STUDIES ON THE EPIDEMIOLOGY AND CHEMOTHERAPY OF *TRYPANOSOMA EVANSI* INFECTIONS IN CAMELS

by

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D E C L A R A T I O N

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

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D E D I C A T I O N

This thesis is dedicated to the late

Prof. K. J. Lindqvist;

A teacher in deed

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A man of science appears to be the only person who has something to say just now, and the only man who does not know how to say it.

-- Sir James Barrie

SUMMARY

The field studies reported in this thesis on epidemiology and chemotherapy of Trypanosoma evansi in camels were conducted for a period of 18 months on two herds of camels located at Ngurunit and Olturot, in Marsabit District, Northern Kenya. In this area, camels provide subsistence for a nomadic population that own them.

During the field studies, data on the disease incidence and patterns of the disease in different age groups was collected. Serum samples were also collected fortnightly and stored at -20^{o}C, to be used later for serological analysis.

The results of these studies show that trypanosomiasis was the most important disease complex in the area and epidemics occurred during and soon after the rains.

Trypanosome infections were most severe in weaner and adult camels. The weaners developed severe clinical disease while in the adults, the effect of the disease was mainly recognised in the pregnant dams which aborted. Camel calves did not show infections until they were weaned and were over one year of age.

Attempts to control the disease by individual animal treatment with quinapyramine sulphate (Trypacide sulphate, May and Baker Ltd, Dagenham, UK) failed while chemoprophylaxis using quinapyramine prosalt (Trypacide prosalt, May and Baker Ltd, Dagenham, UK) reduced infections to manageable level.

Arising from the field studies, a number of questions needed to be answered. The first question was: which species of trypanosomes were responsible for the outbreak of trypanosomiasis in the study area?

From morphometry, the trypanosomes isolated from the camels were of the brucei-type. Because attempts to show presence of tsetse in this area had failed, the trypanosomes would have been termed as T. evansi, in line with Hoare's (1972) criteria of distinguishing T. evansi from other Trypanozoon. The tsetse map of Kenya shows that there are tsetse in the neighbourhood of the study area and because camels cover long distances in search of pasture and water, they could easily have traversed the tsetse infested areas and therefore acquired T. brucei brucei infections.

To investigate further the identity of the stocks collected the following characterization methods were used:

1) Tsetse transmissibility: Each of the trypanosome stocks was raised in irradiated rats and at peak parasitaemia, teneral Glossina morsitans mortisans were allowed to feed on them. The tsetse were then maintained by daily feeding on rabbits. On day 36, the flies were dissected and checked for trypanosome infections in the gut, proboscis and salivary glands. None of the 48 isolates was infective to G.m. morsitans. In comparison, mature infections were found in the flies that had fed on rats infected with a defined T.b. brucei reference strain (KETRI 2502) which had also been obtained from a camel. The

rabbits used to maintain flies infected with the reference T.b. brucei developed chancres and parasitaemia. Thus, by the criterion of tsetse trnsmissibility, the 48 isolates were most probably T. evansi.

2 Isoenzyme typing: Samples of soluble enzymes were prepared from each of the 48 stocks and analysed by thin layer starch gel electrophoresis for the ALAT, ASAT, PGM, ICD, ME and peptidases I and II. Except for PGM, none of the other enzymes revealed consistent differences between the 48 stocks and the reference T.b. brucei strain. However, stocks of T. evansi with a pattern similar to the one seen in T.b. brucei have been described before by Gibson, Marshall and Godfrey (1980). This approach was thus not useful for determining whether these isolates were T. evansi or T.b. brucei.

3) Kinetoplast DNA (kDNA) minicircle analysis: Kinetoplast DNA minicircles were analysed using various restriction endonucleases. Digested samples were then analysed in agarose and polyacrylamide gels. The digested minicircles of the 48 stocks were homogeneous. In contrast, the T.b. brucei reference strains used showed a complex of non-stoicheiometric bands irrespective of the endonuclease used. Analysis of kDNA minicircles was thus able to show unequivocally that the 48 stocks were T. evansi.

4) Chromosome-sized DNA analysis: Each of the trypanosome stocks was embedded in an agarose slab and chromosomes separated using contour-clamped

homogeneous electric field gel electrophoresis. From the karyotype patterns, the intermediate chromosomes and the minichromosomes were bigger in the reference T. evansi and the 48 stocks than those of the reference T.b. brucei. The trypanosome stocks analysed could be grouped into 9 molecular karyotypes. Only one molecular karyotype was found in the herd that was kept under chemoprophylaxis. This herd had a long history of drug use and recurring parasitaemias were often found soon after treatment. When tested for drug sensitivity, the trypanosomes were shown to be four times less sensitive to quinapyramine sulphate than the sensitive stock. It is possible that, the trypanosomes in this herd could have been derived from one drug resistant type.

With regard to the herd kept under individual treatment, nine molecular karyotypes were seen. The majority of the infections that occurred during the second epidemic could be traced to similar karyotypes seen at the beginning of the study. Thus, it appears that karyotyping is a sensitive method for revealing differences between T. evansi isolates and might be useful in revealing multiple re-isolation of the same trypanosome.

The next question that needed to be answered was whether an antigen detection system would have been a better method of detecting infections than parasitological diagnosis. To answer this question, over 3000 serum samples, collected fortnightly for a

period of 18 months, were analysed for the presence of antigens and results compared with the parasitological data. The results can be grouped into four categories:

1) Group one comprised cases in which the presence of trypanosomal antigens could be correlated with parasitological diagnosis. This was observed in 52 out of 61 (85%) instances in which trypanosomes were detected. On treatment, in most of the cases (80%), antigens disappeared from circulation within a period of 30 days further confirming the correlation noted above and also indicating the potential for use of this test to assess efficacy of treatment. In 20% of the instances, antigens remained detectable for a longer period of time, and in five cases even over 500 days . The reasons for persistence of the antigens in the few instances where they did persist could be due to failure of the trypanocides to effect a complete cure either because the trypanosomes were resistant to the drugs used or because the parasites were located in tissues inaccessible to the drugs.

2) Group two comprised those cases in which sera from parasitologically proven infections did not have antigens. This was observed in 9 camels, 7 of which were from a herd that was being examined for the presence of trypanosomes weekly. Two possible explanations were advanced. One was that the antigens might have been mopped up by antibody to form immune complexes and therefore the epitopes recognised by

the trapping antibody masked. Secondly, the trypanosomes could have been detected too early before sufficient parasite destruction had occurred to give detectable levels of antigen in circulation. Attempts to detect immune complexes failed and the second possibility was thought more likely.

3) Group three comprised camels that were at no time parasitaemic despite the presence of antigens. In the herd where control of trypanosomiasis was by prophylaxis, such antigens were noted to disappear from circulation after trypanocide therapy, indicating that, the presence of antigen represented true cases of trypanosomal infections, which could not be detected by the parasitological methods used. That the antigens detected were indeed due to the presence of trypanosomal infection was confirmed by the presence of anti-trypanosomal antibodies in the sera of antigen positive camels.

4) Group four comprised camel calves, in which no trypanosome infections were detected during the early period of their lives. Most of the calves also did not have antigen during this period. The calves appeared to have some form of protection from trypanosome infections. Anti-trypanosome antibodies were not found during this early period. This was suprising for calves born in a trypanosomiasis endemic area. What then was the source of protection? Are there non-specific factors akin to those that contribute to calfhood immunity against babesiosis? These questions remain to be answered.

In six out of 40 calves, occasional antigenaemia was detected but no corresponding antibodies were found indicating absence of a patent infection. This observation is intriguing in the light of the fact that cross-reaction has not been observed between the monoclonal antibody used in the antigen detection and other haemoparasites (Nantulya, Musoke, Rurangirwa, Saigar, and Minja 1987). Does this antigen represent disrupted trypanosomes that were unable to establish infection? Clearly, further work is needed to try and eludicate the nature of calfhood immunity.

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

There are two species of camels in the world, the one- humped dromedary (Camelus dromedarius) and the two-humped bactrian (C. bactrianus). In Africa, there are 13 million dromedary camels, with bactrians being noticeably absent (FAO yearbook, 1986). Kenya alone has some 620,000 camels (FAO yearbook, 1986) which contribute between 1 to 8% of the domestic herbivore biomass (Wilson, 1984, cited by Hjort, 1988). Except for a very small fraction, most of the camels in Kenya are owned by nomadic pastoralists who occupy the arid and semi-arid lowlands in the north and northeast of the country. Three distinct breeds of camels, classified according to body characteristics are kept by the Kenyan pastoralists. These are: Somali, Rendille-Gabra and Turkana.

The arid and semi-arid lands occupy 429,387 km^2 (77%) of Kenya's land surface, and fall within eco-climatic zones Five and Six (Pratt and Gwynne, 1977). Rainfall, 450 to 900 mm per annum in zone Five and 300 to 550 mm in zone Six, is erratic and generally insufficient for conventional agriculture. The evaporative losses are also high, exceeding 2000 mm per annum. The only possible land use is traditional livestock herding.

Until the last 50 years, the traditional herdsmen and shepherds in Africa had their own well-adapted system of managing the dry-land resources, which enabled them to survive successfully in apparent equilibrium with the precarious environment (Tribe, 1977). This equilibrium has been disturbed mainly because the combined impact of drought and disease has been lessened by the provision of

famine relief and improved human and animal health care. Also, with the increase in human population, the hitherto extensive grazing areas of the nomads are no longer available.

Encouraged by the Government's wish to have settled populations, the nomads have tended to settle around a few permanent water places where facilities such as schools, shops, health clinics and administration centres have developed. The resulting permanent nucleic sedentarization of the hitherto nomadic populations has led to continuous use of such desert pastures without allowing for their rest and recovery between grazing periods. This has led to an increased rate of desertification around these human and livestock centres with consequent serious droughts as witnessed in the 1970's and 1980's. As a result, large losses of livestock and a general lowering of living standards of the pastoralists in many parts of Africa occurred (Lusigi, 1986). In Kenya, food aid prevented large scale famines seen in other drought-stricken areas of Africa, where the effects were reminiscent of the 1890's rinderpest epidemics in Maasai country, vividly described by a German traveller (cited by Rower, 1980) in these words:

> "There were women reduced to walking skeletons, out of whose sunken eyes looked the madness of hunger, children resembling deformed frogs rather than human beings, warriors who could hardly crawl on all four, and moronic, emaciated grey beards. These people ate everything available; but they also devoured the

skins, bones and even horns of cattle .....
starvation had depopulated whole districts .....
swarms of vultures followed them awaiting for victims".

It was against this kind of eco-stress in Africa that a global consideration of the desertification took place in Nairobi (UNEP, 1977). Among the recommendations made in this conference was the creation of UN agencies to participate in determining the ecological factors associated with desertification, and remedial courses of action. The UNESCO Integrated Project in Arid Lands (IPAL) located in Marsabit District, Northern Kenya (Figure 1), was thus established. This area was considered representative of Sahelian Africa, both in composition of its vegetation and climate. The IPAL project started within the framework of "Man and Biosphere" (MAB), an organisation that encourages research on environmental problems. Integrated research was conducted in an attempt to provide a scientific basis for the rehabilitation and rational development of arid and semi-arid land ecosystems (IPAL Technical Report Number A-6, 1984).

Livestock was found to be the most important component in the pastoral ecosystem of northern Kenya, and is the main source of food. The pastoralists in northern Kenya can be generally divided into two groups: those who depend primarily on cattle and those who herd camels, although some keep both. Herders of both the cattle and camel also keep large numbers of smallstock. During the droughts of the 1970s and the 1980s, the most affected

animals were the cattle, which either died or ceased lactation and reproduction. Those pastoralists who kept camels fared better (Proceedings of the Camel Workshop, 1986). Cattle-keeping pastoralists in contact with camel-owning nomads realised this and started acquiring camels. Various non-governmental organisations also started camel re-stocking programmes as part of long-term food self-reliance for the nomads who had lost their cattle to drought.

Studies conducted by IPAL's social workers (O'Leary, 1985), in which camel owners were asked to indicate why they preferred camels to cattle, revealed that the camel was considered to be the most reliable of all animals: in drought periods, it is able to provide milk in contrast to other animals whose lactation drops drastically. One Rendille-type camel for example produces four times the amount of milk that local Samburu cattle can produce during the rainy season (Spencer, 1973). Camels provide blood, meat and fat in larger quantities per head than cattle or smallstock and they are valued for their ability to go without water for long periods of time.

This high production can be attributed to the superb adaptation of the camel to degraded harsh environments, and its ability to convert the poor quality arid rangeland forage into milk, meat and fat more efficiently than other animals. Nevertheless, the productivity of the camels kept in northern Kenya was found to be below its estimated potential, due to poor health (IPAL Technical Report Number A-6, 1984).

Among the many diseases to which camels are

susceptible, camel trypanosomiasis is considered to be the greatest cause of morbidity and mortality (Cross, 1917; Gatt-Rutter, 1967). A survey conducted in different areas of Kenya indicated that trypanosomiasis was the most important disease in camels and was widespread (Wilson, Schwartz, Dolan and Olaho, 1983). In that study four disease patterns were identified as shown in Table 1.

The number of camels in a given herd having any of the patterns varied from area to area. For example, at Olturot and Ngurunit (where the present author's studies were conducted, Fig 1), the herds were said to have more animals with type 1 and 4 disease patterns respectively. From these results, Wilson et al. (1983) concluded that, in certain herds where the disease was stable, trypanosomiasis had minimal effect and that, the use of trypanocides could contribute to the conversion from disease "instability" to stable ones. This contention was contrary to the findings of Mahmoud and Gray (1980), who had observed that camels rarely recover from T. evansi infections. Furthermore, data are lacking to show how permanent these disease patterns are. Such data would be helpful in the formulation of strategies for the control of camel trypanosomiasis.

In the same survey, Wilson et al. (1983) observed an apparent calfhood immunity indicated by presence of high levels of trypanosome antibodies in absence of trypanosome infections. This contrasted with the weaned immatures which had a high incidence of trypanosome infections despite high trypanosome antibodies. It is conceivable that the antibodies observed in the calves were of maternal

Table 1: The spectral nature of trypanosomiasis distribution in various camel herds in Kenya (Wilson *et al.*, 1983).

Disease pattern	Trypanosome	Antibody	Mortality	Clinical disease	Disease description
Type 1	+	+	+	+	Unstable
Type 2	+	+	-	+	Unstable
Type 3	+	+	-	-	Stable
Type 4	-	-	-	-	Stable

Key

+ = Present.

- = Absent.

origin while those observed in weaners were actively derived following infections. One question which arises is why maternally derived antibodies would be protective while those from the active immune response would not ? It is possible that the maternally derived antibodies were against majority of the variant surface glycoproteins (VSGs) of trypanosomes common in that area whereas the active immunity was against a few VSGs to which the immature camels had been exposed. No data are available to show whether this immunity in calves is indeed directed towards trypanosome antigens and at which stage in the life of a calf the transition from immunity to susceptibility might occur.

In their studies, Wilson and his colleagues (1983) found that both *brucei*-type trypanosomes and *T. congolense* could infect camels if exposed to tsetse challenge. Forty-nine *Trypanozoon* stocks collected during that survey were screened for isoenzyme patterns (Gibson, Wilson and Moloo, 1983). The majority of them were found to resemble those of *T. evansi* isolates from elsewhere in the world. Four isolates were, however, found to be different and one of them was infective to tsetse and hence designated as *T.b. brucei*. The tsetse-infective isolate (KETRI 2502) came from Rumuruti, an area whose altitude (600 metres) was considered above the tsetse belt (Wilson *et al*, 1983). The other three isolates (KETRI 2460, 2463 and 2479) came from Ngurunit and although their infectivity to tsetse was not established, they were thought to form another group of *T. evansi* found only in East Africa. The work of Gibson *et al*. (1983) demonstrated that the mere isolation of *brucei*

type trypanosome from a camel does not always indicate that it is T. evansi. There is a need therefore to evaluate the methods used to differentiate T.b. brucei and T. evansi with a view to determining those methods which provide reliable results.

The sero-diagnosis of camel trypanosomiasis has undergone major changes over the years. Earlier methods made use of raised euglobulins that occur in the course of trypanosome infections and which could be precipitated by chemicals such as, formalin and mercuric chloride (Knowles, 1924; Bennett, 1929). It was later found that these chemicals precipitated immunoglobulins irrespective of the antigen that stimulated their production and hence, these tests are not specific (Horgan and Bennet, 1929). Later, more specific antibodies were sought (Killick-Kendrick, 1968; Pegram and Scott, 1976; Luckins, Boid, Rae, Mahmoud, El Malik and Gray, 1979). The assays based on antibody detection have one major disadvantage in that, they cannot differentiate present from past infections.

Recently, it has been shown that it is possible to detect circulating trypanosome antigens in sera of camels suffering from trypanosomiasis and that the results correlate well with patent parasitaemia (Rae and Luckins, 1984; Nantulya, Bajyana Songa and Hamers, 1989a). Evaluation of these assays has been confined to sera sampled at an instance. For validation of this test, it is imperative that the antigen assay be performed on serially collected sera from animals in the field where other diagnostic and disease control strategies are being applied. By so doing, the contribution of this test to the

understanding of the epidemiology of T. evansi will be properly evaluated. For the test to be useful, it must also show distinct advantages over techniques already available. An important consideration here is its simplicity and future adaptability to field conditions.

The studies reported here were conducted following earlier general investigations on camel trypanosomiasis in Kenya by various people (Olaho and Wilson, 1981; Wilson et al. 1983; Rutagwenda, 1984), that revealed the varying epidemiological picture of this disease in different areas. The studies were to address in detail the questions of trypanocide usage, the identity of the trypanosomes that cause camel trypanosomiasis in arid areas of Northen Kenya and its diagnosis. These aspects of camel trypanosomiasis were studied as outlined in the study objectives below.

1.2 THE OBJECTIVES OF THE STUDY

1.2.1 To study the disease patterns in two herds of camels living in a trypanosomiasis endemic area and maintained under two different chemotherapeutic control programmes.

1.2.2 To evaluate various techniques with regard to their usefulness in the characterization of trypanosomes found in the two herds.

1.2.3 To compare the results of trypanosome antigen-detection in serial serum samples with those of parasitological findings from the two herds of camels .

1.2.4 To evaluate the trypanocide sensitivity of the trypanosome stocks suspected to be drug fast.

1.3 JUSTIFICATION

It was anticipated that, through this study, adequate information would be obtained to provide a detailed insight into the local epidemiology of T. evansi infections in camels. The application of the findings would enable a more rational use of trypanocides to control disease subsequently and hopefully result in increased productivity of the camels and, therefore, improve the living standards of the camel keeping nomads.

1.4 LITERATURE REVIEW

Trypanosoma evansi, so named by Balbian after its discoverer, Griffith Evans, was the first pathogenic trypanosome to be described (Wenyon, 1926). Evans found the parasites in the blood of horses and camels suffering from a disease the local Indians had known for many years and which they referred to as ''Surra'', meaning emaciated. Following this discovery, the surra organisms were recovered from different mammalian hosts in different countries, and given various local names. Trypanosoma evansi is now the accepted valid name, replacing others like T. evansi var Mborii, T. soudanense. T. morocanum, T. equinum and T. aegyptum, (Hoare, 1972).

Following the discovery of T. evansi, its mode of transmission intrigued investigators. The Indian natives associated the disease with the bite of a large brown fly, but investigators associated it with ingestion of soiled food and water (Hornby, 1921). In 1901, Rogers demonstrated that surra was indeed spread by biting flies.

Trypanosoma evansi belongs to the sub-genus Trypanozoon (Hoare, 1972) and is postulated to have arisen from T.b. brucei, which became adapted to mechanical transmission when infected animals were moved outside the tsetse-infested areas (Hoare, 1940).

The parasite is said to have spread first to the countries north of the zones infested by tsetse (Hoare, 1940), and thereafter, to India by means of camel caravans and military campaigns. From India, the disease then spread to many countries of Asia (Russia, Burma, Malaya and China); the Middle East (Israel, Lebanon, Syria, Iraq and Iran); Islands in the Indian Ocean (Indonesia, Philippines and Mauritius). By the early 1900's, the disease had already reached the United States and Australia where it was subsequently eradicated (Wenyon, 1926). The disease later reached South America where it gained wide geographical distribution (Hoare, 1972). In the "New World" the parasites lost their ability to undergo cyclical development in Glossina, after decades of non-cyclical transmission by biting flies. Despite the inability of T. evansi to survive longer than a few hours in the mouth parts of the biting flies, it established itself outside the tsetse belts of Africa (Hoare, 1940) and today, it is the most widely distributed species of all the pathogenic trypanosomes (Luckins, 1988).

Mechanical transmission is most successful if the feeding time of the biting flies is brief and interrupted, so as to allow quick transfer of the trypanosomes. Among the haematophagous Diptera, Tabanus species are the most efficient mechanical transmitters (Mahmoud and Gray,

1980). They have mouth parts that protect ingested blood films and because of their painful bites, their feeding is quickly interrupted by their hosts and in efforts to complete the blood meal effect transmission of trypanosomes to other hosts (reviewed by Luckins, 1988). Other genera of biting flies that have been shown to be capable of transmitting T. evansi are Stomoxys, Haematopota, Chrysops and Lyperosia (Mahmoud and Gray, 1980). Ticks have also been suggested as possible transmitters, but their feeding behaviour is such that, transmission from one host to another would be difficult. Even the non-blood-sucking flies have been incriminated in carrying trypanosomes from infected meat to open lesions or mucous membranes of susceptible animals such as dogs and horses (Weinman and Ristic, 1968).

Infections of camels with trypanosomes belonging to the genus Trypanozoon are usually attributed to Trypanosoma evansi. The assumption made is that since camels are generally kept in areas removed from the delineated tsetse habitat, the trypanosomes encountered in such areas are not tsetse-transmissible. This however need not be the case as tsetse may be present in an area without being detected (Wells, 1972). In addition, camels may move to areas infested with Glossina and could, therefore, acquire tsetse-borne trypanosomes (Bennett, 1933). For control purposes, it is important to differentiate between infections caused by tsetse-transmissible trypanosomes and those caused by the mechanically transmitted ones. This is because, control of tsetse-transmitted trypanosomes is based on chemotherapy and reduction of the vector

populations (Jordan, 1986), while that of the mechanically transmitted trypanosomes mainly relies on chemotherapy.

The inability of T. evansi to undergo cyclical development in Glossina has been suggested as the best character for distinguishing T. evansi from T. brucei and is used routinely for taxonomic purposes (Hornby, 1921; Hoare, 1972; Gibson Wilson, and Moloo, 1983). In the laboratory, this entails determination of the tsetse transmissibility of the trypanosome stocks in question. Tsetse transmission experiments are, however, difficult to conduct routinely and when done, the percentage of flies infected is low even with known tsetse-transmissible trypanosomes (Duke, 1933). There appear to be several factors that influence the ability of tsetse to become infected, or of trypanosomes to be transmitted (Duke, 1933; Croft, East and Molyneux 1982; Ibrahim, Ingram and Molyneux 1984; Vickerman, 1985; Maudlin and Welburn, 1987; Moloo and Kutuza, 1988). Nevertheless, tsetse transmissibility is likely to remain the preferred criterion for differentiation unless other un-equivocal parameters are found. Lumsden (1974) proposed the use of intrinsic markers (related to the organism itself) such as morphology, isoenzymes, DNA buoyant density and immunological characteristics. He considered these to be more permanent than extrinsic features (not wholly controlled by the organism) such as tsetse transmissibility.

T. evansi is predominantly monomorphic, appearing as long slender and intermediate forms of trypanosomes. Occasionally, short stumpy forms without flagella are seen

(Bruce, 1911; Hoare, 1956). In comparison, T. brucei displays polymorphism whereby, in addition to the long slender types, the short stumpy forms are always present at some stage (Hornby, 1921). Various workers attribute the morphological differences between T. evansi and T. brucei to their different modes of transmission (Bruce 1911; Hornby, 1921; Hoare, 1956). T. evansi, which multiplies exclusively in the mammalian host, has no need for the short stumpy forms which are thought to initiate multiplication in the tsetse (Robertson, 1912; Vickerman, 1985). Polymorphism in T. brucei has, however, been found to vary depending on the course of infection and may disappear completely after prolonged mechanical passage (Fairbain and Culwick, 1947). Such monomorphic T. brucei were found not to be transmissible by tsetse (Ashcroft, 1960). Thus, while morphological features can differentiate brucei-type trypanosomes from the other species (T. congolense, T. simiae and T. vivax) they cannot differentiate T. evansi from the other members of the brucei group (Hoare, 1972). Morphology, although said to be an intrinsic character (Lumsden, 1974), is thus of limited taxonomic value in differentiating T. evansi from T. brucei.

The realisation that phenotypic characters have biochemical causes has been of great value in taxonomic studies. The biochemical methods for parasite characterization have been divided by Newton (1976) into two main categories: those that are concerned with the cell phenotype and those that measure the genotype. One of the most widely used biochemical methods is isoenzyme

typing, a term first introduced by Markett and Moller (1959) to describe techniques that reveal the different molecular forms in which proteins with the same enzymatic specificity may exist. Such differences are genetically functional adaptations to different environments (Masters and Holmes, 1975). Isoenzymes show variations in their molecular structures and, on electrophoresis, can be resolved into separate bands on the basis of differences in charge and size (Holmes and Scopes, 1974). Using this method, it has been possible to show intra-species variations in trypanosomes which are otherwise morphologically indistinguishable, despite different behavioural features (Godfrey, 1979; Miles, Lanham, De Souza and Povoa, 1980; Gibson, Marshall and Godfrey, 1980). For example, Godfrey and Kilgour (1976) examined two aminotransferase patterns of 33 isolates of brucei type trypanosomes and found that, those collected from patients with the chronic gambiense infections had patterns different from those collected from patients suffering from the acute rhodesiense disease or those collected from animals infected with the non-human-infective T.b. brucei.

Studies by Gibson et al. (1980) on isoenzyme profiles of 160 Trypanozoon stocks showed that the brucei trypanosomes show little variation (less than 5% for the enzymes studied). Nevertheless, it was possible to group them into West and East African T. brucei and into human-infective and non-human-infective T. brucei by isoenzyme analysis. These differences were especially pronounced with isocitrate dehydrogenase and phosphoglucomutase. T. evansi stocks were found to form

one homogenous group that resembled West African T. brucei the only difference being with peptidases, PEP 1 and PEP 2. In a later study (Gibson et al., 1983), a T. evansi stock from Kenya could not be differentiated from the West African brucei. Thus, although isoenzymes could differentiate East African T. brucei stocks from T. evansi, they failed to differentiate them from the West African brucei-types. This similarity, however, lends credence to Hoare's (1940) speculation that the West African T. brucei are the progenitors of T. evansi.

Recent advances in molecular biology have made it possible to investigate the evolutionary relationship of species by comparing their genomes. Since the mitochondrial DNA (mtDNA) evolves more rapidly than the nuclear DNA (Steinert, Van Assel, Borst and Newton, 1976; Brown, George and Wilson, 1979), analysis of mtDNA has been found to be the most useful method for showing genetic relationships.

The mtDNA of a typical eukaryote occurs in a form of double stranded circular molecules of about 5 um length (Lima-de-Faria, 1986). The functional equivalent of mtDNA in kinetoplastid protozoa are the maxicircles (Stuart, 1983) which are held in a concatenated minicircle matrix (Simpson, 1972; Borst and Hoeijmakers, 1979; Englund, 1981) which constitutes the kinetoplast DNA (kDNA), within a special organelle, the kinetoplast. The latter is the distinguishing character of the order Kinetoplastida.

The maxicircles make up 10% of the kDNA network, show a high degree of conservation and code for components of the mitochondrial respiratory system (Borst and

Hoeijmakers, 1979; Borst, Hoiejmakers, Frasch, Snijder, Janssen and Fase-Fowler, 1980b). A functional mitochondrial respiratory system is only required by trypanosomes in the insect vector. When in the vertebrate host, they obtain energy from glycolysis alone (Fairlamb, Weislogel, Hoeijmakers and Borst, 1978; Borst and Hoeijmakers, 1979). T. evansi maintenance is independent of cyclical transmission and no maxicircles have been demonstrated in the strains so far studied (Fairlamb et al., 1978; Borst and Hoeijmakers,1979; Borst, Fase-Fowler and Gibson, 1987). The absence of maxicircles cannot, however, be relied upon as a distinguishing characteristic of T. evansi since it is possible for them to be present, albeit in a non-functional state as has been demonstrated in T. equiperdum (Frasch, Hajduk, Hoeijmakers, Borst, Brunel and Davidson, 1980).

The minicircles make up about 90% of the kDNA network but their genetic function has not yet been elucidated. They are thought to play a structural role during kinetoplast division (Simpson, 1972; Borst and Hoeijmakers, 1979; Englund, 1981). In tsetse-transmitted trypanosomes, the minicircles are heterogeneous in base sequence (Donelson, Majiwa and Williams, 1979) while they are homogeneous in some non-cyclically transmitted trypanosomes, such as T. evansi (Riou and Barrois, 1979; Frasch et al., 1980; Borst et al., 1987)

The generation and maintenance of minicircle diversity in T. brucei (as in other genetic heterogeneity) is likely to be the result of somatic and sexual recombinations occurring either singly or together with

somatic mutations. Some workers (Borst et al., 1987) have attributed the difference in minicircle heterogeneity in T. brucei and T. evansi to sexual recombination which has been shown to occur in T. brucei at a certain stage of its development in the tsetse (Tait, 1983; Jenni, Marti, Schweizer, Betschart, Le Page, Wells, Tait, Paindavoine, Pays and Steinert, 1986). This is however questionable, since, sexual recombination, as experimentally observed, has been demonstrated to involve only the nuclear DNA, the inheritance of kDNA being uniparental (Steinberg, Turner, Wells, Ranford-Cartiwright, Le Page and Tait, 1989). The observed difference between the kDNA of these two parasites is therefore likely to be a result of somatic recombination and the balance between factors that tend to generate diversity and those that tend to produce homogenisation. Since T. evansi arose from T. brucei (Hoare, 1940; 1972), homogenisation of its minicircles must have occurred during this transition which also involved loss of tsetse transmissibility. This might have involved processes of gene conversion and unequal segregation. Since homogenisation by these processes, has not occurred in T. brucei, it is conceivable that, minicircle heterogeneity has a functional significance in the tsetse part of the life cycle.

Using restriction enzyme endonucleases, Borst et al. (1987) examined six T. evansi strains and found two types of minicircle patterns. Five of the strains had a type A minicircle which was said to be common in strains from East Africa, West Africa and South America. One strain from Kenya had a different type of mini-circle (type B). The

authors speculated that the two types had arisen independently from two different minicircles that became dominant in the kDNA network of different lineages during the homogenisation of T. brucei minicircles.

An alternative method of studying genetic relationship between individuals is by comparing their intact chromosomes. As early as the 1940s, investigators sought chromosomal differences between trypanosomes in the hope of being able to differentiate the species of the Trypanozoon sub-genus (Vanderplank, 1944). This was, however, unlikely to yield meaningful results because chromosomes of kinetoplastid parasites do not condense at metaphase and are hence invisible by microscopy (Vickerman and Preston, 1970). Recently, a novel technique, pulsed field gradient (PFG) gel electrophoresis (Schwartz and Cantor, 1984) that fractionates chromosome-sized DNA molecules, has enabled some intact chromosomes of kinetoplastids to be visualised (Van der Ploeg, Cornellison, Barry and Borst, 1984a, Van der Ploeg, Cornellison, Michels and Borst, 1984b; Gibson and Borst, 1986; Johnson and Borst, 1986; Kimmel, ole Moi Yoi and Young, 1987; Myler, Aline, Scholler and Stuart, 1988; Dickin and Gibson, 1989).

Fractionation of T. brucei DNA revealed a highly variable karyotype which could be separated into four fractions : a large DNA fraction which enters the gel matrix only under special conditions (Johnson and Borst, 1986); large chromosomes of over 700 kb which comigrate into a compression zone in the gel (Johnson and Borst, 1986); five to seven intermediate sized chromosomes, ranging in sizes from 200 to 450 kb; and about 100

minichromosomes of 50 to 150 kb. The only karyotype of T. evansi so far reported had a chromosome pattern similar to that of T. brucei (Kimmel et al., 1987; Majiwa, Hamers, Van Meirvenne and Matthyssens, 1986a; Shah, Young, Kimmel, Iams and Williams, 1987).

Majiwa, Masake, Nantulya, Hamers and Matthyssens (1985); Majiwa et al (1986a); Gibson and Borst (1986); Masake, Nyambati, Nantulya, Majiwa, Moloo and Musoke (1988) fractionated chromosomes of cloned stocks of T. congolense and showed substantial differences between them. The minichromosomes of isolates from Kilifi, Kenya were much bigger (75-150 kb) and more heterogenous in size compared to those isolated elsewhere in Kenya and from other parts of Africa which were less than 100 kb. Many intermediate sized chromosomes (100-200 kb) were found and were only present in trypanosomes from Kilifi. On the other hand, the medium sized chromosomes (400kb-2Mb) were fewer in the Kilifi isolates. Because of these differences, Majiwa, Young, Hamers and Matthyssens (1986b) suggested a division of T. congolense into karyotypic groups. Earlier work on T. vivax had shown that it lacks both the intermediate- and mini-chromosomes (Van der Ploeg et al., 1984a; Majiwa et al., 1985), although a recent study by Dickin and Gibson (1989) indicated that it might possess one or two mini-chromosomes.

The excitement of finding chromosomal polymorphisms within stocks of the same species led investigators to speculate about a possible correlation between karyotype and phenotypic characters such as drug resistance, virulence and antigenicity (Van der Ploeg et al., 1984a;

Cox, 1985). The hope of finding such a correlation was muted when it was discovered that the karyotypes of trypanosomes undergo frequent translocations and deletions involving the intermediate chromosomes and the minichromosomes (Van der Ploeg et al., 1984b; Majiwa et al., 1986a; Shah et al., 1987). However, a correlation between karyotype and antigenicity was shown to exist in T. congolense isolates from cattle in Kenya (Masake et al. 1988). In that study, clones of T. congolense belonging to one serodeme displayed similar chromosome profiles even after many passages in animals and tsetse. Only in one case was a shift seen in the position of one medium-sized chromosome band following cyclical development in tsetse. These findings have important epidemiological implications, but for them to be generalized, similar studies would have to be done in many different geographical areas and over a long period of time. In T brucei, chromosomal size changes are estimated to occur at a frequency of 10^{-5} to 10^{-6} per trypanosome division (Van der Ploeg et al., 1984b). Similar estimates have not been made in other trypanosome species, but, in T. congolense, out of 117 clones examined (Masake et al.,1988), a minor change in karyotype occurred in one clone that was cyclically transmitted through tsetse. A similar change had been reported by Majiwa et al. (1986b). The roles that such chromosomal alterations play in the biology of trypanosomes are not clear, but those that involve deletions or gain of totally different DNA molecules, might be expected to lead to a change in antigenic repertoire.

As with other trypanosome infections, diagnosis of T. evansi is not without difficulties. The clinical signs are not pathognomonic. In different countries, the host susceptibility to T. evansi differs greatly (Hoare, 1956). For example, while the Asiatic cattle and buffalo are readily infected with T. evansi, African cattle are seldom infected. In Indonesia, the disease is acute in horses and mild in cattle, whereas in the Soviet Union, it is the camels and to a lesser extent, horses, that suffer acute infection. In Sudan and Somalia, horses are refractory, but camels are highly susceptible. In North Africa, horses are the main hosts followed by cattle .

In an attempt to explain these host differences, Hoare (1956), postulated that, there existed a multiplicity of hostal strains of T. evansi. This has however not been proven and the evidence that exists contradicts Hoare's hypothesis. By the criterion of isoenzymes, evansi trypanosomes form one homogenous group (Gibson et al., 1980; Boid, 1988; Al-taqi, 1989; Stevens, Nunes, Lanham and Oshiro, 1989). It is probable that the observed differences in the clinical picture, relate to the level of stress to which different animal species are subjected in different geographical areas.

A classical epidemiological picture of T. evansi shows a definite correlation between the seasonal outbreak of T. evansi infections and the increase in the number of tabanids (Mahmoud and Gray, 1980). A study of the seasonal dynamics of the tabanids in Sudan, conducted by Yagi and Razig (cited by Mahmoud and Gray 1980), showed that, although the number of biting flies is most abundant during

and soon after the rains, some flies survive in small numbers throughout the year. This seasonal variation in the fly numbers explains why sporadic infections are reported during the dry season whereas epidemics commonly occur during and soon after the rains. This classical picture appears to occur in animals experiencing the disease for the first time (Luckins, 1988). Once the disease has become endemic, it assumes low incidence and even when epidemics occur, they tend to be related to factors that produce stress, such as physical exhaustion, inclement weather, pregnancy or lactation (Lohr, Pholpark, Siriwan, Leesirikuj, Srikitjakaran and Staak, 1986).

Trypanosoma evansi has been isolated from camels, horses, donkeys, mules, water buffaloes, dogs, pigs, sheep, goats, elephants, tapirs, deer and capybaras (reviewed by Mahmoud and Gray, 1980; Losos 1980; Stevens et al., 1989). In all these animals, the disease can be acute, chronic or asymptomatic (Losos, 1980). The factors that determine the course that the disease takes are many, but the duration of host-parasite relationship and the prevailing level of stress are considered to be very important (Losos, 1980). Nevertheless, some animal species (camels, horses and dogs) generally suffer a more serious disease, while donkeys, cattle, sheep and goats seem to act as reservoirs.

Various authors have reviewed the clinical signs of T. evansi in various animals (Stephen, 1970; Mahmoud and Gray, 1980; Losos, 1980; Boid, Jones and Luckins, 1985) and the following is a summary of their findings.

In susceptible animals, the acute disease is characterized by intermittent fever, excessive

lachrymation, dullness, enlargement of lymph nodes, petechial haemorrhages on the mucosa and progressive anaemia. Subcutaneous oedematous swellings and urticaria-like eruptions on the skin may be observed. During the early stage of the disease, the parasites are either present continuously or they coincide with elevation of temperature. Pregnant animals may abort, especially if they are in the late stage of gestation (Lohr et al., 1986). The acute form of the disease is almost always fatal unless treated. These signs of acute disease may resemble those seen in anthrax, black-quarter and pasteurellosis (Gatt-Rutter, 1967).

The most common course of the disease is, however, the chronic form which is associated with progressive anaemia, low grade or non-existent parasitaemia and loss of condition. Animals with the chronic syndrome may survive for a number of years; but the disease frequently terminates in death. The signs of the chronic syndrome are also not pathognomonic and may resemble other conditions that cause unthriftiness, for example, malnutrition and helminthiasis.

Anaemia is a cardinal sign of animal trypanosomiasis and is evidenced by reduction in packed cell volume (PCV). During the course of a T. evansi infection in camels, the PCV may drop by 30% (Jatkar and Purohit, 1971). Murray and Dexter (1988) have reviewed the pathogenesis of anaemia in bovine African trypanosomiasis and they point out that anaemia gives a reliable indication of the disease status and can be used to evaluate the productive performance of infected animals. In their review, Murray and Dexter

divided the anaemia into two phases. The first phase (seen in acute trypanosomiasis) is characterised by progressive anaemia and is always accompanied by parasitaemia and usually takes 4 to 12 weeks. Animals that survive this stage progress into phase two which is characterized by transient or complete absence of parasites in the blood, persistently low PCV values (20 to 25%) and generally make no clinical improvement. Animals with this chronic syndrome may remain alive but in poor health and their response to trypanocidal treatment is poor.

The disease can also be mild or asymptomatic. Stress can, however, precipitate relapses or increase the animals' susceptibility to the disease, when apparently healthy animals may suffer severe infections. Stress may be physiological or may be associated with hard work. For example, animals in late pregnancy were noted to be more susceptible to T. evansi infections than those in early stages of gestation (Lohr et al., 1986). Exhaustion, commonly seen in draught animals may constitute stress which may precipitate the disease. Similarly, intercurrent disease, change in climate, or seasonal malnutrition may aid flare-up of clinical signs (Lohr, Pholpark, Srikitjakarn, Thaboran, Betterman and Staak, 1985; Gill, Singh, Gill and Kwatra, 1987).

Age is important in determining the course of the disease. For example, a survey conducted by Wilson, Schwartz, Dolan and Olaho (1983), in Northern Kenya, showed that weaner camels (1-4 years old) developed severe clinical disease compared to adults, while calves (under one year old) showed resistance to infection. These

observations imply that: firstly, calves may be protected by maternal antibodies; secondly, this protection wanes after about one year; and thirdly, older animals acquire immunity. Wilson and colleagues failed to find significant differences in anti-trypanosomal antibodies between the three age groups to explain these differences. They proposed the existence of calfhood immunity not related to the presence of maternal antibodies, but akin to that seen in bovine babesiosis and anaplasmosis. It is probable that with the passage of time, the calfhood immunity wanes and when the animals are weaned, the associated stress makes them highly susceptible. On the other hand, the older animals having had prior exposure to the trypanosomal antigenic types common to an area and survived, would be expected to show resistance. However, if such animals are challenged with trypanosomes antigen types of which they had no prior experience, they would succumb (Murray, Morrison and Whitelaw, 1982).

Because of the varied picture associated with T. evansi infections, diagnosis cannot be based on clinical signs alone. For confirmatory diagnosis, it is important to demonstrate the presence of the trypanosome.

Several parasitological techniques are used to demonstrate the presence of trypanosomes (Killick-Kendrick, 1968; Wilson, 1969; Molyneux, 1975). In routine veterinary diagnosis, depending on the animal species, capillary blood may be taken from the tail, or venous blood taken from an ear vein or the jugular, for examination, either as a wet film under a coverslip to detect motile trypanosomes, or, as stained thick and thin smears, when parasites are

identified on the basis of their morphology.

Examination of wet blood films is quick and the method is, therefore, useful when large numbers of animals are to be examined. This method is however insensitive and half of the infected animals may be missed (Barnett, 1947). In thin films, very little blood is used which results in the method being less sensitive compared to thick blood films (Fiennes, 1952, Killick-Kendrick, and Godfrey, 1963). Before staining, erythrocytes in thick blood films have to be lysed to enable stained trypanosomes to be seen and it is possible that because camel erythrocytes resist lysis to a concentration as low as 0.11% sodium chloride (Speck, 1963), the technique might be less sensitive than with blood from other species. Diagnosis based on microscopic examination is, however, subjective since it depends on the skill of the microscopist, the time spent examining each slide, the level of parasitaemia and the volume of blood examined.

