle with observations on the examination of wet blood preparation as a method of survey. *Annals of Tropical Medicine and Parasitology,* **57**, 117-126.

King, J.M., Heath, R.B. and Hill, R.E. (1977). Game domestication for animal production in Kenya: theory and practice. *Journal of Agricultural Science,* Cambridge, **89**, 445 - 457.

Keoman, J.H. and Hadden, P.M. (1968). Report concerning the side effects of dieldrin spraying on small rodents in South Western Kenya. In: *International Scientific Council for Trypanosomiasis Research,* 1968, Bangui, pp. 257-265, (OAU/STRC Publication No. 102).

Knippling, E.F. (1982). Present status and future trends of the Sterile Insect Technique approach of arthropod pests. In: *Proceedings of symposium on Sterile Insect Technique radiation in insect control.* IAEA, Vienna, pp. 3-23.

Kukla, B.A., Majiwa, P.A.O., Young, J.R., Moloo, S.K. and ole-MoiYoi, O.K. (1987). Use of specific DNA probes for detection and identification of trypanosome infection in flies. *Parasitology,* **95**, 1-16.

Kupper, 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. In: *International Scientific Council for Trypanosomiasis Research and Control,* 18th Meeting, 1985, Harare, Zimbabwe, pp. 364-371, (OAU/STRC Publication No. 113).

Kupper, W. and Wolters, M. (1983). Observations on drug resistance of *Trypanosoma (N)congolense* and *T. (D)vivax* in cattle at a feedlot in Ivory Coast. *Tropenmedizin und Parasitologie,* **34**, 203-205.

Laird, M. and Simmonds, F.J. (1977). Biological prospects in future integrated tsetse control programmes. In: *Tsetse: The future of biological control methods in integrated control*, pp. 177-86, IDRC, Ottawa.

Langley, P.A., Coates, T.W., Carlson, D.A., Vale, G.A. and Marshall J. (1982). Prospects for autosterilization for the tsetse flies *Glossina* spp. (Diptera: *Glossinidae*) using sex pheromones and bisazir in the field. *Bulletin of Entomological Research,* **72**, 319-27.

Langley,, P.A., Trewern, M.A. and Jurd, L. (1982). Sterilization effects of benzyl-1, 3-benzodioxoles on the tsetse fly *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). *Bulletin of Entomological Research,* **72**, 473-481.

Langridge, W.P. and Mgutu, S.P. (1968). Some observations on the destruction of wildlife and insects after spraying with organochloride pesticide for tsetse control. In: *International Scientific Council for Trypanosomiasis Research,* 1968, Bangui, pp. 257-265, (OAU/STRC Publication No. 102).

Laveissiere, C., Couret, D. and Kienon, J-P. (1981). Lutte contre les glossines riverine al'aide de pieges biconiques impregnes d'insecticide, en zone de savana humide. 5. Note de synthese, Cah. ORSTOM Ser. *Entomologie*

medicine et Parasitologie, **19**, 49-54.

Leach, T.M. and Roberts, C.J. (1981). Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the Eastern Hemisphere. *Pharmacology and Therapeutics* **13**, 91-147.

Lee, C.W., Parker, J.D., Baldry, D.T.A. and Molyneux, D.H. (1978). The experimental application of insecticides from a helicopter for the control of riverine populations of *Glossina tachinoides* in West Africa. II. The calibration of equipment and insecticide dispersal. *PANS,* 24,(4), 404-422.

Lee, C.W. and Maurice, J.M. (1983). The African Trypansomiases: Methods of control and eradication in relation to development. Technical paper No. 4. The World bank, Washington D.C.

Letteneur, L. (1978). Crossbreeding N'Dama and Jersey cattle in Ivory Coast. *World Animal Review*, **27**, 36-42.

Llyod, L. and Johnson, W. B. (1924). The trypanosome infections in Nigeria and a new method of estimation. *Bulletin of Entomological Research,* **14**, 265-288.

Logan, L.L., Goodwin,T.J., Tembely, S. and Craig, T.M. (1984). Maintaining Zebu Maure cattle in a tsetse-infested area of Mali. *Tropical Animal Health and Production,* **16**, 1-12.

Lohr, K-F, Omukuba, J.N., Njogu, A.R., Maloo, S.H., Gisemba, F., Okedi, T. and Mwongela, S. (1991). Investigation of the efficacy of flumethrin pour-on for the control of high tsetse and trypanosomiasis challenge in Kenya. *Tropical Medicine Parasitology,* 42, 131-134.

Lorenzini, E., Scott, J.R., Paling, R.W., and Jordt, T. (1988). The effects of *Trypanosoma congolense* infection on the reproductive cycle of N'Dama

and Boran heifers. In: *Livestock production in tsetse affected areas of Africa*, pp. 163-73. International Livestock Centre for Africa and the International Laboratory for Research on Animal Diseases, Nairobi, Kenya, pp. 425-429.

Losos, G.J. and Ikede, B.O. (1972). Review of pathology of diseases in domestic animals caused by *Trypanosoma congolense, T. vivax, T. brucei, T. rhodensiense and T. gambiense*. *Veterinary Pathology,* **9**, (Supplement 1), 1-72.

Lowman, B.G., Scott, N.A. and Sommerville, S.H. (1976). Bulletin No. 6, East Scotland College of Agriculture.

Luckins, A.G. (1977). Detection of antibodies in trypanosome infected cattle by means of a microplate enzyme-linked immunosorbent assay. *Tropical Animal Health and Production,* **9**, 53-62.

Luckins, A.G., Gray, A.R. and Rae, O. (1978). A comparison of the diagnostic value of serum immunoglobulin levels, an enzyme-linked immunosorbent assay and a fluorescent antibody test in the experimental infections with *Trypanosoma evansi* in rabbits. *Annals of Tropical Medicine and Parasitology,* **72**, 429-441.

Luckins, A.G., Boid, R., Rae, P., Mahmoud, M.M., El Malik, K.H. and Gray A.R. (1979). Serodiagnosis of infection with *Trypanosoma evansi* in camels in the Sudan. *Tropical Animal Health and Production,* **11**, 1-12.

Lumsden, W.H.R., Kimber, C.D. and Strange, M. (1977). *Trypanosoma brucei*: detection of low parasitaemias in mice by miniature anion-exchange/centrifugation technique. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **71**, 421-424.

Lumsden, W.H.R., Kimber, C.D., Evans, D.A. and Doig, S.J. (1979). *Trypanosoma brucei*: miniature anion-exchange/centrifugation technique for the detection of low parasitaemias: adaptation for field use. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **73**, 312-317.

MacLennan, K.J.R. (1967). Recent advances in techniques for tsetse fly control. *Bulletin of World Health Organization* **37**, 615-628.

