EPIDEMIOLOGY OF ANIMAL TRYPANOSOMOSIS IN THE KACHIA GRAZING RESERVE, KADUNA STATE, NIGERIA

BY

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A DISSERTATION SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENTS OF THE REQUIRMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN VETERINARY MICROBIOLOGY

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DECLARATION

I declare that the work in the dissertation entitled ' Epidemiology of animal trypanosomosis in the Kachia Grazing Reserve of Kaduna State, Nigeria' has been performed by me in the Department of Veterinary Pathology and Microbiology under the supervision of Professors K. A. N. Esievo and J. U. Umoh and Dr. G. A. Omoogun of Nigerian Institute for Trypanosomiasis Reseasrch.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

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Enwezor, Felicia Nneka Chizoba Date

CERTIFICATION

This dissertation entitled '' EPIDEMIOLOGY OF ANIMAL TRYPANOSOMOSIS IN THE KACHIA GRAZING RESERVE, KADUNA STATE, NIGERIA" By Felicia Nneka Chizoba Enwezor meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

I overwhelmingly dedicate this dissertation as a show of gratitude to the Almighty God for HIS mercies, love and grace that saw me through to the successful completion of the study. Everlasting King of glory, to you is power, honour and glory, now and forever more, amen.

ABSTRACT

This study was designed to determine the tsetse species and trypanosome prevalence in the Kachia Grazing Reserve, identify factors that influence the prevalence as well as characterize trypanosome isolates using molecular methods. Blood samples collected at random from 1,293 cattle, 215 sheep and 130 goats were examined for the presence of trypanosomes using the buffy coat technique and Giemsa thin blood smears. Packed cell volume (PCV) and white blood cell (WBC) and differential leukocyte counts were also determined. Overall parasitological prevalences of trypanosomes were 8.4% in cattle and 17% in sheep and goats. Trypanosome species found were *Trypanosoma vivax* with 105 (96.3%) of 109 infections followed by *T. congolense* (1.9%), *T. brucei* (0.9%) and a mixed infection of *T. congolense* and *T. vivax* (0.9%). Mean PCV of parasitaemic cattle dropped more than those of apparasitaemic being 25.99 ±1.82 %and 29.31 ± 1.70 % respectively. In addition, the PCV of affected and unaffected sheep and goats tested significant at 5% level indicating that anaemia was more on affected animals ($P<0.05$). Total WBC and differential leukocyte counts showed leucopaenia in 105 out of 228 (46.1%) cattle, of which 25 (23.8%) were trypanosome-positive cases and leucocytosis in four tryopanosome-negative cattle. However, 11 of 119 (9.2%) cattle with normal WBC counts were trypanosome-positive cases. Serological prevalence was obtained by inhibition Enzyme Linked Immunosorbent Assay (iELISA) on 545 blood samples from cattle using an anti-Bip monoclonal antibody and a complete fusion protein (hsp 70) expressed in *Escherichia coli*. The test detected antibodies in 359 of 545 (65. 9%) samples. The apparent sensitivity and specificity of the assay were 84.8% and 97.3% respectively. The effects of cattle

movement, herd pen location to watering and grazing points distances were determined by global positioning system (GPS). Cattle movement appeared to influence the prevalence. Returned herds had a higher parasitological prevalence of 16.7%, compared with 11% of resident herds but the results were not statistically significant (P>0.05). Increased prevalence was associated with proximity of herd pens to watering point distances (x^2 for linear trend = 4.447, P<0.05) but no association by grazing point distances (x^2 =2.186, P > 0. 05). Using ITS1 kin primers, the trypanosome isolates were characterized by polymerase chain reaction and shown to be *T. vivax*, *T. congolense* forest and *T. b. brucei*. Results of tsetse trappings using Biconical and NITR designed tsetse (NITSE) traps revealed the presence of two tsetse species: *Glossina morsitans sub morsitans* and *Glossina tachinoides.* Adult tsetse flies (54) were caught comprising 12 *G. m. submorsitans* and 42 *G. tachinoides* but only one *G. tachinoides* was found infected with trypanosome. This study has shown that tsetse flies are still present in the reserve and together with other biting flies contribute to the spread and maintenance of trypanosomosis in the area. The study strongly advocates effective animal health care and prophylaxis against trypanosome and vector control to fight the problem of trypanosomosis and allow permanent raising of livestock in the reserve, thus encouraging sedentarisation.

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ABBREVIATIONS

$0^{o}C$	Degree centigrade
DNA	Deoxyribose nucleic acid
rDNA	Ribosomal- Deoxy ribose nucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
E	East
et al	And others
FAO	Food and Agriculture Organisation of the United Nations
GPS	Global Positioning System
ITS 1	Internal transcribed spacer1
KGR	Kachia Grazing Reserve
Kin 1 primer	Kinetoplast 1 primer
Kin 2 primer	Kinetoplast 2 primer
L	Litre
Min	Minutes
ml.	Milli litre
mM	Millimolar
NLPD	National Livestock Projects Division
N	North
%	Percent
pH	Hydrogen ion
±	Plus or minus
PCR	Polymerase chain reaction
UNDP	United Nations Development Programme
WHO	World Health Organisation

CHAPTER 1

1.0 Introduction

Animals, particularly cattle, play an important role in the Nigerian agricultural economy. Cattle act as a food source for many people, a means of income generation and support crop production (e.g. millet, guinea corn, maize and cassava) by providing manure and draught power. Livestock trading is also an important economic activity. However, trypanosomosis threatens the livelihoods of many of the farmers including the poorer small producers, since the disease makes raising cattle difficult or impossible (FAO, 2002a).

Trypanosomosis occurs in sub-Saharan Africa within the area of distribution of the tsetse fly and causes fever, severe anaemia, weight loss (cachexia), oedema and reproductive disorders (Isoun and Anosa, 1974; Ikede *et al*., 1988; FAO, 2002a) with death ensuing if untreated. Trypanosome infection can lead to severe production losses affecting overall meat supply including individual and community incomes, thus aggravating poverty (Jones *et al*., 2000).

In Nigeria, prevalence studies of animal trypanosomosis have been reported in the range of 3% and 10% (Agu *et al*., 1989; Kalu, 1991, Daniel *et al*., 1994, Abenga *et al*., 2002; 2004). Most studies to date have diagnosed infections with trypanosomes by microscopy of blood samples (Abenga *et al*., 2002). However, clinical diagnosis and parasitological detection of trypanosomes in the blood are insensitive (Majiwa *et al*., 1994). This study was initiated to assess the extent and nature of trypanosomosis in the Kachia Grazing Reserve (KGR) using effective diagnostic methods, such as, enzyme linked immunosorbent assay (ELISA) developed against a trypanosome recombinant antigen, Hsp 70/Bip

(Boulangé *et al*., 2002) and by polymerase chain reaction (PCR) using parasite-specific DNA (Majiwa *et al*., 1994; Desquesnes et al., 2001; Desquesnes and Davila, 2002; Njiru *et al*., 2004). Therefore, the main objectives of the study are to determine the tsetse species and prevalence of trypanosome in the grazing reserve identify factors that may influence the prevalence as well as characterize trypanosome isolates using molecular method.

1.1 **Statement of the problem**

The pathogenic African trypanosomes, the aetiologic agent of trypanosomosis, present an important group of parasitic infections (Kristjanson *et al*., 1999; Picozzi *et al*., 2002). They are not only responsible for severe losses in the agricultural sector but also cause immense sufferings and deaths in affected humans in rural communities of Africa. Trypanosomosis is strictly a problem of rural communities in tropical Africa since it is dependent on tsetse fly for transmission.

In the Kachia Grazing Reserve (KGR) in Kachia Local Government Area of Kaduna State, trypanosomosis has impacted negatively on livestock and mixed farming production systems and consequently led to the abandonment of the grazing reserve in 1985. In 1988 under the auspices of the World Bank/FAO/UNDP assisted Second Livestock Development Project (SLDP), some control measures that involved treatment with trypanocidal drugs and insecticide were applied on livestock to reduce the incidence of the disease and thus encourage the resettlement of pastoralists. However, after 16 years of these

control activities, no new significant efforts had been made to control trypanosomosis in the KGR.

Despite the above, estimates suggested that in the absence of the disease and the vector, the reserve can contribute about one billion Naira (N1.0 billion) annually to the wealth of the nation in terms of milk, meat and agricultural production (NITOR, 2004). Trypanosomosis-induced annual losses in cattle production alone were estimated in the range of 1.0 to 1.2 billion dollars in sub-Saharan Africa (FAO, 2002a). In mixed farming production systems, the disease could reduce the average area planted per household by as much as 50% (Swallow, 1997).

There is therefore a need to obtain current data on the status of tsetse and trypanosomosis in the KGR. For the control of African trypanosomosis in the KGR and Nigeria extensive epidemiological studies are a prerequisite. This study was designed to investigate the epidemiology of animal trypanosomosis in the KGR by detection of trypanosomes in blood smears using the buffy coat microscopic parasitological examination and serology, through detection of parasite-specific antibodies in sera by inhibition ELISA against a trypanosome recombinant antigen (Authié *et al*., 1993a; Boulangé *et al*., 2002) as well as by PCR against parasite-specific DNA (Desquesnes *et al*., 2001).

1.2. **Significance of the Study**

The study will strengthen the capacity and skills of the scientist involved in this project to accurately diagnose bovine trypanosomosis. It will also be of interest

to NITOR, NLPD, Pest Control Services, the Nomadic Education Unit, Kaduna, the Kachia Local Government and the Kaduna State Ministry of Agriculture and Rural Development, as they may need information generated to plan and prioritise areas of targeted work in line with their respective mandates. The findings will enable NITOR scientists to advice livestock owners on risks and appropriate strategies to reduce deaths and loss of productivity among livestock emanating from trypanosomes and their vector.

The findings will also be of interest to the Federal Government of Nigeria, the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) of the African Union. One of the major objectives of the latter is the identification of tsetse and trypanosomosis endemic areas for eradication. Finally, data obtained will be incorporated into the existing disease database in NITOR to update information on the epidemiology of trypanosomosis in Nigeria.

1.3. **Theoretical Framework**

In Africa, the pathogenic trypanosomes which are haemo-parasitic flagellated protozoa vectored by tsetse flies can infect almost all range of domestic animals (Stephen, 1970). The important members of the genus in this context are *Trypanosoma vivax*, *T. congolense*, *T. brucei brucei* and *T. simiae* causing animal trypanosomosis also known as 'nagana' (Zulu; word meaning depression, low spirits).

It is generally believed that circumstantial evidence most probably led African

cattle owners to recognise long ago a link between biting flies and the spread of trypanosomosis in their stock. Four species of tsetse flies are recognized as important vectors of animal trypanosomosis, *Glossina longipalpis*, *G. morsitans*, *G. palpalis* and *G. tachinoides* were then identified by 1850 (Kershaw, 1970). However, not until Evans (1880) showed the parasite now known as *T. evansi* to be the causal organism of 'surra' was a connection between trypanosomes and disease established (Stephen, 1970). Similarly Bruce (1895) in Zulu land established that 'nagana' was caused by a trypanosome and that tsetse fly (probably *G. pallidipes*) could be a vector. Thereafter, Kleine (1909) demonstrated the cyclical development of the parasite in the fly and was confirmed by Bruce (1909).

From the foregoing, it was believed that animal trypanosomosis occured wherever *Glossina* species were found, hence KGR by its geographical location within the tsetse belt, with the enabling environmental and intrinsic factors, the likelihood of trypanosomosis occurring in the reserve would be presumably high.

1.4 **Objectives**

1.4.1 To determine tsetse species, vector capacity and their population density in relation to the occurrence of trypanosomosis in the KGR

1.4.2 To determine the prevalence of trypanosome, assess risk factors (movement, grazing patterns, frequency of disease control, herd location to pen and watering points are associated with prevalence of the disease) and blood pictures of infected bovine herds.

1.4.3 To describe bovine mortality and morbidity and associate them with the

presence of trypanosomes.

1.4.4. To characterize trypanosome species/ isolates circulating in the KGR using the polymerase chain reaction (PCR).

1.5 **Research Questions**

1.5.1 What is the composition of tsetse species found in the KGR?

1.5.2 What is the apparent density of tsetse and vector capacity in the KGR?

1.5.3 What is the prevalence of trypanosome infection in the KGR?

1.5.4 What factors, if any, precipitate the prevalence?

1.5.5 Is there any mortatilty of animals in the KGR due to trypanosomosis?

1.5.6 What are the clinical signs and symptoms observed as a result of trypanosomosis?

1.5.7. Which diagnostic technique is more sensitive?

1.5.8. Are there any trypanosome species in the KGR? If any, what are their subspecies?

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 **African trypanosomosis**

African trypanosomosis is caused mainly by tsetse- transmitted African trypanosomes. The disease is peculiar to Africa affecting both humans and animals and causes fatal sleeping sickness in people and 'nagana'- a fatal wasting disease in domestic livestock.

In Nigeria, African Animal Trypanosomosis (AAT) remains a major threat to livestock in all the agro-ecological zones with cases recorded from all the zones (Onyiah, 1997). The situation was not different from other African countries including Uganda (Picozzi *et al*., 2002; Waiswa and Katunguka-Rwakishaya, 2004), Kenya (Njiru *et al*., 2004) and Burkina Faso (Lefrancois *et al*., 1998) where severe trypanosomosis had been reported. Prevalences, which were said to vary between 3% and 10% had been reported in Nigeria (Agu *et al*., 1989; Kalu, 1991; Daniel *et al*., 1994; Ahmed *et al*., 1994; Ogunsannmi *et al*., 2000; Abenga *et al*., 2002; 2004). However, other studies of outbreak situations had reported a much higher prevalence, 40% to 50% in parts of North Central (Maikaje, 1998) and South Eastern (Anene and Ezekwe, 1998), Nigeria. Furthermore, an outbreak of the disease in Friesan dairy cows reared under intensive (sedentary) management had been reported (Kalu, 1996). In the KGR, the Institute research team sampled bovine herds after the commencement of this postgraduate research work and obtained a prevalence of 17.3% in August 2004 (Lawani *et al*., 2004). The problem of AAT in cattle had been on the increase due to the menace of tsetse infestation, presence of other haematophagous flies and drug resistance, lack of active surveillance of the disease, which was increasingly jeopardized, by lack of funds (Onyiah,

1997).

Economic effects of animal trypanosomosis included loss in agricultural production; estimated annual losses in cattle production alone, excluding the indirect effects engendered by trypanosomosis on total crop production, were in the range of US$1.0 - 1.2 billion (FAO, 2002a). In addition, the disease had influence where people live to manage their livestock and crop agricultural farming, thus further threatening food security (FAO, 2002a). In tsetse-infested areas of sub-Saharan Africa, half of the human population suffered from food insecurity; as approximately 85% of the poor were located in rural areas and more than 80% of the population depended on agricultural production for their livelihood (FAO, 2002a). Therefore urgent control actions are required to curb the problem of AAT on agricultural production.

2.1.1 **Aetiology of African animal trypanosomosis (AAT)**

African animal trypanosomosis, AAT is caused by protozoa in the family Trypanosomatidae and genus *Trypanosoma*. AAT is endemic throughout the humid and semi humid zones of sub-Saharan Africa coincident with the distribution of tsetse, which infest an area embracing 37 countries and covering 10 million km^2 (Hommel, 1991; Black *et al*., 2001; FAO, 2002a; Catley *et al*., 2002).

2.1.1.1 **Trypanosomes species**

Three species of tsetse-fly-transmitted trypanosomes, amonst others, are responsible for the disease in cattle, namely, *Trypanosoma vivax*, *T. congolense* or *T. brucei brucei* producing single or mixed infections. Others are *T. simiae* and *T. suis* in pigs (Hoare, 1972). *T. vivax* also mechanically transmitted by biting flies (tabanid) is a member of the subgenus *Duttonella*, a group of trypanosomes with large terminal kinetoplasts, distinct free flagella and inconspicuous undulating membranes. *T. vivax* is a large (18-26 µm long)

monomorphic organism that is very active in wet-mount blood smears. *T. congolense* resides in the subgenus *Nannomonas*, a group of small trypanosomes with medium-sized marginal kinetoplasts, no free flagella and poorly developed undulating membranes. While *T. b. brucei* is an extremely polymorphic trypanosome occurring as short, stumpy organisms with distinct flagella and intermediate forms that are usually flagellated (Hoare, 1972).

Table 2.1 Morphological Characteristics of Trypanosomes

Species	Site of development In the tsetse fly	Free flagellum	Kinetoplast	Undulating membrane	Size (µm)	Size and motility
T. vivax	Proboscis	Large, Present	terminal	Not prominent	20-26	Large extremely active, traverses the whole field very quickly
T. brucei	Mid gut Salivary glands	Present in all but stumpy forms	Small, Sub-terminal or central	Prominent	13 –35	Large, rapid movement in confined areas

T. cong-olense	Mid gut Proboscis	Absent	Medium, sub terminal	Not Prominent	9-18	Small, sluggish, adheres to red blood cells by anterior end.

Adapted from Hoare (1972).

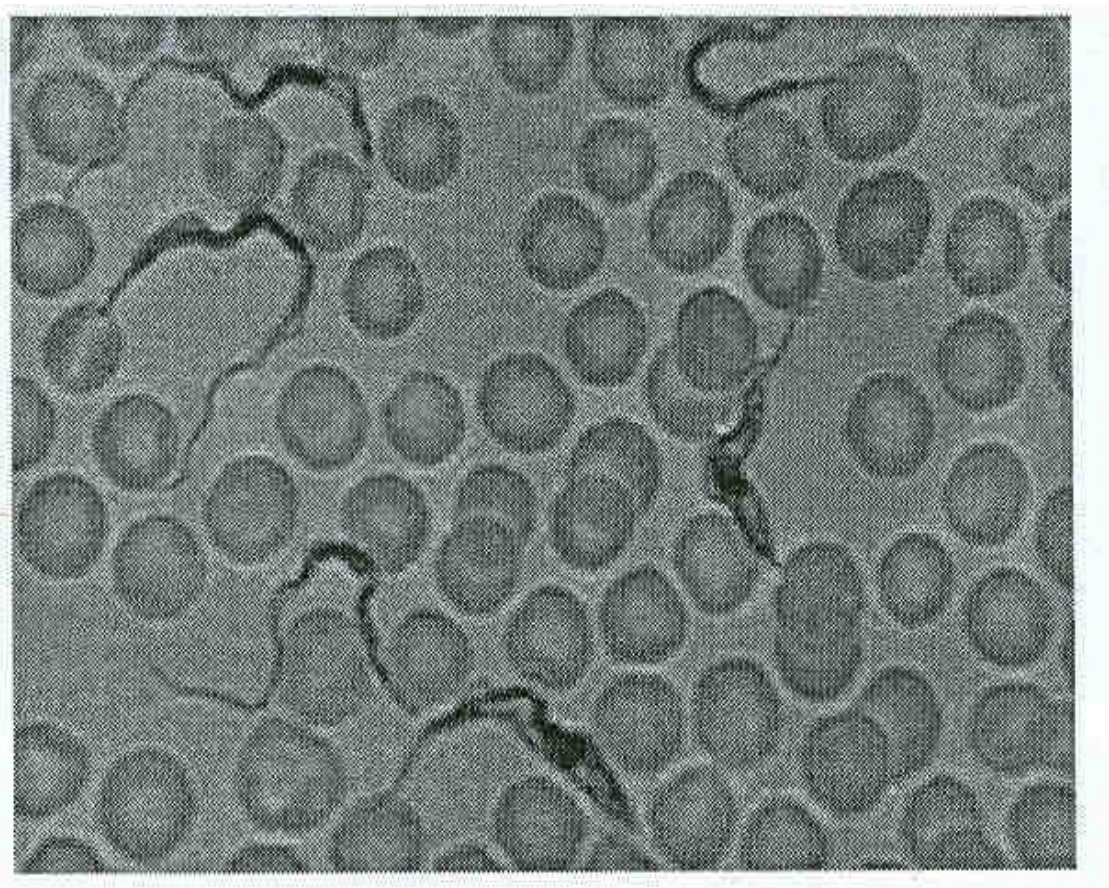

Plate 1. Trypanosomes in blood. (Adapted from the WHO 2000 website).

2.1.2. Prevalence of AAT in Nigeria

The prevalence of AAT in Nigeria based on available literature is presented. In cattle, several studies had reported the prevalence of trypanosomes in various parts of Nigeria. Joshua (1986) during a one-year study of trypanosome infections in cattle from seven locations of the low lying zone of the Jos Plateau obtained trypanosome prevalences of 28%, 14.8%, 11%, 9%, 5.7%, 5% and 0% respectively in the operational zones. In another study, Joshua and Shanthikutmar (1989) recorded trypanosome infection rate of 9.6% in cattle herds around the Jos Plateau. Also on the Jos Plateau, Kalu (1996), obtained a mean prevalence of trypanosome infection of 6.4% in cattle. Agu *et al*. (1989) in a study of 1318 cattle in parts of Kaduna and Plateau States recorded a ruminant trypanosomosis prevalence of 8.2% (108). Furthermore, Kalu *et al*. (1991) recorded 24.6% prevalence in Gboko Local Government Area (LGA) and

in 1996 a study carried out on 320 animals in Katsina LGA showed that among semi-nomadic animals, 21.3% of cattle and 38% of sheep carried matured trypanosome infections and the values were significantly higher ($P<0.05$) than that recorded for peri-domestic animals (Kalu *et al.*, 1996). In an area in Borno State, the trypanosome prevalence of 7.3% in cattle was reported (Ahmed *et al.*, 1994).

In parts of South west Nigeria, infection rates recorded in cattle in Kwara, Oyo and Ondo States were 17.3%, 19.4% and 28.2% respectively while relatively low prevalences of 2.7% and 6.7% were recorded respectively for Ogun and Bendel States (Ikede *et al.*, 1988). Okoli (2003) in a review of 26,763 animal cases in West African Dwarf (WAD) goats treated at Imo State veterinary clinic, South east Nigeria, over a period of 3years (1999-2001) reported a trypanosome infection rate of 55.4% and according to him the disease remained the most common of the diseases encountered in WAD goats treated followed by 25.09% and 19.5% for bronchopneumonia and PPR respectively.

In slaughter animals, low prevalence of trypanosome has been reported: 0.9% in cattle in Anambra State (Ekejindu *et al.*, 1992); 0.6% and 1.14% respectively in sheep and goats in Maiduguri (Basu *et al.*, 1995); 3.83% and 1.83% respectively in sheep and goats in Jos abattoir (Dada *et al.*, 1997); 3.8% in trade cattle at slaughter in Kaduna abattoir (Abenga *et al.*, 2002). However, a higher infection rate of 8.4% was obtained in slaughter cattle at Ibadan abattoir (Reynolds and Opasina (1987); 2.5% in camels infected with *T. evansi*

in Maduguri abattoir (Egbe-Nwiyi and Chaudhry, 1994) and Omeke (1994) reported a high prevalence of 26.8% in pigs during abattoir and field market surveys of 1954 cross bred pigs aged 6-30 months in the endemic Middle Belt zone.

2.1.3 **Aetiologic agents of sleeping sickness (SS)**

Sleeping sickness or human African trypanosomosis (HAT) occurr within the area of distribution of the tsetse fly and is caused by protozoa in the family Trypanosomatidae genus *Trypanosoma*, subgenus *Trypanozoon*. The parasites responsible for causing sleeping sickness belong to a group of closely related trypanosomes in the *Trypanosoma brucei* species complex, namely *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. These two sub species are morphologically indistinguishable from *T. b. brucei*. While *T. b. gambiense* causes a chronic West and Central African sleeping sickness (Gambian SS), *T. b. rhodesiense* causes an acute East African sleeping sickness (Rhodesian SS).

2.1.3.1 **Sleeping sickness**

Unlike AAT, sleeping sickness is typically restricted to historic foci. The earliest recorded major epidemics of sleeping sickness took place in Uganda and Congo between 1896 and 1908 where roughly 500,000 people were estimated to have died in the Congo Basin and approximately 300 000 died in Busoga, Uganda (McKelvey, 1973). With the rift valley transecting the country, Uganda was presumed to be in the precarious position of having foci of both forms of the diseases, which had resulted in two other major epidemics - one in the late 1940's and the other in 1980 (McKelvey, 1973). Throughout West Africa, smaller epidemics of sleeping sickness rapidly spread

from Senegal to Cameroon during the 1920's and then to Nigeria in 1930 to 1960 with infection rate of 14% which declined by the late 1940's and 1968 with infection rate of below 2% due to active surveillance and control (McLetchie, 1948; Duggan, 1962; McKelvey, 1973).

However, when the tsetse population increased and human contact with the flies became frequent, as was presently the case in many African countries, sleeping sickness surged out of its historic foci and did spread through the rural population (WHO, 2000). According to WHO (2000), the disability adjusted life years (DALYs) lost due to this disease were 2.0 million. Estimates also indicated that over 60 million people living in some 250 foci were at risk of contracting the disease, and there were about 300 000 new cases each year (Lancien, 1991; WHO, 1998). However, out of 4 million people under surveillance only about 40 000 were diagnosed and treated, due to difficulty of diagnosis and remoteness of some affected areas (WHO, 1998). Though, these figures were relatively small compared to other tropical diseases, sleeping sickness, without intervention, had the propensity to develop into epidemics, making it a major public health problem (WHO, 2000).

In Nigeria, though sleeping sickness was of low endemicity, the disease had re emerged in the old endemic foci in Gboko (Benue State) and Takum (Taraba State) with new cases being reported in what could be regarded as the new endemic foci in Ethiope East of Delta State, south south Nigeria, an area previuosly known to be sleeping sickness free (Edeghere *et al.*, 1989). In this area, sleeping sickness surveillance carried out by NITOR with support from WHO established a parasitological positive prevalence of 5% and seropositive prevalence of 14.9% from 4966 people who consented to be screened (Elhassan *et al.*, 1993).

Between February 1999 and January 2000, NITOR and the Eku Baptist hospital management treated 27 confirmed voluntary cases under a special

arrangement. Most of the cases were from Ethiope East LGA while others came from adjacent LGAs and nearby States like Edo, Ondo, Ekiti and Lagos (Halid *et al*., 2001). Also in 2000, a case of advanced sleeping sickness in a child from an area in Ethiope East was referred to NITOR from Eku hospital for management but the child later died (Enwezor and Ukah, 2000).

In other African countries, sleeping sickness had reached epidemic proportions, especially in Angola, the Democratic Republic of Congo, Uganda and Sudan. Furthermore, its prevalence was high and increasing in Cameroon, Congo, Cote d' Ivoire, Central African Republic, Guinea, Mozambique, Tanzania and Chad (Black *et al*. 2001; WHO, 2002). The upsurge in sleeping sickness in these countries was probably linked to a breakdown of specialized mobile teams and health facilities as a consequence of war and strife, or changes in health policy (WHO, 2002). These had dismantled the infrastructure of disease surveillance and vector control in many regions of sub-Saharan Africa and resulted in an increase in the exposure of people to infected tsetse flies. Reversal of the current sleeping sickness epidemic would be facilitated by a return of civil order, the widespread application of tsetse traps and odour-baited targets and rehabilitation of disease surveillance and treatment programs (Lancien, 1991; Vale, 1993; WHO, 1998). Therefore, it is important to note that the World Health Organization had organized and supported an international control program to improve sleeping sickness diagnosis and treatment as well as to improve the local disease control infrastructure (Black *et al*., 2001; WHO, 2002). This opportunity could be exploited to control the rising trend in Nigeria

in order to forestall a possible future outbreak of sleeping sickness.

2.2 **The Vector**

2.2.1 **Tsetse fly**

Tsetse fly (genus *Glossina*, family *Glossinidae*), the insect vector of African trypanosomes, infests approximately 10 million km^2 in 37 sub-Saharan countries corresponding approximately to one-third of Africa's total land area and threaten an estimated 50 million people and 48 million cattle (FAO, 2002a). Tsetse flies are largely classified into three subgenera based on morphological differences in the structure of the genitalia: *Morsitans (Glossina), Palpalis (Nomonina)* and *Fusca* (*Austenina*). Tsetse populations are denser in west and central Africa and are found more sporadically to the east and down to the boarders of the Kalahari and Namibian deserts in southern Africa (Jahnke, 1982; Knusden and Sloof, 1992;). Although tsetse fly habitats may vary considerably, climate and altitude (through their direct effects on vegetation, rainfall and temperature) are still the primary determinants for tsetse proliferation. Unlike other insects, there are no seasonal interruptions in the life cycles of tsetse flies. However, both adult longevity and puparial duration are related to temperature and a significant seasonal decline in tsetse populations is normal during the dry season (Jordan, 1974; 1986; Jordan and Langley, 1991).

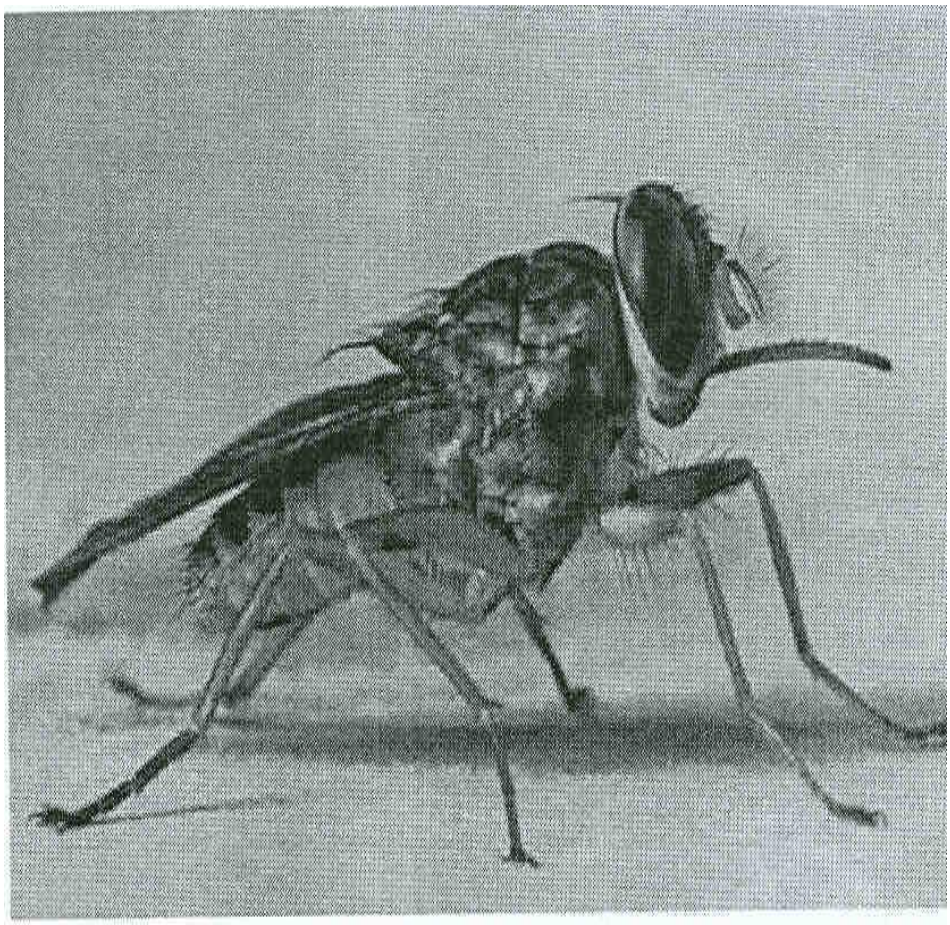

Plate 2. A Tsetse fly of the genus *Glossina*

(Adapted from WHO 2000 website)

2.2.2 **The morsitans group (savanna)**

The species that fall into the morsitans group are listed in Appendix 1. All are potential vectors of both human and animal trypanosomosis. The three *Glossina morsitans* subspecies are exceptionally good vectors of trypanosomes. All species within this group inhabit the savanna woodlands that surround the two major blocks of lowland rain forests in Africa. The distributions of tsetse fly in this group closely follow the distributions of wild animals and water sources. In the wetter areas the flies are observed to roam more widely over the woodland, but in drier areas their movements are restricted to mesophytic vegetation or the watercourses (Glasgow, 1963; Davies, 1977; Jordan, 1986).

2.2.3 **The *palpalis* group (Riverine)**

Eight species in the *palpalis* subgenus are known and of these, five *palpalis* and *fuscipes* subspecies are vectors of both human and animal trypanosomosis. Although flies in this group are continuously found in the lowland rainforests, some are known to extend out to the savanna regions particularly along rivers and streams. The habitat of the *palpalis* flies occurs mainly in the drainage systems leading to the Atlantic or the Mediterranean ocean, extending from the wet mangrove and rain forests along the coastal regions of West Africa to the drier savanna areas, north of the rain forest. The flies in this group were less tolerant to the wide range of climatic conditions of the savanna belt and were therefore restricted to the eco climate of the watercourses from where they

derived their label as the riverine species (Glasgow, 1963; Davies, 1977).

