CLINICAL MANIFESTATION OF EXPERIMENTAL *TRYPANOSOMA EVANSI* INFECTION IN THE DROMEDARY CAMEL AND THE EFFECT OF TREATMENT ON HAEMATOLOGICAL, BIOCHEMICAL AND SEROLOGICAL VALUES.

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A THESIS

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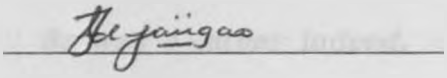
DECLARATION.

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



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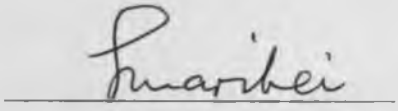

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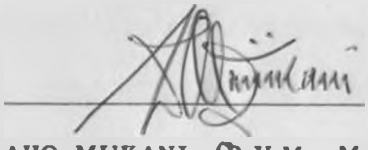

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DEDICATED TO:

This Thesis is dedicated to my late

Father, Peter M. Nyang'ao.

He was a father indeed.

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LIST OF ABBREVIATIONS.

KETRI.....Kenya Trypanosomiasis Research Institute.

PCV.....Packed Cell Volume

BCE.....Buffy coat examination

RBC.....Red blood cells

WBC.....White blood cells

Hb.....Haemoglobin concentration

DLC.....Differential leucocyte count

AP.....Alkaline phosphatase

AST.....Aspartate aminotransferase

ALT.....Alanine aminotransferase

CK.....Creatine kinase

LDH.....Lactic dehydrogenase

TP.....Total proteins

ALB.....Albumin

GLB.....Globulin

BUN.....Blood urea nitrogen

PBS.....Phosphate buffer saline

PSG.....Phosphate saline glucose buffer

ESG.....EDTA saline glucose buffer

EDTA.....Ethylene diamine tetraacetic acid (disodium salt)

DEAE.....Diethylaminoethyl

ELISA.....Enzyme-linked immunosorbent assay

RATE.....Rabbit anti-*T.evansi*

ABSTRACT:

Clinical manifestation of experimental *T.evansi* infection in the dromedary camel and the effect of treatment on haematological, biochemical and serological values.

With the growing awareness of the unique role the camel plays in agriculture and environmental preservation, increasing attention is being focused on improving its productivity and health status, so as to improve and diversify the production capacity of the marginal lands. Information on individual parameters of the camel's haematological and biochemical values is largely inadequate. Because the haematological and biochemical profiles can be useful aids in diagnosis of certain diseases, the norm for these parameters need to be established. *Trypanosoma evansi (T. evansi)* is one of the most economically important diseases of the camel and there is need to study this disease in detail. This study was carried out with the aim of elucidating some of the haematological and biochemical parameters in normal and *T.evansi* infected camels.

Haematological and biochemical values in healthy dromedary camels were determined in eleven camels for a period of 4 weeks. These values correlated well with those established in other countries.

Nine camels were artificially infected with *T. evansi* isolated from a naturally infected camel and passaged through mice. Two camels were used as non-infected controls. Four camels (Group A) were allowed to run with disease throughout the study period, while 5 camels (Group B) were treated with Cymelarsan at the recommended therapeutic dose of 0.25mg/kg body weight by deep intramuscular injection at the onset of the clinical disease. Three out of 4 camels in group A died of the disease, while all camels in group B survived to the end of the

experiment. The clinical signs manifested by the infected camels included inappetance, dullness, enlarged superficial lymph nodes, excessive bilateral lacrimation, ventral oedema, poor hair coat and emaciation. Pyrexia coincided or closely followed parasitaemia crises. Post mortem examination revealed excessive peritoneal and pericardial fluids, haemorrhages in the gastrointestinal tract (especially in the abomasum and small intestines), froth in trachea and lungs, congested meninges and kidneys and oedematous brain.

Following infection PCV, RBC and HB levels dropped rapidly in the first 3 weeks in all the infected animals. In group A camels, PCV, RBC and Hb levels continued to decline until they died or the study was terminated, while in the treated camels these parameters levelled off 4 weeks after treatment and started recovering. There was an increase in some enzyme activity notably AP, AST, CK and LDH after infection even though there was no statistical significant difference in some of the enzyme changes when compared to the non-infected controls.

Parasites disappeared from peripheral circulation within 24 hours after treatment and were no longer detectable to the end of the study. Circulating antigens which were detected 2 weeks after infection were cleared within 10 weeks following treatment. In the 4 non-treated camels, antigens remained detectable throughout the study period.

Thus, in this study baseline data of some haematological and biochemical values of the Kenya dromedary has been established. A comprehensive clinical picture of *T. evansi* infection in the dromedary camel has been recorded. The study has further demonstrated that *T.evansi* infection in camels is fatal, especially when no therapeutic intervention occurs and that the disease can manifest itself as an acute

syndrome with camels dying within the first few weeks of infection. The disease causes changes in haematological and biochemical values. Cymelarsan, as used in this study, elicits complete cure in camels infected with *T. evansi*. Antigen-detection ELISA (Ag-ELISA) has been shown to be a more efficient assay in assessing the patent state of infection in infected camels and in evaluating the success of therapeutic intervention than the antibody-detection (Ab-ELISA) test.

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

There are two species of camels: the one-humped camel, *Camelus dromedarius*, and the two-humped camel, *Camelus bactrianus*. The one-humped camel is found in the desert and semi-desert areas of Northern and Eastern Africa, the Middle East and West-Central Asia. The two-humped camel is found in the colder areas of Southern Russia, Mongolia, East-central Asia and China (Wilson, 1981). *Camelus dromedarius*, the only camel species found in Kenya, is kept by the nomadic pastoralists of the arid and semi-arid parts of northern Kenya. The latest estimation of the population of camels in Kenya is 800,000 (FAO Animal Health Yearbook, 1982) making Kenya to rank 6th in Africa and 8th in the world in camel keeping.

The camel is essential for the livelihood of the nomadic pastoralists in Kenya. It is a hardy animal that is very well adapted physiologically and anatomically to arid and semi-arid conditions where it demonstrates more efficient utilization of the pasture than most of other livestock species. The camel is used mainly to provide milk and as a draft animal. Camel milk, which contains a higher proportion of vitamin C than either cow or human milk, is the staple diet of many nomadic people. Camels also provide meat, blood and skin. Another aspect where the camel is of economic importance in Kenya is the use of this animal in tourism industry. The recent introduction of the Maralal International Camel Derby is drawing increasing participation from other countries of Africa and the Middle East. With the growing awareness of the unique role the camel plays in agriculture, culture and heritage of

the arid and semi-arid environment, increasing attention is being focused on improving its productivity and health status, so as to improve and diversify the production capacity of these marginal lands. Moreover, more and more of the areas traditionally used for cattle are gradually suffering from desert encroachment, particularly in the dry savannahs of Africa South of Sahara.

Trypanosomiasis due to *T. evansi* (surra) is endemic in Kenya and is the most important disease affecting the camels (Fazil, 1977; Olaho-Mukani, 1981; Rutagwenda, 1982). Diagnosis of *T.evansi* infections in the field is largely based on clinical signs, the cardinal ones being anaemia and emaciation, even though they are not pathognomonic. Studies carried out in Kenya on camels naturally infected with surra failed to clearly indicate if the anaemic status, emaciation and deaths observed were due to helminths, trypanosomiasis or malnutrition (Rutagwenda, 1982; Wilson *et al.*, 1983). Thus, there is a need to establish a comprehensive clinical picture of camel trypanosomiasis.

T.evansi is not restricted to the bloodstream and causes vascular damage, disruption of connective tissue and hence the disruption of the integrity of body cells, causing large quantities of cellular enzymes to be released into the blood (Goodwin, 1970). Thus, assaying the serum levels of a number of organ-specific enzymes should allow detection of the host's tissue damage by the parasite because the enzymes reasonably reflect pathological changes in the specific organ(s) or tissue. It is important to note that the haematological and biochemical profiles of normal Kenyan camels are far from complete and the veterinarians and researchers working with these animals rely on data established in countries such as Sudan (Salaheldin *et al.*, 1979), India

(Lakhotia *et al.*, 1964) and Iran (Ghodsian *et al.*, 1978).

The principal practical control method of *T.evansi* is mainly through surveillance and chemotherapy. However, resistant stocks of *T.evansi* strains to the recommended drugs used for camel surra (suramin and quinapyramine) have been reported (Gill, 1971; Luckins *et al.*, 1979; Schillinger *et al.*, 1982). The production of some of these trypanocides has been stopped due to the prohibitive costs of manufacturing, marketing and the low budgets in the target countries (Jeffries, personal communication, 1992). Recently, a novel trypanocide, Cymelarsan® (Rhone Merieux), has been introduced for trials for treatment of camel surra. Intensive studies need to be done pertaining to this drug before it is registered for use in camel trypanosomiasis in this country.

It is hoped that this study will go a long way in improving the diagnostic accuracy of the camel surra and generate baseline data on the Kenyan dromedary camel.

1.2 Objectives

a) To establish the normal haematological and biochemical profiles of the Kenyan camel.

b) To establish the clinical symptoms exhibited by camels experimentally infected with *T.evansi*.

c) To determine clinical, haematological and biochemical parameters in camels during the course of infection with *T.evansi* and after successful treatment with Cymelarsan® (Rhone Merieux).

d) To use antigen ELISA as a tool for evaluating the efficacy of Cymelarsan® (Rhone Merieux).

CHAPTER TWO

LITERATURE REVIEW

2.1. Aetiology

2.1.1. Historical Background

Trypanosoma (*Trypanozoon*) *brucei evansi* (hereafter referred to as *Trypanosoma evansi*) was the first pathogenic trypanosome to be identified and described as the causative agent of mammalian trypanosomiasis (Evans, 1880). It was associated with an epidemic in equines and camels known as 'surra' in India. More discoveries have been made over years since then, of similar diseases caused by trypanosomes indistinguishable from *T. evansi* in various parts of the world and in diverse mammalian hosts. Thirty three different names have been used for these non-tsetse transmitted parasites. Currently, *T.evansi* is the only accepted valid name that is given to the parasite of surra and replaces all the other names previously used for this species (Hoare, 1956). It is presumed that *T.evansi* evolved from *T.brucei* due to continuous mechanical transmission of the latter by blood sucking flies, in the absence of *Glossina*. During the adaptation to the biting flies, *T.brucei* lost cyclical transmission ability and became adapted to mechanical transmission giving rise to a predominantly slender parasite that infects animals outside the tsetse infested areas (Hoare, 1972).

2.1.2. Classification and Morphology.

T. evansi belongs to the phylum Protozoa, order Kinetoplastida, family Trypanosomatidae, genus *Trypanosoma* and subgenus *Trypanozoon*

(Hoare, 1972). The genus *Trypanozoon* is the most homogeneous group of all trypanosomes in that it contains a number of species which are morphologically indistinguishable but differ only in biological and nosological features.

In the blood of its mammalian host, *T.evansi* is represented almost exclusively by slender trypanosomes that are distinguishable from the intermediate and stumpy forms of *T.brucei*. The overall length of *T.evansi* ranges from 15µ to 34µ, with a mean of 24µ. The kinetoplast is sub-terminal, the undulating membrane is well developed and there is a substantially long flagellum (Soulsby, 1986). Although *T.evansi* is typically monomorphic, some strains sporadically exhibit some type and degree of polymorphism. Forms which lack a kinetoplast may arise spontaneously, especially after drug treatment (Hoare, 1954; Killick-Kendrick, 1964).

T.evansi is the main causative agent of camel trypanosomiasis. Other parasites in the subgenus Trypanozoon include *T.brucei brucei*, *T.b.rhodesiense*, *T.b.gambiense*, all of which are tsetse borne and *T.equiperdum* which is sexually transmitted. Tsetse transmitted trypanosomes (*T.brucei* and *T.congolense*) affect camels in regions where these flies exist and have been reported in camels in Somalia, Kenya and Sudan (Bennett, 1933; Wilson *et al.*, 1983; Olaho-Mukani, 1981; Dirie *et al.*, 1989). However, most camels are not found in the tsetse-infested areas, hence the tsetse-borne trypanosomes are less prevalent in these animals. Pellegrini (1948) artificially infected two camels with *T.simiae*, but recent reports show that this species of the subgenus Nanomonnas is not pathogenic to camels (Zweygarth *et al.*, 1987).

2.2. Epizootiology

2.2.1. Distribution and Hosts

Surra has an extremely wide geographical range in countries with hot and warm temperatures, but previous distribution coincided with that of the camels. Transmission by blood-sucking insects other than *Glossina* has enabled *T.evansi* to extend its range into Africa, Asia and America (Soulsby, 1986). In Africa, the disease occurs in regions around the Sahara desert: West, North and East Africa. South of the Sahara it almost merges with the northern limits of the tsetse fly areas (Hoare, 1972). In the Arabian peninsula surra occurs in Saudi Arabia, Yemen, Sultanate of Oman and the United Arab Emirates. In Near and Far East it is reported in Turkey, Lebanon, Israel, Syria, Jordan, Iraq, Iran, Afghanistan, Pakistan, Central Asian republics of USSR, India, Burma, Malay Peninsula, Indo-China, Southern China, the Islands of Indonesia and Philippines. The disease also occurs in South and Central America (Hoare, 1972). Thus, the disease occurs throughout all the areas where camels are commonly found. The disease has, however, been eradicated in Australia, North America and South West Africa as a result of major veterinary control measures (Rottcher *et al.*, 1987).

T.evansi epidemics involve different animal hosts in different parts of the world and the effects of the infection in different localities vary according to the strain of parasite present (Hoare, 1956). In Africa, camels are most affected and in Central and South America the horses are the main hosts. In the Soviet Union and Mid-Asia, the camels are the most affected animals and in India buffaloes are most affected. Natural infections have been reported in camels, horses, mules and donkeys (Bennett, 1933). All camels are susceptible regardless of the

breed or age, so are horses and most carnivores. Young camels however have shown some degree of resistance (Wilson *et al.*, 1983).

Among the domesticated animals, *T.evansi* causes disease in camels, horses, donkeys, mules, cattle, water-buffaloes, sheep, goats, dogs and cats. In the wild animals the disease has been reported in elephants, African-buffalo, deer, antelopes, tapir and capybara (*Hydroechoerus hydrochaeris*) (Mahmoud and Gray, 1980). Horses, dogs and camels usually experience an acute fatal infection with *T.evansi* and die after a brief period of illness (Currason, 1943). Since the disease is fatal in horses and dogs, these animals are unimportant in the maintenance of *T.evansi* infection in nature (Ilemobade, 1971; Horchner *et al.*, 1983).

Evidence from several workers confirms that cattle, sheep and goats undergo a protracted course of a mild clinical disease that results in carrier state and may act as reservoir hosts for the camel (Ilemobade, 1971; Khasanov and Ivanitskaya, 1974; Chand and Singh, 1971; Malik and Mahmoud, 1978). Water-buffaloes, antelopes, deers, and other wild animals are also considered to be asymptomatic reservoirs of the infection for equines and camels. Cattle reservoir hosts in South America provide a source of *T.evansi* for vampire bats (*Desmodus rotundus*) which transmit the organisms to horses. Healthy capybara has been found to harbour *T.evansi* and constitutes a reservoir of infection for horses and dogs in South America (Reveron *et al.*, 1992). Ocelot (*Felis pardalis*) is a reservoir found in lower Amazon Region (Shaw, 1977). Srivastava and Ahluwalia (1972) observed that pigs could be experimentally infected with *T.evansi* showing intermittent fever and rarely parasitaemia. However, recent studies show that pigs are not affected by *T.evansi* parasites (Zweygarth *et al.*, 1987). From an

economic point, the camel is probably the most important victim of surra.

2.2.2: Transmission

Surra is transmitted mechanically from an infected to a healthy animal by a number of haematophagous flies (Gatt-Rutter, 1967; Scott, 1973). The most efficient flies that transmit this disease are the tabanids (horse-flies) (Rogers, 1901; Nieschulz, 1930). The other flies that may transmit the disease but are of minor importance are *Stomoxys*, *Lyperosia* and *Haematobia*. Transmission efficiency of different flies may vary under different geographical conditions (Losos, 1980), but it has been shown that tabanids are the principal vectors of surra in camels under field conditions (Dirie *et al.*, 1989). *T.evansi* undergoes no cyclical development in all these flies. In the case of mechanical transmission by the biting flies, the trypanosome is passed from the infected to a clean host by infected blood in or on the mouthparts as the fly moves from one animal to another between interrupted blood meals (Soulsby, 1986). An interrupted feed on an infected host leaves the fly hungry and when it moves to another host it can transmit the infection through its trypanosome-contaminated mouthparts (Hoare, 1972). Infectivity of *T.evansi* is determined by a time factor, since transmission is effective only during a very short period of survival of the haematozoic forms of the parasite in the proboscis of the vector. Hence, the shorter the time between the two feeds the greater the chances of mechanical transmission (Hoare, 1972). Infectivity is highest minutes after feeding, decreases rapidly and is lost within 8 hours (Losos, 1980). The existence of carrier animals especially goats, sheep and donkeys, in the vicinity of susceptible camels makes transmission by biting flies possible.

Vampire bats have been found to play an important part in the spread of *T.evansi* among horses in Central and South America and have been shown to transmit this disease mechanically (Hoare, 1965). The bats can get the infection, remain carriers and transmit the disease when they bite other animals. The parasites in the bat do not undergo any development, but multiply in their blood as they do in the natural vertebrate hosts (Losos, 1980). Occasionally, the bats die of the infection about a month after an infected blood meal. Horse flies also transmit *T.evansi* in this part of the world (Boehringer and Prosen, 1961). However, bats are more effective in transmission than the biting flies because the trypanosomes multiply in their tissues and infections are maintained for long periods of time.

In dogs and cats, infection occurs by ingestion of tissues from infected carcasses (Soulsby, 1986). Nasal and conjuctival transmission of trypanosomes have also been reported (de Jesus, 1951) but these routes are unimportant in the field. Mosquitoes have been incriminated but evidently their role is very small (Currason, 1947). The transmission of *T.evansi* by ticks from camel to camel has been proved impossible or inefficient (Kirmse and Taylor-Lewis, 1978). Cross and Patel (1922) successfully used ticks to transmit *T.evansi* from camel to rabbit and not from camel to camel. Direct transmission to the foetus in pregnant camels has been reported during the acute stages of infection (Sergent *et al.*, 1920).

Farm practices such as ear notching, tattooing and castrations have been proved to cause transmission of *T.evansi* from infected camels to clean ones. These unsanitary managemental practices when done using the same devices in goats seemingly normal but otherwise sub-

clinically sick, make transmission of the disease from the goat to a healthy camel possible. Pastoral practices e.g. blood letting have also been shown to cause transmission. According to Leese (1927) transmission can also occur when infected camels rub against healthy ones.

2.2.3. Occurrence

Surra is a seasonal and regional disease prevalent during fly season and in areas near water where the flies breed (Higgins, 1983). There is a definite correlation between seasonal outbreaks of *T.evansi* infections and the tropical rainy season. The intensity of the disease is closely associated with seasonal increases in fly numbers at the end of the rains (Yagi and Razig, 1972). Most outbreaks have been shown to occur immediately after the rainy season when the number of tabanids increases (Dirie *et al.*, 1989). In Arabia the disease is prevalent from March to September (Higgins, 1983). Olaho-Mukani (1981) showed that in Kenya, there were elevated point prevalence rates of surra in two peaks, one following the long rains (March to June) and the other after the short rains (September to November). Each peak was followed by a rapid fall as the dry season advanced. Prevailing ecological conditions during and immediately after the rains favour the breeding and prevalence of the tabanids and other haematophagous flies that transmit the disease. The sporadic infections in the dry season are due to other species of tabanids confined to river banks and are prevalent throughout the year. These ensure that transmission of the disease occurs wherever the reservoir hosts, the vectors and the susceptible hosts coexist (Yagi and Razig, 1972).

2.3. Pathogenesis and Pathology

2.3.1. Pathogenesis and clinical signs

2.3.1.1. Clinical signs

a) The disease in camels

Trypanosomiasis in camels usually takes a chronic course, however several phases are recognized: acute, subacute and chronic (Gatt-Rutter, 1967; Richard, 1979; Dirie *et al.*, 1989). The course of infection depends upon the susceptibility of the host, strain of the infecting parasite and the density of the biting flies and therefore the parasite load.

i) Acute form.

The acute form of the disease begins with first appearance of trypanosomes in peripheral blood after the incubation period of 3 to 7 days. The animal stops eating, becomes dull and listless, there is a drop in milk production and pregnant females abort (Higgins, 1983). The parasitaemia is high and although fluctuating, during the course of the disease trypanosomes are always demonstrated in blood. There is persistent fever and some animals may die during this phase of the disease which lasts two to three weeks (Higgins, 1983). Petechial haemorrhages in serous membranes may be observed.

ii) Subacute form.

The subacute disease lasts 3 to 4 months and the animal starts losing body condition, there is progressive development of anaemia and some animals may die. The subacute form of disease was reproduced by subcutaneous inoculation of infective blood (Alongi and Balboni,

1935). Animals that survive the acute and subacute disease develop a chronic disease which is the most common.

iii) Chronic form.

Chronic surra is the most prevalent form of the disease under natural conditions. It is a slow wasting disease that is protracted over 2 to 3 years and is characterized by intermittent fever, low frequency and intensity of parasitaemia, anaemia, progressive emaciation, disappearance of the hump, atrophy of the thigh muscles, oedema of dependent parts and deaths in some animals (Gatt-Rutter, 1967; Mahmoud and Gray, 1980; Singh *et al.*, 1980). Huge production losses occur due to lower milk and meat yields, abortions, premature births and inability to feed the young; all of which reduce reproductive potential in affected herds (Yagil, 1982). The chronic disease appears in a period when the infected animal has exhausted its defence mechanisms. This is the period when the parasites, being tissue invasive, also become established extravascularly and are less numerous in the peripheral blood. Debility, alopecia, keratinization, depletion of subcutaneous fat and facial oedema have also been demonstrated in experimentally infected camels (Raisinghani, *et al.*, 1980). Singh *et al.* (1980) observed diarrhoea and sexual excitement as additional signs. The chronic phase is followed by a period characterized by aparasitaemia when relapses become more rare and animals remain in apparently good health, sometimes for several years. This is the inapparent disease (Wilson *et al.*, 1981). Occasionally, camels may recover spontaneously (Sergent *et al.*, 1920).

b) The disease in other animals

In the horse the average prepatent period is 3 to 4 days after inoculation (Horchner *et al.*, 1983). Relapsing or undulating peaks of parasitaemia with corresponding fever follow. Fever increases up to 41°C. With longer periods of high level parasitaemia, fever is nearly continuous and of moderate degree. There is reduced appetite, watery nasal discharge, slight lacrimation, sweating, hyperesthesia, muscular tremors and unsteady gait (Ilemobade, 1971). A decrease of trypanosomes in the peripheral blood usually coincides with a rapid decline in body temperature to an almost normal. Emaciation and oedema in the horse are the most common clinical signs. The oedema varies from urticarial plaques on the neck and flanks, to oedema of the legs and lower parts of the body. The plaques may undergo necrosis in the center and haemorrhages occur at the junction of the skin and mucous membranes, especially at the nostrils, eyes and anus (Soulsby, 1986). The pathogenicity of *T.evansi* infection was demonstrated by Ilemobade (1971) in donkeys, cattle and horses. All infected horses died, six out of seven donkeys and all cattle infected survived. Thus, he showed that *T.evansi* was very pathogenic to horses, less pathogenic to donkeys and had virtually no effect on oxen. The same pathogenicity in the horse was demonstrated by Horchner *et al.* (1983). Ocular lesions that include serous lacrimation, periorbital oedema, a milky-gray cloudiness and progressive vascularisation that starts at the limbus to cover the whole cornea, have been recorded in horses experimentally infected with surra (Ilemobade, 1971; Horchner *et al.*, 1983)

Canine surra is acute and fatal; pups die within 10 to 25 days, but in adult dogs the disease may last from 36 to 150 days ending

fatally. Oedema is marked, corneal opacity is common and, due to the oedema of the larynx, voice changes similar to those which occur in rabies may be noticed (Soulsby, 1986). In elephants, surra follows a course similar to that seen in camels; animals become emaciated and show marked muscular weakness. In the other domestic animals (cattle, goats and sheep) the disease is usually inapparent. The capybara, although normally a reservoir host for the horse, may suffer an acute infection similar to that in the horse.

2.3.1.2. Pathogenesis

T.evansi, like other members of subgenus *Trypanozoon* spreads to different body compartments. The parasites have been found in joints and the cerebrospinal fluid (CSF) of camels (Leese, 1927) and ponies (Horchner *et al.*, 1983). The occurrence of the trypanosomes in CSF could be the likely cause of motor disturbance (Singh *et al.*, 1980; Horchner *et al.*, 1983). The nervous disturbances include intermittent tremors and clonic cramps of muscles in the shoulders and forelimbs. Animals stand with outstretched splayed forelegs and show progressive ataxia. Paraplegia or paralysis finally occurs (Horchner *et al.*, 1983). *T.evansi* can enter the CNS at a very early stage without necessarily causing any immediate nervous symptoms (Kraneveld and Djaenoedin, 1949). Goel and Singh (1970) observed that the aqueous humor of rabbits infected with *T.evansi* and which were developing conjuctivitis and keratitis, and the aqueous humor of a dog which developed glaucoma, contained *T.evansi* parasites. Aqueous humor from eyes with haemorrhagic alterations have been shown to contain large numbers ($6x10^6$) of viable trypanosomes per milliliter. Trypanosomes in smaller

quantities have also been detected in the aqueous humor of those animals with keratitis or uveitis and even from eyes that showed neither haemorrhages nor macroscopic alterations (Horchner *et al.*, 1983).

2.3.2. Pathology.

2.3.2.1. Clinical Pathology

The main clinico-pathological feature of *T.evansi* infection in camels is anaemia similar in nature to that caused by other species of trypanosomes in other animals. The anaemia in camels has been described cytologically as macrocytic (Jatkar and Purohit, 1971) and aetiologically as haemolytic (Raisinghani *et al.*, 1981b). Haematological studies showed reductions in red blood cells, haemoglobin, packed cell volume and serum levels of calcium, chloride, potassium and sodium. Increase in reticulocytes, eosinophils and organic phosphate were observed (Raisinghani *et al.*, 1981b). The level of blood glucose has been shown to fall with increasing parasite levels (Jatkar and Singh, 1971; Raisinghani *et al.*, 1981b). Jatkar *et al.* (1973) observed that in acute infection albumin remained unchanged but fell below normal during chronic disease; alpha globulin fell during acute infection and increased during chronic disease; beta globulin increased during acute infection but remained unchanged in chronic disease and gamma globulin increased during both acute and chronic diseases. Generally, these authors found that serum proteins were inversely proportional to the trypanosome counts. It was also demonstrated that total protein concentration increased above normal values in camels naturally and experimentally infected with *T.evansi* (Boid *et al.*, 1980a). Albumin in

both cases decreased to below normal, alpha globulins remained unchanged and beta globulin levels were unchanged in experimental camels but decreased in natural infections. Immunoglobulin M (IgM) showed significant increase of about 5 times the pre-infection levels, while IgG did not show any significant changes. In buffaloes, the total serum proteins did not increase as in camel infections, but beta and gamma globulins increased and albumin and alpha globulins decreased (Verma and Gautam, 1978).

Blood glucose level has been shown to decrease to below normal level in camels infected with *T.evansi* (Raisinghani *et al.*, 1981b). The fall in blood glucose level was inversely proportional to the parasitaemia. However, Jatkar and Singh (1974) found increasing blood glucose levels in camels infected with *T.evansi*.

Biochemical profiles have also been studied by various authors. Dwivedi *et al.* (1977) found that dogs infected with *T.evansi* showed no changes in serum glutamic oxaloacetic transaminase (SGOT) and there was a slight rise in serum glutamic pyruvic transaminase (SGPT) between the 6th and the 13th day of infection. Boid *et al.* (1980b) showed increase in the levels of sorbitol dehydrogenase (SDH), SGOT and SGPT above the pre-infection levels in camels infected with *T.evansi*, whereas alkaline phosphatase (AP) decreased during the period of patent parasitaemia. The levels of all enzymes returned to normal after treatment with quinapyramine sulphate.

Urine of camels affected with chronic trypanosomiasis is dark-brown with a very strong typical pungent odour and has small amounts of urinary ketones and urine proteins (Hunter, 1986). The smell of urine of infected animals has a diagnostic bearing and is easily

recognized by most camel owners.

2.3.2.2. Gross Pathology.

There are no absolute typical pathological changes in camels dying of *T.evansi* infection at postmortem. Anaemia is often visible with skeletal and heart muscles becoming pale. There are signs of dehydration, pericardial effusion, enlarged lymph nodes and splenomegally (Rottcher *et al.*, 1987).

2.3.2.3. Histopathology.

The recorded histological changes were of the brain of a horse that died of *T.evansi* infection. Sections of the cerebrum had encephalomalacia. Many gitter cells engulfing both fat and hemosiderin, grey matter had neuronal degeneration and neuronophagia and glial cells were abundant. The other organs were characteristic of a chronic debilitating disease (Ilemobade, 1971).

2.4. Diagnosis

The necessity of a rapid and accurate diagnosis is of supreme importance in any clinical condition. In the field the rapidity and accuracy of any one technique or a combination of techniques is vital if suitable treatment of any individual infected animal is to be effective or if chemotherapeutic control of a selected group of animals is to be attempted (Molyneux, 1975). Provisional diagnosis of camel surra can be made from the characteristic clinical symptoms manifested by this disease i.e emaciation and anaemia. However, these signs are not pathognomonic for this disease.

a) Parasitological Diagnosis.

The best way for diagnosis of a parasitic infection is by finding the parasite itself by the standard parasitological methods available. The parasitological diagnosis of acute camel trypanosomiasis is usually relatively easy since the organisms are readily demonstrable in freshly stained blood smears. Diagnosis in chronic infections may present difficulties because parasitaemia is low (Gatt-Rutter, 1967; Killick-Kendrick, 1968) and intermittent (Mahmoud and Gray, 1980). The standard parasitological techniques available for diagnosis of camel trypanosomiasis include:

i) Microscopic examination of a wet preparation of fresh blood is used to detect motile trypanosomes. Alternatively, Giemsa stained thin blood smears can be used to identify parasites on the basis of their morphology. Examination of wet films is quick and the method is suitable for screening large numbers of animals. This method, however, has limitations in that very low-grade infections are impossible to detect and is insensitive in that more than half of the infections may be missed (Barnett, 1947). Searching thin films is an inefficient means of diagnosing surra infections and its use is best restricted to identification of trypanosomes. Examination of thick blood smears has been shown to be a more efficient way of detecting infection than both thin and wet preparations (Killick-Kendrick and Godfrey, 1963).

ii) Concentration techniques have been used to improve on the sensitivity of direct microscopy. The most recent is the haematocrit centrifugation technique, HCT, (Woo, 1969). This is the most rapid and reliable method for detection of low parasitaemia and the determination of the number of trypanosomes in a given volume of blood.

