BOVINE TRYPANOSOMIASIS: SUSCEPTIBILITY OF EAST AFRICAN CATTLE TO AFRICAN TRYPANOSOMIASIS:

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A THESIS

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DECLARATION.

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

Dedicated to Nkibiyoto ene Olooloilelek,
my mother, who brought me up and who constantly
impressed upon me the need to be patient and
dedicated to duty.

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	- Centimeter.
AE	- Diethylaminoethyl cellulose anion-exchange column.
	- Darkground phase contrast technique.
TA	- Ethylene diaminetetraacetic acid.
S	- Foetal bovine serum.
	- Haemoglobin.
AT	- Indirect fluorescent antibody test.
G	- Immunoglobulin G.
M	- Immunoglobulin M.
V	- Mean corpuscular volume.
HC	- Mean corpuscular haemoglobin concentration.
	- Milliliter.
	- Microliter.
V	- Packed cell volume.
S	- Phosphate buffered saline.
G	- Phosphate saline glucose.
C	- Red blood cell.
A buffer	- Specific buffer containing sodium azide.
IA	- Solid-phase radioimmunoassay.
A	- Trichloroacetic acid.
C	- White blood cell.

SUMMARY.

The objective of this project was to determine by experimental studies if any degree of susceptibility to trypanosomiasis existed in indigenous East African Zebu. If such animals were identified, the study would be extended to investigate the factors that might be involved in affecting susceptibility of cattle to trypanosomiasis.

Three groups of East African Zebu from different tsetse-infested areas of Kenya were selected. They included Zebu from the Coast Province and Zebu from Kitui, two areas known to be under low to medium tsetse-trypanosomiasis risk, and Zebu from Western Kenya, where tsetse-trypanosomiasis risk is considered heavy. The susceptibility of these three groups of Zebu was compared with that of Boran and Ayrshire from tsetse-free areas. The age of the cattle was standardised at 1 - 2 years and they were of both sexes. They were kept in fly-proof accommodation at the Veterinary Research Laboratories, Kabete.

All cattle were screened clinically and by haematological and parasitological methods for trypanosomiasis and other diseases. The presence of anti-trypanosomal antibodies was assessed using the immunofluorescence test. In order to assess whether cattle from tsetse-infested areas had

been previously exposed to the stock or clone of Trypanosoma congolense to be used for experimental infection, pre-infection sera was tested for the capacity to neutralise infection of mice with the test stock or clone of T. congolense.

T. congolense was detected in the blood of one Zebu from the Coast. Trypanosoma theileri was identified in four Zebu from Kitui, while microfilaria were found in two Zebu from Western Kenya. All Zebu from the tsetse-infested areas, i.e., from the Coast, Kitui and Western Kenya possessed anti-trypanosomal antibodies against T. congolense, T. vivax and T. brucei, as assessed by the indirect fluorescent antibody test; the Ayrshire and Boran possessed no detectable anti-trypanosomal antibodies. Schizonts of Theileria parva were found in the superficial lymph nodes of all Zebu from Western Kenya. All cattle from tsetse-infested areas were treated with a single intramuscular injection of the trypanocidal drug Berenil (Farbwerke Hoechst, (M), West Germany) at 7 mg/kg, at least five weeks prior to experimental infection. Pre-infection sera from cattle of all groups collected prior to experimental inoculation possessed no neutralizing activity against the stock or clone of T. congolense used for infection. At the start of the experiment

all cattle were in good body condition and were clinically healthy.

The first part of the study compared the susceptibility of Zebu from the Coast and Kitui with that of Boran and Ayrshire to syringe inoculation with bloodstream forms of a stock of T. congolense IL572 of proven virulence in cattle. Subsequently, Boran and Zebu from the Coast were subjected to challenge by Glossina morsitans centralis infected with T. congolense IL 13E-8, a serodeme which is antigenically distinct from T. congolense IL 572 and is virulent in cattle.

In the second part of the investigation, Zebu from Western Kenya, along with Boran and Ayrshire were infected by syringe inoculation with bloodstream forms of T. congolense IL 1180, a clone derived from the same stock as T. congolense IL 572. They were treated 6 - 12 weeks after infection to prevent death and 10 weeks after the last treatment they were challenged with G.m. centralis infected with T. congolense IL 285, a stabilate derived from T. congolense IL 13E-8.

Following both needle and tsetse challenge, the parameters measured included clinical condition, development of anaemia, parasitaemia, the immune response and survival. Clinical condition was

ssessed by regularly measuring rectal temperature
°C), pulse and respiration rates and body weight.
he progressive development of anaemia was followed
y evaluating packed red cell volume percent (PCV),
aemoglobin concentration, total red blood cells. White
lood cells were also counted. PCV was estimated by the
icrohaematocrit centrifuge technique, and haemoglobin
y the cyanohaemoglobin method, using a haemoglo-
inometer. Red and white cells were enumerated using an
lectronic cell counter, Coulter Electronic Model ZBL.
arasites were detected and quantified by examination
f the blood buffy coat by phase contrast microscopy.
he immune response was assessed using single radial
mmunodiffusion (Mancini test), immune lysis and
olid phase radioimmunoassay.

Following needle inoculation, all animals became
nfected. No differences in the prepatent period
ccurred between breeds except for the Ayrshires in
hich parasites were detected two to three days
arlier. Differences were noted in the ability of
ifferent breeds and individuals to regulate parasite
rowth. Boran, Ayrshire and some individual animals
rom groups coming from tsetse-infested areas
xhibited no ability to control or remit parasitaemia.
hese animals became anaemic rapidly and died or required
arly treatment to survive. In terms of

rvival, the Ayrshires were the least resistant to the
thogenic effects of trypanosomiasis. Zebu from Western
nya showed marked heterogeneity in their trypanoresistance:
me animals were very resistant and survived the infection
r more than 12 weeks following needle inoculation; Some
re intermediate while others had no resistance. The
sistant animals possessed a superior capacity
control parasitaemia, developed less severe
aemia and remained in good clinical condition.
similar pattern of events was observed following
etse challenge.

Differences in the humoral immune response
re noted between breeds. Specific trypanolytic
tibody responses differed between the Ayrshire
d the B. indicus types, with Ayrshire eliciting
lower response. There was no difference in the
ak antibody response between the Boran and East
rican Zebu from the Coast, Kitui and Western
nya. However, trypanolytic antibody persisted
ch longer in resistant individuals from Western
nya, while it disappeared rapidly in the Boran,
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From this work, it was concluded that a
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certain Zebu types in East Africa. The degree

of resistance observed seemed to be related to the intensity of tsetse-trypanosomiasis risk which existed in the area from which the cattle were selected, in that it was in the Zebu from Western Kenya that the most resistant individuals were identified. Furthermore, in these studies, the Ayrshire were found to be more susceptible to trypanosomiasis than the Boran and East African Zebu.

The results obtained in the individual Zebu which exhibited the most marked degree of resistance to trypanosomiasis indicated that this trait was associated with a superior capacity to control parasitaemia and the consequent development of less severe anaemia. At the same time, specific antibody responses to the trypanosome variable antigen type used for infection persisted longer in the less susceptible individuals.

Although there was no evidence that the Zebu from tsetse-infested areas had ever experienced the trypanosome stocks and clones used for infection, care must be taken in attributing the difference in susceptibility observed solely to an innate characteristic, as it may be that previous exposure even to antigenically unrelated serodemes might influence the level of host susceptibility.

This study has shown that trypano-resistant cattle exist in the Zebu breed in East Africa. This could be of advantage to the livestock industry or farmer in that these cattle could be propagated in tsetse infested areas. There is now a need for a further in-depth critical evaluation of trypano-resistance in East African Zebu as well as the need for comparative studies with the West African resistant breeds. There is therefore a possibility of a long-term project aimed at identifying and propagating a resistant East African Zebu type in areas of trypanosomiasis challenge.

1. INTRODUCTION.

African trypanosomiasis in cattle is a disease complex caused by three species of the genus Trypanosoma; Trypanosoma congolense, T. vivax and T. brucei. These parasites are cyclically transmitted by several different species of tsetse fly, genus Glossina, which are widely distributed throughout Africa (Ford, 1971).

1.1. THE DISEASE IN CATTLE.

In cattle, as in other susceptible hosts, the first indication that an animal has become infected is the development in the skin of a raised, indurated, painful swelling, termed a chancre, which appears during the first week following the successful feed of a tsetse fly infected with, T. congolense (Roberts, Gray and Gray 1969; Gray and Luckins 1979, 1980; Emery, Akol, Murray, Morrison and Moloo 1980a;Murray, Morrison, Emery, Akol, Masake and Moloo 1980; Akol and Murray, 1982); T. vivax and T. brucei (Emery et al., 1980a; Akol and Murray, 1983). It is only after this reaction is detected that parasites appear in the blood.

In cattle the disease varies considerably in severity and duration and may take one of several courses depending on factors such as degree of challenge, strain of trypanosome, the level of

nutrition, age and breed (Morrison, Murray and Mclntyre, 1981). The disease syndrome is often considered to be either acute or chronic although the line of demarcation between the two is poorly defined. Thus, following infection with some strains of T. vivax, death may occur within 2 weeks (Hudson, 1944; Mwongela, Kovatch and Fazil, 1981; Morrison et al., 1981). This hyperacute syndrome produced by T. vivax, resembles a septicaemic condition. The animals are febrile, show sustained high levels of parasitaemia and often exhibit massive haemorrhages. On the other hand, isolates of T. congolense considered virulent may result in death of the host 6 - 10 weeks after infection. In trypanosomiasis both of these syndromes would be regarded as acute. The chronic disease might, be arbitrarily defined as occurring in animals infected longer than 3 months (Morrison et al., 1981).

The major feature of the disease in cattle is anaemia, (reviewed by Hornby, 1921; Murray, 1979) which is seen clinically as pallor of the mucous membranes. In the early stages of the infection when parasitaemia is readily detected there is intermittent pyrexia. The superficial lymph nodes become palpably enlarged although in the chronic phase of the disease they may be normal or reduced in size. As anaemia becomes more

severe, there is marked loss of body condition. Animals become wasted, lethargic; their coat is dull and staring and they show a 'hunched-up' appearance. In the terminal stages of the disease, affected animals become extremely weak and are often unable to rise. They may exhibit subcutaneous oedema. Cattle usually die due to congestive heart failure which would appear to result from a combination of anemia and myocardial damage.

Many of the animals that survive, remain unproductive and growth in young animals is stunted. Adult cattle may have reduced fertility or abort. Calves born at full term are often small and weak, increasing the incidence of neonatal mortality.

1.2. IMPORTANCE OF TRYPANOSOMIASIS IN AFRICA.

African trypanosomiasis represents a major constraint to agricultural and socio-economic development in vast areas of Africa. Approximately 10 million square kilometres of the African continent is infested with tsetse. This covers 38 countries and 2 island clusters, Cape Verde Islands and Zanzibar (FAO, 1982). It is considered that 7 million square kilometres of the infested area are suitable for livestock and or mixed agricultural development but this has not been possible because of trypanosomiasis. It is also estimated that the exploitation of this region

could double livestock production in the African continent (Ford, 1971).

In the 38 countries infested by the tsetse fly, there are approximately 147 million cattle of which about 30% (50 million) are at risk (FAO, 1982).

In Kenya and 5 neighbouring countries, 1/3 of the total cattle population of 78 million are at risk to trypanosomiasis (Table 1). It is estimated that of the total land surface of Kenya (570,000 square kilometres) approximately one quarter (138,000 kilometres)is infested by tsetse flies (National Atlas of Kenya, 1969). The distribution is patchy at altitudes ranging from sea-level to approximately 1,800 metres. Wherever cattle come in contact with tsetse flies, the disease is endemic. Cattle are in contact with tsetse mainly along the coastline, some parts of North-Eastern Kenya and isolated areas in Western Kenya (Figure 1).

The annual loss in Africa in meat production due to trypanosomiasis alone is assessed at $5 billion. This excludes losses in milk and mixed agriculture such as manure and traction. As a consequence, trypanosomiasis is regarded as the most economically important disease of cattle on the African continent (FAO, 1982).

The trypanosomiasis problem is compounded by the rapidly increasing human population in Africa with resultant urgent need to increase food production and the availability of animal protein (MacLennan, 1981). It is for this reason that Africa still regards the control/eradication of African trypanosomiasis in animals and man as of major priority.

According to a recent report by the Scientific Working Group (SWG) on African trypanosomiasis, the human trypanosomiases represent a continuing, serious threat to the health and morale of many communities in Africa. Recent estimates suggest that some 45 million people are exposed to the risk of infection. Prior to 1979, there were some 10,000 new cases recorded every year. With the present serious outbreaks in Cameroon, Sudan, and Uganda, the number of new cases recorded every year has increased to at least 20,000. This imposes an enormous effect in loss of manpower. The disease in humans is found throughout the belt of Sub-Saharan Africa infested by tsetse flies of the genus Glossina. The belt covers an area of some four million square miles.

Figure 1:

The distribution of cattle in East Africa in relation to tsetse-fly infested areas.

Distribution of Cattle

Distribution of Tsetse.

1.3. CONTROL METHODS.

The methods currently in use to control African trypanosomiasis are; diagnosis and trypanocidal drug treatment, chemoprophylaxis and control or eradication of tsetse with insecticides. However, many years of these strategies have had little effect on the problem at continental level (FAO, 1982).

Although these control measures are successfully employed either separately or together in several locations, they possess certain disadvantages. While the basic cost of the drugs is relatively cheap, their use cummulatively becomes expensive as repeated treatments have to be carried out while requiring facilities and manpower. Frequent use of drugs can lead to development of chemoresistance and this is becoming a serious problem, particularly as the number of drugs commercially available is very limited.

Success in tsetse control and eradication has been achieved in Nigeria, Zambia, Botswana and South Africa (MacLennan, 1981). However, tsetse control or eradication with insecticides is also faced with high costs in that the eradicated area needs rigorous surveillance to prevent re-invasion. There are also the possible environmental hazards posed by the use of insecticides.

In addition, a number of limiting factors, related to the vector and trypanosome are responsible for the persistence of the trypanosomiasis problem. There are at least 22 species of Glossina (tsetse) capable of transmitting infection. These are adapted to a wide range of habitats thereby contributing to the widespread nature of the disease (Ford, 1971). The three species of trypanosomes causing disease in livestock, T. congolense, T. vivax and T. brucei, exhibit a wide host range for both domestic and wild animals. Furthermore, the phenomenon of antigenic variation (Gray, 1965; Vickerman, 1974; Gray and Luckins, 1976; Doyle, 1977) which leads to persistent parasitaemia provides a continuing opportunity for transmission of infection by tsetse.

At present, there is no effective field vaccine available against African trypanosomiasis. The major constraint to the development of a vaccine include the existence of different species of trypanosomes and different serodemes* within the same species, all with the capability of producing different repertoires of variable antigen types (VATs) (Bulletin WHO, 1978).

In the face of these problems, increasing attention has been focused on the potential use of genetically resistant or trypanotolerant livestock in the vast areas of Africa dominated by the tsetse fly.

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* A serodeme designates a set of VATs all of which c[illegible] from one another.

1.4. OBJECTIVE OF THE STUDY.

The phenomenon of trypanotolerance has been attributed to wild animals and West African taurine breeds of cattle but little attention has been paid to the possibility that degree(s) of genetic resistance to trypanosomiasis may have evolved in other tsetse infested areas of Africa. The objective of this Thesis was to attempt to identify whether any degree of resistance to trypanosomiasis might have evolved in the East African Zebu cattle living in trypanosomiasis endemic areas of Kenya. An attempt was also made to investigate the factors that might be involved in affecting resistance to trypanosomiasis.

This was regarded as the first phase of a feasibility study to determine whether or not genetic resistance to trypanosomiasis existed in breeds of cattle in East Africa, and, if it did, whether it could be exploited or improved.

2. LITERATURE REVIEW.

2.1. Trypanotolerance.

Based on field observations, trypanotolerance has been defined as the capacity of certain breeds of cattle, sheep and goats and some species of wild animals to survive and be productive (ILCA, 1979) in tsetse fly infested areas without the aid of chemotherapy, where other breeds cannot.

The trypanotolerant trait is generally attributed to the taurine breeds of cattle in West and Central Africa, namely the N'Dama and the West African shorthorn (reviewed by Murray, Morrison and Whitelaw, 1982). Trypanotolerant breeds are not truly tolerant in the immunological sense in that they develop immune responses to the trypanosome and can develop severe clinical disease. In the present context, trypanotolerance refers to greater resistance or reduced susceptibility to the development of disease (Wakelin, 1978).

2.1.1. Trypanotolerance in cattle in West Africa.

The first evidence of trypanotolerance in West African livestock was recorded by Pierre in 1906. He noted the apparent ability of certain cattle types to survive in tsetse-infested areas. Subsequently, increased attention was paid to the phenomenon of trypanotolerance and a series of

experimental studies have been carried out over the last 30 years, first to confirm field observations and later to look into the various factors that may affect the trait.

Stewart (1951), described his observations on the 'West African Shorthorn cattle' in Ghana (Gold Coast) from 1929 to 1948. The 'West African Shorthorn' in his context comprises all the unhumped cattle which live in West Africa South of the 12th parallel latitude until Nigeria is reached. They are genetically heterogeneousand comprise Hamitic Longhorn, Shorthorns and Zebu. Subjecting these cattle to needle challenge with both T. vivax and T. congolense, there were no obvious clinical signs of the disease and only transient parasitaemia was noted. These cattle were also resistant to needle challenge with a virulent strain of T. congolense from Tanzania. In another experiment where cattle were exposed to constant natural tsetse challenge, trypanosomiasis never appeared in a clinical form, except where intensity of challenge was high. Despite the lack of clear information on the background of the cattle studied and history of trypanosome strains used, Stewart concludes that "These cattle possess a very high degree of resistance to trypanosomiasis".

The relative resistance of N'Dama, N'Dama-Zebu crosses and the Zebu to a natural tsetse fly challenge was evaluated by Chandler (1952). The N'Dama were found to be more resistant to trypanosomiasis than the N'Dama-Zebu crosses and pure Zebu. All 8 N'Dama survived the infection for 48 weeks while 9 out of 12 Zebu and 4 out of 12 cross-breeds died of the infection. Thus, N'Dama-Zebu crosses appeared intermediate in susceptibility between the pure N'Dama and pure Zebu. In these studies the history of the cattle was not known. Chandler (1958), further tested the susceptibility of N'Dama, previously exposed to trypanosomiasis, to infection with T. vivax and T. congolense strains from a wide geographical origin, transmitted through tsetse. The N'Dama self-cured. He concluded that reduced susceptibility to trypanosomiasis is an inherent quality of the N'Dama and there are indications that previous exposure enhances this tolerance.

Desowitz (1959) assessed the tolerance to infection and the immune response between N'Dama, Zebu and Muturu cattle. The N'Dama were of two groups, those previously exposed to trypanosomiasis and those which had never been exposed. The Zebu were previously exposed but the two Muturu cattle

came from a herd which had been isolated from trypanosomiasis for more than 50 years. All cattle were subjected to challenge with Glossina palpalis reared and infected with T. vivax in the laboratory. The N'Dama that had been previously exposed to trypanosomiasis developed transient infections with infrequent scanty trypanosomes in the blood. N'Dama without previous exposure underwent initial intense parasitaemic attacks and crisis and finally developed chronic trypanosomiasis. They were therefore more severely affected than N'Dama that had been previously exposed. The Zebu also developed severe disease initially but later the disease took a chronic course. The two Muturu developed an acute infection, all dying 25 days post-infection. As most of these experiments were carried out on small numbers of animals which had been previously exposed to trypanosomiasis, the influence which acquired or innate resistance had on the outcome is not clear.

As an extension to these studies, Stephen (1966) evaluated the resistance to trypanosomiasis of N'Dama and white Fulani Zebu at an early age. Both breeds were reared free from tsetse exposure. The challenge was by wild caught Glossina morsitans submorsitans. The N'Dama displayed a greater resistance to trypanosomiasis than the Zebu. However,

even the N'Dama suffered severely from the chronic infections exhibiting anaemia, retarded growth and failure to reach sexual maturity.

A more closely controlled experiment was carried out by Roberts and Gray (1973b) to compare the susceptibility of N'Dama, Muturu and Zebu cattle to trypanosomiasis. These cattle had no previous exposure to pathogenic trypanosomes and had been bred from stock also without previous exposure to pathogenic trypanosomes. All cattle were given a uniform trypanosome challenge with wild-caught Glossina morsitans submorsitans and kept under the same conditions of husbandry and management. Zebu were more severely affected by trypanosomal infections than either the N'Dama or the Muturu. The Muturu were more severely affected than the N'Dama. They concluded that the dwarf cattle possess the ability to withstand the effects of trypanosome infection better than the Zebu cattle. Similar results were obtained by Van Hoeve (1972), when cattle of the same breeds were exposed to a natural tsetse challenge in the field, Esuruoso (1977), showed that Muturu with no previous experience of trypanosomiasis were more resistant than Zebu to needle challenge with T. vivax.

More recently, a series of experiments have been carried out in The Gambia, West Africa (Murray, Morrison, Murray, Clifford and Trail, 1979c; Murray, Murray, Wallace, Morrison and McIntyre, 1979d and e; Murray, Clifford, Gettinby, Snow and McIntyre, 1981a). These studies compared susceptibility of N'Dama and Zebu cattle to both needle inoculation with trypanosomes and to natural tsetse challenge. All animals came from areas believed to be free of tsetse and were serologically negative for trypanosomes. They were first subjected to needle challenge with bloodstream forms of T. brucei or T. congolense and observed for 2-5 months. Survivors were exposed to a natural field challenge of G. palpalis gambiensis for 9 months. Subsequently all animals were moved to an area where they came under challenge with G. morsitans submorsitans for 28 months. The N'Dama under all circumstances of challenge showed a significantly superior capacity to control parasitaemia and developed less severe anaemia; as a result they were more productive and suffered fewer mortalities.