2.2.4 **The *fusca* group (forest)**

In West Africa, the *Fusca* subgenus (Appendix 1) particularly *G. fusca* and *G. mediocorum* are efficient vectors of trypanosomes of livestock. But none was a vector of sleeping sickness (Jordan, 1986; 1993). Distributions of the *fusca* group of tsetse depended primarily on forest vegetation and climatic factors. With the exception of *G. longipennis*, most *fusca* group species inhabited moist, evergreen habitats either in riverine forests within savannas (*G. mediocorum*) or in dense and wet rain forests (*G. tabaniformis* and *G. nigrofusca*). In stark contrast to the rest, the *G. longipennis* species lives in one of the driest habitats of tsetse flies. Due to its pupal adaptation to dry conditions, its primary habitat (consisting of dry deciduous acacia bush) was discontinuously spread throughout East Africa (Glasgow, 1963).

2.2.5 **Tsetse distribution**

In Nigeria, tsetse flies were distributed widely from latitude 4^{o}N to 13^{o}N, an area covering all the six ecological zones and infested 75% of the nation's landmass (Davies 1977, F.M.A., 1981). Eleven of the 22 species of *Glossina* that existed had been identified in Nigeria (Davies, 1977). Four (4) of these species are important as transmitters of trypanosomosis in Nigeria, namely, *Glossina morsitans submorsitans*, *G. longipalpis*, *G. palpalis palpalis* and *G. tachinoides*. Field survey results on the status of tsetse revealed a considerable decline of *G. m. submorsitans* and *G. longipalpis* from most of their defined belts/habitats in the northwest and central agro ecological zones (Onyiah, 1997, Madubunyi,

1990; Omoogun and Akinboade, 2000). On the other hand, an expansion of the *G. palpalis palpalis* species into the highlands of Jos Plateau, an area previously regarded as tsetse and trypanosomosis free had been reported (Onyiah, 1997).

The reason adduced for the decline was an increase in human activities, which led to accelerated clearance of some tsetse habitats for agricultural activities, road and housing estate development, especially the savanna woodland in the sub-humid zone (Jahnke, 1982). The reasons for the extension of *G. palpalis* species were (i) Jos Plateau being contiguous with the known tsetse infested States of Kaduna, Nassarawa and Bauchi, (ii) cattle routes that traversed the Plateau and (iii) changes in climatic conditions, e.g. an increase in environmental temperature which provided a conducive atmosphere for the establishment of the flies (Onyiah, 1997).

Another field observation reported the re-infestation of tsetse in the northeast tsetse corridor of Adamawa, Borno and Taraba States, areas previously reclaimed from tsetse through the use of insecticides (Onyiah, 1997). In Ethiope East Local Government Area of Delta State, *G. palpalis* was the vector responsible for sleeping sickness transmission (Omoogun *et al*., 1995). What is obvious from these reports is the rising trend of tsetse flies in the country. This portends a greater risk of trypanosomosis to man and animals in tsetse-infested areas, as they are likely to be exposed to constant tsetse challenge.

2.2.6 **Biting flies**

Biting flies other than the tsetse, through the transfer of blood from one animal to another, also mechanically transmit AAT. There is no mechanical vector for sleeping sickness transmission. The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota*, *Liperosia*, *Stomoxys* and *Chrysops* flies have also been implicated (Ikede, 1989). These flies inhabit mostly the sub-humid zone of Africa and are ubiquitous during the rainy season while their numbers, which are smaller during the dry season, are restricted to river and stream valleys and cattle, camels' and horses' sheds (Karib, 1961; Rahman *et al*., 1990; Said *et al*., 2001).

The trypanosome species most commonly transmitted mechanically are *T. evansi* and *T. vivax* (Rahman *et al*., 1990). Mechanical transmission of *T. congolense* and *T. b. brucei* by *tabanids*, *stomoxys* and *hippoboscids* has been occasionally reported in cattle and sheep (Rahman *et al*., 1990).

2.3 **Susceptibility factors**

Factors influencing trypanosome infection rates in tsetse can be classified into endogenous, ecological, parasite and host factors (Molyneux and Ashford, 1983; Jordan, 1986; 1993). Known vectoral capacity vary within species, for instance, *G. morsitans* has an infection rate of about 20% and *G. palpalis* about 5% (Davies, 1977; Jordan 1986).

2.3.1 **Endogenous factors**

Endogenous factors are those that are associated with the vector itself and include tsetse species, sex, age at infective feed, age structure of tsetse

population, genetic differences (variations within species), behaviour (host preferences) and concurrent infections (virus, bacteria, fungi), interactions between lectins and rickettsia-like organisms, physiological and biochemical state (Molyneux, 1980; Jordan, 1986; Maudlin, 1990). The intrinsic vectoral capacity of a tsetse fly refers to the intrinsic capability of a fly to develop a metacyclic infection (Le Ray, 1989).

With regard to sex, female tsetse flies usually have higher infection rates than males partially because females live longer than males and therefore have a higher probability of infection (Jordan, 1974). However, it had not yet been determined whether the sex of a fly influenced the infection rate (Kazadi, 1991; Minok, 1992). Within species it was found that infection rates varied greatly depending on individual host factors. It was also shown that concurrent infections, for instance, the presence of intracellular rickettsia-like organisms (RLOs) influenced positively the susceptibility of tsetse flies to trypanosome infection. Tsetse flies carrying RLOs in the midgut were found to be six times more likely to be infected with trypanosomes than those without (Maudlin, 1990). It appeared that trypanosomes ate RLOs. Furthermore, it was observed that within any given species, individuals and sexes of the same species responded differently to infection suggesting some involvement of the genetic factors (Jordan, 1993).

Tsetse flies use visual and olfactory characteristics to recognise potential hosts before initiating host-oriented responses. After landing on the host, heat stimulation cause a probing and feeding response. It was found that feeding activity for the *morsitans* group

tsetse was highest during the early morning and the late afternoon due to a combination of external temperatures (20%) and circadian rhythm (80%), (Kernaghan, 1962; Brady and Crump, 1978). It is thought that tsetse flies originally fed on reptiles living in forests and later became adapted to feeding on mammals. Adaptations to feeding on warthogs were thought to be one of the pathways by which tsetse flies entered the savanna ecosytems, subsequently evolving as the separate morsitans subgenus (Kershaw, 1970; Ford, 1971). Most of the knowledge about species-specific host preferences was derived from blood meal analyses of captured flies (Moloo, 1985)

Factors of the parasite such as numbers available to tsetse, parasite species and its infectivity to tsetse, subspecies/strain, host factors (susceptibility, immune state of host, behaviour and attractiveness to tsetse), ecological /environment (climatic factors) all influenced trypanosome infection rates in tsetse (Moloo, 1985).

2.3.2 **Species of hosts**

Studies have shown that a host may possess natural resistance to a particular species of trypanosome. For instance, pigs and laboratory models were shown to be refractory to infection with *T. vivax* whereas this parasite was most important in infection of cattle, sheep and goats, with reported losses (Stephen, 1970; Hommel, 1991). So also chronic infection with *T. congolense* in domestic dogs resulted often in a carrier state but caused serious economic losses in cattle, sheep and goats (Stephen, 1970). However, *T. b. brucei* produced a fulminant and fatal infection in these two species of hosts. People and some old-world primates are resistant to infection with the trypanosome species that caused AAT, i.e. *T. b. brucei*, *T. vivax* and *T. congolense* (Songa *et al*., 1991; Black *et al*., 2001).

Furthermore, several sub-Saharan mammals including Cape buffalo (*Syncerus caffer*) had been selected through evolution to be highly resistant to

trypanosomosis (Mulla and Rickman, 1988; Reduth *et al*., 1994). For example, Cape buffalo that was bred in captivity from trypanosomosis-free parents showed no signs of disease upon infection with *T. b*. *brucei* (Reduth *et al*., 1994); *T. vivax* (Dwinger *et al*., 1986), *T. congolense* (Grootenhius *et al*., 1990; Olubayo *et al*., 1990) or their blood mixtures that augumented serum trypanocidal activity (Wang *et al*., 1999).

2.3.3 **Breed**

Within a host species, wide ranges of susceptibility to a particular disease are often observed between different breeds and this is true for trypanosomosis. In Africa, for example, certain breeds of cattle, horses, sheep and goats, especially the West African dwarf, were more tolerant of trypanosomosis than others. Field and experimental studies had shown that the pathogenic effects of AAT were more severe in the Zebu cattle breeds (*Bos indicus*) than in the hump-less West African dwarf (*Bos taurus*) cattle breeds (Mattioli and Wilson, 1996; Dolan, 1998; Murray *et al*., 1990; Paling and Dwinger, 1992). Similar observations were reported for sheep and goats (FAO, 1987). This phenomenon of innate resistance to trypanosomosis was termed trypanotolerance. Trypanotolerant cattle could survive in areas of low tsetse and trypanosome challenge without chemotherapy but through self- cure (Bradley *et al*., 1996).

The kinetics and specificity of anti-trypanosome immunity in N'Dama indicated that it was mediated predominantly by anti-variant specific genes (VSG)

antibodies and not by antibodies directed against invariant antigens (Black *et al*., 2001). Trypanosome-infected susceptible hosts typically did not produce trypanodestructive antibodies other than those that were VSG specific (Black *et al*., 2001). This was collaborated by the results of several studies (Akol and Murray, 1983; Wellde *et al*., 1989; Paling *et al*., 1991 a, b; Dwinger *et al*., 1992) which showed that immunity in cattle that had undergone self-cure after trypanosome infection was directed only against the infecting parasites and their progeny. The immuned animals remained susceptible to infection with trypanosomes that differed in VSG from the priming organisms.

Studies had also shown that during the early stages of trypanosome infection, levels of parasitaemia were similar in N'Dama and Boran (Zebu) cattle but the trypanotolerant N'Dama cattle developed less severe anaemia than the more susceptible Boran cattle (Authie *et al*., 1993 a, b). The extent to which this reflects differences in immune responses is unclear. The infected N'Dama developed higher titre IgG1 responses against trypanosome antigens than their Boran counterparts (Authie *et al*., 1993 a, b; Taylor *et al*., 1996), including a response against the *T. congolense* cysteine protease, *congopain* and as suggested, it was possible that antibodies against these non-VSG components affect trypanosome-induced pathology (Authie, 1994).

The ability of N'Dama cattle to sustain health and develop serodeme-specific immunity under low challenge with *T. b. brucei*, *T. congolense* and *T. vivax* attested to the husbandry skills of the African pastoralists who had managed

indigenous herds of cattle whether they were domesticated in Africa or later introduced from other regions (Bradley *et al*., 1996; Hanotte *et al*., 2000). Because resistance was developed by trypanosomes against all the currently available drugs, cattle with reduced susceptibility to trypanosomosis and a lower requirement for drug therapy or prophylaxis may have a major contribution to make towards future sustainable livestock production in the tsetse infested areas of Africa (Dolan, 1998; Black *et al*., 2001).

Attempts had been made to cross, or engineer into improved cattle phenotypes of N'Dama (Dolan, 1998), but this became a difficult task. The mechanisms underlying the sustained health of the trypanotolerant cattle under low to moderate challenge with tsetse-transmitted trypanosomes are complex and unresolved (Kamanga-Sollo *et al*., 1991; Williams *et al*., 1996; Taylor, 1998), may differ among breeds of trypanotolerant cattle (N'Dama, Muturu and Keteku) and may involve several loci. Indeed studies had been carried out at the International Livestock Research Institute (ILRI) to map the genetic basis of trypanotolerant phenotype of N'Dama cattle through segregation analysis (Black *et al*., 2001). However, relevant loci had not yet been identified for cattle and limitations imposed by numbers of progeny may preclude fine mapping of the trait. Similar studies in mice (Kemp *et al*., 1997; Iraqi *et al*., 2000) suggested the involvement of a chromosome region of large effect, possibly comprising more than 1 resistant locus, on chromosome 17, and of further loci on chromosomes 1 and 5 as reviewed by Black *et al*. (2001) and Enwezor and Lawal (2003).

2.3.4 **Age**

It had been suggested at various times that calves were more resistant to trypanosomosis than adults (Wellde *et al*., 1989). These authors reported that the general trend of the chronic disease in calves was towards recovery when they infected calves of less than one year with strains of *T. congolense* at the same time as they infected adult cattle that showed a trend towards death. The reasons for this apparent resistance in calves are not completely known. But a study of tsetse across different seasons and hosts showed that feeding success varied according to host age (adult =7%; calf = 3%) and was negatively correlated with the frequency of host defensive behaviour and the relative abundance of non-biting Diptera (Schofield and Terr, 2002).

2.3.5 **Sex**

The effect of sex on the clinical signs of trypanosomosis are linked to sexual attributes, as in cases of disorders of the reproductive tract rather than with the fact that males may be more susceptible than females. There have been reports of reproductive disorders resulting in abortions and stillbirths in female animals (Elhassan, 1987; Ikede *et al*., 1988; Okech *et al*., 1996) and in male animals, loss of libido including cessation of semen production were reported (Isoun and Anosa, 1974, Ogwu and Njoku, 1987, Sekoni, 1994).

Furthermore, the stress of pregnancy in the cow and of agricultural work in the

bull can result in recrudescence and exacerbation of infections (Edeghere *et al*., 1992). Sometimes, too, one particular sex may be regarded by farmers as being of greater value than the other and will therefore receive a correspondingly greater amount of care and attention when sick (Putt *et al*., 1988).

The nutritional status of the host also influences the degree of severity of disease. For instance, it has been established that cattle on a supplemented diet showed remarkable rates of growth and significant reduction in severity of trypanosomosis compared with ones not fed supplemented diet (Murray, 1989).

2.3.6 **Environment**

Extrinsic determinants of disease in an environment can affect the vector involved in the transmission of a disease and thus determine the type and extent of the disease transmission-taking place (Putt *et al*., 1988). Three major extrinsic factors, namely, climate, soils and man influence tsetse distribution and the influences differ across savannah, riverine and forest tsetse groups (Reid *et al*., 2000).

2.3.6.1. **Effects of climate change**

A distinction is normally made between the macroclimate or weatherand the microclimate. The term microclimate refers to the actual climatic conditions prevailing in the specific restricted environment where the host, agent or vector or intermediate host actually live (Putt *et al*., 1988). Global climate change has

been widely studied in recent years. For example, the Intergovernmental Panel on Climate Change (IPCC) predicted that climate change is likely to influence agriculture generally and livestock production specifically in sub-Saharan Africa (McDermontt *et al*., 2001a). Using length of growing period (LGP), which is function of rainfall and temperature and represents the numbers of days per year with sufficient moisture for crop growth, McDermontt and co-workers estimated current LGP for 10-arc-minute grid squares based on a daily water balance model in 2000. They also went forward to obtain likely daily water balance and LGP changes in between 2000 and 2050 from the worldwide web (http://ipcc-dac.cru.uea.ac.uk\). The study concluded based on LGP, that tsetse distributions and numbers are expected to contract markedly in semi-arid and sub-humid West Africa, though they observed that LGP is a relatively better predictor of tsetse presence for forest and riverine flies than for savannah flies (McDermott *et al*., 2001b). The result appeared to be similar to previous observations which reported that the population dynamics of the riverine tsetse seemed to be principally related to climatic and breeding site factors, such as atmospheric temperature, humidity, volume of rainfall and the moisture content of the development soils for the larvae and pupae (Omoogun *et al*., 1991).

Tsetse flies seem to do best at temperatures near to 25^{o}C. Departures from this temperature produce deleterious effects, culminating in rapid death (Jordan, 1974). In nature, temperature changes are accompanied by changes in atmospheric humidity, in wind and in light and are referred to as season (Glasgow, 1963). In areas with prolonged rainless season, referred to as

savanna, inhabited by various members of the morsitans group, water holes dry up and some mammals, but not all, are restricted to the vicinity of permanent water (Glasgow, 1963). Blood sucking flies of the *Tabanidae* and *Culicidae* whose breeding site is associated with water become scarce but *Hippoboscidae* which breed in same way as tsetse are not affected (Jordan, 1974).

Studies have shown that if seasonal effects or weather effects are known, it would be possible to predict when host populations are at a particular risk of contracting disease and thereby implement appropriate control measures at strategic times (Putt *et al*., 1988). This approach has been used with success in the control of helminthiasis, ticks and tick-borne diseases, trypanosomosis, foot and other nutritional deficiencies (Putt *et al*., 1988).

2.3.6.2 **Soils**

Soils through interaction with climate determine vegetation and the environment in which the livestock are kept. The main effect of vegetation is on nutrition. While macroclimates can have a direct effect on microclimates, the study of macroclimates alone can frequently be misleading in achieving an understanding of the epidemiology of a disease. For instance, regions where existing macroclimatic conditions might be thought unsuitable for the transmission of a disease may contain limited areas where the microclimatic conditions are suitable for the survival of the disease agent and its vector or intermediate host (Putt *et al*. 1988).

Studies have indicated that a water hole or an irrigated pasture in an arid environment may provide enhanced conditions for disease transmission, since they prove attractive to livestock particularly those times of the year when the macroclimate is at its most severe (Jordan, 1986). For instance, in arid areas, the transmission of trypanosomosis may in fact take place during the dry season when the hosts, the agent and the tsetse- vector are all concentrated around permanent water sources (Jordan, 1974; Jordan, 1986). The distribution and classification of tsetse flies in the country is according to the vegetation zones in which they are found. The availability of suitable habitats and host also determine primarily their distribution and abundance (Omoogun *et al.*, 1991).

2.3.6.3 **Man**

Man, by altering the environment through the creation of favourable artificial microclimates for livestock rearing, such as housing, water supplies, irrigation, etc, can alter the determinants of diseases present in that environment (Putt *et al.*, 1988). The impact of sundry human activities on the natural ecosystems of tsetse has, in the main, two consequences to the fly. Firstly to effect alterations which are inimical to its continued survival, by depriving it of its food supply through depletion of the game population and through the progressive transformation of its habitat into pasture and farm land. Secondly to force adaptive changes to unusual habitats and sources of food, in the face of extinction (Turner, 1980).

The distribution of tsetse, particularly that of the savannah species, *G.*

morsitans Westwood and *G. fusca* has been greatly altered by increase in human activities (Omoogun and Akinboade, 2000). For example, selective destruction of game animals, bush clearance and large scale ground aerial glocinicide sprayings led to the disappearance of *G. fusca* and to the collapse of some of the morsitans belts (Born *et al*., 1986; Jordan, 1986; Omoogun and Akinboade, 2000), although, re-invasion due to inability to quickly put into use through human settlement of some of the cleared areas by tsetse has been reported (Jordan, 1986).

Furthermore, instances of the adaptability of tsetse species are becoming more and more familiar. Two examples from West and East Africa have been well documented. In southern Nigeria, Baldry (1964, 1968, 1969) studied, over a number of years, the progressive adaptation of *G. tachinoides* Westwood away from its natural riverine environment, through semi-natural, semi- artificial habitats to completely artificial ones created by man (houses, pig sties and cattle enclosure) with concomitant changes in feeding habits from opportunistic to highly specialized feeding almost solely on pigs and domestic cattle. In Kenya, a similar process of peri-domestication has been observed in *G. fuscipes fuscipes* Newstead (Willet, 1965). This species spread out from its natural but diminishing riverine habitat to colonize man-made vegetation associations, particularly boma-hedges rings of the shrubs *Euphorbia tirucalli* and *Lantana* surrounding hamlets and kraals. These produced conditions of intimate man-fly contact, which resulted in an outbreak of sleeping sickness (Turner, 1981).

Agricultural practices have helped to create new tsetse habitats. Reports of colonization by tsetse of (*G. palpalis palpalis* R-D, *G. fusca* Walker and *G. fusciplerius* in West and Central Africa) commercial or cash crop plantations of various kinds, e.g. sugar cane plantation, orchard fruits, oil palm, rubber, teak, bananas, etc) and the effects this has on the sleeping sickness transmission among plantation workers are well documented (Finelle, 1980, Turner, 1981). Factors reported to have enhanced the colonization included: size of the plantation, its stage of growth, the extent and continuity of the canopy, the presence of un cleared undergrowth, proximity to existing natural habitats of tsetse and the availability of sources of food (e.g. orchard, such as citrus, mangoes, avocadoes, guavas, etc) (Baldry, 1980; Finelle, 1980, Turner, 1981).

2.4 **Reproductive disorders**

Reproductive disorders had been reported in animals infected with tsetse-transmitted African trypanosomes. In a review by Ikede *et al*. (1988), the disorders listed were irregular menstrual (or oestrus) cycle, infertility, abortion and impotence. Intra-uterine infections occasionally occurred and resulted in still birth or neonatal mortality. However, the changes were essentially reversible following treatment, though recovery took several months (Ikede *et al*., 1988)

Studies in *T. vivax* infected pregnant and lactating ewes showed an abortion rate was 15% with lamb mortality rate of up to 85%. Lambs born by infected

dams had lower weaning weights compared with lambs from control dams (Akinbamijo and Reynolds cited by FAO, 1996). Several workers including Isoun and Anosa, 1974; Ogwu *et al*., 1984; Ogwu and Njoku, 1987; Ikede *et al*., 1988; Elhassan *et al*. 1995; Okech *et al*., 1996 had also reported intra-uterine infections which resulted in still birth or neonatal mortality in *T. vivax* infections. In male animals, loss of libido including cessation of semen production were reported (Ikede and Akpavie, 1982; Akpavie *et al*., 1986; Sekoni, 1993; 1994) In addition, *T. vivax*-induced reproductive wastage had been documented. It was shown that during pregnancy and lactation, digestible organic matter intake (DOMI) was significantly lower ($P< 0.05$) in infected ewes compared to controls.

In respect of *T. b. brucei* infections on reproduction, similar observations were made and included abortion, neonatal death and death of infected dams irrespective of the trimester of gestation (Edeghere *et al*., 1992). The study suggested that the stress of pregnancy in the cow and of agricultural work in the bull could result in recrudescence and exacerbation of infections. Polyglandular endocrine failure had been reported in *T. b. brucei* infections of animals (Reinke *et al*., 1998) while Elhassan *et al*. (1994) recorded prolonged low levels of plasma progesterone in *T. vivax* infections. These disorders could devastate livestock industry through reduced livestock productivity.

2.5. **Antigenic variation**

Antigenic variation is defined as a process whereby some species of disease

agent seek to evade the host's defence mechanisms by altering their antigenic characteristics. An extreme case of antigenic variation occurs in trypanosomosis, where infection in the host usually took the form of a series of parasitaemias each one of which involved a form of trypanosome antigenically different from the preceeding one (Putt *et al*., 1988).

Studies had shown that trypanosome-infected mammals produced antibodies that reacted with the immunodominant surface coat antigens of African trypanosomes and caused immune clearance of targeted parasites (Black *et al*., 2001). The surface coat on each trypanosome is composed of about 10 million copies of a single species of variant surface glycoprotein (VSG). African trypanosomes expressed only one VSG at a time and evade immune control by switching off the expressed VSG gene and replacing it with one of their 1,000 or so genomic VSG genes, or their recombinants (Borst *et al*., 1998; Cross *et al*., 1998). This process was sometimes associated with a change in the active VSG gene expression site (Black *et al*., 2001). It could be with growth factor receptors as had been shown that genes that encoded the transferrin receptor were associated with the VSG gene expression site (Steverding *et al*., 1995).

Furthermore, it was reported that the rate of VSG gene-switching varied from around 10^3 to around 10^6 switches per trypanosome per generation depending on the test organism (Turner and Barry, 1989). Consequently parasitaemic waves were comprised of major and minor VSG types (Turner and Barry, 1989). It thus followed that dominant VSG types in each wave stimulated the

production of antibodies and were cleared but allowed growth-competent minority variants to seed the next wave, hence continuing the process (Black *et al*., 2001).

2.6 Immunosuppression

The word 'immunosuppression' means"suppression of the immune response leading to greater susceptibility of an animal to pathogens". It had been suspected that trypanosome infection precipitated unusually severe attacks from other diseases which if present alone ran comparatively mild courses (Reid *et al*., 1979). Trypanosomosis had a harmful effect on the immune (defensive) system of the infected animal. The usual mechanisms for resisting infection are unable to exert their full protective effect and what would normally be mild became acute. This phenomenon had been reported by several workers (Goodwin *et al*., 1972; Holmes *et al*., 1974; Rurangirwa *et al*., 1978; Whitelaw *et al*., 1979; Ilemobade *et al*., 1982; Anene *et al*., 1989; Enwezor and Ekejindu, 1998). According to these authors, immunosuppression lowered the host's resistance to other infections and resulted in secondary diseases that greatly complicated both the clinical and pathological features of trypanosomosis.

Also, vaccination against another infection was shown ineffective with concurrent trypanosome infection (Ilemobade *et a*l., 1982). Thus immunosuppression is an important factor that will be taken into account when considering the overall effects of an outbreak of trypanosomosis.

2.7 **Clinico-pathological features of trypanosomosis**

The clinico-pathological features of AAT is influenced by several factors including those attributed to by the host, the trypanosome species and serodeme of the particular trypanosome responsible for the infection (Masake *et al*., 2002). Because simultaneous infections with more than one trypanosome species are very common (Nyeko *et al*., 1990) and simultaneous infection with trypanosomes and other haemoparasites (*Babesia spp*; *Theileria spp*; *Anaplamosis spp* and *Erlichia spp*) frequently occur, it is difficult to conclude which clinical signs were attributable to each of the infecting parasite (Majiwa *et al*., 1994). Few adequately controlled studies have been made and thus a 'typically' clinical response to each trypanosome was difficult to extrapolate. What followed was a summation of the syndromes observed in field and experimental cases of trypanosomosis caused by each of the three African animal trypanosomes (FAO, 1979).

The cardinal clinical sign observed in AAT is anaemia. Within a week of infection with the haematic trypanosomes (*T. congolense* and *T. vivax*) there is usually a pronounced decrease in packed cell volume, hemoglobin, red blood cell and white blood cell levels and within two months these may drop to below 50% of their pre-infection values (Valli *et al*., 1978; Murray 1978; Aliyu *et al*., 1979; Anosa, 1980; Egbe-Nwiyi *et al*., 2003). Also invariably present are intermittent fever, oedema and loss of condition. Abortion may be seen and infertility of males and females may be sequela with death occurring during the acute phase of the disease (Masake and Nantulya, 1991; Masake *et al*., 2002). Although

infected animals undergo self-cure, a majority remain infected for months or even years exhibiting low levels of fluctuating parasitaemia. These serve as a source of infection for tsetse flies, thus creating a reservoir for the disease (Masake *et al*., 2002).

Anaemia, a red cell disorder occurr when the concentration of haemoglobin fell below what was normal for an individual, age, gender and environment, resulting in a reduction of oxygen carrying capacity of the blood (Cheesbrough, 2000). The anaemia in trypanosomosis could either occur intravascularly in the early acute phase or extravascularly in the sub acute and chronic phase of the disease (Esievo and Saror, 1991). The development of anaemia is multifactorial, for instance, erythrophagocytosis due to a cleavage of the erythrocyte surface sialic (neuraminic) acid by a trypanosome sialidase (Esievo, 1980; Esievo *et al*., 1982), haemolysis of erythrocytes either by haemolytic biologically active materials released by dying or dead trypanosomes (Tizard *et al*., 1978a) or immunological means (Amole *et al*., 1982; Vincendeau *et al*., 1996) have among others been advanced.

T. congolense was called a haematic trypanosome found only in the blood vessels of the animals it infects as it did not localize and multiply outside blood vessels. Infection with *T. congolense* may result in per-acute, acute or chronic disease in cattle, sheep, goats and camels. Pigs often developed a milder disease; chronic disease is common in dogs. The incubation period is followed by intermittent febrile episodes such as depression, lethargy, weakness and

anaemia (Losos and Ikede, 1972). Other signs include loss of condition, salivation, lacrimation and nasal discharge (Stephen, 1970; Murray, 1978). As the disease progresses, loss of condition and changes of hair from black to metallic brown are seen. The back is arched and the abdomen ‘’tucked up^. Accelerated pulse, jugular pulsation and breathing difficult. Anaemia is a prominent sign. Early in the infection, the organisms are readily demonstrable in blood smears but as the disease progresses to its acute and chronic forms, organisms are most demonstrable in lymph node smear (Murray, 1978; Murray and Dexter, 1988; Vale 1993).

T. vivax has a variable incubation period in the host and although it is considered to be less virulent for cattle than *T. congolense* in East Africa (Belot and Leroy, 1998), mortality rates of over 50% could occur. There is marked variation in the virulence of different strains of *T. vivax*, some causing haemorrhagic syndrome (Mwongela *et al*., 1981; Wellde *et al*., 1989; Magona *et al*., 1997) but it remained the most important cause of trypanosomosis of cattle, sheep and goats in West Africa (Luckins, 1992; Daniel *et al*., 1994, Anosa *et al*., 1995; Kalejaiye *et al*., 1995; Abenga *et al*., 2002, 2004). It causes mild diseases in horses and chronic disease in dogs. *T. vivax* was often difficult to find in blood smears and could also be demonstrated in lymph node smears (Luckins, 1992).

T. b. brucei has a relatively short incubation period and causes severe to fatal

infection in horses, camels, dogs and cats. It usually causes mild, chronic or sub clinical disease in cattle, sheep, goats and pigs. A febrile response occurs in the horse 4-14 days after infection, followed by recurrent febrile reactions. The organisms are not always easily perceived in blood smears and are best demonstrated in tissue smears or sections (e.g., lymph nodes). Infected animals die in a few weeks or several months, depending on the virulence of the strain of *T.b. brucei* (Luckins, 1992).

2.8 **Gross lesions**

No pathognomonic change is seen in AAT. Anaemia, oedema and serous atrophy of fat are commonly observed. Subcutaneous oedema is prominent and usually accompanied by ascites, hydropericardium and hydrothorax. The liver may be enlarged and oedema of lymph nodes is often seen in the acute disease but they may be reduced in size in the chronic disease (Leach, 1965; 1973). The spleen and lymph nodes may be swollen, normal, or atrophic. Necrosis of the kidneys and heart muscle and sub serous petechial haemorrhages commonly occurr. Gastro-enteritis is also common and focal polioencephalomalacia may be seen. A localized lesion (chancre) may be noted at the site of fly bite, especially in goats. The anaemic blood changes seen include anisocytosis, poikilocytosis, polychromasia and punctate basophilia. All, some, or none of the above may be seen (FAO, 1979; Murray, 1979).

The lesions caused by the trypanosomes in susceptible host species vary considerably, depending on the species and strain of trypanosome and the

species and breed of host animal affected. The haematic trypanosomes (*T. congolense* and *T. vivax*) cause severe injury to the host mainly by production of severe anaemia, which accompanied in the early stages of the disease by leucopoenia and thrombocytopaenia (Losos and Ikede, 1972). In the terminal stages of the disease caused by the haematic trypanosomes, focal polioencephalomalacia probably resulted from ischaemia due to massive accumulation of the parasites in the terminal capillaries of the brain. The lesions resulting from *T. b. brucei* (a tissue parasite) (Losos and Ikede, 1972) are remarkably different from those seen with the haematic trypanosomes. Anaemia an important lesion but much more dramatic were the inflammation, degeneration and necrosis resulting from cellular invasion of various organs. Marked proliferative changes reflecting immunologic response were observed in most body tissues (Losos and Ikede, 1972; Murray and Dexter, 1988).

2.9 **Diagnosis, treatment and control**

2.9.1 **Diagnosis**

Diagnosis of trypanosomosis is based on demonstrating either the parasite or parasite antibodies against the antigens of trypanosomes. In recent times, the use of polymerase chain reaction (PCR) had been suggested for accurate diagnosis of the disease as well as characterisation of the parasites to species and subspecies levels (Majiwa *et al*., 1994; Desquenes and Davila, 2002). For the direct demonstration of pathogenic African trypanosomes, the techniques that could be employed are as follows:

2.9.1.1 **Conventional field methods**

Blood sampling for diagnosis could be obtained from both the peripheral and deep blood vessels. However, it should be realised that less than 50% of

infected animals may be identified by examination of peripheral blood (Murray *et al.*, 1977). Peripheral blood is obtained by puncturing a small vein in the ear or tail. Deeper samples are taken from a larger vein, e.g. jugular vein by jugular venepuncture. It is important to sterilise instruments or to use disposable instruments between animals so that infection cannot be transmitted by residual blood.

2.9.1.2 **Wet blood films**

A small drop of blood is placed on to a clean glass slide and covered with a cover slip to spread as a monolayer of cells. This is examined by light microscopy (200 fields) to detect any motile trypanosomes.

2.9.1.3 **Stained thick smears**

A large drop of blood is placed on the centre of a microscope slide and spread with a toothpick or the corner of another slide so that an area of approximately 1.0 - 1.25 cm in diameter is covered. This is air dried for 1 hour or longer while protecting it from insects. This is followed by de-haemoglobinization by immersing in distilled water for 5- 10 minutes. Thereafter Giemsa staining is applied diluted in the normal proportion of 1:10 for 30 minutes. Differentiation in water for 20-60 seconds is preferred until the smear assumes a bluish pink colour and later dried by standing upright in a rack. Examination is carried out preferably using the 100x oil immersion.The advantage of the thick smear technique is that it concentrates the drop of blood into a small area and thus less time is required to detect the parasites. The disadvantage is that the trypanosomes may be damaged in the process and the method is therefore not

suited for species identification in case of mixed infections (Cheesbrough, 2000).

