STUDIES ON INFECTIVITY AND PATHOGENICITY OF AN ISOLATE OF *Trypanosoma evans/IN* YANKASA SHEEP

BY

PATRICK AMIDU AUDU
(BSc, MSc)

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PART1AL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN VETERINARY PARASITOLOGY, DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY, FACULTY OF VETERINARY MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

DECEMBER, 1999

DECLARATION

I hereby declare that the work in this thesis was carried out by me in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, under the supervision of Prof. K.A.N Esievo; Dr. G. Mohammed, and Dr. O.J. Ajanusi.

ABUZA

The work is original and has not been submitted in any form to any other university for any degree. Where use has been made of the published work of other investigators, this is duly acknowledged in the appropriate parts of the text and in the list of references at the end of the dissertation.

26.01.2000

Name: Patrick Amidu Audu

DECEMBER, 1999.

CERTIFICATION

This thesis titled "**Studies on Infectivity and Pathogenicity of an Isolate of *Trypanosoma evansi* in Yankasa Sheep**", meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

27th Jan., 2000

Prof. K.A.N. Esievo (DVM, MSc, PhD) — **Date**
Chairman, Supervisory Committee,
Department of Veterinary Pathology and Microbiology,
Ahmadu Bello University, Zaria

27/Jan/2000

Dr. G. Mohammed (DVM, PhD) — **Date**
Member, Supervisory Committee,
Department of Veterinary Surgery and Medicine,
Ahmadu Bello University, Zaria

27-1-2000

Dr. O.J. Ajanusi (DVM, MSc, PhD) — **Date**
Member, Supervisory Committee and Head,
Department of Veterinary Parasitology and Entomology,
Ahmadu Bello University, Zaria

24/03/01

Dean, Postgraduate School, — **Date**
Ahmadu Bello University, Zaria.

DEDICATION

For the immeasurable roles they played in my life and career, this work is deservedly dedicated to the followings:

(a) My father, Audu Ocho (of the blessed memory)

(a) My mother, Mrs. Asmau Audu,

(c) The entire Ocho family,

(d) My darling wife, Vivienne, our children, Ocho, Ojonoma, and those yet unborn.

ACKNOWLEDGMENTS

I am exceedingly grateful to Professor K.A.N. Esievo, my major supervisor, for his involvement and tremendous guidance towards the successful execution of this work. His intelligent and constructive criticisms on all the various stages of this work is very much valued. I have certainly benefitted from this wealth of intellectual experience.

I wish to also extend my sincere gratitude to my second supervisor, Dr G. Mohammed for his invaluable contributions and useful criticism on many aspects of the work. The precious times he spent reading and his scholarly corrections of the manuscript is highly appreciated.

Profound appreciation is extended to Dr. O.J. Ajanusi, who doubled as my third supervisor and Head, Department of Parasitology and Entomology; for his interest, useful suggestions, and for allowing me the use of facilities in the Protozoology Research Laboratory. I am indeed very grateful.

I am much indebted to Miss E. Tangwan for her help in obtaining the *T.evansi* isolate which was used for this study. Her contribution in this regard is highly appreciated.

My gratitude also goes to Dr. A. Sannusi (formerly of the Department of Parasitology and Entomology) Professor R.I.S. Agbede and Dr. I.A. Lawal, for their encouragement, assistance and the stimulating discussions, particularly in the planning of this work.

Dr. N.D.G. Ibrahim of the Department of Veterinary Pathology and Microbiology was particularly helpful. I am really grateful for his enthusiasm and assistance in the examination of the histopathological slides even at short notice. I am grateful to Mrs N.P. Chiezey for her assistance in the statistical analysis of the data collected.

I wish to acknowledge with thanks, the cooperation of the entire members of staff of the Department of Veterinary Parasitology and Entomology, especially to those in the Protozoology Research Laboratory. The generous technical assistance provided by Malam Bala, Messrs Aduak Gaiya, Raphael Idoko, Jerry Bale and Malam Yusuf, to mention but a few, is very well appreciated.

I wish to also thank the technical staff of the Department of Veterinary Pathology and Microbiology and those of the Histology Laboratory in the Department of Veterinary Anatomy; for their cooperation and assistance in the preparation of the histopathological slides. My sincere thanks also goes to Mr. S. Ojih of the Department of Biological Sciences for his technical assistance. I wish to extend my gratitude to Prince Adah Jacob and Mr. J.O. Audu for the typesetting of this work.

The love, support and understanding of the entire members of Ocho family, especially my father (of the Blessed memory) and my mother, towards my intellectual pursuits are most appreciated.

I deeply appreciate the prayers, love, patience, understanding and encouragement of my wife, Vivienne and our children Ocho and Ojonoma for a successful completion of this work.

I sincerely thank all my friends and colleagues in the Faculties of Veterinary Medicine and that of Science, for their encouragement towards the success of this research work.

The present study was supported by a grant from the Ahmadu Bello University Board of Research to which I express my full appreciation.

Finally, and very importantly, I wish to thank the Almighty God for His blessings and protection over me, and seeing me through this tortuous and very rigorous study. May His name be praised for ever - Amen.

ABSTRACT

The infectivity and the induced pathology by an isolate of *Trypanosoma **evansi*** were investigated in one group of eight infected Yankasa sheep in comparison with six non-infected controls. The infected sheep each receieved intravenously, 2 mils of blood containing approximately 2.0 x 10^6 *T. evansi* obtained from naturally infected camels slaughtered at Kano Abattoir.

The parasite was identified prior to inoculation into the experimental sheep. The sheep developed parasitaemia 3 to 6 days post-infection. Subsequently, the degree of parasitaemia varied between and within individual sheep with peak parasitaemia on day 8 pi. Following infection, the inoculated sheep developed intermittent temperature rise with a peak which almost coincided with the first peak of parasitaemia in the infected sheep. The mean rectal temperature of the infected sheep was moderately elevated above the pre-infection values in the acute stage, while the temperature was miled in the chronic stage in comparison with the pre-infection values.

The mean values of haematological parameters specifically packed cell volume (PCV), haemoglobin concentrations (lib), and red blood cell counts declined gradually in the infected sheep; and these values were significant in all infected ly ($P<0.05$) lower compared with corresponding values for the control group. The PCV of the infected sheep dropped by about 42%from pre-infection mean value of 30.50 ± 7.8% to 17.67 ± 4.04% by Day 48 post-infection. There was a lymphocytic leucocytosis which was statistically ($P>0.05$) insignificant. Other clinical signs observed included emaciation, weakness and rough hair coat. The infection produced two forms of the disease, namely, acute form within 11 days from the onset of the disease, and chronic form which persisted for up to 56 from onset of patency..

Four of the infected sheep died of the acute disease within Day 2 and 8 from the onset of clinical signs, while another two died 40 and 56 days from the onset of the clinical signs. The other two sheep tolerated the infection until termination of the study 65 days from on set of patency. Splenomegaly and lymphadenopathy were prominently observed at post-mortem.

Histopathological examination of tissue sections from the Organs of the affected sheep showed erythrophagia, dense proliferation of lymphocytes at germinal centers in the spleen, mononuclear cell infiltration in the kidney, haemosiderosis and haemosiderin-laden macrophages in the spleen and liver. These changes are capable of interfering with the functioning of the vital organs; a situation that can lead to death resulting from multi-organ dysfunction.

TABLE OF CONTENTS

CONTENTS: **PAGE:**

Title Page i

Declaration ii

Certification iii

Dedication iv

Acknowledgments v

Abstract viii

Table of Contents x

List of Tables xiv

List of Figures xv

List of Plates xvi

List of Abbreviations xvii

CHAPTER ONE

1.0 GENERAL INTRODUCTION 1

CHAPTER TWO

2.0 LITERATURE REVIEW 5

2.1 Historical Background 5

2.1.1 Origin of Camels 5

2.1.2 Classification and Distribution of Camels 5

2.1.3 Adaptation of Camels 10

2.1.4 Uses of Camels 11

2.2.0 The Parasite *Trypanosoma evansi* 12

2.2.1 Classification of *Trypanosoma evansi* 13

2.2.2 Vectors of *T.evansi* 14

2.2.3 Geographical Distribution and Host-Range of *T.evansi* 17

2.2.4 Morphology and Motility of *T evansi* 20

2.2.5 Phylogenetic Relationship Between *T.evansi* and *T.brucei* 20

2.3 Clinical Signs of *T.evansi* Infection 22

2.4 Pathogenesis and Pathology of *T.evansi* Infection 23

2.5 Antigenic Variation in *T.evansi* 29

2.6 Diagnosis of *T.evansi* Infection 31

2.6.1 Clinical Diagnosis 31

2.6.2 Parasitological Diagnosis 31

2.6.3 Silicone Centrifugation Technique 33

2.6.4 Mini-Anion Exchange Chromatography Technique 33

2.7 Chemotherapy 36

2.8 Drug Resistance 37

2.9 Immunology 38

2.10 Control of *T.evansi* infection 41

2.10.1 Vector Control 41

CHAPTER THREE

3.0 INFECTIVITY AND PATHOGENICITY OF THE *Trypanosoma evansi* ISOLATE IN YANKASA SHEEP 43

3.1 Summary 43

3.2 Introduction 45

3.3 Materials and Methods 49

3.3.1 Camels 49

3.3.2 Isolation and Identification of Trypanosomes 50
3.3.3 Experimental Animals 51
3.3.4 Experimental Procedures 52
3.3.5 Experimental Infection of the Animals 52
3.3.6 Parameters Investigated 55
3.3.7 Clinical Signs 56
3.3.8 Post-mortem Examination 57
3.3.9 Statistical Analysis 57
3.4 Results 57
3.4.1 Infection 57
3.4.2 Rectal Temperature 58
3.4.3 Parasitaemia 63
3.4.4 Haematological Changes 63
3.4.4.1 Total and Differential Leucocyte Counts 66
3.4.5 Total Protein 69
3.4.6 Weight Changes 69
3.4.7 Other Observed Clinical Signs 71
3.5 Discussion 72

CHAPTER FOUR

4.0 GROSS AND HISTOPATHOLOGIC FEATURES OF *Trypanosoma evansi*-INFECTED YANKASA SHEEP 76
4.1 Summary 76
4.2 Introduction 77
4.3 Materials and Methods 77

4.3.1 Control and Experimental Infection 77

4.3.2 Collections and Examination of Samples 77

4.3.3 Analysis of Samples 78

4.4 Results 78

4.4.1 Post-mortem Changes 78

4.4.2 Histopathological Findings 79

4.4.2.1 Spleen 79

4.4.2.2 Liver 80

4.4.2.3 Kidneys 80

4.4.2.4 Lungs 80

4.4.2.5 Other Findings 81

4.5 Discussion and Conclusion 94

CHAPTER FIVE

5.0 GENERAL DISCUSSION 96

References 100

Appendices 114

LIST OF TABLES

TABLE: **PAGE:**

3.1 *T.evansi*-infected and control Yankasa groups 54

3.2 Summary of outcome of the infection with *T.evansi* in Yankasa.Sheep..... 59

3.3 Rectal temperature, body weight, and haematological parameters in *T.evansi*-infected and control Yankasa sheep..... 60

3.4 Weekly Clinical Parameters in the un-infected (control) Yankasa sheep..... 61

3.5 Weekly Clinical Parameters in the T. evansi-infected Yankasa sheep..... 62

LIST OF FIGURES

FIGURE:		PAGE:
1.	Distribution of sheep in Nigeria	48
2.	Fluctuations in Rectal Temperature and Packed Cell Volume (PCV) with Respect to Parasitemia in Infected and Un-infected Control Sheep	64
3.	Mean PCV, Haemoglobin Concentration(Hb), and Red Blood Cell (Rbc) Counts in *T.evansi*-infected and Un-infected Control Sheep	65
4.	Mean Total and Differential Leucocytes Counts	67
5.	Mean Monocyte and Eosinophil Counts	68
6.	Mean PCV, Total Protein, and Weight Changes in *T.evansi* Infected and Un-infected Control Sheep	70

LIST OF ABBREVIATIONS

Ab-ELISA	- Antibody detection enzyme-linked immunosorbent assay
Ag. ELISA	- Antigens-detection enzyme-linked immunosorbent assay
Ad libitum	- At will
BCE	- Buffy coat examination
CATT	- Card agglutination trypanosomal test
°C	- Degree centigrade
DEAE	- Diethyl amino ethyl cellulose
ELISA	- Enzyme-Linked immunosorbent assay
Hb	- Haemoglobin
HCT	- Haematocrit centrifuge technique
IgG	- Immunoglobulin G
IgM	- Immunoglobulin M
Kg	- Kilogram
MHCT	- Microhaematocrit centrifuge technique
PCV	- Packed cell volume
PBS	- Phosphate buffered saline
%	- Percentage
RBC	- Red blood cell
STDM	- Standard trypanosome detection methods
WAD	- West African dwarf

CHAPTER ONE

GENERAL INTRODUCTION

Infection due to *Trypanosoma evansi*, commonly known as "surra" (Evans, 1880), is a very important disease of camels, dogs, horses, donkeys and other ruminants (Hoare, 1956). In camels, the disease exists in three forms. In the hyper-acute form death occurs rapidly before the development of any clinical signs. The acute form which is characterized by prostration, fever, reduced milk production, abortion, epiphora, oedema, mucosal haemorrhagia, enlarged lymph nodes and death which may occur after several weeks.The chronic form is characterized by weight loss, anaemia, and intermittent fever. Death may occur within 1-2 years or the animal may eventually recover (Mahmoud and Gray, 1980; Losos, 1986).

The chronic disease is the most common and most difficult to diagnose as it could be confused with haemonchosis in cattle. Central nervous system (CNS) disturbances characterized by hyperexcitability and incoordination leading to paralysis have been reported (Losos, 1986). Delayed oestrus, abortion, and still birth are associated with chronic cases. "Surra" (Hunter, 1990).

T.evansi is transmitted mechanically from an infected animal to a susceptible one by haematophagous flies of several different genera including *Tabanus*, *Stomoxys*, *Haematopota*, *Chrysops* and *Lyperosia* (Hunter, 1990; Wilson, 1984). The genus *Tabanus* is particularly important in the transmission of the parasite.

Several methods exist for the diagnosis of *T.evansi* infection and include parasitological techniques such as wet film, thin, and thick smear examinations, microhaematocrit centrifuge technique

(mHCT), and mini-anion exchange chromatography. Animal sub-inoculation is another diagnostic method which is very sensitive in detecting *T.evansi* infection even at very low parasitaemia (Ilemobade, 1971).

Immunological tests like mercuric chloride tests, IgM-titration by precipitation diffusion method, indirect immuno-fluorescent antibody test (IFAT), enzyme linked immunosorbent assay (ELISA) and Suratex tests (Olaho-Mukani *et al.*, 1995) are also used.

T.evansi is known to infect different animal species in different parts of the world. Studies on experimental infection of donkeys and cattle in Nigeria (Ilemobade, 1971), goats and sheep in India (Chand and Singh, 1971) and Russia (Khasanov and Ivanitskaya, 1974) showed that these animals undergo a protracted course of the disease resulting in a "carrier state" thereby acting as reservoir hosts.

T.evansi is assuming greater economic importance because of the significant growth in world camel population, particularly in the dry savannah region of Africa. In Nigeria, camels are increasingly becoming economically important, particularly in the northern parts, due to their increasing use as a source of meat (Falope, 1991). Surra constitutes a major factor limiting the productivity of these animals in Nigeria.

Although extensive work has been carried out on animal trypanosomosis in Nigeria (Anosa, 1977; Saror, 1975, 1980; Sackey, 1998) little has been done in the area of infection due to *T.evansi*. More work is therefore required in this area about the disease because of its economic significance in many parts of the world affecting both domestic as well as wild animals (Mahmoud and Gray,

1980). *T.vivax* infection relapses occur with the blood restocked by trypanosomes of different antigenic identity (Clarkson and Awan, 1969). It is therefore not unlikely that the differences in infectivities of *T.evansi* observed in domestic animals by previous workers (Curasson, 1948 ; Saseendranath *et al.*, 1995) might be related to differences in the characteristics of the isolates.

Obviously there is paucity of information available on the susceptibility of domestic animals to *T.evansi* infection. Preliminary reports on the isolation of *T.evansi* from blood samples obtained from slaughtered camels at Kano Abattoir have indicated that the organism exists in Nigeria (Sani, 1994; Musa, 1994). The isolate was infective for laboratory mice, rats and goats. Infected mice and rats developed parasitaemia following challenge with the *T.evansi* isolate. However, the parasitaemia was very low in infected goats suggesting that goats are relatively resistant to infection with this isolate.

Variations in the pathogenicity of *T.evansi* strains that exist in the field have been reported. For instance, different strains of T. evansi have been found to be pathogenic to goats in East Africa (Ngeranwa *et al.*, 1991), Red Madras sheep in India (Saseendranath *et al.*, 1995), and Friesian Holstein calves in Indonesia (Payne *et al.*, 1992). The importance of the present study is therefore strengthened by the fact that the existence of such strains could be dangerous for animals that are kept in the vicinity of, or herded together with camels.

With increase in consumption of camel meat and fear of outbreak of surra, it is hoped that with a better of the behavior of the parasite in other animals herded with camels, an important contribution could be made towards efforts directed at developing new strategies towards protecting camels and other susceptible animals against the disease.

The present study was designed with following objectives:

a) studying the infectivity of the *T.evansi* isolate in Yankasa sheep;

b) to document the gross and histopathologic changes in the infection with a view to determining the pathogenicity of the *T.evansi* isolate in Yankasa sheep.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Background

2.1.1 Origin of Camels

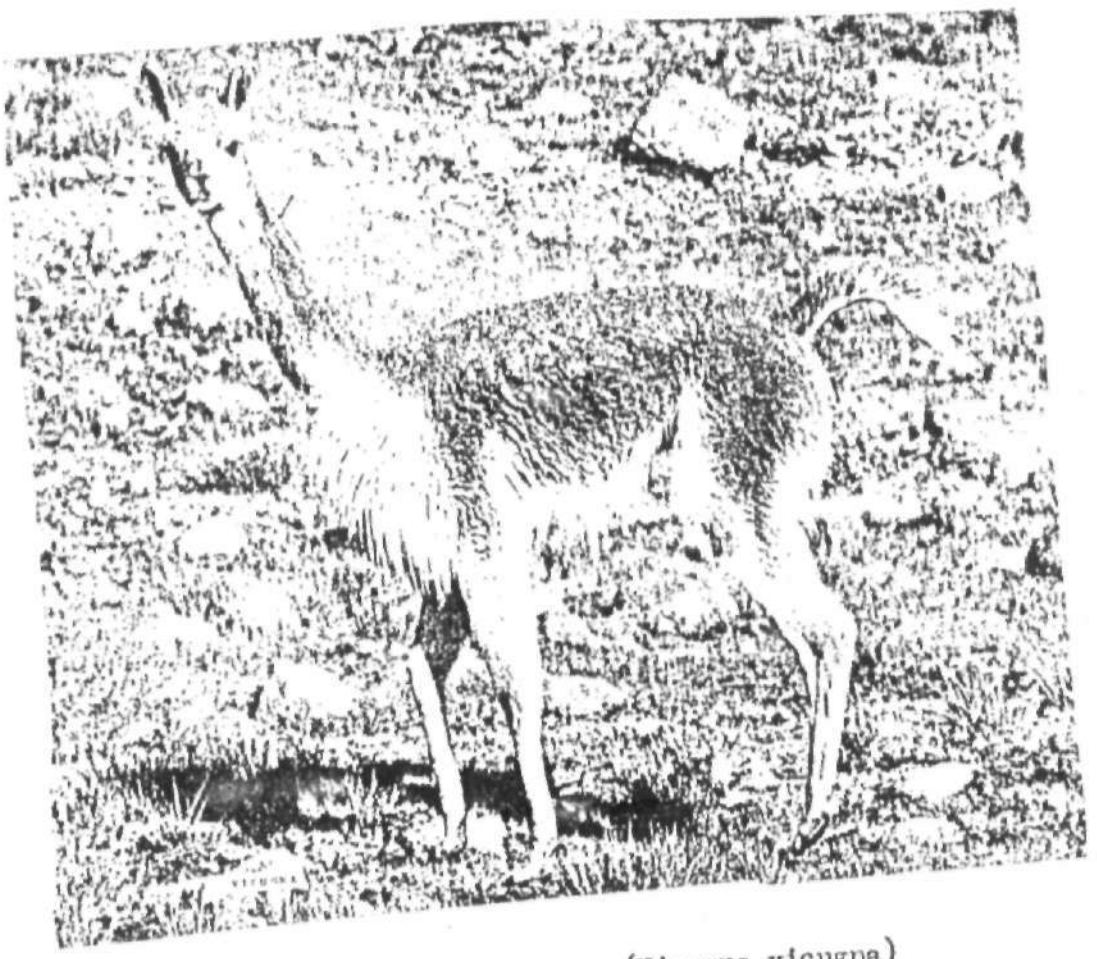
Palaentological evidence suggests that cameloids evolved in North America. From their origin, the cameloids dispersed southward into South America and North West ward across the Bering land-bridge into Asia. They spread westward across the dry lands of the old world until the entire family became extinct in North America (Walker, 1964).

2.1.2 Classification and Distribution of Camels

The taxonomical classification of camels indicates that they belong to the Class *Mammalia*, Subclass *Theria*, Intra-class *Eutheria*, Order *Artiodactyla* (even toed), Suborder *Tylopoda*, and Family *Camelidae* (Walker, 1964).

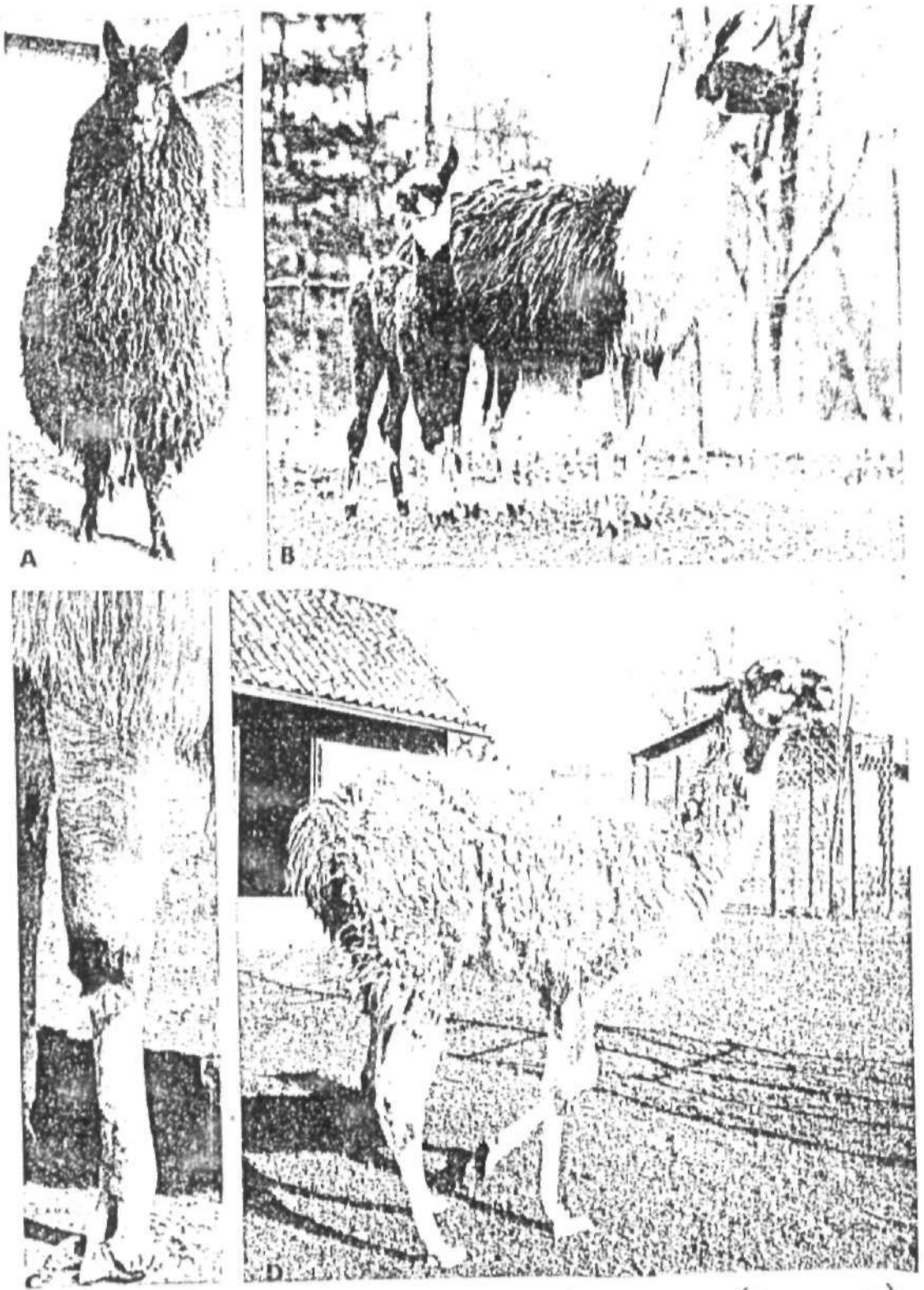
Unlike other domestic animals, the Camelidae are less widely distributed. This is because their environmental tolerance is limited, and their use to man is more specialized. The Camelidae differ from other ruminants in not having horns. They are unique among mammals in the oval shape of their red blood corpuscles (Walker, 1964).

In the new world, the Camelidae are represented by wild species in the high Andes. For example, the Vicuna (*Vicugna vicugna*) is now very rare and are found in the highlands of Peru and Bolivia (Walker, 1964). The Guanaco (*Lama guanacoe*) now has discontinuous distribution from Bolivia to Tierradel Fuego.



Vicuna (Vicugna vicugna)

(SOURCE: Walker, 1964)



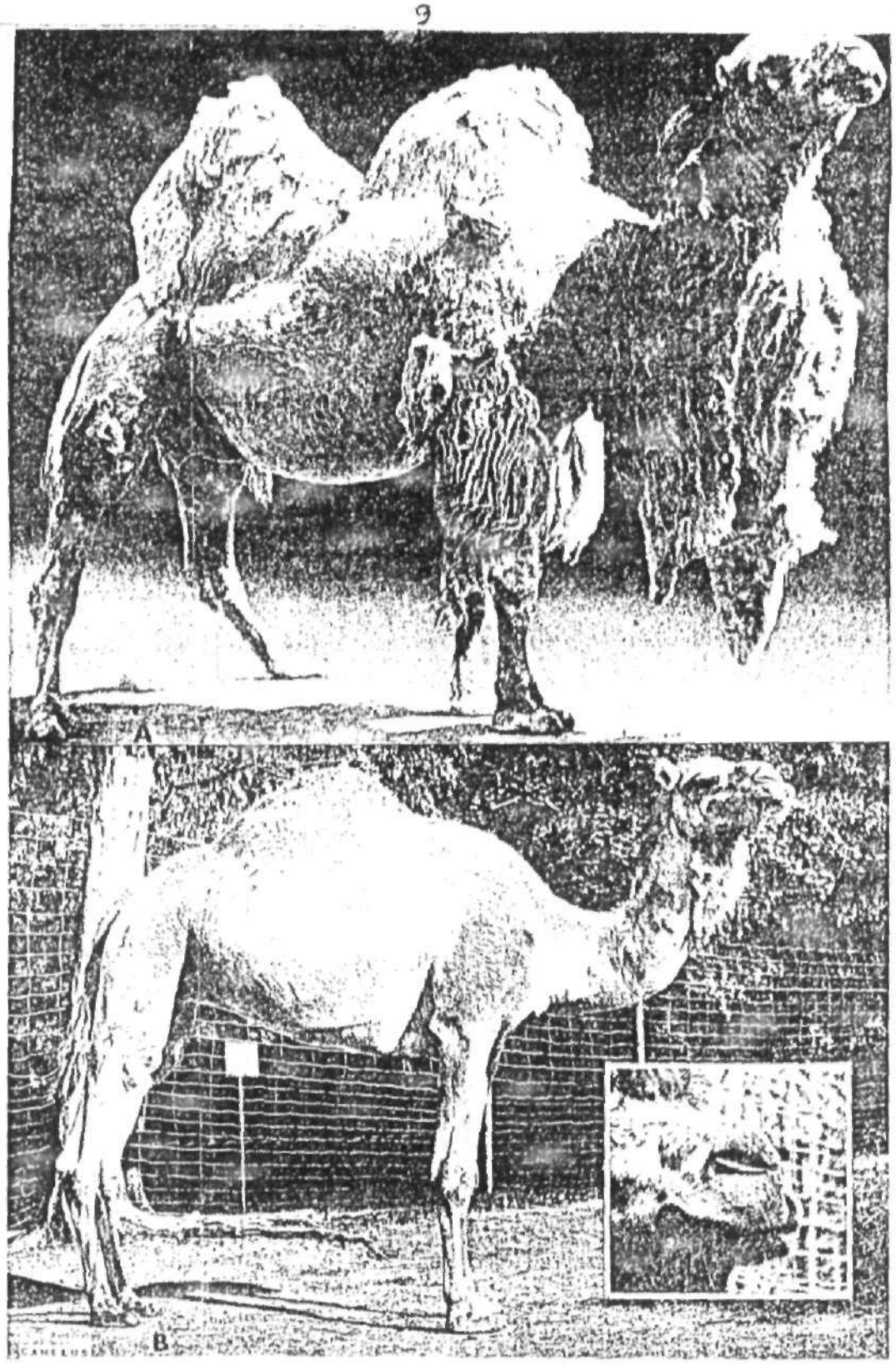
A. Alpaca (Lama pacos), B. Lamas (L. peruana)

C. Leg of Lama; D. Guanaco (L. guanacoe)

(SOURCE: Walker, 1964)



Plate 3: The one -humped camel (*Camelus dromedarius)*



A. Camelus bactrianus

B. Camelus dromedarius

(SOURCE: Walker, 1964)

Two domestic animals, Llama (*Luma glama*) and Alpaca (*Lama pacos*) have evolved from the Guanaco and are now found in Peru and Bolivia.

