EFFECTS OF EXPERIMENTAL *TRYPANOSOMA EVANSI* AND *TRYPANOSOMA BRUCEI BRUCEI*INFECTIONS ON HAEMATOLOGICAL AND SOME REPRODUCTIVE PARAMETERS OF YANKASA RAMS

By

YUNUSA ADAMU WADA

DEPARTMENT OF BIOLOGICAL SCIENCES,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA NIGERIA

MAY, 2015

EFFECTS OF EXPERIMENTAL *TRYPANOSOMA EVANSI* AND *TRYPANOSOMA BRUCEI BRUCEI*INFECTIONS ON HAEMATOLOGICAL ANDSOME REPRODUCTIVE PARAMETERS OF YANKASA RAMS

By

YunusaAdamuWADA[B.Sc. BIOLOGY, ABU, 2009]

(M.Sc./SCIE/01821/2010-2011)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN ZOOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

MAY, 2015

DECLARATION

I declare that the work in this thesis entitled "**Effects of Experimental *Trypanosomaevansi*and *Trypanosomabruceibrucei*Infections on Haematological and some Reproductive Parameters of Yankasa Rams**"has been carried out by me in the Department of Biological Sciences, under the supervision of Professors S.J. Oniye and P.I. Rekwot and Dr. O.O. Okubanjo. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

YunusaAdamu WADA _____ _____

Signature Date

CERTIFICATION

This thesis titled "**Effects of Experimental *Trypanosomaevansi*and *Trypanosomabruceibrucei*Infections on Haematological and some Reproductive Parameters of Yankasa Rams**"by YunusaAdamu WADA meets the regulationsgoverning the award of the degree of Master of Science (M.Sc.) of Ahmadu BelloUniversity, and is approved for its contribution to knowledge and literary presentation.

Prof. S.J. Oniye _____ _____
Chairman, Supervisory Committee Signature Date
Department of Biological Sciences,
Ahmadu Bello University, Zaria

Prof. P.I. Rekwot _____ _____
Member, Supervisory Committee Signature Date
National Animal Production Research Institute,
Ahmadu Bello University, Zaria

Dr. O.O. Okubanjo _____ _____
Member, Supervisory Committee Signature Date
Department of Veterinary Parasitology and Entomology,
Ahmadu Bello University, Zaria

Prof. A.K. Adamu _____ _____
Head, Department of Biological Sciences Signature Date
Ahmadu Bello University, Zaria

Prof. A.Z. Hassan _____ _____
Dean, School of Postgraduate Studies, Signature Date
Ahmadu Bello University, Zaria

DEDICATION

I dedicate this work to the memory of my lovely mum, to my family and whosoever may find the information useful.

ACKNOWLEDGEMENTS

I am most grateful to Almighty God for making me who I am and for His guidance, protection, and keeping me in good health and strength throughout this period. Praise Be to Him for His Mercy

My special and profound gratitude goes to my supervisors, Professors S.J. Oniye and P.I. Rekwot and Dr. O.O. Okubanjo, for the assistance and guidance they rendered during the course of the study. Indeed, they were instrumental to the success of this work through their close supervision and constructive criticisms. Forever I remain thankful and say God bless you.

I am especially grateful to the Head of Department and the Technical Staff of the Department of Veterinary Parasitology and Entomology for their assistance in the Laboratory.

I acknowledge the technical support I enjoyed fromthe staff of Artificial Insemination Unit of the National Animal Production Research, Shika, A.B.U., Zaria, especially, AlhajiSalisuMagaji A. for always being around for technical assistance during the semen analysis in the Fertility Laboratory of the Artificial Insemination unit. MaytheAlmighty God bless you all, Amin.

My never ending appreciation goes to the Head, Department of Veterinary Pathology, Dr. MuhammedBisalla for assisting in interpreting the histopathological findings and teaching me the basics as well. I thank Mr. B. Bitrus of the Histology Laboratory who assisted adequately in the preparation of the various tissues and slides. To you all, I remain forever thankful.

My heartfelt appreciation goes to my lovely parents, most especially to the loving memory of my simple, loving and caring mum. I want you to know that your kind words and memories will forever linger in my heart. I pray that the Almighty grant you JannatulFirdaus as your final abode, Amin.

To my kind and lovely dad also, I remain loyal and grateful for giving me the greatest gift of life - education, and for your support and advice throughout till now. May Almighty Allah continue to grant you sound health, more happiness, wisdom and peace of mind and may paradise be your final destination, Amin.

My entire family members, friends and the brethren all over who stood by me in prayers during the period of the study are highly acknowledged. Your role was invisible but it broke barriers and put everything in place for me. To you all, I say God bless.

Finally, my most precious and innermost appreciation and thanks go to my closest friend and wife, Byntu and my lovely daughter, Fareedah. You are truly a source of blessing and motivation to me. May the Almighty bless our union and family forever, Amin.

ABSTRACT

The effects of experimental *T. evansi* and *T. bruceibrucei*infections on haematology and some reproductive parameters of Yankasa rams were investigated. Sixteen Yankasa rams aged, 24 to 30 months and weighing 22-25kg were acclimatized for a period of two months in a clean fly proof house, adequately fed and given water *ad libitum*. Of the sixteen rams, twelve that were clinically fit for the experiments at the end of the eight weeks acclimatization period were randomly divided into four groups (I, II, III and IV), of three rams each. Groups I and II were each challenged singly with experimental *T. bruceibrucei*(Federe strain) and*T. evansi* (Sokoto strain), respectively, while group III was challenged with mixed *T. bruceibrucei*and *T. evansi*parasites (50% of each species in the infective inoculum) and group IV was left as uninfected control. Each infected ram received 2ml containing $2x10^6$ trypomastigotes via the jugular vein. Parasitaemia in groups I and III appeared 5-9days and 6-11 days post infection, respectively, while it appeared 19-21 days post infection (P.I) in group II. There was significant difference ($P < 0.01$) in the mean parasitaemia levels of groups I and III in comparison to that of group II at the end of the experiment. The pre-infection mean weekly packed cell volume (PCV)of the rams in the experimental groups I, II, III and IV were 33.0 ± 1.2%, 31.0 ± 1.6%, 31.67 ± 1.1% and 32.67 ± 1.5%, respectively.The mean PCV by the end of the experiment in groups I, II, III and IV were18.70 ± 1.4%, 24.75 ± 1.6%, 17.48 ± 1.8%, and 35.01 ± 1.5%respectively. There was a significant difference ($P < 0.01$) in the mean PCV and haemoglobinconcentrations among the groups at the end of the experiment. The clinical signs observed in the infected groups included intermittent pyrexia, pale ocular mucous membranes indicative of anaemia, dullness, lethargy, roughy hair coat, scrotal oedema, scrotal degeneration, loss of libido, increase in semen collection reaction time, emaciation, and death. There was a drastic and progressive deterioration in semen quality in all infected rams manifested by a decrease in volume or cessation of semen production, a sharp decrease in progressively motile sperm, elevated numbers of dead sperm and significant morphological abnormalities of sperm in most animals. The rams especially in groups I and III were all deemed unfit for breeding by the end of the 98 days post infection. Uninfected rams were healthy and had good semen characteristics throughout

the experimental period. There was significant difference ($P < 0.01$) in the gonadal and epipidymal sperm reserves of groups I and III in comparison to group II, and between infected and control rams.The gross lesions observed in the infected rams in group II were moderate and very severe in groups I and III. These lesions included pale carcasses indicative of anaemia, watery blood, lymphadenopathy, splenomegaly and hepatomegaly.No observable gross lesion was found in the organs from rams of the control groupIV. The microscopic lesions observed in the testes of the infected rams were characterized by degeneration of the seminiferous tubules, mononuclear infiltration of interstitial tissues, infiltrations by lymphocytes with minor cellular damages.The results indicate that trypanosomosis due to experimental*T.b. brucei*,*T.evansi* and mixed infections (of both parasites), may cause marked defects in the haematological parameters, severe degenerative changes in the reproductive organs resulting in the rapid deterioration of semen characteristics and associated infertilityproblems. Therefore, pathogenic trypanosomes may be important cause of various grades of infertility problems in Yankasa rams in tsetse and pathogenic trypanosomes-endemic areas of Nigeria.

TABLE OF CONTENTS

TITLE **PAGE**

TITLE PAGE ……..……i

DECLARATION……ii

CERTIFICATION…….iii

DEDICATION ……..….iv

ACKNOWLEDGEMENTS ……...v

ABSTRACT ……....vii

TABLE OF CONTENTS ……..ix

LIST OF TABLES…….…..xix

LIST OF FIGURES …….....xx

LIST OF PLATES ……....xxii

LIST OF ABBREVIATIONS ……..…xxvi

CHAPTER ONE

1.1 INTRODUCTION ……1

1.2 Statement of the Research Problem ……7

1.3 Justification……..8

1.4 Aim and Objectives of the Research……9

1.5 Research Null Hypotheses…….10

CHAPTER TWO

2.0 LITERATURE REVIEW……11

2.1 Introduction……11

2.2 Aetiology and Transmission of African Animal Trypanosomosis (AAT)……12

2.3Trypanosome Morphology and Taxonomy……...14

2.3.1 The subgenus*Nannomonas*……...14

2.3.2 The subgenus*Duttonella*……...15

2.3.3 The subgenus*Trypanozoon*……...16

2.4 Host Range……17

2.5 Transmission and Distribution……...18

2.6 Life Cycles and Modes of Transmission of Trypanosomes……...21

2.7 Antigenic Variation……....23

2.8 Sheep Breeds and Distribution in Nigeria……25

2.8.1 Balami……25

2.8.2 Uda……25

2.8.3 Yankasa……..25

2.8.4 West African Dwarf……...25

2.9 Reproductive Physiology of the Male……26

2.9.1 Anatomy of male genitalia……26

2.9.1.1 *Testes*……..27

2.9.1.2 *Scrotum*……..28

2.9.1.3 *Spermatic cord*……...30

2.9.1.4 *Epididymis*……31

2.9.1.5 *Vas deferens and urethra*……...31

2.9.1.6*Accessory glands* ……..32

2.9.1.7 *The penis* ……..33

2.10 Pathogenesis of *Trypanosoma* infection……34

2.11Prepatent Period and Parasitaemic Waves……..35

2.12Clinical Signs……36

2.13 Anaemia……37

2.14 Various Stages of Anaemia in Trypanosomosis……....38

2.14.1 Phase I: Acute crises……..38

2.14.2 Phase II: Chronic……38

2.14.3 Phase III: Recovery……39

2.16 The Pathogenesis of Anaemia in Trypanosomosis……39

2.16.1 Anaemia through mechanical injury to erythrocytes…….....39

2.16.2 Anaemia through undulating pyrexia…….....40

2.16.3 Anaemia through platelets aggregation (Microangiopathy)……..40

2.16.4 Anaemia caused by trypanosome toxins and metabolites…….....41

2.16.5 Lipid peroxidation…….....42

2.16.6 Idiopathic serum factors……44

2.16.7 Immune complexes……45

2.16.8 Malnutrition……...46

2.16.9 Tumor necrosis factor/Bone marrow nitric oxide (NO)……46

2.17 Devastating Effects of Trypanosomosis on Livestocks…….....47

2.17.1 Immunosupression…….....47

2.17.2 Animal productivity……...48

2.17.3 Work performance…….....48

2.17.4 Reproductive performance……49

2.17.5 Endocrine system……..53

2.18 Pathology of Trypanosomes in Livestock……....54

2.19 Trypanosome-Induced Endocrine-Related Disorders in Both Sexes……...54

2.20 Diagnosis of Trypanosomosis……...56

2.21 Trypanosomosis Control……57

2.21.1 Trypanocidal drugs……...57

2.21.2 Tsetse vector control…….58

2.21.3 Vaccination……59

CHAPTER THREE

3.0 MATERIALS AND METHODS……....61

3.1 Study Area……....61

3.2 Experimental Animals……...61

3.2.1 Housing and screening of experimental animals……..61

3.2.2 Acclimatization and physical examination of animals……..63

3.2.3 Source of trypanosomes ……64

3.3.0 Experimental Design……....64

3.3.1 Grouping of the experimental animals…….....66

3.3.2 Inoculation of experimental animals…….66

3.4.1 Observation of clinical signs…….....67

3.4.1.1 *Determination of rectal temperature*…….....67

3.4.1.2 *Determination of weight*……67

3.4.2 Determination of haematological parameters……67

3.4.2.1 *Determination of the level of parasitaemia*……..68

3.4.2.2 *Determination of Packed Cell Volume (PCV)*……...68

3.4.2.3 *Determination of haemoglobinconcentration*……..69

3.4.2.4 *Determination of total plasma protein*……..69

3.4.2.5 *Thin blood smear for trypanosomes identification*……69

3.5 Reproductive Parameters……...69

3.5.1 Determination of scrotal circumference……70

3.5.2 Semen collection……70

3.6 Semen Evaluation……..71

3.6.1 General examination……..71

3.6.1.1 *Semen collection reaction time*……..71

3.6.1.2 *Semen volume and colour*……..72

3.6.1.3 *Semen pH*……...72

3.6.2 Microscopic examination of semen……..72

3.6.2.1 *Gross sperm motility*……..72

3.6.2.2 *Sperm concentration* ……73

3.6.2.3 *Percentage live- dead spermatozoa* ……..73

3.6.2.5 *Spermatozoa abnormal morphology*……74

3.7 Determination of Gonadal sperm and Epididymal Sperm Reserve……...74

3.7.1 Determination of gonadal sperm/spermatid reserves……74

3.7.2 Determination of epididymal sperm reserves……75

3.8 Histopathological Analysis……76

3.8 Data Analyses……77

CHAPTER FOUR

4.0 RESULTS……78

4.1. General Clinical Observations……...78

4.1.2 Mean parasitaemia levels (+)……85

4.1.3 Mean rectal temperature (^{o}C)……87

4.1.4 Mean weekly live body weight changes……89

4.1.5 Mean weekly scrotal circumference ……....91

4.1.6 Mean weekly plasma protein (g/dl)……...91

4.1.7 Mean weekly pack cell volume (PCV)……..93

4.1.8 Mean weekly haemaglobin concentration (Hb)……96

4.2 Semen Characteristics……96

4.2.1 Semen collection reaction time (sec)……96

4.2.2 Semen volume and Colour (ml)……98

4.2.3 Gross forward motility (%)……98

4.2.4 Semen pH……101

4.2.5 Spermatozoa concentration (10^{6}/ml)……101

4.2.6 Total spermatozoa output ……101

4.2.7 Percent dead sperm (%)……...102

4.2.8 Normal sperm morphology (%)……...102

4.2.9 Spermatozoa abnormalities (%)……...102

4.3.0 Mean Weekly Spermatozoa Abnormalities (%)……..104

4.3.1 Incidence of mid- piece droplets (MPD) (%)……..104

4.3.2 Incidence of detached head (Free head) (DH) (%)……104

4.3.3 Incidence of tail abnormalities (CT) (%)……109

4.4.0 Gonadal and Epididymal Sperm Reserve (X10^{6}/gm)……..112

4.4.1 Gonadal sperm / Spermatid reserve ($X10^6$/gm)……..…..112

4.4.2 Epipididymal sperm reserve ($X10^6$/gm)……..….112

4.5.0 Gross Pathological Findings……113

4.5.1 Post mortem…….113

4.5.2 Histological findings of tissues and organs…….124

4.5.2.1 *Testes*……122

4.5.2.2 *Spleen*……..122

4.5.2.3 *Liver*……..127

4.5.1.4 *Lungs*……..……127

4.5.2.5 *Kidney*……...127

4.5.2.6 *Lymph nodes*……127

4.5.2.7 *Heart*……..132

4.5.2.8 *Brain*……..132

CHAPTER FIVE

5.1 DISCUSSION…….135

CHAPHTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS……146

6.1 Conclusions……..146

6.2 Recommendations……147

REFERENCES……148

LIST OF FIGURES

FIGURE	TITLE	PAGE
Figure 2.1:	Sagittal section of testis illustrating segments of parenchymal tissue which contain the seminiferous tubules, rete testis, vasa efferentis, epididymis, and scrotal portion of the vas deferen (Amann and Schanbacher, 1983)……..	29
Figure 2.2:	Cross section of parenchymal tissue showing relationship between the seminiferous tubules and interstitial tissue containingLeydig cells (Amann and Schanbacher, 1983)……..……..	29
Figure 2.3: The reproductive system of a ram (Albert, 1961)…….		33
Figure 4.1:	Mean parasitaemic scores of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I),*T.evansi* (Grp II), or both parasites (Grp III)……..	86
Figure 4.2:	Mean weekly rectal temperature of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I),*T.evansi* (Grp II), or both parasites (Grp III)……..	88
Figure 4.3:	Mean weekly live body weight of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I),*T.evansi* (Grp II), or both parasites (Grp III)……	90
Figure 4.4:	Mean weekly scrotal circumference of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I),*T.evansi* (Grp II), or both parasites (Grp III)……..	92
Figure 4.5:	Mean weekly plasma protein of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III)……..	94
Figure 4.6:	Mean weekly packed cell volume of uninfected control Yankasa	

rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III).....95

Figure 4.7: Mean weekly haemaglobin concentrationof uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei*(Grp I), *T.evansi* (Grp II), or both parasites (Grp III).....97

Figure 4.8: Mean weekly incidence of cytoplasmic droplets in spermatozoaof uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III)…….....106

Figure 4.9: Mean weekly incidence of detached head in spermatozoa of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III).....108

Figure 4.10: Mean weekly incidence of tail abnormality in spermatozoa of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III)……...111

LIST OF TABLES

TABLE **TITLE** **PAGE**

Table 3.1: Grading of genital degeneration in ram infected with *Trypanosoma*spp……76

Table 4.1: Mean values for eight weeks pre-infection baseline data for all parameters of 12 experimental Yankasa rams……...79

Table 4.2: The semen collection reaction time andsemen characteristics of infected and control Yankasa rams (Mean ± SEM)……...100

Table 4.3: Testicular length, Volume, Weight and Gonadal Sperm Reserves of Yankasa rams infected with Trypanosomes, 98 days P.I (Mean ± SEM)…….114

Table 4.4: Epididymal length, Weight and Sperm reserve of Yankasa rams infected with Trypanosomes, 98 days P.I (Mean ± SEM)…….....115

LIST OF PLATES

PLATE **TITLE** **PAGE**

Plate I: The experimental animals (Yankasa rams) during the acclimatization period. ……...62

Plate II: Giemsa stained *Trypanosomaevansi*at X1000 Magnification (a) and*T. bruceibrucei*(b) at X400 magnification.….....65

Plate III:Electro-Ejaculator mini tube…….71

Plate IVa-b: A clinically healthy Yankasa ram with good body condition fromthe control group IV (a) and one of the *T.b.brucei*-infectedrams in group I withroughyhaircoat and unco-ordinated posture, 35 day p.i. (b)……80

Plate IVc-d: One of the rams infected with *T.evansi* showing very pale ocular membrane (red arrow) with peri-orbital oedema (yellow arrow) at 56 dayp.i. (c) and*T.evansi*infected ram that collapsed and died of the infection by 57 day p.i. (d)……...81

Plate Va-b: A normal and healthy scrotum with complete folds in one of the control rams (a) and a swollen scrotumwith inflammation (arrow) showingloss of folds in *T.b.brucei*-infected ram at 35d.p.i. (b)……...83

Plate Vc-d: Scrotal atrophy in *T.b.brucei*-infected Yankasa ram at day 70 p.i. (c) and rams with mixed infection, 55 d.p.i.(d)…….....84

Plate VI: Normal spermatozoa morphology of experimental Yankasaram (Control) Eosin-Nigrosin stain (X 400)……..103

Plate VII:Cytoplasmic droplets in spermatozoa of experimental Yankasa ram infected with Trypanosomes (X 400 mag)……..105

Plate VIII:Detached head in spermatozoa of experimental Yankasa ram infected with *Trypanosoma* Species (X 400).……...107

Plate X: Tail abnormalities in spermatozoa of experimental Yankasa ram infected with *Trypanosoma* Species (a) bent tail (arrow)(b) coiled tail. Eosin-Nigrosin stain (X400)…….110

Plate XI: Bent tail (arrow) of experimental Yankasa ram infected with *Trypanosoma* Species (X400)…….110

Plate XII: A normal tissue and organ architecture of control Yankasa ram with fat deposits (arrow) on most of the tissues and organs with red and normal carcass composition.…….....116

Plate XIII: A very pale and anaemic carcass (arrow) composition, with watery blood and severe atrophy of body fats inYankasa ram with mixed infection (*T. bruceibrucei* and *T. evansi*).....117

Plate XIV: Testicular atrophy inYankasa ram with mixed infection (*T. bruceibrucei* and *T. evansi*).……..118

Plate XV: A congested and pneumonic lungs with severe haemorragic points (black arrow) and an enlarged liver (red arrow) in Yankasa ram infected with *T. bruceibrucei* (group I).……119

Plate XVI: An enlarged lymph node (black arrow) and a normal lymph node (red arrow) of infected and control Yankasa rams respectively…….120

Plate XVII: An enlarged spleen of Yankasa ram infected with *T. bruceibrucei*. …..121

Plate XVIII: Serous atrophy of intestinal (arrow) and body fats with watery blood (arrow) of Yankasa ram infected with *T. evansi* (group II)……..123

Plate XIXa-b: A typically normal testes (a) of control Yankasa ram showing full tissue architecture with well defined basement membrane, spermatogonic cell layers, 1^{o} spermatocytes, 2^{o} spermatocytes, spermatids and full sperm reserve in the lumen of the seminiferous tubules and a moderately degenerated testes (b) of*T. evansi*-infected Yankasa ram with reduction

in the spermatogonic cell layers, loss of spermatids and less commonlyspermatocytes with absence of gonadal sperm reserve in most of the lumen of the seminiferous tubules (Haematoxylin and Eosin, X400)……124

Plate XIXc-d: Severe testicular degeneration (c) of *T. b. brucei*-infected Yankasa ram showing reduction in number of spermatogenic cell layers, degenerated leydig and sertoli cells with mononuclear cell infiltration in the seminiferous lumen with almost lost of sperm reserve and a section of testes (d) of Yankasa ram with mixed infection showing diffused degeneration of the basement membrane and sertoli cells with severe depletion of epididymal sperm reserve (Haematoxylin and Eosin, X400)……...125

Plate XXa-b: A Normal spleen architecture (a) of a control Yankasaram showing a normal germinal centre (arrow)and proliferation of lymphocytes at the germinal centres of the spleen (b)of .*T. b brucei*-infected Yankasa ram(Haematoxylin and Eosin, X400)…..126

Plate XXI: Congested liver with mononuclear cell infiltration with sinusoidal spaces (arrow) in the liver of Yankasa ram with mixed infection (group III) (Haematoxylin and Eosin, X400).....128

Plate XXIIa: A normal lung architecture (a) with normal alveoli and complete folds (arrow) of control ram andalveolar degeneration with loss of folds in the lumen (b)of the lungs of Yankasa ram withmixed infection (Haematoxylin and Eosin, X400)……129

Plate XXIIIa-b: A normal kidney architecture(a) showing normal renal tubules (arrow) of Control Yankasa rams andrenal tubular degeneration (arrow) with mononuclear cell infiltration (b)in the kidney of Yankasa ram with *T .b. brucei* infection (Haematoxylin and Eosin, X400)…….130

Plate XIV: Proliferation of lymphocytes in the lymph node of Yankasaram infected with *T.evansi* (group II) (Haematoxylin and Eosin, X400)…...131

Plate XV: Mononuclear cell infiltrations (arrow) in the Heart of Yankasa ram infected with *T.evansi* (group II) (Haematoxylin and Eosin, X400)……133

Plate XVI: Mononuclear cell infiltrations on the tissue section of the brain of Yankasa ram infected with *T. b.brucei*(Haematoxylin and Eosin, X400).……134

LIST OF ABBREVIATIONS

a.m = Ante meridian

cm =Centimetre

d.p.i. = Day post infection

dl = Decilitre

^{o}C = Degree centigrade

G = Gauge

Hand E = Haematoxylin and Eosin

Hb = Haemoglobin

kg = Kilogram

L = Litre

Mag = magnification

mg = Milligram

ml = Millilitre

PCV = Packed cell volume

% = Percent

r.p.m = Revolution per minute

sec = Seconds

SEM = Standard error of mean

CHAPTER ONE

1.1 INTRODUCTION

Livestock production plays important roles in the provision of high-quality protein to consumers and regular income to producers. To fulfill their potential sustainably, they must be managed with care. From time immemorial livestock rearing is given much importance not only in developing countries but also in developed countries (Mohammed, 2007).Currently, livestock is one of the fastest growing agricultural subsectors in developing countries where it contributes about 33 per cent of the Gross Domestic Product (GDP). This growth is driven by the rapidly increasing demand for livestock products; which is equally being driven by population growth, urbanization and increasing incomes in developing countries (Delgado, 2005). Livestock therefore plays a major role in the socio-economic development of any nation. Between 70 – 80% of the Nigeria's population of over 140 million are engaged in agriculture and livestock industry as their major occupation(Mohammed, 2007).