MacLennan, K.J.R. and Na'Isa, B.K. (1970). Relapsing of *Trypanosoma vivax* infections in Nigerian Zebu cattle treated with diminazene aceturate. *Tropical Animal Health and Production,* **2**, 189-195.

MacLennan, K.J.R. and N'Aisa, B.K. (1971). The current status and future prospects regarding tsetse extermination in Nigeria. In: *International Scientific Conference for Trypanosomiasis Research and Control*, 13th Meeting, 1971, Lagos, Nigeria, pp. 303-309, (OAU/STRC Publication No. 105).

MacLennan, K.J.R. (1971). The aparasitaemic interval following diminazene aceturate therapy of a relapsing strain of *Trypanosoma vivax* infecting cattle. *Tropical Animal Health and Production*, **3**, 208-212.

MacLennan, K.J.R. and Cook, M.G. (1972). The resting behaviour of *Glossina morsitans submorsitans* Newst. in Northern Guinea vegetation zone in relation to control using insecticides. *Entomologist,* **105**, 144-52.

MacLennan, K.J.R. (1972). A note on the period of aparasitaemia following the subcurative treatment of *Trypanosoma vivax* in the bovine with diminazene aceturate. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **66**, 339-340.

MacLennan, K.J.R. (1980). Tsetse-transmitted trypanosomiasis in relation to ru-

ral economy in Africa. Part I: Tsetse infestation. *World Animal Review* **36**, 2-17.

MacLennan, K.J.R. (1981). Tsetse-transmitted trypanosomiasis in relation to rural economy in Africa. Part II: Techniques in use for the control or eradication of tsetse infestations. *World Animal Review* **37**, 9-19.

Magnus ,E., Vervoort, T. and van Meirvenne, N. (1978). A card agglutination test with stained trypanosomes (CATT) for serological diagnosis of *Trypanosoma b. gambiense* trypanosomiasis. *Annales de la Societe belge de Medecine tropicale,* **58**, 169-176.

Mahmoud, M.M. and Emalik. K.H. (1977). Trypanosomiasis: Goats as possible reservoirs of *Trypanosoma congolense* in the Republic of Sudan. *Tropical Animal Health and Production,* **9**, 167-170.

Maina, A. (1977). The need for joint tsetse control programmes across the national borders. In: *International Scientific Conference for Trypanosomiasis Research and Control* 15th Meeting, 1977, Banjul, Gambia, pp. 582-584, (OAU/STRC Publication No. 110).

Maloo, S.H., Chema, S., Connor, R., Durkin, J., Kimotho, P., Maehl, H., Mukendi, F. Murray, M., Rarieya, M. and Trial, J. (1987). Efficacy of chemoprophylaxis in East African Zebu cattle to exposed to trypanosomiasis in village herds in Kenya. In: *International Scientific Conference for Trypanosomiasis Research and Control,* 19th Meeting, 1987, Lome, Togo, pp. 282-287, (OAU/STRC Publication No. 114).

Maloo, S.H., Chema, S., Connor, R., Durkin, J., Kimotho, P., Maehl, J.H.H., Mukendi, F., Murray, M., Rarienga, J.M. and Trail, J.C.M. (1988). The use of chemoprophylactic drugs in East African Zebu cattle exposed to

trypanosomiasis in Muhaka area, Kenya. In: *Livestock production in tsetse affected areas of Africa.* pp. 283-288. International Livestock Centre for Africa and the International Laboratory for Research on Animal Diseases, Nairobi, Kenya, pp. 425-429.

Masake, R.A. and Nantulya, V.M. (1990). Sensitivity of an antigen detection enzyme immunoassay for diagnosis of *Trypanosoma congolense* infections in goats and cattle. *Journal of Parasitology*, **77** (2), 231-236.

Mason, I. L. and Maule, J. P. (1960). *The indigenous cattle of Eastern and Southern Africa.* Commonwealth Agricultural Bureaux, Farnham Royal.

Maule, J.P. (1990). *The Cattle of the tropics.* University of Edinburgh.

Mbwambo, H.A., Mella, P.N.P. and Lekaki, K.A. (1988). Berenil resistant *Trypanosoma congolense* in cattle under natural challenge at Kibaha, Tanzania. *Acta Tropica,* **45**, 239-244.

McDowell, R.E. (1977). *Ruminant products: More meat than milk.* Winrock International Livestock And Training Centre, Marilton, Arkansas.

Mehlitz D. and Tietjen, U (1988). Trypanosome infection rates in tsetse midguts using a short term *in vitro* culture technique. *Acta Tropica* **45**, 183-184.

Metam, R.W.M. (1934). Annual Report of the Veterinary Department of Uganda Protectorate, pp. 26-28.

Milligan, P.M.J. and Baker, R.D. (1988). A model for tsetse-transmitted animal trypanosomiasis. *Parasitology*, **96**, 211-39.

Mohamoud, M.M. and Gray, A.R. (1980). Trypanosomiasis due to *Trypanosoma evansi.* A review of recent research. *Tropical Animal Health Production,* **12**, 35-47.

Molyneux, D.H. and Ashford., R. (1983). *The Boilogy of Trypanosoma and*

Leishmania, Parasites of man and domestic animals. Taylor and Francis, London.

Molyneux, D.H. (1975). Diagnostic Methods in animal trypanosomiasis. *Veterinary Parasitology,* **1**, 5-17.

Molyneux, D.H. (1986). African trypanosomiasis. *Clinics in Tropical Medicine and Communicable diseases,* **1,** 535-55.

Moloo, S.K. and Gray, M.A. (1989). New observations on the cyclical development of *Trypanosoma vivax* in *Glossina*. *Acta Tropica* **46**, 3, 167-172.

Monirei, J.M., Murray, M., Whitelaw, D.D., Trail, J.C.M., Wissocq, Y.J. and Chema, S (1982). Susceptibility of East African livestock to African trypanosomiasis. In: *Trypanotolerance and animal production*. E. Karbe and E.K. Freitas, Eds., GTZ. No. 116, pp. 33-36.

Morrison, W.I., Roelants, G.E., Meyer-Withey, K.S. and Murray, M. (1978). Susceptibility of inbred strains of mice to *Trypanosoma congolense*: Correlation with changes in spleen lymphocyte population. *Clinical and Experimental Immunology,* **32**, 25-40.

Morrison, W.I. and Murray, M. (1979). *Trypanosoma congolense:* Inheritance of susceptibility to infection in inbred strains of mice. *Experimental Parasitology,* **48**, 364-74.

Morrison, W.I., Murray, M. and McIntyre, W.I.M. (1981a). Bovine trypanosomiasis. In: *Diseases of cattle in the tropics*. M. Ristic and W.I.M. McIntyre, Eds., Martinos Nijhoff publishers, pp. 469-497.