The sensitivity of microscopic examination of blood for trypanosomes can be improved if the parasites are concentrated by centrifugation, following which the expected region of concentration is examined. When blood is spun in a haematocrit centrifuge, the trypanosomes are concentrated at the buffy coat and examination of the latter has become popular since its first description by Bennet (1962). Various modifications of the original method have been described (Woo, 1969; Murray, Murray and MacIntrye, 1977; Kelly and Schillinger, 1983) all of which seem to be superior to examination of blood obtained directly from the animal. The microhaematocrit technique

is particularly useful in that the status of anaemia in animals can be assessed at the same time. The use of this centrifugation technique in the field is however curtailed by the need for electricity. The battery operated minicentrifuge described by Kelly and Schillinger (1983) is an attractive alternative but has not lived up to the initial acclaim (personal observation).

Sub-inoculation of blood from animals suspected to have trypanosome infections, into susceptible laboratory animals is an old technique which earlier workers found useful to reveal inapparent trypanosome infections (Wenyon, 1926). Mice or rats are the most commonly used animals. In brucei type infections, the use of mice for sub-inoculation of blood has been found to be the most sensitive method compared to other parasitological methods (Paris, Murray and MacOdimba, 1982). Sub-inoculation of blood into other animals has an added advantage in that, isolates can be collected for other studies in the laboratory. Unfortunately, the prepatent period can be long, thus delaying diagnosis. Also, the cost of maintaining animals makes the method prohibitively expensive for routine diagnosis, especially in the field.

Therefore, despite the advances and improvements of parasitological techniques, a high proportion of infections go undetected. As indicated above, the majority of the infections are chronic and often aparasitaemic. Also, the phenomenon of antigenic variation (Doyle, 1977; Cross, Hodder, Allen and Boothroyd, 1980; Seed and Sechelski, 1988) results in intermittent parasitaemia and so, the parasites are not always demonstrable even in acute cases

(Murray, Barry, Morrison, Williams, Hirumi and Rovis, 1979). This has necessitated the development of indirect methods based on the host immune response to the parasite.

As early as 1899, Rabnowitsch and Kemp (cited by Wenyon, 1926) showed that sera from animals suffering from trypanosomiasis caused lysis of trypanosomes. Watson (1915, cited by Wenyon, 1926), demonstrated that the lysis was due to the presence of anti-trypanosome antibodies and used the complement fixation reaction to detect specific immunoglobulin to T. equiperdum in horses. The same technique was later used by Schoening (1924) to diagnose T. evansi infections in camels, sheep, rabbits and dogs with an aim of guarding against the introduction of trypanosome-infected animals to the United States. The test was later used to eliminate T. equiperdum infection from horses in Canada (Wenyon, 1926). In the tropics, the test did not become popular because, many sera were found to have anti-complementary activity; there were also difficulties of standardizing the antigen used in the test (Gill, 1970).

By the early 1920s, scientists had begun investigating the nature of reactions of various chemicals in sera collected from patients suffering from various diseases. This was after Gate and Papacostas noted gelatinisation of sera of syphilitic patients on addition of formalin (Spackman, 1923). A similar reaction was later shown to occur in sera from kala-azar patients and in sera from animals infected with T. evansi (Spackman, 1923). The formalin test was adopted as a routine diagnostic method for camel trypanosomiasis in Sudan (Knowles 1924; 1925 and

1927). The basis of this reaction is that formalin precipitates immunoglobulins (Horgan and Bennett, 1929) irrespective of the antigen that stimulated their production. Hence, the test was not specific for any one disease (Bennet, 1933). Earlier on, while working in Sudan, Bennet (1929) had introduced the use of mercuric chloride instead of formalin, which he claimed gave a specific reaction for camel trypanosomiasis. In experimental camel infections, Leach (1961) observed that the mercuric chloride test became positive 10 to 15 days post-infection and negative reactions occurred two to three months following treatment with a trypanocide. Attempts to reproduce these results in other animal species failed (Killick-Kendrick, 1968). Later, investigators also failed to correlate results of this test and patent parasitaemia in camels (Pegram and Scott, 1976; Luckins, Boid, Rae, Mahmoud, El Malik and Gray, 1979).

Gill (1964; 1966) described a modified method for an indirect haemagglutination test (Boyden, 1951) to detect T. evansi antibodies in experimental sera and found the test to be specific, sensitive and easy to perform. The test was later used for diagnosis of natural T. evansi infections in camels (Jaktar and Singh, 1971; Olaho and Wilson, 1981) and other ruminants (Shen, 1974). In sheep infected with T. vivax, this technique was found to give false positive reactions that required rigorous standardization before the non-specific reactions could be eliminated (Clarkson, Beatrice, Cottrell and Enayat, 1971).

The introduction of the immuno-fluorescent assay, which employed antibodies or antigens labelled with a

fluorescent dye, in parasite immunodiagnosis (Sadun, Williams and Anderson, 1960) and its subsequent application to trypanosomiasis (Williams, Duxbury, Anderson and Sadun, 1963) led to a major improvement in the sensitivity of serological methods. The test was first evaluated for diagnosis of surra by Sabanshiev (1972; 1973; cited by Luckins et al., 1979) and later by Luckins, Gray and Rae (1978) and Luckins et al (1979). It was reported to give a rapid and reliable indication of T. evansi infections. The test was adaptable to large scale use and furthermore, antigen preparation was reproducible (Voller, 1977). The technique, however, had one major drawback in that it required an expensive fluoresence light source for the microscope. Also, the reading of the results was based on visual estimation of intensity of the fluorescence and was hence subjective (Voller, 1977).

The development of techniques which utilise enzyme labels (for example, enzyme linked immunosorbent assay, ELISA) which were easy to evaluate, sensitive and adaptable as simple field screening procedures offered an attractive alternative to immunofluorescent techniques (reviewed by Voller, Bidwell and Bartlett, 1976). In trypanosomiasis, the test was first used to detect antibodies to T. rhodesiense infections in man (Voller, Bidwell and Bartlett, 1975). Luckins et al. (1978; 1979) later used the ELISA on sera collected from animals infected with T. evansi and found the test to have specificity and sensitivity comparable to the immunofluorescent technique. Despite the high sensitivity of the ELISA, it suffered one major problem in that the antigen was a crude trypanosome

preparation, the quality of which was ill-defined (Voller and de Savigny, 1981). The ELISA was therefore, difficult to standardize (Van Meirvenne and Le Ray, 1985).

The problem of antigen standardization was solved for T. b. gambiense by introduction of the card agglutination test (CATT; Magnus, Vervoot and Van Meirvenne, 1978) which was based on selected antigenic variants stabilized on whole trypanosomes following the technique described by Nantulya and Doyle (1977). Using the T. b. gambiense CATT test, Zweygarth, Sabwa and Rottcher (1984) determined the presence of anti-trypanosomal antibodies in camel sera and found that, the test worked well only if undiluted sera were used. When tried on sera collected from water buffaloes, the test was found to work well only after inclusion of a specific anti-buffalo immunoglobulin (Bajyana Songa, Hamers-Casterman, Hamers, Pholpark, Leidl, Tachchaitrong, Chaichanopoonpol, Vioorrakool and Thirapatsakum, 1987). The anti-buffalo immunoglobulin binds to the antibody on the erythrocytes and therefore increases the efficiency of agglutination. Further improvement of this test has been reported by the use of T. evansi variant antigenic type (VAT) which is dominant in all T. evansi stocks (Bajyana Songa and Hamers, 1988). Personal experiences with the CATT test in the field, in the dusty environment of Marsabit, northern Kenya were, however, unsatisfactory because agglutination could not be differentiated from the effect of dust that invariably settled on the card.

Antibody detection systems have unfortunately been found to be inadequate since they provide only a

presumptive diagnosis and do not differentiate between current and past infections. This is because anti-trypanosome antibody levels may persist for several months after successful trypanocidal therapy (Luckins et al., 1978; 1979).

An alternative approach to antibody detection is the immunological demonstration of parasite-specific antigen which is analogous to a parasitological diagnosis (Voller and De Savigny, 1981). The first attempt to detect circulating trypanosomal antigens was made in Chagas disease (Araujo, 1982) but the sensitivity obtained was low, compared to, for example, the culture of suspect blood (Araujo, 1982). Later, Rae and Luckins (1984) developed a T. evansi antigen-detection system, which used polyclonal anti-T. evansi antibodies and demonstrated antigenaemia within a week of experimental infections. With this method, the antigens were shown to disappear from circulation within 10 days following a trypanocide treatment. The authors felt that this test might even be useful in predicting relapses following therapy. This polyclonal antibody system has however, been found to have a low specificity because cross-reactions occur with non-targeted trypanosome species as well as other haemoparasitic diseases.

The development of monoclonal antibodies (MoAb) with defined specificity (Parish, Morrison and Pearson, 1985; Richardson, Jenni, Beecroft and Pearson, 1986; Nantulya, Musoke, Rurangirwa, Saigar and Minja, 1987) was a major breakthrough in the diagnosis of African trypanosomiasis.

The MoAbs developed by Nantulya and colleagues (1987), were based on invariant surface antigens and were able to distinguish between brucei type trypanosomes, T. congolense and T. vivax. Also, the MoAbs showed no cross-reactivity with common haemoparasites of animals (Nantulya, et al., 1987) or man (Nantulya, 1989).

Using a brucei MoAb to diagnose rhodesiense sleeping sickness, a 90% correlation between antigenaemia and parasitological diagnosis was reported (Nantulya, 1989). Additionally, the test detected trypanosome antigens in 11% of patients who had no demonstrable parasitaemia. Applying the same MoAb to diagnose T. evansi infections in various animal species, antigens were detected as early as six days following experimental infection and the antigenaemia persisted as long as the animals remained infected (Nantulya, Bajyana Songa, and Hamers, 1989a). As had been found in humans suffering from rhodesiense sleeping sickness, the antigenaemia in T. evansi infections correlated well with parasitological findings and in certain cases, detected antigens where parasites had been missed. With a tube-ELISA as opposed to a microtitre plate-ELISA, the same level of sensitivity was obtained (Nantulya, Lindqvist, Diall and Olaho, 1989b), thus, for the first time, providing a potentially suitable, individual animal diagnostic test in the field.

Despite the high sensitivity of this test, it failed to detect some cases with proven trypanosome infections (Nantulya, 1989; Nantulya et al., 1989a,b). Two hypotheses were advanced as possible explanation for this anomaly. Firstly, in periods of antibody excess, the antigens were likely to be in complexes and therefore, not demonstrable

in the ELISA system used. Secondly, in very early infections, insufficient parasite destruction might produce undetectable levels of circulating antigens. Thus, although the MoAb based antigen-detection system may eventually be the test of choice in diagnosis of trypanosomiasis, some infections will not be detected unless microscopy is used to demonstrate the presence of live trypanosomes (Nantulya, 1989). In certain cases where neither the parasites nor the antigens are demonstrable despite clinical signs, detection of anti-trypanosomal antibodies or immune complexes will be necessary.

The incidence and the economic importance of trypanosomiasis caused by T. evansi are less well documented than that caused by the tsetse transmitted trypanosomes. Nonetheless, T. evansi infections constitute an important constraint to the livestock industry wherever they occur (Lohr et al., 1985; 1986; Touratier, 1988; Luckins, 1988) and effective control of this disease would greatly improve livestock industry.

Animals infected with trypanosomes elicit strong and rapid humoral antibody responses (Holmes and Scott 1982). For this reason, one would expect that an immunological approach to the control of animal trypanosomiasis would be a practical proposition. This would have been the case were it not for the powerful survival strategy of antigenic variation adopted by salivarian trypanosomes, that enables them to survive in an immunized host.

Antigenic variation is the process by which trypanosomes keep varying the antigens that constitute their surface coat. This phenomenon has been known for

many years and may have been described for the first time in 1905 by Franke (cited by Gray, 1965), who found that the serological characteristics of a strain of T. evansi changed in an infected host. Five years later, physicians observed marked periodicity in temperature and parasitaemia of patients infected with trypanosomes and both these parameters coincided with each other (Ross and Thomson 1911, cited by Donelson and Turner, 1985). Ross and Thomson had speculated that, the relapsing parasitaemias were a result of trypanolytic crises following the production of antibodies in the blood. It is only in the last 20 years or so, that these speculations were confirmed and explained.

The subject of antigenic variation has been reviewed by many authors (Vickerman, 1969; Gray and Luckins, 1976; Doyle, 1977; Cross, 1978; Vickerman, 1978; Cross et al., 1980; Donelson and Turner, 1985) and only the relevant practical aspects of it will be dealt with here.

Vickerman (1969) was the first to describe the compact and electron opaque coat that covers the pellicular surface of trypanosomes. Njogu and Humphreys (1972) paved the way with their attempt to purify variable surface glycoproteins (VSGs). Subsequent work by Cross (1973; 1975) revealed that the coat is made of a matrix of identical glycoprotein molecules that differed only on the amino acid sequences. This difference existed even on clones of one parental source, suggesting that, the antigens are coded by different genes. The antigens that cover the surface coat of trypanosomes are now called variable surface glycoproteins (VSGs) and the variant

individual trypanosome variants are referred to as variant antigenic types (VATs). The total repertoire of VATs as determined serologically, that an individual trypanosome possesses is called a serodeme (WHO, 1978). A single clone has been shown to be capable of generating 10^4 to 10^5 VATs (Capbern, Giround, Baltz and Melten, 1977; Van der Ploeg, Valerio, De Lange, Bernads, Borst and Grosveld, 1982) and recent studies indicate that a single trypanosome may have at least 1000 genes that code for VSGs (Van der Ploeg et al., 1982).

The switching of VSG genes appears to be programmed in that, when infections are initiated on separate occasions with the same trypanosome variant, the sequence of successive detectable variants tend to be predictable (Gray and Luckins, 1976; Hudson, Taylor and Elce, 1980) and certain variants tend to predominate (predominant VATs). The predominant VATs are quickly eliminated by the host antibodies. However, in a population of 10^4-10^5 dividing organisms, one trypanosome turns off the genes controlling the expression of the initial VSG and switches to genes for expression of a different VSG (Miller, Allan and Turner, 1984). This leads to the emergence of populations expressing new VSG genes. Thus, during an infection, antibodies are produced against the VSGs of the infecting organisms. A few individual trypanosomes, however, escape because they have acquired a new coat of VSGs to which the available antibodies cannot bind. The variant individuals multiply while the immune system raises another set of antibodies which will eventually kill all the parasites with this coat of VSG, but a few trypanosomes

will have acquired a new coat of VSGs and therefore a new population will proliferate. The parasite in effect remains one step ahead of the host immune response. It is evident that the blood-stream trypanosomes possess such a huge repertoire of reserve antigen coats, that, it is almost impossible to develop a conventional vaccine against them (Nantulya, 1986). T. evansi may possess fewer serodemes than tsetse-transmitted trypanosomes (Hamers, personal communication) and it is, theoretically easier to protect animals immunologically against evansi trypanosomes. To do so however, one would require a cocktail of all the VATS expressed in all different serodemes, a proposition which might be impracticable.

As in T. evansi, the number of VSG's displayed on the surface of metacylic trypanosomes have been shown to be limited (Le Ray, Barry and Vickerman, 1978). Additionally, studies conducted on T. brucei, T. congolense and T. rhodesiense have shown that when clones of one parental source are cyclically transmitted, they tend to revert to a common basic VAT characteristic of the parental clone (reviewed by Nantulya, 1986). These findings have led to speculation that it might be possible to take advantage of the limited VAT repertoire of metacyclic forms in order to develop an effective vaccine (Donelson and Turner, 1985). This approach was considered impracticable by Nantulya (1986) for the following reasons: firstly, although for each serodeme the number of VSGs in the metacyclic trypanosomes are limited, each trypanosome species has very many serodemes and secondly, genetic recombination between parasites of different serodemes may occur during cyclical

transmission, resulting in even greater diversity of metacyclic antigens. For T. evansi this approach would, of course, not be possible since the parasite cannot be transmitted cyclically. Thus, immunoprophylactic control of trypanosomiasis is not at present possible.

In the absence of an immunoprophylactic solution to the problem of trypanosomiasis, control of this disease relies on two approaches: those directed against the vector and those directed against the parasite. For vector control to be effective, a thorough understanding of the vector's biology is essential in order to identify possible weak areas for intervention. In the tsetse- transmitted trypanosomiasis, the fly's biology is well understood and major strides have been made in vector control (Jordan, 1986). Similar claims cannot be made about the biting flies that transmit T. evansi. Hence, control of T. evansi principally relies on the use of trypanocidal drugs.

Despite the numerous years of industrial research into trypanocidal drugs, only five trypanocides are commercially available for the treatment and control of African animal trypanosomiasis (Table 2) and it is now 28 years since the last trypanocide (isometamidium chloride) was introduced onto the market. The slow turn-out of new trypanocides is worrying considering that, in Africa alone, 44 million cattle are exposed to trypanosomiasis (FAO/WHO/OIE, 1982), which represents a large potential market for trypanocidal drugs. Williamson (1976) attributed the slow turn-out of trypanocides to the prohibitive costs of developing and marketing them without an assured market, since the target countries have low

budgets for veterinary drugs and their economic stability is uncertain.

In the absence of new trypanocides, the existing compounds must be used rationally if the much needed increase in livestock production to sustain the rapidly growing human population is to be achieved. Rational use of drugs is, however, subject to severe physical and economic constraints (Holmes and Scott, 1982). In many countries, the veterinary services do not reach the extensively kept livestock, and, therefore, the treatments for trypanosomiasis and other diseases are performed by untrained personnel and often empirically. Leach and Roberts (1981), consider that properly organised mass treatment campaigns may be the method of choice for dealing with trypanosomiasis, since the trypanocide can be administered properly, by qualified personnel, and at the appropriate time. Although this may be the case, the logistical problems that would have to be overcome in organising and conducting such campaigns, are enormous. To have the desired impact on the disease, such campaigns would have to be related to the period of risk. This would require a sound understanding of the epidemiological patterns of the disease - an exacting requirement for countries with small veterinary budgets.

Another important consideration in the use of trypanocides is the target organisms against which the drug is intended for. For example, T. evansi infections, like those of the other members of sub-genus Trypanozoon, are more sensitive to suramin and quinapyramine than other trypanosome species (Leach and Roberts, 1981). The reasons

Table 2. Drugs currently used for the treatment and prophylaxis of animal trypanosomiasis. (Adapted from Kuttler and Kreier, 1986).

Generic name	Dose ($mgkg^{-1}$) and route	Activity	Susceptible trypanosomes
Suramin	7.0-10.0 i.v.	Curative	T.evansi T. brucei T. equiperdum
Quinapyramine dimethyl-sulphate	3.0-5.0 s.c.	Curative	T. evansi T. equiperdum T. brucei T. congolense T. vivax
Quinapyramine prosalt:	3.0-5.0 s.c.	Prophylactic	As for sulphate
Diminazene aceturate	3.5-7.0 i.m.	Curative	T. vivax, T. congolense T. brucei T. evansi
Isometamidium chloride	0.25-0.5 i.m.	Prophylactic	T. vivax T. congolense brucei sp.* T. evansi*

Key

i.m. = intramuscular s.c.= subcutaneous

* = less susceptible i.v. = intravenous

for this differential susceptibility are not clear, but may be related to the organism's propensity to invade body tissues. The host, too, is important when considering what drug to use. For example, the trypanosome curative dose of diminazene aceturate is toxic to camels and is, therefore, not recommended for use in this species (Leach, 1961). These two aspects tend to narrow the range of drugs that can be effectively and safely used for each animal species. For the camel, the only recommended trypanocides are suramin and quinapyramine although intravenous use of isometamidium has been reported (Balis and Richard, 1977).

Suramin was first developed as an offshoot of the dyestuff industry by chemists working for the Bayer Company during the first world war. It was introduced on the market in 1920 to treat human trypanosomiasis but was later shown to be effective against T. evansi infections in camels (Knowles, 1925), horses and cattle (Edwards, 1926)

With the exception of quinapyramine, the efficacy of suramin on trypanosomes of the subgenus Trypanozoon is greater than that of any other trypanocides marketed for animal trypanosomiasis (Leach and Roberts, 1981). It is less effective against T. congolense and T. vivax (Ruchel, 1975).

The recommended dosage is a single injection of 7 to 10 mg kg^{-1} (Kuttler and Kreier, 1986). Generally however, in camels, the drug is given as 10 g in a 10% aqueous solution irrespective of the weight of the camel. Lower dosages (5 g and below) have been tried but were considered likely to result in drug resistance (Gatt-Rutter, 1967). In horses, two intravenous injections

of 10 mg kg^{-1} in 10% aqueous solution at an interval of one week are recommended (Leach and Roberts, 1981).

The mode of action of suramin is still unknown (Williamson, 1976) although it has been shown to inhibit several trypanosomal enzymes (Fairlamb and Bowman, 1977; Fairlamb, 1981). The drug is said to be trypanostatic and its effect is, therefore, dependent on host immunological mechanism to clear the parasites (Leach and Roberts, 1981). Suramin is extremely anionic and binds avidly to plasma proteins in which form it enters the trypanosome via endocytosis (Fairlamb and Bowman, 1977). Once complexed to plasma proteins, it is excreted slowly and may persist in the bloodstream for upto 3 months . This could explain its prophylactic activity (Dewey and Wormall, 1946). Unfortunately, trypanosomes multiplying in blood in which the levels of drug are not curative develop tolerance to the drug and this may have contributed to development of resistance to suramin (Leach and Roberts, 1981).

In the mid 1920s, research in strylquinoline compounds showed some of them to have trypanocidal activity, mainly against the trypanosomes of the brucei group (Browning, Cohen, Ellingworth and Gulbransen, 1953). One such strylquinoline was quinapyramine. Field trials conducted by Davey (1950) showed that quinapyramine had a wide range of action, being active against T. evansi, T. equiperdum, T.b. brucei, T. congolense and T. vivax (Curd and Davey, 1950; Davey, 1950).

The drug was presented as two salts, namely, a readily soluble dimethyl sulphate and the sparingly soluble chloride. After subcutaneous injection, the former was

quickly absorbed, making it better for curative purposes, whilst the latter formed a depot in the subcutaneous tissue from where it was slowly absorbed and thus, would maintain prophylactic levels of the drug (Spinks, 1950). The drug blood levels attained from the depot were, however, too low to be curative (Curd and Davey, 1950). In order to increase absorption, a mixture of the two salts containing three parts of dimethylsulphate and two parts of chloride was found to give high levels of drug in the blood and was subsequently made and given the trade name ''Antrycide'' prosalt (Imperial Chemical Industries, UK). The chloride salt gave the protection while the dimethylsulphate salt destroyed the trypanosomes that might have been present at the time of treatment. The dose to be given was measured in terms of dimethylsulphate so as to ensure enough curative dose was always given. The recommended dosages were 3 and 5 mg kg^{-1} body weight, administered subcutaneously. This formulation was found to provide up to three months protection to cattle under low tsetse challenge and two months in medium challenge situations (reviewed by Davey, 1958).

Davey (1950) found that the trypanosomes "breaking through" the waning concentration of quinapyramine towards the end of its prophylactic period could become resistant to the drug. To overcome this, he advised re-treatment soon enough to prevent emergence of breakthrough resistant strains. Nonetheless, extensive field resistance was eventually developed by T. congolense and T. vivax (Davey 1958) and the drug was withdrawn from the market in 1977.

In 1984, quinapyramine was re-introduced on the

market, under the trade name "Trypacide" (May and Baker Ltd, Dagenham, U.K.) mainly for the treatment and prevention of T. evansi infections in camels and equines.

Quinapyramine when given subcutaneously to camels as either salt causes tissue reaction at the injection site, resulting in oedematous swelling. Also the prosalt depot causes an inflammatory reaction that produces a hard encapsulated swelling at the site of injection. The tissue reaction may be minimised by massaging the injection site (Davey, 1958).

The maximum amount of dimethylsulphate by itself or as contained in the prosalt recommended for any species of animal is 5 mg kg^{-1}, and even at this dosage, some animals show signs of parasympathetic stimulation. These include: increased restlessness, salivation, diarrhoea, sweating, uncontrolled tremors and stamping of feet. The severity of these reactions may be exacerbated by stress (Davey, 1950). In the horse, these symptoms may be marked and death can occur. To minimise these side reactions, it is advisable to divide the dose for horses into two or three equal parts, given at intervals of six hours.

Quinapyramine is trypanostatic and an effective dose may take from two to four days to clear trypanosomes (Ormerod, 1951). Such trypanosomes were found by Ormerod not to be infective 24 hours after treatment. Newton (1964) found that quinapyramine inhibits RNA synthesis. Bacchi (1981) suggested that it might also interfere with polyamine biosynthesis.

Diminazene aceturate was developed from a quinoline derivative (Jensch, 1958) by a chemist at Hoechst, West

Germany. It was introduced in the market under the trade name "Berenil" and gained widespread use.

Fussanger and Bauer (1958) found that a single dose of 3.5 mg kg^{-1} will eliminate infections caused by T. congolense and T. vivax but a higher dose (5.0 mg kg^{-1}) was required to eliminate brucei type trypanosomes (Petrovoski, 1974). Unlike other trypanocides, diminazene aceturate had a higher safety margin and in cattle, up to 21.0 mg kg^{-1} could be administered safely . In the field, however, 7.0 mg kg^{-1} is normally the maximum dosage used. The drug is toxic to camels and deaths occur at doses of 7.0 mgkg^{-1} (Leach, 1961), while lower dosages do not cure trypanosomes. Therefore it is contraindicated in camels.

The other group of trypanocides available on the market are the phenanthridines which include: homidium bromide, homidium chloride and isometamidium. These trypanocides are mainly used for treatment of T. vivax and T. congolense infections; the brucei group trypanosomes being less sensitive to them (Balis and Richard, 1977),

There are various conflicting reports in the literature concerning the use of isometamidium chloride on T. evansi infections in various animals (reviewed by Balis and Richard, 1977; Touratier, 1988). From the review of Balis and Richard, it appears that a dosage of 0.25 to 1.0 mg kg^{-1} is recommended for all species except donkeys, where up to 2 mg kg^{-1} might be necessary.

Both the intramuscular and intravenous routes can be used. When administered intramuscularly, isometamidium chloride produces a severe local reaction. It is important,

therefore, to ensure that intramuscular injections are sited deep within the muscle and that not more than 0.5 mg kg^{-1} of drug should be injected at one site. Intravenously injected drugs must be administered slowly, and not more than 1 mg kg^{-1} should be injected. However, the intravenous route will provide a shorter protective period, but it has the advantage of rapid effect and carcase damage does not occur.

Phenanthridines are intercalating agents and are thought to act on DNA by binding between adjacent nucleotide pairs, thereby preventing DNA replication (Newton, 1974). Additionally, they may interfere with polyamine synthesis (Bacchi, 1981).

The extensive use of these few trypanocides has resulted in the appearance of drug resistant trypanosomes, which has been reported for all the trypanocides available on the market (reviewed by Peregrine, 1987). The situations under which drug resistance develops have been reviewed by Leach and Roberts (1981), and were said to be those that favour the multiplication of trypanosomes in levels of drugs which were insufficient to prevent infection from occurring. Trypanosomes multiplying in such media show loss of response to concentrations which previously would have been toxic. In the field, such situations could occur because of frank underdosing, or when prophylactic treatments are irregular, or stopped while the animals are still at risk. Frank underdosing is likely to be prevalent in areas where veterinary supervision is minimal. The usage of trypanocides on farms where veterinary supervision is permanently available may

also expose trypanosomes to subcurative levels of drugs if treatments are not related to prevailing local epidemiological situations. Determination of the period of risk is, therefore, important in order to ensure that the inter-treatment interval is such that high levels of drug are present at the time of high challenge.

Drug resistance can, however, occur naturally, in which case, it is not dependent on previous exposure to the drug concerned (Leach and Roberts, 1981). For example, brucei group trypanosomes are less sensitive to phenanthridines than T. congolense or T. vivax. It is, therefore, prudent to know the identity of the parasites likely to be in an area, so that proper chemotherapeutic or chemoprophylactic drugs can be given.

Many of the trypanocides for animal trypanosomiasis currently available on the market (except suramin) have been developed by a process of "hybrid synthesis" which involved combining portions of already known trypanocides (Leach and Roberts, 1981), and this process is considered to have favoured the development of cross-resistance. Because of this, Whiteside (1958) advised the use of "sanative drug pair" (pairs of drugs that are chemically unrelated) to combat cross-resistance.

In the field, recurrence of parasitaemia at a period when circumstances would have precluded reinfection is usually attributed to emergence of drug resistant strains of the parasite. This, however, need not be the case always as trypanosomes can be present in tissues inacessible to the drugs (Jennings et al., 1979). The way in which such trypanosomes escape the action of

trypanocides has been shown to involve the existence of certain privileged sites such as the brain, aqueous humor of the eye and pericardial fluid in which the drugs do not reach lethal concentrations (Jennings, Holmes, Chizyuka and Urquhart, 1979; ILRAD, 1984). According to evidence provided by Jennings and colleagues, the timing of treatment relative to the time of infection is crucial in the development of relapse infections. In the field, it is difficult to know how long infections have been ongoing and, therefore, this consideration has no practical value.

A more practical approach is to use trypanocides that can cross tissue barriers whenever relapses from such tissues are suspected. One such drug is melarsoprol which is used for treating late cases of human trypanosomiasis (Apted, 1970). A related compound, Melarsamine chlorhydrate (Cyamelarsan, Rhone Poulenc, France), is at present being considered for registration for treatment of trypanosomiasis in camels with central nervous system involvement (Sones, Rhone Poulenc, personal communication).

Despite the afore-mentioned bleak situation in trypanocide usage, chemotherapy is at the moment the only practical method of managing T. evansi infections and in the trypanosomiasis endemic area of northern Kenya, camels were shown to be more productive when a trypanocide was used than when it was not (Rutangwenda, 1984).

A sound knowledge of the local epidemiological patterns is imperative to any trypanosomiasis control programme. Under nomadic camel husbandry, the development of successful disease control programmes would lead to bigger and more productive herds (Bornstein, 1988) and

would, therefore, improve the living standards of the pastoralists. In their present conditions, the arid lands of Kenya can support more animals (IPAL, Technical report Number A-6, 1984). The camel is more productive than other livestock kept in the same environment and, considering the fragility of the arid land environments, the camel might be the animal of choice for exploitation, since, it has several attributes that minimize the danger of environmental degradation (Hjort, 1988). It has big soft feet, so the vegetation cover is not trampled. It browses in bush rather than grazes on a sparse grass cover. Its selective browsing behaviour leads to an even environmental ''wear and tear''.

For the camel to contribute effectively in alleviation of malnutrition in the arid areas of northern Kenya, rational control programmes of camel trypanosomiasis would have to be formulated. To do so, it is imperative to have accurate epidemiological data pertaining to the trypanosomes prevalent in endemic areas. It was in the light of this that the studies described in the next three chapters were conducted.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 THE STUDY AREA

2.1.1 LOCATION OF STUDY AREA

The investigations were conducted on camel herds located at Ngurunit and Olturot, which were field stations within the UNESCO - IPAL study area (Chapter 1), in Marsabit District, Northern Kenya (Figure 1).

A small laboratory was established at Ngurunit, from where all the specimens were processed and stored.

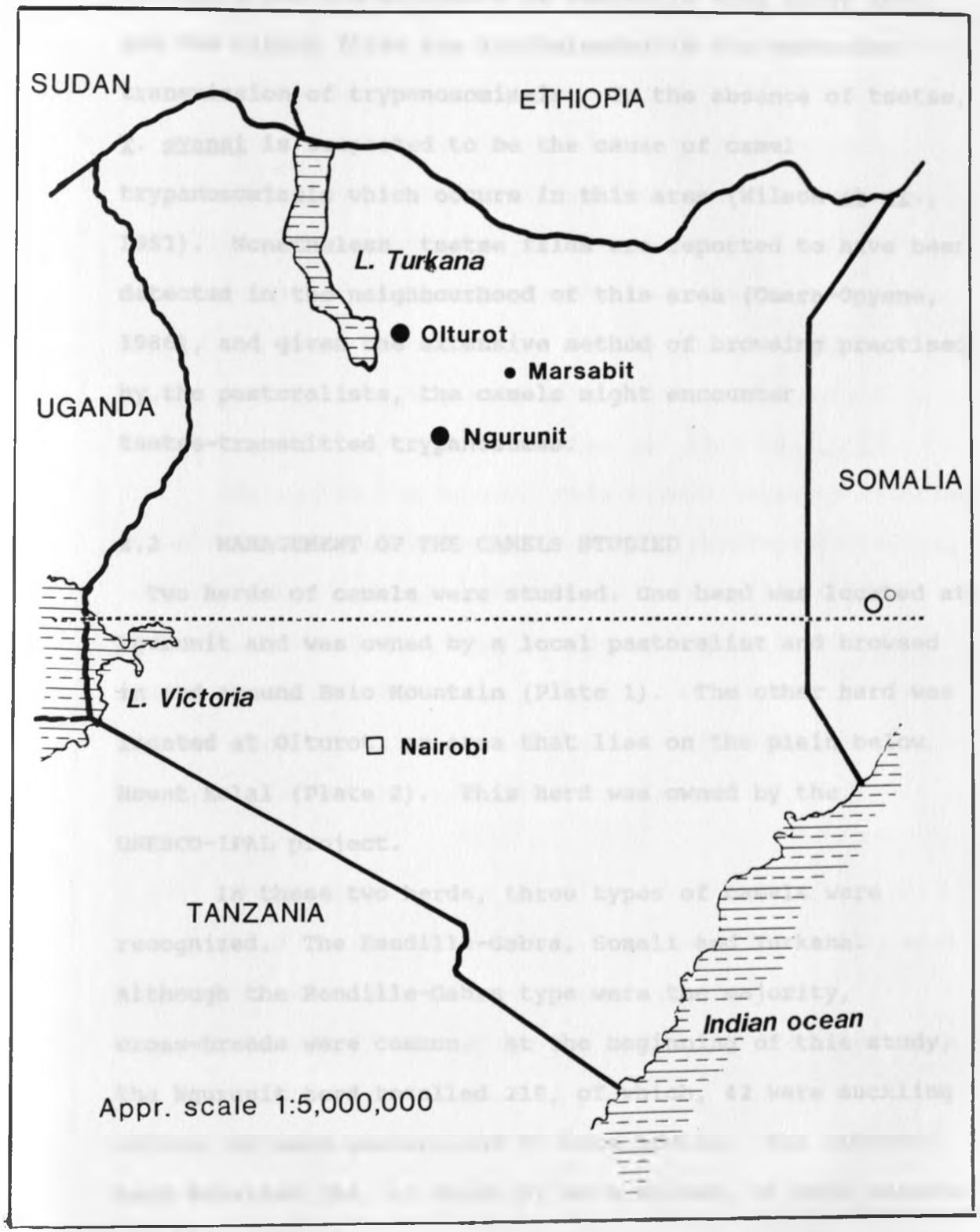
2.1.2 CLIMATE

The area has a typical semi-arid climate (IPAL Technical Report A-6, 1984). The rainfall periods are short with a highly variable distribution. Heavy rains result in floods which last for a few days, usually from late March through May and again from late October through early December. In between these rainy periods, the weather is characteristically hot and dry. The annual total rainfall at Ngurunit and Olturot ranges from 300 to 700 mm and 100 to 600 mm, respectively.

2.1.3. VEGETATION

The principal vegetation types are annual grassland dominated by *Aristida* spp., dwarf shrubland dominated by *Duosperma eremophilum* and *Indigofera spinosa* and shrubland dominated by *Acacia reficiens* (IPAL Technical report A-6, 1984). The *Indigofera* spp. and *Acacia* spp. are the preferred browse for the camel.

Figure 1 : **Location of camel herds in Ngurunit and Olturot, Northern Kenya**



2.1.4 TRYPANOSOME VECTORS

Five genera of biting flies have been identified in this area namely: Tabanus, Philoliche, Atylotus, Hippobosca and Stomoxys (Omara-Opyene, 1986). No evidence is available for the existence of tsetse in this study area and the biting flies are incriminated in the mechanical transmission of trypanosomiasis. In the absence of tsetse, T. evansi is suspected to be the cause of camel trypanosomiasis which occurs in this area (Wilson et al., 1983). Nonetheless, tsetse flies are reported to have been detected in the neighbourhood of this area (Omara-Opyene, 1986), and given the extensive method of browsing practised by the pastoralists, the camels might encounter tsetse-transmitted trypanosomes.

2.2 MANAGEMENT OF THE CAMELS STUDIED

Two herds of camels were studied. One herd was located at Ngurunit and was owned by a local pastoralist and browsed in and around Baio Mountain (Plate 1). The other herd was located at Olturot, an area that lies on the plain below Mount Kulal (Plate 2). This herd was owned by the UNESCO-IPAL project.

In these two herds, three types of camels were recognized. The Rendille-Gabra, Somali and Turkana. Although the Rendille-Gabra type were the majority, cross-breeds were common. At the beginning of this study, the Ngurunit herd totalled 218, of which, 42 were suckling calves, 90 were weaners and 86 were adults. The Olturot herd totalled 154, of which 21 were calves, 38 were weaners and 95 were adults.

From each of the two herds, 20 suckling calves, 20 weaners and 20 dams were selected randomly for the study and double ear-tagged for identificaton purposes.

The selected animals were managed traditionally and grazed together with the others in the herd. They differred only in the application of the anthelmintics and trypanocides (section 2.2.1).

The animals grazed extensively by day and were penned at night. In the herd owned by the pastoralists, the calves were grazed and penned separately from their dams to restrict their milk intake and therefore ensure a supply for human consumption. In the institutionally owned herd, the calves were grazed with the dams and were also penned together at night and hence had milk ad libitum.

During the dry season, camels were taken to watering wells every nine days. Sometimes this involved travelling long distances and the camels would be away for a number of days during which time they would also mix with other camels from different areas. During the wet season, the animals were not taken for watering as the water content of the green forage was considered sufficient.

2.2.1 DRUGS USED

Prior to the start of the experiment and every month thereafter, all animals were treated orally with thiophanate 20% w/v (Nemafax; May and Baker Ltd., Dagenham, UK) at a dosage rate of 66.7 mg kg^{-1}.

Quinapyramine dimethylsulphate (Trypacide sulphate, May and Baker Ltd., Dagenham, UK) was used to treat trypanosome infections. Quinapyramine prosalt (Trypacide

prosalt, May and Baker Ltd., Dagenham, UK) was used for prophylaxis. The drugs were freshly prepared in distilled water as 2% (w/v) solution (for quinapyramine sulphate) and suspension (for quinapyramine prosalt) and were used at a dosage of either 3 mg kg^{-1} or 5 mg kg^{-1} (section 4.3.1). Both quinapyramine sulphate and prosalt were administered by subcutaneous injection in the lower part of the neck using an 18 G x 3.75 cm needle. Prior to drug administration, the injection site was disinfected with 70% ethanol.

Occasionally, Delnav Pye grease (Cooper Ltd, UK), an organophosphate insecticide, was applied to the nostrils and inguinal areas, which were the pre-dilection sites of Hyalomma dromedarius, the tick commonly found in this area.

Camel bodyweights were estimated prior to the administration of drugs. The weights were estimated by measuring (in metres) the shoulder height (SH), the thoracic girth (TG) and the abdominal girth (AG). Using the formula SH x TG x AG x 50 = liveweight (kg), the bodyweights were calculated (Schwartz, Dolan and Wilson, 1983).

2.2.2 COLLECTION, PREPARATION AND STORAGE OF BLOOD SPECIMENS

The camels were restrained by the owners. For the animals with good temperament, the lips were held tightly which effected good restraint. For the animals of bad temperament, it was neccessary, in addition to holding the lips tightly, to flex and tie the forelimb at the carpus joint with a rope such that, the animal stood on three legs.

Blood was collected from the jugular vein which had been occluded by a rope, venipuncture being performed with an 18 G x 3.75 cm needle.

Unclotted blood for packed cell volume (PCV) determination and buffy coat examination for trypanosomes was collected in labelled 10 mls re-used vacutainer tubes (Greiner), containing 0.2 ml of 10% ethylene-diamine tetra-acetate (EDTA). Each blood sample was kept in a cool box containing ice soon after collection and the samples were processed for the PCV determination and for the detection of trypanosomes after the whole herd was sampled.

Serum samples for use in immunodiagnosis were taken into clean universal bottles and allowed to clot. The clot was separated from the sides of the bottles after one hour using a wooden applicator stick and then kept in a refrigerator overnight. Serum from each animal was aliquoted into four 500 ul plastic tubes and stored at -20^{o}C in a kerosene freezer. Every month, the samples were transported in a cool box packed with ice to Kenya Trypanosomiasis Research Institute (KETRI), where they were kept frozen at -20^{o}C until required.

2.2.3 PREPARATION OF TRYPANOSOME ISOLATES

Each stock was raised by intra-peritoneal inoculation of fresh parasitaemic camel blood into two mice. Wet preparations of mouse tail blood were examined three times a week, and at peak parasitaemia, the mice were anaesthetized with diethyl-ether, and blood was collected by cardiac puncture into a 1.0 ml syringe containing

heparin at a concentration of 10 units ml^{-1}. The blood was placed in a glass tube and glycerol was added dropwise and thoroughly mixed to give a final concentration of 10%. The blood was then drawn into plain microhaematocrit capillary tubes (leaving an air space at both ends) and one end was sealed with plasticine. Tubes containing the same population of trypanosomes were placed in 5 ml freezing tubes (Greiner, West Germany) labelled and allowed to freeze for 24 hours in the vapour phase of liquid nitrogen before immersion into the liquid phase (-196^{o}C) where they remained until required.

Plate 1. View of Baio Mountain, around which the herd at Ngurunit browsed. Note the camels and the goats in the background.

Plate 2. View of Mount Kulal, around which the herd at Olturot browsed. Note the camels in the background.

CHAPTER 3

CHARACTERIZATION OF TRYPANOSOMES ISOLATED FROM THE STUDY CAMELS

3.1 INTRODUCTION

Infections of camels with trypanosomes belonging to the genus Trypanozoon are usually attributed to Trypanosoma evansi. The assumption made is that since camels are generally kept in areas removed from the delineated tsetse habitat, the trypanosomes encountered in such areas are not tsetse-transmissible. This, however, need not be the case as tsetse may be present in an area without being detected (Wells, 1972). Besides, the camels in northern Kenya graze far and wide and could have come into contact with Glossina which is present in the neighbourhood (see 2.1.4), and therefore could have acquired tsetse-borne trypanosomes.

In designing a control strategy for T. evansi infections in camels in any given endemic area, it is essential to ascertain the identity of the prevalent trypanosomes. This is because the tsetse-transmitted brucei-types and the mechanically transmitted T. evansi require different control strategies. The control of the former trypanosomes is based on both chemotherapy and reduction of the vector populations (Jordan, 1986), while control of T. evansi is by chemotherapy and chemoprophylaxis. Techniques are now available to address the question of T. evansi identity.

Various techniques were used to characterize the trypanosome stocks isolated from the camels, and the results are reported in this chapter.