As an integral component of agricultural economy of any nation, livestock performs multifarious functions such as provisions of food, nutrition, income, savings, foreign currency earning (by exporting hides and skin, bone and other products), draught power, manure, fuel, transport, social and cultural functions (Bangladesh Agricultural Research Council, 2010). At the household level, livestock plays a critical economic and social role in the lives of pastoralists, agro-pastoralists and smallholder farmers. Livestock fulfills an important function in coping with shocks, accumulating wealth and serving as a store of value in the absence of formal financial institutions and other missing markets (Asfaw*et*

al., 2011). In the case of smallholder mixed farming systems, livestock provides nutritious food, additional emergency and cash income, transportation, farm outputs and inputs and fuels for cooking food. In the case of pastoralists, livestock represents a sole means to support and sustain their livelihoods. Furthermore, available research suggests that with economic growth, consumption patterns tend to change towards high value and high-protein foods, such as those derived from livestock (Delgado *et al*., 1999).

Sheep and goats constitute the world's largest population of livestock with an estimate of 1,078.9 and 861.9 million, respectively. This implies that there is about one goat to approximately 1.25 sheep in the world, but tremendous variations occur among the different parts of the world regarding the number of goats, its ratio to sheep and their percentages (FAOSTAT, 2008). The largest numbers of goats are in Asia, followed by Africa, representing about 59.7% and 33.8% respectively, summing up to 93.5% of the world's total. The lowest numbers of goats are in Oceania, accounting for 0.1% of the world's total number. The ratio of goat to sheep ranged between one goat to 0.8 sheep in the Caribbean to one goat to 119.2 sheep in Oceania (FAOSTAT, 2008). The total number of goats in the world has increased by 146% of the total number (590.1 million) encountered in 1990. During the same period, cattle number increased by 5%, while that of sheep decreased by 10%, reflecting a fall in the productivity of sheep as a major livestock species.Africa alone contributed a total of 287.6 million sheep and 291.1 million goats, representing approximately 26.7% and 33.8% respectively, of the world's total(FAOSTAT, 2008). Within Africa, the distribution of these small ruminants varies widely, with a higher concentration found in dry areas than humid areas (Ademosun,

1988). In West Africa, Nigeria has the largest number of livestock population.In the world, the country is ranked sixth in terms of sheep and goat production with a total production level of 33.9 million sheep and 53.8 million goats (FAOSTAT, 2008). Keeping small ruminants is regarded as an investment as these animals are sold to meet compelling family and financial obligations or slaughtered for consumption. Little capital investment in buildings or other material is required for their upkeep, and space and maintenance requirements are low, with high reproductive efficiency (Ademosun, 1988). This implies that, given the economic growth in Nigeria, the market demand for livestock and livestock products is likely to continue growing in the future (Ademosun, 1988). Paradoxically, the livestock sub-sector tends to be playing a decreasing role in national development in view of its contribution to the country's agricultural Gross Domestic Product (Ifeanyi and Olayode, 2008). Livestock as a percentage of agricultural GDP was as high as 19% in 1983 and 1984, but the share has dropped persistently over time to 10% in 1998 and further to 6% in 2004 and 2005 (Ifeanyi and Olayode, 2008).

One of the most important and debilitating constraints to livestock production is livestock diseases (Firew, 1999; Soudre *et al*., 2013). Diseases are the most significant limiting factors to livestock productivity in sub-Saharan African countries. Diseases such as helminthosis, ectoparasitism, and haemoparasitism as well as bacterial and viral infections constitute impediments to livestock production(Agwuma, 1983).Due to the devastating effect of livestock diseases, animal protein output has not been able to keep up with national demands (Njombe and Msanga, 2009).Prominent among livestock diseases istrypanosomosis, which isone of the most deadly and pathogenic despite the

age-long attempts to control it. Griffin (1978), reported that sheep and goats gave quicker returns and lower per head value than cattle. However, a disease such as animal trypanosomosis poses a great risk to their production. The economic impact of the disease on these animals has been shown to be substantial. It is described as a complex debilitating and often fatal conditions caused by infection with one or more of the pathogenic tsetse-transmitted protozoan hemoflagellate parasites of the genus *Trypanosoma* (Anene *et al*., 2001). The incidence and severity of the disease in different regions are dependent upon local conditions (Anene *et al*., 2001). The impact of trypanosomes on African agriculture is most obviously felt at the herd level leading to reduced milk output, reduced live animal output and reduced efficiency of animals used for cultivation (Swallow, 2000). In susceptible cattle breeds, the disease reduces calving by up to 20% thereby causing death of young stock; meat and milk outputsare reduced by at least 50% (Swallow, 2000). The disease has been a great challenge to the livestock industry where the barrier imposed has been difficult to surmount by any form of chemotherapy, prophylaxis or control (Holmes *et al*., 2004; Van den Bossche and Doran, 2004).

The major pathogenic trypanosome species in livestock are transmitted by the tsetse fly (genus *Glossina*) and include:*Trypanosoma congolense, T.vivax, T.brucei brucei* and *T.evansi*, while the sub species of *T.brucei, T.b.rhodesiense* and *T.b.gambiense* cause sleeping sickness in man and were important causes of death in Africa (Losos, 1986; Okubanjo *et al*., 2014). Nagana and related diseases also caused by *T. congolense, T.vivax* and *T.brucei brucei* in cattle are prevalent in much of sub-Saharan

Africa,especially Nigeria. Surra, another disease caused by *T. evansi*, isa problem wherever camels are or have been. Chagas disease caused by *T.cruzi* (transmitted mechanically) is a serious public health problem in South and Central America, where about 10 million people are infected(Losos, 1986; Okubanjo *et al*., 2014). The pathogenicity of trypanosome infection varies considerably and depends on both the species of trypanosome and the host involved. Trypanosomes are either haematic such as *T. congolense* and *T. vivax* which are found in the plasma or tissue-invasive such as *T.b. brucei, T.b. rhodesiense* or*T.b. gambiense,T. evansi* and *T. equipadum* which are found intravascularly or extravascularly(Murray *et al*., 1982; Awobode, 2006). The severity of the infection is influenced by a number of factors such as virulence of the different species of trypanosomes, age, nutritional status, and the breed of livestock (Murray *et al*., 1982; Awobode, 2006).

Clinically, the effects of trypanosomosis on these animals range from anaemia, immunosuppression, depression with inability to rise, pyrexia directly associated with parasitaemia, paleness of mucous membrane, rapid pulse beat, retarded growth, roughness of haircoats,enlargement of peripheral lymph nodes, low milk production, low meat quality, and weight loss as well as infertility, abortion, stillbirth and depressed reproductive performance and reduced capacity to work leading to morbidity and mortality in the absence of treatment (Sekoni, 1994;Snow *et al*., 1996; Bezerra*et al*., 2008; Batista *et al*., 2007; 2009; 2012; Silva *et al*., 2013).Trypanosomosis can be a highly debilitating and fatal disease in domestic ruminants, mainly due to the haematological disturbances that induce severe anaemia and inflammatory foci in the central nervous

system (CNS), heart, liver, spleen and lymph nodes (Gardiner *et al*., 1989; Desquesnes, 2004; Chamond *et al*., 2010). Further haematological effects include decreased packed cell volume, reduced red blood corpuscles, reduced haemaglobin concentration, decrease in total protein and leucocytes counts (Sekoni *et al*., 1990a; Audu *et al*., 1999; Chamond *et al*., 2010).Histologically, the effects of trypanosomosis on reproductive tissues and organs include testicular lesions and severe degeneration, necrosis and focal calcification of the seminiferous tubules in rams and goats (Edeghere *et al*., 1985; Rodrigues *et al*., 2013). Adamu *et al*. (2007) reported depletion of spermatogonic cells and destruction of interstitial tissues in Zebu bulls experimentally infected with *T. vivax*. Sporadic cases of congenital transmission of trypanosomes have also been reported in humans, sheep and goats (Ikede and Losos, 1972), calves (Batista *et al*., 2012) and mice (Ijagbone and Agbede, 2000). Additionally, *T. vivax* infection has induced abnormal pregnancy, dystocia, abortion, premature and low birth weights, stillbirths, transplacental foetal infection, neonatal death and other pathogenic effects on foetuses and offsprings (Sekoni, 1994; Batista *et al*., 2012). Other pathological effects include splenomegaly, hepatomegaly, genital lesions, congestion of the lungs, inflammatory lesions of the heart and paleness of carcasses (Dargantes *et al*., 2005b).

Direct losses due to trypanosomosis are estimated to amount to between US$ 1-1.2 billion each year, while the indirect impact of African animal trypanosomosison agriculture in sub-Saharan Africa exceeds this amount. A pondered evaluation extrapolated for the total tsetse-infested lands values the total losses, in terms of agricultural Gross Domestic Product, at US$ 4.75 billion per year (FAO, 2004). In

Nigeria, these damaging effects and the manpower and material resources committed to the disease result in financial loss of more than $23.8 million annually (Mulligan *et al*., 1970).

1.2 Statement of the Research Problem

In Nigeria, demand for ramsduring festivity is on the increase but parasites especially trypanosomes, have become a major threat to sustainable livestock production resulting in a decline in production (Soudre *et al*., 2013). Apart from haematological and pathogenic aberrations that do occur in animals infected with the parasites, recent studies show that they cause awide range of reproductive disorders in animals, including degeneration of the hypothalamus, pituitary glands and gonads with consequent disruptions in the secretions and plasma concentrations of the hormones necessary for normal reproductive processes in both sexes (Sekoni,1994). In female animals, the effects include severe genital lesions, temporary or permanent anoestrus, and abnormal oestrous cycles. Additionally, trypanosomal-induced death during pregnancy, abnormal pregnancy, dystocia, abortion, premature birth, low birth weight, stillbirth, transplacental foetal infection, neonatal death and other pathogenic effects on foetuses and offsprings have been reported (Sekoni, 1994; Silva *et al*., 2013).

In the males, the effects include delayed puberty, loss of libido, severe degenerative changes of the genitalia, manifested by the production of very poor quality semen and loss of epididymal and gonadal sperm reserves (Sekoni, 1994; Mbaya *et al*., 2011; Okubanjo *et al*., 2014). Testicular atrophy has also been reported with the presence of

dead spermatozoa in the seminiferous tubules of bulls, gazelles and rams (Sekoni, 1994; Sekoni *et al*., 2004; Bezerra *et al*., 2008; Mbaya *et al*., 2011). Infection of rabbits with *T. brucei brucei* causes scrotal oedema, scrotal inflammation, severe testicular degeneration, abnormal spermatogenesis and incomplete resolution of the genital organs following berenil chemotherapy (Ikede and Akpavie, 1982). Omeke and Igboeli (2000) reported the presence of *Trypanosoma brucei brucei* in the genital tract, soft organs and brain of infected boars with resultant degeneration of testes. There was also loss of libido in infected boars. It has also been reported that animals that survived infection often remained infertile (Sekoni *et al*, 2004).Information on the effect of *T. brucei brucei*, *T. evansi* and mixed infections on the haematology and reproductive physiology of Yankasarams is scanty and basically unavailable.Sheep are often grazed alongside cattle or camels from Northern Nigeria towards the Southern vegetation belts by cattle herdsmen in search of greener pastures thereby exposing them to the risk of trypanosomes.

1.3 Justification

Although *T. brucei brucei* and *T.evansi* are primarily parasites of cattle and camels respectively, there is the likelihood that animals herded together with cattle or camels, especially Yankasa rams, may become infected with these parasites. Consequently, there is an urgent need to investigate factors that may negatively affect the success of sheep breeding especially in line with reproductive problems such as lowered fertility, infertility and or sterility. Therefore, a thorough understanding of the pathogenic trypanosomes which could alter normal reproductive physiology of animals with associated

haematological changes (Biryomumaisho *et al*., 2003), is a pre-requisite for this study in Yankasa rams.This will provide vital information on the progression and degree of degenerative changes that occur in the host's tissues as well as the severity of the infection. Hence the need for this study on the effect of *T.brucei brucei* and *T. evansi* on the haematological and reproductive parameters of Yankasa rams.The study will provide information on the effects of *T. brucei brucei*, *T. evansi* and mixed infections on the haematological indices and reproductive parameters (semen quality and gonadal and epididymal sperm reserves) of Yankasarams in Nigeria. Yankasa rams were selected for this study considering the fact that Yankasa sheep are the most numerous breed of sheep in the Guinea Savanna region. They are also inhigh demand amongst the Muslim populace of Nigeria due to their ceremonial values (naming ceremony), religious importance (used as sacrificial animals during the Eid-El kabir festive periods), social and economic values (e.g. mutton, barbecue, suya and pepper soup).

1.4 Aim and Objectives of the Research

The aim of the research was to evaluate the effects of single and mixed experimental *Trypanosoma evansi* and *Trypanosoma brucei brucei* infectionson thehaematological and some reproductive parameters of Yankasa rams.

The specific objectives of the research were to determine the effects of single and mixed experimental *T. evansi* and*T. brucei brucei* infections ofYankasa rams on:

i. Haematological indices.

ii. Semen characteristics.

iii. Epididymal and gonadal sperm/spermatid reserves.

iv. Anatomy of reproductive organs and tissues.

1.5.1 Research Null Hypotheses

Single and mixed experimental infections of *T. evansi* and *T.b. brucei* ofYankasa rams have no significant effects on:

i. Haematological indices.

ii. Semen characteristics.

iii. Epididymal and gonadal sperm/spermatid reserves.

iv. Anatomy of reproductive organs and tissues.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Trypanosomosis refers to a group of diseases including sleeping sickness in humans and nagana in cattle in Africa, and Chagas disease in South America, which still remain a considerable problem in the 21st century (Wainwright, 2010). Animal trypanosomosis is an economically devastating disease and a major constraint to livestock production in tropical Africa (Tafese *et al*., 2012; Guegan *et al*., 2013). However, its greatest socio-economical effects are seen in sub-Saharan Africa, an area particularly suited to the survival of tsetse fly which is the vector responsible for cyclical transmission of both animal (i.e.*Trypanosoma congolense, T. vivax* and *T. brucei brucei*) and human (*T. b. rhodesiense* and *T. b. gambiense*) trypanosomes (Losos, 1986). Also, mechanically transmitted *T. evansi* in tse-tse fly free area of Africa remains the most important disease of camels.

African animal trypanosomosis (AAT) is most important in cattle but can also cause serious losses in pigs, camels, sheep and goats. Infection of cattle by one or more of the three African animal trypanosomes results in subacute, acute, or chronic disease characterized by intermittent fever, anaemia, occasional diarrhoea, rapid loss of condition and often terminates in death (Mulligan *et al*., 1970). It is considered a major constraint to livestock productivity in sub-Saharan Africa and has profound effects on rural development over vast areas (Tafese *et al*., 2012).The economic losses attributed to AAT

are due to decreased meat and milk production as a result of mortality, morbidity and infertility. When the impact on crop productivity due to reduced animal draught power and manure is considered, the true economic losses could be much higher (Budd, 1999). The epidemiology of trypanosomosis is complicated by the fact that both the human and livestock infective trypanosomes circulate in wild animals and livestock (FAO, 2011).

2.2 Aetiology and Transmission of African Animal Trypanosomosis (AAT)

The most important trypanosomes in terms of economic losses in livestock are the tsetse transmitted species: *Trypanosoma congolense*, *T. vivax*, *T. brucei* and *T.evansi* (Mulligan *et al*., 1970; Audu *et al*., 1999). However, *T. vivax* has been found outside the zone of *Glossina* infestation, where it is transmitted non-cyclically by other species of biting flies (Molyneux and Ashford, 1983). *Trypanosoma evansi* is also acyclically transmitted by biting flies of the genus *Tabanus* and *Stomoxys*. *Trypanosoma equiperdum* on the other hand is transmitted sexually, from host to host (Molyneux and Ashford, 1983).*Trypanosoma brucei brucei*belongs to the subgenus *Trypanozoon.* Horses, dogs, cats, camels and pigs are very susceptible to *T. b. brucei* infection. Infection of cattle, sheep, goats and sometimes pigs results in mild or chronic infection (Mulligan *et al*., 1970). Although this last observation is widely accepted, Moulton and Sollod (1976) indicated evidences that this organism is widespread in East and West Africa and that it can cause serious disease and high mortality in cattle, sheep and goats.In East Africa, *T. congolense* is considered to be the single most important cause of African Animal Trypanosomosis (AAT). This trypanosome is also a major cause of the disease in cattle in West Africa (Mulligan *et al*., 1970). Sheep, goats, horses and pigs may also be seriously

affected. Although *T. vivax* is considered to be less pathogenic for cattle than *T. congolense*, it is nevertheless the most important cause of AAT in West African cattle (Hoare, 1972). This trypanosome readily persists in areas free of tsetse flies (for example, in Central and South America and in the Caribbean), where it is transmitted mechanically by biting flies or contaminated needles, syringes and surgical instruments (Mulligan *et al*., 1970).*Trypanosoma congolense* and *T. vivax* are both regarded as haematic parasites, confined to the circulatory system, although *T. congolense* does undergo early development extravascularly at the site of tsetse bite and in associated lymph nodes. Similarly, *T. vivax* has been found extravascularly in the pituitary gland, cerebrospinal fluid aqueous humour and cardiac muscle (Fiennes, 1970; Blum *et al*., 2008). Trypanosomes of the *T. brucei* group are primarily considered as extravascular parasites (Losos, 1986).

In Nigeria, animal trypanosomosis caused by *T. vivax, T. brucei, T. congolense* and *T. evansi* is one of the most important diseases of livestocks (Bawa *et al*., 2005: FAO, 2011). About 75 – 80% of the land area of Nigeria harbours around 20 (twenty) species of tse-tse flies capable of transmitting pathogenic trypanosomes to animals and man (Anosa, 1990a). The remaining non tse-tse infested area of Nigeria, harbours haematophagous flies which transmit pathogenic trypanosomes mechanically. Trypanosomosis is partially responsible for nomadism, transhumance and their attendant socio-economic problems in Nigeria. Trypanosomosis is also responsible for restricting some breeds of livestock especially cattle, sheep and goats to the Sahel and northern Sudan vegetation zones where rainfall and pastures are limited (trypanosomosis non

endemic areas) and for rendering the lush pastures of the south vegetative zones not available for grazing (trypanosomosis endemic areas) (Bawa *et al*., 2005). Sheep and goats can be infected by trypanosomes but are generally less affected than cattle.

2.3 Trypanosome Morphology and Taxonomy

A sound knowledge of the basic features of the various trypanosomes enables the identification of each species and so the exact cause of the disease.Trypanosomes are classified in the Phylum Sarcomastigophora; the Order Kinetoplastida and the Family Trypanosomatidae(Mulligan *et al*., 1970). The trypanosome consists of a single cell varying in size from 8 to over 50 μm. There are distinct differences in appearance, shape and size between the various species of trypanosomes, allowing specific identification. The salivaria group of trypanosomes may or may not have a free flagellum, the kinetoplast is terminal or sub-terminal, and the posterior end of the body is usually blunt. They develop as trypomastigotes within the mammalian host and are usually pathogenic (Mulligan *et al*., 1970).

2.3.1 The subgenus *Nannomonas*

This is the smallest of the pathogenic trypanosomes, with a length of 8-24 μm. The blood forms are monomorphic, in that they lack a free flagellum. Generally two variants are to be seen, a shorter form (9-18μm), the typical *congolense* type and a longer form (up to 25μm), with individuals intermediate in length between the two (Hoare, 1972). There is evidence that strains with the most forms, the so-called ‘dimorphic’ strains, cause a more severe form of trypanosomosis. In stained specimens of *T. congolense* the cytoplasm

stains a diffuse, even, pinkish colour and is seldom granular.The nucleus is centrally placed. The kinetoplast is of medium size and is usually situated at the margin of the body, just in front of the posterior extremity (marginal and subterminal).The undulating membrane is poorly developed and inconspicuous (Hoare, 1972).*Trypanosoma simiae* species ispolymorphic, with a length of 15-19 μm. In typical cases some are without a free flagellum. The kinetoplastis of medium size, marginal and subterminal, as in *T. congolense* (Hoare, 1972).*Trypanosoma gogfreyi* has been separated recently from *T. congolense* in the Gambia, not only on the basis of isoenzymatic and DNA differences, but also because the disease it causes is different(Hoare, 1972). It is pathogenic in pigs, but the disease is more chronic than the one caused by *T. simiae*. Morphologically it is similar to *T. congolense*, with a length of 9-22 μm, but the undulating membrane is described as being usually conspicuous (Hoare, 1972).