Microhaematocrit centrifuge technique (MHCT) is particularly useful in that trypanosomes in the blood of camels can be detected 6 to 10 days earlier than by wet or thick blood films, the status of anaemia in the test animals can be assessed at the same time and the technique can be carried out in the field using a battery powered minicentrifuge (Kelly and Schillinger 1983). Miniature anion exchange chromatography technique (MAECT) (Lumsden *et al.*, 1979) is a concentration technique used widely to diagnose *T. gambiense* infections in man but is yet to be established as a routine test for diagnosis of animal trypanosomiasis due to its more cumbersome procedure (Nantulya, 1990).

iii) Sub-inoculation of susceptible animals with blood from suspected animals can also be used for parasitological diagnosis. Animal inoculation reveals many subpatent infections of *T. evansi* in camels (Godfrey and Killick-Kendrick, 1962). Rats or mice are commonly used because they are cheaper and more easily transported in the field. This technique is more sensitive than direct microscopic examination of the blood sample or the MHCT (Pegram and Scott, 1976). However, situations have been reported where MHCT gave better results than mouse inoculation (Otsyula *et al.*, 1992). The advantage of this method is that the trypanosome isolates in the rodents can be used later for studies in the laboratory. However, the method is not a useful one for diagnosis due to long prepatent periods in the commonly used laboratory rodents (i.e. diagnosis is not immediate) and also the cost of maintaining the rodents in the laboratory makes the method prohibitively expensive for routine diagnosis. This method is best used for epidemiological studies (Nantulya, 1990).

Thus, in the standard parasitological diagnostic methods a high proportion of infections still go undetected as the majority of infections are chronic or often aparasitaemic.

b) Serological Diagnosis.

Earlier serological tests, such as mercuric chloride test, formol gel test and thymol turbidity test (Knowles, 1924; Bennett and Kenny, 1928) previously used to detect trypanosomes in camels, relied on increases in the levels of euglobulins in the infected animals. These methods were not specific for trypanosomiasis and were unreliable for accurate diagnosis of this disease (Horgan and Bennett, 1929; Pegram and Scott, 1976). Serological tests were later developed to detect trypanosome-specific antibodies in camel serum. Enzyme-linked immunosorbent assay, (ELISA), indirect fluorescent antibody test, (IFAT), capillary agglutination test, (CAT) and passive haemaglutination test (PHT) have all been used as diagnostic tests for camel surra (Jatkar and Singh 1971; Jatkar *et al.*, 1977; Luckins *et al.*, 1979; Olaho-Mukani, 1981). None of these tests however, differentiate between past and present infections owing to the persistence of antibodies for up to 100 days or more after chemotherapy has cleared trypanosomes from the bloodstream or following self cure (Luckins *et al.*, 1978, 1979; Nantulya *et al.*, 1989a). A positive antibody result may indicate current infection or infection within previous several months. Chemotherapeutic intervention relying on positive antibody results may end up being wasteful especially when treating animals with cleared infections. Detection of anti-trypanosomal antibody levels is more useful as an epidemiological tool rather than a diagnostic test (Nantulya, 1990).

The most recent method for serological diagnosis of camel trypanosomiasis is the detection of the presence of trypanosomal antigens in circulation (Rae and Luckins, 1984; Nantulya *et al.*, 1989a; Olaho-Mukani *et al.*, 1992a). This test detects actively infected animals because the antigens detected are products of parasite degradation. Thus, the demonstration of trypanosomal antigens in the blood of the infected animal would be synonymous with parasitological diagnosis (Voller and De Savigny, 1981) and hence an indicator of current infection. Applied to the diagnosis of *T. evansi* infections in camels in an endemic area, the test was able to detect circulating antigens in 92% of the animals with parasitologically confirmed diagnosis (Nantulya *et al.*, 1989b). The test also detected antigens in 55% of the animals in the same herd which had been missed by parasite detection techniques. Camels from trypanosomiasis free areas tested negative on this test, demonstrating that the test has a high specificity. Even though this test could not detect 6-15% cases with proven trypanosome infection parasitologically, it is the only potentially suitable serological diagnostic test for individual animal diagnosis in the field (Nantulya *et al.*, 1989b). This test has potential for adoption in the field for immunodiagnosis of camel trypanosomiasis (Nantulya *et al.*, 1989b). Antigen-trapping assays are easy to perform; the results can be read visually; large numbers of serum specimens can be analyzed at a time; they can be applied to the diagnosis of individual animals and they are more sensitive than the techniques for parasitological diagnosis (Nantulya, 1990). Antigen assays are also suitable for assessing therapeutic success (Olaho-Mukani *et al.*, 1992b).

2.5. Treatment

a) Naphthylamine drugs

Two drugs recommended for treatment of *T. evansi* infections in the dromedaries are suramin and quinapyramine sulphate (Balis and Richard, 1977; Rottcher *et al.*, 1987). In the naphthylamine group there is only one compound, Suramin, which is used as a curative agent for *T.evansi*. Suramin (Naganol, Antrypol, Bayer 205) introduced in the 1920's, was a major advance in chemotherapy of trypanosomiases. In camels, single doses of 10g in aqueous solutions injected intravenously, cured nearly every infected case (Alongi and Balboni 1935). It was reported that 100% cure was obtained with 5g suramin in a single intravenous injection in camels, but reduced doses caused drug resistance in which renewed treatment with the same drug at even higher dosages had no effect. Suramin resistant trypanosome population has been reported by several authors after administration of sub-curative doses. Resistant stocks of *T. evansi* to suramin have been reported in Sudan, Kenya and Russia (Gill, 1971; Mahmoud and Osman, 1979; Luckins *et al.*, 1979; Schillinger *et al.*, 1984). *T. evansi* stocks resistant to suramin were found susceptible to quinapyramine (Leach, 1961). Accidental paravenous injection of this drug has been shown to cause thrombophlebitis at the site of injection (Schillinger *et al.*, 1984). Manufacture of this drug has been discontinued recently.

b) Quinaldine group

Among the quinaldine group, quinapyramine sulphate was shown to have a wide range of action against most trypanosomes of subgenus *Trypanozoon* in all animal species (Davey, 1950). It gave protection to

camels for up to 3 months when administered at a dose rate of 5mg/kg subcutaneously. A mixture of quinapyramine sulphate (3 parts) and quinapyramine chloride (2 parts) (Prosalt) when administered at a dose of 7.4mg/kg subcutaneously gives a prophylactic cover lasting 3-4 months. Severe quinapyramine overdose causes salivation, muscle tremors, stiffness and collapse while moderate overdose causes nephrotoxicity (Davey, 1957). There is an indication of discontinuation of both quinapyramine sulphate and Prosalt in the near future (Jeffries, Rhone Merieux, personal communication). *T. evansi* strains with dual resistance to suramin and quinapyramine exist nowadays (Mahmoud and Osman, 1979; Schillinger *et al.*, 1984).

c) Phenanthridine group

In the phenanthridine group of drugs, isometamidium (Samorin^{R}, May and Baker) is a drug that is effective against trypanosomes in various animal species (Arowolo and Ikede, 1977; Toro *et al.*, 1983). According to Balis and Richard (1977), samorin given at 0.5mg/kg body weight (b.w.) was well tolerated and effective in camels naturally infected with *T. evansi* and no evidence of trypanosomiasis was found 8 weeks after treatment. However, recent work has shown that therapeutic effectiveness of this drug is transient with relapses occurring within three weeks after treatment (Ali *et al.*, 1985). Thus, Samorin has only a moderate effect against *T. evansi.* Intravenous or intramuscular administration of Samorin produces adverse toxic signs such as trembling, lacrimation, salivation, restlessness and defaecation followed by profuse diarrhoea and urination (Schillinger *et al.*, 1985; Ali *et al.*, 1985). This drug is recommended for use in camels only as an

emergency measure where dual resistance against suramin and quinapyramine exist (Rottcher *et al.*, 1987). According to Schillinger *et al.* (1982) isometamidium has minimal effect against *T. evansi* in East African camels.

The other trypanocides in this group of drugs that are used for treatment of tsetse-transmitted trypanosomes (e.g. Homidium and Pyrithidium bromide) are not effective against *T.evansi* treatment (Srivastava and Ahluwalia, 1973).

d) Aromatic arsenicals

Arsenicals, alone or in combination or in combination with other compounds e.g. antimonials, were the first drugs to be used against camel trypanosomiasis but had the disadvantage of having a narrow safety margin (Gatt-Rutter, 1967). Gill and Sen (1971) found that melarsen oxide and diminazene were fully curative to equines infected with *T. evansi*. Melarsoprol (Mel B), another arsenical, is said to be effective against suramin and quinapyramine resistant stocks of *T.evansi*. Clinical trials with melarsoprol in camels, given at doses of 3.5mg/kg b.w., showed encouraging results (Rottcher *et al.*, 1987). Cymelarsan® (Rhone Merieux), a new arsenical compound patented in 1985, has not been used commercially for treatment of animal trypanosomiasis but trials with camels naturally and artificially infected with *T.evansi* have shown that this drug is active against camel surra. The drug acts rapidly as parasites disappear from the bloodstream within 24 hours after injection (Zellecke *et al.*, 1989; Otsyula *et al.*, 1992; Nyang'ao *et al.*, 1992). Cymelarsan has been shown to be fully effective against *T.evansi* strains resistant to suramin and quinapyramine

(Raynaud *et al.*, 1989). Studies on naturally infected camels have shown that the drug is effective at the recommended therapeutic dose of 0.25mg/kg (Nyang'ao *et al.*, 1992). This drug has an added advantage in that it crosses from the peripheral circulation into the central nervous system, especially in the cerebro-spinal fluid (CSF) to clear *T.evansi* parasites which have been shown to invade this compartment (Leese 1927; Horchner *et al.*, 1983).

e) Other drugs

Berenil, an aromatic diamidine, has been found to be too toxic for camels (Fazil, 1977; Homeida *et al.*, 1981) for camels.

2.6 Control

Trypanosoma evansi is harboured by animal reservoirs and spread mechanically by insect vectors. Its eradication is nearly impossible because of the difficultly in eliminating these two important factors (Gatt-Rutter, 1967). For vector control to be effective, a thorough understanding of the vector's biology is essential in order to identify possible areas for intervention. The tsetse fly's biology is well understood and strides in its control have been made (Jordan, 1986), unlike for the other biting flies. Control measures of surra that have been tried are early diagnosis and treatment. To reach the diagnosis, accurate diagnostic tests are needed (Mahmoud and Gray, 1980). Once confirmed, the infected animals should be segregated immediately and treated. Other methods that have been tried are slaughter, especially animals found infected that are old, emaciated or unsound, and thus not

worth treating. Then proper and effective disposal of infected carcasses should be ensured. Regular veterinary examination of all camels and correct treatment of those which are trypanosome-positive are the most important measures to reduce new infections. Sheep and goats which are very often kept close to camels act as asymptomatic carriers of *T. evansi* from which transmission to camels occurs. A knowledge of fly seasons and the distribution of biting flies especially tabanids is essential to protect the camels from these biting flies (Higgins, 1983). During the fly season, fly zones should be avoided, although this is practically difficult. This however, is often impossible and steps have to be taken in some instances to prevent flies from biting camels e.g by the use of repellents (Gatt-Rutter, 1967). Tabanid challenge in sedentary camel herds can be reduced by trapping large numbers of flies using traps (Molyneux and Ashford, 1983). Chemoprophylaxis, using a quinapyramine-suramin combination gives ponies full protection for 6.5 to 23 months against *T. evansi* depending on the dose and the intensity of the infection. Recently, isometamidium chloride has been shown to provide prophylaxis at a dose of 0.5 to 1.0mg/kg body weight for 2 to 4 months in several species of livestock including camels when given by deep intramuscular injection. Treatment of animals at the end of tropical rain season seems to give good protection to camels living in or crossing endemic areas (Higgins, 1983). Quinapyramine sulphate and chloride at the ratios 3:2 (Prosalt) has been used for protecting camels. Production of a vaccine is still remote due to the problem of antigenic variation. The only suitable methods of control of camel trypanosomiasis, currently, is surveillance and chemotherapy, and chemoprophylaxis. Since control of *T.evansi* is mainly

by chemotherapy, there is need for a drug that is fully effective against all strains of the parasite. Cymelarsan has shown promising results in field trials (Zellecke *et al.*, 1989; Otsyula *et al.*, 1992). Further studies should be carried out on this drug under laboratory conditions to evaluate its efficacy for large scale use.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental camels

Twelve young Somali-type camels aged between $2^1/_2$ and 4 years, were used in this study. They were obtained from the Kenya Trypanosomiasis Institute (KETRI) herd at Athi River. Each camel was numbered and assigned an information sheet on which all relevant information was recorded. These included an ear-tag number, sex, date of birth, sampling dates, samples taken and results. Body weights were estimated using the method described by Schwartz *et al.* (1983) i.e. body weight of a camel (in kilograms) is the product of 50, the shoulder height (SH, in meters), the thoracic girth (TG in meters) and the abdominal girth (AG in meters). Fecal examination was done using the modified McMaster technique (Anon, 1979) to determine their worm load. All the animals were thereafter dewormed using Valbazen^R (Kenya-Swiss) at a dose of 7.5mg/kg body weight. Fecal examination of these camels was also done at the start, in the middle and at the end of the experiment and found negative. The camels were then sprayed with an acaricide (Triatix^R, COOPER) to clear all the ectoparasites. The animals were screened for any trypanosome infection using mouse inoculation (MI) and buffy coat examination (BCE), and antibody and antigen-detection enzyme-linked immunosorbent assay (ELISA). Screening was done three times. All the camels were negative for *T.evansi* and other tsetse-borne trypanosomes. Thus the camels were all healthy at the start of the experiment. The animals were housed in fly-proof stables at KETRI-Muguga and fed with hay; water was provided *ad libitum*.

Supplementation with mineral salt and ranch cubes was done three times a week. The animals were randomly divided into three groups. Group A had five camels numbered 149, 155, 156, 159 and 161; group B had five camels (numbers 147, 151, 160, 162 and 261) and groups C had two camels numbered 150 and 163. Pre-infection parameters were established for a period of four weeks. Thereafter, group A animals were infected by intravenous inoculation with 2 x 10^7 trypanosomes of a clone of *T.evansi* isolate KETRI 2468 suspended in 6ml phosphate saline glucose buffer (PSG). This inoculum was within the range of trypanosome concentration used by previous workers (Boid *et al.*, 1980a; Raisinghani *et al.*, 1980a; Ali *et al.*, 1985). The infection was allowed to run throughout the study period. Group B camels were infected with the same isolate and inoculum dose as for group A, but were treated with Cymelarsan® at the recommended therapeutic dose of 0.25mg/kg body weight intramuscularly after the onset of the clinical disease i.e. during the first peak of parasitaemia. Group C camels were used as non-infected controls.

3.2. Trypanosomes

3.2.1. Stabilate History

Whole blood from a camel naturally infected with trypanosomes at Kulal, Marsabit was inoculated into two Swiss-white mice. At peak parasitaemia the mice were bled by cardiac puncture and blood mixed with EDTA saline glucose (ESG) pH 8.0. Thereafter, the trypanosome preparation was mixed in equal volume of 20% glycerol. This primary isolate was dispensed into capillary tubes, sealed with plastoceal and cryopreserved in liquid nitrogen at -196°C, in KETRI's trypanosome

bank. This isolate was later characterized by starch gel isoenzyme electrophoresis (Gibson *et al.*, 1983) and kinetoplast DNA analysis and identified as *T.evansi* and stabilated as KETRI 2468. This bank stabilate was used for cloning as described below (3.2.2).

3.2.2. Clone Preparation

Clones were obtained by the microscopic isolation of a single trypanosome which was allowed to multiply in mice as described by Otieno and Darji (1985). Briefly, stabilate KETRI 2468 was used to infect Swiss-white mice. Blood from a heavily parasitaemic mouse was diluted in phosphate saline glucose buffer (PSG), pH 8.0, and serially diluted to give a concentration of approximately 10^3 trypanosomes per milliliter. Several microdroplets of this diluent were then placed on a cover slip and quickly put into a moistened siliconised grooved microscope slide. The droplets were viewed under a microscope at x160 magnification. Only a microdroplet that had a diameter smaller than the microscope field were examined. A microdroplet containing a single organism (confirmed by a second person) was flooded with PSG buffer, sucked into a 1ml syringe and injected intraperitoneally into a lethally irradiated Swiss-white mouse using a 25G needle. The mouse was monitored daily for parasitaemia and at peak parasitaemia it was anaesthetised with diethyl ether and bled by cardiac puncture. Blood was collected using a 1ml syringe containing EDTA. The trypanosomes were separated from blood using DEAE-52 column as described by Lanham and Godfrey (1970). Briefly, a standard column (9cm diameter by 6cm) was packed with DEAE-52 equilibrated with PSG buffer pH 8.0. Blood from mice at peak parasitaemia was diluted 6:4 with PSG and

applied onto the DEAE-52 column. Trypanosomes were eluted with the same buffer. Using the same buffer, the trypanosomes were washed twice by centrifugation at 1,500 x g. From this trypanosome suspension, several Wistar rats were inoculated intraperitoneally, each rat getting 1 x 10^8. The rats were sacrificed at peak parasitaemia and blood collected into a 10ml syringe containing EDTA and PSG. Trypanosomes were separated on a DEAE-52 column equilibrated with PSG buffer as described above. Thereafter, the trypanosome elute was centrifuged in a 10ml plastic tube (Sterilin, U.K.) for 5 minutes at 1,500 x g, 4°C in an MSE Chilspin bench centrifuge (Fisons, England). The supernatant was discarded and the resulting trypanosome pellet washed three times in PSG, pH 8.0, by centrifugation as above. The final pellet was suspended in 10ml PSG and trypanosomes counted using a haemocytometer. Each camel in groups A and B was inoculated with 2 x 10^7 trypanosomes by intravenous injection.

3.3. Sampling frequency

Pre-infection baseline data was established for four weeks in all the three groups of camels at weekly intervals. Blood was collected aseptically by venipuncture of the jugular vein using an 18 x 1½" gauge needle. From each camel 5ml of blood was collected into a bijou bottle containing 5% EDTA for haematology, 15 ml was collected into a universal bottle for serology, and 5 ml into a heparinized Vacutainer® (Becton Dickinson) for biochemistry. Blood for haematology and serology was collected once per week throughout the study period and that for biochemistry once every two weeks. In addition, 1ml of blood was collected daily from all camels for monitoring parasitaemia by BCE and

MI. After treatment blood from group B camels was also collected daily and examined using the same techniques, to determine the time to disappearance of trypanosomes.

3.4. Samples

3.4.1. Unclotted Blood Samples

A blood sample for haematological analysis was preserved in EDTA at a concentration of 0.05ml of 10% solution per 5ml of blood. Bijou bottles containing EDTA blood were shaken gently to allow thorough mixing of the blood with the anticoagulant. Haematological analysis was carried out on the same day.

Blood for biochemical assays was collected into heparinized vacutainers and separated immediately by centrifugation at 3000 x g. Plasma was collected and used for analysis either immediately or was stored at -20°C until analyzed.

3.4.2. Serum Samples

Blood collected for serology was allowed to stand in universal bottles for three to four hours at room temperature and at +4°C overnight before serum was separated by centrifugation at 3,000 x g for 15 minutes. The serum samples for serology were stored in a pair of bijou bottles at -20°C in a large serum bank until analyzed.

3.5. Laboratory Procedures

3.5.1. Packed cell volume (PCV) determination

Packed cell volume was determined using EDTA-blood soon after bleeding. After thorough gentle mixing, non-heparinized capillaries

(Hawksley, England) were filled with this blood and sealed with plastoceal (Hawksley, England). These tubes were then spun in a microhaematocrit centrifuge (Hawksley, England) for 5 minutes at 1,500 x g. The tubes were then placed in a haematocrit reader (Hawksley, England) and the PCV readings taken. All PVC readings were expressed in percentages.

3.5.2. Buffy coat examination

Buffy coat examination (BCE) was carried out as described by Woo (1969). Briefly, after the PCV's were determined, the capillary tubes were cut between the buffy coat and red blood cell-junction using a diamond pen cutter. The buffy coat was applied onto a glass microscope slide and covered with a 22 x 22mm glass cover slip. Thereafter, the buffy coat smears were examined using a Zeiss microscope at a magnification of x 250 and an 11/7 condenser. At least five fields were examined and the average concentration of trypanosomes per ml scored as reported by Murray *et al.* (1977). Daily parasitaemia levels (i.e. number of trypanosomes per field) were determined throughout the study period.

3.5.3. RBC, WBC and Hb determination

The red blood cell (RBC) and white blood cell (WBC) counts were determined using an electronic coulter counter model ZBI, (Coulter Electronics Inc., Hialeah, Florida). The haemoglobin (Hb) concentration was determined using a haemoglobinometer (Coulter Electronics Inc., Hialeah, Florida).

3.5.4. Differential Leucocyte Count

Thin blood smears were made from the EDTA-blood soon after bleeding on a pair of pre-cleaned blood slides for each camel. The slides were then air dried, fixed with double-distilled methanol, stained using 10% Giemsa and kept until they were examined by microscopy.

3.5.5. Biochemical Analysis

Biochemical analysis was carried out using VETTEST® 8008-Auto Analyzer (Sanofi Animal Health Ltd, England). The following assays were carried out, alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), creatine kinase (CK), blood urea nitrogen (BUN), albumin (ALB), globulins (GLB) and total proteins (TP). Samples analyzed included those from camels bled before infection, during the course of infection and after treatment.

3.5.6. Serological Techniques

Enzyme-linked immunosorbent assays for detecting circulating antigens (Ag-ELISA) and circulating antibodies (Ab-ELISA) were employed.

3.5.6.1 Reagents for enzyme-linked immunosorbent assay

a). Preparation of trypanosomal antigen for Ab-ELISA.

Ten donor mice were infected with *T. evansi* isolate KETRI 2531 (isolated from a camel on Galana Ranch) each mouse getting 1 x 10^6 trypanosomes. When parasitaemia was in log phase (about 1 x 10^7 trypanosomes per ml of blood) the mice were killed and blood collected by cardiac puncture. Trypanosomes were separated from the blood

using a diethylaminoethyl cellulose anion exchange chromatography column (DEAE-52, Whatman) as described by Lanham and Godfrey (1970). The trypanosome suspension was centrifuged at 3,200 g for 15 minutes, the supernate discarded and the resulting pellet washed three times in phosphate buffered saline glucose (PSG), pH 8, by centrifugation at 3,200 x g for 15 minutes. The trypanosomes were re-suspended to 1.0ml in PSG. This was used to infect 50 rats, each getting a 1ml suspension of 1 x 10^8 trypanosomes by intraperitoneal injection. The trypanosomes were harvested from the rat blood at fulminating parasitaemia, separated and washed as above. Thereafter, they were sonicated at maximum amplitude for about 60 seconds, three times with cooling in between on ice using an MSE ultrasonic Disintegrator (Safam Electrical Instrument Co. Ltd., England). The lysate was centrifuged at 10,000 x g for 30 minutes at +4°C. and the protein content of the supernatant determined by Bio-rad protein assay. Briefly, the dye reagent concentrate was diluted 1:4 (v/v) with deionized water and filtered. A standard protein, bovine serum albumin (BSA) of known concentration (70mg/dl) was prepared. Five milliliters of the diluted dye was dispensed into test tubes and 0.1ml of the standard BSA and the unknown protein fractions added to the diluted dye. These were mixed gently and 100µl of each dispensed per well of a micro-titre ELISA plate (NUNC®, USA). The concentration of the proteins was measured using a micro-ELISA Auto Reader (MR 580) at 595nm wavelength. The protein concentration in the supernatant was then calculated using the formula:

$$\text{Concentration of unknown protein (mg/ml)} = \frac{\text{OD of unknown sample x conc. of known sample}}{\text{OD of known sample.}}$$

The optimal concentration of the trypanosomal antigen supernatant was determined using a checkerboard titration. These samples were stored in liquid nitrogen at -196°C. and later used in antibody-ELISA (Ab-ELISA).

b). Preparation Of Trypanosomal Antigens For Raising Polyclonal Rabbit Anti-*T. evansi* Immune Serum

Ten rats were infected with the same isolate KETRI 2531 at 1 x 10^6 trypanosomes per rat and the trypanosomes harvested as described in 3.5.6.1 (a) above. The final trypanosome pellet was mixed with a lysing and protein stabilizing solution (containing 2mM amino carproic acid, 2mM EDTA, 2mM diethiothreitol). Thereafter, the trypanosomal lysate was centrifuged at 12,000 x g (using an MSE Chilspin) bench centrifuge (Fisons, England). The supernatant was then dialysed overnight in 0.01M PBS, pH 8.0. The protein concentration was determined using Bio-rad protein assay as in 3.5.6.1 (a). This antigen was stored in liquid nitrogen at -196°C and later used to raise rabbit anti-*T.evansi* (RATE) hyper-immune serum as described in (c) below.

c). Preparation, purification and conjugation of Rabbit anti-*T. evansi* IgG (RATE/IgG)

Two New Zealand white rabbits were inoculated with the lysate antigens (from (b) above) using the method described by Thalhamer and Freund (1984). Briefly, 2mg of soluble trypanosome proteins were injected subcutaneously at day O and intramuscularly at day 7 and day 21. At day 22, 5mg of the lysate antigens was administered by subcutaneous injection, and at day 23 by intravenous injection. The first dose was administered with Freud's complete adjuvant (Sigma, USA), subsequent injections were administered in 140 mM sodium chloride. Antiserum was collected 10 to 14 days after the last injection. The Ouchterlony double immuno-diffusion technique (DID) (1964) was employed to determine the antibody response and the specificity of the antibody in the rabbit serum. A standard template for gel double diffusion (GDD) was used to punch wells in agarose, (Merck, WG) in 0.01M PBS, pH 7.2 (1% w/v), containing sodium azide. The wells were then filled with antigen (in the central well) and serum (in the peripheral wells), the slides placed in a storage box on a dampened filter paper and left to develop over-night. The gel reactions were read after 24 hours and the serum with the highest antibody titre (up to 1/64 dilution) used.

Purification of RATE-IgG from the antisera was done by salt fractionation. The antisera were precipitated with saturated ammonium sulphate solution (SAS, 100%), reconstituted in 0.01M PBS (pH 7.5) and then extensively dialysed against the same buffer. The IgG was then applied on a DEAE-52 column equilibrated with 0.01M phosphate buffer pH 7.5. Fractions of IgG (5ml) were collected into test tubes and the

protein concentration of each fraction determined using the standard Bio-rad protein assay. The IgG rich protein tubes were pooled into a dialysis tubing and further concentrated using polyethylene glycol (MW 2000) for 8 hours. Finally the protein concentration of the final volume of the immunoglobulin fraction was determined using the Bio-rad protein assay as described earlier.

The immunoglobulin (RATE/IgG) purified above was then conjugated with horseradish peroxidase (Sigma, Type VI) following the method of Nakane and Kawaoi (1974) as modified by Henning and Nielsen (1987).

d) Preparation of Camel IgG and rabbit-anti-camel IgG

Camel serum (50 ml) was warmed to 25°C and purification of the IgG from the serum done by salt fractionation using saturated ammonium sulphate solution as described in 3.5.6.1 (c) above.

The IgG obtained was used to immunize two New-Zealand white rabbits to produce rabbit anti-camel IgG. The rabbits were immunized with 2mg of camel IgG in Freud's complete adjuvant by subcutaneous injection into eight sites on the rabbits' back. Boosting was done on days 21 and 35 with the same dose of camel IgG in Freud's incomplete adjuvant intramuscularly. Serum was collected 10 days after the last boost. Rabbit anti-camel IgG was prepared and conjugated as in 3.5.6.1 (c) above.

3.5.6.2. Antigen-ELISA Technique

The assay system used was based on the double antibody-sandwich assay described by Bidwell and Voller (1981) as modified by

Olaho-Mukani *et al.* (1992a). Briefly, Dynatech microtitre plates (IMMULON^{R}, U.S.A) were presensitized with 1% glutaraldehyde at room temperature for one hour and then rinsed three times with distilled water. The plates were then coated with 100µl/well of polyclonal anti-*T.evansi* (RATE) immunoglobulin at a concentration of 10µg/ml in carbonate/bicarbonate buffer, pH 9.6, and incubated at +4°C overnight. Thereafter, the plates were washed three times (5 minutes per wash) with 0.01M PBS Tween-20, pH 7.4, and blocked with 1% bovine serum albumin (BSA) Tween-20 buffer and incubated for 30 minutes at 37°C. Test and control sera were added to each well (100µl/well) in the dilution 1/4 (previously determined using a checkerboard titration) followed by incubation for 30 minutes at 37°C. The plates were washed again as above and 100µl/well of rabbit anti-*T.evansi* peroxidase conjugate (from 3.5.6.1 (d)) added and the plates incubated and washed as before. Tetramethyl-benzidine (TMB, Sigma) substrate was added and the plates incubated for 15 minutes at 37°C. The reaction was stopped by adding 50µl/well of 2M sulphuric acid (H_2SO_4) and the optical density (OD) values determined at 450nm using a Dynatech micro-ELISA plate reader (MR 580, DYNATECH, U.S.A.). The cut-off OD point for a positive Ag-ELISA was calculated as: mean OD of control animals mean x 2 $\pm$ 1 SD.

3.5.6.3. Antibody-ELISA Technique

Dynatech micro-titre plates (USA) were coated with an optimal concentration of soluble trypanosomal antigen in 100µl/well of carbonate buffer, pH 9.6. The plates were incubated at 37°C for 3 hours, then at +4°C overnight. The plates were washed three times (5 minutes/wash)

with 0.01M PBS Tween-20, pH 7.4. Subsequently, 100µl/well test and control sera diluted at 1/400 in PBS-Tween-20 buffer containing 63mg/l of gelatin was added and the plates incubated for 1 hour at 37°C. The washing procedure was repeated and rabbit anti-camel IgG-peroxidase conjugate diluted optimally at 1:500 in PBS Tween-20 was added at 100µl/well. The plates were again incubated at 37°C for 1 hour and washed as before. The remaining stages of the test were as described for Ag-ELISA.

Optical density reading of 0.150 and above were considered positive for the test, since none of the negative control sera gave an OD reading greater than 0.145.

3.5.7. Mouse inoculation (MI)

0.5ml of EDTA blood from each camel in groups A and B was inoculated into a pair of Swiss-white mice intraperitoneally post-infection daily for three weeks and thereafter once a week to the end of the study. Parasitaemia in the mice was monitored daily by microscopic examination of wet smears of blood from their tails. Once parasitaemia was noted the mice were disposed off. The same procedure of MI was followed to monitor the time to disappearance of parasites, in camels in group B following treatment. Mice that were aparasitaemic were monitored for a total of 60 days after which they were disposed of.