2.9.1.4 **Stained thin smears**

Blood sample collected in a vacutainer is well mixed by repeated inversion for 1 minute. A small drop of the blood from a micro haematocrit capillary tube is applied onto a clean slide approximately 20mm from one end. A spreader is placed on the slide at an angle of 30^{o} and drawn back to make contact with the blood. The blood is allowed to run to each end of the spreader and then spread along the slide in a fairly rapid but smooth motion. Then waving it in the air quickly dries the slide. (Data can be written on to the smear using an ordinary pencil). Fixing in methanol (methyl alcohol) for 1 minute is performed. Thereafter Giemsa staining is carried out by adding 20ml of concentrated Giemsa stain to 180 ml of M/15 pH 7.2 phosphate buffer. Preferably already diluted Giemsa staining solution can be used. The slides are then immersed in an upright position for 30 minutes. And differentiation in phosphate buffer pH 7.2 is performed until smears assume a bluish pink colour. Finally, slides are drained in upright position to dryness and examined under a microscope using oil immersion. These methods referred generally to as the Standard Trypanosome Detection Methods (STDM) are still widely used, though not very sensitive especially when parasitaemia is low (Molyneux, 1975).

2.9.1.5 **Concentration methods**

Concentration methods become necessary especially when parasitaemia is low

in which case it is difficult to demonstrate the parasites. The heamatocrit centrifugation method described by Woo (1970) and buffy coat dark-ground/phase contrast described by Murray *et al*. (1977) have been found rapid and reliable to a considerable extent for diagnosis of trypanosomosis (Waiswa and Katunguka-Rwakishaya, 2004).

2.9.1.6 **Serological tests**

Infection gives rise to specific antibody responses and a variety of methods to detect specific humoral antibodies to trypanosome antigens have been introduced. These have been partially validated but await large-scale evaluation and standardisation. The one that is widely used is ELISA techninique. The principle of this technique is that specific antibodies to trypanosomes can be detected by enzyme-linked anti-immunoglobulins using solid-phase polystyrene plates coated with soluble antigen. The enzyme may be peroxidase, alkaline phosphatase or any other suitable one. The enzyme conjugate binds to the antigen/antibody complex and then reacts with a suitable substrate to yield a characteristic colour change either of the substrate itself or an added indicator (the chromogen).

2.9.1.7 **Detection of trypanosomal DNA**

During the past few years, several research centres have been working on the development of PCR procedures for the detection of minute amounts of trypanosomal DNA sequences (Duvallet *et al*., 1998; Desquesnes and Davila, 2002; Njiru *et al*., 2004). The sensitivity of PCR has increased with the

advancement of technologies for sample preparation to reach a level of 1 trypanosome/ml of blood, which has brought to field samples sensitivity two to three times higher than microscopic observation of the buffy coat (Desquenes and Davila, 2002).

2.9.2 **Treatment and control (chemotherapy and Chemoprophylaxis)**

The use of drugs for the prevention and treatment of trypanosomosis has been important for many decades but the rapidity with which the trypanosomes had developed resistance to each drug introduced had tremendously complicated this approach to controlling the disease (Aliyu, 1990; Kinabo, 1990; Sinyangwe *et al*., 2004). In spite of this, some of the older chemoprophylactic drugs such as the quinapyramine derivatives; antrycide and antrycide prosalt are still being used and give effective protection against *T. b. brucei* infection in horses, camels and cattle for up to three months (Aliyu, 1990).

2.9.2.1 **Chemotherapy**

Chemotherapy means the treatment of disease by the use of chemicals and such chemicals are called drugs. Several drugs had been used with little success but the most widely used chemotherapeutic drug was diminazene aceturate (Berenil), which was effective against all three African animal trypanosomes (Aliyu, 1990; Kinabo, 1990).

2.9.2.2 **Chemoprophylaxis**

The drug pyrithidium bromide (Prothidium) was useful in the prophylaxis of *T.vivax* and *T. congolense* infections in cattle, sheep and goats and could give

protection for up to six months. The most widely used of the newer chemoprophylactic drugs (and also the least expensive) is isometamidium chloride (Ogunyemi and Ilemobade, 1989). This drug had been in use for over 20 years and sold under the trade names, Samorin, Trypamidium and M&B 4180A, was widely, reported as excellent for the prophylaxis of all three African animal trypanosomes and gave protection for 3-6 months. The isometamidium drugs were also excellent chemotherapeutic agents as were the quaternary ammonium trypanocides Antrycide, Ethidium and Prothidium (Ogunyemi and Ilemobade, 1989). However, the development of resistance had been reported in both East and west Africa. Homidium bromide had also been reported to be an effective chemoprophylactic drug, so also the newly introduced arsenical Cymelarsan that was reported to be an effective drug in the treatment of *T. b. brucei* infection (Waiswa and Katunguka-Rwakishaya, 2004). Although extensively used in trypanosomosis control, chemoprophylaxis was an expensive, time-consuming and thus unsatisfactory long-term solution to the problem of African animal trypanosomosis (AN-Henfrey *et al.*, 1992).

2.9.3 **Vector Control**

Past efforts placed on the eradication, either using ground spraying or the sequential aerosol application of insecticides for tsetse vector had only been successful in achieving objective within the project area. However, the actions had not produced sustainable result due to the inability to consolidate and protect in the longer term against reinvasion (FAO, 2002b). There is a need for reforms in technical approach and policy for achieving a sustainable tsetse

eradication result.

2.9.3.1 **Bush clearing**

Discriminative bush clearing, extensively used in early tsetse fly eradication campaigns had been locally useful because it eliminated the breeding sites of the tsetse. Despite the apparent success of these methods, it was widely accepted that bush clearing was unsuitable as a long term control measure due to expense and speed of reinvasion as well as the environmental damage it caused through soil erosion, decreased soil fertility and its adverse effects on water supplies (Jordan, 1986).

Game elimination and thus elimination of the main source of blood meals for tsetse was used in early eradication campaigns. This was an ineffective and wasteful procedure (Jordan, 1986).

2.9.3.2 **Sterile insect technique**

This is a non-insecticidal method of approach. It relies on the mating of wild females with laboratory-bred sterile male flies. Physiologically, female tsetse flies are only required to mate once to store sperm in its spermathecae in sufficient quantity such that fertilization can occur over its entire reproductive life. However, SIT was considered to be impractical for control of high density populations above 1000 males per sq mile due to the large number of sterilized males that would be required. For SIT to be effective, it has been estimated that 10% of the females in the population need to be inseminated and in order to achieve that, the number of sterile males released must contribute 80% of the male population (FAO, 2002b).

2.9.3.3 **Targets and traps**

Traps and targets are mechanical devices used to kill or weaken tsetse flies through

insecticides or various trapping methods. The traps and targets attracted tsetse flies by taking advantage of their primary host-seeking behaviours and visual and olfactory stimulation (Jordan and Langley, 1991; Vale, 1993). The development of potent attractants in the last 20 years as well as the production of second-generation synthetic pyrethroid insecticides made this form of control technique highly successful (Jordan and Langley, 1991; FAO, 2002b). There were many prototypes of traps and targets customized to attract as many tsetse flies as possible in different environments, examples were the NITSE traps used in Nigeria and NGU and NG2B traps that were used successfully in Burkina Faso and Kenya and the widely used biconical traps (Omoogun *et al*., 1994; Amsler *et al*., 1994).

2.9.3.4 The future of trypanosomosis control

Since no vaccine is currently available for African trypanosomosis, future control lies on integrated vector and disease control with participation of local community. Despite the multitude of control strategies, the impact of trypanosomosis control is yet to be felt due to lack of effective surveillance against the disease. However, with the coming on board of the Programme Against African Trypanosomiasis (PAAT) and the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), there is hope for effective control of the disease in the near future. PAAT combines the forces of Food and Agriculture Organisation, World Health Organisation, International Atomic Energy Agency and African Union/InterAfrican Bureau for Animal Resources to promote integrated trypanosomiasis control through coordinated international action. PATTEC, in its concept note highlights the ultimate objective as eradication of tsetse and trypanosomosis from Africa (FAO, 2002b). Thus pursuant to this objective, PATTEC would undertake the organisation and coordination of the campaign and mobilised the necessary human, financial and material resources as endorsed in its summit held in July 2001 in Lusaka, Zambia (FAO, 2002b). It was hoped that PAAT-PATTEC alignment together with the involvement and coordination of stakeholders in the affected African countries would tackle the tsetse and trypanosomosis problem in sub-Saharan Africa with the overall aim of reducing poverty and promote agricultural development in these regions.

Finally, research efforts being made in the development of 'anti-disease' vaccines for trypanosomosis through identification of parasite components that were involved in the induction of host cytokines, known to play an important role in the pathology of the disease should continue (de Baetselier, 1996).

CHAPTER 3

3.0 **Assessment of tsetse situation in the Kachia grazing reserve**

3.1 **Summary**

The current status of tsetse fly (*Glossina* spp) was assessed in the months of March, June and August 2004 in the Kachia Grazing Reserve (KGR), Kaduna State, Nigeria. Tsetse populations were sampled for ten days in the months of March and June and six days in August using traps (Biconical and NITSE). Traps were placed 100 metres apart along all riparian vegetation at the banks of rivers/streams and dams and places suspected of high animal-man-fly contact. Two tsetse species (*Glossina morsitans submorsitans* and *Glossina tachinoides*) were found in the course of the investigation. A total of 54 adult tsetse flies were caught comprising 12 *G. m. submorsitans*, and 42 *G. tachinoides*. Only one *G. tachinoides* upon examination was found infected with *T. vivax*, the rest were negative. In addition, a total of 324 other biting flies of veterinary importance were also caught. Though it was only one fly infected, since it was *T. vivax*, and with other biting flies, it became reasonable to suggest that trypanosomosis may pose a serious danger to animal health in the KGR.

3.2 **Introduction**

KGR which is drained by the Kaduna river system and several streams is a mixed farming system and livestock is an important economic activity with dairy farms in the reserve. Sometimes in the past, the KGR had reported severe

trypanosomosis that affected livestock and measures were taken to control the disease under the SLDP. Animals were treated with trypanocidal drugs and vector control by insecticidal pour-on approach was applied on the animals to reduce trypaosomosis incidence (NLPD, 1988).

Since 16 years of this control activity, no other control activity of this nature had been carried out. The KGR by its location lies within the Guinea Savanna (Fig. 1), which is considered to be one of high trypanosomosis risk. This study was therefore carried out to determine the present status of tsetse flies (*Glossina* spp) and trypanosomosis that they transmit.

3.3 **Objective**

To determine tsetse species, vector capacity and their apparent density in relation to the occurrence of trypanosomosis in the KGR.

3.4 **Materials and methods**

3.4.1 **Study area**

KGR lies in South East (SE) of Kaduna State, some 90 km from Kaduna and between latitudes 10^{o} 03' and 10^{o} 13' N and longitudes 7^{o} 55' and 8^{o} 06' E. The geographical size of the KGR is 33,411 hectares. The land lies approximately 700-790m above sea level and is drained by the Kaduna river system. The KGR is officially gazetted as contained in the Kaduna State of Nigeria Gazette No. 8, vol. 30 and in particular the KGR order of 1996

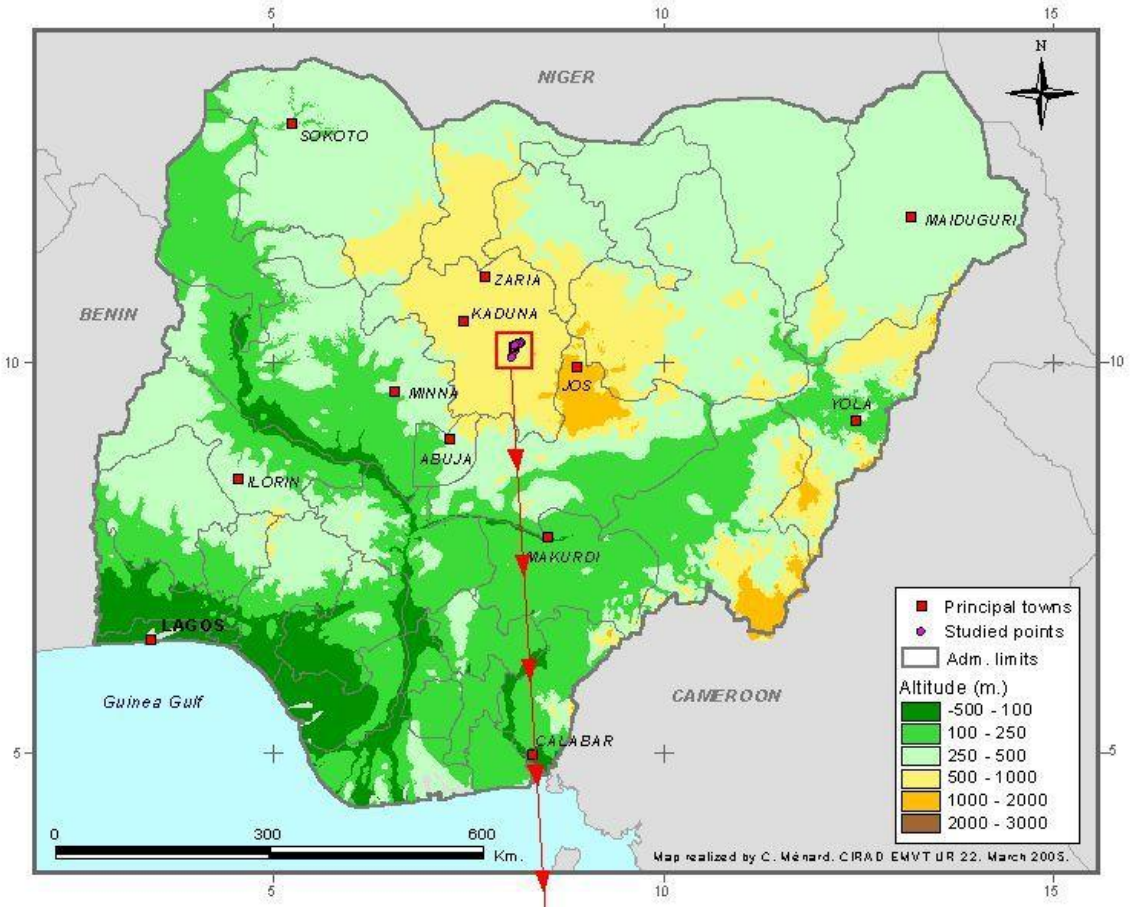

Fig. 3.1: Map of Nigeria showing the study area (Kachia grazing reserve) inset. (source: software arcview 3.2)

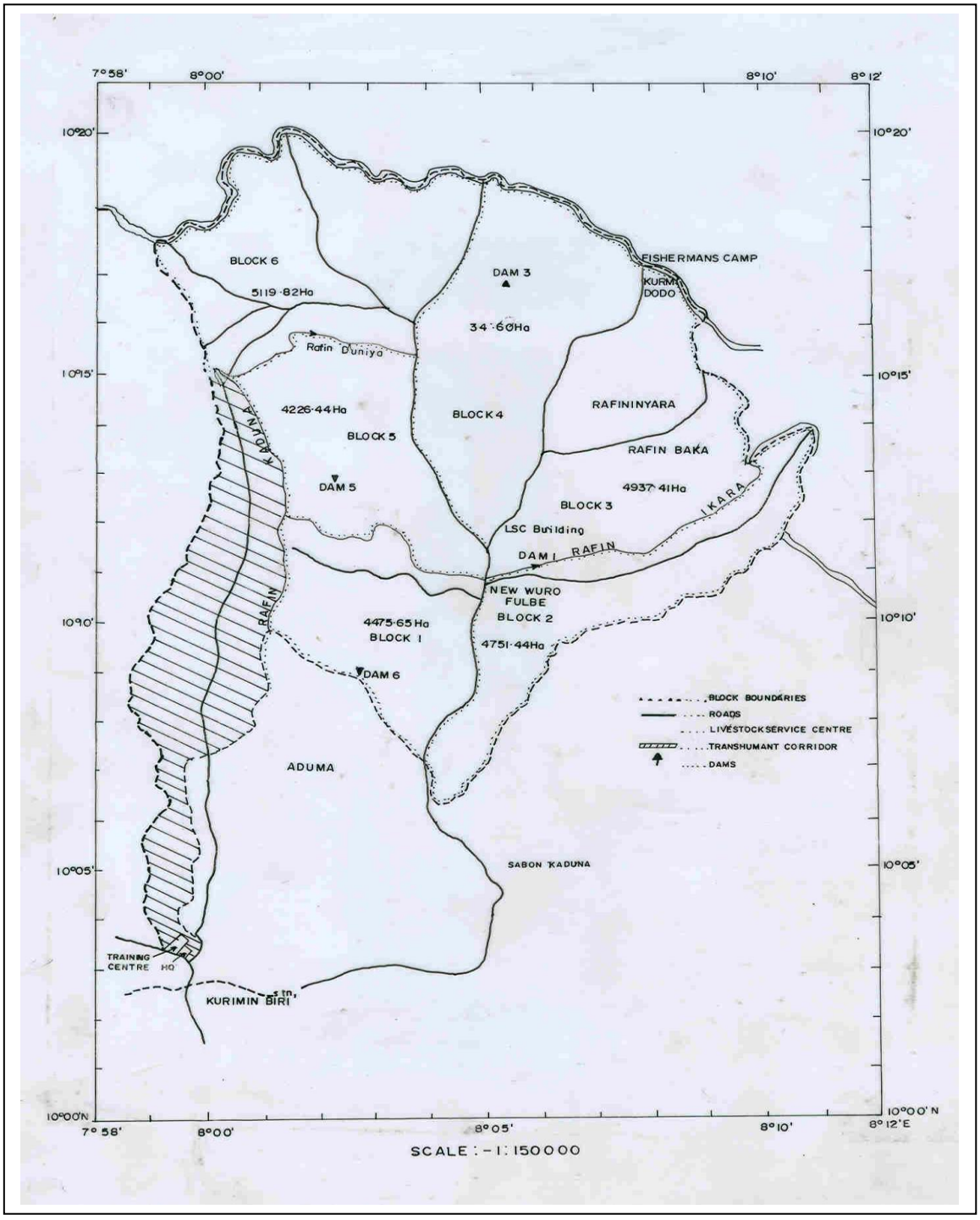
7°58'
8°00'
8°10'
8°12'
10°20'
10°15'
10°10'
10°05'
10°00'N
8°12'E
BLOCK 6
5119·82Ha
DAM 3
34·60Ha
FISHERMANS CAMP
KURMI DODO
Rafin Duniya
4226·44Ha
BLOCK 5
BLOCK 4
RAFININYARA
RAFIN BAKA
DAM 5
4937·41Ha
BLOCK 3
LSC Building
DAM I RAFIN
IKARA
KADUNA
RAFIN
NEW WURO FULBE
BLOCK 2
4751·44Ha
4475·65Ha
BLOCK I
DAM 6
BLOCK BOUNDARIES
ROADS
LIVESTOCKSERVICE CENTRE
TRANSHUMANT CORRIDOR
DAMS
ADUMA
SABON KADUNA
TRAINING CENTRE HQ
KURIMIN BIRI
SCALE :- 1:150000

Fig 3.2: Map showing the Kachia Grazing reserve and settlement in blocks, areas not to be settled and dams. (source: NLPD,1988).

Government No.4 KGR (source: Kaduna State Ministry of Agriculture (see Appendix 11). The annual rainfall is between 1200-1350mm, falling mainly between April and October. The annual temperature lies between 24^{o}C and 28^{o}C (NLPD, 1988). The vegetation is typical of the Northern Guinea Savanna Woodland (NLPD, 1988). The settlement pattern is divided into six (6) blocks of various sizes (Fig.2). The Fulani pastoralists are in Blocks 1- 5.

3.4.2 **Entomological surveys**

Entomological surveys were carried out using two different types of traps, viz biconical (Challier-Laveissiere, 1973) and NITSE (Omoogun *et al.*, 1994) and hand nets. The trapping sites were located along the streams and the dams within the KGR. In the late dry season in March 2004, traps were set along Shagol Wuro Nyako and Dalligi streams; Mayo Jamil/ Rafin Zabi; Mayo Burno and Dams 1, 2 and 6 which serve as watering points for the animals. Traps were also set in the same locations during the June survey and later in similar areas in the August survey of 2004. Survey exercises lasted ten (10) days in the months of March and June and six (6) days in August. The traps were set up 100 metres away from each other daily starting from 08.00 hours and were left in positions ranging from 24 hours to 48 hours before removal and mounting in other locations. The ambient temperature and relative humidity readings were

taken with the aid of whirling hygrometer to relate climate conditions during the period of trap settings and harvesting. Planted traps were inspected after every 12 hours and on collection of the catch; the flies were identified according to species by the method of Davies (1977) and the total numbers recorded.

For determination of apparent density which is defined as the number of flies that seem to be present as indicated by the number that can be caught, the working is as follows:

$$\text{Apparent density} = \frac{\text{No. of flies caught}}{\text{No. of traps set x No. of days of trapping.}}$$

Vector competence is the ability of a tsetse fly to acquire an infection and transmit it.

3.5 Results

3.5.1 Analysis of climate condition

The average monthly values for maximum and minimum temperatures and relative humidity during the months of March, June and August 2004 are presented. Mean temperatures ranged from 24°C to 30.7°C in March, 20.1°C to 24.8° C in June and 22°C to 32°C in August. Mean values for relative humidity were 26.4%, 53.4% and 69% for the above respective months.

3.5.2 Collected flies

G. m. submorsitans and *G. tachinoides* were the two species of tsetse caught. Both species were found distributed in the riverine flood plain. Tsetse apparent

density was low as shown in Table 3.1. Twelve (12) adult male (3) and female (9) *G. m. submorsitans* were caught in the month of March; 10 (83.3%) caught by biconical traps and two (16.7%) during random searches with a hand net.

In the June survey, a total of 8 adult male (5) and female (3) *G. tachinoides* were caught by biconical trap. However, no trypansome infection was detected from the examination of flies collected in March and June. In August 2004, a total of thirty-four (34) *G. tachinoides* were caught. Of this, only one fly was caught with a hand net near a dwelling place. Seventeen of the flies caught were by biconical and 16 by NITSE traps. Eleven of the flies caught died in transit and out of the 23 remaining flies, 3 were teneral. The rest 20 tsetse flies were dissected and examined for trypanosome infection rate. Dissections showed many of the flies to be at various stages of pregnancy with eggs, first and second instar larvae in utero. During the August survey, only one (1) (5.5%) was found positive with *T.vivax* massively invading the labrum and hypopharnyx indicative of a *Trypanosoma vivax* infection. The infection rate for the March, June and August surveys was 2.5%. Biting flies caught were *Tabanus*, *Stomoxys* and *Chrysops* totalling 324. In addition, a total of 405 *Musca domestica*, wasps classified as *hymenoptera* B, 28 and 7moth (butterfly) were caught during the surveys.

Table 3.1: Apparent density of tsetse captured in the KGR (March, June, August, 2004)

Block	No. of Traps			Total No. of tsetse caught			Days of Trapping			Apparent density		
	March	June	Aug	March	June	Aug	March	June	Aug	March	June	Aug
1	2	2	4	0	0	2	1	2	1	0	0	0.5
2	10	6	10	3	0	2	3	2	1	0.1	0	0.2
3	6	18	8	9	8	0	3	3	1	0.5	0.14	0
4	2	4	8	0	0	30	1	2	3	0	0	1.25
5	4^{+}	6	0	0	0	0	2	1	0	0	0	0

+ traps got burnt during bush burning in the KGR.

3.6 **Discussion**

This study has provided scientific evidence on the presence of tsetse flies in the KGR. The species encountered during the period of study were *G. m. submorsitans* and *G. tachinoides*. Both species were found distributed in the riverine flood plain. Though the apparent density is low between 0.1 and 1.25 (Table 3.1) but *G. tachinoides* was a good transmitter of human and animal trypanosomosis (Davies, 1977). The limited days of trapping due to logistics and the effect of human activities which may alter or destroy tsetse habitat could account for this. For instance felling of mahogany wood (*Khaya senegalensis*) for timber in the reserve was evident. Also evident was the burning of bushes and destruction of other non-economic important trees to pave way for farm activities. It is pertinent to note here that two of our traps were burnt in the month of March in the course of bush burning. These activities could reduce tsetse presence and probably contributed to the low apparent density recorded.

The prevalence of tsetse varied within the blocks, with tsetse caught in blocks 2 and 3 only in the month of March; though block 3 recorded a higher number of 9 flies as against 3 in block 2 in March. In June, flies were only caught in block 3 and during the August survey, Blocks 1, 2 and 4 had tsetse caught (Table 3.1). The majority of flies (30/34) (94%) caught by traps occurred in block 4 alone during the August survey. This finding was contrary to what was observed during the March and June surveys when by only blocks 2 and 3

showed evidence of the existence of flies. The reason may be dispersal of flies, streams dried up exposing their beds and flies would concentrate in areas around water bodies. In the month of August, the luxuriant vegetation which provided shades conducive for the flies could account for the high catches.

As to the transmission of the disease in the KGR, the finding of vector competence had clearly shown that cyclical transmission of trypanosomosis occurrred in the study area. The infection rate observed (2.5%), though low, still portends a great danger. This one infected fly may be important in relation to the biting flies, since *T. vivax* was the infecting trypanosome in the tsetse and it is the trypanosome mechanically transmitted.The majority of these biting flies were blood feeders and were considered to be of considerable economic importance due to their known and potential role as vectors of trypanosomosis and their nuisance value (Balfour, 1906; Rahman *et al*., 1990; Djiteye *et al*., 1998).

That a Fulani woman caught few of the tsetse flies, while some Fulani male members also caught some other biting flies with hand nets was interesting and noteworthy as was indicative of the willingness and readiness of the people to participate and accept control programme activities designed for the reserve to fight the problem of trypanosomosis and its cyclical and mechanical vectors in the area.

This study has shown the existence of tsetse flies, the transmitter of trypanosomosis, tabanids and other biting flies in the KGR.

CHAPTER 4

4.0 **Prevalence, risk factors and blood picture of bovine trypanosomosis in the KGR**

4.1 **Summary**

This study assessed the prevalence of trypanosomes in cattle at the KGR in March and June 2004 and in February 2005. A total of 1293 cattle blood samples were collected at random using a table of random numbers. The samples were analysed using the buffy coat technique and Giemsa thin blood films for parasite detection and identification. PCV, total WBC and Differential counts were determined. Also determined were the effects of cattle movement, herd pen location to watering and grazing point's distances (using the global positioning system (GPS)) and frequencies of disease control on prevalence. Overall, the detected prevalence of trypanosomosis was 8.4%. The prevalences in the months of March, June (2004) and February (2005) were 2.3%, 11.6% and 15.4%, respectively. Cattle movement appeared to influence infection rate, returned herds had a higher prevalence of 16.7% compared with 11% of resident herds but the results were not statistically significant ($P>0.05$). Increased prevalence was associated with proximity of herd pens to watering point's distances (x^2 for linear trend = 4.447, $P<0.05$), but no association of herd pens to grazing point distances (x^2 =2.186, $P>0.05$). Treatment of cattle was once every other year as informed by 96.3% of heads of households. Mean PCV of parasitaemic cattle dropped more than those of aparasitaemic and were 25.99 ± 1.82 and 29.31 ± 1.70, respectively. Total WBC and differential leukocyte counts showed leucopenia in 105 out of 228 (46.1%); 119 (52.2%) had normal WBC counts and only four animals had leucocytosis but were trypanosome-negative. Of the leucopaenic cattle, 25 (23.8%) were trypanosome-positive cases while 80 (76.2%) were negative for

trypanosomes. However, 11 (9.2%) of 119 cattle with normal WBC counts were trypanosome-positive. Furthermore, differential counts showed lymphocytopaenia and neutrophilia playing a role in the observed leucopaenia and leucocytosis, respectively. Among the leucopaenic animals, serology test carried out gave 90.9% and 76% anti-trypanosomal antibodies in trypanosome-positive and trypanosome-negative cattle respectively, suggesting strongly that trypanosomosis might be responsible for the lymphocytopaenia that induced the leucopaenia. Since susceptible Zebu cattle were the predominant breeds in the reserve and KGR lies in the morsitans belts, this study strongly advocates effective use of chemotherapeutic and chemoprophylaxis as well as vector control measures to fight the problem of trypanosomosis and allow permament raising of livestock in the reserve, thus encouraging sedentarisation.

4.2 **Introduction**

Tsetse-transmitted African animal trypanosomosis represented a serious problem in sub-Saharan Africa. The disease is a major constraint to livestock production and it is not practical to keep domestic animals in heavily tsetse-infested areas. Where they were kept under lower challenge, productivity was poor (Connor, 1989). Control of animal trypanosomosis therefore was accorded a priority in rural development programmes. In the KGR, livestock is an important economic activity with dairy farms in the reserve. Severe trypanosomosis that erupted in the reserve between 1985 and 1987 had significantly affected livestock production and productivity in the area (NLPD, 1988). To curb the disease, control measures that included treatments with trypanocidal drugs and insecticidal pour-on were used on livestock (NLPD, 1988).

Over 16 years of these control activities, documented reports on trypanosomosis status in the KGR appeared scanty or unavailable. To clarify the epidemiology of

trypanosomosis, surveys of cattle were conducted twice in 2004 and then once in 2005. Knowledge of trypanosome-infection status of cattle and risk posed by the disease to the cattle in the KGR will allow for targeted interventions against trypanosomosis. Indeed the more the targeted interventions, the greater the reduction in the risk of animal disease. Consequently an overall improvement in the livestock and human health of rural Pastoralists is envisaged. Furthermore, the control methods currently in use require a good knowledge of the epidemiology of the disease for a better effectiveness (Duvallet *et al.*, 1999). The epidemiology of animal trypanosomosis in the KGR was therefore carried out simultaneously with entomological survey, which lasted for nine days each for the months of March and June 2004, respectively, while in February 2005, prevalence study was conducted. Presented herein are the results of a longitudinal study that determined the extent and nature of bovine trypanosomosis in the KGR.

4.3 **Objective**

To determine the prevalence of trypanosomes, assess risk factors (whether movement, grazing patterns, frequency of disease control and herd location to pen and watering points are associated with prevalence of the disease) and blood pictures of the host during trypanosomosis.

4.4 Materials and methods

4.4.1 Study design

4.4.2 **Pre-survey visit**

The pre survey visits were made to the National Livestock Project Division of the Federal Livestock Department, the Kaduna State Ministry of Agriculture and

Rural Development, the Nomadic Education Unit, the Chairman, Kachia Local Government Area, the Project Officer in charge of KGR and the District head (Hakimi) of KGR in August 2003 and were intimated of the intents of the study, solicited for their support, cooperation and approval to conduct research activities in the KGR. Preliminary pieces of information were obtained about the KGR size, population size of both the human settlers and animals in the reserve, settlement pattern (in blocks) and infrastructure development services available. Subsequent visits were planned in mid January and February 2004. The two visits involved separate meetings with the Local Government Chairman, Kachia LGA and then with the Project Officer, the District head, the ward leaders (the Ardos), and council in the KGR. The purpose of these meetings was to sensitise and create awareness about the objectives of the study, establish rapport and obtain consent to conduct research and allow blood samples to be obtained from their cattle. Thereafter a date for commencement of the main survey was agreed on, at that meeting, to start in March 2004 (Plate 1, Appendix 5).

4.4.3 **Main survey**

This involved searching for tsetse and determining the prevalence of trypanosomes in the KGR during the dry and wet seasons. One field trip each was undertaken in the months of March and June 2004 and then later in February 2005. Ethical clearance to proceed on this study was obtained from the Kaduna State Ministry of Agriculture and Natural Resources and the pastoralists in the KGR for consenting to bleeding of their animals.

4.4.4 **Sampling frame**

The following sampling and data collection procedures were employed:

The number of settlers per block was determined using the KGR record and this gave a total of 604 human settlers and 12,300 cattle, 2038 sheep and 1008 goat population, as at August 2003.

It was initially proposed to stratify the blocks into two groups based on size of settlers (high and low density) and then to select two (2) blocks at random from each stratum. However, during the March 2004 field activities, many of the settlers had moved out of the blocks with their animals. This situation warranted sampling at random in blocks 1 to 5.

In each Block, a list of households with cattle herds was made and through a simple random process of balloting, 9, 11, 2 and 3 households were selected from blocks 2, 3, 4 and 5. In Block 1, only one household was around and was enlisted in the study. This gave a total of 26 households selected during the March field survey. In the month of June 2004, the same households were again sampled, though few of them had moved out of the reserve with their animals and new ones came in. Data collection on returned herds to assess the effect of movement of animals on the prevalence of trypanosome was done through sampling animals that returned from their transhumance pattern habits. Returned herds from Riyom LGA, Plateau State, Jagibdi and Kafanchan in Jema'a LGA, Kaduna State and Bauchi State were randomly sampled in June 2004. A similar exercise was carried out in February 2005. Cattle were aged between 1 and 12 years.