In the old world, there are two types of camels. The one-humped, and the two-humped which are classified as separate species (Walker, 1964). *Camelus dromedarius* (dromedarian camels) is the one-humped while *Camelus bactrianus* (bactrian camels) is the two-humped. Dromedary, comes from the Greek word *dromos* (which means road) correctly applied to the riding or racing camel. The two-humped variety differs from the one-humped in being woollier, having shorter legs, and darker in color (Walker, 1964).

2.1.3 Adaptations of Camels

The two-humped camel is adapted to the low winter temperature of Asia, while the one-humped type is the typical animal of the deserts of North Africa and Middle East.

The gestation periods are 12 and 13 months for the one-humped and two-humped camel, respectively (Walker, 1964).

The dromedary camels have deserved reputation for being extremely well adapted to life in hot, dry land. Some of the adaptations are anatomical, physiological, and behavioral. Insulation from high atmospheric temperature can be provided by the animal's coat. The light shiny skin that reflects solar radiation and subepidermal layer of fat (Walker, 1964). The hairs are short but lie flat and serve as a very effective barrier against heat gain from the environment.

The legs and the feet of dromedary camel are well suited to the desert environment. The heavy pad of dromedary's foot also furnishes insulation from the hot desert, while the firm elastic tissue gives excellent traction on sand, on gibbers, and can cover considerable distances without drinking water. The long legs also help to support the camels higher up the ground level thereby minimizing the amount of radiated rays reaching the body (Walker, 1964).

Camels have certain physiological adaptations which enable them to withstand dehydration under extremely rigorous environmental conditions. The body temperature rises slowly and perspiration does not set in significantly until the temperature reaches about 105°F, which at night may fall rapidly (Walker, 1964). Camels hardly sweat except during the hottest hours of the day when man, within the same environment, perspires almost profusely from the effect of the hot sun.

Camels have internal reservoir from which water could be withdrawn as needed. For instance, the hump in which a lot of fat is stored, can produce water when oxidized. Less urine is produced by camels and only a small quantity is required in order to get rid of urea from the body. Similarly, fecal water losses are also minimal. A dromedary, however, can tolerate water loss of up to 30 percent of its body weight. It maintains its blood moisture effectively, and under the same conditions of water loss, would experience only 10 percent decrease in blood volume (Walker, 1964).

2.1.4 Uses of Camels

Camels are multipurpose animals with several uses. Traditionally, they serve as the main means of transportation in the desert areas, where vehicular transport is very limited. They could cover

long distances of up to 56 km per hour (Walker, 1964). In addition to this, camels also serve as source of meat. In Nigeria, especially in the northern states of Kano, Sokoto, and Katsina, a high population of camel meat consumers exists. For example, an average of 65 camels are slaughtered daily at the Kano Abattoir. Therefore, these animals are of high economic significance because they serve as source of income to owners. Additionally, cheese can be produced out of camel milk. Their by-products like wool, hides, and skin serve as important raw materials for textiles and leather industries, respectively.

2.2 The Parasite *Trypanosoma evansi*

Trypanosoma evansi was the first trypanosome to be described as the causative agent of mammalian trypanosomosis. The first report on *T.evansi* was published by Griffith Evans when he first saw the parasite in the blood of infected camels and equines in 1880 (Mahmoud and Gray, 1980).

In 1894, McFadyean reported similar observations made by John Henry Steel during an outbreak of a disease in transport mules in Burma in 1885 (Mahmoud and Gray, 1980). The true nature of the organism was discovered by Crookshank in 1888 (Hoare, 1970).

Since then, *T.evansi* was associated with infection in other mammalian host in different countries. The disease is known by a variety of names. The disease surra, caused by *T.ninaekohlyakimovi* (Hoare, 1972) was also later described by Yakimoff in 1921 and 1923 in Russian camels in Turkestan (Mahmoud and Gray, 1980). This description came after the parasite was first detected in Russian camels in Astrakhan and Saratov in 1912 by Feinschmidt.

Similar reports on infections caused by trypanosomes indistinguishable from *T.evansi* occurred in various parts of the world affecting different mammalian hosts. More than thirty synonyms of the trypanosome affecting camels exist outside the well established tsetse belt. Some of these names include *T.soudanense* (Laveran, 1907) *T.venezuelense* (Mesnil, 1910), *T.annamense* (Laveran, 1911) to mention but a few. At one stage about 33 different names were ascribed to this trypanosome. The name *Trypanosoma evansi* is now accepted worldwide (Hoare, 1972). Other disease caused by related trypanosomesinclude Murrina (*T.hippicum*), Malde cadera (*T.equinum*), Derrengadera (*T.venezuelense*) in central and South America, and Su-auru (*T.ninaekohlyakimoiv*) in Russia (Hoare, 1972).

In Nigeria, *T.evansi* was reported in camels in early 1960s (Godfrey *et al.*, 1962). So far no other species of trypanosomes have been found in camels in Nigeria, although elsewhere in Africa, South of the Sahara, there have been reports of infections of *T.vivax*, *T.congolense* and *T.simiae* (Godfrey *et al.*, 1962).

2.2.1 Classification of *Trypanosoma evansi*

In order to distinguish *T.evansi* from other members of the *Trypanozoon* subgenus, Hoare (1956) laid down certain criteria for its identification. These criteria took into consideration the absence of tsetse flies in the areas of infections, the absence of pleomorphism in the blood stream forms, the inability of the trypanosome to develop in *Glossina*, etc.

Hoare (1940) had postulated that *T.evansi* could have arisen from *T.brucei* when carried outside the tsetse zone by camels. The systematic classification of *T.evansi* is given in Appendix 1.

2.2.2 Vectors of *T.evansi*

Trypanosoma evansi lacks the gene necessary for mitochondrial development (Borst *et al* 1987) and therefore is unable to undergo growth and differentiation in the insect vector. Nevertheless this has not preclude transmission by biting flies.

Trypanosoma evansi is transmitted mechanically by biting flies which belong to different genera. The proven natural vectors of *T.evansi* are *Tabanus*, *Stomoxys*, *Haematopota*, *Chrysops* and *Lyperosia* species (Hunter, 1990). *Tabanus* is the most important vector in the transmission. More than 20 different species of Tabanus have been shown experimentally to transmit *T.evansi*. Some of these include: *Tabanus albitriangularis, T. albimedius, T. bicallosus, T. minimus, T. nemoralis, T. immanis, T. stilatus, T.tropica, T. vagus, T. virgo* etc (Cross, 1923; Singh, 1925)

In Indonesia all local strains of *Tabanus* (horse fly) were reported to be vectors of *T.evansi* (Nieschutz, 1930), while *Stomoxys* (stable fly) proved to be ineffective. In Africa, both horse flies and stable flies are vectors of *T.evansi* (Barotte, 1925; Curasson, 1943). The persistent and aggressive feeding habit of these flies normally provoke the host interrupting the flies from feeding. In the cause of feeding on a susceptible host, such flies may transmit the infection mechanically (Hunter, 1990). Transmission of the parasite from an infected to a susceptible host depends on the time interval between the two feedings. The infectivity is greatest minutes after feeding, but decreases rapidly and could be lost totally within eight hours (Losos, 1986).

The severity of the infection in a given locality depends on the density of the fly population and the incidence of the infection in susceptible animals. Yagi and Raziq (1972) observed that most

outbreaks of surra occur during and especially towards the end of the rainy season. Prevailing ecological conditions favoring the breeding and prevalence of Tabanids regarding rainfall, suitable moisture, and surface water pools, also favor the development of suitable camel browsing conditions where *Acacia senegalense* shrubs grow in abundance.

In the Sudan, seasonal outbreaks of *T.evansi* infections correlate with increase in the number of Tabanids during the rainy season. Yagi and Raziq (1972) reported that *Tabanus taeniola* is prevalent throughout the year but the number varies directly with rainfall. While *Tabanus biguttatus* is found throughout the year, *Alylotus agrestis* and *A.fuscipes* appear only at the end of the rainy season. *T.biguttatus* increases in number especially after the rains but it is less numerous compared to *T.taeniola*. *Acala latipes* and *Philoliche magretti* were only observed during the rainy season in small numbers. The prevalence of some species of Tabanids all year round ensures the transmission of *T.evansi*, hence, sporadic infections are observed during the dry season and outbreaks during the rainy season.

The role of tabanid flies other than *Tabanus* species is not well documented because only few studies have been undertaken, bus successful experimental transmission have been obtained with both *Haematopota*, *Lyperosia* and *Chrysops* (Fraser, 1909; Niechulz, 1926b, 1927a, b). The position of *Stomoxys* is equivocal ; a number of experimental transmissions have been carried out with *S. nigra* and *S.calcitrans* (Mitzmain, 1912; Sergent and Donatien 1922), although the success rate is possibly less than that obtained with tabanids. *Stomoxys nigra* was considered to be the principal vectors in Mauritius (Moutia, 1928) but in other studies in the Philippines (Mitz main 1912) *Stomoxys* was thought to be unimportant.

Transmission studies have confirmed the role of capybara (*Hydrochoerus hydrochoerus*) as a reservoir of *T.evansi* infection for horses and dogs in Colombia (Morales *et al.*, 1976). Ocelot (*Felis pardalis*) and Orang-utan (*Pongo pygmaeus*) have been described as important reservoirs for *T.evansi* in lower Amazon region and Sumatra, respectively (Marinkelle, 1976).

Vampire bats (*Demsmodus rotuntus*, chiroptera), found in Central and South America, are also vectors of *T.evansi* (Hoare, 1972). Infected bats transfer the parasite through saliva to cattle or horses while feeding on their blood (Losos, 1986). Dogs can get infected when they consume infected carcasses (Hoare, 1970). It has also been demonstrated that vomit drops from lapping insects, obtained from blood and exudates of wounds, were infective to laboratory rodent (Hunter, 1990).

Transmission studies involving different species of ticks indicate that engorged adults do not harbor viable trypanosomes. However, *T.evansi* survived for hours in the larvae and nymphs of *Hyalomma dromedarii*, *Rhipicephalus sanguineus*, and *Dermacenter andersoni*. Ticks are therefore considered not efficient transmitters of *T.evansi* (Mahmoud and Gray, 1980).

Although the mode of mechanical transmission of *T. evansi* is well established, (Luckins, 1998) reported that its dynamic are not understood, hence a considerable experimentation is required to define quantitatively the effect of the host species, the duration of infection in the host, and the level of parasitaemia, the period between feeds and relative efficiency of different vector species in ensuring successful transmission.

2.2.3 Geographical Distribution and Host-Range of *T.evansi*

The distribution of surra was thought to coincide with that of camels (Hoare, 1967), but is now known to be wide spread. The disease is common in countries with hot or warm climates, most especially, arid deserts and semi-arid steppes. Such areas include the Middle East, Asia the Far-East, Central and South America, and in some areas of Africa. Delineation of the distribution of surra is difficult because it overlaps that of tsetse-transmitted animal trypanosomiasis (Hunter, 1990).

All species of domesticated livestock can be infected with *T.evansi*, although different animal hosts are involved in the epidemics of *T.evansi* in different parts of the world (Luckins, 1998). In Indochina, horses are mainly affected, followed by camels, bovines, and buffaloes. However, in Asia a much wider range of host is involved including bactrian and dromedary camels, and cattle, buffalo, horses and pigs (Pathak *et al.* 1993; Partoutomo *et al* ,1994; Tuntasuvan *et al* 1996). In Somalia, Kenya, Ethiopia, Sudan, Chad, Nigeria, and French West Africa, camels are most susceptible. In Central and South America, horses are the main hosts (Monzon et al, 1995 a; Silva et al . 1995) followed by cattle (Mahmoud and Gray, 1980). Natural infections occur in donkeys (Bennett, 1933). In Africa beyond the northern most limits of the tsetse fly and in parts of eastern Africa camels are the most important host (Dia et al. 1997)

Susceptibility studies in domestic camels revealed trypanosomes in splenectomized but not in intact camels (Dennig, 1972). *T.evansi* obtained from infected camels, horses, mules, and dogs was found to be pathogenic to sheep and goats (Khasanov and Invanitskaya, 1974), but the

clinical signs were milder in sheep and goats. This suggests that sheep and goats might act as reservoir hosts of *T.evansi* in Uzbekistan. In goats, the infection can persist for up to 19 weeks (Chand and Singh, 1971) and detectable only after splenectomy and inoculation of infected blood into susceptible dogs.

Experimental *T.evansi* infections in donkeys and cattle in Nigeria (Ilemobade, 1971) and sheep and goats in Russia (Khasanov and Ivanitskaya, 1974) confirmed that such animals undergo a protracted course of the disease resulting in a "carrier state", and may therefore act as reservoir hosts.

Experimental *T.evansi* infections in rabbits in Britain showed evidence of a vigorous immunological response to the infection (Uche and Jones, 1992). Studies carried out in endemic areas in Indonesia confirmed the presence of antibodies to *T.evansi* in 50% of bovine and 83% of buffalo calves sampled (Payne *et al.*, 1991).

Balakrishnan *et al.* (1994) studied 3 cases of experimental canine trypanosomosis due to *T.evansi*. The main clinical signs being corneal opacity, anorexia, oedema of limbs and general weakness. In another study, 35.4% camels were positive for *T.evansi* (Olaho-Mukani *et al.*, 1992). In India, 31.7% camels were found to be positive for *T.evansi* infection using the double antibody sandwich enzyme-linked immunosorbent assay (ELISA; Pathak *et al.*, 1993).

High incidences of the disease have been reported in Kenya and Sudan. In Kenya it is the most important disease affecting camels. Olaho-Mukani *et al.* (1992) reported 70% mortality, abortion, and weight loss in affected herds. Similarly, in Sudan the disease has been recognized

as an important disease of livestock since the beginning of this century. Other African countries that have high rates of *T.evansi* infection in camels include Somalia, Ethiopia, Chad, Nigeria, and French West African countries (Mahmoud and Gray, 1980). Besides camels, cattle have been reported as the next most highly infected animals in Africa (Mahmoud and Gray, 1980).

It is not clear to what extent, if any, there exists true wild-animal foci of infection that operate independently of the domesticated animals/ranch situation (Luckins, 1998). Infection both natural and artificial have been shown in various animals such as Capy bara, wild dogs and deer (Luckins, 1998). No attempt have been made to investigate such relationships properly, and there are number of factors that might preclude its occurrence . First, *T. evansi* was introduced as a parasite of domesticated livestock, and any wild animals infections are acquired secondarily, hence the disease is often fatal in animals (Clark and Dunn, 1933). Second in some instances the ecological conditions might preclude any close contact with wild animals in different ecological conditions (Luckins 1998). However, *T. evansi* shows a wide range of pathogenicity, and chronic infections in wild animals do sometimes occur, as in South America where the Capybara might be a reservoir (Arias *et al*. 1997; Franke *et al*. 1994a, b; Stevens *et al*. 1989). Some reports indicate the susceptibility of capybara to *T. evansi* (Migone, 1910), but more recent work Franke et al. (1994a) considered capybara were implicated as reservoirs of infection in Pantanal region of Brazil. Dogs, probably acquired infection from being fed capybara and were considered highly suspect since they are associated closely with horses (Luckins, 1998), a situation which requires further investigation especially the relationship between capybara, dogs, cattle and horses, the feeding preferences of flies and the characteristics of the strains of T. evansi isolated from different hosts.

2.2.4 Morphology and Motility of *T.evansi*

Trypanosoma evansi is typically monomorphic in character. Polymorphic forms are not stable and could occur sporadically. Only the slender form, which is similar to *T.brucei*, is constant (Jeffrey and Leach, 1966), ranging from about 15-34 μm in length, with a mean of 24 μm (Hoare, 1970; Hunter, 1990). It has a pointed posterior end, subterminal kinetoplast, a well developed undulating membrane, and a free flagellum which arises from the posterior end. Some strains may loose their kinetoplast spontaneously in nature especially following chemotherapy (Hoare, 1954). The flagellum is long and runs forward along the outer margin of the undulating membrane which is usually conspicuous. The flagellum is free at the anterior end and is used for movement (Jeffrey and Leach, 1966). The nucleus is single, elongated, and centrally positioned. The parasite multiplies by longitudinal binary fission in the blood and other tissue fluids. Movement is achieved through rapid twisting motion as in *T.brucei* but with a relatively slow forward locomotion (Losos, 1986).

2.2.5 Phylogenetic Relationship Between *T.evansi* and *T. brucei*

T.evansi and *T.brucei* have common features. It is hypothesized that these features might have been due to occasional penetrations of camels caravans into tsetse belts thereby exposing them to *T.brucei* infection. On return to tsetse free areas, the *T.brucei* infection become widely spread among other camels through horseflies. *T.brucei* therefore became adopted to mechanical transmission over time, lost its transmissibility by *Glossina* and become monomorphic bearing all the features of *T.evansi* (Hoare, 1970).

Evidence from isoenzyme studies and characterisation of nuclear and kinetoplast support this hypothesis and suggest only limited evolutionary origin of *T. evansi* (Gibson *et al.* 1983; Songa *et al.* 1990) Inspite of this, the parasite has achieved a wide geographical distribution, infects numerous host species and shows a range of pathogenicity that indicate the possibility of a range of genotypes. Stocks of *T. evansi* from different areas of Kenya were found to conform to a homogenous pattern of Zymodemes and were similar to patterns observed in another three isolates from South America, Nigeria and Sudan (Gibson *et al.* 1983) Similar investigation on 12 isolate from different animal hosts in China confirmed that isolates of *T. evansi* formed a homogenous group irrespect of their origin (Lun *et al.* 1992a). These homogeneity have been seen among stocks of trypanosomes were isolated from dogs, and capybara and differed in their virulence (Luckins, 1998).

In South East Asia, Stock of *T. evansi* from Java were shown to form homogeneous group with similar characteristics to stocks isolated elsewhere (Boid 1985). The use of molecular karyotyping improved the level of discrimination between stocks that were in distinguishable by isoenzyme analysis. The stocks were found to fall into several karyotype groups (Lun *et al.* 1992)

Characterisation of stocks from China, Africa and South America and the Philippines by restriction fragment length polymorphism revealed that the Chinese stocks were identical , but differed from isolates from elsewhere (Zhang and Baltz, 1994). Eight different karyotypes were also identified amongst trypanosomes isolated from two herds of camel under different therapeutic regimes (Waitumbi and Young, 1994). *Trypanosoma evansi* resistant to the effects of quinapy ramine were also found to express two different karyotypes (Waitumbi *et al.* 1994)

KASHIM IBRAHIM

Clearly molecular characterisation has considerable potential in understanding the epidemiology of *T.evansi*, particularly in determining the relationship between strains isolated from domesticated livestock and the putative reservoir hosts, the origin of strains and their spread amongst individuals in a herd, their possible association with disease and presence of the parasite, the effects of trypanocidal treatment and the presence of drug resitance . The information may be necessary for developing control methodology. Unfortunately , however only relatively few isolates (Luckins, 1998) have been studied comprehensively, and currently the knowledge of molecular characteristics of *T.evansi* in relation to overal epidemiology of the disease is very limited.

2.3 Clinical Signs of *T.evansi* Infection

The clinical disease produced varies according to the strain of the parasite and the species of the animal host affected. The most severe disease occurs in camels, horses, and dogs (Curasson, 1943). Generally, infection due to *T.evansi* may be hyper-acute, acute, or chronic.

In camels and equines, infection is characterized by an elevation in body temperature which coincides with rising parasitemia, progressive development of anaemia, loss of condition, and weakness. Recurrent episodes of fever occur during the course of the disease. Oedema in the dependent parts of the body including the legs, neck and abdomen also occurs. Petechial haemorrhages in mucous membranes may be observed at the junction of the skin and mucous membrane especially of the nostrils, eyes, and anus. An intermittent fever may be present but in some cases the condition is so acute that this is not apparent. The disease may be fatal within months or may last for a few years. Spontaneous recovery is usually rare (Hornby, 1952; Stephen, 1970).

At post-mortem, there is marked emaciation, enlargement of lymph nodes, and splenomegaly. Petechial haemorrhage occurs on the serous surface and parenchyma of both the liver and kidney.

Chronic infection occurs in camels in Asia with the animals becoming progressively weaker and eventually emaciated. Such chronic infections last about 3 years. In African camels, the disease is less chronic. In the Sudan, where *T.evansi* exclusively affects camels, the disease known as Gufar, could lead to death within a few months of onset of patency. However, a few camels may die in 2-3 weeks. The clinical signs are characterized by fever, progressive emaciation, and anaemia. Oedema of the dependent parts of the body also occurs. Because of the distinct nature of the African form of the infection from that in Asia, the trypanosome was referred to as *T.soudanense*. Occasionally, in the Sudan, horses come down with a chronic infection which may end fatally. However, infected donkeys develop mild fever and may spontaneously recover (Mahmoud and Gray, 1980).

Acute infection is common in dogs in the Middle East with death occurring in 2-4 weeks of infections. Severe oedema, corneal opacity, change in voice due to laryngeal oedema, similar to what occurs in rabies may be observed (Mahmoud and Gray, 1980)..

2.4 Pathogenesis and Pathology of *T.evansi* infection

Following infection in an animal with *T.evansi*, the trypanosomes appears in the peripheral blood within one week. However, prepatent period varies according to the animal species affected and the immune status of such infected animals (Ilemobade, 1971). The severity of the diseases varies with the strain of the parasite and the animal species involved (Curasson, 1948). For

instance, it has been shown that a strain of *T.evansi* was infective to Madras sheep in India following experimental infection leading to the death of all the infected sheep (Saseendranath *et al.*, 1995).

It is believed that *T.evansi* when destroyed within the circulatory system, releases toxic substances which damage the lining of the blood vessels. In some cases, the sudden release of large amounts of such toxins triggers a chain reaction which produces a shock-like syndrome. Therefore, the damage to the host does not depend solely on the production of toxic substances (Mehlhorn and Piekarski, 1989). Thus, the pathogenic effect of the organism, according to Mehlhorn and Piekarski (1989) can further be explained as a syndrome involving the following components:

Pancytopenia as a result of direct influence of the parasite on the cells or the phagocytic defense reaction of the organism which may be on auto-immunological basis. The resulting anaemia which appears with the progressing parasitemia is the classical symptom of the disease (Mehlhorn and Piekarski, 1989).

Metabolic activities of the trypanosomes results in depleting the host's nutrients and the production of toxic metabolites. The consumption of glucose, production of pyruvate and de-amination of amino acids such as tyrosine and tryptophan seem to be especially important. This may result in intermediate metabolic disorder (Mehlhorn and Piekarski, 1989).

The action of certain secretions, such as acid phosphatase which activates the complement system, and pharmacokinetic active substances like serotonin and kinin which have direct pathogenic effect (Mehlhorn and Piekarski, 1989) are also significant.

A trypanosome-induced hypothyroid status which may play a role in the impairment of mitochondrial ATPase, a key enzyme in energy metabolism is also implicated (Mehlhorn Piekarski, 1989).

Autolysis of the pathogen leading to the release of phospholipase which together with free fatty acids, lead to cell damage and immuno-suppressive effects (Mehlhorn and Piekarski, 1989).

Mechanical invasion of the extra-ordinarily strong and mobile pathogens, additional cell damaging lysosomal enzymes and remains of autophagosomes are excreted in the flagella pocket. Hence pinocytosis from the flagella pocket block the plasma proteins of the host (Mehlhorn and Piekarski, 1989).

Immunological mechanisms involving *T.evansi* characterized by antigenic variation, with resultant exhaustion of the antibody production of the host during the IgM phase (Seifert, 1996).

Tissue damage due to infection with *T.evansi* has been described as multifactorial. However, the underlying feature is the progressive anaemia throughout the course of the disease, even in later stages when the parasites are either present in very low numbers or apparently absent altogether (Seifert, 1996). In animals infected with *T.evansi*, the level of anaemia estimated by measurement of erythrocyte counts, packed cell volume, and blood hemoglobin content can be related to the severity of the clinical disease (Seifert, 1996).

In the early stage of the disease, lasting about 12 weeks in cattle, the main features of the disease are fluctuating parasitaemia and anaemia. The cause of the anaemia is complex but is believed

to be haemolytic, due primarily to erythrophagocytosis. This is resulting from the stimulation and expansion of the mononuclear phagocytic systems throughout the reticulo-endothelial system, ultimately leading to splenomegaly. Animals that survive the first parasitemic phase, develop the chronic form of the disease during which the parasitaemia becomes low. The parasites are difficult to detect even though anaemia persists (Seifert, 1996).

Extensive haemorrhages involving mucous serous surfaces, emphysema in the lungs, and mild gastro-enteritis are observed at postmortem examination in *T.evansi*-infected animals (Seifert, 1996).

Acute infection in camels lasts about 2-3 months and is characterized by localization of trypanosomes in the connective tissue. This results in a variety of lesions including subcutaneous oedema of dependent parts and other organs of the body (Losos, 1986).

Visceral forms of *T.evansi* including trypomastigotes, amastigotes, and sphaeromastigotes occur in the heart, optic lobe, cerebrum, and lung (Misra and Chpdhury 1974). The amastigote and sphaeromastigote forms are believed not to be degenerate forms but active visceral phases.

Experimental pathogenicity studies in Nigerian equines revealed that mortality in horses is very high (Ilemobade, 1971). However, *T.evansi* infection in donkeys and cattle appear to be mild without causing ill-effect. Such animals could serve as latent carriers of *T.evansi* for several months. Prolonged passage of *T.evansi* in mice reduces its virulence to guinea-pigs and subsequently in its total elimination (Bhattacharyya and Singh, 1974).

Blood biochemical and hematological changes commonly occur in animals infected with *T.evansi*. In camels, severe hypoglycemia is a common feature (Jatker and Singh, 1974) and changes in levels of plasma proteins have been observed in camels in India (Jatkar *et al.*, 1973).

Serum albumin levels are usually not affected in acute infections. However, in chronic infections decreased levels are encountered (Monzon and Villavicencio, 1990). Alpha-globulin levels fall during acute but become elevated during chronic infections. However, beta-globulin levels increase during acute and remain unchanged in chronic disease. Gamma-globulin levels increase during acute, and chronic infections. Investigations on serum proteins and immunoglobulin of *T.evansi*-infected Sudanese camels revealed an increase in the total protein concentrations, while albumin levels in both natural and experimental infections decreased below normal (Monzon and Villanvicencio, 1990). However, alpha-globulin levels remain unchanged (Luckins *et al.*, 1980). Beta-globulin levels are not affected following experimental infection but decreased in naturally infected camels. Gamma-globulin and IgM levels increased, significantly whereas IgG levels were not significantly affected.

Verman and Gautam (1977) showed that total serum proteins did not increase in *T.evansi*-infected buffaloes. However, an increase in beta and gamma globulin, and a decrease in albumin and alpha-globulin levels did occur. The variations in the different protein fractions in both acute and chronic forms of the disease are indicative of the presence of the disease in the *T.evansi* infected animals.

Studies on changes in serum enzyme levels following *T.evansi* infection in dogs showed no significant change in serum glutamic oxalo-acetic transaminase (SGOT). However, a slight

transcient elevation in serum glutamic pyruvic transaminase (SGPT) level has been observed (Mahmoud and Gray, 1980). Sorbitol dehydrogenase, SGPT and SGOT levels are known to be elevated whereas alkaline phosphatase levels fall during the period of patent parasitemia in infected Sudanese camels. However, following treatment with quinapyramine sulphate (Antrycide), the levels of these enzymes return to normal (Bbid *ei al.*, 1980) suggesting that the presence of the parasites in peripheral circulation is responsible for the changes in the levels of the enzymes observed in the infected animals.

Experimental *T.evansi* infection in rabbits showed evidence of depressed immunological response (Uche and Jones, 1992). The changes observed were indicative of immune response. Nevertheless, changes observed in the kidneys, lungs, and heart were destructive in nature and capable of causing sudden death in the infected animal following multiple organ dysfunction .. The presence of many trypanosomes in the vulval tissue was suggestive of a possible sexual transmission of *T.evansi* (Uche and Jones, 1992).

Pathogenesis of *T.evansi* infection in East African goats, showed that the trypanosomes could be located extra-vascularly in body fluids such as synovial, peritoneal, cerebrospinal fluid, and in the lymph. The carcasses of such infected animals were emaciated and pale. Histopathologically, lymphoid hyperplasia, muscular atrophy, and nephrotic changes occurred (Ngeranwa *et al.*, 1993). Necrotic foci in the liver, kidneys, lymph nodes, spleen, and lungs were also observed. Other changes occurred in the lymphatic tissues which were depopulation of lymphocytes. This was accompanied by hepatic, lymph node, splenic, and renal infiltration by inflammatory cells (Ngeranwa *et al.*, 1993).