3.6. Clinical Examination

All the camels were examined daily and all the clinical signs manifested recorded accordingly. Rectal temperature, body condition, appetite and demeanor were noted and recorded daily at 9.00am. The

body weights were estimated weekly using the method of Schwartz *et al.* (1983).

3.7. Pathology

Post mortem examination was carried out on all camels that died during the experiment. Samples for histopathology were taken from most organs and processed using the standard procedures.y

3.8 Data Analysis

Analysis of the sequential data from each infected group was compared to the data of the non-infected control camels. The difference between the control group and either of the infected groups was analyzed by the student's "*t*" test for unequal means (Snedecor and Cochran, 1982). This design was employed in the analysis of the packed cell volume (PCV), red blood cells count (RBC), haemoglobin concentration (Hb), white blood cell count (WBC) and the biochemical values. For any animal that died during the study, the last value in any variable was used for the analysis. All graphs presented in this study were drawn from the means of all the camels in a particular group.

CHAPTER FOUR

RESULTS.

4.1. Clinical Assessment

4.1.1. Clinical Signs

The first clinical signs observed in all the infected camels were inappetance and dullness. These occurred, on the average, 7 days after infection. The camels had enlarged superficial lymph nodes, the most prominent ones being the inferior cervical (plate 1) and supramammary lymph nodes. Excessive bilateral ocular discharge was observed in all the camels in group A that were not treated (plate 2). All the infected animals developed ventral oedema that started around the umbilical area and spread anteriorly to the sternal pad (plate 3). Posteriorly, the oedema extended to the preputial area in males (plate 4) and the udder in females. Oedema of the feet was observed in only one camel in group A that survived to the end of the experiment. The animal had poor coat quality. One of the camels in group A developed a skin condition characterized by dandruff. These untreated animals lost weight and became emaciated, characterized by sunken flanks, prominent ribs and drooping hump (plate 5) when compared to the treated camels (group B) and the controls (plate 6a and b). Two camels in group A that were infected and not treated died 11 and 12 days, respectively, following infection, while a third camel in the same group died three days before the study was terminated. One camel in this group (C161) had low PCV throughout the pre-infection period and was not used in the experiment. Thus, there were only four camels in group A, of which only one survived to the end of the study and was treated before being

discharged from the stables. Appetite and temperature returned to normal after parasites disappeared from circulation.

Camels in group B showed the same clinical picture as group A. These animals were treated during the first peak of parasitaemia. Recovery was rapid with the animals regaining their appetite one day after treatment. All the camels in group B that were infected and treated survived to the end of the experiment.

Other clinical signs observed in some of the animals included coarse tremors, unsteady gait and tendency to fall down, especially those that developed fulminating parasitaemia by day 12. The camels tended to lie down. Animal number C159 was recumbent five days before it died (plate 7).

Plate 1: A camel infected with *T. evansi* showing an enlarged inferior cervical lymph node 3 weeks post-infection.

Plate 2: A camel infected with *T. evansi* showing excessive serous ocular discharge 3 weeks post-infection.

Plate 3: Ventral oedema extending anteriorly to the sternal pad in a camel infected with *T. evansi* 4 to 6 weeks post-infection.

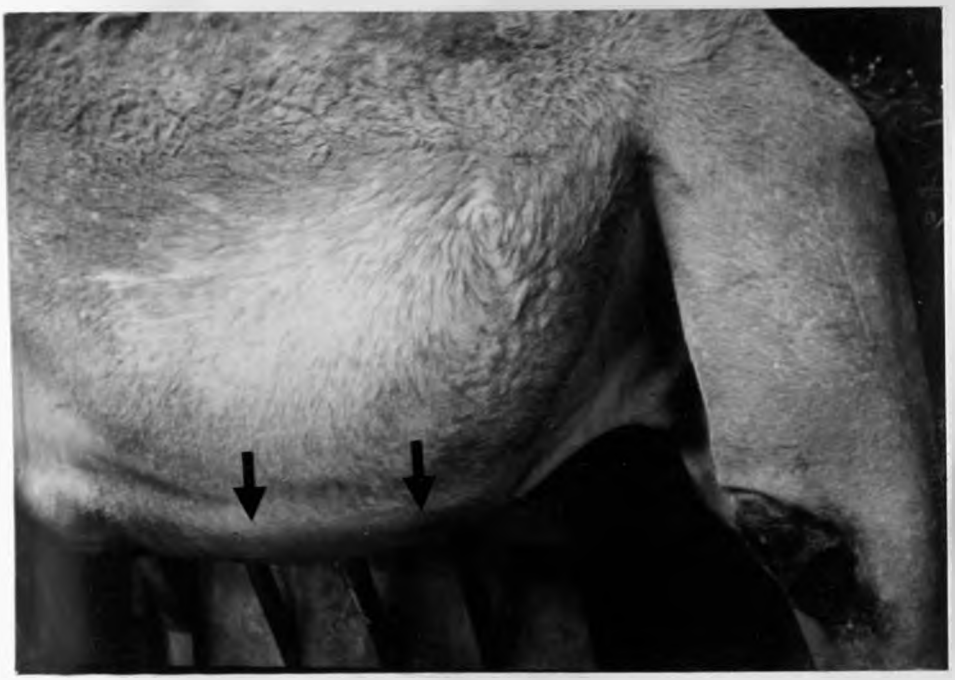


Plate 4: Ventral oedema extending posteriorly to the preputial area in a male camel infected with *T. evansi* 4 to 6 weeks post-infection.

Plate 5: Emaciated camel in the untreated group (A) at the end of the study (16 weeks).

Plate 6(a): Infected and treated camel (Group B) at the end of the study (16 weeks).

Plate 6(b): Control camel (non-infected) at the end of the experiment (16 weeks).

Plate 7: A recumbent camel infected with *T. evansi* (group A) a few days before death (16th week).

4.1.2 Parasitaemia

All infected camels developed parasitaemia between days 4 and 7 after infection. The first fulminating peak of parasitaemia was observed eleven days post infection in all camels. The highest level of parasitaemia attained was +6 scored according to Murray *et al.* (1977). Each camel attained this score at least once in the first 40 days post infection (Table 1).

Two camels in group A (C149 and C156) died during the first peak of parasitaemia. The other two camels in the same group developed several relapsing peaks of parasitaemia (Fig. 1). The relapsing parasitaemia in these animals was milder or scanty, thus not comparable to the first peak. Camel C159 developed intermittent parasitaemia throughout the study period and died shortly before the end of the experiment. The intermittent parasitaemic peaks were accompanied by increases in rectal temperature which were erratic but persistent. One animal in this group survived to the end of the study and was treated before being released from the stable.

In group B the first fulminating parasitaemia was observed during the same period as in group A. During this acute phase, the camels were treated with Cymelarsan at a dose of 0.25mg/kg body weight by deep intramuscular injection. No parasites were observed 24 hours after treatment as monitored by BCE and MI (Fig. 2). These animals remained negative for parasites throughout the study period when monitored using BCE and MI.

Key

d = dead

6+ = Over 100 trypanosomes per microscopic field (x 250)

5+ = 10 to 100 trypanosomes per microscopic field (x 250)

4+ = 1 to 10 trypanosomes per microscopic field (x 250)

3+ = 1 trypanosome per microscopic field (x 250)

2+ = 1 to 10 trypanosomes per slide preparation

1+ = 1 trypanosome per slide preparation

0 = 0 trypanosome per slide preparation

Table 1: Parasitaemia level using buffy coat examination (BCE) for the first 40 days of infection.

Days Post infection

ANIMAL No.	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
GRP A																		
149	0	0	0	0	3+	5+	6+	6+	d									
156	0	0	4+	5+	5+	6+	6+	d										
155	0	4+	5+	6+	0	0	0	0	0	0	0	4+	6+	5+	0	0	0	0
159	0	0	0	1+	2+	4+	5+	0	0	3+	3+	5+	5+	3+	0	5+	5+	6+
GRP B																		
147	0	0	1+	4+	0	0	0	2+	3+	5+	6+	0	0	0	0	0	0	0
151	0	0	0	0	4+	5+	5+	6+	5+	6+	6+	0	0	0	0	0	0	0
160	0	0	0	0	4+	5+	5+	5+	6+	6+	6+	0	0	0	0	0	0	0
162	0	0	0	2+	4+	5+	5+	6+	5+	6+	6+	0	0	0	0	0	0	0
261	0	0	0	2+	3+	5+	5+	6+	6+	6+	6+	0	0	0	0	0	0	0

GRP A	21	22	23	24	25	26	27	28	30	31	32	33	34	35	36	37	38	39
149	d																	
156	d																	
155	0	0	4+	5+	4+	0	0	0	0	0	0	0	1+	0	0	0	0	0
159	6+	0	0	0	0	0	0	1+	0	0	0	1+	0	0	0	0	0	0
GRP B																		
147	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
151	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
162	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
261	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 1: Mean daily parasitaemia level of infected non-treated camels in group A that had intermittent parasitaemia to the end of the study.

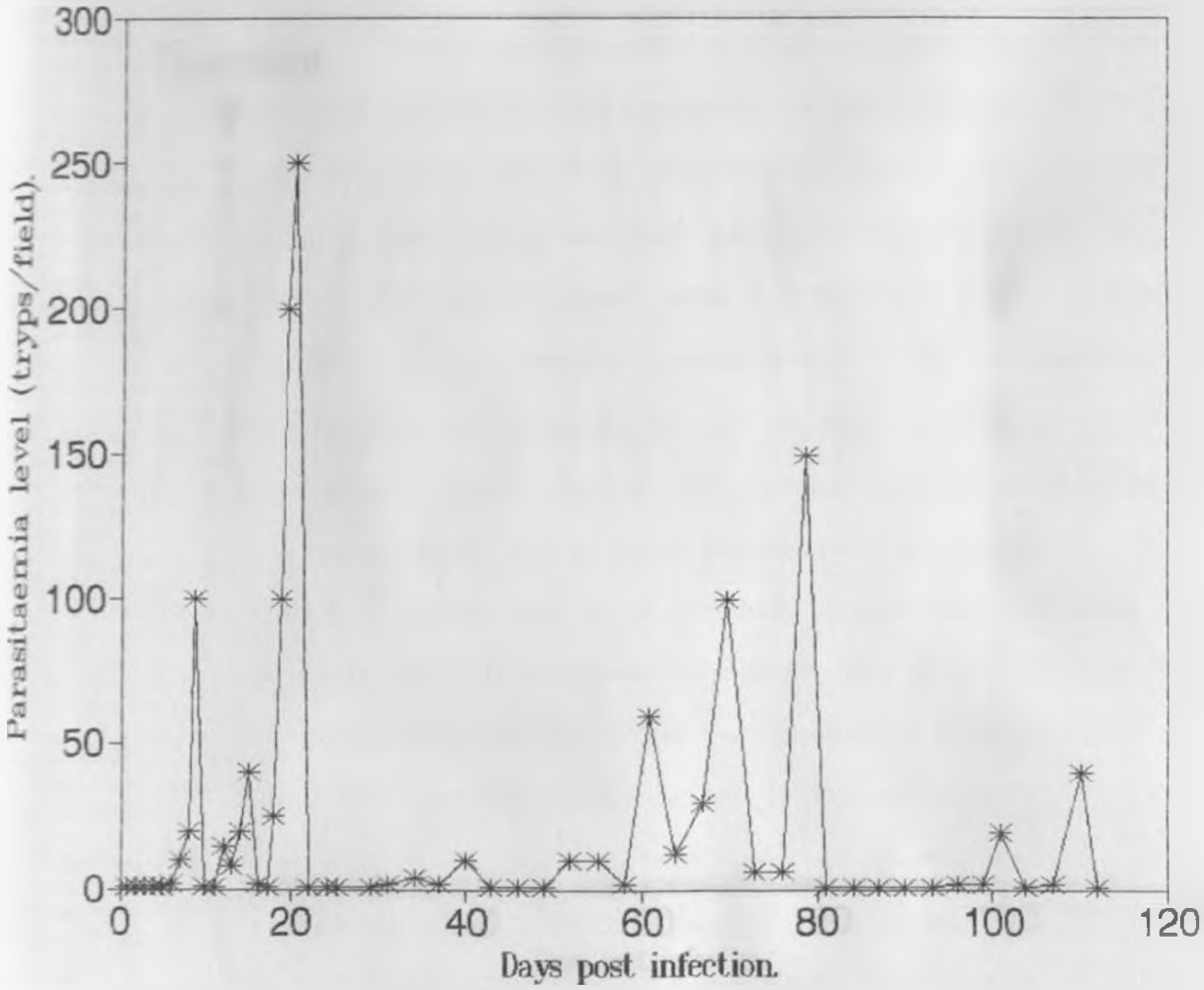
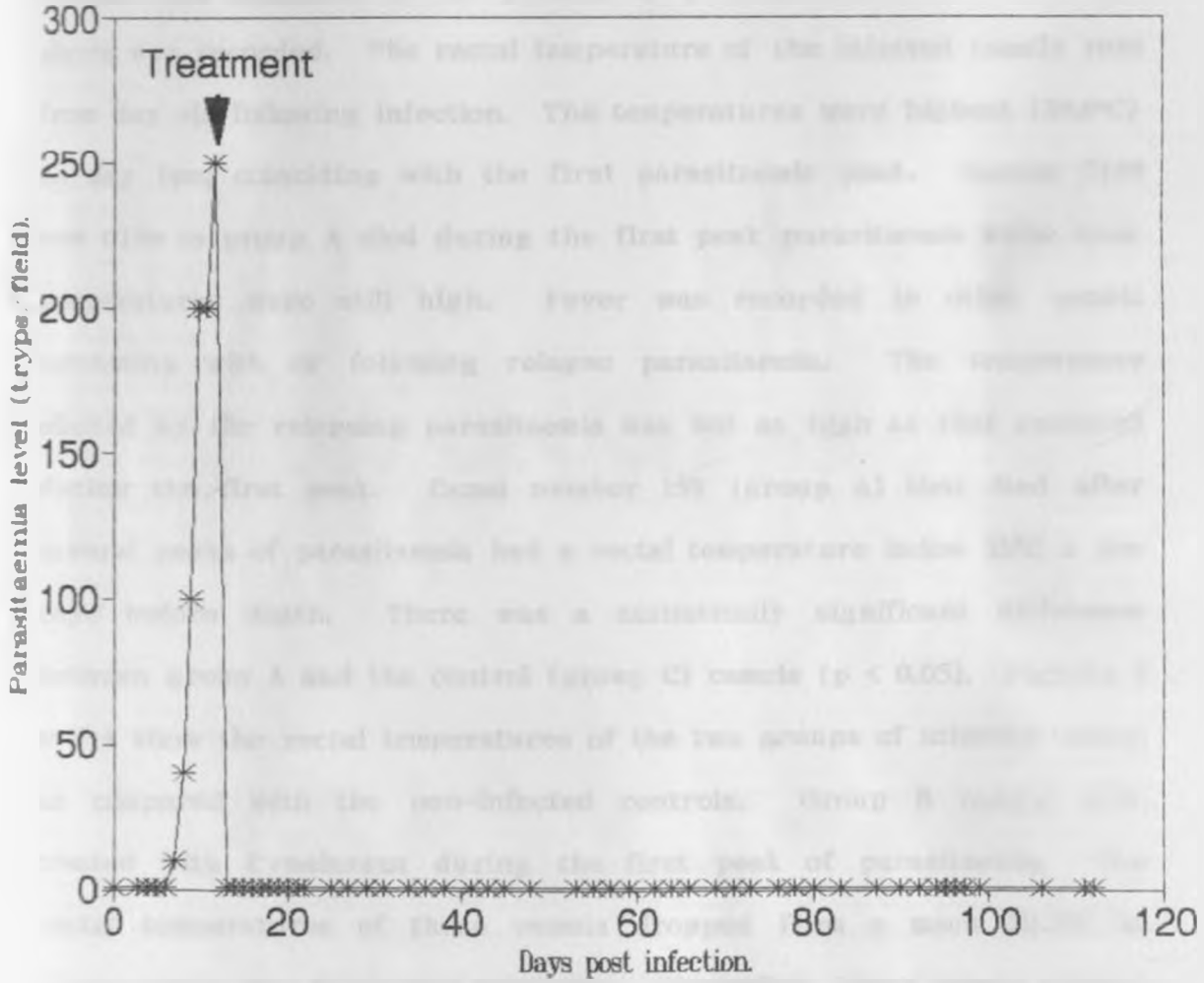


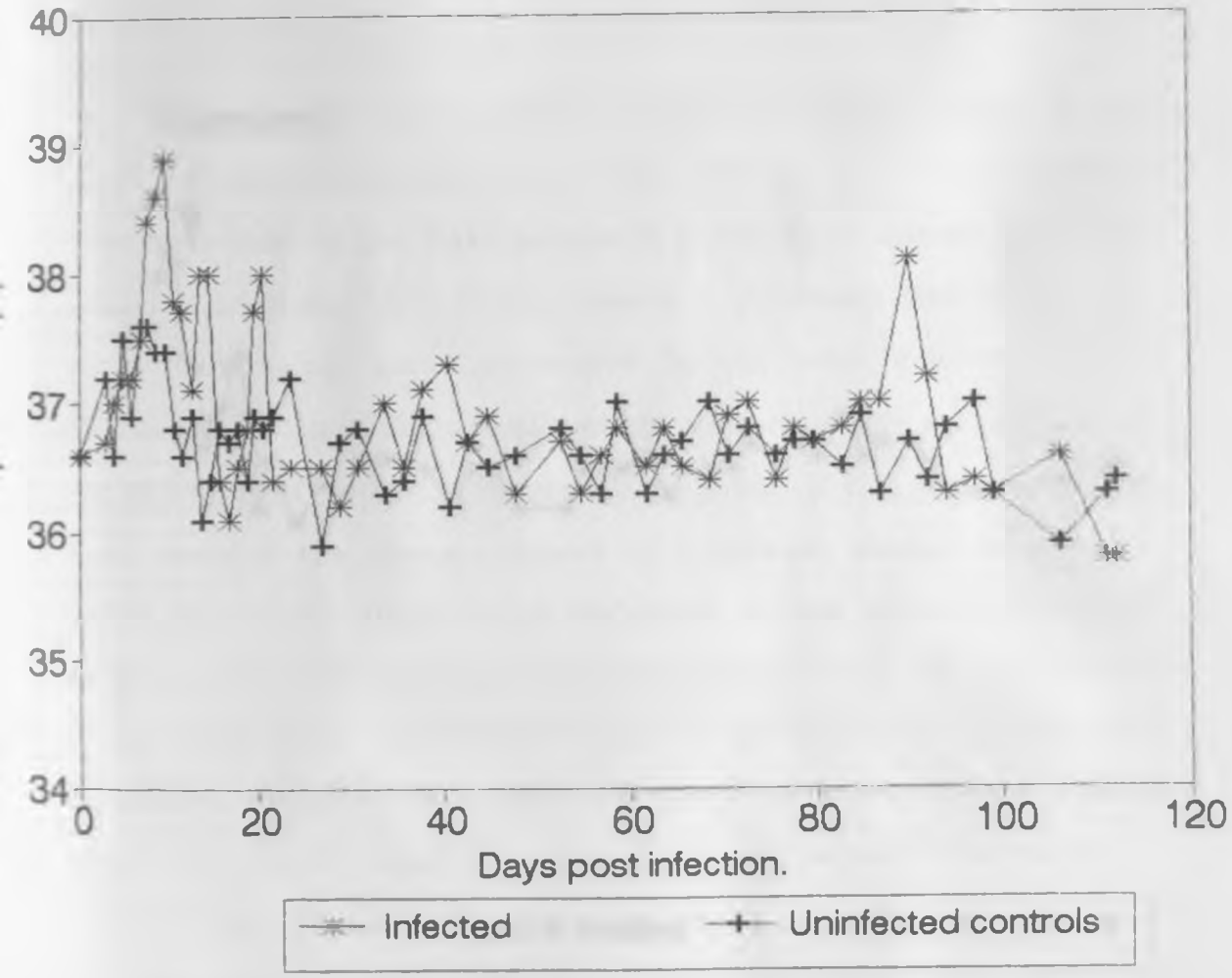
Figure 2: **Mean daily parasitaemia level of infected and treated camels (Group B).**



4.1.3. Rectal temperature

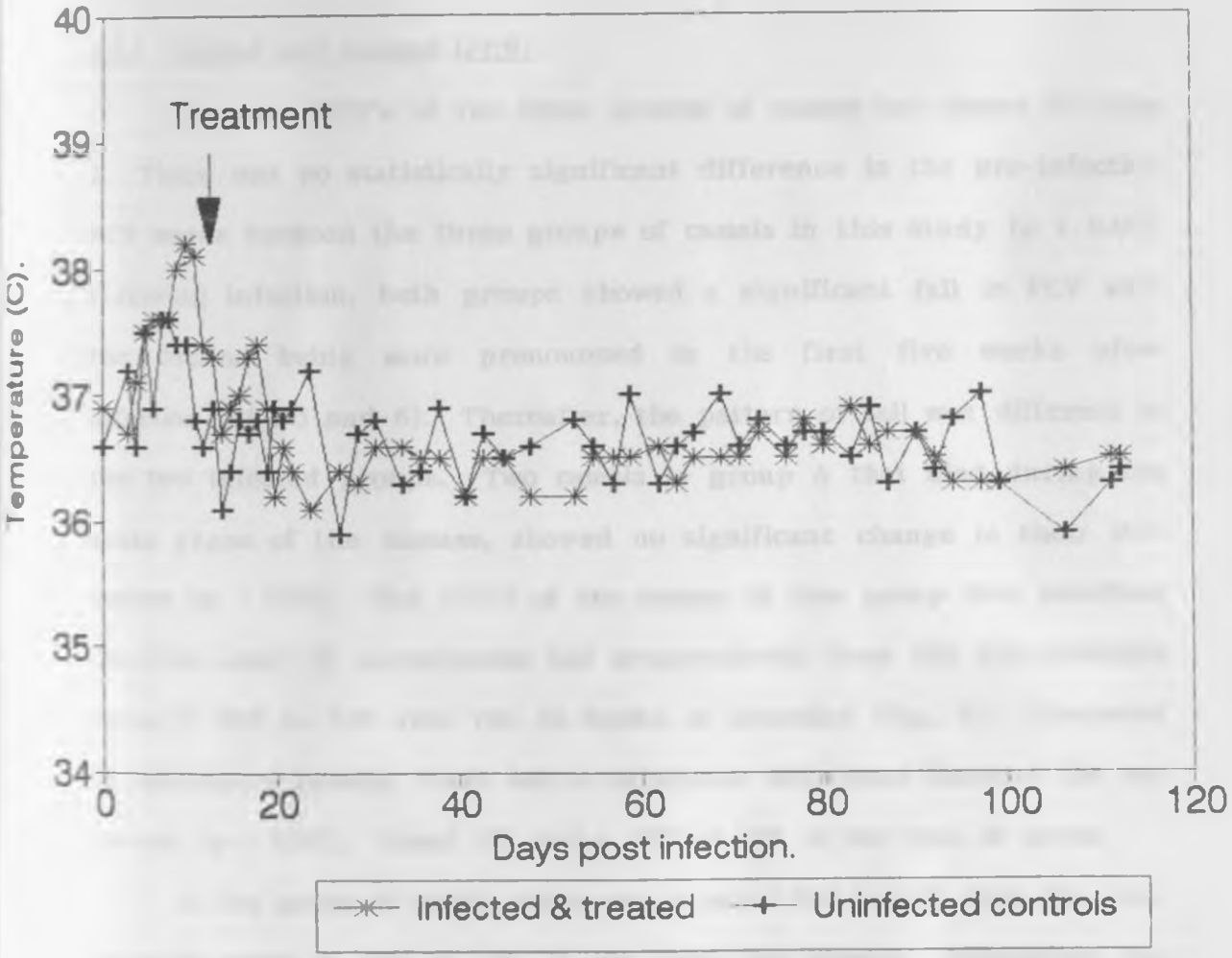
The mean temperature of all the camels used in the study showed no significant difference prior to infection ($p > 0.05$). The highest temperature of the controls was 37.6°C (Appendix I), and in this study, pyrexia was regarded to be present if a temperature of 38.0°C and above was recorded. The rectal temperature of the infected camels rose from day six following infection. The temperatures were highest (39.6°C) on day ten, coinciding with the first parasitaemic peak. Camels C149 and C156 in group A died during the first peak parasitaemia while their temperatures were still high. Fever was recorded in other camels coinciding with or following relapse parasitaemia. The temperature elicited by the relapsing parasitaemia was not as high as that recorded during the first peak. Camel number 159 (group A) that died after several peaks of parasitaemia had a rectal temperature below 35°C a few days before death. There was a statistically significant difference between group A and the control (group C) camels ($p < 0.05$). Figures 3 and 4 show the rectal temperatures of the two groups of infected camels as compared with the non-infected controls. Group B camels were treated with Cymelarsan during the first peak of parasitaemia. The rectal temperatures of these camels dropped from a mean 39.2°C to 37.3°C within two days after treatment. Thereafter, these camels showed no temperature rise throughout the study period. There was no statistically significant difference between group B and C camels. The control camels had a stable temperature throughout the experimental period ranging between 36.1°C and 37.4°C. Following infection and up to the end of the experiment, there was significant difference between group A camels and group B ($p < 0.05$).

Figure 3: Mean daily rectal temperatures in infected non-treated camels (Group A)



Day 0= Mean of 28 days before infection

Figure 4: Mean daily rectal temperatures in infected and treated camels (Group B).



Day 0= Mean of 28 days before infection.

4.2 Haematological Findings

The range of various pre-infection haematological parameters in the present study were compared to normal values for the dromedary camel in literature (Appendix II).

4.2.1 Packed cell volume (PCV)

The mean PCV's of the three groups of camels are shown in table 2. There was no statistically significant difference in the pre-infection PCV means between the three groups of camels in this study ($p > 0.05$). Following infection, both groups showed a significant fall in PCV with the decline being more pronounced in the first five weeks after infection (Fig. 5 and 6). Thereafter, the pattern of fall was different in the two infected groups. Two camels in group A that died during the acute phase of the disease, showed no significant change in their PCV values ($p > 0.05$). The PCV's of the camels in this group that survived the first peak of parasitaemia fell progressively from the pre-infection value of 28% to 21% over the 16 weeks of infection (Fig. 5). Compared to the control camels, there was a significant difference between the two groups ($p < 0.05$). Camel 159 had a PCV of 18% at the time of death.

In the group B camels there was a rapid fall in PCV from the pre-infection mean of 28% to 23%, in the first five weeks. Thereafter, the mean PCV's of these camels rose slightly and leveled off at 24% by the end of the study (Fig. 6 and Table 2). Analysis of the PCV values over the 16 weeks following infection indicated a significant difference between the control group and group B ($p < 0.05$). However, the PCV's had not risen to the pre-infection levels by the end of the experiment. Control animals showed no significant changes in their mean PCV values

throughout the study period. When the PCV of group A (infected and not treated) was compared to group B (infected and treated) there was a significant difference between the two groups only in the last 7 weeks of the study ($p < 0.05$) with the treated camels having a higher mean PCV value.

Figure 5: Mean weekly packed cell volume of infected non-treated camels (Group A).

