CLINICAL AND PATHOLOGICAL OBSERVATIONS IN KENYAN DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE.

BY

DAVIS NJUGUNA R. KARANJA (BVM, U.O.N.)

A THESIS SUBMITTED IN PART FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PATHOLOGY AND MICROBIOLOGY OF THE UNIVERSITY OF NAIROBI.

DEPARTMENT OF VETERINARY PATHOLOGY AND MICROBIOLOGY,

FACULTY OF VETERINARY MEDICINE,

UNIVERSITY OF NAIROBI.

P.O. BOX 29053, NAIROBI.

SEPTEMBER 1992

DECLARATION

I hereby declare that this thesis is my original work and has not been presented in any other university.

.....

D. N. R. Karanja

This thesis has been submitted for examination with our approval as university supervisors;

DR. T. A. NGATIA .....

PROF. J. G. WANDERA .....

From the Department of Veterinary Pathology and Microbiology; University of Nairobi.

ACKNOWLEDGEMENT

I wish to convey my sincere gratitude to the University of Nairobi, Department of Veterinary Pathology and Microbiology and the Faculty of Veterinary Medicine for according me the opportunity to carry out this research. I am particulary grateful to the Deans' committee for funding this project through a research grant; vote number 500-655-219.

I am deeply indebted to my supervisors who dedicated much of their time and energy to this work. Their tireless advices made this research a success.

My sincere gratitude also go to the technical staff in the Departments of Veterinary Pathology and Microbiology and Clinical Studies for their technical advice. Iam grateful to Mr. Henry Gathuo who provided the stabilate on behalf of the director; ILRAD. I feel obliged to thank the Veterinary Pathology animal attendants for taking great care of the animals.

Sincere gratitude also go to my parents, friends and relatives for their moral support. Their endless encouragement was an inspiration throughout the whole course. I thank as well Mr. Peter Mwangi Kamau for typing this work and Dr. C. O. Kimoro for his assistance in statistical analysis.

DEDICATION

Dedicated to my parents; Mr & Mrs Karanja Njuguna
and to my wife Irene Wanjiku Njuguna
with lots of love.

TABLE OF CONTENTS

LIST OF FIGURES.

LIST OF TABLES

LIST OF APPENDICES

ABBREVIATIONS

BUN ..... Blood urea nitrogen

°C ..... Degrees celsius

CO_3^{2-} ..... Carbonate ion

EDTA ..... Ethylene-diamine-tetra-acetic acid

Fl ..... Femtolitres

Hb ..... Haemoglobin

H & E ..... Haematoxylin and eosin

H_2o ..... Water

MCH ..... Mean corpuscular haemoglobin

MCHC ..... Mean corpuscular haemoglobin concentration

MCV ..... Mean corpuscular volume

Mg% ..... Milligrams per cent

Mg/dl ..... Milligram per decilitre

Ml ..... Millitre

Mmol ..... Millimole

MPS ..... Monocyte-phagocyte system.

NH_4^+ ..... Ammonium ion

N:L ..... Neutrophil : lymphocyte ratio

PALS ..... Periarteriolar lymphatic sheaths

Pg ..... Picograms

PCV ..... Packed cell volume

P.i. ..... Post-inoculation

PSG ..... Phosphate saline glucose

RBC ..... Red blood cell

RES ..... Reticulo-endothelial system

R.p.m. ..... Revolutions per minute

µm ..... Micrometre

µmol ..... Micromole

SUMMARY

The study was conducted to establish the type and severity of *Trypanasoma congolense* infection in donkeys, particularly the clinical picture, and to correlate this with the effects on draught power and the role these animals play in the perpetuation of the disease. Five mature donkeys were subcutaneously inoculated with 7.5 x 10^6 blood stream forms of *T. congolense*, strain IL 3575. Three donkeys in adjacent fly proof stalls were kept as controls. The donkeys were monitored clinically on daily basis for a period of three months. Blood was collected from the jugular vein twice a week for haematology and once a fortnight for serum biochemistry. Blood from the marginal ear vein was drawn into heparinized capillary tubes for diagnostic purpose. Thin buffy coat smears were made, stained in 1:5 Giemsa for 15 - 20 minutes and observed under oil-immersion for the presence or absence of trypanosomes. Subinoculations into mice and sheep were done to verify whether the trypanosomes observed in donkeys were viable and pathogenic. The degree of anaemia in sheep and the rapidity at which mice died was the measure of pathogenicity. Donkeys were euthanized after three months and thorough post mortem examinations done. Samples were collected from the lymphoid organs, heart, lungs, skeletal muscles, kidneys, gastrointestinal-tract, brain and glandular organs whether showing gross lesions or not. They were fixed in ten per cent formalin, embedded in paraffin wax, sectioned at thickness of 6μm and stained

with haematoxylin and eosin (H & E).

The infection had a prepatent period of 29 - 41 days in donkeys. The clinical picture indicated a subclinical infection with no signs but a positive diagnosis was made using thin stained buffy coat smears. Pyrexia peaks accompanying parasitaemic phases in ruminants and horses were lacking. The respiratory and heart rates were elevated during the third month of infection when pounding heart beats were picked on auscultation, and the mucus membranes were pale. Attempts to quantify the parasites in blood using a haemocytometer were unsuccessful because they were low in numbers and appeared sporadically. These trypanosomes were morphologically similar to those inoculated into the donkeys.

The results showed red blood cells to decrease by 46.7% ; the packed cell volume by 41.6% haemoglobin concentration by 41.4% in the infected donkeys. In the control group, the red blood cells decreased by 28.6%; the packed cell volume by 22.2% and the haemoglobin concentration by 26.8%. This indicated that a decrease of 18.1%, 19.4% and 14.6% in the red blood cell count, packed cell volume and haemoglobin concentration, respectively in infected animals was attributed to *T. congolense* infection. Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration increased over pre-infection values by 15.8%, 30.1% and 6.3%, respectively in infected animals. Thus the anaemia observed could morphologically be classified as normochromic, macrocytic. The leukocytes

counts showed minimal changes although a relative decrease in neutrophils and a relative increase in lymphocytes was observed in infected animals. Monocytes counts were higher in infected animals. This depicts an efficient regulatory mechanism(s). The fall in total plasma proteins plus a rise in eosinophil count in both groups of animals suggested a helminth infection. This was confirmed at post-mortem when helminth parasites were observed in the gastro-intestinal tract from the stomach, intestines to the colon. The parasites were mainly the small and large strongyles. This concurrent helminthiasis contributed to the decrease of 22.2% in packed cell volume in the two groups. The total bilirubin rose to 0.8mg% from 0.2 - 0.5mg% while blood urea nitrogen increased to 89mg% from 12 - 40mg%. These values were all within the ranges provided by other workers.

At necropsy, the infected donkey carcasses were in fair body condition but moderate amounts of straw - coloured fluid was found in all the body cavities. The liver was slightly enlarged while the spleen displayed prominent Malphigian corpuscles. Microscopically, the lymph nodes had follicular hyperplasia characterized by many prominent follicles. Medullary cords were enlarged and had increased number of plasma cells as well as macrophages. The spleen had follicular hyperplasia of the Malphigian corpuscles, the periarteriolar lymphatic sheaths were expanded while the marginal zones closely resembled the white pulp. Macrophages and plasma cells were encountered in the red pulp while mitotic figures of medium-sized lymphocytes were

occasionally observed in the follicles. The lungs had patches of atelectasis, kidneys displayed areas of interstitial nephritis while the liver showed hydropic degeneration and occasional helminth larval migratory tracts.

The mice peaked in parasitaemia 10 - 15 days after inoculation. Most of these died within 30 days although a few of them survived for more than 60 days. In sheep, a parasitaemia appeared 7 - 17 days after inoculation and persisted in transient peaks to the end of the experiment. A decrease of 17.5% in packed cell volume was attributed to trypanosomiasis. These observations suggests that donkeys may be potential reservoirs of *T. congolense* infections in endemic areas.

INTRODUCTION

Domesticated donkeys *(Equus asinus)* have acclimatized to the warm dry climate; an ecological zone between the true desert and the semi-desert areas. They are tolerant to cool climates of the highlands and the temperate regions as well . In Africa, they are found in the entire northern coastal belt and the fringes of the Sahara, including Egypt. They are also found in southern regions, along the east coast. Commonly, the donkey is found among the nomadic people and less frequently among the settled populations of the West (Epstein, 1984; Fielding, 1987).

Donkeys are well adapted to these regions partly because of their nutritional and water requirements. Their nutrition is modest consisting of coarse pastures, thistles, thorn bush and if nothing else is available, they can feed on paper and dry horse manure. They require water every 2-3 days (Epstein, 1984) or much longer (up to 8 days) with no serious consequences when they return to water (Maloiy and Boarer, 1972).

The World Health Organisation (1981, cited by Epstein, 1984) report on animal populations indicated that there were 38.6 million donkeys in the world. Thirty six million, seven hundred thousands were in developing countries, Africa accounting for 11.9 million. In Africa, it is the most predominant equine constituting 67% of the African equines but only 33% of the world equines.

The Donkey Breed Society of the World (1970, cited by Fraser, 1972)

noted that donkeys are working animals in many countries of the world. Their conformation must therefore be suitable for carrying or drawing a load thus demanding most of the characteristics associated with working horses. It is one of the most important draught animals which exists in millions and serves a key role in the agricultural economy of the developing World (Soulsby, 1986). It has been regarded as a pack transporter particularly in Africa (Oppong, 1979; Epstein, 1984) while in areas where soils are light and sandy, it has been used for riding, carting, threshing and farm cultivation (Fielding, 1987). It has been used for milk production in Egypt and in Kenya (Epstein, 1984). Donkey meat has been used for human consumption in Nigeria (Fielding, 1987) and Central Jordan (Abo-Shehada, 1988). In developed countries, the donkey has been put into the medical approaches in working with physically and mentally disabled whether being driven, ridden or just held on hand to be petted and patted. This has been due to its temperament (Camac, 1986).

In Kenya, shortage of tractors due to various reasons is well known to farmers who are unable to prepare their land in time. Tractors are expensive and their spare parts sometimes miss from the shelves for months on end. An inquest into the appropriate technology that guarantees profitability and being of low-cost, easily available, easily applicable and can be readily adopted has been going on. One of the possibilities considered has been animal power, where the oxen and the donkey are highly placed. The donkey may provide such technology which can be used for rural transport providing social

integration and overall development. Fielding (1987) postulated that the Africa's rural transport needs the increased use of the animals which are free of foreign exchange, are self reproducing and are employment creating.

The donkey has fulfilled all these purposes better than cattle and horses. Unlike cattle , it is not affected by most of the cattle diseases such as foot and mouth disease, and rinderpest which often cause heavy losses. It is rarely affected by the African horse sickness, a fatal disease in horses that requires the annual vaccination, which is expensive (Fielding, 1987).

The donkey is handy for the poor populations of the world who can hardly afford medical care for their children leave alone for the donkey (Svendsen, 1986). As such, being hardy, quiet, intelligent, patient, cheap to buy and modest in its nutritional demands, easy to train for light draught and pack work, has attracted its extensive usage (Epstein, 1984).

The donkey has been a servant of man for several thousand years but little is known on its medicine and hence treatment. In most cases, treatment is based either on basic medical principles or on the assumption that it is just a small horse (Fowler, 1986). This could be attributed to the ignorance and misunderstanding with respect to health and welfare of this animal as observed by Soulsby (1986).

Literature on donkey diseases is scanty and an attempt can only be made to rank them according to the most frequently reported in literature. Parasitism seems to be the most common problem although bacterial, viral, fungal and

hereditary diseases are occasionally encountered (Eaton- Evans, 1986).

Soulsby (1982) compiled a list of parasites which may affect the horse, mule and donkey. This contains parasites ranging from helminths, arthropods to protozoa. Fowler (1986) highlighted the most common parasites encountered in United Kingdom (U.K.). These comprised of helminths and arthropods. Bliss (1986) attempted to discuss parasites in areas outside U.K., but only managed to describe helminth parasites and their strategic drug control.

Helminth parasites are common in donkeys as they are in other domestic animals. Bliss, Svendsen, Geongoulakis, Grosomanidis and Taylor (1985) and Bliss (1986) observed that donkeys put on strategic anthelmintic drug control improved in body condition, their longevity and their immediate health. Several authors have indicated that donkeys are infected with helminth parasites. In Kenya, Round (1962), Ngatia and Kuria (1991) reported the presence of small and large strongyles in these animals. Fraser (1972) and Fowler (1986) reported that donkeys may act as reservoirs for lungworms to horses in Britain. Bliss *et al* (1985) indicated that donkeys in Mediterranean countries suffer from helminth infections and could benefit from anthelmintic dosing. In Zimbabwe, Eysker and Pandey (1987,1989), Pandey and Eysker (1989,1990), observed that donkeys carry heavy helminth loads consisting of both the small and large strongyles. Abo-Shehada (1988) indicated that donkeys grazing on pastures in Central Jordan often show hydatidosis.

In a review of the problems plaguing a donkey stud in U.K.,Eaton-Evans

(1986) observed that bacteria, viruses and fungi were some causes of abortion; venereal and non-venereal causes of misconception. He also noted that bacteria were among the major causes of foal death.

Lloyd (1972) reported an outbreak of streptothricosis in a group of donkeys in Nigeria where *Dermatophilus congolense* was isolated from the lesions. Timoney, O'Reilly, Mcardle, Ward and Harrington (1985) were of the opinion that donkeys could be sources of infections for contagious equine endometritis caused by *Taylorella* (*Haemophilus*) *equigenitalis*.

Viral infections have been reported by a number of workers. Fowler (1986) listed some of the main viral causes of systemic diseases in U.K. He observed the influenza viruses to be the most common. Rose, Round and Beveridge (1970) reported an outbreak of equine influenza which caused death in donkeys. Experimentally, Marthur,Sharma, Kulshrestha and Kumar (1972) demonstrated that the African horse sickness virus could occasionally cause disease with subsequent death in donkeys.

Mycotoxicoses are reported rarely in the donkey. *Fusarium moniliforme* toxicosis was once reported in Egypt (Wilson and Maronpot , 1971).

Donkeys are remarkably free from the hereditary diseases that afflict the horse. Their small size may explain why they are free from roaring and whistling and the rarity of complaints associated with arthritic conditions (Fraser, 1972).

Protozoal diseases have been reported in various parts of the world in

donkeys. *Besnoitia bennetti* has been shown to produce infertility in the donkeys (Terrell and Stookey, 1974). *Babesia equi* may cause fatal infections (Lloyd 1972).

The members of the genus *Trypanosoma* may cause severe diseases in donkeys. *T. brucei* and *T. evansi* have been shown to cause fatal infections in these animals while *T. vivax* has been reported to cause only mild infections (Ikede, Akpokodje, Hill and Ajidagba, 1977; Suryanarayana, Gupta and Singh, 1986).

Donkeys are mainly inhabitants of the tsetse fly infected areas, but their role in trypanosomiasis is not clearly known. *T. congolense* has been encountered in the blood of the donkeys by several workers (Macfie, 1916; Boyt, Mackenzie, Pilson and Leavis, 1972; Oppong, 1979; Stevenson and Mwangi, 1991). After conducting studies in Southern Rhodesia (now Zimbabwe) Boydt *et al* (1972) concluded that the donkeys could act as important sources of food and reservoirs of trypanosomes especially for *Glossina morsitans west w.* Trypanosomiasis caused by *T. brucei* exerts a heavy toll in transport animals of Sudan, more so in the donkey, mule and the camel than the oxen (Webb, 1915). Various reports indicate that this organism causes an insidious disease in donkeys which could be fatal (Head, 1904; Webb, 1915; Defreef, 1919; Currason, 1940; Annon, 1947; Maclennan, 1970; Ikede *et al*, 1977; Annon, 1978). In his report, Webb (1915) stated that little is known on natural recovery from *T. brucei* infections in donkeys because the

disease has a chronic course.

T. evansi produces a subacute to a chronic disease in donkeys (Suryanarayana *et al*, 1986). The insidious onset of this infection could have prompted Bennett, (1933) to arrive at a conclusion that donkeys could be regarded as dangerous reservoirs of the *T. brucei* in camels in Sudan.

T. vivax on the other hand has been shown to produce only a mild infection in donkeys with most of the parameters monitored being within the normal range (Kyewalabye, Kwari, Ajayi and Shinggn, 1989).

T.congolense infection in donkeys has not been intensively studied which means that the features of the disease can only be assumed to parallel that in ruminants and the horse.

Although donkeys have been shown to harbour *T. congolense,* no clinical disease has been recorded. This may imply that they are just "healthy carriers" of the trypanosomes, or alternatively, they may suffer from a clinical disease but has not been reported. This in turn could mean that due to this weakening disease they are unproductive in endemic areas.

1.1 OBJECTIVES OF THE STUDY.

The main objectives of the study were to study the various parameters in donkeys experimentally inoculated with *T. congolense*.The parameters were:

1.1.1 Haematological changes,

1.1.2 Serum biochemical changes,

1.1.3 Clinical signs manifested if any,

1.1.4 Pathological changes (gross and microscopic) and

1.1.5 Pathogenicity of trypanosomes from donkeys in parasitaemia to mice and sheep.

These parameters could assist the clinicians in recognising the disease faster, help verify whether donkeys come down with any form of clinical or subclinical disease, and at the same time find out whether these parameters could be useful in assessing the progress of the disease in these animals. The post-mortem results on the other hand could assist in elucidating both the pathogenesis of the disease in these animals and the mechanism(s) used in the regulation of the level and duration of parasitaemia. The test on pathogenicity could be useful in identifying the reservoir potentiality of the donkeys in trypanosomiasis endemic areas.

2 LITERATURE REVIEW

2.1 AETIOLOGY

Trypanosomiasis in donkeys can be caused by one or more species of the genus *Trypanosoma*. *T. congolense* (Hoare, 1972) is a relatively small trypanosome measuring 8 - 24μm in total length. It has no free flagellum but a minute portion is sometimes visible especially in the long forms. It has a medium sized, subterminal and marginal kinetoplast, and a centrally placed nucleus. The posterior end is rounded or obtusely pointed and has no conspicuous undulating membrane.

T. brucei measures 25 - 35μm in length, has a long free flagellum, a pointed posterior end, and a conspicuous undulating membrane. It has a small terminal kinetoplast and a pointed tail.

T. vivax measures 20 - 27μm in length, has a broad, bulbous posterior end, large and terminal kinetoplast and a short free flagellum. The undulating membrane is usually poorly defined.

T. evansi is indistinguishable from the slender form of *T. brucei*. It measures 15 - 34μm long, has a subterminal kinetoplast, a well developed undulating membrane and a free flagellum. It is morphologically indistinguishable from *T. equiperdum*. *T. equinum* differs from these two by only the lack of a kinetoplast (Soulsby 1982).

The outcome of an infection depends primarily on the species and the

strain of the trypanosome involved. *T.congolense* has a number of strains distinguishable by mean lengths, and the percentage of the short and long forms. They show wide differences in virulence in cattle. Godfrey (1961) observed that the "*congolense* (mean length of 11.2-13.8 µm ; 0-2% long forms) and the intermediate (mean length of 13-14 µm ; 4-11% long forms) types" had high infectivity, and produced high parasiteamia but were of low pathogenicity. On the other hand " *dimorphon*(mean length of 13.8-15.7 µm ; 20-36% long forms) type " showed high infectivity, high parasiteamia and high pathogenicity.

2.2 TRANSMISSION

T. congolense, *T. vivax* and *T. brucei* are cyclically transmitted by tsetse flies (*Glossina spp*) which occupy approximately 10 million square kilometres of subsaharan Africa between the latitudes 14° N and 29° S. Occasionally, they can be transmitted by blood sucking insects particularly the tabanids and *Stomoxys* spp. The rest are transmitted mechanically either through coitus as it is the case with *T. equiperdum* or through mechanical vectors as it is the case with *T. evansi* and *T. equinum* (Soulsby, 1982; Urquhart and Holmes, 1987)

Presence of tsetse flies preclude the rearing of livestock in affected areas while in areas where vectors are not so numerous, trypanosomiasis is rarely a serious problem in domestic animals (Urquhart and Holmes, 1987).

T. congolense can develop in any species of Glossina present in the environment but the most important Glossina species differ from one region to

the other. *G. austeni* is the most important in East Africa, *G. morsitans* in Northern Nigeria and *G. palpalis* in Guinea (Soulsby, 1982). In Kenya, *T. congolense* has been observed in *G. morsitans*, *G. austeni* and *G. pallidipes* (Hoare, 1970).

The infectivity of *T. congolense* for the tsetse flies is usually lower than of *T. vivax* but higher than of *T. brucei* (Hoare, 1970). However, Maudlin and Welburn (1989) observed that a single organism was sufficient to infect a tsetse fly provided that the tsetse fly was susceptible to the trypanosome infection. As a result, these authors concluded that provided there were sufficient organisms to infect each fly, the midgut infection rate could not be increased by increasing the infective dose even 100 folds.

2.3 EPIDEMIOLOGY

Trypanosomiasis is a disease complex affecting both man and his animals in Africa, Asia and South America (ILRAD, 1989). In Equatorial Africa, animal trypanosomiasis constitutes a major obstacle to the development of animal production. In addition to causing a clinical disease resulting in debility, infertility and death for the livestock inhabiting tsetse infested areas, the disease renders vast areas totally unsuitable for maintenance of livestock.

In Tropical areas, only a few species of trypanosomes are of overwhelming importance as a cause of morbidity and mortality in domestic animals. *T. vivax* predominates in West Africa and *T. congolense* in East

Africa (Valli, Forsberg and Robinson, 1978a). *T. brucei* and *T. evansi* are occasionally encountered in these regions as well. In Kenya, trypanosomes encountered in order of importance are; *T. congolense*, *T. vivax*, *T. brucei* and *T. evansi*. The first three are found in Nyanza, Rift Valley, Eastern, North Eastern, Coast and some parts of Central province. Surra (*T. evansi*) of camels transmitted mechanically occurs in Northern desert parts of the country wherever camels are kept (Whiteside, 1958).

The distribution of trypanosomiasis closely follows that of tsetse flies. *T. congolense* is quite common in animals in contact with tsetse flies. No tsetse fly belt is devoid of the disease and every kind of domestic mammal is susceptible to the infection (Willet, 1970). The widespread nature of these trypanosomes has been attributed to a number of factors: (a) Some tsetse fly species are capable of transmitting the disease each of which is adapted to different climatic conditions and vegetations (Ford, 1970). (b) The trypanosomes are capable of infecting not only most of the domestic animals but also many species of wild game (Willet, 1970; Murray, Morris and Whitelaw, 1982; Maloo, 1986). These wild animals and some breeds of cattle such as N'Dama and Muturu usually suffer no clinical disease but become "healthy carriers" which constitute important reservoirs of infection. The latter animals maintain a low parasitaemia which is difficult to diagnose by the routinely employed methods, but may be sufficient enough to infect *Glossina* feeding on them (Gaafar, Urquhart, Euzeby, Soulsby and Lammler, 1971). (c) The

antigenic variation phenomenon makes sure that a persistent infection in the bloodstream is maintained which provides ample opportunity for transmission by tsetse flies.

The outcome of an infection depends on a variety of factors such as the species and strains of the trypanosome, the susceptibility of the mammalian host and certain environmental factors.

N'Dama and Muturu breeds of cattle, Dwarf West African goats and Djallonke sheep are less susceptible to trypanosomiasis than their exotic counterparts. These trypanotolerant livestock are found in trypanosomiasis endemic areas and are thought to have evolved through natural selection. They are known to regulate both the level and duration of parasitaemia.

Environmental factors particularly stress greatly influence the outcome of an infection. During drought, the poor nutritional status of cattle renders them more susceptible to the effects of the disease. In addition, workload of draft animals and reproductive diseases increase the severity of the disease (Murray, Trail and Grootenhuis, 1984).

2.4 PATHOGENESIS

A few days following metacyclic trypanosomal inoculation by infected tsetse flies into clean susceptible cattle, a raised subcutaneous swelling several centimetres in diameter develops. The lesion, " a chancre", specific for the trypanosome persists for 2-3 weeks, and is thought to be the primary site for

multiplication of trypanosomes. It consists of lymphoblasts and plasma cells (Luckins and Gray, 1978) or may consist of lymphoblasts, eosinophils, fibroblasts and few neutrophils (Grootenhuis, Dwinger, Dolan, Moloo and Murray, 1990). Trypanosomes are observed extravascularly within the chancre and are thought to gain access into the bloodstream either directly by migration or via afferent and efferent lymphatics. The chancre size depends on the species of trypanosome involved. In cattle, *T. congolense and T. brucei* induce very large skin reactions while *T. vivax* produces a much smaller lesion. Similar lesions (chancres) have been observed in goats, sheep, wildlife and man (Morrison, Murray and Akol, 1985). The appearance of the chancre precedes detectable parasitaemia by a few days and it is accompanied by the development of fever and marked enlargement of draining lymph node(s). This is followed by generalized lymphadenopathy and spenomegally. The lymph nodes are not only palpably enlarged, but may be visibly enlarged from a distance (Stephen, 1986).

In cattle, anaemia has been regarded as a reliable indicator of the progression of the disease. Two to three weeks post-infection, there is development of a severe anaemia which correlates directly with the appearance and level of parasitaemia. The anaemia is thought to be haemolytic in nature and it is as a result of increased red cell destruction by the phagocytes in the spleen, liver, lungs, haemal nodes, bone marrow and in blood stream (Fiennes, 1970; Stephen, 1986).

Cattle surviving this phase of anaemia or those that do not become re-infected, gradually proceed to another phase of anaemia which appears in cattle any time between 4 and 6 months post-infection. At this time, there is progressively decreasing waves of parasitaemia with parasites becoming more scanty, sporadic, and difficult to detect. However, animals become progressively anaemic and respond very poorly to chemotherapy. The probable mechanisms precipitating this anaemia have been postulated to be failure of haemopoiesis due to marrow dysfunction, haemolysis resulting from toxins and erythrophagocytosis (Fiennes, 1970).

Organ and tissue lesions observed correlate with the invasiveness of the trypanosome. *T. congolense*, an obligate intravascular parasite is thought to produce the lesions observed, partly through anaemic anoxia and partly through the microcirculatory disturbance.

2.5 CLINICAL SIGNS

In cattle, *T. congolense* infection has a prepatent period of 5-10 days. With appearance of trypanosomes in the blood, the body temperature rises to between 39.4° C and 40.0° C, seldomly higher. The disease may consist of a series of crises characterised by relapsing fever. The peaks of temperature recur every 12 days, persisting for 2 - 3 days. After 3 or 4 such peaks, the animals go to a period of elevated temperature, lasting upto 30 days which may terminate in death. The pyrexia tends to parallel the parasitaemia throughout the

course of infection. Appetite is good except during periods of pyrexia when the animal appears dull, inactive and depressed. The superficial lymph nodes may or may not be notably enlarged (Fiennes, 1970; Stephen, 1986).

In cattle, as the disease progresses, anaemia becomes more marked. The mucus membranes become pale and the pulse and respiratory rates increase. The pulse rate rises from 70 beats/minute to 120 beats/minute while the respiratory rate rises from 30 to 55 cycles/ minute (Valli *et al*, 1978a). The haircoat becomes lustreless and starry and there is loss of libido. Terminally, animals are often too weak to rise and eat, have laboured breathing accompanied by grunts, and the jugular pulse may be seen. Mucus membranes appear white. Death occurs in a few weeks but may take several months to a year. Spontaneous recovery may occur. Calves continuously infected show stunted growth and fail to reach maturity (Stephen, 1986).

In the horse, *T. congolense* infection may have a prepatent period of 17 - 19 days (Stephen, 1962). Pyrexia is only observed at peak parasitaemia. The appetite, faeces and urine are unchanged. Some oedema of legs below the carpals and hock joints as well as moderate hydrocele may be observed. Throughout the course of infection, the animal may show no weight loss and may remain alert. Stephen (1962) observed that the horse spontaneously recovered by the 45th day post-infection. The parasites became scanty and sporadic in circulation but they could be detected for a period exceeding one year. Kimberling and Ewing (1973) observed a contrasting picture in two field

cases in Kenya. They noted a generalized oedema, anorexia, fever, pounding heart beat and increased respiratory rate. They recorded a heart beat of 86 beats/ minute and a respiratory rate of 22 cycles/ minute as compared with normal values of 28 - 40 beats/minute and 12 - 18 cycles/minute respectively. The mucus membranes were icteric and congested and treatment was instituted to save the lives of these animals.

T. brucei causes a disease in donkeys similar to that observed in the horse, ruminants and dogs. The disease has a prepatent period of 5 days and a parasitaemia appears in transient peaks although quite persistent. Pyrexia of upto 40.0° C may coincide with peak parasitaemia. The pyrexia persists for 5 days before it fluctuates. The heart beat rises to 68 beats/minute on the highest. The jugular pulse may be prominent during the terminal stages. Anaemia and emaciation are visible terminally. The respiratory rate increases from the day of detectable parasitaemia to 34 cycles/ minute. The increased heart rate persists until the animals become moribund. The breathing may be shallow and partly abdominal while moist rales suggestive of pulmonary oedema are auscultated in terminal stages. The animals have normal faeces and urine and maintain a capricious appetite (Ikede *et al*, 1977).

T. evansi has been shown to cause a similar clinical picture in donkeys to that reported in other domestic animals. The donkeys come down with a chronic disease characterised by anaemia and emaciation while the appetite remain normal or becomes capricious. A subacute to a chronic infection where

animals display intermittent fever, dullness, weakness, emaciation, anaemia, lacrimation, persistent nasal watery discharge and loss of body condition has been reported (Suryanarayana *et al*, 1986).

2.6 CLINICAL PATHOLOGY

2.6.1 HAEMATOLOGY

Anaemia, a reduction below normal of red blood cell (RBC) numbers and/or haemoglobin (Hb) concentration reflects a secondary development to a primary disease (Schalm, 1975). The reduction in packed cell volume (PCV) and the RBC numbers is a characteristic feature in trypanosomiasis and are usually accompanied by red cell morphological changes and haemoglobin content .