2.5 Antigenic Variation in *T.evansi*

The ability of trypanosomes to counter the host's immune response by producing a large number of antigenically different populations throughout the course of infection in the host has fascinated scientists since the beginning of the 20th Century (Ritz, 1916). The biology of antigenic variation has continued to attract research efforts on trypanosomosis, reflecting the rapid expansion in immunology, biochemistry, and molecular biology that characterized the last 25 years (Hyde, 1990; Turner, 1992).

A considerable amount of work on this subject, had established many of the tenets of antigenic variation, particularly the tendency for certain antigen types to appear early in infection irrespective of the infecting variant (predominant antigen types); the reversion to a particular antigen type after cyclical transmission that was a chain characteristics (basic strain antigen), and the classification of trypanosome isolates into groups (serodemes) according to the range of predominant antigen types.

The work carried out on trypanosome antigenic variation has been extensively reviewed (Gray, 1967; Lumsden, 1967; Gray and Luckins, 1976). The results of this formed the basis for further investigations in this area in many laboratories in the last 25 years which was witnessed by a refinement of many of the scientific techniques employed in the 1960s, in particular the use of cloned populations and standardization of terminology (Lumsden, 1982). Much of the work centered on *T.b.brucei* with other species of trypanosomes receiving less attention (Boid *et al.*, 1996). Studies on antigenic variation in *T.gambiense* suggested that antigenic diversity amongst isolates of *T.b.gambiense* was more limited than had been found in *T.brucei*. The study indicated

that isolates of *T.b.gambiense* from widely separated areas of Nigeria, produced a large number of variable agglutinogenic antigens in common (Gray, 1972; 1973). However, further researches carried out on stocks of *T.b.gambiense* from Nigeria, Senegal, Uganda and Zaire were also found to show high degrees of similarity in their antigens (Gray, 1974).

Some technical problems hindered studies on the antigenic variation in *T.congolense*. One of these problems was its tendency to agglutinate in the absence of antibody so much that antigenic analysis relied on the technically more complex neutralization tests rather than the simpler agglutination tests used for *T.brucei* and *T.gambiense*. However, studies on a very small number of metacyclic forms of *T.congolense* suggested a high degree of antigenic diversity between metacyclic populations of 4 stocks of *T.congolense* from East and West Africa (Luckins and Gray, 1979).

In line with the current research, extensive studies on the phenomenon of antigenic variation in *T.evansi* has been on since the mid 1980s (WHO, 1983). Some of these studies aimed at examining the effect of the apparent absence of metacyclic forms on antigenic development and diversity. The results have revealed that antigenic variation in *T.evansi* occurred in a similar way to that in tsetse transmitted trypanosomes, including the presence of predominant antigens (Jones and Mckinnell, 1984; 1985a; 1985b). Furthermore, antigenic diversity between stocks of *T.evansi* was far more limited than in the tsetse transmitted trypanosomes (Jones and Mckinnell, 1985c). The limited antigenic diversity found among *T.evansi* stocks in the Sudan using 30 predominant variable antigenic types (VAT) isolated from six stocks (Jones and McKinnell, 1985b) compared with those found in other species of salivarian trypanosomes suggested that host immunity might play a significant role in the natural regulation of *T.evansi* infection. This

may probably be an important factor in the pattern of infection in many areas known to have recorded cases of *T.evansi* infection.

2.6 Diagnosis of *T.evansi* infection

Several diagnostic methods including clinical, parasitological, serological, etc., are used in the detection of *T.evansi* infections in animals.

2.6.1 Clinical diagnosis

This is based on the identification of clinical manifestations of the disease based on locality. The clinical diagnosis is essentially carried out on suspected animals. Usually, there are no pathognomonic signs and chronic infections do resemble other parasitic or infectious conditions. Such diseases cause physiological stress, anaemia, oedema, lymph node enlargement, conjunctivitis, and keratitis. In camels, the disease could be confused with gastrointestinal strongylosis, haemonchosis, massive hydatidosis, anthrax, chronic infectious diseases, and malnutrition (Jeffrey and Leach, 1966).

2.6.2 Parasitological diagnosis

Definitive diagnosis depends on the demonstration of the trypanosomes in infected blood. The parasitological techniques involved are referred to as standard trypanosome detection methods (STDM). These include the preparation and examination of wet, thin and thick blood films, microhaematocrit centrifuge technique, buffy coat examination, and mini-anion exchange chromatography.

a) Direct microscopic examination: The immediate observation of a drop of fresh blood sample, placed on a clean glass slide and covered with a cover-slip, can be very useful in the field to check for parasitaemia. Identification of the trypanosome species with accuracy is difficult with this technique except in *T.vivax* and *T.congolense* infections in cattle because the parasites can easily be identified through their movement.

b) Micro-haematocrit centrifuge Technique (mHCT): In cases of low parasitemia, it is easier to detect trypanosomes when they are concentrated (Woo, 1969). This is achieved either by the microhaematocrit centrifugation technique or by separation of the trypanosomes through filtration or lysis of red blood corpuscles. The trypanosomes are then examined under phase-contrast or dark field illumination microscopy (Murray *et al.*, 1977). This method is used to detect trypanosomes at the buffy zone and stained with Giemsa. The mHCT is capable of detecting trypanosomes in blood of camels six to ten days earlier compared to wet and thin blood film methods. The technique can be very useful in the field using battery-powered mHCT.

c) Laboratory Animal Inoculation: This is a sensitive method for detecting *T.evansi* and *T.brucei* infections in camels (Godfrey *et al.*, 1962). Blood taken from suspect animals are inoculated into laboratory animals (usually mice and rats) in order to demonstrate trypanosomes that are rare at the time of sampling.

2.6.3 Silicone Centrifugation Technique

The efficiency of this technique depends on the existing difference between the density of the host erythrocytes and that of the trypanosomes. Erythrocytes are usually denser and are pelleted by centrifugation through a layer of silicone fluid (density 1.075) which trypanosomes cannot pass through. Results from this method are comparable to those obtained by the mHCT, and the miniature anion-exchange chromatography technique (Lanham and Godfrey, 1970). This technique is yet to be applied in the diagnosis of trypanosomosis in camels.

2.6.4 Mini-anion Exchange Chromatography Technique

This technique is capable of separating trypanosomes from the host red blood cells by passing infected blood through a column of diethyl aminoethyl (DEAE) cellulose equilibrated at pH8.0 with phosphate buffered saline glucose. The trypanosomes are eluted through the column which retains the host erythrocytes (Lanham and Godfrey, 1970). As a modification of this technique, the eluate is collected in a sealed pasture pipette which is centrifuged. The sealed end is examined for trypanosomes under the microscope (Lumsden *et al.*, 1973). This technique has also not yet been applied in the diagnosis of Surra in camels.

Tests with high degree of sensitivity and specificity have been developed to detect invariant species and subgenus specific circulating antigens in blood as means of diagnosing *T.evansi*, other animal trypanosome species, and human trypanosomes (Nantulya, 1995).

The test involves of latex particles sensitized with specific antibodies. The presence of specific antigens in the specimen leads to the agglutination of the sensitized latex particles.

Similarly, Suratex (a card latex agglutination test) has been developed for the diagnosis of patent and sub-patent *T.evansi* infections in camels. The test, when compared with microhaematocrit and mouse inoculation tests, was found to perform better. Out of 326 camels examined for *T.evansi*infection, 12.7% were positive on mHCT, 30.7% by mouse inoculation (MI), and 33.0% by Suratex test (Nantulya, 1995; Olaho-Mukani *et al.*, 1995).

In another trial involving camels and bulls under different drug regimes, Suratex detected infection while mHCT and MI did not. Results are obtained within 5 minutes and hardly any equipment is required apart from heparinized whole blood and plasma or serum (Nantulya, 1995; Olaho-Mukani *et al.*, 1995).

A formol-gel test has been developed in an attempt to develop an improved method for the detection of *T.evansi* infection (Plantureux, 1923; Knowles, 1924). Another widely used method is the mercuric chloride test (Bennett and Kenny, 1928). These two methods were based on elevation of serum protein levels following infection. The mercuric chloride test was claimed to be more efficient (Bennett, 1933) and sensitive (Mahmoud and Gray, 1980) than the formol-gel test, although some other workers have disputed this based on some experience in Chad and Ethiopia (Pegram and Scott, 1976). Efforts to improve the diagnostic efficiency of blood film preparations and examination were considered at length by Killick-Kendrick (1968). Rabbits and dogs infected with *T.evansi* developed conjunctivitis, keratitis, and glaucoma. The trypanosomes were demonstrated in the aqueous humor.

Studies on *T.evansi* strains of different origins led to the development of two diagnostic procedures which were first tested in the laboratory and subsequently in the field (Touratier, 1992). Thus, the card agglutination trypanosomiasis test (CATT) and the enzyme-linked immunosorbent assay (ELISA) supplement microscopic examination of smears.

In live animals, immunofluorescence is one of the reliable
of the antibody and antigen detection enzyme immunc
infections in camels was undertaken in Kenya. Olaho-M
diagnosis of *T.evansi* infection in camels by the use of a n
linked immunosorbent assay (Ag-ELISA), or in combinati
is a preferred approach in assessing infection compared t
linked immunosorbent assay (Ab-ELISA) test.

Rocket immunoelectrophoresis showed a reduction
complement component (C_3) in the blood of rabbits exp
reduction is associated with C_3 activation in rabbits w
Jones, 1992). No evidence of this activation was detected in uninfected rabbits, rabbits with early stage, light infections, or rabbits cleared of infection by drug treatment.

Evaluation of monoclonal and polyclonal antibody based on antigen detection immunoassay for diagnosis of *T.evansi* infection in dromedary camels, showed that the tests were at least six times more sensitive than the hematocrit centrifuge technique (HCT) and these should be used in routine diagnosis in addition to the parasite detection techniques (Diall *et al.*, 1992).

Serological diagnosis of *T.evansi* has been used to diagnose *'mal'de caderas'* caused by *T.evansi* in horses (Monzon, 1993). Similarly, an Ag-ELISA based on a *T.brucei* group-specific monoclonal antibody has been shown to detect circulating *T.evansi* antigen in horse sera on 7 and 21 days post-infection (Monzon *et al.*, 1995b). It was further shown that a combination of the Ag-ELISA and parasitologic methods detected more animals with *T.evansi* infection (Monzon *et al.*, 1995), which indicates that the Ag-ELISA test is useful both in the diagnosis of *T.evansi* and in assessing the efficacy of drug treatment in horses.

2.7 Chemotherapy of *T.evansi* infection

Much has been reviewed about the chemotherapy of *T.evansi* infection (Braide, 1974; Williamson, 1976). No new effective drugs have been brought into field use for more than 20 years. There are a few chemoprophylactic and chemotherapeutic drugs, some of which are not well tolerated while trypanosomes do develop resistance to some of them (Losos, 1986).

Recent reports on chemotherapy of *T.evansi* infections deal essentially with efforts to enhance drug therapy using different complexes of existing drugs. The sensitivity of thirteen clones of *T.evansi* from China to diminazene acceturate (Berenil®), Suramin (Naganol®), Cymelarsan (Melcy®), quinapyramine (Trypacide®), and Isometamedium chloride (Samorin®) have been determined (Zhang *et al.*, 1991). Clones selected for resistance to Berenil®, were not cross-resistant to Naganol® and Samorin®, but were shown to be more sensitive to Trypacide®, than the normal strain in *in-vivo* tests. The *in-vitro* assay procedure may be of potential use for screening new trypanocides and also for the rapid detection of drug resistant isolates of *T.evansi* and *T.equperdum* (Anon, 1991; Zhang, *et al.*, 1991).

Melcy® is a new curative drug which is very effective against *T.evansi* and *T.brucei*. Melcy® is well tolerated by camels and can be given to pregnant and weak animals. The drug is administered intramuscularly as a 0.5 % solution at 0.1 g 20 ml^{-1} for every 400 kg. The results of field application of Melcy® in 40 buffaloes naturally infected with *T.evansi* in the South of China and treated with a single dose of Melcy (R) at 0.25 mg kg^{-1} to 3.0 mg kg^{-1} intramuscular injection, cured all fourty animals with exception of two which relapsed two months after drug administration (Zhang *et al.*, 1991). These results indicate that Melcy (R) is a very active trypanocidal drug against *T.evansi* (Zhang *et al.*, 1991).

2.8 Drug Resistance

Most of the trypanocidal drugs currently available have been in use for many years and there are widespread reports that of drug-resistance especially when exposed to subcurative doses of the trypanocide insufficient to ensure destruction of the parasites. The situation is quite common with the use of prophylactic drugs in tsetse-transmitted trypanosomosis. Drug-resistant stocks have been reported to persist even after passage through the tsetse-fly (Finelle, 1973). In general, it has been shown that suramin-resistant stocks of *T.evansi* remain sensitive to quinapyramine (Finelle, 1973) and isometharnedium (Stepanova and Petrovskii, 1976).

Drug-resistant stocks of *T.evansi* have been reported in the Sudan (Leach, 1961; Luckins *et al.*, 1979), India (Gill, 1971), and Russia (Petrovskii and Khamieu, 1974). Studies on Suramin-sensitivity to 3 stocks of *T.evansi* isolated from camels in Russia showed that infections in mice required a minimum therapeutic dose of 60 mg kg^{-1} Suramin compared to 10 mg kg^{-1} Suramin required to cure infection with a more susceptible stock from Turkmenia. Luckins *et al.* (1979)

reported that *T.evansi* stocks isolated from the vicinity of Kassala in the Sudan was not responsive in camels after 2 successive treatments with 10 g Suramin and a dose of 3.0 mg kg^{-1} quinapyramine.

Various drug regimes used in the treatment of *T.evansi* outbreak in the Sudan showed that the stock involved was resistant to Suramin but susceptible to 2 quinapyramine sulphate. Berenil® at 3.5 mg kg^{-1} was ineffective while at 7 mg kg^{-1} it proved highly toxic to adult camels (Leach, 1961). Resistance to quinapyramine-suraminate was also reported in some stocks of *T.evansi* in India, with varying degrees of resistance to Suramin and Trypacide® but not to Berenil® (Gill, 1971). In rats infected with resistant stocks of *T.evansi*,8 times the dose needed to cure infections with sensitive *T.evansi was* required (Mathura stock).

The real extent of trypanocide resistance in *T.evansi* is difficult to assess. Only isolated cases have been reported in the literature and it seems that the drug-resistant stocks concerned remain sensitive to other trypanocides. The situation, however, requires continuing assessment because to date no biochemical or genetic markers for drug resistance have been identified in trypanosomes (Boid *et al.*, 1996). The assessment of the sensitivity of trypanosomes to trypanocidal drugs therefore relies on measurements of the growth of parasite populations in the presence and absence of drugs.

2.9 Immunology

The appearance of pathogenic trypanosomes in the blood stream of a host stimulates the development of humoral immune responses directed against the surface coats of the different antigenic variants and induce immunity to the particular variants.

In a study to determine the protective activity of different immunoglobulins following *T.congolense* and *T.vivax* infections in cattle, the typical primary response against a variant was first found in IgM, and later in the IgG fraction of the infected serum.

Other studies have indicated the predominance of antibodies of the IgG class in *T.evansi* infection, where a number of different major antigenic components were identified by sodium dodecyl sulphate-polyacrylemide gel electrophoresis (SDS - PAGE) and Western blotting (Uche *et al.*, 1993).

Protection against homologous challenge was observed to be induced by infection with *T.evansi* followed by treatment with a trypanocidal drug (Uche and Jones, 1994). Response to experimental infection was influenced by the pattern of antibody response to the different components of the parasite. It was observed that where surface antigens were reorganized, absolute protection was conferred. But where only a few components were recognized, as in heterologous serodeme, only partial immunity was conferred (Uche and Jones, 1994). These studies have shown that differences in the quality and quantity of the immune response are important in the determination of the outcome of infection and there were clear differences between animals.

Sera from animals that are resistant to *T.evansi* on the basis of delay in appearance of parasitemia and its level (Uche *et al.*, 1992) contained antibodies that recognized a greater range of antigens earlier in the infection than sera from those that were susceptible.

Cyclical transmission of trypanosomosis may occur via the development of a chancre as has been demonstrated with *T. congolense*.Metacyclic trypanosomes developed at site of their deposition in the dermal collagen before leaving via the draining lymphatics and entering the lymph node (Luckins and Gray, 1978; 1979; Gray and Luckins, 1980). Similar developmental changes have been observed in *T.evansi* infections (Luckins *et al.*, 1991; Uche and Jones, 1993b). This stage of development in the skin appeared to have important consequences in the acquisition of immunity. Infected animals treated with trypanocidal drugs failed to develop reactions when challenged with a homologous serodeme, suggesting the importance of immunity at the level of the skin.

The multiplicity of antigenic types in nature and the capacity of each type of change enables the trypanosomes to thrive in an animal that has been previously immunized (Richardson and Kendall, 1964). Variations in strain virulence and extreme specificity of protective and anti-trypanosomal antibody are some of the major constraints to successful vaccination (Gray, 1965). However, studies on *T.evansi* susceptibility amongst different animals was in mice and lowest in cattle and buffaloes (Gray, 1965).

Guinea-pigs showed considerable resistance but eventually became infected. Mice infected with *T.evansi* were demonstrated to possess agglutinogenic and trypanolytic activities at the time of the disappearances of the trypanosomes from peripheral circulation (Kacker *et al.*, 1974). The serum of these mice agglutinated the parasites within 24 hoursfollowed by lysis after 72 hours. Killed *T.evansi* vaccines prepared from infected mice could offer some degree of protection in susceptible mice (Ryu, 1975). Vaccines containing carbol PBS or 10 % Lugol's Solution gave the best protection.

2.10 Control of *T.evansi* infection

The control or eradication of *T.evansi* infection through surveillance and chemotherapy was attempted in the former USSR (Kunichkkin and Iskalieu, 1975). The control measures employed include the detection and treatment of infected animals using appropriate chemotherapy, vector and herds movement control, as well as other traditional methods.

Control can be achieved through movement of herds away from areas known to be infested by biting flies especially at seasons of the year when the biting fly populations are high (Hunter, 1990). The movement of the herds avoided the hatching of dung breeding flies (*Lyperosia* and *Stomoxys* species).

Wilson (1984) noted that night traveling in known Tabanid infested areas adopted by traditional camels owners, watering in tight groups during the hottest part of the day, and coldest hours of the day are effective in avoiding contact with the vectors of *T.evansi*.

Smudge fire insect repellants are very good protectors of livestock against biting flies (Wilson, 1984; Hunter, 1990).

2.10.1 Vector Control

The spread of the disease can be checked by the use of natural enemies of the vectors. For instance, predators, pathogens, and parasites on the larva, pupa, and adult stages of the vectors have been demonstrated to check the spread of the disease (Chainey, 1993). However, this

method has proved not to be successful in the field because the use of predators has not been very effective. However, the use of parasites on the vector eggs can be very effective (Chainey, 1993). Members of the family, Asilidae, Mantidae, Mantispidae, Carabidae, and the order Odonata are known to be insect predators.

The larvae of Tachinidae and Cynipoidae are parasites of Diptera insects (Imms, 1959). The capturing of adult flies by methods such as hand catching, use of adhesive surfaces, sticky traps and the removal of puparia in localized breeding sites in areas with low population of the vectors is another effective method of controlling the spread of *T.evansi* infections (Chainey, 1993). Cross paring vectors with other species can lead to females being unable to produce offsprings leading to total elimination of the vectors with time..

Another control method is the use of insecticides. However, this has an undesirable effect of causing environmental hazards and its application is usually impracticable because the breeding sites are very diffuse and extensive, coupled with the fact that the larvae are below the soil surface and would therefore require higher doses of the chemical to eliminate them (Chainey, 1993).

It has also been reported that alterations of vegetation or habitats of the vectors through bush clearing, improving environmental and personal sanitation, as well as cultivation of marshy lands areas or drainage of marshy areas are indirect ways of vector control (Chainey, 1993). Removal of vector food sources can lead to their starvation, consequently leading to reduction in the spread of the disease (Baldry and Jordan, 1965).

CHAPTER THREE

3.0 INFECTIVITY AND PATHOGENICITY OF THE *Trypanosoma evansi* ISOLATE IN YANKASA SHEEP

3.1 Summary

Studies on the infectivity and pathogenicity of an isolate of *Trypanosoma evansi* was carried out using two groups (infected and uninfected control) of Yankasa sheep aged between 9 and 13 months.

Each of the experimentally infected sheep was intravenously (jugular vein) inoculated with 2 ml of infected blood containing approximately 2.0 x 10^6 *T.evansi* parasites. The trypanosomes were isolated from naturally infected camels slaughtered at Kano Abattoir, Kano, Nigeria.

Clinical examination of animals included physical examination and laboratory procedures like haematology and blood protein level determination. Haematological parameters determined included levels of parasitaemia, packed cell volume, red blood cell counts, haemoglobin concentration, total leucocytes counts. The infected sheep were positive for *T.evansi* from day 3post-infection, and developed clinical trypanosomosis, characterized by fluctuating pyrexia, parasitaemia, and anaemia.

The prepatent period varied between 3 and 6 days with a mean of 3.3 $\pm$ 1.0 days post-infection from the method employed. Seven of the *T.evansi* inoculated Yankasa sheep developed parasitaemia by day 3. Parasitaemic waves, typical of trypanosome infections, were also recorded.

The mean rectal temperature in the *T.evansi* infected sheep was significantly elevated (P<0.05) between days 2 and 12 post-infection with a peak mean of 40.13 ± 1.06°C on day 6 post-infection, while the mean rectal temperature in the control sheep ranged between 38.47 ± 0.55°C and 38.85 ± 0.98°C.

Anaemia was a distinct feature of the disease and its development coincided with a fall in the PCV, Hb concentration, and erythrocyte counts. The mean values of PCV, Hb concentration and erythrocytes counts in infected animals were significantly (P<0.05) lower when compared with corresponding mean values recorded for the control group. The lowest mean PCV (17.67 ± 4.04 %) was observed on day 51 post-infection (Table 3.5).

The mean blood protein levels in both infected and control Yankasa sheep were generally within normal range, although the values decreased non-significantly (P>0.05) between days 3 and 8 post-infection.

The mean live-weights of the control Yankasa sheep increased gradually (11.75 ± 7.34 kg to 14.9 ± 5.30 kg) during the acute course of the study. However, although the mean weight in the infected group also increased (12.16 ± 2.35 kg to 13.28 ± 2.06 kg) within the same period of study, there was evidence of poor weight gain during the chronic phase. The values decreased gradually but significantly (P<0.05) from 13.28 + 2.06kg to 12.20 ± 1.34kg up to the point when the experiment was terminated (Table 3.5) compared with the un-infected values which increased significantly (P<0.05) from a mean of 14.9 + 5.30 to 16.6 + 5.30 within the same period of study.

Leucocytosis occurred in the *T.evansi*-infected Yankasa sheep between days 12 and 60 post-infection.

Other clinical signs observed included central nervous derangement, enlarged superficial lymph nodes, epiphora, weakness, and rough hair coat.

Most of the clinical and pathological features of the infection observed in the present study are in agreement with previous findings reported in animal trypanosomosis. This indicates that the *T.evansi* isolate used was pathogenic to Yankasa sheep, and as such could be a potentially important threat to sheep production in Nigeria.

3.2 Introduction

Sheep are fairly well distributed all over Nigeria, and play a significant role in the socio-economic life of the people. They are found across the various ecological zones and mostly located in the Guinea Savannah (Fig 1) region. Besides cattle, they are reared along with camels in many areas. Sheep account for over 11% (48,000 metrid tonnes) of the total meat supply in Nigeria (Adu and Ngere, 1979).

Besides their use as source of meat, other products like milk and skins (used as raw materials to feed the local leather industry) are also important. Sheep skin annual production has been estimated to be about 7, 500 tonnes. Sheep also feature very well in many social and religious feasts in Nigeria.



Photograph of Yankasa Sheep (common in the north)

Economic production of sheep in Nigeria is hampered by diseases, with trypanosomosis being one of the most important. Hitherto trypanosomosis was never considered a problem of farm animals in northern Nigeria because the area is generally tse-tse free. Now, the introduction of *T. evansi* that can be transmitted mechanically by biting flies coupled with the influx of camels into several parts of Nigeria, is gradually changing this picture. Available reports have shown that *T.evansi* is pathogenic to different animal species in different parts of the world (Bennett, 1933; Ilemobade, 1971; Khasanov and Invanitskaya, 1974; Mahmoud and Gray, 1980). *T.evansi* is assuming greater economic importance because of the significant growth in the world camel population, particularly in the dry savannah region, where camels are used as such of meat (Falope, 1991)

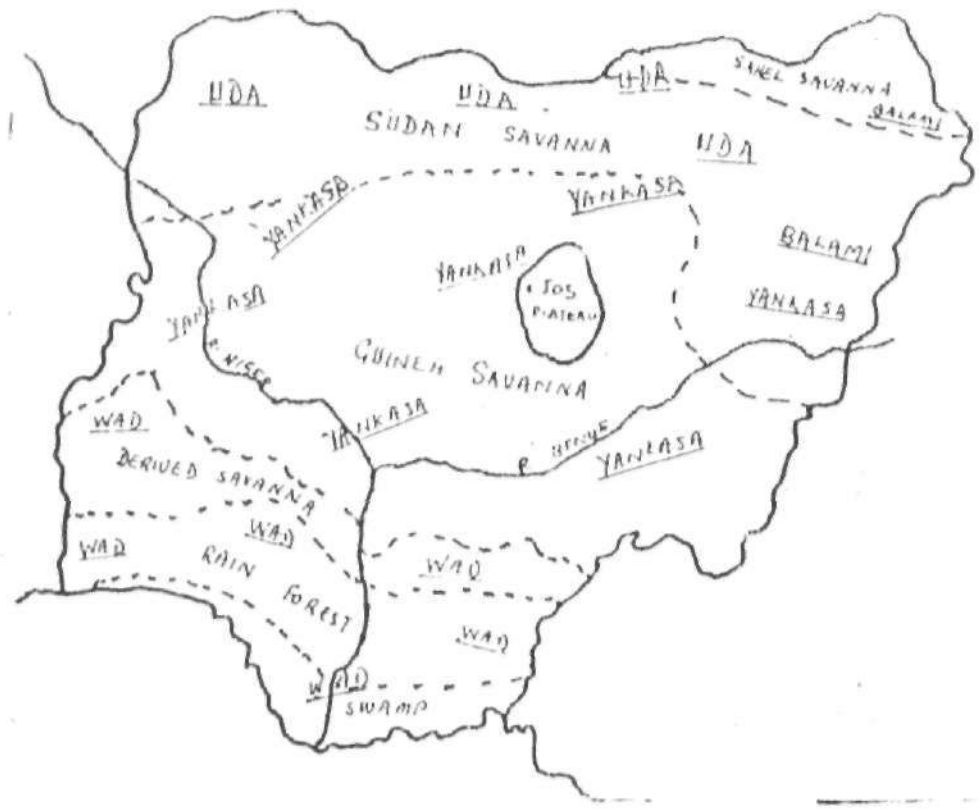


Fig. 1: Distribution of sheep in Nigeria

SOURCE: ÁDU AND UGERE (1979)

Epidemiologically, *T.evansi* may be assuming greater importance because of the cross-infectivity of the trypanosomes between camels, sheep, and goats. The transmission is mechanical through *Tabanids* and other hematophagous flies ubiquitous in the area.

Preliminary reports on the infectivity of *T.evansi* identified from blood samples obtained from slaughtered camels at Kano Abattoir, Nigeria, indicated the isolate to be infective to mice and rats (Musa, 1994). However, goats were relatively resistant to infection with the isolate (Sani, 1994). With the ever increasing demand for camel meat, and the threat of outbreak of the disease in sheep, it is hoped that a complete understanding of the behavior of the parasite in sheep, especially when reared with camels, would provide the frame-work for integrated control measures needed to protect camels and other susceptible animals. The present study was therefore designed to investigate the infectivity and the pathogenicity of the *T.evansi* isolate in Yankasa sheep.

3.3 Materials and Methods

3.3.1 Camels

Camels slaughtered at the Kano abattoir are mostly trade animals coming from the Sahelian regions of Africa. On importation, the animals usually are driven directly into the abattoir premises, where they may be held for up to three weeks prior to slaughter. They are usually fed crop residues and sparingly watered.

Previous history pertaining to the animals were not known, but majority of them brought to the abattoir for slaughter were adult males ranging between 7 and 16 years old. The abattoir records

showed that an average of 65 camels were slaughtered daily. Fat and healthy looking camels, with an average weight of about 450 kg were preferred. Nutritional records were not available. Apart from the use of crop residues, there were no attempts to fatten the animals before slaughter; probably the animals would have been on a free range grazing habit before they were brought for slaughter.

3.3.2 Isolation and Identification of Trypanosomes

The *T.evansi* isolate used in this study was obtained from the blood of a naturally infected camel slaughtered at Kano Abattoir. Kano is said to be tsetse free and is situated close to the northern boundary of Nigeria. The abattoir is located about 3 km North East of Kano metropolis.