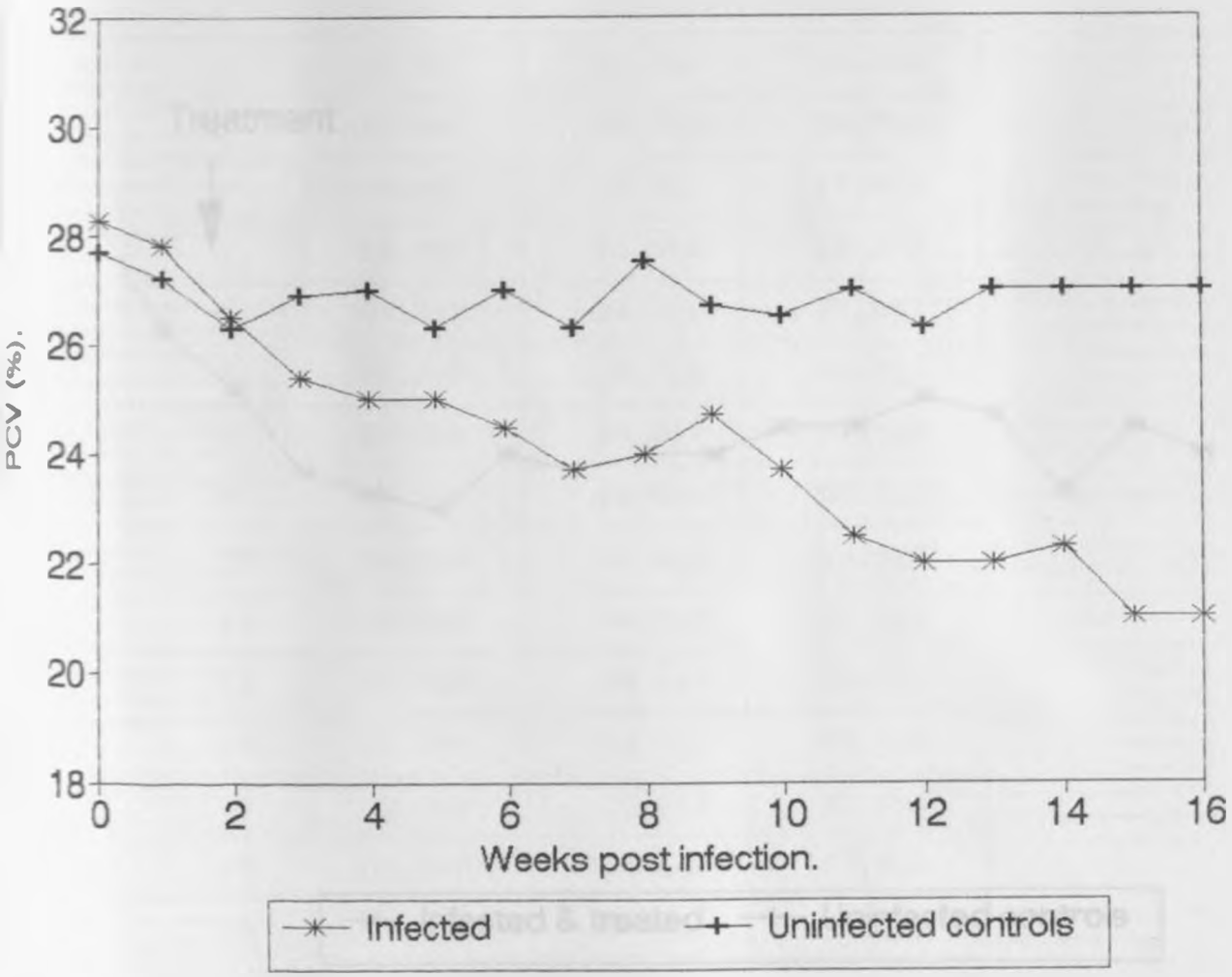
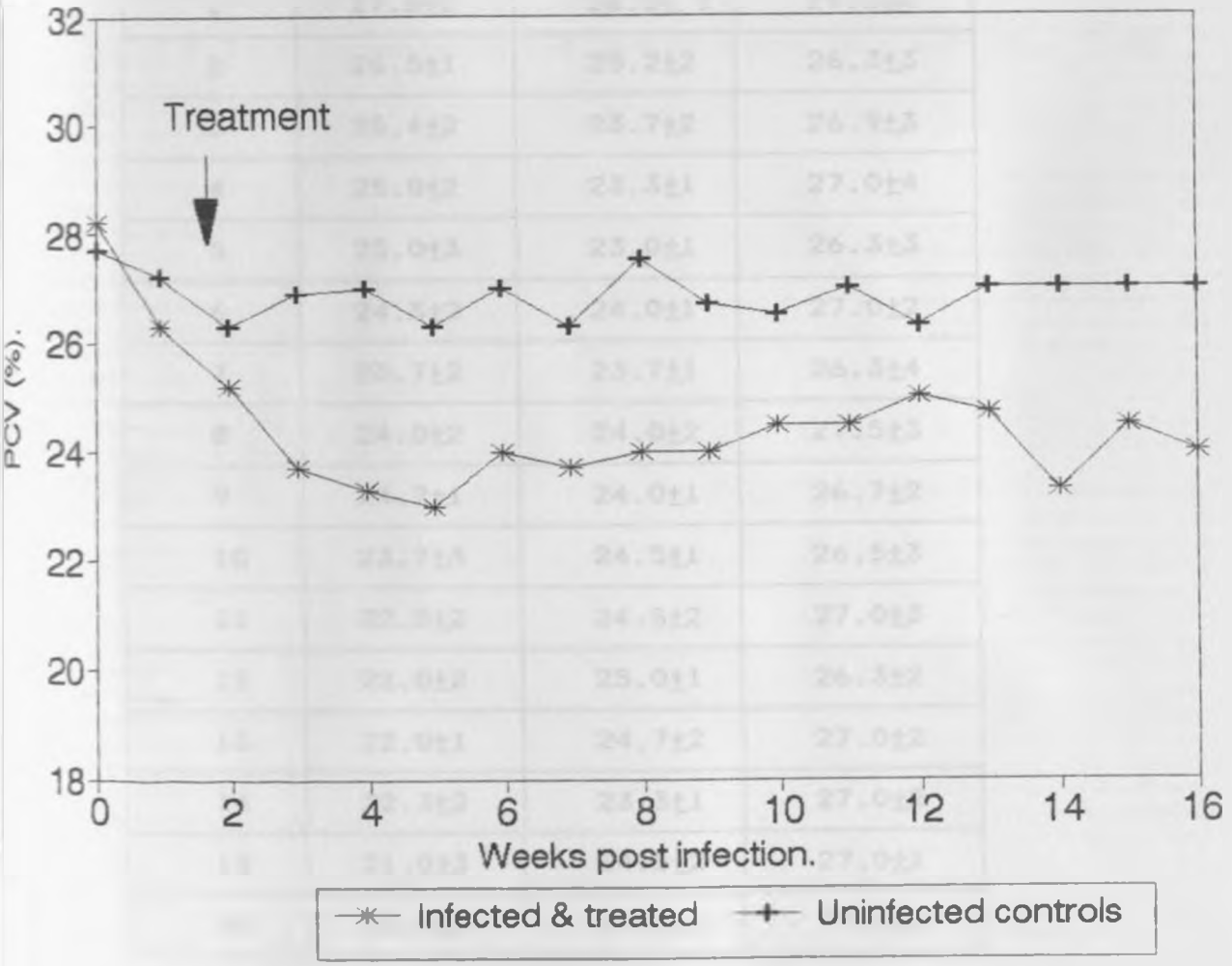


Figure 6: Mean weekly packed cell volume of infected and treated camels (Group B).



Week 0= mean of 4 weeks before infection

Table 2: Mean PCV values (%) (±SD) in infected and control camels throughout the study period.

	Camel Groups		
Weeks PI	A (n=4)	B (n=5)	C (n=2)
0	28.3±1	28.2±2	27.7±2
1	27.8±2	26.3± 1	27.2±4
2	26.5±1	25.2±2	26.3±3
3	25.4±2	23.7±2	26.9±3
4	25.0±2	23.3±1	27.0±4
5	25.0±3	23.0±1	26.3±3
6	24.5±2	24.0±1	27.0±2
7	23.7±2	23.7±1	26.3±4
8	24.0±2	24.0±2	27.5±3
9	24.7±1	24.0±1	26.7±2
10	23.7±3	24.5±1	26.5±3
11	22.5±2	24.5±2	27.0±3
12	22.0±2	25.0±1	26.3±2
13	22.0±1	24.7±2	27.0±2
14	22.3±2	23.3±1	27.0±2
15	21.0±3	24.5±2	27.0±1
16	21.0±3	24.0±1	27.0±3

**Week 0= mean of 4 weeks before infection.
PI = Post-infection

T-test for the hypothesis "mean 1 = mean 2"
(Alpha=0.05).

Pre-infection:
- A vs C $p > 0.05$.
- B vs C $p > 0.05$.
- A vs B $p > 0.05$.

Post-infection:
- A vs C $p < 0.05$.
- B vs C $p < 0.05$.
- A vs B $p > 0.05$.

4.2.2. Red Blood cell count (RBC)

The results of red blood cell (RBC) counts are given in Table 3. Analysis of the values during the 4 weeks prior to infection indicated no significant difference between the three groups ($p > 0.05$). Following infection, however, there was a gradual but significant drop in the RBC values of both the untreated and treated camels ($p < 0.05$) (Fig. 7 and 8).

Figure 7: Mean weekly red blood cell count of infected non-treated camels (Group A).

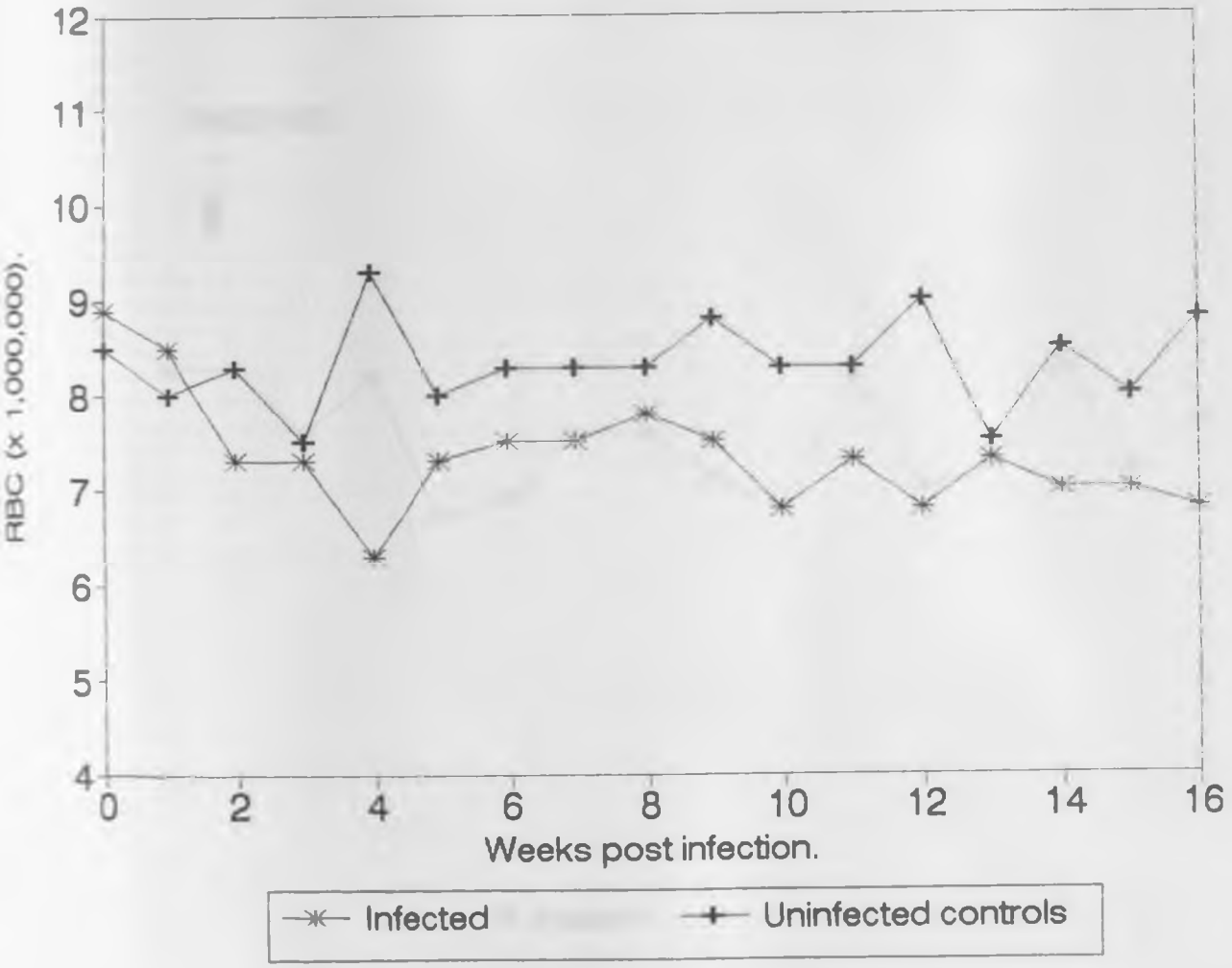
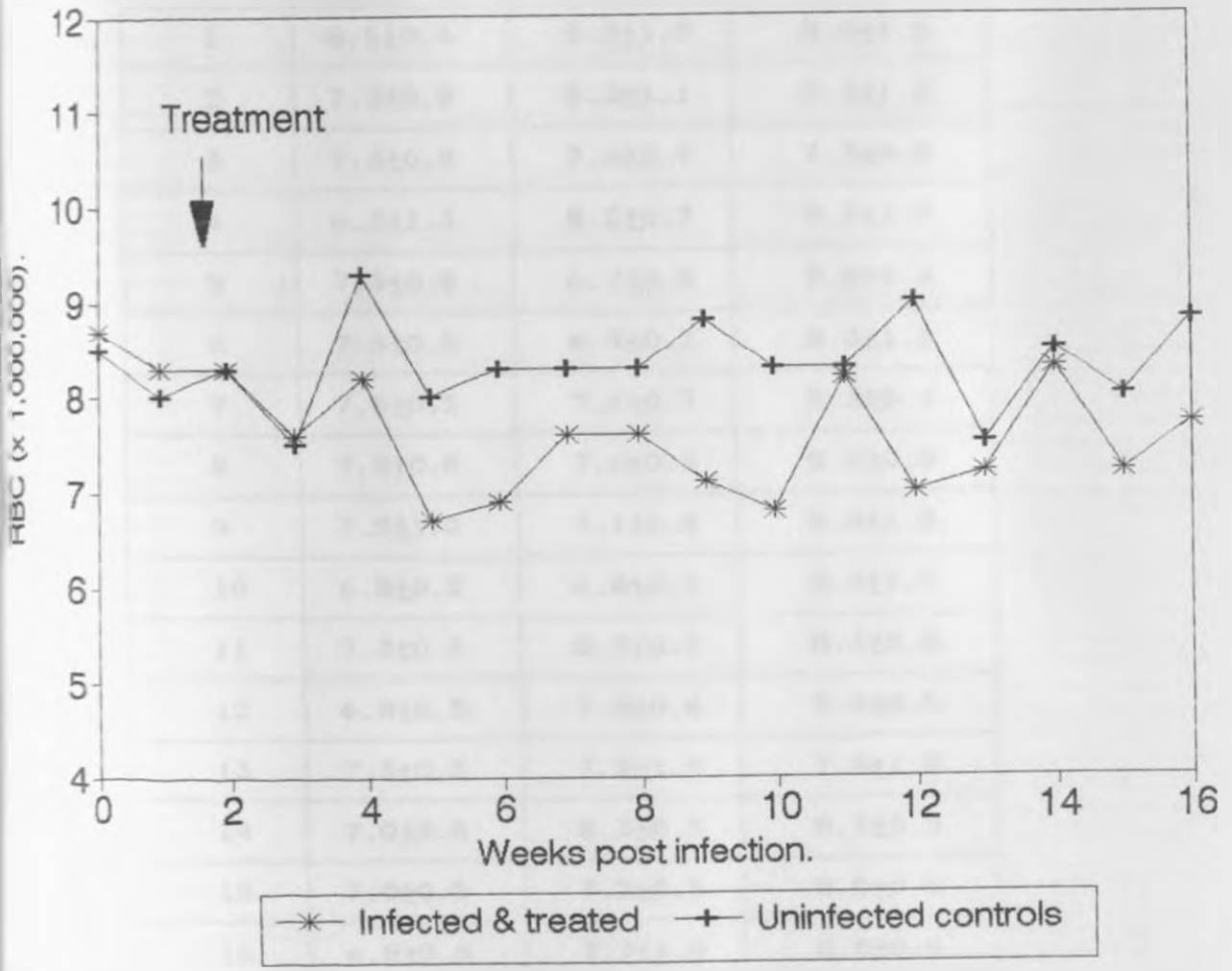


Figure 8: Mean weekly red blood cell count of infected and treated camels (Group B).



Week 0 = mean of 4 weeks before infection.

Table 3: Mean red blood cell count ($x10^6$/ml) (±SD) of infected and control camels throughout the study period.

Weeks PI	Camel Groups		
	A (n=4)	B (n=5)	C (n=2)
0	8.9±0.6	8.7±0.5	8.5±0.8
1	8.5±0.6	8.3±1.0	8.0±1.5
2	7.3±0.8	8.3±1.1	8.3±1.3
3	7.3±0.8	7.6±0.9	7.5±0.5
4	6.3±1.3	8.2±0.7	9.3±1.3
5	7.3±0.8	6.7±0.8	8.0±0.3
6	7.5±0.5	6.9±0.7	8.3±1.3
7	7.5±0.5	7.6±0.7	8.3±0.3
8	7.8±0.8	7.6±0.6	8.3±0.8
9	7.5±1.0	7.1±1.8	8.8±1.8
10	6.8±0.3	6.8±0.5	8.3±1.0
11	7.3±0.3	8.2±0.7	8.3±0.3
12	6.8±0.3	7.0±0.4	9.0±0.5
13	7.3±0.3	7.2±1.0	7.5±1.0
14	7.0±0.0	8.3±0.5	8.5±0.5
15	7.0±0.5	7.2±0.5	8.0±0.5
16	6.8±0.3	7.7±1.0	8.8±0.8

**Week 0= mean of 4 weeks before infection.
PI = Post-infection

T-test for the hypothesis "mean 1 = mean 2" (Alpha=0.05).

Pre-infection:
- A vs C $p > 0.05$.
- B vs C $p > 0.05$.
- A vs B $p > 0.05$.

Post-infection:
- A vs C $p < 0.05$.
- B vs C $p < 0.05$.
- A vs B $p > 0.05$.

4.2.3. Haemoglobin (Hb) concentration

Pre-infection values of haemoglobin content were similar in all groups of animals (Table 4; Figs. 9 and 10). There was a gradual fall in Hb concentration from the pre-infection value up to week three post infection in both groups A and B camels, even though this drop was not significant ($p > 0.05$). Towards the end of the experiment, however, the Hb concentration of group A camels was significantly lower than that of the control camels ($p < 0.05$). During the same period, group B camels had a lower Hb concentration than the control camels, but the results were not statistically significant ($p > 0.05$).

Figure 9: Mean weekly haemoglobin concentration of the infected non-treated camels (Group A).

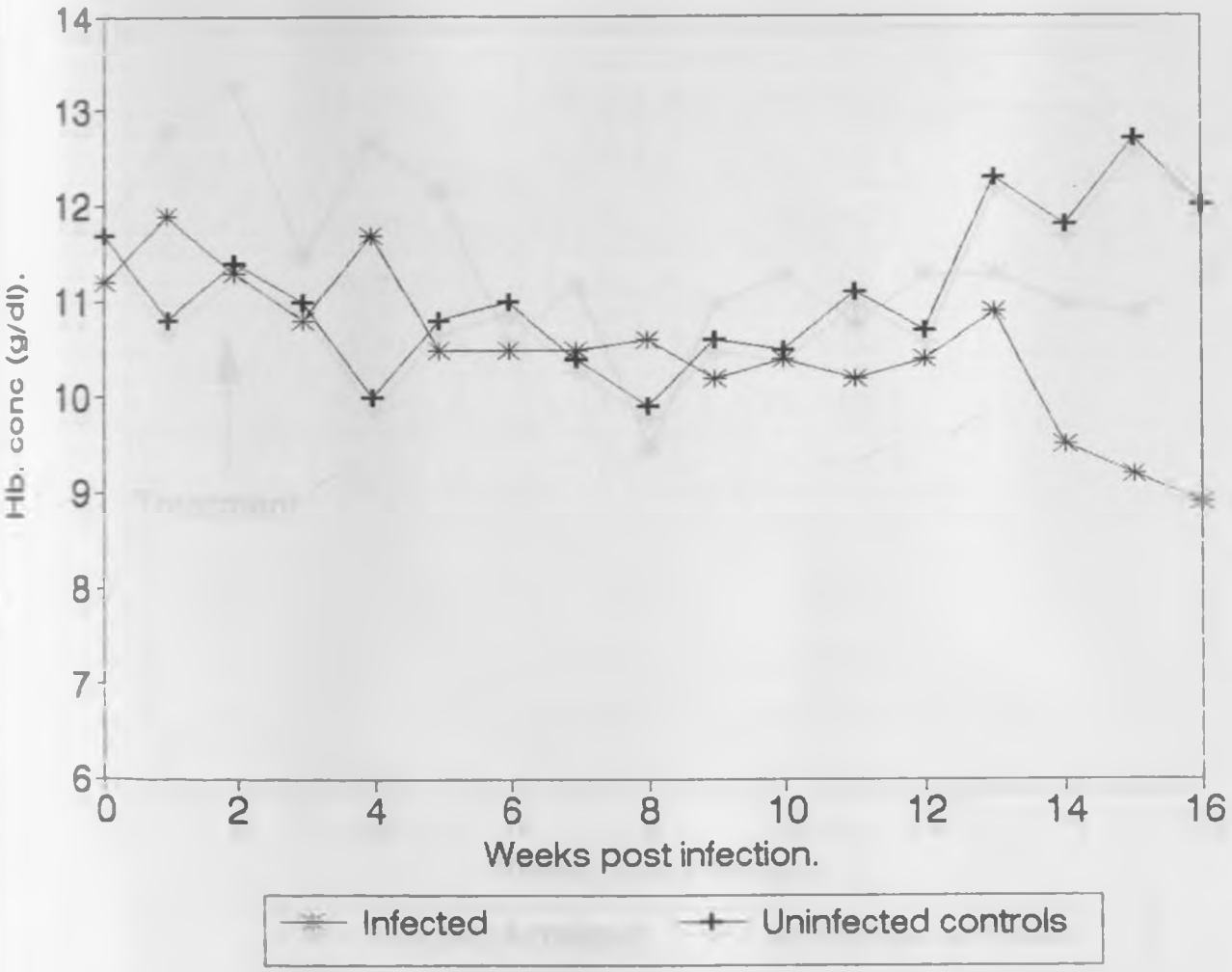
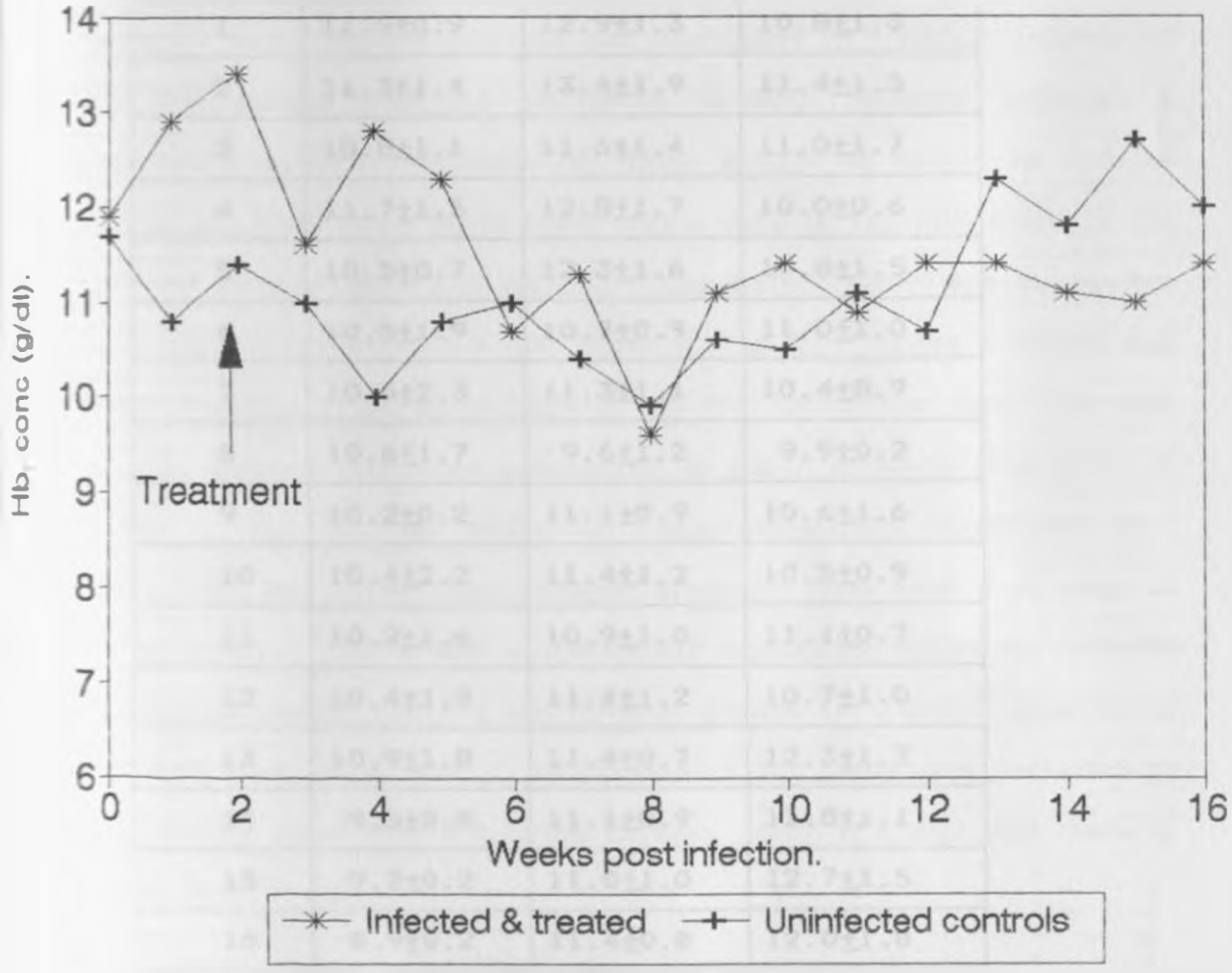


Figure 10: Mean weekly haemoglobin concentration of the infected and treated camels (Group B).



Week 0 = mean of 4 weeks before infection.

Table 4: Mean weekly haemoglobin concentration (g/dl) (±SD) of infected and control camels throughout the experiment.

Weeks PI	Camel Groups		
	A (n=4)	B (n=5)	C (n=2)
0	11.2±0.8	11.9±0.5	11.7±0.6
1	11.9±0.9	12.9±1.3	10.8±1.3
2	11.3±1.4	13.4±1.9	11.4±1.5
3	10.8±1.1	11.6±1.4	11.0±1.7
4	11.7±1.5	12.8±1.7	10.0±0.6
5	10.5±0.7	12.3±1.6	10.8±1.5
6	10.5±1.9	10.7±0.9	11.0±1.0
7	10.5±2.3	11.3±1.1	10.4±0.9
8	10.6±1.7	9.6±1.2	9.9±0.2
9	10.2±0.2	11.1±0.9	10.6±1.6
10	10.4±2.2	11.4±1.2	10.5±0.9
11	10.2±1.6	10.9±1.0	11.1±0.7
12	10.4±1.9	11.4±1.2	10.7±1.0
13	10.9±1.8	11.4±0.7	12.3±1.7
14	9.5±0.8	11.1±0.9	11.8±1.1
15	9.2±0.2	11.0±1.0	12.7±1.5
16	8.9±0.2	11.4±0.8	12.0±1.8

**Week 0= mean of 4 weeks before infection.
PI = Post-infection

T-test for the hypothesis "mean 1 = mean 2"
(Alpha=0.05).

Pre-infection:	A vs C	$p > 0.05$.
	B vs C	$p > 0.05$.
	A vs B	$p > 0.05$.
Post-infection:	A vs C	$p > 0.05$.
	B vs C	$p > 0.05$.
	A vs B	$p < 0.05$.

4.2.4. White blood cell count (WBC)

There was no significant difference in the total WBC counts during the pre-infection period between group A and C camels ($p > 0.05$, Table 5). Group B camels, however, had higher WBC counts than those of group A or C. The difference between group B camels and those in groups A and C before infection was significant ($p < 0.05$). Analysis of the WBC counts over the 16 weeks following infection indicated a significant difference between the three groups of camels ($p < 0.05$). During the first three weeks there was a drop in the WBC count in the infected camels (groups A and B). Thereafter, there was no marked difference between group A and group C (Fig. 11). Group C camels and those in group B had an increase in the WBC count from week 2 to week 4. The increase was more pronounced in group B camels than those of group C (Fig. 12), the difference being statistically significant ($p < 0.05$). The WBC count then dropped gradually from week 4 to week 11 in all groups. There was a statistically significant difference between the control camels and the two infected groups ($P < 0.05$). Analysis of the WBC count from week 11 to the end of the experiment indicated no statistically significant difference between group C camels and those in group B ($p > 0.05$).

Figure 11: Mean weekly white blood cell count of infected non-treated camels (Group A).

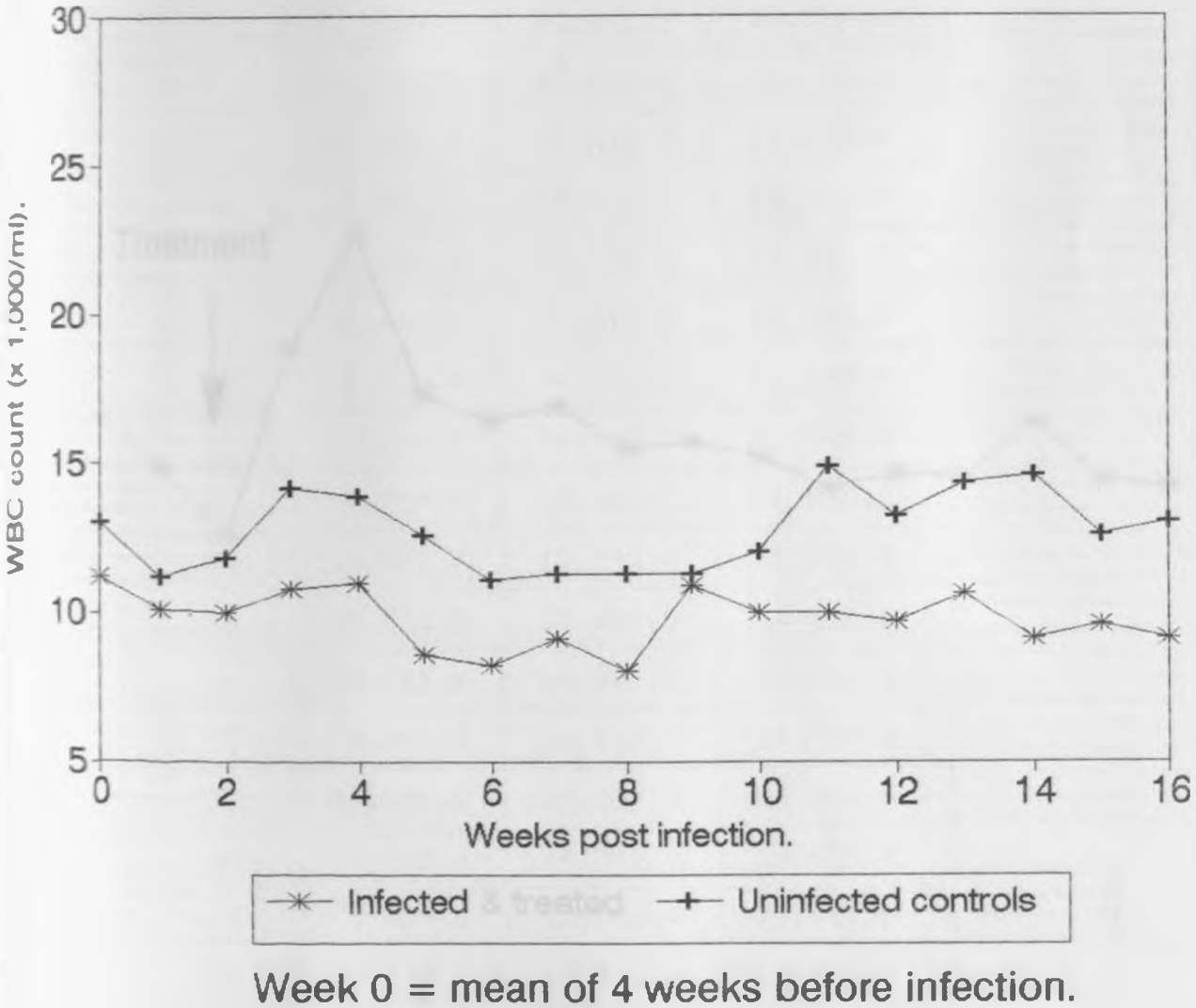


Figure 12: Mean weekly white blood cell count of infected and treated camels (Group B).