The red cell morphology in anaemia may be described according to whether the mean corpuscular volume (MCV) is normal (normocytic), above normal (macrocytic) or below normal (microcytic). In addition, the mean corpuscular haemoglobin concentration (MCHC) indicate whether the haemoglobin content is normal (normochromic) or depressed (hypochromic) (Schalm, 1975). These observations provide a useful morphological classification on which most cases of anaemia in bovine trypanosomiasis have been described (Saror, 1979)

Anaemia has been described as the most significant feature of animal trypanosomiasis. The severity of anaemia depends not only on the trypanosome species, but also, on the host species and the acuteness as well as the chronicity of the infection (Cox, 1979). The significance of anaemia on the overall morbidity of animal trypanosomiasis has been difficult to assess due to concurrent diseases such as parasitic infections and nutritional deficiencies . However, Obi and Anosa (1980) in studying trypanosomiasis in Nigerian cattle indicated that the disease produced a more severe anaemia than most of the blood protozoan and gastrointestinal helminth parasites encountered among the animals. Studies conducted by Mamo and Holmes (1975) in Ethiopian Zebu cattle infected with *T. congolense* also indicated that anaemia is the primary cause of death.

In cattle, *T. congolense* may cause a macrocytic anaemia with an MCV value of 58 fentolitres (fl) on the highest in acute cases, and a microcytic anaemia with a MCV of 50fl on the lowest in chronic cases (Fiennes, 1954). Naylor (1971a); Mamo and Holmes (1975); Maxie, Losos and Tabel (1979); Valli and Mills (1980) and Valli *et al* (1978b) have reported a normochromic normocytic anaemia during early infections. Losos, Paris,Wilson and Dar (1973); Wellde, Lotzsch, Deindl, Sadum, Williams and Warui (1974) observed a normocytic normochromic anaemia which was also described by Valli *et al* (1978b) in chronic infections.

The anaemia in the acute phase of the disease is regenerative in nature.

An increase in MCV of upto 56fl and MCH of 20.7pg (Naylor, 1971a and Losos *et al*, 1973) indicate a responsive bone marrow. Fiennes (1954); Naylor (1971a) and Valli *et al* (1978b) indicated that the reticulocytes in peripheral blood could rarely be observed in *T. congolense* infected cattle. Kaaya (1975); Mackenzie and Cruickshank (1973) observed marked increase in reticulocytes in peripheral blood of sheep. Abnormal erythrocytes have been reported by a number of authors in cattle with *T. congolense* infection. Omuse (1973) and Naylor (1971a) observed anisocytosis; Fiennes (1954) saw punctate basophilic cells and cells with cabot rings, while Wellde *et al* (1974) reported anisocytosis, polychromatophilia, basophilic stippling and normoblasts.

In the horse, *T. congolense* infection causes a decrease in PCV, RBC count and Hb concentration. The RBC decrease to a lowest value of 5.5 x 10^6/ml, PCV to 21% and Hb concentration to 10.6 gm% within the same period. These parameters decrease progressively from the day of infection but later rise to pre-infection values (Stephen, 1962). Kimberling and Ewing (1973) indicated that the PCV at one time of peak parasitaemia reached 9% but a few days after treatment it rose to 35%, which they regarded as normal. A similar progressive anaemia has been reported in *T. vivax* infection in the horse (Stephen and Mackenzie, 1959).

In the donkeys, the PCV, RBC count and Hb concentration decrease progressively from the day of inoculation with *T. brucei* (Ikede *et al*, 1977). The PCV , RBC count and Hb content decrease to 18.6%, 2.89 x 10^6 /ml, and

6.39gm%, respectively, indicating approximately 40% fall in red cell indices. No further alterations in red cell indices were recorded up to the time of death. *T. evansi* infection in donkeys also show a gradual but marked fall in PCV, Hb content and RBC count (Suryanarayana *et al* 1986) while *T. vivax* only produce mild changes in these haematological parameters which appear within the normal ranges (Kyewalabye *et al*,1989).

Leukocytes also show dramatic changes in *T. congolense* infections in cattle. Leukopenia appear in early stages of the disease (Losos *et al* 1973; Omuse, 1973; Wellde *et al*, 1974; Valli *et al*, 1978b). This may be attributed to a decrease in both the neutrophils and lymphocytes. Lymphocyte count as low as 5.00 x 10^3 /ml which later rise to a count of 11.5 x 10^3 /ml relative to a total leukocyte count of 14.0 x 10^3/ml may be seen. The leukopenia may be evident at the onset of patent parasitaemia and persist throughout the course of infection. Low leukocyte counts may be encountered during high levels of parasitaemia (Wellde *et al*, 1974). A relative increase in mononuclear leukocyte counts occur early in infection with relative decrease in segmented neutrophils which increase terminally. Eosinophils, lymphocytes and neutrophils may be depressed early in infection while monocyte levels show little change. On the other hand, basophils tend to disappear but plasma cells increase in number. Leukocytosis may be encountered terminally (Naylor, 1971a).

In the donkey, *T. brucei* infection causes a leukopenia as observed in other animals. A count as low as 7.0 x 10^3/ml may be observed by the 7th day

of infection. At this time of leukopenia, there is a relative lymphocytosis and a neutropenia, while the absolute lymphocyte counts remain relatively constant (Ikede *et al*, 1977).

The pathogenesis of anaemia manifested in natural infections with trypanosomes have been a matter of conjecture. Most workers suggest haemolysis, haemodilution and dyshaemopoiesis as the major mechanisms precipitating the anaemia. They may act singly or in concert (Suliman and Feldman, 1989). In acute infections, anaemia is thought to be due to haemolysis. The progressive fall in Hb and PCV together with the reduction in RBC count,decreased red cell survival time as well as increased percentage of circulating reticulocytes suggest erythrocyte destruction. Several factors have been incriminated to induce the haemolysis. Among these are : released haemolytic factors (Esievo, Saror, Ilemobade and Hallaway,1982); immunological mechanisms (Kobayashi, Tizard and Woo, 1986); non-specific activation of RES (or monocyte-macrophage system ;MPS) (Stephen, 1986); and pyrexia (Suliman and Feldman, 1989).

Haemodilution has been considered as a cause of anaemia in cattle (Fiennes, 1954; Naylor 1971a; Mamo and Holmes, 1975). In chronic or prolonged stages of trypanosomiasis, anaemia manifested is believed to be due to failure of haemopoiesis and bone marrow function. Hypoferreamia, microcystosis (Fiennes, 1970), low plasma iron turnover (Dargie , Murray, Murray, Grimshaw and McIntyre, 1979), increased haemosiderin deposits within

the RES (Valli and Forsberg, 1979), depressed immature red cell numbers in the bone marrow and yellow gelatinous marrow all suggest dyshaemopoiesis. These findings indicate defective iron incorporation into developing red cells of the bone marrow. This dysfunction of bone marrow may explain the poor response to trypanocidal therapy in animals with long-standing infections. Myeloid hypoplasia of the bone marrow with absence of reticulocytes in circulation further supports dyshaemopoiesis as the cause of anaemia observed in chronic cases of trypanosomiasis (Fiennes, 1954; Naylor, 1971b).

2.6.2 SERUM BIOCHEMISTRY

The change in plasma proteins may not be significant in the course of the disease (Suliman and Feldman, 1989). The contrary has been reported in cattle infected with *T. congolense* (Naylor, 1971a; Omuse, 1973; Wellde *et al*, 1974). The total proteins may fall early in infection to 5.4 mg% but may later rise to pre-infection values. Valli, Mills, Lumsden, Rattray, and Forsberg (1980) observed no major changes in serum proteins in neonatal calves while in 6 month old calves, reduced serum proteins were recorded.

Plasma fibrinogen content have been of major concern recently in infected animals as it is involved in the formation of fibrin thrombi in small blood vessels. An initial rise as high as 647 mg% has been recorded early in infection in cattle infected with *T. congolense* but a rapid decrease to values as low as 352 mg% may occur (Forsberg, Valli, Gentry and Donworth,1979).

Organ function tests have been carried out to determine the most severely affected organs as well as try to unravel the pathogenesis of the disease. The serum bilirubin, serum transaminases, blood urea nitrogen , creatine, glucose, total lipids, thyroxine binding globulin (T_3), thyroxine (T_4), cortisol and plasma electrolytes have been estimated in infected animals (Fiennes, 1954; Naylor, 1971a; Wellde *et al*, 1974; Valli *et al*, 1980). Indirect bilirubin may increase from the range of 0.0 - 0.4mg% to 1.2mg% in acute infections but may be within the normal range in chronically infected animals (Fiennes, 1954; Naylor, 1971a). Serum glutamic oxaloacetic transaminases (SGOT;aspartate aminotransferase ;AST), blood urea nitrogen (BUN), creatine and glucose in cattle infected with *T. congolense* have been studied. AST decrease early in infection from 62 Reitman Frankel units (RF) before infection to 34 RF later in infection. AST later rise sharply and remain relatively stable at this level while BUN increase gradually to reach 9 mg% by the 30th week of infection. Creatine display constant levels at 2.5 mg% early in infection. Glucose remain relatively constant except in acute and terminally infected animals,where it decreases from 45 mg% to 35 mg% (Wellde *et al* , 1974).

2.7 GROSS PATHOLOGY

Ruminants dying from *T. congolense* infection have emaciated carcasses and generalized gelatinous fat atrophy. Increased amounts of straw-coloured fluid in body cavities and the subcutis may occur (Omuse, 1973; Kaliner, 1974; Kaaya, 1975; Valli *et al*, 1978a; Mwangi, 1987).

The lymphoid organs are extensively involved. Enlargement of all the lymph nodes in cattle and goats may occur. This enlargement may be due to oedema, haemorrhage or both (Losos *et al*, 1973; Omuse, 1973; Valli *et al*, 1978a; Kanyari, 1981; Moulton, 1986; Mwangi, 1987). In chronic stages,they become firmer as a result of fibrosis. The thymus and haemolymph nodes are enlarged and display prominent lymphocytic follicles (Valli *et al*, 1978a; Moulton, 1986).

The spleen show uniform enlargement (Losos *et al*, 1973; Omuse, 1973; Kaliner, 1974) and display prominent malphigian bodies bulging out on the cut surface (Kaaya, 1975; Valli *et al*, 1978a). It may increase in weight and remain heavy except in chronic cases where it becomes lighter (Moulton, 1986). The liver may be symmetrically enlarged and congested (Omuse, 1973; Kaaya, 1975; Valli *et al*, 1978a). The kidneys may be slightly congested and display prominent glomeruli (Omuse, 1973; Valli *et al*, 1978a) while the testes may be flabby and contracted with extensively wrinkled scrotum (Kaaya, 1975).

In goats, lungs may show pulmonary oedema (Kaaya, 1975; Mwangi,

1987). In some cases, they may be congested, fail to collapse and are more dense on palpation (Omuse, 1973; Kaliner, 1974; Valli *et al*, 1978a; Jubb, Kennedy,and Palmer, 1985), as observed in cattle. Intercurrent anterior bronchopneumonia due to secondary bacterial infections may occur (Jubb *et al*, 1985b) while pneumonic areas and abscesses have also been reported in goats (Mwangi, 1987).

The heart may be flabby and oedematous (Omuse, 1973; Kaaya, 1975). Hypertrophy of the left ventricle , serous atrophy of coronary fat and white foci on epicardium may also be encountered (Kaaya, 1975; Valli *et al* ,1978a; Jubb *et al*,1985b).

In cattle and goats, the marrow of long bones may display marked reddish brown areas indicating erythropoiesis (Losos *et al*, 1973; Kaaya, 1975; Valli *et al*, 1978a). In cattle,the brain may show oedema especially under the meninges and between the gyri (Omuse, 1973; Kaliner, 1974) or display prominent choroid plexus (Valli *et al*, 1978a). Focal polioencaphalomalacia has also been reported (Losos *et al*, 1973).

2.8 HISTOPATHOLOGY

2.8.1 LYMPH NODES

In cattle, the lymph nodes may show haemorrhage and follicular hyperplasia in acute cases (Naylor, 1971b; Losos *et al*, 1973; Omuse, 1973; Valli and Forsberg, 1979; Jubb *et al* 1985b; Moulton, 1986). On microscopic examination, grossly haemorrhagic nodules consist of active lymphatic tissue surrounded by areas of congestion. In more chronic cases, necrosis of lymphocytes and infiltration with plasma cells in the medulla, and extensive fibrosis are consistent features. There is a reduction in density of the large lymphocyte population, increase in large reticuloendothelial cells as well as plasma cells in the parafollicular and medullary parasinusoidal areas. The sinuses are also dilated with macrophages (Naylor, 1971b; Omuse, 1973; Kaliner, 1974; Jubb *et al*, 1985b; Moulton, 1986). Haemosiderosis may be occasionally encountered (Jubb *et al, 1985b).* In goats, hyperplasia of the cortex with secondary follicles but no haemosiderosis may occur (Kaaya, 1975). Phagocytosis of erythrocytes and trypanosomes together with marked haemosiderosis may be seen (Mwangi, 1987).

2.8.2 HAEMAL NODES

In acute infections, in cattle,the haemal nodes may show hypertrophy of the cortex with formation of well defined lymphatic follicles (Losos *et al,*

1973). In chronic stages, a reduction in the sinus width and a general increase in macrophages, plasma cells and haemosiderin may be encountered (Valli and Forsberg, 1979). In sheep, lymphoid hyperplasia, sinusoids packed with macrophages (which have a number of intact erythrocytes and haemosiderin) may be seen (Mackenzie and Cruickshank, 1973).

2.8.3 SPLEEN

In cattle the spleen may be congested and show follicular hyperplasia in acute cases. The follicles may have hyperplastic cells and blast cells replace the small lymphocytes in the periarterial lymphatic sheaths (PALS) and in the marginal zones. Macrophages may be plentiful in the red pulp sinuses (Naylor, 1971b; Losos *et al*, 1973; Moulton, 1986). In chronic cases, the follicles may vary in size with some being large and displaying blast cells, while others are inactive. Germinal centres may show central hyaline changes depicting lymphocytic necrosis (Omuse, 1973; Moulton, 1986). Haemosiderosis may be frequently encountered in the germinal centres and red pulp (Losos *et al*, 1973; Kaliner, 1974). In goats, hyperplasia of the red and white pulp, numerous plasma cells and macrophages may be observed. Mwangi (1987) reported hyperplasia and enlargement of the RES with evidence of phagocytosis of even trypanosomes. Haemosiderosis has also been seen (Kaaya, 1975).

2.8.4 LIVER

In cattle, the liver show distension of sinusoids and central veins with cellular material, haemosiderin in the central veins and extramedullary erythropoiesis in the sinusoids. (Naylor, 1971b; Losos *et al*, 1973). Hypertrophic kupffer cells, centrolobular necrosis, fatty infiltration and atrophy of hepatocytes may occur (Omuse, 1973; Kaliner, 1974). Increased interstitial stroma without changes in lobular organisation and generalised kupffer cell hyperplasia may also occur. In goats, slight enlargement of kupffer cells may be seen. Sinusoids and veins may show large number of neutrophils and small aggregations of lymphocytes and macrophages may be encountered in periportal areas (Kaaya, 1975; Mwangi 1987).

2.8.5 KIDNEYS

In cattle, kidneys may show hypercellular glomeruli and foci of mononuclealar cell infitration (Naylor, 1971b; Losos *et al* , 1973; Kaliner, 1974; Valli and Forsberg, 1979). Periglomerular and perivascular cuffing formed by plasma cells and slight fatty infiltration may be observed (Omuse, 1973). Haemosiderin deposits have also be seen in tubular epithelial cells (Naylor, 1971b; Kaliner, 1974; Valli and Forsberg, 1979). In goats, hypercellularity of the glomeruli, some foci of interstitial nephritis and hyaline casts in the distal and proximal convoluted tubules have been reported (Kaaya, 1975).

2.8.6 PANCREAS AND ADRENAL GLANDS

In cattle, necrosis of islets of Langerhans may occur in the pancreas while the medulla of the adrenal glands may be slightly infiltrated with mononuclealar cells (Omuse, 1973).

2.8.7 TESTES

In goats, complete or almost complete degeneration of seminiferous tubular epithelium except for a few spermatogonia and primary spermatocytes may be observed (Kaaya, 1975). Seminiferous tubules may be markedly shrunken while the epididymal lumina appear empty (Kaaya and Oduor-Okell, 1980; Sekoni, Njoku, Kumi-Diaka and Saror, 1990).

2.8.8 HEART

In cattle, the heart may show myocardial degeneration and small foci of hyaline material (Naylor, 1971b; Omuse, 1973). Interstitial infiltration with macrophages and lymphocytes in the myocardium may be encountered (Kaliner, 1974; Valli and Forsberg, 1979; Jubb *et al*,1985b).

2.8.9 LUNGS

In cattle, lungs may show congestion, increased number of macrophages, presence of haemosiderin and thickened interalveolar septa (Naylor, 1971b; Losos *et al*, 1973; Kaliner, 1974; Valli and Forsberg, 1979). In goats, moderate

congestion, oedema, alveolar emphysema, thickening of interalveolar septa and alveolar walls; together with haemosiderin in alveolar capillaries has been observed (Kaaya, 1975).

2.8.10 BONE MARROW

In acute infections, in cattle, the long bones may show hyperplasia of erythroid tissue with nests of immature red blood cells but no evidence of leukopoiesis, but megakaryocytes may be numerous. Haemosiderin may be encountered occasionally (Naylor, 1971a, Losos *et al*,1973; Valli and Forsberg, 1979). In sheep, Mackenzie and Cruickshank (1973) saw hyperplasia of erythroid tissue with conspicuous erythrophagocytosis, and a decrease in myeloid: erythroid ratio (M:E) together with increased reticulocytes.

2.8.11 BRAIN

In cattle, focal polioencephalo-malacia affecting the grey matter on the apex of the gyri, which also involves primarily the molecular, granular and pyramidal layers may occur. Small blood vessels may be engorged and trypanosomes may be seen in them (Losos *et al* ,1973). Oedema of the brain, coupled with veins distended with blood, vascular wall filled with macrophages and haemosiderin may be seen. Perivascular infiltrations with histocytes, lymphocytes, plasma cells and occasionally, eosinophils have been reported (Kaliner, 1974).

2.8.12 BLOOD VESSELS

In cattle, capillaries in the skeletal muscles and the cerebrum may show hypertrophic endothelial cells often occluding the lumen. Some capillaries in the same locality may be dilated and trypanosomes localized in them (Losos *et al*, 1973; Kaliner, 1974; Valli and Forsberg, 1979).

Ikede *et al* (1977) described the histopathology of an experimental *T. brucei* infection in donkeys. They indicated that the donkeys suffered a non-purulent encephalitis. The liver displayed marked haemosiderin deposition, centrolobular congestion,and fatty change. Erythrophagocytosis by kupffer cells was also observed. The spleen and the lymph nodes were active and displayed massive haemosiderosis. The kidneys showed perivascular cuffing by mononuclear cells and tubular casts (proteinaceous and crystalline casts). These conform quite well with what has been observed in other domestic animals (Stephen, 1986).

2.9 DIAGNOSIS OF ANIMAL TRYPANOSOMIASIS

The diagnostic techniques can be broadly classified into parasitological, serological, artificial culture media, xenodiagnosis, animal subinoculation and chemical tests. Originally, the diagnosis of animal trypanosomiasis was based on the demonstration of trypanosomes in blood and tissue fluids on wet preparations, later on, thin and thick stained smears. However, advances in technology have brought about the development of more sensitive and more

sophisticated techniques. Currently the parasite concentration and deoxyribonucleic acid (DNA) probes are being used. Antibody detection systems have undergone similar changes from the more remote precipitation and agglutination tests to the more elaborate and sensitive techniques such as haemagglutination tests, fluorescent antibody tests and Enzyme Linked Immunosorbent Assay (ELISA) tests. Despite all these enormous changes, the principles of diagnosis have remained the same, namely; the demonstration of the trypanosomes and the detection of increasing antibody titres in infected animals. No single technique no matter how sophisticated it might appear has been able to detect infections all the time; hence a combination of two or more of these techniques often give the best results (Stephen, 1986).

3 MATERIALS AND METHODS

3.1 EXPERIMENTAL DONKEYS

Eight mature donkeys of mixed sexes were purchased from an open market in Limuru, a tsetse fly free area in Kiambu district and transported to the Department of Veterinary Pathology and Microbiology; University of Nairobi. They were selected on the basis of their body condition as well as the age. They were labelled with ear tags as D1 to D8 and divided randomly into two groups; infected group (D1, D2, D4, D5 and D6) and control group (D3, D7 and D8). They were housed in two adjacent fly-proof stalls and dewormed with pyrantel embonate granules (Strongylid plus; Pfizer limited, England) at a dosage rate recommended for the horse to remove gastrointestinal nematodes. They were fed on hay, mineral salts, concentrate supplements and water provided *ad libitum*. The donkeys were allowed to acclimatize for six weeks during which time they were monitored thoroughly for their state of health.

3.2 TRYPANOSOMES AND ANIMAL INOCULATION

Ten Balb C mice in peak parasitaemia with *Trypanosoma congolense*, strain IL 3575 were acquired from International Laboratory for Research on Animal Diseases (ILRAD). Strain IL 3575 was obtained from IL 1180 after a single passage in mice. The latter was previously obtained by one passage in mice from a clone IL 968 (Nantulya, Musoke, Rurangirwa and Moolo, 1984),

a derivative of STIB 212, isolated from a lion in Serengeti, Tanzania (Geigy and Kauffmann,1973).

These mice were anaesthetized with diethyl ether and bled to death from orbital sinuses. Blood from all the mice was pooled and heparin used as the anticoagulant. The trypanosome concentration was estimated using an improved double Neubeur haemocytometer. One millilitre(ml) of this pooled blood contained 1.0×10^7 trypanosomes. The blood was subsequently diluted to a final dilution of approximately 1.5×10^6 trypanosomes per ml using phosphate saline glucose (PSG) of pH 7.8.

Each of the five donkeys D1, D2, D4, D5 and D6 was inoculated subcutaneously around the neck region with 5ml of the blood containing approximately 7.5×10^6 trypanosomes.

3.3 ANIMAL OBSERVATIONS

During the acclimatization period, the rectal temperature, respiratory and heart rates were taken and recorded every morning. Blood was collected once a week for haematology and once a fortnight for serum biochemistry. Faecal samples for parasite egg counts were collected twice during this period to asses the level of helminth infection. Blood was thoroughly screened for any blood parasites using parasitological techniques.

The experimental period consisted of three months. The rectal temperature, respiratory rate and heart rate and their character recorded before

blood samples were collected. These three parameters were taken daily for the first one month of infection and only five times a week thereafter.

3.4 COLLECTION OF BLOOD SAMPLES

Blood for haematology was collected twice a week. Two to five millilitres of blood were collected from the jugular vein using a disposable needle (G18; 1.5 inches long) into Bijon bottles containing sodium-ethylene-diamine tetra-acetic acid(EDTA). Ten to fifteen millitres of blood were taken using an equivalent needle size into clean dry universal bottles twice a month for serum extraction.

3.5 BLOOD ANALYSIS

3.5.1 RBC

Blood was well mixed then diluted with isotonic saline (Isoton; coulter electronics, Kenya Limited, Nairobi) to 1:20 dilution for erythrocyte count, MCV and haematocrit determinations. This diluted blood was drawn into a coulter counter (Model ZM; coulter electronics Inc., Florida). Blood cells being poor conductors of electricity introduce a resistance into the electrical circuit and as they pass through the aperture, pulses appear on an oscilloscope where they are counted. MCV computer and haematocrit accessory units enable the system to perform haematocrit and MCV simultaneously.

3.5.2 LEUKOCYTES

Blood for leukocyte count and haemoglobin determination was diluted with isoton to a 1:200 dilution. Zap-oglobin (Coulter electronics, Kenya limited, Nairobi) was added into this diluted blood to lyse all the erythrocytes and convert all the haemoglobin into cyanmethaemoglobin. The leukocytes were counted as for erythrocytes.

3.5.3 HAEMOGLOBIN

Haemoglobin was estimated by a cyanmethaemoglobin method (Schalm, 1975) and read in a heamoglobinometer (Coultronics, France S.A.; Margency).

3.5.4 PACKED CELL VOLUME (PCV) / MICRO-HAEMATOCRIT

Centrifugally derived packed cell volume (micro-haematocrit) was determined by centrifuging well mixed blood contained in capillary tubes (75mm in length; with an internal diameter of 1.2mm) sealed at one end at 12,500 revolutions /minute (r.p.m.) for 5 minutes to ensure that erythrocytes were well packed without trapping any plasma in between. The haematocrit value was determined on a micro-haematocrit reader (Hawksley and sons Limited, London).

3.5.5 TOTAL PLASMA PROTEINS

Total plasma protein was determined by flooding the plasma on the prism

of a refractometer (model SPR-T_2; Atago, Japan). The value was read after viewing through the eyepiece of the refractometer in natural light and the value recorded as the point where the dividing line between the bright and dark fields crosses the scale (Schalm, 1975).

Plasma in capillary tubes was put into a water-bath at 56-58°C for 3-5 minutes, after which it was centrifuged for the standard 5 minutes as above to precipitate the fibrinogen onto the buffy coat. The remaining plasma was placed onto a refractometer prism and the readings made. The fibrinogen content was regarded as the difference between the total plasma protein reading and the results obtained after heating plasm (Schalm, 1975).

3.5.6 ICTERUS INDEX

Icterus index was estimated by matching the plasma colour in the capillary tubes with standards prepared from potassium dichromate and scored from 0 to 100 units (Schalm, 1975).

3.5.7 DIFFERENTIAL LEUKOCYTE COUNTS

The differential leukocyte counts were done after staining the smears with 1:5 Giemsa stain solution. Smears were made from well mixed blood, air dried, fixed in absolute methyl alcohol for 3 - 5 minutes and stained with freshly prepared 1:5 Giemsa stain solution. They were allowed to stain for 15 - 20 minutes washed in slow running water, dried and observed under oil-immersion.

The battlement technique (Schalm, 1975) consisting of counting three horizontal edge fields followed by two fields towards the centre followed by two horizontal fields, then two fields in the vertical direction to reach the edge again was used until 100 leukocytes were counted as they were differentiated.

3.5.8 RETICULOCYTE COUNTS

New methylene blue stain solution was used to stain for reticulocytes. Two drops of blood were mixed with an equal quantity of the stain, and left in contact for 15 - 20 minutes in small test tubes. A thin smear was prepared from the mixture in the usual manner and allowed to dry. The reticulocytes were counted for every 100 erythrocytes under oil-immersion.

3.5.9 DETERMINATION OF ERYTHROCYTE INDICES

Mean corpuscular volume (MCV) expressed in fentolitres (fl) was read directly from the computer accessory in the coulter counter.

Mean corpuscular haemoglobin (MCH) expressed in picograms (pg) as the average weight of haemoglobin in the erythrocyte population of cells was calculated as follows:-

$$MCH = \frac{\text{Haemoglobin content (g/dl)}}{\text{erythrocyte count } (x10^6/ml)} \times 10 \text{ pg}$$

Mean corpuscular haemoglobin concentration (MCHC) expressed in grams/100ml of blood (gm%) is the ratio of weight of the haemoglobin to the volume of erythrocyte and was calculated as follows:-

$$\text{MCHC} = \frac{\text{Haemoglobin content (g/dl)}}{\text{packed cell volume (\%)}} \times 100 \text{ gm\%}$$

The packed cell volume used was obtained from the coulter counter reading. These formulae were obtained from the work of Dixon and Archer (1974).

3.5.10 SERUM BIOCHEMISTRY

Blood in universal bottles was allowed to stand at room temperature until a clot formed at the base of the bottle and serum separated out at the top. Serum was later extracted by centrifuging this clotted blood at 4,500 r.p.m for 10 minutes and the clear serum pipetted out into clean and dry plastic vials. The serum was used for the estimation of blood urea nitrogen (BUN) and total bilirubin.

BUN was determined by a kit from coultronics (France, S.A. Margency)

using this principle:

$$\text{Urea} + 2H_2O \xrightarrow{\text{urease}} 2NH_4^+ + Co_3^{2-}.$$

The ammonium ions react with phenol and hypochlorite to give a coloured complex whose rate of formation and relative amounts can be estimated by a kinetic method in a spectrophotometer (Model ASA 24; coultronics France S.A.; Margency). The reading was done at 546 nm wavelength.

The total bilirubin was determined by colorimetric method using a kit from Boehringer (Mannhean, France S.A.; Margency). The bilirubin in the sample is coupled with diazotized sulfanilic acid in the presence of caffeine to give an azo dye. The azo dye level can be estimated in a spectrophotometer at 578 nm wavelength.

3.6 POST-MORTEM EXAMINATION

The donkeys were euthanized with pentobarbitone sodium (Euthatal; May and Baker Limited; Dagenham, England) at a total dosage of 4000 milligrams (mg) infused intravenously for a rapid knock down. Whenever this drug was not enough, the unconscious animals were killed by stabbing the spinal cord at the cervical region. The post-mortem was done according to methods described for the horse (Jones and Gleiser, 1954).

All the pathological changes were recorded and samples collected from the heart, lungs, liver, spleen, lymph nodes, stomach, intestines, kidneys, adrenal glands, brain, bone marrow whether or not they were showing pathological changes. The samples were fixed in 10% formalin, embedded in paraffin wax, sectioned at a thickness of 6 µm thick and stained with heamatoxtylin and eosin (H&E).

DIAGNOSIS

For the detection of the trypanosomes, a combination of a modified darkground/ phase contrast buffy coat technique (Murray, Murray and McIntyre; 1977) subinoculation into susceptible laboratory animals was used. Blood from the marginal ear vein was collected into heparinized capillary tubes every morning (except on days when blood was collected for haematology). The capillary tubes were centrifuged for the standard five minutes, PCV read and then cut at the level of the buffy coat to include 1mm of the erythrocytes and 1cm of the plasma. The contents were expelled onto a clean dry slide. The resulting drop of the buffy coat and plasma was mixed thoroughly before spreading it out as a thin smear. The smear was air-dried, fixed in absolute methyl alcohol for 3 - 5 minutes and stained with 1:5 Giemsa stain solution for 15 - 20 minutes. The smear was then examined for trypanosomes. At least 200 oil-immersion fields starting from the tail of the smear were examined before the animals could be declared negative for trypanosomes.