Blood samples collected from the slaughtered camels were each obtained in sterilized Bijou bottles containing disodium salt of ethylene diamine tetra-acetic acid (EDTA) and transported immediately to the Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. In the Laboratory, the blood samples were immediately examined by wet film examination. Two milliliters (ml) of a blood sample positive for *T.evansi* (Plate IV) was immediately inoculated, intravenously, into a *donor* sheep which was used to harvest the trypanosome for subsequent infection of the experimental sheep.

The *T.evansi* isolate was identified according to the techniques described by Jeffrey and Leach (1966) and John *et al.* (1992). The *T.evansi* isolate had a pointed posterior end, with subterminal kinetoplast, and a well developed undulating membrane. The flagellum was free arising from the posterior end, and located forward along the outer margin of the undulating membrane. The

nucleus was single, elongated and centrally positioned (Plate IV). In addition, when compared with other species of trypanosomes like *T.vivax*, *T.congolense*, and *T.brucei*, the distances between the posterior end and the kinetoplast, from kinetoplast to mid-nuclear point, from the anterior end to the tip of the flagellum and free flagellum length were longer thus agreeing with John *et al's.* (1992) description of the parasite.

3.3.3 Experimental Animals

Fourteen intact male and female Yankasa sheep, aged between 9 and 13 months, were purchased and used for this experiment. The sheep were obtained locally from Giwa market, about 28 km north-west of Zaria.

On arrival in the Department, the sheep were routinely de-wormed orally using Levamisole (Worm-care®) at the therapeutic dose of 7.5 mg kg^{-1} body weight. The sheep were sprayed with a 0.2 % water solution of Supona® (Shell, London) against ectoparasites. The sheep were prophylactically treated against coccidiosis using Amprolium.

Also sheep that were positive for *Anaplasma ovis* were treated with Long Acting Terramycin® (Pfizer Products Ltd; Ikeja, Nigeria). They were subsequently kept in a tick- and fly-proofed pen. The sheep were fed grass-hay, grain offals, and cotton seed concentrates. Salt-licks and water were supplied *ad libitum*.

All the Yankasa sheep were examined for hemoparasites twice weekly for 9 weeks using thin blood smear preparations and sub- inoculation of rats prior to infection. At the point of

experimental inoculation with *T.evansi*, all the Yankasa sheep were certified negative for infection with hemoparasites.

3.3.4 Experimental Procedures

All the Yankasa sheep were identified by means of ear tag numbering, and were allowed to acclimatize for 9 weeks after which they were randomly divided into two groups. They were monitored for another 2 weeks to obtain base-line data. Six Yankasa sheep constituted the uninfected control group (Group 1), while the remaining 8 belonged to the experimentally *T.evansi* infected group (Group 2).

3.3.5 Experimental Infection of the Animals

One Yankasa sheep was used as a donor and was infected with 2 ml of *T.evansi*-infected camel blood via jugular venipuncture. The donor sheep was then monitored for parasitaemia. After parasitaemia had been established (Plate 5), the donor sheep was bled via the jugular vein. The blood was collected into a conical flask containing EDTA as anticoagulant. The blood was then diluted with phosphate buffered saline glucose (PSG; Lumsden *et al.*, 1973) prior to inoculation into the experimental sheep.

Each of the infected Yankasa sheep was inoculated with 2 ml of the diluted blood containing about 2.0×10^6 *T.evansi* trypanosomes as estimated using the improved Naubauer haemocytometer (Petana, 1963) using Gram's iodine as diluent.



Plate 5: Photomicrograph of *Trypanosoma evansi* from the infected blood of a naturally infected camel. Note the free and long flagellum with well developed undulating membrane. The nucleus is elongated and centrally placed. (Haematoxylin & Eosin x400magnification)

Table 3.1: ***T.evansi*** **infected and control Yankasa sheep groups**

Control (Group 1)	***T.evansi*** **infected** (Group 2)
P 3030	P 3035
P 3031	P 3036
P 3032	P 3037
P 3034	P 3038
P 3039	P 3040
P 3042	P 3041
-	P 3046
-	P 3047

The inoculated sheep were allowed to go through the full course of the infection without treatment.

3.3.6 Clinical Examination of Experimental Sheep

In order to evaluate the infectivity and pathogenicity of the *T.evansi* isolate in the infected Yankasa sheep, certain parameters were assayed prior to the inoculation. These parameters were then compared with values obtained after infection. The parameters investigated included (a) daily rectal temperature which was determined using a clinical thermometer, (b) parasitaemia which was determined every 3 days weekly using three major parasitological methods (wet film, haematocrit centrifuge technique, and thin blood film), (c) blood protein concentrations (used to determine the nutritive state of the sheep) were estimated every 3 days in each week using Goldberg Refractometer, (d) haematology by daily determination of PCV for one week and subsequently twice every week to estimate the degree of anaemia using the microhaematocrit centrifugation technique (Coles, 1974), Hb concentration was also determined according to the method of Coles (1974), and total RBC, total and differential leucocytes counts were estimated using the improved Neubauer haemocytometer (Baker and Silverton, 1978) every 3 days weekly, (e) live weights (kg) of both the control and experimentally infected Yankasa sheep were determined once every week using the portable foot weighing balance.

The wet blood film was examined after a drop of blood was placed on the center of a clean microscopic slide. This was then covered with a clean cover slip, and viewed under the light microscope at x 40 magnification.

For the HCT test, four micro-haematocrit capillary tubes (one end sealed) were each filled with about 70 µl of blood and centrifuged at 1500 rpm for about 5 minutes using a Hawksley centrifuge. The tubes were then placed on a clean slide. A drop of immersion-oil was put on top of each of the tubes and the buffy coat-plasma interface examined under the light microscope at x 40 magnification (Woo, 1969) for trypanosomes.

To conduct the thin blood smear, a drop of the suspected blood was placed at one end of a clean microscopic slide and using a spreader, and a thin smear was made. The slide was quickly air-dried, fixed in methanol for 3 minutes, stained with 10% Giemsa, and rinsed under a stream of tap water. After it was air-dried, a drop of immersion-oil was placed on it and examined under the light microscope at x 100 magnification.

Parasitaemia was scored as +, ++, +++, and massive (representing 1-5, 6-10, 10-20, and more than 20 trypanosomes per microscopic field) at x 100 magnification.

3.3.7 Clinical Signs

All the infected and control sheep were subjected to detailed clinical examination on a daily basis. Their ocular and buccal mucous membranes were examined. Their physical appearances, appetites, oral secretions, gait posture, appearance of superficial lymph nodes especially the pre-scapula and pre-femoral, cutaneous swellings and appearance of hair-coat.

3.3.8 Post-mortem Examination

Post-mortem examinations were carried out on both groups of Yankasa sheep (infected that were sacrificed *in extremis*, and control).

3.3.9 Statistical Analysis

The data obtained in the present study were presented as Mean (± standard deviation (SD)). The statistical analysis used was the *Student t-test* for the differences between control and infected animals. *Duncan's Multiple Range Test* was used for variables. Means (±SD) of the various parameters were compared and those differences that had a probability of $P<0.05$ were considered as statistically significant.

3.4 Results

3.4.1 Infection

Following mechanical infection, all the *T.evansi*-infected Yankasa sheep became parasitaemic (Table 3.2). Two phases of infection developed namely; acute which lasted for 14 days post-infection, and chronic which persisted for up to 59 days post-infection. Of the eight infected sheep, four were *in extremis* and had to be humanely killed (P3041 and P3038 on day 3, P3046 on day 6, and P3035 on day 14 post-infection). However, two (P3037, P3036) died from the chronic form on days 42 and 59, respectively. The remaining two (P3040, P 3047) survived the infection up to the end (day 68) when the experiment was terminated.

3.4.2 Rectal Temperature

The mean rectal temperature in the control Yankasa sheep ranged between 38.47 ± 0.55°C and 38.85 ± 0.98°C throughout the period of study (Table 3.4). However, the mean rectal temperature in the *T.evansi*-infected Yankasa sheep was moderately elevated (2.0 oC) above the pre-infection levels, and above corresponding values for the control especially between days 2 and 12 post-infection, attaining a peak (40.13 ± 1.06 oC) on day 6 post-infection (Table 3.5). Five Yankasa sheep (P3035, P3036, P3037, P3040, and P3047) had peak rectal temperatures of at least 40°C. The highest observed was 41.2°C in sheep P3047. The mean rectal temperature peaks corresponded with periods of rising parasitaemia (Fig.2).

Table 3.2: Summary of the outcome of the infection with *T.evansi* in Yankasa sheep

Sheep No.	Prepatent Period (days)	Patency Period (days)	Survival PI* (days)	Parasitaemia *in extremis*	Maximum T**(at day) (°C)	PCV *** *in extremis* (%)	Form of Infection
P3046	3	3	4	++	38.8 (-10)	24	Acute
P3038	3	2	5	++	39.2 (-10)	22	Acute
P3041	3	4	7	++	38.8 (-10)	28	Acute
P3035	6	8	14	+++	40.5 (6)	24	Acute
P3037	3	40	43	+++	41.1(6)	17	Chronic
P3036	3	56	59	+++	40.9(6)	10	Chronic
P3040	3	65	68	-	39.7(40)	S	Chronic
P3047	3	65	68	-	40.6(26)	S	Chronic

* PI = Post-infection

** T = Rectal temperature

*** PCV = Packed cell volume

Note: Sheep P3040 and P3047 survived up to the end of the experimental period

Table 3.3: Rectal temperature, body weight, and haematological parameters in *T. evansi*-infected and control Yankasa sheep

Parameter	Control Yankasa Sheep (Group 1) Range	Infected Yankasa Sheep (Group 2) Range
T# (°C)	38.2 - 39.1	37.7 - 40.0
PCV* (%)	23.3 - 28.7	17.7 - 27.80
Hb* (g dl^{-1})	7.9 - 9.1	5.4 - 9.0
Total Protein (g dl^{-1})	6.5 - 8.4	6.9 - 8.83
Total RBC* (x 10^6)	4.3 - 11.5	2.4 - 12.53
Body weight (kg)	13.1 - 16.6	10.7 - 13.60

• SD = Standard deviation

T = *Rectal temperature*

* PCV = Packed cell volume

⊛ Hb = Haemoglobin

• RBC = Red blood cells

Table 3.4: Weekly Clinical Parameters in the Un-infected (Control) Yankasa Sheep

Time of Infection (days)	RT (°C) Mean ± SD	Packed cell volume (%) Mean ± SD	Parasitemia HCT (10^6) Mean ± SD	Hemoglobin (g/dl) Mean ± SD	Total protn (g/dl)	RBC count 10^6 Mean ± SD	Weight(kg) Mean ± SD
-10	38.5 ± 0.55	27.2 ± 5.19	npf	9.1 ± 1.76	7.3 ± 0.90	11.5± 2.33	11.8 ± 7.34
-5	38.5 ± 0.40	25.2 ± 5.19	npf	8.4 ± 1.74	7.1 ± 0.79	9.2± 1.33	
0	38.2 ± 0.39	24.8 ± 5.27	npf	8.2 ± 1.79	7.8 ± 0.78	4.8± 1.43	14.3 ± 4.64
3	38.4 ± 0.20	25.3 ± 4.08	npf	8.5 ± 1.36	7.2 ± 0.56	4.3± 2.41	
6	38.3 ± 0.23	25.3 ± 3.08	npf	8.4 ± 1.34	6.7 ± 0.35	4.8 ± 1.43	
8	38.9 ± 0.99	25.0 ± 3.35	npf	8.1 ± 1.01	7.5 ± 0.42	5.2 ± 2.27	14.8 ± 4.72
12	38.6 ± 0.06	25.8 ± 3.13	npf	8.8 ± 1.04	7.5 ± 0.62	4.5 ± 2.11	
15	38.5 ± 0.18	25.4 ± 3.20	npf	8.5 ± 1.17	7.2 ± 0.60	5.8 ± 2.14	14.9 ± 5.30
19	38.8 ± 0.39	25.4 ± 3.13	npf	8.5 ± 1.05	7.1 ± 0.50	5.4 ± 3.12	
22	38.5 ± 0.48	25.2 ± 3.27	npf	8.5 ± 0.84	7.0 ± 0.84	6.3 ± 2.12	15.9 ± 5.50
26	38.7 ± 0.19	24.6 ± 3.71	npf	8.1 ± 1.00	7.1 ± 0.71	5.6 ± 1.97	
30	38.8 ± 0.72	25.2 ± 3.56	npf	8.1 ± 1.11	7.2 ± 0.43	4.8 ± 3.11	15.2 ±5.66
34	38.7 ± 0.41	24.8 ± 2.39	npf	8.2 ± 0.70	7.0 ± 0.30	5.2 ± 1.22	
37	38.8 ± 0.29	25.0 ± 2.35	npf	8.1 ± 0.40	7.2 ± 0.40	5.8 ± 1.34	
40	36.6 ± 0.24	25.4 ± 1.34	npf	7.9 ± 1.26	7.2 ± 0.32	6.4 ± 2.44	
44	38.4 ± 0.16	25.8 ± 1.10	npf	8.3 ± 1.02	7.3 ± 0.46	5.2 ± 1.56	16.0 ± 5.42
48	38.5 ± 0.19	25.8 ± 1.10	npf	8.3 ± 0.93	7.4 ± 0.32	5.0 ± 4.12	
51	38.5 ± 0.11	25.4 ± 1.07	npf	8.2 ± 1.01	7.3 ± 0.30	5.4 ± 3.12	16.2 ± 5.37
55	38.5 ± 0.20	26.6 ± 1.67	npf	8.7 ± 0.52	7.0 ± 0.44	4.9 ± 2.23	
60	38.6 ± 0.15	26.0 ± 2.55	npf	8.5 ± 0.78	7.5 ± 0.45	5.6 ± 1.32	16.2 ± 5.30
64	38.5 ± 0.15	26.0 ± 0.71	npf	8.7 ± 0.36	7.2 ± 0.67	5.4 ± 2.37	
68	38.6 ± 0.13	26.6 ± 1.14	npf	8.7 ± 0.39	7.1 ± 0.40	6.2 ± 1.57	16.6 ± 5.30

Table 3.5: Weekly Clinical Parameters in the *T.evansi*-infected Yankasa Sheep

Time of Infection (days)	RT (°C) Mean ± SD	Packed cell volume (%) Mean ± SD	Parasitemia HCT (10^6) Mean ± SD	Hemoglobin (g/dl) Mean ± SD	Total protn (g/dl)	RBC count 10^6 Mean ± SD	Weight(kg) Mean ± SD
-10	38.8 ± 0.41	31.0 ± 7.80	-	10.2 ± 2.63	6.7 ± 1.12	12.5 ± 1.78	12.2 ± 2.35
- 5	38.4 ± 0.28	25.8 ± 2.77	-	8.3 ± 0.75	7.0 ± 1.05	12.5 ± 2.66	
0	38.1 ± 0.36	25.5 ± 4.38	-	8.5 ± 1.46	7.4 ± 1.00	11.9 ± 1.74	12.5 ± 2.33
3	38.1 ± 0.69	25.0 ± 1.97	+	8.3 ± 0.60	7.3 ± 0.68	4.7 ± 0.62	
6	40.1 ± 1.06	24.5 ± 2.66	++	8.7 ± 0.71	7.0 ± 0.47	3.4 ± 1.45	
8	39.1 ± 0.54	24.2 ± 1.10	++	8.1 ± 0.40	7.1 ± 0.48	4.5 ± 1.24	13.2 ± 1.96
12	39.4 ± 1.21	24.0 ± 1.41	++	8.3 ± 0.76	7.3 ± 0.72	3.7 ± 0.43	
15	39.1 ± 0.87	24.0 ± 0.71	-	7.9 ± 0.38	7.5 ± 0.42	3.6 ± 0.72	13.3 ± 2.06
19	39.4 ± 0.69	23.0 ± 2.00	+	7.7 ± 0.70	7.4 ± 0.43	3.7 ± 2.89	
22	39.5 ± 0.76	22.0 ± 1.63	+	7.7 ± 0.87	7.5 ± 0.66	3.7 ± 1.42	13.6 ± 2.18
26	39.9 ± 0.68	23.8 ± 4.03	++	7.5 ± 1.29	7.9 ± 0.48	3.7 ± 1.03	
30	39.0 ± 0.44	23.3 ± 3.40	+	7.6 ± 0.84	7.5 ± 0.44	2.9 ± 0.96	13.3 ± 2.17
34	38.9 ± 0.51	21.3 ± 3.40	-	7.3 ± 1.41	7.2 ± 0.37	4.7 ± 1.73	
37	38.8 ± 0.13	21.5 ± 1.73	-	7.4 ± 0.91	7.2 ± 0.40	5.0 ± 0.86	12.8 ± 1.97
40	39.1 ± 0.53	20.4 ± 1.34	+	6.6 ± 0.93	6.9 ± 0.48	5.5 ± 2.14	
44	39.2 ± 0.12	25.0 ± 2.94	-	6.9 ± 1.27	7.9 ± 0.81	5.0 ± 2.13	12.1 ± 1.65
48	39.0 ± 0.15	20.7 ± 3.21	+++	7.0 ± 1.75	7.1 ± 0.74	5.2 ± 1.43	
51	39.1 ± 0.06	17.7 ± 4.04	+	6.2 ± 1.51	7.9 ± 0.51	4.0 ± 2.48	11.0 ± 2.50
55	38.7 ± 0.51	18.3 ± 3.06	+	6.2 ± 1.65	7.4 ± 0.92	3.5 ± 0.94	
60	38.9 ± 0.07	18.0 ± 5.29	-	5.5 ± 1.97	6.8 ± 0.53	3.2 ± 1.64	10.7 ± 2.53
64	38.9 ± 0.21	22.5 ± 4.95	-	7.5 ± 0.21	7.1 ± 0.50	2.9 ± 0.73	
68	38.9 ± 0.14	23.5 ± 2.12	+	7.5 ± 0.71	6.9 ± 0.14	2.4 ± 1.44	12.2 ± 1.34

Temperature (°C) infected group (Group 2)

3.4.3 Parasitaemia

The results of this study showed an average prepatent period of 3.4 days in the *T.evansi*-infected Yankasa sheep. Seven of the infected sheep became parasitaemic on the 3 post-infection, while one (P3035) showed parasitaemia on day 6 post-infection. The infection with this isolate produced parasitaemic waves at an average of 8.3 days interval throughout the duration of the experiments. Mean parasitaemic peaks occurred on days 6, 10, 19, 26, 30, 40, 50, 55, and 68 post infection. Six of the infected Yankasa sheep died by day 59 post-infection with varying levels of parasitaemia. Two sheep (P3040 and P3047) survived the infection. The surviving sheep had very low parasitaemia of "+", and which in some occasions could not be detected from the blood beginning from day 30 post-infection. However, sub-inoculation of the suspected blood into albino rats demonstrated the presence of the parasite in these infected Yankasa sheep.

3.4.4 Haematological Changes

Anaemia was a consistent distinct feature of the infection with this *T.evansi* isolate. The onset of parasitaemia was followed by a significant decrease ($P<0.05$) in the values for the PCV, Hb, and total RBC counts in the infected Yankasa sheep as indicated by the mean values of these parameters when compared with the pre-infection values (Fig. 2) up to day 60 post-infection. The lowest mean PCV (17.67 ± 4.04) was recorded on day 51 post-infection. These significant changes were not observed in the control group. The anaemia observed was progressive and intermittent coinciding with peaks of parasitaemia (Fig. 2).

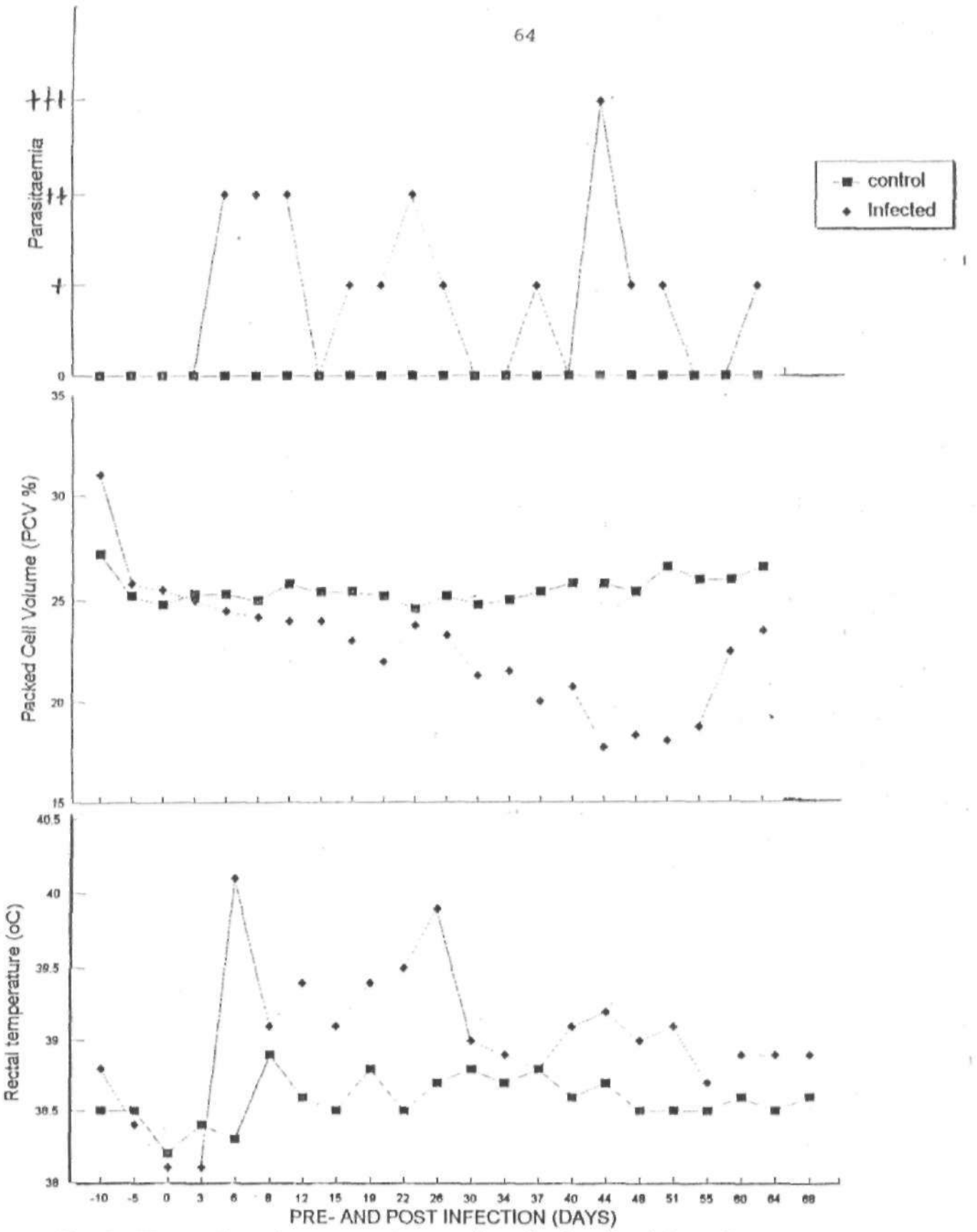


Fig. 2: Fluctuations in temperature and packed cell volume with respect to parasitaemia in T.evansi infected and uninfected sheep.

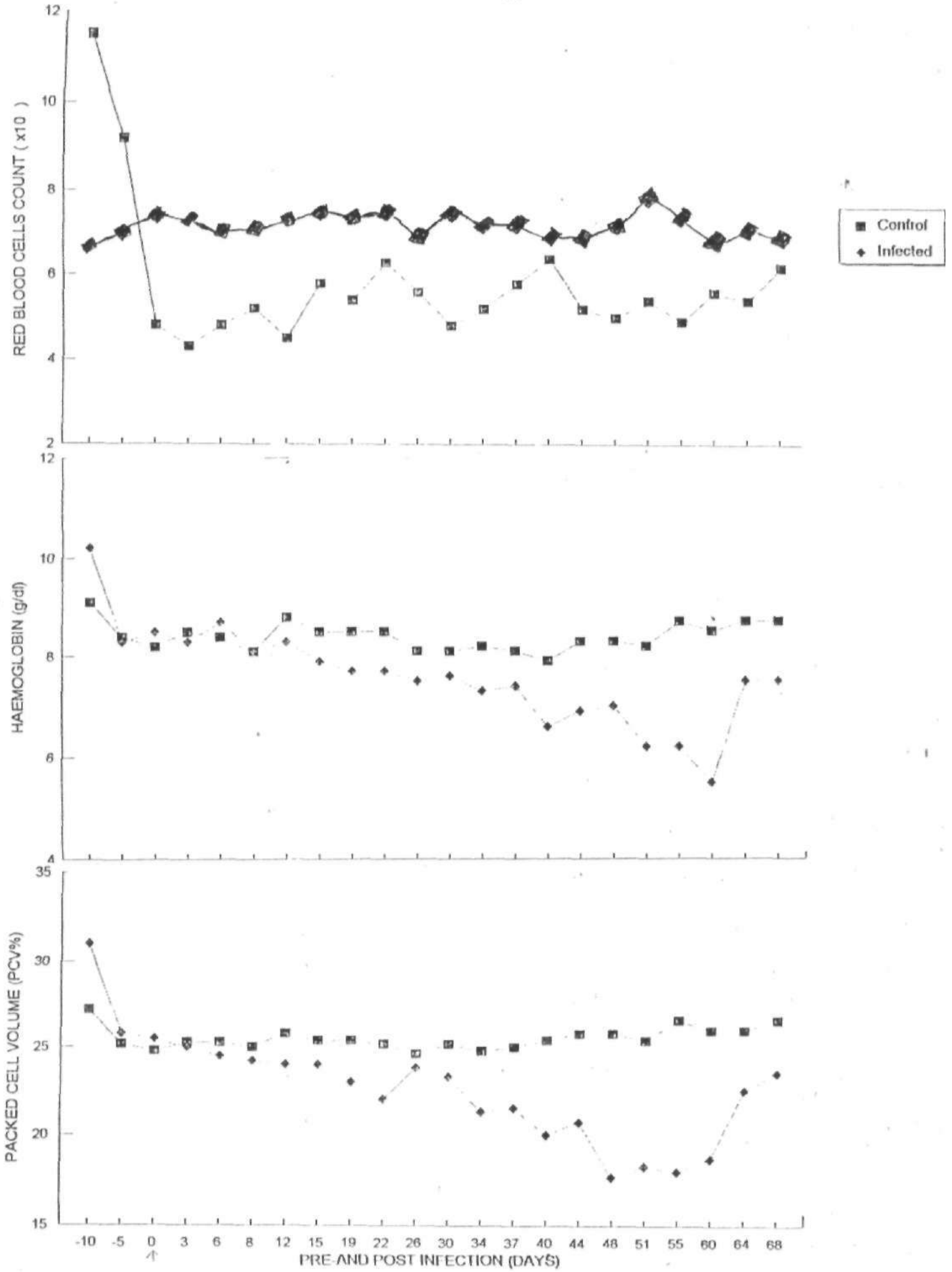


Fig. 3: Mean of Packed cell volume, Haemoglobin (Hb) and Red Blood Cells (RBC) in T.evansi infected and uninfected sheep

3.4.4.1 Total and Differential Leucocytes Counts

Significant (P<0.05) leucocytosis occurred in the infected Yankasa sheep from Day 12 post infection as a result fo lymphocytosis, eosinophilia, and monocytosis. The pre-infection and control counts remained within normal ranges (Fig 4 and 5).

The mean total leucocyte counts in the infected sheep ranged from $16.2 \pm 2.2 \times 10^9 L^{-1}$ (Day 0 post-infection) to $22.2 \pm 3.0 \times 10^9 L^{-1}$ (Day 60 post-infection). In the control sheep, the values ranged from $17.2 \pm 1.5 \times 10^9 L^{-1}$ to $21.2 \pm 2.1 \times 10^9 L^{-1}$ during the same period of investigation (Figs 4 and 5). The mean values of leucocyte counts for the control sheep were higher than the corresponding values for the infected for greater part of the experiment. However, the values were significantly (P<0.05) higher than those of the controls on Days 12 and 60 post-infection, which was associated with significant (P<0.05) increases in the mean lymphocytes and mean monocyte counts. Eosinophil were scarce up to Day 22 post infection but became apparent on Days 26, 64 post-infection but no significant (P<0.05) increase occured. With exception of the increase in mean neutrophils observed in the control sheep on Day 6, the neutrophils of both infected and control sheep remained steady with sligh fluctuations within normal range, both pre- and post-infection (Fig.5).

Lymphocytosis occurred only from Day 36 to the end of the study.