Studies in other areas of West Africa further confirmed the superior resistance of the N'Dama to trypanosomiasis compared with the Zebu (Toure, Gueye, Seye and Mane, 1978; Toure and Seye, 1980; Saror, Ilemobade and Nuru, 1981). Studies in Senegal by Toure and his colleagues where N'Dama and Zebu (with and without previous exposure) were subjected

to natural tsetse challenge, proved the N'Dama to be more resistant than the Zebu although there was some overlap in resistance. N'Dama and Zebu which had been exposed to tsetse showed greater resistance than animals that had not. This was related to less severe anaemia and lower levels of parasitaemia. Saror et al. (1981) further confirmed the above findings by subjecting N'Dama and Zebu to needle challenge with T. vivax. The N'Dama were more resistant than the Zebu as judged by less severe anaemia. N'Dama reared in an endemic trypanosomiasis area were even more resistant than the N'Dama not previously exposed. This was reflected by lower parasitaemia in the most resistant animals.

Recent studies in Upper Volta (CREAT, Annual report 1981) showed that some breeds of cattle in West Africa have more resistant individuals than others but all breeds contain sensitive and resistant individuals. The order of reduced resistance was N'Dama, Muturu, Baoule, Zebu and N'Dama - Simmental crosses. These studies further proved that cattle previously exposed to trypanosomiasis are more resistant than those without previous exposure.

These results confirm that trypanotolerant breeds such as the N'Dama and the West African Shorthorn are innately more resistant than the Zebu and there were indications that the level of resistance

exhibited by various breeds but especially the N'Dama, could be supplemented by previous exposure.

2.1.2. Trypanotolerance in other species.

2.1.2.1. Sheep and goats.

In contrast to the increasing amount of information that has become available on the susceptibility of cattle to trypanosomiasis, the situation in sheep and goats is far less clear. However, it is generally accepted that sheep and goats throughout West, Central and East Africa are apparently able to survive and be productive in tsetse infested areas without the aid of trypanocidal drugs. This is taken as an indication of their trypanotolerance.

The trypanotolerance in sheep in West and Central Africa is attributed to the Djallonke or Fouta Djallon type (Mason, 1951). They are 'dwarf' in stature and comprise two types, dwarf forest type and the Savannah type. A similar variation occurs in goats and the dwarf goat or Fouta Djallon goat also considered trypanotolerant corresponds very closely in name, size and distribution to the dwarf sheep (Mason, 1951). Djallonke sheep and dwarf goats are found throughout the tsetse areas of West and Central Africa where there is no other breed of small ruminant (ILCA, 1979).

While there is evidence that dwarf Djallonke sheep are more resistant than the larger Fulani sheep following syringe challenge with T. congolense (Toure et al., 1983), earlier experimental studies have shown the dwarf goats to be very susceptible to T. brucei (Bungener and Mehlitz, 1976; Ikede, 1979) and also T. vivax (Saror, 1980).

It was not until recently that evidence for genetic resistance was reported in sheep and goats in East Africa under controlled experimental conditions. In Kenya, Griffin and Allonby (1979a) demonstrated that indigenous breeds of sheep, the Red Masai and the Blackhead Persian, were more resistant than the exotic Merino breed to syringe challenge with T. congolense. Indigenous goats, the Galla and East African goat were more resistant than exotic Saanen to the same type of challenge. Indigenous breeds of sheep and goats were also more resistant compared with exotic breeds following natural tsetse challenge (Griffin and Allonby, 1979b).

2.1.2.2. Wild Bovidae and other wildlife.

Wild animals have a reputation for being trypanotolerant or resistant to trypanosomiasis. In some cases it is believed that they might be completely refractory to infection. This is based on observations that wildlife survive in areas

heavily infested with tsetse and also on parasitological surveys (Ashcroft, Burtt and Fairbain, 1958).

Carmichael (1934) carried out a limited study on the susceptibility of the antelope, waterbuck, reedbuck, duiker, bushpig, jackal and multimammate mouse to T. brucei and T. congolense. One antelope and the multimammate mouse died of T. brucei infection. All the other animals developed transient infections.

In a more extensive study, Ashcroft, Burtt and Fairbain (1958) tested the susceptibility to trypanosomiasis of a wide variety of wild animals coming from tsetse-free areas. Animals were challenged with either G. morsitans infected with T. rhodesiense, T. brucei and T. congolense or blood infected with any of the three. The results from these studies showed that there was a marked variation in susceptibility to trypanosomiasis between different species of wildlife ranging from animals that usually died of the infection (jackal, hyrax and monkey); those that were infectible but resistant to infection (eland, bushbuck, oribi); those with scanty infections (warthog, bushpig and porcupine) and those that were completely refractory to infection (baboon).

Roberts and Gray (1972b) showed that duiker were very resistant to T. vivax and developed sporadic parasitaemia with no anaemia or clinical

signs of the disease. T. congolense was more easily detected in the duiker than T. vivax but the infection was sporadic and transient. On the other hand, gazelle behaved similarly to cattle following T. vivax and T. congolense infection. The animals were prostrate and febrile at peak parasitaemia but the effect was transient.

More recently the susceptibility of wild ungulates to T. congolense, T. vivax and T. brucei was compared to that of cattle and goats (Murray, Grootenhuis, Akol, Emery, Shapiro, Moloo, Dar, Bovell and Paris, 1981b; Grootenhuis, Varma, Black, Moloo, Akol, Emery and Murray, 1982). The wildlife species studied included the buffalo, waterbuck, bushbuck, eland and oryx. All except the oryx had never previously been exposed to tsetse flies. The areas investigated included host attractiveness, transmission of infection, skin reactivity and host responsiveness. The oryx and waterbuck attracted very few tsetse flies while cattle controls attracted a large number of tsetse, of which a large proportion successfully engorged themselves with blood. The attractiveness of the eland could be ranked between the above two extremes. The buffalo were as attractive to tsetse as cattle.

In the eland and waterbuck challenged with G.m. morsitans infected with T. congolense, fewer and smaller chancre reactions developed compared with cattle and goats. In contrast to domestic animals, no significant anaemia developed in these wild species.

With T. brucei, the kinetics of the chancre following successful tsetse feeds were similar in the eland, waterbuck, cattle and goats. High parasitaemia was recorded in the eland and waterbuck but no significant anaemia developed. Tsetse-transmitted East and West African stocks of T. vivax failed to infect the eland and waterbuck but caused an acute disease in the goat and a chronic trypanosomiasis syndrome in cattle.

Following intravenous inoculation with stocks of T. brucei, the waterbuck and the buffalo developed no obvious disease, while one cow died of the infection. With each of the trypanosome species, there was also evidence that the kinetics of parasitaemia depended on the stock of trypanosome used.

2.1.3. Evidence of resistance to trypanosomiasis in East African cattle.

It is generally believed that of the African breeds of cattle, Bos indicus types are the most susceptible to African trypanosomiasis. This has

been demonstrated experimentally in Zebu in West Africa, but the possibility that genetic differences in susceptibility to trypanosomiasis might have developed in Bos indicus breeds in certain areas of Africa has been ignored. This is despite the fact that certain populations of Zebu cattle survive in tsetse fly endemic areas without the aid of trypanocidal drug treatment.

There has been no documented experimental studies on the level of resistance to trypanosomiasis exhibited by Zebu in East Africa. However, there is epidemiological evidence that, in some areas of East Africa, Zebu have developed a degree of resistance to trypanosomiasis (Cunningham, 1966). With a view to obtaining evidence to the hypothesis that natural resistance to trypanosomiasis may occur in cattle, diagnostic surveys were carried out in cattle in various areas of Uganda and Kenya. In the survey carried out on the north-east shores of Lake Victoria, very high rates of infection were found in cattle living on the periphery of the fly belt. More than 50 percent of the cattle investigated were parasitaemic. Furthermore, many of the cattle examined were in good condition. Many more cattle were positive serologically than were parasitaemic. For example, at Alego in Central Nyanza, Kenya, 152 cattle were examined using stained blood films,

inoculation of blood into mice and testing sera for agglutinating antibodies against T. brucei group antigens. A 30 percent prevalence of trypanosomes was found, while agglutinating antibody was reported in 90 percent of the cases (Cunningham, 1966). This is despite the fact that serological tests against T. congolense and T. vivax were not done. In conclusion, thousands of cattle around the north-east shores of Lake Victoria are surviving inspite of the fact that they are continuously exposed to trypanosomiasis. By definition, these animals might be considered trypanotolerant.

3. MATERIALS AND METHODS.

3.1. Cattle breeds.

Three different breeds were used in these studies. They were the small indigenous East African Zebu (*Bos indicus*), the Boran (*Bos indicus*) and the Ayrshire (*Bos taurus*) (Figure 2).

Source and background.

The origin of experimental cattle is as shown in Figure 3. Three groups of East African Zebu were selected from three different tsetse infested areas of Kenya.

Group 1 was obtained from Ukunda, South of Mombasa on the Kenya Coast. This area is under tsetse challenge, considered low to medium. The species of tsetse flies reported in this locality include *G. pallidipes*, *G. austeni* and *G. brevipalpis* "Kenya Veterinary Services department Annual report" (1976).

Group 2 came from Kisasi in Kitui, Eastern Province. This area is also under tsetse challenge considered low to medium and *G. pallidipes* is the only species recognised in the area (National Atlas of Kenya, 1969).

Group 3 comprised cattle purchased at Sindo, a small town on the shores of Lake Victoria, in Homa Bay District of Western Kenya. The town is adjacent to the Lambwe Valley and the level of

tsetse challenge is believed to be heavy in some parts of the valley. G. pallidipes is the only species reported inhabiting the Lambwe Valley and its surroundings (Njogu, A.R., personal communications, 1983).

The Boran were born and reared at ILRAD Kabete, a tsetse-free environment.

The Ayrshire originated from Elementeita on the Rift Valley floor, an area known to be free of trypanosomiasis.

Age.

Cattle aged one to two years were selected. Aging was done by assessing the number of permanent incisor teeth.

Sex.

At the age selected, sex was not considered an important factor in selecting animals for susceptibility studies (Max Murray, personal communication), but whenever possible cattle were evenly matched with regard to sex.

Management.

All experimental cattle were housed in fly-proof concrete buildings at the Veterinary Research Laboratory, Kabete. Cattle were allowed to acclimatise to their new environment for at least 5 weeks before the start of the experiment. Hay and water were provided ad libitum and concentrates at 2 percent body weight on a daily basis.

Figure 2.

Breeds of cattle used in these studies.

A - Indigenous East African Zebu.

B - Boran.

C - Ayrshire.

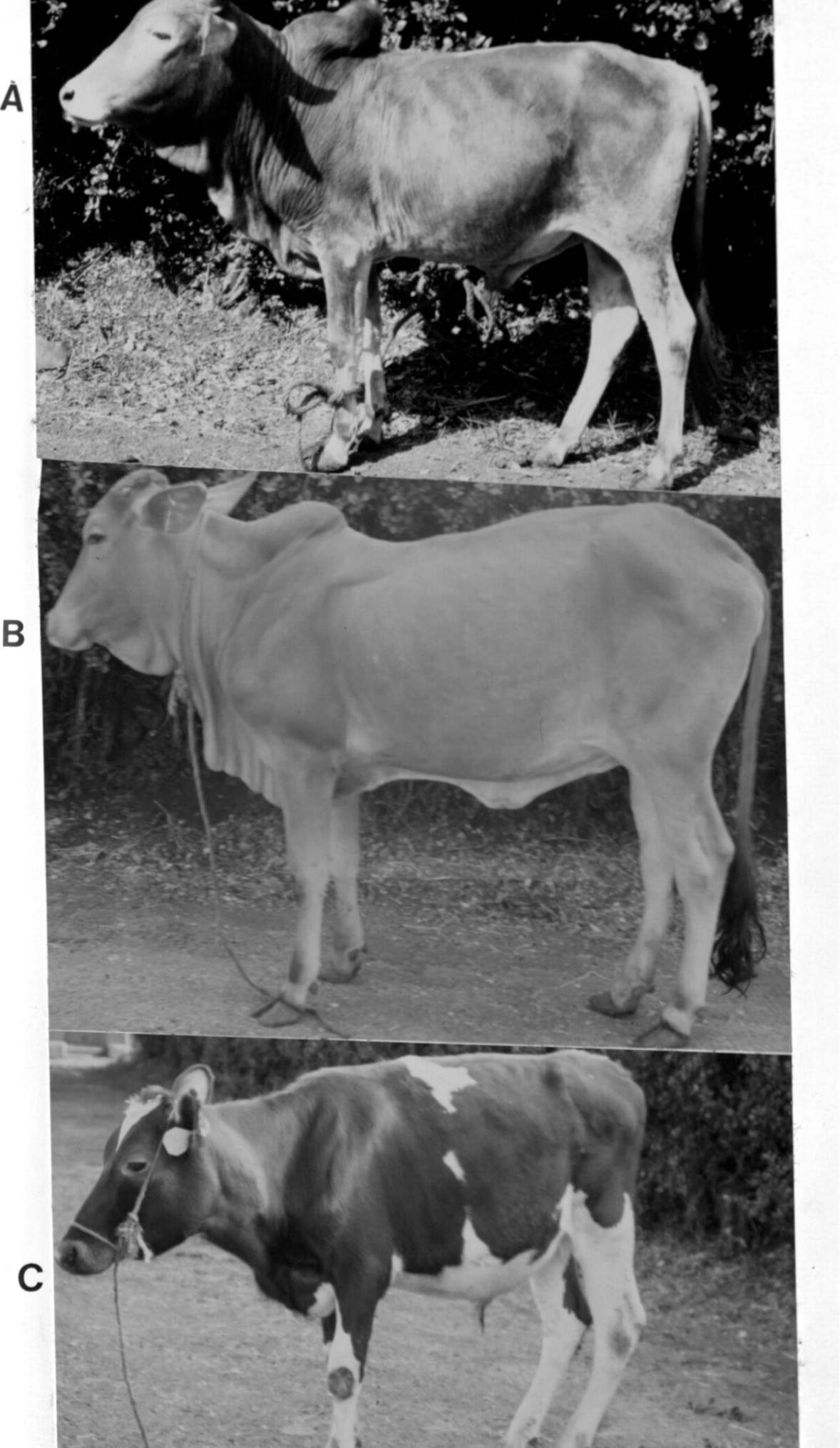

A
B
C

Figure 3.

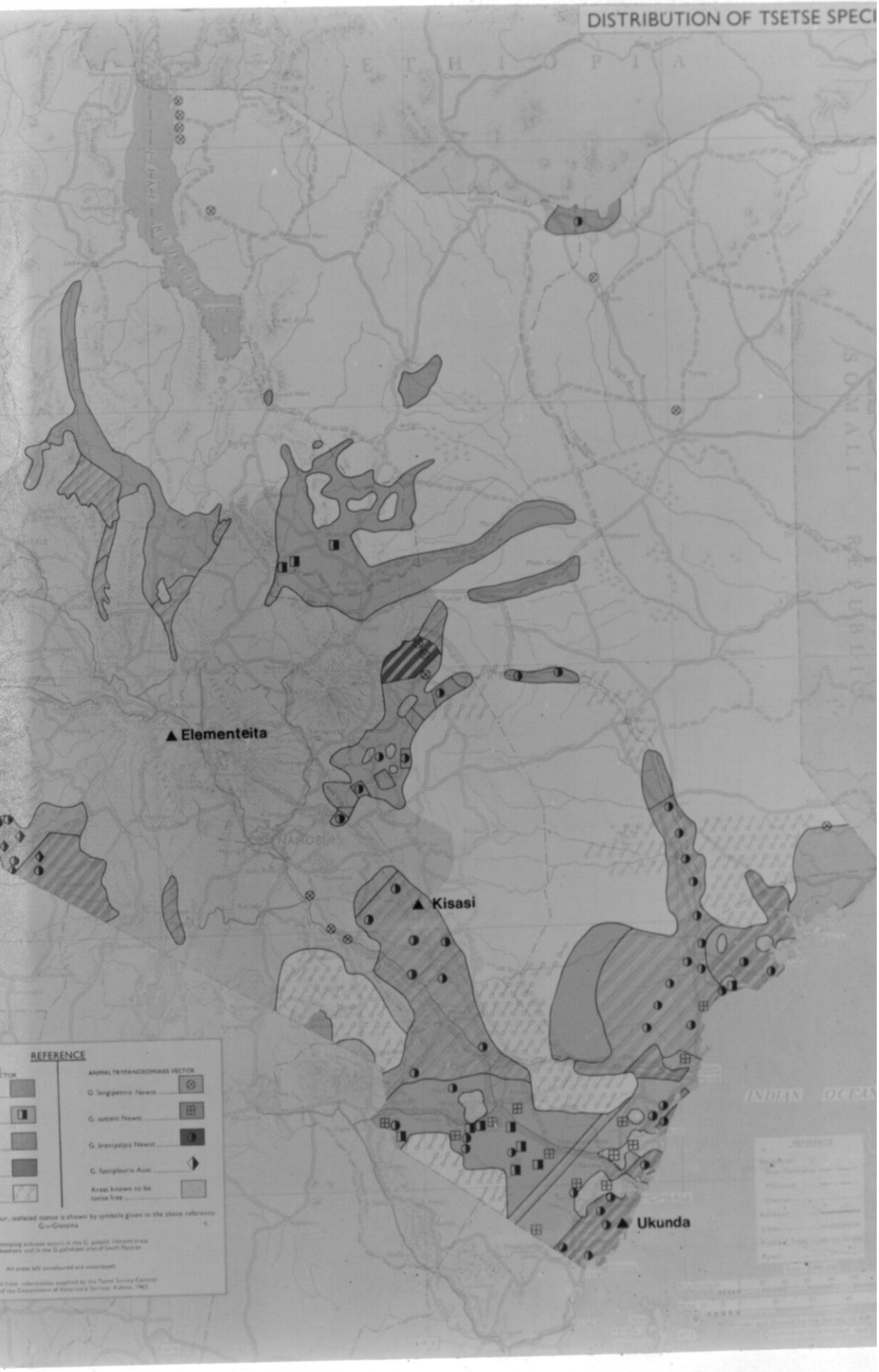
The distribution of tsetse species in Kenya and the origin of experimental cattle.

Ukunda - Zebu from Coast.

Kisasi - Zebu from Kitui.

Sindo - Zebu from Western Kenya.

Elementeita - Ayrshire.


DISTRIBUTION OF TSETSE SPECI
ETHIOPIA
▲ Elementeita
▲ Kisasi
▲ Ukunda
INDIAN OCEAN
REFERENCE
ANIMAL TRYPANOSOMIASIS VECTOR
G. longipennis Newst
G. austeni Newst
G. brevipalpis Newst
G. fuscipleuris Aust
Areas known to be tsetse free
G=Glossina

3.2. Prechallenge screening.

Cattle from tsetse endemic areas (Coast, Kitui, Western Kenya) and the Ayrshire from the Rift Valley were screened clinically and by haematological and parasitological methods for trypanosomiasis and other diseases including anaplasmosis, babesiosis and theileriosis.

A physical examination was carried out on all cattle from the field. This included assessment of parameters such as body condition, lymph node size, pulse rate, mucous membranes, respiration rate and rectal temperatures. Smears were prepared from lymph node aspirates and stained with Giemsa. Jugular blood was collected and packed red cell volume (PCV percent) was measured and thin blood smears were made and stained with Giemsa. The presence of trypanosomes was assessed by the blood buffy coat phase-contrast darkground technique (DG). The presence of anti-trypanosomal antibodies was assessed using indirect immunofluorescence. The effect of pre-infection sera from cattle coming from trypanosomiasis endemic areas, on the stock and clone of T. congolense used in the studies was assessed using the infectivity neutralisation test. All cattle were haemoglobin typed.

3.3. Laboratory animals.

Inbred A/J mice were born and reared at ILRAD. They were used to expand trypanosome populations to infect tsetse and to perform immunological tests. New Zealand white rabbits, obtained locally in Kenya, were used to obtain fresh serum to act as a source of complement for the immune lysis test. The animals were maintained on a commercial pelleted ration.

3.4. Trypanosomes.

T. congolense IL 572. This is a stock derived from an isolate made from a lion at Serengeti National Park, Tanzania by Geigy and Kauffmann(1973) as shown in Figure 4. T. congolense IL 1180 is a clone derived from IL 572 (Figure 4). A large batch of stabilate was made from the stock and clone using irradiated A/J mice (700 rad); this was subsequently used to infect cattle. Parasites used for infection were diluted in PSG, (pH 8.0), to make a concentration of 1×10^6 trypanosomes per ml which was used as the standard infection dose per animal.

T. congolense IL 13 E-8, was derived from an isolate made from a cow in Ikulwe, Busoga, Uganda by Van Hoeve in 1962. Clone IL 285 was derived from it as illustrated in Figure 5. These parasites were used to infect cattle by tsetse transmission as described on section 3.5.

Figure 1.

The source and procedure used to derive T. congolense stock ILRAD 572 and clone ILRAD 1180 used to infect cattle.

* = Number of days trypanosomes grown in rats or mice.