Subinoculation into mice was done twice in the course of the experiment for diagnostic purposes and test of pathogenicity. Mice used for this purpose were 2 - 2.5 months old.

3.8 MOUSE AND SHEEP SUBINOCULATIONS FOR PATHOGENICITY

Blood from all the infected donkeys was inoculated into mice and sheep to try and verify whether the trypanosomes observed on the smears were still pathogenic at least to these animals (mice and sheep). The first subinoculation into mice was done on day 29 post-infection when the first parasitaemia was detected in one of the infected donkeys (D4). Two mice were used for each donkey and 0.5 - 1.0 ml of freshly drawn blood was inoculated intraperitoneally. These mice were monitored for 30 days taking blood from the tail and making a wet film every three days. The wet films were observed in subdued light for the presence of trypanosomes. The donkeys were declared not infected at the time of mice inoculation if the mice remained negative upto the 30th day. A second mice subinoculation was done on day 41 post-infection when three of the infected donkeys (D2, D4 and D6) became parasitaemic. At this time, one of the infected donkeys (D1) had not displayed any parasitaemia and it was therefore necessary to try and propagate them in mice if they were there. The mice were monitored for 30 days as in the first group. All deaths were recorded as a measure of pathogenicity.

Three sheep were acquired from Kinoo, a tsetse fly free area in Kiambu district about 3 kilometres from the Faculty. They were dewormed and monitored clinically for one week before inoculation with blood from mice and donkeys, (D2 was used). One sheep (S3) was inoculated with about 2 ml of blood in EDTA obtained from parasitaemic mice from previous subinoculation. A second sheep (S1) was inoculated with 5 ml of whole blood straight from the donkey (D2) while a third sheep (S2) was inoculated with 15ml of whole blood from the same donkey. The difference in the dosage rate was enevitable in order to establish whether there was a particular blood dosage necessary to cause the infection in the sheep. All the inoculations were done intravenously.

The sheep were monitored clinically every morning. Blood was collected from the jugular vein three times during the one week acclimatization period for haematology and screened for any parasites. During the experimental period blood was collected from the marginal ear vein (for diagnostic purposes) every morning except on week-ends and days when blood for haematology was taken. Blood for haematology was collected from the jugular vein twice a week for the 60 days of experimental period.

Diagnosis of trypanosomiasis in sheep was based on the demonstration of the trypanosomes on thin stained buffy coat smears. Pathogenicity was based on the level of anaemia observed at the termination of the experiment.

3.9 STATISTICAL ANALYSES

The data on haematology and serum biochemistry were subdivided into pre-infection period, first month post-infection, second month post-infection and third month post-infection and analyzed in these categories. Mean values of the parameters for each collection period were compared between the control group and infected group. In the infected group, only those animals that developed a detectable parasitaemia (D2, D4 and D6) were considered. Two tailed student's t-test was used for the analysis in accordance with the formulae below (Steel and Torrie, 1980). The values were considered significantly different at 95% level of significance.

$$D = Y_1 - Y_2$$

$$S^2D = \frac{\Sigma D^2 - (\Sigma D)^2/n}{n-1}$$

$$SD = \frac{\sqrt{S^2D}}{n}$$

$$t = \frac{D}{SD}$$

where Y_1 is the mean value of the parameter monitored in the control group,

Y_2 is the mean value of the parameter monitored in the infected group ,

S^2D is the variance of the sample mean differences,

SD is the standard deviation of sample mean differences,

D is the mean of the sample differences,

n sample size

n-1 are the degrees of freedom.

4 RESULTS

4.1 CLINICAL OBSERVATIONS

During the pre-infection period, the donkeys were observed to be in good body condition. They had a good appetite. The urine and faeces were of normal amounts, colour and consistency. The temperatures were in the range of 36.4°C - 37.8°C, heart rate in the range of 30 - 34 beats per minute while the respiratory rate was in the range of 18 - 22 cycles per minute (Appendices 1-3).

In the course of the experiment, the temperature, respiratory and heart rates showed similar changes in all the infected animals which had parasitaemia. Temperature changes beyond the range indicated above were recorded on a few occasions. D2 showed a temperature of 38.2°C on day 54 post-infection (p.i.) and 38.0°C on day 75 p.i. The first temperature peak came three days after a parasitaemic peak, while the second temperature peak coincided with a parastaemic phase, when parasites could be detected on stained thin buffy coat smears and stained thin blood smears. D4 showed no major temperature changes and most of the recordings were within the range given above. D6 displayed a temperature of 38.2°C, which appeared on day 75 p.i. and coincided with a parasitaemic peak.

The respiratory rates were in the normal range during the first month of infection as shown in Appendix 3. Minor increases were observed during the second month of infection. D2 recorded a reading of 24 cycles per minute on

day 58 p.i. (a 20% increase from the pre-infection value). Similar readings were recorded in D4 and D6 at about the same time.

The heart rate rose to 45 beats per minute in D2 (a 36.4% increase from pre-infection value) on day 54 p.i. The highest reading was 56 beats per minute (a 57.6% increase) during the third month of infection. D4 had a reading of 52 beats per minute (a 62.5% increase) on day 61 p.i. with the highest recorded being 56 beats per minute during the third month. D6 recorded the first noticeable increase on day 75 p.i. when it rose to 48 beats per minute (53.8% increase) with 56 beats per minute being the highest. In all the infected animals, the heart rate remained consistently high after the increase until the termination of the experiment. At the same time, the mucus membranes became pale and the heart beat became pounding in character.

All the infected animals remained alert, bright and maintained a capricious appetite. The faeces and urine indicated no form of abnormality while the mucus membranes showed no form of discoloration.

The control animals showed an average temperature of 36.8°C ± 0.4°C with a range of 36.0 - 37.6°C. The respiratory rate was on average 18.4°C ± 0.8 cycles per minute with a range of 18 - 22 cycles per minute. The heart rate was on average 33.2 ± 3.8 beats per minute with a range of 27 - 44 beats per minute. In the course of the experiment, these parameters with the exception of the heart rate were all in the ranges given above. The heart rate changes were noted particularly during the third month of observation when they were higher

limits of this range indicated.

PATTERNS OF PARASITAEMIA

Following the subcutaneous infection in donkeys, the parasites were first detected on day 29 in D4, day 35 in D6 and day 41 in D2. D5 died on day 19 p.i. with no detectable parasitaemia. D1 on the other hand did not develop any detectable parasitaemia for the entire experimental period. In the three donkeys that developed detectable parasitaemia, transient peaks were observed at particular times as described for each donkey and as shown in Table 1.

D4 first developed detectable parasitaemia on day 29 p.i. observed on stained thin buffy coat smears. The parasitaemia disappeared for 8 days, only to reappear on day 37 p.i. and persisted for 18 days, but at low levels. On day 58 p.i., a high parasitaemia (trypanosomes could be detected in the two types of smears in less than 50 fields) was observed where the parasites could be detected on thin blood smears and thin buffy coat smears. The highest parasitaemia was recorded on day 63 p.i. when two trypanosomes could be observed on each oil-immersion field of stained thin buffy coat smears. After this peak, the parasitaemia became sporadic (trypanosomes were not observed regularly). Another peak of parasitaemia was detected on day 75 p.i. when trypanosomes could again be observed on thin blood smears and thin buffy coat smears. Thereafter , the trypanosomes were detectable regularly until the termination of the experiment.

D6 showed the first detectable parasitaemia on day 35 p.i. which persisted at a low level (trypanosomes could be detected only in stained buffy coat smears after an intensive search) until day 51 p.i. when it disappeared for several days. A second peak was recorded on day 58 p.i. when trypanosomes could be detected on both thin blood smear and thin buffy coat smears. The parasitaemia became even higher on day 61 p.i. when two trypanosomes could be observed in every five oil-immersion fields on thin buffy coat smears and one trypanosome in every 20 fields in blood smears. This parasitaemia persisted for three days after which it disappeared with no trypanosomes being detectable even after intensive search. The trypanosomes could again be detected on day 75 p.i. (on both types of smears) and persisted until the termination of the experiment.

D2 was the last to peak in parasitaemia on day 41 p.i.. The parasitaemia persisted sporadically until day 50 p.i. when the parasites could be observed on both thin blood smears and thin buffy coat smears. Another peak was observed on day 61 p.i. when trypanosomes could be detected regularly on both thin blood smears and thin buffy coat smears for a period of 3 days. Parasitaemia became sporadic until day 75 p.i. when trypanosomes could again be regularly detected on both smears until the end of the experiment.

Attempts to quantify the trypanosomes during the course of the experiment (using a haemocytometer) were unsuccessful even when parasites could be detected on thin blood smears. The low parasiteamia in the infected

animals together with the inaccuracy of the haemocytometer method of counting could account for this.

The trypanosomes measured on average 12.5 ± 1.1 μm in total length with a range of 9.6 to 14.1 μm for 100 organisms. They had no free flagella, had medium sized subterminal and marginal kinetoplast as well as centrally located nuclei. The posterior ends were rounded and no conspicuous undulating membranes could be observed (Fig. 1).

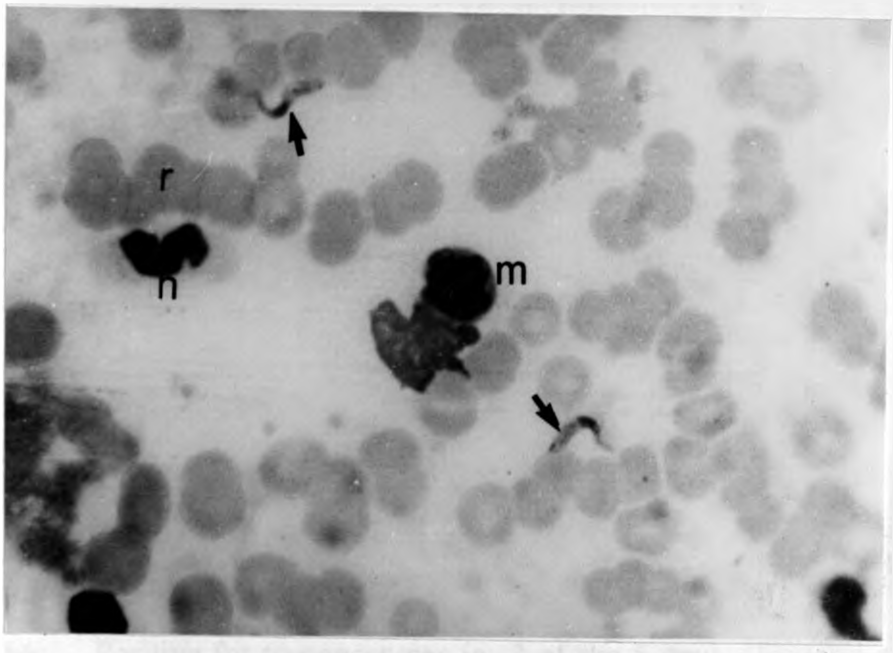


Figure 1: A thin buffy coat smear of blood from a donkey (D4, 75 days p.i.) infected with *T. congolense* showing trypanosome parasites (arrows), rouleaux formation (r), a neutrophil(n) and a monocyte(m). Giemsa X1000.

Table 1. Estimation of parasitaemia in donkeys infected with 7.5 x 10^6 bloodstream forms of *T. congolense*.

Period in Weeks	Donkey identity and state of parasiteamia		
	D2	D4	D6
Pre-infection	-	-	-
P.i. ≤ 4	-	-	-
5	-	1+	-
6	1+	4+	3+
7	3+	3+	4+
8	2+	3+	2+
9	4+	3+	2+
10	3+	2+	1+
11	2+	2+	3+
12	1+	1+	3+
13	2+	3+	1+
14	1+	1+	1+

Key:
- \- Negative for trypanosomes in 5 of 5 days.
- 1+ Positive for trypanosomes in 2 of the 5 days.
- 2+ Positive for trypanosomes in 2-3 of the 5 days.
- 3+ Positive for trypanosomes in 4-5 of the 5 days.
- 4+ Positive for trypanosomes in more than 5 days.

3 CLINICAL PATHOLOGY

3.1 HAEMATOLOGICAL RESULTS

The PCV, RBC count and Hb concentration mean values are given in Appendices 4-6. These parameters were high in the infected group than in the control group at the start of the experiment. However, these differences were not significant. They decreased progressively in the two groups of animals during the course of the experiment. The PCV was 38.7% in the infected group and 37.9% in the control group at the time of infection. These values decreased to 33.9% and 33.7% by the end of the first month p.i. while during the second month p.i. the values decreased further to 26.6% and 31.2% in the infected and control groups respectively. At the termination of the experiment, the values were at 22.6% in the infected group and 29.5% in the control group (fig. 2). This indicated a decrease of 12.4% and 11.1% in the first month,a decrease of 31.3% and 17.7% by the end of second month and a decrease of 41.6% and 22.2% at the termination of the experiment in the infected and control groups respectively. The mean values in the infected group were significantly lower ($P < 0.025$) than in the control group at the close of the experiment.

The RBC count was 6.21 x 10^6/ml in the infected group and 6.43x10^6/ml in the control group at the time of infection. The values came down to 5.68x10^6/ml and 5.71x10^6/ml by the end of the first month and became even lower to 4.06x10^6/ml and 4.96x10^6/ml by the second month in the infected and

control groups respectively. Lower values of 3.25 x 10^6/ml in infected animals and 4.59 x 10^6/ml in control animals were observed at the termination of the experiment (fig. 3). These changes reflected an 8.5% and 11.2% decrease by the first month, 34.6% and 22.9% by the second month and 47.7% and 28.6% by the termination of the experiment in the infected and control groups respectively. The RBC count in the infected group was significantly lower ($P < 0.01$) than in control group at the end of the experiment.

The Hb concentration at the time of infection was 12.8gm% in the infected group and 12.7gm% in the control group. These values however decreased progressively to stand at 10.5gm% and 11.1gm% by the first month; 8.3gm% and 10.1gm% by the second month and 7.5gm% and 9.3gm% by the end of the experiment in the infected and control groups, respectively (fig. 4). This indicated a decrease of 18.0% and 12.6% during the first month, 35.2% and 20.5% in the second month and 41.4% and 26.8% by the third month in the infected and control groups, respectively. The Hb content in the infected group was however significantly lower ($P < 0.05$) than in the control group at the end of the experiment.

Fig. 2: Sequential determination of packed cell volume in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.

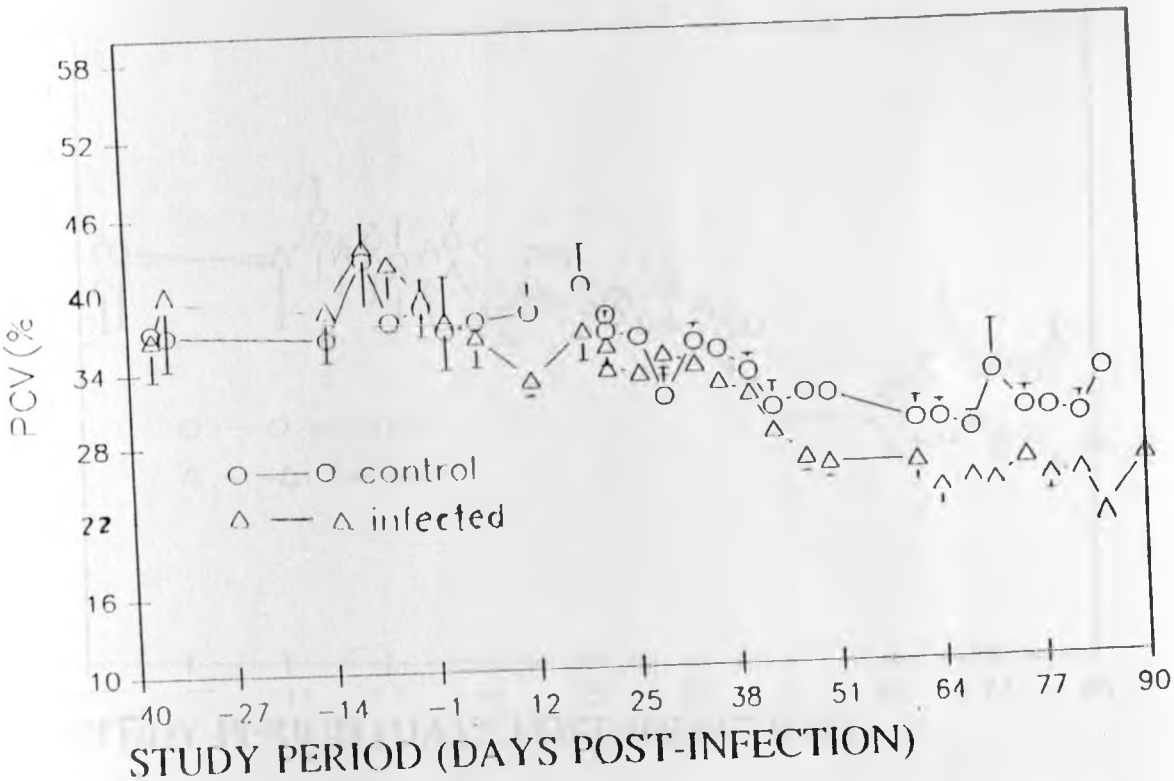
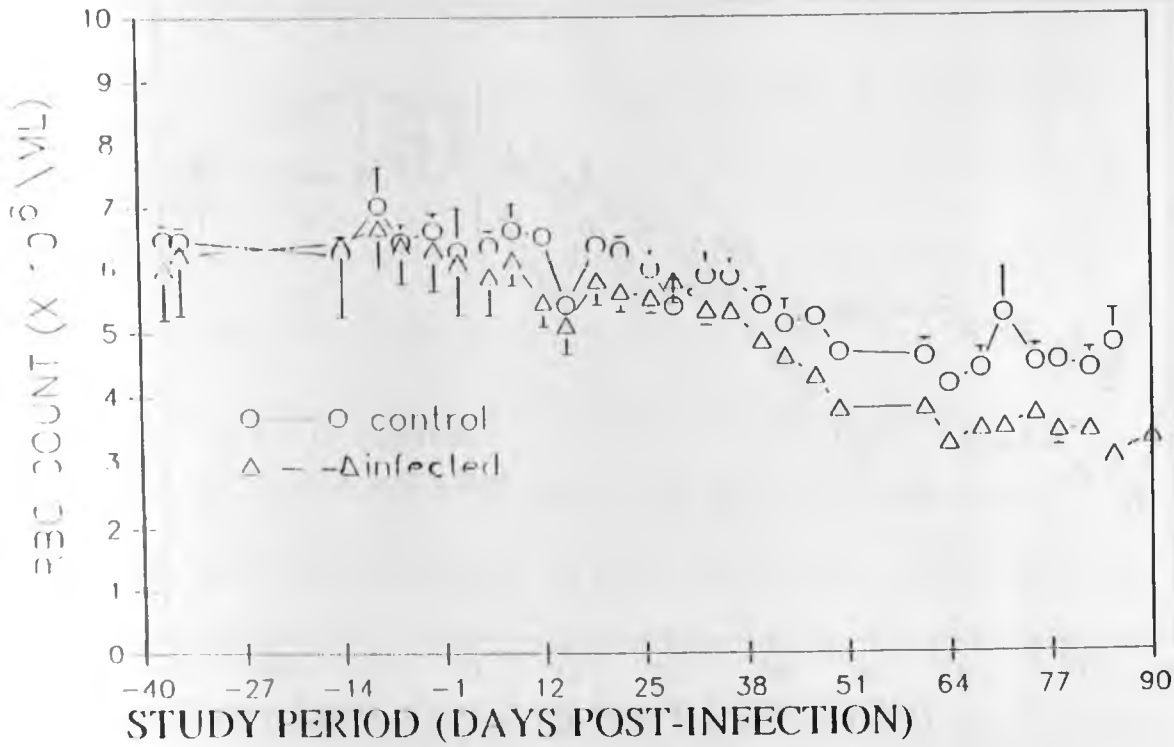
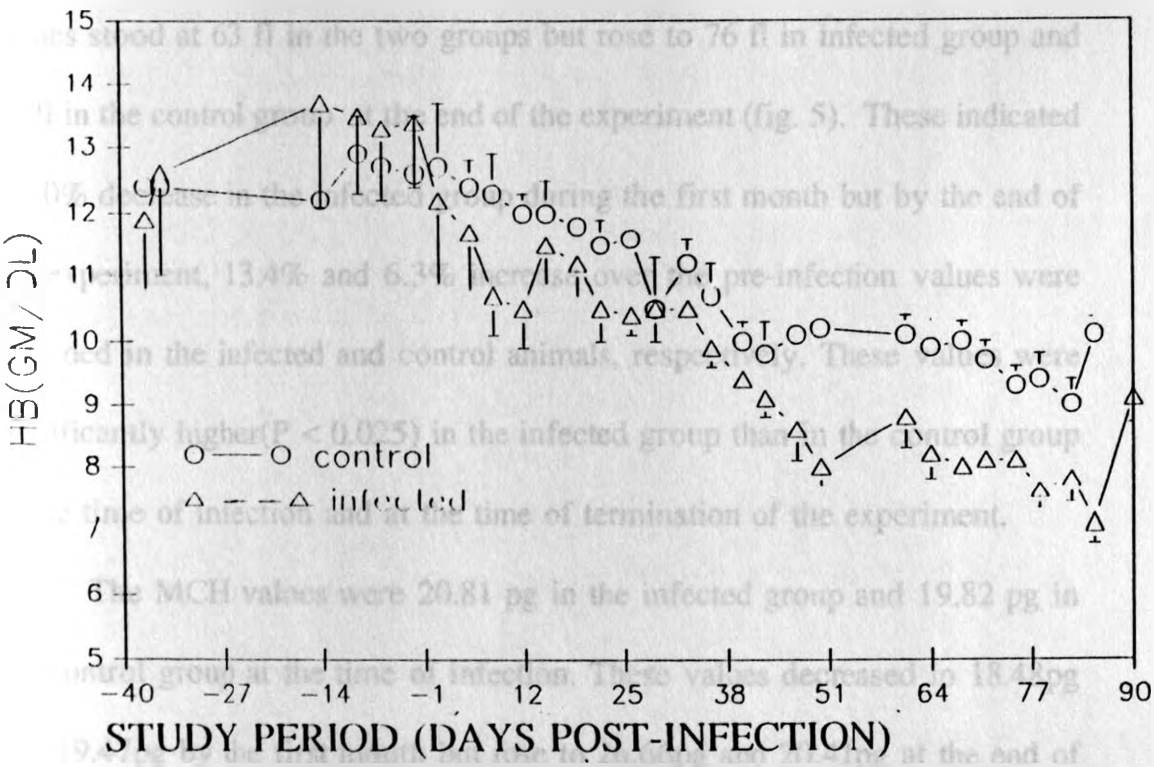


Fig. 3: Sequential determination of red blood cell counts in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.



g. 4: **Sequential determination of haemoglobin concentration in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.**



3.2 OBSERVED ERYTHROCYTE INDICES

The erythrocyte Indices mean values namely; MCV, MCH and MCHC are shown in Appendices 7-9. These parameters decreased progressively from the time of infection in the two groups to reach minimum values by the 4th and 5th week p.i. They later increased gradually to the pre-infection values by the 9th week p.i. and even higher values were recorded at the end of the experiment.

The MCV values were 67 fl and 63 fl at the time of infection in the infected and control groups, respectively. At the end of the first month the values stood at 63 fl in the two groups but rose to 76 fl in infected group and 67 fl in the control group at the end of the experiment (fig. 5). These indicated a 6.0% decrease in the infected group during the first month but by the end of the experiment, 13.4% and 6.3% increase over the pre-infection values were recorded in the infected and control animals, respectively. These values were significantly higher($P < 0.025$) in the infected group than in the control group at the time of infection and at the time of termination of the experiment.

The MCH values were 20.81 pg in the infected group and 19.82 pg in the control group at the time of infection. These values decreased to 18.48pg and 19.47pg by the first month but rose to 26.66pg and 20.41pg at the end of the experiment in the infected and control groups, respectively (fig.6). These reflected a decrease of about 11.2% and 1.8% by the first month but an increase of 28.1% and 3.0% at the end of the experiment in the infected and control

groups, respectively. The values in the infected groups were significantly higher ($P < 0.025$) at the time of infection but not at any other time of the experiment.

The MCHC readings were 33.0gm% in the infected group and 33.5gm% in the control group at the time of infection. These values came down to 30.9gm% and 32.9gm% by the end of the first month but increased gradually to stand at 34.4gm% and 31.4gm% at the end of the experiment in the infected and control groups, respectively (fig. 7). No significant differences were observed between the infected and control groups in the course of the experiment.

Fig. 5: Sequential determination of mean corpuscular volume in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.

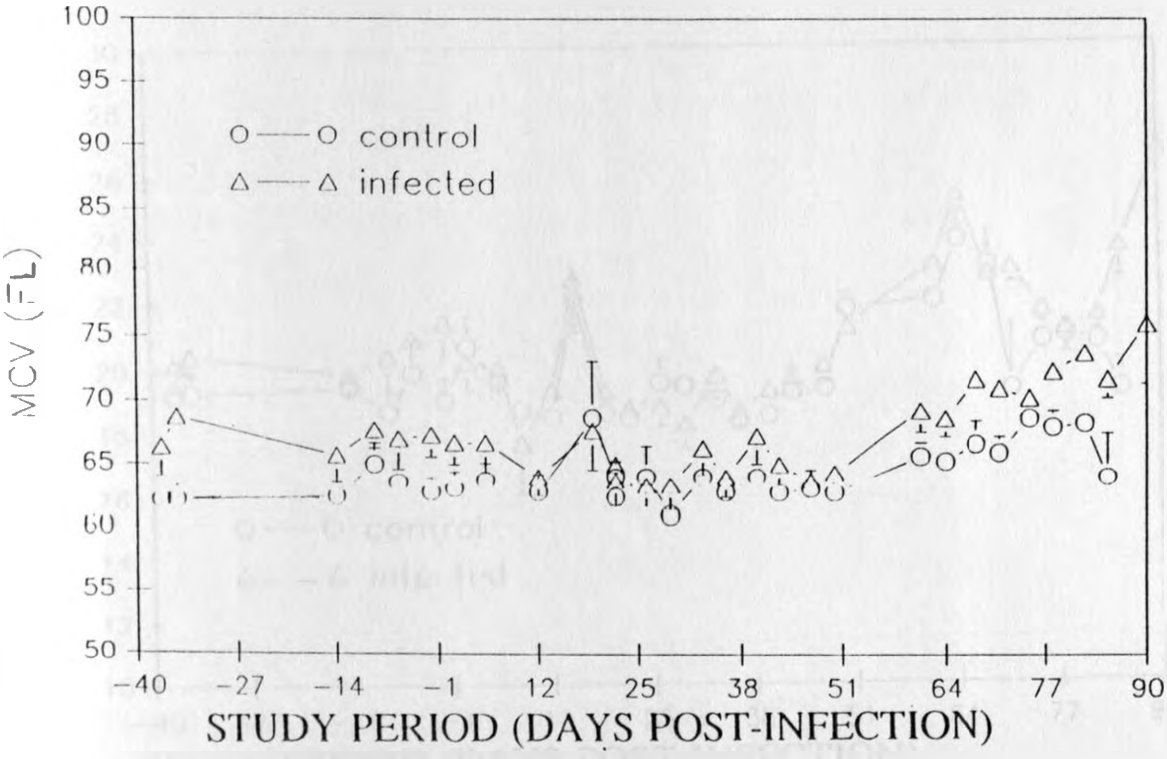


Fig. 6: Sequential determination of mean corpuscular haemoglobin in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.