2.3.2 The subgenus *Duttonella*

Trypanosoma vivax was named because of the vigour of its activity under the microscope when examined in fresh preparations. The parasite moves rapidly across the field of view. *T. vivax* as seen in the blood of mammals and it is also essentially monomorphic, with a free flagellum (Hoare, 1972). Its length, including the free flagellum, varies from 18 to 31 μm. The kinetoplast is large and terminal or almost so and the organism is much larger than in any of the other pathogenic species, and this is a distinguishing feature. The nucleus is centrally placed and the posterior extremity is swollen and blunt. The undulating membrane is inconspicuous. *T. uniforme* are small trypanosomes (from 12 to 20 μm), otherwise similar to *T. vivax* (Molyneux and Ashford, 1983).

2.3.3 The subgenus *Trypanozoon*

The subgenus *Trypanozoon* is the most homogeneous group of salivarian trypanosomes, represented conventionally by species which are morphologically indistinguishable but differ in biological features (Hoare, 1972). This group comprises five members: *T b. brucei, T. b. gambiense, T. b.rhodesiense, T. evansi* and *T. equiperdum*. *Trypanosoma brucei* is polymorphic, with three main forms, all of which have a small kinetoplast and a conspicuous undulating membrane. These three forms are(a) long slender forms (23-30 μm in length) with a free flagellum, which may be up to one half of the length of the organism. The posterior end is pointed and the nucleus is central. The kinetoplast is placed up to 4 μm in front of the posterior extremity (Hoare, 1972). (b) Short stumpy forms (17-22 μm in length) normally without a free flagellum, but in which there may occasionally be individuals with short free flagella. The kinetoplast is usually subterminal. The position of the nucleus varies greatly and it is in some cases in the posterior part of the cell, sometimes so far posterior that the kinetoplast is anterior to it (so-called postero nuclear forms).(c) Intermediate forms, varying in length between the two previously mentioned types. A free flagellum, of varying length, is always present. The nucleus is centrally placed. The posterior end is somewhat variable in shape, but usually blunt. The kinetoplast is close to the posterior extremity (Hoare, 1972).During the course of the infection, there is a change in the trypanosome population from the long thin forms, through the intermediate, to the short stumpy forms, and this altered appearance is accompanied by a change in the type of respiration, as the trypanosome prepares for its period within the tsetse fly. The short stumpy forms are adapted to living

and developing in the tsetse, while long thin forms are the true mature blood forms which die in the gut of the insect (Hoare, 1972).

Trypanosoma evansi is a salivarian trypanosome belonging to the Family trypanosomatidae; Order kinetoplastida. This tissue parasite is thought to have evolved from *T. brucei* and is closely related to the subspecies: *T. brucei brucei, T. brucei rhodesiense* and *T. brucei gambiense,* the aetiologic agents of animal and human trypanosomosis in Africa, respectively (Brun *et al.*, 1998). The current hypothesis is that when *T. brucei*-infected camels were moved outside the tsetse fly zone in Africa via travelling caravans, trypanosomes were spread by other haematophagous flies to other animal species. Recent molecular evidence supports the close genetic relationship between *T. brucei* and *T. evansi*, leading others to suggest that *T. evansi* is a subspecies of *T. brucei* (Jensen *et al.*, 2008; Lai *et al.*, 2008).*Trypanosoma evansi* is morphologically indistinguishable from the slender form of *T. brucei.* It is generally monomorphic in its slender form, although stumpy and intermediate forms may be observed in some isolates (Losos, 1980). *Trypanosoma evansi* is 14-33 μm long and 1.5-2.2 μm wide, with a free flagellum and a subterminal kinetoplast (Brun *et al.*, 1998). Isolates without kinetoplast have been identified in livestock from Brazil (Ventura *et al.*, 2002).

2.4 Host Range

Cattle, sheep, goats, pigs, horses, camels, dogs, cats and monkeys are susceptible to AAT and may suffer syndromes ranging from subclinical, mild or chronic infection to acute fatal disease(Molyneux and Ashford, 1983). Rats, mice, guinea pigs and rabbits are

susceptible and useful laboratory species (Molyneux and Ashford, 1983). More than 30 species of wild animals can be infected with pathogenic trypanosomes, and many of these remain carriers of the organisms. Ruminants are widely known to be active reservoirs of the trypanosomes. Wild equidae, lions, leopards and wild pigs are all susceptible and can also serve as carriers of trypanosomes (Molyneux and Ashford, 1983).

2.5 Transmission and Distribution

In Africa, the primary vector of*T. congolense*, *T. vivax* and *T. brucei* is the tsetse fly (Hoare, 1972). These trypanosomes replicate in the tsetse fly and are transmitted through tsetse fly saliva when the fly feeds on an animal. The three main species of tsetse flies for transmission of trypanosomes are *Glossina morsitans*, which favours the open woodland savanna; *G. palpalis*, which prefers the shaded habitat immediately adjacent to rivers and lakes; and *G. fusca*, which favours the high, dense forest areas (Hoare, 1972). The distribution of the tsetse-transmitted African trypanosomes is governed by that of their tsetse vectors, which infest an area of sub-Saharan Africa that extends from the southern edge of the Sahara desert (latitude 15° N) to Angola, Zimbabwe and Mozambique (latitude 20° S). Trypanosomosis is also mechanically transmitted by tsetse and other biting flies through the transfer of blood from one animal to another. The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota*,*Liperosia*, *Stomoxys* and *Chrysops* flies have also been implicated(Hoare, 1972). In Africa, both *T. vivax* and *T. b. brucei* have spread beyond the "tsetse fly belts" (WHO, 2003), where transmission is principally by tabanid and hippoboscid flies. Of the three African animal

trypanosomes, only *T. vivax* occurs in the Western Hemisphere in at least 10 countries in the Caribbean and South and Central America (Hoare, 1972).

In Nigeria, animal trypanosomosis is distributed from the mangrove forest to the Sudan savanna due to the presence of tsetse flies in these areas. The Sahel savanna, the plateaux, including Mambila, Jos and Obudu areas are classified as tsetse-free, however cases of trypanosomosis have been reported in Jos Plateau and the Sahel north of Maiduguri (Anosa, 1991a). Recent investigations have revealed that between 75- 80 percent of the landmass area of Nigeria is infested with eleven species of tsetse fly, which are capable of being vectors of both animal and human trypanosomosis (Anosa, 1991a).Several surveys have been carried out in Nigeria to evaluate the situation with regards to the incidence of bovine trypanosomosis (Folkers and Jones-Davies, 1966). According to Anosa (1991a), prevalence of trypanosomosis dropped from 89.6 percent in 1915 to between 30-45 percent in the 1950s and 1960s as a result of introduction of trypanocidal drugs. The incidence of bovine trypanosomosis in northern Nigeria was reported as 33 percent from 925 cattle using the blood smear techniques (Glover, 1961) with the relative frequency of *T. vivax* as 92%, *T. brucei*, 5% and *T. congolense* 3%. Tsetse risk and trypanosomosis acquired along a major trade cattle route from Jibiya in the north to the south-west region of Nigeria have been described (Yesufu and Mshelbwala, 1973). The investigators found infection rates of 100%, 42.8% and 28.6% for *T. vivax, T. brucei* and *T. congolense,*respectively. Godfrey *et al*. (1965) also found an infection rate of 42% in another herd of trade cattle at Ilorin. In another study of cattle (4,043) trekked from northern Nigerian to south-west of Nigeria, rates of trypanosomosis acquired along the

trade route were 54.7%, 6.7%, 2.7% and 5.3% for *T. vivax, T. brucei,T.congolense* and unidentified species of trypanosomes respectively (Folkers and Jones- Davies, 1966). The Northern Nigerian Veterinary Tsetse and Trypanosomiasis Unit Report of 1954 –55 showed a marked rise in the relative incidence of *T.congolense*, which were in accordance with results of Folkers and Jones-Davies (1966). Yesufu and Mshelbwala (1973) also confirmed the findings of Godfrey *et al*. (1965) that *T.vivax* was the most common species infecting trade cattle, while *T. congolense* was more common in the migratory cattle in northern Nigeria (Killick–Kendrick and Godfrey, 1963).*Trypanosoma congolense*, which is the major cause of cattle trypanosomosis in East Africa, is the second most important cause of bovine trypanosomosis after *T. vivax* in Nigeria (Anosa, 1991b).According to Griffin (1978), many of the accounts of naturally occurring trypanosomosis in sheep and goats came from Eastern Africa, and are due to *T. congolense* which is clearly the most widespread and economically important species in the region (Robson and Ashkar, 1972). Kalu (1991) in Jos, Plateau State, Nigeriascreened three hundred and fifty-five blood samples for trypanosomes using six diagnostic techniques and recorded prevalence of 51.6% in sheep, 33.3% in goats and 24.6% in cattle. *Trypanosoma vivax* and *T. congolense* were the most prevalent species encountered, accounting for 51.7% and 11.9% of all positive cases, respectively. In another study, using sheep and goats slaughtered at Bodija abbatoir, Ibadan, Kalejaiye *et al.* (1995) reported prevalence of2.28% and 4.20%in goats and sheep respectively. *Trypanosoma vivax*accounted for 1.65%, *T. brucei* 0.4% and *T. congolense* 0.2% of the infections in goats.

2.6 Life Cycles and Modes of Transmission of Trypanosomes

Trypanosomes are parasites with a two-host life cycle: one mammalian and one arthropod (Urquhart *et al.,* 1996; FAO, 2000). Tsetse flies ingest trypanosomes present in the blood or lymph while feeding on an infected host. Thereafter the trypanosomes lose their glycoprotein surface coat, and in the case of *T.brucei* and *T.congolense*, become elongated and multiply in the midgut before migrating forward to the salivary glands (*T.brucei*) and the proboscis (*T.congolense*) (Urquhart *et al.,* 1996). There, they undergo a transformation,losing their typical trypanosome or trypomastigote form and acquire an epimastigote form characterized by the fact that the kinetoplast lies just in front of the nucleus (Urquhart *et al.,* 1996; FAO, 2000). After a further multiplication of the epimastigotes they transform again into small, typically trypomastigote forms with a glycoprotein surface coat. These are the infective forms for the next host and are called metacyclic trypanosomes (Urquhart *et al.,* 1996). The entire process takes at least two or three weeks and the metacyclic trypanosomes are inoculated into the new host when the tsetse fly feeds (Urquhart *et al.,* 1996).With *T.vivax*,a similar process of cyclic development takes place except that it occurs entirely within the proboscis. At the site of inoculation the metacyclic forms multiply locally as the typical blood forms, producing within a few days a raised cutaneous inflammatory swelling called a chancre (FAO, 2000). Thereafter, they enter the bloodstream, multiply and a parasitaemia, detectable in the peripheral blood, usually becomes apparent 1-3 weeks later. Subsequently, the parasitaemia may persist for many months although its levels may change due to the immune response of the host (Urquhart *et al.,* 1996).

Trypanosomes use cyclic transmission where the arthropod vector is a necessary vector, in which the trypanosomes multiply, undergoing a series of morphological transformations before metacyclic forms (trypomastigotes) infectious to the next mammalian host are produced. When multiplication occurs in the digestive tract and proboscis, so that the new infection is transmitted when feeding, the process is known as anterior station development and the various species of trypanosomes which use this process are often considered as a group, the salivaria. All salivaria are trypanosomes transmitted by tsetse flies, the main species being *T.congolense,T.vivax* and *T.brucei* (Urquhart *et al.,* 1996; FAO, 2000). In other trypanosomes, multiplication and transformation occurs in the gut and the infective forms migrate to the rectum and are passed with the faeces.This is posterior station development and the trypanosome species using this process are the stercoraria. The human infective *T. cruzi* of South and Central America belongs to this group. In domestic animals these are all relatively non-pathogenic trypanosomes such as *T.theileria* and *T.melophagium*,transmitted by tabanid flies and keds respectively.

Non-cyclical transmission is essentially mechanical transmission in which the trypanosomes are transferred from one mammalian host to another by the interrupted feeding of biting insects, notably tabanids and *Stomoxys*. The trypanosomes in or on the contaminated proboscis do not multiply and die quickly so that cross contamination is only possible for a few hours (Urquhart *et al.,* 1996).The salivarian trypanosomes, normally transmitted cyclically in tsetse flies, may on occasion be transmitted mechanically (FAO, 1998; 2000). The importance of this latter mode of transmission is

variable from place to place, depending on the numbers of hosts and biting insects present, and also on the species of trypanosome. Large biting insects such as tabanids carry more blood and are more likely to act as mechanical vectors than for example mosquitoes. Tsetse themselves can also act as mechanical vectors (FAO, 1998).The life cycle of *T. evansi* is simple and direct. It does not undergo any development or multiplication in its insect vectors. The parasite multiplies by binary fission only in the animal host (Brun *et al.,* 1998). *Trypanosoma evansi* does not develop in its insect vectors because it lacks maxicircle DNA that is necessary for development in vectors (WHO, 2009).Apart from the classical cyclical and non-cyclical transmissions, dogs, cats and wild carnivores may become infected by eating fresh carcasses or organs of animals which have died of trypanosomosis; the parasite penetrating oral abrasions (Urquhart *et al.,* 1996).

2.7Antigenic Variation

Trypanosomes undergo antigenic variation that enables them to evade the host‘s immune system (Morrison *et al*., 2009). Antigenic variation occurs when a new generation of trypanosome arises that expresses a different variable surface glycoprotein (VSG). The VSG is a glycoprotein with a molecular weight of about 65,000 KDathat forms a 12-15 nm thick external monolayer over the plasma membrane and is attached to it by a glycosyl-phosphatidyl inositol (GPI) anchor (Blum *et al.,* 2008). It covers the entire parasite including the flagellum presumably as a protective shield against host antibodies (Morrison *et al*., 2009). This protective coat determines the antigenic features of the parasite; is easily recognised by the host and is highly immunogenic. Perhaps the most

important aspect of trypanosomosis which accounts for the persistent parasitaemia is the way in which the parasite evades the immune response of the host through antigenic variation (Urquhart *et al.,* 1996). The repeated switching of the glycoprotein coat is now known to depend on a loosely ordered sequential expression of an undefined number of genes, each coding for a different glycoprotein coat. This, together with the finding that metacyclic trypanosomes may be a variation of antigenic types each expressing a different genetic repertoire, explains why domestic animals even if treated successfully, are often immediately susceptible to reinfection (Urquhart *et al.,* 1996). Once a host is infected with a trypanosome manifesting a specific antigen, known as variable antigen type (VAT), the host responds by producing specific antibodies to eliminate the proliferating trypanosomes. Successive waves of parasitaemia occur when a new VAT becomes predominant. These cyclical waves of parasitaemia are characteristic of trypanosome infections and are associated with clinical signs of pyrexia (Losos, 1980; Dargantes *et al.*, 2005a). Two or several VATs may be present during the second and subsequent parasitaemic waves (Dargantes *et al.*, 2005a). Whilst some authors believe that the host‘s immune system triggers antigenic variation, others believe that antibodies selectively regulate rather than initiate VSG changes. This is because VSG switches have been observed *in vitro* in the absence of antibodies, in immuno-compromised animals and in early infections before antibodies are produced. There are over 1,000 genes encoding different VSGs but only 10, one gene coding for a particular VSG, is expressed at any point in time (WHO, 2009; Morrison *et al*., 2009). The expression of a new VSG may also involve genomic rearrangement (Morrison *et al*., 2009).

2.8Sheep Breeds and Distribution in Nigeria

Sheep are kept everywhere in Nigeria, with a broad distinction between their importance and ubiquity in the north, and the more dispersed populations of the humid zone. Sheep and goats are seen as having secondary importance in relation to crops.There are generally considered to be four breeds or races of sheep native to Nigeria namely, the Balami, Uda, Yankasa and West African Dwarf (WAD) (Adu and Ngere, 1979).

2.8.1 Balami

The Balami are the largest bodied native sheep in Nigeria and are confined to the semi-arid north, but it is favoured as a stall-fed breed by Muslims throughout the Nigerian Middle Belt (Aganga *et al*., 1988). It is white and hairy with pendulous ears and a long thin tail; rams have throat ruffs and are horned but ewes are normally polled.Another feature that makes the Balami distinctly recognisable is its Roman nose; a large bulbous nose that distinguishes it from the Yankasa (Aganga *et al*., 1988).

2.8.2 Uda

The Uda is slightly smaller-bodied than the Balami, although their size ranges overlap. It is easily recognised by a distinctive coat colour pattern, entirely brown or black forequarters and white behind. Uda sheep derive their name from a Fulani clan, the Uda'en, who herd large flocks of this breed between Niger and the northern reaches of the Nigerian Middle Belt (Adu and Ngere, 1979).

2.8.3 Yankasa

The Yankasa breed has been the most extensively studied in Nigeria (Aganga *et al*., 1988). The body colour is white with black patches around the eyes and sometimes on the feet. The muzzle and ears are usually black too. Rams have curved horns and hairy white

mane while ewes are polled. Yankasa sheep have been recorded in all parts of Nigeria, though the populations attenuate towards the northern border and the sea-coast (Aganga *et al*., 1988). Yankasa sheep do not need daily watering in the wet season and watering once a day suffices in the dry season (Aganga *et al*., 1988).

2.8.4 West African Dwarf

The West African Dwarf breed of sheep is a small-bodied, compact breed which may be all white, black, brown, or spotted black or brown on a white coat (Devendre and McLeroy, 1982). Its variation in colour and patchy distribution make it difficult to be distinguished clearly from the Yankasa. Adu and Ngere (1979) opined that different types exist, mentioning the 'Pagan' variety on the Jos Plateau, and the 'Umuahia' variety near the Niger-Benueconfluence, but there is no published account of such varieties. Devendre and McLeroy (1982) argued that the WAD breed cannot be subcategorised on the basis of appearance, and no performance data is available.

2.9 Reproductive Physiology of the Male

Proper understanding of reproductive physiology of the male sheep and related pathology requires appreciation of the anatomy and development of the male genital and ductal system, physiology of the testis, hormonal control of the testis, as well as the processes involved in the deposition of the seminal fluid within the female genital tract (Amann and Schanbacher, 1983).

2.9.1 Anatomy of male genitalia

The male genitaliaconsist of the testis, epididymis, vas deferens, ampulla, spermatic cord, vesicular gland, prostate gland, Copwer's gland and penis.

2.9.1.1 *Testes*

The testes are paired organs which descend from the abdominal cavity during foetal development to lie in the scrotum. They are the primary organs of reproduction in males, just as ovaries are primary organs of reproduction in females. Testes are considered primary because they produce the male gametes (spermatozoa) and secrete the male sex hormone, testosterone (Albert, 1961). Testosterone is essential for the development of male characteristics, maintaining normal sexual behaviour and sperm production. Testes differ from ovaries in that all potential gametes are not present at birth. Germ cells, located inthe seminiferous tubules, undergo continual cell divisions, forming new spermatozoa throughout the normal reproductive life of the male (Albert, 1961).

2.9.1.1.1 *Functional morphology of the testes*

The testis of the bull is 10 to 13 cm long, 5 to 6.5 cm wide and weighs 300 to 400 gm. The testis is of similar size in boars, but is smaller in rams, bucks (goats) and stallions. In all species, testes are covered with the tunica vaginalis.Thisoutpocketing of the body wall that surrounds the testes isa serous tissue, which is an extension of the peritoneum. This serous coat is obtained as the testes descend into the scrotum and is attached along the line of the epididymis. The outer layer of the testes, the tunica albuginea testis, is a thin white membrane of elastic connective tissue in which the seminiferous tubules and Leydig cells are encased (Albert, 1961). Numerous blood vessels are visible just under its surface. Beneath the tunica albuginea testis is the parenchyma, the functional layer of the testes. The parenchyma has a yellowish color and is divided into segments by incomplete septa of connective tissues. Located within these segments of parenchyma tissue are the seminiferous tubules. Seminiferous tubules are formed from primary sex cords. They

contain germ cells (spermatogonia) and nursa cells (Sertoli cells). Sertoli cells are larger and less numerous than spermatogonia. With stimulation by follicle stimulating hormones (FSH), Sertoli cells produce both androgen-binding protein and inhibin. Seminiferous tubules are the sites of spermatozoa production. They are small, convoluted tubules approximately 200um in diameter. It has been estimated that the seminiferous tubules from a pair of bull testes, streched out and laid end to end, approach 5 km in length. They make up 80% of the weight of the testes (Albert, 1961). Seminiferous tubules join a network of tubules, the rete testis, which connects to 12 to 15 small ducts, the vasa efferentia, which converge into the head of the epididymis(Figure 2.1).

Leydig (interstitial) cells are found in the parenchyma of the testes between the seminiferous tubules (Figure 2.2). Luteinising Hormone (LH) stimulates Leydig cells to produce testosterone and small quantities of other androgens. Secretion of testosterone by the Leydig cells provides a high concentration of testosterone around the seminiferous tubules which is essential for spermatogenesis (Albert, 1961). Testosterone is needed for the development of secondary sex characteristics and for normal mating behaviour. In addition, it is necessary for the function of the accessory glands, production of spermatozoa and maintenance of the male duct system. Through its effects on the male, testosterone aids in maintenance of optimum conditions for spermatogenesis (Albert, 1961).

2.9.1.2 *Scrotum*

The scrotum is a two-lobed muscular sac enclosing the testes and it supports and protects the testes and also plays a major role in temperature regulation.

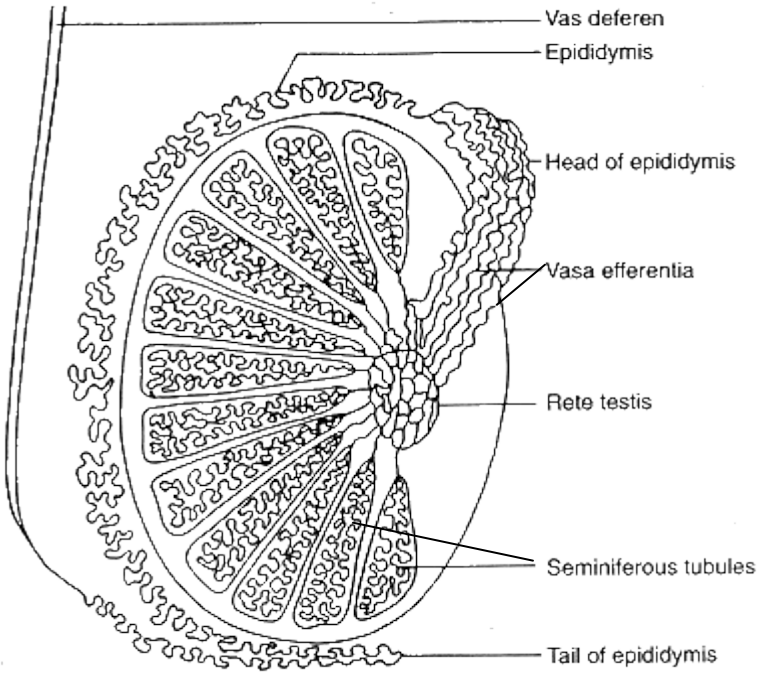


Figure 2.1: Sagittal section of testis illustrating segments of parenchymal tissue which contain the seminiferous tubules, rete testis, vasa efferentis, epididymis, and scrotal portion of the vas deferen (Amann and Schanbacher, 1983).