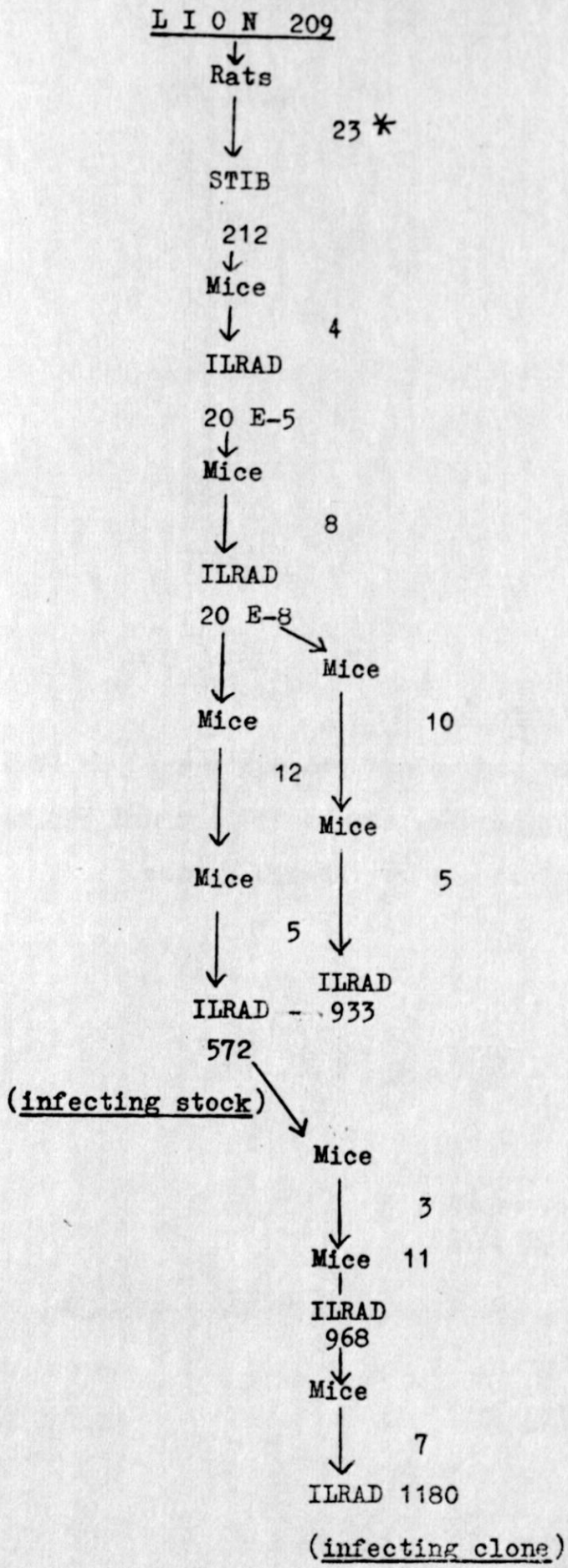
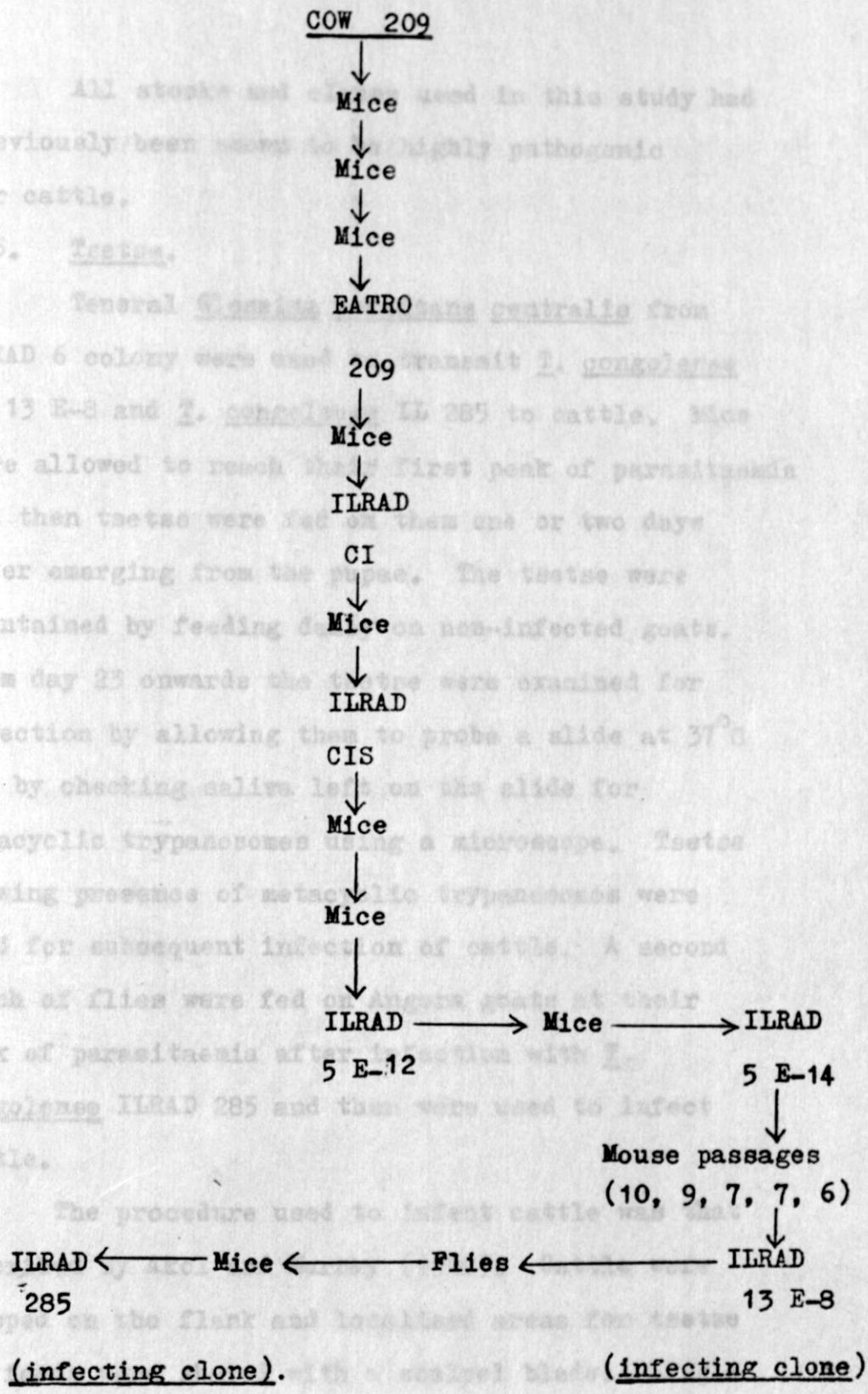

LION 209
Rats
23 *
STIB
212
Mice
4
ILRAD
20 E-5
Mice
8
ILRAD
20 E-8
Mice
Mice
10
12
Mice
Mice
5
5
ILRAD
ILRAD - 933
572
(infecting stock)
Mice
3
Mice
11
ILRAD
968
Mice
7
ILRAD 1180
(infecting clone)

Figure 5.

The source and procedure used to derive *T. congolense*, clones 13E - 8 and 285 used to infect cattle through tsetse.


COW 209
Mice
Mice
Mice
EATRO
209
Mice
ILRAD
CI
Mice
ILRAD
CIS
Mice
Mice
ILRAD
Mice
ILRAD
5 E-12
5 E-14
Mouse passages
(10, 9, 7, 7, 6)
ILRAD
13 E-8
Flies
Mice
ILRAD
285
(infecting clone).
(infecting clone)

All stocks and clones used in this study had previously been shown to be highly pathogenic for cattle.

3.5. Tsetse.

Teneral Glossina morsitans centralis from ILRAD 6 colony were used to transmit T. congolense IL 13 E-8 and T. congolense IL 285 to cattle. Mice were allowed to reach their first peak of parasitaemia and then tsetse were fed on them one or two days after emerging from the pupae. The tsetse were maintained by feeding daily on non-infected goats. From day 23 onwards the tsetse were examined for infection by allowing them to probe a slide at 37°C and by checking saliva left on the slide for metacyclic trypanosomes using a microscope. Tsetse showing presence of metacyclic trypanosomes were used for subsequent infection of cattle. A second batch of flies were fed on Angora goats at their peak of parasitaemia after infection with T. congolense ILRAD 285 and then were used to infect cattle.

The procedure used to infect cattle was that described by Akol and Murray (1982). Cattle were clipped on the flank and localised areas for tsetse fly feeds were shaved with a scalpel blade. Single infected tsetse flies in Geigy - 1 cages were placed on each shaved site and the tsetse allowed to feed

until they had taken a full blood meal. The point of entry of the proboscis (bite site) was marked in each case. Each animal received three tsetse bites.

3.6. Critical parameters in assessing resistance of cattle to trypanosomiasis.

3.6.1. Survival.

Trypanotolerant breeds of cattle such as the N'Dama, have a unique ability not only to control parasitaemia but in some cases even to 'self-cure' following both needle and natural tsetse challenge. As a result, they are able to survive the infection and continue to be productive. In my studies, survival time was taken as the number of days an infected animal took to develop PCV levels of 15 percent or less. Previous experience has shown that under field conditions, cattle with severe anaemia (PCV = 15 percent or less) are severely compromised in their ability to forage and the ensuing stress is liable to lead to death at any point (Morrison et al., 1981).

3.6.2. Anaemia.

Anaemia is the most important pathological feature of bovine trypanosomiasis (Murray, 1979). It has also been shown that resistant species and breeds, such as N'Dama cattle of West Africa and wildlife, develop less severe anaemia than more susceptible animals (Murray et al., 1982). Assessment of anaemia in trypanotolerance studies is of major importance.

3.6.3. Parasitaemia.

Previous studies on susceptibility to trypanosomiasis (reviewed by Murray et al., 1982). In these studies, evidence was provided that increased resistance to trypanosomiasis depends on the inherent capacity to limit parasitaemia. Parasitaemia was therefore chosen as the key parameter in the investigations carried out in this Thesis.

3.6.4. Immune response.

The superior capacity of some breeds of cattle to control parasitaemia has been attributed to innate differences in the immune response, although there is little confirmatory data. Desowitz (1959) believed that the trypanotolerant nature of the N'Dama lay in their capacity to mount a superior secondary immune response to the trypanosome and this was supported by experiments of Roberts and Gray (1973b). In addition, Chandler (1958), stated that the neutralizing antibody response to trypanosomes was greater in N'Dama than in Zebu.

It was, therefore, considered important to compare not only the quantity but also the quality and class of antibody response between the breeds of cattle used in our studies.

3.6.5. Local skin reaction (chancre).

It has been shown that in resistant wild animals such as the eland and waterbuck, chancres induced by T. congolense transmitted by tsetse appear less frequently and are smaller than in susceptible domestic animals (Murray et al., 1981b). These resistant wild animals develop low parasitaemias and little clinical evidence of the disease. The skin may therefore play a role in trypanotolerance and the comparative chancre development of different breeds of domestic animals was considered an important parameter.

3.6.6. Haemoglobin typing.

N'Dama cattle show almost 100 percent gene frequency for haemoglobin A while Zebu are a mixture of A and B. It has therefore been speculated that haemoglobin type could be used as a possible marker for resistance to trypanosomiasis (Bangham and Blumberg, 1958). All experimental cattle were haemoglobin typed in our investigations in order to further clarify the relationship between haemoglobin type and resistance of cattle to trypanosomiasis.

3.7. METHODS.

3.7.1. Clinical and production parameters.

Rectal temperature (^{o}C), pulse rate, respiration rate and general body condition were monitored daily in all experimental cattle following both needle inoculation and tsetse-transmitted infection.

Changes in body weight (kg) were monitored at 2 weekly intervals in all experimental and control cattle using a weighing scale.

Following infection with tsetse-transmitted T. congolense, the progressive development of localised skin reactions (chancres) at the sites of successful tsetse feeds was monitored. Skin thickness (mm) was measured using vernier callipers. This was done before infection and thereafter daily for 30 days.

In all experiments, in addition to death, survival time was assessed by the number of days an infected animal took to develop PCV levels of 15 percent or less. From past experience, this level was considered critical and the possibility of death imminent. When animals reached this level, they were treated with the trypanocidal drug Berenil.

3.7.2. Haematology.

Blood was collected from the jugular vein into 10 ml E.D.T.A. treated vacutainer tubes. The haematological parameters evaluated included, packed red cell volume (PCV percent), erythrocyte haemoglobin concentration (Hb. mg/dl), red blood cell count (RBC, x 10^{12} per litre), mean corpuscular volume (MCV, femto-litre) and total white cell count (WBC, x 10^{9} per litre).

PCV was measured using a microhaematocrit centrifuge. This was monitored in all cattle following both needle and tsetse infection, initially daily for 30 days and then at weekly intervals till the experiment was terminated.

Haemoglobin was estimated as Cyan-methaemoglobin in a Coulter haemoglobinometer. This was evaluated at weekly intervals.

Total erythrocytes and leucocytes were enumerated on an electronic particle Coulter counter (Model ZBL). This was done at weekly intervals.

Mean corpuscular volume was calculated using the formula.

$$MCV = \frac{PCV \times 10}{RBC}$$

The mean corpuscular haemoglobin concentration was calculated using the formula

$$MCHC = \frac{Hb}{PCV} \times 100$$

MCV was expressed in femto-litres and MCHC as a percentage.

3.7.3. Parasitology.

Jugular blood was collected into 10 ml E.D.T.A. treated vacutainer tubes and parasitaemia was evaluated using the blood buffy coat darkground phase contrast technique (DG), as described by Murray, Murray and McIntyre (1977) and Murray, Murray, Murray, Morrison, Pyne and McIntyre, (1979). Microhaematocrit capillary tubes were filled ¾ with blood and spun for 5 minutes. The trypanosomes were concentrated in the buffy coat. The capillary tube was cut with a diamond pointed pen, 1 mm below the upper layer of red cells and approximately 2 cm above to include the plasma. Using a microhaematocrit tube holder, the contents were gently expressed on to a slide, mixed and covered with a coverslip (22x22 mm).

The preparation was examined by phase-contrast microscopy using a combination of Phaco 2 NPL 25/0.5 objective, a Zernicke 402 condenser and Periplan NF x 10 eye-pieces (E. Leitz Wetzlar).

A system of scoring was adapted to allow approximate quantitation of trypanosomes in blood (Paris, Murray and Mc Odimba, 1982). The scores and the interpretation are shown in Table 3. When the score was above 5+, the blood was diluted 1:10 with Ziehl-Neelsen stain and counting was done using an improved NEUBAUER haemocytometer (Paris et al., 1982).

The technique described is currently the most sensitive for the detection of trypanosomes in bovine blood and the only method for quantifying small concentrations (Paris et al., 1982).

In all cases parasitaemia was monitored daily for 30 days and subsequently at weekly intervals.

Table 2: Darkground/phase contrast buffy coat parasitaemia scoring system.

Score	Trypanosomes/field x 250.	Estimated parasitaemia trypanosomes/ml
6+	Swarming >100	$>5 \times 10^6$
5+	>10	$>5 \times 10^5$
4+	1 -10	$10^4 - 5 \times 10^5$
3+	1 per 2 fields to 1 per 10 fields	$5 \times 10^3 - 5 \times 10^4$
2+	1 - 10 per preparation.	$10^3 - 10^4$
1+	1 per preparation.	$10^2 - 10^3$

3.7.4. Immune response.

The humoral immune response following infection was assessed using:-

(i) Single radial immunodiffusion.

(ii) Immune lysis.

(iii) Solid-phase radioimmunoassay (SRIA).

Sera used in these assays were prepared from whole blood and stored in 0.5 ml plastic vials at -20°C. Sera were collected before challenge (day 0 serum), daily after challenge till day 14 and on a weekly basis thereafter.

3.7.4.1. Single radial immunodiffusion (Mancini test).

The technique adopted was that of Fahey and McKelvey (1965), a modification of that described by Mancini, Carbonara, and Heremans (1965). This test was used to estimate total immunoglobulin G (IgG) and immunoglobulin M (IgM) in the sera of cattle infected with T. congolense IL 572.

Miles Kits (Miles Laboratories, Stoke Poges Slough England) were used to estimate IgM. Goat anti-bovine IgG_1 and IgG_2 was purified by absorption and their monospecificity was confirmed by immunoelectrophoresis. The antisera were diluted with PBS, pH 7.0, mixed with 3 percent melted agar and poured as a 1 mm thick layer on to glass plates. Two mm

wide holes were punched out of the agar and diluted sera were dispensed into the holes and incubated in a humid atmosphere.

Reading of the precipitin rings was done 22 hours after sample application. Ring diameters were measured and the corresponding concentration of immumoglobulin (mg/100 ml) was read against a standard curve on semi-logarithmic graph paper.

3.7.4.2. Immune lysis test.

The test was performed as modified by Morrison, Wells, Moloo, Paris and Murray (1982). Trypanosomes used in the test were grown in sub-lethally irradiated, A/J mice, 700 rad. Mice, inoculated intraperitoneally with 10^3 bloodstream forms of T. congolense IL 1180, were bled at peak parasitaemia and trypanosomes were separated from blood on a DEAE-cellulose anion exchange column (Lanham and Godfrey, 1970). Eluted trypanosomes were washed twice with PSG, pH 8.0, and resuspended at 2×10^7 per ml, in PSG containing 10 percent FBS. Two-fold dilutions of test sera from different animals were made in PSG containing 10 percent FBS. Twenty µl of each serum dilution were dispensed in duplicate into round-bottomed microtiter plates. To each well, 20 µl of fresh rabbit serum as a source of complement were added. 20 µl of trypanosome suspension were added to all wells. The plates were incubated at

37°C for 1 hour and were then observed with an inverted microscope using phase-contrast illumination. The results were recorded as complete lysis when no motile trypanosomes were seen and negligible lysis when less than 50 percent lysis occured. The end point was expressed as the reciprocal of the serum dilution at which 90 percent of the trypanosomes were lysed.

3.7.4.3. Solid phase radioimmunoassay (SRIA).

The general principles of SRIAs have been reported in detail by Hunter (1978). Described below is a modification of the basic SRIA designed for use in analysis of humoral immune responses against African trypanosomes in cattle. The assay permits measurement of serum antibodies with activity against conformational determinants of variant specific surface antigen (VSSA) of the trypanosome.

In this assay, whole trypanosomes were used as target antigen. Female sublethally-irradiated (700 rad) A/J mice were inoculated intraperitoneally with 1×10^3 bloodstream forms of T. congolense IL 1180. Parasites inoculated were harvested from mice during the first peak of parasitaemia and purified as already described. The parasites were then bound to a microtitre plate by the following procedure: 50 µl PSG, pH 8, containing 10^5 purified washed parasites were added to each well. The plates

were centrifuged at 500 *g* for ten minutes. The plates were washed three times in PBS. Fifty µl of 0.25 percent glutaraldehyde in PBS, pH 8.0, were added to each well and incubated for 10 minutes. The plates were washed three times in PBS. Fifty µl of PBS containing 1 percent normal rabbit serum and 0.2 percent sodium azide (RIA buffer, pH, 8.0) were added to each well and incubated for 1 hour. The plates were washed three times in RIA buffer and the buffer removed by flicking plates on to absorbent material. Two fold dilutions of each test serum sample were made using RIA buffer. Plates were washed once in RIA buffer and 50 µl aliquots of diluted test sera were added to each well. Plates were incubated for 1 hour at room temperature and were washed three times in RIA buffer. 50 µl of 1^{125} labelled goat anti-bovine IgG or IgM specific serum* (diluted in RIA buffer and containing approximately 20,000 counts per minute) was added to each well. Plates were incubated for 1 hour at room temperature and washed three times in RIA buffer. Wells were excised with a hot wire cutter and counted in a Packard 5360 Auto - Gamma Scintillation Spectrometer.

* Prepared and purified by Mr. Charles Atinda of ILRAD.

Data were plotted as binding curves derived from duplicate analysis of each dilution of test serum. The titre read at 50 percent binding was expressed as $\log_{10}$ reciprocal of the serum dilution.

3.7.5. Indirect fluorescent antibody test (IFAT).

Immunofluorescence was carried out on trypanosomes fixed on slides.

Sublethally irradiated A/J mice (700 rads, from a caesium source) were infected intraperitoneally with stabilates of T. congolense, T. brucei and T. vivax (rodent adapted). At peak parasitaemia, trypanosomes were separated from mouse blood using diethylaminoethyl (DEAE) cellulose anion exchange column (Lanham and Godfrey, 1970). Twenty five percent foetal bovine serum (FBS) in phosphate saline glucose (PSG), pH 8.0, was added to the isolated trypanosomes.

Serial two-fold dilutions of the test sera from $^{1}/10$ to $^{1}/5120$ were made using phosphate buffered saline (FBS), pH 7.2. Ten microliters of the isolated trypanosomes at a concentration of 1×10^{6} trypanosomes per ml were added to each of the 8 zones of the Cooke's microscope slides (Dynatech. Sussex, U.K.). Trypanosomes were fixed on slides with 5 percent acetone for 5 minutes, 2 - 3 drops of each serum dilution were placed in each zone of the Cooke slide. Slides were washed twice in PBS (pH 7.2) and excess PBS was wiped away from the reaction zones. Twenty microliters of diluted ($^{1}/80$) anti-bovine gamma globulin conjugated to fluorescein isothiocyanate was added

to the reaction zones. The edges of the reaction zones were cleaned. Slides were mounted in tris saline glycerol (pH 7.8) and a long cover slip was placed on the slides to cover all the reaction zones. The edges of the slides were sealed with nail varnish. Slides were examined for fluorescence using a Leitz Orthoplan microscope equipped with incident ultraviolet illumination, a Phaco NPL 40/0.7 objective and GW x 6.3 eyepieces (E. Leitz, Wetzlar, West Germany). The serum dilution at which trypanosomes did not exhibit positive fluorescence was taken as the titre. Controls included slides where normal bovine serum, PBS or conjugate were used.

3.7.6. Infectivity neutralisation test.

This test was carried out on pooled serum samples from East African Zebu coming from trypanosomiasis-endemic areas and also on the Ayrshire sera. Serum from Boran which had no previous exposure to trypanosomiasis was used as a control.

— Sera from East African Zebu from the Coast, and Kitui and the Ayrshire were screened against stock T. congolense IL 572. East African Zebu from Western Kenya were screened against clone T. congolense IL 1180. These were the stabilates used for the experimental infections.

For each sample, 6 plastic 3 ml tubes were set up in a rack in ice-water (4°C). 1.5 ml of 10 percent FBS in PSG (pH 8.0) was added into each tube. In the first tube 0.5 ml of control serum was added, mixed and 0.5 ml of the mixture was discarded. 0.5 ml of pooled serum from each group was added and a serial double dilution was carried out to the last tube. Parasites derived from T. congolense IL 572 and 1180 were diluted in 10 percent FBS in PSG (pH 8.0) to give a concentration of 1 x 10^4 trypanosomes/ml. 0.5 ml of this dilution was added to each tube and incubated for 30 minutes at 4°C. 0.2 ml aliquots from each serum dilution was inoculated into each of six A/J mice intraperitoneally. Tail blood was examined for the presence of trypanosomes daily for 30 days.

3.7.7. Haemoglobin typing.

Haemoglobin typing was carried out using cellulose acetate paper electrophoresis of haemolysed blood. This is a modification of the starch gel-electrophoresis described by Bangham and Blumberg (1958). Blood was collected from the jugular vein into 10 ml tubes containing disodium salt of ethylenediaminetetraacetic acid (E.D.T.A.). An equal volume of blood was added to 0.5 ml distilled water and allowed to stand at room temperature for 10 minutes. Osmolysis occured in the red blood cells and

haemoglobin was set free. Drops of haemolysed blood were applied on cellulose acetate strips of Sartorius membrane type (50 x 200 mm) and run in an electrophoretic chamber with voltage 200 - 400 volts, and a constant 15 amperes for 40 minutes. The strips were stained for 5 minutes with Poinceau-S-stain in 3 percent trichloroacetic acid (T.C.A.) and washed 3 times with acetic acid before examination.