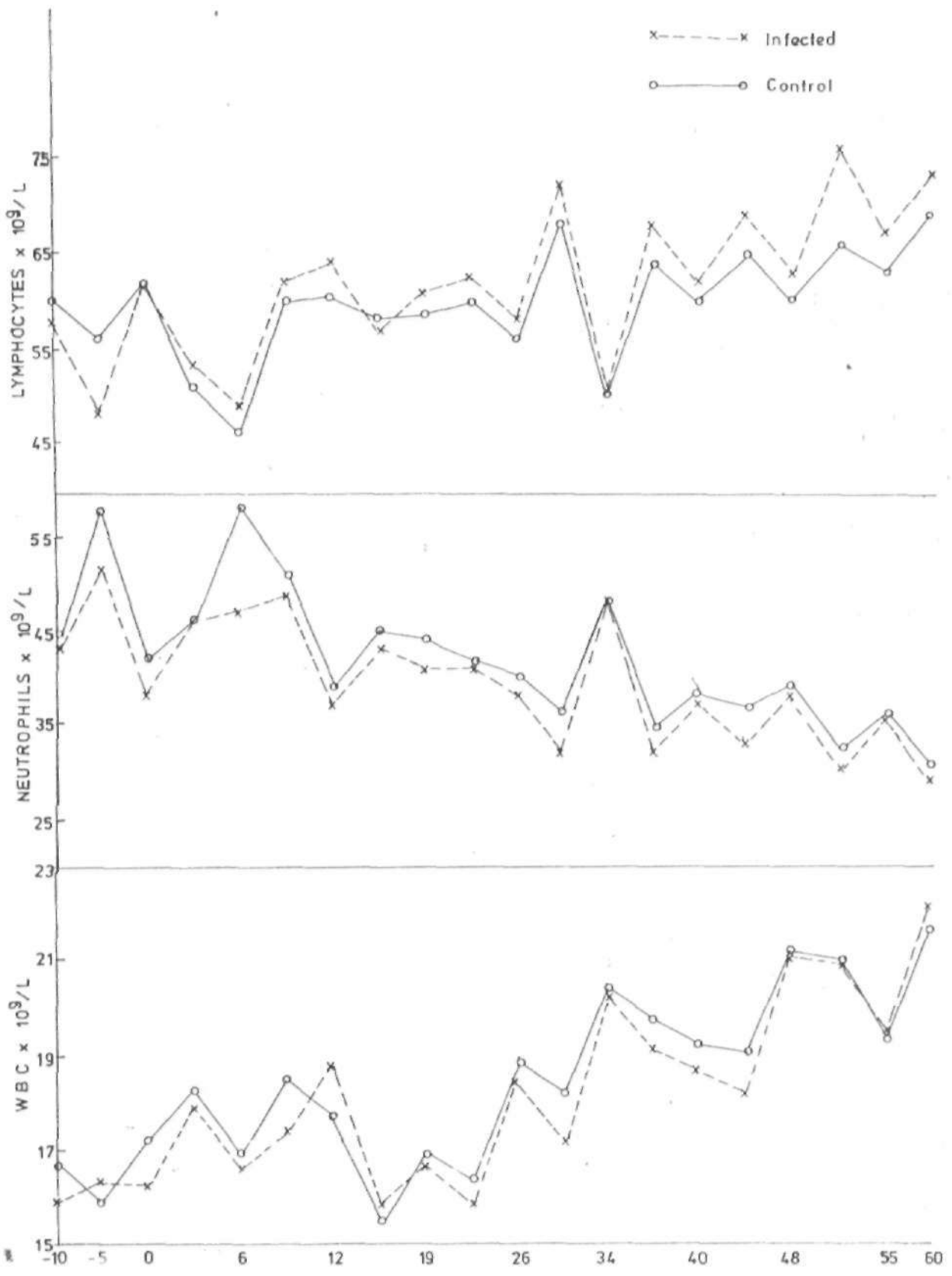


Fig. 4 Mean Total and Differential Leucocytes counts (WBC, Neutrophils and Lymphocytes)

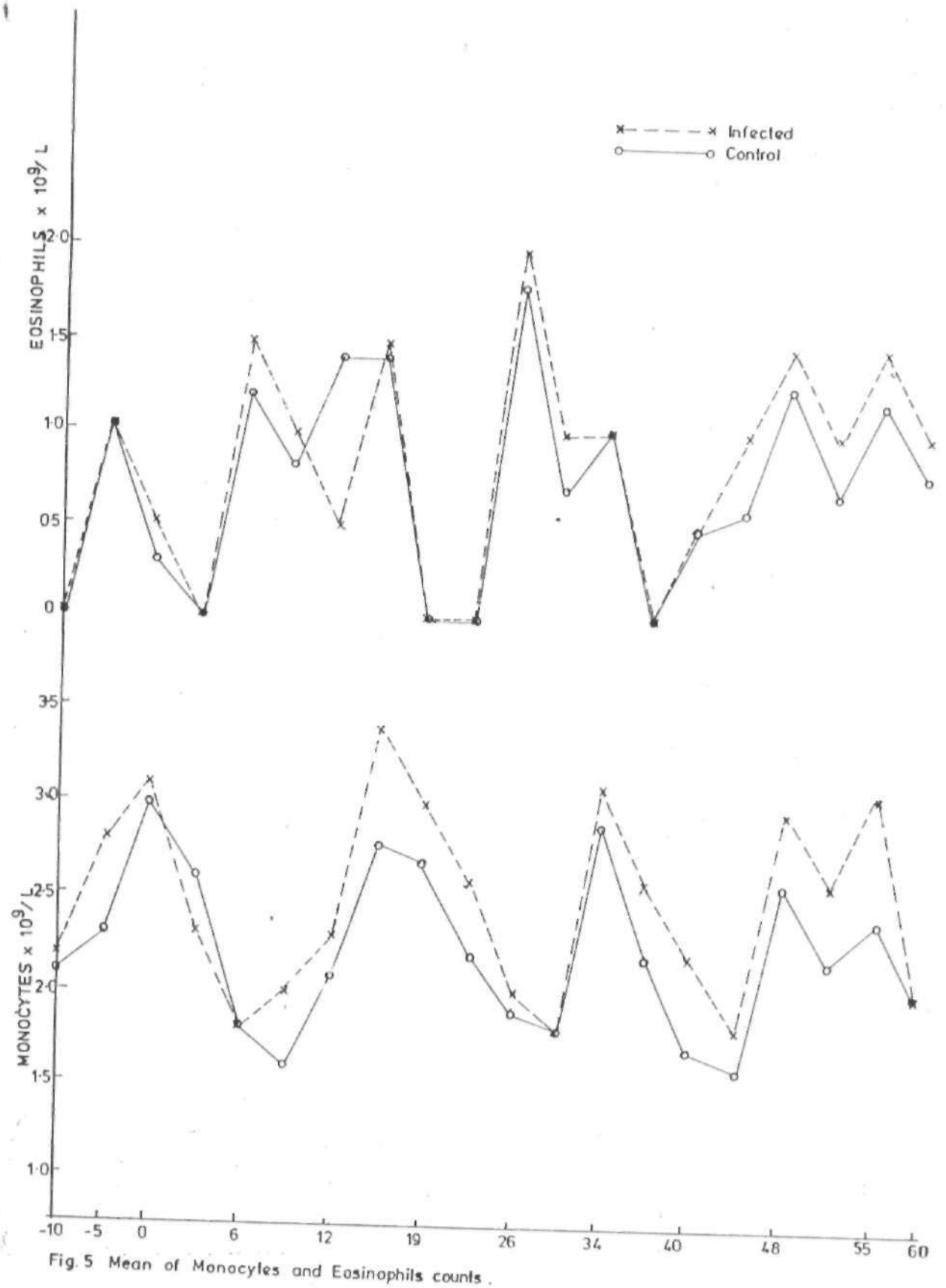


Fig. 5 Mean of Monocyles and Eosinophils counts.

3.4.5 Total Protein

The pre-infection mean blood protein in the control and the infected Yankasa sheep remained within the normal range. However, a non-significant ($P>0.05$) decrease occurred in the infected group between Days 3 and 8 post-infection. Thereafter, the values fluctuated within normal range up to the time when the experiment was terminated.

3.4.6 Weight Changes

The mean live body weights of the control sheep increased gradually from 11.75 ± 1.33kg (Day 10 pre-infection) to 16.64 ± 5.30kg at the end of the experiment (Fig. 6) representing a 29.38% gain in the mean body weight. The mean body weight in the infected Yankasa sheep increased from a pre-infection value of 12.16 ± 2.35kg (Day 10) to 13.28 ± 2.06kg on Day 22 post infection. However, the values decreased significantly ($P<0.05$) later up to the point of termination of the experiment. The infected Yankasa sheep lost 11.78% in body weight by the end of the experiment. Sheep P3036 and P3037 became highly emaciated (Fig. 6).

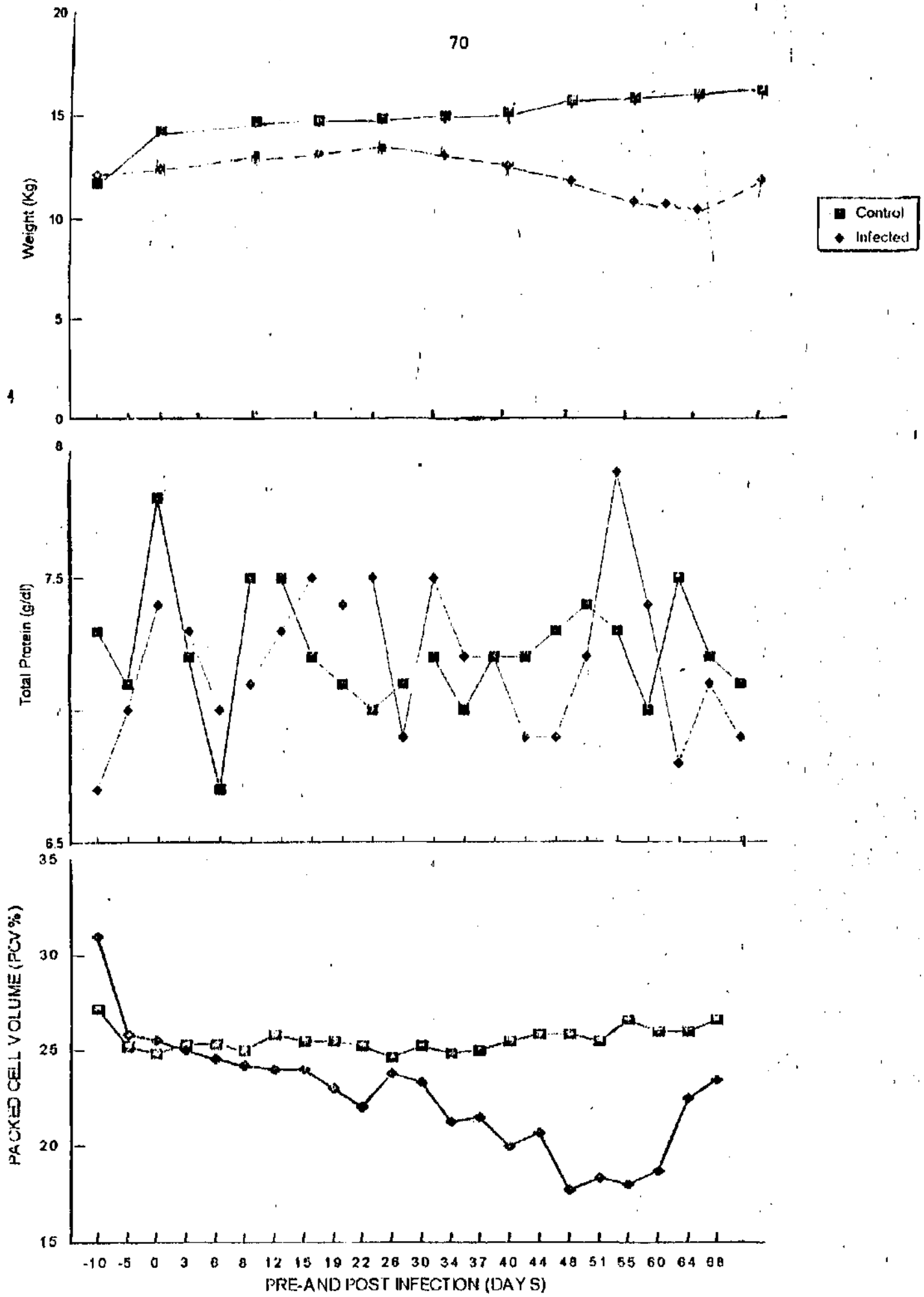


Fig.6 Mean of Packed Cell Volume, Total Protein and Weight Changes in T. evansi infected and uninfected sheep.

3.4.7 Other Observed Clinical Features

Following the development of parasitemia, similar clinical signs were observed in all the infected Yankasa sheep. The initial clinical signs were those of dullness, and recumbency. Other clinical signs included pronounced lacrimation, emaciation, weakness, dehydration, and rough hair-coat. Conjunctivitis, with evidence of blindness manifested by bumping into one another or the feeding troughs. Edematous swellings on the face and particularly the lower jaw were also evident. Superficial lymph nodes were enlarged, and respiratory problems in form of dyspnea and serous nasal discharges also occurred. Nervous disorders, rolling of the eye-lids, and staggering were also observed. General body conditions became poor and eventually death occurred terminally. However, the two sheep that survived the infection regained their body condition later during the course of the experiment with a slight gain in weights.

3.5 Discussion

The results obtained in the present investigation demonstrated that the *T.evansi* isolate produced infection with a variable course and parasitaemia in the Yankasa sheep. The parasitaemia varied in duration and intensity amongst individual sheep.

The observed increase in rectal temperature following the T. *evansi* infection, may bring about metabolic disorder. This is an indication of increased rate of heat production in the body which can be caused by many factors including stress . This could possibly play a significant role in the process of haemolysis and the consequent decrease in PCV. RBC exposed to temperatures a few degrees above normal body temperature are said to exhibit increased osmotic fragility, shortened life-span, and undergo accelerated haemolysis (Kelly, 1974; Murray 1978).

The fluctuations in temperature and the corresponding. undulating nature of the parasitaemia obtained in the present study have previously been reported in calves infected with *T.evansi* (Singh and Misra, 1988). The intermittent parasitaemias have also been attributed to the phenomenon of antigenic variation as reported in trypanosome infections (Edwards *et al.*, 1956).

The *T.evansi* isolate produced pronounced parasitaemic peaks indicating that the parasite is infective to Yankasa sheep. The Parasite was multiplying faster in the early stages of the infection (and shorter peak intervals) because the protozoa was trying to adjust to the new host environment..

The fall in PCV in the infected sheep coincided with peaks of parasitaemia on day 48 PI. This

observation is in agreement with the report of Anosa (1977) that the PCV of trypanosome-infected sheep and goats drops during parasitaemic peaks and then rises slightly during the interventions, suggesting that the red cells destruction is closely related to the presence of parasites in the peripheral circulation. The onset of anaemia which accompanied decrease in mean PCV values as observed in the current study is in agreement with previous findings in goats, sheep, Friesian calves, and Buffaloes infected with *T.evansi* (Ngeranwa *et al.*, 1991; Payne *et al.*, 1991; 1992; Onah *et al.*, 1996). Similar observations were made in goats (Saror, 1980; Maxie *et al.*, 1976) and in sheep and goats (Anosa and Isoun, 1974)infected with *T. vivax*, strongly suggesting that the presence of live trypanosomes in the infected animals was essential for the development of anaemia (Clarkson, 1968; Naylor, 1971; Holmes and Jennings, 1976).

It appears reasonable to suggest that the presence of *T.evansi* in the blood of the infected sheep subsequently followed by parasitaemic waves coinciding with decrease in mean PCV values implies some causal relationships between the parasites and anaemia, further giving evidence for the pathogenicity of the parasites in the Yankasasheep.

The development of anaemia in trypanosomiasis is believed to be multifactorial (Jenkins *et al.*, 1980). The precise mechanism involved in the development of anaemia in the present study was not investigated. However, possible explanations for this include the involvement of specific immunoglobulin against *T.evansi* which form complexes with the antigen and complement on the surface of red blood cells leading to their sequestration and destruction in the reticulo-endothelial system. This has been shown to occur in calves infected with *T.congolense* (Kobayashi and Tizard, 1976) and in rabbits infected with *T.brucei* (Dodd *et al.*, 1978). Other possible factors include the role of a 'non-specific haemolytic factor' which might facilitate the

destruction of normal red blood cells by macrophages (ILRAD, 1984). Some haemolytic factors have been identified which might have contributed to erythrocyte lysis (Fiennes, 1954; Nguyen Huan *et al.*, 1975; Tizard *et al.*, 1978).

A possible explanation for the leucocytosis observed in the infected sheep is that the trypanosomes may be producing pathological changes in the peripheral blood of the sheep in a manner similar to those produced by tsetse-transmitted trypanosomes (Esievo *et al.*, 1984; Sackey, 1998). Therefore one may surmise that lymphocytosis and monocytosis in the current study were responses due to the *T.evansi* infection as previously reported in trypanosome infection in ruminants (Onah *et al.*, 1996; Sackey, 1998).

The fall in total plasma protein concentrations in the infected sheep in the current study agrees with the reports of Losos and Ikede (1972) on trypanosomosis of goats and sheep. These decreases are suggestive of increased protein break down or urea loss, haemodilution, and serum extravasation (Anosa and Isoun, 1974) which could cause oedema in the infected sheep. Oedema of the face, lower jaw, limbs and scrotum have been reported in trypanosomosis of Uda rams (Maikaje *et al.*, 1991). Although the protein fractions were not determined in the current study, most reports on this subject point to the fact that decreases in albumin levels are responsible for oedema found in chronic cases of trypanosomosis (Monzon and Villavicencio, 1990).

The observed interstitial pneumonia in one of the infected sheep is in agreement with similar observations of Krammer (1966) in natural infections of trypanosomosis in West African Dwarf sheep and goats in Eastern Nigeria. This has been attributed to lowered immune response as a result of the infection allowing otherwise normal respiratory microfloral to invade the respiratory

tract.

Very limited information is available on central nervous system involvement in animal trypanosomosis. However, the staggering movement of infected sheep observed in this study suggest that *T.evansi* causes CNS derangement, particularly, since brain squash examination was negative for *Cowdria ruminantium,* a common haemoparasite of ruminants in this locality.

Generally, the infected sheep had appetite, but it was poor throughout the course of investigation including animals that were *in extremis*. Therefore the weight loss and emaciation observed in the infected sheep could possibly be due to impairment of feed conversion efficiency. The fall in live body weights of the infected sheep is also an indication of the wasting nature of trypanosomosis. The decrease in the body weight was more pronounced in sheep that died in the chronic phase indicating muscle wastage in trypanosomosis (Sackey, 1998).

The fall in the body weights of the infected sheep is an indication of the wasting nature of trypanosomosis. The decrease in the body weight was more pronounced in sheep that died in the chronic phase indicating muscle wastage as reported by Sackey (1998).

Both the acute and chronic infections observed in this study and the severity of the infection within 59 days post-infection , agrees with similar observation by Saseendranath *et al.* (1995). The results of the present study have revealed the susceptibility of Yankasa sheep to the *T.evansi* isolate. Therefore, it seems apparent that sheep needs to be protected like Zebu cattle found in the same vegetational zone.

CHAPTER FOUR

4.0 GROSS AND HISTOPATHOLOGIC FEATURES OF *Trypanosoma evansi*-INFECTION IN YANKASA SHEEP

4.1 Summary

The pathology of *Trypanosoma evansi* infection in Yankasa sheep was studied using an isolate of the parasite obtained from the infected blood of a camel slaughtered at Kano abattoir. Pathological changes affecting various organs and the clinical effects on the Yankasa sheep were noted. These changes included pale anaemic carcasses, serous atrophy of adipose tissue, enlarged superficial lymph nodes and splenomegaly.

Histopathological changes in the infected Yankasa sheep included hepatocyte fatty degeneration, erythrophagia, and haemosiderosis in association with focal areas of hepatic necrosis in chronic cases. Glomerular, proximal, and distal convoluted tubular necrosis, mononuclear cell infiltration, and congestion were observed in the kidneys of the *T.evansi*-infected Yankasa sheep. Other lesions associated with the infection included emphysema, pulmonary congestion, and diffuse mononuclear cell infiltration into the lung parenchyma. No similar significant findings were observed in the organs of the control sheep.

The results of this pathological study have further indicated that Yankasa sheep are susceptible to the *T.evansi* isolate. However, the full genetical characterization of the isolate has not been carried out.

4.2 Introduction

Pathological changes associated with ruminant trypanosomosis have been subjected to intensive research (Edwards *et al.*, 1956; Ikede, 1979, Saror, 1980; Sekoni, 1992). However, most of the reports are those concerning *T.vivax*, *T.brucei*, and *T.congolense* (Ikede and Losos, 1972; Saror, 1980; Sekoni, 1992), with little information on *T.evansi* infections in ruminants. Some of the few reports were on pathological changes in *T.evansi*-infected rabbits and goats which involved degenerative changes in lymphoid organs with immune response derrangement (Uche and Jones, 1992). Similarly; lymphoid hyperplasia, muscular atrophy, bronchopneumonia, and hepatic, renal, splenic, and pulmonary necrotic foci have been reported in goats infected with *T.evansi* (Ngeranwa *et al.*, 1993).

The present study was designed and carried out to investigate the pathological changes in Yankasa sheep following infection with the *T.evansi* isolate.

4.3 Materials and Methods

4.3.1 Control and Experimental Infection

The same sets of Yankasa sheep used in the infectivity and pathogenicity study (Section 3.3.3) were used for the pathological study.

4.3.2 Collection and Examination of Samples

Four of *the T.evansi*-infected Yankasa sheep that died as a result of the acute infection, and two

others that were *in extremis*, and humanely killed ; were immediately necropsied. For the purpose of comparison, two of the control Yankasa sheep were also sacrificed, and post-mortem examinations, involving gross pathological evaluations on various organs were carried out.

The cerebrospinal fluid, liver, bone marrow impressions, and brain squash smears were prepared, stained with Giemsa, and microspically examined under x 40 objective for identifying trypanosomes, *Cowdria ruminantium* and possibly other haemoparasites.

4.3.3 Analysis of Samples

Organ samples of the liver, bone marrow, lymph nodes, kidneys, heart, and lung were obtained and fixed in 10% buffered neutral formalin and processed using Technicon Tissue Processor. The tissues were sectioned 5 μm thick and stained using Haematoxylin and Eosin. Samples obtained from two control Yankasa sheep were similarly processed.

4.4 Results

4.4.1 Post-Mortem Changes

The carcasses of the infected sheep were pale and showed severe anaemia as indicated by thin watery blood, especially in those with chronic infection. Splenomegaly and enlarged superficial lymph nodes were observed in all the *T.evansi*-infected sheep.

The mucosal surface of the abomasum of sheep P3046 and P3041 had petechial haemorrhages. However, sheep P3035 had congested lung with evidence of pneumonia, hepatomegaly, catarrhal enteritis, enlarged adrenal glands and edematous lymph nodes. Sheep P3036, P3037, and P3038 had moderately enlarged lymph nodes and splenomegaly.

4.4.2 Histo-pathological Findings

Marked histo-pathological changes occurred in the tissues and organs of the infected Yankasa sheep. However, such changes were not observed in the tissues of the two control Yankasa sheep that were examined.

4.4.2.1 Spleen

Histological section of the spleen of uninfected control Yankasa sheep showed normal tissue architecture (Plate 7) while that of the infected Yankasa sheep showed severe lymphocytic proliferation at the germinal centres and peri-arteriolar lymphatic sheath which extended into the red pulp. Haemosiderosis and haemosiderin-laden macrophage occurred in the spleen of the infected sheep. These features were more pronounced in sheep P3036 and P3037 which had cronic infection (Plates 8 and 9).

4.4.2.2 Liver

Histological section of the liver of uninfected control Yankasa sheep showed normal tissue architecture (Plate 10), while the liver of the *T.evansi*-infected Yankasa sheep showed centrilobular necrosis in the hepatocytes and other focal areas of necrosis. Hepatic macrophagic erythrophagia, haemosiderosis, and haemosiderin-laden macrophages occurred in sheep P3036 and P3038 (Plates 11 and 12).

4.4.2.3 Kidney

Histological section of the kidney of uninfected control Yankasa sheep showed apparently normal tissue architecture (Plate 13) while non-suppurative glomerulonephritis involving the proximal and distal collecting tubules with a few mononuclear cell infiltration and congestion were observed in the kidneys of the infected Yankasa Sheep (P3037 and P3036; Plates 14 and 15), which died in the chronic phase of the infection.

4.4.2.4 Lungs

The lungs of infected Sheep (P3035) showed congestion, emphysema, diffuse macrophage infiltration into lung parenchyma and pulmonary oedema (Plates 16 and 17). There were no significant histological changes in the lung of the control Sheep P3038.

4.4.2.5 Other Findings

There were no significant histo-pathological findings in the intestine, testes, ovaries, epididymis, and heart of Sheep P3046, P3038, P3041, P3035, P3037, and P3036.

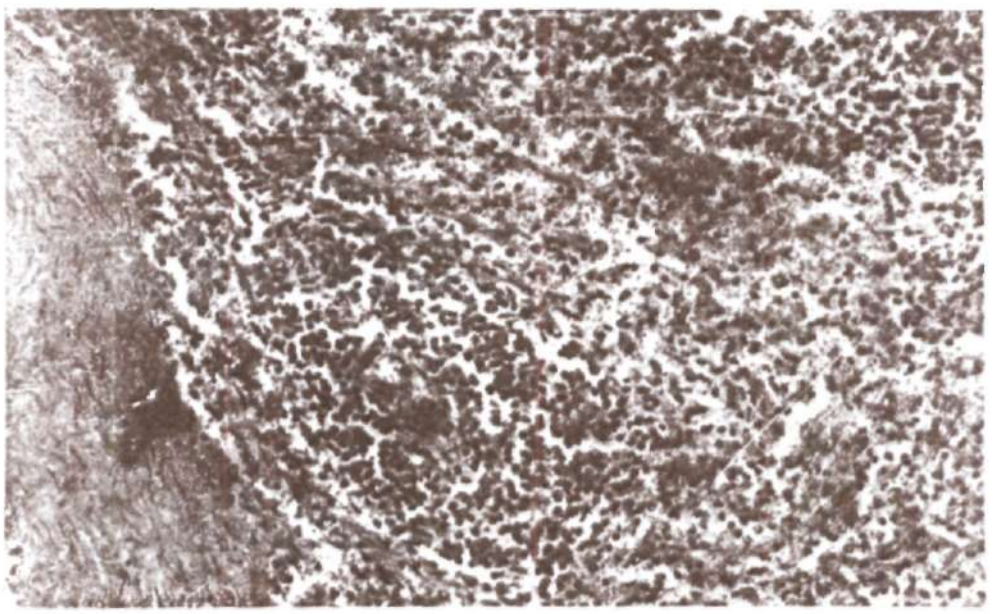


Plate 7: Histological section of spleen of control sheep showing normal tissue architecture. (Haematoxylin & Eosin x 4000)

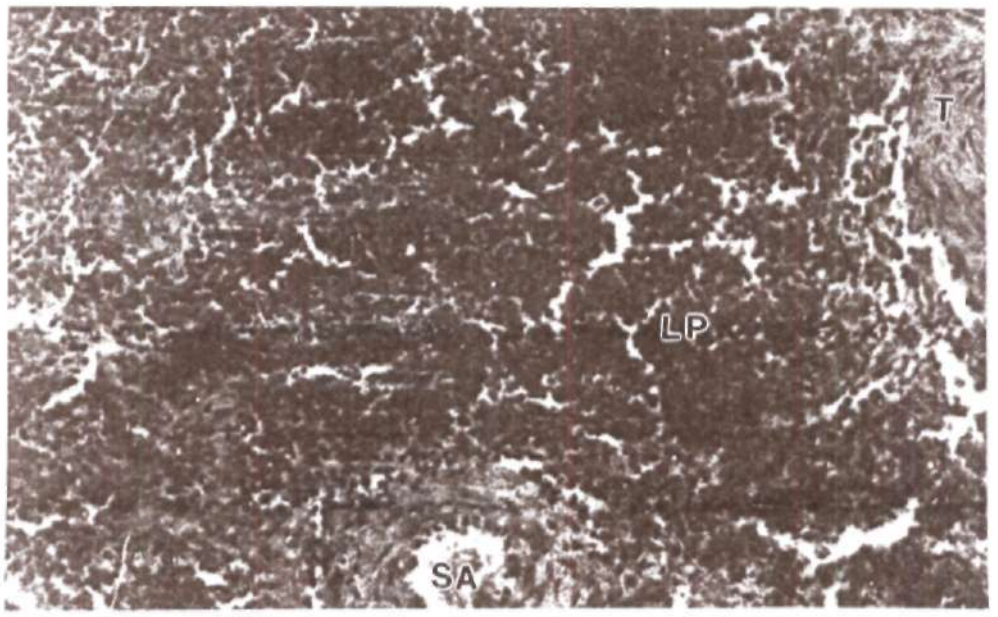


Plate 8: Histological section of the spleen of *T.evansi*-infected Yankasa sheep (P3036) showing dense lymphocytic proliferation (LP) involving the germinal centers and the red pulp. Note splenic artery (SA) and trabeculae (T). (Haematoxylin & Eosin x 4000)