3.2 EXPERIMENT 1 - CHARACTERIZATION OF THE TRYPANOSOME ISOLATES BY MORPHOLOGY AND TSETSE TRANSMISSION.

3.2.1. MATERIALS AND METHODS

3.2.1.1. EXPERIMENTAL ANIMALS

Mice: Male and female outbred Swiss mice, aged 8 to 12 weeks, were obtained from the KETRI breeding colony.

Rats: Large ex-breeder female Sprague Dawley rats were obtained from the ILRAD colony.

Rabbits: Male and female New Zealand white rabbits, aged between two to three months, were obtained from the KETRI colony.

3.2.1.2. TRYPANOSOMES

Forty-eight trypanosome stocks (Table 3) collected between July 1986 and December 1987 as described under section 2.2.3 from two separate camel herds in northern Kenya (Figure 1) were studied.

3.2.1.3. TSETSE

Teneral male and female Glossina morsitans morsitans were obtained from the KETRI tsetse colony.

3.2.1.4. EXPERIMENTAL DESIGN

Each of the 48 trypanosome stabilates (Table 3) was thawed and aspirated from the capillary tube into a 1.0 ml syringe containing 0.3 ml phosphate saline glucose (PSG), pH 8.0 (57.0 mM Na_2HPO_4, 2.3 mM NaH_2PO4, 42.8 mM NaCl, 55.5 mM glucose) and inoculated intra-peritoneally

Table 3 : A summary of information on the origin of trypanosome populations used.

Camel number	Isolate designation	Location	Month of isolation
902	NGT, 902	Ngurunit, Kenya	July, 1986
920	NGT, 920	,,	,, ,,
921	NGT, 921	,,	,, ,,
928	NGT, 928	,,	,, ,,
929	NGT, 929	,,	,, ,,
932	NGT, 932	,,	,, ,,
907	NGT, 907	,,	August, 1986
922	NGT, 922	,,	October, 1986
803	OLT, 803	Olturot, Kenya	August, 1986
807	OLT, 807	,,	,, ,,
808	OLT, 808	,,	,, ,,
811	OLT, 811	,,	,, ,,
813	OLT, 813	,,	,, ,,
857	OLT, 857	,,	,, ,,
819	OLT, 819	,,	,, ,,
812	OLT, 812	,,	December, 1986
916	NGT, 916	Ngurunit, Kenya	April, 1987
934	NGT, 934	,,	,, ,,
935	NGT, 935	,,	,, ,,
958	NGT, 958	,,	,, ,,
904	NGT, 904	,,	May, 1987
937	NGT, 937	,,	,, ,,
938	NGT, 938	,,	,, ,,

Table 3 continued.....

Camel number	Isolate designation	Location	Month of isolation
941	NGT, 941	Ngurunit, Kenya	June, 1987
943	NGT, 943	,,	June, 1987
944	NGT, 944	,,	,, ,,
901	NGT, 901	,,	July, 1987
903	NGT, 903	,,	,, ,,
906	NGT, 906	,,	,, ,,
908	NGT, 908	,,	,, ,,
918	NGT, 918	,,	,, ,,
923	NGT, 923	,,	,, ,,
927	NGT, 927	,,	,, ,,
930	NGT, 930	,,	,, ,,
931	NGT, 931	,,	,, ,,
939	NGT, 939	,,	,, ,,
942	NGT, 942	,,	,, ,,
945	NGT, 945	,,	,, ,,
946	NGT, 946	,,	,, ,,
948	NGT, 948	,,	,, ,,
950	NGT, 950	,,	,, ,,
959	NGT, 950	,,	,, ,,
960	NGT, 960	,,	,, ,,
810	OLT, 810	Olturot, Kenya	March, 1987
820	OLT, 820	,,	July, 1987
833	OLT, 833	,,	,, ,,
840	OLT, 840	,,	,, ,,
854	OLT, 854	,,	,, ,,

into two rats which had been immunosuppressed by irradiation (650 kilorads) from a caesium source at ILRAD. A well-defined T.b. brucei stock, (KETRI 2502, Gibson et al., 1983) was inoculated into other rats to provide controls. At peak parasitaemia, thin blood smears were made from the rats' tails, fixed with methanol and stained with Giemsa's stain. Thereafter, teneral male and female G.m. morsitans, were allowed to feed on the rats. To facilitate uninterrupted feeding by tsetse, the rats were restrained in wire cages that allowed minimal movements by the animals. For each stabilate, 30 tsetse were fed on the flanks of the rats (15 flies per rat) until all flies were visibly engorged. Thereafter, the flies were maintained by daily feeding for 15 min, on a normal rabbit's ears (one rabbit per group of tsetse infected with a particular stabilate). On day 36, the flies were killed with chloroform, dissected and examined for trypanosomes in the gut, proboscis and salivary glands (Baker, 1970). Flies that died before day 36 were also dissected and checked for trypanosome infection. The transmission experiment for the reference T. brucei was repeated twice.

The rabbits used for feeding the flies were bled every other day from a punctured marginal ear vein and unclotted blood was collected directly into heparinised microhaematocrit capillary tubes (Hawskley and Sons Ltd, U.K.) and examined for the presence of trypanosomes by the haematocrit centrifugation technique (Murray et al., 1977).

3.2.2. RESULTS

3.2.2.1. PARASITE MORPHOLOGY :

Examination of Giemsa stained thin blood smears from camel and rats (the latter being done at peak parasitaemia) showed that all the 48 trypanosome stabilates were monomorphic and predominantly of the long, slender forms (Plate 3a and 3b). Examination of smears from rats, inoculated with the reference T. b. brucei stock (KETRI 2502), taken at peak parasitaemia revealed polymorphic trypanosome populations, containing the short stumpy forms, the intermediate forms and long, slender forms.

3.2.2.2. TRYPANOSOME INFECTIONS IN TSETSE :

None of the 48 stabilates was infective to G.m. morsitans. Of the 30 flies that were fed on T.b. brucei infected rats, two flies developed mature infections, which were detected in salivary glands and mouth parts by day 36,when they were dissected. In the two repeat experiments, 4 and 3 flies developed mature infections respectively. Of the flies that died before day 36, only those infected with the reference T.b. brucei stock had live trypanosomes in the midguts (Plate 4).

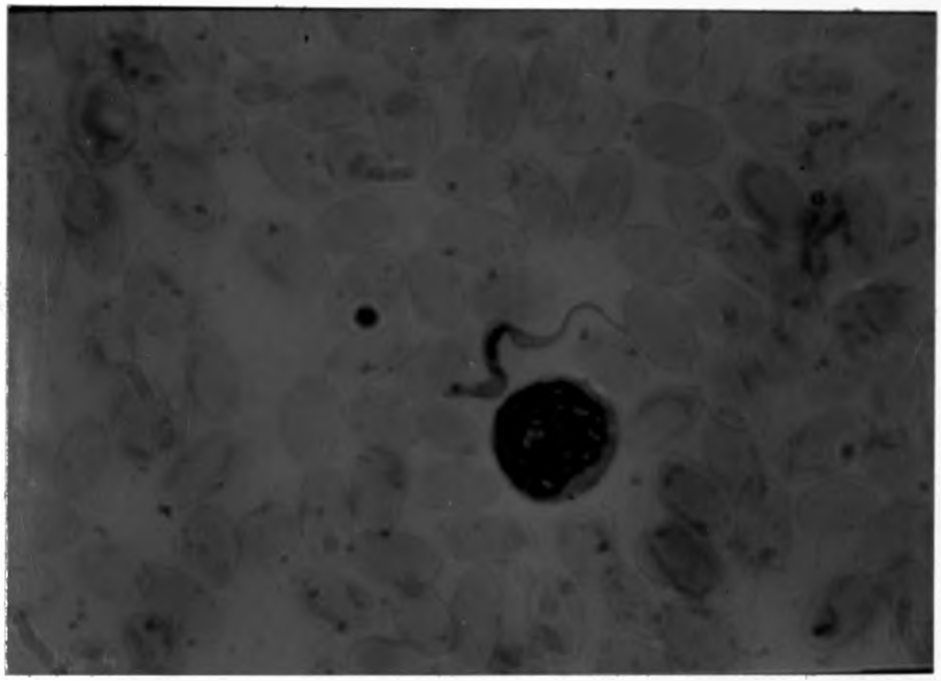
3.2.2.3. RABBITS :

The rabbits used for feeding of tsetse flies infected with T.b. brucei, developed chancres on the ears (Plate 5) by day 21 and became parasitaemic by day 24. In contrast, no chancres developed in the rabbits used for feeding tsetse which had the initial blood meal from rats

infected with any of the 48 T. b. brucei-like isolates from camels. In addition, none of these rabbits became parasitaemic.

Plate 3a. Long slender forms of brucei-type trypanosomes in camel blood, photographed at X1000 magnification. Note the oval shaped camel erythrocytes

Plate 3b. Long slender forms of brucei-type trypanosomes in rat blood, photographed at X1000 magnification.



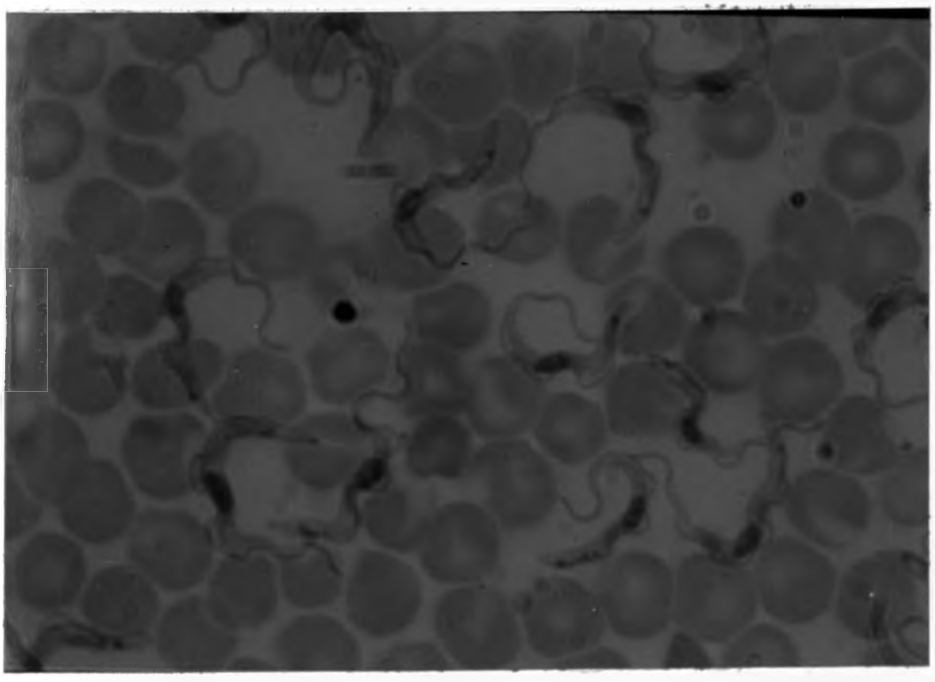
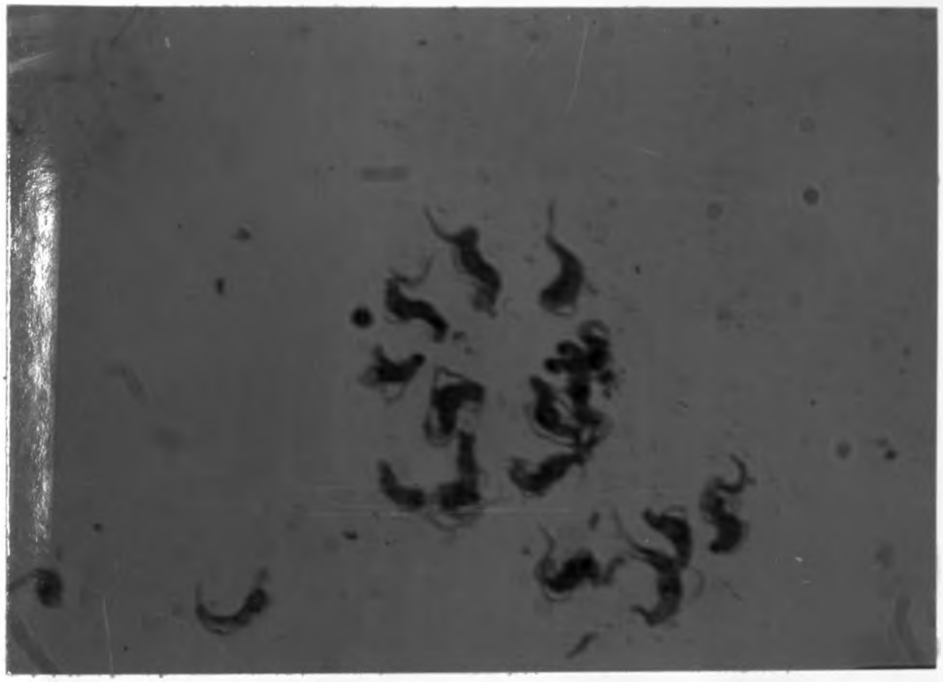
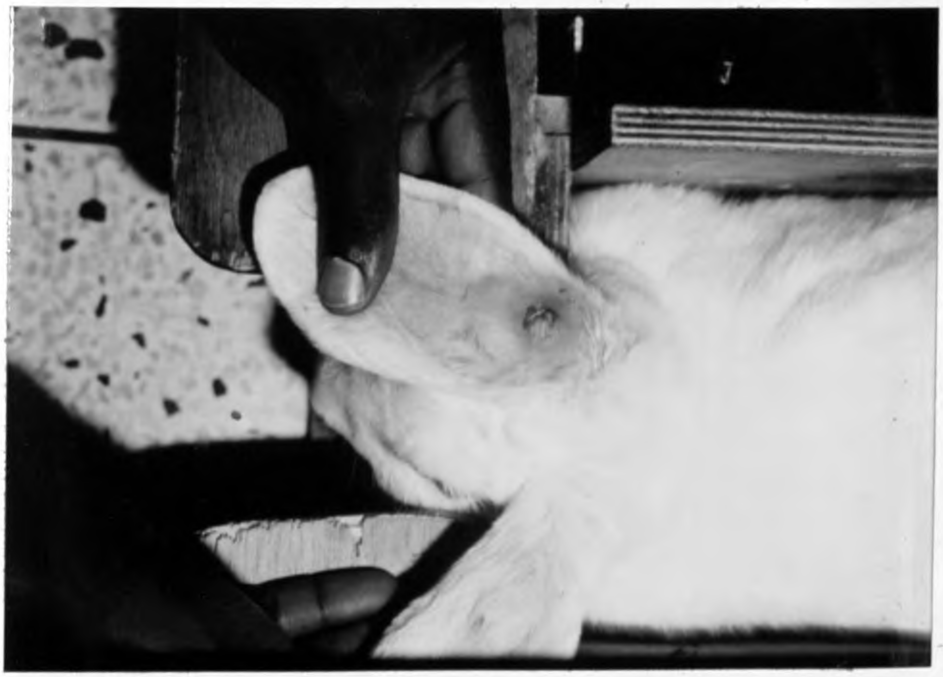


Plate 4. Insect form trypanosomes (procyclics) in the tsetse midgut, photographed at X1000 magnification.

Plate 5. Rabbit showing local reaction, or 'chancre' at the site of tsetse bite, where trypanosomes multiply.





3.3 EXPERIMENT 2 - CHARACTERIZATION OF THE TRYPANOSOME ISOLATES BY ISOENZYME ANALYSIS

3.3.1. MATERIALS AND METHODS

3.3.1.1. GROWTH AND ISOLATION OF TRYPANOSOMES

In addition to the 48 trypanosome stocks from the camels (Table 3), two other stocks (a well defined T.b. brucei IL 2280; Onyango, Van Hoeve and De Raadt, 1966 and T. evansi KETRI 2472; Gibson et al., 1983) were included. A stabilate of each trypanosome stock (section 3.2.1.2.) was thawed and aspirated from the capillary tube into a 1.0 ml syringe containing 0.3 ml PSG, pH 8.0. When live trypanosomes were detected by microscopy, the 0.3 ml trypanosome suspension was inoculated intraperitoneally into one adult rat (3.2.1.1.) which had been immunosuppressed by irradiation (650 kilorads). Wet preparations of tail blood from the rat were monitored daily and at peak parasitaemia (usually between 4-7 days), the rat was anaesthetised with diethyl-ether and blood was collected into a 10 ml syringe containing 0.5 ml of sodium citrate glucose (100 mM trisodium citrate, 40 mM glucose, pH 7.7) by cardiac puncture, which was performed with an 18 gauge hypodermic needle. The blood was centrifuged at 2500 rpm for 10 min at 4^{o}C in a" Minifuge" 2 (Heraeus Christ, West Germany) in a 10 ml plastic tube (Sterilin, U.K.) whose internal diameter did not exceed 1.5 cm. The buffy coat containing the majority of trypanosomes was taken and suspended in 5 ml PSG. Trypanosomes were separated from the blood cells by diethylaminoethyl (DEAE) cellulose anion exchange chromatography using the method of Lanham and Godfrey

(1970). Briefly, the method was as follows : DEAE cellulose (DE-52, Whatman, England) was equilibrated with PSG and placed in a 10 ml syringe barrel plugged at the nozzle with glass wool. The trypanosome suspension was layered on top of the cellulose and trypanosomes were eluted with PSG while the blood cells remained adsorbed to the cellulose.

3.3.1.2. PREPARATION OF TRYPANOSOME LYSATES -

Each of the 50 stocks was passaged in rats and trypanosomes isolated (section 3.3.1.1) at peak parasitaemia through a DEAE-cellulose column. The trypanosomes were washed twice with PSG by centrifugation at 2500 rpm for 10 min. in a "Minifuge" 2. The pelleted trypanosomes in a 5 ml freezing tube (Greiner, West Germany), were lysed with an equal volume of distilled water containing enzyme stabilizers (1 mM dithiothreitol, 1 mM aminocaproic acid and 1 mM di-sodium ethylene diaminetetra acetate, final concentrations in lysate) followed by freezing in liquid nitrogen and thawing at room temperature. The freeze-thaw cycle was repeated three times. Thawed lysates were centrifuged at 14,000 rpm for 30 min at 4^{o}C in a refrigerated micro-centrifuge (Tomy MR-150, Japan) and the supernatant containing the soluble enzymes was stored as 25 ul frozen droplets ("beads") in liquid nitrogen. Also, lysates of red blood cells from normal rats were made as described above.

3.3.1.3. PREPARATION OF THIN LAYER STARCH GELS

For each run, 50 ml of 9% starch gels were prepared by boiling hydrolysed starch (Connaught Laboratories Ltd., Canada) in gel buffer appropriate for each enzyme (Table 4). The resulting solution was de-aerated using a water aspirator pump, poured onto a 14 cm x 18 cm glass gel-forming plate and then spread into a thin layer. The solution was allowed to gel for 10 min at room temperature and then left at 4°C for at least one hour before use.

Sample slots were made along a straight line on one side of the gel with a scalpel blade. For each sample, one "bead" (section 3.3.1.1) was removed from liquid nitrogen into 1.5 ml polypropylene micro-centrifuge tube (Eppendorf, West Germany), thawed and placed on ice. Boiled cotton threads, cut to fit the size of the slot, were soaked in the lysates and then tucked in the slots with a pair of fine forceps.

3.3.1.4. ELECTROPHORESIS

The loaded gels were transferred to the electrophoretic tank containing the appropriate buffer for each enzyme (Table 4) and rested on a cooling plate at 4°C.

Two wicks were placed 10 cm apart over either side of the gel with one end of each wick immersed in the tank buffer. The wicks were then held in position by placing another glass plate over them. With an LKB (Bromma, Sweden) power supply, the samples were run from cathode to anode under selected electrophoresis conditions as shown in Table 5.

TABLE 4 : ENZYME DEVELOPERS USED

Enzyme	Buffer	Substrate	Co-enzyme	Linking enzyme	Additional ions	Visualisation method*
AlA1	6 ml 0.019M NaH_2PO_4 0.081M Na_2HPO_4, pH 7.4	10 mg x-ketoglutaric acid 120 mg L-alanine	3 mg NADH	80 U LDH	None	UV, filter paper
ASA1	As in AlA1	10 mg x-ketoglutaric acid acid 25 mg L-aspartic acid	3 mg NADH	80 U MDH	None	As for AlA1
ME	5 ml 0.6 M Tris/HCl pH 7.4	2.5 ml 0.63 M Malic acid pH 7.0	10 mg NADP	None	2 ml 0.1M $mgCl_2$	4 mg MTT 2 mg PMS 180 mg agar in 17 ml H_2O
PGM	8 ml 0.3 M Tris/HCl pH 7.4	32 mg glucose 1-phosphate with 1% glucose 1,6 diphosphate	20 mg NADP	50 U of glucose 6-phosphate dehydrogenase	5 ml of $MgCl_2$	5 mg MTT 2 mg PMS 180 mg agar in 17 ml H_2O
PEP-1	2 ml 0.1 M Na_2HPO_4 pH 7.4	10 mg L-leucylglycylglycine	None	1 mg peroxidase 1.7 mg L-amino acid oxidase	0.2 ml 0.1M $MnCl_2$	1 ml saturated solution of 3-amino a-ethyl carbazole in ethanol 180 mg agar in 17 ml H_2O
PEP-2	As for PEP-1 "	7 mg L-leucyl-L-alanin	None	As above	As above	
ICD	5 ml 0.135 M Tris/0.042M citric acid, pH 7.0	10 mg isocitric acid (trisodium salt)	3 mg NADP	-	3 ml 0.1M $MgCl_2$	4 mg MTT 2 mg PMS 6 ml water 180 mg agar in 17 ml H_2O

*Gels were incubated at 37°C until the bands were visible

TABLE 5 : SUMMARY OF ELECTROPHORETIC CONDITIONS FOR THE ENZYMES ANALYSED

Enzyme	Tank buffer (TB)	Gel buffer TB:H_2O	Volts/cm	Time (hours)	Reference
+ALAT	0.15M Tris 0.007 M Citric acid pH 9.0	1:9	45	3	Miles et al., 1980
ASAT	0.15M Glycine NaOH, pH 9.5	1:9	25	2	Miles et al., 1980
ME	0.2M PO_4 pH 7.0	3:40	20	2.5	Young and Godfrey, 1983
PGM	0.2M PO_4 pH 7.0	see below*	20	2.5	Young and Godfrey, 1983
PEP-1	0.18M Tris 0.02M KH_2PO_4 pH 9.0 with HCl	1:9	20	3	Young and Godfrey, 1983
PEP-2	same as PEP-1				
ICD	0.661M Tris 0.083 citric acid pH 8.6	1:4	20	3	Gibson et al., 1978

* Gel buffer was made of 10 mM Tris, 10 mM maleic acid, 1 mM EDTA, pH 7.4

+ For ALAT, the "beads" were diluted by adding an equal volume of lysing buffer before loading onto the gel

Chemicals used to make electrophoretic buffers were: tris (Riedel-de Haen, West Germany), citric acid (May and Baker Ltd, U.K.) and glycine (Merk, West Germany). The following analytical grade chemicals obtained from BDH, U.K., were used : disodium hydrogen phosphate, mono-sodium dihydrogen phosphate, potassium dihydrogen phosphate, Maleic acid and di-sodium ethylene diaminetetra acetate (EDTA).

3.3.1.5. STAINING OF GELS

Enzyme bands were visualised using a specific chromogenic reaction for each enzyme (Table 5). The following six enzymes were stained for : Aspartate aminotransferase (ASAT), EC 2.6.1.1; Alanine aminotransferase (ALAT), EC 2.6.1.2; Malic enzyme (ME), EC 1.1.1.40; Phosphoglucomutase (PGM) EC 2.7.5.1; Isocitrate dehydrogenase (ICD), EC 1.1.1.42; two peptidases: substrate L-Leucylglycylglycine, substrate Leucyl-L-alanine, EC 3.4.11.

Chemicals used for revealing enzyme bands were obtained from Sigma (U.K.). These were; DL-malic acid, phenazine methosulphate (PMS), 3-[4, 5-Dimethylthiazol-2-41]-2, 5-diphenyltetrazolium bromide, Thiazolyl blue (MTT), DL-isocitric acid (trisodium salt), B nicotinamide adenine dinucleotide phosphate (NADP, acid free), B nicotinamide adenine dinucleotide, disodium salt (NADH, disodium salt), -ketoglutaric acid (monopotassium salt), L-alanine, L-aspartic acid, lactate dehydrogenase (porcine muscle, 50% glycerol), malic dehydrogenase (porcine heart 50% glycerol), glucose-6-phosphate dehydrogenase (Bakers Yeast, Type XV), x-D-glucose

1-phosphate dehydrogenase (disodium salt) with 1% glucose-1,6-diphosphate, peroxidase (from horseradish), L-amino acid oxidase (venom from Crotalus adamanteus), 3-amino-a-ethylcarbazole. The following analytical grade chemicals were also used : Ethanol (May and Baker, U.K.), Magnesium chloride (BDH, U.K.), Manganese chloride (BDH, U.K.).

3.3.2. RESULTS

The isoenzyme profiles of the trypanosome stocks examined are summarized in Plates 6 to 12. Good band resolution was obtained with ICD, PGM, ME and ASAT. Poor resolution was seen with peptidases and ALAT.

The 48 trypanosome stocks collected during this study, produced similar enzyme banding patterns for ICD (Plate 6), PGM (Plate 7) ASAT (not shown) and ME (Plate 8). Three enzymes, ALAT (not shown), PEP 1 (Plate 9) and PEP 2 (Plate 10) showed various banding patterns between different isolates. Overall, these enzymes did not show consistent differences between the 48 stocks and the T.b. brucei reference strains. However the phosphoglucomutase isoenzyme pattern of the 48 stocks was similar to that of the reference T. evansi stock, and differed from that of the reference T.b. brucei.

Plate 6. Electrophoretic pattern obtained for ICD.

* = Reference T.b. brucei.

● = RBC lysate

- = Cathode

+ = Anode

↑ =Direction of enzyme migration

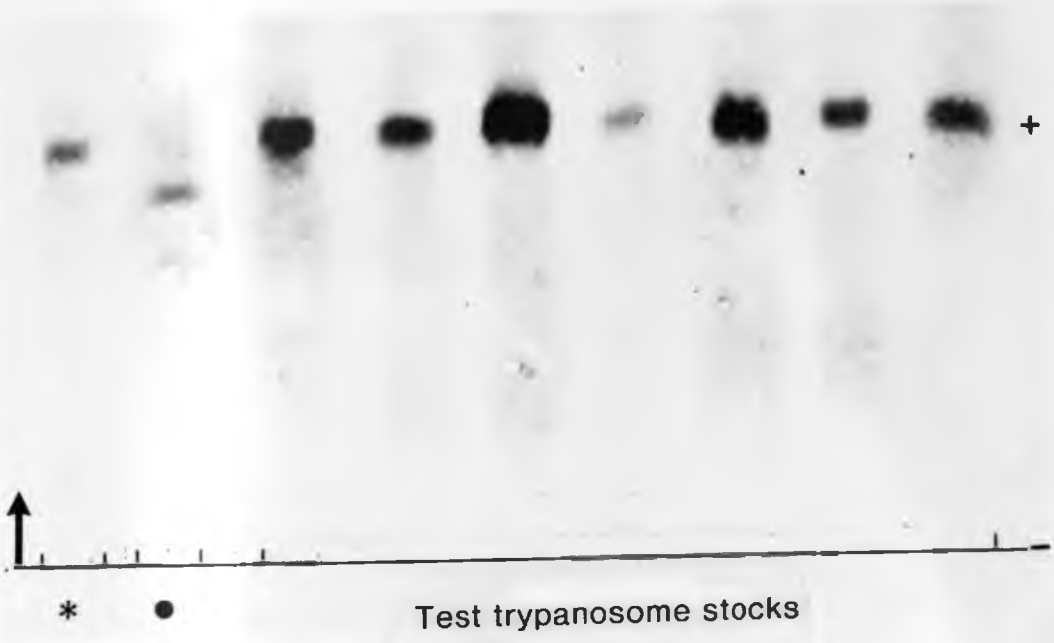

+
-
*
●
Test trypanosome stocks

Plate 7. Electrophoretic pattern obtained for PGM.

* = Reference T.b. brucei.

● = RBC lysate.

- = Cathode

+ = Anode

↑ =Direction of enzyme migration

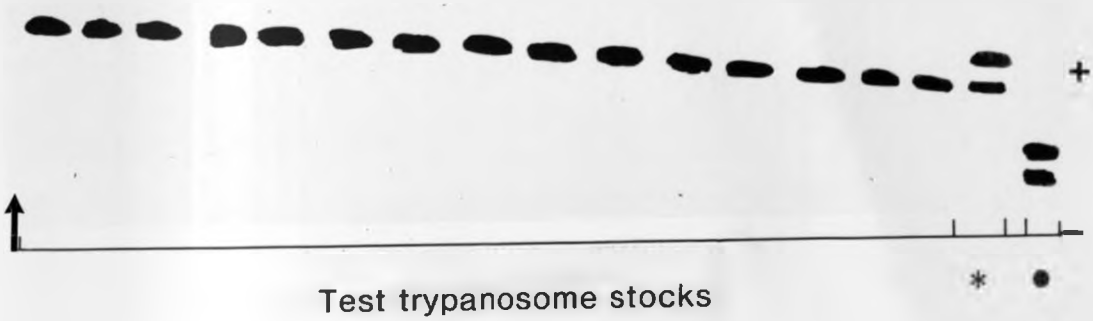

+
*
Test trypanosome stocks

Plate 8. Electrophoretic pattern obtained for ME.

- = Cathode

+ = Anode

↑ =Direction of enzyme migration

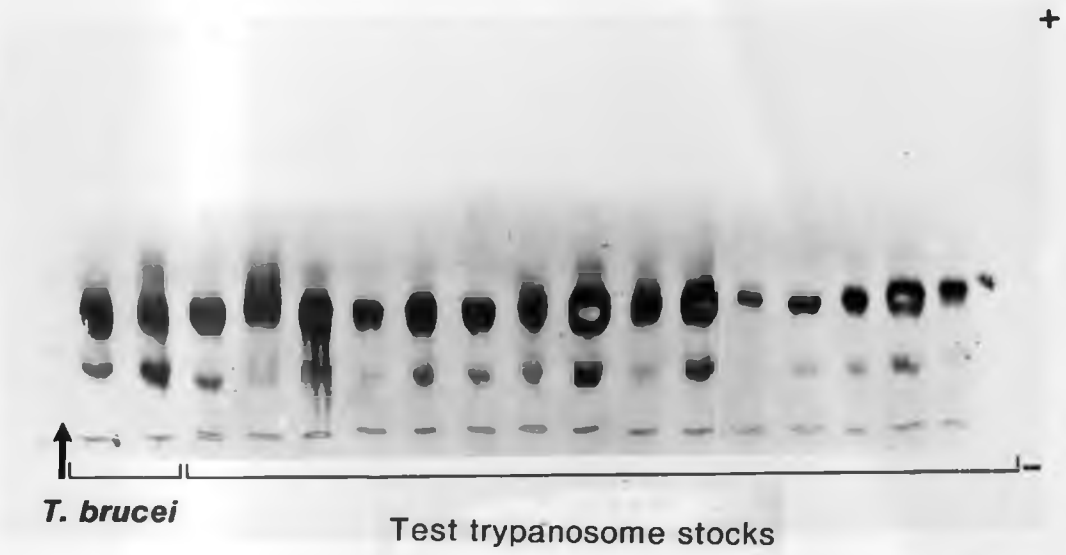

+
T. brucei
Test trypanosome stocks

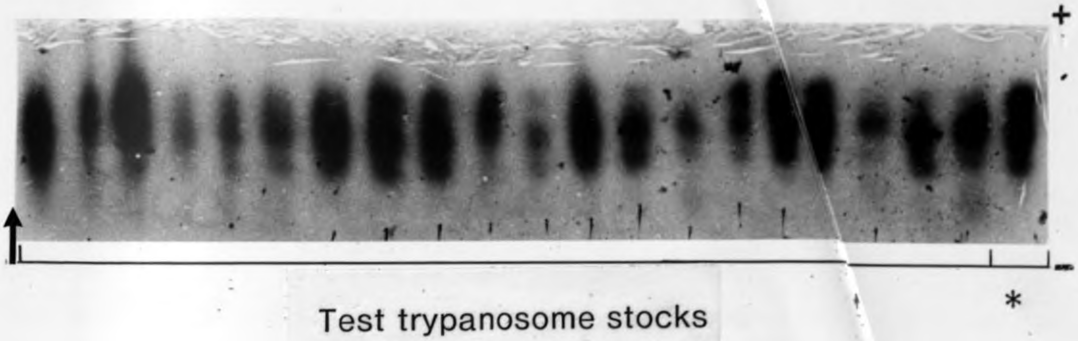
Plate 9. Electrophoretic pattern obtained for PEP-1.

* = Reference T.b. brucei

- = Cathode

+ = Anode

↑ =Direction of enzyme migration



Test trypanosome stocks

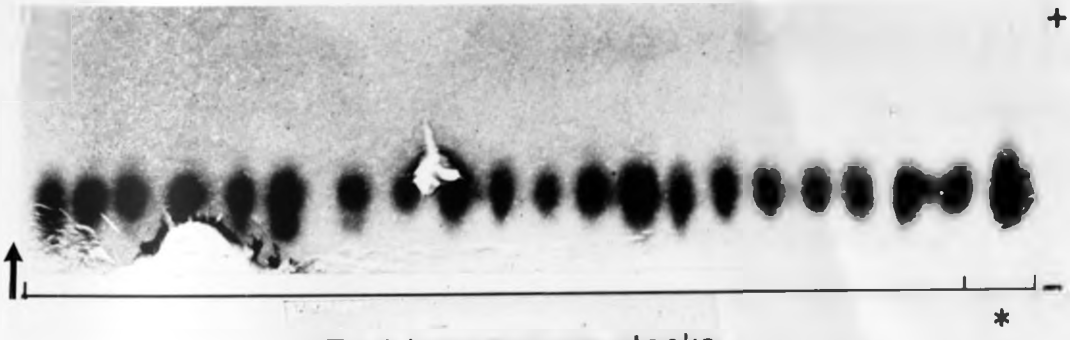
Plate 10. Electrophoretic pattern obtained for PEP-2.

* = Reference T.b. brucei.

- = Cathode

+ = Anode

↑ = Direction of enzyme migration



Test trypanosome stocks

3.4. EXPERIMENT 3 - CHARACTERIZATION OF THE TRYPANOSOME ISOLATES BY KINETOPLAST DNA (kDNA) ANALYSIS

3.4.1. MATERIALS AND METHODS

3.4.1.1. ISOLATION OF TRYPANOSOME kDNA

Each of the 48 trypanosome stocks (Table 3) was passaged in rats and the parasites isolated at peak parasitaemia through a DEAE-cellulose column (section 3.3.1.1.). The parasites were collected in a 45 ml plastic tube (Falcon, Switzerland), counted using the improved Neubuer haemocytometer and washed by centrifugation in PSG at 2500 rpm for 10 min at 4^{o}C in a "minifuge" 2 centrifuge. For comparative purposes, the kDNA of a *T.b. brucei* (ILRAD 430) and *T. evansi* (KETRI 2472) was also isolated. The pelleted trypanosomes (section 4.3.1.2) were carefully suspended in SE buffer (100 mM NaCl, 100 mM EDTA, 10 mM Tris, pH 8.0) at a concentration of approximately 10^{9} trypanosomes ml^{-1} (at least 10^{8} trypanosomes were used). Sodium dodecyl sulphate (SDS) was then added to a final concentration of 1% and mixed with the suspension by rolling until the lysate turned clear and mucoid. The lysate was then digested for 12 to 15 h at 50^{o}C with proteinase K (Bethesda Research Lab., USA) at 100 $ugml^{-1}$ final concentration.

The linear genomic DNA was rapidly sheared by passing the lysate through a 23G X 25mm needle from a 10 ml syringe barrel using maximum hand pressure. This was repeated 10 times. Intact kDNA network was recovered by centrifugation for 30 min, 15,000 rpm at 20^{o}C in a Beckman JA-21 rotor

(USA). The pelleted kDNA was resuspended in 1.0 ml TE buffer (10 mM Tris, 1 mM EDTA pH 8.0) and washed three more times in PSG by centrifugation. The pellet recovered after the final wash was resuspended in 200 ul of TE buffer and collected in an "Eppendorf" tube and incubated for 30 min at 37^{o}C with ribonuclease (RNAse A, Sigma, U.K.) at a final concentration of 100 ug ml^{-1}. Prior to use, the RNAse dissolved in 10 mM Tris·Cl(pH 7.5) and 15 mM NaCl at a concentration of 10 mg ml^{-1} and had been boiled for 30 min. and then stored at -20^{o}C. SDS and proteinase K were then added to 0.1% w/v and to 100 ug ml^{-1} respectively. The solution was mixed by slow vortexing and then incubated for 30 min at 37^{o}C.

Phenol was re-distilled and stored at -20^{o}C. For use, it was equilibrated with TE buffer pH 8.0 and an equal volume of chloroform was added. The proteinase K treated trypanosome lysate was deproteinized by shaking with phenol/chloroform (1:1 v/v) mixture. The mixture was then centrifuged for five min. at 14000 rpm in a "Microfuge" centrifuge (Eppendorf 5414, West Germany). The aqueous phase containing the DNA was removed. The interphase containing denatured protein was re-extracted with 200 ul TE to recover more kDNA. The process was repeated until the interphase was clear.

Traces of phenol in the DNA sample were removed by the addition of an equal volume of chloroform, thorough mixing and spinning for 1 min in an Eppendorf 5414 "Microfuge" at 14000 rpm. Chloroform was removed with an "Eppendorf" pipette from the bottom of the tube.

The kDNA was precipitated overnight at -20^{o}C with

1/10 volume of 3 M sodium acetate pH 7.5 and 2.5 volumes of absolute ethanol.

The precipitated kDNA pellets were recovered by centrifugation at 14000 rpm for 1 min at 0°C in a refrigerated micro-centrifuge (Tomy MR -150, Japan).

The pellet was washed once with 70% ethanol by centrifugation for half a minute, at 14000 rpm in an Eppendorf 5414 Microfuge at room temperature, and then dried in a vacuum dessicator for 5 min after which 100 ul TE buffer was added, and the suspension was incubated for 5 min at 37°C. The mixture was then vortexed slowly and the dissolved kDNA was stored at -20°C until required.

3.4.1.2. RESTRICTION ENZYME DIGESTIONS

Digestions of kDNA were carried out with the following restriction endonucleases :

Restriction enzyme	Recognition sequence
Ase 1	ATTAAT
Hinf 1	GANTC
Mbo 11	$GAAGA(N)_8$
Mse 1	TTAA
Mbo 1 + Taq 1	GATC + TCGA

All the enzymes were obtained from New England Biolabs, USA. The following four enzyme buffers (Table 6) were prepared as 10x concentrated stocks, and were diluted ten-fold for use.

The choice of buffer was as recommended in the New England Biolab Catalogue (1988). All the reactions were

Table 6 : Composition of enzyme buffers

Solution	1	2	3	4
Tris-HCl (mM)pH 7.5	10	10	10	10
MgCl2 (mM)	10	10	10	10
NaCl (mM)	0	50	100	150
BSA (ug/ml)	100	100	100	100

carried out in sterile 1.5 ml plastic tubes (Eppendorf, West Germany) at 37°C for 3 h except for reactions using Taq 1 which were carried out at 60°C. Because of temperature incompatibility between Mbo1 and Taq 1, reactions were first carried out with Mbo 1 at 37°C and then with Taq 1 at 60°C. To avoid evaporation during Taq 1 digestions, the reaction mixture was covered with 2 drops of mineral oil (Sigma, U.K.).

The concentration of kDNA was not determined prior to digestion due to the small volume of DNA available. Generally, 2.5 ul of DNA were used for each reaction. Two units of enzyme ml^{-1} of DNA were used and if partial digestion occurred, repeat digestions were done with additional enzyme. To avoid excess glycerol in the reaction mixture, the volume of enzyme used was kept to 1/10 of the reaction volume.

3.4.1.3. ELECTROPHORESIS

All the samples were analysed in agarose gels except Mse 1 digests which were analysed in polyacrylamide gels.

3.4.1.3.1. Agarose gel electrophoresis

Two per cent agarose gels were prepared in 1 x TAE buffer (40 mM Tris acetate and 1 mM EDTA disodium salt, pH 8) according to the method described by Maniatis et al.(1982). Gels were made by boiling the agarose (Ultra-pure, Bethesda Research Laboratories, USA) in 1 x TAE buffer until the agarose was completely dissolved; cooling the agarose solution to 50°C in water bath; and, pouring the solution into a gel tray fitted with 1 to 2 mm

sample comb. After the gels had set, they were transferred to a horizontal electrophoretic apparatus (LKB, Sweden) containing 1 x TAE buffer and the comb was then removed.

Samples in 10 to 20 ul containing 1 x gel-loading buffer (prepared as a stock containing 5 x TAE, 50% glycerol, 0.25% bromophenol blue and 0.25% xylene cyanol) were loaded into the slots and electrophoresed at either 100 volts for 3 h or 30 volts for 12 to 13 h.

Fragments of Phi x 174 RF DNA digested with Hinc II endonuclease (New England Biolabs, USA) were used as reference DNA size marker.

3.4.1.3.2. Polyacrylamide gel electrophoresis

A 12% acrylamide gel (29:1, acrylamide: bisacrylamide) was prepared by dissolving 8.7 g of acrylamide (Serva, Germany) and 0.3 g of N'N' methylene bisacrylamide (Serva, Germany) in 7.5 ml of 10 x TBE buffer (0.89 M Tris-borate, 0.89 M boric acid and 0.02 M EDTA) and water was added to bring the final volume to 75 ml (Maniatis et al., 1982). The resulting solution was de-aerated and sterilized by filtering through a 2 um pore size filter (Nalgene, USA). Five ml of this solution was caused to polymerize by addition of 25 ul of freshly prepared 10% ammonium persulphate and 2.5 ul TEMED (N,N,N'N'-tetramethylethylene diamine, Sigma, UK) and quickly poured into a glass sandwich to seal any leaks at the bottom end.

To the rest of the solution was added 0.25 ml of freshly prepared 10% ammonium persulphate and 37.5 ul TEMED which started the polymerization of the acrylamide. A 2 mm

sample comb was inserted and polymerization was allowed to proceed for 45 min after which the gel was transferred to a vertical electrophoretic apparatus (LKB, Sweden) containing 1 x TBE buffer. The comb was removed and 20 ul of each sample loaded. The samples were electrophoresed at 50 volts for 12 hours with an LKB (Sweden) power supply.

Fragments of Phi x 174 RF DNA digested with Hinc II endonuclease were used as size markers.