Morrison, W.I., Murray, M., Sayer, P.D. and Preston, J.M. (1981b). The pathogenesis of experimentally induced *Trypanosoma brucei* infection in the dog. I: Tissue and organ damage. *American Journal of Pathology,* **102,**

168-181.

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.

Mortelmans, J. and Kageruka, P. (1976). Trypanotolerant cattle breeds in Zaire. *World Animal Review,* **19**, 14-17.

Munyua, W.K. (1985). Goat trypanosomiasis: trypanotolerance and epidemiology among goat breeds in Kenya. In: International Scientific Council for Trypanosomiasis Research and Control, 18th meeting, Harare, Zimbabwe. OAU/STR Publication No. 113, pp. 284-292.

Murray, M. (1974). The pathology of African trypanosomiasis In: *Progress in Immunology*. L. Brent and J. Holborrow, Eds., North Holland publishing company, Amsterdam, Part II, Vol. 4, pp. 181-192.

Murray, M. (1979). Anaemia in bovine trypanosomiasis: An overview. In: *Pathogenicity of trypanosomes*. G. Lossos and A. Chouinard, Eds., IDRC, Ottawa, No. 132e, pp. 121-127.

Murray, M. (1989). Factors affecting duration and intensity of trypanosome infection of domestic animals. *Annales de la Societe belge de Medecine tropicale,* **69**, 189-196.

Murray, M., Murray, P.K. and McIntyre, W.I.M. (1977). An improved parasitological technique for the diagnosis of African trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **71**, 325-326.

Murray, M. and Urqurhart, G.M. (1977). Immunoprophylaxis against African

trypanosomes. In: *Immunity to blood parasites of animals and man.* L.H. Miller and J.J. McKelvey, Eds., Plenum press, New York and London, pp. 209-241.

Murray, P.K., Murray, M., Wallace, M., Morrison, W.I. and McIntyre, W.I.M. (1979a). Trypanosomiasis in N'Dama and Zebu cattle. I. An experimental investigation of susceptibility to *Trypanosoma brucei, Trypanosoma congolense* and mixed infections. In: *International Scientific Conference for Trypanosomiasis Research and Control,* 15th Meeting, 1977, Banjul, Gambia, pp. 470-481, (OAU/STRC Publication No. 111).

Murray, M., McIntyre, W.I.M., Murray, P.K., Urquhart, G.M., Jennings, F.W., Greig, W.A., Clifford, D.J., N'Dow, W.S.M., Toure, B.N., Sanyang, B.T. and Bray, R.S. (1979b). Cattle diseases and trypanosomiasis in The Gambia: I. Clinical Studies. In: *International Scientific Conference for Trypanosomiasis Research and Control,* 15th Meeting, 1977, Banjul, Gambia, 1979, pp. 83-91, (OAU/STRC Publication, No. 110).

Murray, M, McIntyre, W.I.M., Murray, P.K., Urquhart, G.M., Jennings, F.W., Greig, W.A. Clifford, D.J., N'Dow, W.S.M., Toure, B.N., Sanyang, B.T. and Bray, R.S. (1979c). Cattle diseases and trypanosomiasis in The Gambia: II. Pathological Studies. In: *International Scientific Conference for Trypanosomiasis Research and Control,* 15th Meeting, 1977, Banjul, Gambia, pp. 92-98, (OAU/STRC Publication No. 110).

Murray, M., Clifford, D.J., Gettinby, G., Snow, F. and McIntyre, W.I.M. 1981a. A study of susceptibility to African trypanosomiasis of N'Dama and Zebu cattle in areas of *Glossina morsitans submorsitans* challenge. *Veterinary Record,* **109**, 503-510.

Murray, M., Grootenhuis, J.G., Akol, G.W.O., Emery, D.L., Shapiro, S.Z., Moloo, S.K., Dar, F.K., Borell, D.L. and Paris, J. (1981b). The potential application of research on African trypanosomiasis in wildlife and preliminary studies on animals exposed to tsetse infected with *Trypanosoma congolense*. In: *Wildlife disease research and economic development*. L. Karstad, B. Nestel and W. Graham, Eds., IDRC, Ottawa, pp. 40-45.

Murray, M., Morrison, W.I., Clifford, D.J., Murray, P.K. and McIntyre, W.I.M. (1981c). Susceptibility to African trypanosomiasis. Studies in cattle and mice. In: *International Scientific Conference for Trypanosomiasis Research and Control,* 16th Meeting, 1979. Younde, Cameroon, pp. 265-271.

Murray, M. Morrison, W.I. and Whitelaw, D.D. (1982). Host susceptibility to African trypanosomiasis: Trypanotolerance. *Advances in Parasitology,* **21**, 1-68. J.R. Baker and R.Muller, Eds., Accademic Press, London and New York.

Murray, M. and Trail, J.C.M. (1982). Trypanotolerance: Genetics, environmental influence and mechanisms. In: *Second World Congress on Genetics applied to Livestock Production,* Madrid, Spain, pp. 293-306.

Murray, M., Trail, J.C.M., Davis, C.E. and Black, S.J. (1984a). Genetic resistance to African trypanosomiasis. *Journal Infectious Diseases,* **149**, (3), 311-319.

Murray, M. and Gray, A.R. (1984). The current situation on animal trypanosomiasis in Africa. *Preventive Veterinary Medicine,* **2**, 23-30.

Murray, M., Trail, J.C.M. and Gootenhuis, J.G. (1984b). Trypanotolerant livestock: Potential and future exploitation. *Outlook on Agriculture,* **13**, 43-51.

Murray, M. and Trail, J.C.M. (1984c). Genetic resistance to animal

trypanosomiasis in Africa. *Preventive Veterinary Medicine,* **2**, 541-51.

Murray, M., Trail, J.C.M. and Maloo, S.H. (1987). African trypanosomiasis in cattle: Improved performance by chemoprophylaxis. *World Veterinary Congress.* 16A-1.

Murray, M. and Dexter, T.M. (1988). Anaemia of bovine African trypanosomiasis: A review. *Acta Tropica,* **45**, 398-432.

Murray, M. and Njogu, A.R. (1989). African trypanosomiasis in wild and domestic ungulates: the problem and its control. In: *the biology of large African mammals in their environment.* P.A. Jewell and G.M.O. Maloiy, Eds., Symposium of Zoological Society of London, **61**, 217-240. Oxford, Claredon Press, 1989.

Murray, M., Stear, M.J., Trail, J.C.M., d'Iteren, D.G.M., Agyemang, K. and Dwinger, R.H. (1991). Trypanosomiasis in cattle: prospects for control. In: *Breeding for Disease Resistance in farm animals.* J.B. Owen and R.F.E. Axford, Eds., CAB International, Wallingford, pp. 203-223.