3.7.8. Pre-challenge treatment.

All experimental cattle were treated with Berenil (Diminazene aceturate, Farbwerke Hoechst, Frankfurt, West Germany), at a "dose of 7 mg" per kilogram body weight and observed for 5 weeks. To eliminate possible anaemia causing helminths, each animal was drenched with Nilzan (Levamisole hydrochloride + Oxyclozamide, Wellcome, Cooper Veterinary Division, Kabete) at a dose of 1 ml/3 kg body weight, 5 weeks before experimental infection.

3.8. EXPERIMENTAL DESIGN.

3.8.1. Experiment 1.

The susceptibility of Zebu from the Coast, Zebu from Kitui, Boran from ILRAD and Ayrshire from Elementeita were compared. The susceptibility of 8 animals from each group was tested and 4 were kept as uninfected controls. The animals were infected by intravenous needle inoculation of 1×10^6 bloodstream forms of T. congolense IL 572.

The following parameters were monitored at regular intervals throughout the experimental period.

Clinical parameters including rectal temperature (^{o}C), pulse rate and respiration rate were monitored daily for 30 days and weekly, thereafter for 10 weeks in all experimental and control cattle. Progressive changes in body weight (Kg) were monitored at 2 weekly intervals throughout the course of infection.

Survival time was assessed by the number of days an infected animal took to develop PCV levels of 15 percent or less.

Anaemia was assessed by measuring PCV percent. This was done daily for 30 days and weekly thereafter for 10 weeks in all experimental cattle.

The first appearance of parasites in blood and the subsequent parasitaemic wave pattern was monitored daily for 30 days initially and weekly thereafter, for 10 weeks using the DG phase contrast technique.

Total serum immunoglobulin G (IgG) and immunoglobulin M (IgM) were estimated at weekly intervals by Mancini test over an 8 week period in all experimental cattle.

3.8.2. Experiment 2.

Four East African Zebu from the Coast and 4 Boran, which had previously been infected with T. congolense IL 572 (experiment 1) were treated with Berenil (7 mg/kg) at 8 - 10 weeks post-infection. When the haematological values had returned to normal, they were challenged with G.m. centralis infected with T. congolense IL 13E-8, a different strain from IL 572. Each animal received 3 tsetse bites. The following parameters were evaluated regularly.

The progressive development of the chancre was followed. Skin thickness was measured using vernier callipers. This was done before challenge and daily thereafter for 30 days.

Survival time was assessed by the number of days an infected animal took to develop PCV levels of 15 percent or less.

The progressive development of anaemia was assessed using PCV percent. This was monitored daily for 30 days initially and later on a weekly basis. Parasitaemia was evaluated daily for 30 days and weekly thereafter.

3.8.3. Experiment 3.

Seven Zebu from Western Kenya, 4 Boran and 3 Ayrshire were infected by intravenous needle inoculation of 1×10^6 bloodstream forms of T. congolense clone IL 1180. All parameters were evaluated for 6 months depending on the survival time of individual cattle.

Clinical parameters evaluated include, rectal temperature (^oC), pulse rate, respiration rate and general body condition. Progressive changes in body weight (kg) were monitored at 2 weekly intervals throughout the course of infection.

Survival time was assessed by the number of days an infected animal took to develop PCV levels of 15 percent or less.

The haematological parameters evaluated included PCV, erythrocyte haemoglobin concentration, total red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin concentration and total white cell count.

PCV was monitored daily for 30 days and weekly thereafter. Haemoglobin concentration, total erythrocyte count, total white blood cell count were monitored on a weekly basis throughout the course of infection.

The appearance of parasites in blood and the subsequent parasitaemic wave pattern was monitored

daily for 30 days and on a weekly basis thereafter.

The immune response was assessed by the trypanolysis test and SRIA.

3.8.4. Experiment 4.

Seven Zebu from Western Kenya, 4 Boran and 3 Ayrshire which had been previously infected with T. congolense clone IL 1180, were treated with Berenil (7 mg/kg) and observed for 6 weeks. When haematological values had returned to normal, all cattle and 4 Boran without previous exposure were challenged with G.m. centralis infected with T. congolense IL 285, a different serodeme from IL 1180. Each animal received 3 tsetse bites.

The progressive development of the chancre was followed for 30 days.

Survival time was assessed by the number of days an animal took to develop critical PCV of 15 percent or below.

Haemotological parameters evaluated included; PCV, Hb, MCV, MCHC, RBC and WBC counts.

Parasitaemia was monitored daily for a month and on a weekly basis thereafter.

3.9. STATISTICS.

The samples analysed at any one time in these studies did not exceed forty. Therefore, non-parametric tests were used for statistical analysis. The TWO SAMPLE MANN-WHITNEY TEST was used (Siegel, 1956) to compare all observations in this study (appendix 11).

4. RESULTS.

4.1. Results of pre-challenge screening.

All cattle from trypanosomiasis endemic areas were in good body condition and physically healthy.

One animal from the Coast had a PCV of 23 percent and *T. congolense* was demonstrated by buffy coat dark ground phase contrast technique. Four cattle from Kitui had the non-pathogenic *Trypanosoma theileri*. All cattle from Western Kenya were chronic carriers of *Theileria parva*, with schizonts being demonstrated in all superficial lymph nodes. Two out of 10 had microfilaria.

Cattle coming from trypanosomiasis endemic areas (Coast, Kitui, Western Kenya) had anti-trypanosomal antibodies against *T. congolense*, *T. vivax* and *T. brucei*. However, none of the sera from these cattle had any neutralizing effect on the stock or clone of *T. congolense* used in these studies.

All the Ayrshire were exclusively haemoglobin A (Table 2). Indigenous East African Zebu and the Boran had a gene frequency of 0.6 - 0.7 Hb. A and 0.3 - 0.4 Hb. B.

Table 3: Haemoglobin type and gene frequencies in experimental cattle.

Group	Haemoglobin type			Gene frequency		
	AA	AB	BB	Hb.A.	Hb.B.	Total number of cattle
East African Zebu (Coast)	4	6	-	0.7	0.3	10.
East African Zebu (Kitui)	3	7	1	0.6	0.4	11.
East African Zebu (Western Kenya)	5	3	2	0.7	0.4	10
Boran	4	5	2	0.6	0.4	11.
Ayrshire	12	-	-	1	-	12

4.1.1. SUSCEPTIBILITY OF ZEBU (COAST AND KITUI), BORAN AND AYRSHIRE TO NEEDLE INOCULATION WITH T. CONGOLENSE IL 572.

4.1.2. Clinical condition and survival.

Following intravenous inoculation with 10^6 T. congolense IL 572, all East African Zebu (Coast and Kitui), Boran and Ayrshire cattle became infected.

Fluctuating low grade fever (39.5 - 40.1°C) was recorded in experimental cattle of all groups during the course of infection. The first significant increase in rectal temperatures was observed on the time of the first peak of parasitaemia. An increase was subsequently noted at every other parasitaemia peak. There was no significant variation in the pattern of rectal temperature observed between the Ayrshire, Boran and the indigenous East African Zebu. Animals that became recumbent and were about to die exhibited temperatures below normal (35°C). There were no significant changes in daily rectal temperature in the non-infected controls; temperatures ranged between 37.5 - 39.2°C over the period of observation.

One week after cattle attained the first parasitaemic peak, elevated pulse and respiration rates were noted. As anaemia became more severe, with PCV of 20 percent or below, evidence of cardiac

insufficiency such as tarchycardia and a prominent jugular pulse were recorded.

Associated with the appearance of parasitaemia, a significant drop in PCV occured in cattle from all groups. The anaemia was progressive in all groups and became obvious clinically as pallor of the unpigmented mucous membranes. Following the development of parasitaemia, superficial lymph nodes were palpably enlarged. Lacrimation was evident especially in the Ayrshire group. Rough and harsh hair coats, emaciation and sub-mandibular oedema were other common clinical signs. Cattle continued eating throughout the course of infection giving the impression that the appetite was not severely affected. Terminally cattle were extremely lethargic and usually became recumbent. Animals which were recumbent, usually failed to respond to treatment with the trypanocidal drug Berenil and died.

There were differences in the pre-infection average body weight between and within breeds of cattle (Table 4). The Boran from ILRAD, had the highest pre-infection body weight, 200$\pm$20.2 kg. The rate of loss in body weight varied between all groups of cattle. By 2 weeks after infection, weight loss was obvious in the Boran, Zebu from Kitui and the Ayrshires. It was not until the sixth week

that loss in body weight commenced in the E.African Zebu from Coast. The surviving Ayrshire lost 30.8 percent of their average body weight by week 6 of challenge, a loss that was significantly higher than that of the E. African Zebu and Boran. Ten weeks post-challenge, the Boran lost 22.5 percent of their body weight while E. African Zebu from Coast lost 37.9 percent and Zebu from Kitui 34.0 percent. The difference between the Boran and E.African Zebu was statistically signficant (5 percent level by Mann-Whitney test). Throughout the course of infection there was no significant change in the body weight of control cattle from all groups.

The Ayshire required earlier treatment to survive than the Boran and E. African Zebu from the Coast and Kitui (Table 5). By week 3 post-inoculation, one Ayrshire had a PCV of 12 percent and had to be treated to survive. The rest had to be treated on or before day 49 to survive (Table 5). There was no significant difference in survival time between Boran, E.African Zebu from the Coast and Kitui; all cattle from these groups required treatment by the tenth week post-infection having developed critical PCV values of 15 percent and below. Thus Ayrshire cattle were sign-ificantly more susceptible to T. congolense than animals from the other 3 breeds, although all cattle suffered severely from the infection.

Table 4: Percentage drop in body weight from pre-infection values in cattle inoculated with bloodstream forms of *Trypanosoma congolense*, IL 572.

Weeks after infection.	Breed			
	East African Zebu (Coast)	East African Zebu (Kitui)	Boran	Ayrshire
2	0	2.3	0.8	5.1*
4	0	7.5	2.5	10.9
6	3.4	24.9	5.5	30.8*
8	31.0	30.2	15.0 *	-
10	37.9	34.0	22.5*	-

Pre-infection weights (Kg): East African Zebu (Coast) = 145 ± 14.8
East African Zebu (Kitui) = 132.5 ± 25.6
Boran = 200 ± 20.2
Ayrshire = 142.5 ± 22.4

* - Significantly different from other breeds at 5 pecent level (Mann-Whitney test).

Table 5: Survival time in cattle inoculated with bloodstream forms of *Trypanosoma congolense* IL 572.

Breed	Number of cattle	SURVIVAL* Days after infection							
		28	35	42	49	56	63	70	77
East African Zebu (Coast)	8	8	8	8	8	7	6	5	0
East African Zebu (Kitui)	8	8	8	8	8	6	5	5	0
Boran	8	8	8	8	8	6	5	5	0
Ayrshire	8	7	5	3	1	0	0	0	0

* = reduction in number means either the animal required treatment to survive or died of the infection.

4.1.3. Haematology.

Prior to infection, there were significant differences in the average PCV between breeds. The Ayrshire had the lowest value of 30.8±3.4, Zebu from kitui, 34.8±3.4, Zebu from Coast 40.5±4.0 and Boran 43±4.6.

To allow proper comparison of the development of anaemia between breeds, anaemia was assessed as the percentage drop in PCV from pre-infection values. The onset of anaemia coincided with the appearance of parasites in the blood. Thus, a significant drop in PCV occured 2 weeks post-infection in cattle of all groups (Table 6). The rate of development and the degree of anaemia reflected the level of parasitaemia and was significantly greater in the Ayrshire compared with the Bos indicus types. Three weeks post-infection the percentage drop in PCV from pre-infection values was; 40.1±7.1 in the Ayrshire, Boran 22±10.8, Zebu from the Coast 23.4±9.0 and Zebu from Kitui 26.8±7.1. Throughout the course of infection there was no significant difference in the degree of anaemia between the Boran, Zebu from Coast and Kitui and, by 5 to 6 weeks, all breeds including the surviving Ayrshires exhibited similar drops in PCV of between 46 and 54 percent. Subsequently, the PCV levels remained around this level with all individual animals requiring treatment at PCV = 15 percent or below or death occuring.

Table 6: Percentage drop in packed cell volume from pre-infection values in cattle inoculated with bloodstream forms of T. congolense, IL 572.

Days after infection	Breed			
	East African Zebu (Coast)	East African Zebu (Kitui)	Boran	Ayrshire
7	4.1±2.0	4.8±3.0	4.5±4.3	9.4±5.0
14	12.0±5.5	15.6±6.3	9.7±7.6	24.4±7.5
21	23.4±9.0	26.8±7.1	22±10.8	40.1±7.1
28	37.1±14.6	31.2±9.3	38.0±9.0	40±9.1
35	45.9±7.0	45.6±8.5	50.0±8.4	46.3±6.0
42	49.0±7.6	53.8±7.5	51.2±7.3	48.4±7.5

Pre-infection PCV: East African Zebu (Coast) = 40.5±4.0
East African Zebu (Kitui) = 34.8±3.4
Boran = 43±4.6
Ayrshire = 30.9±4.0

4.1.4. Parasitological findings.

Following intravenous needle inoculation with T. congolense all animals became infected. The parasites were usually detected in the Ayrshire on days 5 to 6 after infection, which preceeded detection in the Boran and East African Zebu from the Coast and Kitui by 2 to 3 days.

Once parasites were demonstrated in blood, the number progressively increased till peak parasitaemia was reached in cattle of all groups (Table 7). However, in the Ayrshires the average time taken to reach peak parasitaemia was significatly shorter compared with the Boran and East African Zebu from the Coast and Kitui. The time to first parasitaemic peak was similar in the Boran, East African Zebu from Coast and Kitui.

The level of the first peak varied considerably between individual cattle of a breed. The levels achieved in the Ayrshires of $>5x10^6$ trypanosomes per ml were significantly higher than in the other breeds (Table 7). There was no significant difference in the level of the first peaks of parasitaemia between the Bos indicus breeds. Once peak parasitaemia was attained, the level remained high and did not relapse in the Boran, Ayrshire and Zebu from Kitui (Figure 6). Animals from these groups had no ability

to remit parasitaemia. On the other hand all individual cattle of Zebu from the Coast showed some ability to remit parasitaemic waves (Figure 6).

Table 7: The prepatent period, time to first peak and level of first peak parasitaemia in cattle inoculated with bloodstream forms of *T. congolense* IL 572.

Breed	Prepatent period (days)*	Time to first peak (days)*	Level of first peak as judged by parasitaemia score**
East African Zebu (Coast)	8.1±1.1	12.6±1.4	4.5±0.9
East African Zebu (Kitui)	8.1±1.2	13.3±4.4	5.0±0.7
Boran	8.6±2.0	13.6±1.8	4.5±0.5
Ayrshire	5.6±0.8	8.6±1.5***	5.5±0.7***

* = arithmetic mean ± one standard deviation.

** = darkground phase/scoring system (Table 3).

*** = significantly different from the other breeds at 5 percent level (Mann-Whitney test).

Figure 6.

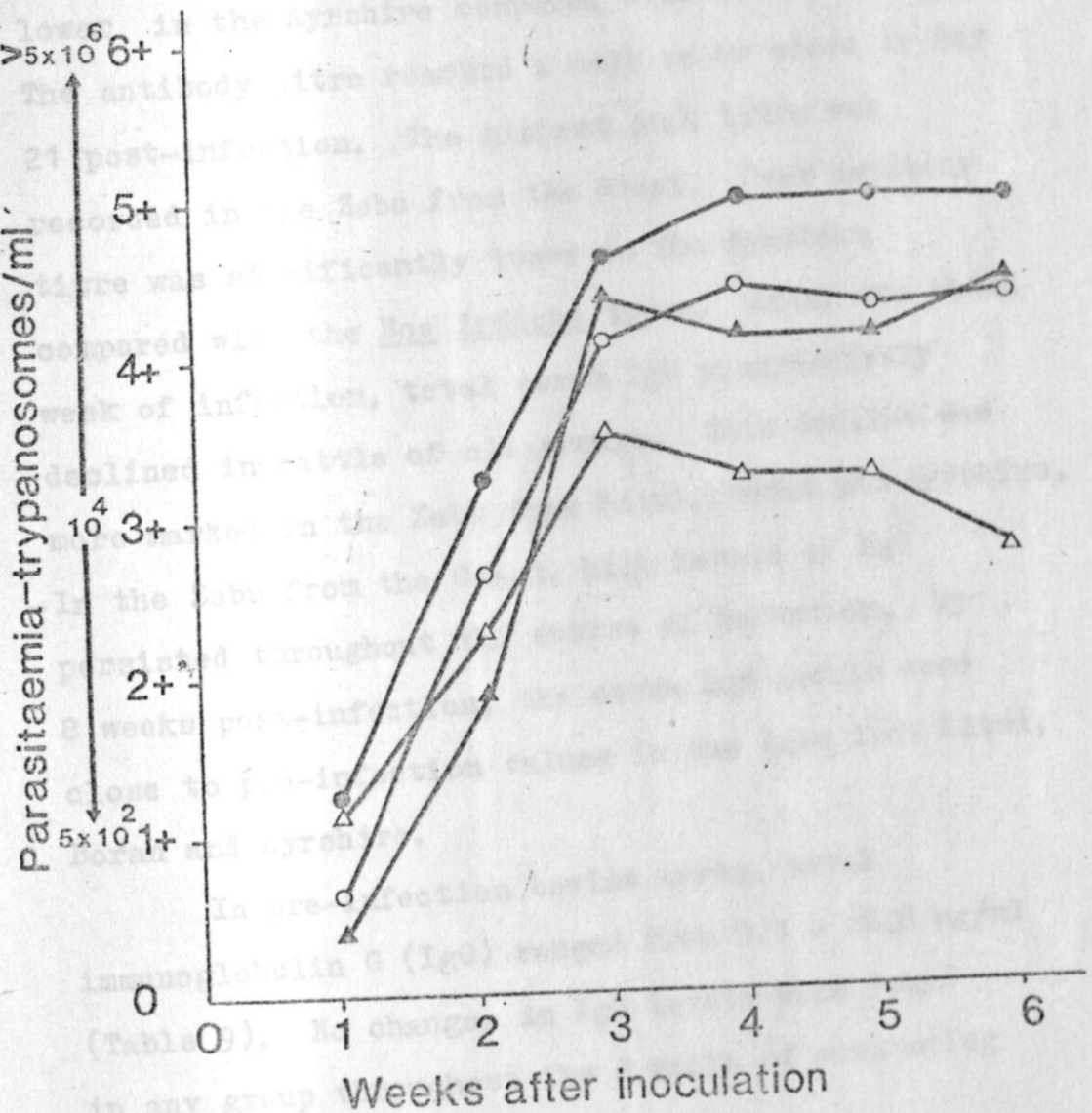
Parasitaemic profile in cattle infected with bloodstream forms of T. congolense IL 572. The results represent the average weekly parasitaemia score for each breed.

(△) Zebu, (Coast)

(○) Zebu (Kitui)

(▲) Boran

(●) Ayrshires


Parasitaemia-trypanosomes/ml
5x10^6
6+
5+
4+
10^4
3+
2+
5x10^2
1+
0
0
1
2
3
4
5
6
Weeks after inoculation

4.1.5. Immune response.

4.1.5.1. Single radial immunodiffusion (Mancini test).

The total serum immunoglobulin M (IgM) in a normal bovine is 2.7-4 mg/ml. A significant increase in total serum IgM occurred on day 14 of infection (Table 8). This initial increase was significantly lower in the Ayrshire compared with the other breeds. The antibody titre reached a peak on or close to day 21 post-infection. The highest peak titre was recorded in the Zebu from the Coast. Peak antibody titre was significantly lower in the Ayrshire compared with the Bos indicus types. After the third week of infection, total serum IgM progressively declined in cattle of all groups. This decline was more marked in the Zebu from Kitui, Boran and Ayrshire. In the Zebu from the Coast, high levels of IgM persisted throughout the course of infection. By 8 weeks post-infection, the serum IgM levels were close to pre-infection values in the Zebu from Kitui, Boran and Ayrshire.

In pre-infection bovine serum, total immunoglobulin G (IgG) ranged from 9.7 - 26.8 mg/ml (Table 9). No changes in IgG levels were found in any group throughout the 8 weeks of monitoring following infection.

Table 8: Mean values of total serum immunoglobulin M (IgM) in cattle infected with T. congolense IL 572 (mg/ml ± Sd).

Breed	Days after infection						
	Pre-infection	7	14	21	28	35	56
East African Zebu (Coast)	2.7±1.4	4.2±1.7	8.54±2.4	10.1±1.6	8.2±2.4	8.8±2.6	8.00±3.7
East African Zebu (Kitui)	4.0±2.0	4.5±1.2	8.3±1.7	9.0±1.0	8.5±1.3	5.7±3.0	5.8±3.9
Boran	3.5±0.9	4.5±0.9	9.2±1.0	9.7±0.8	4.6±0.7	2.7±1.1	3.9±1.4
Ayrshire	3.4±0.8	4.4±1.3	5.6±2.0*	8.1±1.0*	5.2±0.2	3.7±1.5	3.2±1.2

* - significantly lower than the mean of the other breeds at 5 percent level (Mann-Whitney test).

Table 9: Mean values of total serum immunoglobulin G (IgG) in cattle infected with *T. congolense* IL 572 (mg/ml ± Sd).