3.4.1.4. STAINING OF GELS AND PHOTOGRAPHY

Gels were stained for 15 to 30 min with 0.25 $ugml^{-1}$ of ethidium bromide in the appropriate electrophoretic buffer. The gels were photographed following UV transillumination using Type 57 polaroid film. The optimal exposure time was 12 sec at f16.

3.4.1.5. MOLECULAR WEIGHT DETERMINATION

The molecular weight of the sample DNA fragments were determined by interpolation on a plot of log molecular weight of reference DNA plotted against migration in the gel. The size bands of the reference DNA were 1060, 770, 610, 500, 390 (340) x 3, (300) x 2, 210, 160 and 80 base pairs (bp).

3.4.2. RESULTS

The types of restriction patterns obtained with the 48 trypanosome stocks isolated from camels are summarized in Table 7.

All the isolates produced identical mini-circle restriction patterns with the fragment lengths summing to

about 1 kb. Ase 1 produced two bands of 340 and 660 bp (Plate 11), Hinf 1 produced two bands of 107 and 950 bp (Plate 12), Mbo II produced one band of about 1 kilobase, (Plate 13), Mse 1 produced eight bands of 250, 185, 150, 120, 90, 79, 76 and 64 bp (Plate 14) while Mbo 1 and Taq 1 double digestion produced two bands of 635 and 365 bp (Plate 15). In total, 15 recognition sites with 65 base pairs were examined.

In contrast to the discrete pattern obtained with the mini-circle digests of the camel trypanosome isolates and the reference T. evansi (KETRI 2472), the reference T.b. brucei (ILRAD 430) showed a complex of non-stoicheiometric bands irrespective of the endonuclease used.

Table 7: Restriction sequences, the number of bands obtained and the number of base-pairs examined with the different endonucleases

Enzyme	Recognition sequence	Number of sites	Base-pairs
Ase 1	ATTAAT	2	12
Hinf 1	GANTC	2	8
Mbo II	GAAGA(N)8	1	5
Mse 1	TTAA	8	32
Mbo 1	GATC	1	4
Taq 1	TCGA	1	4
		—	—
Total		15	65

▼ = enzyme cleavage site

A = Adenine

T = Thiamine

G = Guanine

C = Cytosine

Plate 11. Ase 1 kDNA minicircle restriction pattern obtained with the test trypanosome stocks. The arrow-heads show un-digested 1 Kb minicircles.

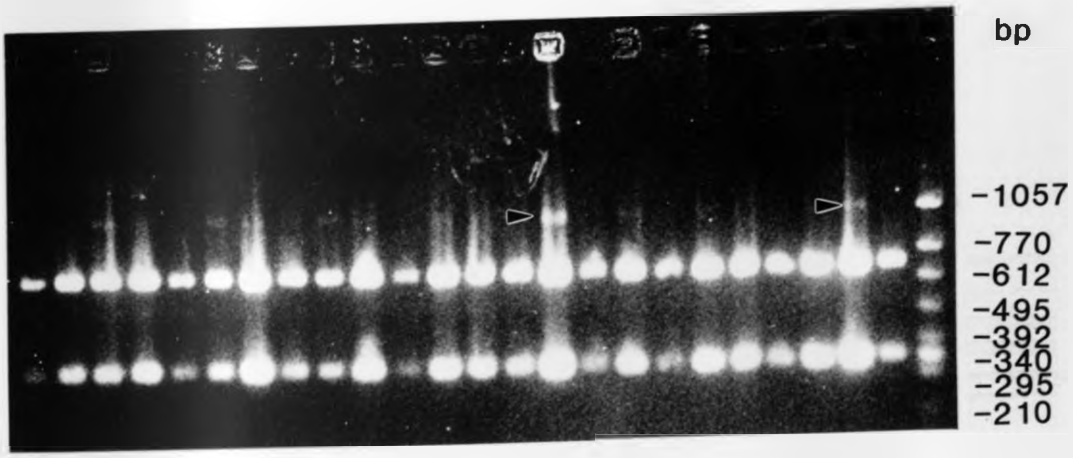

bp
-1057
-770
-612
-495
-392
-340
-295
-210

Plate 12. Hinf 1 kDNA minicircle restriction pattern obtained with the test trypanosome stocks.

* = Reference T.b. brucei minicircle restriction pattern.

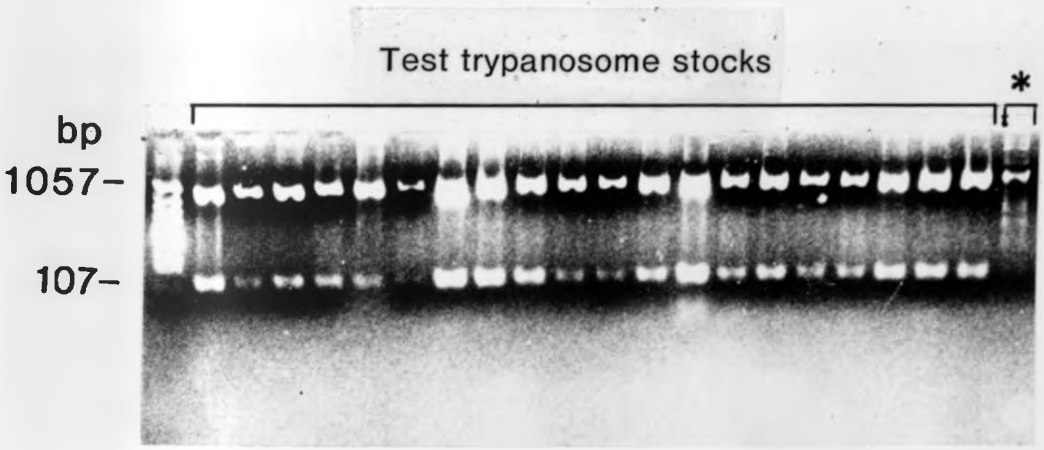

Test trypanosome stocks
*
bp
1057–
107–

Plate 13. Mbo II kDNA minicircle restriction pattern obtained with the test trypanosome stocks.

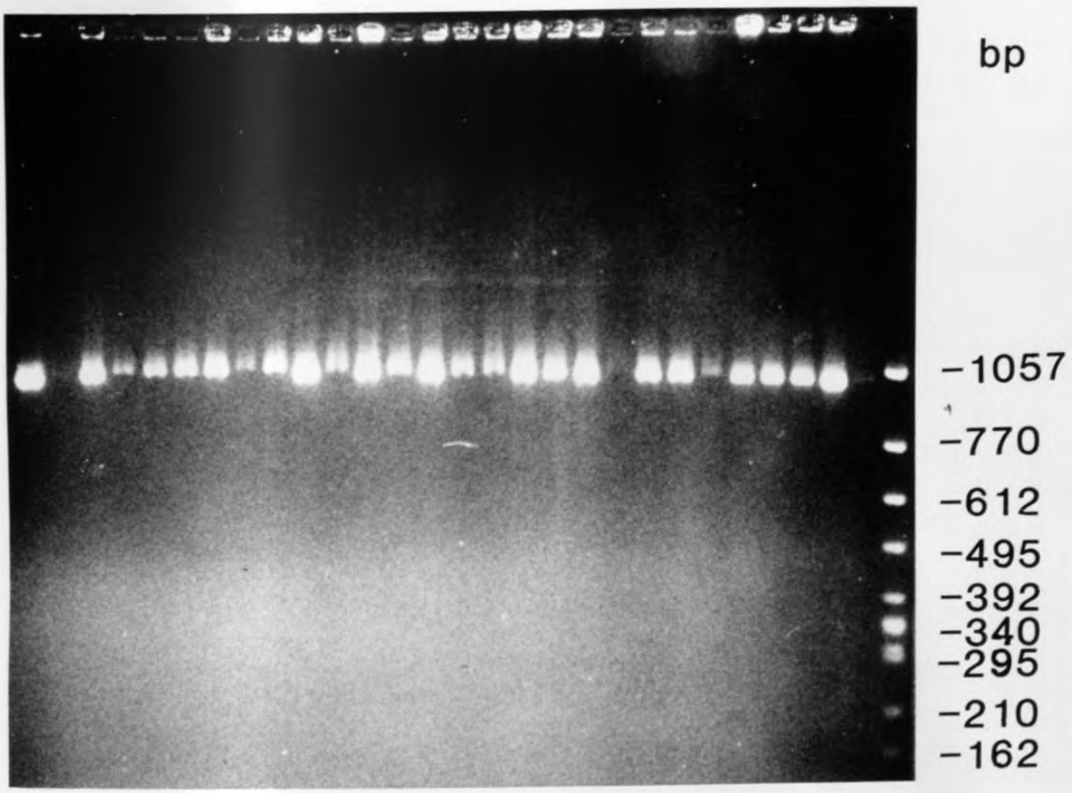

bp
1057
770
612
495
392
340
295
210
162

Plate 14. Mse 1 kDNA minicircle restriction pattern obtained with the test trypanosome stocks.

* = Reference T.b. brucei minicircle restriction pattern.

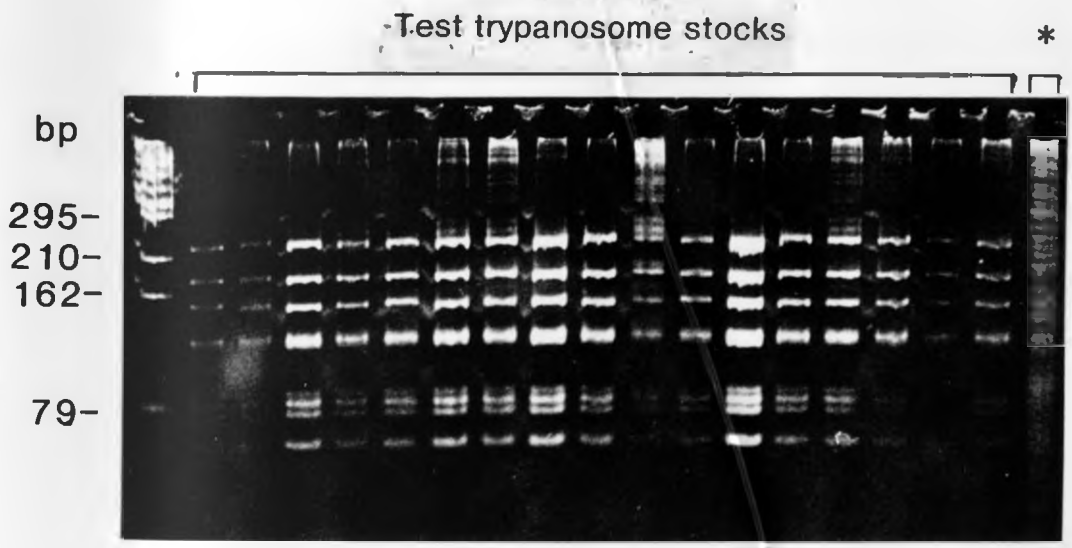

Test trypanosome stocks
*
bp
295-
210-
162-
79-

Plate 15. Mbo 1 X Taq 1 kDNA minicircle restriction pattern obtained with test trypanosome stocks.

* = Reference T.b. brucei minicircle restriction pattern.


Test trypanosome stocks
*
bp
-1057
-770
-612
-495
-392
-340
-295
-210
-162

3.5 EXPERIMENT 4- STUDIES OF CHROMOSOME-SIZED DNA WITH PULSED FIELD GRADIENT (PFG) GEL ELECTROPHORESIS

3.5.1. MATERIALS AND METHODS

3.5.1.1. PREPARATION OF TRYPANOSOMES

3.5.1.2. TRYPANOSOMES

A selected sample of 36 trypanosome stocks isolated from the camels during the period of the study and another 8 from KETRI trypanosome bank were used. A summary of the information on 40 trypanosome stocks used is shown in Table 8. A reference T.b. brucei (ILTat 1.3) of known chromosome size (Shah et al., 1987) was used as a size marker.

Trypanosomes were prepared for PFG gel electrophoresis using a modification of a method originally described for yeast by Schwartz and Cantor (1984). One and half per cent low melting point agarose (Ultra pure, Bethesda Research Labs, USA) was completely dissolved in PSG by boiling and left to cool to 42^{o}C in a water bath. Meanwhile, trypanosomes (3.2.1.2.) were separated from blood cells by DEAE-cellulose column (3.3.1.1.), counted in a haemocytometer and pelleted by centrifugation at 2500 rpm for 10 min at 4^{o}C in a "Minifuge" 2 centrifuge. The trypanosome suspension was adjusted to approximately 1 x 10^{9} ml^{-1} in PSG.

The trypanosome suspension was warmed briefly to 42^{o}C and 0.35 ml of the suspension was taken in a 1.0 ml pipettor and quickly mixed by swirling with an equal volume of the warm agarose. The mixture was quickly pipetted into a plastic mould made of two 1.0 mm-thick plates separated by 1 mm spacers which had been left cooling on ice. The

Table 8 : A summary of information on the origin of trypanosome stocks analysed by PFG gel electrophoresis

Camel number	Isolate designation	Place of isolation	Date of isolation
902	NGT, 902	Ngurunit, Kenya	July, 1986
920	NGT, 920	,,	,,
921	NGT, 921	,,	,,
928	NGT, 928	,,	,,
907	NGT, 907	,,	,,
932	NGT, 932	,,	,,
922	NGT, 922	,,	,,
763	NGT, 763	,,	December, 1986
	NGT, 763a*	,,	January, 1987
935	NGT, 935	,,	April, 1987
934	NGT, 934	,,	,,
	NGT, 934a*	,,	July, 1987
958	NGT, 958	,,	April, 1987
	NGT, 958a*	,,	June, 1987
904	NGT, 904	,,	May, 1987
937	NGT, 937	,,	,, ,,
941	NGT, 941	,,	June, 1987
944	NGT, 944	,,	,, ,,
901	NGT, 901	,,	July, 1987
927	NGT, 927	,,	,, ,,
939	NGT, 939	,,	,, ,,
959	NGT, 959	,,	,, ,,
Ngutaiyo	NGT, Ngutaiyo	,,	,, ,,
Nguto	NGT, Nguto	,,	,, ,,

Table 8 continued.....

Camel isolate	Isolate designation	Place of isolation	Date of isolation
807	OLT, 807	Olturot, Kenya	August, 1986
49	OLT, 49	,,	,, ,,
62	OLT, 62	,,	,, ,,
63	OLT, 63	,,	,, ,,
188	OLT, 188	,,	,, ,,
42	OLT, 42	,,	,, ,,
819	OLT, 819	,,	,, ,,
	OLT, 819a*	,,	October, 1986
812	OLT, 812	,,	December, 1986
803	OLT, 803	,,	,, ,,
833	OLT, 833	,,	July, 1987
840	OLT, 840	,,	,, ,,
NA	KETRI, 2416	Garrissa, Kenya	NA, 1968
NA	KETRI, 2445	NA	NA,
NA	KETRI, 2540	Carimagua, South	NA, 1973
NA	KETRI, 2540a	America**	
NA	KETRI 2472	Khartoum, Sudan	NA, 1979
NA	KETRI 2472(a)**		
NA	KETRI 2447	Olturot, Kenya	NA,1980
NA	KETRI 2447(a)**		

Key

NA = information not available

* = Stock isolated from reccurring parasitaemia

**= Stock derived from mice

agarose-trypanosome mixture was left to set on ice for 15 min.

The trypanosomes, now embedded in the agarose slab, were lysed and deproteinized *in situ* in 10 ml of 250 mM EDTA pH 8.0, 500 $ugml^{-1}$ proteinase K (Bethesda Research Labs., USA) and 0.5% SDS, for 16 h at 50°C. The digested slab was washed four times at room temperature with 10 mM EDTA pH 8.0, each wash being performed on a slow shaker for 30 min.

The agarose slab was then cut into small rectangular blocks (about 4.5 mm x 3 mm) and stored in 10 mM EDTA, pH 8.0 at 4°C until required.

3.5.1.3 ELECTROPHORESIS

Gels of 1.5% agarose were prepared in 120 ml of 0.375 x TBE buffer, cooled to 50°C and poured into a 14 cm x 10 cm gel tray fitted with a continuous 1 mm sample well former. After the gel had set, the comb was removed and the trough so made was filled with 0.375 x TBE buffer. The sample blocks were placed vertically into the trough leaving a 2 mm gap between them.

The gel containing the samples was removed from the tray and transferred into a contour-clamped homogeneous electric field (CCHEF) electrophoretic apparatus (designed by Dr. John R. Young, ILRAD, according to Chu, Vollrath and Davis, 1986) containing 2.0 l of 0.375 x TBE buffer. The buffer was circulated through coiled tubing in a refrigerated water bath to maintain the buffer temperature at 13 to 15°C. The chromosomes were separated using an electrophoretic power supply (Pharmacia, Sweden) at 50

volts (voltage gradient being 2.5 v/cm) for the first 2 h and then at 200 volts (voltage gradient being 10 v/cm) for a further 15 h, the direction of the electric field being alternated between 120^{o} every 20 sec using an MPH programmable power inverter and alternating field adapter (MPH, U.K.).

3.5.1.4 STAINING OF THE GEL AND PHOTOGRAPHY

Gels were stained with 0.25 ug ml^{-1} ethidium bromide in TBE buffer overnight at room temperature. The gels were photographed using Type 57 Polaroid film following UV trans-illumination. Exposure times were 12 to 15 seconds at f22.

3.5.2. RESULTS

The molecular karyograms of the 44 trypanosome stocks are shown in plates 16a,b and c.

In general, the DNA of each stock separated into four fractions. Fraction One was composed of molecules that hardly moved from the gel slot. Fraction Two was a single band of large molecules of over 600 kb. Fraction Three comprised 3 to 7 intermediate size molecules of between 200 to 600 kb. Fraction Four contained many small DNA molecules of between 50 and 200 kilobases. The majority of isolates had the mini-chromosomes in the range of 100 and 200 kb. In two isolates (KETRI 2416 and 2445, Plate 16c, L), the sizes of the mini-chromosomes (between 50 and 100 kb) were similar to that of the reference T.b. brucei.

Table 9 summarises information on the 38 trypanosome isolates and the type of chromosomal patterns to which they

were allocated. The allocation of a pattern to a particular group was based on the number of intermediate chromosomes and their relative positions compared to the size marker (Plates 16a,b and c).

Fourteen distinct patterns: 8 in Ngurunit, 1 in Olturot and 5 from the other areas were obtained. All the isolates from the Olturot herd had a type F pattern and were indistinguishable from each other, while those collected from Ngurunit had heterogenous karyotype patterns. Isolates collected from Ngurunit during the beginning of the study showed four chromosomal patterns, (E, F, G and H). Three of these, (E, F and H), were later seen in trypanosomes collected during a trypanosomiasis epidemic that occurred in the Ngurunit herd 12 months after the beginning of the study (July 1987). This time, it was the type E pattern that was predominant. Four other distinct patterns (D, I, J and K) were obtained during this outbreak, three of which were from trypanosomes collected from camels that had been introduced into the Ngurunit herd from other areas.

The isolates from other geographical locations showed distinct patterns (A, B, C, L and M).

The A pattern (isolated from a camel in Khartoum, Sudan), was surprisingly similar to the Olturot pattern, and differed only in the absence of one medium sized DNA molecule.

TABLE 9 A SUMMARY OF INFORMATION ON THE TRYPANOSOME ISOLATES FROM NGURUNIT, AND THE KARKYOTYPE PATTERNS TO WHICH THEY WERE ALLOCATED TO.

Isolate designation	Location	Time M/Y	PATTERNS			
			E	F	J	Others (unique)
902	Ngurunit	7 - 86				*
920	"	7 - 86	*			
921	"	7 - 86			*	
928	"	7 - 86			*	
907	"	8 - 86			*	
932	"	8 - 86		*		
922	"	10 - 86			*	
763	"	12 - 86				*
763a	"	1 - 87				*
903	"	4 - 87	*			
934	"	4 - 87	*			
934a	"	7 - 87	*			
935	"	4 - 87		*		
958	"	4 - 87	*			
958a	"	7 - 87	*			
904	"	5 - 87	*			
937	"	5 - 87		*		
941	"	6 - 87	*			
944	"	6 - 87		*		
901	"	7 - 87			*	
927	"	7 - 87				*
939	"	7 - 87	*			
959	"	7 - 87	*			
Ngutaiyo	"	7 - 87				*
Nguto	"	7 - 87				*

Key

M = Month of isolation

Y = Year

Plate 16a. Molecular karyotypes of some of the stocks studied, listed from left to right.

* = T.b. brucei (ILTat 1.3), used as a size marker.

A = KETRI 2447

B = KETRI 2472

C = KETRI 2540

C = KETRI 2540a

D = NGT 763

D = NGT 763a

E = NGT 934

F = OLT 833

F = OLT 62

G = NGT 902

I = NGT Nguto

F = OLT 812

The letters A,B,C,D,E,F,G,I refer to the type of chromosomal patterns to which the isolates were allocated.

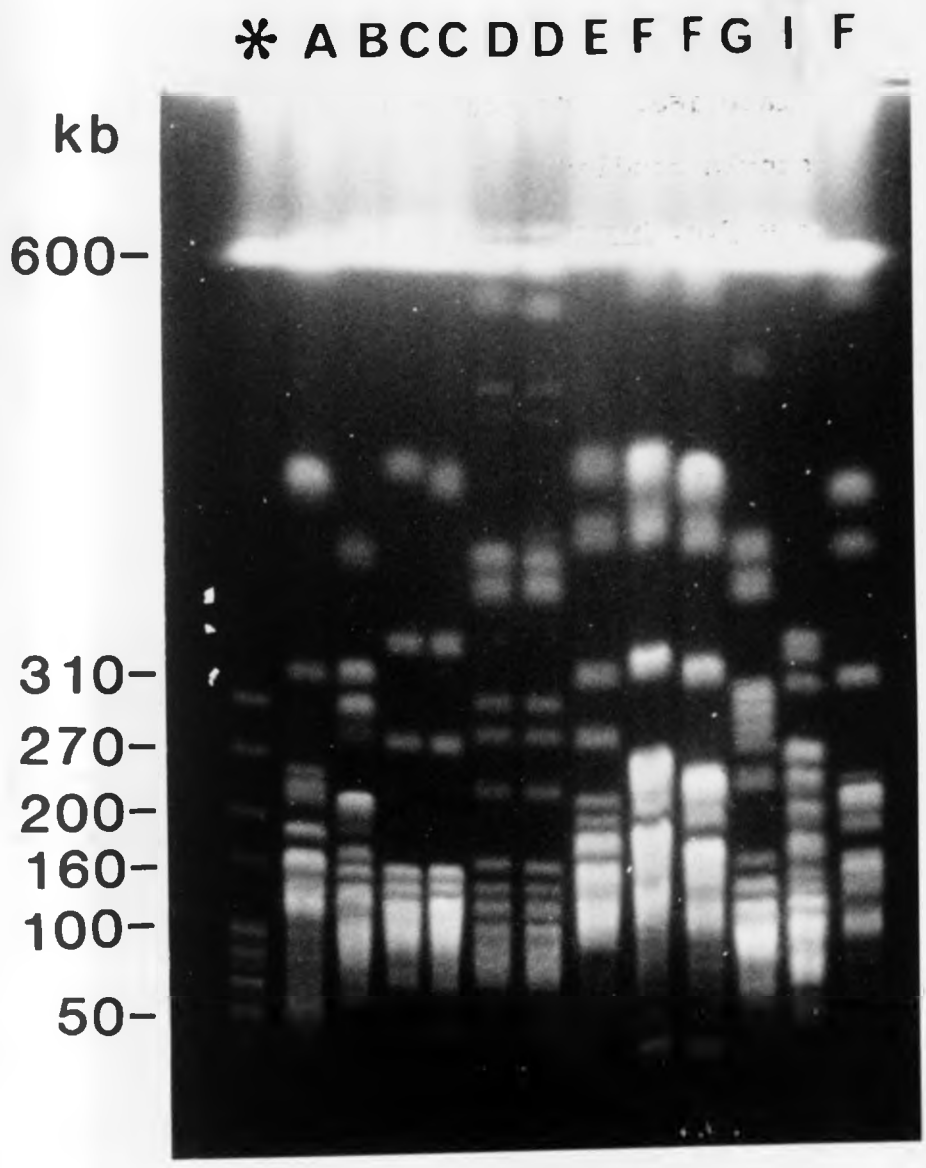

* A B C C D D E F F G I F
kb
600-
310-
270-
200-
160-
100-
50-

Plate 16b. Molecular karyotypes of some of the stocks studied, listed from left to right.

* = T.b. brucei (ILTat 1.3), used as a size marker.

I = NGT Nguto

F = OLT 803

O = degraded sample

A = KETRI 2447a

B = KETRI 2472a

E = NGT 904

F = NGT 937

F = NGT 932

H = NGT 901

E = NGT 959

H = NGT 921

The letters A,B,E,F,H,I alphabets refer to the type of chromosomal patterns to which the isolates were allocated.

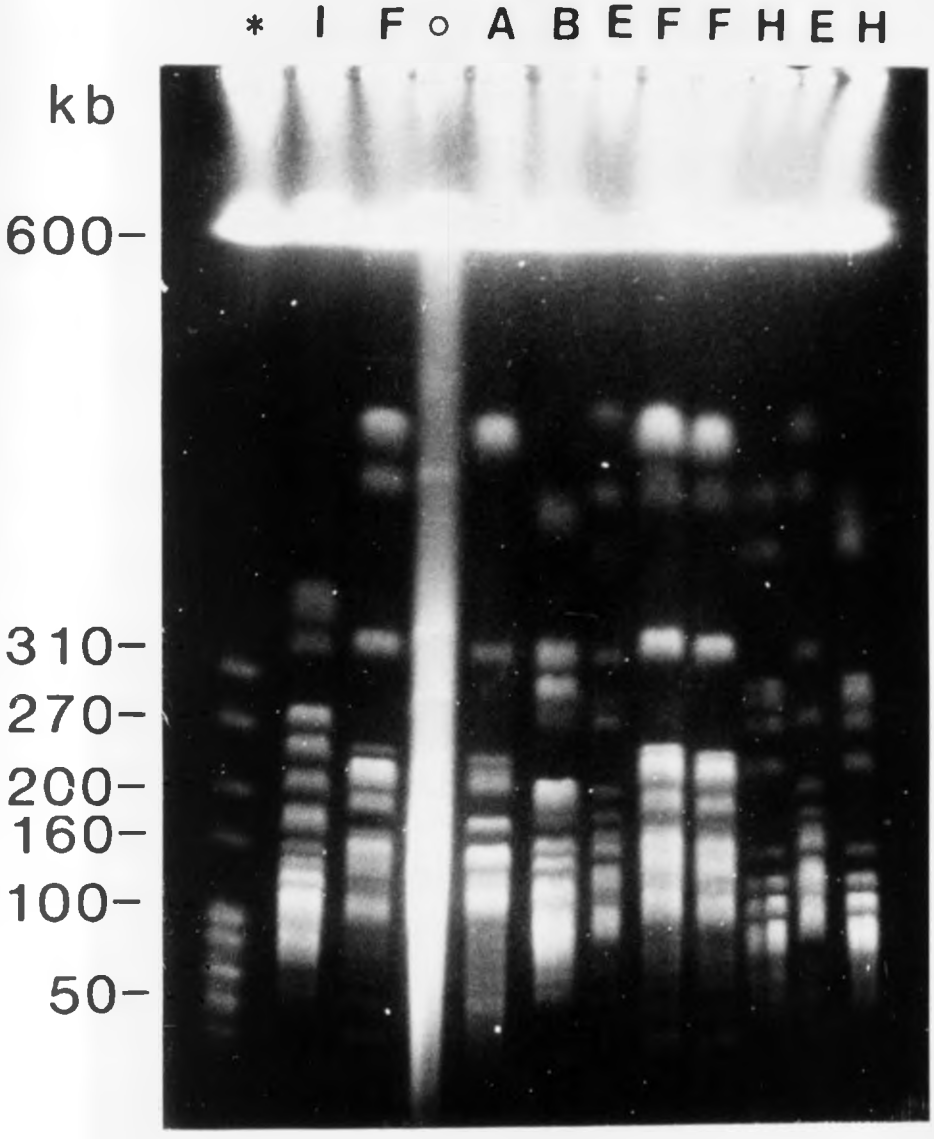

* I F ○ A B E F F H E H
kb
600–
310–
270–
200–
160–
100–
50–

Plate 16c. Molecular karyotypes of some of the stocks studied, listed from left to right.

* = T.b. brucei (ILTat 1.3), used as a size marker.

H = NGT 920

F = OLT 840

▼ = T. vivax (ILRAD 1392)

F = OLT 819

L = KETRI 2416

E = NGT 934

E = NGT 934a

F = OLT 819a

J = NGT Ngutaiyo

M = KETRI 2445

K = NGT 927

E = NGT 939

The letter E,H,F,J,K,L,M alphabets refer to the type of chromosomal patterns to which the isolates were allocated.

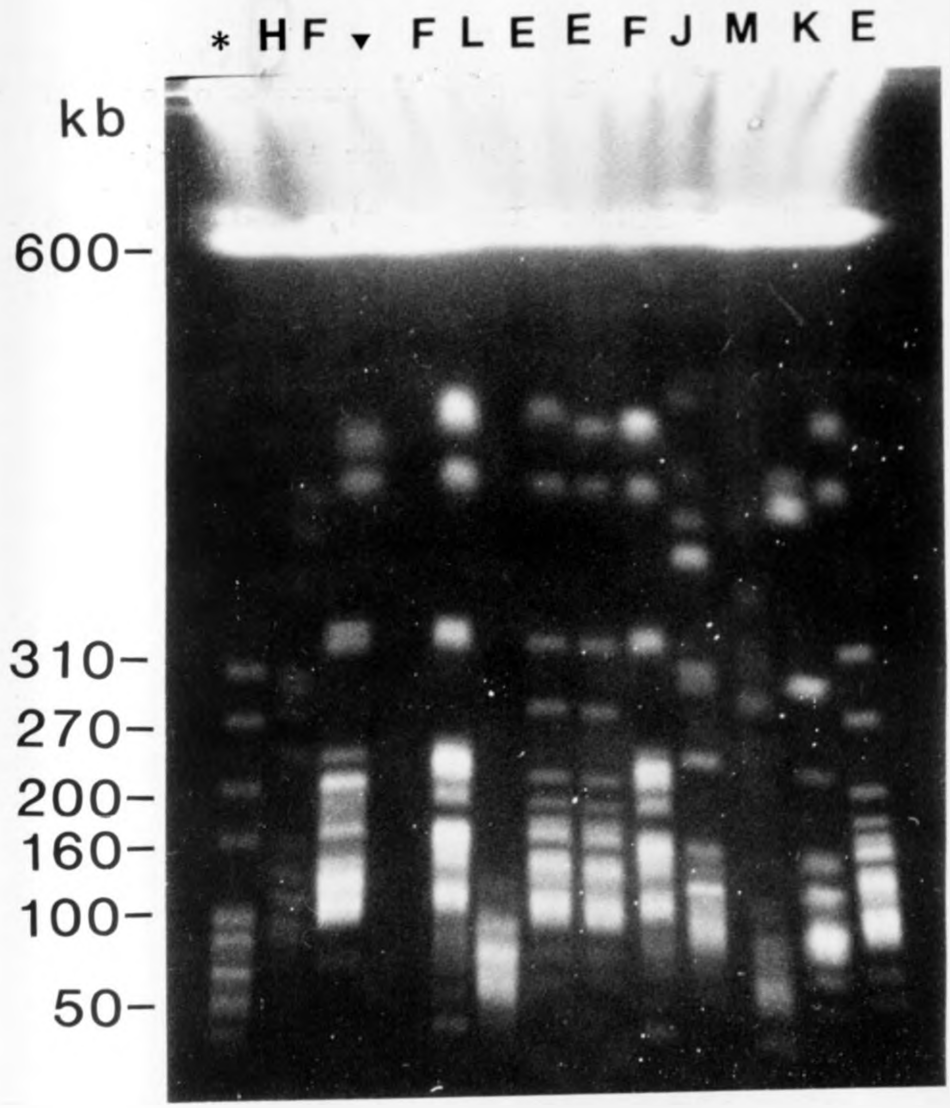

* H F ▾ F L E E F J M K E
kb
600–
310–
270–
200–
160–
100–
50–

Plate 16d. Molecular karyotypes of some of the stocks studied listed from left to right.

* = T.b. brucei (ILTat 1.3), used as a size marker.

F = OLT 63

F = OLT 49

E = NGT 904

E = NGT 958

O = Degraded sample

F = OLT 807

H = NGT 922

H = OLT 188

H = NGT 928

H = NGT 907

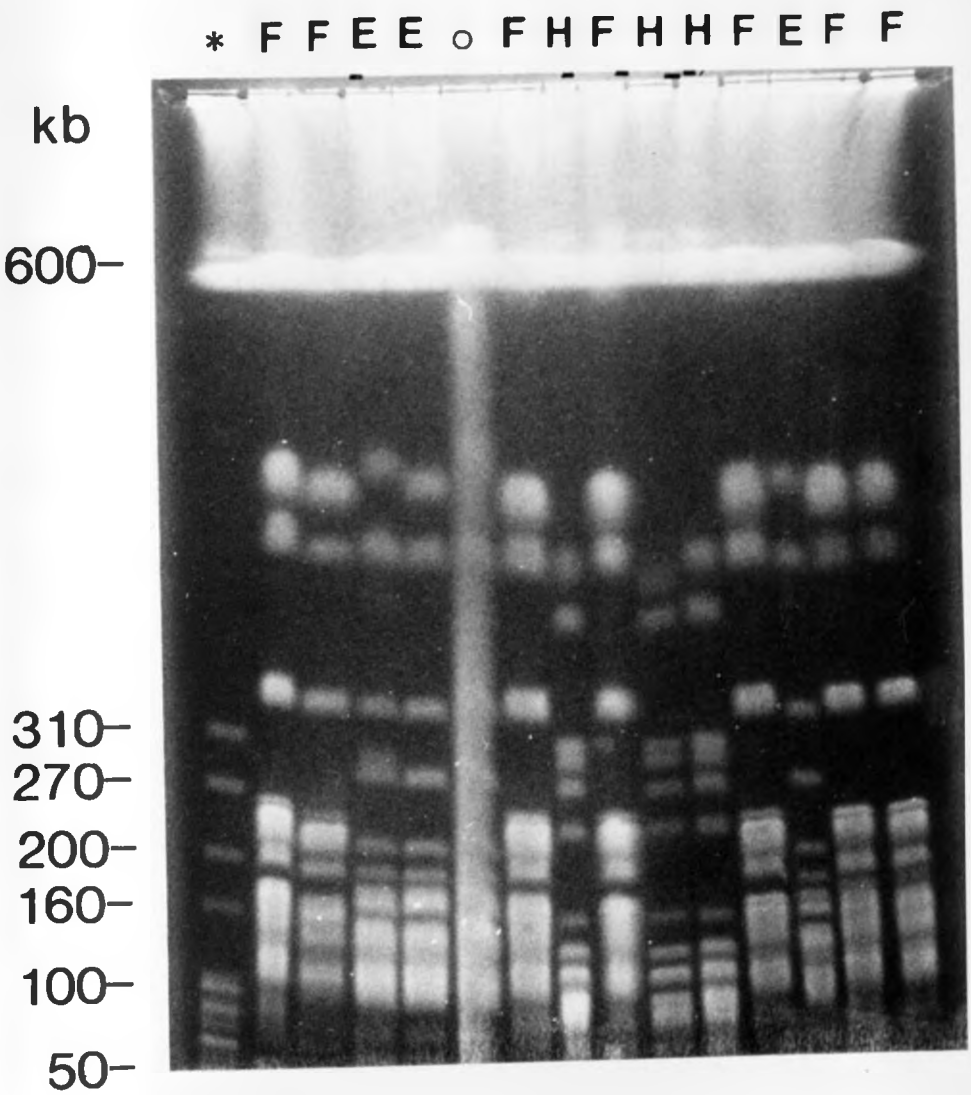
F = OLT 42

E = NGT 941

F = NGT 935

F = NGT 944

The letters E,F,H refer to the type of chromosomal patterns to which the isolates were allocated.


* F F E E ○ F H F H H F E F F
kb
600–
310–
270–
200–
160–
100–
50–

3.6 DISCUSSION

T. evansi and T.b. brucei are indistinguishable, both morphologically and on the basis of the disease they cause. Their only difference is that, the geographical distribution of T.b. brucei is correlated with that of Glossina, while that of T. evansi is not. For this reason, controversy exists as to how different these two parasites are. Hornby (1921) stated that :

> " ..... until it is proved that a true strain of surra organism from, say a horse in India, can likewise multiply in the tsetse flies we must distinguish between the two parasites".

In the studies on tsetse transmissibility of trypanosomes collected from camels in Marsabit area, no isolate developed in tsetse, while the reference T.b. brucei, also originally isolated from a camel, completed cyclical development. Tsetse transmissibility experiment with the reference T. b. brucei was repeated twice, and in both instances infections were transmitted. However, the percentage of flies found infected with T. brucei was low, a finding that has been reported by other workers (Duke, 1933). It was also noted that in those flies that died after their first blood meal, live trypanosomes were demonstrable only in those that had fed on rats infected with the reference T.b. brucei.

The failure of the camel trypanosome isolates to develop in Glossina reflects the probability that they were T. evansi. These findings would be in agreement with those

of Duke (1933) who failed to transmit a T. evansi strain from Abyssinia (present day Ethiopia) using 276 laboratory-bred G. palpalis dissected 30 days after the infective feed. Duke also failed to find trypanosomes in the gut or proboscis of 157 flies that had died before the 30 days were over. In another study, Hoare (1940) found that the majority of T. evansi die within six hours of being ingested, and the few that remain alive do not undergo any morphological change and they even die within 24 h. With tsetse transmissible trypanosomes, although high mortality of the parasites occurs (Bruce, 1911; Hoare, 1940) in the hostile environment of the tsetse gut (Croft et al., 1982; Ibrahim et al., 1984; Maudlin and Welburn, 1987), a few trypanosomes escape death by transforming into trypomastigotes (procyclics) which migrate to the salivary glands and mouthparts. T. evansi on the other hand, fails to transform into procyclics even in culture (Zweygarth and Kaminsky, 1989).

Unfortunately, trypanosome infection rates in Glossina vary so much even with known tsetse transmissible species (Duke, 1933; Moloo and Kutuza, 1988) that one cannot with certainty classify all brucei-type trypanosomes isolated from camels as T. evansi, on the basis that they fail to develop in tsetse.

The successful adaptation of T. evansi to different environments of the world (Africa, Asia and South America) is unequalled by its T. brucei progenitors (Hoare, 1972). In their new niches, evansi trypanosomes are likely to have evolved biochemical characteristics that would allow their differentiation from their brucei relatives. Isoenzyme

studies by Gibson et al. (1980) showed that such differences indeed exist but are of limited taxonomic value in that they can only differentiate T. evansi from the East African T.b. brucei but not from the more closely related West African types from which they are thought to have recently evolved (Hoare, 1940; 1972).

Out of the seven enzymes tested in the present studies, only PGM showed an unequivocal difference between trypanosomes collected from camels and the East African T.b. brucei reference strain. However T.b. brucei PGM banding pattern had also been seen in other T. evansi isolates examined by Gibson et al. (1983). Thus it would appear that isoenzyme typing on its own is not a reliable method for differentiating T. evansi, either from the West African T.b. brucei or from the East African types. Gibson et al. (1983) reported enzyme differences in T. evansi isolates with regard to ME, PEP-1, PEP-2, ALAT, PGM and ASAT. In the results reported in this chapter, differences between the isolates collected from camels were only seen in ALAT, PEP-1 and PEP-2. It is worth noting that some of the isolates described by Gibson et al. (1983) were collected from the same areas as those used in this study. Boid (1988), while working on isolates collected from the Sudan, found no enzyme differences in ASAT, PGM or PEP-2. Such discrepancies point to the unreliability of isoenzyme analysis in distinguishing T. evansi from T. b. brucei. Also, in the absence of definite biochemical markers, some of the isolates referred to as T. evansi, simply because they were collected from camels, might have been T.b. brucei.

If the inability of T. evansi to complete cyclical development in Glossina is accepted as the fundamental difference from T. brucei, examination of genetic material that controls trypanosome transmission through tsetse may be more informative than tsetse transmission per se. The kDNA of trypanosomes contains essential genes for activation of the trypanosome mitochondrial respiratory system whose expression is believed to be necessary in trypanosome developmental stages occurring in Glossina (Benne, 1985). T. evansi is said to lack maxicircles (Fairlamb et al., 1978) which may explain their inability to multiply in tsetse (Borst and Hoeijmakers, 1979; Borst et al., 1980b; Borst et al., 1987). It can, however, be argued that the absence of maxicircles is not diagnostic for T. evansi because they can be retained in a non-functional state for long periods (Borst et al. 1987), as has been demonstrated with a strain of T. equiperdum (Frasch et al., 1980).

Kinetoplast DNA of trypanosomes contains another group of circles, the minicircles, the genetic function of which is unknown. They may play a structural role during cell division, and possibly hold the maxicircles in place (Simpson, 1987). The minicircles of T. evansi have been shown to differ from those of T. brucei by their sequence homogeneity (Borst and Hoeijmakers, 1979; Borst et al., 1987).

The results of the minicircle restriction enzyme analyses reported here showed that all the 48 trypanosome isolates had homogeneous minicircles confirming that, by this criterion, they were T. evansi. In contrast, the T.

brucei used for comparative purposes showed minicircle heterogeneity, a finding that has been reported by other workers (Steinert et al., 1976; Fairlamb et al, 1978; Borst et al., 1980a; Simpson and Simpson, 1980; Borst, Fase-Fowler and Gibson, 1981). A similar minicircle pattern for T. evansi had been reported by Borst et al. (1987) and is said to be widespread. Such isolates with similar minicircle sequences were considered as clones of a single ancestral T. evansi (Borst et al., 1987) which arose by one of the minicircles becoming dominant. If this is true, trypanosomes with similar minicircle patterns might express a similar variant antigen type (VAT) repertoire, and vaccination against antigens expressed early in infection might be possible in areas where a single T. evansi sub-group is responsible for the infections (Borst et al., 1987). The results of Experiment 3, show that trypanosomes with similar minicircles were isolated more than once from the same group of animals indicating that a T. evansi infection of a particular minicircle type, if treated, does not render animals immune to all T. evansi infections of that minicircle type. Thus, there may not be a direct correlation between minicircle type and serodeme.