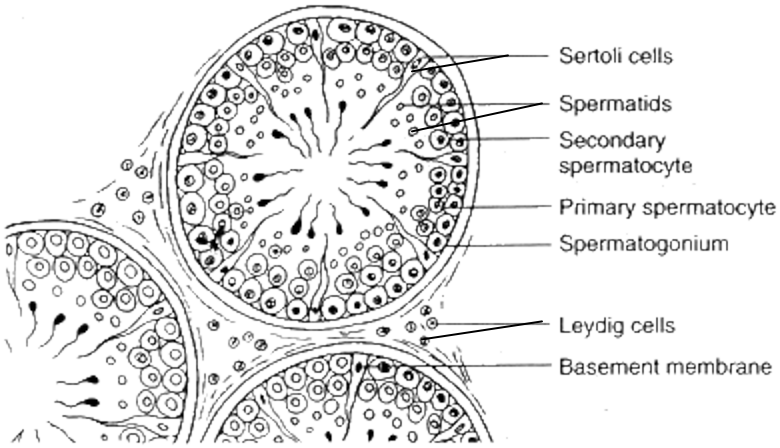


Figure 2.2: Cross section of parenchymal tissue showing relationship between the seminiferous tubules and interstitial tissue containing Leydig cells (Amann and Schanbacher, 1983).

It maintains thetemperature 3 to 5^{o}C below body temperature for optimal function.It is located in theinguinal region between the rear legs of most species. The scrotum has the same embryonic origin as the labia majora in the female. It is composed of an outer layer of thick skin with numerous large sweat and sebaceous glands. This outer layer is lined with a layer of smooth muscle fibres, the tunica dartos, which is interspersed with connective tissue. The tunica dartos divides the scrotum into two pouches and is attached to the tunica vaginalis at the bottom of each pouch (Bedford, 1978).

2.9.1.3 *Spermatic cord*

The spermatic cordconnects the testis to its life support mechanisms, the convoluted testicular arteries and surrounding pampiniform plexus, and nerve trunks. In addition, the spermatic cord is composed of smooth muscle fibres, connective tissue, and a portion of the vas deferens. Both the spermatic cords and scrotum contribute to the support of the testes. Also, they have a joint function in regulating the temperature of the testes (Bedford, 1978). Several examples can be given to illustrate the importance of temperature control of the testes. If a ram's scrotum is insulated, or the testes are tied against the abdomen, sterility results. The higher temperature causes degeneration of the cells lining the wall of the seminiferous tubules. Fertility will be restored if the testes and scrotum are returned to their natural state before total degeneration occurs. However, a few weeks will be required before fertile semen is again produced. The bilateral crytorchid is sterile, again illustrating that production of spermatozoa stops when the temperature inside the testes is as high as normal body temperature(Bedford, 1978). Low fertility semen produced by several species of farm animals during the summer has been

attributed to the inability of the body's cooling mechanisms to keep the testes cool enough (Bedford, 1978).

2.9.1.4 *Epididymis*

The epididymis is located in the testes and is a long and convoluted tube in which sperm cells produced by the testicles are stored and mature to a stage capable of fertilization. This maturation change occurs as sperm cells move from the head to the body of the epididymis with mature sperm being stored in the tail of the epididymis. The first external duct leading from the testis is fused longitudinally to the surface of the testis and is encased in the tunica vaginalis with the testis (Bedford, 1978). The single convoluted duct is covered with an extension of the tunica albuginea testis. The captut (head) of the epididymis is a flattened area at the apex of the testis, where 12 to 15 small ducts, the vasa efferentia, merge into a single duct. The corpus (body) extending along the longitudinal axis of the testis is a single duct which becomes continuous with the cauda (tail). The total length of this convoluted duct is about 34 metres in the bull and longer in the ram, boar and stallion. The lumen of the cauda is wider than the lumen of the corpus. The structures of the epididymis and other external ducts (vas deferens and urethra) are similar to that of the tubular portion of the female tract. The tunica serosa (outer layer) is followed by a smooth muscle layer (middle) and an epithelial layer (innermost) (Bedford, 1978).

2.9.1.5 *Vas deferens and urethra*

The vas deferens is a pair of ducts, each leading from the distal end of the cauda of each epididymis. Initially supported by folds of the peritoneum, it passes along the spermatic

cord, through the inguinal canal to the pelvic region, where it merges with the urethra at its origin near the opening to the bladder. The enlarged end of the vas deferens near the urethra is the ampulla. The vas deferens has a thick layer of smooth muscles in its walls and appears to have single function of transport of spermatozoa (Bedford, 1978). Removal of a section of the vas deferens in each testis is known as vasectomy; preventing passage of sperm from the epididymis. Some have suggested that ampullae serve as a short term storage depot for semen. However, spermatozoa age quickly in the ampullae. It seems more likely that spermatozoa may pool in the ampullae during ejaculation before being expelled into the urethra. The urethra is a single duct which extends from the junction of the ampullae to the end of the penis. It serves as an excretory duct for both urine and semen (Bedford, 1978).

2.9.1.6 *Accessory glands*

The accessory glands are located along the pelvic portion of the urethra, with ducts which empty their secretions into the urethra. They include the vesicular glands, the prostate gland and the bulbourethral glands. Accessory glands secrete additional fluids, which when combined with the sperm and other secretions from the epididymis, form the semen. They contribute greatly to the fluid volume of semen. Some of the secretions contain nutrients like fructose while others produce alkali secretion to raise the pH of the ejaculate (Bedford, 1978). These secretions are added quickly and forcibly during the mating to propel sperm into the urethra. In addition, their secretions are solution of bufers, nutrients, and other substances needed to assure optimum motility and fertility of semen (Bedford, 1978).

2.9.1.7 *The penis*

The penis functions as the organ of copulation in males. It forms dorsally around theurethra from the point where the urethra leaves the pelvis with the external urethral orifice at the free end of the penis(Bedford, 1978). Bulls, boars and rams havesigmoid flexure, an S-shaped bend in the penis which permits it to be retracted completely into the body. These three species and the stallion have retractor penis muscles, a pair of smooth muscles which relax to permit extension of the penis and contract to draw the penis back into the body. These retractor penis muscles arise from the vertebrae in the coccygeal region and are fused to the ventral penis just anterior to the sigmoid flexure(Bedford, 1978).The glans penis, which is the free end of the penis, is well supplied with sensory nerves and is homologous to the clitorisin the female. In most species the penis is fibro elastic, containing small amounts of erectile tissues. The penis of stallion contains more erectile tissues than are found in bulls, boars, bucks and rams (Bedford, 1975; 1978).

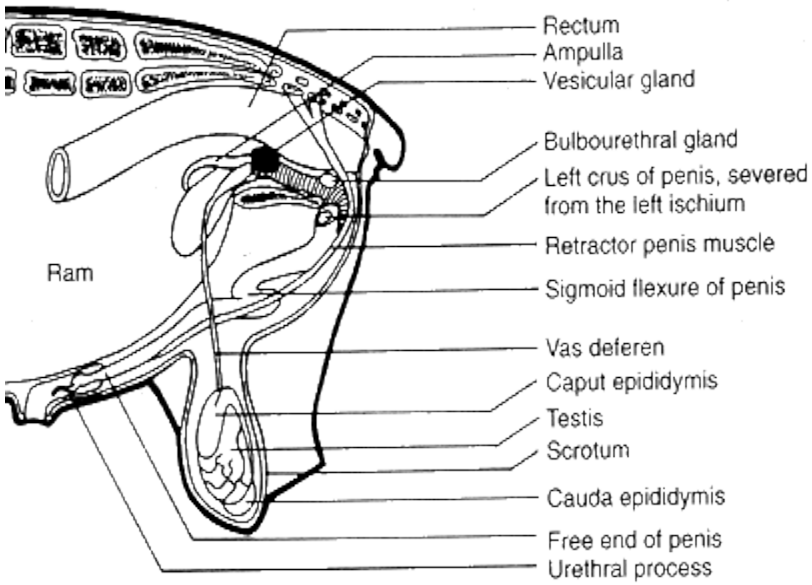


Figure 2.3:The reproductive system of a ram (Albert, 1961)

2.10 Pathogenesis of Trypanosomes

Initial replication of trypanosomes is at the site of inoculation in the skin which results in a swelling and a sore (chancre) and then spread to the lymphnodes and blood and continues to replicate (FAO, 2000). *Trypanosoma vivax, T.congolense* and *T.brucei* are characteristically present in the bloodstream. *Trypanosomabrucei* is also found extravascularly in tissues, for example, the myocardium, the central nervous system and the reproductive tract (FAO, 1998). Lymphoid enlargement and splenomegaly develop, associated with plasma cell hyperplasia and hypergammaglobulinaemia, which is primarily due to an increase in immunoglobulin M. Concurrently there is a variable degree of suppression of immune responses to other antigens such as microbial pathogens or vaccines (FAO, 1998). Ultimately, in infections of long duration, the lymphoid organs and spleen become shrunken due to exhaustion of their cellular elements. Anaemia is a cardinal feature of the disease, particularly in cattle, and initially it is proportional to the degree of parasitaemia (FAO, 2000). It is haemolytic in that the red blood cells are removed from circulation by the expanding mononuclear phagocytic system. Cell degeneration and inflammatory infiltrates occur in many organs such as the skeletal muscles and the CNS, but perhaps most significantly in the myocardium, where there is separation and degeneration of the muscle fibres (Urquhart *et al.*, 1996). The responses of antibodies developed against the glycoprotein coat of the trypanosomes kill the parasites and result in the development of immunocomplexes. Antibodies however do not clear the infection, since trypanosomes have genes that can code for a number of different surface-coat glycoproteins and therefore change their surface antigenic makeup to evade the antibodies. Thus there is a persistent infection that results in a continuing cycle of

trypanosome replication, antibody production, immunocomplex development and changing surface-coat glycoproteins(Urquhart *et al.*, 1996).

2.11 Prepatent Period and Parasitaemic Waves

The prepatent period varies from species to species and depends on the susceptibility of the host, pathogenicity of the isolate, route and dose of infection and the diagnostic method used to detect parasitaemia. The prepatent period in mice infected with approximately 10^4 trypanosomes was 4 to 12 hours, 1 day and 2 to 5 days post-infection using polymerase chain reaction (PCR), micro-haematocrit centrifugation technique (MHCT) and direct blood examination, respectively (Ijaz *et al.*, 1998; Fernández *et al.*, 2009). Likewise, data from experimental studies indicated that trypanosomes could be detected in blood as early as 24 hours in horses (Wernery *et al.*, 2001), 48 hours in goats and cattle (Payne *et al.*, 1993; Dargantes *et al.*, 2005b), 3 days in Rusa deer, pigs and sheep (Audu *et al.*, 1999; Reid *et al.*, 1999), 6 days in Australian wallabies (Reid *et al.*, 2001), and 14 days in buffalo (Holland *et al.*, 2001a) by MHCT. The prepatent period is 3 days in buffalo if mouse inoculation test (MIT) is used (Holland *et al.*, 2001a). The prepatent period of as early as 2 days, have been reported in South American coati (Herrera *et al.*, 2002), 5 days in rats (Al-Mohammed, 2006), and 9 days in dogs (Aquino *et al.*, 2002) after intravenous infection with *T.evansi*. Raina *et al*. (1985) observed a prepatent period of 8 to 12 days and 2 to 10 days, respectively, following oral challenge of dogs and mice with *T. evansi*. The characteristic fluctuating parasitaemia in animals infected with *T. evansi* is due to the interplay of the immune response of the host and the ability of the parasite to evade it by antigenic variation. These waves of parasitaemia are

directly related to the rise and fall of the body temperature of the host and occur at intervals of 6 to 7 days in cattle (Payne *et al.*, 1993), 8.3 days in sheep (Audu *et al.*, 1999) and 13 to 19 days in goats (Dargantes *et al.*, 2005b).

2.12 Clinical Signs

Because simultaneous infection with more than one trypanosome species are very common and simultaneous infection with trypanosomes and other hemoparasites (*Babesia* spp,*Theileria spp, Anaplasma* spp.and *Ehrlichia* spp.) frequently occurs it is difficult to conclude which clinical signs are attributable to a given parasite (FAO, 1998; FAO, 2000). Anaemia is for example seen in a whole series of diseases caused by blood parasites (in particular *Babesia* and *Anaplasma*) as well as in certain gastrointestinal helminthes (*Haemonchus contortus*) and it is therefore not typical of trypanosomes by itself (Nyenko *et al.,* 1990).The major clinical sign in African Animal trypanosomosis is anaemia. Also invariably present are intermittent fever, roughy haircoats, edema and loss of condition. Abortion is seen and infertility of males and females may be a sequel. The severity of the clinical response is dependent on the species, the breed of the affected animal, the dose and virulence of the infecting trypanosome. Stress such as poor nutrition, or concurrent disease, plays a prominent role in the disease process. Within a week of infection with the haematic trypanosomes (*T.congolense* and *T.vivax*) there is usually a pronounced decrease in packed cell volume, haemoglobin, red blood cells and white blood cell levels and within 2 months these may drop to below 50% of their pre-infective values (FAO, 1998).

2.13 Anaemia

At an early stage there is phagocytosis of red cells by the white cells of the host. The red cells apparently become coated with toxins and material from trypanosomes which tricks the phagocytes into mistaking them for foreign invaders and remove them (auto-immunity). It is possible that the anaemia caused by phagocytosis is increased by toxic substances emanating from the trypanosomes, which destroys cells directly by lysis (FAO, 1998).The haematopoietic system tries to compensate for loss of erythrocytes by increasing its activity but later in the chronic stages of trypanosomosis, other toxins from the parasite have a depressing effect on the haematopoietic system and the host is unable to produce as many red cells as are removed. The anaemia means a reduction in haemoglobin and therefore in the oxygen carrying capacity of the blood. Insufficient oxygen is available to the cells and the efficiency of their normal activities is reduced leading to deterioration of health condition (FAO, 1998).

The mechanism or pathophysiology of anaemia in trypanosomosis is complex and multifactorial in origin (Naessens *et al*., 2005). It initiates a cascade of events leading to haemolytic anaemia and cardiovascular collapse (Anosa, 1988). In human trypanosomosis, disseminated intravascular coagulation has been reported (Barret-Connor *et al*., 1973).Among the complex and multifactorial etiologies associated with the anaemia is haemolysin, a sensory/excretory product of living trypanosomes. This product is known to lyse red blood cells in the absence of antibodies (*in-vitro*) and causes haemodilution (*in-vivo*). This mechanism has been adequately described in gold fish (*Carassius auratus*) infected with *Trypanosoma dahilewskyi* (Nazrul-Islam and Woo,

1991) and in murine models infected with *T. b. rhodesiense* (Naessens *et al*., 2005). Haemolytic anaemia caused by animal and human trypanosomes has been reported in *T. brucei* infection of red fronted gazelles (*Gazellarufifrons*) (Mbaya *et al*., 2009a), sheep and goats (Ikede and Losos, 1972; Anosa, 1977). Similarly, it was reported in vervet monkeys (*Cercopethicus aethiopes*) (Abenga and Anosa, 2006), and baboons (*Papio anubis*) (Mbaya *et al*., 2009b).

2.14 Various Stages of Anaemia in Trypanosomosis

Three phases of anaemia have been reported to occur in trypanosomosis. These include Phase I (acute crises), phase II (chronic) and phase III (recovery) (Anosa, 1988).

2.14.1 Phase I: Acute crises

This phase begins with the initial appearance of trypanosomes in peripheral circulation. The parasitaemia in this case is usually high, fluctuating and evident in most days (Abenga and Anosa, 2006; Mbaya *et al*., 2009a; Mbaya *et al*., 2010;Mbaya *et al*., 2011). During this phase the anaemia is morphologically classified as macrocytic and normochromic (Maxie and Losos, 1979; Anosa and Isoun, 1980). At this stage death commonly occurs due to severe pancytopoenia and other pathologies (Anosa, 1988). Sub-acute cases have been produced experimentally in rodents infected with *T. congolense* (Isoun and Esuroso, 1972) and with *T. brucei* (Mbaya *et al*., 2011).

2.14.2 Phase II: Chronic

This phase follows the acute crises phase and is characterized by low levels of parasitaemia with low to moderate erythrocyte values at this point persists with minor fluctuations. This period ranges from several weeks to months. With the *T. brucei* groups

which mostly invade tissues, this is the aparasitaemic phase when the parasites establish extravascularly and are less numerous in peripheral circulations (Rabo, 1995) or absent (Mbaya *et al*., 2007, 2009a). In this chronic phase, the morphological classification of the anaemia is normochromic and normocytic (Maxie and Losos, 1979).

2.14.3 Phase III: Recovery

This phase is characterized by the low, infrequent or absence of parasitaemia. At this point, declined erythrocyte values begin to return towards pre-infection values and other pathological changes undergo resolution (Anosa, 1988) leading to self-recovery as commonly encountered in trypanotolerant wildlife (Mbaya *et al*. 2009a).

2.16 The Mechanism of Anaemia in Trypanosomosis

The interplay of several factors acting either individually or synergistically contributes to the development of haemolytic anaemia in human and animal trypanosomosis. Most common among these factors are erythrocyte injury caused by lashing action of trypanosome flagella, undulating pyrexia, platelets aggregation, toxins and metabolites from trypanosomes, lipid peroxidation and malnutrition (Saror, 1982; Igbokwe, 1994).

2.16.1 Anaemia through mechanical injury to erythrocytes

Anaemia caused by mechanical injury to erythrocyte occurs by the lashing action of the powerful locomotory flagella and microtubule reinforced bodies of the millions of the organisms during parasitaemia (Vickerman and Tetley, 1978). Erythrocyte membrane damage has also been associated with adhesion of erythrocytes, platelets and reticulocytes to trypanosome surfaces via sialic acid receptors leading to damages to

erythrocyte cell membranes (Bungener and Muller, 1976; Banks, 1980; Anosa and Kaneko, 1983; Shehu *et al*., 2006). As such, several areas of discontinuity occur along the surface of erythrocyte membranes where they adhere to the trypanosomes. Mechanical damage to vascular endothelium has been reported when tissue-invading trypanosomes such as the *T. brucei* group penetrate tissues via the interstices (Anosa and Kaneko, 1983).

2.16.2 Anaemia through undulating pyrexia

In trypanosomosis, a direct relationship exists between undulating pyrexia and fluctuating parasitaemia (Nwosu and Ikeme, 1992; Igbokwe, 1994; Mbaya *et al*., 2009a). Under laboratory conditions, Karle (1974) exposed erythrocytes to temperatures above the normal body temperature and found out that the osmotic fragility and permeability of erythrocytes were greatly enhanced. It was also reported that increased body temperatures decreased erythrocyte plasticity and longevity invivo (Woodruff *et al*., 1972). Consequently, temperature elevation increased the rate of immunochemical reactions thereby initiating lipid peroxidation of erythrocytes (Igbokwe, 1994).

2.16.3 Anaemia through platelets aggregation (Microangiopathy)

Intact trypanosomes or fragments of trypanosomes may cause platelet aggregation commonly called microangiopathy (Davies *et al*., 1974). This can lead to the release of platelets autoantibodies that in turn releases procoagulants and thereby causing fibrin deposits. Subsequently, microthrombi formation or disseminated intravascular coagulation occurs (Igbokwe, 1994). During trypanosomosis, erythrocytes with weak cell

membranes become fragmented and lysed as they squeeze through the fibrin deposits of the microthrombi (Anosa and Kaneko, 1983; Murray and Dexter, 1988). Disseminated intravascular coagulation has been reported in *T. b. gambiense* infection of the baboon (*Papio Anubis*) (Mbaya *et al*., 2009b), *T. vivax* infection of cattle (Isoun and Esuroroso, 1972) and in goats (Vanden Ingh *et al*., 1976; Anosa and Isoun, 1983).

2.16.4 Anaemia caused by trypanosome toxins and metabolites

Living and dead trypanosomes can produce various forms of active chemical substances, which can elicit erythrocyte injury (Zwart and Veenendal, 1978; Naessens *et al*., 2005). Common among these chemical substances are proteases, neuraminidase, phospholipase, free fatty acids, pyruvates and aromatic byproducts. Neuraminidase has been generated *in-vitro*by *T. vivax* during periods of parasitaemia, making erythrocytes prone to phagocytosis (Esievo, 1979; 1983). One of the factors that make erythrocytes prone to phagocytosis by the expanded mononuclear phagocytic system (MPS) during trypanosomosis is associated with the activity of neuraminidase. This enzyme cleaves off sialic acids on the surface of erythrocytes and thereby disabling them (Verma and Gautam, 1978; Igbokwe, 1994; Adamu *et al*., 2009) and by damaging erythropoietin (Igbokwe *et al.*, 1989).Trypanosomes are capable of releasing proteolytic lysosomal enzymes (proteases) from pockets on their flagella and from damaged or dead trypanosomes (Vickermon and Tetley, 1978; Rautenberg *et al*., 1982; Londsdale-Eccles and Grab, 1986; Igbokwe, 1994). The enzyme, when released into the general circulation is capable of damaging erythrocytes and vascular endothelium by cleaving sialic acid fractions from the cell membrane in the form of glycopeptides (Cook *et al*., 1966). It was

also reported that aromatic amino acids could be metabolized by trypanosome to produce toxic by-products, which acts directly on the erythrocyte cell membrane to cause osmotic fragility and lyses (Igbokwe, 1994). Similarly, phenylalanine could be catabolized to phenylpyruvate, which is proteolytic in nature and inhibitory to mitochondrial gluconeogenesis (Igbokwe, 1994). Tryptophan can also be broken down during trypanosomosis to indole-ethanol, which damages erythrocyte cell membranes (Igbokwe, 1994).