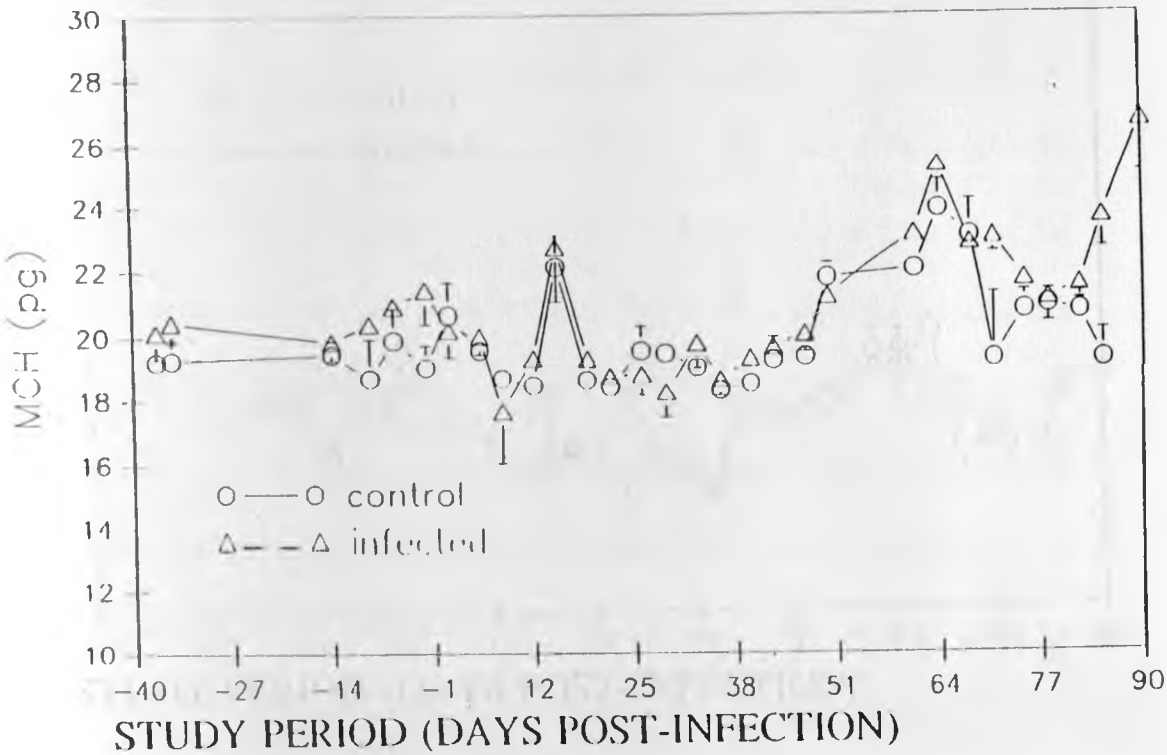
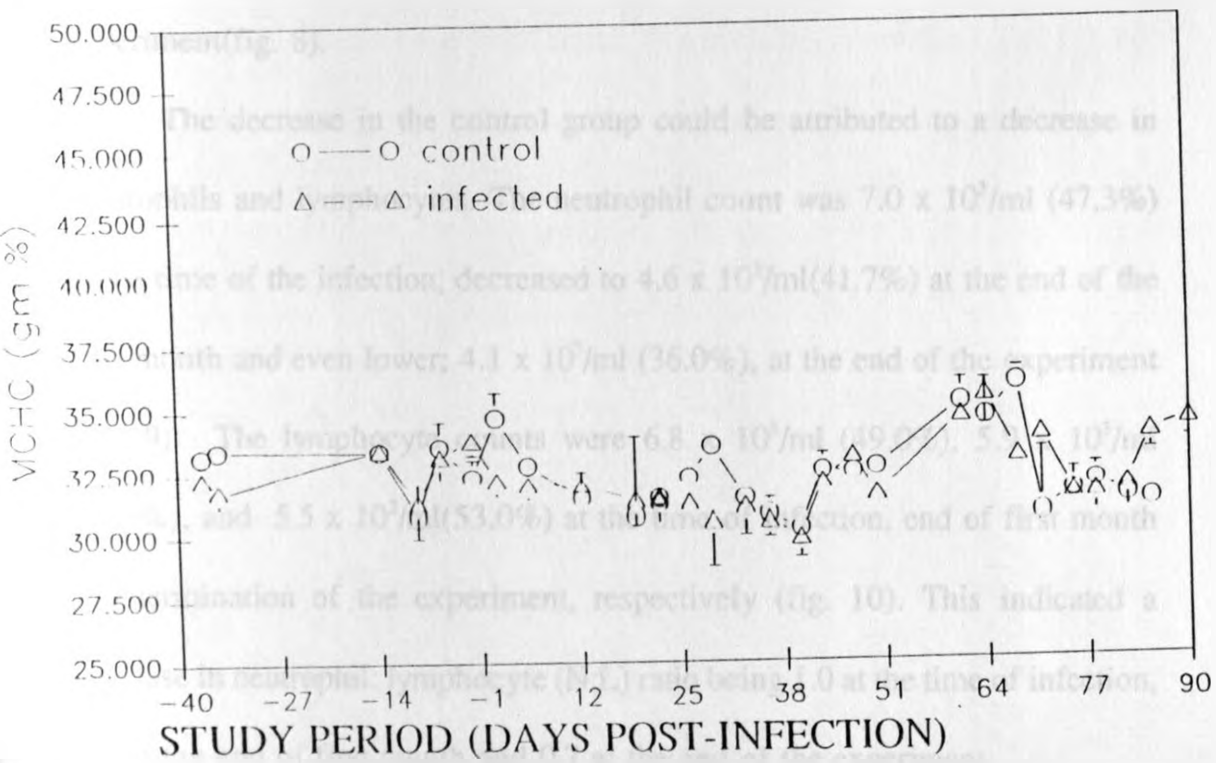


Fig. 7: Sequential determination of mean corpuscular haemoglobin concentration in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.



3.3 TOTAL AND DIFFERENTIAL LEUKOCYTE COUNTS

The total and differential leukocyte counts are shown in appendices 10-14. The total leukocyte counts stood at 15.3 x 10^3/ml in the infected group and 14.4 x 10^3/ml in the control group at the time of infection. These values however decreased in both groups of animals to stand at 10.6 x 10^3/ml and 11.0 x 10^3/ml by the first month, and even lower; 10.3 x 10^3/ml and 7.4 x 10^3/ml during the 10th week p.i. in the infected and control groups respectively. In the control group, the level remained lower than pre-infection values while in the infected group, the levels rose up to stand at 17.0 x 10^3/ml at the end of the experiment(fig. 8).

The decrease in the control group could be attributed to a decrease in neutrophils and lymphocytes. The neutrophil count was 7.0 x 10^3/ml (47.3%) at the time of the infection, decreased to 4.6 x 10^3/ml(41.7%) at the end of the first month and even lower; 4.1 x 10^3/ml (36.0%), at the end of the experiment (fig. 9). The lymphocyte counts were 6.8 x 10^3/ml (49.0%), 5.9 x 10^3/ml (45.3%), and 5.5 x 10^3/ml(53.0%) at the time of infection, end of first month and termination of the experiment, respectively (fig. 10). This indicated a decrease in neutrophil: lymphocyte (N:L) ratio being 1.0 at the time of infection, 0.8 at the end of first month and 0.7 at the end of the experiment.

In the infected animals the neutrophils stood at 6.8 x 10^3/ml(41.3%) at the time of infection, decreased to 3.3 x 10^3/ml(42.3%) by the end of the first month and even lower; 1.7 x 10^3/ml(15.0%) at the end of the experiment (fig.

9). The lymphocytes started at 6.8 x 10^3/ml(52.7%), decreased to 6.4 x 10^3/ml(50.0%) by the end of the first month but higher values of up to 8.9 x 10^3/ml(80.7%) were recorded at the termination of the experiment (fig. 9). This indicated that the N:L ratio was 1.0 at the time of infection, decreased to 0.5 by the end of the first month and even lower; 0.2 at the end of the experiment.

Eosinophil count remained in the normal range in the infected group but rose beyond the pre-infection levels in the control group during the third month of infection (fig. 11). Monocytes were observed to be higher in the infected group than in the control and even higher than pre-infection values (fig. 12) but they were not significantly different. Basophils displayed no major changes.

Fig. 8: Sequential determination of total leukocyte counts in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.

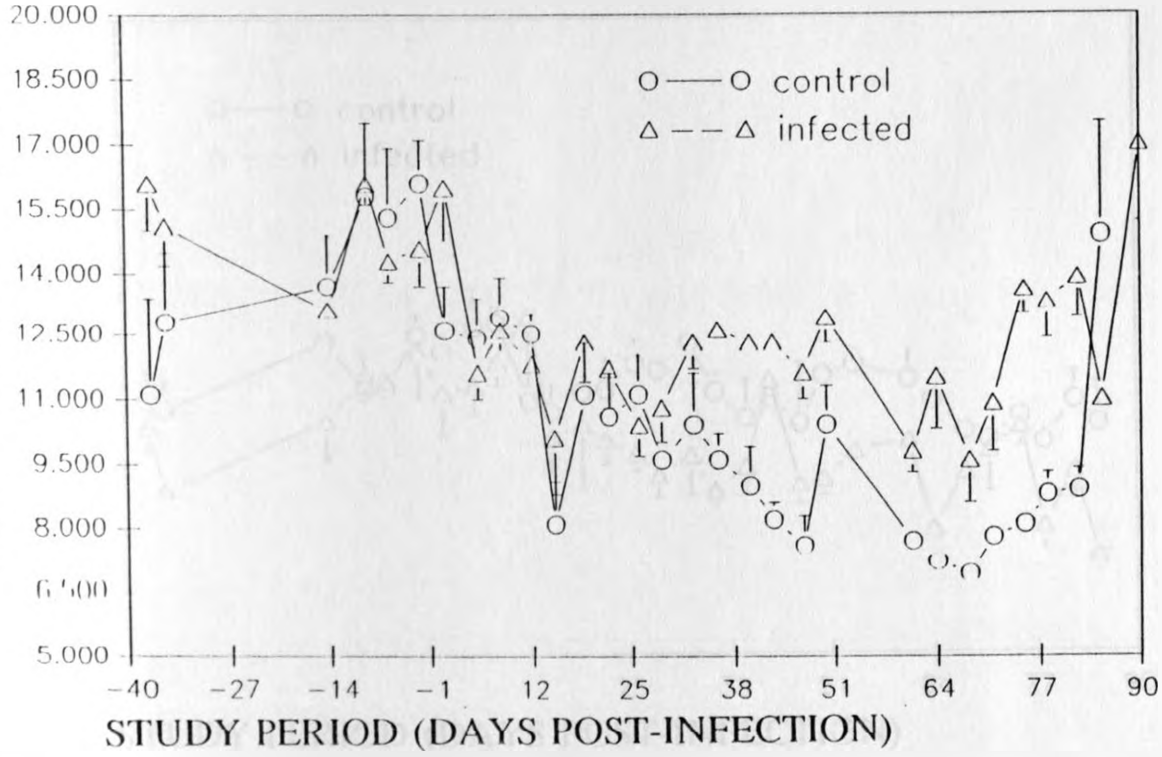


Fig. 9: Sequential determination of neutrophil counts in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.

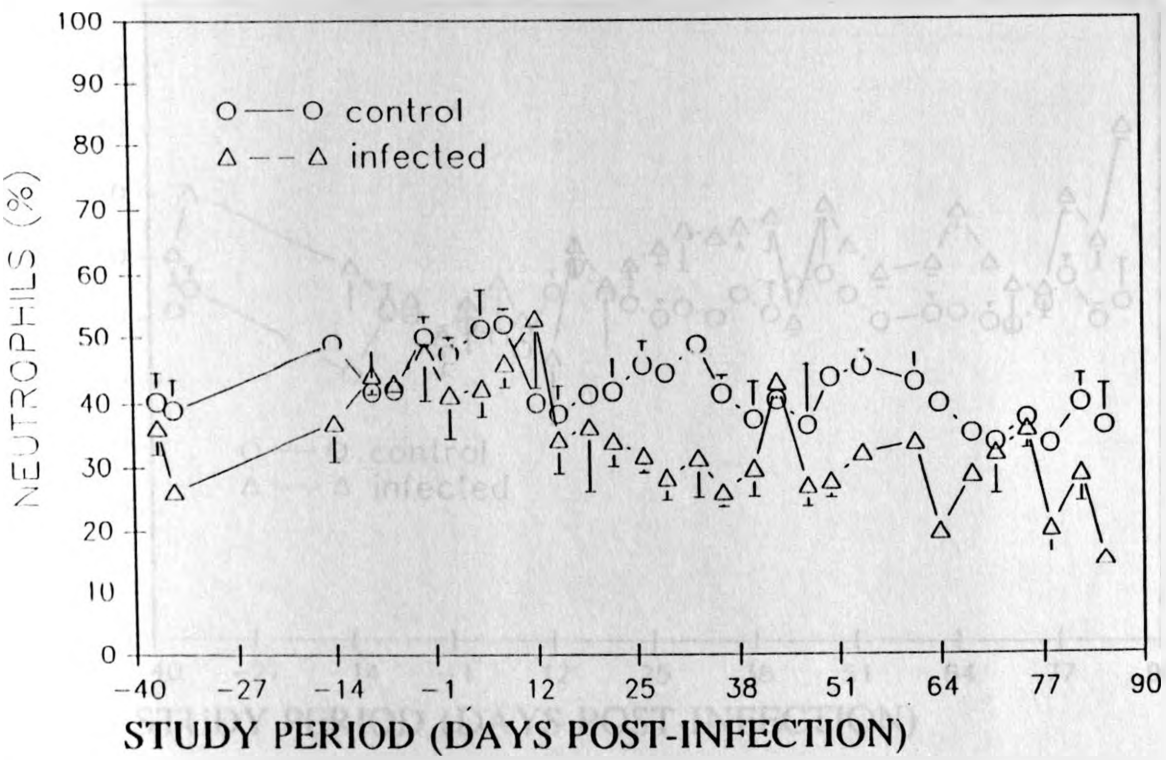


Fig. 10: Sequential determination of lymphocyte counts in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.

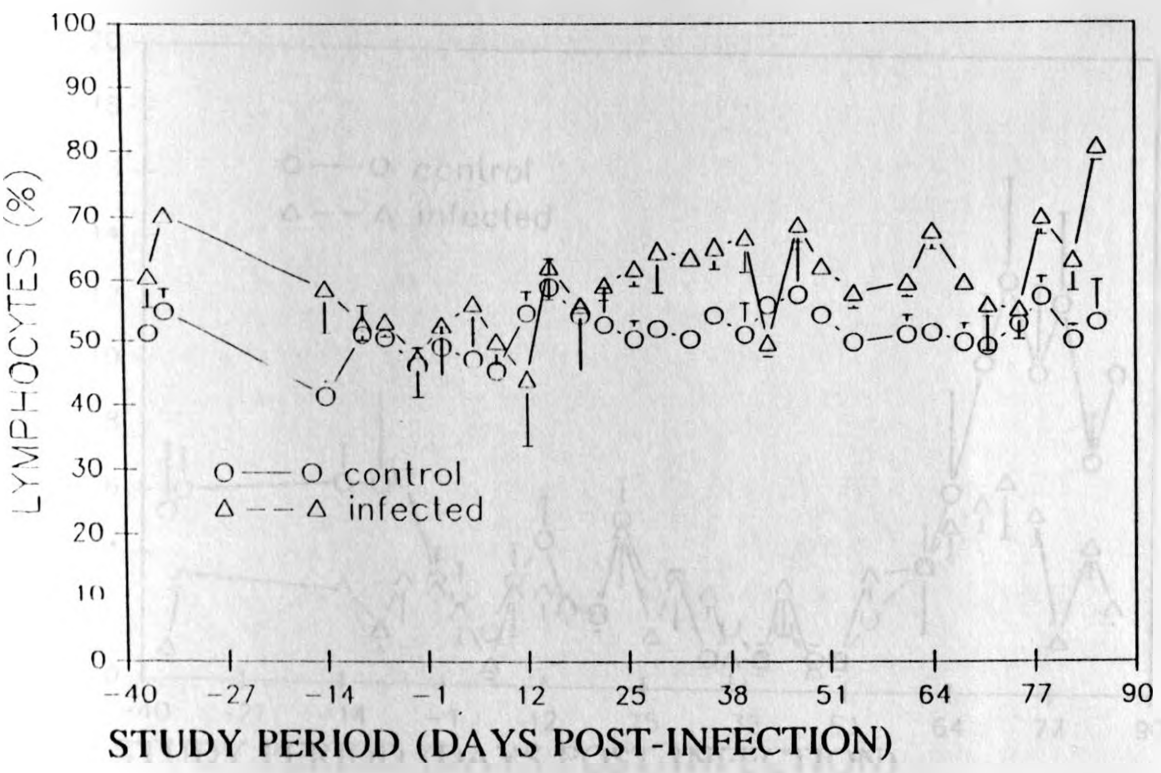


Fig. 11: Sequential determination of eosinophil counts in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.

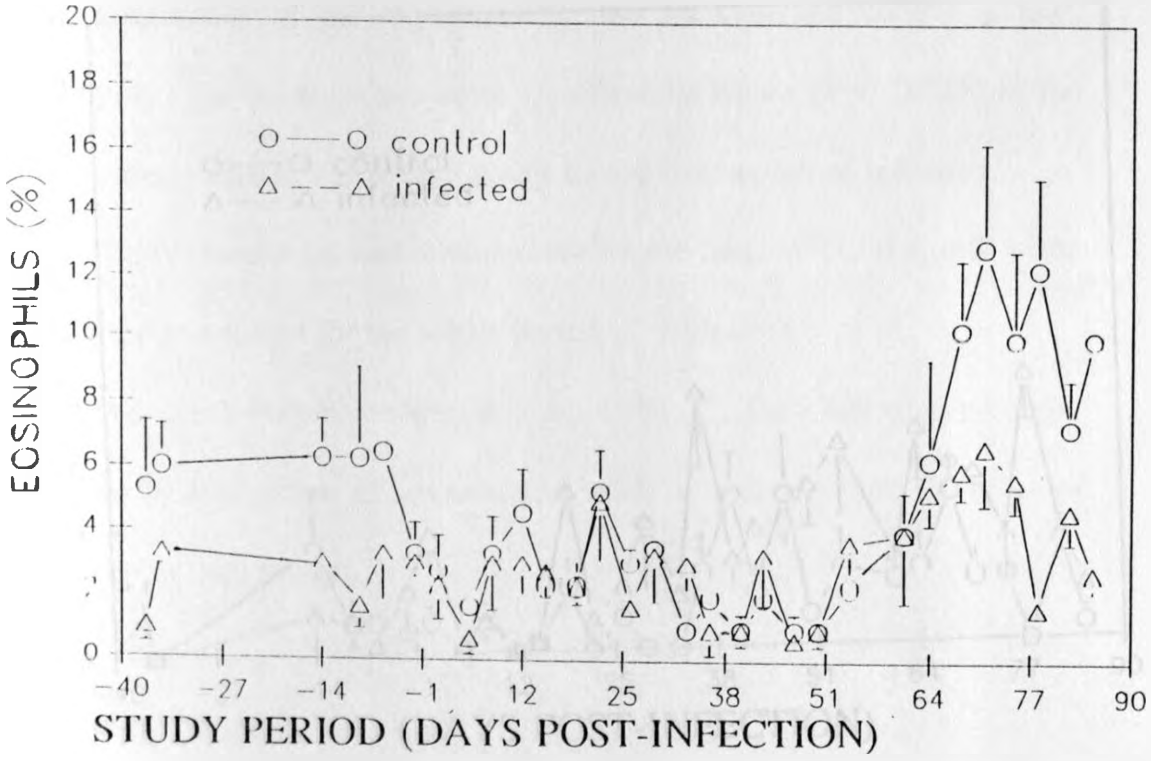
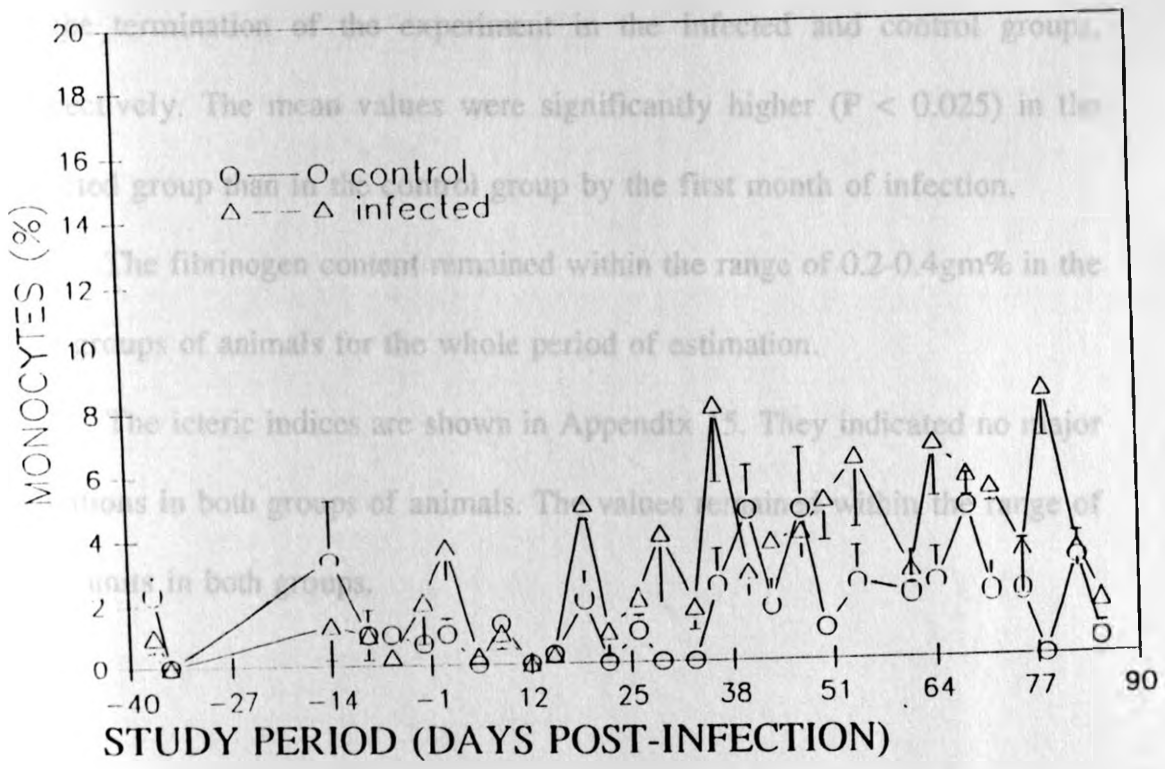


Fig. 12: Sequential determination of monocyte counts in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.



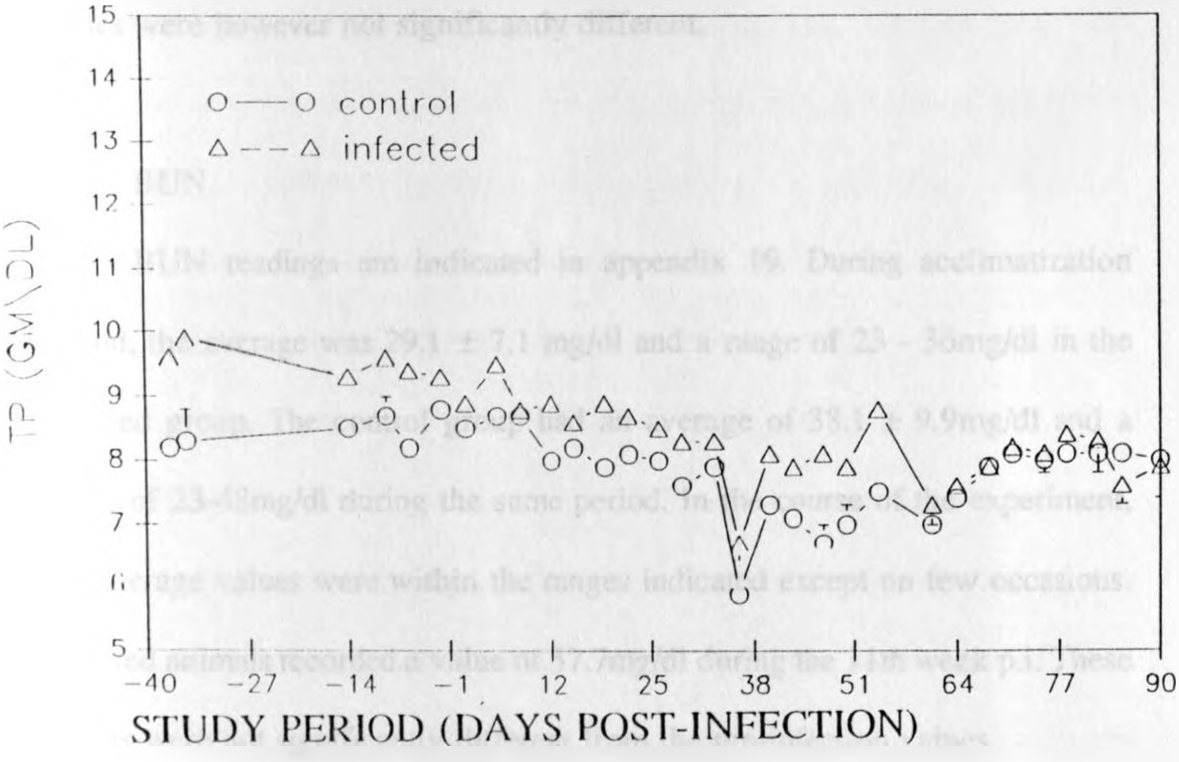
4 PLASMA PROTEINS

The total plasma proteins and fibrinogen contents are given in Appendices 16 and 17.The total plasm proteins were 9.1gm% in the infected group and 8.6gm% in the control group at the time of infection. They decreased progressively in the two groups of animals; being 8.4gm% and 7.8gm% at the end of the first month and 7.8gm% and 8.1gm% at the end of the experiment in the infected and control groups respectively (fig. 13). These values reflected a decrease of 7.7% and 9.3% at the end of the first month and 13.2% and 5.8% at the termination of the experiment in the infected and control groups, respectively. The mean values were significantly higher ($P < 0.025$) in the infected group than in the control group by the first month of infection.

The fibrinogen content remained within the range of 0.2-0.4gm% in the two groups of animals for the whole period of estimation.

The icteric indices are shown in Appendix 15. They indicated no major variations in both groups of animals. The values remained within the range of 2-15 units in both groups.

Fig. 13: Sequential determination of total plasma proteins in the control donkeys and donkeys experimentally infected with *Trypanosoma congolense*.



3.5 SERUM BIOCHEMISTRY

3.5.1 Total bilirubin

The total bilirubin mean values are show in appendix 18. The pre-infection values were on average 0.3 ± 0.1 mg/dl and a range of 0.2-0.5mg/dl in both groups of animals. In the course of the experiment, the average values were within this range in the control group while in infected group, the levels rose to 0.8mg/dl on few occasions during the second and third months. These values were however not significantly different.

3.5.2 BUN

BUN readings are indicated in appendix 19. During acclimatization period, the average was 29.1 ± 7.1 mg/dl and a range of 23 - 36mg/dl in the infected group. The control group had an average of 38.1 ± 9.9mg/dl and a range of 23-48mg/dl during the same period. In the course of the experiment, the average values were within the ranges indicated except on few occasions. Infected animals recorded a value of 57.7mg/dl during the 11th week p.i. These values were not significantly different from the pre-infection values.

PATHOLOGICAL FINDINGS

GROSS - PATHOLOGY

The infected donkey carcasses of D2 and D4 were in fair body condition while D6 carcass was in poor condition characterized by lack of adequate muscle cover over the bony prominences and the extensive serous atrophy most evident in the coronary groove and perirenal areas. The control animal carcasses were in good body condition. D8, one of the control animals despite the good body condition was severely icteric with marked yellowish discoloration of the subcutaneous , omental, coronary and perirenal fat.

The superficial lymph nodes namely; prescapular, precrural, submandibular and the parotid were small and firm in both the infected and control animals, although slightly larger in the infected animals. The mesenteric lymph nodes, were enlarged and oedematous in the two groups.

Spleens in the infected animals D2, D4 and D6 were markedly enlarged. They had blunt edges and firm nodules protruded above the splenic capsule. On cutting, these spleens looked fleshy and the malphigian corpuscles were prominent. D4 spleen had petechial haemorrhages on the capsule. In the control group, the spleens were of normal size. However, one of the control animals D8 had an opaque capsule which also had petechial haemorrhages which could not be explained.

The livers in the infected group were slightly enlarged but firm. D1 had

a liver with whitish specks all over the surface which on cutting were observed to be abscesses. D8, one of the control animals had round depressed scars on the surface that felt fibrotic on cutting. D8 had a severely damaged liver and occasionally, abscesses close to the bile ducts pressed on them leading to accumulation of bile and hence the jaudice observed in the whole carcass. D1 and D8 had thickened bile ducts and abscesses in the liver parenchyma which contained dark-red pus. In some of these abscesses, larvae of *Strongylus* spp were recovered in these two animals.

Kidneys appeared grossly normal in all the animals. The cranial mesenteric arteries and their branches in all cases had aneurysms. On few occasions, two aneurysms could be observed in one artery as was the case with D2. In some of these aneurysms, larval stages of *Strongylus vulgaris* were found with no major differences observed between the two groups.

The gastro-intestinal tract of all these donkeys had a parasitism of both botfly larvae and helminths. *Gastrophilus nasalis* larvae were evident in the glandular stomach and occasionally extended to the duodenum. At the margo-plicatus on the glandular portion were infestations of *Habronema muscae*. The caecum and the colon of all the animals were infested with *Cyathostomum tetracanthum*. D1, D3 and D8 on top of these helminths contained other species as well. D1 and D3 had *S. vulgaris* while D8 had *Strongylus edentatus* as well. D1 also had *Oxyuris equi* in the colon.

The intestinal tract had many nodules, hard in consistency,and protruded

into the intestinal lumen and were visible on the serosal surface. The nodules were most prevalent in the small intestines and decreased towards the colon and none found in the rectum. Some of these nodules contained brown coloured helminth larvae coiled inside them.

Moderate amounts of straw-coloured fluid was found in the peritoneal cavity and pericardial sacs of all the infected animals (D2, D4 and D6). In the control animals, only small quantities of similar fluids were observed in the peritoneal cavity. D8 also had small amounts of the straw-coloured fluid in the pericardial sac.

The heart in the two groups of animals were normal in size. The lungs of the infected animals did not collapse easily. D4 and D6 had lungs with pulmonary oedema while those from control animals were relatively normal.