Mutayoba, B.M., Gombe, S., Waindi, E.N. and Kaaya, G.P. (1989). Comparative trypanotolerance of the Small East African breed goats from different localities to *Trypanosoma congolense* infection. *Veterinary Parasitology,* **31**, 95-105.

Mwambu, P.M. and Mayende, J.S.P. (1971a). Occurence of Berenil resistant strains of *Trypanosoma vivax. Transactions of the Royal Society of Tropical Medicine and Hygiene,* **36**, 414-418.

Mwambu, J.P. and Mayende, J.S. (1971b). Berenil resistant *Trypanosoma vivax* strains isolated from naturally infected cattle in Teso District, Eastern Uganda. In: *International Scientific Conference for Trypanosomiasis*

Research and Control, 13th Meeting, Lagos, Nigeria, pp. 133-138, (OAU/STRC Publication No. 105).

Mwambu, P.M. (1971). The effect of a block treatment regime using ethidium on cattle trypanosomiasis in an endemic area. *East African Agricultural and Forestry Journal,* **36**, 414-418.

Mwongela, G.N., Kovatch, R.M. and Fazil, M.A. (1981). Acute *Trypanosoma vivax* infections in dairy cattle in the Coast Province, Kenya. *Tropical Animal Health and Production*, **13**, 63-69.

Nantulya, V.M. (1981). *Annual Report of the International Laboratory for Research on Animal Diseases,* Nairobi, Kenya.

Nantulya, V.M. (1989). An antigen detection enzyme immunoassay for the diagnosis of *T. rhodesiense* sleeping sickness. *Parasite Immunology,* **11**, 69-75.

Nantulya, V.M. (1990). Trypanosomiasis in domestic animals: the problem of diagnosis. *Revue Scientique et Technique Office International des Epizooties,* **9**, 357-367.

Nantulya, V.M., Hammers, R. and Bajyanga Songa, E. (1989). Detection of circulating trypanosomal antigens in *Trypanosoma evansi* infected animals using *Trypanosoma brucei* group specific monoclonal antibody. *Tropical Medicine Parasitology,* **40**, 263-266.

Nantulya, V.M. and Lindqvist, K.J. (1989). Antigen detection enzyme immunoassays for the diagnosis of *Trypanosoma vivax, Trypanosoma congolense,* and *Trypanosoma brucei* infections in cattle. *Tropical Medicine Parasitology,* **40**, 267-272.

Nantulya, V.M., Lindqvist, K.J. Diall, O. and Olaho-Mukani, W. (1989). Two

simple antigen detection enzyme immunoassays for the diagnosis of *Trypanosoma evansi* in the camel (*Camelus dromedarius*). *Tropical Medicine Parasitology,* **40**, 415-418.

Nantulya, V.M., Musoke, A.J., Rurangirwa, F.R. and Moloo, S.K. (1984). Resistance of cattle to tsetse-transmitted challenge with *Trypanasoma brucei* or *Trypanosoma congolense* after spontaneous recovery from syringe passaged infections. *Infection and Immunity,* **43**, 735-738.

Nantulya, V.M., Musoke, A.J., Rurangirwa, F.R., Minja, S. and Saigar, N. (1985). Diagnosis of African trypanosomiasis using monoclonal antibodies. In: *Proceedings of the VII International congress of Protozoology,* Nairobi, Kenya, p. 173.

Nantulya, V.M., Lindqvist, K.J., Stevenson, P. and Mwangi, E.K. (1992). Application of a monoclonal antibody-based antigen detection enzyme-linked immunosorbent assay (antigen ELISA) for field diagnosis of bovine trypanosomiasis at Nguruman, Kenya. *Annals of Tropical Medicine and Parasitology*, **86,** 225-230.

Nash, T.A.M. (1970). Control by parasite predators. In: *The African Trypanosomiases.* H.W. Mulligan, Ed., George Allen and Unwin, London, pp. 521-532.

Nicholson, M.J. and Butterworth, M.H. (1986). A guide to condition score scoring of Zebu cattle. ILCA 1986.

Njau, B.C., Mkonyi, P.M. and Kundy, D.J. (1983). Berenil resistant *Trypanosoma congolense* isolated from infected goats in Tanga region, Tanzania. In: *International Scientific Conference for Trypanosomiasis Research and Control,* 17th Meeting 1983, Arusha, Tanzania, pp. 289-298.

Njogu, A.R., Dolan, R.B., Sayer, P.D., Wilson, A.J. and Alushula, H. (1985a). Strategic chemoprophylaxis for the control of bovine trypanosomiasis. In: *International Scientific Conference for Trypanosomiasis Research and Control* 18th Meeting, 1985. Harare, Zimbabwe, pp. 199-204.

Njogu, A.R. Dolan, R.B., Wilson, A.J. and Sayer, P.D. (1985b). Trypanotolerance in East African Orma Boran. *Veterinary Record,* **117**, 632-636.

Njogu, A.R., Ishmael, A.A., Dolan, R.B., Okech, G, Sayer, P.D. Opiyo, E.A. and Alushula, H. (1988). Trypanotolerance in East Africa: a summary of studies in the Orma Boran cattle. In: *Livestock Production in Tsetse Affected areas of Africa.* International Livestock Centre for Africa and the International Laboratory for Research on Animal Diseases, Nairobi, Kenya, pp. 447-448. ILCA/ILRAD, 1988.

Oduye, O.O. and Okunaiya, O.A. (1971). Haematological studies on the White Fulani and N'Dama breeds of cattle. *Bulletin of Epizootic Diseases of Africa*, **19**, 213-218.

Ogwu, D., Njoku, C.O. and Osori, D.I.K. (1985). Effects of *Trypanosoma vivax* infections on pregnancy and fertility of heifers. In: *International Scientific Conference for Trypanosomiasis Research and Control,* 18th Meeting, Harare, Zimbabwe, pp. 191-192.

Oludumade, M.A. Takken, W. Dengart, S.O. Tenabe, S.O., Feldmann, H.U. and Hamman, H.J. (1977). Status of tsetse control by sterile insect technique in Nigeria: I. The eradication of *Glossina palpalis palpalis* from four riverine forests. In: *International Scientific Conference for Trypanosomiasis Research and Control*, 17th Meeting, 1977, Harare, Zimbabwe, pp.

351-363.

Omerold, W.E. and Rickman, L.R. (1988). Sleeping sickness control: How wildlife and man could benefit. *Oryx,* **22**, 36-40.