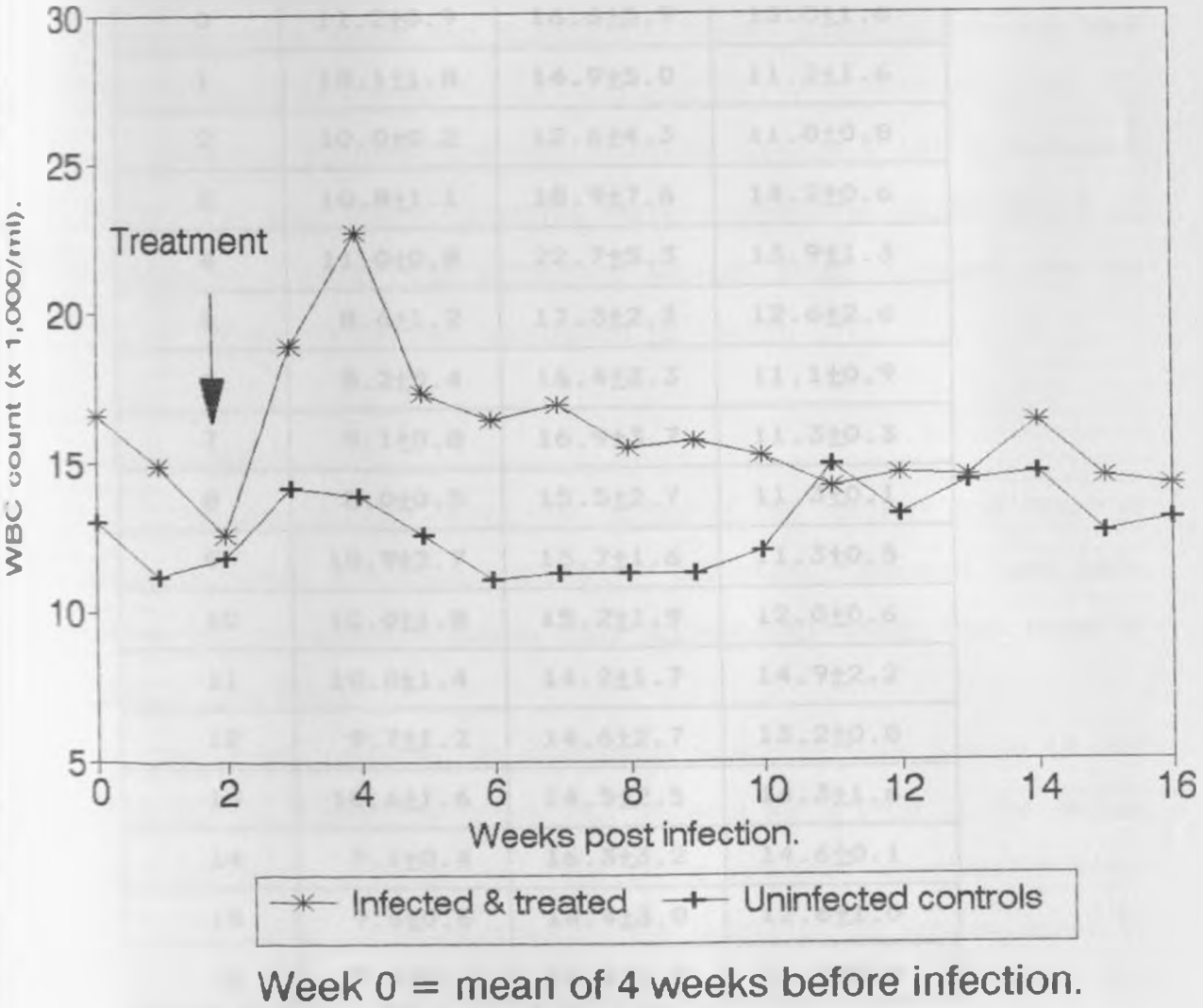


Table 5: Mean weekly white blood cell count ($x10^3$/ml) (±SD) of infected and control camels throughout the experiment.

Weeks PI	Camel Groups		
	A (n=4)	B (n=5)	C (n=2)
0	11.2±0.9	16.6±5.9	13.0±1.6
1	10.1±1.8	14.9±5.0	11.2±1.6
2	10.0±0.2	12.6±4.3	11.8±0.8
3	10.8±1.1	18.9±7.6	14.2±0.6
4	11.0±0.8	22.7±5.3	13.9±1.3
5	8.6±1.2	17.3±2.3	12.6±2.6
6	8.2±0.4	16.4±3.3	11.1±0.9
7	9.1±0.8	16.9±3.7	11.3±0.3
8	8.0±0.5	15.5±2.7	11.3±0.1
9	10.9±2.7	15.7±1.6	11.3±0.5
10	10.0±1.8	15.2±1.9	12.0±0.6
11	10.0±1.4	14.2±1.7	14.9±2.2
12	9.7±1.2	14.6±2.7	13.2±0.8
13	10.6±1.6	14.5±2.5	14.3±1.6
14	9.1±0.4	16.3±3.2	14.6±0.1
15	9.6±0.6	14.4±3.0	12.6±1.0
16	9.1±0.4	14.2±1.8	13.0±0.6

**Week 0= mean of 4 weeks before infection.
PI = Post-infection
T-test for the hypothesis "mean 1 = mean 2"
(Alpha=0.05).

Pre-infection:
A vs C $p > 0.05$.
B vs C $p < 0.05$.
A vs B $p < 0.05$.

Post-infection:
A vs C $p < 0.05$.
B vs C $p < 0.05$.
A vs B $p < 0.05$.

4.2.5 Differential Leucocyte count

(a) Lymphocytes

Analysis of the lymphocyte counts during the pre-infection period showed no significant difference between group A and C camels while there was a statistically significant difference between group B and C camels. Group A camels had significantly higher lymphocyte counts than group B camels ($p < 0.05$).

Following infection, the cell counts in groups A and B increased significantly, with group A showing higher lymphocyte counts than B or C camels ($p < 0.05$). The difference between group A camels and the controls (group C) was also significant ($p < 0.05$).

(b) Neutrophils

The pre-infection neutrophil counts of the controls and those of group B camels were not statistically different. However, the mean counts for the group A camels were significantly lower than those of group B ($p < 0.05$).

Following infection, the neutrophil counts in both groups (A and B) showed an initial rise which was followed by a drop in the second and third week, respectively. The levels remained significantly below the pre-infection status ($p < 0.05$) The counts in the treated group of animals, did recover steadily towards the pre-infection level by the tenth week (Figs. 15 and 16).

(c) Monocytes

There was no significant difference between the mean monocyte counts of the three groups of camels throughout the study period.

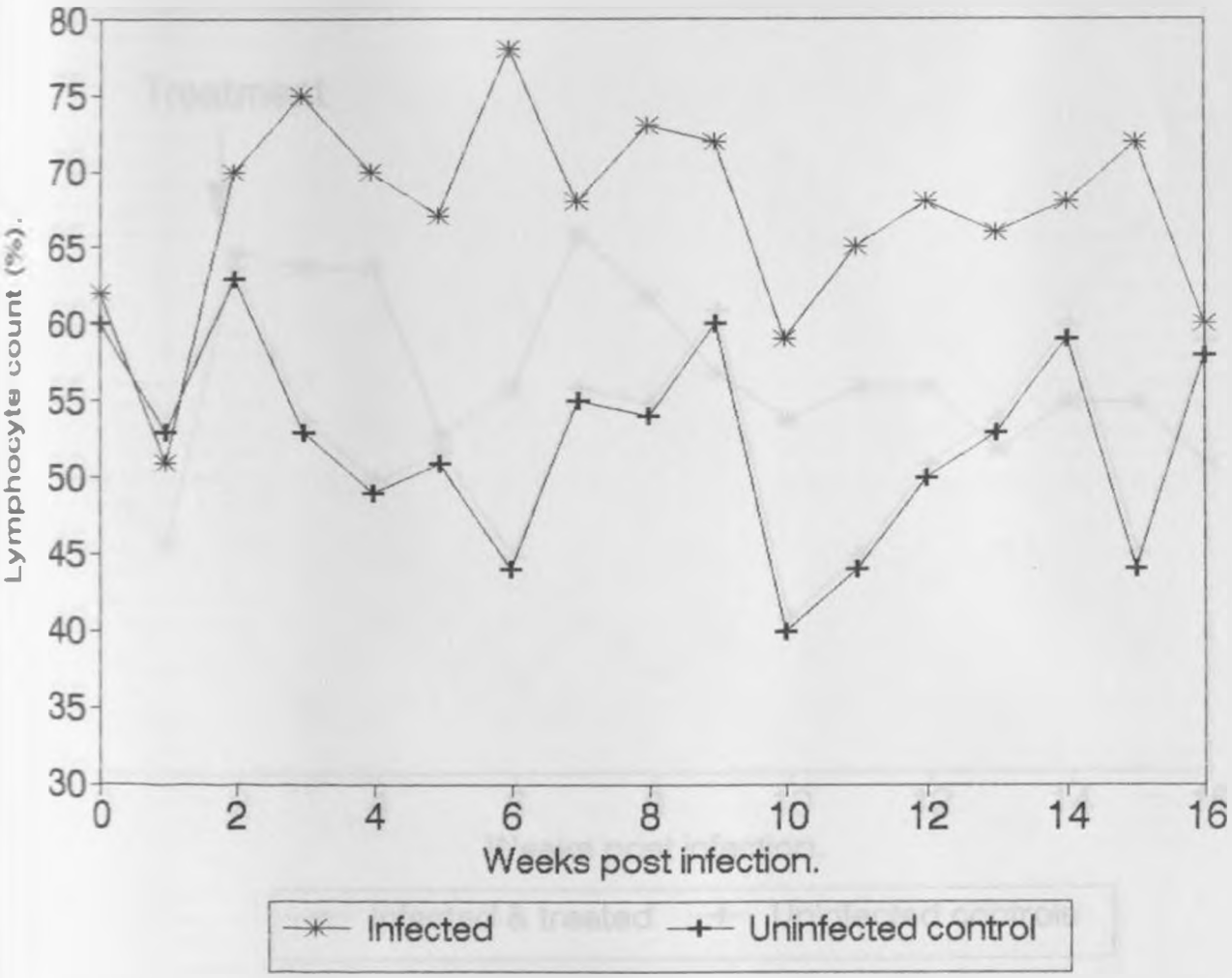
(d) Eosinophil

The values of the eosinophil count in the infected groups remained lower than that of the control camels throughout the observation period, but these results were not statistically significant.

(e) Basophils

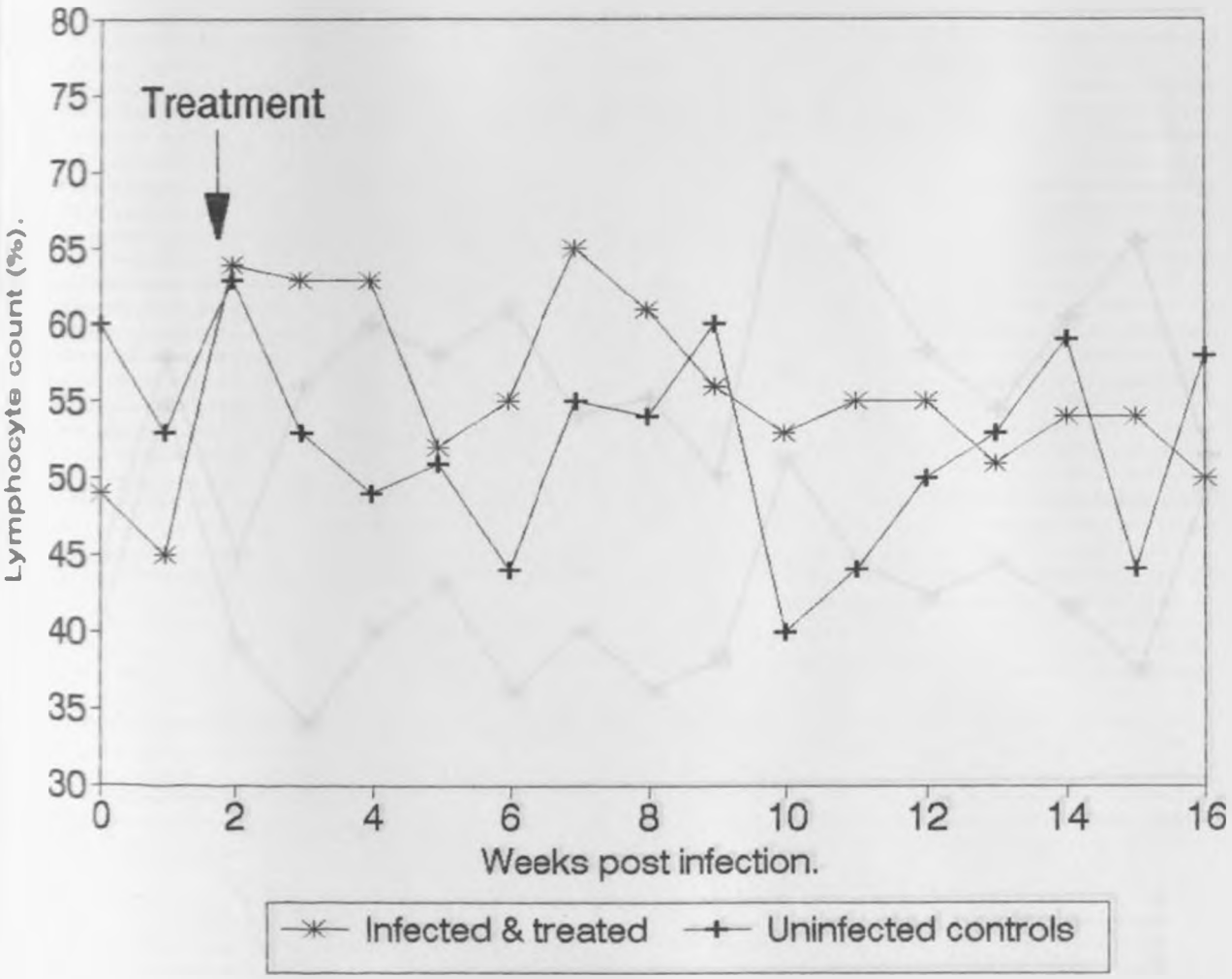
There were no basophils observed in all the smear preparations in both the pre-infection and post-infection periods.

Figure 13: Mean weekly lymphocyte count in infected non-treated camels (Group A).



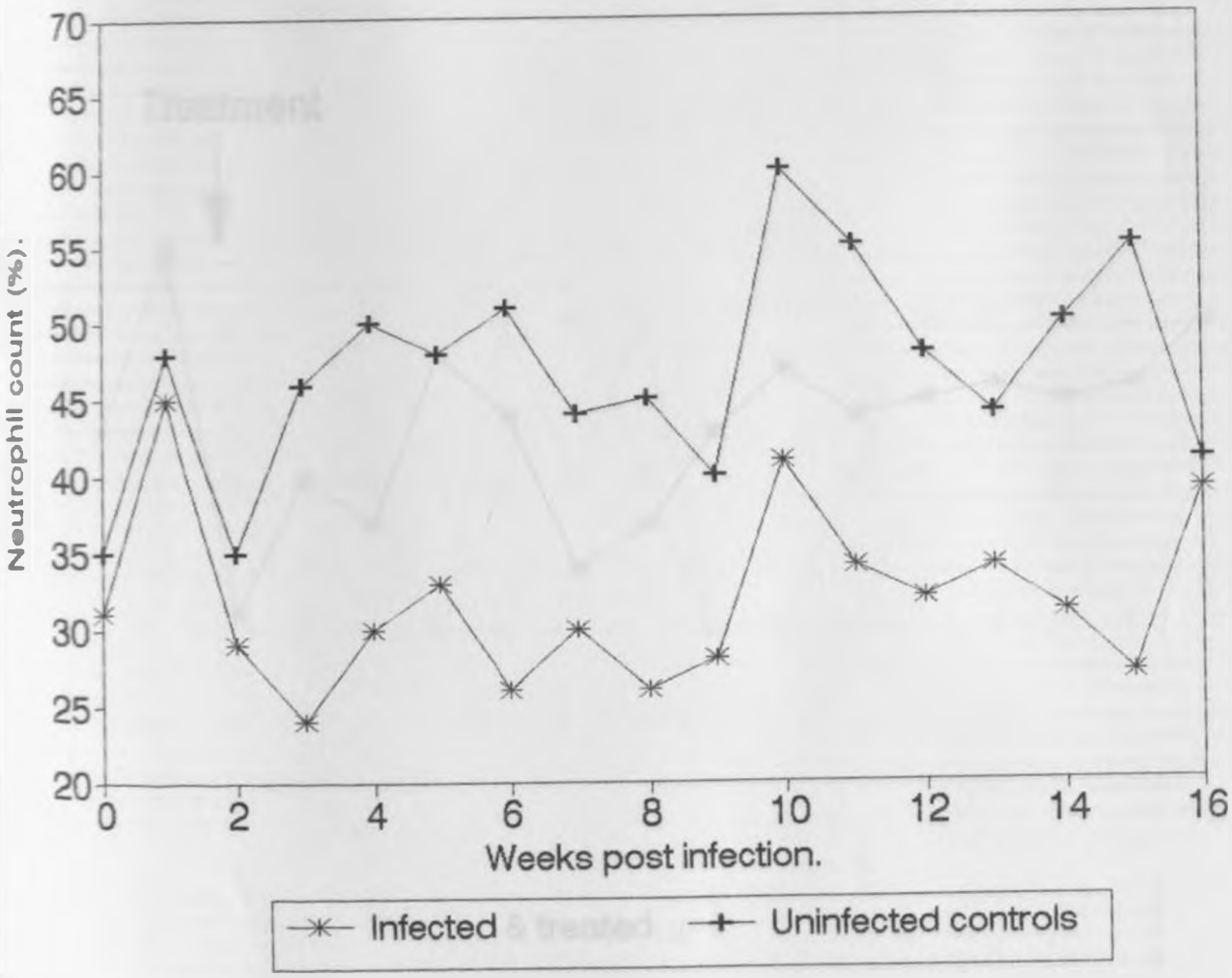
Week 0 = mean of 4 weeks before infection.

Figure 14: Mean weekly lymphocyte count in infected and treated camels (Group B).



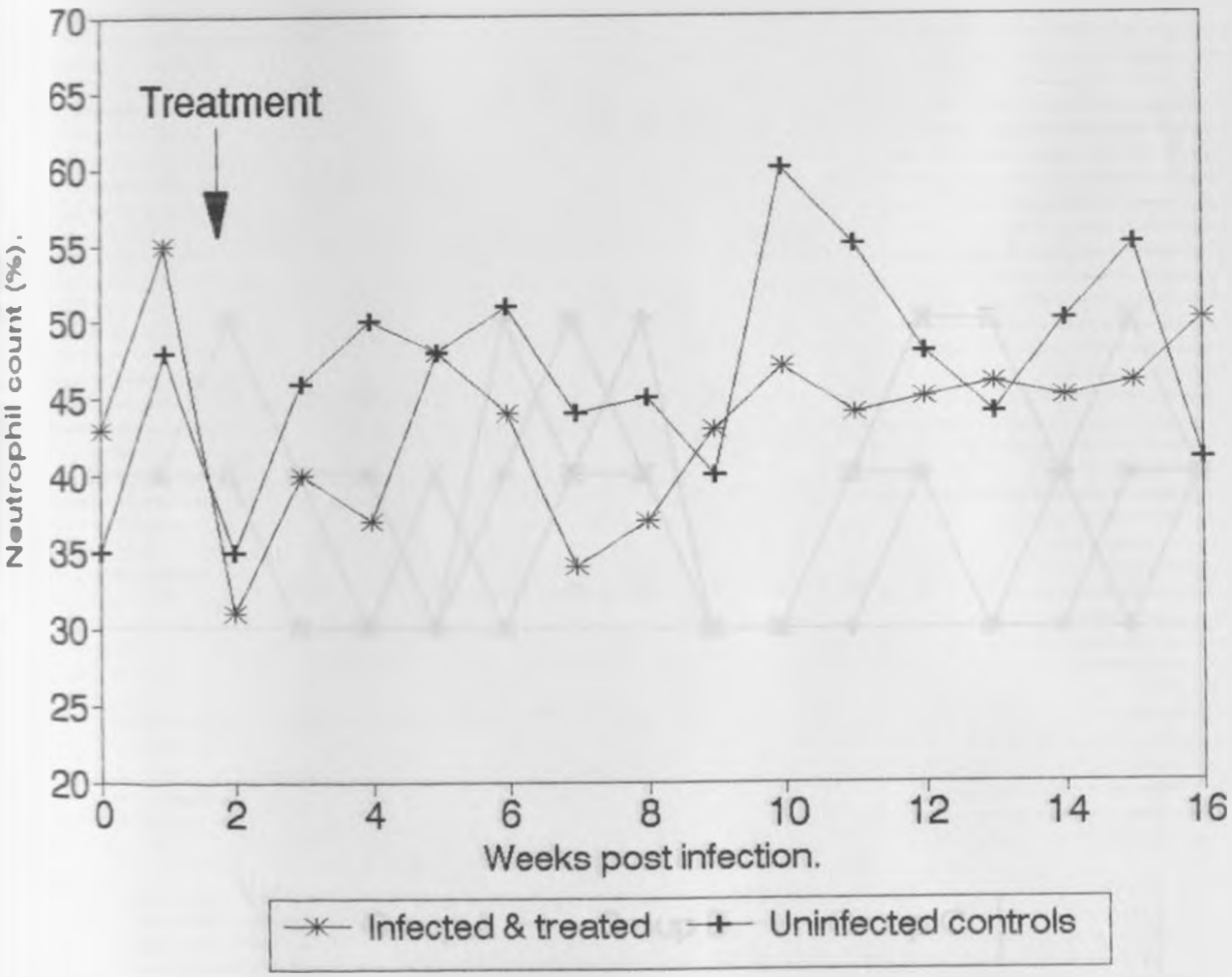
Week 0 = mean of 4 weeks before infection.

Figure 15: Mean weekly neutrophil count in infected non-treated camels (Group A).



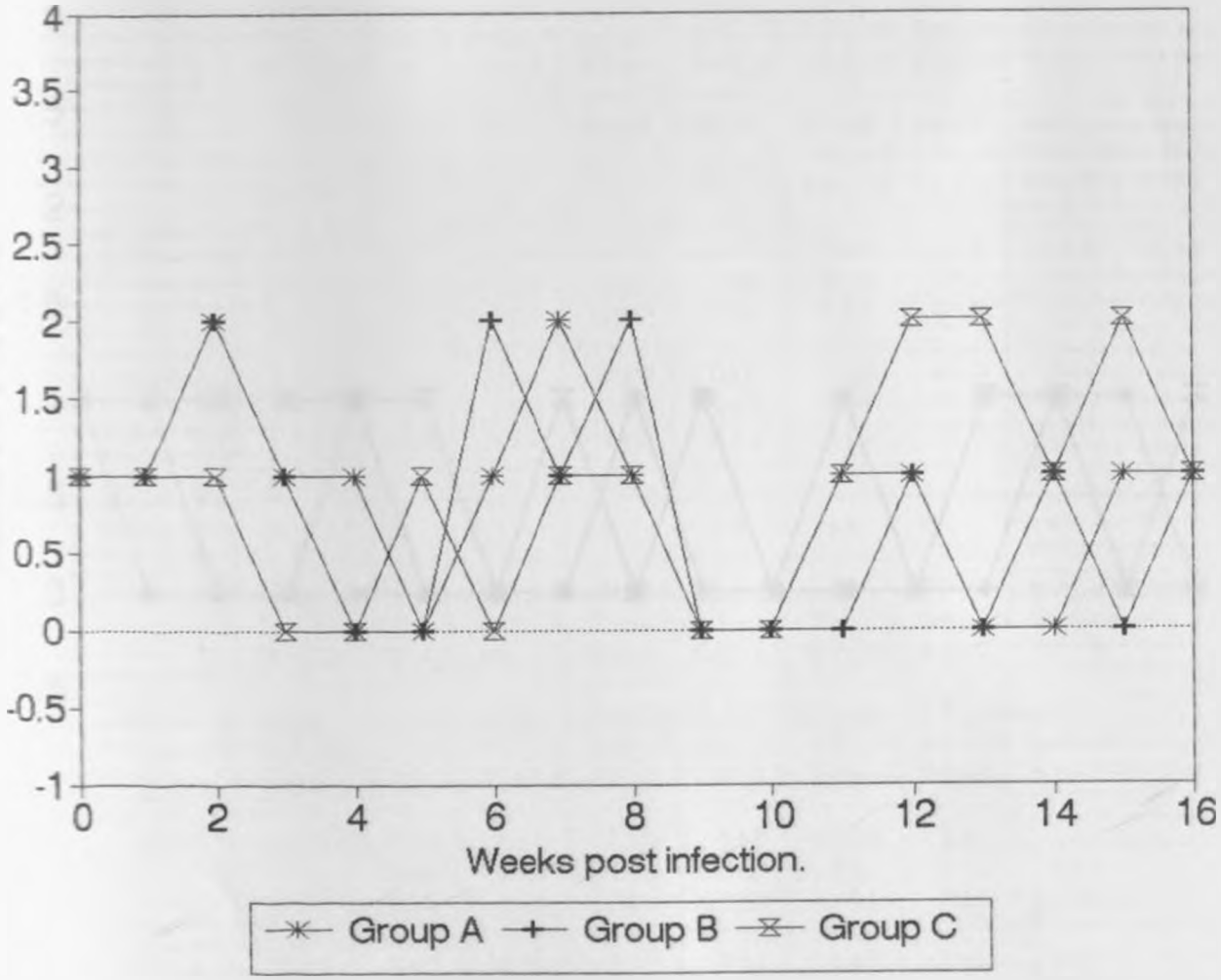
Week 4 = mean of 4 weeks before infection.

Figure 16: Mean weekly neutrophil count in infected and treated camels (Group B).



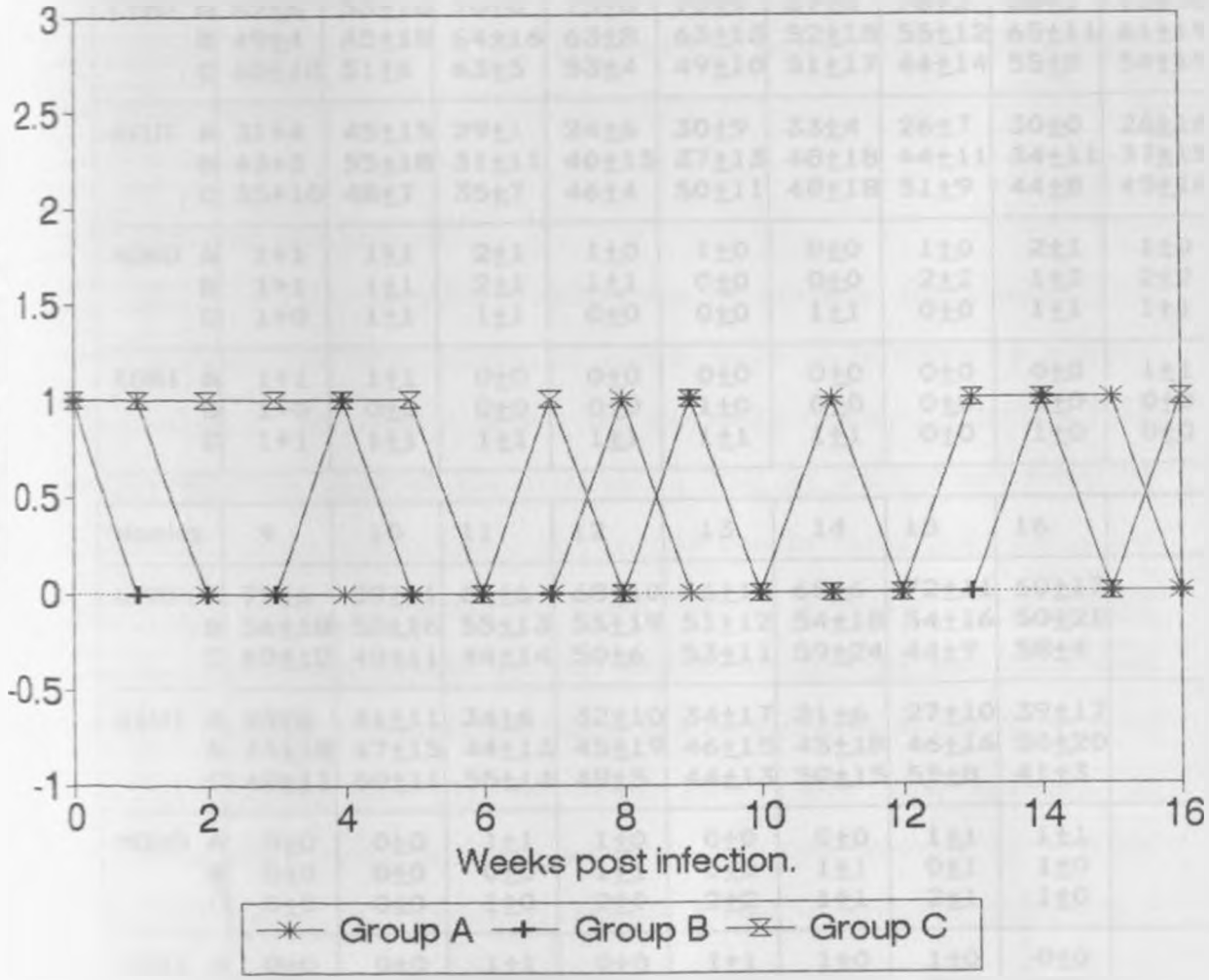
Week 0 = mean of 4 weeks before infection.

Figure 17: Mean weekly monocyte count in infected non-treated (Group A), infected and treated (Group B) and control (Group C) camels.



Week 4 = mean of 4 weeks before infection.

Figure 18: Mean weekly eosinophil count in infected non-treated (Group A), infected and treated (Group B) and control (Group C) camels.



Week 4 = mean of 4 weeks before infection.

Table 6: Mean differential leucocyte count (%) (±SD) of infected and control camels throughout the experimental period.

Weeks		0	1	2	3	4	5	6	7	8
LYMP	A	62±6	53±16	70±0	75±6	70±9	67±4	78±2	68±1	73±16
	B	49±4	45±18	64±16	63±8	63±13	52±18	55±12	65±11	61±15
	C	60±10	51±6	63±5	53±4	49±10	51±17	44±14	55±8	54±15
NEUT	A	31+4	45±15	29±1	24±6	30±9	33±4	26±7	30±0	26±16
	B	43+3	55±18	31±11	40±15	37±13	48±18	44±11	34±11	37±15
	C	35+10	48±7	35±7	46±4	50±11	48±18	51±9	44±8	45±16
MONO	A	1+1	1±1	2±1	1±0	1±0	0±0	1±0	2±1	1±0
	B	1+1	1±1	2±1	1±1	0±0	0±0	2±2	1±2	2±2
	C	1+0	1±1	1±1	0±0	0±0	1±1	0±0	1±1	1±1
EOSI	A	1+1	1±1	0±0	0±0	0±0	0±0	0±0	0±0	1±1
	B	1+0	0±0	0±0	0±0	1±0	0±0	0±0	0±0	0±0
	C	1+1	1±1	1±1	1±1	1±1	1±1	0±0	1±0	0±0

Weeks		9	10	11	12	13	14	15	16
LYMP	A	72±6	59±11	65±6	68±10	66±17	68±6	72±11	60±17
	B	56±18	53±16	55±13	55±19	51±12	54±18	54±16	50±21
	C	60±12	40±11	44±14	50±6	53±11	59±24	44±9	58±4
NEUT	A	28±6	41±11	34±6	32±10	34±17	31±6	27±10	39±17
	B	43±18	47±15	44±13	45±19	46±15	45±18	46±16	50±20
	C	40±11	60±11	55±14	48±5	44±13	50±15	55±8	41±3
MONO	A	0±0	0±0	1±1	1±0	0±0	0±0		
	B	0±0	0±0	0±0	1±1	0±0	1±1		
	C	0±0	0±0	1±0	2±0	2±2	1±1		
EOSI	A	0±0	0±0	1±1	0±0	1±1			
	B	1±1	0±0	0±0	0±0	0±0			
	C	1±0	0±0	0±0	0±0	1±1			

LYMP = Lymphocyte count (%)
NEUT = Neutrophil count (%)
MONO = Monocyte count (%)
EOSI = Eosinophil count (%)

Group A: n=4
Group B: n=5
Group C: n=2

4.3 Biochemical Profile

Appendix III shows the pre-infection levels of the biochemical values obtained in the present study as compared to results of various workers, while Tables 6a and 6b show the mean (±SD) plasma chemistry values (of common enzymes and important non-enzymatic blood components, respectively) of the infected and control camels throughout the study period.