2.16.5 Lipid peroxidation

The mechanism of anaemia in trypanosomosis is greatly associated with the generation of free radicals and super oxides following lipid peroxidation. These oxidative products generally attack the cellular integrity of erythrocytes during trypanosomosis (Anosa and Kaneko, 1983; Igbokwe, 1994; Umar *et al*., 2007). They also particularly attack erythrocyte membrane polyunsaturated fatty acids and proteins (Slater, 1984) or red blood cells directly leading to oxidative haemolysis (Umar *et al*., 2007). Sialic acids consist of about four derivatives of nine-carbon sugar neuraminic acids (Varki, 1992; Schauer and Kamerling, 1997). It was therefore concluded that anaemia in trypanosomosis might occur due to erythrophagocytosis (Holmes and Jennings, 1976) and may be associated with the formation of antigen-antibody complexes with sialic acids (Audu *et al*., 1999). Esievo *et al*. (1982) pointed out that trypanosomosis may cause a deficit in the systematic antioxidant capacity of the infected host. This has been demonstrated in acute *T. b. gambiense* infection in rats (Ameh, 1984), *T. evansi* (Wolkmer *et al*., 2009) and in *T. brucei* infected mice (Igbokwe *et al*., 1989), where

erythrocytes were susceptible to free radical-damage following hydrogen peroxidation, leading to enhanced oxidative haemolysis in mice. Peroxidation caused the erythrocytes to produce large quantities of lipid peroxidation by-products. This is suggestive therefore that erythrocytes of the infected animals possessed decreased antioxidant ability, leading to its inability to withstand oxidative stress (Igbokwe, 1994). *Trypanosoma vivax* produced neuraminidase enzyme, which had a direct relationship with parasitaemia. It was therefore concluded that neuraminidase produced by trypanosomes invivo, cleaved off erythrocyte surface sialic acid, making the red cells prone to phagocytosis. Similarly, Nok and Balogun (2003) showed a progressive increase in the level of serum sialic acid corresponding with anaemia and parasitaemia in *T. congolense* infected mice. *Trypanosoma vivax* was observed to be highly erythrogenic in mice, which was probably associated with depressed erythropoietin activity following the cleaving of sialic acid fragments (Igbokwe *et al*., 1989). It has also been reported that glycolysis in trypanosomosis leads to the accumulation of pyruvate *in-vivo* as parasitaemia increases (Grant and Fulton, 1957; Coleman and Von, 1957). A ten-fold increase of pyruvate has been observed in *T. brucei*-infected rabbits (Goodwin and Guy, 1973). In as much as the influence of pyruvate is debatable, it may not reach toxic levels in the blood during trypanosomosis (Igbokwe, 1994) however, Newton (1978) suggested that pyruvate might lead to acidosis and a lowered affinity of haemoglobulin for oxygen. It also inhibited the tricarboxylic acid cycle (TCA) in human mitochondria during *T.b*. *gambiense* infection (Seed and Hall, 1985). After death and autolysis, trypanosomosis releases large quantities of phospholipase A1 and lysophospholipase A1 (Tizard *et al*., 1978b). These chemical substances can cause erythrocyte degradation, damage to vascular endothelial cells and

haemolysis (Colley *et al*., 1973). Phospholipase A1 was demonstrated in extreme proportions *in-vitro* in tissue fluids and less in plasma of rabbits infected with *T.brucei* (Hambrey *et al*., 1980). Tizard *et al*. (1978a) observed that phospholipase released free fatty acids (FFA) from phosphatidylcholine *in-vivo*. Most common of them were palmitic, stearic and linoleic acids (Tizard and Holmes, 1977). Tizard *et al*. (1978b) reported that linoleic acid possessed a detergent - like activity, which produced severe haemolysis and cytotoxicity invitro. It was however believed that free fatty acids are easily bound by albumin and may not cause haemolysis invivo. The author however pointed out that during high parasitaemia in *T. congolense* infection, the FFA released exceeded the binding capacity of albumin and thereby leading to cytotoxicity and haemolysis. Similarly, it was reported that even the albumin bound FFA may cause haemolysis due to the activities of its oxidized products. Nok *et al*. (1992a, b) reported that trypanosomes could cause certain alterations that invariably affected erythrocyte membrane fluidity hence a decrease in erythrocyte membrane-bound enzymes (NaK-ATpase and CaMg-ATpase). Lipid peroxidation of membranes has been associated with decrease in membranes fluidity and in the activities of membrane-bound enzyme (McCay and King, 1980; Slater, 1984; Igbokwe, 1994). It was however suggested that a comprehensive study in ruminants is needed to highlight the extent of anti-oxidant deficiency and the degree of susceptibility of red cells to oxidative damage during trypanosomosis (Igbokwe, 1994).

2.16.6 Idiopathic serum factors

In trypanosomosis, an unknown (idiopathic) serum factor, not of a trypanosome origin but a heat stable-protein has been demonstrated to inhibit activities of erythropoiesis

(Kaaya *et al*., 1979; 1980). It was reported that serum from cattle infected with *T. congolense* and *T. vivax* did not depress colonies of erythrocytes invitro. However, an unknown serum factor entirely different from those reported by Kaaya *et al*. (1979; Kaaya and Odour-Okelo, 1980) had effect on anerythroid colony (Igbokwe *et al*., 1989).

2.16.7 Immune complexes

Immunological mechanisms in trypanosomosis have been advanced as a major reason for the removal of erythro autolologous immunoglobulin (IgM and IgG) antibodies and complement (C3) on the surface of red cells (Kobayashi *et al*., 1976; Facer *et al.*, 1982; Assoku and Gardiner, 1992; Naessens *et al*., 2005). The mechanism suggested that autoantibodies appeared after the first peak of parasitaemia that correlated with the decline in packed cell volume (PCV). Red cell surfaces may bind auto or poly reactive antibodies, or may be sensitized by absorption of immune complexes (Naessens *et al*., 2005). Alternatively, erythrocytes may passively absorb trypanosome molecules followed by binding of antitrypanosome antibodies with subsequent removal from the system (Murray and Dexter, 1988; Rifkin and Lansberger, 1990; Naessens *et al*., 2005). Several authors (Ikede and Losos, 1972; Mackenzie and Cruickshank, 1973; Mackenzie *et al*., 1978; Anosa and Isoun, 1980; Igbokwe, 1994) reported an overwhelming proliferation of tissue macrophages during trypanosomosis. The activation of macrophages was through lymphokines, antigen-antibody complexes and C3b complement fragments (Allison, 1978). It was suggested therefore, that cytokines mediated loss of erythrocytes in trypanosomosis (Naessens *et al*., 2005). Similarly, strong evidence suggested that anaemia in trypanosomosis was mediated by TNF-a, IFN gamma and other inflammatory

cytokines (Jelkmann, 1998). Nemeth *et al*.(2004) suggested that anaemia in trypanosomosis involving hypofferaemia was caused by IL-6 and hepeidin. Other studies concluded that this is unlikely to cause anaemia in trypanosomosis, but some weak evidence of the role of THF-a-in the severity of anaemia in trypanosome-infected cattle, mice infected with *T. brucei*,and in *T. brucei gambiense* infected mice (Silegham *et al.*, 1994a; Magez *et al*., 1999; Naessens *et al*., 2005).

2.16.8 Malnutrition

Trypanosomosis may cause a drop in feed intake hence there is energy deficit and loss of tissue associated with catabolism of body fat, deficiencies of vitamin C, B and essential amino acids (Igbokwe, 1994). Inadequate energy supply to erythrocytes may alter the erythrocyte membrane surface therefore leading to weakening of the cell membrane, increased osmotic fragility and haemolysis (Jennings, 1976).

2.16.9 Tumor necrosis factor/Bone marrow nitric oxide (NO)

It has been reported that tumor necrosis factor (TNF) production by monocytes from cattle infected with *Trypanosoma* (*Duttonella*) *vivax* and *T.* (*Nannomonas*) *congolense*, was found to play in concert in the severity of anaemia associated with trypanosomosis (Silegham *et al*., 1994b). Bone marrow cell population from *T. brucei* infected mice exhibited levels of bone marrow nitric oxide production. This was found to coincide with suppressed bone marrow T-cell proliferation in response to stimulation with mitogen concanavalin *invivo*and *invitro*.It was therefore concluded that nitric oxide might inhibit proliferation of haemopoitic precursors leading to anaemia in trypanosomosis (Mabbot

and Sternberg, 1995). A similar synthesis of nitric oxide and cytokines leading to anaemia in human trypanosomosis has been reported (MacLean *et al*., 2001).

2.17. Devastating Effects of Trypanosomosis on Livestocks

2.17.1 Immunosupression

Host immunosuppression is a well-known feature of bovine, human, and murine trypanosomiasis In some cases, suppression is specific, involving only the host response to the parasite; in others, generalized suppression occurs in the infected animals, marked by a decrease in T cell proliferation and cytokine production (Taylor and Mertens, 1999). This is why the immunological response to various heterologous and non-parasitic antigens is weak in cows (Sileghem *et al*., 1994a). The occurrence of secondary bacterial infections in *T. vivax*-infected ruminants lends support to the features described above (Anosa and Isoun, 1980). As noted earlier, immunosuppression was first identified based on increased susceptibility of trypanosome-infected hosts to secondary infections and has been further studied in this light. This is a relevant phenomenon, as cattle infected in the field often die of opportunistic infections. Disease-associated suppression also affects host immune response to trypanosomes. Nonetheless, the role of suppression in parasite control is not clear (Sileghem *et al*., 1994a). Several studies have been conducted on the humoral response to parasite antigens. Williams *et al*. (1996) and Taylor *et al*., (1996) found that trypanosome-susceptible cattle produce lower levels of IgG against trypanosome-specific antigens than do tolerant cattle. Two hypotheses have been generated to try to explain the decreased antibodies titers observed in infected cattle. The first of these is that the mechanism involves the increased catabolism of serum

immunoglobulins (Nielsen *et al*., 1978) or antigenic competition (Nantulya *et al*., 1982). It has also been proposed that macrophages play a central role in initiating immunosuppression by interacting with the parasite (Sileghem *et al*., 1994b).

2.17.2 Animal productivity

The temperature rise in infected animals suggests an increase in metabolic rate and maintenance requirements by 20 and 30%. Increase in maintenance requirements would decreases net energy required for growth, faetal development and milk production. Trypanosomiasis reduced body weight (due to reduced feed intake), milk production and feed conversion efficiency and caused reproductive wastage, abortion, dam mortality and poor weaning weight (Zwart*et al*., 1991).

2.17.3 Work performance

Infection with *T. evansi* can result in reduced work output of draught animals(Payne *et al*., 1991). High mortality in buffaloes infected with *T. evansi* has been associated with periods of intense work(Payne *et al*., 1991). The stress induced by work probably results in increased corticosteroid secretion that could, in turn, aggravate the outcome of infection by depressing the host's immune response. *T. evansi* infection had a marked effect on the packed cell volume (PCV) and temperature profiles of the buffaloes (Payne *et al*., 1991). Prolonged fall in PCV in *T. evansi*-infected animals could result in a decline in the oxygen-carrying capacity of the blood. Maintenance of an elevated body temperature in *T. evansi*-infected animals results in a reduction in energy available for metabolic activity and work. The estimated energy expenditure ranged from 1.22-1.32

times maintenance energy when the buffaloes traveled a distance of 12- 15 km/day at a speed of 1.11-1.39 m/s (Payne *et al*., 1991).

2.17.4 Reproductive performance

Reproductive disorders in both males and females are part of the clinical features of African trypanosomosis in man and his domestic animals (Apted, 1970).Osaer *et al*. (1998) suggested that trypanosome infection can reduce ewe productivity by acting directly on the pregnant uterus and also by disrupting cyclicity and preventing a return to estrus. Both energy-restricted and protein-restricted diets can aggravate the detrimental effects of *T. congolense* infection on growth and productivity in sheep (Katunguka *et al*., 1993; 1995). Supplementation has beneficial effect in pregnancy in trypanosome-infected dams including reduced ewe and prenatal lamb mortality. Reduction in both luteal and placental progesterone secretion might include gonadal atrophy due to thrombosis in the gonadal blood vessels, severe anaemia and thermal effects of pyrexia (Mutayoba *et al*., 1988a). Trypanosomes have been shown to reduce secretion of, and gonadal sensitivity to, gonadotropins (Mutayoba *et al*., 1996) possibly as a result of hypothalamic-pituitaryadrenal activation. Haematic forms of trypanosomes cross the placental barrier possibly leading to disturbance of placental progesterone production and fetal death.Genital lesions have been reported in female animals infected with trypanosomosis. Studies in ruminants infected with *T. brucei*, *T. congolense* or *T. vivax* have shown that infection can lead to irregular oestrus cycles and intra- uterine infections with abortions in females (Ikede and Losos, 1972; Ige and Amodu, 1975). Pathologic changes include cystic degeneration in cattle, sheep and goats (Vohradsky, 1971; Mutayoba *et al*., 1988a).

Static ovaries, characterised by the absence of follicles or corpora lutea have been reported in Zebu cows (Ogwu *et al*., 1984). Ileweyn *et al*. (1987) reported in Boran cows infected with *T. congolense* stoppage of cyclical activity. Zebu cows similarly infected with *T contgolense* by Ogwu and Njoku (1992) developed atrophic ovaries with multiple follicular cystic degeneration and total absence of corpora lutea. These reports were in contrast to presence of persistent corpora lutea reported in other studies (Luckins *et al*., 1986). In cattle, flaccidity and atrophy of the uterine horns have been reported (Ogwu *et al*., 1984). Other reported pathological findings include reduced kidding percentage in *T. congolense*-infected goats (Kanyari *et al*., 1983), abortions in various stages of pregnancies, retained placenta and premature births, birth of weaklings and neonatal mortalities (Sekoni, 1994; Silva *et al*., 2013). Pathogenesis of tissue and organ damage in trypanosomosis that result in reproductive disorders are not well understood (Sekoni, 1994). Anomalies of the oestrous cycle as a result of trypanosome infections have been reported. In sheep and goats, anomalies observed include; silent estrous, irregar estrous, temporary and permanent anestrous. Ogwu *et al*. (1984) showed that pyrexia adversely affected the uterus, ova and the embryo, resulting in conception failure and embryonic death.Direct effects of the trypanosomes on the embryo and other vital organs have also been reported. Direct ivasion of offspring shortly after birth through trans-placentatal transmission has been reported in sheep (Ikede and Losos, 1972). Trypanosomes have been known to cause lesions in the higher centres (hypothalamus, pituitary) that control reproduction, resulting in depressed plasma progesterone, elevated corticosteroids (Mutayoba *et al*., 1988b; Ikede and Losos, 1975).

In males,the infection is characterized by elevated sperm morphological abnormalities (Sekoni, 1994; Okubanjo *et al*., 2014). Morbid changes or functional disturbances in the testis, epididymis, and vas deferens or in the accessory glands can lead to a significant increase in the number of morphological sperm abnormalities in the ejaculate (Morrow, 1980; Sekoni, 1992; Okubanjo *et al*., 2014). Testicular hypoplasia, degenerative changes of the seminiferous epithelium and inflammatory changes have been associated with defective spermatogenesis (Hafez, 1980). Increased scrotal diameters, scrotal inflammation with scab formation, peri-orchitis, epididymitis, severe testicular degeneration were among the many pathological changes observed in *T.brucei brucei* infected rabbits (Ikede and Akpavie, 1982). In *T. vivax* infected rams and bucks, several authors (Isoun and Anosa, 1974; Agu *et al*., 1986; Sekoni, 1992; 1993; Okubanjo *et al*., 2014) reported a massive scrotal enlargement, severe testicular, epididymal degeneration and deterioted semen characteristics. Deteriorated semen characteristics have also been reported in West African short horn bulls infected with *T. congolense* (Grundler and Djabakou, 1985; Grundler *et al.*, 1988). Mulligan e*t al*. (1970) reported that in cattle with chronic infections of *T. congolense*, there may be loss of libido and interference with reproduction and that infected calves may fail to reach maturity. An investigation on the effect of *T. vivax* and *T. congolense* infections on reproduction in Zebu bulls revealed the *T. congolense* to be more pathogenic (Sekoni *et al*., 1988; 1990). In these studies, there was progressive elevation of semen ejaculation time and poor semen quality manifested by all or some of the following: lack or decreased volume of semen, oligospermia, aspermia, more than 70% dead spermatozoa, very poor sperm motility and 100% morphological sperm abnormalities (Sekoni *et al*., 1988; 1990a; 1990b). Chemotherapy

with homidium chloride 12 weeks post infection failed to reverse the deteriorated semen characteristics to their pre-infection level (Sekoni *et al*., 1988; 1990a; 1990b). Osaer *et al*. (1997) in a study involving *T. congolense* infection in Djallonke rams reported reduced libido with a higher rate of refusals and production of poor quality semen with some adverse effects on mass motility, percentage of live sperm cells and minor sperm cell morphology.

Histopathological examination of infected male genitalia revealed severe degeneration of seminiferous tubules and interstitial leydig cells, there was lack of epididymal sperm reserve (Sekoni *et al*., 1990). In *T. congolense* infections in small ruminants, testicular degeneration (Kaaya and Oduor-Okelo, 1980; Anosa and Isoun, 1980) and reduction in plasma testosterone levels have been reported (Waindi *et al*., 1986; Adeyemo *et al*., 1990). Osaer *et al*. (1997) observed that the inflammatory changes in the genital organ due to the invasive character of the parasites in *T. brucei* infections are not found in infections with *T. vivax* and *T. congolense*, where testicular degeneration is well marked, mainly as the result of thrombosis in the spermatic blood vessels and prolonged fever. They reported that testicular and epididymal lesions lead to drastic and progressive deterioration in semen quality as revealed by a decrease in motile sperm, a sharp increase in percentage of dead sperm and a higher percentage of abnormal spermatozoa in rams. The mechanisms of damage to the male genitalia that result in reproductive disorders partly involve the direct effect of the trypanosomes (Sekoni, 1994). Trypanosomes have been shown to be present in the interlobular tissue of severly degenerated testes and in the epididymides of rabbits, mice and rams infected with pathologic trypanosomes (Ikede

and Akpavie, 1982). The resultant effect has been decreased or complete cessation of sperms production resulting from impairment of spermatogenesis (Sekoni *et al*., 1988). Waindi *et al*. (1986) suggested the mechanisms of trypanosome individual testicular dysfunction as pituitary hormonal defects, fever and stress. Chronic intermittent pyrexia following trypanosome infection has been incriminated (Anosa and Isoun, 1980). Some of the mechanisms of tissue and organ damage involve physiological disturbances due to the chronic and fluctuating pyrexia, massive and persistent immunological reactions and direct trypanosome invasion of tissues (Sekoni, 1994).

2.17.5 Endocrine system

The thyroid gland is one of the endocrine organs, which is affected during trypanosomiasis.*T. congolense* infection rapidly impaired the function of the thyroid gland in goats as defined by considerable plasma thyroxin (T4) decrease (Mutayoba *et al*., 1996) and in human sleeping sickness showed decreased in T3 and T4 without TSH variation(Mutayoba *et al*., 1994; 1996). Plasma concentration of thyroid hormone correlated positively with energy intake. Decrease T4 level in trypanosome-infected animals could have an adverse effect on feed intake and efficiency of feed conversion in spite of apparent good appetite. The effect of trypanosomiasis infection on the endocrine system in general can be direct or indirect(Hublart *et al*., 1990). The direct effect might be mediated by biologically active factors of parasite origin such as phospholipase A, protease and peptidase. Proteases of parasitic origin were suggested to cause gonadotrophic dysfunction in rats infected with *T. brucei* (Hublart *et al*., 1990). The indirect effect could be mediated through a highly activated immune response where cells

such as macrophages, activated during infection, could release cytokines activating and down regulating the endocrine system. IL-1 and IL-6 are known to be stimulants of the hypothalmic-pituitary axis while TNF (cachectin) is known to inhibit the secretion of pituitary hormones such as growth hormone and TSH(Hublart *et al*., 1990).

2.18 Trypanosome-induced pathology in Livestock

Numerous studies have been carried out on the pathogenic effects of trypanosomosis on domestic animals amongst which are the three most important trypanosome species(Griffin, 1978). A lot of the authors of these studies have divided these species into those restricted to the circulatory system, namely *Trypanosoma congolense, Trypanosoma vivax* and *Trypanosoma brucei* group of organisms, which occur in the intercellular tissue fluids and fluids of the body cavity as well as in the plasma (Griffin, 1978). Subcutaneous edema is particularly prominent and is usually accompanied by ascites, hydropericardium and hydrothorax (FAO, 1998). The liver may be enlarged, and edema of lymph nodes is often seen in the acute disease but they may be reduced in size in the chronic stage(FAO, 1998). The spleen may be swollen, normal or atrophic. Necrosis of the kidneys and heart muscle and subserous petechial hemorrhages commonly occur(FAO, 1998). Gastroenteritis is also common, and focal polioencephalomalacia may be seen(FAO, 1998).

2.19 Trypanosome-induced Endocrine-related Disorders in Both Sexes

Reproduction in both male and female is under neuro-endocrine control. Trypanosomosis have pathological effects on the endocrine glands, which inturn interefere with endocrine

control of reproduction and reproductive processes in both sexes (Sekoni, 1994). Anterior pituitary lesions have been reported in domestic animals including horses, sheep and dogs that were infected with *T. brucei* (Ogwu *et al*., 1992). Trypanosome infections are said to be associated with depression in the levels of gonadal, pituitary and thyroid hormones and these could be characterized clinically by a decline, and or cessation of normal reproductive function and depressed metabolism(Mutayoba and Gombe, 1989). Luckins *et al*. (1986) reported increase in plasma cortisol levels with simultenous suppression of the activity of the thyroid gland with resultant decrease in T4 levels in *T. cogolense* infeted goats. Reduced responsiveness of the hypothalamic-pituitary- adrenal axis in Boran cattle infected with *T. congolense* was reported by Abebe *et al*. (1993).Progesterone levels have been reported to be elevated in female goats infected with *T.congolense* (Luckins *et al*., 1986).In the report of Ileweyn *et al*. (1987), depressed levels of plasma progesterone were observed, while Elhassan *et al*. (1991) also reported a prolonged plasma progesterone depression in *T. vivax* infected ewes. The most consistent anomalous consequence of trypanosomosis, according to Sekoni (1994), is a decline or stoppage of progesterone production, which is somewhat partly responsible for the cessation of cyclicity or abortion in non-pregnant and pregnant animals, respectively.

In male animals, the single most studied endocrine hormone is testosterone. Some studies have reported reproductive-endocrine changes in male ruminant animals and a decrease in plasma testosterone concentration during *T. congolense* (Waindi *et al*., 1986) and a mixed *T. congolense* and *T. b .brucei* (Adeyemo *et al*., 1990) infections. The result of these two studies were however, questioned by Mutayoba *et al*. (1994) in a study on the

effects of *T. congolense* infection in rams on the pulsatile concentration of LH and testosterone and its response to injection of Gonadotropin Releasing Hormone. In a series of studies, they (Mutayoba *et al*., 1994; 1996) attempted to find out the exact status and sequence of events in the testis. They came to the following conclusions after using thesame strain of *T. congolense* in all the series of studies:

- That the decline in plasma LH concentration in infected rams was caused by reduced Gnrh stimulation of the pituitary, whereas the decline in plasma testosterone was partly caused by reduced sensitivity of the leydig cells to circulating LH.
- That the impairment of testosterone secretion within the early stages (4 weeks) of *T. congolense* infection in sheep may be due to testicular rather than pituitary effects.
- That trypanosome-induced pyrexia might be involved in leydid cell steroidogenesis and subsequent decline in plasma testosterone levels, probably, by impairment of enzymes involved in steroid biosynthesis.