In each selected household, data on number of animals, number of cattle, herd composition of cattle, where cattle were quartered, where they grazed, watering points, movement of cattle (along the river, stream, watering point, etc) were obtained. Where an owner had two herds, only one that was selected at random was sampled.

A questionnaire was used to collect information on the grazing patterns and whether animals were allowed to graze freely or restricted. The same was done for trypanosomosis awareness by respondents, frequency of disease control, whether treatment was given regularly (at least yearly), sporadic (not every year) or never (Appendix 4). Distances from blocks to watering and grazing points were taken using a vehicle kilometre distance and ranged 1km to 3km and 1 km to 7 km, respectively. Animals reported sick by their owners and those apparently healthy were also sampled.

For each individual herd sampled, demographic data on each animal location, age, breed, physical condition (examined for the physical appearance of body condition (emaciation), mucous membrane of the eyes and mouth, by a Veterinarian were obtained on a one-page questionnaire filled for each animal to record the information and others

such as deaths that had occurred as a result of trypanosomosis (Appendices 4a & 4b). Within the herd, the cattle were drawn at random without replacement. Altogether, 1293 cattle made up of 529 in March and 536 in June 2004 and 228 in Feruary 2005 were sampled and their blood specimens collected.

The geographic positions of each dwelling and the watering point or points (two at most) were determined by global positioning system (GPS) (Garmin TM).

4.4.5 **Clinical examination**

Clinical examination under the supervision of a Veterinarian was carried out on all the animals sampled in the reserve. Samples were collected from blocks 1 to 5 as aforementioned. A map showing settlement pattern by blocks is shown in Fig. 2. The body conditions, the mucous membranes of the eyes and mouth, physical and mental alertness of the animals were recorded. In the months of March and June 2004 and February 2005, 1293 heads of cattle made up of 529, 536 and 228 for the months mentioned respectively were clinically examined.

4.4.6 **Parasite detection by microscopy**

Each animal examined was bled from the jugular vein using syringe and needle and 8 ml of blood was collected. 1ml was discharged into labeled EDTA vacutainer bottles while the remaining 7ml was retained in the syringe, labeled appropriately and used for serum harvesting. Samples collected were put in cold boxes to avoid deterioration and then examined in the location under a tree shade in the field. A microhaematocrit capillary tube was filled with about 70 µl of blood and centrifuged for 5 mins at 10,000 rev per min. Two microhaematocrit capillary tubes were filled for each blood specimen. The centrifuged blood was also used for packed cell volume estimation (PCV). The

capillary tube was then cut 1mm below the buffy coat to include the uppermost layer of red cells and 2mm above to include the plasma. The contents of the capillary tube were gently expressed on a clean slide, mixed and covered with a 22x 22 mm cover slip. The preparation was examined for trypanosomes in the field using a dark ground phase contrast microscopy. 200 fields were examined for each sample.

4.4.7 **Treatment of animals**

Animals found to be infected with trypanosomes were treated with diminazene aceturate (Dimina vet) at 3.5 mg/kg.

4.4.8 **Data analysis**

Data were analysed using Chi Square, odds ratio, paired t-test and one-way ANOVA, as appropriate (Armitage, 1980).

4.5 **Results**

4.5.1 **Clinical diagnosis**

Out of a total of 1293 cattle examined 354 (27.4%) were emaciated and majority of them (292; 82.5%) also had pale mucous membranes. The remaining 939 (72.6%) were in good body condition. However, there was no statistical difference implying that animals are equally likely to be infected whether emaciated or satisfactory (OR =0.65; CI = 0.39 – 1.09; x^2 = 2.72, P value = 0.0993) (Appendix 9). Analysis of body condition by seasons indicated that in the 529 cattle examined in the March survey (dry season) 299 (56.5%) were emaciated while the rest 230 were judged as satisfactory. In the June survey (wet season) only 55 (10.3%) of 536 cattle were emaciated while in the February survey of 2005 (dry season) all the animals (100%) examined were in satisfactory health condition. Analysis of possible association of body condition with trypanosomosis showed no statistical relationship suggesting no seasonal effects on the animals

investigated. (OR=0.65; CI= 0.39 –1.08; X^2= 2.72; P value= 0.0993) (Appendix 10). From the result, the probability that a cow with poor body condition was positive for trypanosomosis was 0.017.

4.5.2 **Prevalence of trypanosomes using the buffy coat method**

Among 1293 cattle sampled, 882 (68.2%) were females and 411 (31. 8%) males. The buffy coat examination showed that 109 cattle were infected with trypanosomes giving an overall prevalence of 8.43%. Out of these positive cases, 105 (96.3%) was due to *T. vivax*, 2 (1.9%) to *T. congolense,* 1 (0.9%) to *T. b. br*ucei and a mixed infection of *T. congolense* and *T. vivax* (1; 0.9%).

Analysing for sex as a factor of trypanosome infection showed no statistical difference, for even though there were more infections in cows, there was no association between sex and trypanosomosis (odds ratio= 1.01; C.I. = 0.65 - 1.58; x^2 =0.0, P value=0.9528) (Appendix 11). Further analysis by season showed that in the dry season (March survey) only 12 cattle from a total of 529 were detected positive for trypanosomes with a prevalence of 2.3%. All these 12 positive cattle were emaciated. In the wet season (June survey), out of 536 cattle examined, 62 were detected positive for trypanosomes with a prevalence of 11. 6%. In another dry season survey of the following year (February 2005), 35 of 228 cattle were positive for trypanosomes and prevalence 15.4%. However, there was no statistiscal significant difference for seasonal effect observed in this study (t= -0.789; df= 1; P value= 0.575) (Appendix 10)

4.5.3 **Analysis of trypanosome prevalence by blocks of residence**

Trypanosome prevalence varied across the blocks of residences with block 3 having the

highest prevalence of 9.7% followed by blocks 1 and 4 with prevalences of 9.4% and 8.2% respectively. Blocks 2 and 5 were almost at par with 7.3% and 7.2% prevalences respectively (Fig. 3). However, the prevalences between the blocks were not statistically significant (t = 2.71; df = 4; P value= 0.054) (Appendix 12).

4.5.4 **Analysis of trypanosome prevalence by age**

The age distribution of trypanosomosis is shown in Fig. 4. Infections were observed in all the age groups studied with 0-1 year's age group having the highest percentage of 17.0% trypanosome positive cases. Statistical analysis showed that the infections in the different age groups differed significantly ($P<0.05$) and that the pattern of infection as shown by coefficient of determination decreased with increasing age ($r= -0.94$, $r^2=89.1\%$, $\vartheta \neq 0$).

4.5.5. Effect of movement of cattle on trypanosome prevalence

To examine whether movement of cattle had effect if any on the prevalence of trypanosomosis in the KGR the study compared the prevalences obtained between resident and returned cattle. Out of a total of 401 resident cattle sampled, 44 were positive for trypanosomes with an overall prevalence of 11% (Fig. 5). Among the returned cattle sampled, 53 of 318 were trypanosome positive giving a prevalence of 16.7%. (Fig. 5).

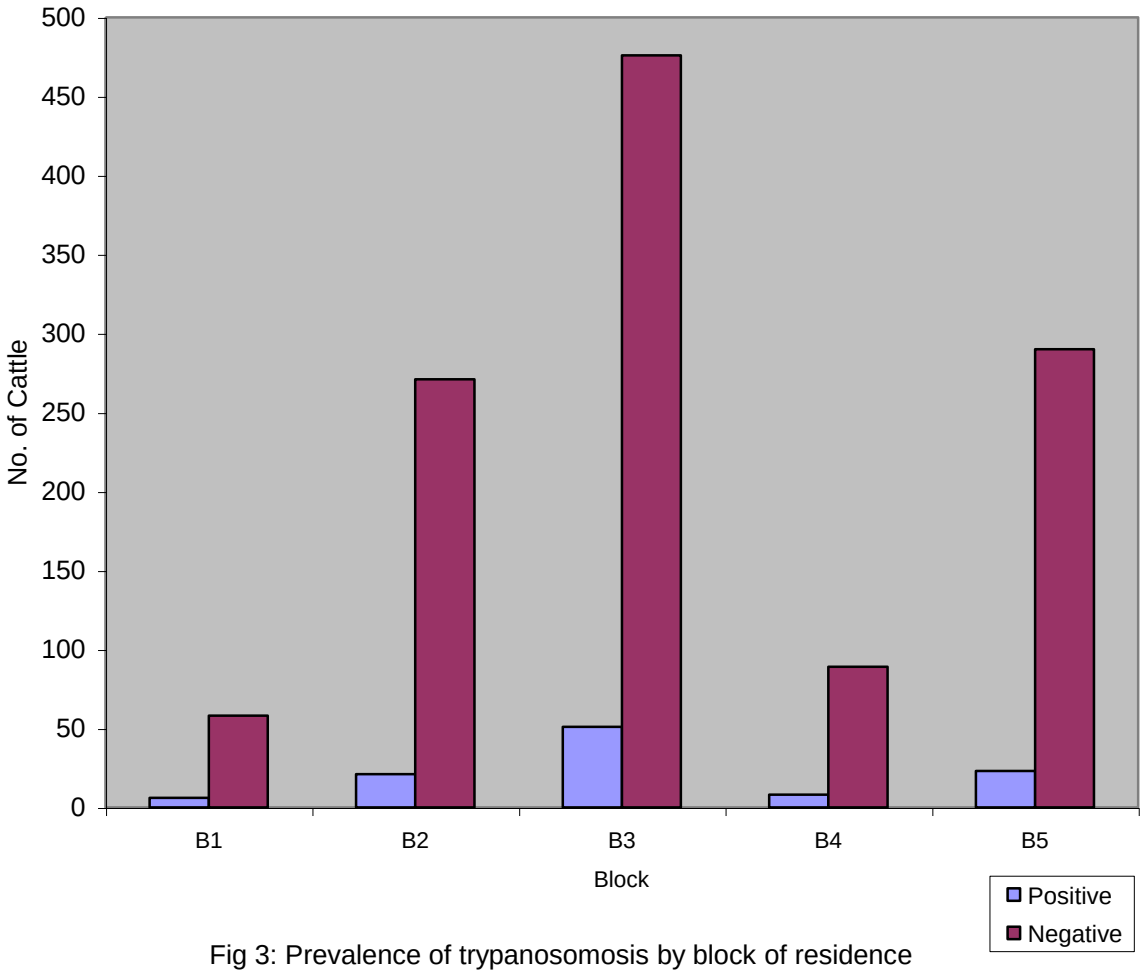

Fig 3: Prevalence of trypanosomosis by block of residence

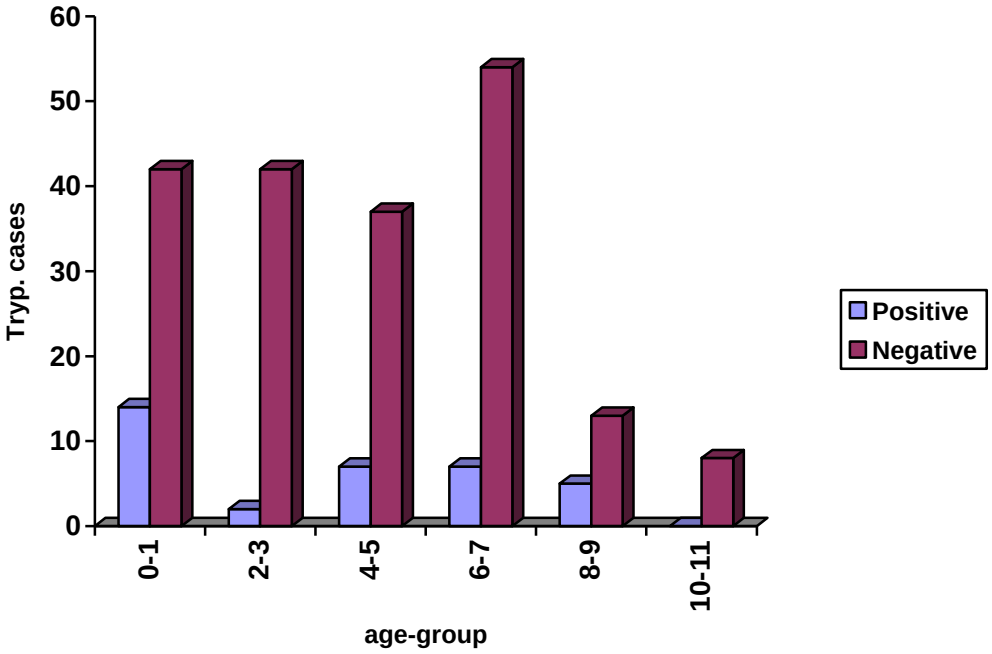

Fig.4: Prevalence of trypanosomisis by age group

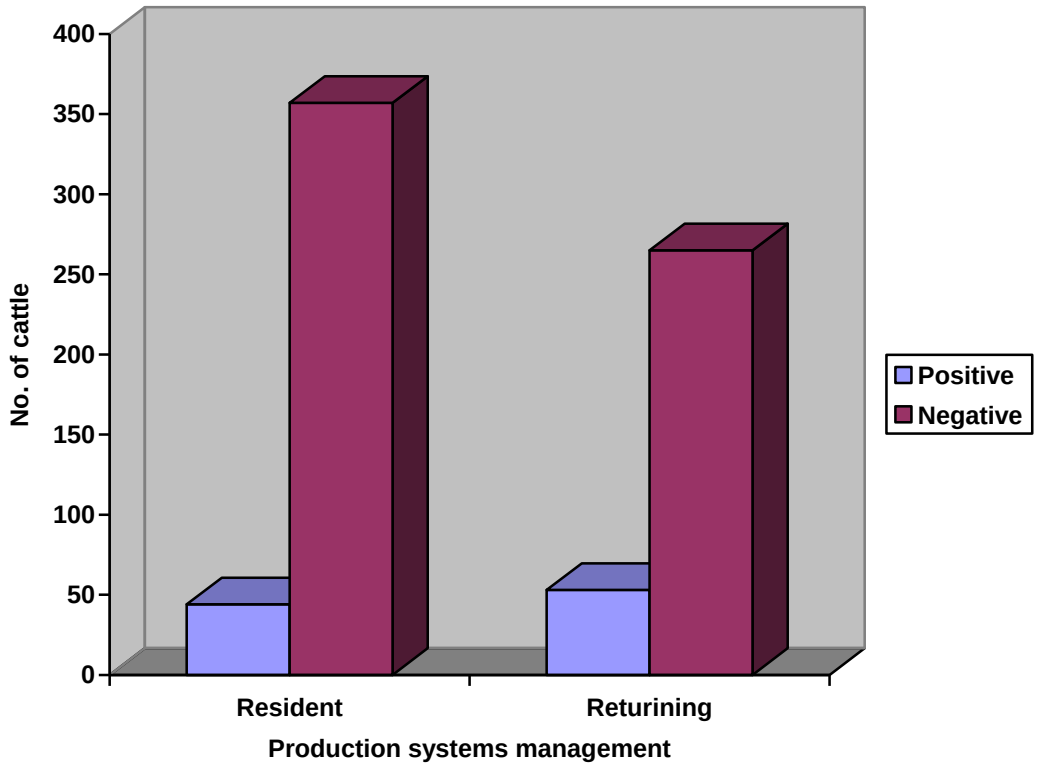

Fig. 5: The effect of movement on trypanosome prevalence between resident and returned cattle in the KGR (June, 2004)

4.5.6 Analysis of trypanosome prevalence by watering points distances (from 1km to 3km)

Fig. 6 shows the GPS mapping of the coordinates realised. As is evident cattle in Blocks 3, 4 and 5 were quartered proximal to the watering points. Spatial distributions of specific locations at risk of trypanosome infections showed that the hydrological network played an important part in trypanosomosis.

A total of 529 cattle investigated, 325 trekked 1km distance from pen to watering points before returning to pen (Fig 7) followed by 143 and 61 that trekked 2km and 3km distances. Out of this total, 12 were positive for trypanosomes. Eleven (91.7%) of these positive cases trekked 1km and only one (8.3%) positive case trekked 2km distances to and from pen to watering points. Statistical analysis showed that there was an association between trypanosome infection and decreased watering point distances Appendix 13).

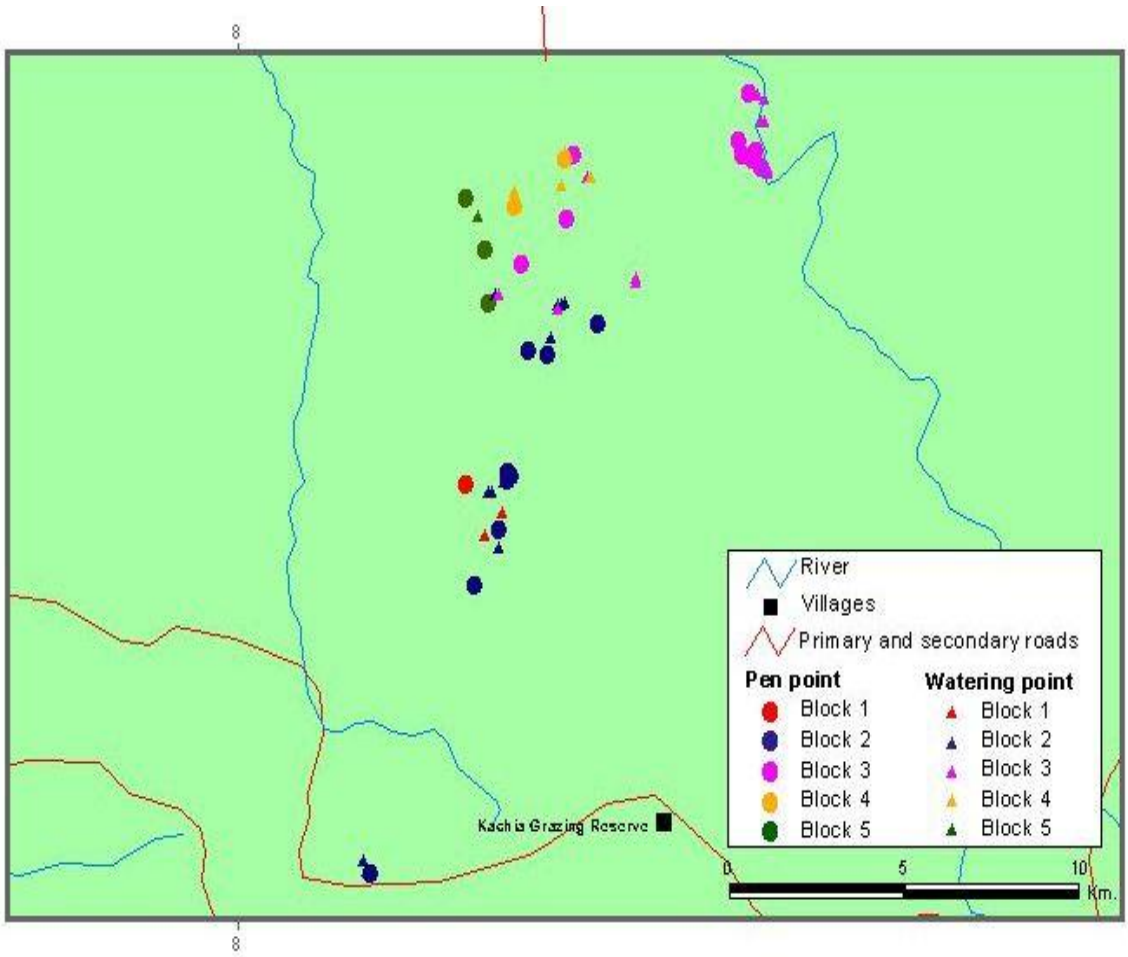

Fig. 6. Map showing the effect of watering points on trypanosomosis prevalence in the KGR (Feb, 2004) (source: software arch view 3.2)

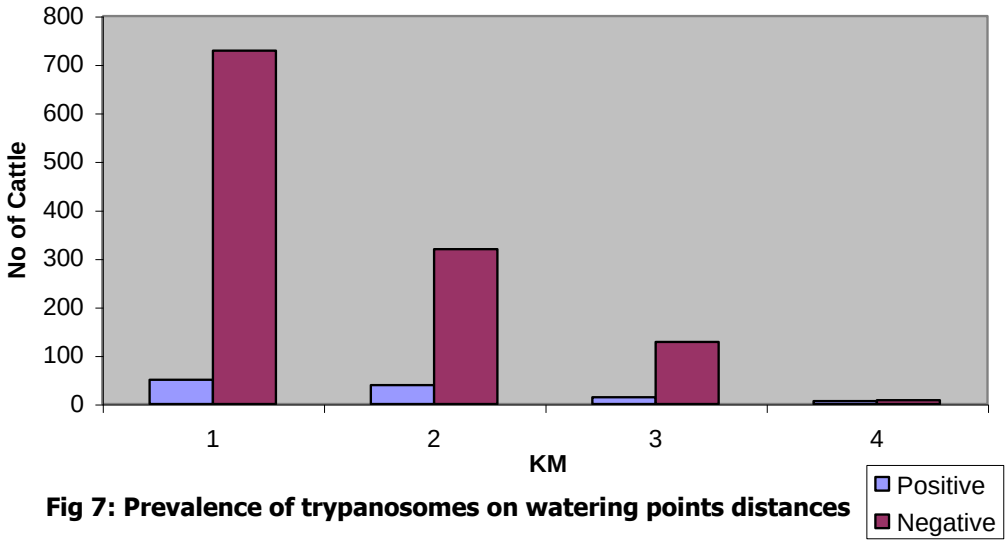

Fig 7: Prevalence of trypanosomes on watering points distances

4.5.7 Analysis of trypanosome prevalence by grazing points

distances (from 1km to 7km)

The results showed that there were high infections by grazing points in cattle that trekked 1 and 2 km from pen to grazing points. Out of a total of 12 positive cases, 4 (33.3%) and 6 (50%) trekked 1 and 2 km respectively from pen to grazing point and the remaining two positive cases trekked 3 km distance. However, the infection did not significantly increase in any of the grazing points (x^2 for linear trend= 2. 186; P value= 0.1234) (Appendix 14).

4.5.8a Awareness of trypanosomosis by respondents

All (100%) of the 27 heads of households interviewed said that they were aware of trypanosomosis, which they called 'samore'. Samore is the Fulani vernacular name for the disease. They also said that the disease could be obtained by the riverside following bites by an infected tsetse fly, known as 'kudan tsando'. They further informed that their parents told them the history of this disease.

4.5.8b Management practices by respondents

When asked frequency of trypanosomosis control, 26 out of 27 (96.3%) heads of households indicated that they treated their cattle sporadically, meaning treatment given not every year. Only one (3.7%) head of household said he treated his cattle regularly. Furthermore all (100%) of the heads of households answered that due to the high cost of trypanocidal drugs, priority attention was given only to cattle in terms of treatment coverage compared to sheep and goats. With regard to supplemental feeding, if any, during the dry season, 9 of 27 (33.3%) said they gave maize shaft (dusa) and potash, 4 of 27 (14.8%) gave only maize shaft while 14 of 27 (51.9%) gave nothing. Thus from a total of 529 cattle examined in the dry season, 349 cattle received supplementary feeding; out of which 12 (3.4%) were positive for trypanosomes. The remaining 178 cattle received nothing.

4.5.9 Analysis of haematological parameters

The haematological parameters evaluated were the PCV, total WBC and differential leukocyte counts. There were decreases in the mean PCV of parasitaemic compared to aparasitaemic cattle, which were 25.99 ± 1.82% and 29.31 ± 1.70% respectively.

However, the decrease was more in trypanosome-positive cattle. Further analysis by age groups showed that the decrease in PCV was most pronounced in the 0-2 year age group; mean PCV was 23.47% ± 3.10% and was statistically significant ($P< 0.05$) (Fig. 8). Thus the anaemia manifested by drop in PCV values was most pronounced in this age group (Appendix 15).

The total WBC and differential leukocyte counts were carried out on 228 blood samples collected from cattle in the KGR during the month of Feb. 2005 survey. From this total, 105 (46.1%) were leucopenic as they had WBC counts of 4 x 10^9/L or less, 119 (52.2%) had normal WBC counts that were in the range of above 4 x 10^9/L to 10 x 10^9/L. Only four (1.7%) animals had counts above the normal range, that is, above 10 x 10^9/L. Of the number 105 (46.1%) that had WBC counts 4 x 10^9/L or less, 25 (23.8%) were trypanosome-positive cases while 80 (76.2%) were parasitologically negative for trypanosomes. In the 119 animals with above 4 x 10^9/L and 10 x 10^9/L WBC counts, 11 of them (9.2%) were trypanosme-positive cases and 108 (90.8%) were trypanosome-negative. All the animals with WBC counts above 10 x 10^9/L were parasitologically negative for trypanosomes (Appendix 21)

Furthermore the results of the differential leukocyte counts of all the 228 animals analyzed are summarized in Tables 4.1 and 4.2. The animals with 4 x 10^9/L and below WBC counts were leucopenic and the differential counts showed that lymphocyte cells were responsible for the observed leucopenia as these cells were reduced below their normal reference values. Among the leucopaenic animals, serology test carried out gave 90.9% and (76%) anti-trypanosomal antibodies in trypanosome-positive and trypanosome-negative cattle. The group with normal values did not appear to have any problem yet as their values were within the normal range except of course for those with positive trypanosmes. The differential leukocyte counts of the four with WBC above 10 x 10^9/L showed neutrophilia to be responsible for leucocytosis.

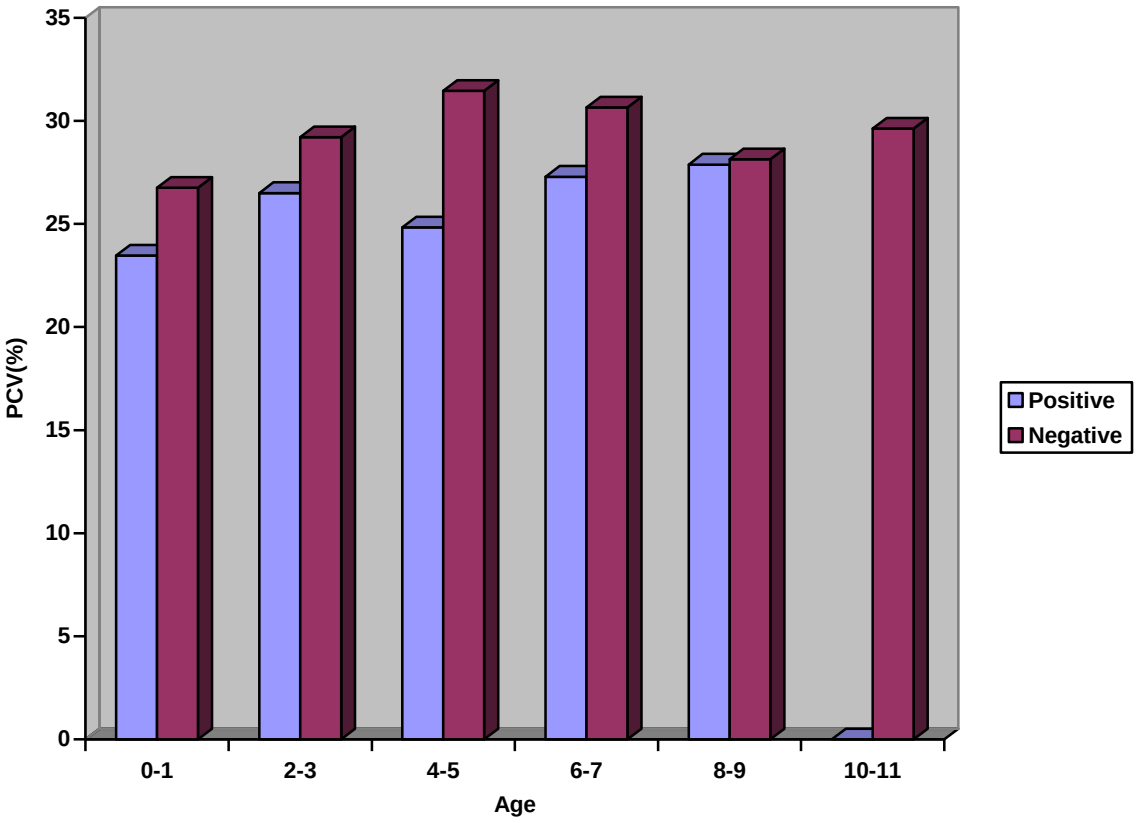

Fig.8: Haematological parameters by age group (in years).

Table 4.1: Mean ± SD differential leukocyte counts of cattle (105) 4x10^9/L Total WBC counts and less in the KGR (Feb. 2005)

Mean differential. leucocyte Counts		Number. of cattle	Neutrophil	Lymphocyte	Eosenophil	Monocyte	Basophil
4x10^9/L and less	Pos.	25	1.64 ± 0.22	0.92 ± 0.15	0.14 ± 0.03	0.12 ± 0.03	0.00
	Neg.	80	1.63 ± 0.34	0.98 ± 0.12	0.12 ± 0.03	0.08 ± 0.00	0.00

Table 4.2: Mean ± SD differential leukocyte counts of cattle (119) above 4x10^9/L **Total WBC counts in the KGR (Feb. 2005)**

Mean differential. leucocyte Counts		Number. of cattle	Neutrophil	Lymphocyte	Eosenophil	Monocyte	Basophil
Above 4X10^9/L	Pos.	11	3.36 ± 0.43	2.14 ± 0.18	0.19 ± 0.12	0.00	0.00
	Neg.	108	3.32 ± 0.73	2.10 ± 0.45	0.45 ± 0.10	0.20 ± 0.52	0.00

Table 4.3: Mean ± SD of differential leukocyte counts of cattle (4) above $10X10^9/L$ Total WBC counts in the KGR (Feb. 2005)

Mean differntial. leucocyte Counts		Number. of cattle	Neutrophil	Lymphocyte	Eosenophil	Monocyte	Basophil
Above	Pos	0	0.00	0.00	0.00	0.00	0.00
$10x10^9/L$	Neg.	4	6.85 ±0.39	3.15 ±0.08	0.46±0.22	0.30 ±0.11	0.00

4.6 Discussion

This study has shown that trypanosomosis with a prevalence of 8.4% constitutes a health problem to cattle rearing in the KGR. Our results support the findings of a sociological survey, which reported severe trypanosomosis in the KGR (NLPD, 1988) and compares favourably with the prevalences of trypanosomes 9.4% and 9.1% reported in cattle by Agu *et al*. (1989) and Abenga *et al.* (2004) respectively in other areas of Kaduna State. Factors that may have facilitated the prevalence were movement of cattle in and out of the reserve (transhumance-pastoralism), the breed which was essentially the white Fulani (trypanosusceptible Zebu), the mode of transmission (cyclical and mechanical) given the presence of tsetse and other biting flies in the reserve and proximity of herd pens to watering point's distances.

Movement of cattle in and out of the reserve was found to influence the prevalence of the disease minimally in this study, as returned herds had a higher prevalence of 16.7% than resident herds that had 11% prevalence, though the difference was not significant ($P>0.05$). Exposure to areas where the animals could easily pick up more infections might be responsible for the result. On the other hand, the KGR from the point of view of grazing reserve development towards improved livestock development is supposed to be a safe-haven for livestock raising, however this does not appear to be so in the light of the present result. Since the main objective of grazing reserve establishment is sedentarisation, the finding of non-significant difference between the

prevalence rates of trypanosomes in returned and resident herds, suggests that a lot needs to be done in the KGR to clear the problem of trypanosomosis.

Analysis of the prevalence of trypanosome by season appeared to suggest seasonal variations in prevalences as 11.6% was obtained in the month of June compared with 2.3% obtained in March of the same year, 2004, since the result was statistically significant ($P< 0.05$) and this is in agreement with other workers who reported seasonal variations in prevalences of diseases (Jordan, 1986; Putt *et al.*, 1988). Although a similar survey conducted in February 2005 revealed a much higher prevalence of 15.4% which appears to contradict the role of season as advanced above; as returning herds, from the southern areas of the country, into the KGR, included in this survey, might have contributory factor in this higher prevalence of February 2005.

Analysis of the prevalence of trypanosome by blocks of residence indicated prevalence variations with block 3 being the highest, though the result was not statistically significant (Appendix 12). Block 3 was closest to watering points of the animals. That hydrological network played an important part in trypanosomosis was illustrated in the following results. There were associations between increased trypanosome infections with decreasing watering point's distances to herd pen (x^2 for linear trend = 4.447, $P<0.05$) but no association of grazing point's distances to herd pen on prevalence (x^2 for linear trend = 2.186; $P>0.05$). It therefore appears reasonable to suggest that 1 km distance, the shortest pen to watering distance, that provided the highest infection, was within the flying strength abilities of the flies; hence the flies could fly in search of blood meal and return to watering point habitats.