Breed	Days after infection					
	Pre-infection	7	14	21	28	56
East African Zebu (Coast)	24.6±5.3	24.2±5.1	25.1±4.8	24.5±5.0	24.0±4.5	26.2±5.0
East African Zebu (Kitui)	25.6±6.5	25.5±5.0	24.9±6.0	26.3±6.2	23.0±6.0	26.0±6.5
Boran	24.6±5.4	25.6±5.2	25.5±5.0	24.5±4.0	24.6±5.4	23.5±5.0
Ayrshire	22.8±2.8	22.6±2.8	23.6±3.2	22.9±3.4	24.6±3.0	23.5±2.5

4.2. SUSCEPTIBILITY OF ZEBU (COAST) AND BORAN TO T. CONGOLENSE IL 13E - 8. TRANSMITTED BY G.M. CENTRALIS.

4.2.1. Clinical condition and survival.

Following infection with T. congolense IL 13E - 8 transmitted by tsetse, all East African Zebu (Coast) and Boran cattle developed detectable skin lesions by day 5. This was a firm discrete nodule 2 - 3 mm in diameter. The skin thickness progressively increased, reaching a maximum between day 10 and 14 (Table 10). At this stage the lesion appeared as a circumscribed indurated swelling which was painful to the touch.

In the Boran, the average skin thickness before challenge was 11.3 ± 1 mm. By day 13 of infection, it was 29 ± 0.8 mm. This was an increase of 157 percent. In the East African Zebu from the Coast, the pre-infection skin thickness was 7.1 ± 2 mm. The average skin thickness increased to 15 ± 2 mm by day 13. This was an increase of 114 percent over the pre-infection average skin thickness. The skin thickness subsequently regressed and by day 25 post-infection, it was barely detectable (Figure 7). Thus while there was no fifference between the 2 breeds in the kinetics of development of the chancre, magnitude of the chancre reaction was greater in the Boran than in the East African Zebu from the Coast.

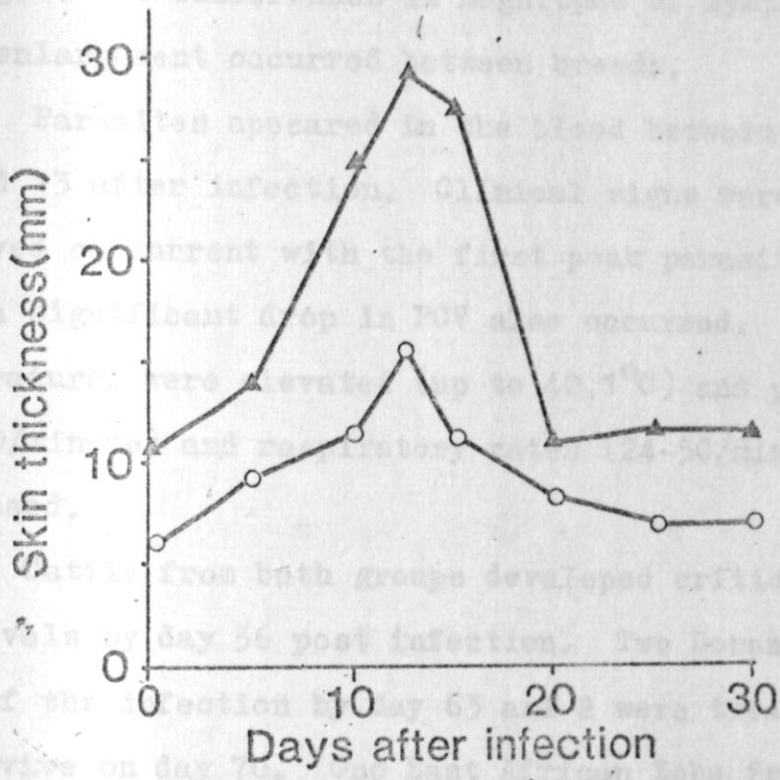
Table 10: Chancre reactions in cattle infected with T. congolense IL 13
transmitted by G.m. centralis.

Breed	Number of animals	Skin thickness (mm)(percent in Day 0	5.	13.	
East African Zebu (Coast)	4	7.1±2	8.5±1(20*)	15±2(114)	8
Boran	4	11.3±1	13.5±.5(19.5)	29±0.8(156.6)	12

* = percentage increase in skin thickness from pre-infection thickness.

Figure 7.

Changes in skin thickness as a measure of skin reactivity in the Boran (▲) and East African Zebu from Coast (○) infected with T. congolense 13E-8 transmitted by G.m. centralis. The results represent the mean of 4 cattle from each group.


Skin thickness(mm)
30
20
10
0
0
10
20
30
Days after infection

Accompanying development of the chancres, rescapular and prefemoral lymph nodes of the psilateral flank became markedly enlarged. They eached largest size at maximum chancre reaction. ubsequently, they regressed but for more than 30 ays after infection they were still significantly nlarged. No differences in magnitude of lymph ode enlargement occurred between breeds.

Parasites appeared in the blood between day 0 and 13 after infection. Clinical signs were bserved concurrent with the first peak parasitaemia hen a significant drop in PCV also occurred. Rectal temperatures were elevated (up to 40.1°C) and pulse (60-80/minute) and respiratory rates (24-50/minute) increased.

Cattle from both groups developed critical PCV levels by day 56 post infection. Two Boran died of the infection by day 63 and 2 were treated to survive on day 70. One East African Zebu from the Coast was treated on day 56, one died on day 63 and 2 others were treated on day 70.

Table 11: Survival time of cattle following infection with T. congolense 13 E-8 transmitted by G.m. centralis.

Breed	Number of cattle.	Survival*							
		Days after infection							
		28	35	42	49	56	63	70	77
East African Zebu (Coast)	4	4	4	4	4	4	3	2	0
Boran	4	4	4	4	4	4	2	2	0

* = reduction in number means either the animal required treatment to survive or died of the infection.

4.2.2. Haematology.

The PCV began to decline significantly 14 days after challenge, following the initial detection of parasitaemia (Table 12). Individual variation in the onset and rate of development of anaemia was minimal in cattle of both breeds. Six weeks after infection, cattle from both groups had an average PCV of 20 percent. One East African Zebu from the Coast had a PCV of 13 on day 56 and was treated. One died on day 63 with a PCV of 12 and 2 others were treated on day 70 with PCV's of 14 and 15 respectively. Two Boran died on day 63 and 2 others were treated on day 70 having developed critical PCV ($\leq 15\%$) (Table 12). There was no breed variation in the onset or severity of the anaemia observed.

Table 12: Changes in packed cell volume in cattle infected with *T. congolense* IL 13E - 8 transmitted by *G.m. centralis*.

Days after infection	East African Zebu (Coast)				Boran			
	ILE	ILF	ILH	ILJ	895	899	968	971
0	33	30	29	38	31	30	29	31
7	31	29	30	36	30	29	26	27
14	32	27	28	30	23	28	24	27
21	26	23	22	28	20	23	22	21
28	25	23	20	28	19	23	20	20
35	24	20	20	26	18	20	20	20
42	20	20	20	23	19	21	17	18
49	14	19	18	20	20	19	16	15
56	13	19	17	20	20	20	14	13
63	-	17	-	19	20	18	-	-
70	-	14	-	15	14	15	-	-

4.2.3. Parasitological findings.

Following successful tsetse feeds, all cattle became infected. Parasites were detected microscopically in blood between day 10 and 13, coinciding with the period of maximum chancre reactivity.

The prepatent period in the East African Zebu from the Coast was 11 ± 0.5 and Boran 12.8 ± 0.8. The difference is not statistically significant. The time to first parasitaemic peak was similar in both breeds although the level was slightly lower in the Coast Zebu (Table 13). The subsequent levels of parasitaemia in the Zebu from the Coast was lower than in the Boran, and the Zebu from the Coast were able to remit wawes of parasitaemia (Figure 8). On the other hand, in the Boran, once peak parasitaemia was achieved the level remained persistently high throughout the course of infection. All surviving animals remained parasitaemic throughout the 10 weeks of monitoring.

Table 13: The prepatent period, time to first peak and level of first peak parasitaemia in cattle infected with *T. congolense* IL 13 E-8 transmitted by *G.m. centralis*.

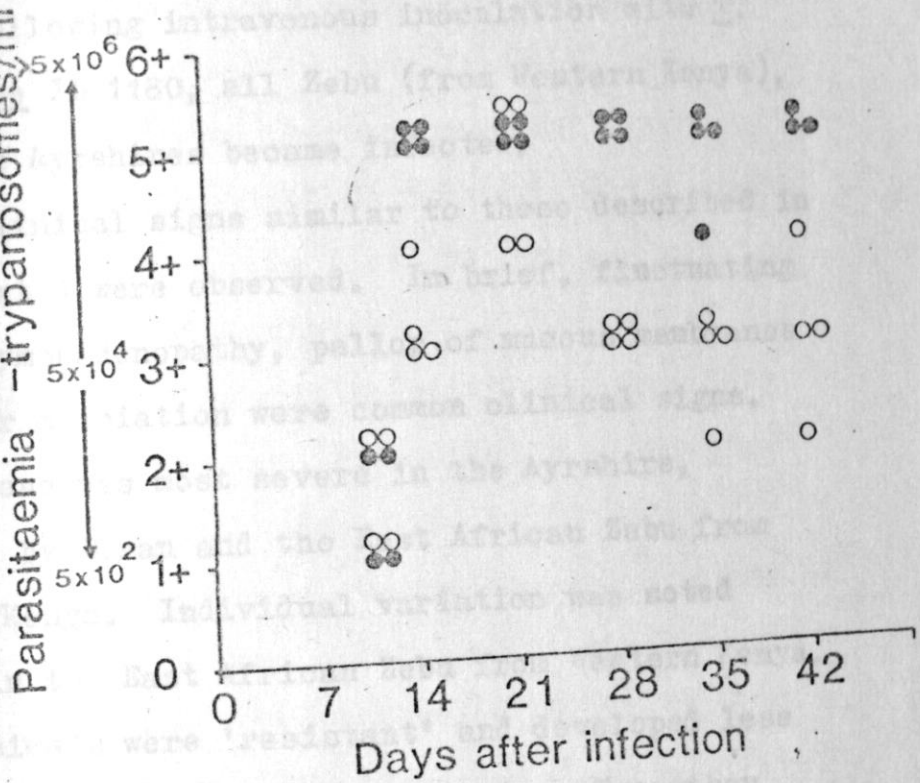
Breed	Number of cattle.	Prepatent period (days).	Time to first peak (days).	Level of first peak judged by parasitaemia score.
East African Zebu (Coast)	4	11.0 ± 0.5	16 ± 0.8	4 ± 0.7
Boran	4	12.8 ± 0.8	15 ± 0	5 ± 0

Figure 8.

Profile of parasitaemia in the Boran and East African Zebu from the Coast infected with *T. congolense* 13E-8 transmitted by *G.m. centralis*. The results represent individual animal score at time of patency and on a weekly basis thereafter.

(O) Zebu from Coast.

(●) Boran.


Parasitaemia -trypanosomes/ml
5x10^6
5x10^4
5x10^2
6+
5+
4+
3+
2+
1+
0
0
7
14
21
28
35
42
Days after infection

4.3. SUSCEPTIBILITY OF ZEBU (WESTERN KENYA) BORAN AND AYRSHIRE TO NEEDLE INOCULATION WITH T. CONGOLENSE IL 1180.

4.3.1. Clinical condition and survival.

Following intravenous inoculation with T. congolense IL 1180, all Zebu (from Western Kenya), Boran and Ayrshires became infected.

Clinical signs similar to those described in experiment 1 were observed. In brief, fluctuating fever, lymphadenopathy, pallor of mucous membranes and later emaciation were common clinical signs. The disease was most severe in the Ayrshire, followed by Boran and the East African Zebu from Western Kenya. Individual variation was noted mainly in the East African Zebu from Western Kenya. Three animals were 'resistant' and developed less severe clinical disease and anaemia. Two other animals were intermediate in susceptibility and 2 were as susceptible as the Boran.

There were significant (5 percent level) differences in the pre-infection body weights between the three breeds of cattle. The Boran, reared at ILRAD had the highest average body weight, 158.8 ± 32.1 kgs, Ayrshire 136.6 ± 26.2 and the 'dwarf' East African Zebu from Western Kenya weighed 101.4 ± 17.3. Body weight changes after infection has been established, varied from animal to animal

(Table 14). In the Ayrshire group, individual cattle lost approximately 20 percent of their pre-infection body weights within 6 weeks of infection. The Boran with the highest pre-infection body weight lost significantly less weight compared with the Ayrshire and some individual cattle from the Zebu group. In the East African Zebu, there were marked individual differences in body weight changes during the course of infection. During the first 30 days of infection losses in body weight occurred in all cattle of the group. Two very susceptible individuals from this group lost 13 percent and 20 percent of their pre-infection body weight, respectively, by week 6 of infection, when they were treated to prevent death. Two others from the same group lost 21 percent and 27.2 percent of their pre-infection body weight 10 weeks post-infection. Despite minor changes during the early phase of the disease 3 cattle from Western Kenya showed no weight loss by week 14. Two of these cattle had put on an extra 5 and 11 kilograms body weight.

Marked differences in survival time were noted between breeds. Individual variation in the ability to withstand infection was minimal in the Boran and Ayrshire although as in experiment 1, Boran survived longer. All 3 Ayrshire required

treatment by day 42 (Table 15). All the Boran developed critical PCV levels and had to be treated between weeks 6 and 9 after infection. Marked heterogeneity in survival time was noted in the East African Zebu from Western Kenya. Two cattle from this group were very susceptible and required treatment on day 27 and 40. Two other animals were intermediate in susceptibility needing treatment on days 77 and 80. Three cattle from this group were judged as resistant; they survived beyond 5 months of infection, in good body condition, without requiring any treatment. All cattle survived after treatment at critical PCV levels and were subsequently used in part 4 of these studies.

Table 14: Body weight (kg) changes in cattle inoculated with bloodstream forms of *T. congolense* IL 1180.

Week of infection	East African Zebu (Western Kenya).							Boran				Ayrshire		
	Group 1.			Group 2		Group 3								
	707	711	712	708	710	715	716	55	972	973	967	555	556	551
0	90	105	85	110	120	75	125	240	200	150	205	100	150	160
4	85	96	75	95	105	65	100	235	195	135	190	80	120	140
6	80	100	80	90	105	-	-	230	195	-	185	-	-	110
8	90	100	82	90	100	-	-	225	190	-	-	-	-	-
10	86	95	80	80	95	-	-	-	-	-	-	-	-	-
12	90	105	85	-	-	-	-	-	-	-	-	-	-	-
14	95	116	85	-	-	-	-	-	-	-	-	-	-	-
percent decrease from pre-infection values.	0	0	0	27	21	13	20	6	5	10	10	20	20	23

Table 15: Survival time of cattle inoculated with bloodstream forms of *T. congolense* IL 1180.

Breed	Number of cattle	Survival* Days after infection.						
East African Zebu (Western Kenya)	7	27	40	77	80	>150	>150	>150
Boran	4	49	50	60	63			
Ayrshire	3	30	38	42				

* = Survival was assessed on the basis of treatment of animals with PCV = 15 percent or less or the animal died.

4.3.2. Haematology.

Coinciding with the development of parasitaemia a significant drop in PCV occurred 14 days post inoculation in the Boran, Ayrshire and certain individual Zebu from Western Kenya. In 3 cattle from Western Kenya, a significant decrease in PCV occurred only after week 3 of infection.

As in experiment 1, the Ayrshire was most severely affected and became rapidly anaemic within the first 3 weeks of infection (Table 16). Within this time the Ayrshire had a PCV drop of 41 percent, Boran 20 percent and East African Zebu from Western Kenya 17 percent. While at week 3 there was no significant difference in the severity of anaemia between the East African Zebu from Western Kenya and the Boran, thereafter, the Boran became progressively more anaemic with a 50 percent drop in average PCV value by the fifth week of infection. At his time, the East African Zebu from Western Kenya had dropped only 29 percent.

While as a group, the East African Zebu from Western Kenya developed less severe anaemia than the Boran and Ayrshire, marked heterogeneity in the onset and progressive development of anaemia was noted in this group of animals. Those individual

animals with a greater ability to control parasitaemia developed significantly less severe anaemia. Occasionally the PCV levels rose slightly following remissions (Figure 9). In the more susceptible individuals the PCV drop was much more rapid; in these cattle the onset and severity of the anaemia was similar to that of the Boran.

The other haematological parameters paralleled the changes in PCV. Thus a significant drop in total red blood cells occurred in cattle of all groups within the first week of infection (Figure 10). The drop was more sharp in the Ayrshire. After the first week, red cell loss became progressive in all breeds. However, marked differences in the total red blood cell count were noted within Zebu from Western Kenya (Figure 11). The most resistant Zebu lost significantly less red blood cells compared with Zebu displaying intermediate susceptibility and the most susceptible Zebu. The most susceptible Zebu displayed a rapid and sharp decrease in total red blood cells between weeks 3 and 5 of infection and had to be treated to survive.

For seven weeks after infection, a progressive decrease in erythrocyte haemoglobin occurred in both the Boran and the East African Zebu from

Western Kenya (Figure 12a). There was no statistical significant difference in haemoglobin value between the two breeds over the 7 weeks period of observation. The Ayrshire displayed a rapid drop in haemoglobin compared with East African Zebu (Figure 12b). There was no heterogeneity in the Ayrshire and minimal variation in the Boran.

There was no significant change in the mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in cattle of all breeds throughout the course of infection. MCV values ranged between 41 - 50 and MCHC ranged between 22 - 30 percent. These values showed little change throughout the course of infection.

During the first week of infection, a sharp drop in total white cell count occurred in cattle of all breeds. The magnitude of the decrease was more marked in the Ayrshire and Boran compared with East African Zebu (Figure 13). After the first week, a progressive increase in the total leucon occurred in cattle of all groups, and in the Ayrshire total white blood cells had reached pre-infection values by week 4 of infection. During the course of infection differences were noted in total white cell count between individual cattle from the Zebu group. Mild leucopaenia

occurred within the first week of infection. This was more marked in the most susceptible individual (Figure 14). Thereafter, occasional increases which were more marked in the most resistant "individuals" occurred (Table 17).

Table 16: Percentage drop in packed cell volume from pre-infection values in cattle inoculated with bloodstream forms of T. congolense IL 1180.

Days after infection	Breed		
	East African Zebu (Western Kenya)	Boran	Ayrshire
7	3.1±3.6	5.5±2.3	8.5±4.5
13	9.5±6.8	9.7±7.6	23.8±7.4
21	17.0±11.1	20±9.8	41.1±7.3*
28	25.9±13.3	39.0±9.2	40±9.0
35	28.5±14.2*	50.0±8.0	45±6.5
42	31.2±14.3*	50.2±7.0	45±6.5

Pre-infection PCV: arithmetic mean ± one standard deviation. East African Zebu (Western Kenya) = 28.6±3.9
Boran = 29.3±1.9
Ayrshire = 27.3±3.4

* = significant difference than that of the other breeds at 5 percent level(Mann-Whitney, test).

Figure 9:

Comparative profiles of parasitaemia and pattern of anaemia between a resistant Zebu (707; Δ o) and a susceptible Zebu (716; ▲ ●).

The susceptible Zebu had to be treated with the trypanocidal drug Berenil at week 5 to survive.

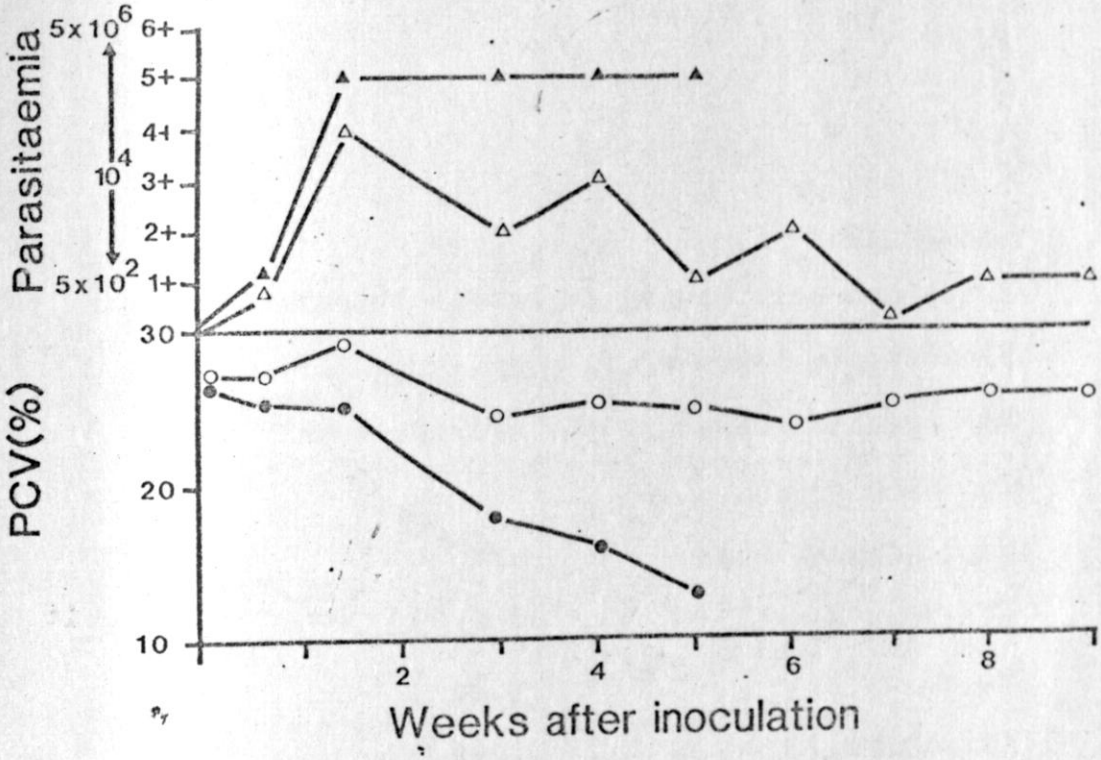

Parasitaemia
5x10^6
6+
5+
4+
10^4
3+
2+
5x10^2
1+
PCV(%)
30
20
10
2
4
6
8
Weeks after inoculation

Figure 10.

Red blood cell counts in cattle inoculated with bloodstream forms of T. congolense IL 1180. The results represent the average weekly mean for each breed.