2.20 Diagnosis of Trypanosomosis

Routine diagnosis of trypanosomosis in the field is undertaken via clinical signs and knowledge of the endemicity of the disease in the area (WHO, 1998; FAO, 2000). However, other more accurate methods of diagnosis include: parasitology, serology and molecular biology. Some of the parasitological methods currently in use are: stainedthin blood films, the buffy coat dark ground-phase contrast technique (BCT), the haematocrit centrifugation technique (HCT) and the miniature-anionexchange centrifugation

technique (mAECT) (FAO, 2000). These methods areconfirmatory since they depend on demonstration of trypanosomes. However, these parasitological detection methods have a limited analytical sensitivity (i.e. lower detection limit and may lead to under-reporting of the prevalence of disease (FAO, 2000).More sensitive diagnostic methods, including Polymerase chain reaction (PCR), Indirect fluorescent antibody test and the detection of trypanosome-specific antibodies and antigens have been developed (FAO, 2000).

2.21 Trypanosomosis Control

Attempts to control trypanosomosis have been ongoing for the last hundred years.Early methods involved a combination of clearing vegetation, exterminating wildlife hosts and spraying of bushes(WHO, 1998; FAO, 2000)

2.21.1 Trypanocidal drugs

The control of trypanosomosis in domestic livestock depends mainly upon the use of drugs, either curatively or as a prophylactic(WHO, 2005). Resistance to the available drugs is on the increase and their continued use is expensive for livestock owners, it has been estimated that at least US$ 20 million (approximately 50 million doses) is spent annually to treat or protect animals exposed to trypanosomes in Africa. The actual amount of trypanocides used is difficult to estimate, particularly in recent years, since the distribution of trypanocides has become more decentralized, with a number of generic brands being sold, increasingly through traders and shopkeepers and less through official veterinary channels (WHO, 2005).Drugs currently recommended for chemotherapy of animal trypanosomosis come from only three closely related groups. These are the

phenanthridines, isometamidium and homidium, and the aromatic diamidine, diaminazone. Only isometamidium and homidium are recommended for prophylaxis. The incidence of resistance to these drugs is apparently increasing and the main means of controlling the disease is therefore under threat (Peregrin, 1994). Drug resistant trypanosomes develop through; under dosing which may occur for a variety of reasons such asunderestimation of animal body weight, over diluted solutions or incorrectly calculated dosevolume, incorrect (and therefore ineffective) injection or an incorrect strategy of drug use. Measures that may delay the development of drug resistance are to reduce the selection pressure on trypanosome populations by avoiding exclusive reliance on drugs for trypanosomosis control and avoiding mass treatment of livestock at short intervals (Leak, 1998).

2.21.2 Tsetse vector control

Tsetse vector control methods relying on large scale bush clearing and aerialspraying methods are no longer used due to environmental concerns(Weidhaas and Haile, 1978). Tsetse control currently relies on two bait systems: insecticide-treated traps and targets and insecticide treated livestock. Sterile Insect Technique (SIT) has also been used in efforts to eradicate tsetse flies in some areas. Because of the stability of tsetse populations and their low reproductive rate, little sustained mortality pressure (additional to natural mortality) needs to be exerted on a population to cause its extinction (Weidhaas and Haile, 1978).That makes them good candidates for traps and target control methods. Not to be forgotten though are the risk of reinvasion or immigration into an area already cleared of tsetse flies. The theory behind this control method is simple: the flies are

visibly attracted to a trap or target.This attraction may be further helped by the use of olfactory attractants. When the tsetse lands on a trap or target they either receive a lethal dose of insecticide, or are caught in the trap and subsequently die (Leak, 1998). The effectiveness of traps and targets will depend on when the flies are active, how they move in their active state, whether they will move into the vicinity of a trap or target and finally, whether they are trapped or killed (FAO, 2001).Insecticide-treated livestock was developed as a method of tsetse control from the concept of baited traps and targets (Baylis and Stevenson, 1997). It is widely accepted by a majority of stockowners in Africa. The method has been used to control tsetse and trypanosomosis with varying results in Kenya (Stevenson *et al*., 1991). Most commonly used are the synthetic pyrethroids, and of these deltamethrin appears to be the most potent and it is also low in mammalian toxicity and has minimal environmental impact (Thompson *et al*., 1991). Extensive use of insecticides on cattle for tsetse control appears to have the potential to interfere with endemic stability immunity of cattle to several tick borne diseases. Thus the long term use of these products may jeopardize control of tick borne diseases (FAO 2001).

2.21.3 Vaccination

Attempts to vaccinate cattle against trypanosomosis started at an early stage in trypanosomosis research(FAO, 2001). For example, efforts have been made to protect the animals by deliberately infecting them with known strains of trypanosomes and then treating them at various intervals following infection. These animals were said to be tolerant or to some extent “premunized” but the resistence broke down relatively

easy(FAO, 2001). Attempts were also made to vaccinate cattle with killed trypanosomes by giving part-curative doses of trypanocidal drugs after infection, or by injection of small numbers of living trypanosomes(FAO, 2001). These attempts failed and it is now recognized that the major obstacle to immunization lies in the phenomenon of antigenic variation in trypanosomes. Trypanosomes are covered by a dense coat of variant surface glycoproteins that stimulate antibody production in the host. The surface coat changes successively during the course of an infection, thus avoiding the immune response of the host. At present there are no drugs available that have the ability to interfere with antigen switching (FAO, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out at the animal fly-proof pen located at the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.Zaria is located between Latitude 11^{o} 11'N and Longitude 07^{o} 38'E, at an altitude of 686 metres above sea level. It lies within the Guinea Savanna zone, and has 3 distinct seasons including harmattan (Nov- Feb), hot (Mar -May) and rainy (Jun- Oct) (Ayo *et al*., 1998). Its annual rainfall, average temperature and relative humidity are 1055millimetres, 24.55^{o} C and 43.6%, respectively (Meteorological Unit IAR, 2009).

3.2 Experimental Animals

Sixteen apparently healthy and intactYankasa rams (Plate I), aged between 24 and 30 months and weighed between 22 to 25 kg were purchased from Sheme local market, an apparently tsetse free zone, in Katsina State of the Nigerian Sudan-Guinea Savannah. The ages of the animals obtained from the market were confirmed by the method of temporary and permanent incisors as described by Wilson and Durkin(1984) (i.e. by the eruption of permanent incisors at permanent corners of the cheek).

3.2.1 Housing and screening of experimental animals

The animals on arrival were housed in an insect-proof animal pen at the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello



Plate I: The experimental animals (Yankasa rams) during the acclimatization period.

University, Zaria, where they were screened forthe presence of haemoparasites, as well as endoand ectoparasites. The rams were thereafter treated with Oxytetracycline (Tridax®) intramuscularly, at a dose of 20 mg/kg body weight and Albendazole (Sambezole®, Sam Pharmaceutical Ltd; Animal Care, Nig. Ltd) orally, at a dose of 7.5 mg/kg body weight. The rams were sprayed against ectoparasites with Diazinon (Diaznol®, Animal Care, Nig. Ltd.), at concentration of 2 ml/litre of water.They were acclimatized for eight weeks and neck-tagged for the purpose of identification (Plate I).

3.2.2 Acclimatization and physical examination of animals

The rams were fed with wheat offal, ground-nut hay "harawa", cowpea hay, fresh grasses (whenever available) and salt licks. Water was supplied *adlibitum* (Plate I). During the eight weeks acclimatization period, they were subjected to routine handlings, such as physical examination, determination of the body weight, rectal temperature, scrotal circumference, semen collection and collection of blood samples for screening of haemoparasites and determination of baseline semen and haematological indices. Before commencement of the experiment, the rams were ensured to be clinically free of trypanosomes and other haemoparasites in their blood using buffy coat centrifugation technique (Woo, 1969; Biryomumaisho *et al*., 2013).

3.2.4 Source of trypanosomes

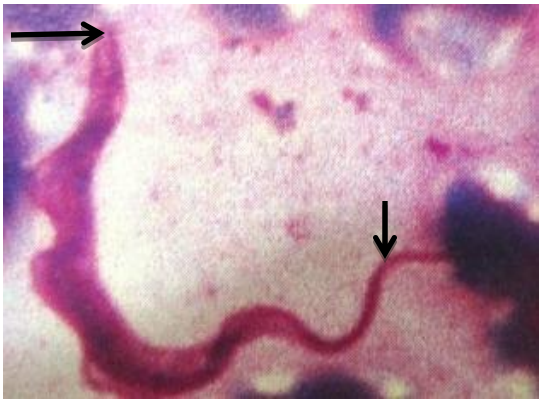
Trypanosoma evansi was obtained from an infected camel that was slaugtered at the Sokoto Abattior, Sokoto State, while *Trypanosoma brucei brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria, originally isolated from a natural infection in cattle, in Kaduna State. Both parasites were

maintained in Wistar rats by serial passage and were transported to the Protozoology Laboratory, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for proper identification using the Giemsa-stained thin blood smear diagnostic technique (Hoare, 1972).*Trypanosoma evansi* was identified morphologically by the characteristic free and long flagellumwith well developed undulating membrane, subterminal kinetoplast and an elongated and centrally placed nucleus (Plate II-a) while *Trypanosoma brucei brucei* was identified by the presence of short posterior kinetoplast,long and conspicuous undulating membrane with no free flagellum(Plate II-b). Both parasites were sub-inoculated intraperitoneally into ten Wistar rats each and were kept in separate cages in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The rats were fed with commercial pelleted feed and water supplied *ad libitum*. Prior to inoculation into experimental rams, blood samples were collected daily from each of the rats to determine the level of parasitaemia using the haematocrit centrifugation technique (HCT) as described by Woo (1969) and Biryomumaisho *et al*. (2013).

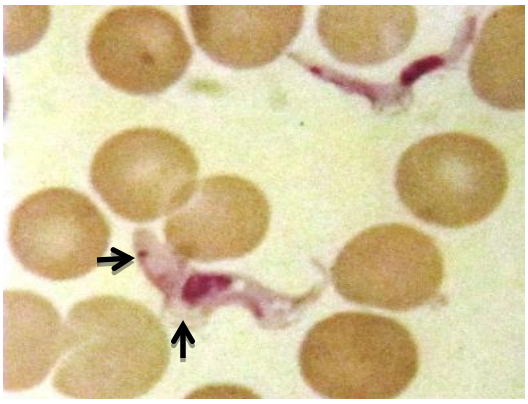
3.3.0 Experimental Design

3.3.1 Grouping of the experimental animals

By the end of the eight weeks acclimatization period, four of the sixteen rams that were deemed unfit (due to poor semen characteristics) for the experiment were eliminated. The remaining twelve that were clinically fit (due to good semen characteristics and higher haematological values) for the experiment were randomized into four experimental groups (GI, GII, GIII and GIV)



a



b

Plate II: Giemsa stained *Trypanosoma evansi*at X1000 Magnification (a) and *T. brucei brucei*(b) at X400 magnification.

of three rams each, based on their mean packed cell volumes (PCV) and weights. The rams in groups I, II and III were experimentally infected with *T.brucei brucei, T. evansi* and mixed inoculum of both parasites, respectively while those in group IV served as the uninfected control.

3.3.2 Inoculation of experimental animals

The trypanosome parasites after detection in the blood of the inoculated rats were monitored to their peak value (30 – 40 in buffy coat layer per field). All the infected rats became parasitaemic within 3-14 days post inoculation. The rats were bled using sterilized surgical blades through cardiac (heart) puncture to collect sufficient blood into Bijou bottles, containing 2 mg of Ethylene Diamine Tetraacetic Acid (EDTA) for the inoculation of the rams in groups GI, GII and GIII. The dosage of *T.evansi* and *T. b. brucei*usedfor inoculation was estimated using the rapid matching wet-examination technique described by Herbert and Lumsden (1976).A drop of mouse blood was examined under the X40 magnification of a microscope, the number of trypanosomesin each field counted and matched with log figureobtained from a reference table (Herbert and Lumsden, 1976).

Group I – each ram was inoculated via the jugular vein with 2mls of blood containing $2x10^6$*Trypanosoma brucei brucei.*

Group II – each ram was inoculated via the jugular vein with 2mls of blood containing $2x10^6$*Trypanosomaevansi*.

Group III – each ram was inoculated via the jugular vein with 2mlsof blood containing $1x\ 10^6$*Trypanosoma evansi* and $1x10^6$ *Trypanosoma brucei brucei.*

Group IV - served as the uninfected control.

The rams were thereafter observed daily for clinical signs and parasitaemia. The inoculated rams were allowed to go through the full course of the infection (98 days post infection), during which feed and water were supplied *ad libitum*.

3.4.1 Observation of clinical signs

Clinical signs that were investigated during the study included rectal temperature, feed intake, body condition, weaknessand dullness, roughy hair coat, packed cell volume (PCV), haemoglobin concentration, total plasma protein and anaemia.

3.4.1.1 *Determination of rectal temperature*

The rectal temperature for each experimental animal was assessed daily in the morning between 7.00 am - 8.00am using a clinical digital thermometer (KRIS-ALOY CE **0197**). The thermometer was inserted into the rectum and tilted to touch the rectal mucosa. After a beep, the thermometer was removed and the body temperature changes were read and recorded in degrees centigrade (^{o}C).

3.4.1.2 *Determination of weight*

A portableweighing scale (HANA J1108001129) was used to determine the weights of the animals on weekly basis and these were recorded in kilogramme (kg).

3.4.2 Determination of haematological parameters

Blood samples were collected once daily, from day 2 post-infection (p.i) upto 23 days post infection when parasitaemia had established in all infected groups, thereafter, every 3 days upto the end of the98-days post infection. Two mls of blood was collected from each of the experimental animals via the jugular vein using sterile 5 ml syringe and

21Guage X 1½ inch needle. The blood was carefully dispensed into clean, labeled Bijou bottles containing EDTA, for the determination of PCV, haemaglobin concentration, total plasma protein and the determination of parasitaemia (Coles, 1986).

3.4.2.1 *Determination of the level of parasitaemia*

The level of parasitaemia was estimated using haematocrit centrifugation technique (HCT) as described by Woo (1969). The procedure involved filling two heparinised micro-capillary tubes (75x1.5mm) to approximately two-third of their volumes with each of the infected blood.The tubes weresealed with a sealer and thereafter, placed in a micro-haematocrit centrifuge in an opposite direction to be balanced, while the sealed ends were allowed to face outwards. They were spinned for 3 minutes at 1500 revolutions per minute (rpm). The spun capillary tubes were thereafter placed on a glass slide and oil immersion was applied on the buffy coat area and viewed under the objective lens (X40) to determine parasitaemic scores as described by Woo(1969):

1. + = less than 10 trypanosomes in buffy coat or plasma layer seen per field.
2. ++ = 10 - 20 trypanosomes in buffy coat or plasma layer seen per field.
3. +++ **=** Numerous (20 – 30) trypanosomes in buffy coat or plasma layer seen per field**.**
4. ++++ = massive (30 – 40) trypanosomes in buffy coat or plasma layer seen per field.

3.4.2.2 *Determination of packed cell volume (PCV)*

The PCV was determined using standard microhaematocrit centrifugation technique and the values were read by Hawksley microhaematocrit reader (Gellman Hawksley Ltd,

England) as described by Coles (1986). The packed cell volume was determined daily for the first 23 days; thereafter, at every 3 days upto the end of the 98 days post infection**.**

3.4.2.3 *Determination of haemoglobin concentraion*

Haemoglobin (g/dl)was estimated by calculation. Its value approximates to one- third (1/3) of the PCV value (Coles, 1986).

3.4.2.4 *Determination of total plasma protein*

Total Plasma Protein (g/dl) was determined from the plasma portion of the centrifuged capillary tube and the value was read from a Goldberg hand refractometer (Coles, 1986).

3.4.2.5 *Thin blood smear for identification of trypanosomes*

A drop of blood was placed on a clean and grease-free microscope slide. A thin smear of blood was made by sliding another slide on the first slide. The smear was air-dried and fixed in methanol for about 3 minutes. The slide was stained in 10 % Giemsa stain for 25 to 30 minutes, rinsed in distilled water, dried and examined microscopically under oil immersion at X100 objective (Coles, 1986).The identification of the trypanosomes was done based on their morphological features as described by Hoare (1972).

3.5 Reproductive Parameters

Reproductive parameters measured were scrotal circumference, semen collection reaction time, semen characteristics (colour), pH, concentration of semen, total sperm output, ejaculate, viability (life/dead%), sperm morphology (normal sperms, head abnormalities

and tail abnormalities) according to the method of Zemjanis (1970).Gonadal and epididymal sperm/spermatid reserves were also determined according to the method of Sekoni *et al*. (1981), Rekwot *et al*. (1994), Alabi (2005) and Ogunlade *et al*. (2006).

3.5.1 Determination of scrotal circumference

Scrotal circumference was measured in centimetres (cm) using a flexible measuring tape at the point of the widest scrotal diameter by applying pressure with a hand above the head of the epididymides thereby gently forcing the testes into the scrotum (Osaer *et al.*, 1997).

3.5.2 Semen collection

Semen collection was done weekly between 9am and 10am, by electrostimulation with the help ofa portablebattery-powered electro-ejaculatory mini tube for small ruminants (Plate III)as described by Morar *et al*. (2010).The rams were adequately restrained; the prepuce was washed and driedusing cotton wool soaked in diluted chloroxylenol (0.002%; Dettol^{R}) to remove dirts and debris. The probe of the electro-ejaculator was lubricated using petroleum jelly and inserted into the animal's rectum and switched on, this resulted in erection and subsequently, ejaculation. Semen began to flow once the animal has achieved excitation by the stimulatory action of the electroejaculatory device. The impulses consisted of applying the stimulus at an interval of 5 seconds, with 5 seconds break (Morar *et al*., 2010). The ejaculates were collected into pre-warmed, sterile and graduated transparent collection tube, labelled and kept in a water bath at a

temperature range of 35-37ºC. This was done to prevent temperature changes which may affect the quality of semen, before analysis (Rao, 1971).



Plate III: Electro-Ejaculator Mini Tube

3.6 Semen Evaluation

Each ejaculate was evaluated for volume, motility, pH, concentration, sperm morphological abnormalities and live/dead differential sperm count, to determine the effects of trypanosomes on the seminal parameters.General semen examination was done at the point of collection while the microscopic examination was done at the Fertility Laboratory of theArtificial Insemination Unit, National Animal Production Research Institute (NAPRI), Ahmadu Bello University,Shika, Zaria, usingstandard laboratory methods (Bitto *et al*., 1988).

3.6.1 General examination

Parameters such assemen collection reaction time (sec), semen volume (ml)and semencolour and semen pH were promptly evaluated.

3.6.1.1*Semen collection reaction time*

The semen collection reaction time, defined as the time between when the electroejaculator was first inserted into the rectum of the ramto the time the ramfirst ejaculated, was determined using a digital stop watch and recorded in seconds.

3.6.1.2 *Semen volume and colour*

The volume of semen (ml) ejaculated was measured directly from the calibrated tube used for collection. The semen colour was noted immediately after collection for milky, creamy and watery appearance.

3.6.1.3 *Semen pH*

This was determined by inserting a pH meter into the collection tube and the value was then taken.

3.6.2 Microscopic examination of semen

Microscopic examination of semen sample was done under a light microscope in the laboratory to examine the gross sperm motility, the sperm concentration, and sperm morphology.

3.6.2.1 *Gross spermmotility*

This was determined according to the methods described by Zemjanis (1970) and Rao (1971)by placing a drop of raw undiluted semen on a pre-warmed slide then cover-slipped and viewed using a field microscope at X40 magnification.The results were scored objectively using the scoring pattern described by Omalaka (1992) in percentages as presented below:

90-100%	Excellent, continuous progressive motility
80-89%	Very good, continuous progressive motility
70-79%	Good, continuous progressive motility
60-69%	Shift continuous progressive motility
50-59%	Very active-none-progressive motion
40-49%	Shift none-progressive motion

10-19% No motion

3.6.2.2 *Sperm concentration*

Sperm concentration was evaluated by visual count under the microscope using improved Neubauer Haemocytometer. The sperm cells were immobilized using a 1% formaldehyde solution prior to counting. The raw semen was mixed thoroughly and filled into the unopette capillary tube with a dilution ratio of 1:10. The diluted semen was thereafter transferred unto the haemocytometer chamber and counted under the microscope.

The number of sperm cells counted using the haemocytometer multiplied by a million (10^6) was the concentration per ml of the raw semen.

The total spermatozoa per ejaculate were estimated by calculation as described by Coffin (1953) and Egbuka (1995):

Total spermatozoa per ejaculate = Sperm concentration per ml × Volume per ejaculate

3.6.2.3 *Percentage live- dead spermatozoa*

The percentage live sperm and spermatozoa morphological abnormalities were determined using Eosin-Nigrosin stain technique, applied on a glass slide (Michael *et al*., 2009). The staining mixture consisted of 1% Eosin B and 5% of nigrosin in 3% sodium citrate dehydrate solution.One drop of raw semen was added to one drop of the stain, thereafter it was mixed thoroughly and a fresh smear was made from it. The slide was then examined under a light binocular microscope at X40 magnification. A minimum of 100 spermatozoa were counted and the percentage of each estimated. The live-dead staining principle was based upon the observation that Eosin-B penetrated the dead sperms (thereby making them appear pink),while the viable sperm cells repelled the stain and appeared unstained (white).

3.6.2.5 *Spermatozoa abnormal morphology*

Morphological abnormalities of the spermatozoa that were examined includedMid Piece Droplets (MPD), incidence of Detached Head (DH), Bent Tail (BT) and incidence of Coiled Tail (CT). These abnormalities were determined by making a thin smear of the semen sample on clean grease-free glass slide and fixed with buffered formal saline. Semen samples stained with eosin-nigrosin were used to determine the morphological abnormalities of the sperm head. A minimum of one hundred sperm cells were counted per slide using light microscopy at X40 magnification and were classified and calculated as described by Blom (1972)and Sekoni *et al*.(1981).

3.7 Determination of Gonadal sperm andEpididymal Sperm Reserve

3.7.1 Determination of gonadal sperm/spermatid reserves

At the end of the experiment (day 98 post infection), a total of eight rams(two (2) from each group) were randomly selected from the experimental rams. They were slaughtered to determine their gonadal sperm/spermatid reserves; that is, their integrity to support gonadal functions. The two testes of each of the 8 rams were carefully removed and labeled for proper identification. A small portion of the testes from each of the sacrificed ram was removed and preserved in Bouin solution for histopathological examinations.The weight, length and volume of each testis were determined. Gonadal sperm/spermatid reserves were determined according to the standard methods of Rekwot *et al*. (1994), Alabi (2005) and Ogunlade *et al.* (2006). Briefly, the tunica albugenia of each testis was removed using a scapel blade to expose the testicular parenchyma which was weighed, sliced and homogenized with a high speed blender for two minutes with 50

mls of 0.9% sodium chloride (NaCl) containing antibiotics (sodium penicillin G, 100 IU/ml and streptomycin sulphate, 1 mg/ml) to prevent bacterial growth.The homogenate volume, after rinsing the blender container with 20ml of saline and adding the effluent, was measured. About 5ml of the homogenate was transferred to a conical flask and further dilution was made with 40ml of saline and the homogenate was stored overnight at 5^{o}C to allow sperm cells to ooze out of the tissues. Finally, the gonadal sperm/spermatid concentration was determined with a haemocytometer according to the method of Coles (1974). The concentration of spermatozoa was determined using the erythrocyte counting chamber of a haemocytometer that was crossed with microscopic grids containing small squares. Sperm cells and spermatids were counted diagonally from top left to bottom right in five large squares according to the method of Rekwot *et al*. (1994).