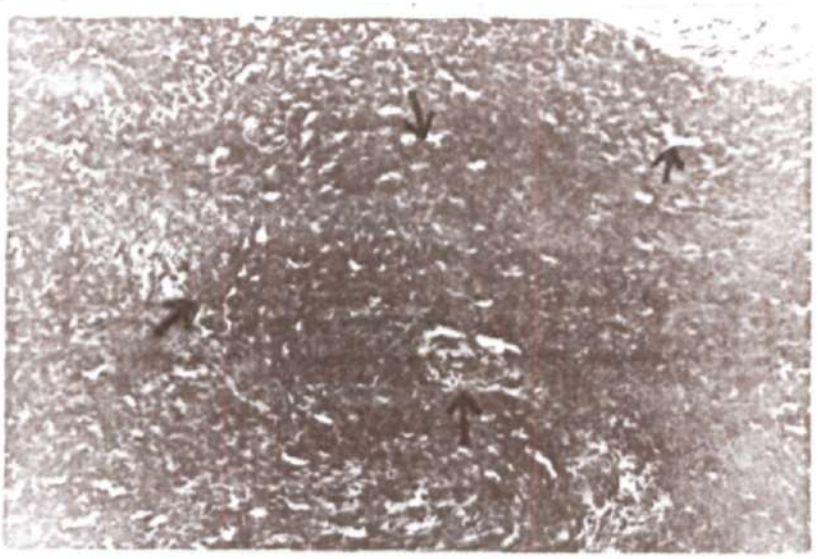


Plate 9: Histological section of the spleen of *T.evansi* infected Yankasa sheep (P3037) showing lymphocyte proliferation (arrow) at germinal center. (Haematoxylin & Eosin x 4000)

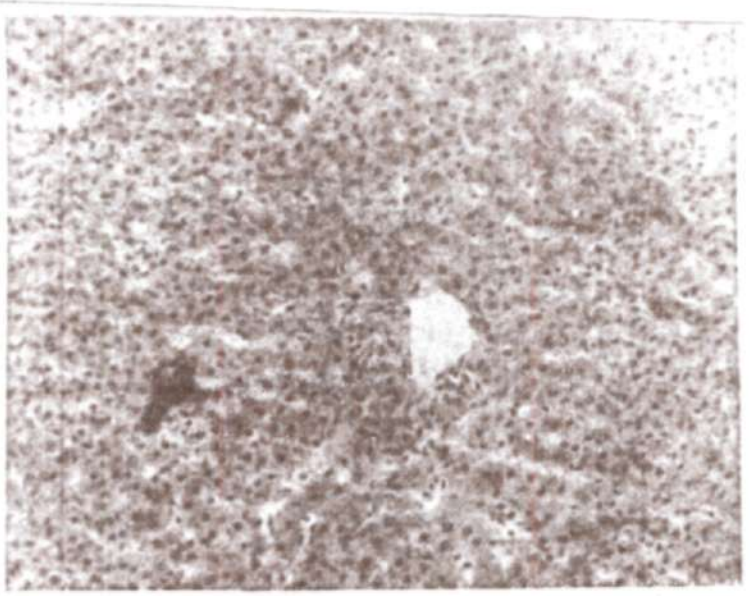


Plate 10: Histological section of the liver of control sheep showing apparently normal tissue architecture. (Haematoxylin & Eosin x 4000)

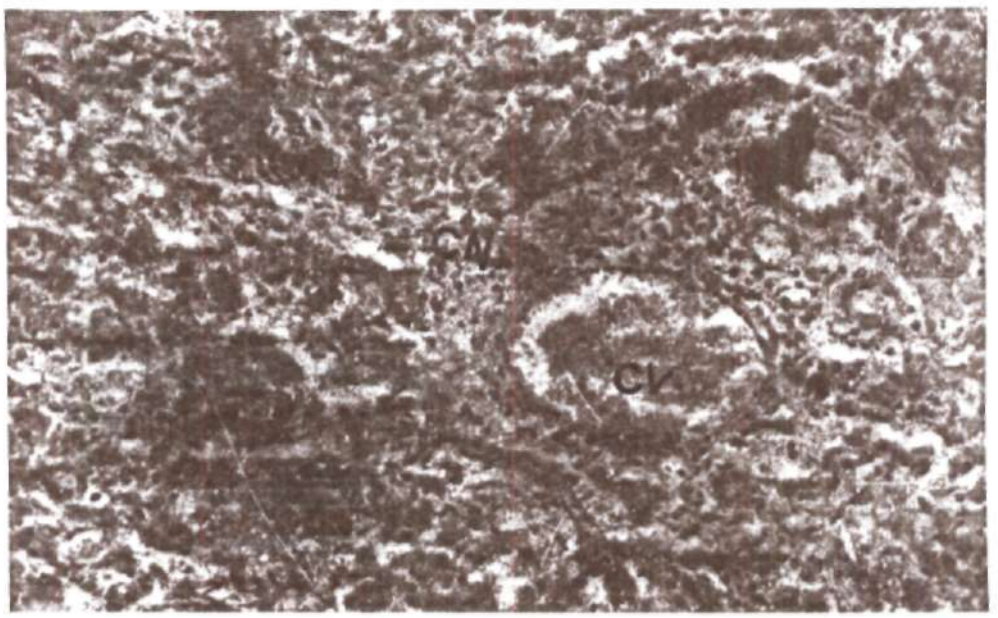


Plate 11: Histological section of the liver of *T.evansi*-infected Yankasa sheep (P3036) showing centrilobular hepatocyte necrosis (CN). Note central vein (CV). (Haematoxylin & Eosin x 4000)

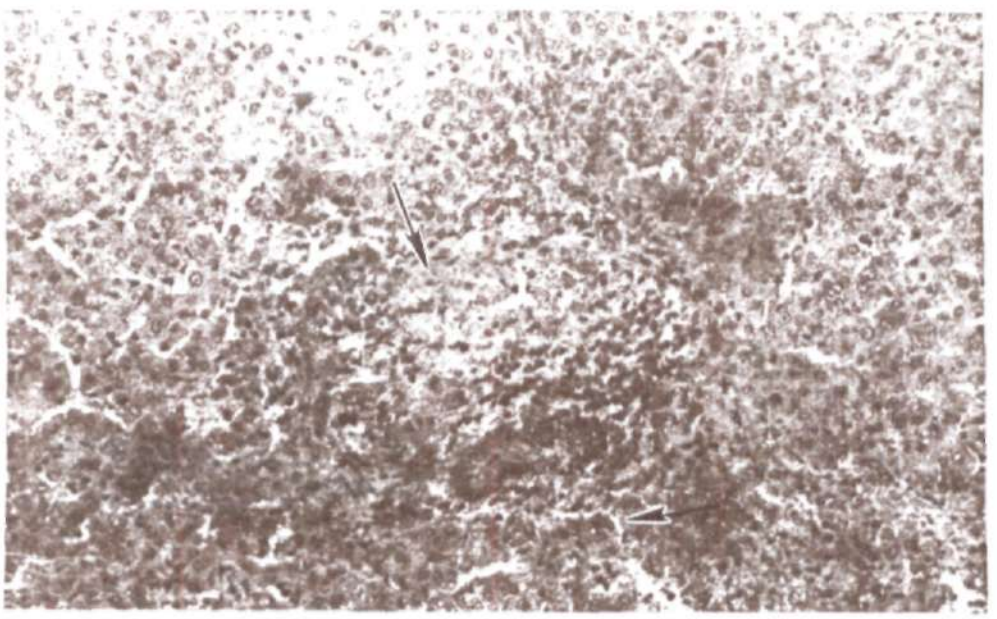


Plate 12: Histological section of the liver of *T.evansi*- infected Yankasa sheep (P3038) with centrilobular necrosis (arrow). (Haematoxylin & Eosin x 4000)

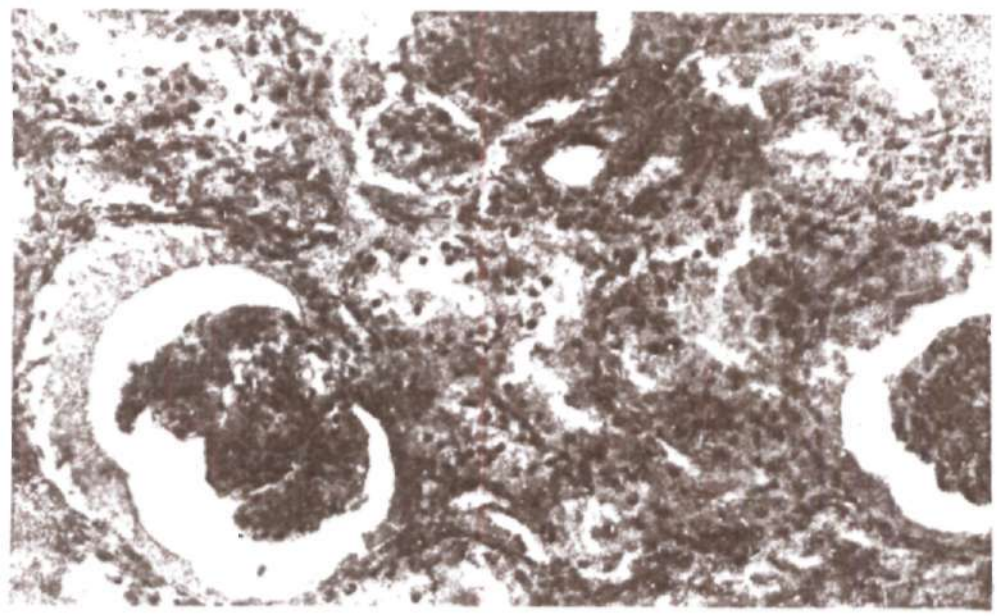


Plate 13: Histological section of the kidney of control Yankasa sheep showing apparently normal tissue architecture. (Haematoxylin & Eosin x 4000)

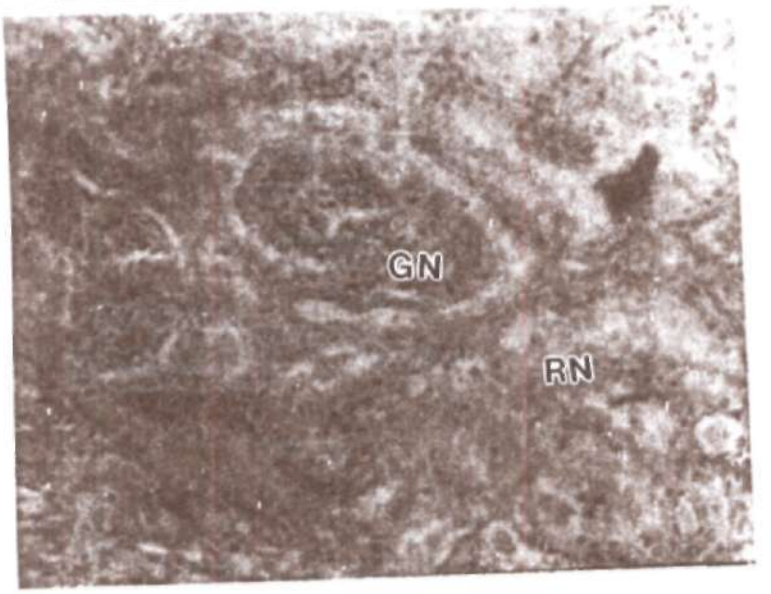


Plate 14: Histological section of the kidney of *T.evansi* infected Yankasa sheep (P3037) showing non-suppurative glomerulonephritis and renal tubular necrosis (RN). (Haematoxylin & Eosin x 4000)

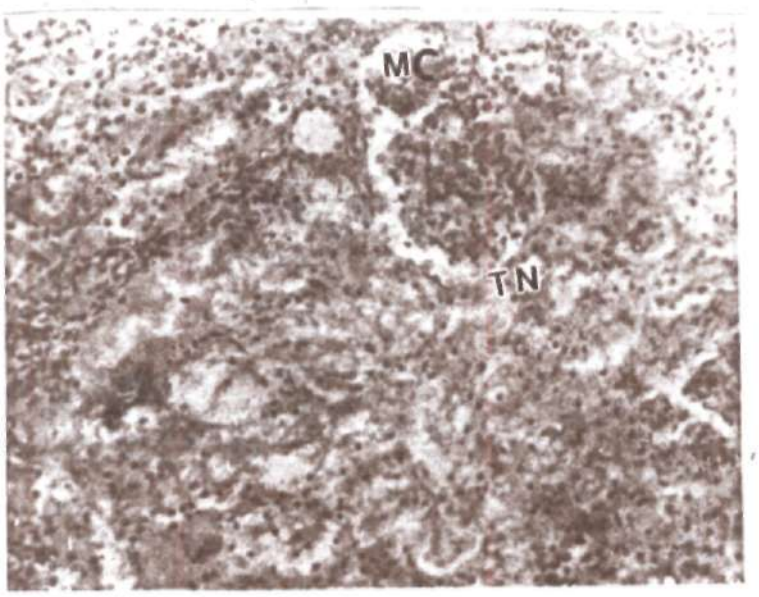


Plate 15: Histological section of the kidney of *T.evansi*-infected Yankasa sheep (P3036) showing tubular necrosis (TN), and Tubular degeneration with mononuclear cellular (MC) infilteration (Haemotoxylin & Eosin x 4000).

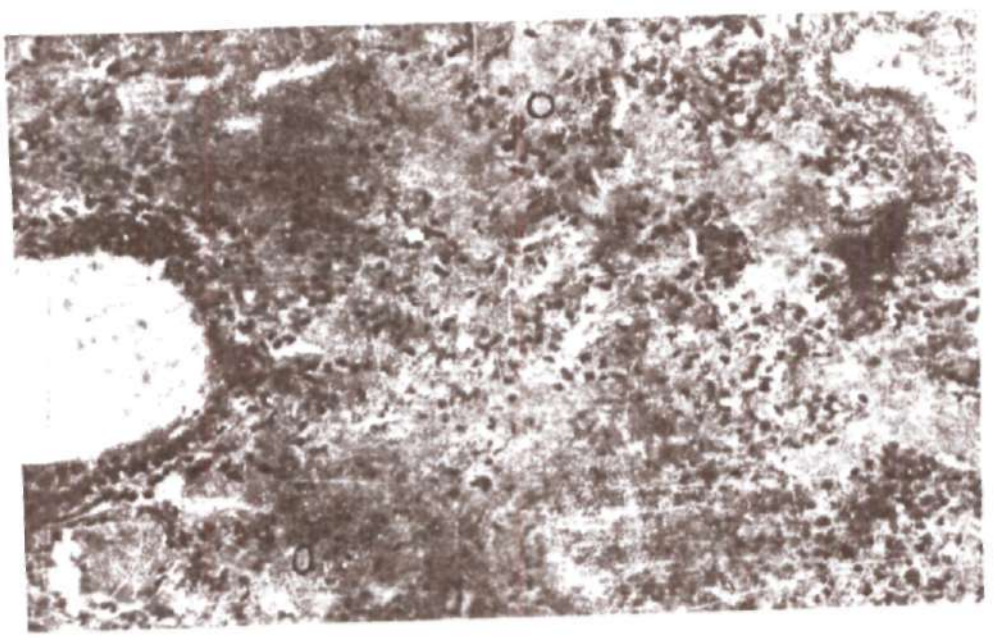


Plate 16: Histological section of the lung of *T.evansi*-infected Yankasa sheep (P3035) showing mononuclear cell infiltration into lung parenchyma and pulmonary oedema (O). (Haematoxylin & Eosin x 4000)

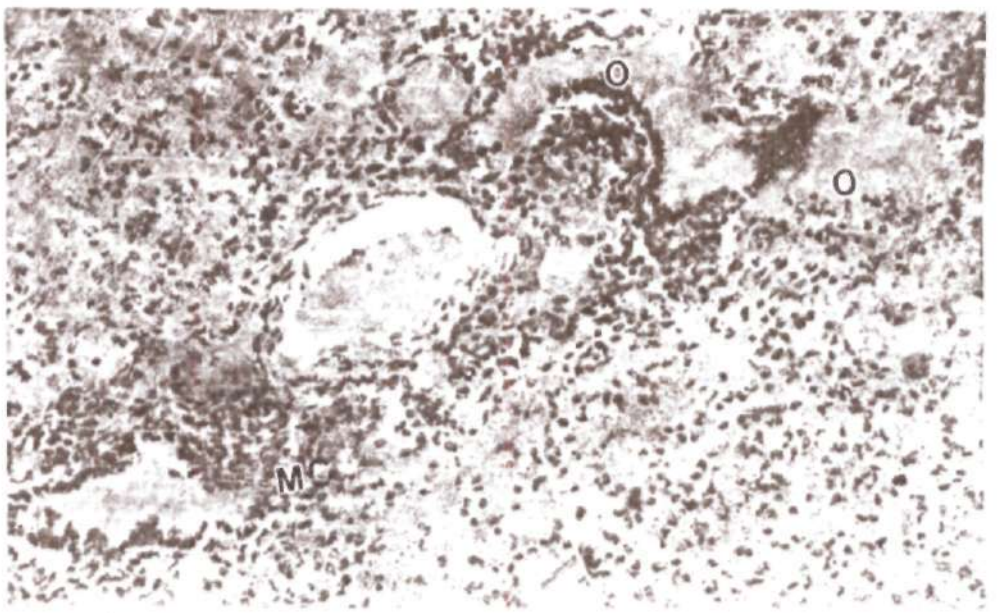


Plate 17: Histological section of the lung of *T.evansi*-infected Yankasa sheep (P3035) showing mononuclear cell (MC) infiltration into lung parenchyma and pulmonary oedema (O). (Haematoxylin & Eosin x 4000)

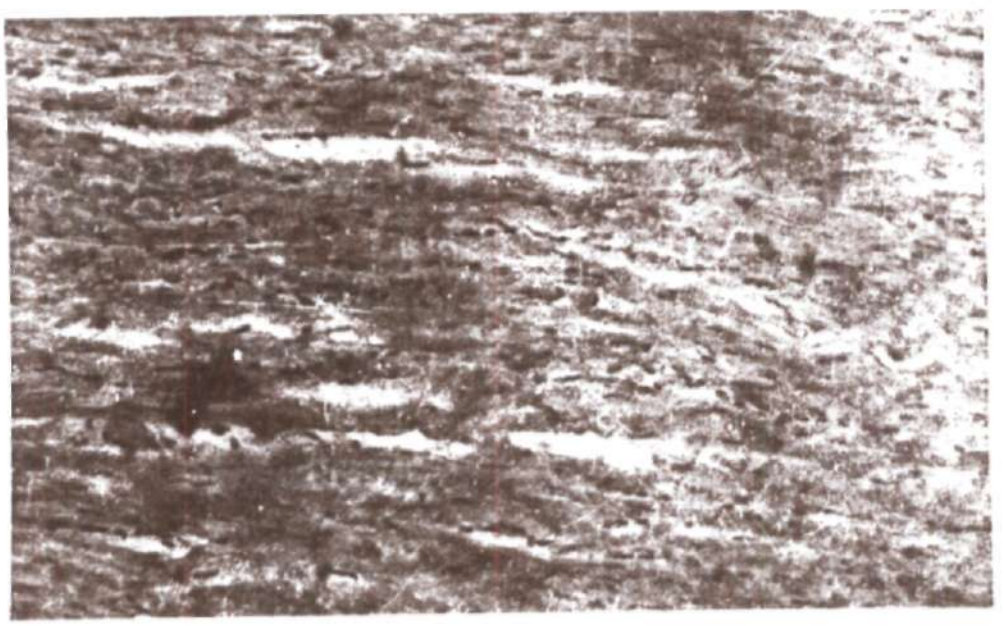


Plate 18: Histological section of the heart of *T.evansi*-infected Yankasa sheep (P3038) showing no significant histo-pathological changes. (Haematoxylin & Eosin x 4000).

4.5 Discussion and Conclusion

The results of the present study have clearly shown that the most prominent histo-pathological features of the infection in the Yankasa sheep with the isolate of *T.evansi* include lymphocytic proliferation in germinal centers of the spleen and lymph nodes, haemosiderosis, and haemosiderin-laden machrophages as observed in chronic stage of the infection. These changes are indicative of extensive mononuclear cell reaction due to extravascular localization of trypanosomes in the interstitial connective tissue as observed in rabbits infected with *T.evansi* (Uche and Jones, 1992), Uda Sheep infected with *T. vivax* (Maikaije, 1991), rats infected with *T.brucei* (Nwaorgu *et al.*, 1981), Sheep and goats infected with *T. vivax* (Anosa, 1977) and in Sheep infected with *T. brucei* (Ikede and Losos, 1972).

The observed generalized degenerative changes such as lymphadenopathy, splenomegaly, and lymphoid hyperplasia featuring in most of the organs of the infected Yankasa sheep agree with the findings of Saseendranath *et al.* (1995) in *T.evansi*-infected Madras red sheep and in *T. congolense* infected cattle and Sheep(Luckins and Gray, 1979) .These changes also showed similarity with those reported in acute trypanosomosis due to *T. vivax* in cattle, sheep and goats (Losos and Ikede, 1972).

The observed necrotic foci in the liver, kidney, and also pneumonic lesions seen in one Yankasa sheep with chronic infection agree with the findings of Ngeranwa *et al.* (1993) in *T.evansi*-infected goats.

The occurrence of haemosiderin-laden macrophages in the kidney and lung of the infected Yankasa sheep are attributed to haemolysis involving the expanded mononuclear phagocytic system as reported in *T.brucei*-infected sheep (Ikede and Losos, 1972) and in goats infected with *T.vivax* (Saror, 1980).

The results of the present study also revealed the occurrence of severe anaemia in the infected Yankasa sheep. This was represented by pale carcasses, and watery blood.

In conclusion, the histo-pathological changes that occurred as a result of *T.evansi*-infection, especially in the case of the two animals that had chronic infection, have indicated that the Yankasa breed of sheep are susceptible to infection with the isolate, and therefore gives support to the findings on its pathogenicity.

A seventy five percent mortality was recorded in the infected sheep in the present study as compared with the 100 percent mortality reported for madras red sheep in India similarly infected with *T. evansi*. Although *T. evansi* might pose serious treat to the livestock industry, especially in the north. The fact that 2 (25%) of the infected Yankasa sheep survived the infection raises some hope for the control of *T. evansi* infection in Yankasa sheep in the field. Hence, further investigation is required to understand features of special interest that might have aided the survival of the two infected sheep.

CHAPTER FIVE

5.0 General Discussion

The acute and chronic trypanosomosis observed in the current study, indicate that the *T.evansi* isolate was pathogenic to Yankasa breed, hence the parasite is potentially of great economic importance, just like other pathogenic trypanosome species of sheep like *T. vivax* (Maikaje *et al* ., 1991), *T. congolense* and *T. brucei* (Losos and Ikede, 1972). It is therefore, reasonable to suggest that Yankasa sheep succumb to *T.evansi* infection, a situation which has also been reported in *T.evansi*-infected goats (Ngeranwa *et al.*, 1993). Sascendranath *et al* (1995) also showed that all Madras red sheep infected with *T. evansi* died of the infection, while 75 % mortality were recorded in the current study. The slight difference in mortalities might be due to difference in breed of sheep.

The anaemia observed in the current study coincided with the peak of parasitaemia in the chronic phase (48 days P I) and is in agreement with previous reports in goats, sheep, Friesian calves, and buffaloes infected with *T.evansi* (Ngeranwa *et al.*, 1991; Payne *et al.*, 1991; 1992; Onah *et al.*, 1996). It is also reasonable to suggest that since *T.evansi* like *T.* brucei is tissue invasive, it caused sequestration and destruction of red blood cells in the reticuloendothelial system as reported in *T.congolense* infected calves (Kobayashi and Tizard, 1976) and in rabbits infected with *T.brucei* (Dodd *et al.*, 1978).

The pyrexia that occurred in the infected Yankasa sheep might be responsible for the haemolysis. It is known that elevated body temperatures play a significant role in haemolysis and fluctuations in rectal temperature as observed in current study coincided with peaks of parasitaemia as previously reported (Edward *et al.*, 1956).

The decrease in total plasma protein concentrations as observed in the infected Yankasa sheep is in line with the observations of Losos and Ikede (1972), and suggestive of increased protein break-down, urea loss, haemodilution, or serum extravasation (Anosa and Isoun, 1974). However, the actual mechanism resulting in the fall in plasma protein levels have not been investigated in the current study.

Loss in body weights occurred in all the infected Yankasa sheep. This observation has similarly been made in association with *T.vivax*-infected Uda rams (Maikaje *et al.*, 1991) and in cattle and goats (Griffin and Allonby, 1979). The weight loss observed in the infected Yankasa sheep may be attributable to many factors including the wasting nature of the infection (Sackey, 1998). However, because no feed intake study was carried out, it is rather impossible to determine exactly the mechanism responsible for the weight loss. For obvious reasons, the loss in weight was more pronounced in those Yankasa sheep that had chronic infection.

The pneumonic lesions such as mononuclear cell infilteration and pulmonary oedema observed in the lung of one of the infected Yankasa sheep have been previously reported by Krammer (1966) in trypanosome infected West African Dwarf sheep and goats in Eastern Nigeria. This condition has been attributed to stress of infection leading to the lowering of immunity and thereby allowing opportunistic micro-organisms in the respiratory tract to invade the respiratory

tissues causing nasal discharges, coughing, etc. (Sackey, 1998). It is possible that the pneumonic lesions observed in the present study are suggestive of the possibility of the trypanosome infection predisposing to respiratory tract infection.

Other observed clinical signs in the infected sheep such as central nervous system disorder, emaciation, dullness, rough hair coat, are suggestive of pathogenic effect of the *T.evansi* isolate in Yankasa sheep. The carcasses of infected sheep were anaemic as evident by the pale mucous membrane. The most prominent histopathological changes in the lymphoid organ, particularly haemosiderosis and haemosiderin - laden macrophages in various organs of the infected sheep, indicate and activated mononuclear phagocytic system as observed in rabbits infected with *T.evansi* (Uche and Jones, 1992), and *T.brucei* (Dodd *et al* 1978). Similar degenerative changes have been reported in *T.evansi* infected madras red sheep (Saseendranath *et al.*, 1995).

Necrotic foci were evident in the liver and kidneys of the infected sheep in the chronic stage of the infection. These findings are in agreement with those of Ngeranwa *et al.* (1993) in *T.evansi*-infected goats, and of Fiennes (1954) in *T.congolense*-infected cattle.

The results of this study have indicated that the *T.evansi* isolate was pathogenic to the Yankasa sheep as evidenced by the development of parasitaemia, anaemia, and gross and histo-pathological lesions. The anaemia and lesions observed in this study are consistent with lesions typically associated with animal trypanosomosis.

There are reports that sheep and other domestic animals only serve as carriers of *T.evansi* (Khasanov and Ivanitskaya, 1974). However, results from this study show that the Yankasa

sheep may be more than just a carrier for *T. evansi* as previously reported (Khasanov and Ivanitskaya, 1974). The same may be true of certain breeds of some other domestic animals. Furthermore, the existence of antigenic differences in trypanosomes, and the fact that different animal hosts and different strains of the parasite are involved in the epidemics of the disease in different parts of the world, (Bennett, 1933; Mahmoud and Gray, 1980) further strengthens the point that the isolate is pathogenic for sheep.

Also, the findings of the present work have very important epidemiologic and economic implications to the livestock industry in Nigeria. Camels are becoming more and more popular as beasts of burden and for their meat, particularly in the northern parts of the country bordering Niger Republic and Chad. The *T.evansi* isolate used in this study came from the blood of a slaughtered camel, as such the possibility of infection becoming important in sheep (especially the Yankasa breed) should be taken into consideration while formulating policies for the control of animal trypanosomosis in tse-tse free ecological zones of Nigeria.

Thus, a study to investigate the possible existence of the infection in sheep in the northern parts of Nigeria would provide a fair assessment of the threat posed by *T.evansi* in the country. The mechanical vectors of the disease are also abundantly found in the region. It is also hoped that a complete understanding of the behavior of *T.evansi* in other animals, particularly those herded with camels (sheep and goats), would provide a frame-work for integrated control measures to protect not only camels, but possibly prevent massive economic losses in sheep and goats.

REFERENCES

Adu, I.F. and Ngere, L.O. (1979) The Indigenous sheep of Nigeria. World Review of Animal Production. Vol. 3. Jul - Sept 1979.

Anonymous (1991) A new treatment for trypanosomiasis in camels (CYMELARSAN). *Revue d'Appuipour L'Elevage*. 2:13-14.

Anosa, V.O. (1977) Studies on the mechanism of anaemia and the pathology of experimental *Trypanosoma vivax* infection in sheep and goats. Ph.D. Thesis, University of Ibadan. Ibadan, Nigeria.

Anosa, V.O. and Isoun, T.T. (1974) Experimental *T.vivax* infection of sheep and goats, the relationship between the parasitaemia, the growth rate and anaemia. *Nigeria Journal of Veterinary Medicine*. 3: 101-108.

Arias, J. F., Garcia, F., Riveria, M. and Lopez, R. (1997). *Trypanosoma evansi* in capybara from Venezuala. *Journal of Wildlife Diseases*. 33: 359-361.

Balakrishnan, V.S.; Alex, P.C.; Babu K.M.J. and Saseendranath, M.R. (1994) Canine trypanosomiasis. *Cheiron*. 23: 93-96.