Pagot, J. (1974). Les races trypanotolerantes. In: *Les Moyens de Lutte Contre les Trypanosomes et eurs vecteurs: Actes du Colloque, Paris 1974,* Instut d'Elevage et de Medicine Veterinaire des Pays Tropicaux, Paris, pp. 235-248.

Paling, R.W., Moloo, S.K., Scott, J.R., Gettinby, G., McOdimba, F. and Murray, M. (1991). Susceptibility of the N'Dama and Boran cattle to sequential challenges of tsetse-transmitted clones of *Trypanosoma congolense. Parasite Immunology,* **13**, 427-445.

Paris J., Murray M. and McOdimba, F. (1982). A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle. *Acta Tropica,* **39**, 307-317.

Payne, W.J.A. (1964). The origin of domestic cattle. *Empire Journal of Experimental Agriculture*, **126**, pp. 98-113.

Pierre, C. (1906). L'elevage dans l'Afrique Occidentale Francaise. Gouvernment General de l'Afrique Occidentale Francaise, Paris.

Pinder, K., Libean, G., Hirsch, W., Tomboura, I., Bauer, R.H. and Roelants, G.E. (1984). Antitrypanosome specific immune responses in bovine of differing susceptibility to trypanosomiasis. *Immunology,* **51**, 247-258.

Pinder, M. and Authie, E. (1984). The appearance of isometamidium resistant *Trypanosoma congolense* in West Africa. *Acta Tropica,* **41**, 247-252.

Pinder, M., Chassin, P. and Fumoux, F. (1986). Mechanisms of self cure from *Trypanosoma congolense* infection in mice. *Journal of Immunology,* **136**,

1427-1434.

Pinder, M., Fumoux, F., Van Melick, A. and Roelants, G.E. (1987). The role of antibody response in natural resistance to African trypanosomiasis. *Veterinary Immunology and Immunopathology,* **17**, 325-332.

Pinder, M., Bauer, J., Fumoux, F. and Roelants, G.E. (1987). *Trypanosoma congolense*: Lack of correlation between resistance of cattle subjected to experimental cyclic infection or to field challenge. *Experimental Parasitology,* **64**, 410-417.

Plimmer, H.G. and Thomson, J.D. (1908). Further results of the experimental treatment of trypanosomiasis in rats. Proceedings of the Royal Society, **80**, 1-2.

Politzar, H. and Cuisance, D. (1982). Sterile Insect Technique in the control and eradication of *Glossina palpalis gambiensis.* In: *Proceedings of symposium on Sterile Insect Technique and Radiation in insect control.* 1982. IAEA, Vienna, pp. 101-109.

Poznasky, M.J. and Juliano, R.L. (1984). Biological approaches to the delivery systems of drugs: A critical review. *The American Society of Pharmacology and Experimental Therapeutics*, **36**, 227-325.

Rae, P.F. and Luckins, A.G. (1984). Detection of circulating trypanosomal antigens by enzyme immunoassay. *Annals of Tropical Medicine and Parasitology,* 78, 587-596.

Rawlings, P., Dwinger, R.H. and Snow, W.F. (1990). An analysis of survey measurements of tsetse challenge to trypanotolerant cattle in relation to aspects of analytical models of trypanosomiasis. *Parasitology,* **102**, 371-377.

Raynaud, J.P., Sones, K.R. and Friedheim, E.A.H. (1989). A review of

Cymelarsan^{R} - a new treatment proposed for animal trypanosomiasis due to *Trypanosoma evansi* and other trypanosomes of the *Trypanosoma brucei* group. In: *International Scientific Council for Trypanosomiasis Research and Control*, 20th Meeting, Mombasa, Kenya, OAU/STRC Publication No. 115. pp. 334-338.

Reed, C.A. (1984). The beginnings of animal domestication. In: Evolution of domesticated animals. I.L. Mason, Ed., Longman, London. pp. 1-6.

Richardson, J.P., Jenni, L., Beecroft, R.P. and Pearson, T.W. (1986). Procyclic tsetse fly midgut forms and culture forms of African trypanosomes share stage-specific surface antigens identified by monoclonal antibodies. *Journal of Immunology,* **136**, 2259-2264.

Roberts, C.J. and Gray, A.R. (1973). Studies on trypanosome resistant cattle. II. The effect of trypanosomiasis on N'Dama, Muturu and Zebu cattle. *Tropical Animal Health and Production,* **5**, 220-223.

Roberts, L.W., Bhoghal, M.S. and Karstad, L. (1980). I. Trypanosomiasis A. Resistance of oryx to tsetse and trypanosomes. In: *Wildlife Disease Research. Semi-Annual Progress Report.* January-June, 1980. Wildlife section, Veterinary Research Laboratories, Government of Kenya, pp. 2-3.

Robson, J. and Rickman, I.R. (1973). Blood incubation infectivity test results for *Trypanosoma brucei* subgroup isolates in the Lambwe Valley, South Nyanza, Kenya. *Tropical Animal Health and Production,* **5**, 187-191.

Roedler, P.L., Scott, J.M. and Pegram, R.G. (1984). Acute *Trypanosoma vivax* infection in cattle in apparent absence of tsetse. *Tropical Animal Health and Production,* **16**, 141-147.

Roelants, G.E. and Pinder, M. (1982). Susceptibility to trypanosomiasis amongst

a range of cattle breeds. In: *Trypanotolerance and Animal production*. E. Karbe and E.K. Freitas, Eds., GTZ. No. 116, pp. 29-32.

Roelants, G.E., Tomboura, I, Sidiki, D.B., Bassinga, A. and Pinder, M. (1983). Trypanotolerance: An individual not a breed character. *Acta Tropica,* **40**, 99-103.

Roelants, G.E. and Pinder, M. (1984). Immunology of the African trypanosomiasis. *Contemporary Topics in Immunobiology,* **12**, 225-274.

Roelants, G.E. (1986). Point of view: Natural resistance to African trypanosomiasis. *Parasite Immunology,* **8**, 1-10.

Roelants, G.E., Fumoux, F., Pinder, M., Quival, R., Bassinga, A. and Authie, E. (1987). Identification and selection of cattle naturally resistant to African trypanosomiasis. *Acta Tropica,* **44**, 55-66.

Rogers, D.J. (1983). Trypanosomiasis 'risk' or 'challenge' - A review with recommendations. In: *Livestock productivity and trypanotolerance. Network Training Manual,* pp. 101-127, ILCA, 1983. M. Murray, J.C.M. Trail, D.A. Turner and Y. Wissocq, Eds., ILCA, Addis Ababa, Ethiopia.

Rogers, D. J. (1985). Trypanosomiasis 'risk' or 'challenge': A review. *Acta Tropica,* **42**, 5-32.

Royal Statistical Society (1987). Generalized Linear Modelling system (GLIM release 3.77).