Analysis by age showed a significant difference of trypanosome infections in the age groups studied and that 0-2 year age group was most vulnerable (Fig. 4). Likely explanations for this result could be that the infections were still recent and also that particular age group was most in number. Furthermore, haematological parameters showed anaemia characterized by depressed PCV values. This was most manifest in the 0-2 year age group with a mean PCV of 23.43 ± 3.10% (Appendix 15) and was statistically significant ($P< 0.05$). The anaemia might be due to the effects of trypanosomes on host erythrocytes and tissues (Fiennes, 1970; Losos, 1986; Anosa, 1988). In addition, the leucopaenia observed, though a common feature of trypanosomosis especially in the acute crisis (Ogunsanmi *et al.*, 1994) was due to lymphocytopaenia. Marked emaciation may be due to muscular degeneration as reported by Fiennes (1970).

Furthermore, serological test carried out showed 90.9% of trypanosome-positive and 76% trypanosome-negative respectively of these leucopaenic animals with anti-trypanosomal antibodies, suggesting that trypanosomosis was largely responsible for the lymphocytopaenia that induced the leucopaenia. Severe anaemia and leucopaenia in this study observed were in agreement with the findings of other investigators (Naylor, 1971; Maxie *et al.*, 1979; Anosa, 1980; Esievo, 1983) who reported leucopenia as a common feature of trypanosome-infections especially in the acute crisis. It is reasonably well to suggest that the 105 leucopaenic cattle were sampled during the acute infections. Those trypanosome-negative animals even though leucopaenic could

be at the fluctuating parasitaemic phase commonly associated with the disease. For the 11 trypanosme-positive animals with normal WBC values, it may be either that the infections were still quite early to have had effect on the blood cells or a revamp of the blood cells, in which case the infections were chronic. The neutrophilia (Table 4. 2) observed might be due to secondary bacterial infections that could follow immunosuppression that is characteristic of the disease. Although the four animals with neutrophilia were negative for trypanosomes, they might be in the chronic stage of infection when parasites become scanty and difficult to detect in the blood.

From the questionnaire administered, it was obvious that the respondents were aware of trypanosomosis with 100% answering in the affirmative. Almost all the respondents treated their animals sporadically with trypanocidal drugs paying priority attention to cattle due to high cost of the drugs. These practices may have accounted for the high prevalence overall of trypanosomosis obtained in sheep and goats (17%) (page 88 , Chapter 5) compared to that for cattle (8.4%) as found in this study.

Finally, the study has shown that trypanosomosis is a threat to animal health in the KGR. Awareness of the disease by the heads of households interviewed makes it easier for implementation of effective control measures against the disease with community participation in mind. However, the practice of movement of animals in and out of the reserve by the Pastoralists is considered inimical to livestock development as it may contribute to increased prevalence

of trypanosomosis. It is, therefore, a likely factor in the maintenance and spread of trypanosomosis in the reserve and should be seriously discouraged. Also, trypano-susceptible Zebu cattle are the predominant breeds in the reserve. This study suggets effective use of chemotherapeutic and chemoprophylaxis as well as vector control measures to fight the problem of trypanosomosis and allow permament raising of livestock in the reserve, thus encouraging sedentarisation.

CHAPTER 5

5.0 Determination of the prevalence of trypanosomes in sheep and goats in the KGR

5.1 Summary

Trypanosome prevalence in sheep and goats was assessed in June 2004 in a farm settlement in southern Kaduna, northern Nigeria. The study aimed at ascertaining the importance of animal trypanosomosis in these two animal species and also assessed the probability of obtaining a positive sample in blood of animals. Blood samples from a total of 345 sheep and goats exposed to natural trypanosomosis challenge were collected and examined using the buffy coat technique and Giemsa-stained thin blood films. The prevalence was 20% and 13%, respectively in sheep and goats with an overall prevalence of 17%. Infection was observed across the age groups of sheep and goats. There was a significant difference ($P < 0.05$) in the level of PCV between the affected and unaffected sheep and goats indicating that anaemia were more on affected animals. The Mean PCV regressed on age group showed a fair but no significant relationship at 5% level ($r = 0.54$, $r^2 = 28.9\%$, $\ell = 0$). The trypanosome species seen were *Trypanosoma vivax*, constituting the highest percentage of 98% and *T. congolense* and *T. brucei* of 1% each. This study has recorded a high prevalence of trypanosomes in the study animals suggesting that trypanosomosis may be an important disease in the study area. Since sheep and goats contribute about 35% of the total meat supply in Nigeria, a programme to improve small ruminant production will be beneficial to the nation.

5.2 **Introduction**

Animal trypanosomosis represents a major constraint in sub Saharan Africa and it is considered the most important vector-borne cattle disease transmitted by tsetse fly vector in this region (FAO, 1996). The disease is a major impediment to livestock production and agricultural productivity in vast areas of sub Saharan Africa (FAO, 1996). In Nigeria, the agricultural sector contributes 37 percent of the Gross Domestic Product (GDP) and employs 65 percent of adult labour force (CBN, 1995). Besides its pivotal role in meeting the food and fiber needs of a large and growing population, it provides the raw materials for the agro-induastrial sector and is the largest contributor (88%) to non oil foreign exchange earnings (Falusi, 1996).

Inspite of the above, Nigeria, with current human population estimated at 120 million, still has animal protein supply as one of her critical food problems. Among the factors restricting livestock production in Nigeria, tsetse-transmitted African animal trypanosomosis unquestionably plays an important role. Unlike cattle, sheep and goats are kept in a very broad range of agro-ecological zones (Luckins, 1992) where they contribute a lot much to the rural economies as a source of meat, milk, manure and readily disposable income (Griffin and Allonby, 1979, Nga'yo *et al*., 2005). In Nigeria, small ruminants contribute an estimated 35% to the total meat supply (Ngere *et al*., 1984). Some workers have underestimated the importance of trypanosomosis in small ruminants, considering the infection as seldom (Leach, 1973; Stephen, 1970). However, recent accounts have shown that sheep and goats acquire natural

trypanosomosis infections and suffer severe economic losses (Griffin and Allonby, 1979; Snow *et al*., 1996; Masiga *et al*., 2002; Nga'yo *et al*., 2005). Given the economic importance of livestock, if trypanomosis problem could be solved, the average carrying capacity of livestock including the small ruminant population would increase by between 2.5 to 3.5 times the current carrying capacity of cattle and for small ruminants by five fold (FAO, 2002a). This screening exercise aimed at assessing the level of trypanosomosis risk in a grazing reserve settlement was undertaken in order to improve livestock development. This work reports the results of a cross-sectional study that assessed trypanosome prevalence in sheep and goats in the reserve.

5.3 **Objective**

i. To determine the prevalence of trypanosomosis in sheep and goats in the KGR.
ii. To determine the probability of obtaining at least one positive case in random samples.

5.4 **Materials and Methods**

5.4.1 **Study Area**

KAchia Grazing Reserve (KGR) is situated between latitudes 10^{O} 03' and 10^{O} 13'N and longitudes 7^{O} 55' and 8^{O} 06'E. The land lies approximately 700 – 790m above sea level and is drained by the Kaduna river system and several streams that feed into them. KGR is a mixed farming system with many livestock species namely, cattle, sheep and goats. The vegetation is typical of

the Northern Guinea Savanna woodland. However, because of the effects of annual bush fires, there are now many species of both the northern and the derived savanna zones found in the area.

The study was carried out in June 2004 over a period of ten (10) days. The sheep and goats from which samples were taken graze freely in the reserve. Altogether, sampling was carried out in 21 households. 15, of these households kept sheep and cattle together, 3 households kept goats and cattle together and 2 households kept sheep, cattle and goats together while only 1 household kept goats alone. The breeds predominant in the reserve were the Red Sokoto goats and Uda sheep. The KGR was chosen because it had in the past (about 16years ago) reported severe trypanosomosis and treatment with trypanocidal drugs and pour-on applied on animals to reduce the incidence of the disease (NLPD, 1988). Over long periods of this control activity and considering that no new significant efforts have been made to control trypanosomosis in the area, this study sought to investigate the prevalence of trypanosomosis in small ruminants in the study area.

5.4.2 **Animal sampling and diagnosis**

A list of households with sheep and goats was made using the KGR record. Through a simple random process of balloting, 6 households for goats and 15 households for sheep giving a total of 21 households were selected from Blocks 2, 3, 4 and 5. A total of 345 animals were randomly screened made up of 215 sheep and 130 goats. Clinical examination by a veterinarian was carried out on

the animals sampled. Clinical conditions looked out for were those involving the body condition (emaciation) and the paleness of the mucous membrane of the eyes and mouth and physical and mental alertness. About five millimeters of blood were collected from the jugular vein of each animal into specimen bottles containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The EDTA was dispensed as one-milligram powder per millimeter of blood. The samples were analyzed using the buffy coat dark ground/phase contrast technique (Murray *et al.*, 1977) and Giemsa stained thin smears. Trypanosome species were identified based on their morphological structure from Giemsa-stained thin films. Packed cell volume (PCV) was used to quantify anaemia using a haematocrit centrifuge and reader (Murray *et al.*, 1977). The mean PCV of affected and unaffected small ruminants were compared using t-test. The pattern of the PCV across the age was also examined using linear regression analysis. Ethical clearance to proceed on this study was obtained from the Kaduna State Ministry of Agriculture and Natural Resources and the Pastoralists in the KGR for consenting to bleeding of their animals.

5.4.3 **Probability considerations**

The probability of picking up a trypanosome infection in a random sample of 345 animals was assessed using the binomial probability distribution function (Armitage, (1980). We considered the random selection of 345 sheep and goats exposed to natural trypanosomosis challenge in a farm settlement in southern Kaduna, northern Nigeria. Infection of an animal is either positive or negative. That is, if the chance of an infection is p, then the chance of no infection is q

given by (1-p). The infection of more than one animal calls for the binomial probability function, which describes the outcome of this type of event that has two possible complementary results. Here it was postulated that the probability of an infection in randomly selected animals (sheep and goats) was given the presence of trypanosomes while the probability of no infection was the fraction of a unity.

This completes the identification of all the parameters needed to solve the binomial probability distribution function:

$f(x; n, p) = ({}^{n}_{x})p^{x} (1-p)^{n-x}$

Where n = number of trials (number of the animals in this sample)

x = number having trypanosomes

P = probability of being infected (p = x/n)

1-p = probability of no infection

The probability of 0, 1, 2,…n infections for 1 to 345 trials was considered. For any given n, the most likely number of infections is x/n (since p = 0.17). The underlining showed the minimum n to be reasonably certain of getting an np infection. We also sought to determine the minimum sample number needed to obtain at least one positive result from a sample for this binary variable, that is, to find at least one affected carrier in a random sample or what sample size is required to give a good likelihood of sampling affected individual animal.

5.4.4 **Treatment of animals**

Animals found infected with trypanosomes were treated with diminazene aceturate (Dimina vet) at 3.5 mg/kg.

5.4.5 Data analysis

Data were analysed using Chis Square, odds ratio, paired t-test and one-way ANOVA, where appropriate.

5.5 Results

5.5.1 Clinical diagnosis

Of a total of 345 sheep and goats comprising 215 sheep and 130 goats examined clinically, 22 (10.2%) sheep and 11 (8.5%) goats showed signs of emaciation with rough hair coat.

5.5.2 Parasitological diagnosis

In the 215 sheep and 130 goats sampled, 42 (20%) sheep and 17 (13%) goats were found positive for trypanosomes. The prevalence in both animals was 17%. Table 5.1 shows the results of prevalence of trypanosome infections with regard, to age. The modal age group was the 2 under 3year group with a frequency of 19 and 6 respectively in sheep and goats. The respective age prevalence in sheep and goats are as follows, the under 1 age group had a frequency of 5 and 1, 1 under 2 and 3 under 4 age groups had 9 and 6 and 6 and 1 respectively while 4 and above age groups had a frequency of 2. Furthermore, Table 5.2 shows the mean PCV of affected and unaffected sheep and goats. Generally, anaemia manifested by low PCV values was observed in sheep and goats but it was more significantly pronounced in affected sheep and goats ($t = -4.98$, $df = 4$, p-value = 0.008). The result of regression of PCV on age showed no statistically significant pattern across the age groups. Even though the infection increased with increasing age, the increase was not significant (Fig.8, $r = 0.54$, $r^2 = 28.9\%$, $\ell = 0$).

The trypanosome species seen were *Trypanosoma vivax* constituting the highest percentage of 98% and *T. congolense* and *T. brucei brucei* of 1% each. The findings here have indicated that trypanosomosis presents a serious risk to animal health in the KGR as evidenced by the overall trypanosome infection rate of 17%.

5. 5.3 Probability testing

The binomial probability result showed that there were no chances of not getting a single infection out of 345 trials with regard to high infection of 17%. Since $p = 0.17$ is a probability of getting a trypanosome infection for each animal examined, the chance that a sample of 345 animals will fail to find any infection is 0.0000 (Table 5.3; App. 11).

Table 5.1: Age distribution of trypanosomosis in sheep and goat in the KGR (June 2004).

Age (years)	Sheep		Goat		Sheep/Goat		Total
	+ve	-ve	+ve	-ve	+ve	-ve	
0 under 1	5	11	1	9	6	20	26
1 under 2	9	33	6	36	15	69	84
2 under 3	19	80	6	53	25	133	158
3 under 4	8	42	1	18	9	60	69
≥ 4	2	6	0	0	2	6	8
Sub-total	42	173	14	116	57	288	345
G.Total	**215**		**130**				

Prevalence = 57/345 = 0.17 or 17%

+ve = positive

-ve = negative

Table 5.2: Mean $\pm$ PCV of infected and uninfected sheep and goats by age in the KGR (June 2004)

Age (years)	PCV	
	Positive	Negative
0 under 1	21.67 ± 3.50	26.17 ± 3.66
1 under 2	21.67 ± 3.67	27.33 ± 3.50
2 under 3	23.22 ± 5.74	27.67 ± 2.96
3 under 4	24.17 ± 5.23	27.22 ± 1.72
$\geq$ 4	15.50 ± 0.00	24.33 ± 2.73

Even though the PCV of the animals are generally low, t-test showed that there was more anaemia in infected animals than uninfected at 5% level (t = -4.98, df = 4, p-value = 0.008).

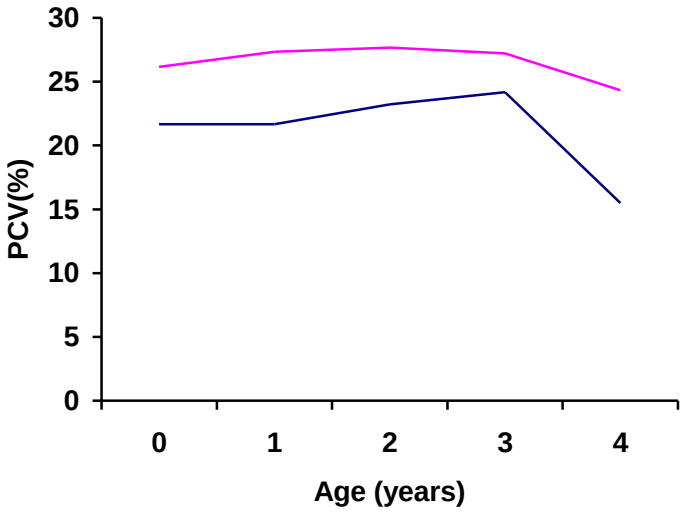

Fig.9: Pattern of PCV of infected animals across the age group

□ = PCV of uninfected animals

□ = PCV of infected animals

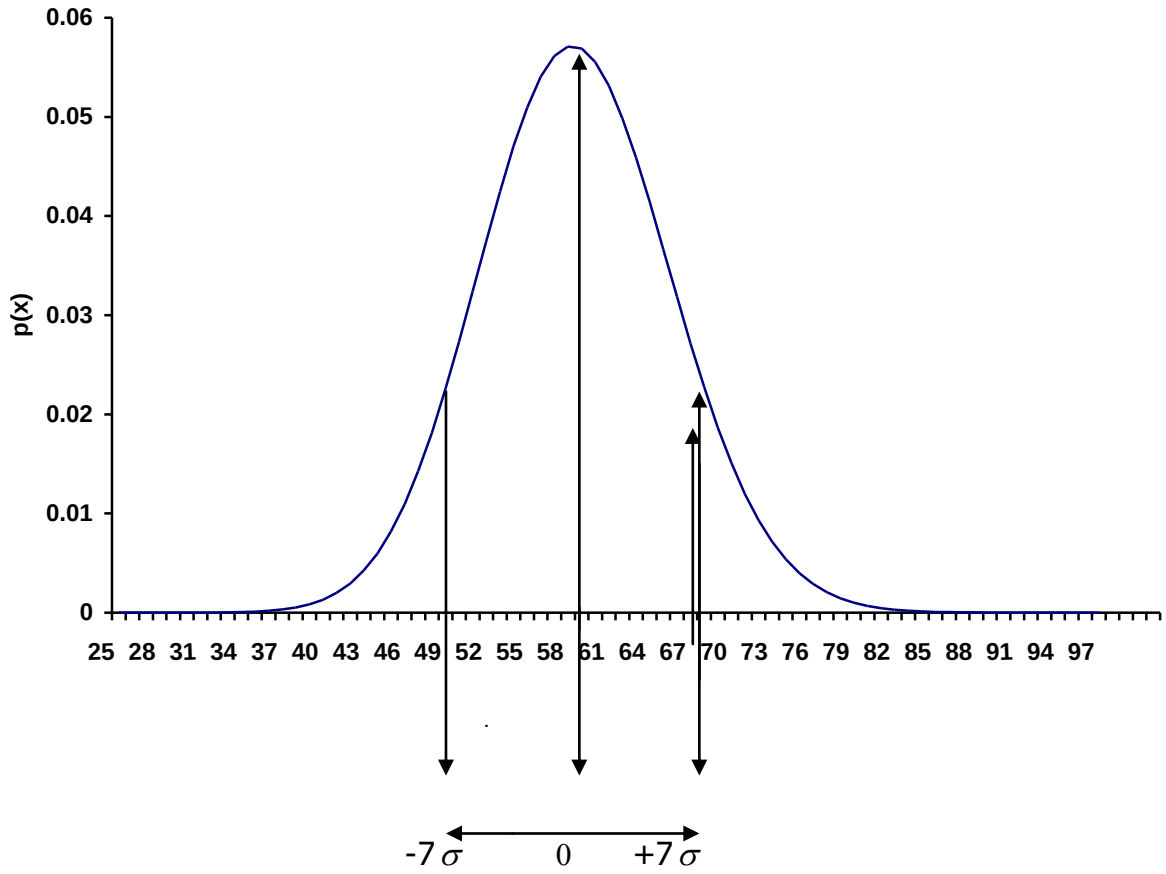

Fig.10: Probability distribution of trypanosomosis for mean = 59

number of trial successes and standard deviation = 7.

5.5 **Discussion**

This study has clearly demonstrated that pathogenic African trypanosomes are prevalent in small ruminants in the KGR and that sheep and goats acquire natural infections. In the present study we have obtained an overall prevalence rate of 17% in sheep and goats. This figure was comparatively much higher than the range of 5 to 6% reported for sheep and goats by some investigators in parts of northern Nigeria (Joshua and Ige, 1982; Daniel *et al*., 1994). The difference could be due to host factor as trypano-susceptible breeds were the predominant breeds in the KGR. The increased prevalence recorded here suggests a rising trend in the disease and supports the observation by Abenga *et al*. (2004) who described a general increase in trypanosome prevalence in the State. This study also obtained a similar high prevalence of trypanosome in a survey of cattle in the KGR.

There was an indication of infection across the age groups studied. The affected small ruminants tested more anaemic than the unaffected at 5% significant level ($P < 0.05$). The anaemia observed in the affected sheep and goats could be due to the effects of trypanosomes on the erythrocyte. Several investigators including Kobayashi *et al*. (1976); Losos (1986); Anosa (1988) and Ogunsanmi *et al*. (1994) had reported anaemia to be a feature of trypanosome infection.

The binomial probability distribution function revealed that due to the high prevalence of 17% infection, the chance that a sample of 345 animals will fail to find any infection becomes lower as the sample size increases. Similarly, as the affected animals drop the probability of missing such infections in a sample of 345 animals or more increase. In a sample of 345 animals from KGR, the probability of getting at least one positive infection was 1 and the probability of missing infection was 0. In Fig 9, the distribution is very nearly symmetrical. It has the maximum at 59 trial successes.

Although earlier reports by several investigators including Stephen (1970), Hoare (1972) and Esuruoso (1974) had observed that cattle were preferred feeding hosts for tsetse flies in areas where both small ruminants and cattle co-existed probably due to low prevalence reported for the small ruminants, however, this study has debunked the observations and demonstrated that small ruminants are important hosts as well and are also exposed to the risk of tsetse and trypanosomosis just as cattle are. More credence to these findings comes from the results of Nga'yo *et al*. (2005), using polymerase chain reaction PCR) they recorded a trypanosome prevalence rate of 25.3% in sheep, 20% in goats and 19.2% in pigs in western Kenya.

From the foregoing, since small ruminants contribute about 35% of the total meat supply (Ngere *et al*., 1984; FMA, 1988) a programme to improve small ruminant production through control of animal trypanosomosis should be considered important given the high prevalence of trypanosomes recorded in this study.

CHAPTER 6

6.0 Determination of serological prevalence of animal

trypanosomosis in the KGR by inhibition ELISA

6.1 Summary

A recombinant hsp 70/Bip antigen for detection of bovine trypanosomosis was evaluated as a means of diagnosis using bovine field serum samples from Kachia grazing reserve, Nigeria. Circulating anti trypanosomal antibodies was detected in 359 of 545 in inhibition ELISA in cattle with apparent serological prevalence of 65.9% as against 6.1% prevalence overall by buffy coat method. Of 33 serum samples from cattle with parasitologically confirmed diagnosis, 28 had circulating anti-trypanosomal antibodies giving an apparent sensitivity of 84.8%. Furthermore, returned herds and resident herds had significant anti trypanosomal antibodies. The test detected antibodies due to the three trypanosomes species *T. vivax*, *T. congolense* and *T. b. brucei*. The specificity of the assay was based on the results of analysis performed on sera samples from cattle infected with *Theileria parva*, *Anaplasma marginale* and *Babesia bigemina*, which tested all negative in the assay. The test offers promise as a useful tool in sero diagnosis of cattle trypanosomosis. However, improvement in test sensitivity and further evaluation of the assay are suggested.

6.2 Introduction

The diagnosis of African trypanosomosis by parasitological technique is limited in sensitivity. Consequently, several attempts had been made to introduce other improved sensitive methods of diagnosis such as serology and DNA- based technologies to enhance the diagnosis of the disease. Given the current high cost of DNA-based techniques, such as PCR, the demand remains high for serological tests that could be applied on a large scale, at low cost and still discriminate between different tyrpanosome species (Bossard *et al.*, 2003). Meeting these goals and requirements rely on the identification of suitable trypanosome antigens and the use of recombinant technology. Antigen-detection assays based on monoclonal antibody for the detection of species-specific invariant antigens of *Trypanosoma congolense*, *T. vivax* or *T. brucei* in experimentally and naturally infected cattle had been developed and evaluated

for sensitivity and specificity (Nantulya *et al*., 1992; Bengaly *et al*., 1995). These authors reported apparent specificity of 100% and 98% respectively but the sensitivity was much lower, about 75%.

In the continuing search for suitable trypanosome antigens that could detect infections of bovine due to the three livestock trypanosomes in a single test and thus serve as a pan trypanosomosis test, without compromising the assay sensitivity and specificity, a recombinant cDNA encoding a 69kDa immunodominant antigen of *T. congolense* was developed (Authié *et. al*., 1992; 1993a, Boulangé and Authié, 1994). The test antigen had been screened in a variety of assays by inhibition ELISA (iELISA) (Boulangé *et al*., 2002; Bossard *et al*., 2003). This work further evaluates the performance of this test assay in iELISA using an anti-Bip monoclonal antibody and a complete fusion protein expressed in *Escherichia coli*.

6.3 Objective

To determine the performance of a 69kDa immunodominant protein of *T. congolense* identified as a member of the Heat Shock Protein (hsp) 70 family as a diagnostic antigen for animal trypanosomosis.

6.4 Materials and Methods

6.4.1 Source of diagnostic antigen

The target antigen used is an immunodominant recombinant 69kDa of *T. congolense* identified as a member of the heat shock protein 70 family (70kDa

hsp) related to mammalian immunoglobulin binding protein (Bip) (Boulangé and Authié, 1994). This antigen was differentially recognized by cattle of differing susceptibility to trypanosomosis (Boulangé and Authié, 1994) and was kindly provided by Drs. Gerard Cuny, Edith Authié and Géraldine Bossard of IRD, Montepellier, France.

6.4.2. Source of serum samples

Serum samples collected during the survey of animal trypanosomosis in the KGR in March and June 2004 and February 2005 were used for the assay. All 228-serum samples collected in February and some 200 and 117 of June and March surveys, respectively, were used for the assay.

6.4.3 Determination of parasite- specific IgG in inhibition ELISA assay

Trypanosome (*T. congolense, T. vivax* and *T. brucei*) – specific IgG antibodies were measured in inhibition ELISA assays using as target antigen the recombinant cDNA encoding a 69kDa immunodominant antigen of *T. congolense* (Authié et al., 1993a, Boulangé and Authié, 1994). This antigen is a member of the hsp 70 family (70kDa heat shock protein), which is closely related to mammalian immunoglobulin binding protein (Bip) (Boulangé and Authié, 1994) and is also differentially recognized by cattle of differing susceptibility to trypanosomiasis (Boulangé and Authié, 1994). Optimum assay conditions were established with respect to concentrations of the antigen, sera, monoclonal antibody (mAb) and the anti mouse peroxidase in preceding titration experiments. Conditions were taken as optimal when normal sera (negative control sera) resulted in no background reaction but only gave colour relative to the Kblue substrate (blue). Briefly described, the optimised

conditions used were as follows. Microtiter plates were coated with 100µl / well of antigen (0.1ng/µl, final concentration 10ng/well) diluted in 50mM PBS pH 7.2 and incubated for 1 hour at 37 ^{o}C. Blocking buffer containing 0 .2% BSA and 0.05 % Tween 20 in 50 mM PBS pH 7.2 dispensed 100µl/ well was used to block unspecific binding sites, followed by incubation for 1hr at 37^{o}C. 100µl / well of serum testes in duplicates (1: 40 dilution) in blocking buffer were incubated for 1hr at 37^{o}C. Thereafter the plates were washed once with PBS followed by the addition of monoclonal antibody (mAb), 100µl/well of mAb at 1: 2000 dilution in blocking buffer were added in all wells except Kblue and control conjugate and incubation for 1hr at 37^{o}C.

Subsequently, goat anti mouse peroxidase (1: 2500) dilution in blocking buffer were added 100µl/ well in all wells except Kblue and incubated for 1hr 37^{o}C. Note, only blocking buffer (100µl/well) was added and in the previous step in wells that did not contain mAb. After incubation, plates were washed twice with wash buffer containing 0 .1 % Tween 20 in 50mM PBS pH 7.2 and once with PBS. Finally, Kblue (Neogen) was added as substrate for the peroxidase (100µl/well) and plates kept away from sunlight for 30 mins afterwhich the reaction was read as OD at 620nm in ELISA machine Reader (Victor Wallac, Perkin Elmer 1420 Multilabel coulter). All sera were tested in duplicates. Cut-off values to decide for a positive or negative result were calculated from mean OD- values plus 2 standard deviations from the percentage inhibition given by 165 sera from healthy cattle (21%). Sera from cattle infected with *Theileria*

parva, *Anaplasma marginale*, *Babesia bigemina* gave inhibition below the cut-off value (Bossard *et al*., 2003).

6.5 **Results**

Of 545 cattle sera samples screened, the test detected antibodies in 359 (65.9%. as against 6.1%) overall by the buffy coat method. Also of 33 serum samples from cattle with parasitologically confirmed diagnosis, 28 sera were detected positive with circulating anti trypanosomal antibodies giving an apparent sensitivity of 84.8%. The specificity of the assay was 97.3%. Further, evidence for the specificity of the test was provided by analysis of sera from cattle infected with *Theileria parva*, *Anaplasma marginale* and *Babesia bigemina*, which were all negative in the test assay. The test detected antibodies due to the three tyrpanosomes species *T. vivax*, *T. congolense* and *T. b. brucei*. Also, both returned and resident herds had significant anti-trypanosomal antibodies.

Table 6. Sensitivity and specificity of serological diagnosis of trypanosome infections in cattle by iELISA based on HSP70/BIP Recombinant Antigen

	iELISA		
	+	-	Total
BCM +	28	5	33
-	331	181	512
Total	359	186	545

Sensitivity= 28/33 x100%= 84.8%

Specificity = 181/186 x100% = 97.3%.

Sero-prevalence=359/545 x100 = 65.9%

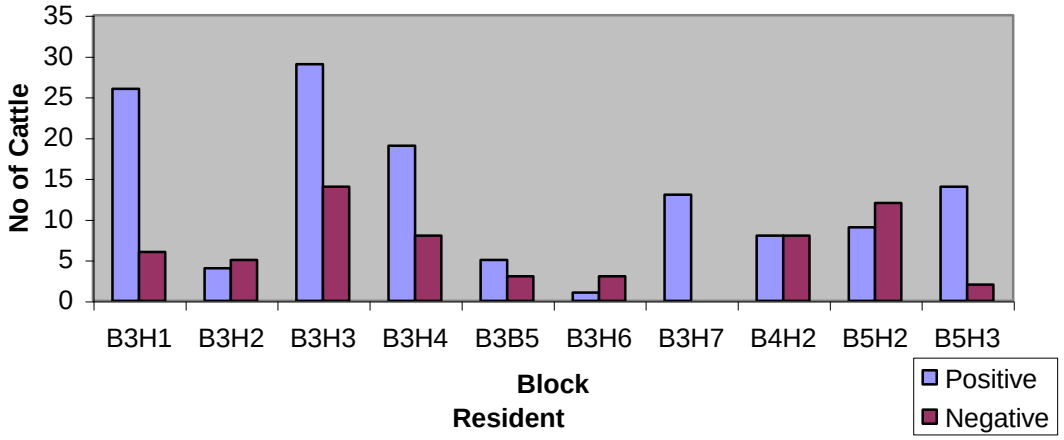

Fig 10: Sero Prevalence of Trypanosomosis in ResidentCattle

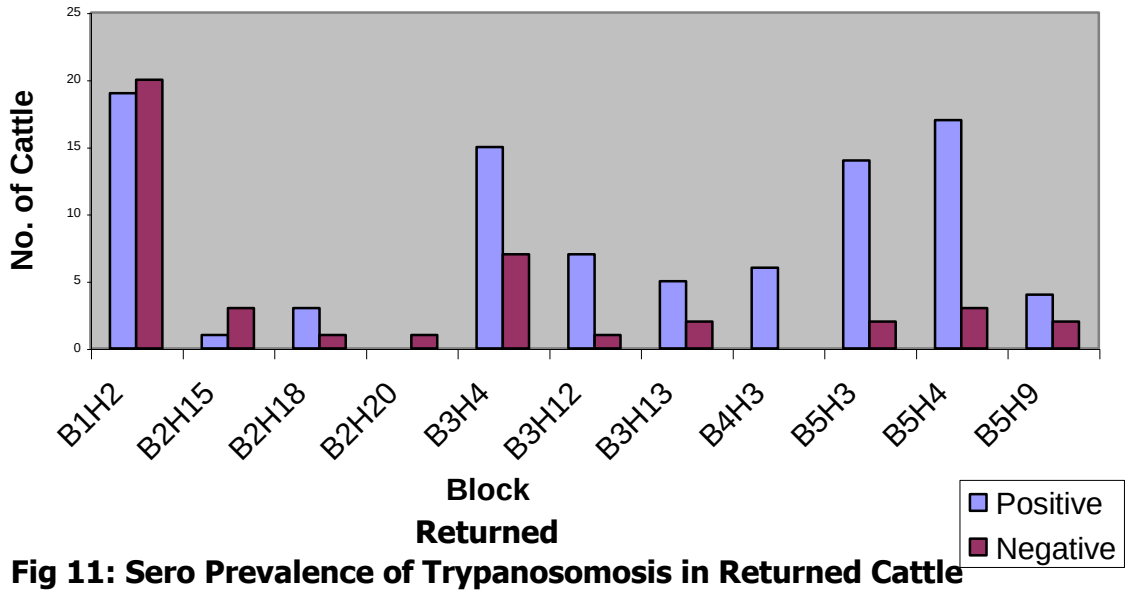

Fig 11: Sero Prevalence of Trypanosomosis in Returned Cattle

6.6 Discussion

This study has assessed the current status of bovine trypanosomosis in the KGR as well as ascertained its prevalence by serological technique. The distribution of bovine antitrypanosomal antibodies indicated that the distribution of bovine trypanosomosis was more widespread (65.9%) than the parasitological prevalence (6.1%) suggested. The specificity of 97.3% obtained for the test is excellent but the sensitivity of 84.8% recorded appears good but need to be improved upon. Boulangė *et al*. (2002) had similarly suggested improvement in the test sensitivity. According to the authors, the test assay was very good in detecting secondary challenge but not so sensitive for detection of primary challenge infections.