Baker, F.J. and Silverton, C.A. (1978) *Introduction to Medical Laboratory Technology*. Section 5: Haematology. ELBS Edition. Pp 549-626

Baldry, D.A.T. and Jordan, K. (1965) Notes on Entomology. Report of Nigerian Institute of Trypanosomiasis Research (NITR). 70-77.

Barotte J. (1925) Les trypanosomiases de L'Afrique du Nord Meimo Soc. Science Nature Maroc. II: 1.

Bennett, S.L.J. (1933); The control of camel trypanosomiasis. *Journal of Comparative Pathology and Therapeutics*. 46: 67-77.

Bennett, S.L.C. and Kenny, P.A.C. (1928) Mercuric chloride as a diagnostic agent for trypanosomiasis in camels. *Journal of Comparative Pathology and Therapeutics*. 41: 341-353.

Bhattacharyya, W.K. and Singh, P.K. (1974) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A research. *Tropical Animal Health and Production*. 12: 35-47.

Boid, R (1985). Iso enzyme Characterisation of Stocks of trypanosomes isolated from cattle in Indonesia. *Research in Veterinary Science*. 39:380-389.

Boid, R. Luckins, A.G. Ray. P.F., Gray, A.R., Mahmoud, M.M. and Malik, K.H. (1980) Serum Immunoglobulin levels and electrophoretic patterns of serum proteins in camels infected with *Trypanosoma evansi*. *Veterinary Parasitology*. 6: 333-345.

Boid, R. Hunter, A.G. Jones, T.W. Ross, C.A. Sutherland, and Luckins, A.G. (1996) Trypanosomosis research at the Centre for Tropical Veterinary Medicine (CTVM) 1970 to 1995. *Tropical Animal Health and Production*. 28: 5-22.

Borst, P., Fase-Fowler, F. And Gibson, W.C. (1987). Kinetoplast DNA of *Trypanosoma evansi* Molecular Biochemistry and Parasitoloty 23:31:38

Braide, V.B. (1974) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888) A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Brown, L.A. and Losos, G.J. (1977) A comparative study of the responses of the thymus, spleen, lymph nodes, and bone marrow of the albino rat to infection with *Trypansoma congolense* and *Trypansoma brucei*. *Research in Veterinary Science*. 23: 196-203.

Chainey, J.E. (1993) Horseflies, Deerflies and Kegs (Tabanids) In: *Medical insects and Arachnids*, Lane, R.P. and Crosskey, R.W. (Editors) Chapman and Hall, British Museum, London. 325-327, 399.

Chand, K. And Singh, R.P. (1971) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Clark, H.C. and Dunn, L. H. (1933). Animal susceptibility to *Trypanosoma hippicum* the equine trypanosome of Panama with speicial refernce to cattle as an unharmed host and probable reservoir of importance. *American Journal of Tropical Medicine*. 13: 273-281.

Clarkson, M.J. (1968) Blood and Plasma volumes in sheep infected with *Trypanosoma vivax*. *Journal of Comparative Pathology*. 78: 189-193.

Clarkson, M.J. and Awan, M.A.O. (1969): The immune response of sheep to *Trypanosoma vivax*. *Annals of Tropical Medicine and Parasitology*. 63: 515-528.

Coles, H.E. (1974) Veterinary clinical pathology. P.W.B. Saunders Company, Philadelphia and London. pp 72-165.

Cross H.E. (1923). A further note on Surra Transmission Experiments with *Tabanus albimedus* and ticks.*Transaction of Royal Society for Tropical Medicine and Hygiene*. 26:469 - 474.

Curasson, G. (1943) Trypanosomes. Traite de protozoologie veterinaire et compre. Tonel paris Vigot-freres.

Curasson, G. (1948) Cited by Mahmoud, M M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Dennig, H.K. (1972) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Dia, M.L., Van Meirvenne, N., Magnus, E., Luckins, A.G., Diop. C., Thiam., Jacquiet, P. and Hammers, R. (1997). Evaluation de quarte tests de diagnostic: frottis sanguins, CATT, IFI et ELISA-Ag dans L'fetude de l'fepidemiologic de la trypanosomes cameline a *Tryparosoma evansi* en Mauritanie. *Revue D'Elevage et de Medicine Veterinaire de pays Tropicaux*. 50 29-36.

Diall, O. Nantulya, V.M. Luckins, A.G., Diarra, B., Kouyate, B (1992) Evaluation of mono- and polyclonal antibody-based antigen detection immunoassay for diagnosis of *Trypanosoma evansi* infection in the dromedary camel. *Revue D'Elevage et de Medicine Veterinaire de Pays Tropicaux*. 45: 149-153.

Dodd, B.E., Jenkins, G.C., Lincoln, P.J. and Mc Crorie, P. (1978) The advantage of a built-up antiglobulin technique for the detection of immunoglobulin on the red cells of rabbits infected with trypanosomes *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 72. 501-505.

Edwards, E.E. Judd, J.M. and Squire, F.A., (1956) Observations on trypanosomiasis in domestic animals in West Africa I. The daily index of infection and weekly haematological values in goats and sheep infected with *T.evansi* and *T.brucei*. *Annals of Tropical Medicine and Parasitology*. 50:223-241.

Evans, G. (1880) Report on "Surra" disease in the Dera Ismáil Khan district. Punjab Government Military Department, No. 493, p.446.

Esievo, K.A.N; Saror. D.I; Alafiyatayo, R.A. and Jamu, A.S. (1984). Effects of Age and Sex on some Haematological Values of kids of Sokoto Red Goats. *Nigerian Veterinary Journal* 13 (2): 48-51.

Falope, O.O. (1991) Camel trypanosomiasis in Nigeria. New prevalent rate. *Bulletin of Animal Health and Production in Africa*. 39: 1-2.

Franke, C.R., Greiner, M. And Mehlitz D. (1994a). Investigations on naturally occuring *Trypanosoma evansi* infections in horses, cattle, dos and capybaras (*Hydrochaeris* hydrochaeris) in Pantanal de pocone (Mato Grosso, Brazil) *Acta Tropica* 58: 159-169.

Franke, C.R., Greiner, M. And Mehlitz D. (1994b). Monitoring of clinical, Parasitological and serological Parameters during an experimental infection of Capybaras (*Hydrochaeris hydrochaeris)* with *Trypanosoma evansi Acta Tropica*. 58: 171-174.

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

Finelle, P. (1973) Chimiotherapic et chimiosprevaution de la trypanosomiase animale. Acquisitions recentes et situation actuelle. Les cahiers de medicine veternaire 42:215-226. *World Animal Review*. 7: 1-6.

Fraser. H. (1909). Surra in the Federated Malay States. *Journal of Tropical Veterinary* Science 4: 345-386.

Gibson, W.C., Wilson, A.J. and Moloo, S.K. (1983). Characterisation of *Trypanosoma*(Trypanoz *evansi* from camels in Kenya using isoenzyme electrophoresis. Research ih Veterinary Scienee. 34:114-118

Gill, B.S. (1971). Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. Tropical *Animal Health and Production.* 12:35-47.

Godfrey, D.G.; Killick-Kendrick, R and Leach, T.M. (1962) Recent findings on the incidence of bovine trypanosomiasis in Nigeria. *9th meeting of International Scientific Committee on Trypanosomiasis Research* (Conakry) C.C.T.A. Publication No. 88, p. 111.

Gray, A.R. (1965) Antigenic variation in a strain of *T.brucei* transmitted by *Glossina morsitans* and *G.palpalis*. *Journal of General Microbiology*. 44: 195-214.

Gray, A.R. (1967) Some Principles of Immunology of Trypanosomosis. *Bulletin of the World Health Organisation*. 37: 177 - 193.

Gray, A.R. (1972) Variable agglutinogenic antigens of *Trypanosoma gambiense* and their distribution among isolates of the trypanosomes collected in different places in Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 66: 263-284.

Gray, A.R. (1973) Antigenic variation in cyclically transmitted isolates of *Trypanosoma gambiense*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 67: 269-270.

Gray, A.R. (1974) Antigenic Similarities among isolates of *Trypanosoma gambiense* from different countries in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 68: 150-151.

Gray, A.R. and Luckins, A.G. (1976) Antigenic Variation in Salivarian trypanosomes. In: The Biology of the Kinetoplastida. (Eds W.H.R. Lumsden and D.A. Evans). Academic Press, London. pp 493-542.

Gray, A.R. and Luckins, A.G. (1980) The initial stage of infection with cyclically transmitted *Trypanosoma congolense* in rabbits, calves and sheep. *Journal of Comparative Pathology*. 90:499-512.

Griffin, L. and Allonby, A.H. (1979) Trypanotolerance in breeds of sheep and goats with an experimental infection of *Trypanosoma congolense*. *Veterinary Parasitology*. 5: 97-105.

Hoare, C.A. (1940) Studies on the behaviour of *Trypanosoma evansi* in tsetse flies, with special reference to its phylogeny. *Parasitology*. 32: 105.

Hoare, C.A. (1954) The loss of kinetoplast in trypanosomes with special reference to *Trypanosoma evansi*. *Journal of Protozoology*. 1: 28-33.

Hoare, C.A. (1956) Morphological and taxonomic studies on mammalian trypanosomes. VIII. Revision of *Trypanosoma evansi*. *Parasitology*. 46: 130-172.

Hoare, C.A. (1967) Evolutionary trends in Mammalian trypanosomes. *Advances in Parasitology*. 5: 47.

Hoare, C.A. (1970) Systematic Description of Trypanosomes In: The African Trypanosomiasis, Mulligan, H.W. (Editor) First edition, Allen-UnWin, London, 51-54.

Hoare, C.A. (1972) The Trypanosomes of Mammals. A zoological Monograph, Black Well Scientific Publications, Oxford and Edinbourgh pp 555-593.

Holmes, P.H. Jennings, F.W. (1976) The effect of treatment on the anaemia of African Trypanosomiasis. In: Pathophysiology of parasitic infections. (Soulsby, E.J.L., ed). Academic Press, New York and London. pp 199-210.

Hornby, H.E. (1952) Animal Trypanosomiasis in Eastern African. His Majesty's Stationary Office, London. pp. 10-11.

Hunter, A.G. (1990) "Surra" In: Handbook on Animal Diseases in the Tropics. 4th Edition. (Sewell, M.M. and Brocklesby, D.W., Editors). Bailliere Tindall, London, 217-221.

Hyde, J.E. (1990) Molecular Parasitology. Open University, Press: Milton Keynes, 302 Pp.

Ikede, B.O. (1979) Genital Lesions in Experimental chronic *Trypanosoma brucei* infection in rams; *Research in Veterinary Science*. 26: 145-151.

Ilemobade, A.A. (1971) Studies on the incidence and pathogenicity of *Trypanosoma evansi* in Nigeria. II: The pathogenicity of *T.evansi* for equine and bovine species, ISCTRC, OAU/STRC publication No. 105, pp 107-114.

International Laboratory for Research on Animal Diseases (ILRAD) (1984) Anaemia in Trypanosomiasis: Haemaorrhagic *T.vivax* may provide a clue. *ILRAD Reports*. pp 1-3.

Imms, A.D. (1959) Outlines on Entomology. 5th Edition. Methuen and Co. Ltd., London. pp 174-175, 183.

Jatkar, P.R., Ghosal, A.K. and Singh, M. (1973) Pathogenesis of anaemia in *Trypanosoma evansi* infection III Studies on serum proteins. *Indian Veterinary Journal*. 50: 634-643.

Jatkaar, P.R. and Singh, M. (1974) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Jeffrey, H.C. and Leach, R.M. (1966) Atlas of Medical Helminthology and Protozoology. pp. 67.

Jenkins, G.C., McCrorie, P., Forsberg, C.M. and Brown, J.L. (1980) Studies on the anaemia in rabbits infected with *Trypanosoma brucei brucei*. *Journal of Comparative Pathology*. 90: 107-121.

John, M.C. Nedunchelliya, S. and Venkataraman, K.S. (1992) Biometrical observations on different strains of *Trypanosoma evansi*. *Veterinary Parasitology*. 43:143-145.

Jones, T. W. and Mckinnell, C.D. (1984) Antigenic Variation in *Trypanosoma evansi*: Isolation and characterization of variable antigen type population from rabbits infected with a stock of *T.evansi*. *Tropenmedizin Und Parasitologie*, 35: 237-241.

Jones, T. W. and Mckinnell, C.D. (1985a) Antigenic Variation in *Trypanosoma evansi*: Variable antigen type development in mice, sheep and goats. *Tropical Medicine and Parasitology*. 36: 53-57.

Jones, T. W. and Mckinnell, C.D. (1985b) Antigenic variation in *Trypanosoma evansi*: Variable antigen type (VAT) composition of first relapse populations in mice. *Tropical Medicine and Parasitology*. 36: 127-130.

Jones, T. W. and Mckinnell, C.D. (1985c) Antigenic variation in *Trypanosoma evansi*: A comparison of the predominant variable antigen type repertoire of stocks from Sudan. *Tropical Medicine and Parasitology*. 205-209.

Kacker, M.L.; Lodha, K.R. and Jatkar, P.R. (1974) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Kelly, W.R. (1974). Veterinary Clinical Diagnosis. Second Edition. Macmillan Publishers Ltd., London. Pp.174-175, 183.

Khasanov, I and Ivanitskaya, E.D. (1974) Trudy Uzbeksogo Naucho-issledovatel'skogo Veterinarnogo institute. 22: 98-100.

Killick-Kendrick, R. (1968) The diagnosis of trypanosomiasis of livestock: A review of current techniques. *Veterinary Bulletin*. 38: 191-197.

Knowles, R.H. (1924) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Kobayashi, A and Tizard, I.R. (1976) The response to *Trypanosoma congolense* infection in calves: Determination of immunological IgG_1, IgG_2, IgM, and C levels and the complement fixing antibodies titres during the course of infection. *Tropenmedizin und parasitologie*. 27: 411-417.

Krammer, J.W. (1966) Incidence of Trypanosomiasis in West African Dwarf Sheep and Goats in Nsukka Eastern Nigeria. *Bulletin of Epizootic Diseases in Africa*. 14:423-428.

Kunichkin, G.I. and Iskaliev, T.E. (1975) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Lanham, S.M. and Godfrey, D.G.; (1970) Isolation of Salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Experimental Parasitology*. 28: 521-534.

Laveran, A. (1907) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Laveran, A. (1911) 'Les trypanosomes out-ils des formes latentes chez leurns hote vertebres *C.R. Academy of Science*. 15: 649.

Leach, T.M. (1961) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Losos, G.J. (1986) Trypanosomiases. In: *Infectious Tropical Diseases of Domestic Animals*. Churchill Livingstone Inc.; New York. Pp. 182-318.

Losos, G.J. and Ikede, B.O. (1972) A review of Pathology of diseases in domestic and laboratory animals caused by *Trypanosoma congolense*, *T.brucei T.rhodesiense* and *T.gambiense*. *Veterinary Pathology*. 9: 1-71.

Luckins, A.G. (1976) Detection of antibodies in trypanosome infected cattle by means of a microplate enzyme-linked immunosorbent assay. *Annals of Tropical Medicine and Parasitology*. 70:479-480.

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

Luckins, A.G. and Gray, A.R. (1979) Trypanosomes in the lymph nodes of cattle and sheep infected with *T.congolense*. *Research in Veterinary Science*. 27: 129-131.

Luckins, A.G.; Rae P.F.; Mahmoud, M.M.; Malik, K.H. and Gray, A.R. (1979) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Luckins, A.G.; Boid, R.; Rae P.F.; Mahmoud, M.M.; Malik, K.H. and Gray, A.R. (1980) Serodiagnosis of infection with *Trypanosoma evansi* in camels in the Sudan. *Tropical Animal Health and Production*. II: 1-12.

Luckins, A.G., McIntyre, N and Ray, P.F. (1991) Multiplication of *Trypanosoma evansi* at the site of infection in skin of rabbits and cattle. *Acta Tropica*. 50:19-27.

Luckins, A.G. (1998). Epidemiology of Surra: Unanswered Questions. *Journal of* Protozoology *Research*. 8: 106-119.

Lumsden, W.H.R. (1967) Trends in research on the immunology of trypanosomosis. *Bulletin of the World Health Organisation*. 67: 167-175.

Lumsden, W.H.R., Herbert, W.J. and McNeillage, G.J.C. (1973) Techniques with trypanosomes. Edinburgh: Churchill Livingston.

Lun, Z.R., Allingham, R., Brun, R, and Lanham, S.M. (1992a). The isoenzyme characteristics of *Trypanosoma evansi* and *Trypanosoma equiperdum* isolated from domestic stocks in China. *Annals of Tropical Medicine and Parasitology*. 86: 333-340.

Lun, Z.R, Brun, R. and Gibson, W. (1992b). Kinetoplast DNA and Molecular Karyotypes of *Trypanosoma evansi* and *Trypanosoma equiperdum* from China. *Molecular* Biochemistr *and Parasitology*. 50: 189-196.

Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885). Balbani 1888. A review of recent research *Tropical Animal Health and Production*. 12: 35-47.

Maikaje, D.B.; Sanusi, A.; Kyewalabye, E.K. and Saror, D.I. (1991) The course of Experimental *Trypanosoma vivax* infection in Uda sheep. *Veterinary Parasitology*. 38:267-274.

Maxie, M.G., Losos, G.J. and Tabel, H. (1976) A comparative study of the haematological aspects of the diseases caused by *Trypanosoma vivax* and *T.congolense* in cattle. In: Pathophysiology of Parasitic Infections. (Soulsby, E.J.L., ed). Academic Press, New York. Pp. 183-198.

Marinkelle, C.J. (1976) The biology of the trypanosomes of non-human primates. Biology of the Kinetoplastida. (Lumsden, W.H.R. and Evans, D.A., eds) Academic Press. p. 232.

Mehlhorn, H. and Piekarski (1989) Trypanosomiasis. In: Part II. Animal Diseases in the Tropics. Tropical Animal Health. (Seifert, H.S.H., ed; 1996) Khuwer Academic Publishers. Dordrechts/Boston/London. Pp. 151-168.

Mesnil, M. (1910) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production.* 12: 35-47.

Migone L.E. (1910). Le role des Carpinchos comme reservoir de virus dans la conservation du mal de Cadeiras. *Bulletin of Society of Pathology Exotic Diseases,* 3: 524-525.

Misra, K.K. and Chaudhury, A. (1974) Multiplication of visceral forms in "Surra" trypanosomes. *Proceedings of the Third International Congress of Parasitology.* 1: 204-205.

Mitzmain, M.B. (1912). The role of *Stomoxys cacitrans* in the transmission of *Trypanosoma evansi. Philippians Journal of Sciences.* 7: 475-519.

Monzon, C.M. (1993) Serological diagnosis of *Trypanosoma evansi* (Steel, 1885) in horses using a direct agglutination test. *Veterinary Parasitology.* 47: 25-35.

Monzon, C.M. and Villavicencio I. (1990) Serum proteins in guinea-pigs and horses infected with *T.evansi* (Steel, 1885). *Veterinary Parasitology.* 36: 295-301.

Monzon, C.M. Hoyos, C.B. and Jara, G.A. (1995a). Outbreaks of equine trypanosomosis caused by *Trypanosoma evansi* in Formosa Province, Argentina. *Review of Scince and Technology.* OIE, 14: 747-752.

Monzon, C.M. Jara, G.A. and Nantulya, V.M. (1995) Sensitivity of antigen ELISA test for detecting *Trypanosoma evansi* antigen in horses in the sub-tropical area of Argentina. *Journal of Parasitology,* 81: 806-808.

Moutia, A. (1928). Surra in Mauritius and its principal vector, *Stomoxys nigra. Bulletin of Entomology Research.* 19: 211-216.

Morales, G.A. Wells, E.A. and Angel, D. (1976) The capybara (*Hydrochoerus hydrochoerus*) as a reservoir host for *Trypanosoma evansi. Journal of Wild Life Diseases.* 12: 572-574.

Murray, M. (1978) Anaemia of bovine African trypanosomiasis: an overview: In: Pathogenicity of trypanosomes. (Losos, G. and Chouinard, A., eds). *Proceedings of a workshop held at Nairobi,* Kenya, 20-23, November, pp. 121-127.

Murray, M. Murray, P.K., McIntyre, W.I.M. (1977) An improved parasitological technique for the diagnosis of African trypanosomiasis. *Transactions of the Royal society for Tropical Medicine and Hygiene.* 71: 325-326.

Musa, A.H. (1994) Isolation of *Trypanosoma evansi* from a goat inoculated with pooled camel blood from Kano Abattoir. Final year DVM project, Ahmadu Bello University, Zaria, Nigeria.

Nantulya, V.M. (1995) The development of latex agglutination antigen tests for diagnosis of African trypanosomiasis. In: OAU/STRC. p. 37.

Naylor, D.C. (1971) The haematology and histopathology of *Trypanosoma congolense* infection in cattle. Part II. *Tropical Animal Health and Production* 3: 159-168.

Ngeranwa, J.J. Mutiga, E.R., Agumbah, G.J.O., Galthumbi, P.K., and Munyua, W.K., (1991) The effects of experimental *Trypanosoma (brucei) evansi* infection on the fertility of male goats. *Veterinary Research Communications*. 15: 301-308.

Ngeranwa J.J. Gathumbi, P.K; Mutiga, E.R. Agumkar G. J. (1993) Pathogenesis of *Trypanosoma evansi* in small East African goats. *Research in Veterinary Science*. 54: 283-289.

Nguyen Huan, C, Webb., Lambert, P.H. and Miescher, P.A. (1975) Schiveizerische Medizinische Wochenschrift. 105: 1582.

Nieschutz, O. (1926b). Zoologische bijolragen tot het surra problem. V. Overbrengings proeven met *Haematopota cingulata* Wied. *Veeartsenij Kunige Medicine*. 57: 1-10.

Nieschutz, O. (1927a). Zoologische bijolragen tot het surra problem. XI Enkele proeven met *Haematopota truncata* Sehumro. Stekh., *H. irrorata* Macq. en *Tabanus brunnipes Sch. Stekh. Veeartsenijkunige Medicine*. 61: 1-13.

Nieschutz, O. (1927b). Zoologische bijolragen tot het surra problem. XIX Overbrengingsproev Met *Stomokys, Lupeerosia, Musca en Stegomyia. Veeartsenijkunige* Medicine 64:1-24

Nieschutz, O. (1930) Surrauebertragungsversuche auf Java and Sumatra. *Veeartsenijk Meded* (Utrecht) No. 75.

Nwaorgu, O.; Iwuala, M.O.E. and Okpala, I. (1981) Studies on the histo-pathology of *Trypanosome brucei* in Albino rats. *Nigerian Journal of Parasitology*. 2: 15-29.

Olaho-Mukani, W. Mungua, W.K. Njogu A.R. Mutugi, M.W. Omuse, J.K. and Sayer, P.D. (1992) Application for an antigen-enzyme linked immunosorbent assay for the diagnosis of trypanosomosis in camels in Kenya. In: *Proceedings of the First International Camel Conference*. Dubai. 2-6 February, 1992. Newmarket (UK). R.W. Publications.

Olaho-Mukani, W; Munyua, W.K., Mutugi, M.W. and Njogu, A.R. (1993b) Comparison of antibody and antigen detection enzyme immuno-assays for the diagnosis of *Trypanosoma evansi* infections in camels. *Veterinary Parasitology*. 45: 231-240.

Olaho-Mukani, W., Nyang'ao, J.M.N., Tengekyon, K.M. and Omuse, J.K.(1995) Evaluation of the card indirect agglutination test for trypanosomiasis using stored sera from *Trypanosoma brucei rhodesiense* infected patients. In: OAU/STRC. pp 43-45.

Onah, D.N., Hopkins, J. and Luckins, A.G. (1996) Haematological changes in sheep experimentally infected with *Trypanosoma evansi*. *Parasitology Research*. 82: 659-663.

Partoutomo. S., Soleh, M., Politedy, F., Day, A., Stevenson, P., Wilson, A.J. Copeman, D.B. and Owen, I. (1994). The epidemiology of *Trypanosoma evansi and Trypanosoma theileri* in cattle and Buffalo in small holder farms in Java. *Penyakit Hewan.* 26: 48. 41-46.

Pathak, K.M; Arora, J.K and Kapoor, M. (1993) Camel trypanosomosis in Rajasthan, India. *Veterinary Parasitology*. 49: 319-323.

Payne, R.C., Sukanto, I.P., Djauhari, D. and Jones, T.W. (1991) *Trypanosoma evansi* infectins in bovine and buffalo calves in Indonesia. *Veterinary Parasitology*. 38: 253-256.

Payne, R.C. Sukanto, I.P Partoutomo, S, and Polytedi, F. (1992) Experimental infection of Friesian Holstein calves with an Indonesian isolate of *Trypanosoma evansi*. *Tropical Medical Parasitology*. 43: 115-117.

Pegram, R.G. and Scott, J.M. (1976) The prevalence and diagnosis of *Trypanosoma evansi* infection in camels in Southern Ethiopia. *Tropical Animal Health and Production*. 8: 20-27.

Petana, W.B. (1963) A method of counting trypanosomes using Gram's iodine as diluent. *Transaction of Royal Society of Tropical Medicine and hygiene*. 57: 397.

Petrovskii, V.V. and Khamiev, S.K.H. (1974) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Plantureux, E. (1923) La "Formol - Gelification "dans la trypanosomiases des dromadaires. *C.R. Seanc Societie de Biologie*. 38: 1189-1190.

Richardson, J.F. and Kendall, S.B. (1964) Veterinary Protozoology. ELBS Edition. Oliver and Boyd Ltd., Britain. 34, 38, 51-55.

Ritz, H. (1916) Uber rezidive bei experimenteller trypanosomiasis 11 Miteilung. *Archiv fur Schiffs-Und Tropen-Medizine*. 20: 397-420.

Ryu, E. (1975) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production*. 12: 35-47.

Sackey A.K. (1998) Comparative study of trypanosomosis experimentally induced in savannah brown bucks by *Trypanosoma brucei*, *T.congolense* and *T.vivax*. Ph.D Thesis. Ahmadu Bello Univesity, Zaria, Nigeria.

Sani, I.A. (1994) Isolation of *Trypanosoma evansi* from rats and mice inoculated with pooled camel blood from Kano Abattoir. Final year DVM project. Ahmadu Bello University, Zaria, Nigeria.

Saror, D.I. (1975) Haematology, serum iron and iron binding capacity of apparently normal and trypanosome infected Zebu cattle. Ph.D Thesis. Ahmadu Bello University, Zaria, Nigeria.

Saror, D.I. (1980) Observations on the course and pathology of *Trypanosoma vivax* in Red Sokoto goats: *Research in Veterinary Science*. 28: 36-38.

Saseendranath, M.R; Ramkrishna, J, and Dhinakaran, M (1995) Pathology of experimental *Trypanosoma evansi* infection in sheep. *Indian Journal of Animal Research*. 29: 65-66.

Seifert, H.S.H. (1996) Animal Diseases in the tropics part II. Tropical Animal Health. Kluwer Academic Publishers Dordrecht, Boston London, pp.151-168.

Sekoni, V.O. (1992) Effect of *Trypanosoma vivax* infection on semen characteristics of Yankasa rams. *British Veterinary Journal*. 148: 501-506.

Sergent, E and Donatien, A. (1922). Transmission naturelle ex experimentale de la trypanosomia des dromadaires par les *Stomoxes*. *Arch. Institute Pasteur L'AFAfrique du nord 2:291-315*

Silva, R. A.M.S., Arosemena, N.A.E, Herrera, H.M., Sahib, C.A. and Ferreira M.S.J. (1915). Outbreak of trypanosomosis due to *Trypanosoma evansi* in horses of Panatela Mato-Grossense, Brazil. *Veterinary Parasitology*. 60: 167-171.

Singh, K. (1925). Surra Transmission experiments. *Department of Agriculture and* Veterinary *Bulletin*. Punjab 16.

Singh, B.P. and Misra, S.K. (1988) A study on the clinical course of *Trypanosoma evansi* in experimentally infected cow-calves. *Indian Veterinary Medical Journal*. 12: 127-128.

Songa, E.B., Paindavoine P., Wittoukk, E, Visseshakul, N., Muldermans S., Steinert, M. And Hamers, R. (1990). Evidence of Kinetoplast and nuclear DNA homogeneity in Trypanoson *evansi* isolates. *Molecular Biochemistry and Parasitology*. 43: 167- 180.

Stephanova, N.I. and Petrovskii V.V. (1976) Trudy Veesoyuznogo Instituta Eksperimental noi Veterinarrii. Moscow. 44: 128-137.

Stephen, L.E. (1970) Clinical manifestations of the trypanosomiases in livestock and other domestic animals In: The African Trypanosomiases (Mulligan, H.W., ed). George Allen and Unwin, London. Pp. 774-794.

Stevens, J.R., Nunes, V.L.B., Lanham, S.M. and Oshiro, E.T. (1989). Isoenzyme characterisation of *Trypanosoma evansi* isolated from capybaras and dogs in Brazil. *Acta Tropica*. 46: 213-222.

Tizard, I.R. Holmes, W.L. and Nielsen, K. (1978) Mechanisms of the anaemia in trypanosomiasis. Studies on the role of the haemolytic fatty acids derived from *Trypanosoma congolense*. *Tropenmedizin and Parasitologie*. 29: 108-114.