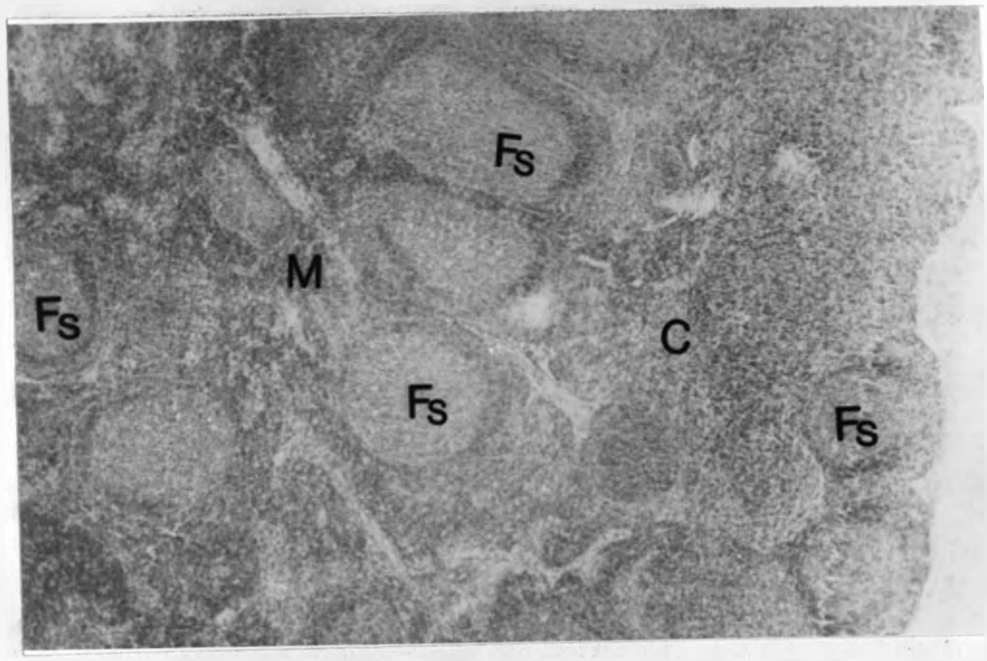
.4.2 HISTOPATHOLOGY

.4.2.1 Lymph nodes

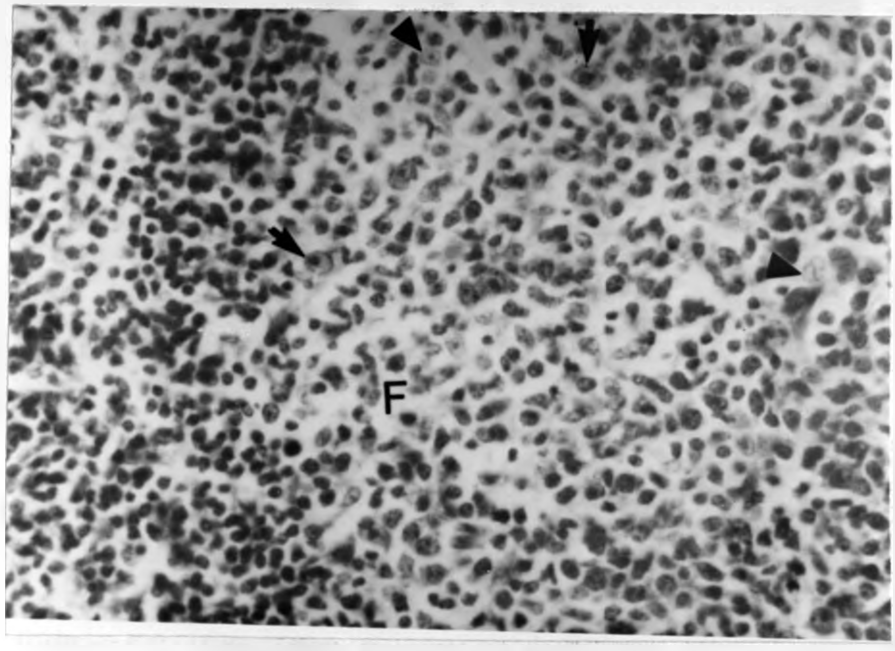
In the infected group, superficial lymph nodes showed relatively similar pictures. Follicular hyperplasia characterized by the presence of secondary follicles both in cortex and medulla was a consistent feature. The secondary follicles were increased both in size and number (Fig. 14). For an average of 20 fields (x40) in the lymph nodes of each animal, 16-19 follicles could be

observed in D2 and 18-20 follicles in D4 and D6. These secondary follicles consisted of germinal centres which contained mainly the large lymphocytes (Fig. 15). Mitotic figures of the large lymphocytes were frequently encountered. The medullary cords were expanded with increased number of macrophages and plasma cells while the sinusoids had increased number of macrophages some of which were laden with haemosiderin . Plasma cells were encountered occasionally in the sinusoids.

In the control group , the lymph nodes displayed a consistent picture of primary follicles consisting of dark homogenously staining centres with densely packed small lymphocytes (Fig.16 and 17). The mesenteric lymph nodes depicted a picture similar to that observed in the infected group.



gure 14: Prescapular lymph node from *T. congolense* infected donkey (D4), 97 days p.i., displaying secondary follicles (Fs) in the cortex (c) and the medulla (m). H&E X40.



igure 15: Prescapular lymph node from a donkey (D4)infected with *T. congolense* (7.5 x 10^6 dosage), 97 days p.i.,focusing into the secondary follicle (F). Note the increased large lymphocytes (arrows) and numerous medium sized lymphocytes (arrow heads). H&E X400.

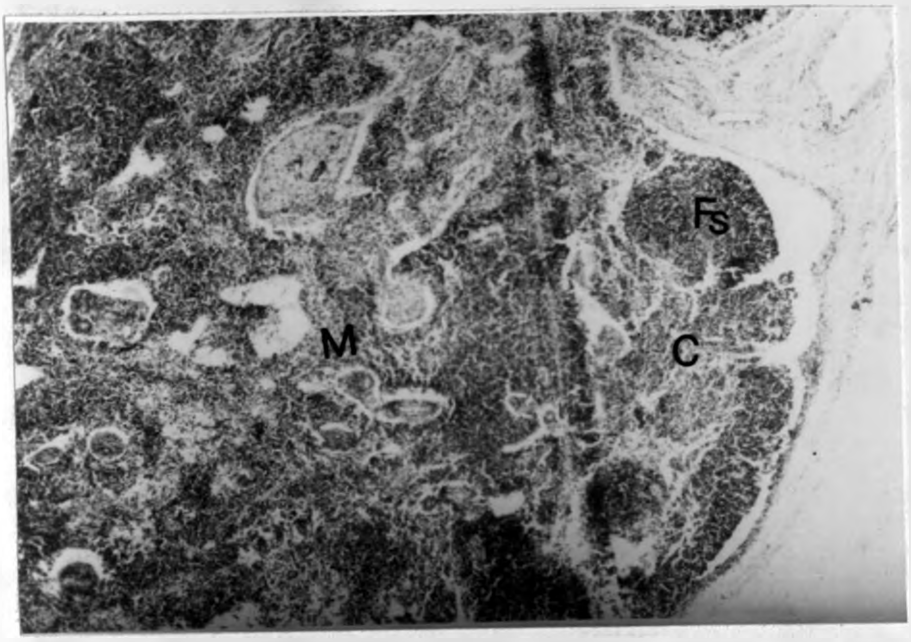


Figure 16: Prescapular lymph node from control donkey (D7) showing few secondary follicles (Fs) in the cortex(c) and none in the medulla(m). H&E X40.

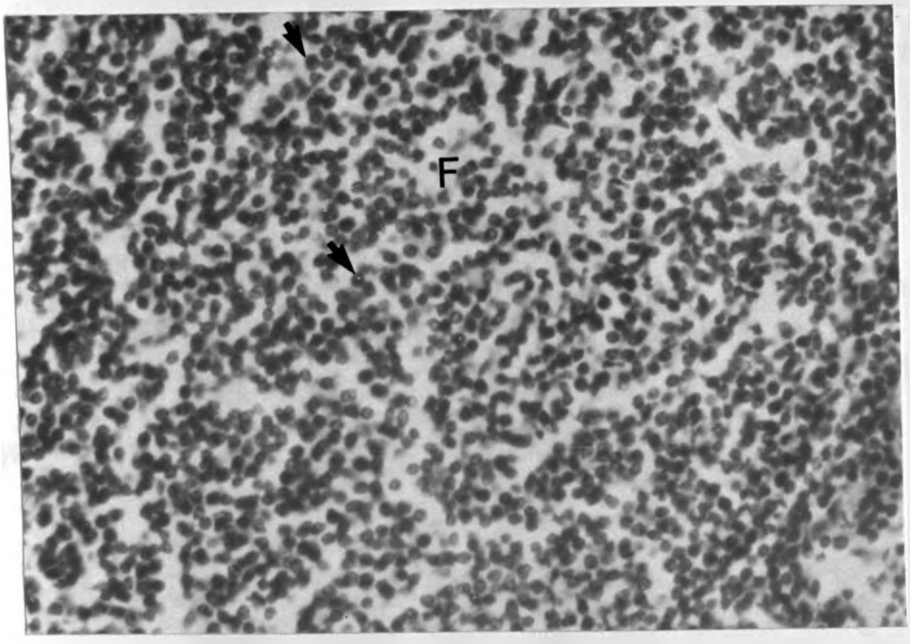


Figure 17: Prescapular lymph node from control donkey (D7) focusing into the follicle(F). Note that the major cells are mainly medium-sized lymphocytes (arrows). H&E X400.

4.2.2 Spleen

The red pulp displayed extensive congestion in all the animals due to the effect of barbiturates used for euthanasia. In the infected animals, the red pulp sinuses contained many macrophages some of which had ingested haemosiderin while others were actively engaged in erythrophagocytosis. Free lymphocytes were also numerous in the red pulp. Hyperplasia of malphigian corpuscles was quite evident in all the infected animals (Fig.18). The secondary follicles were markedly increased in size and numbers with 17 - 20 follicles (for an average of 20 fields at x40 magnification) visible in each animal. The follicles had germinal centres similar to those observed in lymph nodes. The periarterial lymphatic sheaths (PALS) were similar to the secondary follicles while the marginal zones were compressed around the expanded germinal centres and contained many macrophages (Fig. 19).

In the control group, the primary follicles were the majority while the secondary follicles were few (Fig. 20 and 21). Six to eight small, unreactive follicles were observed at x40 magnification for an average of 20 fields in each animal.

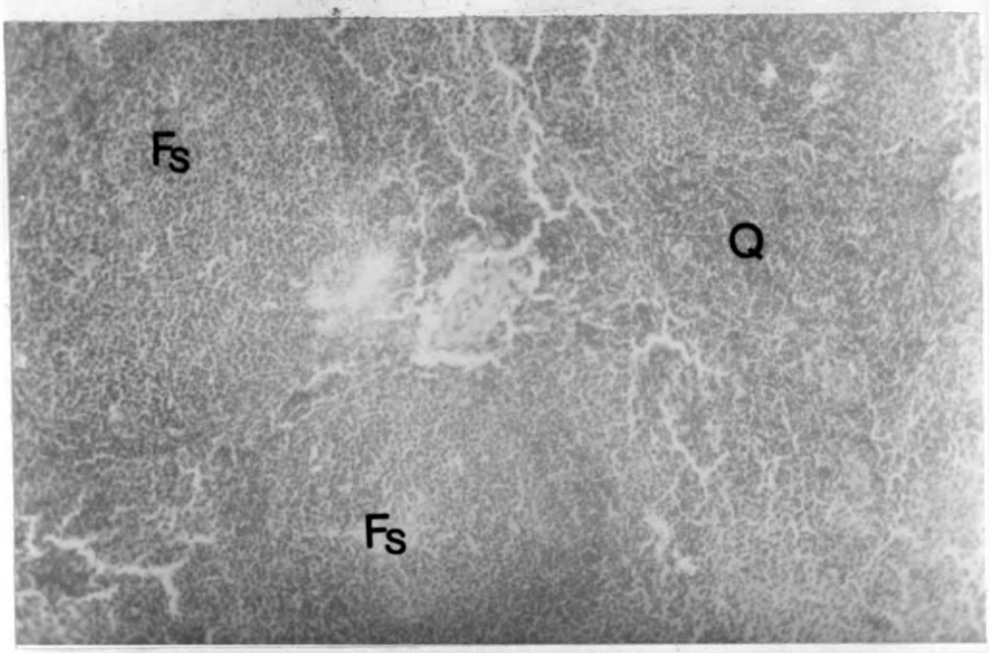


Figure 18: Spleen from donkey (D2) [96 days p.i.] infected with $7.5x10^6$ trypanosomes depicting increased prominence of secondary follicles (Fs) and periarteriolar lymphatic sheath(Q). H&E X40.

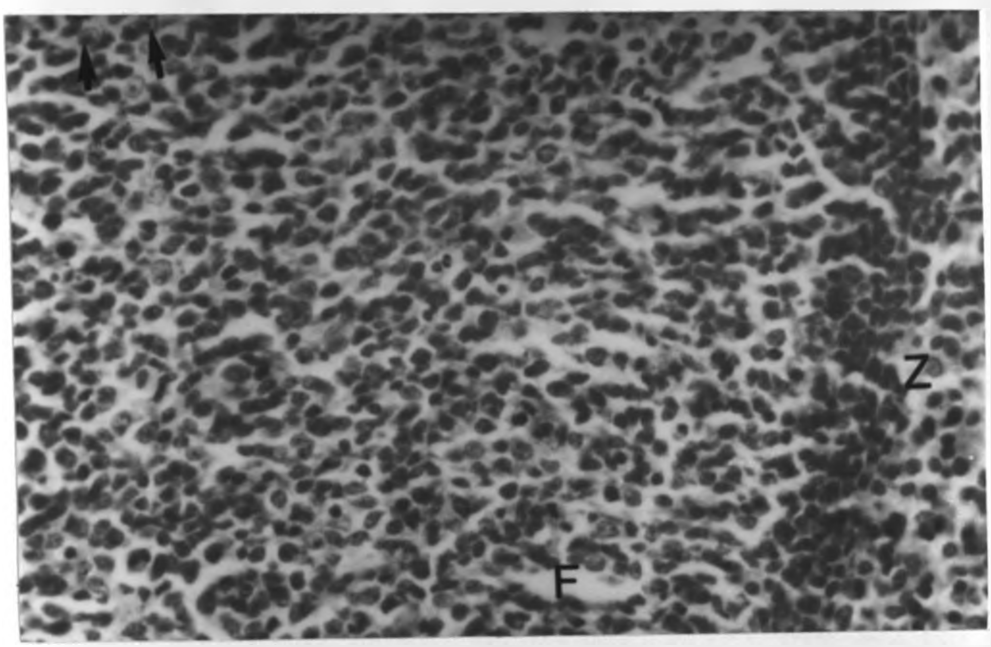


Figure 19: Spleen from D2 [96 days p.i.]infected with $7.5x10^6$ trypanosomes (*T. congolense)* focusing into the follicle(F) and displaying the increased number of large lymphocytes (arrows) and the compressed marginal zone(Z). H & E X400.

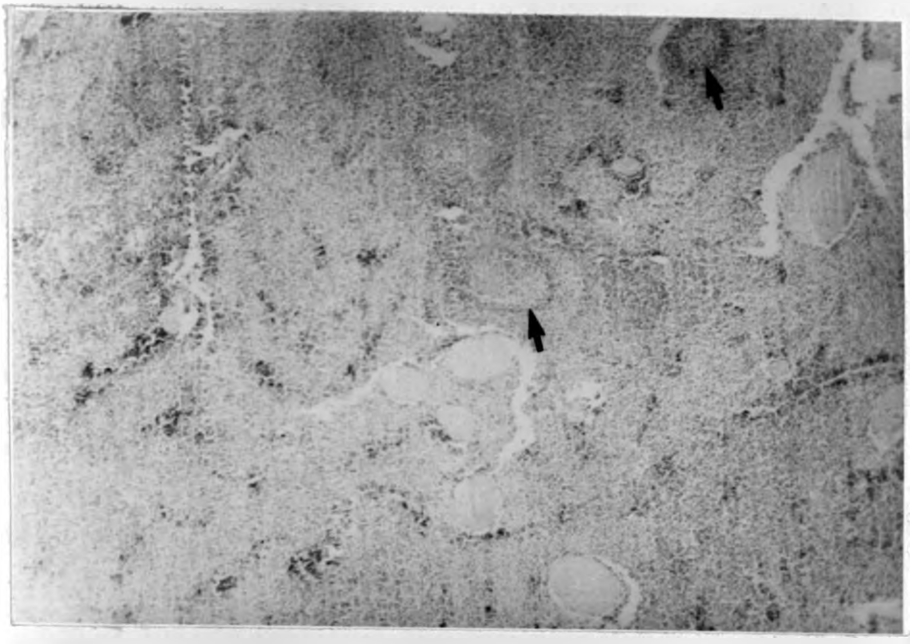


Figure 20: Spleen from control donkey (D3) showing few secondary follicles(arrows) which are quite small. H&E X40.

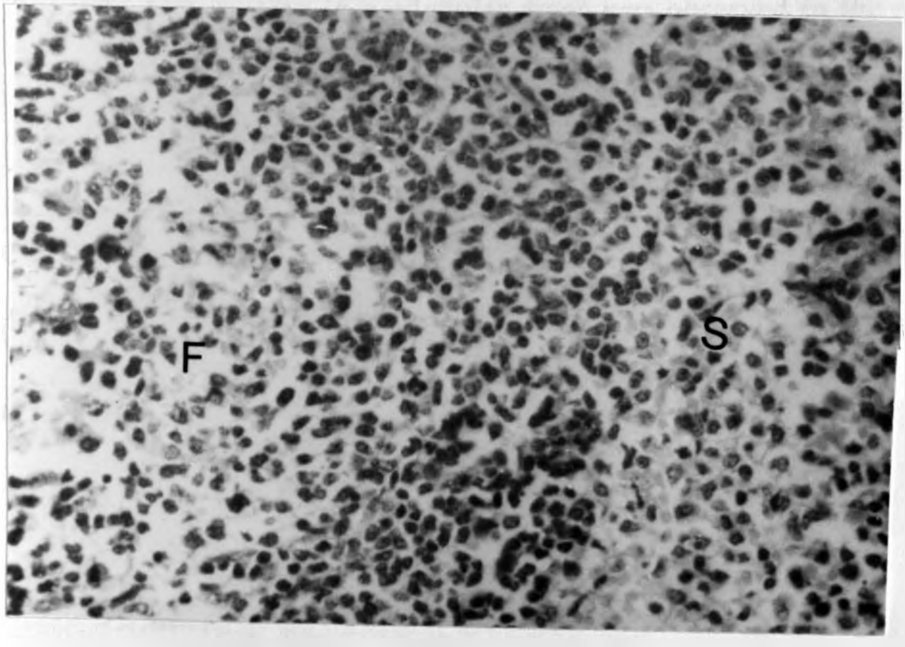


Figure 21: Spleen from control donkey (D3). Note the rarefied cells in the follicle(F) and in the red pulp(S). H&E X400.

.4.2.3 Liver

Hydropic degeneration was a common feature in all the infected animals and was more intense in perilobular areas (Fig. 22). D2 and D4 had hyperplasia of the bile ducts. D4 and D6 displayed congestion where in D6, the vessels were additionally impacted with many neutrophils. Cellular infiltrations were observed in periportal areas in D6, which had in addition, focal neutrophil infiltrations in the parenchyma. The cellular infiltrations in the spaces of Disse were mainly lymphocytes. D4 had eosinophils in these periportal areas as well. Kupffer cells were prominent in all these animals (Fig. 23) and were found to contain haemosiderin in D2 and D6.

Hydropic degeneration in perilobular areas was observed in the control animals. It was more marked in D3 (Fig. 24 and 25). Kupffer cells were prominent in D3 and D8. D1 and D8 had markedly thickened bile ducts and areas of sclerosis that suggested migratory tracts of *Strongylus* larvae. Eosinophils were many in the sinusoids of D8. D7 showed few periportal areas infiltrated by lymphocytes.

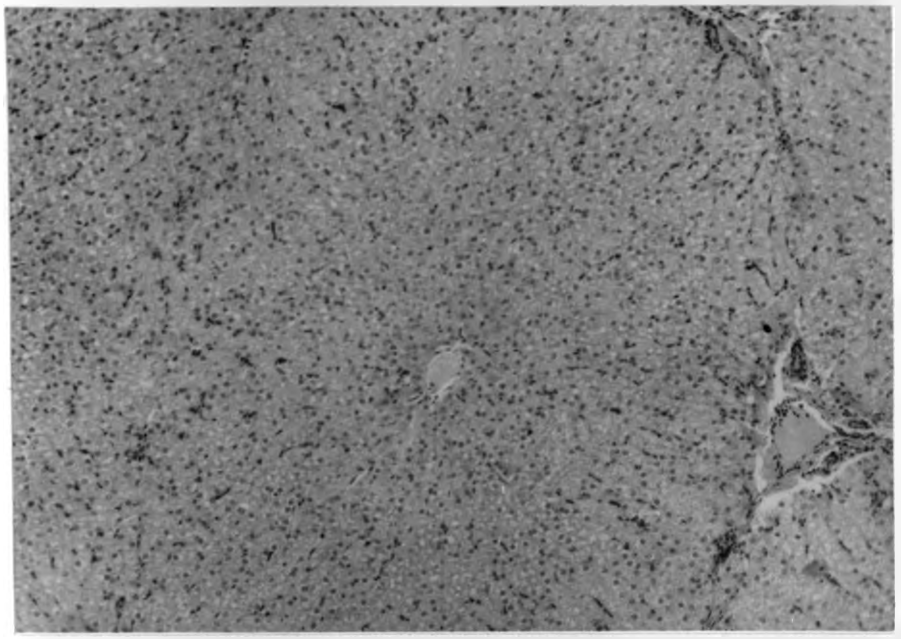


Figure 22: Liver from donkey, D4, (97 days p.i.) infected with $7.5x10^6$ trypanosomes (*T. congolense*), showing hydropic degeneration in perilobular areas [clear spaces]. H&E x40.

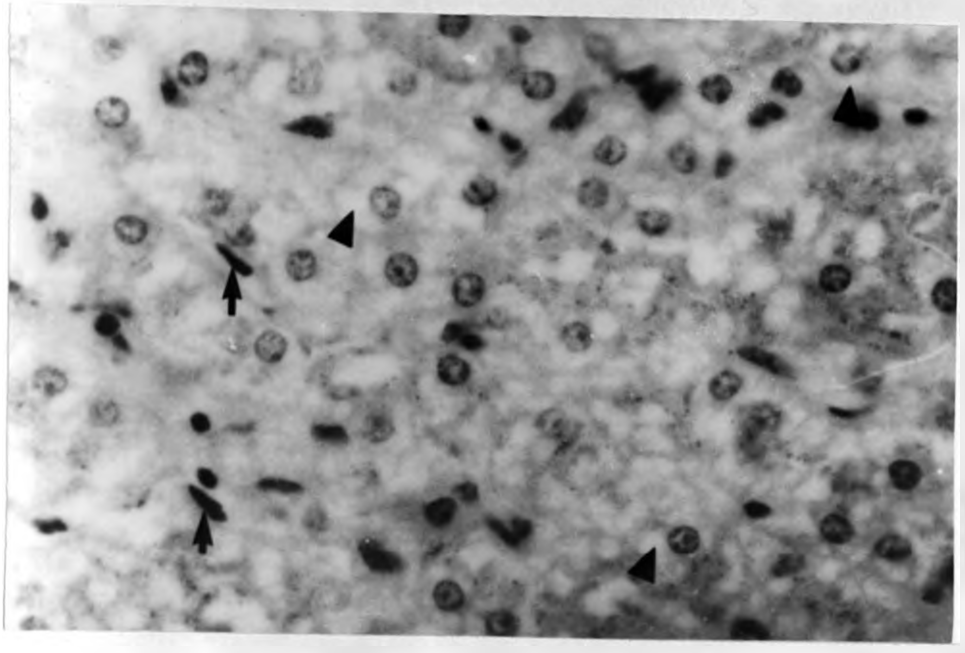
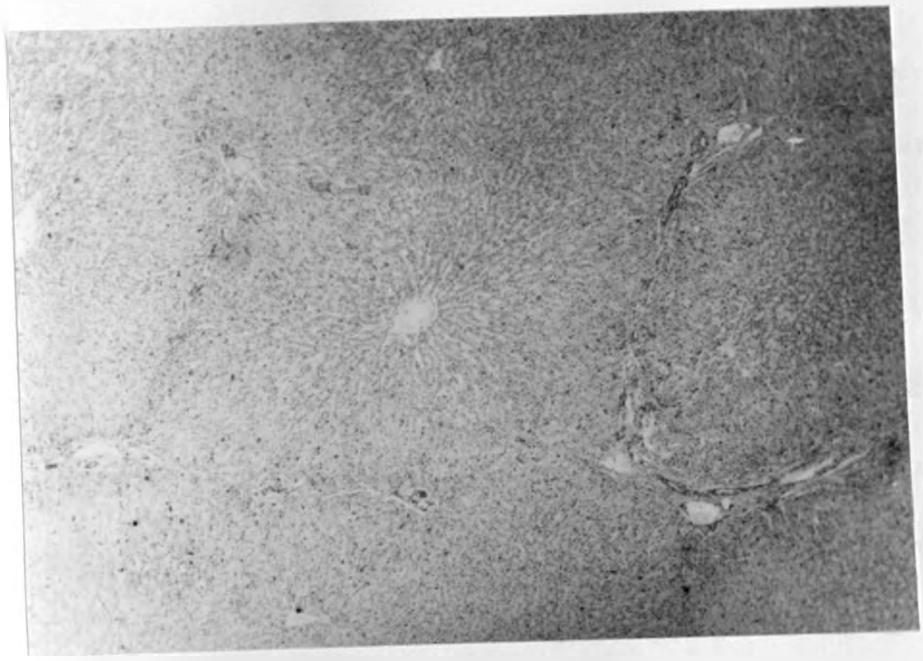
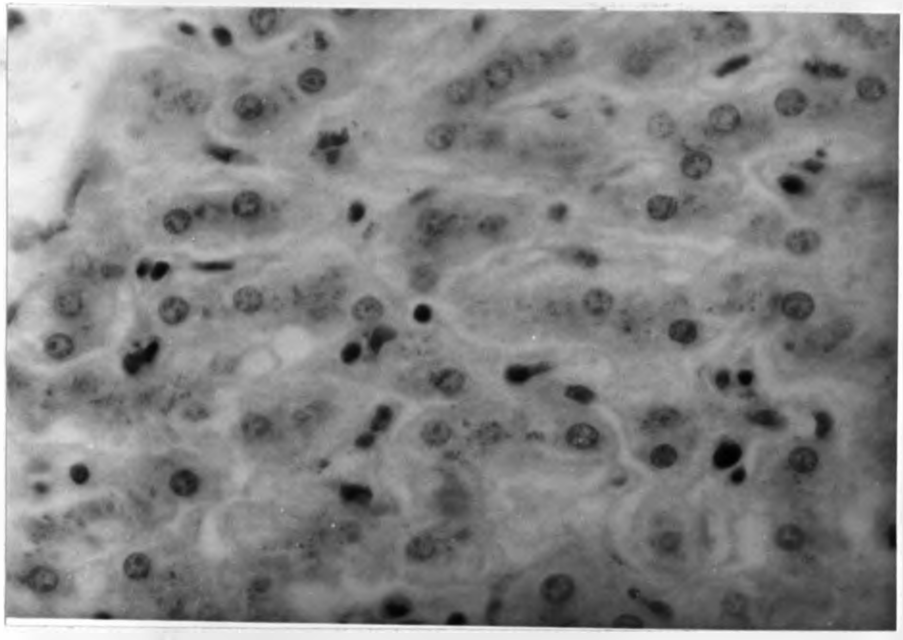


Figure 23: Liver from donkey, D4, [97 days p.i.] infected with $7.5x10^6$ trypanosomes (*T. congolense*) displaying prominent kupffer cells(arrows). Hydropic degeneration of hepatocytes is indicated by the clear empty spaces(arrow heads). H&E x400.



gure 24: Liver from control donkey (D3). Note the hydropic degeneration in perilobular areas (clear spaces). H&E x40.



igure 25: Liver from control donkey (D3) depicting hydropic degeneration of hepatocytes (clear empty spaces). H&E X400.

4.2.4 Kidneys

The glomeruli were congested and compressed onto the Bowman's capsule in D4 and D6, while in D2, they were hypercellular. The cortico-medullary junction was infiltrated by mononuclear cells in D4 and D6 (Fig. 26) whereas in D2, focal areas of mononuclear cell infiltration into the interstitium was evident in the cortex. Developmental stages of *Klossiella equi* were numerous in the tubules and collecting ducts. Some of these stages were still attached onto the epithelial cells in D2 and D4 (Fig. 27).

In the control animals, D7 showed congested glomeruli and cortico-medullary blood vessels. The cortico-medullary junction in D8 was infiltrated by lymphocytes and eosinophils could be observed in the intertubular connective tissue.

4.2.5 Intestines

Focal infiltrations of mucosa and submucosa often involving deeper layers of enteric wall by eosinophils, macrophages, lymphocytes and occasional plasma cells were observed in the intestinal sections . Worm larvae sections could be seen enclosed in thick fibrous capsule and bathed in a protein rich fluid (Fig.28). In some instances, moderate villus atrophy was encountered. Marked hyperplasia of the Peyer's patches was evident in all the animals. D8 and D2, had developmental stages of *Eimeria leukarti* in the lamina propria of the intestinal villi (Fig. 29).

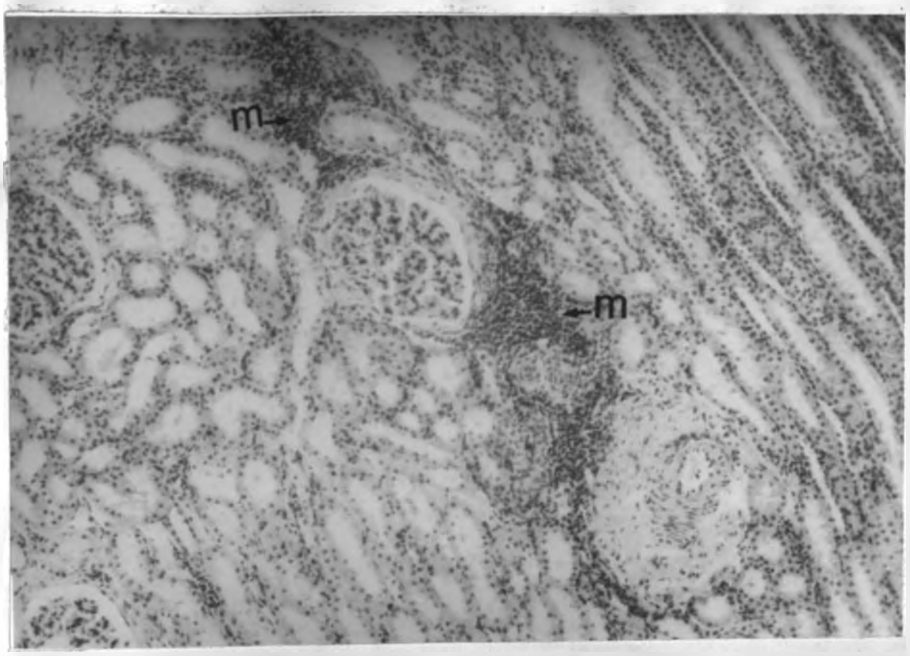


Figure 26: Kidney from D6 [98 days p.i.] infected with $7.5x10^6$ *T. congolense* bloodstream forms displaying cellular infiltration into intertubular areas (m) in the cotrico-medullarly junction. These cells are mainly lymphocytes. H&E X40.