Furthermore, the test detected antibodies due to the three trypanosomes species *T. vivax*, *T. congolense* and *T. b. brucei*. In addition, both returned and resident herds had significant anti trypanosomal antibodies. The finding suggests that a lot needs to be done in the KGR to clear the problem of trypanosomosis. The anti trypanosomal antibody detection by iELISA proved to be a useful tool in establishing the distribution of bovine trypanosomosis and has identified KGR as an area with a high risk from bovine trypanosomosis. The test offers promise as a useful tool in sero diagnosis of cattle trypanosomosis but improvement in test sensitivity and further evaluations of the assay are suggested.

CHAPTER 7

7.0 Association of trypanosomes with mortality and morbidity of cattle in the KGR

7.1 Summary

The effects of trypanosomes as a major cause of mortality and morbidity in the cattle were investigated between the months of March and June 2004 in the Kachia Grazing Reserve following reports of trypanosome-attributable deaths in the study area. In this study, a questionnaire was used to collect information from 26 heads of households about trypanosome-attributable deaths that had

occurred within the last one year. Buffy coat technique and giemsa thin blood films were employed to check for trypanosomes in blood collected from 163 cattle examined clinically by a veterinarian. Of this number, 63 were reported ill by their owners and 98 had no report of illness. Haematological indices were also determined. Results indicated that 9 out of 26 (35%) heads of households interviewed reported death of cattle in their herds. Mortality and case fatality rates were 15.7% and 7.1%, respectively, while trypanosome prevalence was 34.4%. The morbidity manifested by emaciation was 66.9%, anaemia and leucopenia characterized by low PCV and WBC counts were, respectively, 27.49 ±5.59 % and 28.04 ± 5.46%; 3.36 ± 1.02 $x10^9$/L and 3.78 ± 0.76 $x10^9$/L in trypanosome-positive and trypanosome-negative animals. Trypanosomosis was more likely to occur in emaciated animals than non-emaciated (OR=2.4434; 95% C.I.= 1.19<OR<5.02), however, the differences between the mean PCV and mean WBC counts of infected and uninfected cattle were not statistically significant (P> 0.05). It was concluded that trypanosomosis was the main cause of death of cattle in the KGR.

7.2 **Introduction**

The African continent, especially sub-Saharan Africa is saddled with the problem of poverty. From World Bank records, the human population in this region grew at an average annual rate of 3.1 per cent between 1980 and 1987; while the average annual per capita gross domestic product (GDP) declined by 2.6 per cent during the same period (World Bank, 1989). In addition, an estimated 100 million people in the region are hungry and to improve regional food security a

4.0 per cent annual increase in food production and a reduction in population growth are pre requisites (World Bank, 1989; Connor, 1989).

Tsetse-transmitted animal trypanosomosis is one of the major factors slowing the pace of rural development (FAO, 2002b). The disease depresses every aspect of livestock production including denial of the use of animal traction over wide areas. Consequently people have to rely on manual tillage of the land, which is associated with much lower crop yields and poorer food production (Connor, 1989).

In the KGR located in southern Kaduna, Nigeria, livestock development has recorded only few signs of improved production in the past two decades (NLPD, 1988). The limiting factors responsible for this lack of success have been identified, prominent among which are livestock diseases, particularly trypanosomosis. With emphasis on poverty reduction, control of African animal trypanosomosis therefore has a high priority in rural development programmes. Thus following reports of deaths among cattle in the KGR, the study was initiated to identify possible cause of disease and death and proffer solutions to improve animal health and livestock development in the reserve.

7.3 **Objective**

To describe bovine mortality and morbidity and associate them with the presence of trypanosomes.

7.4 **Materials and Methods**

7.4.1 **Study area**

Kachia Grazing Reserve (KGR) is situated between latitudes 10^0 03^1 and 10^0 13^1 N and longitudes 7^0 55^1 and 8^0 06^1. The land lies approximately 700-790m above sea level and is drained by the Kaduna river system and several streams that feed into them. KGR is a mixed farming system with many livestock species namely, cattle, sheep and goats. The vegetation is typical of the Northern Guinea Savanna woodland. However, because of the effects of annual bush fires, there are now many species of both the northern and the derived savanna zones found in the area.The study was carried out in the months of March and June 2004 following reports of trypanosome associated deaths in cattle in the KGR.

7.4.2 **Assessment of mortality and morbidity**

In describing bovine mortality and morbidity in the KGR and associating them with the presence of trypanosomes a questionnaire was used to collect information from the household heads about trypanosome-attributable deaths that had occurred in their different herds as well as clinical and microscopic examination of blood samples and blood films for establishing the presence of trypanosomes in the animals their owners reported of illness and from apparently healthy ones. Altogether twenty-six (26) heads of households were interviewed using the questionnaire Appendix 4b). With regard to morbidity rate, blood samples were collected in herd their owners reported illness and

those not reported of any illness. Altogether, blood was collected individually from 163 cattle via the jugular vein. A veterinarian checked the animals clinically and blood samples collected were checked for the presence of trypanosome infections using the buffy coat/dark ground method (Murray *et al*., 1977). Packed cell volume (PCV) was determined with a microhaematocrit centrifuge and total and differential white blood cell counts were also carried out using the improved Neubauer haemocytometer (Schalm *et al*., 1975). The mortality rate given by crude death rate is the total number of deaths occurring in a specified population during a specified time period divided by the average number of individuals in that population during the specified time period. The case fatality ratio that occurred during the periods of the investigation was given by

$$= \frac{\text{No. of new deaths recorded}}{\text{Total number of animals with cases (infected) during the specified time period.}}$$

7.4.3a **Preparation of thin film**

A drop of blood (EDTA anti coagulated blood) was placed on the end of a clean dry slide using a capillary or Pasteur pipette. The blood was allowed to extend along the edge of the spreader and was spread in such a way as to make a thin film with a smooth tail. The spreader was held at an angle of about 30^{o} and the drop of blood was spread to make a film about 40-50mm in length (two thirds of the slide). The animal detail was written on the end of the slide and the blood film was dried. When completely dried and within a few minutes of making the blood film, the blood was fixed in absolute methanol for about 2 minutes.

7.4.3b **Staining Thin Blood film by Leishman stain**

The pre-fixed dried blood film was covered with undiluted Leishman stain using a dropper bottle. Twice the volume of buffered water pH 6.8 was added to the stain to dilute it. Care was taken to ensure that the diluted stain did not overflow and that the water was well mixed with the stain. It was then allowed to stain for 10 minutes. Thereafter the stain was washed off with tap water and the back of the slide was wiped clean and allowed to stand in a draining rack for the smear to dry. The blood film should appear neither too pink nor too blue microscopically.

7.4.4a **White cell count examination**

A white blood cell (WBC) count is used to investigate infections and unexplained fever and to monitor treatments that can cause leucopoenia. In most situations when a total WBC count is requested it is usual to also perform a differential WBC count. The principle of the test lies in the fact that whole blood (EDTA anti coagulated) is diluted 1 in 20 in an acid reagent which haemolyses the red cells, not the nucleus of nucleated red cells, leaving the white cells to be counted. White cells are counted microscopically using an improved Neubauer ruled counting chamber (haemocytometer) and the number of WBCs per litre of blood calculated. A suitable diluent for the purpose of lysing erythrocytes is 2% glacial acetic acid tinged with gentian violet. Besides destroying the erythrocytes, it will also stain the white cell nuclei (Baker *et al*., 2001).

7.4.4b **Differential white blood cell count**

A differential white blood cell count (WBC) provides information on the different white cells in

the circulating blood, i.e. neutrophils, lymphocytes, monocytes, eosinophils, basophils (rarely seen). Provided the total WBC count is known, the absolute number of each white cell type, i.e. the number of each cell per litre of blood can be calculated and an assessment made of whether the number of a particular cell type is increased or decreased (compared with the accepted reference range).

Steps followed in staining of thin blood film have already been described. After the blood has completely dried it was examined. A drop of oil immersion was placed on the lower third of the blood film and covered with a clean cover glass. The film was then examined microscopically by focussing the cells using 10x objective with the condenser iris closed sufficiently to see the cells clearly and then later use was made of 40x objective to allow more opening of the iris diaphragm. The blood film was examined systematically and the different white cells seen in each field were counted using an automatic differential cell counter. Thereafter the absolute number of each white cell type was calculated by multiplying the number of each cell counted (expressed as a decimal fraction) by the total WBC count.

The blood sample was diluted by washing 50µl of blood into a 950 µl of diluent to give a final dilution of 1 in 20. The dilute sample was then mixed and loaded into the counting chamber. Using the Improved Neubauer chamber, the white cells present was counted in the 4 corner $1mm^2$ areas and those in the central $1mm^2$ area (Baker *et al.*, 2001). The same margin rule was applied for including or excluding cells lying on the peripheral lines.

7.4.5 **Statistical analysis**

Calculation of odds ratio and 95% confidence interval on animals reported sick by the herdsmen and apparently healthy ones were used to assess whether: (i) emaciation (progressive loss of body condition) resulted in greater risk of disease; (ii) animals reported sick resulted in a greater risk of trypanosomosis infection. The student T-test was also carried out to compare the mean WBC and differential counts of infected and uninfected animals.

7.5 **Results**

7.5.1 **Analysis of mortality**

Nine (9) out of 26 heads of households interviewed, i.e. 35% of respondents informed of having lost a total of 38 cattle within the last one year. At the time the team first visited in March 2004, the nine heads of households that reported deaths of cattle had a total of 238 cattle and 244 as at June 2004 owing to new births recorded. Also, four (4) deaths were recorded between the periods under review (Table 7.1). The crude death rate as calculated was 15.7%, though the cause of deaths was unknown and the case

fatality was 7.14%.

Table 7.1: Number of households that reported mortality in the KGR (March 2004)

House holds	Deaths recorded within one year before the study	Size of herds (March 2004)	Size of herds (June 2004)	New births	Deaths between March and June
1	3	19	16	-	3
2	8	52	55	3	0
3	6	25	27	2	0
4	2	10	12	2	0
5	5	31	31	0	0
6	4	17	17	0	0
7	3	28	29	1	0
8	5	45	46	1	0
9	2	12	11	1	1
Total	**38**	**239**	**244**	**10**	**4**

7.5.2 Analysis of morbidity

7.5.2a **Physical examination of body condition and parasitological diagnosis**

Of 163 cattle examined, 109 (66. 9%) were emaciated. Out of this, 38.5% were positive for trypanosomes and 61.5% were negative. Furthermore, 54(33.1%) of the animals were certified as satisfactory by the Veterinarian, of which 14 (25.9%) were trypanosome-positive while 74.1% were negative for trypanosomes. Altogether, 56 cattle were trypanosome-positive giving a 34.4% prevalence rate (Table 7.2). However, test of association of trypanosomosis infection was not dependent on the body condition of the animals investigated at 5% level (χ^2= 2.02, p>0.05). The odds ratio of the physical examination at 95% confidence interval showed an association between emaciation and occurrence of trypanosomosis, though not significant (OR=1.79, 95% C.I= 0.820 - 3.93). Also, results as presented in Table 7.2 showed that 30 (53.6%) of the 56 positive cases diagnosed were reported ill by their owners while 46.4% was not reported ill prior to examination but was detected with trypanosomes in the course of our investigation.

Table 7.2: Morbidity in the KGR (March and June, 2004)

Examination	Tryps. Positive	Tryps. Negative	Total
Emaciated	42	67	109
Satisfactory	14	40	107
Total	56	107	163

Animals with emaciation are more likely to have the disease than animals with satisfactory body condition and there is no association between the factor and the disease (OR = 1.79, 95% C.I = 0.82 – 3.93).

Table 7.3: Cattle Reported sick by their owners and apparently healthy ones

(March and June, 2004).

	Sick	Apparently healthy	Total
Trypanosome-positive	30	26	56
Trypanosome-negative	35	72	107
Total	65	98	163

The animals reported sick are twice more likely to have trypanosomosis infection than the apparently healthy ones and there is association between the factor and the disease (OR = 2.37, 95% C.I.=1.16 – 4.86; x^2= 5.83; P value= 0.015).

7.5.2b Analysis of PCV and total WBC and differential leucocyte counts

The PCV, the total and differential white cell counts were assessed in order to determine proportional changes in the red blood cells and these leucocyte types relative to normal reference values. The results showed the parameters tested to be generally low. The mean PCV values were 27.49 $\pm$ 5.59% and 28.04 $\pm$ 5.46% in infected and uninfected animals respectively but the results were not statistically significant (P > 0.05). But the PCV even declined to below 19 in some animals, suggesting severe anaemia in those animals (data not shown).

Similarly, the animals were leucopenic as shown by decreases in mean total WBC counts, which were 3.36 $\pm$ 1.02x10^9/L and 3.78 $\pm$ 0.76 x10^9/L respectively for trypanosome-positive and trypanosome-negative animals. The results though slightly lower in trypanosome-positive animals compared with the negative counterpart were not statistically significant (P> 0.05). In addition, the results of the mean differential white cell counts in trypanosome-positive and trypanosome- negative animals followed a similar trend and were respectively; Neutrophil (2.34 $\pm$ 0.48 x10^9/L as against 2.35 $\pm$ 0.40 x10^9/L); lymphocyte (1.18 $\pm$ 0.40 x10^9/L as against 1.12 $\pm$ 0.43 x10^9/L); monocyte (0.19 $\pm$ 0.09 x10^9/L as against 0.14 $\pm$ 0.03 x10^9/L) and eosinophil (0.83 $\pm$ 1.23 x10^9/L as against 0.17 $\pm$ 0.07 x10^9/L). Comparison of the results between trypanosome-positive and trypanosome-negative animals showed no statistical significance difference at 5% level (P> 0.05). Also the test of association revealed no statistical association (P > 0.05) (Table 7.4).

Table 7.4: Mean WBC and differential leukocyte counts of cattle reported sick by their owners and apparently healthy ones (March and June, 2004).

Total blood counts		$0 \pm$ sd	t	df	p-value	χ^2	df	p-value	
WBC x 10^9/L	+ve	3.36 ± 1.02	-0.65	2	0.58 NS	0.41	2	0.8146	
	-ve	3.78 ± 0.76							NS*
Neutrophil	+ve	2.34 ± 0.48	-0.02	2	0.98 NS	0.57	2	0.7520	NS
x 10^9/L	-ve	2.35 ± 0.40							
Lymphocyte	+ve	1.18 ± 0.40	0.00	2	1.00 NS	1.65	2	0.4382	NS
x 10^9/L	-ve	1.12 ± 0.43							
Monocyte	+ve	0.19 ± 0.09	0.92	2	0.45 NS	0.01	2	0.9950	NS
x 10^9/L	-ve	0.14 ± 0.03							
Eosinophil	+ve	0.83 ± 1.23	0.93	2	0.45 NS	0.08	2	0.9610	NS
x 10^9/L	-ve	0.17 ± 0.07							
Body	+ve	28.01 ± 19.80	-1.31	1	0.41 NS				
condition	-ve	53.51 ± 10.10							

*NS = not significant.

7.6 Discussion

In this study infection of cattle with pathogenic trypanosomes resulted in a case fatality rate of 7.1% between the months of March and June 2004 when the team visited. The mortality rate as determined from the questionnaire administered at the onset was 15.8%. The finding of case fatality rate of 7.1% corroborates the report of severe trypanosomosis in the KGR recorded in the past, which presumably resulted in deaths of the animals (NLPD, 1988). Although the major cause of deaths in animals infected with pathogenic trypanosomes remains obscure, however, evidence of biological active materials (BAM) released by trypanosomes, immune complexes and micro-vascular injury as possible pathogenetic mechanisms for trypanosomosis have been presented (Kobayashi and Tizard, 1976; Tizard *et al*., 1978b; Edeghere, 1980).

The study recorded tryopanosome prevalence of 34.4%. Of this percentage, 53.6% was reported sick while 46.4% was not reported sick before our investigation began. From our results, there was an association between the animals reported sick by the herdsmen and trypanosomosis infection at 5% level of significance (χ^2 =6.21, $p<0.05$). It appeared that the report of herdsmen about the health problem of their animals could be very much relied upon for making decisions on the management of animal trypanosomosis in the reserve.

Anaemia manifested by low PCV values were observed in the animals investigated. This finding supports the findings of (Kobayashi *et al*., 1976;

Tizard *et al.*, 1978a; Esievo and Saror, 1991; Waiswa and Katunguka-Rwakishaya 2004) which observed that emaciation and anaemia are important clinical features of trypanosomosis. Also, WBC and differential counts were decreased and the observed decrease is consistent with leucopenia, a known feature of trypanosomosis in the acute phase (Anosa, 1988). Emaciation characterized by weight loss or growth failure despite good appetite may be attributed to combine effect of reduced feed utilization and impaired efficiency of conversion. Failure to detect trypanosomes in 67 out of 109 (61.5%) emaciated animals could be ascribed to either poor sensitivity of the diagnostic technique used or fluctuating parasitaemia. The odds ratio result which indicated that trypanosomosis was more likely to occur in emaciated than animals satisfactory (OR=1.79, 95% C.I.= 0.82-3.93, $\chi 2$ = 2.02, p>0.05) suggests that emaciation is a good indicator of trypanosomosis under field condition but there was no association between the factor and the disease.

It is evident from the foregoing that African animal trypanosomosis poses a serious risk to animal health and is the main cause of death of animals in the KGR. Possible factors that led to the problem of trypanosomosis are inadequate surveillance and control activities. In order to reduce reports of disease and deaths and consequently improve animal health in the reserve, active surveillance through case detection, treatment and vector control where appropriate are urgently recommended. To this end, use of more sensitive diagnostic tools to complement the buffy coat technique towards improved case detection that will allow a better management decision is advocated.

CHAPTER 8

8.0 Molecular characterization of bovine trypanosomosis in the KGR

8.1 Summary

Until recently the detection and characterization of pathogenic African trypanosomes by molecular methods rely on species-specific primers, warranting several PCR tests on

each sample owing to diverse species of the pathogenic trypanosomes. The cost is enormous and is a draw back for such studies. Primers (Kin1 and Kin 2) designed to amplify the internal transcribed spacer 1(ITS1) of rDNA and primarily to serve as a universal diagnostic test for all pathogenic trypanosomes have been previously evaluated for PCR diagnosis specific for kinetoplastids and shown to detect and differentiate the *Trypanosoma brucei* complex and *T. cruzi* as well as livestock trypanosomes. Here the primers (Kin1 and Kin 2) were further evaluated. Blood collected from 121 cattle of which 35 were positive for trypanosomes and 86 negative and 3 positive blood specimens from sheep were used for the investigation. The PCR assay allowed detection and characterization of three *Trypanosoma* livestock species namely *T. vivax, T. b. brucei and T. congolense* forest based on size of the PCR products obtained using the kin primers which were 147bp, 480 bp and 710 bp respectively. The kin primers detected cases of mixed infections and appeared very sensitive (100%) in detecting the one *T. brucei, T. congolense* and one mixed-infection of *T. congolense* and *T. vivax* and another mixed infection of the same multi-taxa not shown by the buffy coat.

8.2 **Introduction**

Animal trypanosomosis is a significant major health problem in sub-Saharan Africa including Nigeria. The disease is considered the most important vector borne bovine disease transmitted by vectors in this region (FAO, 1996). Although three major trypanosome species, *Trypanosoma vivax*, *T. congolense* and *T. brucei* cause the disease in bovine and had been documented, their distribution is not precisely elucidated due to limitations in detection and identification techniques. The parasitological detection of trypanosomosis, which is based on the detection of trypanosomes in blood of animals, has a low sensitivity and is hampered by fluctuating parasitaemia (Truc *et al*., 1994). Although several serological tests have been developed, many proved much more sensitive than the parasitological diagnosis (Desquesnes, 1996; Kayang *et al*., 1997; Eisler *et al*., 1998); as also seen in the present study. The existence of sero positives but parasitologically unconfirmed subjects may have epidemiological implications. Some of them might actually harbour the parasite and thus contribute to the persistence of infection and of the foci (Solano *et al*., 1999). Since bovine trypanosomosis is fatal if untreated and more especially since the drugs for curative treatment are costly besides the problem of toxicity, reliable diagnosis is of tremendous importance.

Polymerase chain reaction (PCR) has been introduced for detection of trypanosome DNA in biological samples. In animal trypanosomosis, PCR has been used as a tool allowing precise identification of the infecting trypanosome taxa (Majiwa *et al*., 1994), or to detect trypanosomes in the blood (Katakura *et*

al., 1997; Clausen *et al*., 1998; Solano *et al*., 1999). PCR has also been used on humans as a more sensitive alternative to parasite detection for primary diagnosis of *Trypanosoma brucei gambiense* infection (Simo *et al*., 1999; Kyambadde *et al*., 2000; Penchenier *et al*., 2000), or for stage determination of sleeping sickness by amplifying trypanosome DNA from the cerebrospinal fluid (Truc *et al*., 1999; Kyambadde *et al*., 2000). The present study was initiated in order to characterize the trypanosome species and sub-species that circulate in bovine and evaluate the possibility of realizing molecular epidemiological studies in the KGR.

8.3 **Objective**

To characterize trypanosome species circulating in the KGR using the polymerase chain reaction (PCR).

8.4 **Materials and Methods**

8.4.1 **Origin of samples**

During an epidemiological survey carried out in February 2005 in the KGR (latitudes 10^{o} 03 ◌́ and 10^{o} 13 ◌́ N and longitudes 7^{o} 55 ◌́ and 8^{o} 06 ◌́ E), in which 228 blood samples were selected at random from cattle, dried blood sample of animals found positive for trypanosome or for every individual animal with a haematocrit value below 30% were collected on filter paper. The six serum samples from sheep evaluated were obtasined from one of the households' sample that had sheep in its herd. Examination by buffy coat and giemsa thin film showed that 36 (15.8%) cattle and 3 (50%) sheep were found infected by trypanosomes. Furthermore, dried blood samples of 86 trypanosome-negative cattle and one trypanosome-negative sheep whose haematocrit fell below 30% were subjected to PCR assay.

8.4.2 **Origin of primers**

Drs. Marc Desquesnes and Gérard Cuny of the International Laboratory for Research

and Development (IRD), Montpellier, France provided primers used in this work. Sequences were by Barbara Descamps and M. Desquesnes. The internal transcribed spacer-1 (ITS-1) of ribosomal DNA primers was used. For the first PCR, the sequences of primers TRYP 18.2C and TRYP4R are respectively 5' G C A A AT T G C C C A A T G T C G 3 '

5' G C T G C G T T C T T C A A C G A A3'

For the second PCR, the sequences of primers were IRFCC and TRYP% RCG respectively 5' C C T G C A G C T G G A T C A T 3'

5' A T C G C G A C A C G T T G T G T 3'.

8.4.3 Preparation of blood samples

Blood was collected in heparinized vacutainers during the epidemiological survey and processed as follows. Blood was spotted on filter paper, a small piece (about $5mm^2$) of filter paper with a 10µl blood spot. Blood of all parasitologically positive cattle and sheep were individually spotted on filter paper, allowed to dry and stored at -4^oC until required. In a similar manner, samples of 88 negative cattle, which were selected at random, were also individually spotted on filter paper.

8.4.4 Preparation of samples and PCR assay

Dried blood sample from animals on filter paper was initially soaked in 0.5% saponin (Sigma, USA) in PBS pH 7.2, put in a 1.5 ml micro centrifuge tube for removal of red blood cells and allowed to stand for 5-10 min on ice. The resultant cell was added to 200 µl of 5% chelex-100 (Bio-Rad, USA) in distilled water, incubated at 56^oC for 1h and subjected to boiling water for 30min for genomic DNA extraction (Penvhenier *et al*., 2000). The chelex was removed by centrifugation at 10,000 rpm for one min and the resultant supernatant was put in an eppendorf tube and stored at -20^oC until required.
The principle of ITS1 primers is that the ITS-1 is situated between genes 18S and 5.8S, with primers hybridising in conserved regions of 18S and 5.8S genes, PCR allows amplification of the ITS-1 which length is variable amongst Trypanosoma species but constant among each species.

For PCR amplification, the test was carried out in 25µl reaction mixtures containing 10x reaction buffer (670mM Tris-HCL) pH8.8; 166mM $(NH_4)_2$ SO4, 4.5% TritonX-100, 2mg/ml gelatine) (Promega, UK), 2mM $MgCl_2$, 200µM of

each of the four deoxynucleoside triphosphates (dNTPs), primers at 1µM, 2µl aliquot of chelex isolated DNA solution and 0.5U of Taq DNA polymerase (Promega, UK). The DNA was first denatured at 96°C for one min, and then it was subjected to 30 cycles consisting in one min at 96°C, 50°C 40s, 72°C for one min, followed by a terminal elongation for 7 min at 72°C. The amplification was performed in duplicates to ensure reproducibility and a positive control (with reference DNA was added in each type of trypanosome) and a negative control (without DNA) was added in each PCR assay. When the amplification reaction gave a signal of the expected size of DNA with primers used, without signal in the negative control, the trypanosome was considered as characterized. Amplification products were resolved in 2% molecular grade agar rose (Fischer Biotech).

8.4.5 **Sequencing**

PCR products were purified from the agar rose using a freeze-squeeze method. Briefly, the PCR band was cut from the gel and frozen at -20°C for 4h. The frozen gel slice was placed between Parafilm, squeezed and approximately 50 µl of liquid collected. The collected volume was mixed with 1µl of 3 M sodium acetate and 100µl of 70% ethanol, and then left on ice for 20 min. The mix was then centrifuged at 14,000 rpm for 10 min, the supernatant discarded and the resulting pellet vacuum-dried and re-suspended in 10 µl PCR water. A total of 1µl of this final sample was used in running the sequencing PCR reaction using either forward or reverse ITS1 primers. Sequencing was done in an ABI automatic DNA sequencer (Applied Biosystems).

8.4.6 **Alignment**

The sequences obtained were blasted using the basic BLAST search tool (http://www.ncbi.nlm.nih.gov/BLAST/) and alignment made with specific ribosomal products found in the GenBank using DNAman (Lynnon Biosoft,

Canada).

8.5 Results

8.5.1 Detection of *T. congolense*, *T. brucei* and *T. vivax*

Thin blood smears examinationof the 36 cattle and 3 sheep in which microhaematocrit method detected trypanosomes in the buffy produced 2 of *T. congolense* and 1 mixed infection of *T. congolense* and *T. vivax* sub- species and 33 of *T. vivax* in cattle, in sheep, 2 of *T. vivax* and one of *T. brucei* infections. Using the ITS1 primers, species-specific PCR amplifications were detected. The PCR revealed 1 of 2 *T. congolense* riverine forest type and the other one it detected as *T. vivax*. The one mixed-infection was detected the way it was as *T. congolense* and *T. vivax* (100%) plus another mixed infection of *T. congolense* and *T. vivax*, which was not revealed by the buffy coat and 13 of 33 (39.4%) were detected as *T. vivax*. Finally, in the 86-trypanosome negative thin film smears, 82 (95.3%) were still negative for trypanosomes and they are the 'true negatives' while 4 (4.7%) were detected with *T. vivax* infection. Overall the sensitivity of PCR was 42.9% and specificity was 95.3%. These results indicated that the PCR was very sensitive in detecting and characterizing *T. brucei* and *T. congolense* infections but not so much for *T. vivax* in that the 59.4% false negatives as recorded in this assay were due to *T. vivax*. However, the PCR test is highly specific because the specificity obtained in this result was 95.3% and the kin primers are suitable in type characterization of livestock isolates through a single PCR.

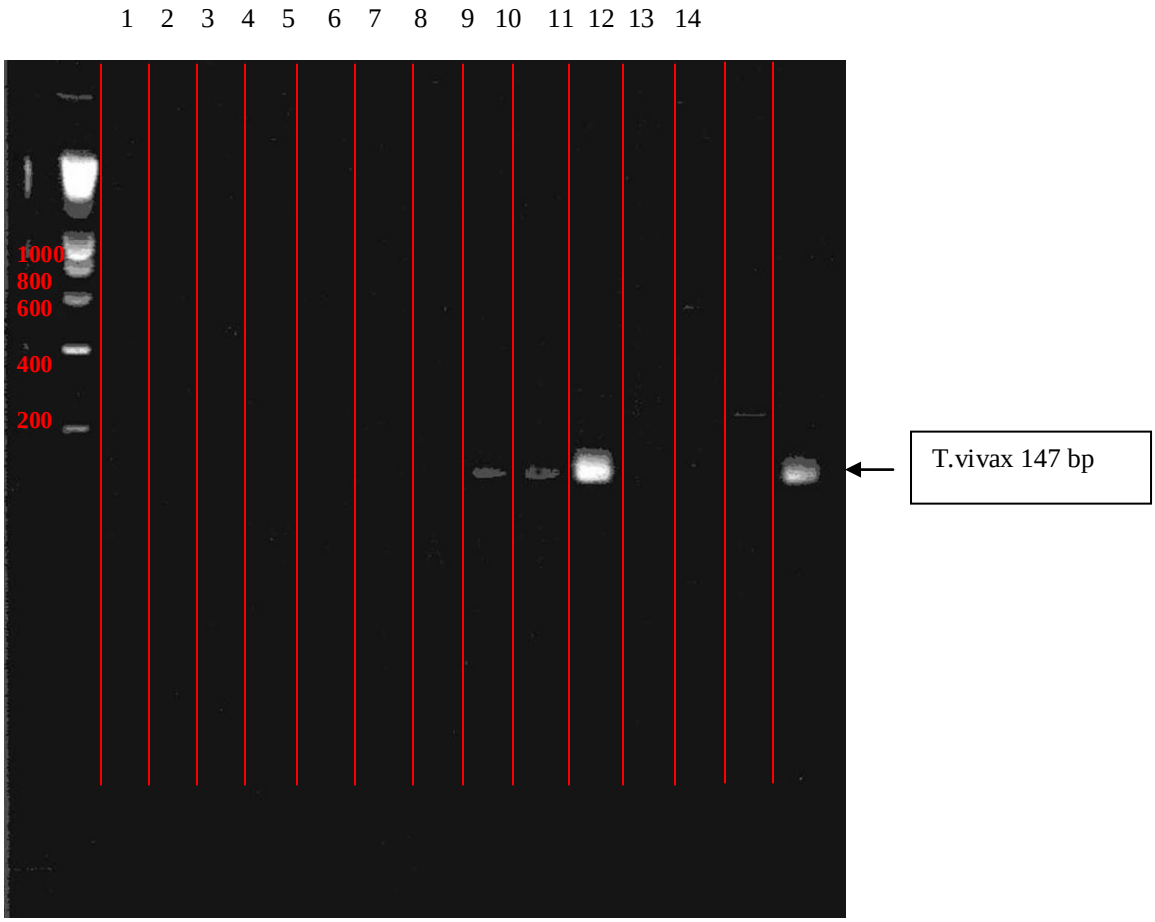

Fig. 13: Lanes of PCR assay

DNA marker: smart leader (eurogentec)

1: negative control

2: B3H4 11

3: B3H5 11

4: B3H4 14

5: B3H4 8

6: B1H2 2

7: B1H2 15

8: B1H2 12

9: B1H2 38

10: B5H1 11

11: B5H1 39

12: B3H6 7

13: Sheep 6

14: positive control

T. congolense 12.14

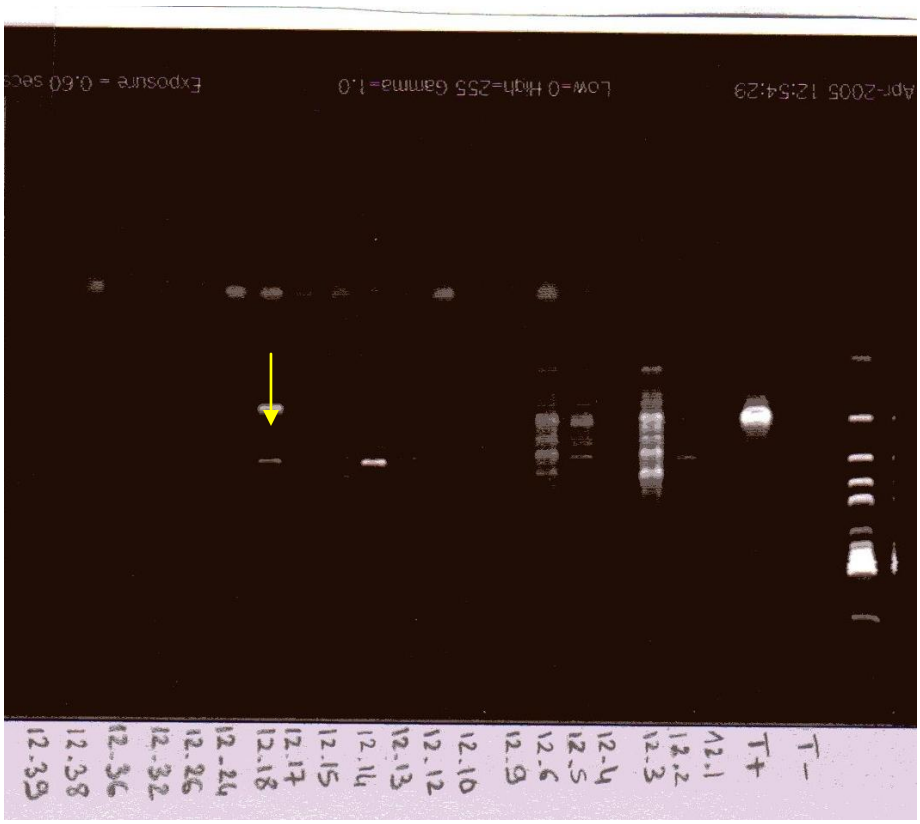

Fig. 14: Lanes of PCR assay

8.6 **Discussion**

This study has shown that PCR was a useful and specific test (95.3%) for characterization (typing) and detection of *Trypanosoma* species. The PCR characterized *T. b. brucei*, the riverine forest of *T. congolense* and *T. vivax*. These subspecies and strains are the types circulating in the KGR. This clarifies one of the objectives that sought to detect and characterize the trypanosome species in the study area, a task that was hitherto difficult to perform.