4.3.1 Alkaline phosphatase

There was no significant difference in alkaline phosphatase (AP) levels between the three groups of camels prior to infection ($p > 0.05$). The level of AP in group A camels fell gradually following infection from the pre-infection mean of 10.6 IU/l to 6.1 IU/l by week 2 and remained significantly lower than that of the control camels throughout the study period ($p < 0.05$). In group B camels, the AP level also fell gradually from the pre-infection mean of 13.8 IU/l to 8.0 IU/l by the third week following infection. Thereafter, there was an increase in the AP activity from 8.0 IU/l to 13.0 IU/l by week 13. There was, however, no significant difference between group B and the controls ($p > 0.05$). When group A AP activity was compared to that of group B, there was a significant difference ($p < 0.05$).

4.3.2 Alanine aminotransferase

Alanine aminotransferase (ALT) levels showed no appreciable difference in all the camel groups prior to and after infection and ranged between 0 and 4 IU/l throughout the study period.

4.3.3 Aspartate aminotransferase

Analysis of aspartate aminotransferase (AST) profile prior to infection showed no significant difference between the three groups of camels ($p > 0.05$). AST in group A camels fell from the pre-infection value of 6.4 IU/l to 2.8 IU/l by the third week, while the level in group B camels fell to 1.9 IU/l by the second week. The level of AST in the control camels also dropped from an initial value of 5.7 IU/l to 4.2 IU/l by week 3 and thereafter, remained constant up to week 13 before rising to 5.8 IU/l at week 14. The AST value in group A rose gradually from 2.8 IU/l in week 3 to 4.5 IU/l in week 9 followed by a drop to 3.7 IU/l in week 13. Eventually there was a final rise to 4.6 IU/l in week 15. After the initial fall in AST activity in group B camels in week 2 there was a rise to 5.7 IU/l by the third week. This was followed by a fall to 4.0 IU/l in week 9 and a gradual rise to 4.9 IU/l by week 13. Statistically, there was no significant difference between the infected camels and the controls throughout the study period ($p > 0.05$).

4.3.4 Creatine Kinase

There was no significant difference in creatine kinase levels among the three groups of camels prior to and following the infection despite the fluctuations observed.

4.3.5 Lactic dehydrogenase

Lactic dehydrogenase (LDH) showed no difference between the three groups of camels prior to infection ($p > 0.05$). After infection, there was a slight decline in the LDH activity to the second and third week in groups A and B, respectively. This was followed by a gradual

increase in the LDH level to the end of the experiment in group B and to week 13 in group A. However, this increase in LDH activity in the infected camels showed no significant difference when analyzed ($p > 0.05$).

4.3.6 Blood urea nitrogen

Pre-infection data of blood urea nitrogen (BU) level showed no significant difference in all groups of camels ($p > 0.05$). After infection, group A camels had a gradual fall in the BU level to the ninth week and then a rise from the ninth week to the end of the experiment. The decline and rise were, however, not significant ($p > 0.05$). In group B camels there was an increase in BU levels following infection up to the second week. This was followed by a decline. The level of BU in group B camels did not drop below that of group C camels and the results between the two groups were not significantly different ($p > 0.05$). In the control camels BU levels were almost constant throughout the experiment with only minor fluctuations.

4.3.7 Total proteins, albumin and globulin

The pre-infection total protein (TP) level showed no significant difference in all the three groups of camels ($p > 0.05$). The TP level in groups A and B increased from the second week of infection and levelled at the fourth week. The total proteins then remained almost constant in these two groups up to the thirteenth week when there was a decline. The increase from the second week to the thirteenth week showed a significant difference between the infected group of camels and the controls ($p < 0.05$). The TP level from week 13 to the end of

the experiment, however, did not drop to below that of the control camels. Total protein in the control camels remained almost constant with only minor fluctuations.

The mean albumin concentration in all the three camel groups showed no difference in pre-infection data. Albumin levels in infected camels and the non-infected controls remained almost constant throughout the experimental period with slight fluctuations but within the normal range (2 to 3 mg/dl). Statistically however, there was a significant difference between the infected groups and the controls ($p < 0.05$). Comparison between group A and B showed no significant difference.

Globulin levels increased significantly in the infected camels of group A and B from the second week and reached a peak level in the third week. From the third week the globulin level remained almost constant in these two infected groups up to week thirteen, when there was a drop, followed by another increase in week fourteen. In the control group, the globulin level remained almost constant with minimal fluctuations. There was a significant difference in globulin level between the infected groups and the controls ($p < 0.05$).

Albumin level was compared to the globulin level in the infected groups. At the start of the experiment (before infection), the albumin level was higher than that of the globulin. However, by the second week following infection, there was a switch in which the globulin level became predominant. The globulin level remained higher from the second week to the end of the study and was more pronounced in group A than in group B.

Table 7a: Mean plasma enzyme activity (IU/l) (±SD) of infected and control camels throughout the study period.

		Weeks post infection						
ENZYME		0	2	3	9	13	14	15
AP	A	10.6±1	6.1±1	5.5±.5	5.9±.4	7.4±0	4.3±.7	5.0±.5
	B	13.8±1	10.4±4	8.0±.8	11.1±4	13.0±1	11.1±1	10.9±7
	C	9.8±.2	10.8±3	9.8±2	11.0±1	7.2±1	9.7±.4	8.9±2
ALT	A	2 ± 1	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	6 ± 2
	B	4 ± 4	0 ± 1	5 ± 7	1 ± 2	0 ± 0	1 ± 1	1 ± 1
	C	1 ± 1	2 ± 2	2 ± 2	0 ± 0	0 ± 0	4 ± 4	0 ± 0
AST	A	6.4±.7	2.9±.5	2.8±1	4.5±1	3.6±1	3.7±1	4.6±.2
	B	5.5±.9	1.9± 1	5.7±2	4.0±1	4.8±2	4.9±2	3.9±.8
	C	5.7±.8	4.7±.4	4.2±.2	4.8±.3	4.8±.7	5.8±.8	3.3±.9
CK	A	55±13	36±18	22±5	32±7	147±36	70±24	49±5
	B	56±3	45±10	94±74	130±92	133±65	74±8	122±80
	C	53±15	110±16	90±11	100±20	87±38	62±12	96±3
LDH	A	130±7	103±7	90±10	139±24	213±47	152±2	120±8
	B	144±5	98±18	108±28	156±55	158±65	153±67	156±39
	C	136±1	150±10	110±9	151±17	60±10	130±15	103±43

**Week 0 = mean of 4 weeks before infection.
PI = Post-infection

T-test for the hypothesis "mean 1 = mean 2"
(Alpha=0.05).

Alkaline phosphatase (AP):-

Pre-infection: "mean 1 = mean 2" in all groups i.e. $p > 0.05$.

Post-infection: A vs C $p < 0.05$.
B vs C $p < 0.05$
A vs B $p < 0.05$

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), creatine kinase (CK) and lactic dehydrogenase (LDH) had p values > 0.05 both pre- and post-infection.

Table 7b: Mean plasma non-enzymatic biochemical values (±SD) of infected and control camels throughout the experiment.

	Weeks Post infection						
	0	2	3	5	9	14	15
ALB A	3.1±.3	2.7±0	3.0±0	3.0±0	2.9±0	2.9±0	2.6±0
(g/l)B	2.7±.1	2.7±.3	2.8±.3	2.8±.2	2.5±.4	2.7±.3	2.2±.7
C	2.8±.2	2.6±0	2.5±.2	2.6±0	2.0±.2	2.7±0	1.8±1
GLB A	2.5±.2	2.6±0	3.0±0	3.1±1	2.8±1	3.1±0	2.7±0
(g/l)B	3.0±.1	2.7±.3	3.0±.4	2.8±.3	2.6±.4	3.0±.8	2.6±.4
C	2.2±.1	2.3±0	2.3±0	2.1±0	2.3±0	2.1±0	2.1±0
TP A	5.4±.2	5.3±0	6.0±0	6.1±1	6.1±0	6.0±0	5.3±0
(g/l)B	5.9±.5	5.3±.6	5.7±.6	5.7±.4	5.2±.4	4.9±1	4.9±1
C	4.8±.2	4.9±.4	4.8±.2	4.7±0	4.2±.3	4.8±0	3.9±1
BUN A	7.0±2	8.1±0	7.6±2	6.4±1	6.9±0	7.3±4	8.6±2
mg/l B	6.6±2.3	14 ±2.5	12 ±3	8.5±4.6	6.4±2.6	5.8±1.9	6.1±2
C	6.1±0.9	5.2±1.8	4.1±1.7	6.4±0.4	4.7±0.6	8.2±2.2	3.9±2

**Week 0 = mean of 4 weeks before infection.

T-test for the hypothesis "mean 1 = mean 2" (Alpha=0.05).

Albumin (ALB), globulin (GLB) and total proteins (TP) had:

pre-infection: p values > 0.05 between all camel groups

post-infection: p values < 0.05 between all camel groups.

Blood urea (mg/l) (BU) had p values > 0.05 both in pre- and post-infection samples between all groups.

4.4. Serological Assays

4.4.1. Antigen Detection

Prior to infection none of the camel sera had an OD reading higher than 0.05 in the ELISA test. The threshold value was thus set at 0.10 which was twice the pre-infection OD and twice the highest OD of the control camels. Following infection, a significant rise in OD was demonstrated in eight out of the nine infected camels. Circulating antigens were first detected by Ag-ELISA in three camels by the first week and in three other camels by the second week after infection. The remaining two camels showed elevated antigens in week 3 and 4, respectively. However, antigens were not detected in one camel (C156) that died during the acute disease, 11 days post infection.

The OD values of camels in group A subsequently showed a progressive increase in circulating antigens and remained detectable throughout the experimental period with some fluctuations (Fig. 19). Following treatment, camels in group B showed an initial sharp rise in the ELISA OD values which remained high up to week 4. This was followed by a gradual decrease and the antigens became undetectable by week 10 after treatment (Fig. 20). Individual camels showed varied times of antigen disappearance following treatment. Antigens were not detectable by the third week following treatment in one camel (number C261) while in another (C162) they persisted up to 10 weeks after treatment and disappeared thereafter.

4.4.2 Antibody Detection

The antibody ELISA profile of the three groups of camels is shown in Figures 21 and 22. Sera from pre-infection period and of the non-infected control camels were all negative (OD cut-off point was 0.15 since none of the pre-infection sera and the non-infected camel sera gave OD readings more than 0.145). Antibodies in two infected camels were first detected 7 days following infection. On average, the antibodies were detected by week 4 in all camels (Figs. 21 and 22). Antibody levels in group A increased gradually, thereafter, reaching a plateau in the fifth week. There was no decline in antibody levels in group A camels. However, in the treated camels (group B) antibody levels increased to detectable levels 2 weeks post treatment and reached a peak in the seventh week (five weeks post-treatment). Thereafter, there was a gradual fall but the levels were still higher than the cut-off point by the end of the study period (14 weeks after treatment).

Figure 19: Mean weekly antigen-ELISA profile of the infected non-treated camels (Group A).

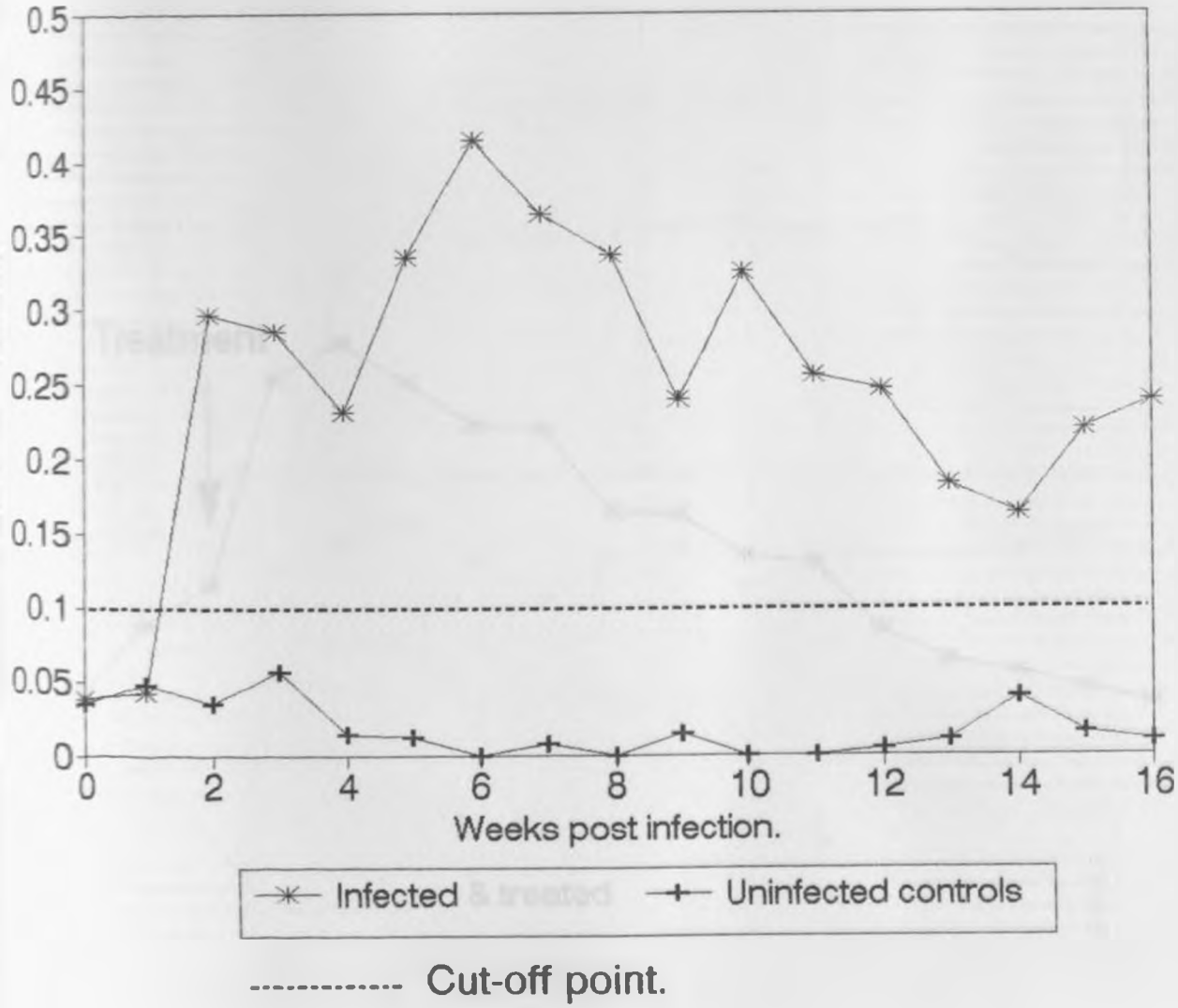


Figure 20: Mean weekly antigen-ELISA profile of the infected camels before and after treatment (Group B).

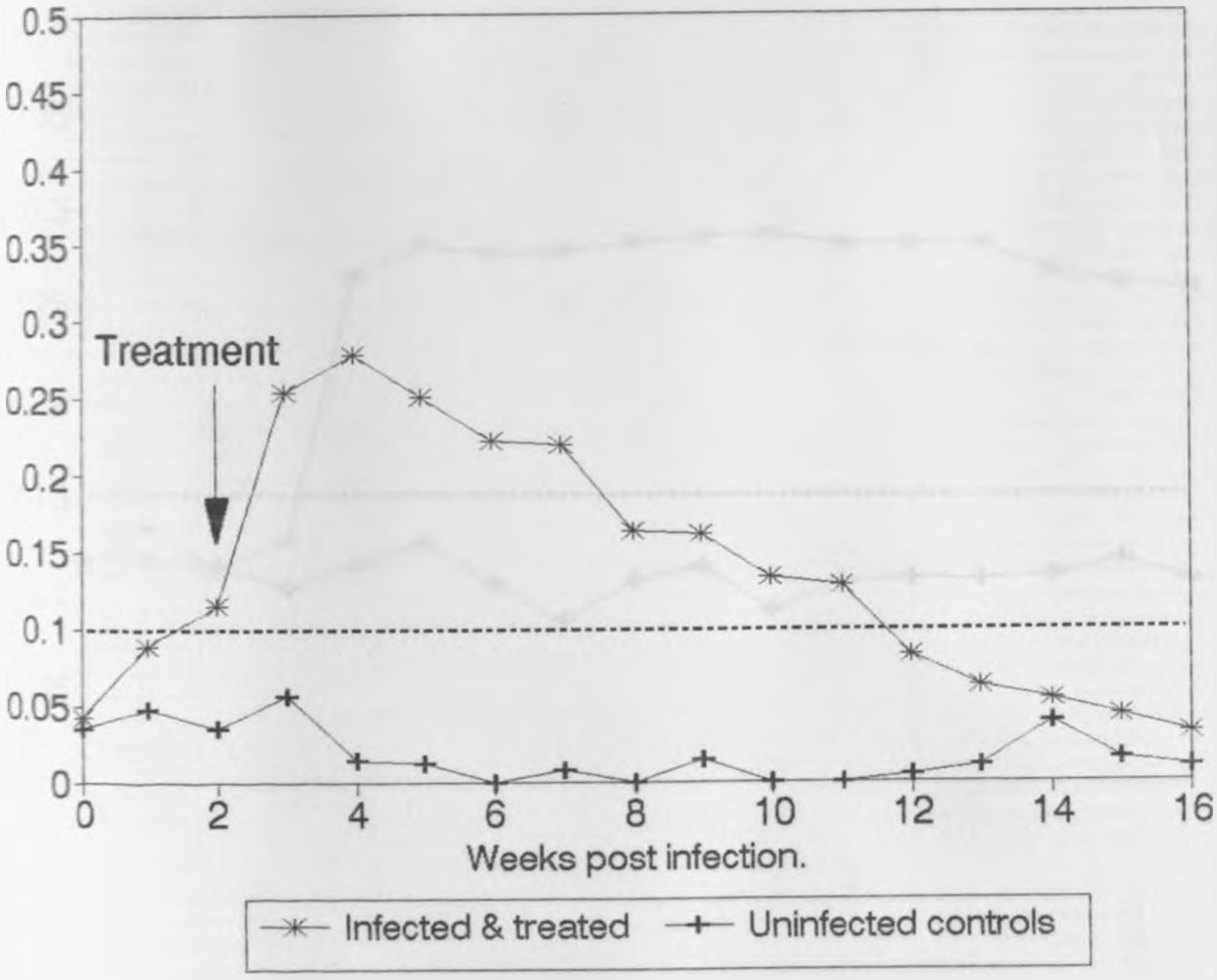


Figure 21: Mean weekly antibody-ELISA profile of infected non-treated camels (Group A)

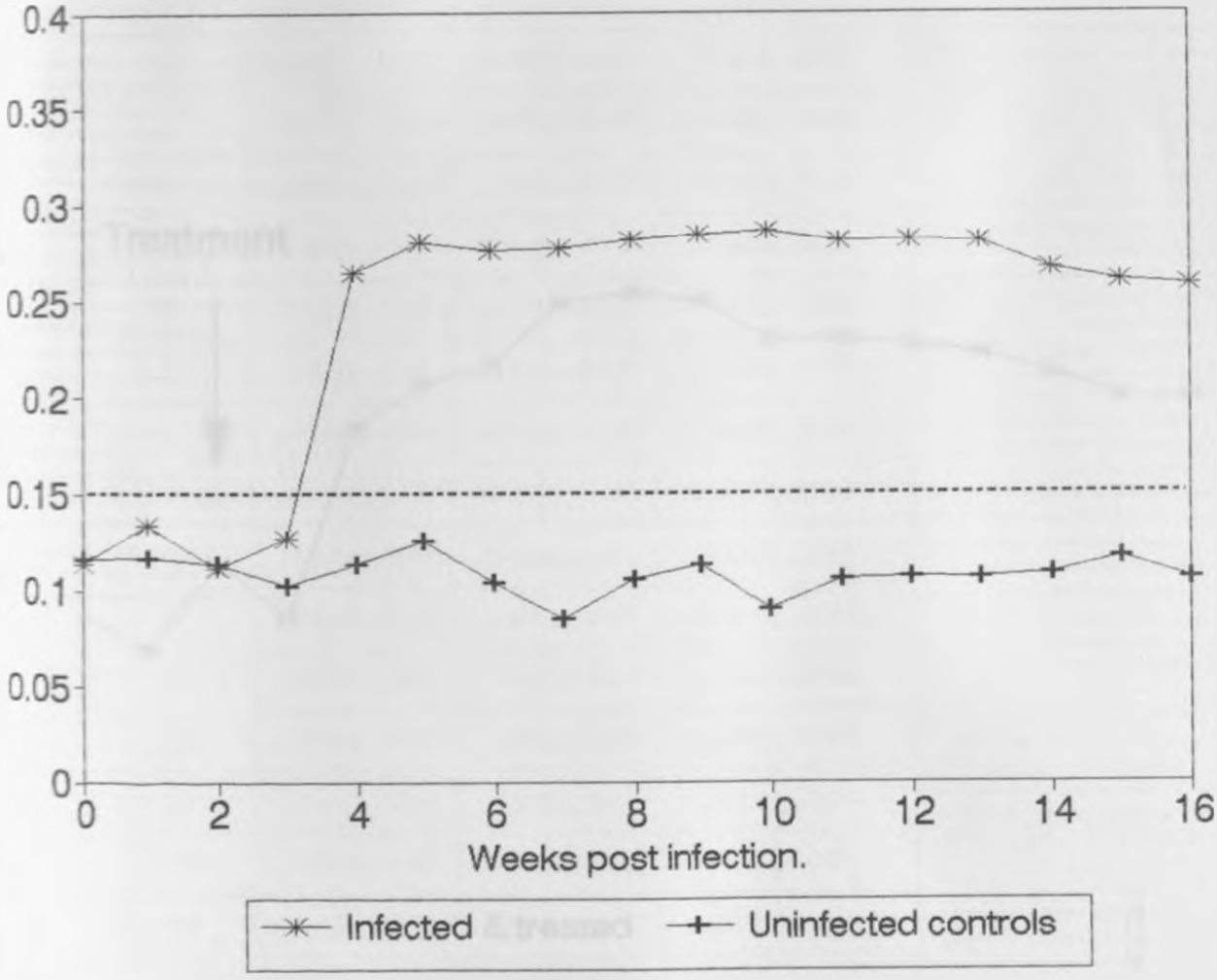


Figure 22: **Mean weekly antibody-ELISA profile of infected camels before and after treatment (Group B)**

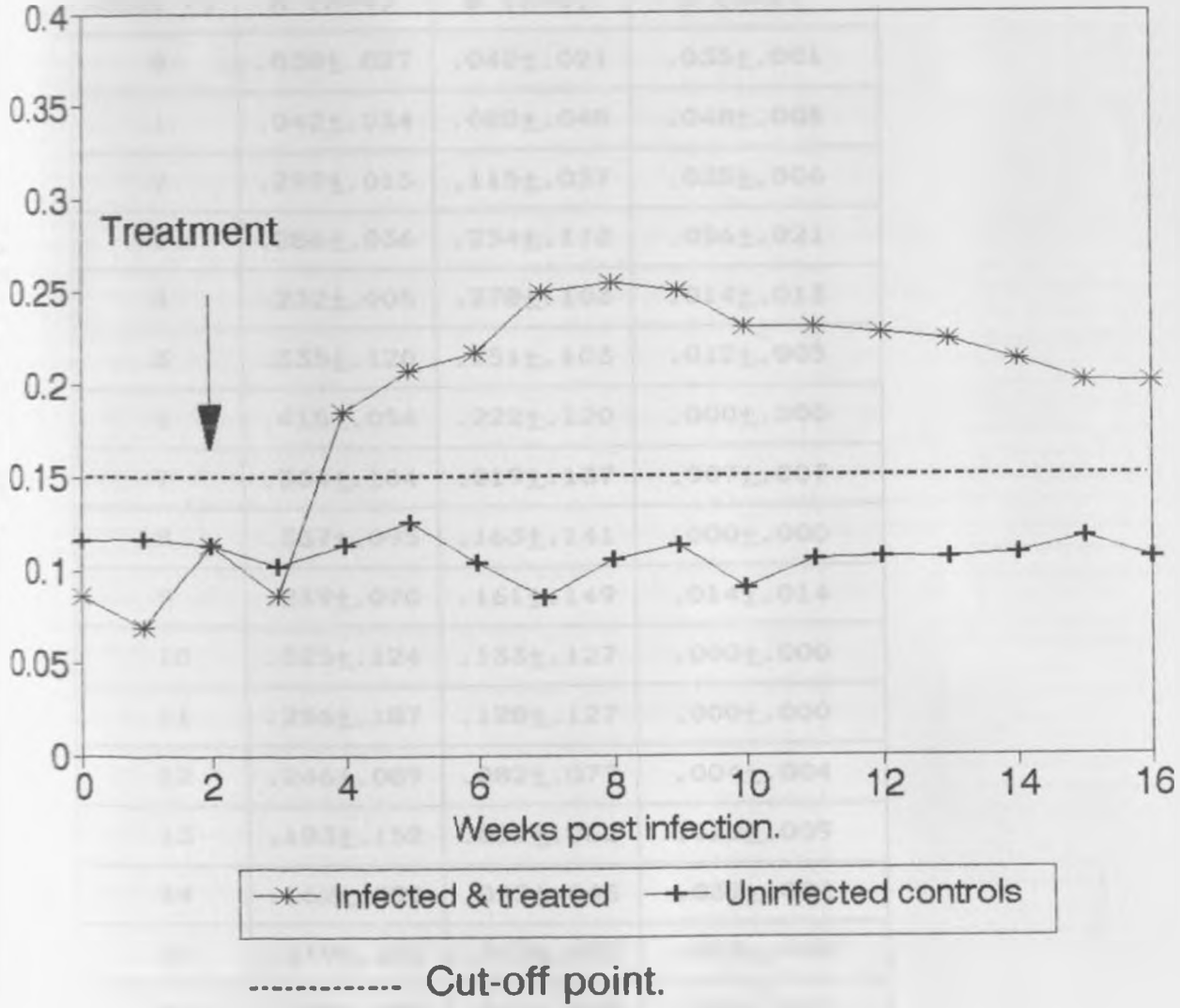


Table 8a: Mean Ag-ELISA OD values (±SD) of infected and control camels throughout the experiment.

	Camel Groups		
Weeks PI	A (n=4)	B (n=5)	C (n=2)
0	.038±.027	.042±.021	.035±.001
1	.042±.034	.088±.048	.048±.005
2	.297±.015	.115±.027	.035±.006
3	.286±.036	.254±.173	.056±.021
4	.232±.005	.278±.103	.014±.013
5	.335±.120	.251±.103	.012±.005
6	.415±.054	.222±.120	.000±.000
7	.364±.104	.219±.137	.007±.007
8	.337±.095	.163±.141	.000±.000
9	.239±.070	.161±.149	.014±.014
10	.325±.124	.133±.127	.000±.000
11	.256±.187	.128±.127	.000±.000
12	.246±.089	.082±.077	.004±.004
13	.183±.152	.062±.062	.010±.009
14	.162±.096	.053±.045	.039±.032
15	.219±.103	.043±.022	.015±.002
16	.238±.093	.031±.008	.009±.002

PI = Post-infection

Table 8b: Mean Ab-ELISA OD values (±SD) of infected and control camels throughout the experiment.

	Camel Groups		
Weeks PI	A (n=4)	B (n=5)	C (n=2)
0	.114±.003	.086±.039	.116±.010
1	.134±.020	.069±.033	.117±.001
2	.111±.008	.0114±.045	.114±.016
3	.127±.033	.086±.033	.102±.002
4	.265±.008	.185±.076	.114±.017
5	.280±.010	.207±.082	.126±.014
6	.276±.009	.217±.072	.104±.011
7	.277±.005	.249±.028	.085±.001
8	.282±.015	.254±.012	.105±.024
9	.284±.014	.250±.037	.113±.013
10	.286±.002	.230±.035	.090±.027
11	.281±.001	.230±.031	.105±.013
12	.282±.011	.227±.031	.107±.024
13	.281±.005	.223±.031	.106±.013
14	.266±.023	.212±.037	.108±.010
15	.260±.026	.201±.035	.117±.012
16	.258±.028	.200±.034	.105±.002

PI = Post-infection

4.5 Pathological Findings

4.5.1 Gross Pathology

Camels C149 and C156 which died of the acute disease had good body condition. The peritoneal cavity was filled with serosanguineous fluid. The rumen was impacted with ingesta while the large and small intestines were gas filled. The mucosa of the abomasum had diffuse and ecchymotic haemorrhages running along the longitudinal folds (Plate 8). The intestines were congested and had diffuse haemorrhages, especially on the mucosa (Plate 9). Haemorrhagic lymphadenopathy of the superficial and deep lymph nodes was pronounced. The trachea and bronchi had froth and were congested (Plate 10). The lungs were oedematous, congested and enlarged as showed by the rounded edges (Plate 11). The pericardial fluid was blood-tinged and the pericardial sac slightly congested. The epicardium and myocardium had petechial haemorrhages (Plate 12) while the heart of the animal that died at the end of the experiment had petechial haemorrhages on the epicardium and depleted coronary fat (Plate 13). The spleens of the animals that died from the acute disease were enlarged, but that of the animal that died towards the end of the study was flabby and pale (Plate 14). The meninges were heavily congested (Plate 15) and the brain oedematous. The kidneys were congested and the pelvis had ecchymotic haemorrhages. The bladder was massively congested and contained yellowish urine.

The camel that died at the end of the study had substantial subcutaneous oedema and yellow gelatinous exudate under the skin, especially on the ventral side of the body. Its muscles were pale and the small intestinal loops had adhered to one another and had

haemorrhages on the serosal surface (Plate 16).