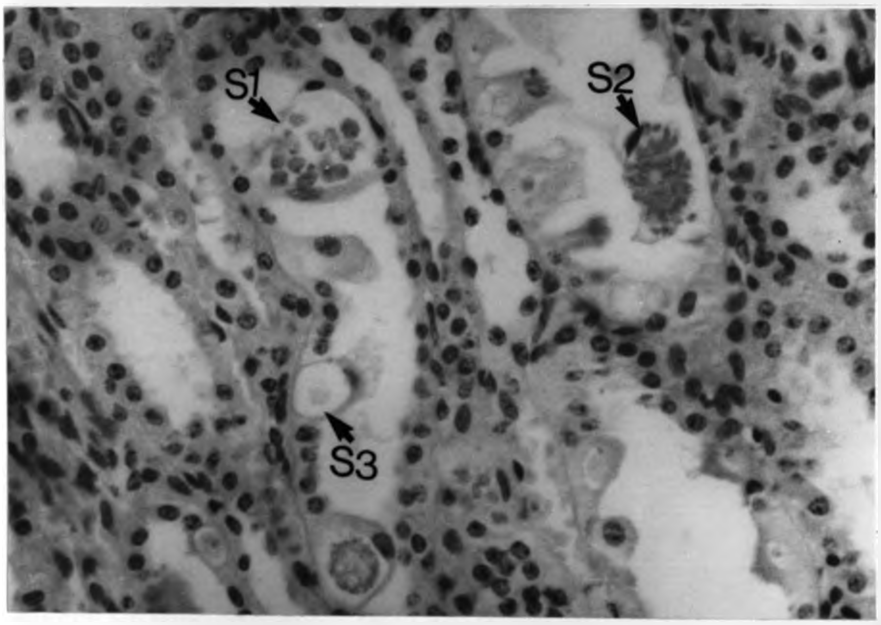
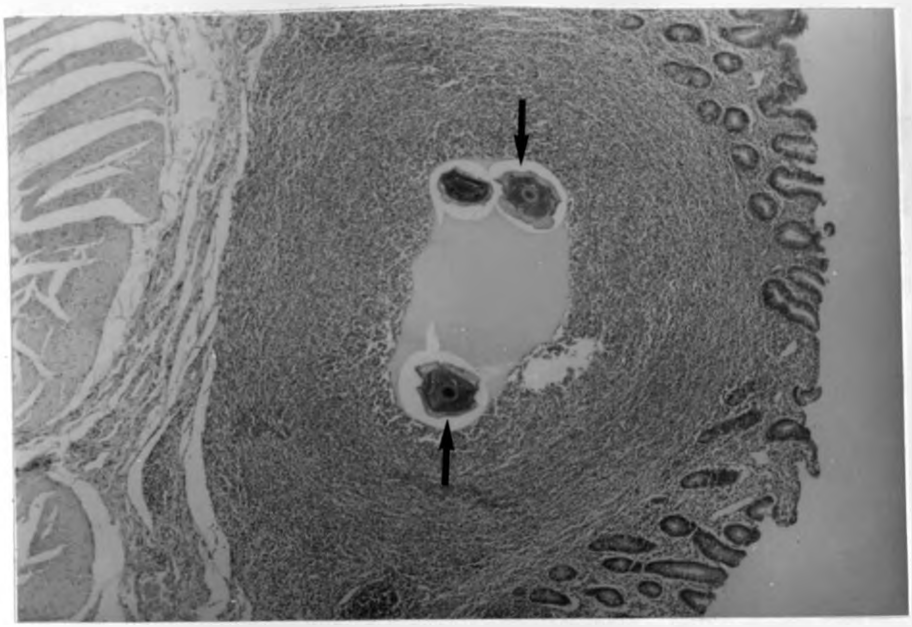
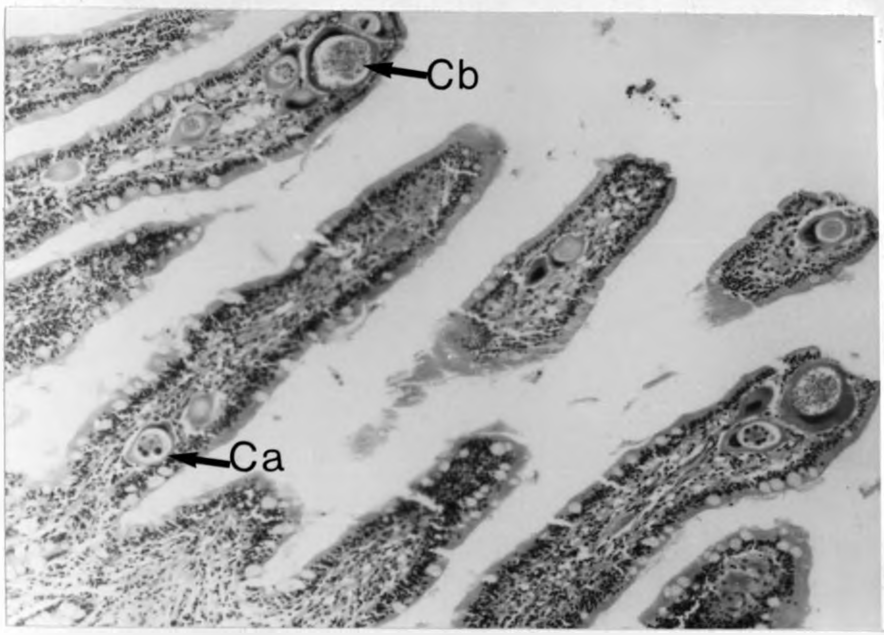


Figure 27: Kidney from D4 [97 days p.i.] infected with $7.5x10^6$ trypanosomes (*T. congolense*) depicting coccidial developmental stages of *Klossiella equi* (an incidental finding). S1 and S2 are sporocyts while S3 is a gamont. H&E X400.



gure 28: Intestines from control animal (D3) showing helminth larval stages (arrows) trapped in the intestinal wall (an incidental finding). H & E X100.



igure 29: Intestine from control animal (D8) displaying coccidian developmental stages of *Eimeria leukarti* (an incidental finding). Cb is a microgamatocyte and Ca is a macrogamatocyte. H&E X100.

4.2.6 Heart

In most of the animals, the heart was of normal microscopic appearance apart from one case. D2 had few focal areas of mononuclear cells infiltration in the myocardium. Myocardial cells were showing degenerative changes and the infiltrating cells were mainly lymphocytes (Fig. 30).

4.2.7 Lungs

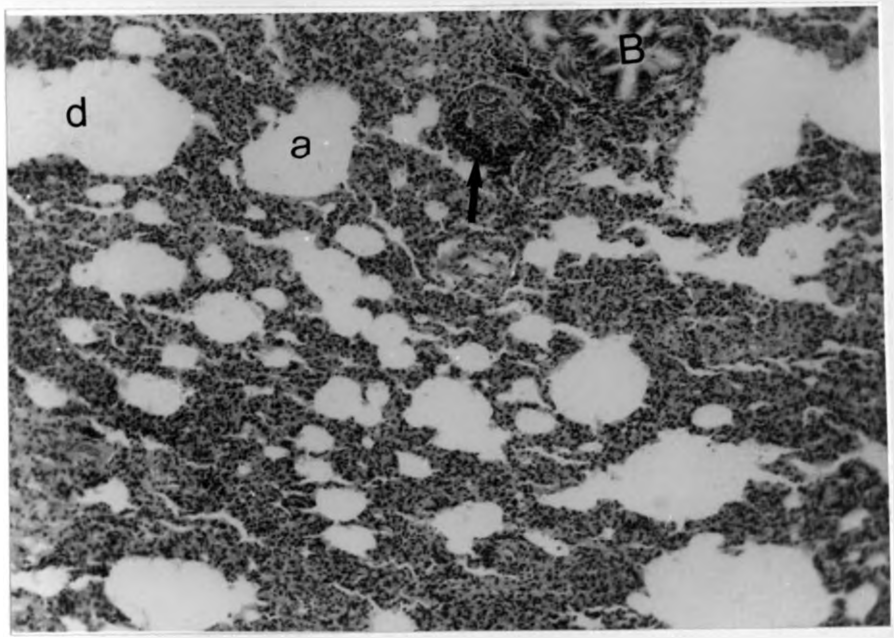
In the infected animals D4 and D6, patches of oedema containing many neutrophils and few lymphocytes were observed. Areas of thickened interalveolar septae indicating interstitial pneumonia were occasionally encountered. Hyperplasia of lymphoid tissue in peribronchiolar areas was evident (Fig. 31). Atelectasis was evident in some portions in all the infected donkeys. Haemosiderin was observed occasionally in macrophages of these infected animals.

The control animal D7 had portions of atelectasis (Fig. 32), D3 was relatively congested while D8 had few patches of atelectasis, many eosinophils in the capillaries and a compensatory emphysema in some portions.

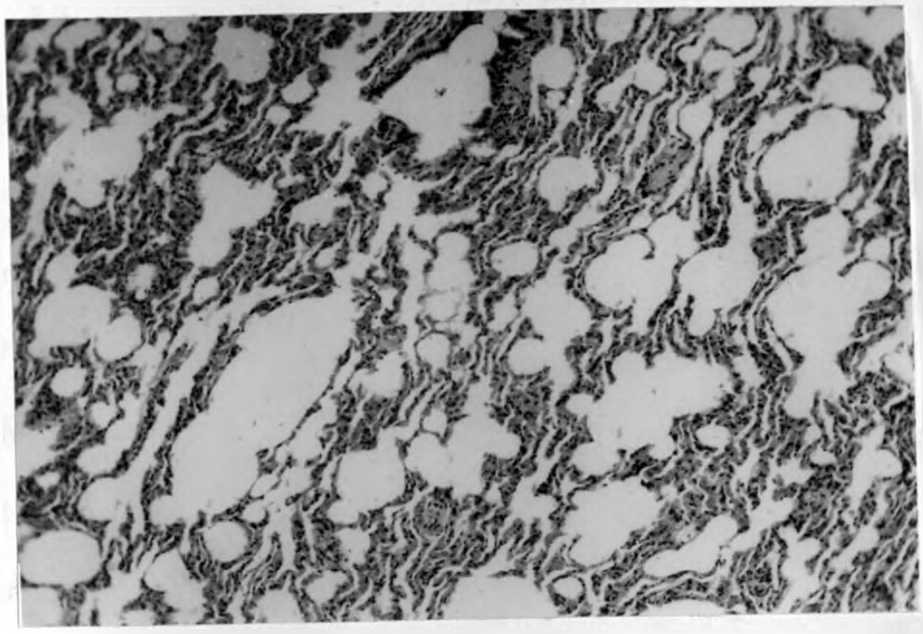
4.2.8 Brain

Mild congestion was observed in infected animals but no parasites were seen.

gure 30: Heart from D2 [96 days p.i.] infected with $7.5x10^6$ trypanosomes (*T. congolense*) showing focal cellular infiltration(F) into the myocardium. H&E X40.



igure 31: Lung from D4 infected with 7.5 x 10^6 trypanosomes (*T. congolense*) depicting thickened interalveolar septae and some degree of atelectasis, dilated alveolar sacs (a) and alveolar ducts (d) as well as hypertrophic peribronchiolar lymphoid tissue (arrows). H & E x100.



gure 32: Lung from control animal (D7) showing some degree of atelectasis. H&E X100.

SUBINOCULATION RESULTS

.1 MICE

In the first subinoculation, only the two mice inoculated with blood from D4 developed parasitaemia by the 10th day. The rest were negative for the 30 days of observation.

In the second subinoculation, mice inoculated with blood from D2, D4 and D6 developed detectable parasitaemia but at different times. Mice inoculated with blood from D4 and D6 took 18 days to peak in detectable parasitaemia while those inoculated with blood from D2 took 30 days. Although the two mice inoculated with blood from D6 were used in the sheep inoculation, the remaining parasitaemic mice survived upto 60 days post-inoculation. Mice inoculated with blood from D1 never peaked in parasitaemia even after 60 days.

The organisms on average measured 13.2 ± 1.6μm in length and a range of 10.8-16.8μm for 50 organisms observed. They had no conspicuous undulating membranes but a medium-sized subterminal marginal kinetoplast were present (Fig. 33).

5.2 SHEEP

All the three sheep developed a parasitaemia but at different times. S3, inoculated with blood from parasitaemic mice developed a peak parasitaemia on

day 7. S1 and S2 inoculated with blood from D2 developed a parasitaemia on days 11 and 17 p.i., respectively. The parasitaemia appeared in transient peaks in all sheep to the end of the experiment.

Temperature increases were observed 2-3 days before parasites peaked in the circulation. Temperatures of upto 41.3°C were observed during these transient peaks. The pattern of pyrexia closely followed that of parasitaemia.

The sheep remained strong, bright and with good appetite. No discoloration of the mucus membranes, faeces or urine were observed.

The haematology results are show in appendices 20-25. The PCV and the Hb indicated a progressive decrease while the RBC count increased gradually. The PCV was 34.3% at the time of inoculation, decreased gradually to stand at 28.3% at the termination of the experiment. This indicated a 6.0% decrease from pre-infection values.

The Hb concentration was 13.4gm% at the start of experiment but declined gradually to 11.0gm% at the end of the experiment. This depicted 17.9% decrease from the pre-infection value. The RBC count increased gradually from a pre-infection value of 10.51 X 10^6/ml to stand at 15.56 x 10^6/ml at the end of the experiment which indicated an increase of about 48.0%.

The MCV decreased slightly from the pre-infection value of 30.0 fl to 29.7 fl at the end of the first month but it rose again to 32.0 fl at the end of the experiment indicating a 6.7% increase. MCH decreased from 12.8 pg at the start of the experiment to 10.7 pg by the end of the first month depicting a

16.5% decrease. Lower MCH values were observed during the second month to stand at 7.4pg at the close of the experiment. MCHC value was 39.42gm% at the time of inoculation but by the end of the first month, it had decreased to 33.98 gm%. However, at the end of the experiment, the values had risen back to pre-infection level.

Leukocyte count gradually increased from a pre-infection value of 10.6 x 10^3/ml to 11.7 x 10^3/ml at the end of the experiment. This indicated a 10% increase. The lymphocytes increased from 5.4 x 10^3/ml at the start of experiment to 9.0 x 10^3/ml in the course of the experiment. This depicted a 51.4% increase. The neutrophils on the other hand decreased from 4.3 x 10^3 at the beginning of the experiment to reach 2.5 x 10^3/ml at the close of the experiment. This reflected a 47.8% decrease. The N:L ratio stood at 0.6 at the start of the experiment but at the end of the experiment, it had decreased to 0.3. Monocytes, eosinophils and basophils showed no major changes.

The total plasma proteins decreased from 7.4gm% at the time of inoculation to the lowest value of 6.4gm% by the 8th week of infection. This indicated a 13.5% decrease. The icteric index remained relatively constant at the level of 2 - 5 units throughout the experiment.

The trypanosomes had a total length of 13.7 ± 2.2µm and a range of 9.6 - 20.4µm for 50 trypanosomes. They had no conspicuous undulating membranes but marginal and subterminal kinetoplasts were present(Fig. 34).

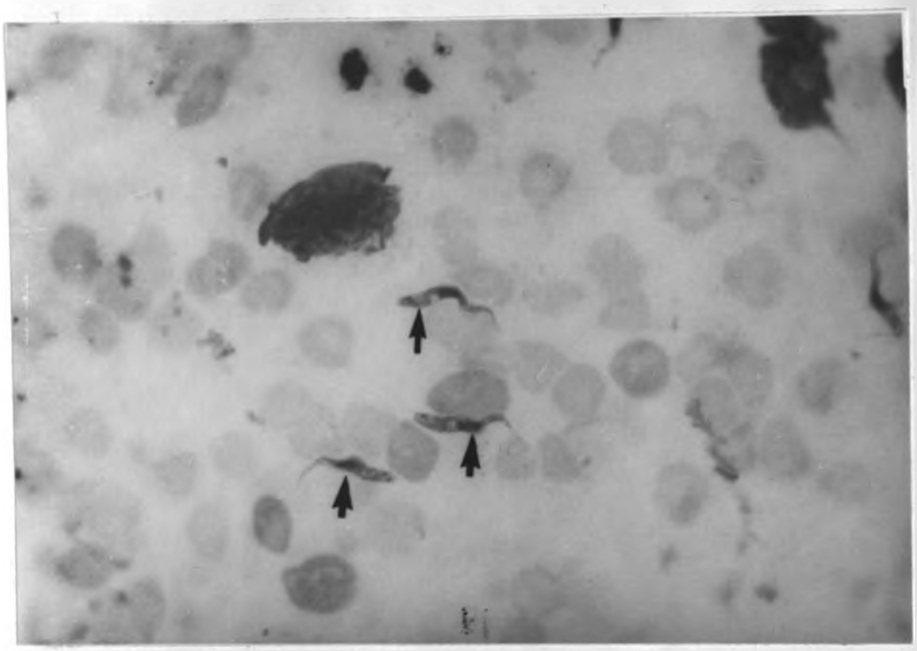


Figure 33: A thin blood smear from a mouse [20 days p.i.] infected with *T. congolense* showing trypanosomes(arrows). Giemsa X1000.

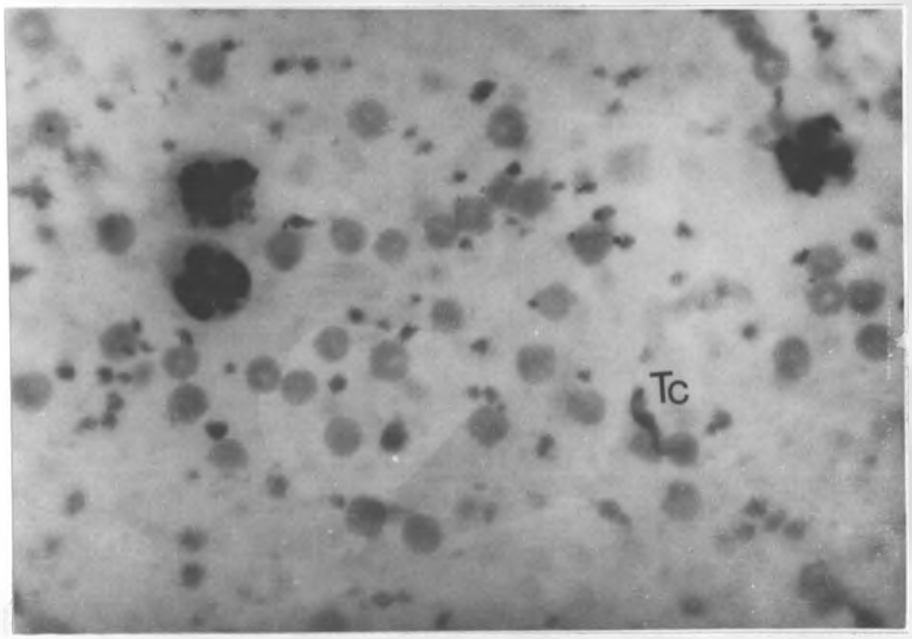


Figure 34: A thin blood smear from sheep (S3, 30 days p.i.) infected with *T. congolense* showing trypanosomes (Tc). Giemsa X1000.

5 DISCUSSION

5.1 HAEMATOLOGY

The results show that, during the acclimatization period, the PCV, total plasm proteins and lymphocyte counts were higher than observed by other authors. The rest of the haematology parameters were on the upper margins of the ranges indicated by these authors (Fowler, 1986; Hill, 1989; Zinkl, Nae, Merida, Farver, and Humble 1990). This could be attributed to emotional stress due to handling during samples collection. This is expected since it was their first time to be subjected to such kind of work.

Most of the parameters were higher in the infected than in the control group although, they were not significantly different except for the MCHC and total plasm proteins. The animals were all adults but the sexes were different and apparently the two female donkeys were in the same group (infected group). Zinkl *et al*(1990) reported higher MCHC, total leukocyte and neutrophil counts in female donkeys higher RBC counts with cells that are smaller in size but low Hb content have been reported (Hill, 1989). The latter author also observed lower total leukocyte, monocyte and lymphocyte counts in the female animals. From these it is difficult to speculate precisely the cause(s) of these differences but the sexes being different could have accounted for the observations.

The fall in total plasma proteins and the rise in eosinophil count observed

in the two groups of animals could suggest helminthiasis with or without nutritional deficiencies being involved in the causation of the anaemia observed. In horses, persistent low grade normocytic or macrocytic anaemia without jaundice indicate a possibility of a chronic haemorrhage. The most common cause of such an anaemia has been attributed to strongylid nematodes (Dixon and Archer, 1974). Eosinophilia accompanied by normocytic, normochromic anaemia has also been observed in young horses with *Strongylus vulgaris* infection (Archer and Poynter, 1957). In this study, the donkeys were observed to harbour *Strongylus* spp at various stages of development. Larvae were observed in intestinal section, cranial mesenteric artery and its branches, and occasionally trapped in the liver. Adult worms were encountered in the caecum and colon in some cases. This observation suggests that the anaemia encountered in the two groups of animals could partly be attributed to helminths. However, the significant differences observed in PCV, RBC counts, and Hb content at later stages of the experiment could be attributed to trypanosomiasis. This concurs with the observations made by Jenkins and Facer (1985) that it is difficult to asses the significance of anaemia in the overall morbidity of African trypanosomiasis as other concurrent helminth infections as well as nutritional deficiencies are encountered. Obi and Anosa (1980) cited by Jenkins and Facer (1985) noted that trypanosomiasis produces a more severe anaemia than most of the blood protozoan and gastrointestinal helminth infections encountered among animals. Similarly, Mamo and Holmes (1975)

observed that Zebu cattle infected with *T. congolense* died of pronounced anaemia.

At the end of the experiment, the PCV values had decreased by 41.6% and 22.2% from the pre-infection levels in the infected and control groups, respectively. This decline in PCV would be an indication of anaemia which was observed even earlier in the second month of infection in the infected group. If all factors contributing to the fall in PCV in the control animals had similar effects on the infected animals, then, it can be deduced that a 19.4% difference at the end of the experiment would be attributable to trypanosomiasis alone.

In the infected animals, the anaemia would be classified as hypochromic, microcytic in the second month of infection when it was characterized by a decrease in MCV by 6.2%, MCH by 9.8% and MCHC by 4.7% from pre-infection values. At the termination of the experiment, increases of MCV by 15.8%, MCH by 30.1% and MCHC by 6.3% over the pre-infection levels were observed. The latter could categorize the anaemia as normochromic, macrocytic. This then concurs partly with what has been observed in cattle infected with *T. congolense* by other workers (Fiennes, 1954; Naylor, 1971a; Mamo and Holmes, 1975; Maxie *et al* 1979; Valli *et al,* 1978b).

The anaemia could be termed as regenerative particularly during the third month of infection. The rising MCV values indicated the presence of primitive cells which are usually larger than mature cells and are highly suggestive of a

functional bone marrow (Coles, 1967). Reticulocytes were not observed in all the donkeys. Dixon and Archer (1974) and Schalm (1975) indicated that reticulocytes are rarely observed in horses even in severe anaemia. Reticulocytes were not observed during the course of bovine trypanosomiasis (Fiennes, 1954; Naylor, 1971a; Valli *et al*, 1978b) as well.

The aetiology of the anaemia observed was not intensively investigated but on the basis of the data obtained in the course of the experiment, some theories can be put forward. The macrocytic, normochromic anaemia observed during the third month of infection may be clearly suggestive of a functional bone marrow, thus, ruling out bone marrow dysfunction as is usually the case in chronic stages of the disease in bovine (Fienne, 1954, 1970; Naylor, 1971a; Valli and Forsberg, 1979;). Although haemolysis could be considered as a probable cause, the icteric indices did not show any major increase from the pre-infection values an observation that tends to rule out intravascular haemolysis. Suliman and Feldman (1989) indicated that bilirubinanaemia and haemoglobinuria are not consistent in trypanosomiasis.

Extravascular haemolysis could be a probable cause of the anaemia observed in the donkeys with trypanosomiasis in this study. Erythrophagocytosis and haemosiderosis were observed in some sections of the spleen and lymph nodes as has been reported in other animals (Fiennes, 1954; Mackenzie and Cruickshank, 1973; Ikede *et al* 1977; Mwangi, 1987).

PCV has been used in the classification of trypanotolerance in the wild

bovidae. The percentage decrease from pre-infection level and whether it returns to the pre-infection level or not after a duration of infection has been the commonly used criterion. Grootenhuis, Varm, Black, Moloo, Akol, Emery and Murray (1982) observed the buffalo to have a maximum drop of 5% ; eland, 24%; orynx 20%; Waterbuck 24%; cattle 37% and goat 50%. The cattle and goats acted as controls in this case. They also noted that the fall in the PCV was inversely related to the level of parasitaemia. The buffalo and orynx recovered to pre-infection levels, the eland almost recovered while the waterbuck, cattle and goats, did not and in the last two, a chronic and severe anaemia developed and culminated in death. Grootenhuis *et al* (1990), Olubayo, Grootenhuis and Rurangirwa (1990) observed that the buffaloes infected with *T. congolense* produced no anaemic response. A similar phenomenon was observed in a wild beast infected with *T. brucei* (Rurangirwa, Musoke, Nkonge, Njuguna, Mushi, Karstad and Grootenhuis, 1986). In cattle, N'Dama have shown a decrease in PCV of 23-25% compared to 41% in the zebu (Dargie *et al*, 1979; Murray, *et al*, 1982).

On the same line of argument, the donkeys in this study had a 19.4% decrease in PCV which was attributable to *T. congolense* infection. No ill-health was observed in these animals except for the moderate loss of body condition. This could imply that the donkeys infected with *T. congolense* are more closer in response to the N'Dama cattle than the African buffalo. The experimental period was rather short to observe whether death or recovery

would have been the end result. In the horse, Stephen (1962) observed that the stallion spontaneously recovered from the disease but parasites could still be observed in the blood for periods exceeding one year. There was no indication of spontaneous recovery in the donkeys in this study and the features of a severe disease observed by Kimberling and Ewing (1973) were absent.

The outcome of an infection depends not only on the virulence of the pathogenic strain of a trypanosome but also on the host species involved (Fiennes, 1970; Jubb *et al*, 1985). Some animals are dramatically affected and die from the infection, others become carriers due to a premune state, while others clear off the infections spontaneously. Coles (1967) was of the opinion that the leukocyte count could be useful in predicting the outcome of an infection. He indicated that where the infecting organism is less virulent, or in resistant animals with virulent organisms, there might be no major changes on leukocytes. There is evidence of leukopenia in cattle infected with *T. congolense* early in infections (Losos, *et al*, 1973; Omuse, 1973; Wellde *et al*, 1974; Valli *et al* 1978b) which are susceptible to the infection. The differential leukocyte counts show a decrease both in neutrophils and lymphocytes indicating a compromised defence mechanism even to concurrent infections. In donkeys, *T. brucei* produce a leukopenia (Ikede *et al* 1977) which also indicate high susceptibility to *T. brucei* infection. The *T. congolense* infection in this study produced a minor leukocyte count response with most of the values being within the range particularly during the pre-infection time. This could suggests

that the trypanosomes, to some extent, produce no noticeable immunosuppression in the donkeys. The almost intact defence system could probably account for the observation of no ill-health.

5.2 SERUM BIOCHEMISTRY

Total bilirubin of 0.3mg/dl (0.16 - 0.40mg/dl) and BUN of 34.8mg/dl (12 - 40gm/dl) were almost within the ranges observed by Fowler (1986), Zinkl *et al* (1990). No major changes from the pre-infection values were observed in the two groups of donkeys in this study. This could on one hand indicate no evidence of intravascular haemolysis which is usually accompanied by a rise in bilirubin. No significant tissue lesions in the donkeys could be expected with this level of BUN. This is further supported by the post-mortem results.

5.3 PATHOLOGICAL FINDINGS

The pathological findings were complicated by several factors other than trypanosomes. Helminth parasites were observed in the stomachs, small intestines, the colons and also in extra-intestinal locations (the liver and cranial mesenteric arteries). According to Archer and Poynter (1957); Dixon and Archer (1974), the large strongyles especially *S. edentatus* and *S. vulgaris* contribute to the development of anaemia and therefore make the interpretation of the results rather difficult particularly when parenchymatous organs are affected. The presence of extra-intestinal stages particularly in the liver

distorted the architectural picture of these organs in both groups of animals. The strict confinement with a likelihood of ammonia gas build up and the dusty hay are probable predisposing factors to pneumonia and other related respiratory conditions. In such conditions bacterial, viral, chlamydial or fungal infections could easily occur. Dietary deficiencies as depicted by the fall in total proteins inspite of supplementation could to some extent account for the lesions observed in the liver. However, the hydropic degeneration in the liver coupled with haemosiderin in kupffer cells could point at trypanosomiasis as has been observed in cattle infected with *T. congolense* (Fiennes, 1954; Naylor, 1971b).

The lymphoid organs namely; the lymph nodes and the spleen were definitely involved. A clear cut difference was observed between the two groups of donkeys. The expansion of the follicular and parafollicular areas in the lymph nodes; the red pulp , the malphigian corpuscles and the periarteriolar lymphatic sheaths in the spleen tend to suggest an immunoproliferative response to an antigenic stimulus (possibly trypanosomes). The mitotic figures of the medium-sized lymphocytes, an indication of blast formation, suggests an enhanced defense mechanism to regulate or eliminate the trypanosomes. The increased number of macrophages and plasm cells in the organs definitely show a degree of both cellular and humoral response. If this assumption is true, it can be deduced that these two mechanisms are involved in the defense mechanism to regulate the level of parasitaemia in donkeys, and needs a further

investigation.

5.4 ROLE OF DONKEYS IN TRYPANOSOMIASIS

In this study, *T. congolense* infection in the donkeys produced a low parasitaemia which was detectable by the routinely employed diagnostic methods. The significance of this parasitaemia was tested in two test systems; mice and sheep. The mice and sheep peaked in parasitaemia as a result of which mice were observed to die. In some cases, mice could live for periods exceeding 30 days in the parasitaemic phase, and this could probably indicate a degree of attenuation although no morphological changes were observed. In the sheep, the PCV, Hb concentration indicated 6.0% and 17.9% decrease from pre-infection values respectively. This could indicate a degree of anaemia in these animals.