With regard to sensitivity, the PCR appeared very sensitive (100%) in detecting the one *T. brucei* and mixed-infections of *T. congolense* and *T. vivax*, though the sample size for both was too small to draw specific conclusion to the results. As for the two parasitologically diagnosed *T. congolense* infections, for which one was confirmed *T. congolense* forest and the other *T. vivax* by PCR, which was contrary to parasitological findings, was difficult to explain. In respect of *T. vivax*, the PCR test appeared not sensitive enough in that the test detected only 13 of 32 (40.6%) of the infections as true positives while the rest 59.4% were classified as false negatives by the kin primers. A number of explanations could account for this wide difference in the results. It has been reported that false negatives could represent recently infected individuals in whom few number of parasites in the initial sample were often present. It could also result from DNA extraction processes in which case the samples could not contain enough DNA to allow for a positive result (Solano *et al*., 2002). Furthermore, it could be that sample collection; storage, DNA extraction and inhibitors affected the result through insufficient detection limit. Indeed, several workers had raised

observations that wet blood or buffy coat instead of dried blood on filter paper is preferred because the latter system had shown poor sensitivity and reproducibility ((Solano *et al*., 2002). In addition, heparin is reported to be more appropriate than EDTA when it is intended to perform PCR on blood sample based on studies that had compared heparin, EDTA and citrate (Burchardt, 1994; Solano *et a*l., 2002). In this study blood collected on EDTA and filter paper dried blood were used because EDTA is a cheap anti coagulant and filter paper collected blood eases a great deal of storage difficulty and transport problems. About DNA extraction and inhibitors, it has been reported that the presence in blood of components that inhibit PCR amplification has been a major problem associated with direct amplification of DNA in lysates (Higuchi, 1989). Above all, it could be that the primers of ITS1 used were not very sensitive in detecting *T. vivax*. For instance, Desquesnes *et al*. (2001) and Njiru *et al*. (2004) had shown that ITS1 primer appeared very sensitive in detecting and identifying *T. congolense* and *T. brucei* but less sensitive for *T. vivax*.

On the whole, the overall sensitivity of the PCR in this study was 42.9% and its specificity 95.3%. While the specificity was very good there are pitfalls with sensitivity especially with *T. vivax* detection. Further studies are needed using ITS1 and species-specific *T.vivax* primers to confirm or refute these preliminary results with *T. vivax* detection. In addition, improvement of DNA preparation and new primer designs are important considerations for enhancement of *T. vivax* detection in field samples.

CHAPTER 9

9.0 CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusions

The epidemiology of animal trypanosomosis was conducted in selected months from 2004-2005 at the Kachia Grazing Reserve of Kaduna State. The aim was to assess the extent and nature of trypanosomosis in the grazing reserve using effective diagnostic tools. For poverty alleviation and reduction to be successfully tackled, effective and accurate diagnosis of infection in livestock is paramount in order to offer quality animal health services towards improved animal production. Trypanosomes present a very significant group of parasitic infections and each year over 3 million cattle and other domestic livestock are lost through deaths caused by the parasites, which are tsetse-borne (FAO, 2002). Similarly losses due to tsetse-transmitted trypanosomosis in crop and livestock production amount to over US$ 4 billion annually (Swallow, 2000). It became imperative to ascertain these objectives, among others, (i). tsetse species, vector competence and population density in the KGR; (ii). Prevalence of animal trypanosomosis in the KGR and (iii) characterize by molecular methods the trypanosome isolates circulating in the KGR. From the results obtained the following conclusions were drawn:

9.1.1 Tsetse flies are still present in the grazing reserve. Two tsetse species, namely, *Glossina morsitans submorsitans* and *Glossina tachinoides* were found during this study. One *G. tachinoides* was found infected with *T. vivax*. Other biting flies of veterinary importance, such as tabanids, stomoxys, chrysops and pangonia, are also present in the grazing reserve and together with tsetse they

contribute to the spread and maintenance of trypanosomosis in the area.

9.1.2 Transmission of trypanosomosis in the KGR may be both cyclical and mechanical, given the *T. vivax* and other biting flies of veterinary importance found in the reserve. In addition, the study was able to establish vector competence, though the infection rate observed was low (5.5%). Tsetse apparent density was also low.

9.1.3 Intra block variations in tsetse catches were observed. Blocks 2 and 3 had flies caught during the March and June surveys, though block 3 recorded a higher number of flies caught. Only block 4 had tsetse caught during the August survey.

9.1.4 Factors that may have influenced the low tsetse density are limited days of trapping due to logistics and the effect of human activities, such as, bush burning and felling of trees.

9.1.5 Cattle movement appeared to influence the prevalence. Returned herds had a higher prevalence of 16.7% compared with 11% of resident herds.

9.1.6 Proximity of herd pens to watering point distances was associated with increased prevalence of trypanosomosis in the animals but there was no association of herd pens to grazing point distances.

9.1.7 Intra block variations in prevalences, though not significant, were obtained with block 3 having the highest prevalence. Block 3 is very proximal to watering points (riverside).

9.1.8. Trypanosomosis awareness in the KGR was very high. All (100%) of the 27 heads of households interviewed said that they were aware of the disease, which they called 'samore'- the Fulani vernacular name for the disease.

9.1.9 Almost all (97.3%) the respondents interviewed said they treated their animals sporadically. One said he treated his animals regularly. But all (100%) informed that due to the high cost of trypanocidal drugs, priority attention was given to cattle in terms of treatment coverage. This practice may have influenced the higher prevalence obtained in sheep and goats than cattle in the grazing reserve.

9.1.10. Small ruminants (sheep and goats) are important hosts as well and are also exposed to the risk of tsetse and trypanosomosis just as cattle are. The prevalence overall of 17% recorded in sheep and goats in the KGR is a clear evidence of this.

9.1.11 The distribution of anti trypanosomal antibodies against a recombinant hsp 70/Bip antigen indicated that the distribution of cattle trypanosomosis was more widespread (65.9%) than the parasitological prevalence (6.1%) suggested.

9.1.12 Trypanosomes cause blood cells abnormalities. Anaemia shown by a decrease in the packed cell volume (PCV) was observed. Leucopenia and leucocytosis manifested by a drop and an increase respectively in total white blood cell and differential counts relative to normal were also observed.

9.1. 13 Trypanosome isolates circulating in the KGR have been characterized and shown to be *T. brucei brucei*, *T. congolense* forest and *T. vivax*.

9.1.14 The Kin1 and Kin 2 primers used in the PCR assay were very suitable for isolate typing/characterization through a single PCR thus saving costs in terms of money and time of having to run several PCR tests for each sample considering the different trypanosomes species in trypanosomosis endemic areas.

9.1.15. While kin primers appeared very sensitive for detection of *T. b. brucei* and *T. congolense*, the primers were not sensitive enough for detection of T. vivax.

9.2 **Recommendations**

The recommendations from the studies are detailed as follows:

9.2.1 Further research needs to be done to determine the breeding sites of tsetse in the KGR.

9.2.2 Use of more sensitive tools (serology) in conjunction with the classical parasitological techniques (such as buffy coat method) should be employed to enhance trypanosomosis detection.

9.2.3 The hsp 70/bip recombinant antigen that proved very useful in detecting circulating anti trypanosomal antibodies in this study should be validated in a variety of field studies conducted in numerous locations in order to ascertain its diagnostic utility.

9.2.4 The use of the polymerase chain reaction (PCR) for livestock trypanosomosis diagnosis needs to be studied extensively using new primer designs.

9.2.5 The strengthening of NITOR in planning, monitoring and trypanosomosis surveillance is important. This obviously will involve a need for increased funding.

9.2.6 Since susceptible Zebu cattle are the predominant breeds in the reserve and KGR lies in the morsitans belts, this study strongly advocates effective use of chemotherapeutic and chemoprophylaxis as well as vector control measures to fight the problem of trypanosomosis and allow permament raising of livestock in the reserve, thus encouraging sedentarisation.

9.2.7 There is an urgent need for active surveillance and systematic elimination of tsetse flies and other biting flies from areas where they are endemic in Nigeria including the grazing reserve.

9.2.8 For the KGR if government intervenes there is need for ground spraying of insecticides in selected blocks (2, 3, 4 and 5) as a short-term measure. This will effectively control tsetse and other biting flies of economic importance in the reserve towards improved animal health and livestock productivity.

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Appendix1: Classification of tsetse fly into three sub genera based on morphological differences in the structure of the genitalia (adapted from Davies, 1977).

Morsitans Group

Glossina longipalpis

Glossina morsitans

Glossina pallidipes

Glossina austeni

Glossina swynnertoni

Palpalis Group

Glossina palpalis

Glossina fusipes

Glossina tachinoides

Glossina pallicera

Glossina caliginea

Fusca Group

Glossina fusca

Glossina nigrofusca

Glossina mediocorum

Glossina tabaniformis

Glossina brevipalpis

Glossina frezlli

Glossina severini

Glossina haningtoni

Glossina fuscipleuris

Glossina vanhoofi

Glossina nashi

Glossina longipennis

Appendix 2: Summary of ecological zones in Africa (adapted from Janke, 1982).

Characteristics	**Ecological zones**				
	Arid	Semi-arid	Sub-humid	Humid	Highlands
Area (1000 km^2)	8327	7050	4858	4137	930
Rainfall (mm)	<500	500-1000	1000-1500	>1500	Variable
Moisture index	36	20	0-20	0	Variable
Growing days	<90	90-180	180-240	<240	Variable
Area of tsetse%	4.2	50.3	68.2	89.7	1.9
Predominant tsetse group	Morsitans	Morsitans, Palpalis	Palpalis, Fusca	Fusca. Palpalis	None

Appendix 3: Determination of animal sample size

Methods

Sample size

For the purpose of assessing objective 2, the sample size of the animals was determined before the various methodologies were applied.

Sample size:

$N = \frac{Z^2PQ}{L^2}$ where N = sample size, Z = 95% confidence level, = 1.96

P = prevalence, Q =1-P

L = allowable error of 3% (just 3% error from the real figure).

Prevalence, P is obtained by taking the average prevalence from works of Agu *et al* (1989) and Kalu (1991). P = (9% + 38%) /2 = 47%/2 =23.5% =24%. The prevalence was from non-outbreak situations. Although Kalu (1991) worked in Plateau State, the study area was near to Kaduna State and Agu *et al* (1989) worked in Kaduna State. It is thought that the mean of these two non outbreak situations will reflect the mean prevalence useful in obtaining the **minimum** required sample number in a non outbreak study as this.

Substituting in the above equation, $N = \frac{(1.96)(1.96)(0.24)(0.76)}{(0.03)(0.03)}$

$N = \frac{0.70070784}{0.0009} = 778.56 = 800$, correct to 3 sig. fig.

Appendix 4a: Questionnaire data on individual animals sampled

Family No. ( ) Number of animals ----------

Number of cattle ---------- Herd composition of cattle ----------

Herd No. ( ) Herd size (large >31)

Medium (11-30) Small (<10) ----------

Grazing pattern : Free or Restricted

Frequency of disease control application:

Regular (at least yearly); Sporadic (not every year); or Never (Please tick)

Breeding method: Natural only or artificial insemination (Please tick)

Supplemental feeding:concentrates and other items; salt only or none (Please tick)

What do they feed the animals with during the dry season? ----------

Animal No. ( )

Age ---------- Sex: Male ---------- Female ----------

Breed type: Zebu ---------- Taurine ----------, ----------

State of health/Body condition:thin/emaciated ----------or satisfactory-----

Mucous membrane: Pale ---------- or not----------

Nasal discharge ---------- or not ----------(Please indicate)

Location: Distance from where cattle are quatered to grazing point ----------

Distance from where cattle are quatered to watering point ----------

Movement of cattle along the river, stream, watering point etc ----------

(The geographic positions of each dwelling and the watering point or points (two at most) will be determined by global positioning system) (GPS) (Garmin TM).

Appendix 4b: Awareness of tsetse by Respondents:

Tsetse: Do you know what tsetse fly (Hausa: Kudan tsando) is? Yes/No (Please tick)

Have you ever seen tsetse fly? Yes/No ----------

Where can one find tsetse in your community? ----------

Does tsetse fly cause any harm? Yes/No/ Don't know (Please tick)

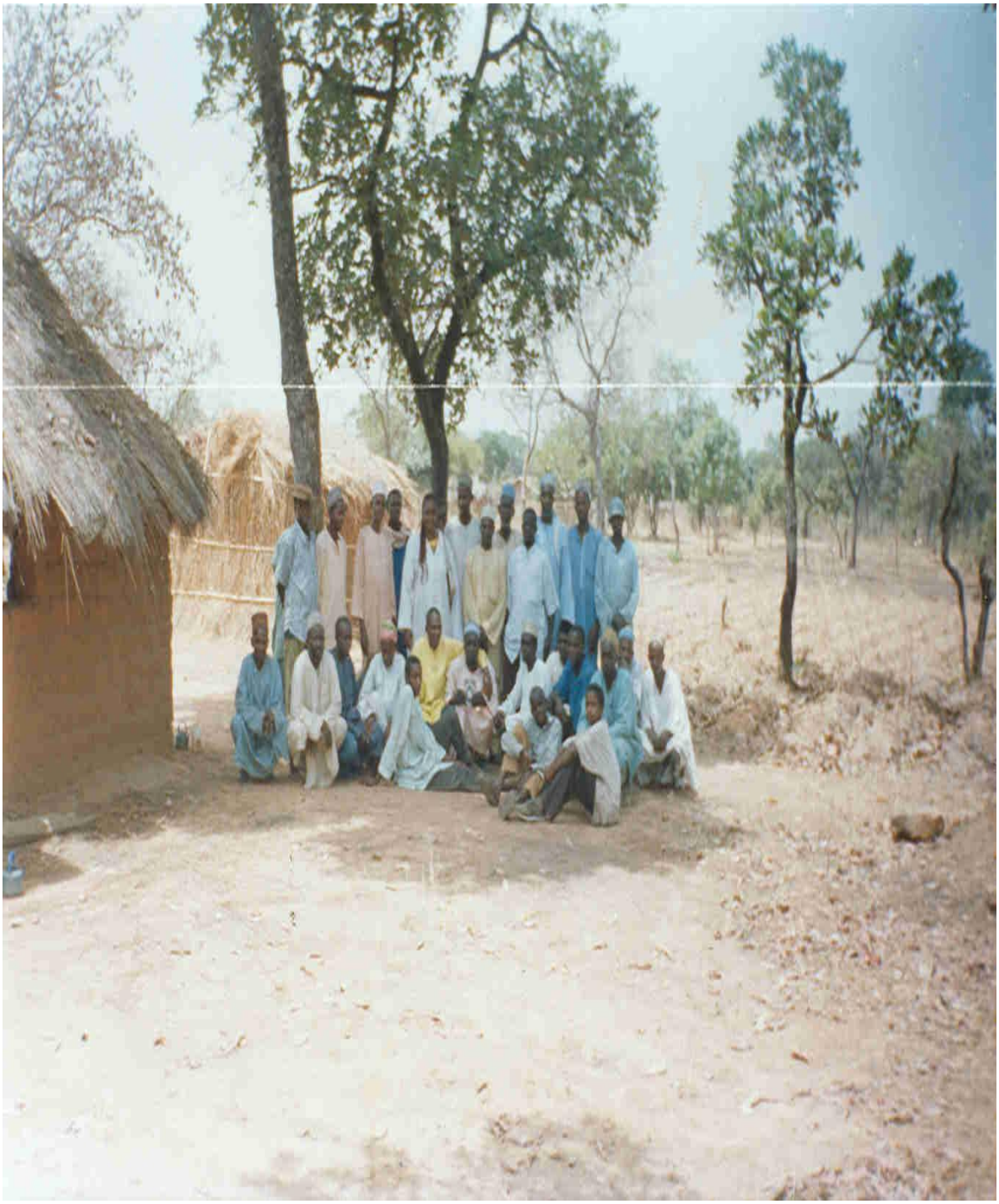
If yes, what harm does it cause in your community and your animals in particular? ----------

Please supply the following information on disease occurrence and death if any in your herd?

Disease	**Type of animal**	**No. of incidence**	**No of animals involved**	**When**	**Amount spent**	**No. of deaths**
Trypanosomiasis						
Rinderpest						
Brucellosis						
Blackquater						
Helminthiasis						
FMD						
Liver flukes						
Others specify						

Appendix 5: Picture taken with Ardos and some pastoralists in the

KGR



Appendix 6: Realization of sampling sites from coordinates obtained with

Global Positioning System (GPS)

The software Arcview 3.2 was used for the map of Nigeria. The data on the map come from DCW and DIVA showing rivers, altitudes etc. DCW and DIVA are free sites of map and geographic data on internet, http://www.maproom.psu.edu/dcw/. The coordinates were converted to decimal degree before integration into the software Arcview.

Appendix 7: Tsetse identification

Briefly explained, a collecting cage was used to collect the tsetse flies, then after each tsetse fly was placed in normal saline and viewed under the microscope. Size is a major feature of morphological identification as each species is slightly different. For *Glossina morsitans*, the middle of the dorsal surface is roughly square in shape, the bulb of the proboscis is dark brown and the last two segments of the tarsus of the hind leg are black socks. *G. tachnoides* is a small fly, also roughly square in shape and has a black stocking on the hind legs. The mouthparts and guts of fresh flies were dissected out and examined under the microscope for the presence of trypanosomes.

Appendix 8: Data ananlysis

i. The final WBC for the whole blood sample was counted as follows using the basic formula:

Number of cells counted (N) =200, dilution factor (DF) =20, area counted (A) =5mm2 and the depth of the counting chamber (D) =0.1mm.

$$WBC = \frac{200 \times 20 \times 10^6}{5 \times 0.1} = 8.0 \times 10^9/L.$$

Appendix 9: Analysis of Body condition

Body condition	Positive	Negative	Total
Emaciation	22	332	354 (27. 4%)
Satisfactory	87	852	939 (72. 6%)
Total	109	1184	1293

OR =0.65; CI = 0.39 – 1.09; x^2 = 2.72, P value = 0.0993. No statistical difference implying that animals are equally likely to be infected whether emaciated or satisfactory.

Appendix 10: Analysis by season (2004 and 2005)

Body condition	March		June		Feb. 2005	
	Positive	Negative	Positive	Negative	Positive	Negative
Emaciation	12	287	10	45	0	0
Satisfactory	0	230	52	429	35	193
Total	529		536		228	

OR=0.65, 95% C.I. = 0.39- 1.08; x^2 = 2.72, df= 1, p value = 0.0993.

Appendix 11: Sex Distribution of Trypanosomosis

Sex	Positive	Negative	Total
Cow	75	812	887
Bull	34	372	406
Total	109 (8.4%)	1184	1293

OR = 1.01, CI = 0.65 – 1.58, x^2 0.0, **P value**= 0.9528. Even though there was more infection in cows, there was no association between sex and trypanosomosis.

Appendix 12 Distribution of Positive animals by buffy coat and by

blocks

Blocks	Dry	Wet	Total
B1	6	0	6
B2	3	18	21
B3	18	33	51
B4	3	5	8
B5	17	6	23
Total	47	52	109

F= 0.19, d f=1, 8, P value = 0.672. Though the prevalences varied across the blocks with block 3 having the highest prevalence, the prevalences were however not statistically significant (t = 2.71; df = 4; P value= 0.054).

Appendix 13 Trypanosome Prevalence by watering point

distances

Km	*Positive*	*Negative*	*Total*
1	11 (91. 7%)	314 (60. 7%)	325
2	1 (8.3%)	142 (27.5%)	143
3	0 (0.0)	61 (11.8%)	61
Total	12 (2.3%)	517 (97.7%)	529

(x^2 for linear trend = 4.447, p-value= 0.03496).
There were greater infections in cattle that trekked 1km from herd pen to watering points, suggesting that trypanosome infections increased with proximity to watering points.
The linear trend statistical analysis performed revealed an association between increased trypanosome infections with decreasing watering point distance.

Appendix 14: Distribution of trypanosomosis by grazing point distances

km	*Positive*	*Negative*	*Total*
1	4	143	147
2	6	184	190
3	2	73	75
4	0	55	55
7	0	62	62
Total	12	517	529

X^2 for linear trend = 2.37; P value = 0.1234.

- *Out of a total of 12 positive cases, 4 (33.3%) and 6 (50%) trekked 1 and 2 km respectively from pen to grazing points and the remaining two positive cases trekked 3 km distance.*

Appendix 15. Mean PCV by Age group

Age Group	Positive	Negative
0-1	23.47 ± 3.10	26.76± 6.15
2-3	26.50 ± 5.43	29.21 ± 4.84
4-5	24.83 ± 3.11	31.45 ± 5.16
6-7	27.29 ± 7.48	30.65 ± 5.68
8-9	27.88 ± 3.83	28.13 ± 3.23
10-11	0.00	29.63 ± 6.59

Appendix 16 Summary of Total WBC counts

WBC x 10^9/L	Trypanosome-Positive	Trypanosome-negative	Total
4 x 10^9/L and below	25 (23.8%)	80 (76.2%)	105 (46.1%)
Above 4 to 10x 10^9/L	11 (9.2%)	108 (90.8%)	119 (52.2%)
Above 10x 10^9/L	0	4	4 (1.7%)

Total	36	192	228

Appendix 17: Mean Total WBC Counts of Cattle (105) 4 x10^9/L and Below In the KGR (Feb. 2005)

Block No	Total WBC x10^9/L	No of Cattle
B1H2	1.81	12
B3H1	3.22	6
B3H2	3.23	8
B3H3	2.94	9
B3H4	3.31	11
B3H5	2.91	9
B3H6	2.60	8
B5H1	3.15	34
B5H2	2.52	8

Appendix 18: Mean Differential leukocyte Counts of Cattle (105) 4 x10^9/L and Below In the KGR (Feb. 2005)

Lymp	Eos	Lymph	Eos	Mono	Baso	NO TRYPS POS	BLOCK NO	Neut	Lymph	Eos	Mono	Baso	NO TRYPS NEG
B1H2	1.74	1.07	0.15	0.14	0.00	1	B1H2	0.89	0.87	0.06	0.08	0.00	11
							B3H1	1.77	1.15	0.10	0.14	0.00	6
							B3H2	1.89	1.11	0.12	0.11	0.00	8
							B3H3	1.72	0.92	0.16	0.15	0.00	9
B3H4	1.96	1.11	0.14	0.11	0.00	9	B3H4	1.82	0.93	0.14	0.11	0.00	2
B3H5	1.49	0.99	0.17	0.15	0.00	2	B3H5	1.50	0.91	0.16	0.13	0.00	7
							B3H6	1.48	0.88	0.10	0.14	0.00	8
B5H1	1.43	0.94	0.13	0.11	0.00	9	B5H1	1.93	1.10	0.15	0.14	0.00	25
B5H2	1.55	0.75	0.09	0.08	0.00	4	B5H2	1.76	0.91	0.11	0.14	0.00	4

Appendix 19: Mean TotalL WBC Counts of Cattle (119) Above 4 to 10 x10^9/L In the KGR (Feb. 2005)

Block No	Mean WBC x10^9/L	No Positive For Trypanosomes	Block No	Mean WBC x10^9/L	No Negative For Trypanosomes
B1H2	6.21	5	B1H2	6.3	20
B3H1	5.0	1	B3H1	5.73	17
			B3H2	5.66	8
			B3H3	5.74	14
B3H4	5.75	2	B3H4	5.02	9
			B3H5	5.47	16
			B3H6	6.39	11
			B5H1	5.09	8
B5H2	6.1	3	B5H2	6.02	5

Appendix 20: Mean Differential Leukocyte Counts of Cattle above 4 To 10 x10^9/L In the KGR (Feb. 2005)

Block No	Neut	Lymp	Eos	Mono	Baso	No Tryp Pos	Block No	Neut	Lymp	Eos	Mono	Baso	No Tryp Neg
B1H2	3.91	2.03	0.33	0.23	0.00	5	B1H2	2.73	1.94	0.33	0.11	0.00	17
B3H1	2.85	2.43	0.00	0.15	0.00	1	B3H1	3.63	2.53	0.30	0.22	0.00	10
							B3H2	3.28	2.05	0.21	0.18	0.00	8
							B3H3	3.41	1.94	0.91	0.18	0.00	14
B3H4	3.13	2.21	0.22	0.20	0.00	2	B3H4	3.19	1.91	0.28	0.26	0.00	9
							B3H5	3.24	1.77	0.27	0.21	0.00	16
							B3H6	3.8	3.13	0.31	0.23	0.00	11
							B5H1	3.01	1.67	0.22	0.15	0.00	8
B5H2	3.67	2.04	0.21	0.11	0.00	3	B5H2	3.57	1.96	1.21	0.27	0.00	4

Appendix 21: Total WBC Counts Of Cattle (4) Above 10 x10^9/L In the KGR(Feb. 2005)

Block No	Total WBC x10^9/L	NoNegative for trypanosomes
B1H2	10.6	2
B3H1	11.3	1
B3H6	11	1

Appendix 22: Mean Differential leukocyte counts of Cattle (4) above 10 x10^9/L In the KGR (Feb. 2005)

BLOCK	Neut	Lymp	Eos	Mono	Baso	Parasitaemia negative
B1H2	6.63	3.06	0.52	0.21	0	2
B3H1	7.23	3.16	0.57	0.34	0	1
B3H6	6.82	3.08	0.66	0.44	0	1

Appendix 23: Binomial distribution probabilities with n=345 and p=0.17

0.0000001	0.0000001	0.0000003	0.0000008	0.00000017
0.0000037	0.0000077	0.0000155	0.0000302	0.0000568
0.0001033	0.0001822	0.0003116	0.0005173	0.0008341
0.0013069	0.0019912	0.002952	0.0042605	0.0059894
0.0082056	0.0109609	0.014282	0.0181608	0.0225458
0.0273375	0.0323878	0.0375056	0.0424677	0.0470347
0.0509705	0.054063	0.0561428	0.0570991	0.0568892
0.0555412	0.0531498	0.0498654	0.0458792	0.0414053
0.0366623	0.031857	0.0271709	0.0227516	0.0187074
0.0151076	0.0119851	0.0093418	0.0071555	0.005387
0.0039868	0.002901	0.0020758	0.0014608	0.0010112
0.0006887	0.0004615	0.0003043	0.0001975	0.0001262
0.0000793	0.0000491	0.00003	0.000018	0.0000106
0.0000062	0.0000036	0.000002	0.0000011	0.0000006
0.0000003	0.0000002	0.0000001		

~~Appendix 24:~~ Total WBC and differential counts carried out on 228 cattle samples in the KGR (February 2005)

Total WBC Units X x 10^9/L	Total WBC	Neut	Lymp	Eos	Mono	Baso	Parasitaemia
B1H2							

1	4.3	2.49	1.46	0.22	0.13	0	Negative
2	2.5	1.50	0.73	0.15	0.13	0	Negative
3	10.2	6.32	3.06	0.61	0.2	0	Negative
4	5	2.85	1.8	0.15	0.2	0	Negative
5	4.9	2.35	2.11	0.25	0.15	0.00	Negative
6	11	7.04	3.3	0.44	0.22	0	Negative
7	3.8	2.36	0.91	0.34	0.19	0	Negative
8	7.32	4.32	2.18	0.36	0.5	0	positive++
9	6	3.42	2.18	0.36	0.5	0	Negative
10	5.4	3.19	1.62	0.38	0.22	0	Negative
11	9.6	6.24	2.11	0.77	0.48	0	Negative
12	3.7	2.59	0.74	0.11	0.22	0.00	Negative
13	2.9	1.94	0.7	0.17	0.09	0.00	negative
14	8.1	5.02	2.43	0.65	0	0	positive+
15	2.6	1.17	1.09	0.18	0.13	0.00	negative
16	6.3	3.47	2.33	0.32	0.19	0	negative
17	1.9	0.89	0.87	0.06	0.08	0	positive+
18	4.7	2.82	1.6	0.19	0.09	0	positive+
19	5.6	3.81	1.4	0.34	0.06	0	negative
20	4	2.36	1.4	0.12	0.12	0	negative
21	3.7	2.00	1.41	0.07	0.22	0	negative
22	8.2	4.92	2.54	0.41	0.33	0	negative
23	4.8	2.88	1.4	0.29	0.24	0	negative
24	8.2	5.41	1.72	0.9	0.16	0	negative
25	6	3.66	2.1	0.24	0	0	negative
26	5.3	3.18	1.75	0.16	0.21	0	positive+
27	8.8	5.44	1.94	0.79	0.53	0	negative
28	4.5	2.70	1.26	0.23	0.32	0.00	negative

		29	3.8	1.94	1.14	0.23	0.11	0 negative
		30	5.1	3.01	1.79	0.2	0.1	0 negative
		31	5.8	3.19	2.32	0.12	0.17	0 negative
		32	7	4.20	2.17	0.28	0.35	0 positive+
		33	3.5	1.93	1.33	0.11	0.14	0 negative
		34	4.4	2.60	1.54	0.18	0.09	0 negative
		35	6.7	3.75	2.95	0	0	0 negative
		36	8.9	5.34	2.76	0.27	0.53	0 negative
		37	1.8	0.77	0.79	0.16	0.07	0 negative
		38	3.4	1.39	1.7	0.14	0.17	0 negative
		39	4.3	2.45	1.55	0.22	0.09	0 negative
B3H1	40		7.9	4.74	2.37	0.47	0.32	0 negative
		41	4.6	2.62	1.52	0.32	0.14	0 negative
		42	9.4	5.83	2.91	0.47	0.19	0 negative
		43	7.1	4.33	2.13	0.21	0.43	0 negative
		44	4	2.32	1.32	0.08	0.28	0 negative
		45	2.3	1.27	0.92	0.07	0.05	0 negative
		46	5.9	2.89	2.18	0.53	0.24	0.00 negative
		47	11.3	7.23	3.16	0.57	0.34	0 negative
		48	6.9	4.14	2.35	0.28	0.14	0 negative
		49	3.2	1.82	1.18	0.06	0.13	0 negative
		50	1.8	0.88	0.67	0.16	0.13	0 negative
		51	3.7	2.00	1.48	0.15	0.07	0 negative
		52	4.6	2.76	1.66	0.14	0.05	0 negative
		53	5	2.85	2	0	0.15	0 positive+
		54	4.9	2.99	1.62	0.1	0.2	0 negative
		55	4.4	2.16	2.16	0.09	0	0 negative
		56	8	4.96	2.24	0.32	0.48	0 negative

	57	6.4	3.71	2.18	0.45	0	0.00	negative
	58	5.2	3.12	1.61	0.16	0.31	0	negative
	59	3.9	2.34	1.33	0.08	0.16	0	negative
	60	5.6	3.19	1.68	0.62	0.11	0	negative
	61	7.5	3.96	2.7	0.6	0.23	0	negative
	62	5.8	3.48	1.86	0.06	0.41	0	negative
	63	6	3.06	2.4	0.24	0.3	0	negative
	64	7.3	4.53	2.19	0.37	0.22	0	negative
B3H2	65	4.5	2.97	1.35	0.09	0.09	0	negative
	66	2.8	1.68	1.09	0.01	0	0	negative
	67	3.3	1.65	1.35	0.2	0.1	0	negative
	68	8.4	4.79	2.86	0.34	0.42	0	negative
	69	5	2.75	2	0.15	0.1	0	negative
	70	6.2	3.91	1.98	0	0.31	0.00	negative
	71	5.4	3.24	1.89	0.22	0.05	0	negative
	72	3	1.92	0.9	0.06	0.12	0	negative
	73	4.4	2.11	1.89	0.13	0.26	0	negative
	74	2.1	1.26	0.65	0	0.19	0	negative
	75	5.2	2.91	1.82	0.16	0.31	0	negative
	76	3.9	2.38	1.29	0.08	0.16	0	negative
	77	3.7	2.22	1.15	0.22	0.11	0	negative
	78	8	3.92	3.36	0.56	0.16	0	negative
	79	3	1.80	1.02	0.12	0.06	0	negative
	80	5.2	3.22	1.77	0.21	0	0	negative
	81	4	2.20	1.4	0.24	0.16	0	negative
	82	5	3.00	1.6	0.25	0.1	0.00	negative
B3H3	83	2.4	1.44	0.82	0.05	0.1	0	negative
	84	3.4	1.97	1.16	0.2	0.07	0	negative
	85	5.1	3.06	1.53	0.2	0.26	0.00	negative