3.7.2 Determination of epididymal sperm reserves

In determining the epididymal sperm reserves, briefly, the epididymis was carefully separated from the testis with a scapel and the lengths and weightsof the caput (head), corpus (body) and cauda (tail) epididymides were determined, minced separately into20 ml of 0.9% NaCl solution with a pair of sharp scissorsand the homogenates were storedovernight at 5^{o}C to allow sperm cells to ooze out of the tissues. The samples were thereafter, filtered through gauze and the filtrate volume was measured and recorded.One ml each of epididymal portion was diluted separately with 2 mls of saline solution.Finally, the concentration of sperm reserve was determined using a

haemocytometer and light microscopy (Rekwot et al., 1994; Alabi, 2005; Ogunlade *et al*., 2006).

3.8 Histopathological Analysis

At the end of the experiment, the selected eight (8) rams were sacrificed and subjected to post mortem examinations and biopsies were taken for histopathology. Tissue samples collected from the spleen, kidney, liver, lungs and pancreas were preserved in 10% buffered neutral formalin (BNF) while those collected from the brain and testes were preserved in Bouin solution. The Bouin fluid mixture was prepared by mixing 75ml of picric acid saturated aqueous solution (2.1%), 25ml of 40% formaldehyde and 5ml of glacial acetic acid. After 48 hours of fixation, the tissue samples were processed (washed in 50% and 70% alcohol), embedded in paraffin wax and sectioned at 3 to5 microns using a microtome. The sections were mounted on clean grease-free glass slides and stained with Haematoxylin and Eosin (H and E) stains as described by Luna (1968). The stained slides were examined microscopically atX40 objective. Histopathological lesions were observed, recorded and photomicrographed with the aid of a digital camera. Sections from the testes were examined and the degrees of testicular degeneration (table 3.1) were scored by method of Sekoni et al. (1990b).

Table 3.1: Grading of genital degeneration in ram infected with *Trypanosoma* spp

Grade	Testes	Epidymides
Severe (+++)	Germinal epithelial layers reduced to one or two with predominantly Sertoli cells. Sixty (60%) plus tubules affected	Lesions observed in 60% or more tubules
Moderate (++)	Germinal epithelial layers reduced to 3	Lesions observed in at

	with at least 50% of tubules affected	least 50% of tubules
Mild (+)	Most tubules normal with slight reduction in the germinal layers	Most tubules are normal

3.8 Data Analyses

The data obtained from the study were prepared for analysis using Microsoft Office Excel (2010). The weekly mean haematological values, weekly mean weights, scrotal circumferences, rectal temperatures and weekly mean parasitaemic scores of all the groups were represented and compared on multiple line graphs using Microsoft Excel Chart Wizard (2010).Inter group comparisons of mean semen parameters, mean gonadal and epididymal sperm reserves were analysed by One-Way analysis of variance (ANOVA), and where significant differences existed, Duncan Multiple Range Test (DMRT) was used to separate the means. Statistical analysis (ANOVA) was carried out using the Superior Performing Statistical Software (SPSS) for windows, version 20 (2011).The values of $P < 0.05$ were considered statistically significant (Steel and Torrie, 1980).

CHAPTER FOUR

4.0 RESULTS

4.1. General Clinical Observations

The mean pre-infection base-line data for all the parameters studied (for the eight weeks acclimatization period) before the rams were grouped and challenged with trypanosomes are presented in table 4.1.

Generally, for the rams that served as control, there were no significant clinical changes associated with the group throughout the study period. Rather, there was observed weight gain, increase in total plasma protein with good body condition (Plate IVa). However, observed clinical signs among the rams in the infected groups I, II and III were similar and include intermittent pyrexia, roughy hair coat (Plate IVb), reduced and or selective feed intake, reduced body weight gain, pale ocular membrane with peri-orbital oedema (Plate IVc), loss of body condition, poor semen output, loss of libido, drowsiness and death (PlateIVd).

By day 49 post infection (p.i.), one ram (R6) in group II (*T. evansi*) began to lose its body condition, and balance. It couldn't stand on its limbs. The packed cell volume decreased severely (from pre-infection value of 30.0% to post-infection value of 13.5% by 56 days

p.i.), its total plasma protein value also decreased significantly (from the pre-infection value of 6.0 to 3.0 by day 56 p.i.). Itsbody weight was significantly ($P < 0.01$) reduced from a pre-infection value of 22.95kg at day 0 to post infection value of 16.95kg by day 56 p.i. The ram totally lost its balance and couldn't ejaculate during semen collection.

Table 4.1: Mean values for eight weeks pre-infection baseline data for all parameters of 12 experimental Yankasa rams

Parameters	Range	Mean	S.D
Life weight (kg)	21.6 - 24.80	23.64	±0.86
Scrotal circumference (cm)	22.41 - 25.93	24.31	±1.02
Packed cell volume (%)	30.77 - 36.14	32.25	±1.94
Haemoglobin conc. (g/dl)	10.20 - 12.30	10.83	±0.65
Total protein (g/dl)	5.80 - 6.85	6.24	±0.33
Rectal temperature (^{o}C)	37.6 - 38.5	38.25	±0.22
Semen collection reaction time (sec)	9.56 -21.45	14.25	±3.39
Semen volume (ml)	0.41 - 0.98	0.78	±0.01
Mass motility (%)	65.60 - 90.20	80.55	±10.15
pH	6.08 - 8.21	7.08	±0.49
Sperm concentration ($X10^{6}$/ml)	698.56 -1152.33	887.67	±98.88
Dead sperm (%)	8.51 - 15.23	12.08	±3.78
Sperm morphological abnormalities (%)			
Detached Heads	0.51 - 1.34	0.92	±0.34
Mid-piece	0.11 - 0.62	0.43	±0.01
Sperm tail	2.36 - 7.64	5.76	±2.11
Total abnormalities (%)	3.79 - 5.87	6.02	±2.87

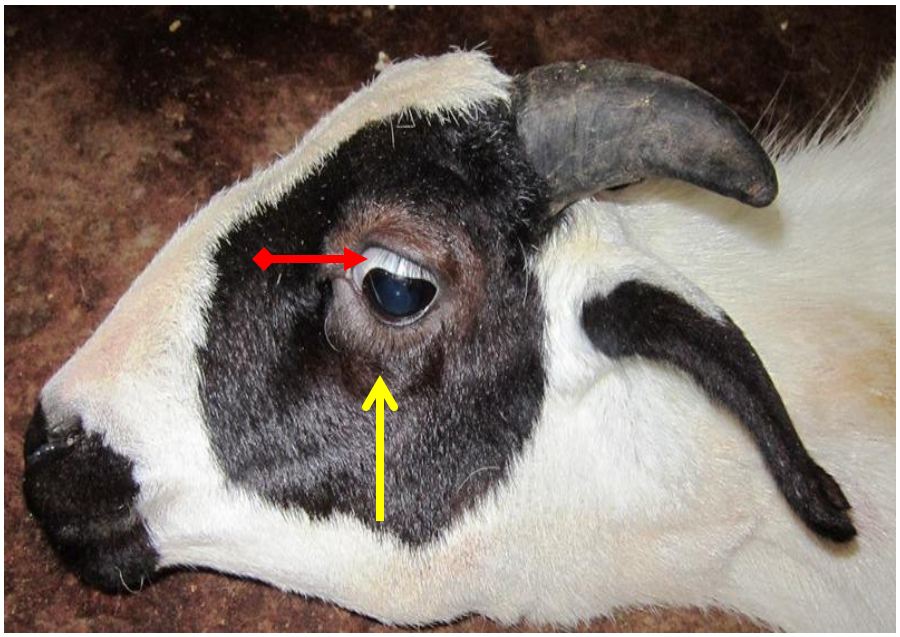


a



b

Plate IVa-b: A clinically healthy Yankasa ram with good body condition from the control group IV (a) and one of the *T.b.brucei*-infectedrams in group I with roughy haircoat and unco-ordinated posture, 35 day p.i. (b)



c



d

Plate IVc-d: One of the rams infected with *T.evansi* showing very pale ocular membrane (red arrow) with peri-orbital oedema (yellow arrow) at 56 day p.i. (c) and *T.evansi* infected ram that collapsed and died of the infection by 57 day p.i. (d).

Animal neck became very stiff and thereafter, died by the evening of day 57 p.i. Post mortem examination was done promptly. Necropsy revealed the presence of congested lungs, very pale and anaemic carcass, serious atrophy of fats in the kidney, heart and intestine, severe lymphomegaly and hepatomegaly. However, the remaining two rams within the group (*T. evansi*) survived the infection upto the end of the experiment as they began to recover, evidenced by gradual gain in weight, gradual increase in PCV value and the animals appeared normal by the end of the experiment (day 98 p.i) with absence of parasitaemia in peripheral blood circulation.

By day 70 p.i., there was severe scrotal atrophy of ram R7 in group I (*T.b. brucei*) as compared to those of the control group IV that had a normal scrotum throughout the study period (Plate V-a). Prior to degeneration, the scrotum was swollen (Plate Vb-c),soft to feel and painful to the animal when touched. As the infection progressed, another ram (R8) in group I(*T.b. brucei*) died by day 77 p.i. Prior to its death, there was decrease in weight (from pre-infection value of 23 .40kg to post infection value of 21.44kg at day 70 p.i.). The ram completely lost its appetite, and couldn't stand on its limbs. The PCV decreased as low as 15% (from pre-infection value of 34% at day 0.) and the ram died overnight (day 77 p.i.). Post mortem result also revealed the presence of severely pale carcass, swollen lymphnodes, and swollen liver and serous atrophy of body fats, with severely degenerated testes (at histology).

All the infected rams in group I (*T.b. brucei*) and group III (mixed inoculum of *T.b.brucei* and *T. evansi*) showed significant ($P < 0.01$) clinical symptoms than those rams infected in group II (*T.evansi*) that showed mild symptoms at the chronic stage of the experiment. At few weeks (day 63 to day 98 p.i) to the end of the experiment, semen characteristics of



a



b

Plate Va-b: A normal and healthy scrotum with complete folds in one of the control rams (a) and a swollen scrotum(arrow)showing loss of folds in *T.b.brucei*-infected ram at 35d.p.i. (b)



c



d

Plate Vc-d: Scrotal atrophy in *T.b.brucei*-infected Yankasa ram at day 70 p.i. (c) and rams withmixed infection, 55d.p.i.(d).

all the infected rams especially those of group I and group III, were observed to be significantly (P <0.01) deteriorated when compared to those of the uninfected control (group IV) that had good semen characteristics. Semen volume was observed to have decreased and deteriorated in colour- watery, as compared to creamy white to milky white in the control rams.Rams infected with either*T.b. brucei* or mixed inoculum of the parasites (*T. brucei and T. evansi*) completely lost the ability to display sexual urge (libido) when they were exposed to two (2) ewes to service in the pen. However, those rams infected with *T. evansi* and those of the uninfected group that served as control were able to display a very good libido towards the ewes but service was not allowed to take place to avoid transmission.

4.1.2 Mean parasitaemia levels (+)

Following inoculation with thetrypanosome species, all the infected groups developed parasitaemia at varying pre-patent periods as shown in Figure4.1. The animals in group I (*T.b.brucei*- infected rams) had the shortest pre-patent period of 5-9 days, which was not significantly (P > 0.01) different from those of group III (mixed infection) that had a prepatent period of 6-11 days, but the difference was significant (P < 0.01) when compared to those of Group II (*T. evansi*-infected rams) that revealed the longest pre-patent period of 19-21 days. The parasites (*T.b.brucei*) were first observed in the peripheral circulation by day 5 post infection (p.i.) in one of the infected rams (R7) in group I, with a low parasitaemic score of one plus (+). Thereafter, there was an observed progressive increase in parasitaemia, attaining a peak by day 28 p.i. (Figure 4.1) with a massive parasitaemic score of three plus(+++).

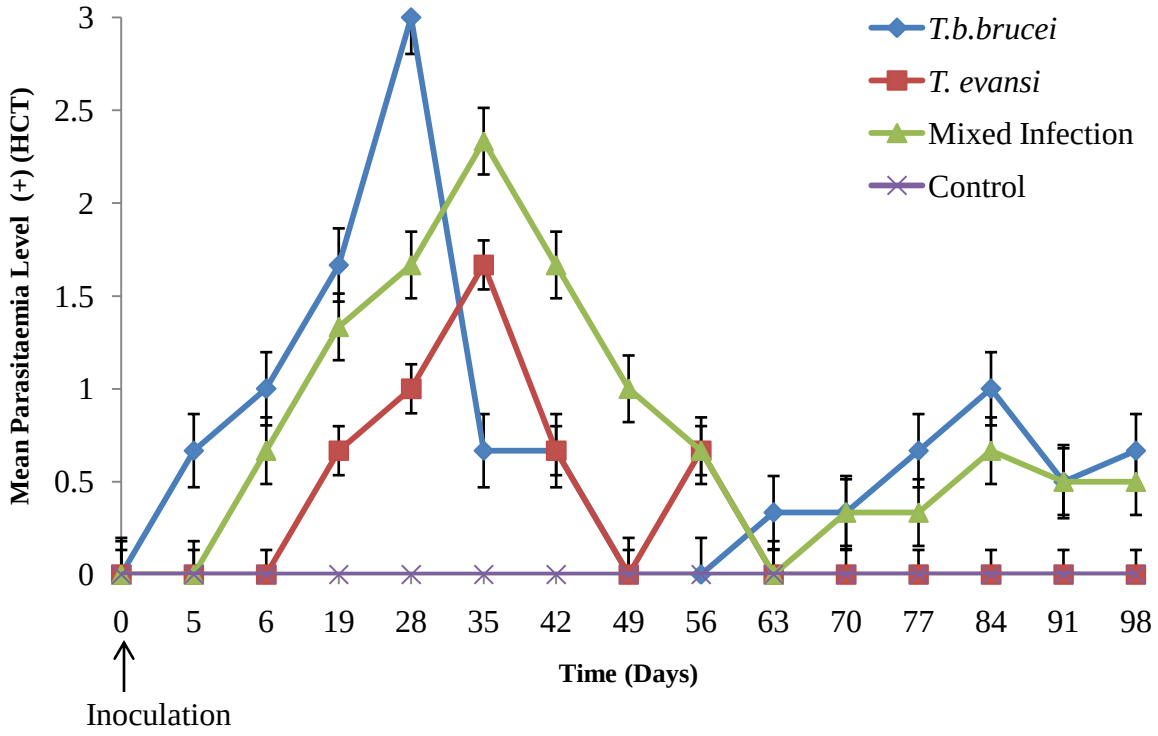


Figure 4.1: Mean parasitaemic scores of uninfected control Yankasarams and rams experimentally infected with either*T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III)

The parasites disappearedfrom the peripheral blood circulation by day 49 and 56 p.i. respectively, with resurgence at day 63 p.i. but at a very low parasitaemic score of one plus (+) up to the end of the experiment (day 98 p.i.). For those rams infected with *T. evansi* (group II), one ram (R5) had low parasitaemic score of one plus (+)at day 19 p.i. and by 21days p.i., all rams in the group developed parasitaemia. Rams in group II attained the peak of parasitaemia by day 35 p.i. (Figure 4.1) with a parasitaemic score of two plus (++). The parasites disappeared from the peripheral blood of the rams by 63 days p.i.upto the end of the experiment. One of the Yankasa rams (ram R3) with mixed infections (group III) revealed parasitaemia by day 6 p.i. and by 11 days p.i. all rams in the group became parasitaemic with a progressive increase to a peak of two plus (++) by 35 days p.i. followed by a continuous decrease in parasitaemia that disappeared completely by day 63 p.i and reappeared by day 70 post infection. The parasitaemia fluctuated at a very low level upto the end of the experiment (Figure 4.1). Among all the infected groups, there was high significant differences ($P < 0.01$) in the level of parasitaemia, with *T.b brucei* infected rams having the highest level of parasitaemic score which was not significantly different ($P > 0.01$) from those of groups III (mixed infection), but differed significantly ($P < 0.01$) from those of *T. evansi* infected group II. All the rams in the uninfected control group IV remained aparasitaemic throughout the experimental period (Figure 4.1).

4.1.3 Mean rectal temperature (^{o}C)

The mean weekly rectal temperatures of all experimental groups during the 98 days post-infection period arepresented in Figure4.2.At the beginning of the experiment, the pre-infection mean rectal temperaturevalues of the rams in groups I, II, III and IV as

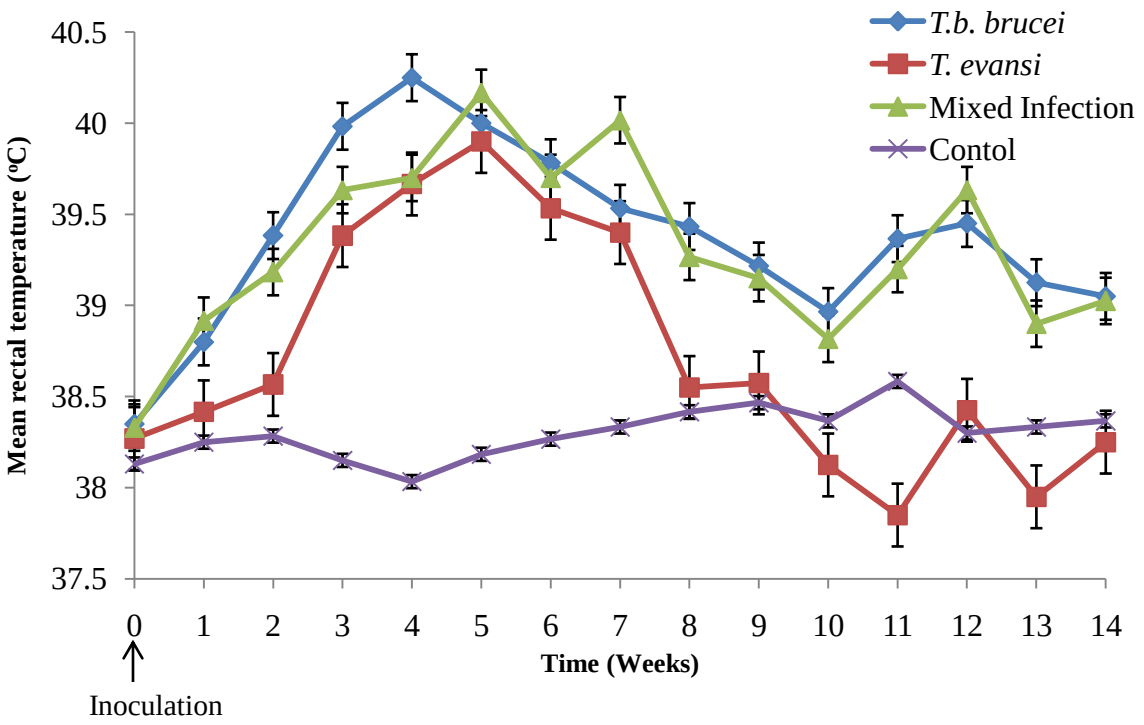


Figure 4.2: Mean weekly rectal temperature of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III).

presented in Figure 4.2 were 38.35 ± 0.0 ^{o}C, 38.27 ± 0.2 ^{o}C, 38.33 ±0.1 ^{o}C and 38.14 ± 0.1 ^{o}C, respectively. By day 2 p.i., the mean rectal temperature values of the infectedgroups began to rise steadily and by day 28 p.i, the mean rectal temperature values ingroups I, II and III reached 40.25 ± 0.1 ^{o}C, 39.67± 0.0 ^{o}C and 39.70 ± 0.4 ^{o}C, respectively. Thereafter,there were fluctuations in the mean rectal temperature values of the infected groups I, II and IIIthat continued upto the end of the experiment. The mean rectal temperature values for thecontrol group remained and fluctuated within the normal range throughout the experimental period. The maximum mean temperature ingroups I, II and III were 40.25 ± 0.1 ^{o}C at 28 days p.i., 39.90 ± 0.2 ^{o}C and 40.17 ± 0.0 ^{o}Cat 35 days p.i., respectively, which were significantly ($P < 0.01$) higher when compared to those of the uninfected control group IV (38.5±0.2 ^{o}C). However, for those of group II, the mean rectal temperature dropped and fluctuated within the normal range upto the end of the experiment (Figure4.2).

4.1.4 Mean weekly live body weight changes

The pre-infection mean weekly weights of the rams in the experimental groups I, II, III and IV were 23.60±1.4kg, 22.57±1.6kg, 23.85±1.5kg and 24.92±1.3kg, respectively. By day 7 p.i., the mean weekly body weight of the infected groups I, II and III began to decline at different levels of significance to the end of the experiment when compared to that of the control group, IV (Figure 4.3). At the end of the experiment, the mean weekly body weights of the infected groups I and III declined to 18.73 ± 1.9kg and 18.85 ± 1.3kg respectively, while that of the rams in group II began to gain weight progressively by day 56 post infection after an initial decrease but the value was not above the initial preinfection value at the end of the experiment (Figure 4.3). There was a significant ($P <$

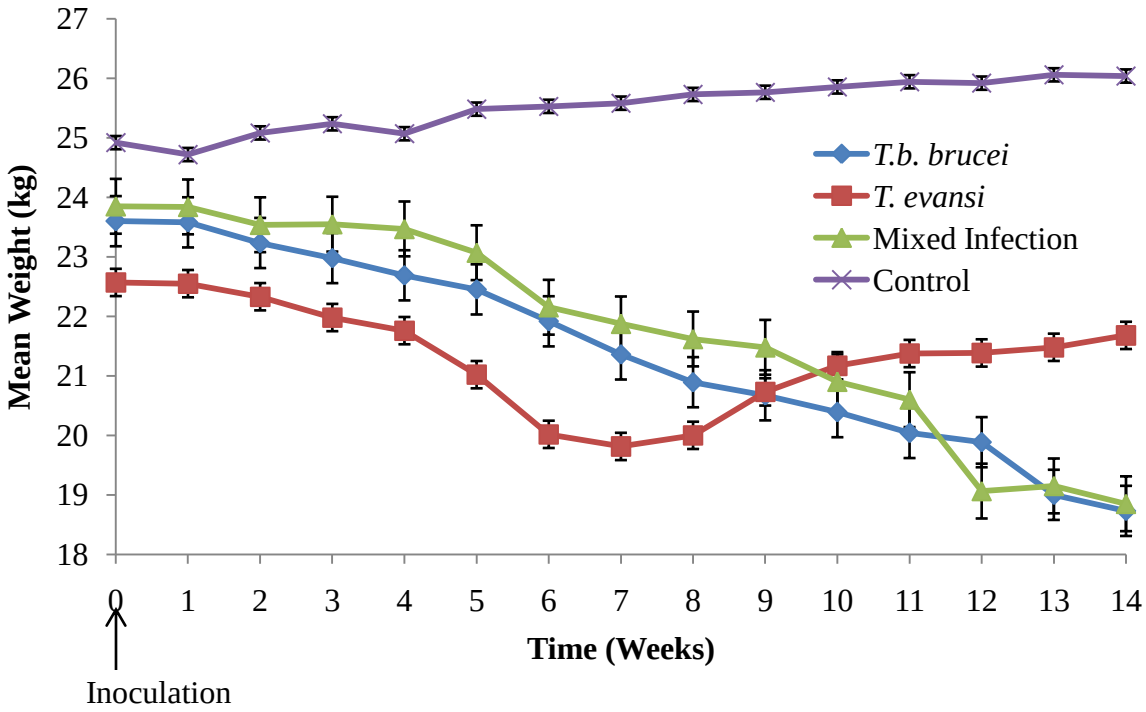


Figure 4.3: Mean weekly live body weight of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III)

0.01) reduction in the live body weight of the infected rams in comparison to the uninfected control group IV that gained weight (26.04 ± 1.7kg) at the end of the experiment (Figure 4.3).