East African Zebu (Western Kenya). (○)

Boran. (△)

Ayrshires. (●)

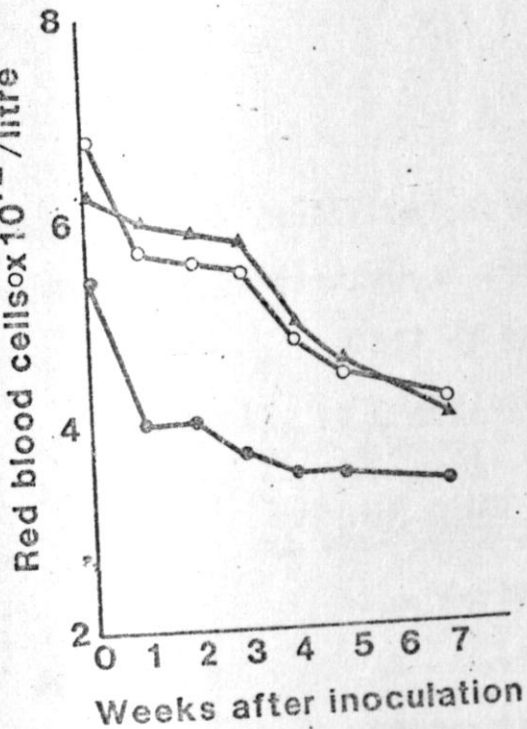

Red blood cells $\times 10^{12}$ /litre
8
6
4
2
0 1 2 3 4 5 6 7
Weeks after inoculation

Figure 11:

Red blood cell counts in individual East African Zebu from Western Kenya inoculated with bloodstream forms of T. congolense IL 1180.

Animals assessed as resistant on clinical and parasitological grounds. (●)

Animals assessed as intermediate in susceptibility on clinical and parasitological grounds. (▲)

Animals assessed as susceptible on clinical and parasitological grounds. (○)

Red blood cells x10^12/litre
7
6
5
4
3
2
0
1
2
3
4
5
6
7
Weeks after inoculation

Figure 12:

Changes in erythrocyte haemoglobin concentration in cattle inoculated with bloodstream forms of *T. congolense* IL 1180.

East African Zebu (Western Kenya). (O)

Boran. (▲)

Ayrshires. (●)

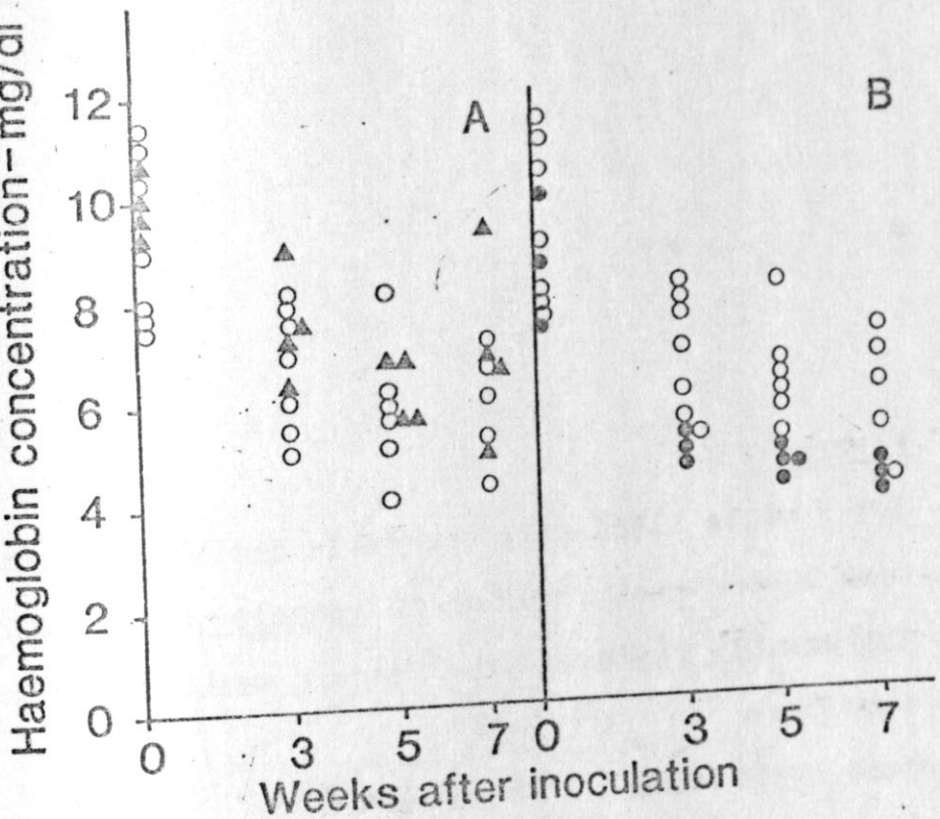

Haemoglobin concentration - mg/dl
12
10
8
6
4
2
0
A
B
0
3
5
7
0
3
5
7
Weeks after inoculation

Figure 13:

Total white blood cell counts in cattle inoculated with bloodstream forms of T. congolense IL 1180. The results represent the average weekly mean for each breed.

East African Zebu
(Western Kenya). (O)

Boran. (▲)

Ayrshires. (●)

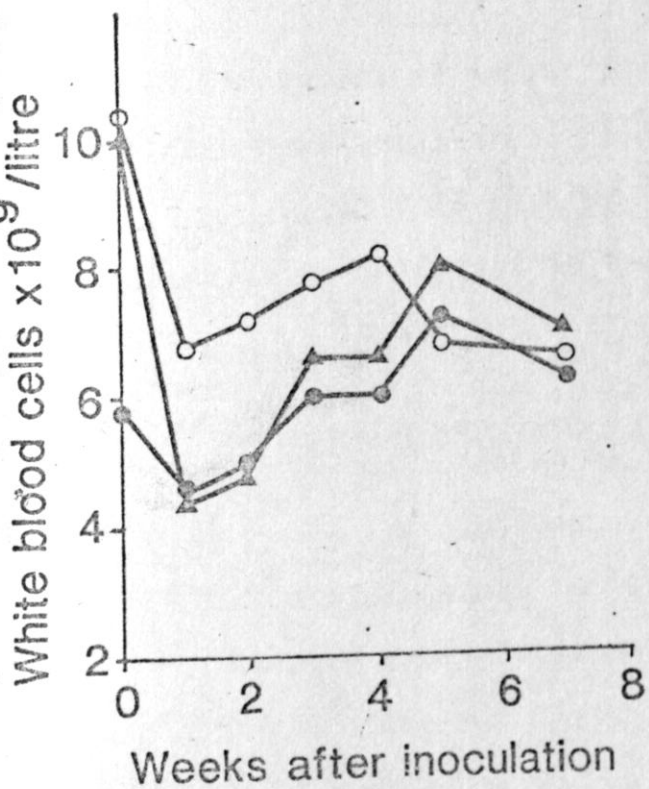

White blood cells x10^9/litre
10
8
6
4
2
0
2
4
6
8
Weeks after inoculation

Figure 14:

Total white cell counts in individual East African Zebu from Western Kenya inoculated with bloodstream forms of T. congolense IL 1180.

Animals assessed as resistant on clinical and parasitological grounds. (●)

Animals assessed as intermediate in susceptibility on clinical and parasitological grounds. (▲)

Animals assessed as susceptible on clinical and parasitological grounds (○)

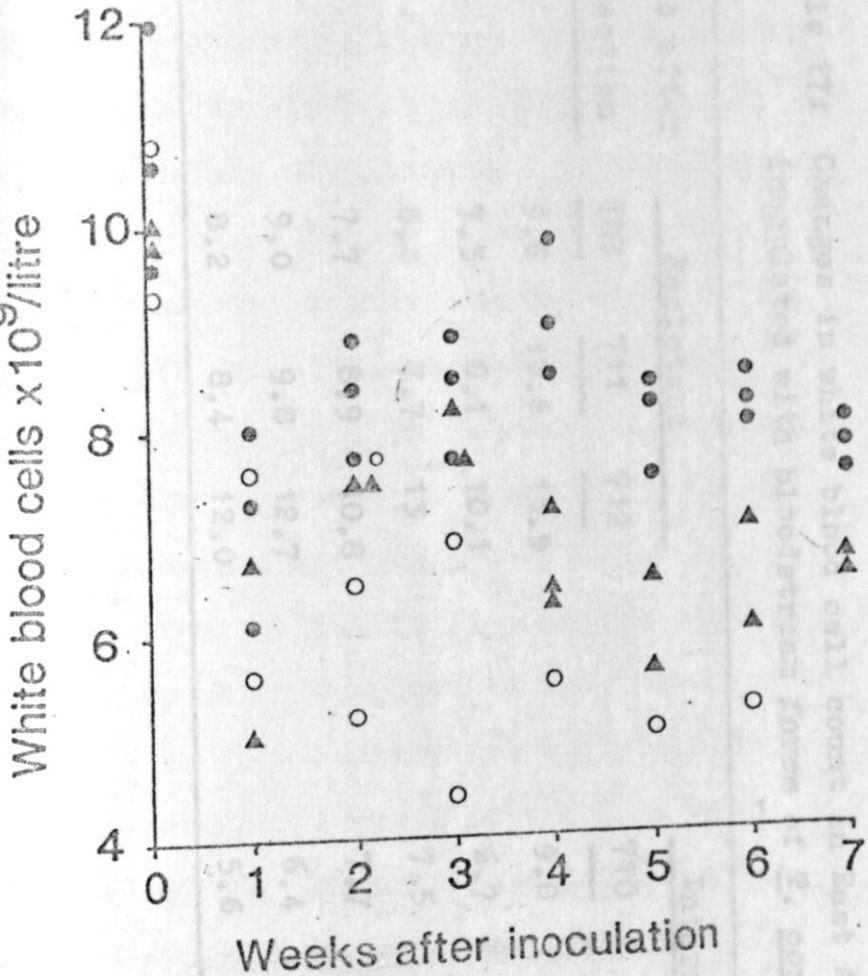

12
10
8
6
4
White blood cells x10⁹/litre
0
1
2
3
4
5
6
7
Weeks after inoculation

Table 17: Changes in white blood cell count in East African Zebu from Western Kenya inoculated with bloodstream forms of T. congolense IL 1180.

Days after infection	Resistant			Intermediate		Susceptible	
	707	711	712	710	708	715	716
0	9.6	10.6	13.9	9.8	10.0	9.3	10.8
7	7.3	6.1	10.1	6.7	5.0	7.6	5.6
14	8.4	7.7	13	7.5	7.5	5.2	6.5
21	7.7	8.9	10.8	7.7	8.2	4.4	6.9
28	9.0	9.8	12.7	6.4	7.2	5.5	6.3
35	8.2	8.4	12.0	5.6	6.5	5.0	-

4.3.3. Parasitological findings.

All cattle became parasitaemic by 5 days of infection. Only minor differences in the prepatent period were noted within breeds. The prepatent period did not differ significantly between breeds, although the Aryshires became parasitaemic first. In contrast to experiment 1 in which the stock of T. congolense IL 572 was used, the prepatent period was significantly shorter with the clone T. congolense IL 1180. The time to first parasitaemic peak was similar in all breeds and occurred 2 - 3 days of patency. The level of the first peak was consistently higher in the Ayrshire compared to the Boran and the East African Zebu from Western Kenya (Table 18).

Significant differences occurred between breeds on the ability to control parasite growth. The Boran and Ayrshire had no ability to remit parasitaemic waves. Once peak parasitaemia was reached, it persisted till the animal died or required treatment to survive (Figure 15).

East African Zebu from Western Kenya were heterogenous in their ability to control parasitaemia. Three animals were able to remit the first and subsequent peaks of parasitaemia; sometimes the parasitaemia was reduced to a +1 or to non-detectable levels in the blood. Two other cattle from this group developed high levels of parasitaemia

and had no capacity to remit parasitaemia (Figure 16). They behaved like the Ayrshire and Boran. Two other cattle were intermediate with a limited ability to control parasitaemia.

Table 18: The prepatent period, time to first peak and level of first peak parasitaemia in cattle inoculated with bloodstream forms of T. congolense, IL 1180.

Breed	Prepatent period (days)	Time to first peak (days)	Level of first peak
East African Zebu (Western Kenya)	3.8±0.3	6.5±1.4	4.3±0.7
Boran	4.5±0.5	6.±0.	4±0.
Ayrshire	3±0.	6±0.3	5.3±0.5*

* = significantly higher than that of the other breeds at 5 percent level (Mann-Whitney test).

Figure 15:

Profiles of parasitaemia in the Boran, Ayrshire and East African Zebu from Western Kenya inoculated with bloodstream forms of T. congolense IL 1180. The results represent the average weekly parasitaemia score for each breed.

Zebu (Western Kenya). (O)

Boran. (▲)

Ayrshires. (●)

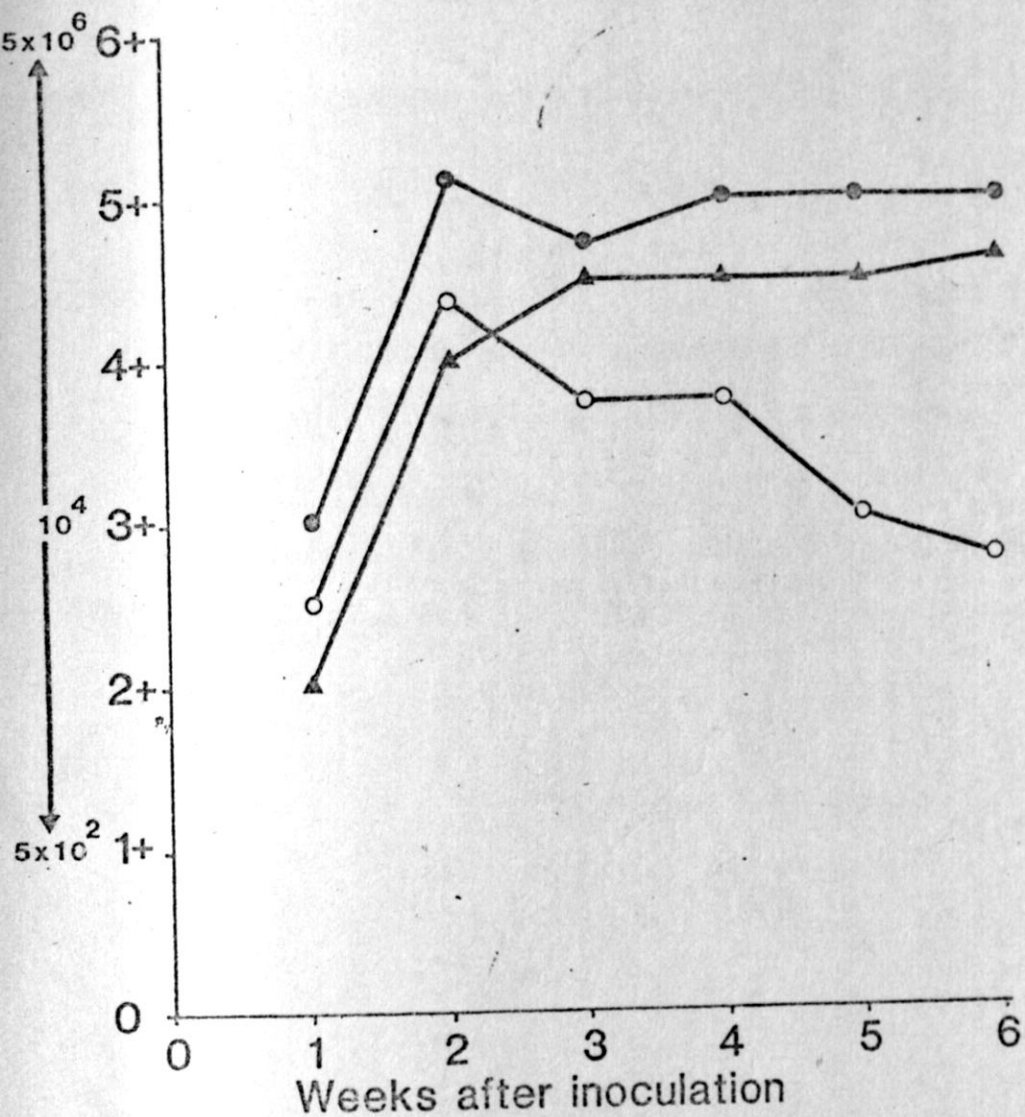
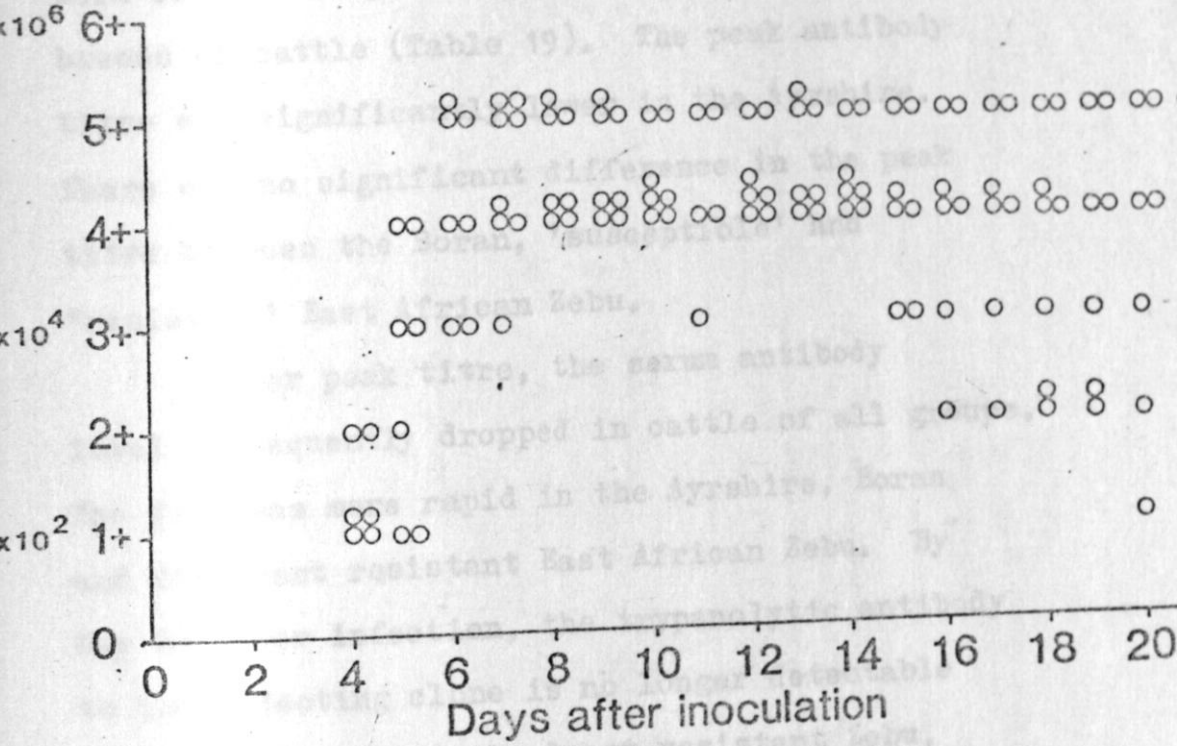

5x10^6
6+
5+
4+
10^4
3+
2+
5x10^2
1+
0
0
1
2
3
4
5
6
Weeks after inoculation

Figure 16:

Individual variation in the ability to control parasitaemia in the East African Zebu from Western Kenya inoculated with bloodstream forms of *T. congolense* IL 1180.


x10^6 6+
5+
4+
x10^4 3+
2+
x10^2 1+
0
0 2 4 6 8 10 12 14 16 18 20
Days after inoculation

4.3.4. IMMUNE RESPONSE.

4.3.4.1. Trypanolysis test.

Trypanolytic antibody was detected in the sera of cattle by 14 days after infection in all breeds of cattle (Table 19). The peak antibody titre was significantly lower in the Ayrshire. There was no significant difference in the peak titre between the Boran, 'susceptible' and 'resistant' East African Zebu.

After peak titre, the serum antibody level subsequently dropped in cattle of all groups. The drop was more rapid in the Ayrshire, Boran and the least resistant East African Zebu. By day 28 after infection, the trypanolytic antibody to the infecting clone is no longer detectable in the Ayrshire and the least resistant Zebu, and barely detectable in the Boran. In the most resistant East African Zebu, trypanolytic antibody persisted and was still detectable by day 42 of infection.

Table 19: Trypanolytic antibody in sera from cattle inoculated with bloodstream forms of T. congolense IL 1180.

Breed	Antibody titre							
	Days after infection							
	0	7	10	14	21	28	35	42
Zebu (R)**	0	0	0	96	96	24	24	12
Zebu (S)***	0	0	0	192	96	0	0	0
Boran	0	0	0	384	384	6	0	0
Ayrshire	0	0	0	12	12	0	0	0

** = resistant.

*** = susceptible.

4.3.4.2. Solid-phase radioimmunoassay (SRIA).

Analysis of sera for specific class of antibody by SRIA showed that IgM was first detectable on day 7, reaching a peak on day 14 (Figure 17a). The peak antibody titre did not differ between the resistant Zebu from Western Kenya, Boran and Ayrshire. After peak titre, elevated IgM levels persisted in the resistant Zebu, sharply dropped in the Boran and to undetectable levels in the Ayrshire. IgG levels were detected and reached a peak on day 14. It persisted in the resistant Zebu and dropped to undetectable levels by day 21 in the Boran and Ayrshire (Figure 17b).

When the IgM response was compared between a resistant and susceptible Zebu over a 6 week period, increased titre was first detected on day 7, and reached a peak on day 14. This elevated titre persisted to day 42 in the resistant Zebu but dropped to undetectable levels by day 21 in the susceptible Zebu (Figure 18).

Figure 17:

Serum immunoglobulin levels as measured by the solid-phase radioimmunoassay (SRIA), in cattle inoculated with bloodstream forms of T. congolense IL 1180

(a) IgG.