Since the development of pulsed field gradient (PFG) gel electrophoresis, the technique has become a popular tool for studying protozoan genomes. Using this method, Van der Ploeg and colleagues (1984a) studied the molecular karyotypes of kinetoplastid parasites and found large differences between trypanosome species. T. brucei was found to have about 100 minichromosomes of between 50 and 150 kb whereas only few were found in T. equiperdum; T.

vivax on the other hand had no DNA smaller than 200 kb. Van der Ploeg et al. (1984a) concluded that the technique of PFG gel electrophoresis can be used to identify subspecies indistinguishable by other means. Giannini, Schittini, Keithly, Warburton, Cantor and Van der Ploeg (1986) studied the karyotypes of Leishmania from different geographical locations and found that within the same subspecies, the karyotype was similar. They concluded that the technique of PFG gel electrophoresis can be used for typing of clinical isolates. Kemp, Corcoran, Coppel, Stahl, Bianco, Brown and Anders (1985) studied the karyotypes of different isolates of Plasmodium falciparum and found polymorphism. They speculated that such differences may be important clinically and antigenically. Cox (1985) suggested that the observed chromosomal polymorphism in trypanosomes and malaria parasites may be related to virulence and drug resistance.

From the karyotypes studied here, chromosomal polymorphism was observed in isolates of T. evansi collected from camels. The general pattern of chromosomes resembled that reported for T. brucei (Van der Ploeg et al., 1984a; Gibson and Borst, 1986). The minichromosomes of the isolates were however generally bigger than those recorded for T. brucei. Two isolates (KETRI 2416, 2445) that had minichromosomes of similar sizes as the reference T.b. brucei (ILTat 1.3) were later shown to have heterogeneous kDNA mini-circles and, therefore, were T.b. brucei. There is a need, however, to examine more isolates in order to clarify whether this difference in the minichromosome sizes between T.b. brucei and T. evansi can

be generalized.

The karyotype homogeneity of isolates collected from Olturot compared to the heterogeneity observed in isolates from Ngurunit was striking and was possibly related to the different drug use in the two herds. The camel herd at Olturot which was owned by a research institution had a long history of routine trypanocide use. During the studies reported here, the herd was again maintained under trypanocidal chemoprophylaxis throughout the 18 months observation period. It is conceivable that the routine drug use in the Olturot herd eliminated drug sensitive trypanosomes leaving resistant ones. Indeed, one of the camels was found infected for a second time a month after it had been treated with a quinapyramine prosalt. When tested for quinapyramine sulphate sensitivity in mice, the infecting trypanosomes were shown to have a four-fold resistance to the trypanocide compared to the sensitive stock (section 6.3.2). Given the karyotype homogeneity of the isolates from Olturot, therefore, they were probably derived from a recent common progenitor.

The camel herd in Ngurunit which was owned by a pastoralist was not under any veterinary health care programme until initiation of the studies reported here, when the camels were given individual trypanocide therapy only on confirmation of diagnosis. This meant that the camels were always at risk of acquiring new trypanosome infections and in the absence of intense drug related selective pressure, the trypanosomes remained karyotypically heterogeneous. Four isolates from Ngurunit displayed the same karyotype (type F) as the drug resistant

Olturot isolates. Drug sensitivity of this isolate was not quantified but the infected camels responded well to trypanocidal therapy and no recurring infections were detected for the period they were under observation. Two isolates with a type E karyotype were detected for a second time in the same camels shortly following treatment and, in mice, they were shown to have a four- to five-fold resistance to the quinapyramine sulphate compared to the sensitive stock. It was also noted that trypanosomes isolated from the recurring parasitaemia had a similar karyotype to the first one. However, not all the camels infected with trypanosomes of type E karyotype had recurring parasitaemia after trypanocide therapy.

From the similarity of the karyotype patterns of the majority of trypanosomes isolated during the second outbreak of trypanosomiasis and those isolated at the beginning of the studies, it is probable that some camels remained as reservoirs after the trypanocide therapy and were, therefore, the source of the second outbreak. The fact that the karyotype patterns that had not been seen earlier were found in trypanosomes that were collected from camels introduced from outside the herd, indicates that karyotyping can be used to differentiate multiple re-isolations of the same trypanosomes from those that are different.

A correlation of chromosome profile and antigenicity was found in T. congolense (Masake et al., 1988). In cross immunisation studies, clones belonging to one molecular karyotype expressed a similar VAT repertoire. These findings must however, be seen in the light that the

trypanosomes studied by Masake et al. (1988) were collected from an isolated herd which had been recently introduced onto a ranch. It is therefore, likely that in the short period of that study, the trypanosome clones that had similar chromosomal profiles were effectively the same trypanosome strain isolated many times. This means that the observed correlation of karyotype and VAT repertoire may have been no more than a reflection of the multiple isolation of each type. While the results of Masake et al. (1988) show that karyotype patterns may predict antigenic similarity in a restricted geographical location and temporal situation, the extent to which this correlation may be generalised in a wider context depends on factors which have not been examined. The major question is that of the relative rates of change of the characteristic examined (karyotype) and that for which a predictive value is desired (VAT repertoire). If karyotype patterns evolve more rapidly than the VAT repertoire changes, then similarity of karyotype should imply antigenic similarity, but karyotypic differences will not imply antigenic differences. On the other hand, if the rates of change are reversed, karyotype patterns will not be useful as predictors of VAT repertoire. Estimates of the stability of VAT repertoire and karyotype patterns in particular trypanosome species will be necessary for generalisation of their expected correlation. A means for the quantitative estimation of similarity in karyotype patterns would be most useful in attempts to evaluate such general correlation, but is lacking so far.

Four different methods have been used to characterise

T. brucei-like isolates collected from camels in Marsabit, northern Kenya and a summary of the characteristics of the field isolates with reference trypanosomes are shown in Table 10 . The tsetse transmission studies showed the inability of the organisms to undergo cyclical transmission in Glossina which was in agreement with the definition of T. evansi (Hoare, 1972). Tsetse transmission experiments were, however, time-consuming and laborious. Also, given the low numbers of tsetse used and the low infection rates observed with the reference T.b. brucei, it can be argued that, had tsetse transmission been the only criterion used, it could not have been relied upon as a definite distinguishing characteristic.

Isoenzyme typing showed polymorphism between the isolates but was unreliable as a method of differentiating T. evansi from T. brucei. No correlation was found between the different zymodemes and phenotype. Analysis of kDNA minicircles showed unequivocal similarity between the camel-derived stocks and the reference T. evansi: the pattern was, however, different from the reference T.b. brucei. From these results and those reported by Borst et al. (1987), analysis of kDNA minicircles revealed the camel-derived isolates to be T. evansi and the technique appears, therefore, to be the only reliable and unequivocal method of differentiating T. evansi from T. brucei.

Highly repetitive DNA sequences have been identified in certain fragments of T. evansi minicircles and on hybridization to total radiolabelled DNA of other brucei group trypanosomes, they showed no homology (Masiga and Gibson, personal communication; Bajyana Songa, Wittouk and

Table 10. Summary of characteristics of field trypanosome stocks with reference T. brucei.

	Tsetse transmission	Isoenzyme patterns	kDNA minicircles	PFGE patterns	Identity of trypanosome
Ngurunit	-	±	Hm	D,E,F,G, H,I,J,K	T. evansi
Olturot	-	±	Hm	F	T. evansi
Reference T. brucei	+	±	Ht	Unique*	T. brucei
Reference T. evansi	ND	±	Hm	B	T. evansi

Key

- = Not transimissible

+ = Transimissible

± = Not distinguishing

Hm = Homogeneous

Ht = Heterogeneous

* = Smaller minichromosomes

ND = Not done

Hamers, personal communication). In future, these highly repetitive DNA sequences may be made into recombinant probes in which case, the testing for putative T. evansi would be less laborious than direct analysis of minicircles using restriction endonucleases. Before gaining widespread use, such probes would have to be tested on large collections of T. brucei and T. evansi in order to confirm their specificity. Also, to be affordable and safe to handle for the laboratories in the countries where the problem of T. evansi exists, the probes would have to be labelled non-isotopically.

Considering the homogeneity of the kDNA minicircles observed in all the stocks examined in this study, and also from the report of Borst et al (1987), it appears that the rate of change of kDNA sequences is slow compared to that of significant phenotypes such as antigenicity and drug resistance.

The karyotypes of T. evansi examined in PFGE showed marked polymorphism. Karyotyping was the most sensitive method for showing differences between the T. evansi isolates. Within the limited geographical location and time of this study, the karyotypes were useful in revealing the most likely source of trypanosomes isolated at various instances. These observations cannot, however, be generalised since karyotypes are bound to change as has been shown to happen in T. brucei (Van der Ploeg et al., 1984a, b). Differences in minichromosomes were observed between stocks that had been identified as T. brucei by kDNA minicircle analysis and those identified as T. evansi. Many more isolates from different geographical

areas would have to be analysed before these observations could be applied in a wider context.

CHAPTER 4

DIAGNOSIS AND CONTROL OF T. EVANSI INFECTIONS

4.1 INTRODUCTION

In routine veterinary practice, diagnosis of T. evansi infections in camels and subsequent treatment is largely based on clinical signs. The cardinal sign of trypanosomiasis is anaemia. Thus, the haematocrit level is popularly used as an aid to diagnosis, and in combination with an aetiological diagnosis, it gives a good indication of the disease status and herd performance (Murray and Dexter, 1988).

Clinical signs of trypanosomiasis, however, are not pathognomonic as a number of other diseases (for example helminthiasis and malnutrition) produce similar signs and the aetiological diagnosis is also a complex issue. The characteristic fluctuating nature of the parasitaemia and the low parasite numbers means that unless regular and repeated sampling is carried out, many infections would be missed. Attempts to overcome the the low sensitivity due to scarcity of parasitaemia have led to development of more sensitive parasitological techniques such as the buffy coat examination (Woo, 1969) and inoculation of suspect blood into laboratory animals (Paris et al., 1982). This however has not obviated the need for repeated sampling. Moreover, many infections still go undetected, partly because trypanosomes can be present in tissues but not in peripheral circulation.

Many immunological methods have been developed for antibody tests but they suffer from three major drawbacks. First, they cannot differentiate between current and past trypanosome infections; second they lack the desired specificity; and third, some of the techniques cannot be

adapted easily to situations that exist in the field (see page 31 to 40).

A new generation of immunological tests for diagnosis of trypanosomiasis has been developed which detect the presence of antigens rather than antibodies and, therefore, are synonymous with parasitological diagnosis (Rae and Luckins, 1984, Nantulya, 1989; Nantulya and Lindqvist, 1989; Nantulya et al., 1989,a and b). These tests have been found to correlate with parasitological diagnosis in both experimental and natural infections. For these tests to gain widespread use, however, they must show distinct advantages over the techniques already available . An important consideration here would be their sensitivity, simplicity and adaptability to field conditions.

In the absence of an immunoprophylactic solution to the problem of trypanosomiasis, control of this disease may be effected by removing the biting flies; by modifying management so that animals are kept away from high risk areas at certain times of the year; and by the use of trypanocides.

The standard methods of vector control, as applied to tsetse, cannot, however, be used for eradicating the biting flies that transmit T. evansi. Removal of the biting flies' habitat cannot be contemplated in the fragile environments of the arid zones. Moreover, the habitats in which the flies exist are the prime pastures for the camels. The flies could also be attacked directly through the application of insecticides, a method which has been used with a good measure of success, for control of tsetse (Jordan, 1986). In order to succeed, this method requires

an enormous capital outlay. Added to the consequent environmental pollution, such an expensive operation may not be justifiable among the nomadic societies whose economies are not integrated with the national economy. Biological methods and use of attractants and traps, as used for tsetse control, require a thorough understanding of the vector's biology, a claim that cannot be made about the biting flies that transmit T. evansi.

The management practice of keeping animals away from the risk areas is widely practised among the nomadic people (Lengima, 1987, personal communication). The nature of pastoralism is, however, such that it is difficult to avoid completely such areas since they often happen to be the areas with abundant pasture.

Under nomadic situations, increase in the number of camels is slow compared to that of cattle and small stock (Lengima, 1987, personal communication). For example, it takes about six years for female camels to reach breeding age. The gestation period is 12 to 13 months and the calving interval is two to three years. Any disease that retards the already slow reproductive rate of camels warrants serious consideration. Camel trypanosomiasis is one such disease. It causes low milk yields, wasting, abortions and deaths, losses that retard herd build up, and, therefore, increase malnutrition among the nomadic people.

The use of trypanocides is, consequently, the mainstay of T. evansi control. Its application is widespread and it is an important aspect of veterinary practice.

Chemotherapy is, however, beset by many problems, amongst which logistic and financial considerations are the most important (Holmes and Scott, 1982). Chemotherapy is justifiable only if it will lead to improved productivity, either in terms of meat, milk or draught power. There is ample evidence to indicate that, in trypanosomiasis endemic areas, cattle are more productive if kept under trypanocide therapy, be it in big ranches or in rural mixed economies (ILCA/ILRAD, 1988). In the pastoral economy, animal husbandry is not seen in monetary terms, and the quantification of the economic benefit of any disease control programme is difficult. Evidence indicates, however, that, the use of a simple veterinary input in camel husbandry leads to improved productivity (Rutagwenda, 1984), and in the study cited trypanocide use was a major input.

A survey conducted in different trypanosomiasis endemic areas of Kenya indicated that different camel herds require different control programmes (Wilson et al., 1983). This observation reiterates the importance of a thorough knowledge of the local epidemiological situations: a pre-requisite to proper drug use. In the field, the use of trypanocides is not always rational. Rarely is it related to the period of risk or greatest challenge (Griffin and Allonby, 1979).

Whatever control strategy is found appropriate, care must be taken in order to avoid emergence of drug resistant strains. The situations under which drug resistance develops have been reviewed by Leach and Robert (1981). In the field, emergence of drug resistant strains is suspected

when the recurrence of parasitaemia occurs at a period when animals are supposed to be under drug protection. While this might be so, recurrence of parasitaemia could also occur because the trypanocides were used incorrectly, for example by underdosing; use of expired or fake drugs; individual animals may have escaped treatment during mass prophylaxis and only later found to be infected. To ascertain that animals with relapse infections had enough levels of trypanocide at the time of such relapses, it would be necessary to determine the plasma drug concentration. Unfortunately, there are, as yet, no simple, sensitive assay methods for trypanocides that can be used routinely. Relapses could also originate from trypanosomes in tissues inacessible to the drugs (Jennings et al., 1979), the trypanosomes, in such cases, emerging once the drug levels have waned to concentrations that are not curative.

The only way to prove drug resistance is to demonstrate the response of the suspect trypanosomes to the trypanocide in question. Ideally such experiments should be performed in the definitive host (in this case the camel), since, as was found by Sones et al. (1987), there are differences in drug sensitivity determined in large animals (bovine, in that experiment) and mice. Owing to the considerable logistical problems and expense of performing such tests in large animal species, mice are generally used, but the results obtained can only provide broad indications of the sensitivity of a stock, and not to predict curative doses for the definitive host (Sones et al., 1987).

Two drug dosages are conventionally used in sensitivity tests; the minimum effective dose (MED), a term that denotes the lowest drug dose which will temporarily clear an infection from the blood of a designated percentage of the test animals, and minimum curative dose (MCD), a term that denotes the lowest dose which will achieve a sterile cure in a designated proportion of the test animals. For practical purposes in the field, MED is of no significance.

In the studies described in this chapter, data on four methods of diagnosis of T. evansi , namely, clinical signs, haematocrit level, parasite detection and trypanosomal antigen detection, were compared. Also both the curative and prophylactic approaches to the control of trypanosomiasis were evaluated. The antigen detection test was carried out 17 months after the serum collections and the results are of interest in retrospect. Serum samples obtained from animals with proven trypanosome infection but without antigenaemia were analysed for the presence of immune complexes. Likewise, samples with antigenaemia but without parasitological confirmation were checked for anti-trypanosomal antibodies.

The broad quinapyramine sulphate sensitivity values of trypanosome stocks that had been shown to either recur after trypanocide treatment or had persistent antigenaemia were investigated in mice. Their sensitivity values were compared to those of two trypanosome stocks, one which was collected from the same area but had not recurred after treatment, and the second was a stock known to be resistant to the major trypanocides available on the market (Gitatha,

KETRI, unpublished information).

It was anticipated that, through this study, adequate information would be obtained to provide an insight into the local epidemiology of the disease. This would subsequently enable a more rational use of trypanocides to control the disease, with the ultimate aim of increasing productivity of the camels and thus improving the living standards of the camel-keeping nomads.

4.2 EXPERIMENT 1- EXPERIMENTAL T. EVANSI INFECTION

4.2.1 MATERIALS AND METHODS

4.2.1.1 PARASITES USED.

Trypanosoma brucei- type trypanosomes, later confirmed by KDNA studies to be T. evansi (section 3.4) were obtained from a naturally infected camel and inoculated intraperitoneally into two female outbred Swiss mice. Wet preparations of the mouse tail blood were examined daily and at peak parasitaemia (about 100 parasites per field, x400 magnification), the two mice were anaesthetised with diethyl-ether and blood was collected by cardiac puncture into a 1.0 ml syringe containing heparin at a concentration of 10 units ml^{-1}. Blood from one of the mice was used to infect the camels while trypanosomes from the other mouse were cryopreserved (section 3.2.1.2) for characterisation (chapter 3).

4.2.1.2 CAMELS USED AND EXPERIMENTAL DESIGN.

Five healthy entire male yearlings (Numbers 763, 764, 765, 766 and 767) aged 2 to 3 years, belonging to a local pastoralist were used. The 5 animals were part of a herd of 30 camels that grazed around the Ngurunit area. For the purpose of identification, the 5 camels were double ear-tagged. Subsequently they were treated orally with an anthelmintic, thiophanate 20% (Nemafax, May and Baker Ltd, Dagenham, UK.) at a dosage rate of approximately 66.7 mg kg^{-1}.

Two of the 5 camels were used as controls while the other 3 were infected. In order to ascertain that the

experimental camels did not have trypanosome infections prior to the experiment, the PCV was determined and the buffy coat examined for the presence of trypanosomes (Murray et al., 1977).

The 3 camels were infected via the jugular vein with 1 x 10^6 trypanosomes. Blood for PCV determination (section 4.2.1.3), antigen detection (section 4.2.1.5), and for examination for presence of trypanosomes (section 4.2.1.4) was subsequently collected every other day for a period of 87 days. Also, the 3 infected camels were examined clinically at the time of sample collection. All the blood samples were collected before 0930 h.

To avoid the rest of the animals acquiring trypanosome infections from the experimentally infected camels, the former were protected with quinapyramine prosalt (Trypacide prosalt, May and Baker Ltd, Dagenham, UK.) at a dosage rate of 5 mg kg^{-1}.

4.2.1.3 DETERMINATION OF PACKED CELL VOLUME (PCV).

Whole blood was mixed gently and thoroughly for half a minute and then drawn up to 3/4 full in plain microhaematocrit capillary tubes (Hawksley and Sons Ltd., U.K.), which were sealed at one end with plasticine and centrifuged for 4 min in a Gelman Hawskley microhaematocrit centrifuge. The haematocrit level was read using the Hawskley haematocrit reader and recorded in l/l .

4.2.1.4 DETECTION OF TRYPANOSOMES

The buffy coat technique (Murray et al.,1977) was used for detecting parasitaemia. Briefly, once the PCV

was read, the capillary tubes were cut with a diamond pointed pen 1.0 mm below the buffy coat in order to incorporate the uppermost layer of erythrocytes. This, together with some plasma, was expressed onto a clean slide and then covered with a coverslip. The preparation was examined for the presence of trypanosomes using an Olympus microscope with a combination of 40x objective lens and a 10x eyepiece. A mirror fitted to the microscope to reflect the ambient light provided illumination.

4.2.1.5 DETECTION OF ANTIGEN BY SANDWICH ELISA TECHNIQUE.

Test reagents.

A T. brucei group specific monoclonal antibody (MoAb), TR7/47.34.16, prepared by Dr. V.M. Nantulya, ILRAD, was used. The antibody was a partially purified fraction of an IgM isotype raised in mice against T. rhodesiense procyclic trypomastigotes (Nantulya et al., 1987). The enzyme-antibody conjugate used was the same MoAb, TR7/47.34.16, conjugated to horseradish peroxidase enzyme (type VI, E.C 1.11.1.7, RZ 3.2. Sigma, UK) prepared by Prof. K. Lindqvist, ILRAD.

As negative controls, serum samples obtained from camels which had been shown to be parasitologically negative by mouse inoculation, were used. Of these sera, 30 came from Athi River and 90 came from Rumuruti. Positive control sera were obtained from three weaner camels that had parasitologically confirmed trypanosome infections of over 30 days duration.

Chequerboard dilutions of the MoAb, control sera and peroxidase conjugate were prepared to determine the dilutions which gave the lowest absorbance with negative sera and the highest absorbance with the positive control sera. When MoAb was used at a concentration of 0.5 ug/well, the conjugate at a dilution of 1:2000 and sera at 1:2, the positive control serum samples gave readings that ranged between 0.800 and 1.500 and all the negative sera read below 0.050.

The test procedure.

The method used was as devised by Engvall and Perlmann, (1971); Bidwell and Voller, (1981). Briefly, the micro-ELISA method used was as follows : Flat bottom microtitre plates (M 129 A or B, Dynatech, U.K.) were coated by placing in each well 100 ul of 5ug/ml MoAb in a carbonate/bicarbonate buffer pH 9.6 (14.6 mM Na_2CO_3, 34.9 mM $NaHCO_3$) with 0.02% sodium azide. The coated plates were sealed and left at $4^{o}C$ for at least 12 h before use. The excess coating solution was drained off just before carrying out the assay and the plates washed by three quick rinses with Dulbecco's PBS, pH 7.4 (Dulbecco and Vogt, 1954) containing 0.5% Tween 80 (washing buffer). The plates were drained dry by tapping them on dry tissue paper.

To each of the wells was added 50 ul of the washing buffer and 50 ul of test serum in duplicates. For each plate, duplicates of negative and positive control sera were included. Also, two wells without sera were used as reagent controls. The plates were then incubated for 10 min at room temperature and then washed as before.

To each of the wells was then added 100 ul of the conjugate diluted to 1:2000 in 1% normal mouse serum in washing buffer . The plates were then incubated at room temperature for 10 min after which they were rinsed once before being filled with washing buffer and left for 10 min. This was repeated twice to remove the excess conjugate, and the excess buffer was drained off as before.

One hundred microlitres of substrate and chromogen was then added to each of the wells. The chromogen used was 223 ug/ml 2, 2'-azino bis 3-ethyl-benzothiazine-6-sulforic acid (ABTS) in citric acid buffer pH 4.0 (49.9 mM citric acid, 0.82 mM benzoic acid and 0.3% glacial acetic acid, containing 0.01% substrate (hydrogen peroxide). The plates were incubated in the dark at room temperature and the optical densities (O.D.'s) read after 30 min at 414 nm wavelength in a Titertek Multiscan micro-ELISA autoreader (Type MCC 340, Titertek, Finland).

4.2.2 RESULTS

4.2.2.1 CLINICAL FINDINGS

Three days post infection (p.i), the 3 infected camels, (763, 765 and 766) which had hitherto been alert and eating, were reported not to be doing well. They were said to be off their feed and preferred to lie in direct sunlight. On the day the camels were reported sick (day 3), they had sunken flanks and their temperatures before 0930 hours were 38.0, 38.1 and 37.5^{o}C. These temperatures are considered high in a camel because its diurnal physiological regulation is such that the body temperature is low early in the morning (average 36 to

36.5°C), and rises steadily throughout the day to about 39°C in the evening. Therefore, a camel with a temperature of 37.5 to 38°C early in the morning was considered febrile (Higgins and Kock, 1986). Figure 2 shows the changes in rectal temperatures following the infection. From day 3, the rectal temperatures of the three infected camels were noted to rise and were highest (40°C) on day 4. They remained high until day 7. Thereafter, the fever fluctuated, until a trypanocide, quinapyramine sulphate, at a dosage rate of 3mg kg^{-1} was administered on day 27.

Camel 766 was noted to be limping on the left fore limb on the third day. By the following day, all the animals showed excessive bilateral lachrymation. On day 6, camel 766 developed a swelling at the coronet of the left fore limb. On day 7, camel 763 was also found to be limping. Examination of this animal did not reveal any obvious cause of the swelling. From day 14 onwards, other than occasional fever, these camels appeared to be well.

Both the control camels developed a slight fever (38.2°C) two days after they received quinapyramine prosalt (day 2). The fever subsequently subsided and they remained well throughout the observation period.

4.2.2.2 PARASITAEMIA.

Heavy parasitaemias were detected on day 2 p.i and remained so until day 27, when trypanocide treatment was administered. Camel 763 and 766 had relapses 39 and 47 days post treatment, respectively. Camel 765 had no relapse. The relapsing parasitaemia was fulminating for

camel 763, while that of 766 was scanty. These relapses did not elicit pyrexia and the camels continued to do well.

4.2.2.3 PACKED CELL VOLUME.

Four days p.i, the mean PCV of the 3 infected camels fell from a pre-infection level of 0.29 l/l, the haematocrit reaching the lowest level recorded of 0.20 l/l by day 21 (Figure 3). Following the quinapyramine sulphate treatment, the PCV rose slowly, and reached 0.23 l/l by day 41 p.i. The relapse parasitaemia was not accompanied by a drop in PCV.

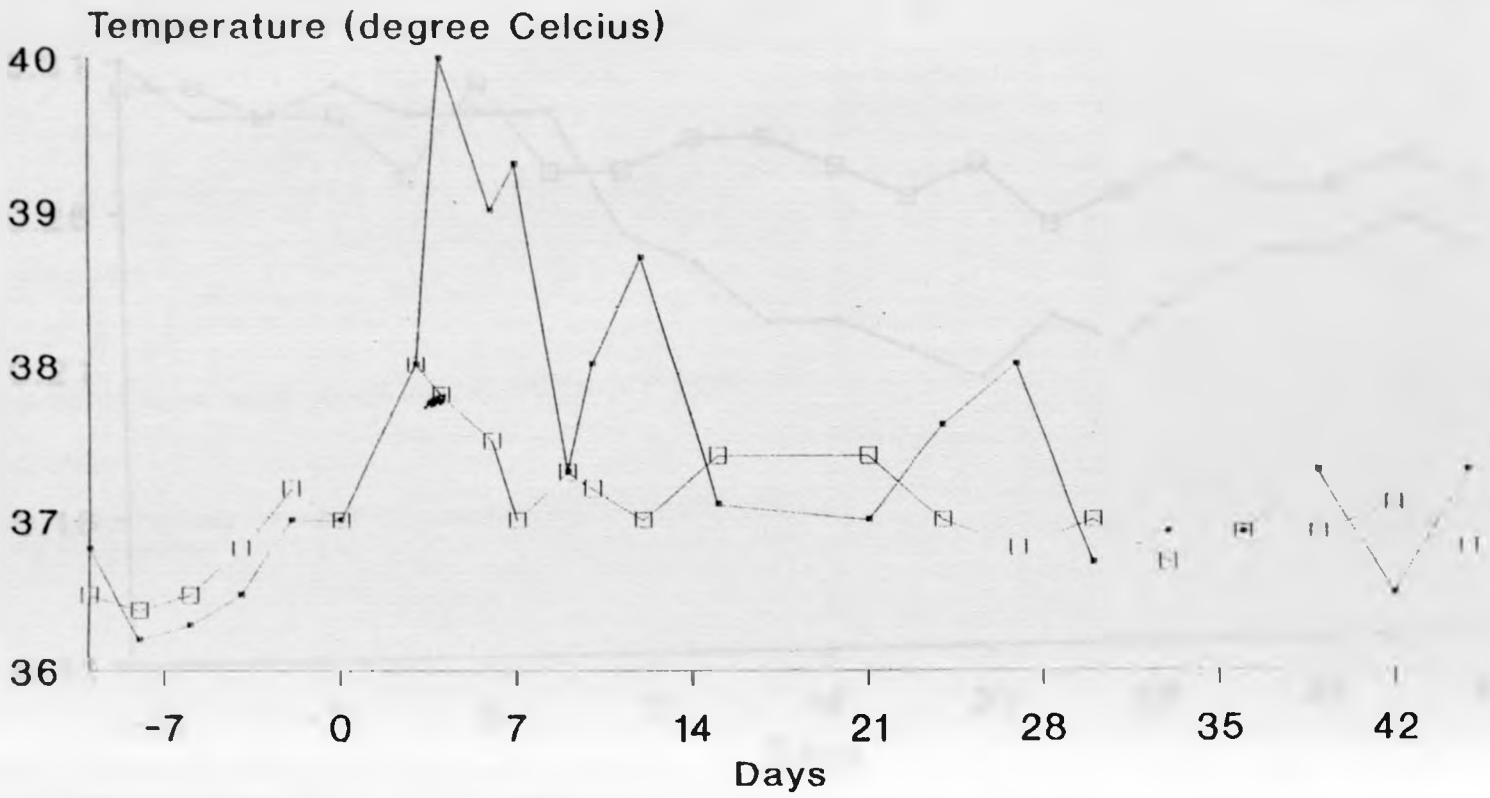
4.2.2.4 ANTIGEN DETECTION

Data on the antigen detection in sera from the 5 camels is summarised in Table 11.

Prior to infection, none of the camels' tested sera had an O.D reading higher than 0.024. The threshold value was thus set at 0.05 which was twice the pre-infection O.D. value.

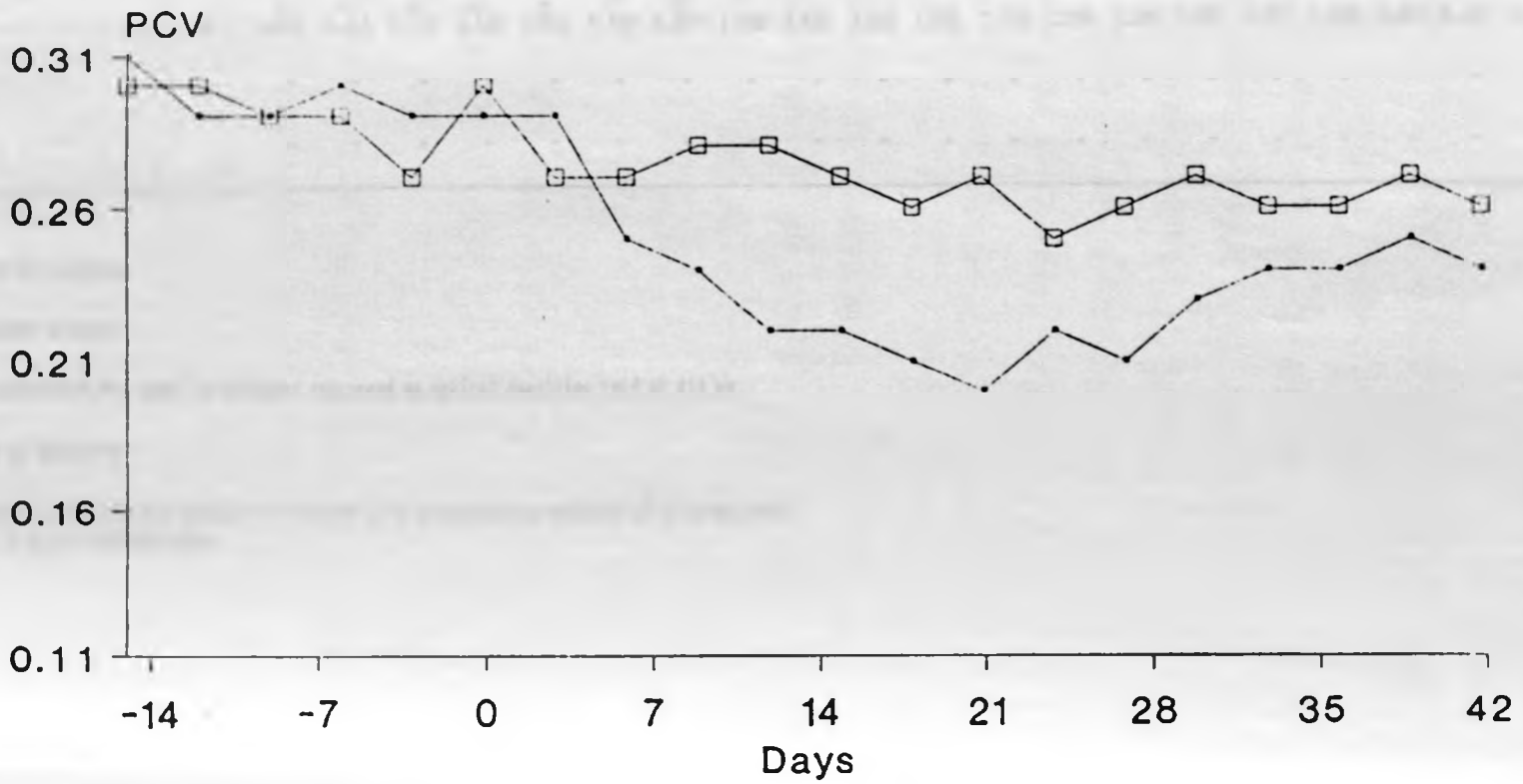
Following the infection, a significant rise in the O.D. value was demonstrated in the serum of one of the camels on day 9 p.i. (camel 766, O.D value of 0.05). Thereafter, the O.D. values of the 3 camels rose and remained high throughout the observation period, even when patent parasitaemia could no longer be demonstrated. Unlike the parasitaemia, the O.D. did not decline following administration of the trypanocide. Parasitaemia relapses were later observed in two of the camels (763 and 766).

Fig. 2. Group mean rectal temperatures of camels experimentally infected with T. evansi.



Day 0 = Day of infection Day 27 = Day of treatment

Fig. 3. Group mean Packed Cell Volume (PCV, l/l) of camels experimentally infected with T. evansi.



Day 0 = Day of infection Day 27 = Day of treatment

Table 11. Antigen detection results and parasitological findings in camels experimentally infected with trypanosomes.

Days	-30	0	2	4	9	11	13	16	19	22	25	28	31	34	37	40	46	49	52	55	58	61	64	67	70	73	79	87
Camel																												
763	-	-	+	+	+	+	0.125	0.365	0.044	1.088	0.861	0.879	0.790	0.624	0.239	0.256	0.356	0.236	0.324	0.369	0.258	0.481	0.343	0.378	0.465	0.697	0.602	0.464
765	-	-	+	+	+	0.248	0.423	0.789	0.933	0.425	0.461	0.355	0.203	0.488	0.351	0.358	0.456	0.158	0.423	0.598	0.687	0.700	0.721	0.568	0.638	0.522	0.403	0.742
766	-	-	+	+	0.049	0.164	0.352	0.125	0.072	0.415	0.588	1.400	1.424	1.421	1.351	1.378	1.100	1.005	0.954	0.874	0.745	0.607	0.412	0.365	0.284	0.293	0.201	0.124
764	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
767	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key

- = Antigens not detected

+ = Trypanosome detected

Numerical values give the level of antigens expressed as optical densities read at 414 nm.

Day 0 = Day of infection

Day 27 = The day the infected camels were treated with quinapyramine sulphate at a dosage rate of 5 mg/kg subcutaneously

4.3 EXPERIMENT 2- NATURAL T. EVANSI INFECTION

4.3.1 STUDY DESIGN

Two herds of camels were studied; one herd was located at Ngurunit and the other at Olturot (see 2.1). The Ngurunit herd was located 38 km away from the field laboratory, while the Olturot herd was 100 km away. Because of the distances involved in travelling to the field laboratory, sampling and examination were more frequent in the herd at Ngurunit than in the herd at Olturot.

Clinical data were obtained from camels at Ngurunit. Blood samples were collected from the camels for a period of 18 months, covering July, 1986 to December, 1987. Whole blood for parasitological diagnosis was collected weekly from camels at Ngurunit and monthly from camels at Olturot. Serum samples for use in immunodiagnosis were collected fortnightly from camels at Ngurunit and monthly from those at Olturot.

Because the Ngurunit herd was nearer, the camels were, therefore, treated on an individual basis with quinapyramine dimethylsulphate at a dosage rate of 5 mg kg^{-1} bodyweight whenever trypanosomes were detected. The camels at Olturot were kept under prophylactic treatment using quinapyramine prosalt at a dosage rate of either 3 mg kg^{-1} or, 5 mg kg^{-1} bodyweight, the dose used being dependent on the anticipated duration of the risk period. The latter is high during the long rainy season when the biting fly population is also high (Mahmoud and Gray 1980). Prophylactic cover was applied when trypanosomes were detected in at least 5% of the population studied.

4.3.1.1 CLINICAL EXAMINATION

Clinical data were obtained whenever possible from camels that were reported to be sick in the herd at Ngurunit.

4.3.1.2 DETERMINATION OF PACKED CELL VOLUME.

This was done as described under section 4.2.1.3

4.3.1.3 DETECTION OF TRYPANOSOMES

4.3.1.3.1 Buffy coat method. Parasitaemia was assessed by the buffy coat method as described under section 4.2.1.4

4.3.1.3.2 Inoculation of camel blood into mice

Once a month, 0.25 ml of fresh whole blood from each camel was inoculated intraperitoneally into one individually marked mouse. This technique is more sensitive in trypanosome detection than the microhaematocrit centrifugation method (Paris et al. 1982). After inoculation, wet preparations of the mouse tail blood were examined three times per week for 30 days before the animals were declared free from infection.

Thin blood smears were also made from samples found to have trypanosomes. These were fixed for one minute with methanol and stained with Giemsa's stain for 30 min and the preparations examined for trypanosomes under x100 oil immersion objective lens of an Olympus microscope.

4.3.1.4 RAINFALL RECORDS

Rainfall data were obtained from rain gauges located at Ngurunit and Olturot field stations.

4.3.1.5 DETECTION OF TRYPANOSOME ANTIGEN.

This was carried out as described under section 4.2.1.5

4.3.1.6 DETECTION OF ANTI-TRYPANOSOME ANTIBODIES

Some of the serum samples collected were tested for the presence of anti-trypanosome antibodies by ELISA.

4.3.1.6.1 Preparation of antigen for use in antibody ELISA

The antigen used in this test was an extract of sonicated T. evansi (KETRI 2540) originally isolated from a horse in South America. Trypanosomes were removed from storage in liquid nitrogen, and inoculated into rats which had been immunosuppressed by irradiation. At peak parasitaemia, the trypanosomes were separated from blood on a DEAE cellulose column (DE 52, Whatman, U.K.) and washed three times with phosphate buffered saline glucose (PSG) pH 8.0. The trypanosomes were concentrated by centrifugation and the pellet resuspended in 5 ml PSG pH 8.0. The trypanosomes were disintegrated by ultrasonic oscillation for 6 min at 20 KHz, 100 W in an ice-bath. The extract was centrifuged at 14,000 rpm for 15 min at 4^{o}C in a refrigerated micro-centrifuge (Tomy MR-150, Japan) and the soluble extract stored at -20^{o}C until required. Before use, protease inhibitors were added to the following final concentration (1 mM Phenylmethylsulfonylfluoride, 0.2 mM N-x-P-Tosyl-l-phenylalanine chloromethyl ketone, 0.05 mM N-Tosyl-l-lysine chloromethyl ketone and 10 ug/ml leupeptin).

4.3.1.6.2 Enzyme conjugate for antibody ELISA

A goat anti-camel IgG horseradish peroxidase conjugate prepared by Prof. K. Lindqvist, ILRAD was used. As negative control, 30 serum samples collected from a herd of camels located at Athi River were used. These animals had no history of trypanosome infection and were parasitologically negative as determined by mouse inoculation and by microhaematocrit centrifugation technique. As positive controls, sera obtained from three camels with parasitologically confirmed trypanosome infections of over 30 days duration were used. A chequerboard titration of antigen, control sera and horseradish peroxidase conjugate were performed to determine the dilutions which gave the lowest absorbance with negative sera and the highest absorbance with the positive control sera. When antigen was used at a dilution of 1:25, the conjugate at 1:10,000 and serum at 1:2, the mean O.D. ($\pm$ S.D.) for the 30 negative control sera analysed for the presence of antibodies was 0.031 $\pm$ 0.009. Values above twice the mean O.D. of the negative control sera were regarded as positive. All the positive control sera gave readings above 0.100.

4.3.1.6.3 Antibody ELISA

The microELISA method was performed as follows : Flat bottom microtitre plates (Dynatech, U.K.) were coated with 100 ul of 1:25 dilution of trypanosome antigen in carbonate buffer pH 9.6 (14.6 mM Na_2CO_3, 34.9 mM $NaHCO_3$) with 0.02% sodium azide. The coated plates were sealed and stored at 4^{o}C until required, when excess coating antigen

was drained off and the plates rinsed three times in Dulbecco's PBS containing 0.5% Tween 80 (washing buffer), and drained dry.

To each of the wells was added 100 ul of washing buffer and 2 ul of each test serum was then added into duplicate wells. On each plate, duplicates of negative and positive control sera were included. Also, two coated wells without sera, were used as reagent controls. The plates were then incubated for 30 min at room temperature and then washed as before.

To each of the wells was added 100 ul of goat anti-camel IgG horseradish peroxidase conjugate diluted to 1:10,000 in 1% normal goat serum in washing buffer. The plates were incubated at room temperature for 10 min. after which they were rinsed once, then filled with the washing buffer and left for 10 min. This washing procedure was repeated twice.

To each of the wells was then added 100 ul of substrate and chromogen and the O.D.'s read after 30 min. (section 4.2.1.5).

4.3.1.7 DETECTION OF IMMUNE COMPLEXES

4.3.1.7.1 <u>The test sera</u>

Serum samples collected from Ngurunit in which antigens were not detected despite a positive parasitological diagnosis, were tested for presence of complexed trypanosomal antigens. The rationale of this assay was that, the failure to detect trypanosomal antigens in certain cases was that the antigens could have been complexed to antibodies. A microELISA assay was,

therefore, designed such that the complexed trypanosomal antigens would bind to a TR7/47.34.16 MoAb coated plate. The MoAb-bound immune complexes could then be revealed and quantified with an enzyme-labelled anti-camel IgG antibody after addition of chromogen and substrate.

4.3.1.7.2 The test procedure

Plates coated with 0.5 ug/well of TR7/47.34.16 MoAb were used. As negative control sera, the samples used as negative controls in the antibody detection assays (section 4.3.1.6.2) were used. As positive control, serum samples which had been shown to have high levels of both antigens and antibody were used. A chequerboard titration of control sera and conjugate (goat anti-camel IgG horseradish peroxidase) was performed to determine the dilutions which gave the lowest absorbance with negative sera and highest absorbance with positive sera. When the conjugate was used at a dilution of 1:10,000 and control sera at 1:50, the mean O.D. ($\pm$ S.D.) for the 30 negative control sera was 0.195 $\pm$ 0.032 and the positive sera tested gave readings of over 0.500. Values above twice the mean O.D of the negative control sera were regarded as positive.

The micro-ELISA method used was as follows : Flat bottom microtitre plates were coated with TR7/47.34.16 MoAb and after washing off the excess antibody (section 4.2.1.7), the wells were filled with 100 ul of Dulbecco's PBS with 0.5% Tween 80 pH 7.4 (washing buffer),and 2 ul of each test serum added in duplicate. Duplicates of negative and positive control sera were also included. Two wells without sera served as reagent controls. The plates

were incubated for 10 min. at room temperatue and then washed by three quick rinses in washing buffer.