	86	6	2.94	2.04	0.48	0.54	0 negative
	87	7	4.41	2.24	0.14	0.21	0.00 negative
	88	2	1.34	0.4	0.08	0.14	0 negative
	89	7.1	4.05	2.49	0.36	0.21	0 negative
	90	4.8	2.93	1.58	0.19	0.1	0 negative
	91	3.9	2.30	1.25	0.2	0.16	0 negative
	92	4.7	3.20	1.27	0.14	0.09	0 negative
	93	8.1	4.86	2.84	0.16	0.24	0 negative
	94	3.6	2.05	1.12	0.29	0.14	0 negative
	95	6.1	3.78	2.01	0.18	0.12	0 negative
	96	5.2	3.07	2.08	0.05	0	0 negative
	97	2.5	1.50	0.8	0.13	0.08	0 negative
	98	1.7	1.07	0.51	0.07	0.05	0 negative
	99	4.2	2.73	1.3	0.08	0.08	0 negative
	100	5.1	2.50	2.24	0.1	0.26	0 negative
	101	3.8	1.90	1.25	0.27	0.38	0 negative
	102	3.2	1.92	0.96	0.13	0.19	0 negative
	103	6.3	3.91	1.95	0.19	0.25	0 negative
	104	5.4	3.24	1.78	0.27	0.11	0 negative
	105	5.2	3.02	1.87	0.16	0.16	0 negative
B3H4	106	2.2	1.32	0.68	0.13	0.07	0 negative
	107	3.8	2.32	1.18	0.15	0.15	0 negative
	108	5.1	3.01	1.68	0.15	0.26	0 negative
	109	3.8	2.30	1.18	0.11	0.23	0 positive+
	110	4.5	2.48	1.62	0.23	0.18	0 positive+
	111	7	3.78	2.8	0.21	0.21	0 positive++
	112	4	2.00	1.56	0.24	0.2	0 positive++
	113	5.2	3.17	1.56	0.21	0.26	0 negative
	114	2.9	1.80	0.78	0.2	0.12	0 positive+

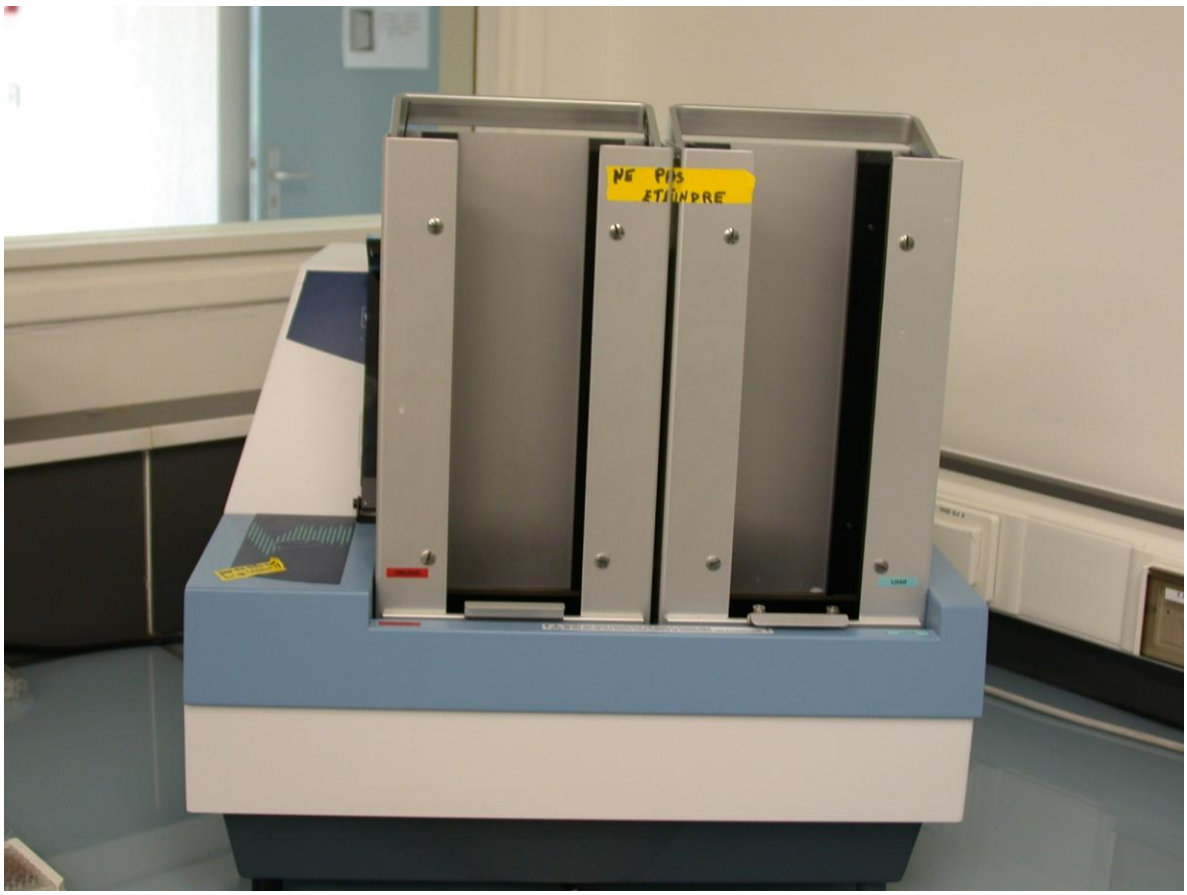
	115	6.2	3.72	1.98	0.31	0.19	0	negative
	116	4.7	2.35	1.83	2.8	0.24	0	negative
	117	3.9	2.15	1.37	0.2	0.2	0	positive+
	117	6.5	3.90	1.95	0.26	0.39	0	negative
	118	3.4	1.90	1.16	0.1	2.38	0	positive+
	119	4	2.44	1.2	0.16	0.2	0	Positive+
	120	7.3	3.65	2.77	0.51	0.37	0	negative
	121	1.9	1.14	0.59	0.1	0.08	0	positive+
	122	3.8	2.28	1.29	0.11	0.11	0	positive+
	123	2.7	1.67	0.89	0.08	0.05	0	positive+
	124	5.6	3.25	2.07	0.22	0.06	0	negative
	125	6	3.24	2.1	0.3	0.36	0	negative
	126	4.2	2.45	1.26	0.29	0.17	0	negative
B3H5	128	4.3	2.24	1.63	0.17	0.26	0	negative
	129	5.6	3.53	1.68	0.28	0.11	0	negative
	130	3.2	1.92	0.99	0.19	0.1	0	negative
	131	5	3.10	1.6	0.5	0.25	0	negative
	131	4.3	2.58	1.46	0.09	0.17	0	negative
	133	2.8	1.60	1.2	0.11	0.17	0	positive+
	134	3.6	2.09	1.15	0.11	0.25	0	negative
	135	4.1	2.46	1.23	0.12	0.25	0	negative
	136	7	4.20	2.17	0.28	0.35	0	negative
	137	5.9	3.66	1.77	0.3	0.18	0	negative
	138	5	2.85	1.75	0.3	0.1	0	negative
	139	4.8	2.88	1.54	0.24	0.14	0	negative
	140	5.4	3.24	1.73	0.27	0.16	0	negative
	141	8	4.88	2.64	0.24	0.24	0	negative
	142	6.1	3.60	1.89	0.24	0.31	0	negative
	143	5.4	2.70	2.11	0.27	0.32	0	negative

	144	3.2	1.92	0.96	0.19	0.13	0	negative
	145	2.7	1.57	0.81	0.16	0.14	0	negative
	146	4.4	2.51	1.41	0.31	0.13	0	negative
	147	5.8	3.48	2.03	0.17	0.11	0	negative
	148	3	1.89	0.9	0.12	0.09	0	negative
	149	1.6	0.96	0.5	0.08	0.06	0	negative
	150	6.4	3.97	1.66	0.51	0.26	0	negative
	151	2.5	1.38	0.78	0.23	0.13	0	Positive+
	152	3.6	2.09	1.08	0.25	0.18	0	negative
B3H6	153	9.2	5.80	3.31	0.55	0.23	0	negative
	154	7	4.20	2.38	0.28	0.14	0	negative
	155	6.6	4.09	2.31	0.13	0.2	0	negative
	156	2.4	1.49	0.67	0.1	0.14	0	negative
	157	5.5	3.30	1.65	0.28	0.28	0	negative
	158	11	6.82	3.08	0.66	0.44	0	negative
	159	1.4	0.67	0.56	0.06	0.1	0	negative
	160	3.1	1.71	1.09	0.16	0.12	0	negative
	161	4.7	2.63	1.55	0.42	0.09	0	negative
	162	3	1.71	0.96	0.15	0.18	0	negative
	163	4.8	2.88	1.63	0.19	0.1	0	negative
	164	1.9	0.84	0.95	0.06	0.06	0	negative
	165	3.5	2.10	1.05	0.18	0.18	0	negative
	166	7.1	3.91	2.27	0.43	0.5	0	negative
	167	6	3.72	1.8	0.24	0.24	0	negative
	168	5.4	3.35	1.78	0.16	0.11	0	negative
	169	7.6	4.10	2.66	0.38	0.46	0	negative
	170	2.2	1.34	0.68	0.04	0.13	0	negative
	171	3.3	1.98	1.09	0.07	0.17	0	negative mf+ve
B5H1	172	2.4	1.20	0.94	0.14	0.12	0	negative

173	1.6	0.98	0.48	0.06	0.08	0 mf+ve
174	4.2	2.48	1.43	0.13	0.15	0 mf+ve
175	5	3.00	1.6	0.25	0.08	0 negative
176	4.1	2.58	1.27	0.16	0	0 negative
177	2.7	1.46	0.95	0.14	0.16	0 positive+
178	3	1.77	0.9	0.12	0.21	0 negative
179	1.9	1.14	0.63	0.06	0.06	0 negative
180	4	2.20	1.52	0.2	0.08	0 negative
181	2	1.06	0.68	0.16	0.1	0 negative
182	3.8	1.98	1.41	0.19	0.23	0 negative
183	2.7	1.65	0.81	0.19	0.05	0 positive+
184	3.9	2.46	1.01	0.21	0.12	0 negative
185	2.6	1.35	1.01	0.13	0.1	0 negative
186	5	3.00	1.5	0.2	0.25	0 negative
187	4.9	2.55	1.96	0.29	0.1	0 negative
188	7	4.20	2.17	0.42	0.21	0 negative
189	6.1	3.66	2.07	0.12	0.24	0 negative
190	2.7	1.57	0.86	0.11	0.11	0 negative
191	1.9	0.99	0.76	0.1	0.06	0 negative
192	1.4	0.85	0.42	0.04	0.08	0 positive+
193	1.9	0.97	0.78	0.1	0.06	0 negative
194	2.6	1.56	0.91	0.05	0.08	0 negative
195	4	2.56	1.2	0.16	0.08	0 negative
196	3.4	1.94	1.19	0.17	0.1	0 positive+
197	1.8	1.08	0.61	0.07	0.04	0 negative
198	2	1.00	0.82	0.1	0.08	0 positive+++
199	3.1	1.86	0.93	0.16	0.16	0 negative
200	3.2	0.96	1.22	0.19	0.19	0 positive+
201	4	2.40	1.32	0.08	0.2	0 positive+

	202	2	0.96	0.86	0.12	0.06	0	positive+
	203	2	1.20	0.7	0.04	0.04	0	negative
	204	3.4	2.07	1.02	0.1	0.2	0	negative
	205	1.7	0.94	0.58	0.07	0.12	0	negative
	206	2	1.04	0.74	0.06	0.16	0	negative
	207	3.4	2.07	1.19	0.17	0.14	0	negative
	208	2.7	1.62	0.89	0.11	0.08	0	positive++
	209	1.6	0.99	0.53	0.05	0.03	0	negative
	210	2.8	1.68	0.9	0.14	0.08	0	negative
	211	3.4	1.84	1.36	0.07	0.14	0	negative
	212	1.8	1.08	0.63	0.05	0.04	0	negative
	213	4.4	2.64	1.36	0.22	0.13	0	negative
B5H2	214	7.2	4.32	2.23	0.36	0.29	0	positive++
	215	1.6	0.96	0.59	0.03	0.02	0	positive++
	216	4.9	2.89	1.67	0.2	0.15	0	negative
	217	3	1.80	0.9	0.15	0.15	0	positive+
	218	2.4	1.37	0.79	0.15	0.1	0	negative
	219	6.3	3.84	2.02	0.25	0.19	0	negative
	220	5	3.00	1.7	0.15	0.15	0	positive+
	221	3.4	2.14	1.02	0.07	0.17	0	negative
	222	7	4.20	2.17	0.21	0.35	0	negative
	223	2.8	1.74	0.92	0.06	0.08	0	positive++
	224	6.1	3.70	2.2	0.12	0.12	0	positive+
	225	5.3	3.29	1.7	0.21	0.11	0	negative
	226	1.9	1.71	0.57	0.11	0.08	0	positive+
	227	6.6	3.63	2.24	0.46	0.26	0	negative
Total average		**4.62**	**2.71**	**1.53**	**0.22**	**0.18**	**0.00**	

Appendix 25: ELISA Reader

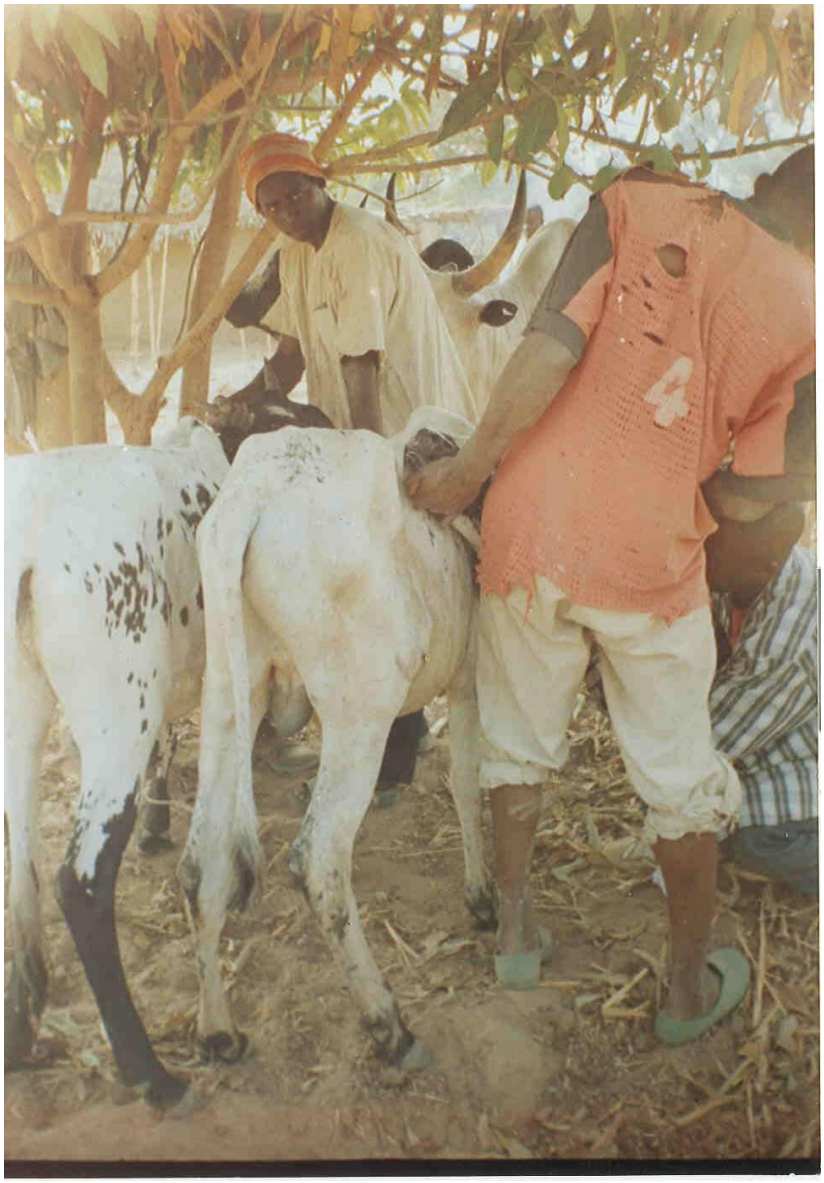

NE PAS
ETEINDRE

Appendix 26: Computer connected to ELISA reader

Appendix 27: Thermocycler 1

Appendix 28: Thermocycler2

Appendix 29: Animal sampling with the Pastoralists restraining



Appendix 30: Front view of Campus Internationale de Baillarguet (Cirad-Emvt, Montpellier, France. (inset: Felicia and colleague Matthieu).

Appendix 31: Gazette of the KGR

Kaduna State Notice No. 29

IN THE HIGH COURT OF JUSTICE
PROBATE DIVISION

WHEREAS the persons whosenames are set out in the first column hereunder died intestate on the date and at the place state d in the said column.

AND WHEREAS the persons whose addresses and claimed relationship to the said deceased are set out in the second column hereunder have applied to the High Court of Justice Kaduna for a grant of 'Letters of Administration' of the personal property of the said deceased.

NOTICE IS HEREBY GIVEN that the said 'Letters of Administration will be granted to such persons unless a 'NOTICE TO PROHIBIT' the grant thereof is filed in the Probate Registry, High Court of Justice, Kaduna within twenty-one days from the date of publication of this notice.

Deceased	*Applicants for Grant*
AGNES HAUWA TAGWAI, late of Ministry of Edcuation, Kaduna, died on the 28th day of October, 1995 at A.B.U. Teachnig Hospital, Kaduna.	Danjuma. D. Tagwai and Daniel. D. Tagwai, of Medical Services Kaduna Polytechnic, the lawful Son and Husband respectively of the said deceased.
JOSEPH BAHAGO, late of Kakuri Kaduna, died on the 14th day of November, 1995 at Kurmin Gwari Kakuri, Kaduna.	Habiba J. Bahago and Malaki Yari, of Kakuri Police Station, Kaduna, the lawful Widow and Brother respectively of the said deceased.
MICHEAL IBRAHIM, late of Kaduna, died on the 4th day of January, 1996 at St. Gerald's Hospital, Kaduna.	Umaru M. Ibrahim and Amina Ibrahim of No. C. 28 Dadinkowa Street, Nasarawa Kaduna, the lawful Son and Widow respectively of the said deceased.

DATED at Kaduna this 26th day of March, 1996.

The Probate Registry,
High Court of Justice,
Kaduna State,
Kaduna.

MRS. MAIRO L. U. MOHAMMED
Probate Registrar

Kaduna State Notice No. 30

IN THE HIGH COURT OF JUSTICE
PROBATE DIVISION

WHEREAS the persons whose names are set out in the first colu nn hereunder died intestate on the date and at the place stated in the said column.

AND WHEREAS the persons whose addresses and claimed relationship to the said deceased are set out in the second column hereunder have applied to the High Court of Justice, Kaduna for a grant of 'Letters of Administration' of the personal property of the said deceased.

NOTICE IS HEREBY GIVEN that the said 'Letters of Administration' will be granted to such persons unless a 'NOTICE TO PROHIBIT' the grant thereof is filed in the Probate Registry, High Court of Justice, Kaduna within twenty-one days from the date of publication of this notice.

Deceased	*Applicants for Grants*
DAUDA GALADIMA, late of Prisons Services, Kaduna, died on the 31st day of October, 1995 at Dr. Gwamna Awan Hospital, Kaduna.	Danladi Galadima and Dyenis Galadima of E.C.W.A. Church, Nassarawa, Kaduna, the lawful Brothers of the said deceased.
HASSAN HARUNA (Cleaning Service), late of Kaduna, died on the 27th day of March, 1996 at U/Sunday, Kaduna.	Fatima Hassan and Hussaini Haruna, of G. 35 Sani Bello Street, U/Sunday, Kaduna the lawful Widow and Brother respectively of the said deceased.
JOSIAH OKOYE OGBUKA, late of Kaduna, died on the 18th day of July, 1989 at Kaduna.	Florence Ogbuka and Samson Ogbuka of No. 54/56 Majalisa Street, Tejevision Kaduna, the lawful Widow and Son respectively of the said deceased.

Deceased	*Applicants for Grant*
LAI M. ALI, late of Nigerian Army, Kaduna, died on the 25th day of March, 1995 at 44 Military Hospital, Kaduna.	Ibrahim Hassan and Hanatu Hassan, of No. 2 Madugu Road, Hayin Banki Kawo, Kaduna the lawful Son and Widow respectively of the said deceased.
BAWA MOMOH, late of Kaduna, died on the 22nd day of March, 1995 at Express Hospital, Kaduna.	Mrs. Bose Bawa and Williams Aigboruon of No. AY 2 Market Road, Nassarawa, Kaduna, the lawful Widow and Brother respectively of the said deceased.

DATED at Kaduna this 3rd day of April, 1996.

MRS. MAIRO L. U. MOHAMMED,
Probate Registrar

The Probate Registry,
High Court of Justice
Kaduna State,
Kaduna.

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KD.S.L.N. No. 10 *of* 1996

THE KADUNA STATE GRAZING RESERVES LAW
(CAP. 62 1991)

THE KACHIA GRAZING RESERVE ORDER 1996

Date of Commencement: 1st *July*, 1988

In the exercise of the powers conferred by section 12 of the Grazing Reserves Law (Cap. 62) 1991 and all other powers enabling him in that behalf, the Military Administrator of Kaduna State hereby makes the following order:

Title. 1. This order may be cited as the Kachia Grazing Reserve Order, 1996.

Creation of Kachia Grazing Reserve 2. "All that piece of land, the situation and limits where of are set forth in the fisrt schedule hereto, subject to the rights affecting the same as set forth in second schedule hereto, shall constitute a Government Grazing Reserve within the meaning of the Law, which Reserve shall be known as Government No. 4 Kachia Grazing Reserve".

FIRST SCHEDULE

All that portion containing three hundred and thirty four decimal one, square kilo or thereabouts situated in Kufana in Chikun Local Government, Kachia in Kachia Local Government and Kamuru Ikulu in Zangon Kataf Local Government Area of Kaduna State and bounded as follows:

Starting from a point marked KC 64s runs on a straight line cut on bearing 131-55', distance 399.79m to KC 1009 whcih is located on the right side of Kaduna to Kachia motor road and 1500m to where the road crosses the railway thence on a bearing of 27-58' for a distance of 163.93m to KC 1010; thence on bearing of 28°00' for a distance of 5.5.75m to KC 1012; thence on series of cut lines on bearings of 325°00', 324°43', 3.5°31 324°52', 325°02', 324°51', 325°17' beacons nos KC 1013,p1, p2, p3, KC 1015, p4, to KC 1016, distance 1873.74m; thence on bearings of 30°02' 29°58' and 30°08' from KC 1017 through p5, KC 1018, p6 to KC 1019 distance 999.24m; thence on bearing of 01°53' from KC 1020 through KC 1021, p7, KC 1021A, p8 to KC 1022, distance 1500.03m; thence on beairng 345°31', 345°33', 34 5°31', 345°32', 345°29', 345°31' and 34532 from KC 1022 through p9 KC 1023, p10, KC 1024, KC 1025, p11, KC 1026, p12, KC 1027, p13, KC. 1028, p14, to KC 1029, distance 2611.06m; thence on bearings of 46°13', 45°48', 46°15', 45°59', 45°57', from p15 through p16, KC 1030, p17, p18, KC 1031, p19, KC 1032 to KC 1033; distance 1574.99m; thence on bearings of 67°26', 67°28', 67°10'

67°35', 67°26',from p20 through KC 1034, p21, KC 1035, to KC 1036, distance 1148,80m; thence on bearing of 20°41, 20°40', 20°39, 20°48', 20°34, 20°39', 20°38', from p22 through KC 1037, p23, KC 1038, p24 p25, KC 1039, p26, KC 1040 to KC 1041, distance 1847.64m; thence on bearings of 343°24', 343°23', 343°24', from p27 through KC 1042, p28, KC 1043, to KC 1045, distance 1400.45m; thence on bearings of 92°24' 92°21, 92°27, 92°25', 92°26', from KC 1046, through p30 KC 1047,' p31 to KC 1048 distance 1198.97m; thence on bearings of 92°25' 92°24' 92°23, from p32 through p33, KC 1049 to KC 1050, distance 599.16m' thence on bearings of 155°38 155°37, 155°33, 155°39', 155°37, 15536, from KC 1057 through KC 1051A, p34, KC 1052,KC 1054 distanc 1399.47m; thence on bearing of 129°20, to beacon No KC 1055, distance 132 70m; thence on bearings of 39°11 39°30', and 39°23 from p36 to p37 distance 599.52m; thence on bearings of 129°20, 129°21', 12928, 129°10, 129°29', 129°23 from p38 through p39, p40, p41, p42 to p43, distance 1067.86m; thence on bearings of 39°23, to p44 distance 199.95m; thence on bearings of 129°22, 129°22, and 129°24, ,from p45 through p46 to p47 distance 849.43m, thence on bearings of 219°22', 219°23', and 219°19, from p48 through p49 to KC 1060, distance 801.44m; thence on bearing of 119°56, to p50 distance 157.40m; thence on bearing of 181°40, to p51, distance 57.71m; thence 60°31 to KC 1061, distance 59.22m, thence 119°54, to p52, distance 342.73m; thence on 169°32 to p53, distance 118.30m thence on 74°19, to KC 1062; thence on bearing 74°19, to KC 1063 distance 125.93m; thence on bearings of 119 °513, 119°52', 119°53, 119°54', 119°53, 54°53, 54°54, to 119°55 from KC 1063 through KC 1064, KC 1065, KC 1066, 1067, 1068, 1069, p54 1070, 1071 1072, 1073, 1074, 1075, 1076, 1077, 1078, 1079 to 1080, distance 4158.18m; thence on bearings of 283°33', 283°32', KC 64S, p123, p124, and KC 1008, distance 499,24m; thence on bearing of 31°39', to p125, distance 71.52m; thence on 291°07, to p126 distance 131.52m; thence on 344°04, to KC 1007, distance 268.34; thence 335°59, to p127, distance 174.06m; thence on 336°55, 336°22, 336°23, 336 09, 336°23, 336°20, 336°22, 336°14, 336°26, from KC 1006 through p128, KC 1005; KC 1004, KC 1003, KC 1002, KC 1001, KC 1000 to KC 999, distance 1395.96m; thence on 321°37, to p129, distance 142.58m; thence on bearng 258°21, to p130, distance 129.23m;

From Beacon No.	*Bearing*	*Distance Metres*	*To Beacon No.*
KC 64S	131°58'	399.79	KC 1009
KC 1009	27°58'	163.98	KC1010
KC 1010	28°00'	235.87	KC1011
KC 1011	28°00'	299.88	KC 1012
KC 1012	325°00'	199.36	KC 1013

From Beacon No.	*Bearing*	*Distance Metres*	*To Beacon No.*
KC 1013	324°43'	65.57	p1
p1	325°31'	60.06	p2
p2	324°52'	54.60	p3
p3	325°02'	220.03	KC 1014
KC 1014	325°02'	400.19	KC 1015
KC 1015	324°51'	151.91	p4
p4	325°51'	97.84	KC 016
KC 1016	30°02'	399.59	KC 1017
KC 1017	29°57'	209.36	p5
p5	30°09'	190.52	KC 1018
KC 1018	29°58'	120.86	p6
p6	30°08'	78.91	KC 1019
KC 1019	01°47'	399.78	KC 1020
KC 1020	01°46'	400.08	KC 1021
KC 1021	01°44'	222.32	p7
p7	01°50'	177.99	KC 1021A
KC 1021A	01°42'	205.09	p8
p8	01°53'	93.77	KC 1022
KC 1022	345°31'	292.74	p9
p9	345°33'	107.31	KC 1023
KC 1023	345°31'	278.70	p10
p10	345°31'	120.87	KC 1024
KC 1024	345°31'	399.98	KC 1025
KC 1025	345°32'	210.86	p11
p11	345°32'	199.23	KC 1026
KC 1026	345°32'	245.94	p12
p12	345°29'	153.65	KC 1027
KC 1027	354°29'	310.85	p13
p13	345°41'	89.19	KC 1028
KC 1028	345°31'	275.46	p14
p14	345°32'	74.28	KC 1029
KC 1029	46°13'	125.60	p15
p15	45°48'	196.08	p16
p16	46°15'	95.90	KC 1030
KC 1030	45°59'	28.72	p17
p17	45°57'	166.93	p18
p18	45°57'	186.16	KC 1031
KC 1031	45°57'	266.62	p19
p19	45°56'	133.55	KC 1032
KC 1032	45°57'	249.83	KC 1033
KC 1033	67°26'	241.43	p20
p20	67°28'	158.35	KC 1034
KC 1034	67°16'	144.12	p21

From Beacon No.	*Bearing*	*Distance Metres*	*To Beacon No.*
p21	67°35'	255.26	KC 1035
KC 1035	67°26'	349.64	KC 1036
KC 1036	20°41'	273.98	p22
p22	20°40'	130.15	KC 1037
KC 1037	20°39'	129.28	p23
p23	20°39'	265.01	KC 1038
KC 1038	20°48'	121.46	p24
p24	20°34'	171.70	p25
p25	20°39'	106.44	KC 1039
KC 1039	20°39'	312.92	p26
p26	20°38'	87.10	KC 1040
KC 1040	20°38'	249.76	KC 1041
KC 1041	343°24'	299.46	p27
p27	343°24'	99°95'	KC 1042
KC 1042	343°24'	348.95	p28
p28	343°23'	31.97	KC 1043
KC 1043	343°23'	199.71	p29
p29	343°24'	220.59	KC 1044
KC 1044	343°24'	199.87	KC 1045
KC 1045	92°24'	399.85	KC 1046
KC 1046	92°21'	199.93	p30
p30	92°27'	199.50	KC 1047
KC 1047	92°25'	150.12	p31
p31	92°26'	249.57	KC 1048
KC 1048	92°25'	90.02	p32
p32	92°24'	161.85	p33
p33	92°24'	147.51	KC 1049
KC 1049	92°23'	199.78	KC 1050
KC 1050	155°38'	291.81	KC 1051
KC 1051	155°37'	255.31	KC 1051A
KC 1051A	155°33'	150.99	p34
p34	155°39'	101.70	KC 1052
KC 1052	155°37'	399.82	KC 1053
KC 1053	155°36'	199.84	KC 1054
KC 1054	129°20'	132.70	KC 1055
KC 1055	39°11'	258.04	p35
p35	39°30'	263.53	p36
p36	39°23'	77.95	p37
p37	129°20'	209.64	p38
p38	129'21'	119.90	p39
p39	129°28'	118.98	p40
p40	129°10'	197.29	p41
p41	129°29'	267.35	p42
p42	129°23'	154.70	p43
p43	129°23'	154.70	p44

From Beacon No.	*Bearing*	*Distance Metres*	*To Beacon No.*
p44	129°22'	399.97	p45
p45	129°22'	322.15	p46
p46	129°24'	127.31	p47
p47	219°22'	443.35	p48
p48	219°23'	255.60	p49
p49	219°19'	102.49	KC 1060
KC 1060	119°56'	157.40	p50
p50	181°40'	57.71	p51
p51	60°31'	59.22	KC 1061
KC 1061	19°54'	342.73	p52
p52	169°32'	118.30	p53
p53	74°19'	125.93	KC 1062
KC 1062	119°51'	399.96	KC 1063
KC 1063	119°51'	299.96	KC 1064
KC 1064	199°52'	141.99	KC 1065
KC 1065	199°52'	257.49	KC 1066
KC 1066	119°53'	200.42	KC 1067
KC 1067	119°53'	199.10	KC 1068
KC 1068	119°53'	230.48	KC 1069
KC 1069	199°54'	89.42	p54
pp54	199°53'	313.62	KC 1070
KC 1070	199°54'	166.29	KC 1071
KC 1071	199°53'	84.95	KC 1072
KC 1072	199°54'	314.61	KC 1073
KC 1073	199°54'	399.80	KC 1074
KC 1074	119°54'	199.61	KC 1075
KC 1075	119°54'	200.71	KC 1076
KC 1076	119°54'	299.60	KC 1077
KC 1077	199°54'	236.33	KC 1078
KC 1078	119°54'	119.51	KC 1079
KC 1079	119°55'	93.68	KC 1080

Located on the left bank of the river Kaduna; thence along the bank down stream to where it is joined by Aduma stream.

KC 64S	283°32'	143.58	p123
p123	283°33'	255.83	p124
p124	283°32'	99.83	KC 1008
KC 1008	31°39'	71.52	p125
p125	291°07'	131.52	p126
p126	344°04'	268.34	KC 1007
KC 1007	335°59'	174.06	p127
p127	336°55'	224.90	KC 1006
KC 1006	336°22'	254.85	p128
p128	336°23'	144.51	KC 1005
KC 1005	336°09'	44.32	KC 1004