Resistant Zebu. (○)

Boran. (▲)

Ayrshire. (●)

(b) IgM

Resistant Zebu. (○)

Boran. (▲)

Ayrshire. (●)

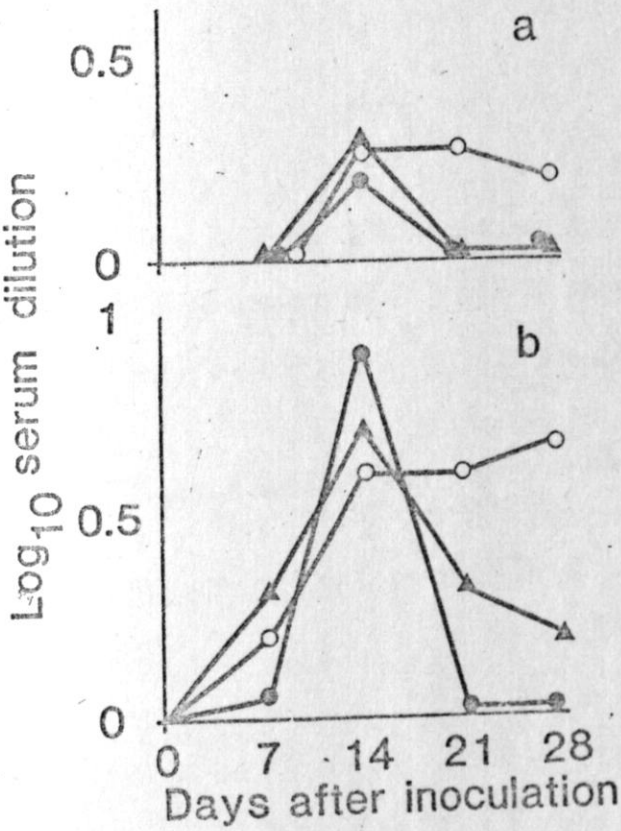
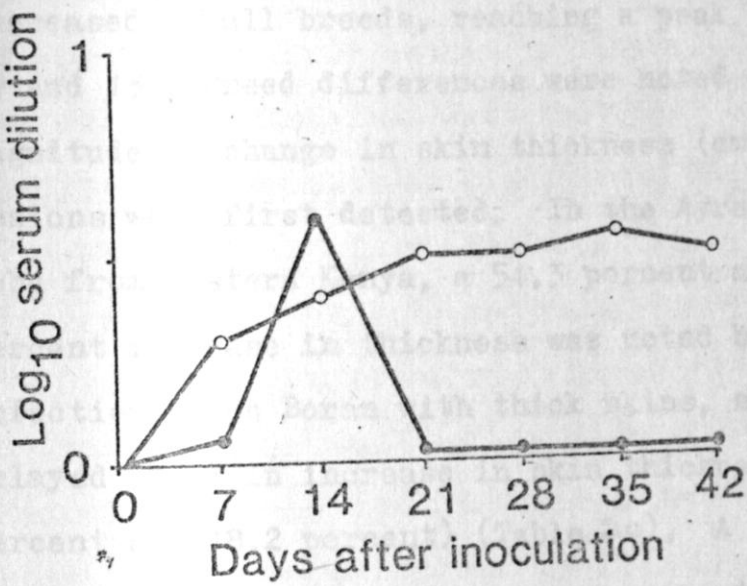

a
0.5
0
1
b
0.5
0
Log10 serum dilution
0
7
14
21
28
Days after inoculation

Figure 18:

Serum IgM levels as measured by the solid-phase radioimmunoassay (SRIA) in a resistant Zebu (O) and susceptible Zebu (●) from Western Kenya.


Log10 serum dilution
1
0
0
7
14
21
28
35
42
Days after inoculation

4.4. SUSCEPTIBILITY OF ZEBU (WESTERN KENYA), BORAN AND AYRSHIRE TO T. CONGOLENSE IL 285 TRANSMITTED BY G.M. CENTRALIS.

4.4.1. Clinical condition and survival.

Following infection transmitted by tsetse infected with T. congolense IL 285, cattle from all groups developed detectable skin lesions on all the 3 bite sites by day 5. Skin thickness progressively increased in all breeds, reaching a peak between day 10 and 13. Breed differences were noted on the magnitude of change in skin thickness (cm) when lesions were first detected. In the Ayrshire and Zebu from Western Kenya, a 54.3 percent and 50 percent increase in thickness was noted by day 5 of infection. The Boran with thick skins, showed a delayed onset in increase in skin thickness (8 percent and 18.2 percent) (Table 2c). A slightly higher increase in skin thickness was noted in Boran without previous exposure compared with those with previous exposure. Maximum skin thickness was reached between day 10 and 13 and the percentage increase was greater in the Boran than the other breeds. The skin thickness subsequently regressed and by day 30 post infection it was barely detectable.

Accompanying development of the chancres, prescapular and prefemoral lymph nodes of the ipsilateral flank became substantially enlarged. They reached largest size at maximum chancre reaction. Subsequently they regressed but for more than 30 days after infection they were still significantly enlarged.

Parasites were first detected in blood between day 7 and 13 after infection. Clinical signs were observed concurrent with the first peak parasitaemia, including a significant drop in PCV. Elevated rectal temperatures (up to 40.1°C), pulse rate 60 - 80/minute and respiratory rate from 24 - 50/minute were observed. The onset of these clinical signs was much earlier in the Ayrshire compared with the other two breeds. Following tsetse transmitted infection, as in needle inoculation, Zebu from Western Kenya displayed the same pattern of heterogeneity in severity of clinical disease, with same animals responding in the same way. Thus, 3 animals were more resistant and clinical disease was less severe. Two other cattle were 'intermediate' in susceptibility and developed severe clinical disease much earlier than the resistant ones. Two cattle from the same group behaved clinically like the Boran.

In this experiment, survival was monitored for 8 weeks only. The trend was similar to that following needle challenge. Three Zebu from Western Kenya (707, 712, 711) survived beyond 8 weeks with PCV values above 20 percent. Two others (710, 708) developed critical PCV values of less than 15 percent and required treatment between weeks 7 and 8. One of the two very susceptible cattle (716) required treatment on week 5 and the other (715) on the sixth week post-infection. One Ayrshire died 4 weeks after infection with persistently high parasitaemia. The remaining two were treated on days 37 and 40 after developing PCV levels of 15 percent or less. With the Boran, one animal died 8 weeks after infection while the rest required treatment between weeks 6 and 8 after infection.

Table 2o: Chancre reactions in cattle infected with T. congolense IL 285 transmitted by G.m. centralis.

Breed	Number of cattle	Skin thickness (cm) (percent increase) 0	5	13	20
East African Zebu (Western Kenya)	7	0.7±0.1	1.08±(54.3*)	1.5±0.2(114.3)	0.96±0.12(37.1)
Boran (i) previously exposed	4	1.3±0.2	1.4±0.2(8)	2.75±1.1(111.5)	1.85±0.45(42.3)
(ii) not exposed	4	1.1±0.1	1.3±0.1(18.2)	2.6±0.2(136.4)**	1.58±0.8(43.6)
Ayrshire	3	0.3±0.1	0.45±0.9(50)**	0.65±0.2(116.7)	0.45±0.15(50)

** = significantly different from other breeds.

* = figures in brackets indicate percentage increase in skin thickness from pre-infection thickness.

Table 21: Survival time in cattle following infection with T. congolense IL 285 transmitted by G.m. centralis.

Breed	Number of cattle	SURVIVAL* Days after infection				
		28	35	42	49	56
East African Zebu (Western Kenya)	7	7	6	6	5	3
Boran	8	8	8	8	6	1
Ayrshire	3	2	2	-	-	-

* = reduction in number means either the animal required treatment to survive or died of the infection.

.4.2. Haematology.

Changes in the blood picture coincided with
ne appearance of parasites in the blood. A
ignificant drop in PCV occurred 2 weeks after
arasites were detected in the blood. This was
ost marked in the Ayrshire, Boran and susceptible
ast African Zebu. These cattle developed a much
ore rapid and severe anaemia compared with resistant
ndividuals from Western Kenya (Table 22).

All Ayrshire developed critical PCV levels
of 15 percent and below by day 35 of infection. One
died and 2 others were treated to survive. All
Boran developed critical PCV level between days 49
and 56 (Table 22). Seven cattle were treated within
this period and 1 died with a PCV of 13 on day 56.
East African Zebu from Western Kenya were heterogenous
and fell into 3 groups. Group 1 cattle were resistant
and had PCV values of above 20 on day 56 of infection.
Group 2 were intermediate in susceptibility and the
progressive drop in PCV was less marked. They
developed PCV values of 15 percent and below between
days 49 and 56. Group 3 were very susceptible. One
animal had to be treated on day 35 with a PCV of
13 percent to survive. The other required treatment
on day 42 of infection.

Total erythrocytes, haemoglobin concentration aralleled the rate of PCV drop and showed a similar reed variation as described following needle inoculation. hus a significant drop in total red blood cells ccurred in cattle of all groups a week after parasites ere detected in blood. This drop was more marked in he Ayrshire, Boran and susceptible Zebu. A progressive ecrease in erythrocyte haemoglobin occurred in cattle f all breeds throughout the eight weeks of observation.

There was no significant change in the mean orpuscular volume (MCV) and mean corpuscular haemoglobin oncentration (MCHC) in cattle of all breeds throughout he course of infection. MCV values ranged between 9-53 and MCHC ranged between 20-25 percent.

During the first week of patent parasitaemia, sharp drop in total white cell count occurred in attle of all breeds. Thereafter a progressive ncrease in the total leucon occurred in cattle of ll groups and this increase appeared sharper in the yrshire.

As a group, East African Zebu from Western Kenya eveloped less severe anaemia compared with the Boran nd Ayrshire. The rate of development and severity of anaemia was significantly more marked in the Ayrshire compared with the Boran and East African Zebu.

Table 22: Changes in packed cell volume in cattle infected with T. congolense IL 285 transmitted by G.m. centralis.

	Zebu (Western Kenya)							Boran								Ayrshires		
	Group																	
Days after	I			II		III												
infection	707	711	712	708	710	715	716	718	721	722	723	971	972	55	967	555	556	551
0	37	30	31	32	33	30	30	33	33	32	28	30	30	29	34	30	32	30
7	32	28	30	30	33	30	30	34	33	34	30	31	31	30	34	26	28	29
14	34	33	27	27	30	28	30	27	28	31	30	28	26	30	30	21	24	22
21	26	29	25	22	25	20	25	25	27	28	26	26	24	28	27	18	20	20
28	26	28	24	20	24	19	15	21	26	26	20	21	20	17	20	14	15	17
35	26	27	23	20	22	20	13	21	22	21	20	20	17	17	20	–	15	16
42	25	25	23	19	20	15	–	20	21	21	18	19	17	16	20	–	–	–
49	27	26	24	17	16	–	–	17	–	20	–	16	18	16	19	–	–	–
56	25	24	23	–	–	–	–	–	–	13	–	–	–	–	–	–	–	–

4.4.3. Parasitological findings.

Parasites were first detected in the blood of the Ayrshires 7 days after infection (Table 23). In the East African Zebu from Western Kenya and the Boran, parasites were detected around day 10. The time taken to reach a parasitaemia peak differed in individual animals of Zebu and Boran breeds but on average this time was similar between these two breeds. With the Ayrshires, peak parasitaemia occurred 3 to 5 days earlier, reflecting the shorter prepatent period. While no significant differences could be demonstrated in the level of the first peak parasitaemia in the three breeds of cattle, the Ayrshires had the highest level and the Zebu the lowest (Table 23).

As with needle inoculation, there were significant differences between breeds and within a Zebu type in the ability to control parasite growth. The Ayrshire, Boran and susceptible East African Zebu had no apparent ability to control parasite growth (Appendix III). Once peak parasitaemi was reached, it persisted at 5+ or above till the animals required treatment to survive or died. There was no differenc in the pattern of parasitaemia that developed between Boran previously exposed and those without previous exposure.

However, the 3 resistant Zebu from Western Kenya had a superior capacity to control levels of parasitaemia. After the first peak of parasitaemia, significant remissions occurred and at times to almost undetectable levels (Appendix III). The 2 most susceptible cattle from this group had no apparent ability to control parasite growth and after peak parasitaemia, high levels persisted throughout the course of the disease, as with the Ayrshires and Borans.

Table 23: The average prepatent period, time to first peak and level of first peak parasitaemia in cattle infected with *T. congolense* IL 285 transmitted by *G.m. centralis*.

Breed	Number of Cattle	Prepatent period (days) *	Time to first peak (days) *	Level of first peak
East African Zebu (Western Kenya)	7	10.2 ± 0.4	13 ± 0	3.5 ± 0.5
Boran	8	10.4 ± 0.9	14.8 ± 1.4	4 ± 0.5
Ayrshire	3	6.3 ± 0.5	10 ± 0	5.4 ± 0.5

* - arithmetic mean $\pm$ standard deviation.

5. DISCUSSION.

The present study demonstrated that Ayrshire cattle, a dairy Bos taurus breed, was of all breeds tested the most susceptible to trypanosomiasis. Following syringe or tsetse transmitted infection with 3 different T. congolense serodemes, the Ayrshire rapidly became infected, developed high levels of persistent parasitaemia and a correspondingly severe anaemia. All animals of this breed had to be treated with the trypanocidal drug Berenil within a few weeks of infection to prevent death. These results confirm previous preliminary reports of increased susceptibility to trypanosomiasis in the Bos taurus breeds of European origin (Mwongela et al., 1981; Trail et al., in press; CREAT, Annual Report, 1981).

On the other hand, there was evidence of increased resistance to trypanosomiasis in a type of East African Zebu. Thus the majority of cattle coming from Sindo in Western Kenya, an area of heavy tsetse challenge, were more resistant to trypanosomiasis than East African Zebu from the Coast and Kitui, two areas considered to be under low to medium tsetse challenge and Boran without previous exposure to trypanosomiasis. Individual

Zebu from Sindo possessed a superior capacity to control parasitaemia following both syringe inoculation and tsetse-transmitted infection. Furthermore, these individuals developed less severe anaemia and survived for more than 5 months without requiring treatment. Other cattle from the same group were as susceptible to infection as the Boran and other Zebu types.

Except for field reports of possibly acquired resistance to local trypanosome strains (Cunningham, 1966; Njogu, A.R. personal communication, 1983), these studies are the first experimental investigations to provide evidence of differences in susceptibility to trypanosomiasis between breeds of cattle in Kenya.

Increased rectal temperature is one of the clinical features in animal African trypanosomiasis. In these experiments, fluctuating rectal temperatures ranging from 39.5 - 40.1^{o}C were observed in the Ayrshire, Boran and East African Zebu. This followed both syringe and tsetse-transmitted infection. Fluctuating temperature had been recorded in cattle following syringe inoculation with trypanosomes (Wellde et al., 1974), and natural or experimental infection with infected tsetse (Murray et al., 1981a; Akol and Murray, 1982).

As with Wellde et al. (1974) the detection of ever coincided with appearance of the first wave of arasitaemia and subsequently at every other peak. hile the role of thermoregulation in increased esistance to disease is speculative, it has been ointed out that N'Dama infected with trypanosomes uring field exposure to tsetse rarely became febrile ompared with Zebu which showed high rectal temperature Murray et al., 1981a). It was suggested that ossibly the N'Dama was never febrile because of a ower 'parasite' load or a different thermoregulatory ystem. In our studies, there was no evidence of reed variation in rectal temperature, following ither syringe infection or tsetse transmitted nfection.

In the current study, the progressive evelopment of other clinical signs agree with hose described by Fiennes (1970), Akol and Murray, 1982) and Morrison et al. (1981). They include, levated pulse (60 - 80/minute), respiration (24 - 0/minute), generalised lymph node enlargement, followed y emaciation in terminal cases. The severity of any f these signs depended on the stage of clinical isease, the breed (Ayrshire compared to Bos indicus ypes) and individual animals (East African Zebu rom Western Kenya).

Loss of body condition following infection with African trypanosomes is a common clinical observation in cattle, whether the disease takes an acute or chronic course (Fiennes, 1970). In our studies, we evaluated progressive weight loss in different breeds of cattle following syringe inoculation with blood-stream forms of T. congolense. In the first experiment, significant weight loss had occurred by week 2 in the Boran, Ayrshire and East African Zebu from Kitui while losses in weight did not commence untill week 5 in the East African Zebu from the Coast. It is possible that the delay in developing severe loss in body condition in the Zebu from the Coast was because of a better ability to control parasite growth. Differences in the rate of body weight loss during the initial phases of trypanosome infection between different breeds of cattle of differing susceptibility have been reported by Murray et al. (1981). N'Dama exhibited a slow decrease in weight initially, while in the Zebu, loss in body weight was much greater. In our experiments, the surviving Ayrshire lost 30.8 percent of their body weight 6 weeks post inoculation. This value is significantly higher than that of the East African Zebu and the Boran. Ten weeks after infection the Boran had lost 22.5 percent, East African Zebu from Coast 37.9 percent and Zebu from Kitui 34 percent, of their

body weight. This difference might be explained by the fact that the Ayrshire developed an acute infection with persistently high parasitaemia and severe anaemia, while these parameters were less severe in the Bos indicus types. However, weight loss was not associated with reduced appetite even when cattle were terminally ill clinically. Possibly this could be due to hypercatabolism of protein in trypanosome infected cattle as pointed out by Nielsen, Sheppard, Tizard and Holmes, (1978a). One interesting finding in these studies was that the Boran lost significantly less weight than the Zebu from the Coast or Kitui. This could be explained by the fact that the Boran used in these studies were reared at ILRAD from birth with exceptionally high nutrient provision and at the time of inoculation they had the highest pre-infection weights despite similar age with the East African Zebu from the field. This ties with the widely held belief that "nutritional status of the host undoubtedly influences the severity of the disease (Morrison et al., 1981)". Thus, in areas of poor pasture or during times of drought the poor condition of the cattle renders them more susceptible to the effects of the disease.

Similarly following tsetse-transmitted infection, the severity of body weight loss was still in the order Ayrshire, East African Zebu (Western Kenya) and Boran.

Although striking breed differences have been reported (reviewed by Murray et al., 1982), individual variation in susceptibility to trypanosomiasis within breeds has also been described. It has been noted in the N'Dama and Zebu in West Africa (Murray et al. 1981b), in Baoules and Zebu from Upper Volta (CREAT, Annual report 1981). Marked heterogeneity in susceptibility to trypanosomiasis was noted along the East African Zebu from Western Kenya. Seven cattle from this group fell into 3 categories as assessed by progressive weight loss. Two cattle were as susceptible as the Boran and lost 13.3 and 20.0 percent of their body weight 4 weeks after infection. Another 2 cattle from Western Kenya were intermediate in susceptibility and lost 20.8 and 27.2 percent of their body weight 10 weeks after infection. The remaining 3 cattle were resistant and except for minor changes during the early phase of infection, they did not loose weight during 14 weeks of infection.

The ability of cattle to survive trypanosome infection, whether following syringe inoculation or tsetse-transmitted infection, probably depends on many factors (reviewed by Murray et al., 1982; Murray et al., 1979a).

In current experiments, by standardizing age, plane of nutrition, dose of organisms and using a strain of T. congolense of known pathogenicity, most variables except breed of cattle were eliminated. Significant differences in survival time between breeds, and individuals within a breed were evident. The Ayrshire breed was the most susceptible requiring treatment between day 21 and 42 post infection in order to survive. Pinder (CREAT, Annual report, 1981) reported similar high susceptibility of Bos taurus crosses (Simmental/N'Dama) to needle inoculation with T. brucei. Some cattle required treatment as early as day 18.

The Boran without previous exposure to trypanosomiasis showed similar survival time as the East African Zebu from the Coast and Kitui, two areas considered to be under low to medium tsetse trypanosomiasis risk. Except for a superior capacity to control parasite growth in the Zebu from Coast compared to the Boran and Zebu from Kitui, previous exposure to trypanosomes did not seem to confer better survival under renewed challenge. While there is no quantitative data on the level of tsetse challenge in the Coast and Kitui areas, selection pressures exerted by trypanosomiasis in these areas may not have been

long enough or sufficiently rigorous for the evolution of a genotype exhibiting significant resistance. On the other hand, some East African Zebu from Western Kenya had a superior capacity to resist the effects of trypanosomiasis compared with the other groups and breeds. Three cattle proved highly resistant to trypanosomiasis, 2 others were intermediate and 2 were highly susceptible. These studies provide the first indication that significant differences in susceptibility, as judged by survival, exist between and within cattle breeds in East Africa.

Differences in susceptibility as judged by weight changes and survival were associated with variation in the capacity of different breeds and individuals within a breed to control parasitaemia and resist anaemia.

The major feature of trypanosomiasis in cattle is anaemia (Hornby, 1921; Murray, 1979). One general observation as regards anaemia is that resistant groups of animals such as N'Dama cattle, and wildlife develop less severe anaemia than more susceptible animals (reviewed by Murray et al., 1982). In the current studies the onset and progressive development of anaemia coincided with detectable parasitaemia. Cattle that developed high levels of persistent parasitaemia, became rapidly anaemic. The order of

decreasing severity of anaemia which followed both syringe and tsetse-transmitted infection was Ayrshires, Borans, East African Zebu from Coast and Kitui and East African Zebu from Western Kenya. These findings are in general agreement with those of Dargie et al., (1979a, b). In their erythrokinetic and ferrokinetic studies of N'Dama and Zebu infected with T. congolense or T. brucei, they found that anaemia was less severe in the N'Dama and concluded that the underlying processes of anaemia reflected the number of parasites in the blood and that the differences between the 2 breeds was due to differences of their ability to control parasitaemia rather than in innate erythropoetic responsiveness. In our studies, both the Boran and the Ayrshire developed high levels of persistent parasitaemia yet the onset and severity of anaemia was more marked in the Ayrshire. It could be speculated that the red blood cells of the Ayrshire may be more prone to the pathogenic effects of trypanosomes or trypanosome products. It has also been pointed out that the Red Maasai sheep and the susceptible Merino, despite similar levels of parasitaemia, displayed different rates of development of anaemia, and this again may have some relation with differential red blood cell fragility (Whitelaw, D.D., unpublished observations).

Differences in total white cell counts occured during the course of infection. Initially there was a sharp drop in total WBC from pre-infection values within the first week of infection when animals were just developing patent parasitaemia. This finding is consistent with observations of Maxie and Valli (1979) who reported a 30 - 50 percent decrease in the total leucocyte concentration in cattle infected with T. congolense and T. vivax. Wellde et al. (1974) reported an intial leucopaenia in cattle needle inoculated with T. congolense, but contrary to the findings in the studies reported here, these authors found that the initial drop in total WBC persisted throughout the course of the disease. In the present studies, an initial leucopaenia was noted during the first week of infection. The decrease was marked in the Ayrshire and susceptible East African Zebu but much less in the resistant Zebu. Thereafter, increase in the total leucon occured in cattle of all groups with slightly higher increases in the most resistant Zebu. The difference between Wellde's finding and ours may be due to the trypanosome strain or breed of cattle used.