One of the donkeys (D1) did not show a detectable parasitaemia inspite of being inoculated with similar dosage. This could probably have been as a result of a previous exposure to *T. congolense.* Although the donkeys were acquired from a tsetse fly free region, there are chances that this particular donkey could have been brought from Narok or Kajiado by livestock traders. The high demand for the donkeys for transport purposes in Limuru and surrounding areas, has prompted a number of livestock traders to go deeper in Maasailand for these animals. If this is the case, probably the donkey had been infected earlier, cleared the infection and resisted the re-infection.

It seems that donkeys may act as important reservoirs of *T. congolense* to the animals in the same environment. Boydt *et al* (1972) conducted studies in Zimbabwe and noted that donkeys could act as food for the *Glossina morsitans* as well as reservoirs for trypanosomes. Scanty and sporadic parasitaemia was a feature of the infection in this study and this could probably imply that chances of transmitting the infections may be minimal. However Maudlin and Welburn (1989) indicated that a single trypanosome could establish an infection in a Glossina feeding on them, thus compounding the problem further.

5.5 EFFECTS ON DRAUGHT POWER

The haemoglobin content in the blood in health is related to the propensity of the animal for sustained muscular activity or ability to meet demands for sudden bursts of speed (Schalm, 1975). Donkeys in many parts of the world are used as working animals. The Donkey Breed Society of the World having observed this, decided that the donkeys must be assumed to be working animals and should therefore meet the characteristics associated with working horses (Fraser, 1972). Irvine (1958) observed that the best performing horses had PCV, MCH and MCHC values higher than the overall averages. He noted that anaemia could be most significant when maximum activity is required.

In the present study, *T. congolense* infection in the donkeys caused a

decrease in red cell mass and Hb content. This observation implies that the disease can interfere with the draught power. Loss of body condition, fatty degeneration and hydropic degeneration of parenchymatous organs could point to a compromised health. This becomes important in the donkeys considering that these animals are usually overburdened and overworked with no medical attention.

T. brucei in the donkeys have been shown to cause weakness (Ikede *et al* 1977). The animals are also observed to fatigue quickly despite the capricious appetite. If the situation is the same in *T. congolense* infections, then their profitability in draught work will be minimal and man will for ever remain a slaving labourer in the tsetse fly infested areas.

6 CONCLUSIONS

1. Donkeys suffer a subclinical *T. congolense* infection which is difficult to detect clinically but a positive diagnosis can be made using the routinely employed methods in cattle. A low parasitaemia appearing sporadically is a characteristic of the infection. Comparative studies on the best diagnostic tests in these animals could be done in order to establish the best method for diagnosing such infections.

2. The red cell mass decreased progressively in the period considered. It is imperative that further studies covering longer durations of time be done to establish the clinical picture in chronic stages of the disease. The mechanism(s) of anaemia modulated by the activated RES system also needs further investigations.

3. Donkeys are potential reservoirs of *T. congolense* infections and may pose a to danger other animals in the same environment. The reservoir problem in areas where donkeys are widely needs to be re-visited and the control measures may be adjusted to cover the donkeys if the disease is to be eradicated.

4. The donkeys may not be profitably utilized in areas where

trypanosomiasis is endemic. The anaemia associated with the infection minimizes not only the longevity of the animals but also the working speed and efficiency.

5. Compared to the susceptible cattle breeds like the Zebu, the donkey displays a better defence mechanism against the *T. congolense* infection. The minimal leukocyte count change depicts an efficient regulatory mechanism particularly of the level of parasitaemia. The prolonged prepatent period of upto 30 days indicates a possible involvement of the host's defence mechanisms in the multiplication of the trypanosomes. This superiority of the donkey over the cattle needs further studies to clearly bring out a good picture.

REFERENCES

Abo-Shehada, M.N. (1988). Prevalence of hydatidosis in donkeys from central Jordan. Veterinary Parasitology *30:* 125 - 130.

Annon (1947) Nigeria: Annual report; sleeping sickness service (1944 - 1946). Tropical Disease Bulletin *44*: 973.

Annon (1978) Kenya: Annual reports; Ministry of livestock development (1976 -1989).

Archer, R.K and Poynter, D. (1957). Anaemia and eosinophilia associated with helminthiasis in young horses. Journal of Comparative Pathology. *67*: 196 - 207.

Bennett, S.C.J. (1933). The control of camel trypanosomiasis. Journal of Comparative Pathology *46*: 67 - 77 and 174 -185.

Bliss, D.H. (1986). Parasites abroad. Svendsen, E. D. (Compilor): *The Professional Handbook of the Donkey*. Sovereign printing groups, England (1986). Pp 85 - 90.

Bliss, D.H.; Svendsen, E.D.; Geongoulakis, I.E.; Grosomanidis, S and Taylor, F. (1985). Strategic use of anthelmintics in working donkeys in mediterranean climatic conditions. Veterinary Record *117*: 613 - 614.

Boydt, W.P; Mackenzie, P.K.I.; Pilson, R.D. and Leavis, H. (1972). The importance of the donkey *(Equus asinus)* as a source of food and a reservoir of trypanosomes for *Glossina morsitans west* w.
Rhodesia Science News 6:18-20.

Camac, O.R. (1986). Introduction and origins of the donkey. Svendsen, E. D. (Compilor): *The Professional Handbook of the Donkey.* Sovereign printing group, England (1986). Pp 1 - 8.

Coles, E.H. (1967). *Veterinary Clinical Pathology.* Coles, E.H. (Editor). W.B. Saunders company, Philadelphia (1967).

Cox, F.E.G. (1979). The pathogenesis of animal trypanosomiasis.
Nature (London) *277*: 603 - 604.

Currason, G. (1940). Social and economic importance of animal trypanosomiasis. Veterinary Bulletin (1941) *11*: 666 - 667.

Dargie, J.D.; Murray, P.K.; Murray, M.; Grimshaw, W.R.T. and McIntyre, W.I.M. (1979). Bovine trypanosomiasis. The red cell kinetics of N'Dama and Zebu cattle infected with *Trypanosoma congolense*. Parasitology *78*: 271 - 286.

Defreef, G. (1919). The nervous symptoms and persistence of trypanosomes in the cerebrospinal fluid of mules affected with Nagana due to *T. brucei* variety *ugandale*. Tropical Veterinary Bulletin *8*:6

Dixon, J.B. and Archer, R.K. (1974). Interpretation of equine aneamias. In: The Veterinary Annual. Wright Scentechnica, Bristol *15*: 185 - 190.

Eaton-Evans, W. (1986). Stud medicine. Svendsen, E. D. (Compilor): *The Professional Handbook of the Donkey*. Sovereign printing group, England. Pp 113 - 120.

Epstein, H. (1984). Ass, Mule and Onger. Magon, I. L. (Editor): *Evolution of Domesticated Animals*. Longman, London (1984). Pp 174 - 184.

Esievo, K.A.N.; Saror, D.I.; Ilemobade, A.A. and Hallaway, H.W. (1982). Variation in erythrocyte and free serum sialic acid concentrations during experimental *T. vivax* infection in cattle. Research in Veterinary Science *32*: 1-5.

Eysker, M. and Pandey, V.S. (1987). Overwintering of non-migratory stronyles in donkeys in the high-Veld of Zimbabwe. Research in Veterinary Science. *42*: 262 - 263.

Eysker, M. and Pandey, V.S. (1989). Small strongyle infections in donkeys from the High-Veld in Zimbabwe. Veterinary Parasitology *30*: 345 - 349.

Fielding, D. (1987). Donkey power in African rural transport. World Animal Review *63*: 22 - 30.

Fiennes, R.T.N.W. (1954). Haematological studies in trypanosomiasis of cattle. Veterinary Record *66*: 423 - 434.

Fiennes, R.T.N.W. (1970). Pathogenesis and Pathology of animal trypanosomiasis. Mulligan,H. W. (Editor): *The African Trypanosomiases*. George and Unwin, Ltd, Great Britain (1970). Pp 729 - 750.

Ford, J. (1970). The geographical distribution of Glossina. Mulligan, H. W. (Editor): *The African Trypanosomiases*. George and Unwin Ltd, Great Britain (1970). Pp 274 - 279.

Forsberg, C.M. Valli, V.E.O.; Gentry, P.W. and Donworth, R.M. (1979). The Pathogenesis of *Trypanosoma congolense* infection in calves. IV. The kinetics of blood coagulation. Veterinary Pathology *16*: 229 - 242.

Fowler, J.N. (1986). Medical . Svendsen, E. D. (Compilor): *The Professional Handbook of the Donkey*. Sovereign Printing group, England. Pp 91 - 99.

Fraser, A.C. (1972). The examination of donkeys. Veterinary Record *90*: 478 - 480.

Gaafar, S.M.; Urquhart, G.M.; Euzeby, J.; Soulsby, E.J.L. and Lammler, G. (1971). Pathobiology of trypanosomiasis. Lumsden, W. H. R. (Editor): *Pathology of Parasitic Diseases*. Pardine University Studies, Indiana (1971). Pp 1 - 14.

Geigy, R. and Kauffmann, M. (1973). Sleeping sickness survey in the Serengeti area (Tanzania) 1971. Part 1: Examination of large mammals for trypanosomes.

Acta Tropica *30*: 12 - 23.

Godfrey, D.G. (1961). "Types of *Trypanosoma congolense*". II. Differences in the course of infection. Annals Tropical Medicine and Parasitology *55*: 154 - 166.

Grootenhuis, J.G.; Dwinger, R.H.; Dolan, R.B.; Moloo, S.K. and Murray, M. (1990). Susceptibility of African buffalo and Boran cattle to *Trypanosoma congolense* transmitted by *Glossina morsitans centralis*. Veterinary Parasitolgy *35:* 219-231.

Grootenhuis, J.G.; Varma, Y.; Black, S.; Moloo, S.K.; Akol, G.; Emery, D.L. and Murray, M. (1982). Host response of some African wild bovidae to experimental trypanosome infection. Karbe, E. and Frectas (Editor): *Trypanotolerance and Animal Production.* Eschborn, West Germany (1982). Pp 37 - 42.

Head, A.S. (1904), Tsetse fly disease among mules in the Sudan. Journal of Comparative Pathology and Therapeutics. *17*: 206-208.

Hill, F.W.G. (1989). Haematological and clinical chemistry values for donkeys in Zimbabwe. Zimbabwe Veterinary Journal *20*: 113-120.

Hoare, C.A. (1970). Systematic description of mammalian trypanosomiases of Africa. Mulligan, H. W. (Editor): *The African Trypanosomiases*. George and Unwin Ltd, Great Britain (1970). Pp 24-59.

Hoare, C.A. (1972). The trypanosomes of mammals. *A Zoological monograph*. Hoare, C.A. (Editor). Blackwell Scientific Publications, Oxford (1972).

Ikede, B.O.; Akpokodje, J.U., Hill, D.H. and Ajidagba, P.O. (1977). Clinical, haematological and pathological studies in donkeys experimentally infected with *Trypanosoma brucei*. Tropical Animal Health and Production 9: 93-98.

International Laboratory for Research on Animal Diseases (1989). Trypanosomiasis. In: Annual reports of ILRAD, NAIROBI. Pp 33-51.

Irvine, G.H.G. (1958). The blood picture in the race horse. I. The normal erythrocyte and haemoglobin status. A dynamic concept. Journal of Animal Veterinary Association *133*: 97-101.

Jenkins, G.C. and Facer, C.A. (1985). Haematology of African trypanosomiasis.

Tizard, I. (Editor): *Immunology and Pathogenesis of Trypanosomiasis*. C.R.C. Press Inc., Florida (1985). Pp 13-44.

Jones, T.C. and Gleiser, C.A. (1954). *Veterinary Necropsy Procedures*. Jones, T.C and Gleiser, C.A. (Editors). Sir Isaac, Pitman and Sons Ltd. London (1954).

Jubb, F.; Kennedy, P.C. and Palmer, N. (1985). Trypanosomiasis: Jubb, Kennedy and Palmer (Editors): *Pathology of Domestic Animals*. Academic Press, Inc., Harcourt (1985) vol 3. Pp 153-159

Kaaya, G.P. (1975). Experimental trypanosomiasis: A study of pathology of *Trypanosoma congolense*, with a special emphasis on the pathogenesis of anaemia. M. Sc. thesis (University of Nairobi; Kenya).

Kaaya, G.P. and Oduor - Okello, D. (1980). The effect on *T. congolense* infection on the testis and epididymis of the goat. Bulletin of Animal Healthand Prodution of Africa *28*: 1-5.

Kaliner, G. (1974). *Trypanosoma congolense*. Histopathological findings in experimentally infected cattle. Experimental Parasitolgy *36*: 20-26.

Kanyari, P.W.N. (1981). Trypanosomiasis: A study on the development and control in three breeds of goats. M. Sc. thesis (University of Nairobi; Kenya).

Kimberling, C.V. and Ewing, J.R. (1973). A case history of *T. congolense* infection in two horses. Bulletin of Epizootic Diseases of Africa *21*: 249-251.

Kobayashi, A. Tizard, I.R. and Woo, P.T.K. (1976). Studies on the anaemia in experimental African trypanosomiasis. II. The pathogenesis of the anaemia in calves infected with *Trypanosoma congolense*. American Journal of Tropical Medicine and Hygiene *25*:401-408.

Kyewalabye, K.E., Kwari, H.D.; Ajayi, M.O. and Shinggn, P. (1989). Clinical parameters of donkeys before and after *T. vivax* infection. Zaria, Nigeria. Revue d'Elevage et de Me'dicine Ve'te'rinariae des Pays Tropicaux 41:271-276.

Lloyd, D.H. (1972). Streptothricosis in the domestic donkey (*Equus asinus asinus*). I. Clinical observations and clinical pathology. British Veterinary Journal *127*: 572-581.

Losos, G.P.; Paris, J.; Wilson, A.J. and Dar, F.K. (1973). Pathology of the disease in cattle caused by *Trypanosoma congolense*. Bulletin Epizootic

Diseases of Africa *21*: 239-248.

Luckins, A.G. and Gray, A.R. (1978). An extravascular site of development of *Trypanosoma congolense*. Nature (London) *272*: 613-614.

Macfie, J.W.S. (1916). Babesiosis and trypanosomiasis at Accra, Gold Coast, West Africa. Annals of Tropical Medicine and Parasitolgy *9*: 457-494.

Mackenzie, P.K.I and Cruickshank, J.G. (1973). Phagocytosis of erythrocytes and leukocytes in sheep infected with *Trypanosoma congolense* (Broden, 1904). Research in Veterinary Science *15*: 256-262.

Maclennan, K.J.R. (1970). The epizootiology of trypanosomiasis in livestock in West Africa. Mulligan, H. W. (Editor): *The African Trypanosomiasis*. George and Unwin Ltd, Great Britain (1970). Pp 751-765.

Maloiy, G.M.O. and Boarer, C.D.H. (1972). Response of the donkey to dehydration: haematological changes. American Journal of Physiology *221*: 37-41.

Maloo, S.H. (1986). Trypanosomiasis of the East African Coast. Kenya Veterinarian *10*: 41-42.

Mamo, E. and Holmes, P.H. (1975). The erythrokinetics of Zebu cattle chronically infected with *Trypanosoma congolense*. Research in Veterinary Science *18*: 105-106.

Marthur, B.B.L.; Sharma., R.N,; Kulshrestha, S.B. and Kumar, S. (1972). Some observations on the behaviour of African horse sickness virus (Jaipur strain) in donkeys. Indian Journal of Animal Science 41:1135-1137.

Maudlin, I and Welburn, S.C. (1989). A single trypanosome is sufficient to infect a tse tse fly. Annals of Tropica Medicine and Parasitology. *83*:431-433.

Maxie, M.G.; Losos, G.L. and Tabel, H. (1979). Experimental trypanosomiasis (*Trypanosoma vivax* and *T. congolense*). 11. Symptomatology and clinical pathology. Tropical Medicine and Parasitology *30*: 274 - 282.

Moulton, J.E. (1986). Structural and functional changes in the lymphoid organs of cattle infected with *Trypanosoma congolense*. Tropical Veterinarian *4*: 97-106.

Morrison, W. I. ; Murray, M. and Akol, G. W. O. (1985). Immune response of cattle to African trypanosomes. Tizard, I. (Editor): Immunology and Pathogenesis of Trypanosomiasis. CRC Press Inc. Florida. Pp 103-131.

Murray, Max; Murray, P.K . and McIntyre,W.I.M. (1977).An improved parsitological technique for the diagnosis of African trypanosomiasis. Trans Royal Society of Tropical Medicine and Hygiene 7: 325-326.

Murray, M.; Morrison, W.I. and Whitelaw, D.D. (1982). Host susceptibility to African trypanosomiasis: Trypanotolerance. Advances in Parasitology *21*: 1-68.

Murray, M.; Trail, J.C.M. and Grootenhuis, J.G. (1984). Trypanotolerant livestock: Potential and future exploitation. Outlook on Agriculture. *13*: 43-51.

Mwangi, D.M. (1987). Immunosuppression in experimental *Typanosoma congolense* infection in goats: Studies using anthrax vaccine. M. Sc. thesis (University of Nairobi; Kenya).

Nantulya, V.M.; Musoke, A.J.; Rarangirwa, F.R. and Moloo, S.K. (1984). Resistance of cattle to tse tse transmitted challenge with *Trypanosoma brucei* or *Trypanosoma congolense* after spontaneous recovery from syringe passaged infections. Infection and Immunity *43*: 735 - 738.

Naylor, D.C. (1971 a). The haematology and histopathology of *Trypanosoma congolense* infection in cattle. Part 11: Haematology (Including symptoms).

Tropical Animal Health and Prodution *3*:159-168.

Naylor, D.C. (1971 b). The haematology and histopathology of *Trypanosoma congolense* infection in cattle. Part 111: Discussion and conclusions. Tropical Animal Health and Prodution *3*: 203-207.

Ngatia, T.A. and Kuria, J.K.N. (1991). Some diseases of donkeys in Kenya as seen during post-mortem examination. Bulletin of Animal Health and Prodution of Africa *39*: 237-240.

Obi and Anosa (1980) cited by Jenkins, G.C. and Facer, C.A. (1985). Haematology of African trypanosomiasis. Tizard, I. (Editor): *Immunology and Pathogenesis of Trypanosomiasis*. C.R.C. Press Inc.,
Florida (1985). Pp 13-44.

Olubayo, R.O.; Grootenhuis, J.G. and Rurangirwa, F.R. (1990). Susceptibility of African buffalo and Boran cattle to intravenous inoculation with *Trypanosoma congolense* bloodstream forms. Tropical Medicine and Parasitology *41*: 121-124.

Omuse, J.K. (1973). Pathogenesis of *Trypanosoma congolense* in cattle. M. Sc. thesis (University of Nairobi; Kenya).

Oppong, E.N.W. (1979). Diseases of horses and donkeys in Ghana (1909 - 1975). Bulletin of Animal Health and Prodution of Africa *27*: 47-49.

Pandey, V.S. and Esyker, M. (1989). *Strongylus vulgaris* in donkeys (*Equus asinus*) from the High-Veld of Zimbabwe. Veterinary Parasitology *32*: 173-179.

Pandey, V.S. and Esyker, M. (1990). Internal parasites of donkeys from the High-Veld of Zimbabwe. Zimbabwe Veterinary Journal *21*: 27-31.

Round, M.C. (1962). The helminth parasites of domesticated animals in Kenya. Journal of Helminthology *36*: 375-449.

Rose,M.A.; Round, M.C. and Beveridge, W.I.B. (1970). Influenza in horses and donkeys in Britain, 1969. Veterinary Record *86*: 768 - 769.

Rurangirwa, F.R.; Musoke, A.J.; Nantulya, V.M.; Nkonge, C.; Njuguna, L.; Mushi, E.Z.; Karstad, L. and Grootenhuis, J.G. (1986). Immune effector mechanisms involved in the control of parasitaemia in *Trypanosoma brucei* infected wildbeast (*Connochaetes taurinus*). Immunology *58*: 231-237.

Saror, D.I. (1979). Classification of the anaemia of bovine trypanosomiasis.

Veterinary Record *105*: 96-98.

Schalm, O.W. (1975). *Veterinary Haematology*. Schalm, O.W. (Editor) Lea and Febiger, United States of America (1975).

Sekoni, V.O; Njoku, C.O.; Kumi-Diaka, J. and Saror, D.I. (1990). Pathological changes in male genitalia of cattle infected with *Trypanosoma vivax* and *T. congolense*. British Veterinary Journal *146*: 175-180.

Soulsby, E.J.L. (1982). Protozoa: Phylum: Sarcomastigophora. Honigberg and Balmuth, 1963. Soulsby, E. J. L. (Editor): *Helminths, Arthropods and Protozoa of Domesticated Animals* . Balliere Tindall, Eastbourne (1982). Pp 514-555.

Soulsby, E. J. L. (1986). Foreword. Svendsen, E. D. (Compilor): *The Professional Handbook of the Donkey*. Sovereign Printing group, England. Pp 1V-V

Steel, R.G.D. and Torrie, J.H. (1980). Comparisons involving two sample means. Steel, R. G. D.and Torrie, J. H. (Editor): *Principles and Procedures of Statistics*: *A Biometrical Approach*. Mcgraw-hill Kogakusha Ltd, Tokyo (second edition, 1980). Pp 86-121.

Stephen, L.E. (1962). Experimental *T. congolense* infection in a horse. Veterinary Record *74*: 853-855.

Stephen, L.E. (1986). *Trypanosomiasis: A Veterinary perspective*. Stephen, L.E. (Editor) Pergamon Press, Oxford (1986).

Stephen, L.E. and Mackenzie, C.P. (1959). Experimental *Trypanosoma vivax* infection in the horse. Veterinary Record *71*: 527-531.

Stevenson, P. and Mwangi, E. (1991). The epidemiology of trypanosomiasis in domestic livestock constantly exposed to tse tse flies. *12th Annual Medical Scientific Conference of KEMRI and KETRI, 4th -8th February, 1991.*

Suliman, H.B. and Feldman, B.F. (1989). Pathogenesis and aetiology of anaemia in trypanosomiasis with special reference to *T. brucei* and *T. evansi.* Veterinary Bulletin *59*: 99-107.

Suryanarayana, C.; Gupta, S.L. and Singh, R.P. (1986). Studies on clinico-haematological changes in donkeys experimentally infected with *Trypanosoma evansi.* Indian Veterinary Journal 10:199-203.

Svendsen, E.D. (1986). Preface. Svendsen, E. D. (Compilor): *The Professional*

Handbook of the Donkey . Sovereign Printing group, England (1986). Pp 111.

Terrell, T.G. and Stookey, J.L. (1974). *Besnoitia benetti* in 2 Mexican burros. Veterinary Pathology *10*: 177 - 184.

Urquhart, G.M. and Holmes, P.H. (1987). African trypanosomiasis. Soulsby, E. J. L. (Editor): *Immune Response in Parasitic Infections*: *Immunology, immunopathology and Immunoprophylaxis. Protozoa*. CRC press Inc., Florida (1987). Vol.3. Pp 1 - 23.

Valli, V.E.O. and Forsberg, C.M. (1979). The pathogenesis of *Trypanosoma congolense* infection in calves. Quantitative histological changes. Veterinary Pathology *16*: 334-368.

Valli, V.E.O.; Forsberg, C.M. and Robinson, G.A. (1978 a). The pathogenesis of *Trypanosoma congolense* infection in calves. I. Clinical observations and gross pathological changes. Veterinary Pathology *15*: 608 - 620.

Valli, V.E.O.; Forsberg, C.M. and McSherry, B.J. (1978 b). The pathogenesis of *Trypanosoma congolense* infection in calves. 11. Anaemia and erythroid response. Veterinary Pathology *15*: 732 - 745.

Valli, V.E.O. and Mills, J.N. (1980). The quantitation of *Trypanosoma congolense* in calves. I. Haematological changes. Tropical Medicine and Parasitology. *31*: 215 - 231.

Valli, V.E.O.; Mills, J.N.; Lumsden, J.H.; Rattray, J.B. and Forsberg, C.M. (1980). The quantitation of *T. congolense* in calves. 11. Biochemical changes. Tropical Medicine and Parasitology. *31*: 288 - 298.

Webb, E.C. (1915). Trypanosomiasis of donkeys and mules in the Anglo-Egyptian Sudan. Journal of Comparative Pathology and Therapeutics *28*: 1 -20.

Wellde, B.; Lotzsch, R.; Deindl, G.; Sadum, E.; Williams, J. and Warui, G. (1974). *Trypanosoma congolense*. I. Clinical observations of experimentally infected cattle. Experimental Parasitology *36*: 6-19.

Whiteside, E. F. (1958). The control of animal trypanosomiasis in Kenya. Zoological section. Department of Veterinary Services in Kenya.

Wilding, J.L.; Kimbali, A.W.; Whitaker. M.W.; Trum, B.F. and Rust, J.H. (1952). Some blood values of the South western burro (*Equus asinus asinus*). American Journal of Veterinary Research *13*: 509 - 513.

Willett, K.C. (1970). Epizootiology of trypanosomiasis in livestock in East and Central Africa. Mulligan, H. W. (Editor): *The African Trypanosomiases*. George and Unwin Ltd, Great Britain (1970). Pp 766 - 773.

Wilson, B.J. and Maronpot, R.R. (1971). Causative fungus agent of leukoencephalomalacia in equine animals. Veterinary Record *88*: 484 - 486.

Zinkl, J.G.; Mae, D.; Merida, P.G.; Farver, T.B. and Humble, J.A. (1990). Reference ranges and the influence of age and sex on haematologic and serum biochemical values in donkeys (*Equus asinus*). American Journal Veterinary Research. *51*: 408 - 413.

APPENDICES

APPENDIX 1

TEMPERATURE CHANGES(°C) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TYRPANOSOSOMA CONGOLENSE.

Days p.i.	Infected Group		Control Group	
	mean	±sd (n=3)	mean	± sd(n=3)
-8	37.3	0.4	36.9	0.6
-6	37.0	0.7	37.4	0.4
-5	37.3	0.3	36.7	0.5
-2	36.8	0.3	37.0	0.2
0	36.5	0.1	36.8	0.4
1	36.7	0.2	37.3	0.6
2	36.5	0.2	36.4	0.1
3	36.1	0.1	36.5	0.4
4	36.6	0.5	36.4	0.2
5	36.2	0.3	36.3	0.2
6	37.1	0.5	36.3	0.4
7	36.9	0.3	37.3	0.3
8	37.2	0.0	37.1	0.2
9	36.3	0.3	36.9	0.2
10	36.7	0.3	36.4	0.2
11	37.3	0.3	36.4	0.1
12	37.5	0.3	37.1	0.2
13	37.1	0.0	37.3	0.2
14	36.9	0.2	37.1	0.1
15	36.5	0.2	37.0	0.2
16	37.0	0.4	36.8	0.1
17	36.9	0.3	37.2	0.1
18	36.8	0.3	36.8	0.1
19	36.9	0.2	36.5	0.1
20	37.2	0.4	36.6	0.1
21	36.4	0.3	36.8	0.2
22	36.9	0.1	37.1	0.2
23	36.8	0.2	36.8	0.1
24	36.6	0.4	36.8	0.1
25	36.5	0.2	36.6	0.1
26	37.0	0.4	36.4	0.2
27	36.9	0.1	36.7	0.4
28	37.1	0.1	36.9	0.1
29	37.4	0.1	36.9	0.1

TEMPERATURE CHANGES(°C) IN DONKEYS cont'd

30	36.3	0.1	37.1	0.1
31	36.9	0.4	36.2	0.1
32	36.4	0.3	36.7	0.4
33	36.8	0.4	36.7	0.3
34	36.6	0.2	36.5	0.4
35	36.6	0.2	36.4	0.2
36	36.4	0.3	36.4	0.4
37	36.6	0.4	36.4	0.2
40	36.6	0.4	36.5	0.3
41	36.6	0.4	36.6	0.5
42	36.9	0.1	36.8	0.2
43	37.4	0.1	37.2	0.7
44	37.6	0.1	37.4	0.3
45	37.1	0.2	37.0	0.3
47	36.7	0.5	36.7	0.2
48	36.9	0.3	36.4	0.1
49	36.6	0.2	36.4	0.1
50	36.7	0.3	37.2	0.1
51	37.0	0.2	36.9	0.1
54	37.3	0.8	37.1	0.2
55	36.7	0.4	37.0	0.1
56	36.8	0.2	36.8	0.1
57	36.9	0.2	36.9	0.2
58	37.0	0.0	37.1	0.2
61	37.6	0.3	37.0	0.4
62	37.3	0.2	37.0	0.3
63	37.0	0.4	36.9	0.1
64	36.5	0.3	36.9	0.4
65	36.6	0.2	36.7	0.2
68	37.2	0.3	37.2	0.2
69	37.0	0.5	37.0	0.2
70	36.9	0.3	37.0	0.1
71	36.8	0.1	36.7	0.1
72	37.5	0.3	37.0	0.1
75	37.9	0.1	37.2	0.1
76	37.1	0.2	36.6	0.2
77	36.8	0.6	36.9	0.5
79	36.8	0.2	37.0	0.2
81	37.2	0.2	36.5	0.2
82	36.8	0.2	37.0	0.3
83	37.0	0.2	37.4	0.2

APPENDIX 2

HEART RATE CHANGES(BEATS/MINUTE) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE.