4.5.2 Histopathology

Histopathological changes in the two camels (C149 and C156) which died of the acute disease were confined to the heart, kidneys, lungs and liver. The heart had severe myocardial haemorrhages and oedema which was characterized by separation of muscle fibres. Venous congestion was pronounced in cardiac vessels (plate 17). The lungs had generalized marked venous congestion with interstitial and alveolar oedema in localized regions. There were, however, some with severe alveolar oedema. In one camel (C156) there was mild generalized lymphocytic infiltration in the lungs (plate 18). The kidneys showed marked venous congestion (plate 19) with occasional haemorrhagic foci. Some areas had severe congestion and haemorrhages. The brain of camel 156 showed histopathological reaction characterized by venous congestion, mild perivascular lymphocytic infiltration and mild gliosis. There was also oedema in the brain stem characterized by vacuolation of the brain tissue (plate 20).

Camel C159 which died at the end of the experiment had histopathological lesions in several organs. The brain had marked oedema, the lung had alveolar oedema with moderate venous congestion plus a few lymphocytic foci. The spleen had a marked expansion of the white pulp and trabeculae at the expense of the red pulp. There was fibrous tissue formation signifying splenic fibrosis. The liver had severe generalized cirrhosis with venous congestion. The heart had a few localized areas of venous congestion. The kidneys had increased glomerular mononuclear lymphocytic infiltration.

Plate 8: Abomasum of an infected non-treated camel that died during the acute phase of the disease showing diffuse and ecchymotic haemorrhages (arrows 1 and 2, respectively)

Plate 9: Small intestines of an infected non-treated camel that died of the acute disease showing diffuse haemorrhages.

Plate 10: Trachea of an infected non-treated camel that died during the acute disease showing froth (arrows 1 and 2) and congestion.

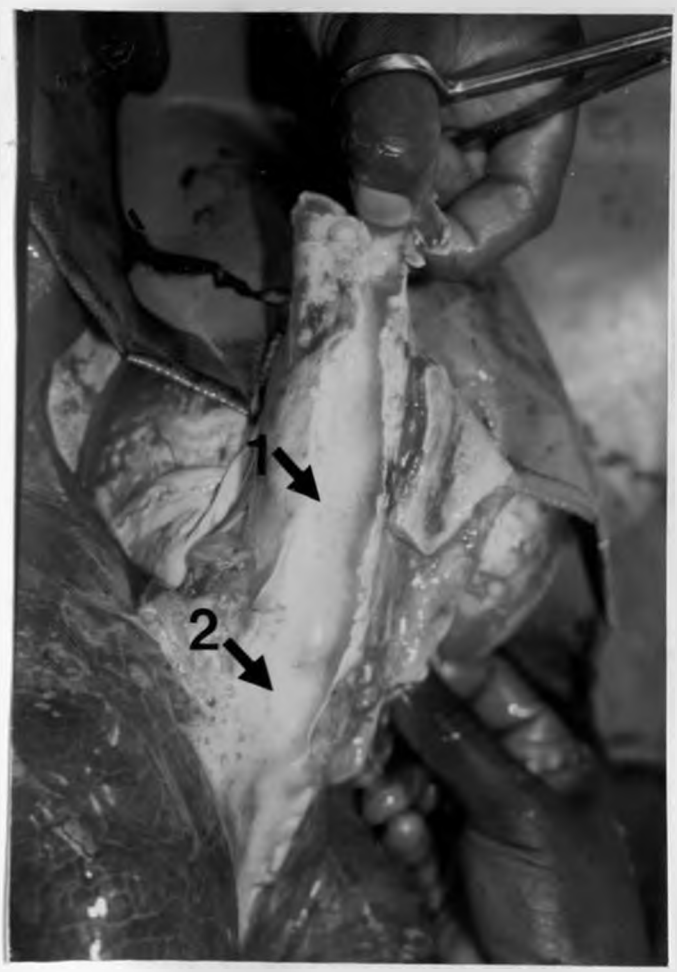


Plate 11: Lung lobes of an infected non-treated camel that died during the acute disease showing rounded edges (arrows).

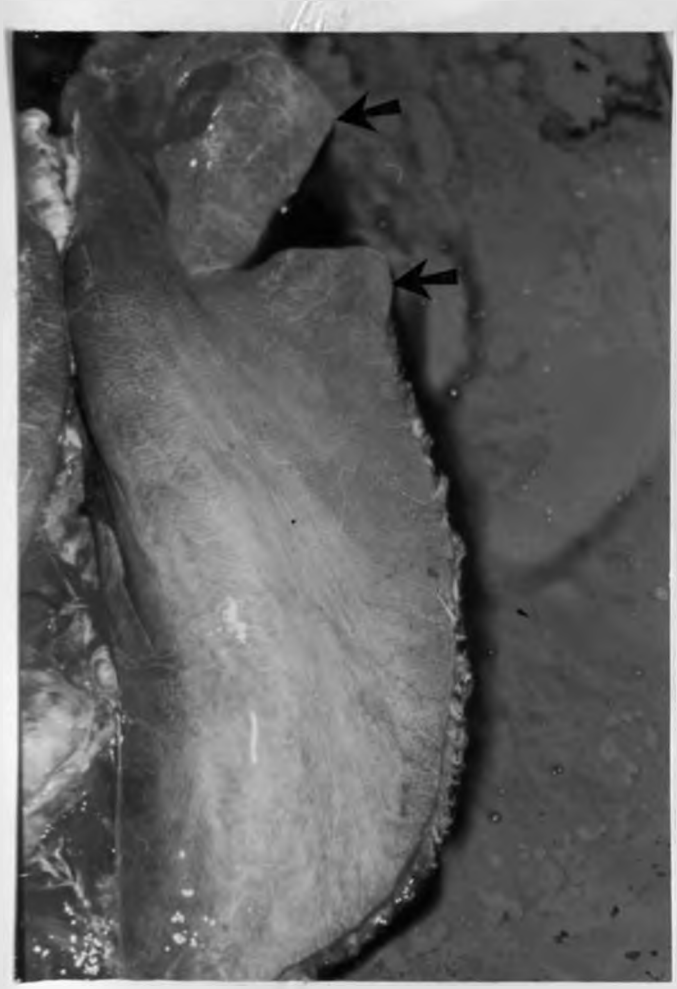


Plate 12: Heart of an infected non-treated camel that died of the acute disease showing petecchial haemorrhages (arrow).

Plate 13: Heart of an infected non-treated camel that died at the end of the study showing depleted coronary fat (arrow).

Plate 14: Spleen of an infected non-treated camel that died at the end of the study showing paleness.

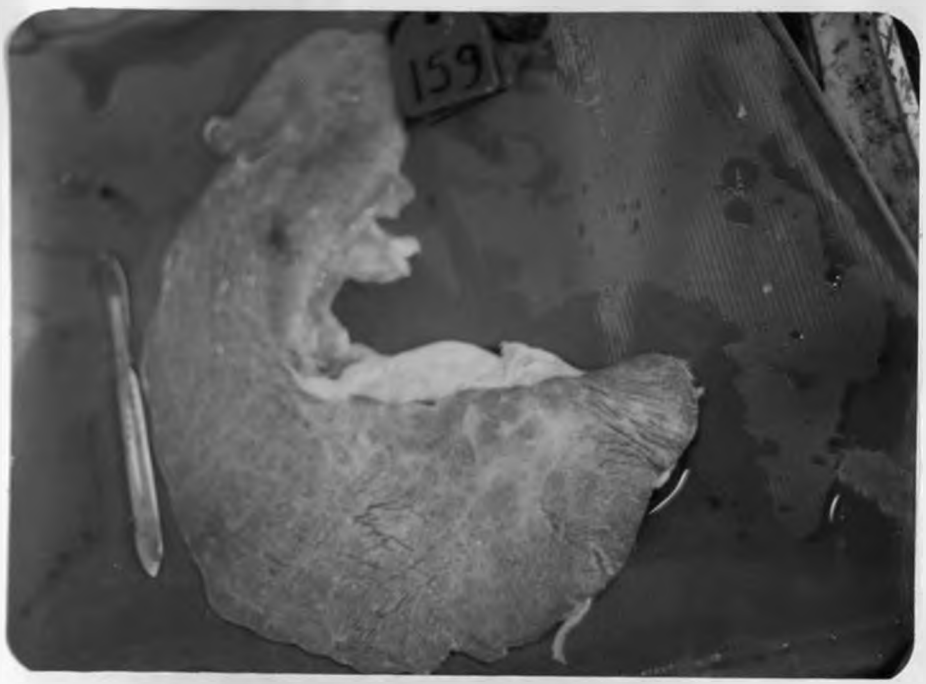


Plate 15: Brain of an infected camel that died of the acute disease showing congested meninges (arrows).

Plate 16: Intestinal loops of an infected non-treated camel that died at the end of the study (note the haemorrhages and adhesions shown by arrows 1 and 2, respectively).

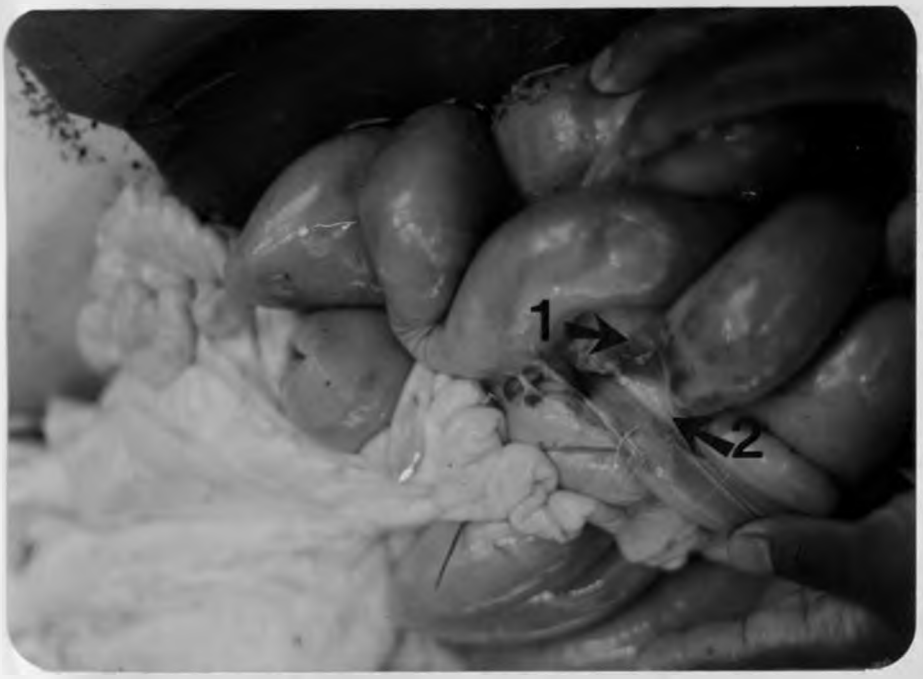


Plate 17: Transverse section of the heart muscle of a camel that died 2 weeks post-infection showing venous congestion (arrows 1 & 2) and myocardial haemorrhage (arrow 3) (H & E, x 100).

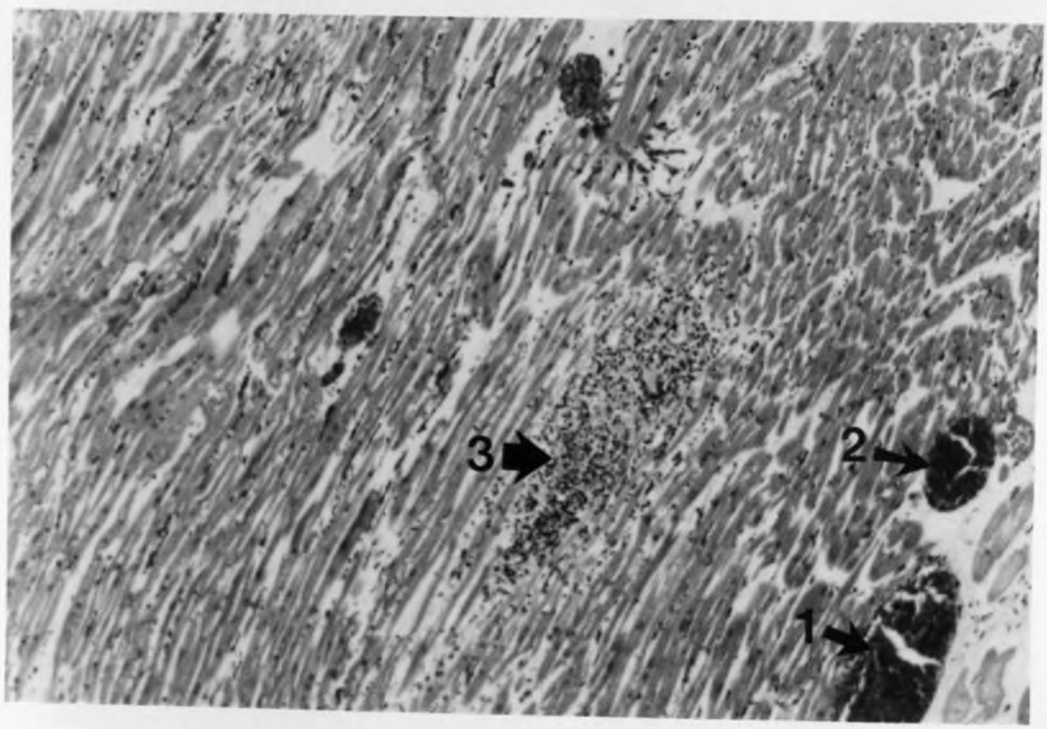


Plate 18: Cross section of the lungs of a camel that died 2 weeks post-infection showing venous congestion (arrows 1 & 2 and mild generalized lymphocytic infiltration (H & E, x 100).

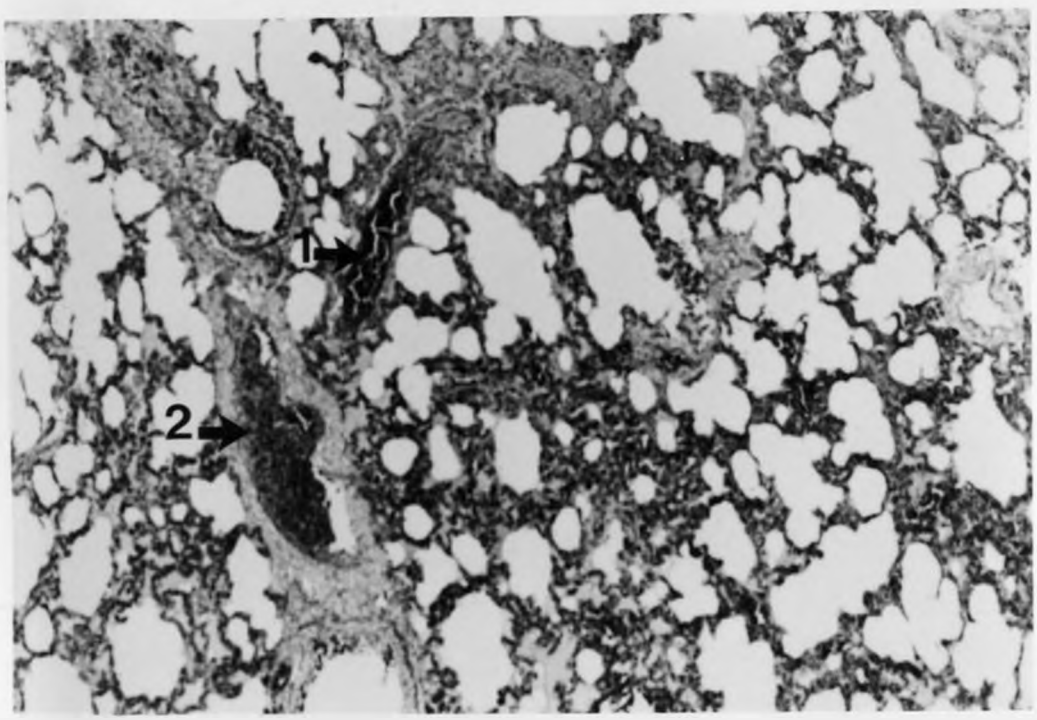


Plate 19: Cross section of the kidney of a camel that died 2 weeks post-infection showing marked venous congestion (arrows) (H & E, x 100).

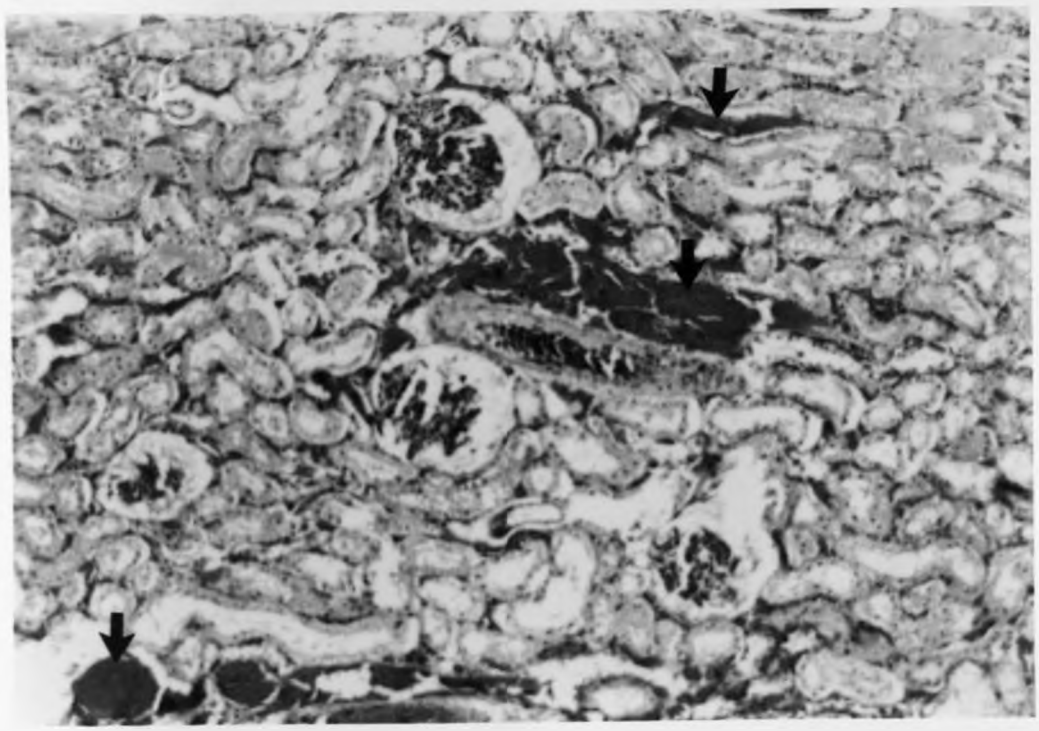
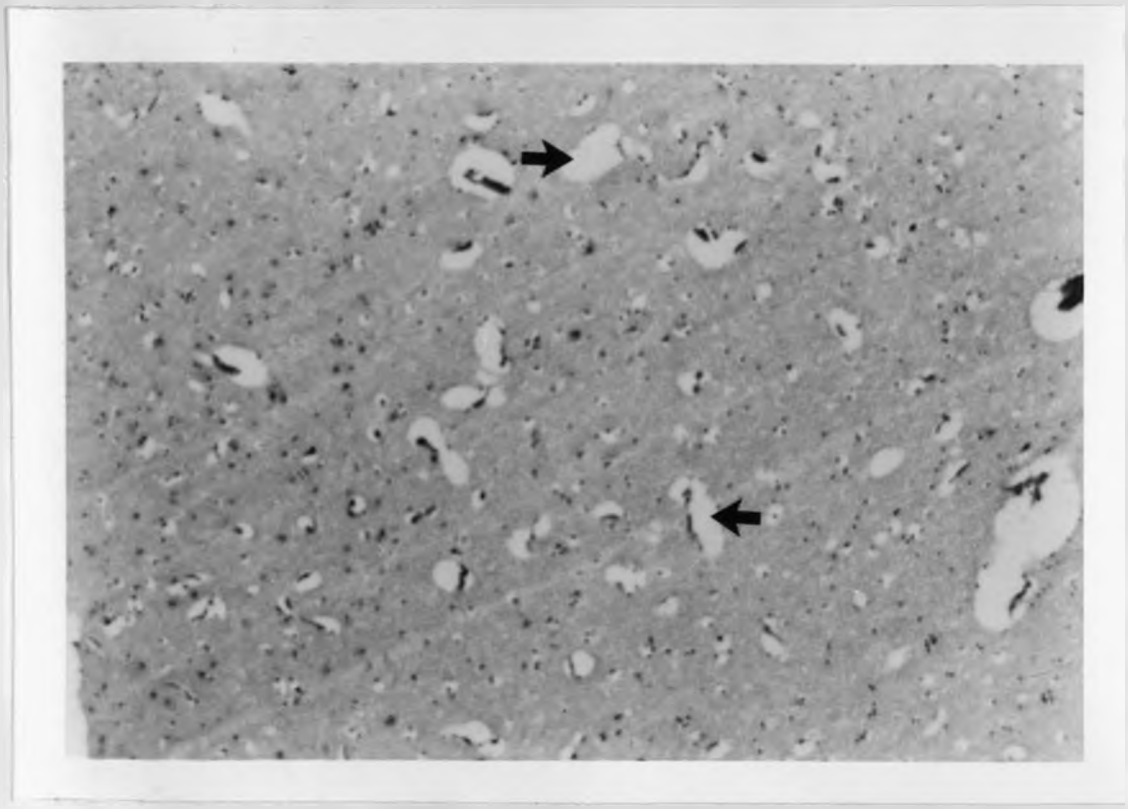


Plate 20: Cross section of the brain of a camel that died at week 16 showing oedema in the brain stem characterized by vacoulation (arrows) (H & E, x 100).



CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Since the review of Gatt-Rutter (1967), little has changed to diminish the importance of *T.evansi* infection on the well being of the world's camel population (Boid *et al.*, 1985), necessitating more impetus to be put in the areas of camel clinical pathology, immunology and related disciplines in order to elucidate the diagnosis and therapeutic management in the control of this important disease. Clinical manifestation of *T.evansi* infection in camels in this study showed significant evidence of changes in health, haematological, biochemical and pathological status.

All infected camels in this study developed an acute or subacute disease, 7 days post infection which was characterized clinically by inappetance, dullness, heavy parasitosis, pyrexia, excessive lacrimation, enlargement of superficial lymph nodes, reduction in packed cell volume and death. Similar signs have been reported elsewhere (Stephen, 1970; Losos, 1980; Higgins, 1983).

Trypanosomiasis is usually characterized by fluctuation of parasitaemia as evidenced by the infected, non-treated camels that survived up to the end of the study. Relapsing parasitaemias are either accompanied with or without pyrexia. In this study, trypanosomes appeared in peripheral circulation in large numbers initially, thereafter parasite numbers were often low and sometimes difficult or impossible to detect using BCE or MI. The appearance of swarming parasites in blood without marked external symptoms of the disease occurred during the

first stage of *T.evansi* infection in a course of 5 stages (Pease, 1906). Persistent high rectal temperatures, in the present study, were observed from day 7 for a period of 3 consecutive days following intravenous inoculation of trypanosomes and thereafter, fever fluctuated coinciding with or immediately after parasitaemia, especially in the infected non-treated camels.

Theiler (1906) reported that the shortest incubation period in *T.evansi* infection was 3 days after which the temperature rose and the parasites appeared. A fever which persisted for some days and then disappeared was noted. The same phenomenon was repeated several times. Similar observations were made in this study, especially in the infected non-treated camels. The same findings have been observed in other camel studies by several workers (Pease, 1906; Mahmoud and Osman, 1979). These series of exacerbations and remission of the temperature curve are characteristic of trypanosomiasis and are typical picture in animals suffering from trypanosome infection (Losos, 1980). Thus, the present study has demonstrated the principal clinical signs of trypanosomiasis, i.e., fever and relapsing parasitaemia.

Trypanosomiasis in camels is a slow wasting disease that is protracted over 2 to 4 years. In the natural disease, the mortality rate is around 3% (Gatt-Rutter, 1967). In the current study the disease caused approximately 80% mortality in the infected non-treated group. This agrees with the observation that trypanosomiasis in the camel is nearly always fatal. If untreated, the disease produces over 90% mortality (Luckins, 1992). Mahmoud and Osman (1979) also reported rapid deaths in some camels infected with *T.evansi*. Verma and Gautam (1978) observed subacute and chronic form of the disease in buffalo and

cow calves. The high mortality rate is reduced greatly by intervention with trypanocides. According to Gatt-Rutter (1967), acute disease may resemble that seen in anthrax, blackquarter and pasteurellosis. The course of the disease manifested in the present study was an acute one with three out of four infected non-treated camels dying within 4 months. Several other authors have managed to reproduce both acute and subacute diseases in the camel (Leese, 1927; Alongi and Balboni, 1935). The factors that determine the course the disease takes are many, but the host-parasite relationship, nutritional status, age, concomitant infection, immunosuppression and prevailing level of stress are considered important (Losos, 1980; Rottcher *et al.*, 1987). In this study the manifestation of the acute disease could have been related to the dose of the trypanosomes used to infect the camels (2×10^7 trypanosomes/camel), the route of infection (intravenous) or the virulence of the trypanosome strain used. Pease (1906) noted an extremely variable course of *T.evansi* infection in camels. The author observed that in some cases the course was rapid taking only a few months. In others he noted a more chronic form, the camels living and remaining in fair condition for long periods. Camel keepers have sometimes given accounts of the disease lasting only a week and seldom exceeding 4 months. Camels under natural challenge usually receive a lower inoculum dose from the biting flies and the route of infection is usually intradermal. Thus, the level of parasitaemia in these field animals is lower compared to what was shown in the current experiment. From this study and other previous work, it is apparent that while *T.evansi* infection in camels generally takes a chronic form, the acute or subacute forms also occur.

Parasites disappeared following treatment within 24 hours as demonstrated by mouse inoculation and buffy coat examination. There were no relapse infections throughout the study period. The disappearance of the trypanosomes from peripheral blood in this study is similar to that reported by other workers (Otsyula *et al.*, 1992; Nyang'ao *et al.*, 1992). There were no relapses in the camels in the present experiment as in previous studies done in the field where relapse parasitaemia occurred following treatment with Cymelarsan (Zellecke *et al.*, 1989; Otsyula *et al.*, 1992). Under field conditions camels are usually under constant exposure to trypanosome challenge from the reservoir animals by the biting flies and therefore it may be difficult to differentiate relapse from new infections. Relapses of *T. evansi* infections in camels treated with other trypanocides especially the recommended drugs, quinapyramine and suramin, have been shown to occur (Mahmoud and Osman, 1979; Luckins *et al.*, 1979; Schillinger *et al.*, 1984). These other drugs do not cross into the occult sites of the body in high enough concentrations to effect cure. Thus, the trypanosomes remain in hiding and eventually relapse into peripheral circulation. Cymelarsan, being an arsenical, can cross into the occult sites and clear all the trypanosomes that could be hiding there. Thus, in this study Cymelarsan could have crossed into the hiding sites and cleared any trypanosomes present there as evidenced by disappearance of parasitaemia and antigenaemia in the treated camels.

A number of workers have found normal blood values for the dromedary camel as shown in appendix II. The pre-infection PCV values of 28.1 ± 0.3 determined in the present study is in reasonable agreement with the results established in several previous studies (Lakhotia *et al.*,

1964; Abdelgadir *et al.*, 1979; Banerjee *et al.*, 1962; Ghodsian *et al.*, 1978; Higgins and Kock, 1985). However, these authors' values and those established in the current study are lower than those reported by Raisinghani *et al.* (1980) and Soliman and Shaker (1976). Soliman and Shaker (1976) gave a PCV range of 42.9 to 44 in the blood of adult she camels in India, while Raisinghani *et al.* (1981a) also working with Indian camels gave a range of 30 to 31. These high PCV values could be attributed to differences in the breed of camel and the geographic location.

Anaemia is an inevitable consequence of trypanosome infection (Murray and Dexter, 1988). This is well demonstrated in the present study, where the PCV, Hb and RBC were all reduced following infection. The best indicator for anaemia was PCV which dropped rapidly in both groups from the pre-infection mean of 27% to 23% within 3 weeks. Similar observations have been reported by Jatkar and Purohit (1971) in camels and mice. According to Murray and Dexter (1988) this rapid drop of PCV is always correlated closely with the appearance (height and duration) of parasitaemia. The degree and severity of anaemia in the current study was closely associated with the development of parasitaemia such that in the first three weeks when parasites were always present in the peripheral circulation, PCV drop was rapid. Subsequent drop in the PCV of the infected non-treated camels in this study was not as rapid as that in the first three weeks. The level of parasitaemia was also scanty. In one review, Murray and Dexter (1988) showed that the initial rapid fall of PCV that accompanies the first wave of parasitaemia is the first phase of anaemia in trypanosomiasis, while the second phase is characterized by absence or scant parasites. In

the infected and treated camels the PCV levelled off at week 6 and remained constant (at an average of 24%) to the end of the experiment, unlike in the non-treated camels where the PCV continued to fall. The non-treated camels, thus, progressed into phase two of the disease syndrome (Murray and Dexter, 1988). The PCV in the treated camels, however, did not rise to pre-infection level by the end of the study as observed in bovine that complete recovery of PCV occurs within 2 to 4 months. The slow rate of recovery of the red blood cells and PCV in this study could be due to the susceptibility of the animals to *T. evansi* infection as shown in certain cattle breeds which are susceptible to tsetse-borne trypanosomiasis such as the zebu (Murray and Dexter, 1988).

The pre-infection RBC level of 8.7 ± 0.5 reported in this study follows a similar pattern and in agreement with that reported by other workers and compares well with previously established values. Banerjee *et al.* (1962) reported that the average number of erythrocytes present in blood samples of dromedaries they examined was 6 to 9 x 10^6/ml. A similar range has been recorded in the present study and by Al-Ali *et al.* (1988), Abdelgadir *et al.* (1979), Ghodsian *et al.* (1964), Lakhotia *et al.* (1964) and Higgins and Kock (1985). However, Majeed *et al.* (1980) and Soliman and Shaker (1976) presented RBC results which were lower than those reported here and by other workers. Majeed *et al.* (1980) showed that RBC values were influenced by seasons such that during winter they recorded a higher value of RBC levels than in other seasons.

The pre-infection Hb level (11.6 ± 0.3) presented in the current study was lower than that established by most investigators. It is in agreement with the values established by Lakhotia *et al.* (1964),

Ghodsian *et al.* (1974) and Abdelgadir *et al.* (1979). A significant fall in RBC numbers and haemoglobin content was observed in group A and B camels in the first three to four weeks after infection. The fall followed a similar pattern to that of the drop in the PCV. The recovery of RBC was observed in the treated group in week 11.