Maxie and Valli (1979), pointed out that leucopaenia seen in trypanosomiasis may be important in the development of immunodepression. While there is evidence for severe immunodepression in rodents infected with

trypanosomes (Sacks et al., 1980; Selkirk and Sacks, 1980; Murray et al., 1982), the phenomenon is less well established in cattle. Little evidence of immunodepression of responses to mitogens and mixed leucocyte reactions of cells from different lymphoid organs was found in trypanosome infected cattle (Masake and Morrison, 1981) and little disorganization or derangement of lymphoid organs occured in trypanosome-infected cattle (Murray et al., 1980).

Parasites were detected in the blood of Ayrshire 2 to 3 days earlier than the Boran and East African Zebu. However, there was no significant difference in the average prepatent period between and within breeds of the Bos indicus type following both syringe and tsetse-transmitted infection. These findings agree with previous reports in N'Dama and Zebu (Toure et al., 1978; Murray et al., 1979; Murray et al., 1981). Similarly, no significant differences in prepatent period had been reported in inbred strains of mice of differing susceptibility (Morrison, Roelants, Mayor-Withey and Murray, 1978). Similarity in the prepatent period in cattle of differing susceptibility excluded any contributory effects of differences in the infection rates.

A consistent observation by workers on trypanotolerance over the last 20 years has been the superior capacity of reputed trypanotolerant breeds such as the N'Dama and West African Shorthorns to control parasite growth (Reviewed by Murray, Morrison and Whitelaw, 1982) compared with the Zebu. Early workers (Chandler, 1952; Stephen, 1966; Roberts and Gray, 1973) did not attach a great deal of importance to this fact and it was not until recently (Murray et al., 1981; CREAT, Annual Report, 1981) that attention was paid not only to breed differences but also to individual variation in the capacity to control parasite growth. In our studies, the Bos indicus types had a superior capacity to control parasite growth compared with the Ayrshire. Differences in the ability to control parasitaemia between the Boran and Ayrshire, both of which had never been previously exposed to trypanosomiasis, could be attributed partly to innate physiological processes which influence parasite differentiation rates in vivo. This may include, among others, the availability of host - derived molecules which stimulate parasite multiplication (Murray and Black, 1984, in press).

It has been suggested that the superior capacity of the more resistant breeds to control parasitaemia is due to a better innate immune response

(Desowitz, 1959). The humoral immune response evaluated using the single radial immunodiffusion test to estimate total immunoglobulin levels revealed a significant increase in total serum IgM by day 14 in all breeds. It reached a peak on or close to day 21. Peak levels were significantly lower in the Ayrshire and of shorter period of elevation. After the third week, serum IgM levels began to decline. The decline was much more rapid in the Boran, Ayrshire and Zebu from Kitui but remained high in the Zebu from the Coast. Similar results were reported by Luckins (1976) in Zebu infected with T. congolense and T. vivax and by Nantulya et al., (1982) using an indirect immunofluorescence test on sera from cattle infected with T. congolense.

There were no significant changes in total serum IgG over the 8 week period of study. Using the same technique, Luckins (1976) and Nielsen et al. (1978b) found no detectable increase in total IgG in cattle infected with T. congolense. Similarly Clarkson et al., (1975b) reported no changes in total IgG in cattle infected with T. vivax.

The reason(s) for persistence of high IgM levels in the sera of cattle from the Coast with a superior capacity to control parasitaemia is not clear. Nielsen et al. (1978a) showed that cattle

infected with trypanosomes show increased catabolism of serum immunoglobulins (Ig) and one may speculate that the rate of catabolism of these immunoglobulins may be different in cattle of differing susceptibility.

Specific trypanolytic antibody was first detectable on or close to day 10. On day 14 antibody titre peaked in cattle of all groups. The Boran and East African Zebu showed the highest titre and the lowest titre was recorded in the Ayrshire. The difference between the peak titre in the Ayrshire and the other breeds was statistically significant. Taking into account that by day 14 most cattle from the Ayrshire group were already experiencing high levels of parasitaemia (up to 6+), the above finding may or may not indicate that the Ayrshire was inferior in it's immune response to the trypanosome. It could be that specific immunoglobulins were adsorbed by the massive parasites hence the low titre in the sera. Alternatively, there may be some defect induced by the parasite in B-cell maturation to antibody - secreting plasma cells, as proposed by Murray and Black, (in press) for mice which are highly susceptible to trypanosome infections; such mice develop high parasitaemias but poor antibody responses.

After day 21, specific trypanolytic antibody persisted in the most resistant Zebu but completely disappeared in the Boran, susceptible Zebu and Ayrshire.

Using solid phase RIA, the pattern of change in immunoglobulins was similar to that observed using the other two assays. Increases in IgM antibody were first detected on day 7, reached a peak on day 14 and either persisted or subsequently declined. This was also true for IgG. These changes were similar in the Boran, Ayrshire and least resistant Zebu. In the most resistant Zebu the peak antibody titre did not differ from that of the Boran and the Ayrshire. However, IgM persisted at high levels for more than 6 weeks post infection.

From their work, Desowitz (1959; 1970) and Weitz, (1970) concluded that resistance has an immunological basis but the findings of these earlier workers was limited by small animal numbers involved, poorly known historical backgrounds, antigenically undefined trypanosome species or strains, and the absence of modern assay techniques. However, recent preliminary investigations on N'Dama and Zebu challenged with T. Brucei revealed no significant difference in the antibody levels between the two breeds but there was an indication

that antibody persisted longer in the N'Dama (Murray, unpublished observations). Studies in Upper Volta (Pinder, Libeau, Hirsh, Tamboura, Hauckbauer and Roelants, 1984) revealed no difference in the primary and secondary immune responses between the N'Dama and Simmental-N'Dama F1 despite the resistance of the former and the high susceptibility of the latter. Our findings and those of workers in Upper Volta indicate that mechanisms other than circulating antibody production against trypanosomes are responsible for differences in susceptibility.

In mice, it has been shown that resistant strains produce a more consistent antibody response to T. congolense than susceptible strains (Murray et al., 1981c), and with T. brucei the antibody response reaches much higher levels in resistant strains (Black et al., 1983). In this respect, an important finding in T. brucei infected mice is that the rate of parasite differentiation influences the kinetics of antibody production. Antibody responses are stimulated by stumpy but not by dividing slender forms of the parasite (Black et al., 1982; Sendashonga and Black, 1982). Similarly, Mahan (unpublished observations, 1983) has shown that mice resistant to T. vivax developed lower levels of parasitaemia and better antibody responses. These

findings suggest that parasite growth is probably controlled by host regulated factors and that these factors most probably act by regulating the rate of parasite differentiation/multiplication. Prostaglandins have been suggested as one of these factors (Jack et al., 1984).

In resistant wild animals such as eland and waterbuck, chancres appear less frequently and are smaller than in susceptible domestic animals (Murray et al., 1981b). It has been suggested that the skin may play a role in trypanotolerance by affecting transmission and initial growth rate of the parasite. To evaluate the role of the chancre in susceptibility to trypanosomiasis, we subjected Boran, Ayrshire and East African Zebu to tsetse-transmitted infections. In the Ayrshire, the breed with the thinest skin, the onset of detectable parasitaemia was 2 - 3 days earlier than in thick skinned Bos indicus cattle. However, maximum chancre reaction in terms of skin diameter and thickness was similar in all breeds. Thus, the role of the skin in trypanotolerance in cattle is not clear. Recent work in Upper Volta failed to demonstrate any difference in the kinetics of chancre development between the N'Dama, Zebu and Baoule cattle (Akol, unpublished observations, 1983); however, in the susceptible Zebu, the intensity of

the skin reaction was much greater than in the resistant Baoule and N'Dama.

Bangham et al., (1958) pointed out that physiological characteristics, such as the absence of haemoglobin B (in the N'Dama) might contribute to the resistance of the breed. It was proposed that resistant animals could be detected by haemoglobin type because N'Dama show almost 100 percent gene frequency for haemoglobin type A (Hb A) and Zebu are a mixture of A and B. Our haemoglobin typing results showed a 100 percent gene frequency for haemoglobin type A (Hb A) in the Ayrshire (B. taurus) and the Bos indicus types gene frequencies of 0.65 (Hb A) and 0.35 (Hb B) respectively. This excludes any association between haemoglobin type and susceptibility to trypanosomiasis, as hypothesised by Bangham and Blumberg, (1958).

There is evidence to suggest that trypanotolerant breeds may also be resistant to several other important infectious diseases. N'Dama have been reported to be more resistant to tick-borne diseases, including heartwater (Cowdria ruminantium), anaplasmosis and babesiosis (Epstein, 1971). N'Dama and West African Shorthorn have been shown to be resistant to streptothricosis (Stewart, 1937; Coleman, 1967) and are also said to be more resistant to helminthiasis

(A.A. Ilemobade, personal communication, 1983). In the Zebu, we found that all cattle from Western Kenya were chronic carriers of Theileria parva, with schizonts being demonstrated in all superficial lymph nodes. Taking into account the absence of chemotherapy and judicial tick control methods, we concluded that these cattle not only developed resistance to trypanosomiasis but also to theileriosis.

In summary, we have shown that breeds of cattle exotic to the tsetse zones, such as Bos taurus, are highly susceptible to African trypanosomiasis. Rearing these dairy cattle in tsetse-infested areas is likely to be difficult in terms of high mortalities such as reported at the Kenyan Coast by Mwongela et al. (1981). Also, it is likely to be more expensive in terms of surveillance and number of treatments required (Trail et al., in press). Thus it was found that the 1/3 Ayrshire - 2/3 Sahiwal breeding females required less than half the number of treatment for trypanosomiasis needed by the 2/3 Ayrshire - 1/3 Sahiwal during the 13 month interval from one calving to another. Therefore, while the productivity was similar in the two crossbreeds, it is more expensive to maintain the 2/3 Ayrshire - 1/3 Sahiwal. We have also obtained evidence that

increased resistance to trypanosomiasis has a genetic basis. Thus, Boran cattle without previous exposure to trypanosomiasis were significantly more resistant to the pathogenic effects of the disease than Ayrshires, also without previous exposure. This finding is supported by the analytical results of Trail et al. (in press) which showed that cross-breeding between the Ayrshire and Sahiwal resulted not only in a suitable dairy cow with the added advantage of heat tolerance but offsprings with a higher proportion of the Sahiwal genetic component ($^2/_3$ Sahiwal - $^1/_3$ Ayrshire) were significantly more resistant to trypanosomiasis compared with the $^1/_3$ Sahiwal - $^2/_3$ Ayrshire cross-breed. We have obtained evidence that the intensity of tsetse/trypanosomiasis risk in an area, may influence the ability of animals to survive subsequent trypanosomal challenge, even to serodemes unrelated to local strains of their area of origin. East African Zebu from Coast and Kitui, two areas considered to be under low to medium tsetse-trypanosomiasis risk were more able to control parasite growth. However, the extent to which this was achieved was not sufficient to increase survival time over the Boran without previous exposure. While pre-infection screening showed that cattle from Coast and Kitui had had

previous exposure to T. congolense, T. brucei and T. vivax, selection pressures exerted by trypanosomiasis in these areas may not have been long or rigorous enough for the evolution of the resistant genotype. On the other hand, we have shown that a strain of East African Zebu from Western Kenya, an area considered to be under heavy tsetse challenge, produced some cattle that were highly resistant to trypanosomiasis. These cattle displayed a superior capacity to control parasite growth, developed less severe anaemia and clinical disease and had survived the infection, irrespective of whether it was delivered by the syringe or by tsetse. In many trypanosomiasis endemic areas, the challenge is hardly ever uniform (Ford, 1971). Some foci in the same geographical area may have constant heavy tsetse challenge and others marginal or negligible challenge. This applies to Western Kenya and may explain the heterogeneity in susceptibility encountered in Zebu from this area. In addition, Cunningham (1966) demonstrated what appeared like a significant degree of acquired resistance to trypanosomiasis in Zebu cattle exposed to constant natural tsetse challenge. Our findings suggest that genetic resistance to trypanosomiasis has also evolved in this area.

6. CONCLUSION.

From this work it can be concluded that a degree of resistance to trypanosomiasis does exist in certain Zebu type in East Africa. The distribution of this degree of resistance seems to be related to the intensity of tsetse-trypanosomiasis risk in that it was in the East African Zebu from Western Kenya where resistant individuals were identified. We have also shown experimentally that breeds exotic to tsetse zones such as the Ayrshire, are significantly more susceptible to trypanosomiasis than the Boran or East African Zebu.

Increased resistance to trypanosomiasis was found to be associated with a superior capacity to control parasite growth and correspondingly develop less severe anaemia. The Ayrshire elicited significantly lower immunoglobulin and trypanosome specific antibody responses than the Bos indicus types. Evidence was also obtained for longer persistence of specific humoral antibody in more resistant cattle.

The degree of resistance to trypanosomiasis displayed by certain Zebu types in East Africa remains to be critically compared with that of the recognized trypanotolerant breeds of West and Central Africa. Even if, as is probable, Zebu are less resistant than trypanotolerant breeds, it is

likely that the degree of reduced susceptibility to trypanosomiasis demonstrated in certain Zebu in this study could be of advantage to the farmer providing the more resistant animals were as productive. It would appear that the development of *B. indicus* breeds with a significant degree of resistance is a long term possibility and an important objective.

Future priority areas for research would be to further identify resistant strains of Zebu cattle from trypanosomiasis endemic areas on a large scale basis, to compare their productivity to that of susceptible strains under the same conditions of management and husbandry and to further evaluate the possible factors involved in increased resistance to trypanosomiasis, such as host factors regulating the rate of parasite differentiation or multiplication, the differential susceptibility of host red blood cells to lysis by trypanosomes or trypansome products. Such knowldge might provide markers that could be used to identify resistant animals without subjecting them to challenge and form the basis of long term breeding programmes.

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8. APPENDICES.

Appendix I.

The details of individual experimental cattle.

Breed	Animal number	Age (yrs)	Sex	Body weight (Kgs)	Experiment
East African Zebu (Coast)	ILA	1½	Male	140	
	ILB	2	M	125	
	ILC	2	M	170	
	ILD	1	M	140	1
	ILE	2	M	160	
	ILF	2	M	150	
	ILG	2	M	130	
	ILJ	2	M	140	
East African Zebu (Kitui)	A490	1½	M	135	
	492	1½	M	105	
	495	2	M	95	
	496	2	M	110	
	497	2	M	140	1
	498	2	M	165	
	497	1½	M	170	
	500	1	M	140	
Boran	A895	1½	M	185	
	899	1½	F	190	
	967	1½	M	240	
	968	1½	F	200	
	969	1½	F	85	1

The details of individual experimental cattle.

Breed	Animal number	Age (yrs)	Sex	Body weight (Kgs)	Experiment
Boran	970	1½	M	170	
	971	1½	M	220	
	972	1½	M	200	
Ayrshire	A546	2	M	175	
	547	2	M	115	
	548	2	F	110	
	549	2	F	155	1
	552	2	M	140	
	553	2	F	125	
	554	2	M	155	
	557	2	F	155	
East African Zebu (Coast)	ILC	2½	M	ND	
	ILE	2½	M	ND	
	ILF	2¼	M	ND	
	ILJ	2¼	M	ND	2
Boran	A895	1¾	M	ND	
	A968	1¾	F	ND	
	A899	1¾	F	ND	2
	A971	1¾	M	ND	
East African Zebu (Western Kenya)	A707	1½	F	90	
	708	2	M	110	
	710	2	M	120	
	711	2	M	105	3 and 4.

e details of individual experimental cattle.

eed	Animal number	Age (yrs)	Sex	Body weight (Kgs)	Experiment
st African Zebu					
estern Kenya)	712	2	F	85	
	715	1½	M	75	
	716	2	F	125	
ran	A 55	2½	F	240	
	972	2	M	200	
	971	2	F	150	3 and 4
	967	2	M	205	
rshire	555	2½	M	100	
	556	2½	M	150	3 and 4
	551	2½	M	160	
ran	718	2	M	ND	
	721	2	M	ND	
	722	2	M	ND	4
	723	2	M	ND	

Appendix II.

MANN-WHITNEY TEST:

The following are suggested steps for carrying t the Mann-Whitney test.

1. State the Null and Alternative hypotheses.
2. Choose the significance level of the test.
3. Arrange all the observations in ascending order.
4. Obtain the ranks of the ordered observations.
5. Sum the ranks of the X observations, S_x.
6. Calculate the test statistic.

$$u = S_x - \frac{n_x (n_x + 1)}{2}$$

7. Determine, with the use of the Mann-Whitney able whether U falls in the critical region, and if so eject the Null hypothesis.

The use of S_x, the sum of the ranks of the X bservations, to reflect the level of interspersion f the X and Y observations can be replaced by S_y he sum of the ranks of the Y observations. The est statistic then becomes

$$U = S_y - \frac{n_x (n_x + 1)}{2}$$

nd the critical region remains unchanged.

xample: The data show the length of time in days hat control mice, X, and inoculated mice, Y, live.

X 20 23 28 30 31 32 44 33

Y 20 29 23 48 41 32 36 43 42 46

Is there a difference in survivorship between ontrol and inoculated mice?

Here $n_x = 8$, $n_y = 10$ and the hypotheses are

H_0: X and Y have the same distribution of life times.

H_1: X and Y have different distribution of life times.

ranging the data in ascending order gives

20 20 23 23 28 29 30 31 32 33 36 41 42 43 44 46 48

d by assigning scores the corresponding ranks for e X and Y values become

x y x y x y x x x y x y y y x y y

$1\frac{1}{2}$, $1\frac{1}{2}$ $3\frac{1}{2}$ $3\frac{1}{2}$ 5 6 7 8 $9\frac{1}{2}$ 11 12 13 14 15 16 17 18

mming the ranks of X,

$$S_x = 1\tfrac{1}{2} + 3\tfrac{1}{2} + 5 + 7 + 8 + 9\tfrac{1}{2} + 11 + 16 = 61\tfrac{1}{2}$$

lculating the test statistic,

$$U = S_x - \frac{n_x (n_x + 1)}{n} = 61\tfrac{1}{2} - \frac{8 \times 9}{2} = 25\tfrac{1}{2}$$

From the Mann-Whitney table* for $n_x = 8$ and $n_y = 10$ start of the lower tail critical region at a 5 percent nificance level is 18. The upper tail critical ion will start at $n_x n_y - 18 = 62$. Since $U = 25\frac{1}{2}$

es not fall in the critical region the Null hypothesis
accepted, and it is believed that control and
oculated mice have the same survivorship.

* - Mann-Whitney table can be checked in any
non-parametric textbook.

Appendix III

Individual animal parasitaemia score (DG) in cattle infected with *T. congolense*, IL 285 transmitted by *G.m. centralis*.

	Zebu (Western Kenya)							Boran								Ayrshires		
	Group																	
Days after	I			II		III												
infection	707	711	712	708	710	715	716	718	721	722	723	971	972	55	967	555	556	551
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1+	2+	1+
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2+	2+	2+
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4+	4+	4+
9	-	-	-	-	-	-	-	-	-	1+	1+	1+	1+	-	1+	5+	5+	5+
10	-	1+	-	-	-	-	-	-	-	2+	2+	3+	1+	-	2+	5+	5+	5+
11	1+	1+	1+	2+	1+	1+	3+	1+	1+	3+	3+	3+	2+	2+	2+	4+	5+	6+
12	3+	3+	2+	4+	1+	4+	5+	2+	2+	3+	2+	3+	3+	3+	2+	4+	5+	5+
13	5+	4+	5+	5+	3+	5+	5+	3+	4+	3+	3+	4+	4+	4+	4+	5+	5+	5+
14	5+	4+	5+	5+	4+	5+	5+	4+	4+	4+	3+	4+	4+	4+	4+	5+	5+	5+

15	4+	3+	4+	5+	5+	4+	5+	5+	4+	4+	3+	2+	5+	5+	5+	4+	5+	5+	
16	2+	2+	1+	4+	3+	5+	4+	5+	4+	3+	5+	4+	5+	4+	5+	5+	5+	5+	
17	1+	1+	2+	4+	4+	5+	5+	3+	4+	3+	5+	5+	4+	5+	5+	5+	5+	5+	
18	3+	2+	3+	5+	5+	5+	4+	4+	4+	5+	5+	5+	4+	5+	4+	4+	5+	6+	
19	1+	2+	4+	5+	4+	4+	5+	5+	4+	5+	4+	5+	4+	4+	4+	5+	5+	5+	
20	1+	1+	2+	4+	5+	4+	5+	4+	4+	5+	3+	4+	3+	4+	5+	5+	5+	4+	
21	2+	3+	4+	5+	4+	5+	4+	4+	4+	5+	3+	4+	3+	4+	5+	5+	4+	5+	
22	4+	4+	5+	5+	4+	5+	5+	5+	5+	4+	5+	4+	5+	4+	5+	5+	4+	5+	
23	3+	4+	5+	4+	5+	3+	5+	3+	4+	4+	5+	4+	5+	4+	5+	5+	4+	5+	
24	4+	5+	5+	5+	4+	5+	5+	5+	4+	4+	5+	4+	5+	4+	5+	4+	5+	5+	
25	5+	3+	3+	4+	4+	5+	4+	4+	5+	5+	5+	4+	5+	4+	5+	4+	5+	5+	
26	5+	3+	4+	5+	5+	5+	5+	5+	4+	5+	3+	4+	5+	5+	5+	5+	5+	5+	
27	2+	4+	3+	4+	5+	5+	5+	5+	5+	5+	4+	5+	4+	5+	4+	5+	5+	6+	
28	2+	2+	1+	3+	4+	5+	4+	5+	5+	5+	4+	5+	4+	5+	4+	5+	5+	–	
35	1+	3+	1+	4+	3+	4+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	–	
42	3+	4+	2+	4+	4+	4+	–	5+	5+	5+	5+	5+	5+	5+	5+	–	–	–	
49	-ve	1+	4+	5+	4+	–	–	–	–	5+	5+	5+	5+	5+		–	–	–	
56	2+	3+	1+	–	–	–	–	–	–	–	1+	–	–	–		–	–	–	