To each of the wells was then added 100 ul of goat anti-camel IgG horseradish peroxidase conjugate diluted to 1:10,000 in 1% normal goat serum in washing buffer. The plates were then incubated for 10 min. at room temperature after which they were rinsed once then filled with the washing buffer and left for 10 min. This was repeated twice.

To each of the wells was then added 100 ul of substrate and chromogen (section 4.2.1.5) and the O.D.'s read after 30 min.

4.3.2 RESULTS

4.3.2.1 CLINICAL FINDINGS

In the 76 weeks of observation of the camel herd at Ngurunit, 30 camels out of the 60 were reported to be sick and examined clinically. Their various clinical features are summarised in Table 12.

The majority of the animals became sick towards the end of the rainfall period (July 1987). Of the 30 sick camels, 11 were just weaned calves, 9 were yearlings and 10 were dams. Generally, there was a history of vague illness: animals were said to be off colour, inappetant and not chewing the cud. Among the sick pregnant dams, abortion was said to be widespread. The affected lactating dams were said to have reduced milk production. In the yearlings and the weaned calves, there was an additional history of "Khanid", a local name used to describe swollen lymph nodes, a condition that was widespread in the area.

When these animals were examined clinically, the main presenting signs were dullness, sunken flanks, copious bilateral lachrymation (Plate 17) and swollen submandibular lymph nodes; the latter sign being more conspicuous among the immatures camels (Plate 18.)

Most affected animals were febrile, with a rectal temperature above 37.5^{o}C before 0930 hours in the morning, or more than 39.5^{o}C in the hot hours of the day when ambient temperatures were high. Despite the excessive lachrymation the only obvious eye lesion was conjunctival congestion. The superficial lymph nodes commonly found to be enlarged were the submandibular, prescapular and precrural. The camels with "Khanid" had dry oral mucosae and halitosis was evident.

Three dams (ear tag numbers 903, 906 and 909) showed signs of threatened abortion indicated by premature relaxation of the caudal pelvic ligaments. The three camels eventually aborted 7 to 8 month-old foetuses (Plate 19) and one other had a still birth (Plate 20). One of them, (ear tag number 909) aborted the day after it had been reported sick; it had also been treated with quinapyramine sulphate four days before, for acute trypanosomiasis after a confirmatory diagnosis. The camel 909 did not improve following treatment and died 24 days thereafter. Another dam, ear tag number 903 aborted one day after treatment with quinapyramine sulphate and another one (906) aborted two days after trypanosomes had been detected but before treatment could be administered.

Table 12. A summary of the significant clinical findings in camels reported sick.

CAMEL NUMBER		902	903	904	906	907	912	916
AGE GROUP		Dam	Dam	Dam	Dam	Dam	Dam	Dam
HISTORY	Time of year	August 1986	July 1987	May 1987	July 1987	August 1987	July 1987	April 1987
	Reported signs	Off food Drop in milk	Off food Threatened abortion	Off food Stomach pain	Off colour Off food	Never thrived	Off colour Not cudding	Off colour Off food
MAIN CLINICAL FEATURES	Demeanour	Dull	Dull	Dull	Dull	Dull	Dull	Dull
	Lymphnodes	Enlarged	Not enlarged	Not enlarged	Not enlarged	Not enlarged	Not enlarged	Enlarged
	Lachrymation	Excessive	Excessive	Excessive	Excessive	Not excessive	Excessive	Excessive
	Temperature°C	38.7	39	39	38.8	36.8	37.9	38.3
	Other findings	-	Aborted	Threatened abortion	Aborted	Emaciated		
CONFIRMATION OF DIAGNOSIS	Trypanosomes	+	+	+(75)	+	+	-	+
	Antigen	+	+	+	+	-	+	+
	Haematocrit l/l	0.22	0.23	0.20	0.25	0.22	0.22	0.23

Foot Note:
+ = Present
- = Absent
(x) = Number of days from the first report of sickness to the detection of trypanosome

The lymphnodes commonly found enlarged were submandibular, prescapular and precural.

Table 12 continued....

CAMEL NUMBER		932	934	918	920	922	926	928
AGE GROUP		Dam	Dam	Weaner	Weaner	Weaner	Weaner	Weaner
HISTORY	Time of year Reported signs	August 1986 Never thrived Drop in milk	July 1987 Bouts of Sickness	July 1987 Off food Off colour	July 1986 Off colour Swollen lymph nodes	October 1986 Off colour Off food	June 1987 Off food	August 1986 Off colour Off food
MAIN CLINICAL FEATURES	Demeanour Lymphnodes Lachrymation Temperature°C Other findings	Dull Not enlarged Excessive 36.5 Emaciated and died	Dull Enlarged Excessive 38 Died	Dull Enlarged Excessive 38.8 Conjunctival congestion	Dull Enlarged Excessive ND -	Dull Enlarged Excessive 37.5 Ulcerated dental pad	Dull Enlarged Excessive 37.9 Petechiations of anal mucosa and gum ulcers	Dull Enlarged Excessive ND -
CONFIRMATION OF DIAGNOSIS	Trypanosomes Antigen Haematocrit l/l	+ + 0.21	+(30) + 0.21	+ + 0.23	+ + 0.20	+ + 0.22	- - 0.25	+(60) + 0.25

Foot Note:

+ = Present

- = Absent

(x) = Number of days from the first report of sickness to the detection of trypanosome

The lymphnodes commonly found enlarged were submandibular, prescapular and precural.

Table 12 continued....

CAMEL NUMBER		929	931	939	941	947	949	950
AGE GROUP		Weaner	Weaner	Weaner	Weaner	Calf	Calf	Calf
HISTORY	Time of year	August 1986	JuYy 1987	July 1987	June 1987	June 1987	June 1987	July 1987
	Reported signs	Off food Off colour	Drop in milk	Off food Off colour	Off colour Not cudding	Off food Very sick	Swollen submandibular lymph nodes	Swollen submandibular lymph nodes
MAIN CLINICAL FEATURES	Demeanour	Dull	Dull	Dull	Dull	Dull	Dull	Dull
	Lymphnodes	Enlarged	Not enlarged	Enlarged	Enlarged	Enlarged	Enlarged	Enlarged
	Lachrymation	Excessive	Not excessive	Excessive	Excessive	Excessive	Excessive	Excessive
	Temperature°C	38.4	36.7	39	39.6	39	38	37.8
	Other findings	-	-	-	-	Foul breath	-	-
CONFIRMATION OF DIAGNOSIS	Trypanosomes	+	+(30)	+(30)	+(30)	-	-	+(30)
	Antigen	+	+	+	+	-	+	-
	Haematocrit l/l	0.24	0.25	0.29	0.21	0.18	0.21	0.20

Foot Note:

\+ = Present

\- = Absent

(x) = Number of days from the first report of sickness to the detection of trypanosome

The lymphnodes commonly found enlarged were submandibular, prescapular and precural.

Table 12 continued....

CAMEL NUMBER		953	955	957	958	959	960
AGE GROUP		Calf	Calf	Calf	Calf	Calf	Calf
HISTORY	Time of year	June 1987	June 1987	June 1987	April 1987	April 1987	July 1987
	Reported signs	Swollen submandibular nodes	Swollen submandibular nodes	Swollen submandibular nodes	Off colour Off colour	Off colour Off food	Off food
MAIN CLINICAL FEATURES	Demeanour	Dull	Dull	Dull	Dull	Dull	Dull
	Lymphnodes	Enlarged	Enlarged	Enlarged	Enlarged	Enlarged	Enlarged
	Lachrymation	Excessive	Excessive	Excessive	Excessive	Excessive	Excessive
	Temperature°C	38.6	39	39.3	39.4	39.4	ND
	Other findings	-	-	-	-	-	-
CONFIRMATION OF DIAGNOSIS	Trypanosomes	-	-	-	+(30)	+	+(45)
	Antigen	+	+	+	+	+	+
	Haematocrit 1/1	0.23	0.21	0.18	0.19	0.24	0.21

Foot Note:
+ = Present
- = Absent
(x) = Number of days from the first report of sickness to the detection of trypanosome
nd = Not done
The lymphnodes commonly found enlarged were submandibular, prescapular and precural.

Plate 17 Copious lachrymation in a camel suffering from acute T. evansi infection.

Plate 18 Swollen submandibular lymphnodes in an immature camel suffering from acute T. evansi infection.

In two females that were suffering from chronic trypanosomiasis, emaciation and poor coat quality were the main features seen. In both animals, patent parasitaemia was demonstrated.

Out of the 30 camels reported as being sick, 21 were found to have trypanosome infections (Table 12). Twelve of the 21 infections were found on the same day that the camels were reported sick, while the other 9 developed parasitaemia within a period of 2 months after they had been reported sick.

The majority of the camels reported as being sick had low haematocrit level (less than 0.24 l/l) on the day they were examined.

Nine animals never developed patent parasitaemias, but 5 of them showed the presence of trypanosomal antigens (Table 12). Of the four remaining animals without parasitaemia or antigenaemia, two were calves (956 and 961) that were stunted and emaciated and were suspected to be suffering from malnutrition. Mineral supplementation given orally failed to invigorate the calves. Both calves eventually died by which time their PCV had dropped to 0.15 l/l and 0.16 l/l respectively. Their carcases were not available for necropsy. The other two camels; a weaner (ear-tag number 926) and a calf (number 947) had a clinical syndrome similar to 'Khanid'. The calf eventually died. The day before it died, it was noted to be very sick, pyrexic, and, all visible lymph nodes were grossly enlarged. The calf's oral mucosa was dry and halitosis was evident. The PCV had fallen to 0.18 l/l from the previous mean of 0.20 l/l. Post-mortem examination revealed focal

areas of ecchymoses on the ventricular epicardium, and the presence of both fresh and black blood in the posterior part of the large intestine. The mucosa of this part of the intestine had diffuse pin-point petechiae running along the longitudinal folds. Haemorrhagic lymphadenopathy of both the superficial and deep lymph nodes was present.

It is important to note that there were 16 other camels which, though never reported as being sick, were found parasitaemic during the routine examination of blood.

4.3.2.2 PARASITOLOGICAL FINDINGS IN THE HERD AT NGURUNIT

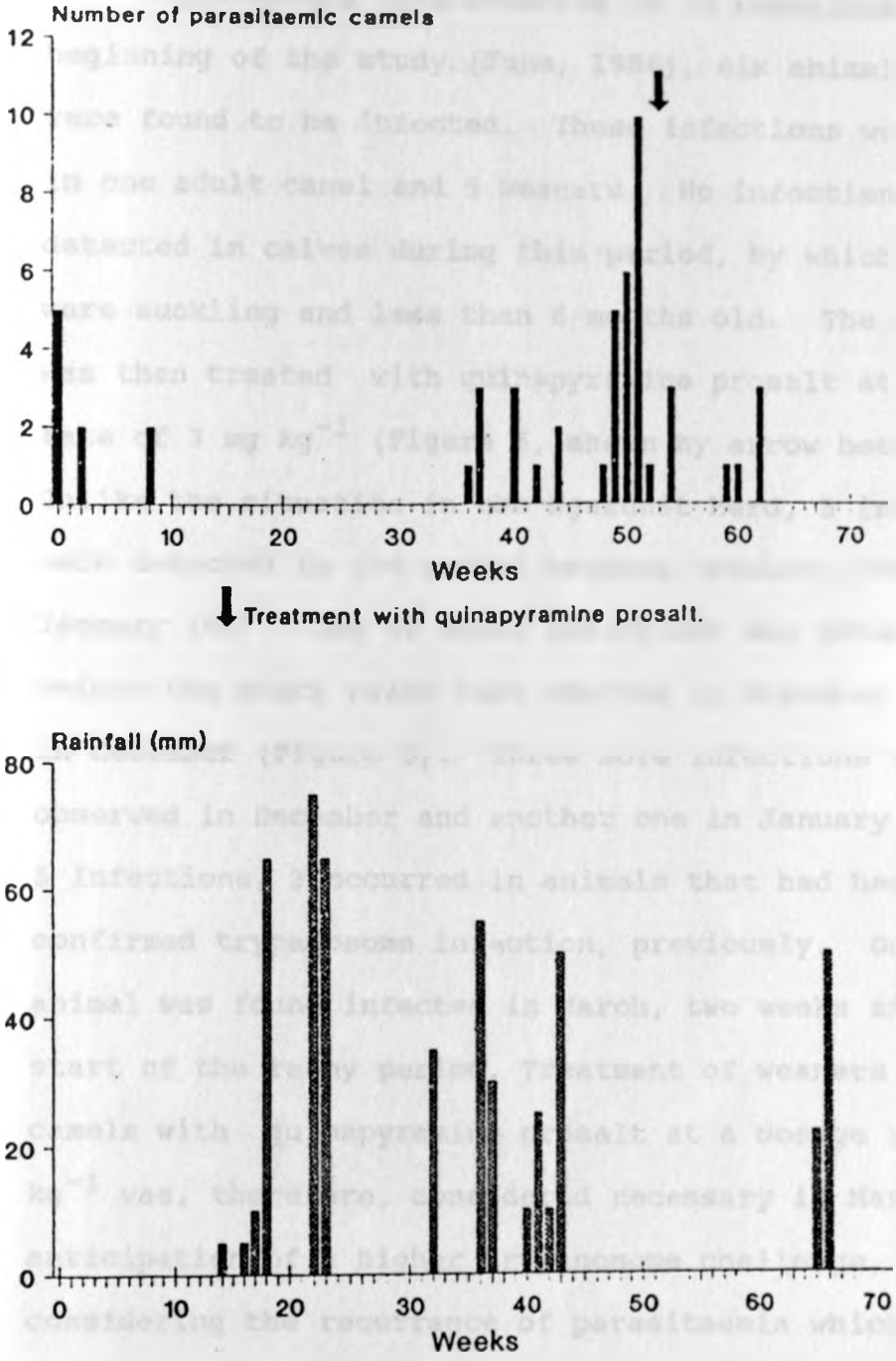
The data on the weekly parasitological findings in relation to the rainfall pattern of the herd at Ngurunit which was not on prophylaxis are summarised in Figure 4.

Trypanosomes were detected on 50 occasions. At the beginning of the study (which was during the dry period), only eight animals out of 60 were found infected: three were adult camels and 5 were weaners. During this period no infections were detected in calves which by then were suckling and were about 6 months old.

The majority of infections (42 out of 50) were detected during an epidemic that started in week 36 (April 1987) and continued up to week 52 (July 1987). The first infection was detected in April, one month after the beginning of the rains (March 1987). By mid-June, the infections had reached an alarming figure (40 out of the 60 study camels). The outbreak was associated with acute clinical disease, abortions and deaths. Because of the severity of the outbreak and the owners' reaction, it was arrested by prophylactic treatment of the whole herd (281

camels) with quinapyramine prosalt (Figure 4, shown by an arrow), at a dosage rate of 5 mg kg^{-1} bodyweight, except for those cases that had already been treated individually with quinapyramine sulphate. It was during this epidemic that the calves were found to be infected for the first time. During the dry months of August and October (1987), 8 cases of trypanosome infections were detected in animals that had been previously infected and treated.

Fig. 4. Weekly incidence of trypanosome infections in camels at Ngurunit in relation to rainfall.



Footnote: Camel herd maintained under individual treatment of parasitaemic animals.

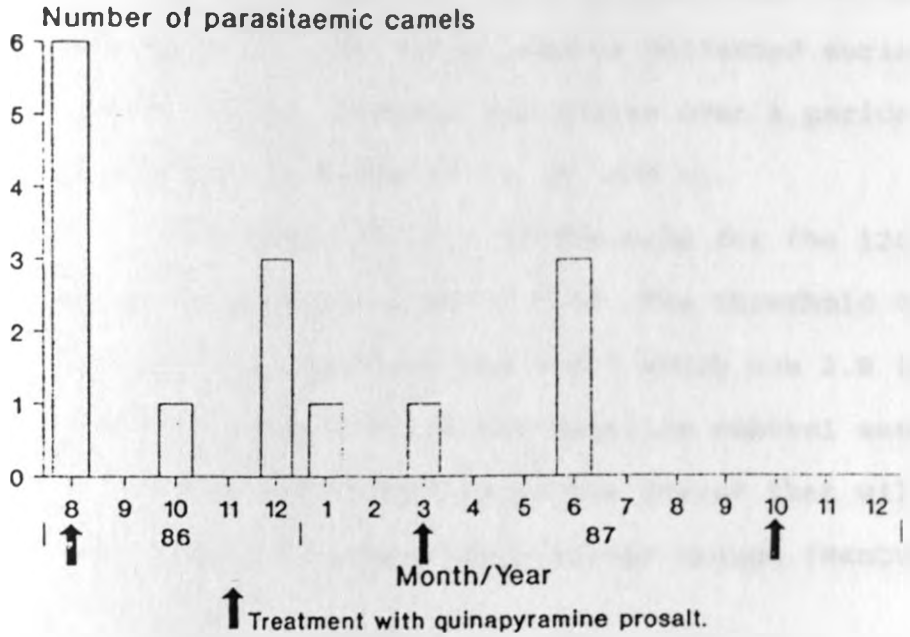
4.3.2.3 PARASITOLOGICAL FINDINGS IN THE HERD AT OLTUROT

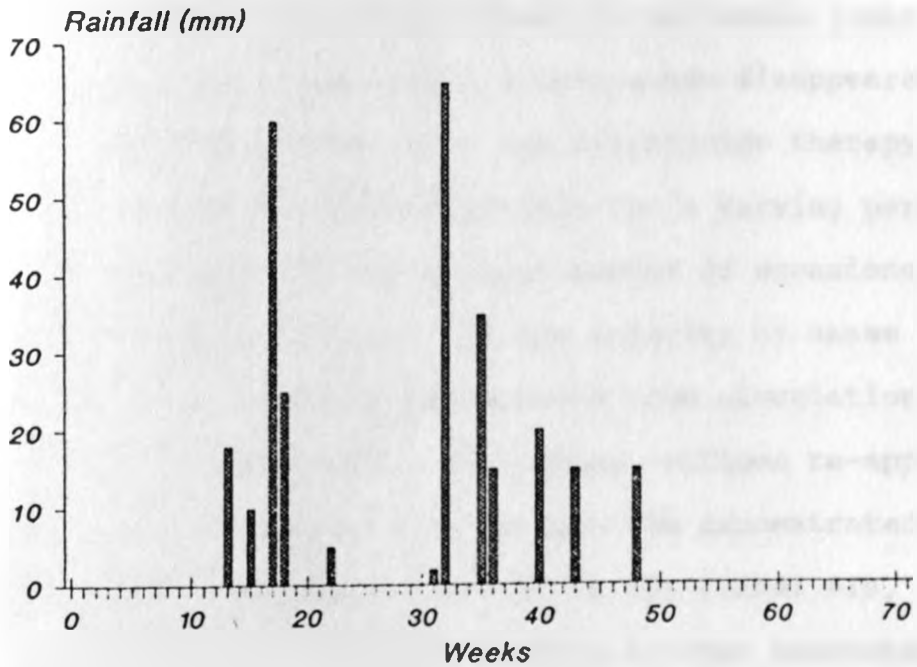
The data on the parasitological findings in relation to the rainfall patterns of the herd at Olturot which received prophylactic treatment are summarised in Figure 5.

Trypanosomes were detected on 16 occasions. At the beginning of the study (June, 1986), six animals out of 60 were found to be infected. These infections were detected in one adult camel and 5 weaners. No infections were detected in calves during this period, by which time they were suckling and less than 6 months old. The whole herd was then treated with quinapyramine prosalt at a dosage rate of 3 mg kg^{-1} (Figure 5, shown by arrow heads). Unlike the situation in the Ngurunit herd, 5 infections were detected in the period between October, 1986 and January 1987. One of these infections was detected just before the short rains that started in November and ended in December (Figure 5). Three more infections were observed in December and another one in January. Of these 5 infections, 3 occurred in animals that had had a confirmed trypanosome infection, previously. One other animal was found infected in March, two weeks after the start of the rainy period. Treatment of weaners and adult camels with quinapyramine prosalt at a dosage rate of 5 mg kg^{-1} was, therefore, considered necessary in March in anticipation of a higher trypanosome challenge, and considering the recurrence of parasitaemia which had been detected during the short rainy season. The calves did not merit treatment since no infections had been detected in them.

Three months after chemoprophylactic treatment, infections involving one weaner and two calves were detected, in June. This was the first time that the calves became infected. The four infected animals were treated on an individual basis with quinapyramine sulphate at a dosage rate of 5 mg kg^{-1} bodyweight. Re-treatment of the whole herd was not considered necessary until October (shown in Figure 5 with an arrow-head), just before the anticipated short rains (November to December).

Fig. 5. Monthly incidence of trypanosome infection in camels at Olturot in relation to rainfall.





Footnote: Camel herd maintained under chemoprophylaxis.

4.3.2.4 CORRELATION BETWEEN PARASITOLOGICAL AND IMMUNOLOGICAL FINDINGS IN THE CAMEL HERD AT NGURUNIT

Comparisons between the parasitological findings and the antigen detection results (expressed as optical density) of 2500 serum samples collected serially from camel calves, weaners and adults over a period of 517 days are shown in Table 13 (a, b, and c).

The mean (± S.D.) of the O.Ds for the 120 negative-control sera was 0.018 ± 0.07. The threshold O.D. reading regarded as positive was 0.050 which was 2.8 times higher than the mean O.D. of the negative control sera. This value had been found to be the lowest that will give a clear visually observable colour change (Nantulya *et al.*, 1989 a,b).

In general, trypanosomal antigens were found on more occasions (273) than patent parasitaemia (only 50). It was also found that, while trypanosomes disappeared from circulation soon after the trypanocide therapy, the antigens remained detectable for a varying period of time, accounting for the greater number of occasions on which they were detected. In the majority of cases (31 out of 42), the antigens disappeared from circulation within 30 days of treatment. In 7 cases, antigen re-appeared and in 2 of these animals, a relapse was demonstrated parasitologically. In six camels (camel 918, 920, 922, 937, 938 and 958) circulating antigen persisted for longer periods and in one camel (Fig. 6), circulating antigen persisted throughout the 517 days of observation.

In the 42 instances in which parasitaemia could be correlated with antigenaemia, in the majority of cases (23), the parasites were detected earlier than the antigen. In one such a case (camel 901), an acute trypanosome infection, without antigenaemia, occurred, and although the camel was treated, it died a week after therapy. Serum obtained from the dead camel revealed the presence of low grade antigenaemia. In 13 instances, the circulating antigen and trypanosomes were detected simultaneously, while only in six instances was the circulating antigen detected first.

Only on 5 occasions (camel 907, 916, 944, 950, and 960, Table 13), was a proven trypanosome infection not accompanied by the presence of circulating antigen. Serial serum samples from three of these camels (907, 916 and 944) did not show presence of anti- trypanosomal antibodies, neither were trypanosomal immune complexes demonstrated.

There were however, many occasions on which antigenaemia was not accompanied by the patent parasitaemia. For example, out of the 273 instances in which antigenaemia was detected, circulating antigen could not be correlated with detectable parasitaemia on 147 occasions. In 13 camels (7 calves, 1 weaner and 5 adults), the antigen level was low and occasional. In two calves (942 and 955), the circulating antigen was not detectable after two months, even though no treatment had been administered. In four adult camels (905, 915, 933 and 936),the antigen level was high and persisted throughout the observation period. Anti-trypanosomal antibody levels

in these four adult camels were also high and detectable throughout the observation period (Table 14).

Calves did not show patent parasitaemia early in life (July 1986 to March 1987) when they were still suckling, and less than one week old. The majority (14 out of 20) did not have detectable trypanosomal antigen level either during this period. Likewise, anti-trypanosomal antibodies were not detected during this period. Following weaning, patent infections were seen and anti-trypanosomal antibodies and trypanosomal antigens were detected (Table 13c and 15). One calf (number 942, Table 13a) showed trypanosomal antigens without an accompanying parasitaemia early in its life (days 31 upto 140), but anti-trypanosomal antibodies were not detected during this period (Table 15 and Fig. 7). Later in life, when the calf developed patent infection, both circulating antigen and anti-trypanosomal antibodies were demonstrated.

Table 13,a. A comparison of the antigen detection results and parasitological findings in camel calves at Ngurunit.

Year	1986								1987																				
Months		Aug		Oct			Dec		Jan	Feb		Mar	Apr	May		June		July			Aug		Sep	Oct	Nov			Dec
Days	0	21	31	67	81	94	127	140	166	189	214	228	256	281	305	317	327	341	354	367	385	399	421	455	471	483	495	'07
Camel																												
942	-	-	0.23	0.60	0.90	0.77	0.23	0.19	-	-	-	-	-	-	-	-	-	-	+	0.77	0.05	-	0.05	+	-	-	-	-
943	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+0.24	0.27	-	-	-	-	-	-	-	-	-	-	-
944	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
945	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	0.77	0.20	0.21	-	-	-	-	-	-
946	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	0.16	-	-	-	-	-	-	-	-	-
947	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Died											
948	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	0.08	-	-	-	-	-	-	-	-
949	-	-	-	-	-	-	-	0.08	-	-	-	-	0.09	-	-	-	-	-	0.08	-	-	-	-	-	-	-	-	-
950	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
951	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.56	-	-	-	-	-	-
952	0.16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
953	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-
954	-	-	-	Died																								
955	-	-	-	-	-	-	-	0.10	-	0.14	0.09	0.10	0.07	NB	0.11	-	0.07	0.47	0.11	0.09	-	-	-	-	-	-	-	-
956	-	-	-	0.24	0.09	-	-	-	-	Died																		
957	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
958	-	-	-	-	-	-	-	-	-	-	-	-	+0.52	-	-	0.07	-	0.24	0.09	0.08	+0.70	0.09	-	-	-	-	-	-
959	0.10	-	0.06	-	0.08	-	-	-	-	-	0.07	0.12	-	-	-	-	-	-	+	0.38	0.18	-	-	-	-	-	-	-
960	-	-	-	-	-	NB	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+0.09	0.07	-	-	-
961	-	-	-	-	-	Died																						

Key

- = No antigen detected

+ = Trypanosome detected

Numerical values give the level antigenaemia expressed as optical densities

NB = Not bled

Table 13,b. A comparison of the antigen detection results and parasitological findings in weaner camels at Ngurunit.

Year	1986								1987																		
Months		Aug		Oct			Dec		Jan	Feb		Mar	Apr	May		June		July			Aug		Sep	Oct	Nov		Dec
Days	0	21	31	67	81	94	127	140	166	189	214	228	266	281	305	317	327	341	354	367	385	399	421	455	471	483	495	507
Camel																												
918	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	1.16	1.34	1.68	2.00	0.49	0.15	0.34	-	-
920	+0.36	0.20	0.80	0.15	0.26	0.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
921	+0.11	0.22	0.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
922	1.34	1.14	1.30	+0.85	0.72	0.60	0.24	0.11	0.12	0.20	0.16	0.12	0.51	-	0.23	-	0.08	0.05	0.07	-	-	0.39	0.07	-	0.47	0.49	0.28	0.41
923	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	0.98	0.12	0.06	-	-	+	0.11	-	-
924	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
925	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
926	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
927	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	0.07	0.09	-	-	-	+0.37	0.21	0.06	-	-
928	+0.07	0.53	0.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+0.83	0.12	0.24	-	-	-	-	-	-
929	+0.84	0.70	0.41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
930	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	0.26	0.12	0.12	-	-	-	+0.19	-	-
931	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.09	-	+	0.10	-	0.08	-	-	-	-	-	-
937	-	-	-	-	-	-	-	-	-	-	-	-	0.11	+0.44	1.22	0.97	1.04	0.49	NB	0.10	-	0.13	0.13	0.18	-	-	-	-
938	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+0.65	0.83	0.82	0.42	0.09	-	+	0.24	-	-	-	-	-	-
939	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1.34	0.95	0.84	0.06	-	-	-	-
940	-	-	-	-	-	-	-	-	Died																			
941	-	-	-	-	-	-	-	-	-	-	-	0.07	NB	1.22	+0.75	0.68	0.15	-	-	-	-	-	-	-	-	-	-	-

Key

- = No antigen detected

+ = Trypanosome detected

Numerical values give the level antigenaemia expressed as optical densities

NB = Not bled

Table 13,c. A comparison of the antigen detection results and parasitological findings in adult camels at Ngurunit.

Year	1986								1987																			
Months		Aug		Oct			Dec		Jan	Feb		Mar	Apr	May		June		July			Aug		Sep	Oct	Nov			Dec
Days	0	21	31	67	81	94	127	140	166	189	214	228	266	281	305	317	327	341	354	367	385	399	421	455	471	483	495	507
Camel																												
901	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	0.80	0.19	0.37	-	-	-	-	-	-
902	+	0.10	0.06	-	-	-	Died																					
903	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	0.13	-	-	-	-	-	-	-	0.13
904	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+0.13	0.11	-	-	-	-	-	0.15	NB	-	-	-	-	-
905	0.62	0.54	0.44	0.25	0.11	0.16	0.22	0.10	0.19	0.40	0.19	0.31	0.39	-	0.12	0.20	0.13	0.35	0.24	0.20	0.17	0.50	0.13	0.10	0.12	0.22	0.17	0.15
906	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+0.06	-	-	-	-	-	-	-	-	-
907	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
908	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.62	+0.29	-	-	-	-	-	-	-	-
909	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	0.07	Died							
910	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
911	-	-	-	-	-	-	-	-	0.08	-	-	-	-	0.16	-	-	-	-	-	-	-	0.15	-	-	-	-	-	-
912	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.22	0.11	0.17	-	-	-	-	-	-
913	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-
914	-	-	-	-	-	-	-	-	-	-	-	-	0.11	0.12	-	-	-	-	-	0.11	0.09	0.22	-	-	-	-	-	-
915	-	-	0.10	0.13	0.13	0.30	0.34	0.13	0.26	0.25	0.25	-	NB	-	0.39	0.27	0.36	0.18	0.79	0.80	0.38	0.99	-	0.11	0.13	0.10	-	-
916	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+0.06	0.19	-	-	-	-	-	-
917	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
932	0.13	+0.11	0.12	-	-	NB	-	-	-	-	-	-	Died															
933	0.36	0.91	0.86	0.65	0.57	NB	0.16	0.23	0.11	0.29	0.36	-	0.54	-	0.20	0.25	-	0.25	0.14	0.14	-	0.32	-	-				
934	0.08	-	0.06	-	-	NB	-	-	-	-	+0.05	-	0.20	+ NB	0.34	-	-	-	+ -	-	+	0.15	-	-		Removed from the		
935	-	-	-	-	-	NB	-	-	-	-	-	-	+0.50	-	-	0.05	0.05	-	0.05	-	-	0.13	-	-		experiment by the		
936	0.32	0.27	0.21	0.14	0.12	NB	0.13	0.09	0.08	-	0.08	-	0.14	-	0.09	0.10	0.07	0.10	0.17	1.09	1.28	1.65	1.30	0.44		owner.		

Key

- = No antigen detected

+ = Trypanosome detected

Numerical values give the level antigenaemia expressed as optical densities

NB = Not bled

Table 14. Anti-trypanosomal antibody levels in four adult camels found to have persistent antigenaemia but no parasitaemia.

Year	1986								1987																		
Months		Aug		Oct			Dec		Jan	Feb		Mar	Apr	May		June		July			Aug		Sep	Oct	Nov			De
Days	0	21	31	67	81	94	127	140	166	189	214	228	266	281	305	317	327	341	354	367	385	399	421	455	471	483	495	50
Camel																												
905	0.87	0.88	0.93	0.86	0.87	0.82	0.70	0.68	0.63	0.61	0.67	0.53	0.43	0.48	0.36	0.40	0.44	0.43	0.41	0.43	0.32	0.41	0.34	0.37	0.36	0.36	0.32	0.35
915	0.47	0.43	0.45	0.40	0.43	0.40	0.44	0.41	0.41	0.45	0.42	0.60	0.82	0.00	0.74	0.75	0.74	0.70	0.72	0.75	0.92	0.87	0.87	0.64	0.66	0.67	0.58	0.46
933	0.59	0.75	0.76	0.71	0.72	0.64	0.56	0.49	0.67	0.45	0.49	0.47	0.48	0.52	0.39	0.37	0.29	0.29	0.31	0.30	0.24	0.26	0.21	0.21	0.24	0.22	0.26	0.30
936	0.61	0.50	0.59	0.52	0.51	0.40	0.32	0.29	0.31	0.29	0.25	0.27	0.23	0.22	0.18	0.18	0.20	0.18	0.15	0.74	0.83	0.82	0.69	0.69	0.70	0.68	0.72	0.60

NB

Numerical values give the level of antibody expressed as optical density read at 414nm.

Table 15. Anti-trypanosomal antibody levels in seven camel calves before and afer trypanosome infection.

Year	1986																1987											
Months	July	Aug		Oct			Dec		Jan	Feb		Mar	Apr	May		June		July			Aug		Sep	Oct	Nov		
Days	0	21	31	67	81	94	127	140	166	189	214	228	266	281	305	317	327	341	354	367	385	399	421	455	471	495	517
Camel																											
942	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	0.11	0.16	0.17	+ 0.15	ND	ND	ND
943	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	0.27	0.16	0.18	ND	ND	ND	ND
958	-	-	-	-	-	-	-	-	-	-	-	-	+	0.10	0.15	0.16	0.15	0.17	0.16	0.21	0.22	0.36	0.43	0.48	0.38	0.26	0.25	0.17
959	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	0.10	0.17	0.14	0.15	0.16	0.17	0.14	0.10	0.09
960	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	0.09	0.10	0.10	0.48	0.46	0.45	0.18

Key

- = Antibodies not detected

+ = Trypanosome detected

ND = Not done

Numerical values give the level of antibodies expressed as optical densities read at 414 nm.

Fig 6. Trypanosomal antigen profile in camel number 922 found to have persistent antigenaemia

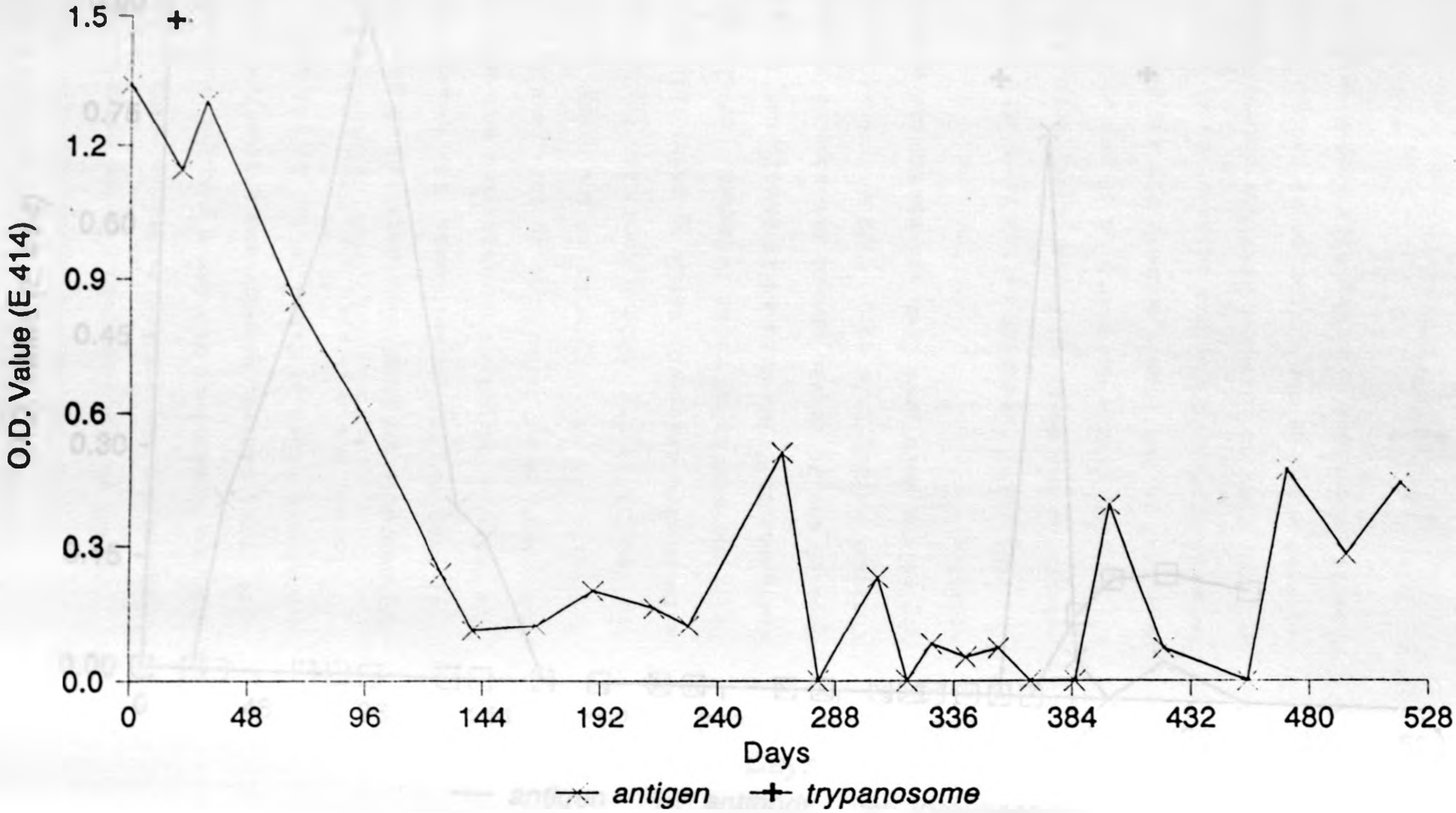
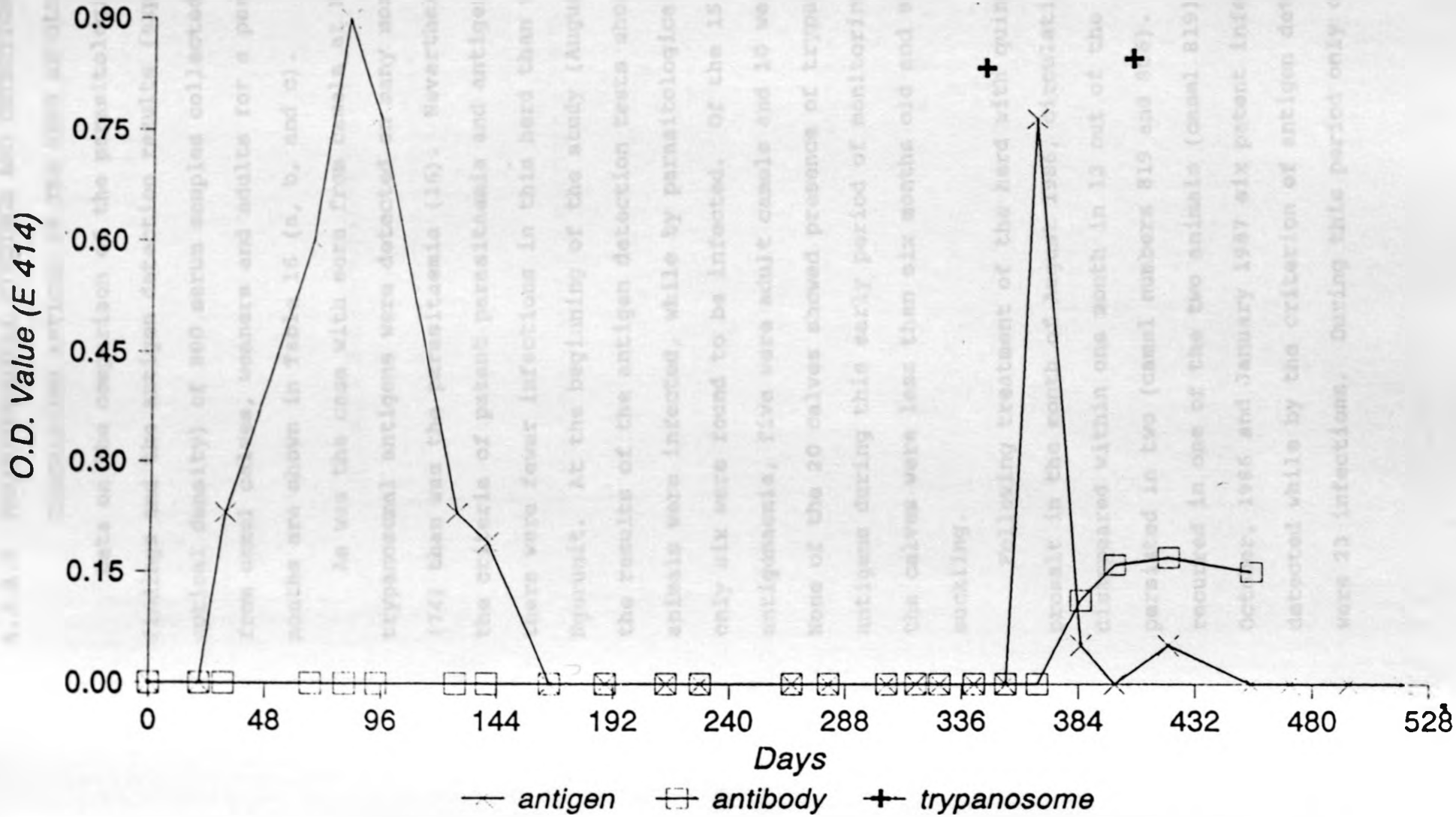


Fig 7. Trypanosomal antigen and antibody profile in camel calf 942 before and after a confirmed trypanosome infection.



4.3.2.5 PARASITOLOGICAL FINDINGS AND DETECTION OF CIRCULATING ANTIGEN IN THE HERD AT OLTUROT.

Data on the comparison of the parasitological findings and the antigen detection results (expressed as optical density) of 800 serum samples collected serially from camel calves, weaners and adults for a period of 15 months are shown in Table 16 (a, b, and c).

As was the case with sera from camels at Ngurunit, trypanosomal antigens were detected on many more occasions (74) than was the parasitaemia (16). Nevertheless, by both the criteria of patent parasitaemia and antigen detection, there were fewer infections in this herd than were at Ngurunit. At the beginning of the study (August, 1986), the results of the antigen detection tests showed that 15 animals were infected, while by parasitological diagnosis, only six were found to be infected. Of the 15 animals with antigenaemia, five were adult camels and 10 were weaners. None of the 20 calves showed presence of trypanosomal antigens during this early period of monitoring. By then, the calves were less than six months old and still suckling.

Following treatment of the herd with quinapyramine prosalt in the month of August 1986, circulating antigen disappeared within one month in 13 out of the 15 camels and persisted in two (camel numbers 819 and 806). Parasitaemia recurred in one of the two animals (camel 819). Between October, 1986 and January 1987 six patent infections were detected while by the criterion of antigen detection, there were 23 infections. During this period only on two

Table 16,a. A comparison of the antigen detection results and parasitological findings in camel calves in Olturot.