Anaemia in trypanosomiasis has been attributed to various causes such as haemodilution, extravascular hemolysis and hemolysis due to toxins from the trypanosomes and bone marrow failure (Holmes, 1976; Dargie *et al.*, 1979; Murray *et al.*, 1980; Jenkins and Facer, 1985). For one to determine the cause of anaemia it is important to monitor erythropoietic activities in the bone marrow, spleen and liver. Dargie *et al.* (1979) showed that there was an increase in erythropoietic activity in animals infected with trypanosomes and concluded that the anaemia observed was due to the extravascular destruction of erythrocytes rather than inhibition of haemopoietic activity. In the present study bone marrow activity was not monitored hence it is difficult to determine what the cause of anaemia was.

Normal total WBC value of 13.6 $\pm$ 2.9 obtained in this study was in excellent agreement with nearly all the normal values published by previous workers. The results are only in disagreement with the values established by Higgins and Kock (1985) who showed the normal range of total WBC for dromedary camel to vary from 2.9 to 9.7 x 10^3/ml. According to these authors, occasionally the number of white cells in the blood of clinically normal adult dromedary camel can be very low. However, the camels these authors used were not kept in their traditional environment, and this could have been the cause for the low WBC encountered in their study. A comparison of the differential

leucocyte counts obtained in this study with those of other authors reveal that the mean pre-infection differential counts fall within the generally established normal ranges (Appendix II). The predominant white blood cells in this study were the lymphocytes, followed by neutrophils. The ratio between the lymphocytes and neutrophils was about 1:1 (Table 6). This has also been shown by Majeed *et al.* (1980). Monocyte and eosinophil percentages in the current study were relatively low when compared to other previous studies. This could have been due to environmental or breed influences.

In this study significant leukopenia was observed during the first peak of parasitaemia which occurred concurrently with the development of anaemia. The reduction in the total WBC was more pronounced in group B camels and occurred up to the second week. This concurrent reduction in the total WBC and onset of anaemia has been documented by several authors in different animal species (Welde *et al.*, 1974; Valli and Mills, 1980). Anosa and Isoun (1980) also found that the maximum drop in the total WBC counts of sheep and goats infected with *T.vivax* took place during the acute phase of the disease. Thereafter, the leucocyte numbers recovered steadily until the end of the experiment. In the present study, the leukocyte numbers recovered more rapidly and leucocytosis which developed peaked in the fourth week. The leucocytosis in the infected camels in the current study was due to lymphocytosis as they are the only cellular population that increased significantly in the observation period. Similar results have been reported by Valli and Mills (1980) who demonstrated that lymphocytosis was responsible for the rise in the total leucocytes in animals infected with trypanosomes. The leucocytosis observed during this study is in

agreement with the findings of Jatkar and Purohit (1971) in camels and mice.

Several studies have been carried out to establish the normal enzyme activity, but there is sparse data on values of trypanosome infected camels. The enzymes assayed in this study included alkaline phosphatase (AP, EC 3.1.3.1), creatine kinase (CK, EC 2.7.3.2), alanine aminotransferase (ALT, EC 2.6.1.2, previously SGPT), aspartate aminotransferase (AST, EC 2.6.1.1, previously SGOT) and lactic dehydrogenase (LDH, EC 1.1.1.27). These enzymes can be organ specific or occur in one tissue in a much higher concentration that in another.

AP, which catalyses the dephosphorylation of ATP, is located in most cells, but has high specific activity in the brush borders of the intestines, in the bones, kidneys, placenta and liver. Serum AP activity has diagnostic value of hepatic and bone diseases in dogs and cats (Cole, 1986; Duncan and Prasse, 1986). In large animals it has a broad range of references, and therefore may not be diagnostic. The normal values of AP of 8.2 IU/l ± 2.9 in racing camels was obtained by Beaunoyer (1992). This level of activity is in reasonable agreement with the pre-infection value of 11.4 IU/l ± 1.8 obtained in this work. These values are however, higher than those established by Al-Ali *et al.* (1988) of 6.3 IU/l ± 0.1. Boid *et al.* (1980a) and Eldirdiri *et al.* (1987) obtained much higher values (31.5 IU/l ± 9.1 and 34.8 IU/l ± 10.3 respectively) than that reported in the present study and by other authors. AP in the infected camels in this study declined gradually in the first two to three weeks after infection. The same observation was reported previously by Goodwin and Guy (1973) and Boid *et al.* (1980b). The cause of the fall of AP during patent parasitaemia is not known. After

the fall, there was an increase in the AP activity from the third week in the present experiment. Considering severe haemorrhage in the intestines and cardiac muscle, there could have been damage of these two tissues which would have resulted in the increase in the AP level as from week 3 onwards. Beaunoyer (1992) could not show significant changes in AP after exercise in racing camels. In the present study, following treatment, the difference in AP between groups B and C was not as marked as that between groups A and C. Thus, the infection caused damage in the intestines, kidneys and liver of the infected non-treated group of camels as shown by the gross pathology and histopathology of the camels that died.

Aspartate aminotransferase (AST) catalyses the transamination of L-aspartate and 2-oxalogutarate to oxaloacetate and glutamate, respectively. Presence of AST in many tissues makes serum levels of this enzyme a good marker of soft tissue damage (Cole, 1986; Kaneko, 1989). AST is considered as a diagnostic enzyme for liver and muscle disease because of its high activity in these organs. Serum AST activity also increases with changes in muscular permeability (sublethal injury and necrosis) so it is used as a diagnostic aid in neuromuscular disorders of domestic animals (Kerr, 1989). The plasma level of AST of 5.9 IU/l ± 0.4 recorded in this study is within the normal range as that reported by Eldirdiri *et al.* (1987) and Al-Ali *et al.* (1988), but lower than that of 11.0 IU/l ± 1.7 reported by Beaunoyer (1992). AST is mostly bound to the mitochondria and therefore marked elevation in the blood indicates serious cell damage if the cause of elevation is cell lysis (Beaunoyer, 1992). In the current study there was a marked increase in AST activity in group B by the third week. The rise closely followed

the first peak parasitaemia. Raised levels of serum AST have also been reported in cattle infected with *T.congolense* (Welde *et al.*, 1974), in horses with myocardial lesions and in rabbits infected with *T.brucei* (Goodwin and Guy, 1973). The elevated levels of AST can thus be attributed to the host cell damage by trypanosomes especially, as it was closely associated with massive parasitosis. The cell damage caused by trypanosomes of the *brucei* group is mainly in connective tissue, including perivascular tissue (Goodwin, 1970). The vascular damage probably results in a fall in pH sufficient to activate lysosomal enzymes thereby causing further tissue damage (Goodwin, 1970). The change of AST observed in this study seemed to relate to host parasitaemia as the levels started to decline and return to normal after treatment of group B with Cymelarsan, which quickly cleared the trypanosomes from circulation. The same was observed in camels following treatment with quinapyramine sulphate and suramin by Boid *et al.* (1980b). According to Boid *et al.* (1980b), AST increase occurs in the second step of pathological process that occurs after the acute crisis. The increase of AST level in this study can be attributed partly to cellular damage caused by trypanosomes and partly to trypanosome lysis (Gray, 1963). The latter could be the cause of the rapid principal increase in AST activity since it occurred immediately following treatment in group B camels.

Alanine aminotransferase (ALT) catalyses the reversible transamination of L-alanine and 2-oxaloglutarate to pyruvate and glutamate (Cole, 1986). This enzyme is a specific indicator of liver damage in primates, dogs, cats, rabbits and rats (Cole, 1986; Kaneko, 1989). ALT in tissues of large animals is too low to be of diagnostic

value. The pre-infection ALT value of 2.02 IU/l ± 0.01 compares well with that of Boid *et al.* (1980b) and Eldirdiri *et al.* (1987). The ALT levels remained low following infection in the present study. Gray (1963) and Boid *et al.* (1980b) found elevated levels of serum ALT in cattle infected with *T.vivax* and camels infected with *T.evansi*, respectively. According to Gray (1963) the increased serum level of this enzyme was due to release of trypanosomal ALT into circulation after destruction of the trypanosomes by the host. *T.evansi* contains ALT:AST in the ratio 5:1. It would be expected that destruction of *T.evansi* by the camels immune system in the two studies would produce an increase in serum ALT that was observed by Gray (1963) and Boid *et al.* (1980b). The observations made in the present study do not seem to support the observations of the these authors.

Creatine kinase (CK) catalyses the reversible phosphorylation of creatine by ATP to form creatine phosphate, required by muscles. CK is found in many types of cells, but has highest specific activity in skeletal muscle, cardiac muscle and brain (Cole, 1986; Kaneko, 1989). CK is a sensitive indicator of muscle damage (Kerr, 1989). The pre-infection CK value obtained in the present study of 54.7 IU/l ± 1.5 was higher than that obtained in previous studies, of 35.6 IU/l ± 9 (Al-Ali *et al.*, 1988), but less than that recorded by Beaunoyer (1992) in racing camels, of 81 IU/l ± 7. Following infection, there was a slight decline of CK activity in groups A and B camels up to the second and third weeks, respectively. Thereafter, the CK activity in these two groups of camels was significantly higher than the pre-infection values and those of control camels. The levels did not return to pre-infection level until week 14 in both groups. The increase in the infected non-treated

group (A) was very rapid while that of infected and treated camels was gradual. CK, being a sensitive indicator of muscle damage, the rapid increase in its activity could imply that there was muscle damage; the damage being more severe in group A than B camels. Studies on exercise stress on racing camels also showed a significant increase in CK a few hours following exercise (Beaunoyer, 1992) and the levels did not return to pre-exercise levels until 36 hours later.

Lactic dehydrogenase (LDH) catalyses the reversible oxidation of pyruvate L(+)-lactate with co-factor NAD in all tissues. Elevated LDH activity is associated with skeletal muscle, liver and heart damage (Kaneko, 1989; Cole, 1980). The normal level of LDH recorded in the present work was 136.6 IU/l ± 5.8. This value is lower than that recorded in all other previous studies. Al-Ali *et al.* (1988) recorded the normal LDH value of 262 IU/l ± 15, Eldirdiri *et al.* (1987) obtained a value of 344 IU/l ± 98, while Beaunoyer (1992) reported a much higher value of 427 IU/l ± 26. Following infection, LDH increased in camels of groups A and B even though the increase was not statistically significant. The increase in the infected group could mean that the trypanosomes caused tissue damage in these two groups of camels. The increase in group A camels was higher than that of group B camels, which could imply that the trypanosomes in the untreated group of camels caused protracted severe muscle damage. Group B camels were treated, thus stopping the trypanosomes from causing further damage.

Urea nitrogen (often called blood urea nitrogen) is formed in the liver and represents the principal end product of protein catabolism. Clinically, urea nitrogen is commonly used as an indicator of renal function in most animals. The main causes for increase in urea nitrogen

concentration are dietary and renal insufficiency (Cole, 1986; Kerr, 1989). The dietary causes of increased urea nitrogen levels are excess dietary protein and carbohydrate deficiency. The causes of renal insufficiency include poor renal perfusion due to fairly severe dehydration or cardiac insufficiency or renal failure. Catabolic breakdown of tissues as a consequence of fever, trauma, infection or toxaemia may also result in a moderate increase in urea nitrogen concentration (Cole, 1986). The pre-infection value obtained in the current experiment was comparable to that recorded by Higgins and Kock (1985). Following infection, the urea nitrogen levels in group A camels fell gradually to the ninth week and then rose to above the pre-infection level up to the end of the experiment. In group B camels there was an increase to the second week then a gradual fall up to the end of the study. In this experiment, the catabolic breakdown of tissues could have been the cause of the moderate increase of the urea nitrogen. Thus, in both groups, there was an overall increase of urea nitrogen above the pre-infection level and the levels in control camels. The increase in the urea nitrogen was slight in both infected groups of camels and lasted a few weeks. In group B camels the increase occurred during the pyrexic period. This could mean that there was tissue breakdown during this period that resulted in the moderate increase in urea nitrogen. Following treatment, the urea nitrogen in group B camels fell gradually to the pre-infection level. In group A camels there was a rise from the ninth week to the end of the experiment. In this group of camels the cause of increased urea nitrogen concentration could have been damage of kidney parenchyma that resulted in decreased renal perfusion. Histopathological examination of the kidneys showed some areas with

marked venous congestion and severe haemorrhages. This could cause damage of kidney parenchyma which could have resulted in decreased glomerular filtration rate and, thus, an increase in the urea nitrogen concentration.

The pre-infection level of total proteins in this experiment of 5.4 g/dl ± 0.4 is in excellent agreement with the data of several other authors (Ghodsian *et al.*, 1978). Following infection, total protein concentration increased above the normal values. The elevation of total serum proteins in the present study was attributed solely to hyperglobulinaemia, since the level of albumin in the infected camels remained nearly constant, with very slight increase compared to the controls. There was elevated globulin concentration from the second week. This elevation of globulins could be attributed to the antibody response stimulated by the trypanosomal antigens. This is further supported by the high levels of total proteins in the infected camels when compared to the control camels from the second week which is the time taken for free antibodies to be detected in circulation (Boid *et al.*, 1980b). The globulin levels increased significantly during infection and this agrees with the observations of Jatkar *et al.* (1973) and Boid *et al.* (1980a). The increased total protein levels in the present study refutes earlier studies by Jatkar *et al.* (1973) that indicated no changes in total protein.

In general, even though the established pre-infection biochemical and haematological values in this study are broadly in agreement with the normal values reported by other workers in literature, the few discrepancies in present findings and those of other authors could be attributed to age, sex, breed of camels used, nutrition, husbandry and

geographical location, since these are some of the factors that have been shown to influence haematological and biochemical profiles of animals. Other factors that could cause the differences are variations in sampling procedures and analytical techniques for determining these parameters, especially the techniques used for enzyme assay. Following infection, there was a marked increase of some enzyme activity notably AP, AST, CK and LDH. These enzymes have been shown to increase in other studies and may be regarded as the most important diagnostic enzymes during pathological changes in the dromedary camels. The increase of the enzyme activity in this study was recorded from the third and fourth week. This could be the time when trypanosomes are invading various tissues from peripheral circulation.

Circulating antigens were detected as early as one week after infection. Rae and Luckins (1984) found that in goats experimentally infected with *T.vivax*, *T.brucei* and *T.evansi* circulating antigens were found within 10 to 14 days which is in agreement with the present study. Other authors have demonstrated circulating antigens as early as 6 days post infection (Nantulya *et al.*, 1989a). During the first peak of parasitaemia (first week) circulating antigens were demonstrated in only three camels. This is the phase when trypanosomes are dividing and no sufficient parasite degradation occurs to produce detectable antigens (Nantulya *et al.*, 1989b). This could have been the reason why antigens were not detected in the first week after infection in camel 156 that died during the acute disease and in the other five camels in which the antigens were demonstrated in the second, third and fourth weeks. After the acute phase, circulating antigens were initially detected in low levels and increased with time in the two surviving camels in group A

and all the camels in group B. The peak OD Ag-ELISA value coincided with the time of maximum parasite destruction. In the acute phase of the disease, when parasitaemia is high and parasites are easily demonstrated by microscopic examination, antigen ELISA test may not be a very useful diagnostic tool because the antigens are usually insufficient in circulation (Nantulya *et al.*, 1989b). Ag-ELISA is most important as a diagnostic tool in the chronic stage of the disease because that is when the parasites are scarce or even absent in the peripheral circulation and are hard to detect. Thus, for diagnostic purposes, parasitological diagnosis and Ag-ELISA assay should be used to complement one another (Nantulya, 1990).

Following treatment of group B camels there was a high elevation of circulating antigens. This was followed by a gradual decline of Ag-ELISA OD values to pre-infection levels by an average of 63 days. Several authors have found different periods of the disappearance of the antigens after treatment. Olaho-Mukani *et al.* (1992b) found no detectable antigens in goats and camels 12 and 41 days after treatment, respectively, while Rae and Luckins (1984) did not detect circulating antigens 7 days following treatment in rabbits, and Liu *et al.* (1988) observed that in treated monkeys antigens could not be detected as early as 27 days. Individual camels had different periods of disappearance of antigens in this study such that in some camels antigens were not detectable by the third week and in one camel they persisted to week 10 following treatment. It appears that after treatment trypanosomes die releasing antigens into circulation. Many are strong immunogens and immediately react with antibodies which had been produced against them to form immune complexes. However, the

less immunogenic antigens or antigens whose epitopes remain exposed are then detectable in circulation for some time after treatment (Olaho-Mukani, 1989). Thus it would be expected that the serum levels of such antigens will be elevated for some time after the treatment of infected animals. These are the antigens detected by Ag-ELISA. Sequential assays of serum from such animals gives a decreasing profile of antigens and thus a better post-treatment picture. The decline in antigens to negative values after treatment in this study shows the usefulness of the Ag-ELISA test in predicting chemotherapeutic success. In the current study Ag-ELISA was successfully used to assess the efficacy of Cymelarsan in treatment of *T.evansi* infection in the dromedary camels as shown in group B. Persistently detectable circulating immune complexes in infected and treated animals for a long period of time could indicate that there is impairment of clearance mechanisms for immune complexes in this disease (Nantulya and Lindqvist, 1989). This could have been the case in camel number 162 that had detectable antigens for about 10 weeks after treatment. In the non-treated group, antigens remained detectable and above pre-infection level until the end of the experiment. Antigenaemia in this group of camels correlated well with parasitological findings, similar to observations by Nantulya and Lindqvist (1989).

The negative Ag-ELISA OD values following treatment showed the ability of Cymelarsan to effect cure by clearing parasites in the peripheral circulation and in the occult sites where other trypanocides fail to reach in therapeutic concentrations (Jennings *et al.*, 1979).

Antibodies were detected starting from the first week following infection in some animals. The antibody levels showed less fluctuation

in group A camels while in group B camels there was a slight decline in the Ab-ELISA values towards the end of the experiment but the level remained higher than the pre-infection values. This indicates that antibodies persisted over 100 days following treatment. This has been documented by several workers (Olaho-Mukani *et al.*, 1992b; Luckins *et al.*, 1978, 1979). Due to the persistence of antibodies to over 100 days post-treatment, they are not a useful indicator of therapeutic efficacy.

The general post mortem picture in this study was a haemorrhagic syndrome, especially in the camels that died of the acute disease. Generalized lymphadenopathy, enlarged spleen, depletion of fat from the subcutaneous tissue and the coronary groove and the haemorrhages in the cardiac muscles and the mucosa of the intestines have been reported in camels by several other authors (Rottcher *et al.*, 1987) and in horses and donkeys (Ilemobade, 1971). Pease (1906) also recorded similar post mortem findings which included considerably large quantities of fluid in the peritoneal and pericardial cavities, oedema of lungs, depletion of fat in the usual fat depots and yellow gelatinous exudate under the skin. Thus, in acute *T.evansi* infection, the expected pathological picture is that of haemorrhagic syndrome while in the chronic disease, it is a wasting syndrome. Similar histological changes, especially in the brain, have been recorded by Ilemobade (1971).

5.2 Conclusions

i) The present study has established fairly comprehensive baseline data for biochemical and haematological values of the normal Kenyan dromedary.

ii) The study has highlighted the salient feature of the clinical disease in the dromedary camel caused by *T. evansi* infection which can be used to provide a provisional diagnosis of surra.

iii) This study has demonstrated that *T.evansi* infection in camels is fatal and that the disease can manifest itself as an acute syndrome with camels dying within the first three weeks of infection.

iv) Anaemia was the main sequel of *T.evansi* infection in camels in this study as demonstrated by a rapid fall in PCV, RBC and Hb within the first three weeks following infection. Leucopenia was observed after the acute disease. Post the acute disease leucocytosis, mainly due to lymphocytosis, developed. Treatment reverted the haematological values to normal.

v) There was evidence that the acute disease does not cause marked changes in the biochemical profile, but that there is increased activity of AP, AST, CK and LHD from the third week. This increase could signify that it is during this time that the trypanosomes are invading the tissues, thereby causing tissue damage which resulted in increased enzyme activity. Total proteins and globulin were noted to increase from the second week in all infected camels. Treatment caused the levels of the biochemical parameters to return to normal.

vi) The main diagnostic enzymes in the dromedary as indicated by this study are AP, AST, CK and LDH. Alanine aminotrasferase (ALT) is not a very useful diagnostic enzyme in the dromedary due to its low activity.

vii) Cymelarsan, the novel trypanocide used in this study, is effective in the treatment of camels infected with *T.evansi*. This was indicated by the disappearance of parasites, antigens and the animals remained aparasitaemic for over 100 days following treatment. The efficacy of Cymelarsan was further supported by improvement of the health status, haematological and biochemical parameters of the camels subsequent to treatment.

viii) Antigen ELISA (Ag-ELISA) was shown to be an efficient test in assessing the patent state of infection in camels and in evaluating the success of therapeutic intervention.

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APPENDICES

Appendix I: Mean daily temperature (°C) (±SD) of infected and control camels throughout the study.

Days PI	Camel Groups A (n=4)	B (n=5)	C (n=2)
0	36.6±.1	36.9±.4	36.6±.3
3	36.7±.5	36.7±.6	37.2±.8
4	37.0±.6	37.1±.9	36.6±.4
5	37.2±.7	37.5±1.2	37.5±.3
6	37.2±.2	37.6±.9	36.9±.4
7	37.5±.5	37.6±.9	37.6±.3
8	38.4±.9	37.6±.7	37.6±.2
9	38.6±1.2	38.0±1.3	37.4±.1
10	38.9±1.2	38.2±1.1	37.4±.1
11	37.8±.9	38.1±1.1	36.8±.3
12	37.7±.5	37.4±1.3	36.6±.3
13	37.1±.9	37.3±.7	36.9±.1
14	38.0±.4	36.7±.4	36.1±.0
15	38.0±1.0	36.9±.9	36.4±.1
16	36.4±.4	37.0±1.0	36.8±.3
17	36.1±.4	37.3±1.3	36.7±.1
18	36.5±.3	37.4±1.3	36.8±.1
19	36.8±.7	36.9±.5	36.4±.1
20	37.7±.7	36.2±.6	36.9±.1
21	38.0±1.3	36.6±.6	36.8±.3
22	36.4±.1	36.4±.5	36.9±.1
24	36.5±.1	36.1±.0	37.2±.4

Appendix 1: Mean daily temperature (°C) (continued)

27	36.5±.1	36.4±.5	35.9±.1
29	36.2±.1	36.3±.4	36.7±.4
31	36.5±.5	36.6±.3	36.8±.3
34	37.0±.6	36.6±.3	36.3±.1
36	36.5±.5	36.5±.2	36.4±.1
38	37.1±.2	36.5±.4	36.9±.1
41	37.3±.3	36.2±.2	36.2±.2
43	36.7±.6	36.5±.4	36.7±.1
45	36.9±.1	36.5±.4	36.5±.2
48	36.3±.2	36.2±.3	36.6±.1
53	36.7±.1	36.2±.2	36.8±.3
55	36.3±.1	36.5±.1	36.6±.4
57	36.6±.1	36.5±.3	36.3±.2
59	36.8±.2	36.5±.3	37.0±.2
62	36.5±.0	36.6±.3	36.3±.8
64	36.8±.0	36.3±.2	36.6±.1
66	36.5±.1	36.5±.1	36.7±.2
69	36.4±.4	36.5±.3	37.0±.7
71	36.9±.1	36.5±.4	36.6±.2
73	37.0±.6	36.7±.2	36.8±.7
76	36.4±.1	36.5±.4	36.6±.3
78	36.8±.3	36.8±.5	36.7±.1
80	36.7±.1	36.6±.5	36.7±.1
83	36.8±.2	36.9±.5	36.5±.1
85	37.0±.1	36.6±.6	36.9±.1

Appendix 1: Mean daily temperature (°C) (continued)

87	37.0±.0	36.7±.6	36.3±.0
90	38.1±.9	36.7±.1	36.7±.2
92	37.2±.4	36.5±.2	36.4±.1
94	36.3±.3	36.3±.2	36.8±.3
97	36.4±.0	36.3±.3	37.0±.3
99	36.3±.0	36.3±.1	36.3±.3
106	36.6±.3	36.4±.2	35.9±.3
111	35.8±.8	36.5±.2	36.3±.1
112	35.8±.8	36.5±.2	36.4±.1

**Day 0 = mean of 4 weeks before infection.

PI = Post-infection.

T-test for the hypothesis "mean 1 = mean 2" (Alpha=0.05).

Pre-infection:
A vs C $p > 0.05$.
B vs C $p > 0.05$.
A vs B $p > 0.05$.

Post-infection:
A vs C $p < 0.05$.
B vs C $p < 0.05$.
A vs B $p < 0.05$.

Appendix II : Ranges of the normal haematological values for the dromedary camels.

RBC	HB	PCV	WBC	LYMP	NEUT	EOSI	MONO	BASO	SOURCE
5.24 - 7.84	8.5 - 14.5	27.3 - 34.7	7.8 - 14.8						Lakhotia et al (1964)
6.1 - 9.3	10.6 - 15.1	28 - 33	10.5 - 28.3	33 - 58	30 - 60	2.0 - 17.5	1.5 - 6.0	0 - 0.5	Banerjee at al (1962)
7.12 - 7.28	12.4 - 14	42 - 44	11.6 - 13.4						Soliman & Shaker (1976)
4.2 - 10.3	7 - 15	18 - 44	6.2 - 30.5	14 - 78	13 - 77	0 - 18	1 - 8	0 - 4	Ghodsian et al (1978)
	9.02 -10.42	30 - 31							Raisinghani et al (1981b)
6.52 - 9.14	9.32 -12.88	27.4 - 32.6	11.2 - 15.2						Abdelgadir et al (1979)
8.25 - 9.45	13 - 16		12.8 - 29.4						Al-Ali et al (1987)
6.55 - 6.89	10.81-11.41		9.15- 11	46 - 49	43 - 46	1 - 8	1 - 2	0 - 1	Majeed et al (1988)
7.6 - 11.0	11.4 - 14.2	24 - 42	2.9 - 9.7	21 - 62	33 - 70	0 - 4	0 - 7		Higgins & Kock (1985)
3.8 - 12.6	10.6 - 20.3	12.9 - 27.2	26.5 - 65.4	21.1- 56.3	0 - 18.9	0 - 12.2	0 - 1		Soni & Aggarwala (1958)
8.2 - 9.2	11.3 - 11.9	27.6 - 28.2	12.7 - 18.5	45 - 70	27 - 50	0 - 2	0 - 1	0	This study

KEY

RBC ($X10^6$ / ml) - Red blood cells

HB (gm/dl) - Haemoglobin content

BASO (%) - Basophils

PCV (%) - Packed cell volume

WBC (10^3/ml) - White blood cells

LYMP (%) - Lymphocytes

NEUT (%) - Neutrophils

EOSI (%) - Eosinophils

MONO (%) - Monocytes

Appendix III: Ranges of the normal serum/plasma chemistry values for the dromedary camels.

AP	CK	ALT	AST	LDH	ALB	GLB	TP	BUN	SOURCE
							5.1 - 9.3		Ghodsian et al (1978)
21.5 - 41.5		1.24 - 2.84	2.6 - 5.0						Boid et al (1988 a)
					3.0 - 4.4	6.3 - 8.7		2.6 - 8.85	Higgins & Kock (1985)
17 - 51	11.3 - 75.4	1.0 - 3.3	2.0 - 8.6	198 - 522					Eldirdiri et al (1987)
5.2 - 7.4	26.6 - 44.6		4.4 - 11.0	211 - 313					Al- Ali et al (1988)
5.3 - 11.1	74.0 - 87.0		9.3 - 12.7	401 - 453					Beaunoyer (1992)
9.7 - 13.2	53.2 - 56.2	1.02 - 4	5.5 - 6.3	130.0-142.4	2.6 - 3.0	2.3 - 2.9	5.0 - 5.9	5.3 - 7.9	This study

KEY

AP - Alkaline phosphatase (IU/L)

CK - Creatine kinase (IU/L)

ALT - Alanine aminotransferase (IU/L)

AST - Aspartate aminotransferase (IU/L)

ALB - Albumin (g/l)

GLB - Globulin (g/l)

TP - Total Proteins (g/l)

BUN - Blood urea nitrogen (mg/l)

Appendix IV: Buffers.

(a) 0.01M PBS pH 7.2.

Solution	A:	0.2M NaH_2PO_4	14.0ml.
Solution	B:	0.2M Na_2HPO_4	36.0ml.
Solution	C:	1.5M NaCl	100.0ml.

To make 0.01m PBS pH 7.2, mix 14ml of solution A, 36 ml of solution B and 100 ml of solution C. Add 850ml distilled water to make up to 1 litre. Adjust pH to 7.2.

(b). 0.01 M PBS-Tween 20, pH 7.4 (Washing buffer).

Solution	A:	0.2M NaH_2PO_4	9.5ml
Solution	B:	0.2M Na_2HPO_4	40.5ml
Solution	C:	1.5M NaCl	100.0ml
Solution	D:	Tween-20	0.5ml

To make 0.01M PBS-Tween 20, pH 7.4 (washing buffer) mix 9.5ml of solution A, 40.5ml of solution B, 100ml of solution C and 0.5ml of Tween - 20. Add 850ml of distilled water to make up to 1 litre. Adjust pH to 7.4

(c). Carbonate/Bicarbonate buffer pH 9.6 (Coating/Coupling).

$NaCO_3$	1.59g
$NaHCO_3$	2.93g

To make coating buffer pH 9.6, dissolve A and B in 1000ml of distilled water.

(d). 0.01 M PBS - Tween 20, pH 7.4 and 1% BSA (Blocking buffer).

Solution	A: 0.2M NaH_2PO_4	9.5ml
Solution	B: 0.2M Na_2HPO_4	40.5ml
Solution	C: 1.5M NaCl	100.0ml
Solution	D: Tween-20	0.5ml
Bovine serum albumin (BSA)		10g

To make blocking buffer ph 7.2, mix 9.5ml of solution A, 40.5ml solution B, 100ml of solution C and 0.5ml of Tween-20 then add 10g of bovine serum albumin and make the solution up to 1000ml using distilled water.

(e). Substrate buffer

Sodium acetate	8.2g
Citric acid	266g

Add distilled water to 8.2g of sodium acetate and 266g of citric acid to make 1000ml. 50mg of tetramethylbenzidine (TMB) is dissolved in 5ml dimethyl sulfoxide (DMSO) to prepare the substrate. To 10ml of substrate buffer, 4µl of TMB and 40µl of H_2O_2 are added and used for one micro-titre plate.

(f). Phosphate buffered glucose saline (PSG), pH 8.0.

A: $NaOH_2PO_4$	0.414g
B: Na_2HPO_4	8.088g
C: NaCl	2.550g
D: Glucose (Dextrose)	10.00g

Dissolve compounds A, B, C and D in 1000ml of distilled water and adjust the pH to 8.0.

(g). EDTA saline glucose buffer (ESG).

A: KH_2PO_4	0.3g
B: NaCl	8.0g
C: D-Glucose	2.0g
D: EDTA (di-sodium salt)	3.0g
E: Phenol red (1:5000)	5.0ml

Dissolve compounds A, B, C and D in distilled water add solution E and make up to 1000ml then adjust the pH to 8.0