Tuntasuvan, D., Chompoochan, T., Vongpakorn, M. And Mohkaew, K. (1996). Detection of *Trypanosoma evansi* antibodies in pigs using enzyme linked immunosorbent assay. *Journal of Thailand Veterianry Medical Association*. 47: 45-53.

Turner, M.J. (1992) The biochemistry of the surface antigen 6f the African trypanosomes. *British Medical Journal*, 41: 137-143.

Touratier, L. (1992) Report of the twelfth international meeting of the oie ad-hoc group on non-tsetse transmitted animal trypanosomiasis. Paris, 15th May, 1991. *Review Scientifique et technique del office International des Epizooties*. 11:943-975.

Uche, U.E. and Jones, T.W. (1992) Pathology of Experimental *Trypanosoma evansi* infection in rabbits. *Journal of Comparative Pathology*. 106: 299-309.

Uche, U.E. and Jones, T.W. (1993b) Effect of complement (C3) depletion on the generation and memory in rabbits primed with antigens of *Trypanosoma evansi*. *Veterinary Parasitology*. 47:205-213.

Uche, U.E. Jones T.W. and Boid, R (1992) Antibody patterns in rabbits showing different levels of susceptibility to an experimental *Trypanosoma evansi* infection. *Acta Tropica*, Basel. 52: 139-147.

Uche, U.E. Jones T.W. and Boid, R (1993) Classic-specific antibody response in rabbits experimentally infected with *Trypanosoma evansi*. *Tropical Medicine and Parasitology*. 44:27-31.

Uche, U.E. and Jones, T.W. (1994) Protection conferred by *Trypanosoma evansi* infection against homologous and heterologous trypanosome challenge in rabbits. *Veterinary Parasitology*. 52:21-35.

Van den Ingh., T.S.G.A.M.; Zwart, D.; Schotman, A.J.H.; Van Miert, A.S.J.P.A.M and Veanendaal, G. (1976) The pathology and pathogenesis of *Trypanosoma vivax* infection in the goat. *Research in Veterinary Science*. 21: 264-270.

Verman, B. B and Gautam, O.P. (1977) Serological diagnosis of experimental bovine sura (*Trypanosoma evansi* infection - a comparison of passive haemaglutination, gel diffusion, and indirect fluorescent antibody test. *Indian Veterinary Journal*. 54: 809-813.

Waitumbi, J. And Young, J.R. (1994). Electrophoretic Karyotyping is a sensitive epidemiological tool for studying *Trypanosoma evansi* infections. *Veterinary Parasitology*. 52: 47-56.

Walker E.P. (1964) Mammals of the world. Vol.II The Johns Hopkins Press, Baltimore pp. 1371.

WHO (1983) African Trypanosomiasis. In: *Tropical Diseases Research*. Seventh Programme Report. Pp. 3-11.

Williamson, J. (1976) Chemotherapy of African trypanosomiasis. *Tropical Diseases Bulletin.* 73: 531-542.

Wilson, R.T. (1984) The camels. Longman Group Ltd., UK. Pp 122-127, 157-169.

Woo, P.T.K. (1969) The haematocrit centrifuge for the detection of trypanosomes in blood. *Canadian Journal of Zoology.* 47: 921-923.

Yagi, A.J. and Raziq, M.T.A. (1972) Cited by Mahmoud, M.M. and Gray, A.R. (1980) Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885, Balbani 1888). A review of recent research. *Tropical Animal Health and Production.* 12: 35-47.

Zhang, Z. Q. and Baltz, T. (1994). Identification of *Trypanosoma evansi*, *Trypanosoma equiperdum* and *Trypanosoma brucei brucei* using repetitive DNA probes. *Veterinary Parasitology.* 53: 197-208.

Zhang, Z.Q, Giroud, C and Baltz, T. (1991) *In vivo* and *in vitro* sensitivity of *Trypanosoma evansi* and *T.equiperdum* to diminazene, suramin, Melcy, quinapyramine, and isometamidium. *Acta Tropica.* Basel. 50(2): 101-110.

APPENDIX 1

Classification of *T.evansi*

Phylum	*Protozoa* (Diesing, 1966)
Subphylum	*Sarcomastigophora* (Honigberg and Balamuth, 1963)
Super class	*Mastigophora* (Diesing, 1986)
Class	*Zoomastigophora* (Calkins, 1909)
Order	*Kinetoplastida* (Honigberg, 1963)
Suborder	*Trypanosomatina* (Kent, 1980)
Family	*Trypanomatidae* (Doflein, 1901, emend, Grobben, 1905)
Genus	*Tryponosoma* (Gruby, 1843)
Sub-Genus	*Trypanosoma* (Luihe, 1906)
Species	*Trypanosoma evansi* (Steel, 1885)
Group	Salivarian

Source: Hoare (1972)

APPENDIX 2

Mean Total and Differential Leucocyte Counts in the *T.evansi*-infected Yankasa Sheep

Time of Infection (days)	Total WBC x 10^9 L^{-1} Mean ± SD	Neutrophils x 10^9 L^{-1} Mean ± SD	Lymphocytes x 10^9 L^{-1} Mean ± SD	Monocytes x 10^9 L^{-1} Mean ± SD	Eosinophils x 10^9 L^{-1} Mean ± SD
-10	15.7 ± 2.2	43.0 ± 3.2	58.3 ± 2.1	2.2 ± 0.4	0.0
- 5	16.3 ± 1.5	52.2 ± 1.5	48.2 ± 5.2	2.8 ± 0.2	1.0 ± 0.5
0	16.2 ± 1.4	38.3 ± 4.3	61.2 ± 4.3	3.1 ± 1.2	0.5 ± 0.7
3	17.9 ± 1.3	46.2 ± 4.0	53.1 ± 2.4	2.3 ± 1.4	0.0
6	16.6 ± 2.1	47.2 ± 2.1	49.3 ± 3.4	1.8 ± 1.2	1.5 ± 0.5
8	17.4 ± 2.6	49.6 ± 0.5	62.4 ± 3.8	2.0 ± 1.0	1.0 ± 0.2
12	18.8 ± 3.1	37.2 ± 7.9	63.4 ± 4.1	2.3 ± 0.2	0.5 ± 0.2
15	15.8 ± 4.2	43.4 ± 4.6	57.2 ± 3.5	3.4 ± 0.1	1.5 ± 0.2
19	16.6 ± 3.2	41.2 ± 3.1	60.9 ± 4.3	3.0 ± 1.3	0.0
22	15.8 ± 1.4	41.2 ± 2.6	62.0 ± 2.6	2.6 ± 0.3	0.0
26	18.4 ± 1.6	38.1 ± 6.2	58.0 ± 2.0	2.0 ± 0.2	2.0 ± 0.2
30	17.1 ± 3.2	32.1 ± 5.1	72.3 ± 2.1	1.8 ± 1.0	1.0 ± 0.4
34	20.2 ± 4.2	48.2 ± 2.3	51.4 ± 3.4	3.1 ± 1.3	1.0 ± 0.5
37	19.1 ± 3.5	32.1 ± 4.6	68.2 ± 2.0	2.6 ± 0.6	0.0
40	18.7 ± 6.4	37.2 ± 3.1	62.3 ± 4.2	2.2 ± 0.4	0.5 ± 0.5
44	18.2 ± 3.7	33.1 ± 2.0	69.1 ± 2.3	1.8 ± 0.3	1.0 ± 1.0
48	21.1 ± 4.8	38.8 ± 3.1	63.3 ± 3.0	3.0 ± 0.8	1.5 ± 0.5
51	20.9 ± 6.0	30.1 ± 2.1	76.2 ± 5.1	2.6 ± 1.6	1.0
55	19.5 ± 4.1	35.3 ± 1.7	67.3 ± 3.4	3.1 ± 1.2	1.5 ± 0.5
60	22.2 ± 3.0	29.1 ± 2.4	73.4 ± 4.4	2.0 ± 1.0	1.0 ± 0.7
64	20.2 ± 4.1	38.2 ± 2.7	69.1 ± 3.2	1.9 ± 0.8	1.5 ± 0.5
68	18.5 ± 3.0	36.4 ± 2.6	60.4 ± 2.6	2.1 ± 0.3	1.0 ± 0.5

APPENDIX 3

Mean Total and Differential Leucocyte Counts in Un-infected (Control) Yankasa Sheep

Time of Infection (days)	Total WBC x 10^9 L^{-1} Mean ± SD	Neutrophils x 10^9 L^{-1} Mean ± SD	Lymphocytes x 10^9 L^{-1} Mean ± SD	Monocytes x 10^9 L^{-1} Mean ± SD	Eosinophils x 10^9 L^{-1} Mean ± SD
-10	16.8 ± .2	45.0 ± 3.2	60.2 ± 2.6	2.1 ± 0.2	0.0
- 5	15.9 ± .3	58.3 ± 1.5	56.7 ± 3.1	2.3 ± 0.2	1.0 ± 0.4
0	17.2 ± 1.5	42.3 ± 2.7	62. ± 2.7	3.0 ± 0.8	0.3 ± 0.6
3	18.3 ± 1.1	46.8 ± 3.2	51.3 ± 1.7	2.6 ± 1.2	0.0
6	16.9 ± 1.8	48.3 ± 1.7	46.2 ± 2.5	1.8 ± 1.0	1.2 ± 0.3
8	18.5 ± 2.1	51.1 ± 1.5	60.3 ± 2.7	1.6 ± 1.0	0.8 ± 0.1
12	17.7 ± 2.4	38.4 ± 4.4	60.8 ± 3.2	2.1 ± 0.2	1.4 ± 0.2
15	15.4 ± .2	45.3 ± 4.9	58.1 ± 2.9	2.8 ± 0.4	1.4 ± 0.0
19	16.9 ± .7	44.1 ± 3.4	58.6 ± 2.5	2.7 ± 1.2	0.0
22	16.3 ± 1.3	42.7 ± 1.9	60.1 ± 1.5	2.2 ± 0.2	0.0
26	*18.8 ± 1.5*	*40.1 ± 4.7*	*56.1 ± 1.5*	*1.9 ± 0.8*	*1.8 ± 0.3*
30	18.2 ± 2.4	36.3 ± 3.2	68.2 ± 1.8	1.6 ± 1.1	0.7 ± 0.0
34	20.4 ± 1.7	48.8 ± 2.4	50.2 ± 2.8	2.9 ± 1.0	1.0 ± 0.5
37	19.7 ± 2.5	34.7 ± 2.1	64.3 ± 1.6	2.2 ± 0.9	0.0
40	19.2 ± 4.2	37.6 ± 2.5	60.1 ± 2.6	1.7 ± 0.4	0.5 ± 0.5
44	19.1 ± 2.7	36.4 ± 1.6	65.3 ± 2.3	1.6 ± 0.2	0.6 ± 1.0
48	21.2 ± 3.1	39.3 ± 2.4	60.2 ± 2.1	2.6 ± 0.6	1.3 ± 0.0
51	21.0 ± 4.5	32.2 ± 2.3	66.2 ± 4.1	2.2 ± 1.2	0.7 ± 0.0
55	19.6 ± 4.4	36.3 ± 2.7	63.5 ± 2.4	2.4 ± 1.4	1.2 ± 0.6
60	22.8 ± 2.9	31.3 ± 2.1	69.4 ± 2.4	2.0 ± 1.0	0.8 ± 0.7
64	21.2 ± 2.1	39.4 ± 2.8	70.2 ± 1.9	1.9 ± 0.8	1.3 ± 0.5
68	19.5 ± 1.9	37.3 ± 1.6	58.1 ± 2.1	1.9 ± 0.4	1.1 ± 0.5

APPENDIX 4

Rectal Temperature in the *T.evansi*-infected Yankasa Sheep

Time of Infection (days)	Yankasa Sheep Identification Number							
	P3035	P3036	P3037	P3038	P3040	P3041	P3046	P3047
-10	39.2	38.2	38.4	39.2	38.4	38.8	38.8	39.2
-5	38.6	38.5	38.7	37.9	38.3	38.5	38.2	38.7
0	38.7	38.3	38.0	38.1	37.5	37.9	38.2	38.4
3	38.1	38.1	38.8	37.5*	38.1	37.0	38.3	39.2
6	40.5	40.9	39.9	-	40.1	-	38.2*	41.2
8	38.8	38.6	38.9	-	39.4	-	-	39.9
12	37.5	40.2	40.7	-	39.0	-	-	39.6
15	38.2*	39.0	40.5	-	38.7	-	-	38.8
19	-	38.9	39.4	-	39.0	-	-	40.4
22	-	39.1	39.0	-	38.7	-	-	40.3
26	-	39.2	39.7	-	39.3	-	-	40.6
30	-	38.5	39.3	-	38.7	-	-	39.4
34	-	38.4	39.2	-	38.6	-	-	39.5
37	-	39.0	38.7	-	38.8	-	-	38.8
40	-	38.8	39.9*	-	39.7	-	-	39.0
44	-	39.3	-	-	39.1	-	-	39.1
48	-	38.8	-	-	39.0	-	-	39.1
51	-	39.1	-	-	39.1	-	-	39.0
55	-	38.1*	-	-	38.8	-	-	39.1
60	-	-	-	-	39.9	-	-	38.8
64	-	-	-	-	39.0	-	-	39.0
68	-	-	-	-	38.8	-	-	38.7

Note: Rectal temperature above 39.5°C is considered as pyrexia

APPENDIX 5

Packed cell volumes (%) in the *T.evansi*-infected Yankasa Sheep

TIME OF INFECTION (Days)	INFECTED YANKASA SHEEP IDENTIFICATION NUMBER							
	P3035	P3036	P3037	P3038	P3040	P3041	P3046	P3047
-10	48	24	29	30	31	32	22	32
-5	30	24	24	25	26	30	23	24
0	20	23	24	24	27	35	25	26
3	25	26	25	22*	26	28*	24	24
6	20	24	25	-	26	-	24*	28
8	24	26	24	-	23	-	-	24
12	24	24	22	-	24	-	-	26
15	24*	24	23	-	24	-	-	25
19	-	22	22	-	22	-	-	26
22	-	20	22	-	24	-	-	22
26	-	19	22	-	28	-	-	26
30	-	23	22	-	30	-	-	28
34	-	20	21	-	18	-	-	26
37	-	21	21	-	20	-	-	24
40	-	19	17*	-	20	-	-	24
44	-	17	-	-	22	-	-	23
48	-	14	-	-	17	-	-	22
51	-	15	-	-	19	-	-	21
55	-	12	-	-	20	-	-	22
60	-	10*	-	-	22	-	-	24
64	-	-	-	-	19	-	-	26
68	-	-	-	-	22	-	-	25

* - Animal died

APPENDIX 6

Haemoglobin Concentrations (g dl^{-1}) in the *T.evansi*-infected Yankasa Sheep

TIME OF INFECTION (days)	INFECTED YANKASA SHEEP IDENTIFICATION NUMBER							
	P3035	P3036	P3037	P3038	P3040	P3041	P3046	P3047
-10	16.0	8.0	9.7	10.0	10.3	10.3	7.1	10.2
-5	10.0	8.7	8.0	8.3	8.3	7.7	7.7	8.0
0	6.7	7.7	8.0	8.0	9.0	11.7	8.3	8.7
3	8.1	8.7	8.3	7.3*	8.7	9.3*	8.0	8.0
6	9.7	8.0	8.3	-	8.7	-	8.0*	9.3
8	8.0	8.7	8.0	-	7.7	-	-	8.0
12	9.3	8.0	7.3	-	8.0	-	-	8.7
15	7.3*	8.0	7.7	-	8.0	-	-	8.3
19	-	7.3	7.3	-	7.3	-	-	8.7
22	-	6.8	7.1	-	8.0	-	-	8.7
26	-	6.3	7.0	-	9.3	-	-	7.3
30	-	7.7	7.3	-	6.7	-	-	8.7
34	-	6.8	7.1	-	6.0	-	-	9.3
37	-	7.0	7.0	-	6.7	-	-	8.7
40	-	6.3	5.6*	-	6.8	-	-	7.8
44	-	5.8	-	-	6.7	-	-	8.3
48	-	5.2	-	-	7.1	-	-	8.7
51	-	4.8	-	-	6.0	-	-	7.8
55	-	4.4	-	-	6.7	-	-	7.6
60	-	3.3*	-	-	6.3	-	-	7.0
64	-	-	-	-	7.3	-	-	7.6
68	-	-	-	-	7.0	-	-	8.0

* – Animal died

APPENDIX 7

Levels of Parasitemia in the *T.evansi*-infected Yankasa Sheep Using HCT/Thin Smear

TIME OF INFECTION (days)	INFECTED YANKASA SHEEP IDENTIFICATION NUMBER							
	P3035	P3036	P3037	P3038	P3040	P3041	P3046	P3047
-10	N	N	N	N	N	N	N	N
-5	N	N	N	N	N	N	N	N
0	N	N	N	N	N	N	N	N
3	N	++	-+	++*	+	++*	+	+
6	+++	++	++	-	++	-	++	++
8	++	++	++	-	++	-	-	++
12	+	+++	+++	-	+	-	-	+
15	+++*	+	+++	-	N	-	-	N
19	-	++	++	-	N	-	-	++
22	-	N	++	-	+	-	-	++
26	-	+	++	-	+	-	-	+++
30	-	+	N	-	N	-	-	+
34	-	++	N	-	N	-	-	+
37	-	N	++	-	N	-	-	N
40	-	N	+++*	-	++	-	-	N
44	-	+	-*	-	+	-	-	N
48	-	N	-	-	+	-	-	+
51	-	++	-	-	N	-	-	N
55	-	++	-	-	N	-	-	N
60	-	+++*	-	-	N	-	-	N
64	-	-	-	-	+	-	-	+
68	-	-	-	-	+	-	-	N

N - No parasite found
+ - *Trypanosoma evansi* present
* - Sheep*in extremis*

APPENDIX 8

Live Weights of *T.evansi*-infected Yankasa Sheep

Time of Infection (days)	YANKASA SHEEP IDENTIFICATION NUMBER P3035	P3036	P3037	P3038	P3040	P3041	P3046	P3047
-10	11.8	13.4	9.7	13.5	13.9	7.8	12.4	14.8
0	12.0	14.0	10.1	14.0	14.1	8.2*	12.8*	15.0
8	12.2	-	12.2	14.1	10.2	-	14.2--	15.1
15	12.2	14.3	10.2	-	14.4	-	-	15.3
22	-	14.2	10.4	-	14.5	-	-	15.3
30	-	13.0	10.3	-	14.5	-	-	15.2
37	-	12.8	10.2	-	13.1	-	-	15.0
44	-	12.4	10.0	-	12.0	-	-	14.0
51	-	11.0	8.2	-	11.4	-	-	13.6
55	-	8.4	*	-	11.1	-	-	13.4
60	-	8.0	-	-	11.4	-	-	13.0
64	-	-	-	-	11.2	-	-	
68	-	-	-	-	7.0	-	-	15.1

* Sheep *in extremis*

Total serum protein of Infected Group (Group 2)

DAYS	P3035	P3036	P3037	P3038	P3040	P3041	P3046	P3047
-10	8.3	7.5	5.2	5.3	6.0	6.3	7.5	7.4
- 5	8.1	7.2	6.0	5.4	6.2	3.5	7.3	7.0
0	7.7	6.8	8.8	8.2	5.5	7.0	7.6	7.2
3	8.3	6.8	8.0	6.8*	6.8	7.2*	7.4	7.0
6	7.5	6.2	7.4	-	6.8	-	7.0*	6.8
8	7.4	7.8	7.8	-	7.0	-	-	6.6
12	8.0	6.4	8.0	-	7.2	-	-	6.8
15	8.2*	7.4	7.2	-	7.6	-	-	7.2
19	-	7.2	7.4	-	8.0	-	-	7.0
22	-	7.0	7.8	-	8.2	-	-	6.8
26	-	6.6	7.2	-	7.4	-	-	6.4
30	-	7.2	8.0	-	7.6	-	-	7.0
34	-	7.0	7.6	-	6.8	-	-	7.4
37	-	7.0	7.8	-	7.0	-	-	7.0
40	-	6.4	7.2*	-	7.4	-	-	6.6
44	-	6.0	-	-	7.6	-	-	7.0
48	-	6.5	-	-	7.0	-	-	8.0
51	-	7.5	-	-	7.8	-	-	8.5
55	-	6.4	-	-	8.2	-	-	7.6
60	-	6.2*	-	-	7.0	-	-	7.2
64	-	-	-	-	7.4	-	-	6.7
68	-	-	-	-	7.0	-	-	6.8

* Sheep died

Temperature (°C) uninfected control group (Group 1)

Sheep	P3030	P3031	P3032	P3034	P3039	3042
-10	37.5	38.4	38.3	38.7	38.8	39.1
-5	38.6	38.4	37.9	38.4	39.1	38.7
0	38.0	38.1	38.7	38.2	38.5	37.6
3	38.5	38.4	38.7	38.3	38.4	38.1
6	38.2	38.0	38.2	38.2	38.7	38.3
8	37.2	38.8	39.9	38.6	38.8	39.8
12	38.6	38.4	38.7	38.7	38.8	38.5
15	38.4	38.4	38.2		38·8	38.6
19	38.8	39.2	38.3		38·6	39·2
22	37.8	38.4	38.3		38·7	39·1
26	38.8	38.9	38.4		38·7	38·6
30	38.4	38.7	38.4		38·6	40·1
34	38.4	39.4	38.4		38·1	38·8
37	38.7	38.8	38.8		38·4	39·2
40	38.5	38.5	38.4		38·6	39·0
44	38.5	38.5	38.8		38·8	38·8
48	38.4	38.4	38.7		38·3	38·7
51	38.4	38.4	38.6		38·6	38·6
55	38.4	38.3	38.4		38·4	38·8
60	38.5	38.8	38.4		38·6	38·6
64	38.3	38.4	38.5		38·7	38·5
68	38.4	38.6	38.6		38·7	

Fever Temperature > 39.5°C

Packed cell volume (%) of uninfected control sheep (Group 1)

DAYS	P3030	P3031	P3032	P3034	P3039	3042
-10	27	25	30	22	23	36
-5	24	22	35	25	25	20
0	23	21	31	32	22	20
3	26	21	30	30	24	21
6	25	23	30	28	24	22
8	24	23	29	28	26	20
12	24	25	32	24	26	24
15	27	24	31	*	24	24
19	23	26	30		26	22
22	25	25	28		28	20
26	24	23	30		26	20
30	26	21	28		29	22
34	25	26	28		22	23
37	25	23	29		24	24
40	26	24	27		26	24
44	27	26	26		26	24
48	26	26	27		24	26
51	27	24	28		25	23
55	26	27	28		28	24
60	27	25	28		28	22
64	27	26	26		26	25
68	26	28	27		27	25

* Animal died

Haemoglobin (Hb) concentration (g/dl) uninfected control Group (Group 1)

DAYS	P3030	P3031	P3032	P3034	P3039	3042
-10	9.0	8.3	10.2	7.3	7.7	12.0
-5	8.0	7.3	11.7	8.3	8.3	6.7
0	7.1	7.0	10.3	10.7	7.3	6.7
3	8.7	7.0	10.0	10.0	8.0	7.0
6	8.3	7.7	7.3	9.3	7.3	10.7
8	8.0	7.7	9.7	8.0	8.7	6.7
12	8.0	8.3	10.7	9.3	8.7	8.0
15	9.0	8.0	10.3	*	8.0	7.3
19	7.7	8.7	10.0	-	8.6	7.3
22	8.3	8.3	9.3	-	9.3	7.3
26	8.0	7.7	9.3	-	8.7	6.7
30	8.7	7.0	9.7	-	7.7	7.3
34	8.3	8.7	9.0	-	7.3	7.7
37	8.7	7.7	8.2	-	8.0	8.0
40	8.6	6.0	9.1	-	8.6	7.3
44	9.0	6.9	9.3	-	8.8	7.6
48	8.4	7.0	9.5	-	8.0	8.8
51	9.0	6.8	9.3	-	8.4	7.7
55	8.4	8.8	8.8	-	9.4	8.0
60	8.8	8.3	9.4	-	8.7	7.3
64	8.7	8.7	9.1	-	9.0	8.2
68	8.3	8.3	*	-	9.2	8.5

* Animal died

Total serum protein (g/dl) of unifected control Group (Group 1)

DAYS	P3030	P3031	P3032	P3034	P3039	3042
-10	6.8	7.6	7.5	7.4	8.5	5.8
-5	6.4	7.0	7.2	7.0	6.3	8.5
0	8.3	8.8	8.0	7.5	6.5	7.8
3	6.8	7.5	7.5	7.4	6.3	7.8
6	6.7	6.0	6.8	7.0	6.8	6.6
8	6.8	7.4	7.6	7.8	7.4	8.0
12	7.2	7.8	8.2	8.0	6.8	6.8
15	7.0	7.0	8.0	-	6.4	7.4
19	6.4	7.6	7.6	-	7.0	7.0
22	6.2	8.2	7.5	-	6.4	6.6
26	6.7	7.8	7.8	-	6.8	6.2
30	6.8	7.4	7.8	-	7.0	6.8
34	6.8	6.8	7.4	-	6.7	7.2
37	7.0	7.4	7.8	-	6.8	7.0
40	7.0	7.6	7.4	-	6.8	7.2
44	6.8	7.0	7.8	-	7.2	7.8
48	7.2	7.4	7.8	-	7.0	7.6
51	6.9	7.6	7.4	-	7.0	7.4
55	6.6	7.0	7.6	-	6.6	7.3
60	6.8	7.8	7.8	-	7.3	7.8
64	7.2	8.2	7.2	-	6.4	6.8
68	6.6	7.4	7.6	-	7.2	6.9

Weights (kg) uninfected control group (Group 1)

DAYS	P3030	P3031	P3032	P3034	P3039	3042
-10	8.4	21.8	15.9	12.8	9.0	11.6
-5						
0	9.2	22.0	16.9	14.0	10.5	13.2
3						
6						
8	9.4	22.1	18.0	15.0	10.8	13.2
12						
15	9.5	18.2	18.2	-	11.0	13.3
19						
22	9.5	23.0	18.1	-	11.2	13.3
26						
30	9.8	25.5	18.4	-	11.2	13.2
34						
37	10.0	23.8	18.4	-	11.5	13.5
40						
44	10.3	24.0	18.8	-	13.0	14.0
48						
51	10.4	24.1	19.0	-	13.4	14.3
55						
60	10.5	24.1	19.2	-	13.7	14.6
64						
68	10.5	24.2	19.5	-	14.1	14.9

WCT/Thin smear of uninfected group (Group 1)

DAYS	P3030	P3031	P3032	P3034	P3039	3042
-10						
-5						
0						
3						
6		NPF				
8						
12						
15						
19						
22						
26						
30						
34						
37						
40						
44						
48						
51						
55						
60						
64						
68						

NPF - No Parasite found

Summary of Analysis of variance for clinical parameters of Infected and control sheep

---

1. **ONE WAY ANALYSIS OF VARIANCE FOR Temperature**

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	1.978	1.978	15.44	0.000
Error	42	5.381	0.128		
Total	43	7.358			

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	-------+--------+--------
Tem contr	22	38.597	0.160	(---------*-------
Tem infec	22	39.021	0.480	(-----*----------

-------+-------+-------+--

Pooled StDev = 0.358 38.60 38.80 39.00

M

---

2 **ONE WAY ANALYSIS OF VARIANCE FOR PCV**

2. ONE WAY ANALYSIS OF VARIANCE FOR PCV

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	107.42	107.42	20.71	0.000
Error	42	217.85	5.19		
Total	43	325.28			

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	-------+--------+-------+--
contr pc	22	25.627	0.723	(-----*-----
infec pc	22	22.502	3.139	(-----*------)

-------+-------+------+----

Pooled StDev = 2.277 22.5 24.0 25.5

MTB > AOVOneway contr hb infec hb.

3. **ONE WAY ANALYSIS OF VARIANCE FOR Haemoglobin**

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	7.487	7.487	14.14	0.000
Error	42	22.242	0.530		
Total	43	325.28			

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	-------+--------+-------+--
contr pc	22	8.4028	0.2829	(-----*-----
infec pc	22	7.5778	0.9885	(-----*------)

-------+-------+------+----

Pooled StDev = 0.7277 7.60 8.00 8.40

MTB > AOVOneway TPcontr TP infec.

---

4. **ONE WAY ANALYSIS OF VARIANCE FOR Total Protein**

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	0.0172	0.0172	0.24	0.626
Error	42	2.9909	0.0712		
Total	43	3.0081			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	-------+--------+-------+--
TP contr	22	7.2111	0.2366	(---------*-------)
TP infec	22	7.1716	0.2940	(-----*-------------)

-------+-------+------+----

Pooled StDev = 0.7277 7.120 7.200 7.280

MTB > AOVOneway TPcontr TP infec.

5. **ONE WAY ANALYSIS OF VARIANCE FOR Weight**

Analysis of Variance.

Source	DF	SS	MS	F	P
Factor	1	43.84	43.84	30.42	0.000
Error	20	28.83	1 44		
Total	21	72.67			

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	------+--------+--------+
Wt contr	11	15.151	1.357	(---------*-------)
Wt infec	11	12.328	1.021	(-----*----------)

-------+-------+------+----+

Pooled StDev = 1.201 12.0 13.2 14.4 15.6

MTB > AOVOneway TPcontr TP infec.