Year	1986									
Month	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
Days	0	21	31	67	81	94	127	140	166	189
Camel										
836	-	-	-	NB	-	-	NB	-	-	-
837	-	-	-	NB	-	-	NB	-	-	-
838	-	-	-	NB	-	-	NB	-	-	-
839	-	-	-	NB	0.07	-	NB	-	0.06	-
840	-	-	-	NB	-	-	NB	-	-	-
841	-	-	-	NB	-	-	NB	0.07	-	-
842	-	-	-	NB	-	-	NB	-	-	-
843	-	-	-	NB	-	-	NB	-	-	-
844	-	-	-	NB	-	-	NB	-	-	-
845	-	-	-	NB	-	-	NB	-	-	-
846	-	-	-	NB	-	-	NB	-	-	-
847	-	-	-	NB	-	-	NB	-	-	-
848	-	-	-	NB	-	-	NB	-	-	-
849	-	-	-	NB	-	-	NB	-	-	-
850	-	-	-	NB	-	-	NB	-	-	-
851	-	-	-	NB	-	-	NB	-	-	-
852	-	-	-	NB	-	-	NB	-	-	-
853	-	-	-	NB	-	-	NB	-	-	-
854	-	-	-	NB	-	-	NB	-	-	-
855	-	-	-	NB	-	-	NB	-	-	-

Key

- = No antigen detected

+ = Trypanosome detected

Numerical values give the level of antigenaemia expressed as optical density, read at 414 nm.

NB = Not bled

1987						
JUN	JUL	AUG	SEP	OCT	NOV	DEC
214	228	266	281	305	317	327
-	-	0.13	-	-	-	-
-	-	-	-	-	-	-
-	0.46	-	-	-	-	-
-	-	0.18	-	-	-	-
-	+1.04	0.74	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	+0.14	-	-	-	-	-
-	-	-	-	-	-	1.52
-	-	-	-	-	-	-

Table 16,b. A comparison of the antigen detection results and parasitological findings in weaner camels at Olturot.

Year	1986						1987			
Month	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
Days	0	21	31	67	81	94	127	140	166	189
Camel										
781	0.17	-	-	NB	-	-	NB	-	-	-
801	0.15	-	-	NB	-	-	-	-	-	-
802	0.06	-	-	NB	-	-	NB	-	0.07	0.06
803	-	-	-	NB	+0.40	+0.36	NB	0.10	0.07	-
804	-	-	-	NB	-	-	NB	-	-	-
805	-	-	-	NB	-	-	NB	-	-	-
806	0.52	0.44	0.32	NB	0.14	-	NB	0.09	0.10	-
807	+0.38	-	-	NB	-	-	NB	-	-	-
808	+0.42	-	-	NB	+0.25	0.18	NB	0.14	-	-
809	-	-	-	NB	-	-	NB	-	-	-
810	-	-	-	NB	-	-	NB	+0.15	-	-
811	+0.18	-	-	NB	-	-	NB	-	-	-
812	0.14	-	-	NB	+0.27	-	NB	0.80	-	-
813	+0.27	-	-	NB	-	-	NB	-	-	-
814	-	-	-	NB	-	-	NB	-	-	-
815	-	-	0.06	NB	0.54	-	NB	-	0.05	-
856	-	-	-	NB	-	-	NB	-	-	-
857	+0.24	-	-	NB	-	-	NB	-	-	-
858	-	-	-	NB	-	-	NB	-	-	-

Key

- = No antigen detected

+ = Trypanosome detected

Numerical values give the level of antigenaemia expressed as optical density, read at 414 nm.

NB = Not bled

1987						
JUN	JUL	AUG	SEP	OCT	NOV	DEC
214	228	266	281	305	317	327
-	-	-	-	-	-	-
-	-	-	-	0.23	0.22	0.10
0.05	0.05	0.09	0.07	0.05	0.00	0.00
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
0.80	-	0.29	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-

Table 16,c. A comparison of the antigen detection results and parasitological findings in camel dams at Olturot.

Year	1986									
Month	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
Days	0	21	31	67	81	94	127	140	166	189
Camel										
816	-	-	-	NB	-	-	NB	-	-	-
817	0.07	-	-	NB	-	-	NB	-	-	-
818	-	-	-	NB	-	-	NB	-	-	-
819	+0.35	0.12	+0.23	NB	0.30	-	NB	0.21	-	-
820	-	-	-	NB	0.07	-	NB	0.22	0.07	-
821	-	-	-	NB	-	-	NB	-	-	-
822	-	-	-	NB	-	-	NB	-	-	-
823	-	-	-	NB	-	-	NB	-	-	-
824	-	-	-	NB	-	-	NB	-	-	-
825	-	-	-	NB	-	-	NB	-	-	-
826	-	-	-	NB	-	-	NB	-	-	-
827	-	-	-	NB	-	-	NB	-	-	-
828	-	-	-	NB	-	-	NB	-	-	-
829	0.10	-	-	NB	-	-	NB	-	-	-
830	0.17	-	-	NB	-	-	NB	0.58	-	0.15
831	-	-	-	NB	-	-	NB	-	-	-
832	-	-	-	NB	-	-	NB	-	-	-
833	0.09	-	-	NB	-	-	NB	0.12	-	-
834	-	-	-	NB	-	-	NB	-	-	-
835	-	-	-	NB	-	-	NB	-	-	-

- = No antigen detected
+ = Trypanosome detected
Numerical values give the level of antigenaemia expressed as optic density, read at 414 nm.
NB = Not bled

---1987---

JUN	JUL	AUG	SEP	OCT	NOV	DEC
214	228	266	281	305	317	327
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	+0.19	0.10	0.14	-	0.11	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	0.52	-	-	-	-	0.50
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	0.29	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
0.19	-	0.09	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	+	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-

occasions were the antigens detected in calves (Number 839 and 841) and even then the levels of circulating antigen were low.

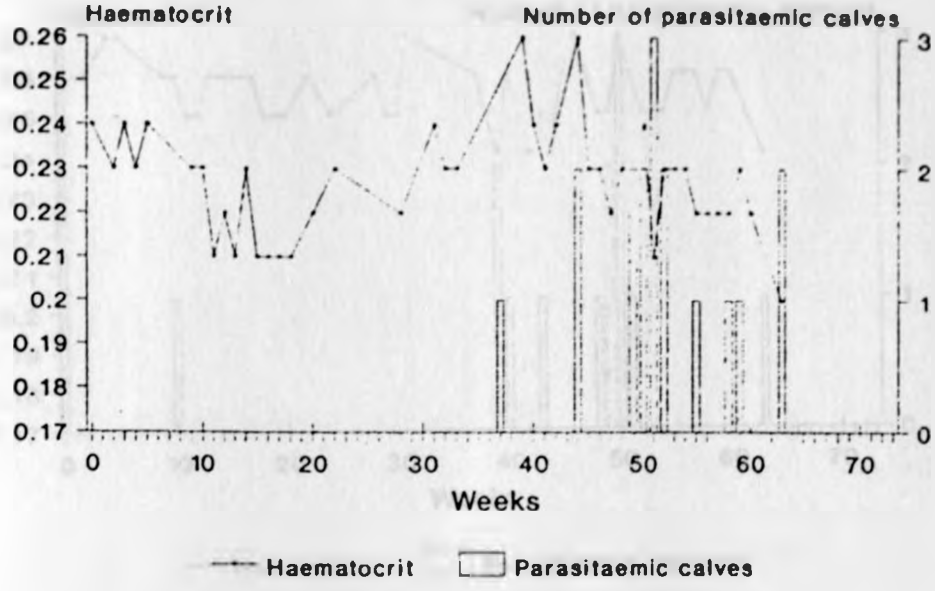
In March 1987, the weaners and adult camels were retreated with quinapyramine prosalt at a higher dosage rate (5 mg kg^{-1}). Again, circulating antigen disappeared from the majority of the camels that had them at the time of treatment. Nevertheless, on many more occasions (Table 16a and b), antigens were demonstrated and during this period two cases of parasitaemia were detected (Table 16a). The calves which were not treated in March 1987 showed the presence of antigens on eight occasions, and patent parasitaemia was demonstrated in two calves (Table 16c). In one of the two calves with patent parasitaemia (number 854), circulating antigen was not detected. This was the first time that the calves were found to be infected.

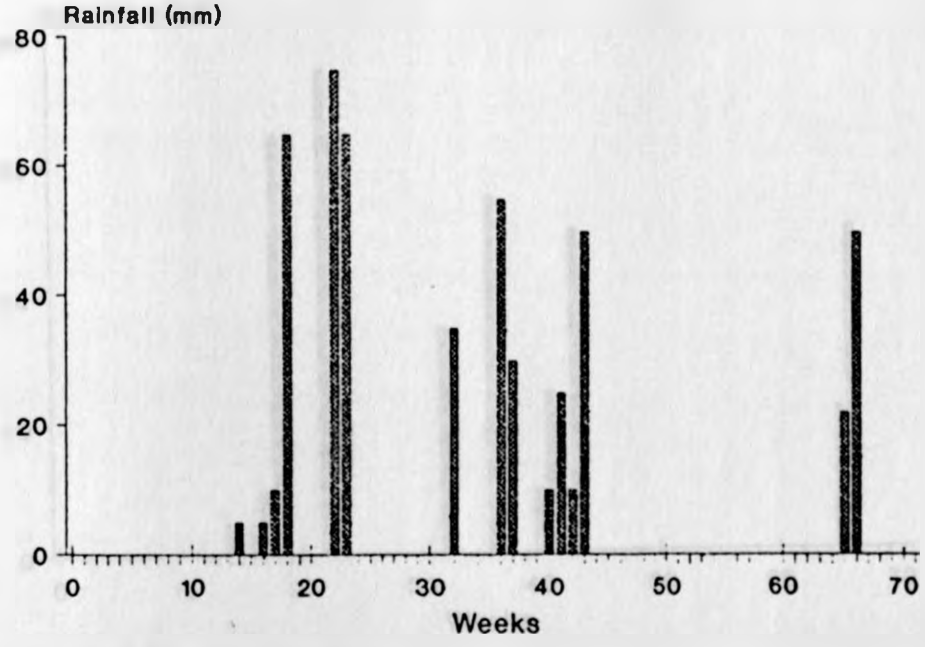
4.3.2.6 HAEMATOCRIT FINDINGS IN THE NGURUNIT HERD

Data on the weekly packed cell volume in relation to rainfall patterns and trypanosome infections in the calves, weaners and dams are summarised in Figure 8, 9 and 10 respectively.

The calves' haematocrits showed four distinct phases: an initial phase of moderately low PCV levels (0.23 to 0.24 l/l) was noted in the first 10 weeks of observation. A second phase of even lower haematocrit (0.21 to 0.23 l/l) was observed from week 11 upto 22. In the third phase, the PCV increased gradually and reached 0.26 l/l by week 45. This phase coincided with an improvement in pasture following the December rains. A fourth phase of declining PCV coincided

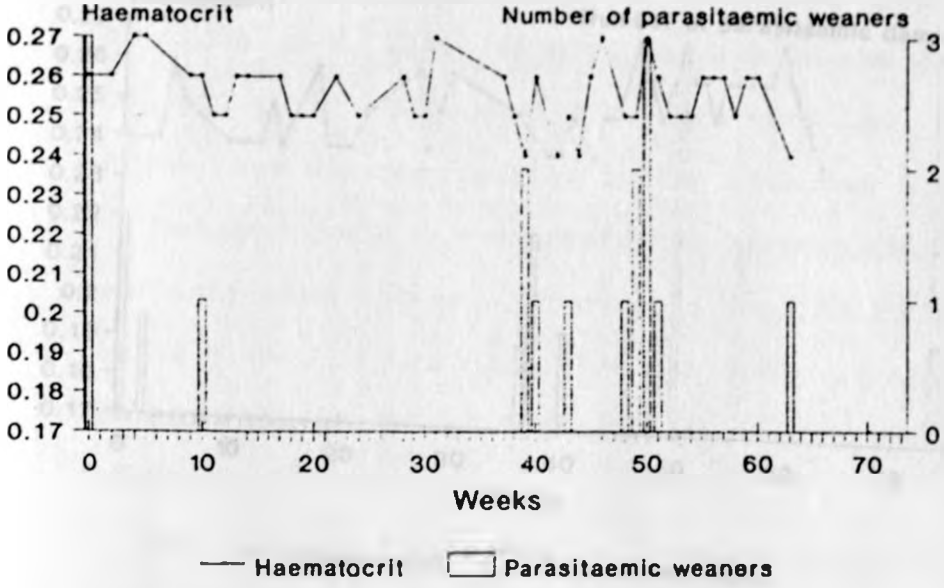
Figure 8. Weekly mean haematocrit (l/l) of camel calves at Ngurunit in relation to parasitaemia and rainfall.

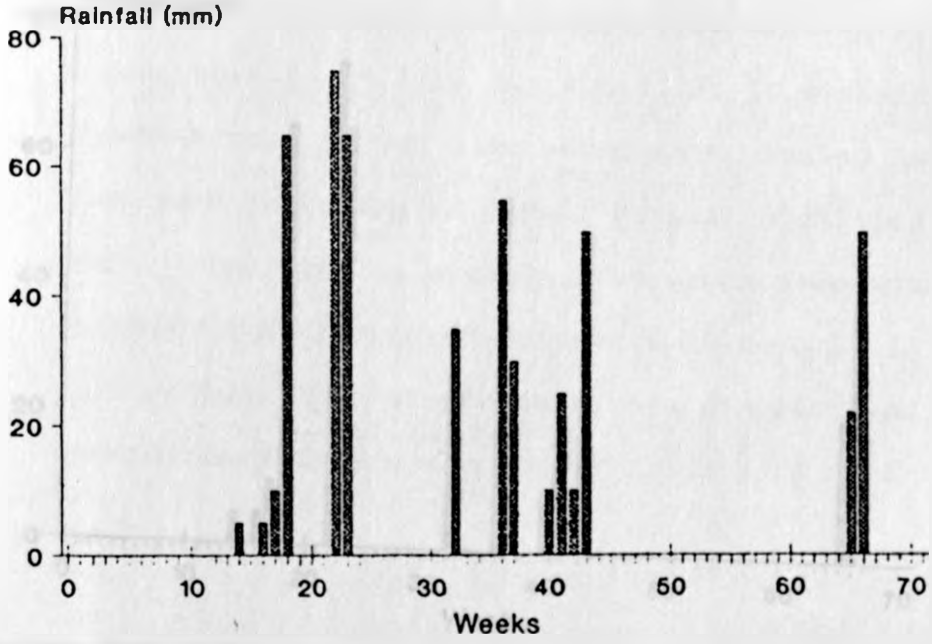




Footnote: Camel herd maintained under individual treatment of parasitaemic animals.

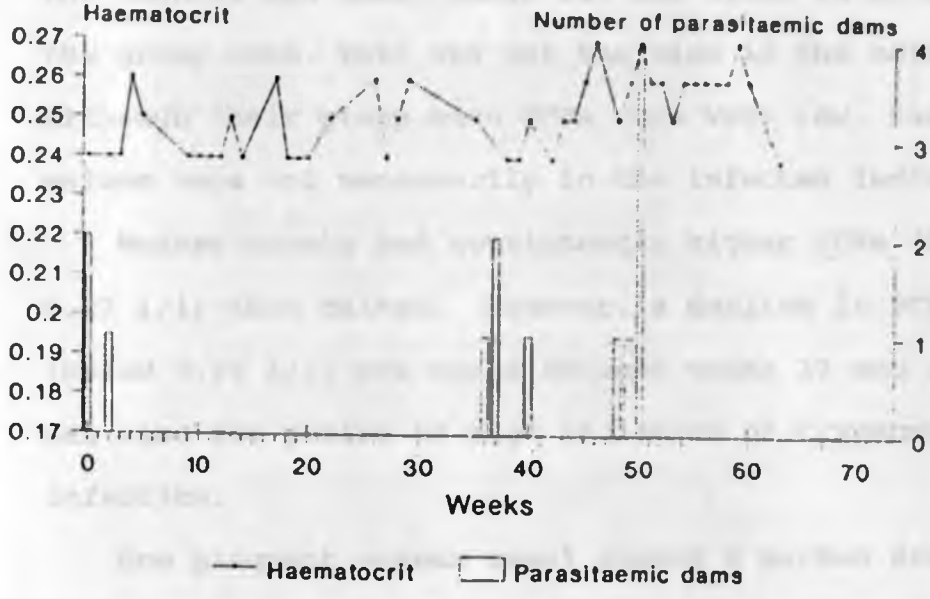
Figure 9. Weekly mean haematocrit (l/l) of weaner camels at Ngurunit in relation to parasitaemia and rainfall.

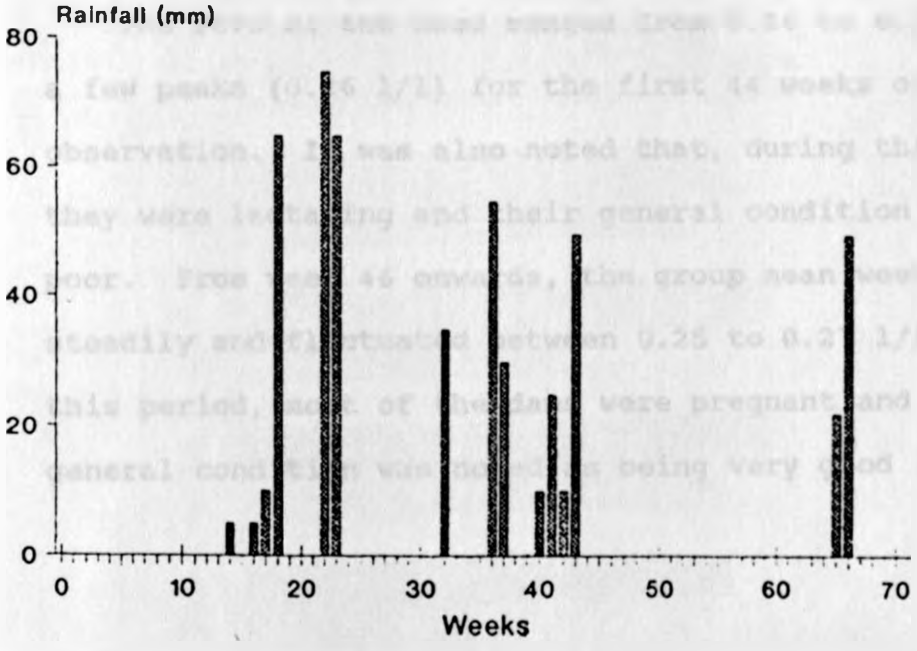




Footnote: Camel herd maintained under individual treatment of parasitaemic animals.

Figure 10. Weekly mean haematocrit (l/) of camel dams at Ngurunit in relation to parasitaemia and rainfall.





Footnote: Camel herd maintained under individual treatment of parasitaemic animals.

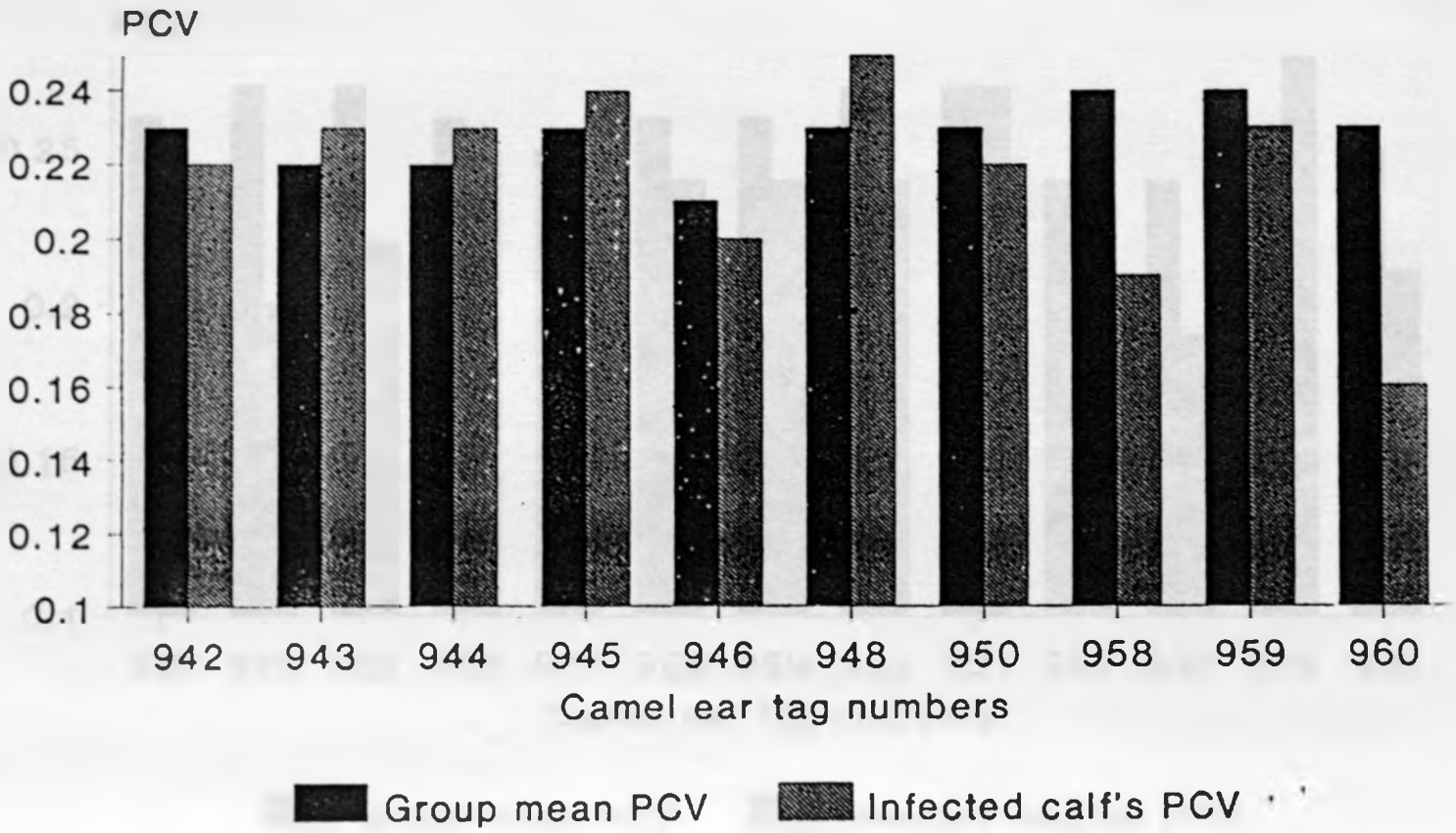
with the period of high trypanosome infection rate. The haematocrit values of the parasitaemic calves, yearlings and dams are shown in Figures 11, 12, and 13 respectively. It was found that, whenever trypanosomes were detected in the weaners and dams, their PCV was found to be lower than the group mean. This was not the case in the calves and although their group mean PCVs were very low, the low values were not necessarily in the infected individuals.

Weaner camels had consistently higher PCVs (0.25 to 0.27 l/l) than calves. However, a decline in PCV levels (below 0.25 l/l) was noted between weeks 37 and 44. This was also the period of high incidence of trypanosome infection.

One pregnant weaner camel showed a marked drop in PCV (from 31% to 21%) within a period of one week following blood letting (Plate 21).

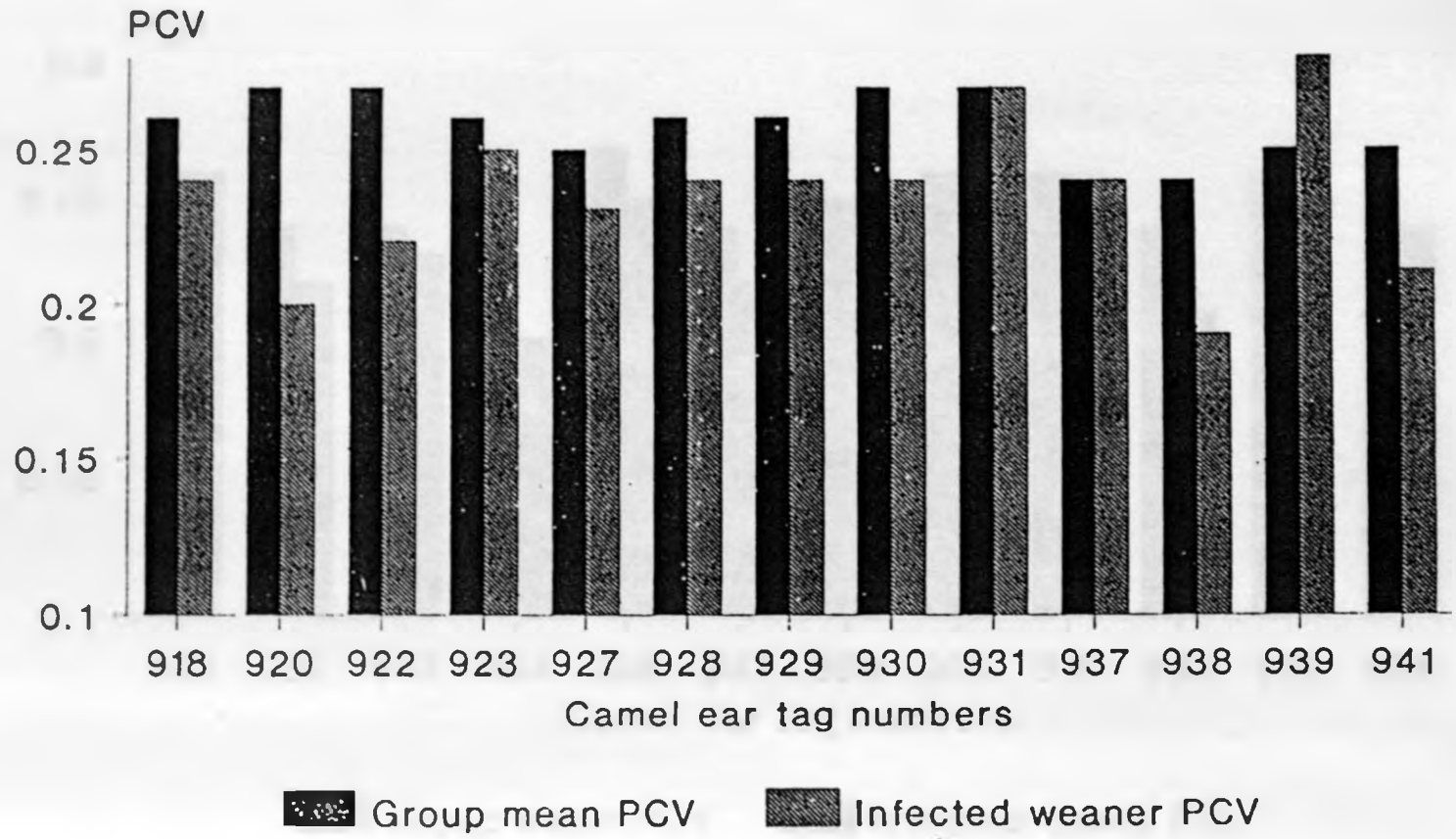
The PCVs of the dams ranged from 0.24 to 0.25 l/l with a few peaks (0.26 l/l) for the first 44 weeks of the observation. It was also noted that, during this period, they were lactating and their general condition was also poor. From week 46 onwards, the group mean weekly PCV rose steadily and fluctuated between 0.25 to 0.27 l/l. During this period, most of the dams were pregnant and their general condition was noted as being very good (Plate 22).

Figure 11. Calf group mean PCV (I/I), compared to that of an infected calf at the time of parasitaemia.



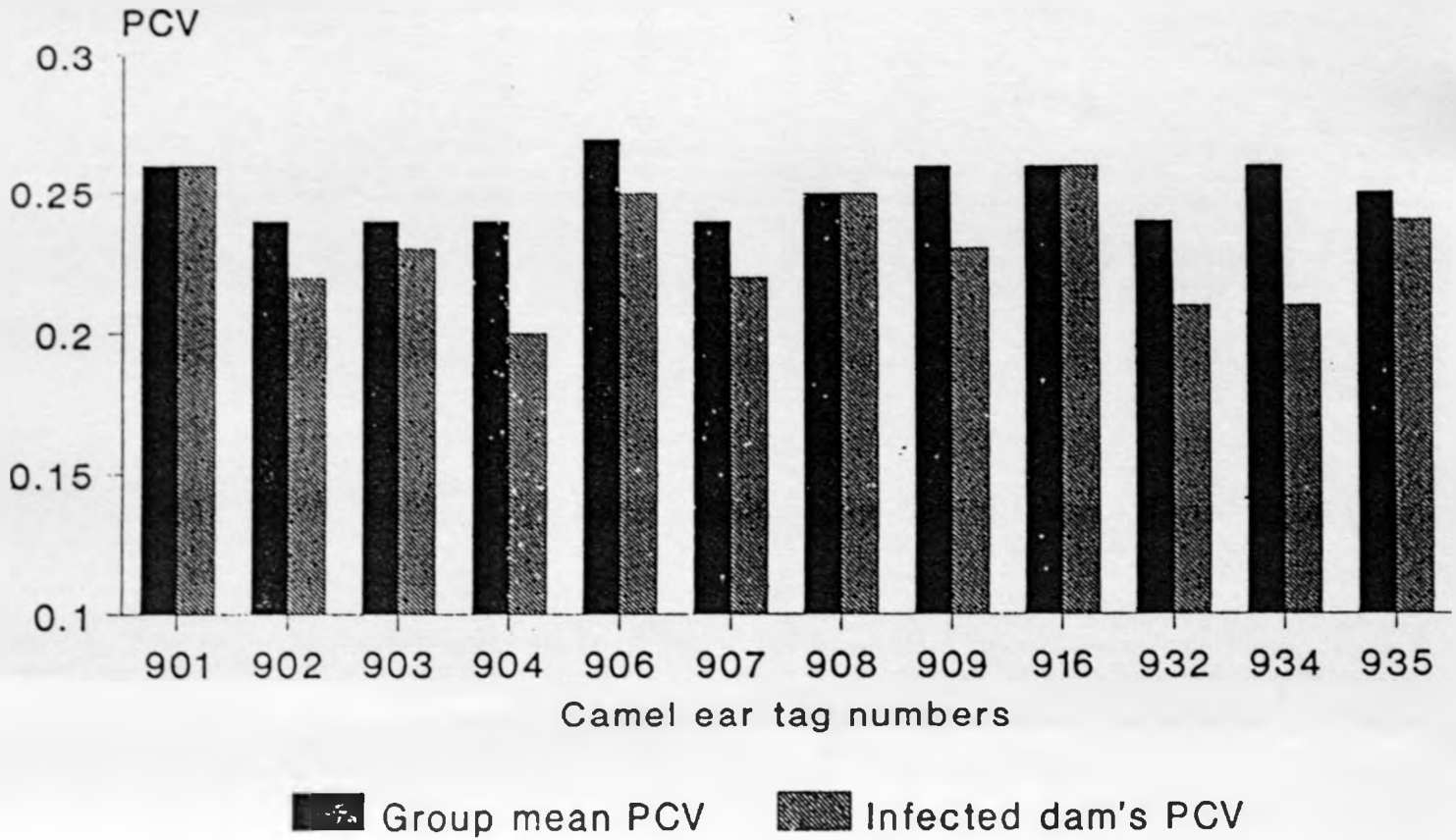
Footnote: Camel calves at Ngurunit

Figure 12. Weaner group mean PCV (I/I), compared to that of an infected weaner at the time of parasitaemia.



Footnote: Weaner camels at Ngurunit

Figure 13. Dam group mean PCV (I/I), compared to that of the infected dam at the time of parasitaemia.



Footnote: Camel dams at Ngurunit

Plate 21 Blood letting as practised by Samburu people. Note the amount of blood bled from the camel.

Plate 22 A pregnant camel in exceedingly good condition.

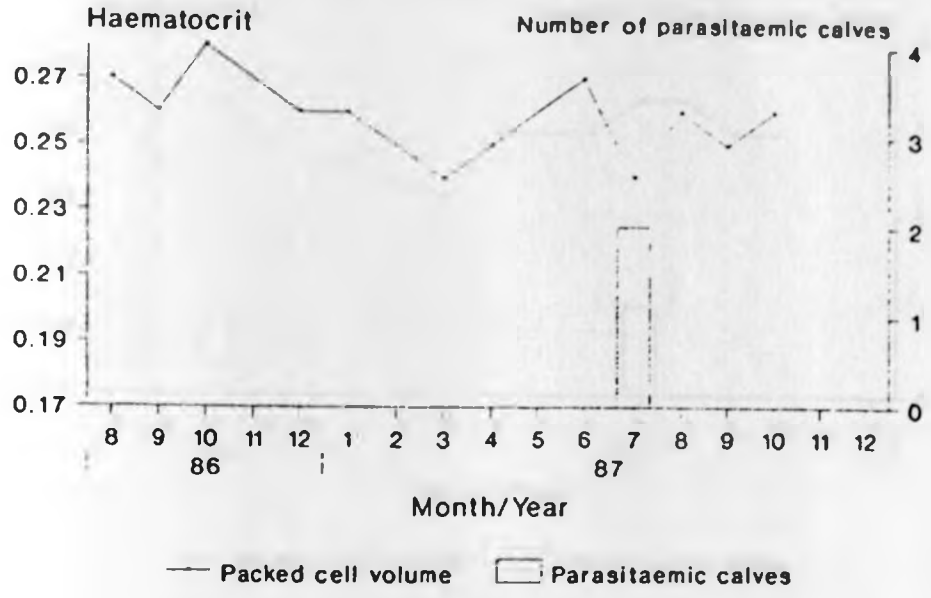
4.3.2.7 THE HAEMATOCRIT FINDINGS IN THE OLTUROT HERD

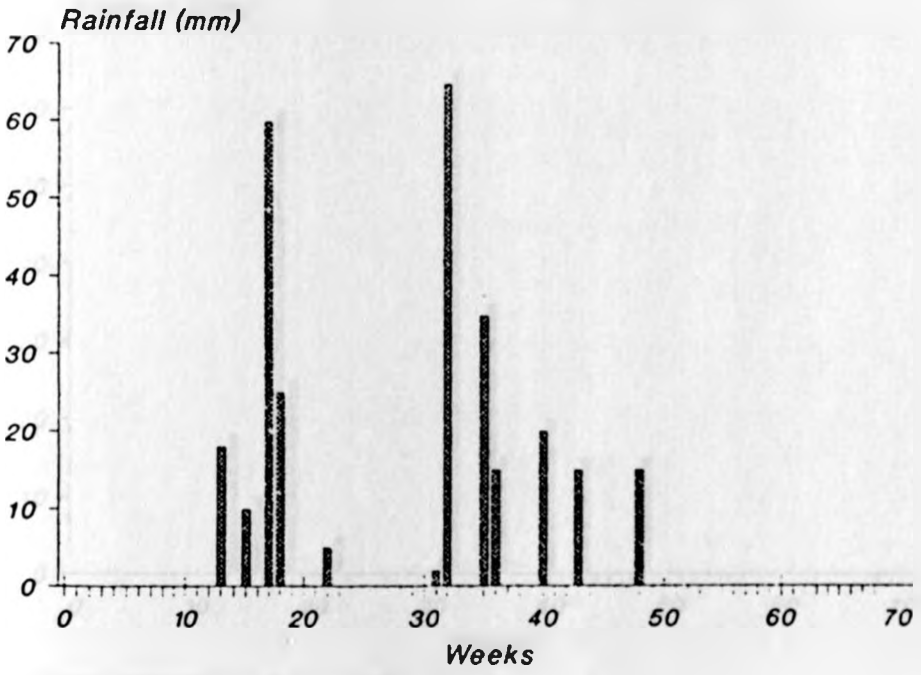
The monthly group mean PCVs of the calves, dams, and weaners in relation to parasitaemia and rainfall are shown in Figures 14, 15 and 16, respectively.

In general, the PCV values in calves showed three phases (Figure 14). There was a phase of high PCV ($\geq$ 0.26 l/l), evident up to the December, 1986. This was followed by a period of reduced haematocrit level ($\leq$ 0.24 l/l), from January to March, 1987. This period coincided with an outbreak of a specific syndrome (locally referred to as ''Khanid'') which was characterized by lymphadenopathy especially of the submandibular lymph nodes and concurrent anaemia. No trypanosomes were detected in affected calves, although calves in the neighbourhood with a similar syndrome did show intercurrent trypanosome infections. A third phase of a gradually rising PCV was interrupted in the month of July 1987. During this month, trypanosomes were also detected. This was the first time that the calves had been found to have trypanosome infections although these were detected in only two calves. The rainfall pattern did not seem to influence the haematocrit level.

The mean PCV of the dams (Figure 15) was generally lower than that of the calves. Again three phases of PCV levels were apparent. Between August and December, 1986, the haematocrit level ranged between 0.24 to 0.26 l/l. From January to March 1987, the PCV values of the dams were even lower and ranged between 0.25 l/l to 0.23 l/l. During this phase, the dams were lactating and their general

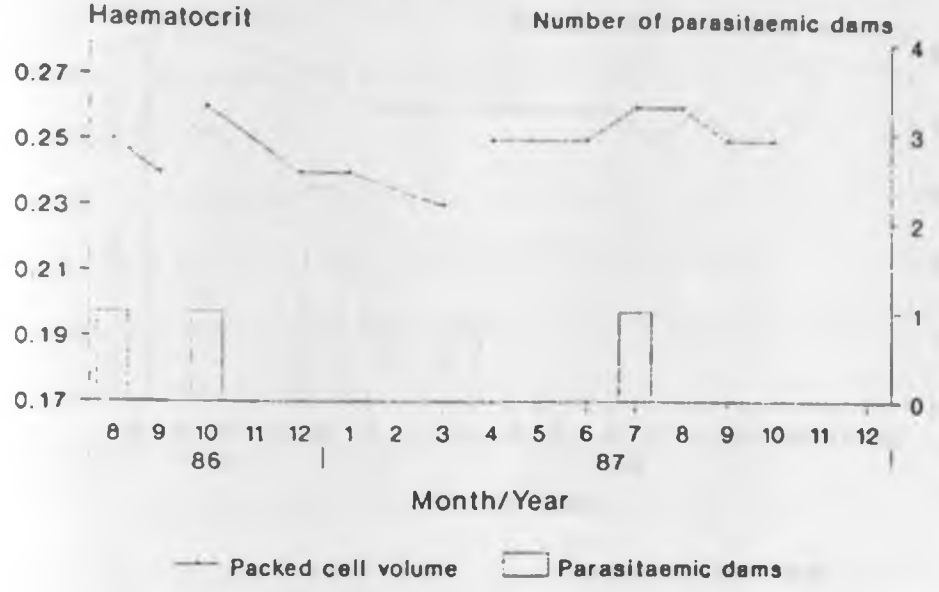
Fig. 14. Monthly Mean haematocrit (l/l) of camel calves at Olturot in relation to parasitaemia and rainfall.

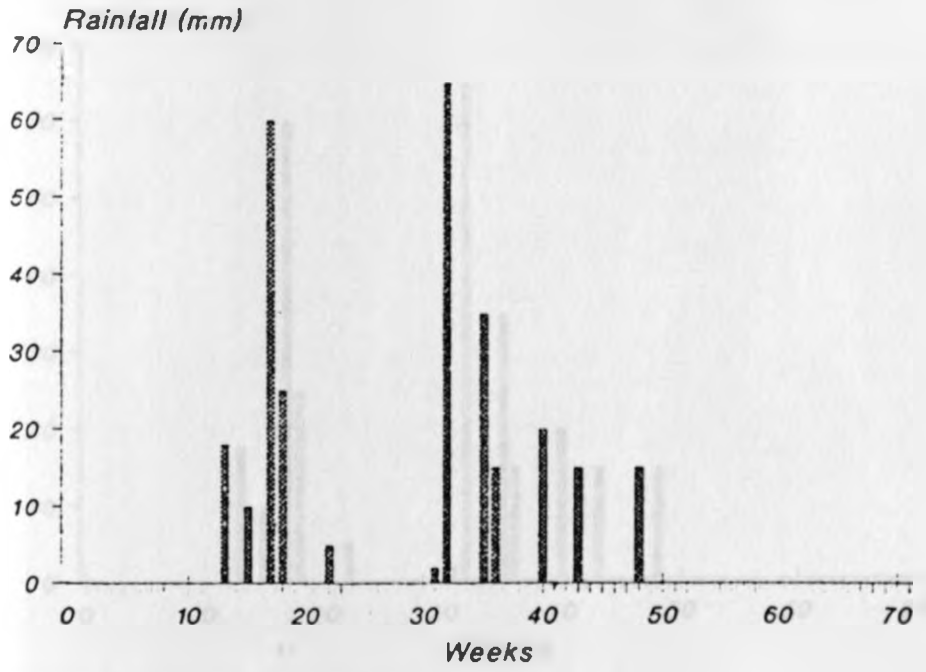




Footnote: Camel herd maintained under chemoprophylaxis.

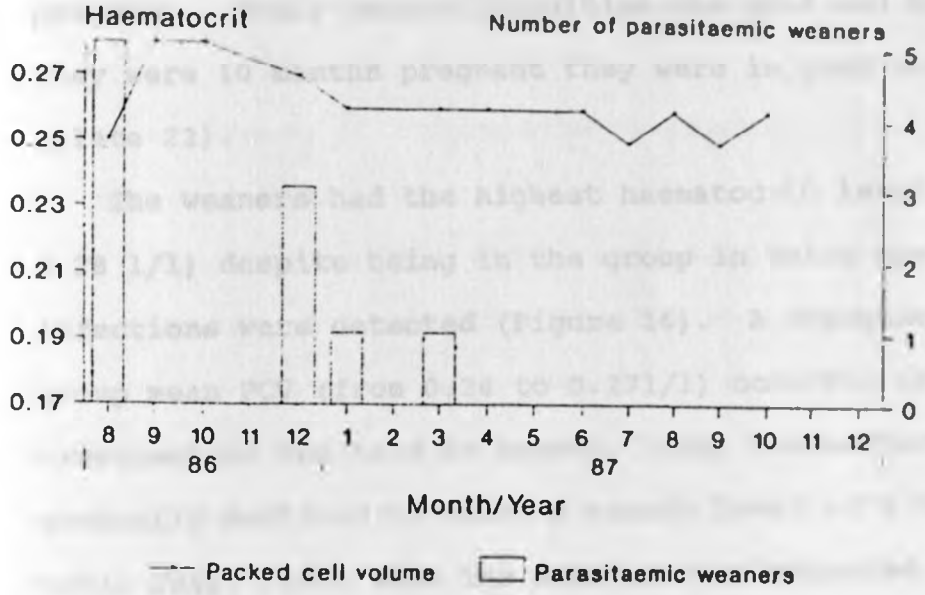
Fig. 15. Monthly Mean haematocrit (l/l) of camel dams at Olturot in relation to parasitaemia and rainfall.