Ryan, B.F., Joiner, B.L. and Ryan, T.A. (1985). Minitab Handbook. PWS-KENT, Publishers.

Saror, D.I., Ilemobade, A.A. and Nuru, S. (1981). The hematology of N'Dama and Zebu infected with *Trypanosoma vivax.* In: *International Scientific Conference for Trypanosomiasis Research and Control,* 16th Meeting, 1979,

Younde, Cameroun, pp. 287-294, (OAU/STRC Publication No. 111).

Schillinger, D. (1984). The problem of drug resistance. Field aspects of trypanosomiasis in Kenya. In: *Proceeding of the Kenya Veterinary Association meeting*, Mombasa, 9-10th August, 1984.

Schoenfield, A. (1988). Tsetse control with insecticide treated cattle in Zanzibar. Joint meeting of the Panels of Experts on the Ecological/Technical and development aspects of the programme for the control of Animal Trypanosomiasis and related development. Accra, Ghana 1988.

Scott, J.M. and Pegram, R.G. (1974). A high incidence of *Trypanosoma congolense* strains resistant to homidium bromide in Ethiopia. *Tropical Animal Health and Production,* **6**, 215-221.

Scott, J.M., Pegram, R.G., Holmes, P.H., Pay, J.W.F., Knight, P.A., Jennings, J.W. and Urquhart, G.M. (1977). Immunosuppression in bovine trypanosomiasis: Field studies using FMD vaccine and Clostridial vaccine. *Tropical Animal Health and Production,* **9**, 159-165.

Sendashonga, C. and Black, S.J. (1982). Induction and regulation of Anti-VSSA humoral immune responses by slender and stumpy forms of *Trypanosoma brucei* parasites. *Parasite Immunology,* **4**, 245-257.

Selkirk, M.E. and Sacks, D.L. (1980). Trypanotolerance in inbred mice: An immunological basis for variation in susceptibility to infection with *Trypanosoma brucei*. *Tropenmedizin und Parasitologie,* **31**, 435-438.

Shapiro, S.Z. and Murray, M. (1982). African trypanosome antigens recognized during the course of infection in N'Dama and Zebu cattle. *Infection and Immunity,* **35**, 410-416.

Smith, I. M. and Rennison, B. D. (1960). Some factors concerned in trypanosome

challenge. In: *International Scientific Council for Trypanosomiasis Research,* 1958, Brussels, pp. 63-66.

Soltys, M.A. (1955). Studies on resistance to *Trypanosoma congolense* developed by Zebu cattle treated prophylactically with Antrycide Pro-salt in an enzootic area of East Africa. *Annals of Tropical Medicine and Parasitology* **49**, 1-8.

Sombroek, W.C., Braun, H.M. and van der Pouw, B.J A. (1982). Explanatory soil map and Agro-climatic zone map of Kenya. Report E1. National Agricultural Laboratories, Soil Survey Unit, Nairobi, Kenya.

Stephen, L.E. (1966). Observations on the resistance of the West African N'Dama and Zebu cattle to trypanosomiasis following challenge by wild *Glossina morsitans* from an early age. *Annals of Tropical Medicine and Parasitology,* **60**, 230-246.

Spielberger, U., Na'Isa, B.K., Koech, K., Manno, A., Skildmore, P.R. and Coutts, H.H. (1979). Field trials with synthetic pyrethroids: permethrin, cypermethrin and decamethrin against *Glossina* spp. (Diptera: *Glossinidae*) in Nigeria. *Bulletin of Entomological Research,* **69**, 667-89.

Stephen, L.E. (1970). Clinical manifestations of the trypanosomes in livestock and other domestic animals. In: *The African Trypanosomiases.* H.W. Mulligan, Ed., George Allan and Unwin, London, pp. 774-794.

Stewart, J.L. (1937). The cattle of the Gold Coast. *Veterinary Record,* **49**, 1289-97.

Stewart, J.L. (1951). The West African Shorthorn cattle. Their value to Africa as trypanosomiasis resistant animals. *Veterinary Record*, **63**, 454-457.

Stevenson, P., Munga, L., Makumi, M., Baylis, M. and Alushula, H., The control of trypanosomiasis by deltamethrin treatment of ranch cattle in Kenya. In:

International Scientific Conference for Trypanosomiasis Research and Control, 21st Meeting, 1991, Yamoussoukro, Cote d'Ivoire, *in press*.

Tacher, G. (1982). The use of drugs in the development of livestock production in the tsetse-infested areas. *World Animal Review,* **44**., 30-43.

Takken, W., Oludunmade, M.A., Denwart, L., Fieldmann, H.U., Onah, J.A. Tenabe, S.O. and Hamann, J. (1986). The eradication of *Glossina palpalis palpalis* (Diptera: *Glossinidae*) using traps, insecticide impregnated targets and the sterile insect technique. *Bulletin of Entomological Research,* **76**, 275-286.

Tarimo, S.A. Golder, T.K., Dransfield, R.D. Chaudhury, M.F.B. and Brightwell, R. (1985). Preliminary observations on trypanosome infection rates in *Glossina pallidipes* and the factors affecting them at Nguruman, Kenya. In: *International Scientific Council for Trypanosomiasis Research and Control,* 18th meeting, 1985. Harare, Zimbabwe. OAU/STRC Publication No. 113. pp. 276-283.

Templeton, J.W., Rogers, S. and Adams, G.L. (1988). Natural disease resistance in domestic animals. *Journal American Veterinary Medicine Association,* **192**, 1306-1315.

Thompson, J.W., Mitchell, M., Rees, R.B., Shereni, W., Schoenfield, A.H. and Wilson, A. (1991). Studies on the efficacy of deltamethrin applied to cattle for the control of tsetse flies (*Glossina* spp.) in Southern Africa. *Tropical Animal Health and Production* **23**, 221-226.

Toure, S.M., Seye, M., Dieye, T. and Mbengue, M. (1983). Trypanotolerance: Comparative pathological survey on Djallonke and Fulani sheep of the Sahel. In: *International Scientific Conference for Trypanosomiasis Research*

and Control 17th Meeting, 1981. Arusha, Tanzania, (OAU/STRC Publication No.112), pp. 326-337.

Toure, S.M., Gueye, A., Seye, M., Ba, M.A and Mane, A. (1978). A comparison between the pathology of natural trypanosomiasis in Zebu and N'Dama cattle. *Revue d'Elevage et de Medecine Veterinary des Pays Tropicaux* **31**, 293-313.