Days p.i.	Infected group		Control group	
	mean ±	sd(n=3)	mean ±	sd(n=3)
-8	32.7	0.9	30.7	2.5
-6	31.3	1.9	32.0	0.0
-5	30.7	0.9	29.3	0.9
-2	34.0	1.6	31.3	3.4
0	32.0	1.6	31.3	1.6
1	33.0	2.4	32.0	2.5
2	33.3	1.9	30.0	2.8
3	38.3	3.9	32.6	3.3
4	30.3	2.1	32.0	1.9
5	37.3	5.0	32.0	3.3
6	30.3	4.0	29.3	2.5
7	34.7	3.4	32.0	2.1
8	33.0	2.4	32.6	2.4
9	32.7	2.1	30.3	2.1
10	31.3	3.4	30.0	3.7
11	33.0	2.4	30.0	1.4
12	34.0	2.8	30.3	2.4
13	37.3	1.2	32.0	0.9
14	39.7	7.9	30.0	2.1
15	34.0	2.8	30.6	0.9
16	36.7	3.4	30.3	0.9
17	33.3	1.9	30.6	2.1
18	31.0	2.2	29.3	3.7
19	33.3	5.0	29.3	0.9
20	35.3	5.3	30.3	2.1
21	31.3	3.4	32.0	3.7
22	28.7	1.9	32.3	3.3
23	30.7	2.6	30.3	2.1
24	30.0	2.4	28.7	0.9
25	30.0	2.1	30.0	2.4
26	31.3	3.4	28.3	1.2
27	37.0	2.8	32.3	3.3
28	40.3	3.7	33.0	2.4
29	35.0	6.2	35.0	6.2
30	43.0	9.9	37.0	6.2
31	40.0	9.3	32.3	3.3

HEART RATE CHANGES (BEATS/MINUTE) IN THE DONKEYS cont'd

32	35.0	1.4	32.7	4.9
33	34.0	3.7	30.3	2.1
34	31.0	3.7	28.7	0.9
35	30.3	2.1	29.0	5.1
36	32.3	4.8	31.3	2.4
37	28.3	1.2	27.3	0.5
40	29.0	1.4	30.0	2.4
41	31.3	2.4	31.0	3.7
42	40.0	2.8	33.0	4.2
43	40.0	6.2	37.0	9.9
44	47.0	2.4	37.0	1.4
45	33.0	1.4	31.0	3.7
47	37.0	3.7	28.0	1.4
48	38.0	2.4	30.0	0.0
49	33.0	3.7	39.0	8.5
50	37.0	2.1	34.0	5.7
51	42.3	5.0	33.0	2.4
54	41.3	1.9	33.3	1.9
55	41.3	3.8	32.0	1.4
56	41.3	5.0	33.3	1.9
57	38.7	3.8	33.3	1.9
58	46.7	3.8	41.3	1.9
61	49.3	8.6	37.3	6.8
62	44.0	3.3	37.3	5.0
63	40.0	3.3	41.0	1.9
64	40.0	1.9	34.5	5.1
65	42.7	1.9	40.0	1.9
68	41.3	3.3	38.7	6.5
69	44.0	3.8	36.0	3.8
70	42.7	1.9	40.0	3.3
71	45.3	3.8	40.0	3.3
72	50.7	1.9	36.0	3.3
75	54.7	3.3	44.0	5.7
76	52.0	3.8	37.3	1.9
77	49.3	3.8	36.0	0.0
79	42.7	1.9	36.0	3.3
81	41.3	1.9	36.0	3.3
82	45.3	3.3	37.3	1.9
83	44.0	1.9	41.3	3.8

APPENDIX 3

RESPIRATORY RATE CHANGES (CYCLES/MINUTE) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group		Control group	
	mean ±	sd(n=3)	mean ±	sd(n=3)
-8	20.7	0.9	22.7	0.9
-6	20.7	0.9	20.7	2.5
-5	18.0	0.0	19.3	0.9
-2	18.7	0.9	19.3	1.9
0	20.0	1.6	19.3	0.9
1	18.0	0.0	18.0	0.0
2	18.0	0.0	18.0	0.0
3	17.3	0.9	18.0	0.0
4	19.0	1.0	18.0	0.0
5	20.0	0.0	18.0	0.0
6	18.0	0.0	18.7	0.9
7	19.0	1.0	18.0	0.0
8	18.0	0.0	18.0	0.0
9	18.0	0.0	18.0	0.0
10	18.0	0.0	18.0	0.0
11	18.0	0.0	18.0	0.0
12	18.0	0.0	18.0	0.0
13	18.7	0.9	18.0	0.0
14	18.0	0.0	18.0	0.0
15	18.0	0.0	18.0	0.0
16	19.3	0.9	18.0	0.0
17	18.0	0.0	18.7	0.9
18	18.7	0.9	18.0	0.0
19	18.0	0.0	18.0	0.0
20	18.7	0.9	18.7	0.9
21	18.0	0.0	18.0	0.0
22	18.7	0.9	18.0	0.0
23	18.7	0.9	18.0	0.0
24	18.0	0.0	18.0	0.0
25	18.0	0.0	18.0	0.0
26	18.0	0.0	18.0	0.0
27	18.7	0.9	18.0	0.0
28	18.0	0.0	18.0	0.0
29	18.7	0.9	18.0	0.0
30	18.0	0.0	18.0	0.0
31	19.3	0.9	18.0	0.0

RESPIRATORY RATE CHANGES (CYCLES/MINUTE) IN DONKEYS cont'd

32	18.0	0.0	19.0	1.4
33	18.0	0.0	18.0	0.0
34	18.0	0.0	18.0	0.0
35	19.0	1.4	18.7	0.9
36	18.0	0.0	18.0	0.0
37	18.0	0.0	18.0	0.0
40	18.0	0.0	18.0	0.0
41	18.0	0.0	18.0	0.0
42	18.0	0.0	20.0	2.8
43	18.0	0.0	18.0	0.0
44	18.0	0.0	18.0	0.0
45	18.0	0.0	18.0	0.0
47	18.0	0.0	18.0	0.0
48	18.0	0.0	18.0	0.0
49	18.0	0.0	18.0	0.0
50	18.0	0.0	18.0	0.0
51	18.0	0.0	18.0	0.0
54	19.3	0.9	19.0	1.4
55	17.3	0.9	18.0	0.0
56	18.7	0.9	18.0	0.0
57	18.0	1.6	18.0	0.0
58	20.7	2.5	18.0	0.0
61	22.7	1.9	19.3	0.9
62	20.0	2.8	19.3	0.9
63	20.0	0.0	18.7	0.9
64	19.3	0.9	18.0	0.0
65	18.7	0.9	18.0	0.0
68	19.3	0.9	18.0	0.0
69	19.3	0.9	18.7	0.9
70	19.3	0.9	18.7	0.9
71	20.0	0.0	19.3	0.9
72	20.0	1.6	18.7	0.9
75	22.7	1.9	18.7	0.9
76	19.3	0.9	18.7	0.9
77	19.3	0.9	18.0	0.0
79	18.7	0.9	18.0	0.0
81	18.7	0.9	18.0	0.0
82	18.0	1.6	19.3	0.9
83	22.7	1.6	18.0	0.0

APPENDIX 4

MICRO-HEAMATOCRIT[1] (PACKED CELL VOLUME[2]) CHANGES (%) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH T. CONGOLENSE.

Days p.i.	Infected group		Control group	
	mean ±	sd(n=3)	mean ±	sd(n=3)
-37	32.7/36.8	5.3/5.8	33.7/33.7	0.9/1.4
-35	33.0/40.0	6.5/9.8	32.3/32.3	1.2/1.4
-14	32.7/38.8	5.9/7.1	34.3/34.3	3.4/1.2
-9	33.7/43.6	3.1/8.0	34.0/34.0	2.8/5.2
-6	33.3/42.2	4.9/4.6	33.5/33.5	0.5/1.8
-2	33.0/39.5	4.3/5.1	33.3/33.3	1.9/3.4
1	32.3/37.5	5.6/6.8	32.3/32.3	3.7/7.0
5	29.7/36.5	3.9/4.4	32.7/32.0	1.4/1.9
12	28.3/32.9	3.3/2.2	30.7/30.7	0.8/3.3
15	31.3/36.7	2.5/4.1	32.0/32.0	3.3/3.3
19	31.3/35.5	1.7/3.0	31.3/31.3	0.8/6.0
22	30.0/33.6	0.8/1.3	36.6/35.6	2.9/1.2
26	28.7/33.2	0.9/1.3	36.1/34.9	0.8/2.7
29	30.0/34.7	1.4/2.9	31.3/33.1	2.6/1.3
33	30.0/33.8	1.4/0.5	35.6/30.2	2.1/3.8
36	25.7/32.4	0.9/0.6	34.9/31.3	3.1/2.4
40	26.0/31.4	0.8/0.8	33.1/31.2	1.9/1.5
43	24.3/28.3	0.9/1.2	30.2/28.9	3.1/2.2
47	25.7/26.1	3.3/2.1	31.3/28.9	0.8/3.4
50	22.0/25.6	2.2/2.2	31.2/28.0	0.8/1.2
61	22.0/25.6	2.2/3.1	28.9/32.6	1.4/0.8
64	20.7/23.4	4.0/3.2	28.9/29.6	2.1/3.2
68	24.3/24.1	1.9/1.1	28.0/29.6	0.5/2.5
71	22.3/23.8	0.9/0.5	32.6/28.9	2.4/2.0
75	21.0/25.6	0.8/0.3	29.6/32.5	2.1/6.8
78	19.0/24.1	1.6/2.8	29.5/31.0	2.1/2.6
82	19.3/24.4	1.2/1.2	28.9/28.9	2.7/1.4
85	21.1/20.8	4.1/1.8	32.5/32.5	1.2/2.7

[1] Micro-heamatocrit or centrifugally derived PCV.
[2] PCV obtained from the computer reading.

APPENDIX 5

RED BLOOD CELL COUNT CHANGES (X 10^6/ML) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	6.00		1.36	6.45		0.46
-35	6.23		1.64	6.43		1.07
-14	6.40		2.00	6.28		0.51
-9	6.65		1.12	7.03		0.52
-6	6.36		1.00	6.41		1.26
-2	6.30		1.10	6.60		0.44
1	6.11		1.44	6.26		0.31
5	5.89		1.08	6.34		0.27
12	5.48		0.68	6.50		0.00
15	5.12		0.79	5.43		0.40
19	5.82		0.68	6.35		0.47
22	5.64		0.55	6.26		0.75
26	5.54		0.42	5.99		0.59
29	5.81		0.60	5.41		0.45
33	5.35		0.42	5.90		0.49
36	5.33		0.29	5.88		0.68
40	4.86		0.17	5.42		0.08
43	4.62		0.26	5.12		0.11
47	4.31		0.25	5.23		0.45
50	3.80		0.22	4.68		0.33
61	3.82		0.35	4.61		0.52
64	3.27		0.37	4.18		1.26
68	3.49		0.11	4.42		0.45
71	3.51		0.03	5.27		0.45
75	3.72		0.11	4.51		0.45
78	3.45		0.44	4.53		0.35
82	3.45		0.19	4.40		0.52
85	3.04		0.36	4.78		0.92

APPENDIX 6

HAEMOGLOBIN CONCENTRATION CHANGES(MG%) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	11.9		1.9	12.4		0.4
-35	12.6		2.7	12.4		0.2
-14	13.7		2.7	12.2		0.4
-9	13.5		2.0	12.9		1.1
-6	13.3		1.9	12.7		0.1
-2	13.4		1.8	12.6		1.5
1	12.2		2.2	12.7		1.8
5	11.7		1.5	12.4		0.7
12	10.5		1.0	12.0		0.5
15	11.5		1.1	12.0		0.9
19	11.2		0.9	11.8		0.3
22	10.5		0.8	11.5		0.7
26	10.4		0.5	11.6		0.1
29	10.5		0.9	10.5		1.3
33	9.9		0.1	11.2		0.7
36	9.4		0.5	10.7		0.8
40	9.1		0.2	10.0		0.5
43	8.6		0.5	9.8		0.8
47	8.0		0.8	10.1		0.4
50	8.8		0.5	10.2		0.2
61	8.2		0.9	10.1		0.7
64	8.0		0.7	9.9		0.3
68	8.1		0.3	10.0		0.5
71	8.1		0.3	9.7		0.6
75	7.6		0.3	9.3		0.6
78	7.8		0.6	9.4		0.7
82	7.1		0.7	9.0		0.7
85	9.1		0.6	10.1		0.4

APPENDIX 7

MEAN CORPUSCULAR VOLUME CHANGES(FL) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	66.3		3.9	62.0		1.6
-35	68.7		0.9	62.0		2.2
-14	65.7		3.7	62.3		2.1
-9	67.7		2.5	65.0		2.9
-6	67.0		4.1	63.5		1.5
-2	67.3		2.9	62.7		2.1
1	66.7		4.1	63.0		2.4
5	66.7		2.8	63.7		2.5
12	64.0		5.4	62.7		2.6
15	67.7		1.6	68.7		7.6
19	65.0		3.4	64.0		2.2
22	63.7		2.9	62.3		1.2
26	63.3		3.3	64.0		4.3
29	63.3		3.7	61.0		0.8
33	66.3		2.9	64.0		1.6
36	64.0		3.8	62.7		2.1
40	67.3		2.9	64.0		1.6
43	65.0		1.7	62.7		1.2
46	63.7		1.7	63.0		2.8
50	64.3		2.9	62.7		1.2
61	69.3		2.5	65.7		1.9
64	68.7		0.9	65.3		0.5
68	71.7		1.6	66.7		3.1
71	71.0		0.5	66.0		2.2
75	71.0		1.2	68.7		1.2
78	70.2		1.7	68.0		1.2
82	72.3		2.4	68.3		1.7
85	71.7		8.0	64.0		6.0

APPENDIX 8

MEAN CORPUSCULAR HAEMOGLOBIN CHANGES(PG) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	20.15		1.41	19.23		0.68
-35	20.46		0.92	19.28		0.97
-14	19.83		1.01	19.42		0.78
-9	20.38		0.55	18.68		2.13
-6	20.91		0.26	19.87		1.66
-2	21.40		1.79	19.01		1.22
1	20.18		1.47	20.64		1.72
5	19.99		0.98	19.54		0.50
12	19.29		2.75	18.43		0.39
15	22.69		0.65	22.08		1.71
19	19.27		2.85	18.59		0.45
22	18.72		0.73	18.38		0.15
26	18.77		0.54	19.51		1.33
29	18.21		1.12	19.43		0.37
33	19.74		1.35	19.00		0.72
36	18.61		1.31	18.26		0.11
40	19.27		0.89	18.47		0.74
43	19.62		0.47	19.22		1.21
46	20.01		0.45	19.32		0.82
50	21.16		0.93	21.73		0.79
61	23.11		0.55	21.98		0.73
64	25.25		0.60	23.86		1.52
68	22.81		0.66	23.03		1.92
71	23.01		0.45	19.24		3.44
75	21.66		0.89	20.71		1.01
78	21.08		0.09	20.87		0.77
82	21.48		1.26	20.67		1.04
85	23.58		0.81	19.20		1.58

APPENDIX 9

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION CHANGES (MCHC%) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	32.29		0.36	33.19		0.30
-35	31.78		1.15	33.42		0.70
-14	33.44		0.01	33.36		0.11
-9	31.25		2.29	30.72		2.26
-6	33.15		1.58	33.55		1.73
-2	33.80		1.03	32.30		1.24
1	32.16		1.08	34.70		1.74
5	31.98		1.04	32.77		0.56
12	31.93		0.94	31.40		1.93
15	31.33		1.65	30.69		5.60
19	31.44		0.14	31.14		0.50
22	31.31		1.40	31.42		0.43
26	31.22		0.39	32.26		0.90
29	30.53		3.08	33.52		0.30
33	31.07		0.19	31.38		0.11
36	30.54		1.11	30.75		1.05
40	29.86		1.35	30.21		0.47
43	32.04		0.80	32.49		1.22
47	33.07		0.66	32.42		0.57
50	31.49		0.88	32.63		0.74
61	34.65		0.85	35.15		1.52
64	35.45		2.07	34.58		2.45
68	33.04		0.83	35.86		0.98
71	33.89		0.50	30.70		4.49
75	31.47		0.71	31.54		1.54
78	31.49		1.30	31.92		1.20
82	31.77		1.75	31.29		0.92
85	33.89		1.28	31.12		0.13

APPENDIX 10

TOTAL LEUKOCYTE COUNTS(X 10^3/ML) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	16.1		1.9	11.1		4.0
-35	15.1		1.5	12.8		2.9
-14	13.1		0.2	13.7		2.1
-9	16.1		0.8	15.8		2.9
-6	14.3		0.9	15.3		2.8
-2	14.6		1.6	16.1		1.8
1	16.0		2.0	12.6		1.9
5	11.6		1.1	12.4		1.8
12	11.8		0.6	12.5		0.9
15	10.1		2.3	8.1		1.7
19	12.3		1.6	11.1		2.0
22	11.7		1.6	10.6		1.7
26	10.4		1.2	11.1		1.6
29	10.8		1.4	9.6		0.2
33	12.3		1.1	10.4		2.0
36	12.6		0.2	9.6		1.0
40	12.3		0.5	9.0		1.5
43	12.3		0.5	8.2		0.7
47	11.6		1.1	7.6		1.2
50	12.9		1.1	10.4		1.5
61	9.8		0.8	7.7		0.5
64	11.5		2.0	7.2		0.2
68	9.6		1.7	6.9		0.5
71	10.9		1.9	7.8		0.5
75	13.6		1.1	8.1		0.0
78	13.3		1.5	8.8		0.8
82	13.9		1.7	8.9		0.8
85	11.0		0.6	14.9		4.5

APPENDIX 11

NEUTROPHIL COUNT(%) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	36.3		7.4	40.3		7.6
-35	26.3		2.1	39.0		8.0
-14	37.0		10.6	49.0		2.9
-9	44.3		5.0	41.7		10.3
-6	43.0		3.6	42.0		1.0
-2	47.7		12.7	50.0		5.7
1	41.3		11.9	47.3		4.7
5	42.3		7.6	51.3		11.1
12	53.0		18.5	40.0		2.9
15	34.3		9.1	38.3		7.5
19	36.3		17.6	41.3		1.9
22	34.0		6.7	41.7		8.3
26	31.3		4.5	45.7		6.2
29	28.3		6.2	44.5		2.5
33	31.7		10.7	48.7		3.4
36	26.0		4.2	41.3		4.8
40	29.7		7.8	37.3		10.1
43	43.0		4.3	40.3		2.4
47	27.0		5.9	36.3		16.2
50	27.7		4.7	43.7		2.1
61	33.7		0.9	43.0		7.1
65	19.7		2.1	39.7		1.7
68	28.7		2.5	35.0		2.2
71	32.0		11.3	33.7		0.9
75	35.7		5.3	37.3		3.1
78	20.0		6.5	33.3		3.7
82	28.7		7.9	39.7		7.4
85	15.0		2.9	36.0		11.0

APPENDIX 12

LYMPHOCYTE COUNT CHANGES IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TYRPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	60.7		8.6	51.3		3.4
-35	70.3		2.4	55.0		6.4
-14	58.7		12.8	41.3		4.2
-9	53.0		5.0	51.0		8.6
-6	53.3		4.5	50.5		2.5
-2	47.3		10.5	46.0		5.1
1	52.7		14.1	49.0		5.9
5	50.0		6.4	45.3		3.7
12	44.0		17.6	54.7		6.3
15	62.3		9.2	59.0		7.8
19	56.0		18.1	54.3		2.6
22	59.3		4.2	52.7		10.8
26	62.0		5.1	50.3		5.4
29	64.7		11.4	52.0		3.0
33	63.7		12.8	50.3		3.4
36	65.3		6.0	54.3		3.7
40	66.7		9.4	51.0		9.4
43	49.7		3.9	56.0		2.2
47	68.7		6.5	57.7		14.4
50	62.3		1.7	54.3		2.1
61	59.7		4.0	51.0		5.7
65	67.7		4.9	51.3		3.1
68	59.7		2.6	49.7		5.3
71	56.0		13.4	49.0		8.6
75	55.3		9.0	52.7		0.9
78	70.0		5.0	57.3		5.6
82	63.3		8.6	50.0		4.3
85	80.7		3.8	53.0		12.0

APPENDIX 13

MONOCYTE COUNT CHANGES(%) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	1.0		0.8	2.3		0.9
-35	0.0		0.0	0.0		0.0
-14	1.3		1.9	3.3		3.3
-9	1.0		1.4	1.0		1.4
-6	0.3		0.5	1.0		1.0
-2	2.0		2.2	0.7		0.5
1	3.7		1.9	1.0		0.8
5	0.3		0.5	0.0		0.0
12	1.0		0.8	1.3		0.5
15	0.0		0.0	0.0		0.0
19	0.3		0.5	0.3		0.5
22	5.0		0.8	2.0		1.4
26	1.0		1.4	0.0		0.0
29	2.0		0.0	1.0		0.8
33	4.0		3.6	0.0		0.0
36	1.7		1.2	0.0		0.0
40	8.0		4.2	2.3		2.1
43	2.7		1.2	4.7		2.4
47	3.7		0.5	1.7		1.2
50	4.0		1.6	4.7		3.3
61	5.0		2.2	1.0		0.0
65	2.7		0.9	2.0		2.2
68	6.7		2.9	2.3		0.9
71	5.7		1.2	4.7		1.7
75	5.3		0.9	2.0		1.4
78	3.3		2.4	2.0		2.8
82	8.3		2.6	0.0		0.0
85	3.3		1.2	3.0		1.4

APPENDIX 14

EOSINOPHIL COUNT CHANGES(%) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	1.0		0.8	5.3		3.2
-35	3.3		0.9	6.0		2.2
-14	3.0		0.0	6.3		2.1
-9	1.7		1.2	6.3		4.8
-6	3.3		2.5	6.5		0.5
-2	3.0		0.8	3.3		1.7
1	2.3		1.7	2.7		2.1
5	0.7		0.9	1.7		0.5
12	3.0		1.6	4.6		2.5
15	2.7		1.2	2.3		0.9
19	2.3		0.9	2.3		1.2
22	5.0		3.3	5.3		2.3
26	1.7		0.5	3.0		0.8
29	3.3		2.5	3.5		0.5
33	3.0		1.4	1.0		0.0
36	1.0		1.4	2.0		0.0
40	1.0		0.8	1.0		0.8
43	3.3		2.6	2.0		0.8
47	0.7		0.5	1.0		0.8
50	1.0		0.8	1.0		0.0
61	4.0		3.6	4.0		2.2
64	5.3		1.7	6.3		5.4
68	6.0		1.6	10.3		4.0
71	6.7		3.1	13.0		5.7
75	5.7		1.7	10.6		5.1
78	1.7		0.5	12.3		5.0
82	4.7		1.7	7.3		2.6
85	2.7		0.9	10.0		0.0

APPENDIX 15

ICTERIC INDICES IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	2-5		0.0	2-5		0.0
-35	2-5		0.0	2-5		0.0
-14	2-5		0.0	2-5		0.0
-9	2-5		0.0	2-5		0.0
-6	2-5		0.0	2-5		0.0
-2	2-5		0.0	2-5		0.0
1	2-5		1.9	5-10		1.9
5	5-10		1.9	2-5		0.0
12	2-5		0.0	2-5		0.0
15	10-15		0.0	2-5		0.0
19	5-10		4.7	2-5		1.9
22	5-10		0.0	2-5		1.9
26	2-5		1.9	2-5		0.0
29	5-10		1.9	2-5		1.9
33	5-10		1.9	2-5		1.9
36	2-5		1.9	2-5		1.9
40	2-5		1.9	2-5		1.9
43	2-5		1.9	5-10		1.9
47	5-10		1.9	2-5		1.9
50	2-5		0.0	2-5		1.9
61	2-5		1.9	2-5		1.9
65	5-10		1.9	5-10		0.0
68	2-5		0.0	2-5		0.0
71	2-5		0.0	2-5		1.9
75	2-5		1.9	2-5		1.9
78	2-5		1.9	2-5		1.9
82	2-5		1.9	5-10		4.1
85	5-10		1.9	10-15		9.5

APPENDIX 16

TOTAL PLASMA PROTEIN CHANGES(GM%) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-37	9.6		0.2	8.2		0.3
-35	9.9		0.4	8.3		0.8
-14	9.3		0.2	8.5		0.4
-9	9.6		0.4	8.7		0.6
-6	9.4		0.4	8.2		0.2
-2	9.3		0.1	8.8		0.0
1	8.9		0.4	8.5		0.1
5	9.5		0.2	8.7		0.2
12	8.9		0.1	8.0		0.3
15	8.6		0.4	8.2		0.4
19	8.9		0.4	7.9		0.3
22	8.7		0.4	8.1		0.1
26	8.5		0.2	8.0		0.0
29	8.3		0.4	7.6		0.4
33	6.7		0.3	7.9		0.2
36	8.1		0.5	5.9		0.1
40	7.9		0.4	7.3		0.1
43	8.1		0.3	7.1		0.1
47	7.9		0.2	6.7		0.6
50	7.3		0.5	7.0		0.6
61	7.6		0.3	7.0		0.2
64	7.9		0.3	7.4		0.0
68	8.2		0.4	7.9		0.1
71	8.1		0.5	8.1		0.4
75	8.4		0.4	8.0		0.2
78	8.3		0.8	8.1		0.2
82	7.6		0.6	8.1		0.1
85	7.9		0.8	8.1		0.1

APPENDIX 17

FIBRINOGEN CONTENT CHANGES(GM%) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
43	0.4		0.0	0.5		0.1
47	0.3		0.0	0.3		0.1
50	0.3		0.1	0.3		0.1
54	0.4		0.0	0.4		0.1
61	0.3		0.1	0.2		0.0
64	0.4		0.0	0.4		0.0
68	0.3		0.1	0.3		0.0
71	0.4		0.0	0.3		0.1
75	0.4		0.0	0.3		0.1
78	0.3		0.1	0.4		0.0
82	0.3		0.1	0.3		0.1
85	0.3		0.1	0.2		0.0

APPENDIX 18

TOTAL BILIRUBIN CHANGES(MG%) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-14	0.3		0.1	0.2		0.0
-9	0.3		0.1	0.3		0.1
-6	0.3		0.1	0.5		0.1
5	0.5		0.0	0.2		0.2
19	0.3		0.1	0.3		0.2
33	0.4		0.0	0.3		0.2
47	0.6		0.1	0.5		0.2
61	0.4		0.0	0.1		0.0
75	0.8		0.0	0.3		0.1

APPENDIX 19

BLOOD UREA NITROGEN(MG%) IN CONTROL DONKEYS AND DONKEYS EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	Infected group			Control group		
	mean	±	sd(n=3)	mean	±	sd(n=3)
-14	25.3		0.9	36.0		11.3
-9	26.0		2.9	34.7		8.3
-6	39.5		6.5	46.5		1.5
5	29.3		4.0	33.7		8.7
19	34.7		4.9	62.0		14.4
33	23.7		4.0	35.0		17.7
47	57.7		25.3	37.0		12.4
61	26.7		2.5	29.3		4.1
75	36.3		3.3	30.7		6.1

APPENDIX 20

PACKED CELL VOLUME CHANGES(%) IN SHEEP EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	mean ±	sd(n=3)
-2	32.7	3.9
-1	35.0	2.8
0	35.3	2.1
3	35.3	1.7
6	35.0	0.8
10	35.5	0.5
13	34.3	1.2
17	32.7	2.1
20	34.0	1.6
25	33.3	4.1
28	32.3	3.3
32	29.0	5.0
34	34.0	3.6
38	30.0	4.5
41	28.3	3.9
45	30.0	3.7
48	28.0	4.5
53	27.3	3.8
55	27.3	3.3
59	28.3	2.5
61	28.3	3.3

APPENDIX 21

RED BLOOD CELL COUNT CHANGES(X 10^6/ML) IN SHEEP EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	mean ±	sd(n=3)
-2	10.85	1.82
-1	10.50	0.78
0	10.00	0.50
3	11.43	0.74
6	9.01	6.89
10	12.83	0.43
13	16.08	2.79
17	16.87	0.18
20	13.60	0.78
25	9.90	0.85
28	11.08	1.50
32	9.25	1.10
34	13.45	1.30
38	13.82	3.39
41	9.42	3.23
45	15.48	6.96
48	9.07	1.85
53	11.93	1.39
55	16.17	2.80
59	15.25	2.99
61	15.87	5.60

APPENDIX 22

HAEMOGLOBIN CONCENTRATION CHANGES(GM%) IN SHEEP EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	mean ±	sd(n=3)
-2	12.3	1.3
-1	14.6	0.6
0	13.1	0.4
3	13.0	1.3
6	14.3	0.1
10	14.6	0.1
13	14.1	0.4
17	12.7	0.5
20	13.9	1.0
25	11.4	0.4
28	10.8	1.9
32	11.6	2.1
34	14.0	1.3
38	11.9	1.4
41	11.9	1.5
45	12.5	1.6
48	11.8	1.8
53	11.6	1.6
55	11.3	1.5
59	10.0	0.7
61	11.9	1.8

APPENDIX 23

EAN CORPUSCULAR VOLUME(FL) IN SHEEP EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	mean ±	sd(n=3)
-2	29.8	0.2
-1	30.2	1.0
0	33.2	0.9
3	30.2	1.7
6	30.8	0.8
10	29.8	0.3
13	30.5	0.4
17	29.3	0.2
20	30.7	0.5
25	30.0	1.4
28	31.2	0.2
32	29.3	0.8
38	30.7	1.4
41	30.8	11.2
45	30.5	0.7
48	31.2	0.6
53	30.5	0.4
55	31.0	0.8
59	30.8	1.7
61	30.7	0.5

APPENDIX 24

MEAN CORPUSCULAR HAEMOGLOBIN(PG) IN SHEEP EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	mean ±	sd(n=3)
-2	11.53	1.35
-1	13.94	0.48
0	13.07	0.31
3	11.78	0.86
6	25.41	12.54
10	11.21	0.27
13	9.02	1.48
17	7.55	0.40
20	10.75	0.85
25	11.56	0.53
28	9.87	1.85
32	12.40	0.74
38	10.60	1.89
41	8.93	1.28
45	13.94	4.59
48	9.51	3.20
53	14.26	1.45
55	9.47	0.32
59	7.27	1.91
61	8.06	1.59

APPENDIX 25

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION CHANGES (GM%) IN SHEEP EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CONGOLENSE

Days p.i.	mean ±	sd(n=3)
-2	37.70	0.42
-1	41.85	1.67
0	38.38	0.10
3	36.62	2.04
6	38.47	3.48
10	41.00	0.72
13	41.08	0.38
17	39.05	1.44
20	40.75	0.97
25	34.71	4.28
28	33.24	2.79
32	39.79	0.21
38	41.24	0.63
41	39.99	3.05
45	41.97	0.98
48	45.68	2.73
53	42.42	0.69
55	37.72	3.23
59	35.73	5.16
61	41.82	1.53