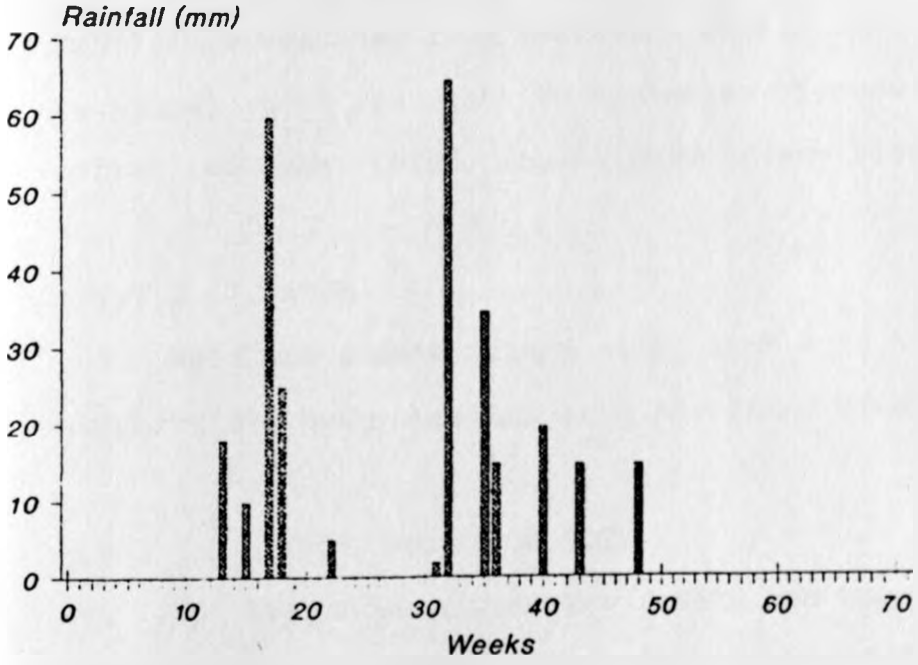




Footnote: Camel herd maintained under chemoprophylaxis.

Fig. 16. Monthly Mean haematocrit (l/l) of weaners camels at Olturot in relation to parasitaemia and rainfall.





Footnote: Camel herd maintained under chemoprophylaxis.

condition was also poor. A phase of consistently higher PCV (0.25 - 0.26 l/l) was noted from the month of April, 1987 onwards. By this time, all the calves had been weaned and the dams were about four to five months pregnant. Their general condition was good and by the time they were 10 months pregnant they were in good condition (plate 22).

The weaners had the highest haematocrit level (0.25 to 0.28 l/l) despite being in the group in which most infections were detected (Figure 16). A dramatic rise in group mean PCV (from 0.24 to 0.271/l) occurred after the treatment of the herd in August, 1986. Thereafter, the PCV gradually declined to reach a steady level of 0.26 l/l, until July, 1987, when the mean value fluctuated between 0.25 and 0.26 l/l.

4.4 EXPERIMENT 3- QUINAPYRAMINE SULPHATE SENSITIVITY OF STRAINS OF T. EVANSI ISOLATED FROM RECURRING PARASITAEMIA IN CAMELS

4.4.1 MATERIALS AND METHODS

4.4.1.1 TRYPANOSOME STOCKS

A summary of information on trypanosome stocks used is shown in Table 17.

Nine trypanosome stocks were tested. Six (NGT 934,938, 958, 763 and OLT 803, 819) were obtained from camels found to have been parasitaemic shortly after the trypanocide treatment. One stock (Ngurunit 922) was from a camel that showed a persistent antigenaemia of 450 days after trypanocide therapy. For comparative purposes, a stock (Ngurunit 929) that did not show relapse following trypanocide treatment was used as a sensitive strain. KETRI 2429 was used as a resistant strain. The latter was a T. evansi stock resistant to the major trypanocides on the market (Gitatha, KETRI, unpublished information).

4.4.1.2 MICE

Male and female Balb/c mice, aged 8 to 10 weeks, weighing 20g were obtained from the ILRAD breeding colony.

4.4.1.3 EXPERIMENTAL DESIGN

The trypanosome isolates (which had been cryopreserved, section 2.2.3), were thawed and aspirated from the capillary tubes into a 1.0 ml syringe containing 0.3 ml PSG, pH 8.0 (section 3.2.1.4). Thereafter, the

Table 17. A summary of information on the trypanosome stocks used.

Stock designation	Comment about the camel from which the stock was isolated.
NGT 934	Parasitaemia recurred 13 days after quinapyramine sulphate administration.
NGT 938	Parasitaemia recurred 24 days after quinapyramine sulphate administration.
NGT 958	Parasitaemia recurred 51 days after quinapyramine sulphate administration.
NGT 763	Parasitaemia recurred 39 days after quinapyramine sulphate administration.
OLT 803	Parasitaemia recurred 13 days after quinapyramine prosalt administration.
OLT 819	Parasitaemia recurred 60 days after quinapyramine prosalt administration.
NGT 929	No recurrence of parasitaemia or persistence of antigenaemia after trypanocide therapy.
NGT 922	Antigenaemia without parasitaemia persisted for 450 days after trypanocide therapy.

trypanosome suspension of each stock was inoculated intra-peritoneally (i.p.) into two mice. At peak parasitaemia, the mice were anaesthetised with diethyl-ether and blood was collected into a 1.0 ml syringe containing a drop of sodium citrate glucose, pH 7.7, by cardiac puncture, which was performed with a 25 gauge hypodermic needle.

Thirty five mice were injected i.p. on the left flank with each stock, at dose of 1×10^6 trypanosomes/mouse.

The mice were then allocated to 7 treatment groups as shown in Table 18. All mice were injected i.p on the right flank with an appropriate quinapyramine dose, 6 hours after infection. Dilutions of quinapyramine were made as follows: 1 mg ml^{-1} stock of the drug was made in sterile distilled water. Two hundred microlitres of this solution, containing 200 ug of the drug (equivalent to 10 $mgkg^{-1}$ for a 20g mouse) was used to treat Group 1 mice. This dose had been found by Gill (1971) to be the maximum amount tolerated by 80% mice. Groups 2 to 7 received similar volumes of the drug at doubling dilution. Group 8 was an untreated control.

4.4.2 RESULTS

The quinapyramine sensitivity of the nine trypanosome stocks over a dosage range of 0.156 to 10 mg kg^{-1} is summarised in Table 19.

For all the stocks, the dose required to cure all the mice was above 1.25 mg kg^{-1}. Two stocks (Ngurunit 922 and Olturot 819), had MCD 100 of 2.5 mg kg^{-1}, which was also the MCD 100 of the reference sensitive stock (Ngurunit

929). For the reference resistant stock (KETRI, 2429) and three others (Ngurunit 938, 934 and 958), the maximum dose used (10 mg kg^{-1}) failed to cure the mice. Two stocks (803 and 763) had MCD 100 values between the two extremes.

Table 18: Groups of mice and the quinapyramine dosages tested.

Group	Number of mice	Quinapyramine dose (mg kg^{-1})
1	5	10
2	5	5
3	5	2.5
4	5	1.25
5	5	0.625
6	5	0.3125
7	5	0.1565
8	5	Untreated controls

Table 19: Quinapyramine sensitivity tests of nine stocks of T. evansi collected from camels in northern Kenya.

Dose mg kg^{-1}	Camel numbers from which the trypanosome stocks were collected.								
	803	819	922	929	938	934	958	763	KETRI 2429
10	0/5	0/5	0/5	0/5	1/5	2/5	1/5	0/5	2/5
5	1/5	0/5	0/5	0/5	2/5	2/5	0/5	0/5	5/5
2.5	3/5	0/5	0/5	0/5	4/5	5/5	5/5	1/5	5/5
1.25	5/5	1/4	1/5	3/5	5/5	5/5	5/5	1/5	5/5
0.625	5/5	5/5	1/5	5/5	5/5	5/5	5/5	5/5	5/5
0.3125	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
0.1565	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

Key

n/5 = Relapse rate

4.4 DISCUSSION

In this chapter, studies to elucidate various diagnostic and control methods of T. evansi infections in camels under both experimental and natural conditions of challenge have been described.

The experimentally infected camels developed an acute disease three days post infection which was characterized by inappetance, heavy parasitosis, pyrexia, excessive lachrymation, enlargement of superficial lymph nodes, oedema, and reduction in packed cell volume. Similar clinical signs have been reported in the literature (Stephen, 1970 and Losos, 1980).

The sudden and acute illness is perhaps related to the dose of trypanosomes used ($1x10^6$) and the route of inoculation (intravenous). Under natural challenge, the number of parasites that are contained in about 0.01 ul of blood retained in the mouth parts of haematophagous flies are few (reviewed by Luckins, 1988) and because they are inoculated intradermally, the prepatent period is longer. Nevertheless, acute natural infections were commonly observed during the studies reported here.

Persitently high rectal temperatures were observed for a period of seven days following the intravenous inoculation of the trypanosomes and, thereafter, the fever fluctuated. This is the typical picture in animals suffering from acute trypanosome infections (Ross and Thomson, 1911, cited by Donelson and Turner, 1985). Parasitaemias, however, remained detectable up to the time

of trypanocide treatment. Ordinarily, trypanosomiasis is characterized by fluctuation of parasitaemia, but possibly because of the heavy dose inoculated directly into the blood, the parasitosis could have been so high that, fluctuation in actual numbers due to the phenomenon of antigenic variation was not obvious. The relapsing parasitaemias that occurred after the trypanocide treatment were not accompanied by pyrexia, nor did the camels show signs of illness, unlike the preceding parasitaemic waves. The reason for this is not clear, but it appears that, for some reason, the virulence of relapsing parasitaemia was low.

''Anaemia is an inevitable consequence of trypanosome infections'' (Murray and Dexter, 1988), a statement that was well illustrated in the experimentally infected camels. Their PCV dropped by 31% (from 0.29 to 0.20 l/l) within a period of 22 days. A similar drop was reported by Jatkar and Purohit (1971). The rapid drop occurs in the acute phase and, as was seen in this experiment, is associated with parasitaemia (Murray and Dexter, 1988). Following trypanocide therapy, the PCV did not improve to pre-infection level in the 87 days of observation. Similar observations were noted in the bovine (Murray and Dexter, 1988).

Trypanosome infections in camels exposed to natural challenge were also associated with low PCV values. This was most evident during the trypanosomiasis epidemic that occurred from April to July, 1987, when the group mean PCVs dropped markedly. The drop was more marked in the recently weaned calves which had no prior experience of the

disease. However, because of the chemotherapeutic interventions, the full effect of the disease on PCV was probably reduced.

The basis of anaemia in trypanosomiasis has been reviewed recently and is thought to be largely due to increased rate of erythrocyte destruction by an expanded mononuclear phagocytic system (Murray and Dexter, 1988). A second cause of anaemia is thought to be related to the hypo-proteinaemia in animals suffering from trypanosomiasis, and as a consequence, the incorporation of iron into red blood cells is reduced. This second cause of anaemia is important since it explains why other conditions (for example, helminthiasis and malnutrition) that are characterised by hypoproteinaemia result in anaemia. These two conditions are commonly found in camels in northern Kenya (Rutangwenda, 1984), and if anaemia is to be used as an indicator of trypanosomiasis, it would have to be considered as possible differential diagnosis.

Data on PCV in the camel herds at Ngurunit and Olturot showed that, apart from trypanosomiasis, three other factors influence haematocrit levels. These were: nutrition, physiological status and management practices.

Poor nutrition was considered to be the most important cause of low PCV in the traditionally managed calves. They were grazed and penned separately from their dams in order to restrict suckling so that the dams could provide milk for human consumption. The calves were also grazed in areas near the homesteads which were generally overgrazed. In the institutionally owned herd, the calves were grazed with the dams and were also penned at night with them and

hence had milk ad libitum. Their good nutrition was reflected in the higher PCV values compared to their counter-parts.

The changes in physiological states of female camels affected PCV. The PCVs were at their lowest during the lactation period (sections 4.3.2.6. and 4.3.2.7). Later on when the dams were heavily in-calf, their general condition was very good and their PCVs high. Another physiological factor that was suspected to influence the level of PCV was watering. This aspect was not investigated but it was commonly observed that, following watering, the camels' PCV rose. In the areas in which these studies were conducted, camels were watered every nine days. A severely dehydrated camel can take upto 200 litres of water (Guthier-Pilters and Dagg, 1981; cited by Dorman, 1986). To be able to cope with such an amount without suffering erythrolysis, the camel's red blood cells are oval, a shape that enables them to expand by up to 200 times (Meyerstein, Mazor, Etzion and Yagil, 1981). It is likely that such an expansion causes the PCV to rise.

Packed cell volume was also influenced by practices such as blood letting. The latter was commonly practised as a method of treatment for various conditions and also to provide blood for human consumption. In one camel (931), which was bled 10 litres as a traditional treatment for threatened abortion, the PCV dropped from a value of 0.31 l/l to 0.20 l/l within a period of one week: the camel did not abort following this "treatment".

From the foregoing, it is obvious that, although anaemia is a cardinal sign of trypanosomiasis, it is not

pathognomonic for the disease. Nevertheless, as Murray and Dexter (1988) point out, when accompanied by an aetiological diagnosis, it gives a reliable indication of the disease status and the productive performance of such infected animals.

There was a striking difference in the number of infections detected in the herd kept under individual animal treatment (50) and that kept under chemoprophylaxis (15). This difference was also reflected in the number of clinical cases (30), abortions (3) and deaths (3), all of which were confined to the herd maintained under individual animal therapy. The observed differences can be attributed to either the different control programmes practised or, the different levels of trypanosome challenge in the two environments. The latter possibility was considered unlikely because earlier studies by Wilson and others (1983) had shown that incidences of trypanosomiasis and the severity of clinical disease were higher at Olturot than at Ngurunit.

Wilson and colleagues (1983) had recommended the use of chemoprophylaxis in herds with an unstable disease (indicated by presence of clinical disease associated with trypanosome infections), a practice they felt would introduce disease stability. This was confirmed in the present study. In herds where the disease was stable, they recommended the use of individual treatments as opposed to chemoprophylaxis which they felt would lower endemic stability. In the present study, individual treatments using quinapyramine sulphate eliminated the infections found in the herd during the initial period of the study,

when the weather was dry and parasite transmission was low. However, this strategy failed to maintain the initial stability observed at the beginning of the study, a phenomenon which Wilson and colleagues (1983) had also reported.

Soon after the start of the long rains in March, 1987, new infections started to appear in both herds and in anticipation of a higher trypanosome challenge than the one seen during the short rains and, considering the observed relapses, the whole herd at Olturot was treated prophylactically with quinapyramine prosalt at the maximum dose (5 mg kg^{-1}) recommended by the manufacturers (Davey, 1958). This seemed to have eliminated the infections since none was detected until three months later. Two months is the expected duration of protection with quinapyramine prosalt (Gill and Malhotra 1971).

Unlike the situation observed in the herd at Olturot, new infections which were associated with clinical disease, were continuously detected in the camels at Ngurunit. Despite the prompt treatment of the parasitaemic individual camels, the epidemic threatened to run out of control. Only after the administration of chemoprophylactic drug to the whole herd did the emergence of new infections stop. Thus, for some reason, the 'enzootic' stability had reverted to a state of instability and the recommendation of individual treatments in such herds as put forward by Wilson *et. al.* (1983) appears to be misleading. It would thus appear that, during the period of high trypanosome challenge, group prophylaxis with quinapyramine prosalt was more suitable than treatment of individual parasitaemic

camels with quinapyramine sulphate. The reasons for this conversion from a state of apparent 'enzootic' stability to instability are probably numerous, but introduction of trypanosomes from other areas and the possibility of there being stress factors that increased the camels' susceptibility were considered. The former possibility was confirmed by karyotype studies (section 3.5.2). Whatever the explanation, it is evident that individual animal treatment failed while chemoprophylaxis reduced infections to a manageable level.

The relationship between outbreaks of T. evansi infections, rainfall and vector population is a well known phenomenon (reviewed by Mahmoud and Gray, 1980). In the present study, vector dynamics were not investigated and in the absence of fly data, this study must be regarded as incomplete. However, from the data available, a few epidemiological observations that have relevance to trypanosomiasis control can be made.

Trypanosomiasis was found to be endemic in the herds studied and control was warranted. At the beginning of the study the trypanosome incidence was about the same in both herds and although different control strategies were adopted, the level of control achieved during the proceeding dry period (July to November, 1987) was the same. It could then be argued that during the dry periods, it would have been more economical to treat the individual parasitaemic animals instead of the whole herd, as was done at Olturot. While this might have been so, it is difficult to predict what would have happened to the herd at Olturot, which was 100 km away and where the surveillance and prompt

treatment would not have been possible.

Following the short rains (November to December, 1986), cases were detected in the herd on prophylaxis but not in the herd under individual animal treatment, confirming earlier observations by Wilson and others (1983), that trypanosome incidence was higher at Olturot.

Surveillance of the incidence of new infections in order to determine risk is important in determining the inter-treatment interval (Rogers, 1980). In practice, other factors such as the cost of the drugs and expected fly population may influence the treatment interval. In the present study, the number of new infections detected at Olturot during the short rains was considered too low to warrant re-treatment of the whole herd with a prophylactic drug. It was considered that individual treatments of the infected animals would be sufficient until after the beginning of the long rains. This indeed appeared to have been successful. Although this was the case, there was an inherent risk in this approach in that, because of the low sensitivity of the present diagnostic methods, a much greater percentage of the herd was probably infected without being detected by the end of the wet season, and many camels might have been carrying chronic infections. Because of the problem of tissue invasion in *brucei*-type infections (Jennings *et al*., 1979), chronic infections are more difficult to treat. Also, delaying the inter-treatment period for too long, exposes trypanosomes to declining drug levels which enhance emergence of drug resistant strains (Whiteside, 1960). The relapse infections found in camels 803 and 819 could have been as a

result of drug resistance, a situation which was investigated further (see below).

Recurrence of parasitaemia in animals that had been treated previously was observed in both herds and depending on the length of time between the trypanocidal treatment and the recurrence of parasitaemia, they were attributed to either re-infections or relapses. Re-infections were suspected when parasitaemia recurred in the same animal at a time when the drug concentrations would not have been curative. For quinapyramine sulphate this was taken to be one month after its application and for quinapyramine prosalt, three months (Davey, 1958). Relapses were suspected when parasitaemia occurred soon after treatment, at a time when the animals were supposed to be under drug protection. In practice, such relapses are best treated with a sanative drug (Whiteside, 1958), and for camels, a good alternative to quinapyramine is suramin. At the time this study was conducted, suramin was not available, and up to the time of writing the thesis, it was still unavailable commercially in Kenya.

Perhaps the most striking finding of these experiments was that, the calves did not require trypanocidal treatment before they were weaned and over one year old. Under traditional management, the calves were subjected to severe nutritional stress, and in a trypanosomiasis endemic area, one would have expected them to succumb to clinical disease in absence of chemotherapy. A low plane of nutrition is a major factor in reducing trypanotolerance (MacLennan,1974). The factors that may have been responsible for the apparent calfhood immunity are discussed later.

Prior to the experimental inoculation of camels with trypanosomes, the animals were free of the disease as indicated by the absence of patent parasitaemia, they had high PCV values and had no detectable anti-trypanosomal antibodies or circulating antigen. Following infection, clinical disease developed and the circulating antigen became demonstrable soon after. During the early acute phase of the disease, the circulating antigen was not demonstrable despite the high parasitaemia. Similar observations were made by Nantulya (1989) who proposed that, during the multiplication phase of trypanosomes, there may be insufficient parasite destruction to produce detectable levels of antigen in the circulation and, therefore, the test would produce false negative results. This, indeed, appeared to be the case because soon after the acute phase was over (indicated by a reduction in rectal temperature), the circulating antigen was detectable albeit initially at low levels. The absence of detectable antigenaemia at the time when the parasitaemia is high would however not prejudice the test, since simple microscopy would suffice to make a diagnosis. It is at the chronic stage, during which time the parasitaemia is scarce, that the ELISA antigen test is likely to be most useful. The persistence of antigenaemia long after the trypanocide therapy in animals that subsequently relapsed is a clear indicator of the usefulness of the test in predicting chemotherapeutic success. Thus it appears that the antigen detection assay, as used in this study, is valid as a diagnostic method which can also be used for assessment of the efficacy of chemotherapeutic

interventions.

A comparison of the parasitological findings and the antigen detection results of the camels exposed to natural trypanosome challenge revealed four important features.

Firstly, in most of the instances in which trypanosomes were detected, the antigen detection test gave a positive result (52 out of 61). A comparable sensitivity was reported in another study of camel trypanosomiasis in Mali and Kenya (Nantulya et al., 1989b). In nearly all these cases (see results of experimental infections, Table 11), the parasite was detected first and the circulating antigen a week or two later. This observation has the important implication for any future development of this test, that antigen detection will be more useful for established infections rather than for the early stages of the disease. The strategy, therefore, will be to combine parasitological diagnosis with the antigen detection assay.

Secondly, there were cases in which sera from parasitologically proven infections did not show detectable circulating antigen, even after trypanocide therapy. Similar findings have been reported (Nantulya, 1989), and two explanations were proposed. One, as alluded to earlier, during the trypanosome multiplication phase, there may be insufficient parasite destruction to produce detectable levels of antigen in the circulation and, therefore, the test would produce false negative results. This is likely to occur in a herd which is examined for the presence of trypanosomes by microscopy frequently and at short intervals and, therefore, many cases are likely to be detected soon after the infection. This was the case in the

studies reported here, whereby, 7 out of the 9 camels with parasitaemia but no antigenaemia were seen in the herd at Ngurunit. This herd was being examined for the presence of trypanosomes weekly and, therefore, many cases were likely to have been detected at the multiplication phase. Why the antigens were not detectable following trypanocidal therapy is not clear but it is conceivable that the antigens could have been cleared from circulation before the next serum sampling which was usually fortnightly.

Nantulya's (1989) second explanation was that, in cases of antibody excess, the antigens would exist in the form of immune complexes and would, therefore, be undetected, since the epitopes would be masked by the antibodies. This possibility was evaluated in four camels which had no detectable antigen despite the presence of trypanosomes. In none of these four camels were immune complexes detected. Parasitaemia without antigenaemia was, therefore, most likely due to the infections being at the multiplication phase when trypanosome destruction was minimal.

The third observation was that, following trypanocide therapy of proven trypanosome infection, the antigens remained detectable for varying lengths of time. In the majority of instances (87% in the herd at Ngurunit and 71% in the herd at Olturot), antigens disappeared from the circulation within a period of 30 days. Persistence of antigens for a similar length of time after trypanocidal treatment was reported in monkeys experimentally infected with T .b. rhodesiense (Liu, Pearson, Sayer, Gould, Waitumbi and Njogu, 1988) and in cattle experimentally

infected with T. congolense, T. vivax and T. brucei (Nantulya & Lindqvist, 1989). Thirty days is probably the maximum period that antigens remain in the circulation following successful trypanocide therapy. Compared to the delay observed in the clearance of antibodies (up to three months), this is a major improvement on accurate diagnosis of current infections (Luckins et al, 1978; 1979). In certain other cases, circulating antigen persisted longer, sometimes for over 500 days. Whether this represents a failure of the animal to clear antigens from the circulation or the release of antigens from trypanosomes hidden in various tissue foci is not clear. No attempts were made to evaluate these possibilities but from the data on the experimental infections in which relapses were observed in camels with persistent antigenaemia, the latter possibility seems most plausible.

The failure of a trypanocide to effect a complete cure can emanate from low dosage rates, resistance of trypanosomes to the drug used or because the parasites are located in tissues inaccessible to the drug. For the purpose of treatments, the weights of the camels were estimated by measuring (in metres) the shoulder height (SH), the thoracic girth (TG) and the abdominal girth (AG). Using the formula SH x TG x AG x 50 = liveweight (kg), the bodyweights were calculated. This method has a 10% accuracy rate (Schwartz, Dolan and Wilson, 1983). This level of accuracy is adequate and under-dosing due to low dosage rate calculations was considered unlikely. The finding of trypanosomes shortly after trypanocidal treatment, at a time when the camels would have been under

drug protection (camels 803 and 819 at Olturot and 934 at Ngurunit) indicated a possibility of drug resistance. Drug sensitivity tests carried on trypanosomes isolated from these camels showed resistance to quinapyramine sulphate at the maximum dose tested (see section 4.3). In cases where trypanosomes exist in privileged locations, persistence of antigens without a patent parasitaemia would be expected after trypanocidal treatment. This was indeed the case in six camels in Ngurunit and eight in Olturot. However, it is possible that the presence of drug resistant trypanosomes led to chronic infections and subsequent invasion of the body tissues. Therefore, a distinction between an infection with drug resistant trypanosomes and that emanating from trypanosomes hidden in tissues cannot be determined by analysis of antigens alone.

The fourth observation was that, in certain cases, trypanosomal antigens were the only indicator of infection even when parasitological examination was performed as frequently as at weekly intervals. If indeed such antigens represented low grade trypanosome infections, one would have expected their disappearance soon after treatment. At Olturot, where control of trypanosomiasis was by prophylaxis, antigens disappeared from the circulation in 15 out of 20 occasions when antigens were detectable at the time of trypanocide therapy. Reports by other workers also seem to indicate that, antigenaemia can be equated with parasitaemia (Nantulya, 1989; Nantulya and Lindqvist, 1989; Nantulya et al, 1989 a,b.). If these observations are correct, then, antigen detection does not only provide a more reliable picture of the herd infection status, but

also provides useful information regarding the animals that are likely to be the reservoirs of infection, and, therefore candidates for culling on the basis of their failure to respond to therapy.

The Ngurunit herd (which was not treated with a trypanocide until parasitological diagnosis was made) had five adult camels in which high levels of circulating antigens remained detectable throughout the observation period (over 500 days). It is likely that these camels had been infected long before the initiation of the study and hence the trypanosomes had already invaded the tissues from which antigens were continually being released following disruption of trypanosomes. Why such trypanosomes would remain hidden for such a long period of time is not clear, but it can be that, in their concealed state, the trypanosomes do not change their antigenic coat and hence the host humoral immune mechanism is able to kill them once they enter in the blood stream. Only if the immune mechanism is for some reason lowered will such infections establish themselves. In experimental T. b. rhodesiense infections in monkeys, relapses have been known to occur after a period of two years of aparasitaemia (Dr. P.D. Sayer, personal communication). There were other camels in both Olturot and Ngurunit in which low levels of antigens were occationally detected. Such occational antigenaemia could represent occasional disruption of trypanosomes as they enter in the blood stream.

From the parasitological and antigen data relating to the calves, it appears that they possessed some form of protection from trypanosome infections in their early

life. In cattle calves, such an immunity has been demonstrated by Wellde, Hockmeyer, Kovatch, Bhogal and Diggs (1981) who attributed it to maternally derived immunity. The nature of this protection in the camel calves was not clear considering that no anti-trypanosomal antibodies were detected in the calves before they became infected. The camel has a diffuse epitheliochorial type of placenta (Sumar, 1988) which does not allow the transfer of immunoglubulins (Jacobson, 1977), and therefore, the bulk of maternal antibodies are derived from colostrum. The colostral immunoglubulins represent antibodies to antigens in the environment of the dam (Herbert, 1970). The reason for the absence of trypanosomal antibodies in calves born of dams living in a trypanosomiasis endemic area was surprising, but the following is suggested.

It is possible that the passively acquired anti-trypanosomal antibodies were catabolised within a short period of time, such that, by the time the calves were recruited for the studies (about six months of age), the maternally derived immunoglobulins had already waned. In general, maternally-derived antibodies last only 3 months. Considering that such immunoglobulins would have been directed against the VSGs of trypanosomes common in that area, they would have protected the calves from infection during their early period of life. It should be noted also that the commencement of the studies coincided with the dry period when both the vector population and the incidence of trypanosome infections in the whole herd was low. Moreover the traditional management of calves could also have contributed to lack of exposure to infections:

the calves were grazed separately from the other age groups. This was primarily to restrict milk intake from their dams since it was needed for human consumption as well. The calves were grazed in areas near the homesteads, which were generally overgrazed and therefore, fewer biting flies would have been present. Later on, when they were weaned and allowed to join others in search of pasture and water, the immunologically naive calves (as far as trypanosomiasis was concerned) would have been expected to suffer acute disease on challenge. This was indeed observed in the subsequent wet season when the majority of infections that occurred were in the recently weaned calves. Some adult camels were also found to lack trypanosomal antigens and antibodies. In one such adult camel (901), a peracute infection occurred and the camel died within a week of being infected.

It is, however, unlikely that the calves would not have been exposed to trypanosome infections for over one year, considering that the older camels and other animal species returned to the homestead in the evenings where vectors, albeit few, were always present and, therefore, some transmission was bound to occur. It is probable that in addition to the protection afforded by maternal antibodies and the lack of exposure resulting from management, there are other non-specific factors akin to those that contribute to calfhood immunity against babesiosis (Riek 1963). Wilson et al. (1983) also reported a very low incidence of trypanosome infections in camel calves, compared to the older age groups. Wilson and colleagues did not find a difference in the prevalence rate of

anti-trypanosomal antibodies between the calves, weaners and adults. They made an assumption that the high antibody levels conferred protection to the calves. It is, however, difficult to explain why the anti-trypanosomal antibodies did not protect the older age groups as well. Clearly, further work is needed to elucidate the nature of calfhood immunity.

From the foregoing, it appears that there are two pertinent questions that need to be addressed to if the detection system for circulating antigen is to gain widespread use. One is whether the presence of antigen constitutes an unequivocal basis for making a diagnosis of a trypanosomal infection and two, whether its absence represents a lack of the infection.

So far, no cross-reaction has been observed between the monoclonal antibody used, and other haemoparasites, or, with negative sera from non trypanosomiasis endemic areas (Nantulya, 1989). Thus, it appears as though the test has absolute specificity. If this is so, then the presence of antigens can be taken to represent the presence of a trypanosomal product, which in itself would mean an active infection. However, since the release of antigens will depend on the destruction of trypanosomes from the preceeding parasitaemic waves, repeated sampling would be essential in those cases that are negative at first sampling. As some animals may die of sudden acute infections before antigens are demonstrable as happened to camel number 901, it is noteworthy that parasitological techniques can detect the infection in these situations. Clearly the two diagnostic approaches are complementary.

The failure of the trypanocide to cure the infections even after the correct dosage was used is worrying because it could mean that, either, the parasites were resistant to the trypanocide used, or, the parasites had already invaded tisues inacessible to the drug by the time the treatment was instituted. Jennings and collegues (1979) are of the opinion that, tissue invasion by T. brucei occurs fairly early in infection (at least by day 21 in mice). Resistance to quinapyramine because of previous misuse was likewise possible since the drug had been on the market before. Additionaly, because of the relatedness of the trypanocides (Leach and Roberts, 1981), cross resistance could have ocurred.

It is difficult to differentiate relapses originating from trypanosomes located in tissues inaccessible to the drugs and those occurring because the trypanosomes are resistant to the drugs. There are, at present, no simple and sensitive assay methods for measurement of plasma levels of quinapyramine in treated animals which could have been used in the present study to ascertain whether the relapses observed occured in the presence of curative plasma levels of the drug. Drug sensitivity trials were therefore conducted to find out whether a correlation existed between the observed recurrences of parasitaemia in camels and susceptibility of the trypanosome isolates to various dosages of quinapyramine.

The quinapyramine sulphate MCD 100 for the sensitive stock (Ngurunit 929) was 2.5 mg kg^{-1}, a dose that Gill (1971) regarded as curative for normal T. evansi strains. Two other stocks (Ngurunit 922 and Olturot 819) had similar

MCD 100 to the sensitive stock. Ngurunit 922 had been isolated from a camel that had persistent antigenaemia in the absence of parasitaemia for 450 days following trypanocidal treatment. The source of these circulating antigens could have been from trypanosomes located in tissues other than those (trypanosomes) in peripheral circulation. While this might be so, the absence of patent parasitaemia for such a length of time is intriguing. Another stock (Ngurunit 763), which had been used for the experimental infection in camels and subsequently relapsed (see 4.2.2.2), also showed an unexpectedly lower level of resistance in mice. Since the infected camels had been treated 27 days after infection, it was possible that by the time of treatment, the parasites had already invaded privileged sites such as brain, aqueous humor of the eye and pericardial fluid (MacLennan, 1971), tissues which quinapyramine does not enter in trypanocidal concentrations. The relapse parasitaemia could have originated from such tissues. The Olturot 819 stock had been collected from a camel that had breakthrough infections 31 days after it had received quinapyramine prosalt, a trypanocide said to have a prophylactic period of two months (Gill and Malhotra, 1971). In the absence of data on quinapyramine plasma concentration at the time of such a relapse, it is difficult to explain this observation. The possibility that the camel escaped treatment during mass prophylaxis cannot be ruled out.

Another stock (Olturot 803) collected from a camel that had a breakthrough infection one month after administration

of quinapyramine prosalt could not be cured even at 10mg kg^{-1}) in mice, and hence was considerd to be resistant. The apparently sensitive stock (Olturot 803) and the resistant one (Ngurunit 819), had been collected during the same period and had a similar karyotype pattern (see page 87); such findings suggested that they were genetically related, although different in sensitivity to the drug.

Studies conducted at ILRAD (Peregrine, Knowles, Ibitayo, Scott, Moloo and Murphhy, 1991) have also shown that clones with a similar genetic background could express significantly different levels of resistance.

The maximum dose used (10 mg kg^{-1}) in mice failed to cure the infections with the reference drug resistant stock (KETRI 2429) and three isolates from Ngurunit (934, 938, 958). Two of these stocks (Ngurunit 934 and Olturot 803) had been noted to relapse in camels very soon (13 days) after the trypanocidal treatment. The other two (Ngurunit 938 and 958) had, in addition to relapses seen 24 and 51 days respectively after trypanocide administration, a detectable antigenaemia was observed long after treatment. An interesting observation with all these trypanosome relapses was that, after a second trypanocidal treatment in the camels with the same drug at the same dosage rate, the recurrence of parasitaemia was not seen. It is possible that, by the time the second treatment was done, the animals were able to mount an effective immune response that cleared the infections. Why this was not possible following the initial treatment is difficult to explain. Could it also be that drug resistance is not a permanent characteristic ? More work is needed to elucidate further the mechanisms of drug resistance in trypanosomes.

In conclusion, four methods namely: clinical signs, haematocrits, parasitology and serology were used to diagnose and to describe the local epidemiological picture of T. evansi infections in the two herds of camels. The clinical signs that developed following both the experimental and natural acute T. evansi infections were characterized by inappetance, excessive lachrymation, lymphadenopathy, oedema, abortions and pyrexia. These signs were however not consistently present in all the infected animals. On their own, these signs would only enable a provisional diagnosis. In the chronically infected individual camels, it was the weight loss that was most characteristic of the disease. In the field, the nature of general practice is such that, provisional diagnosis is made, treatment instituted and the response to therapy used for the confirmation of the diagnosis. This approach has merit in that therapy is not delayed while awaiting laboratory confirmation; but can be expensive if the diagnosis turns out to have been incorrect.

At the same time, although anaemia is a cardinal sign of trypanosomiasis, low PCV only correlated with trypanosome infection in weaner camels. In camel calves and dams, the level of haematocrit was markedly influenced by the level of nutrition (in calves) and the physiological state (pregnancy and stage of lactation in dams). Parasitological diagnosis was the only sure way of confirming a diagnosis. Examination of blood smears by light microscopy is the method routinely used for making a parasitological diagnosis of trypanosomiasis in the field. To be reliable, the examination has to be done frequently,

a proposition which is not practical in the present day veterinary practice in the areas where camels are kept. Ideally, what is then needed is a serological test which findings are synonymous to a parasitological diagnosis. The trypanosomal antigen detection test as used in this study was found to complement that of parasitological diagnosis and moreover, it detected more infections than parasitological tests. The presence of trypanosomal antigen in absence of a parasitological diagnosis may make the interpretation of the test difficult, a problem that can be solved by demonstration of anti-trypanosome antibodies. The finding of trypanosome antigen in absence of both the antibody and the immunecomplexes as was found in the studied cases is intriguing but would require further investigation before ascribing such antigens to non-specific reaction inherent in the test system. The point to remember when evaluating the trypanosomal antigen detection test is that, the "litmus test" (demonstrating the presence of a trypanosome) is in most cases impossible due to the fluctuation and scarcity of parasitaemia and existence of trypanosomes in ''occult'' forms. In addition, these studies have also shown that, following trypanocidal treatment, the antigens are cleared from the circulation within a month and any persistence beyond this period may indicate unsuccessful therapy.

Finally, the field studies have shown that it is possible to maintain camels in a healthy state amidst T. evansi challenge, provided rational control measures are adopted. Individual treatments with quinapyramine sulphate sufficed only when the trypanosome challenge was low (dry

season) but failed during the high risk period (long wet season). During the latter period, chemoprophylaxis was necessary to control the disease. Quinapyramine prosalt at 3 mg kg^{-1} just after the short rains (November), and 5 mg kg^{-1} shortly after the long rains (April) provided adequate protection to camels against T. evansi infections. Continued surveillance of new trypanosome challenge is important in order to detect emergence of drug resistant stocks and to economise on drugs. Although the sensitivity tests conducted in mice indicated the presence of quinapyramine resistant stocks of T. evansi in the herds studied, most of the losses emanated from failure to use chemoprophylaxis rather than from failure of drugs used to remove established infections.

CHAPTER 5

GENERAL CONCLUSION

The work reported here set out to identify the trypanosomes that cause camel trypanosomiasis and assess different methods of its diagnosis and control. These are crucial factors in the understanding of the epidemiology of the disease and are important consideration if a rational control programme of camel trypanosomasis is to be devised.

Trypanosomiasis is the most important disease of camels in the arid areas of Kenya and is widespread (Wilson *et al.*, 1983). These findings were confirmed in the present study. The disease was found to be seasonal. Epidemics occurred during and soon after the long rains of April, while sporadic infections occurred during the dry season. It was during the wet season epidemic that the threat of camel trypanosomiasis to traditional camel husbandry and the subsequent lowering of the standards of living of the camel keeping nomads was appreciated. The weaner camels developed severe clinical disease, pregnant dams aborted and lactating ones reduced milk production. Chemotherapy was essential inorder to control the disease.

By the criteria of tsetse transmissibility and kinetoplast DNA minicircle analysis, all the isolates were *T. evansi*. The homogeneity of their kDNA minicircles on digestion with restriction endonucleases compared to the heterogeneity of *T. b. brucei* kDNA minicircles was an easy and reliable criterion of distinguishing the two, otherwise, indistinguishable species.

Karyotyping was found to be a sensitive method of revealing differences within *T. evansi* isolates. These differences could not however be attributed to any useful phenotype. The method, nevertheless, was found to be

useful in revealing strains of trypanosomes that were likely to be re-isolations of the same parasites. Further work is needed to determine how stable the karyotypes of T. evansi are and whether they could be useful in interpreting the varied epidemiological observations that characterise different outbreaks that commonly occur in T. evansi endemic areas.

The clinical picture associated with T. evansi varied from one age group to another. The effect of the disease was mainly noted in the pregnant and the lactating camels and the weaners. The camel calves did not show infections until they were weaned and were over one year of age.

Much as anaemia was commonly associated with trypanosome infections, other factors such as level of nutrition (in calves) and physiological status (pregnancy and stage of lactation in dams) were found to markedly influence the level of haematocrit. Packed cell volume was also influenced by practices such as blood letting. Only in the weaner camels were the low PCVs nearly always correlated with trypanosome infections.

A comparison of the parasitological diagnosis and antigen detection revealed that, antigen detection is more sensitive than the other diagnostic methods. In cases where the antigenaemia correlated with parasitaemia, the trypanosome was detected first. If such cases were treated soon after their detection, the circulating antigens were not demonstrable. Following a successful trypanocide therapy, circulating antigens were cleared from circulation within a period of one month. In a few cases, antigens persisted for a long time, even following what appeared to

be a successful therapy. It is not clear whether this represents a failure of the animal to clear the antigen or whether this represents treatment failure, leading to continued release of antigens by trypanosomes in various tissue foci.

In order to keep camels healthy in a trypanosomisis endemic area, chemotherapy was found necessary. Individual treatments sufficed during the dry season when the infections were sporadic but failed during the long wet season epidemics. During the latter period, chemoprophylaxis was necessary to control the disease.

The adult and weaner camels were found to be the most susceptible animals to T. evansi infections and needed treatment to prevent lowering of their productivity. Suckling calves were found to be trypanotolerant and did not require treatment before they were weaned and over one year old. More studies are needed to identify the factors involved in the apparent calfhood immunity.

Cases that occurred during the wet season epidemics were found to be acute while the sporadic ones that occurred during the dry season were of a chronic nature. Two factors could have been responsible for this. Firstly, because the majority of the calves are weaned in the period between December and March, by the beginning of the long rainy period (April to June), there existed a large pool of susceptible immatures, which develop clinical disease once infected. Secondly, following the rains, camels that were in satelite camps (refered to as Fora camels in the Rendille language) in search of better pastures come home, and with them, they bring new strains of trypanosomes which

other camels had not experienced before and therefore tend to cause severe disease.

Sensitivity tests to quinapyramine sulphate conducted in mice indicated the existence of drug fast stocks of T. evansi. While the presence of such stocks are worrying, most of the losses that occured emanated from the failure to use chemotherapeutic drugs appropriately, rather than from the failure of trypanosomes to respond to the drugs used.

On the basis of the results of these studies, to improve control of camel trypanosomiasis, the following recommendations are suggested: one, that kDNA minicircle analysis be used routinely to differentiate cases of T. evansi from those of T. b. brucei. This differentiation is important in determining the vectors involved in the spread of infections. This is crucial when planning control programmes. The differentiation is also important in delineating the distribution of the two species and in tracing the movements and potential establishments of the two species in new ecological zones. Two, karyotyping should be used to determine the molecular karyotypes of T. evansi in different endemic areas inorder to possibly monitor the introduction of foreign T. evansi strains. This has a potential in linking karyotypes to phenotype. Three, although the trypanosome antigen detection ELISA is a more sensitive method in detecting trypanosome infections than the conventional diagnostic techniques, antigenaemia alone should not be used routinely as a basis of treatment. This is because the presence of trypanosome antigen does not always indicate the presence

of a live trypanosome infection and because it is not yet clear what the antigenaemia in absence of parasitaemia represents in terms of host-parasite relationship. However, because the technique detects more cases than parasitological method, it should be used to reveal the true extent of trypanosomiasis in a herd and to assess the effect of therapeutic interventions on the disease incidence.

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