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

Trail, J.C.M., Colardelle, C, d'Iteren, G.D.M, Dumont, P., Itty, P., Jeannin, P., Maehl, J.H.H., Nagda, S.M., Ordner, G., Paling, R.W., Rarieya, J.M., Thorpe, W. and Yangari, G. (1988). Evaluation of criteria of trypanotolerance. In: *Livestock Production in Tsetse Affected areas of Africa.* International Livestock Centre for Africa and the International Laboratory for Research on Animal Diseases, Nairobi, Kenya, pp. 425-429.

Trail, J.C.M., d'Iteren, G.D.M., Feron, A., Kakiese, O., Mulungo, M. and Pelo, M. (1991a). Effect of trypanosome infection and control of anaemia development on productivity of N'Dama cattle. *Acta Tropica*, **48**, 37-45.

Trail, J.C.M., d'Iteren, G.D.M., Colardelle, C. Maille, J.C., Ordner, G., Sauveroche, B. and Yangari, G. (1991b). Evaluation of a field test for trypanotolerance in young N'Dama cattle. *Acta Tropica*, **48**, 47-57.

Trail, J.C.M., d'Iteren, G.D.M. and Murray, M. (1991c). Practical aspects of developing genetic resistance to trypanosomiasis. In: *Breeding for Disease Resistance in farm animals.* J.B. Owen and R.F.E. Axford Eds., CAB

International, Wallingford, pp. 224-234.

Trail, J.C.M., d'Iteren, G.D.M., Viviani, P., Yangari, G. and Nantulya, V.M. (1992). Relationship between trypanosome infection measured by antigen detection enzyme immunoassays, anaemia and growth in trypanotolerant N'Dama cattle. *Veterinary Parasitology*, **42**, 213-223.

Turner, M.J. (1982). The biochemistry of the variant surface glycoprotein of salivarian trypanosomes. *Advances in Parasitology,* **21**, 69-153.

Vale, G. A. (1974). The response of tsetse fly (Diptera: *Glossinidae*) to mobile and stationary baits. *Bulletin of Entomological Research,* **64**, 545-588.

Vale, G.A. (1974b). New field methods for studying the responses of tsetse flies (Diptera: *Glossinidae*) to hosts. *Bulletin of Entomological Research,* **64**, 199-208.

Vale, G.A. and Hargrove, J.W. (1979). A method of studying the efficiency of traps for tsetse flies (Diptera: *Glossinidae*) and other flies. *Bulletin of Entomological Research*, **69**, 183-93.

Vale, G.A. (1980). Field studies of the responses of tsetse flies (*Glossinidae*) and other diptera to carbon dioxide, acetone and other chemicals. *Bulletin of Entomological Research*, **70**, 563-70.

Vale, G.A. (1982a). The trap-oriented behaviour of tsetse flies (*Glossinidae*) and other diptera to carbon dioxide and other chemicals. *Bulletin of Entomological Research*, **72**, 71-93.

Vale, G.A. (1982b). The improvement of traps for tsetse flies (Diptera: *Glossinidae*). *Bulletin of Entomological Research*, **72**, 95-106.

Vale, G.A. (1982c). Prospects of using stationary baits to control and study populations of tsetse flies (Diptera: *Glossinidae*). In: *Sterile Insect Technic*

for Radiation in insect control, pp. 191-203. IAEA-SM-225/34.

Vale, G.A., Bursell, E. and Hargrove, W. (1985). Catching out the tsetse fly: A review. *Parasitology Today,* **1**, 106-110.

Vale, G.A. and Hall, D.R. (1985a). The role of 1-octen-3-ol, acetone and and carbon dioxide in the attraction of tsetse flies, *Glossina* spp. (Diptera: *Glossinidae*) to the ox-odour. *Bulletin of Entomological Research*, **75**, 209-217.

Vale, G.A. and Hall, D.R. (1985b). The use of 1-octen-3-ol, acetone and carbon dioxide to improve baits for tsetse flies *Glossina* spp. (Diptera: *Glossinidae*). *Bulletin of Entomological Research*, **75**, 219-231.

Vale, G.A., Flint, S. and Hall, D.R. (1986). The field responses of tsetse flies, *Glossina* spp. (Diptera: *Glossinidae*) to odours of host residues. *Bulletin of Entomological Research*, **76**, 685-693.

Van den Ingh, T.S.A.M., Zwart, D., Vanmiert, A.S. and Schotman, A.J.H. (1976). Clinicopathological and Pathomorphological observations in *Trypanosoma vivax* infections in cattle. *Veterinary Parasitology,* **2**, 237-250.

Van den Ingh, T.S.G.A.M. and De Neij-Bakker, M.H. (1979). Pancarditis in *Trypanosoma vivax* infections in cattle. *Tropenmedizin und Parasitologie,* **31**, 239-243.

van Hoeve, K., Cunningham, M.P. and Graenge, E.B. (1964). Some observations on the treatment of cattle with Berenil. In: *International Scientific Council for Trypanosomiasis Research* (1964), pp. 27-29.

Vickerman, K. (1984). Trypanosome survival mechanisms. Biological basis of trypanosome antigenic variation. In: *Molecular Parasitology*. T.J. August, Ed., Accademic press, Orlando, 1984, pp. 3-18.

Voller, A. (1977). Serology of African trypanosomias. *Annales de la Societe belge de Medecine tropicale,* **57**, 273-280.

Voller, A., Bidwell, D.E. and Bartlett, A. (1975). A serological study on human *T. rhodesiense* infections using microscale ELISA. *Tropenmedizin und Parasitologie,* **26**, 247-251.

Waltkins, T.I. and Woolfe, G. (1952). Effect of changing the quartenizing group on the trypanocidal activity of dimidium bromide. *Nature*, **169**, 506.

Weissenhutter, E. (1969). Untersuchungen uber den diagnostichen Wert des indirekten Coons Test for Rindertrypanosomiasis nach Chemoprophylaxe. Z. *Tropenmedizin und Parasitologie,* **20**, 131-139.

Weissenhutter, E. (1973). Verglichende Untersuchugen uber den Wert des indirekten Fluoreszenz-Antikorper-Tests als Hilfsmittel Zur Diagnose der Rinder Africa. Z. *Tropenmedizin und Parasitologie,* **24**, 60-66.

Weitz, B. (1963). Feeding habits of *Glossina. Bulletin of World Health Organization,* **28**, 711-729.

Wellde, B.T., Lotzsch, R., Deindl, G., Sadun, E.H., Williams, J.S. and Warui, G. (1974). *Trypanosoma congolense.* I. Clinical observations of the experimentally infected cattle. *Experimental Parasitology*, **36,** 6-19.

Wellde, B.T., Chumo, D.A., Adoyo, M., Kovatch, R.M., Mwongela, G.N. and Opiyo, E.A. (1983). Hemorrhagic syndrome in cattle associated with *Trypanosoma vivax* infection. *Tropical Animal Health and Production,* **15**, 95-102.

Wells, E.A. (1972). The importance of mechanical transmission in the epidemiology of 'Nagana': A review. *Tropical Animal Health and Production,* **4**, 74-88.

Wells, E.A. (1984). Animal trypanosomiasis in South America. *Preventive Veterinary Medicine,* **2**, 31-44.

Wells, E.A., Bentacourt, A. and Raminez, L.E. (1982). *Trypanosoma vivax* in Columbia: Epidemiology and economic impact. *World Animal Review,* **43**, 17-23.

Wells, E.A., Bentacourt, A, Raminez, L. E. (1984). Animal trypanosomiasis in Columbia. *Preventive Veterinary Medicine,* **2**, 31-44.

Wery, M., van Wettere, P., Wery-Paskoff, S., van Merveinne and Masatewa, M. (1970). The diagnosis of human trypanosomiasis (*T. b. gambiense*) by the use of the fluorescent antibody test. 2. First results of field application. *Annales de la Societe belge de Medecine tropicale,* **50**, 711-730.

Whitelaw, D.D., McAskill, J.A., Holmes, P.H. Jennings, F.W. and Urquhart, G.M. (1980). Genetic resistance to *Trypanosoma congolense* infections in mice. *Infection and Immunity,* **27**, 707-713.

Whitelaw, D.D., Kaaya, G.P., Moulton, J.E., Moloo, S.K. and Murray, M. (1985). Susceptibility of different breeds of goats in Kenya to experimental infection with *Trypanosoma congolense. Tropical Animal Health and Production,* **17**, 155-165.

Whiteside, E.F. (1955). In: *Kenya Annual Report,* 1954. Department of Veterinary services. Colony and Protectorate of Kenya, pp. 30-36.

Whiteside, E.F. (1960a). The maintainance of cattle in tsetse-infested country. In: *International Scientific Council for Trypanosomiasis Research and Control,* 1958, Brussels, pp. 83-90.

Whiteside, E.F. (1960b). Recent work in Kenya on the control of drug resistant cattle trypanosomiasis. In: *International Scientific Council for*

Trypanosomiasis Research, 8th Meeting, 1960. Jos, Nigeria. CCTA No. 62, 141-154.

Whiteside, E.F. (1962a). Interactions between drugs, trypanosomes and cattle in the field. In: *Drugs, Parasites and Hosts.* L.G. Gordwin and R.M. Nimmo-smith, Eds., Churchill, London, pp. 116-141.

Whiteside, E.F. (1962b). The control of trypanosomiasis with drugs Kenya: methods and costs. *East African Agricultural and Forestry Journal,* **28**, 67-73, (1962 b).

Williamson, D.L., Dame, D.A., Gates, D.B., Cobb, P.E., Bakul, B. and Warner, P.V. (1983). Integration of insect sterility and insecticides for control of *Glossina morsitans morsitans* West. (Diptera: *Glossinidae*) in Tanzania: V. The impact of sequential releases of sterilized tsetse flies. *Bulletin of Entomological Research,* **73**, 391-404.

Williamson, J. (1970). Review of the chemotherapeutic and chemoprophylactic agents. In: *The African Trypanosomiases.* H.W. Mulligan, Ed., George Allen and Unwin, London., pp. 125-221.

Williamson, J. (1976). Chemotherapy of African trypanosomiasis. *Tropical Diseases Bulletin,* **73**, 531-542.

Williamson, J. (1980). Present situation of research for the new trypanocidal drugs: In: *Report of expert consultation on research on trypanosomiases.* FAO, Rome. AGA-801., pp. 90-96.

Wilson, A.J. (1969). Value of indirect fluorescent antibody test as a serological test aid to diagnosis of *Glossina*-transmitted bovine trypanosomiasis. *Tropical Animal Health and Production,* **1**, 89-95.

Wilson, A.J., Dar, F.K. and Paris, J. (1972). A study on the transmission of

salivarian trypanosomes isolated from wild tsetse flies. *Tropical Animal Health and Production,* **4**, 14-22.

Wilson, A.J., Paris, J. and Dar, F.K. (1975a). Maintenance of a herd of breeding cattle in an area of high trypanosome challenge. *Tropical Animal Health and Production,* **7**, 63-71.

Wilson, A.J., LeRoux, J.G., Paris, J., Davison, C.R. and Gray, A.R. (1975b). Observations on a herd of beef cattle maintained in a tsetse area. I. Assessment of chemotherapy as a method for the control of trypanosomiasis. *Tropical Animal Health and Production,* 7, 187-199.

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**, 1-12.

Wilson, A.J., Njogu, A.R., Gatuta, G., Mgutu, S.P. and Alushula, H. (1983). An economic study on the use of chemotherapy to control trypanosomiasis in Galana Ranch, Kenya. In: *International Scientific Conference for Trypanosomiasis Research and Control* 17th Meeting, 1981, Arusha, Tanzania, pp. 306-319.

Wilson, A.J., Gatuta, G.M., Mgutu, S.P. and Alushula, H. (1986). A simple epidemiological method for animal trypanosomiasis to provide relevant data for effective financial decision making. *Veterinary Parasitology,* **20**, 261-274.

Wissocq, Y.J., Trail, J.C.M., Wilson, A.D. and Murray, M. (1983) Genetic resistance to trypanosomiasis and potential economic benefits in cattle in East Africa. In: *International Scientific Conference for Trypanosomiasis*

Research and Control, 17th Meeting, Arusha, Tanzania, 1981, (OAU/STRC Publication No. 112), pp. 361-367.

Woo, P.T.K. (1969). The haematocrit centrifuge technique for the detection of trypanosomes in blood. *Canadian Journal of Zoology,* **47**, 921.

Woo, P.T.K. (1970). The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Tropica,* **27**, 384-386.

Wooff, W.R. (1968). The eradication of tsetse *Glossina morsitans* Westw. and *G. pallidipes* Aust., by hunting. In: *International Scientific Conference for Trypanosomiasis Research and Control,* 12th Meeting, 1968, Bangui, pp. 267-286.

Zwart, D., Perie, N.M., Keppler A. and Goedbloed, E. (1973). A comparison of the methods of diagnosis of trypanosomiasis in East African domestic ruminants. *Tropical Animal Health and Production,* **5**, 70-86.

Zwart, D. (1979). A review of studies of three mouse infective *Trypanosoma vivax* strains. *Veterinary Science Communication,* **3**, 187-206.

Zweygarth, E., Sabwa, C. and Rottcher, D. (1984). Serodiagnosis of trypanosomiasis in dromedary camels using card agglutination test (testtryp CATT). *Annales de la Societe belge de Medecine tropicale,* **64**, 309-313.