4.1.5 Mean weekly scrotal circumference

The pre-infection mean scrotal circumferences of the rams in the experimental groups I, II, III and IV were 24.37±1.1cm, 23.94±1.0cm, 24.52±1.3cm and 24.51±0.3cm, respectively. By day 21 p.i. the mean weekly scrotal circumference of the infected groups I and III showed a transient sharp increase in scrotal circumference to a value of 24.87±0.1cm and 24.72±0.2cm, respectively. There was thereafter,an observed sharp and progressive decrease in scrotal circumference which set in by day 28 p.i. and day 35 p.i. to a value of 22.29±0.2cm and 22.23±0.1cm for the infected rams in groups I and III, respectively. These values decreased upto the end of the experiment (Figure 4.4). For rams infected with *T. evansi* (group II), there was slow and gradual decrease in scrotal circumference from a pre-infection value of 23.94±0.7cm to 23.27±0.1cm by the end of the experiment (98 days post infection). There was a statistical significant ($P < 0.01$) reduction in the mean weekly scrotal circumference of all the infected rams in comparison to the uninfected control rams that maintain a relatively normal and increased scrotal circumference (24.64 ±0.3cm) by the end of the experiment (Figure 4.4).

4.1.6 Mean weekly plasma protein (g/dl)

The pre-infection mean weekly plasma protein of the rams in the experimental groups I, II, III and IV were 6.33 ± 0.4 g/dl, 6.42 ± 0.6 g/dl, 5.98 ± 0.2 g/dl and 6.27 ± 0.7 g/dl

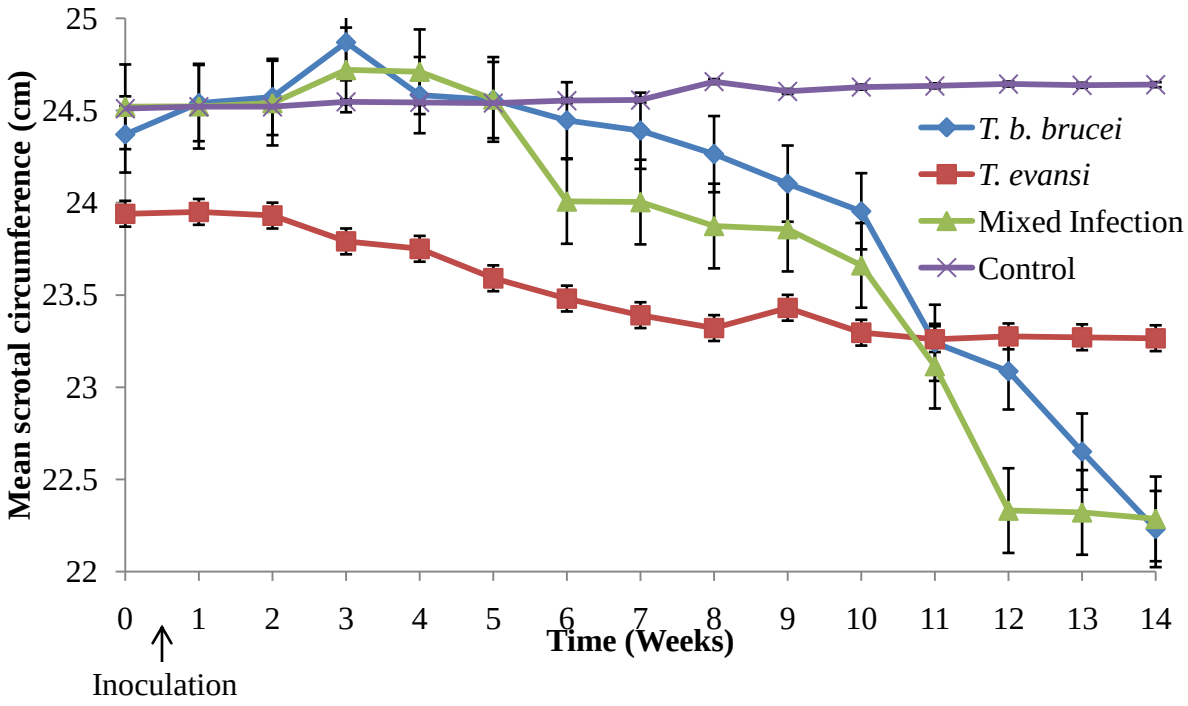


Figure 4.4: Mean weekly scrotal circumference of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III).

respectively. By day 7 p.i., the mean total plasma protein of the infected groups I and III began to decline at different levels of significance upto the end of the experiment (98d.p.i) when compared with that of the control group IV. Rams infected with *T. evansi* (group II) showed that the blood plasma protein were within the normal range though with a slight decrease at the initial stage but maintained a normal value upto the end of the experiment. By the end of the experiment, the final mean plasma protein of all the experimental groups I, II, III and IV were 3.83 ± 0.2 g/dl, 6.01 ± 0.0 g/dl, 3.74 ± 0.3g/dl and 6.23 ± 0.6g/dl, respectively. Statistically, the result showed that the mean total plasma protein decreased significantly ($P<0.01$) in group I and III when compared to group IV, while that of group II was not statistically different ($P > 0.01$) from that of the control group, IV (Figure 4.5).

4.1.7 Mean weekly pack cell volume (PCV)

The pre-infection mean weekly PCV of the rams in the experimental groups I, II III and IV were 33.0 ± 1.2%, 31.0 ± 1.6%, 31.67 ± 1.1% and 32.67 ± 1.5%, respectively. By day 7 p.i., the mean weekly PCV of the infected groups I and III began to decline progressively upto the end of the experiment. By the end of the experiment (98 d.p.i), the mean PCV of the infected groups I and III declined to 18.70 ± 1.4% and 17.48 ± 1.8%, respectively, while that of group II had an initial decrease in PCV (18.00 ± 1.4% by day 49 p.i.), thereafter the value began to increase up to a mean value of 24.75 ± 1.6% by the end of the experiment (Figure 4.6). Generally,there was a significant reduction ($P <0.01$) in the mean weekly PCV for rams in groups I and III when compared to groups II and IV, respectively.

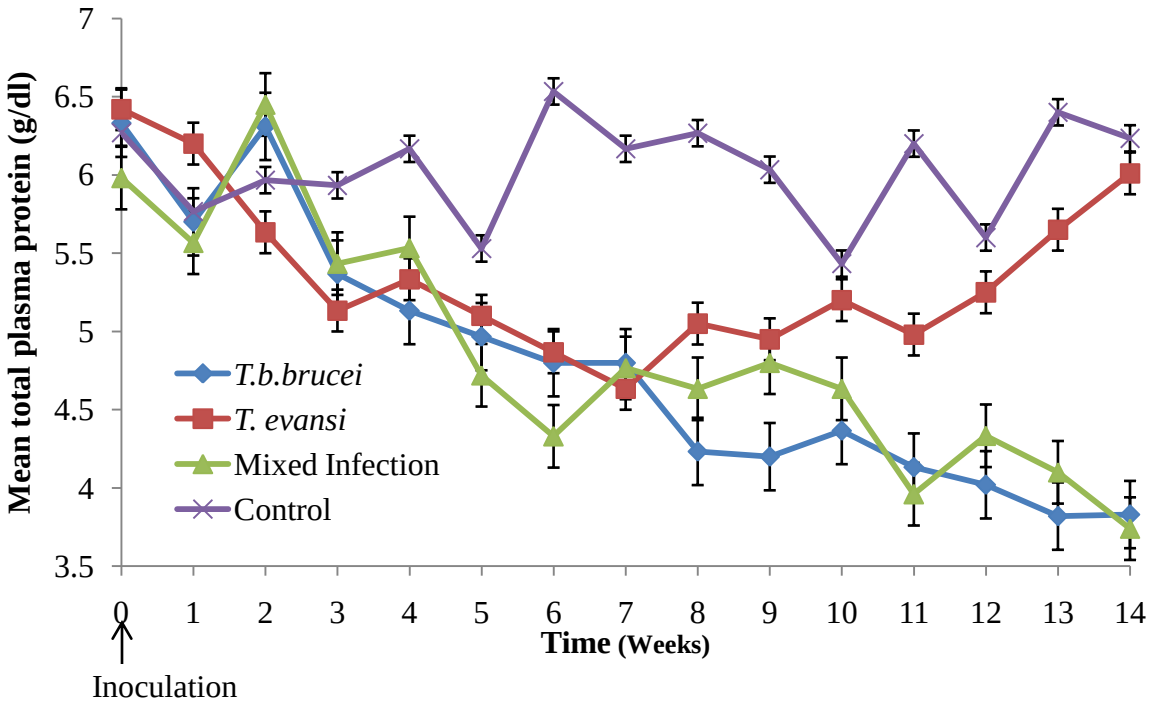


Figure 4.5: Mean weekly plasma protein of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III).

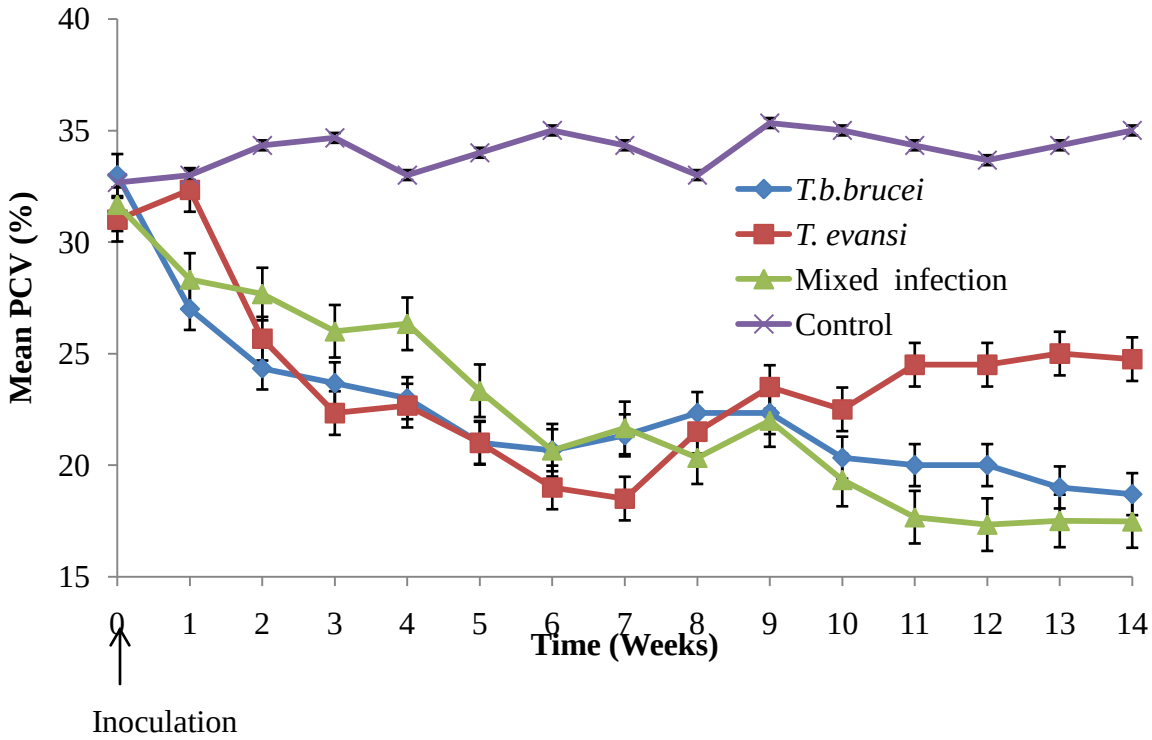


Figure 4.6: Mean weekly packed cell volume of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III).

4.1.8 Mean weekly haemaglobin concentration (Hb)

The pre-infection mean weekly haemaglobin (Hb) values of rams in the experimental groups I, II III and IV were 11.0 ± 1.1 g/dl, 10.33 ± 1.3 g/dl, 10.56 ± 1.1g/dl and 10.89 ± 1.2 g/dl, respectively. By day 7 p.i., the mean Hb value of the infected groups I and III began to decline progressively to post-infection values of 6.23 ± 1.1 g/dl and 5.83 ± 1.0 g/dl at the end of the experiment respectively, while that of the infected rams in group II had an initial decrease (6.17 ± 1.2 g/dl by day 49 p.i.), thereafter the value started increasing up to mean value of 8.25 ± 1.0 g/dl by the end of the experiment (Figure 4.7). Generally, significant reduction ($P < 0.01$) in the mean weekly haemaglobin value was observed in group I and group III when compared to thoseof groups II and IV, respectively (Figure 4.7).

4.2 Semen Characteristics

Table 4.2 shows the semen characteristics of experimental Yankasa rams. For all the semen parameter studied, the mean values for the infected groups I, II and III differed significantly ($P < 0.01$) from those of the control group IV, which remained within normal range throughout the duration of the study (Table 4.2).

4.2.1 Semen collection reaction time (sec)

There was a sharp increase ($P < 0.01$) in the mean semen collection reaction time of rams in all the infected groups, in comparison to the control rams which remained within the normal range by the end of the experiment (Table 4.2).

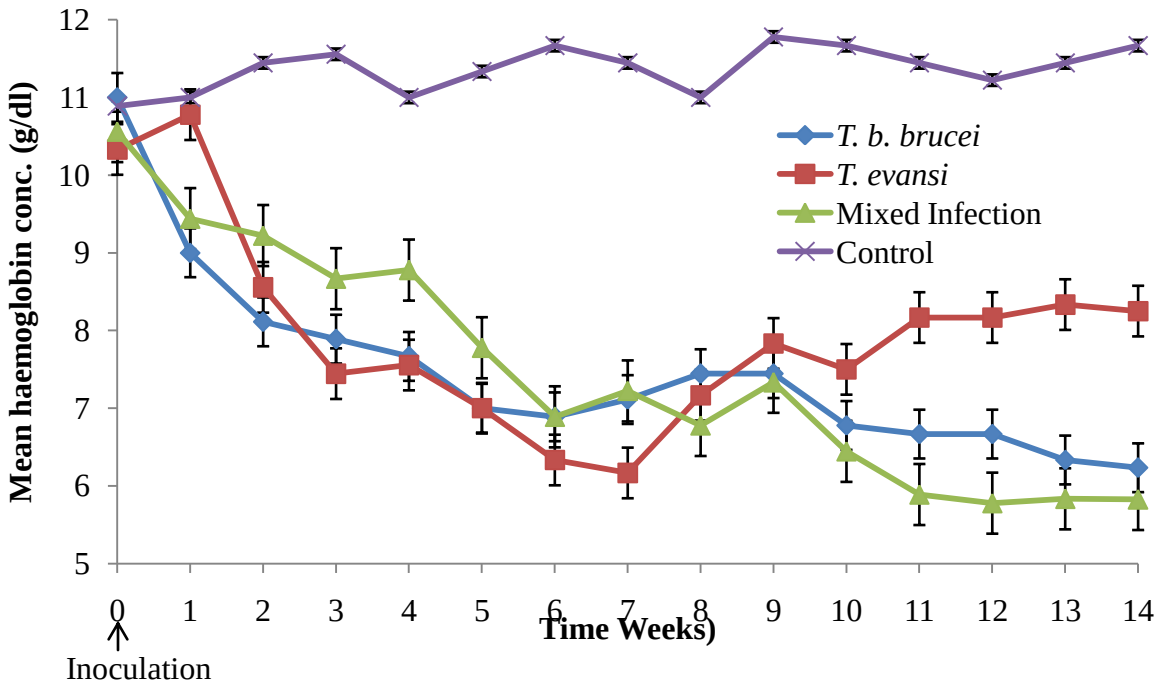


Figure 4.7: Mean weekly haemaglobin concentrationof uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III).

Rams in group I had the highest mean value (62.95±3.21sec) of semen collection reaction time, followed by those of group III (62.13±3.316sec) and group II (46.09±3.41sec) while those of the uninfected controlgroup IV maintained the least mean semen collection reaction time (26.98±3.05sec). Statistical analysis (ANOVA) revealed no significant difference (P> 0.05) existed between the values for infected rams in groups I and III but were significantly different(P < 0.01) from those of groups II and IV at the end of the experiment respectively (Table 4.2).

4.2.2 Semen volume and Colour(ml)

Rams in the control group IV had the highest mean semen volume (0.72±0.02 ml), followed by those of groups II (0.49±0.02 ml), I (0.37±0.03ml) and III (0.36±0.02 ml), respectively(Table 4.2). The mean semen volumes of the infected groups were significantly lowers (P < 0.01) in comparison to that of the control group IV. However, the valuesof groupsI and III were not significantly different (P > 0.01) from each other, but differed significantly (P < 0.01) from those oframs in groups II and IV at the end of the experiment (Table 4.2). The semen colour of all the infected groups differed significantly (P < 0.05) from those of the control groups. The colour ranged from milky white in the control, creamy white in *T. evansi*-infected group, to watery colour (seminal fluids) and in *T brucei brucei*-infected rams and those with mixed infection (*T. b. brucei* and *T. evansi*) (Table 4.2).

4.2.3 Gross forwardmotility (%)

The percent gross forward (wave pattern) motility of spermatozoa in the semen was significantly higher (P < 0.01) in the control group IV (82.83±1.15%) when compared to

all the infected groups I (55.90±3.43%), II (61.86±3.27%) and III (45.87±3.65%),repectively. Among the infected groups, percent gross forward motility was significantly higher ($P < 0.01$) in group II in comparison togroups I and III.

Table 4.2: The semen collection reaction time andsemen characteristics ofinfected and control Yankasarams (Mean ± SEM)

Parameter	GI (*T. b. brucei*)	G II (*T.evansi*)	G III (Mixed)	G IV (Control)	Level of sig.
Collection reaction time (sec)	62.95 ± 3.21^{a}	46.09 ± 3.41^{b}	62.13 ± 3.16^{a}	26.98 ± 3.05^{c}	< 0.001**
Volume (ml)	0.37 ± 0.03^{c}	0.49 ± 0.02^{b}	0.36 ± 0.02^{c}	0.72 ± 0.02^{a}	< 0.001**
Progressive motility (%)	55.90 ± 3.43^{c}	61.86 ± 3.27^{b}	45.87 ± 3.65^{d}	82.83 ± 1.15^{a}	< 0.001**
pH	6.98 ± 0.14	7.26 ± 0.16	7.01 ± 0.16	7.24 ± 0.08	0.233
Sperm concentration ($x10^{6}$ /ml)	381.00 ± 15.00^{b}	409.00 ± 13.00^{b}	372.00 ± 14.00^{b}	891.00 ± 19.00^{a}	< 0.001**
Total sperm/ejaculate ($x10^{6}$ /ml)	141.00 ± 5.00^{bc}	201.00 ± 5.00^{b}	134.00 ± 4.00^{c}	641.43 ± 5.00^{a}	< 0.001**
Dead sperm (%)	36.41 ± 2.88^{b}	27.71 ± 2.37^{c}	44.62 ± 3.03^{a}	12.14 ± 0.82^{d}	< 0.001**
Total abnormality (%)	43.51 ± 2.48^{a}	35.94 ± 2.16^{b}	40.80 ± 2.15^{ab}	8.57 ± 0.55^{c}	< 0.001**

Means with different superscripts across the same row differed significantly. W.H.O, 1992 Standard values for fertile rams: <20% abnormality: < 25% dead sperm.** = High significant difference exists at $P \leq 0.01$
GI (*T.b. brucei*); GII (*T. evansi*); GIII (*T. b. brucei* and *T. evansi* Mixed); GIV (Uninfected Control)

4.2.4 Semen pH

Although, rams infected with *T.brucei brucei* (group I) and those with mixed infection (*T brucei brucei* and *T. evansi*) (group III) had a higher mean pH values, there was no significant differences ($P > 0.01$) in the semen pH of all the experimental groups under study (Table 4.2).

4.2.5 Semen concentration (10^6/ml)

There was high significant decrease ($P < 0.01$) in spermatozoa concentration in millions per ml of all the infected groups I, II and III when compared to the control group IV. Among all the infected groups, spermatozoa concentration was lowest in group III (372.00 ±14 x $10^{6/}$ml) and was highest in group II (409.00 ±13 x $10^{6/}$ml) (Table 4.2). However, ANOVA revealed no significant difference ($P > 0.05$) between all the infected groups I, II and III but were statistically different ($P < 0.01$) in comparison to the control group, IV (Table 4.2).

4.2.6 Total spermatozoa output

The total spermatozoa per ejaculate was significantly higher ($P < 0.01$) in the control group IV (641.52 ±5.00 x $10^{6/}$ml) when compared to all the other infected groups I (141.00 ±5.00 x $10^{6/}$ml), II (201.00 ±5.00 x $10^{6/}$ml) and III (134.00 ±4.00 x $10^{6/}$ml). No difference exist ($P > 0.01$) between infected group I incomparison to groups II and III, respectively. However, there is significant different between group II and III (Table 4.2).

4.2.7 Percent dead sperm (%)

The percent dead spermatozoa were highest in group III (44.62± 3.03%), followed by those of group I (36.41±2.88%) and group II (27.71±2.37%) respectively, while those of the control (group IV) had the least percent (12.14±0.82%) dead spermatozoa at the end of experiment (Table 4.2).

4.2.8 Spermatozoa abnormalities (%)

The mean total spermatozoa abnormalities of all the infected groups I, II and III differed significantly ($P < 0.01$) from that of the control group IV at the end of the experiment (Table 4.2). The highest mean percent spermatozoa abnormalities were observed in group I (43.51±2.48%), followed by those of group III (40.80±2.15%) and finally group II (35.94±2.16 %). The value was lowest (8.57±0.55%) in the control group, IV.

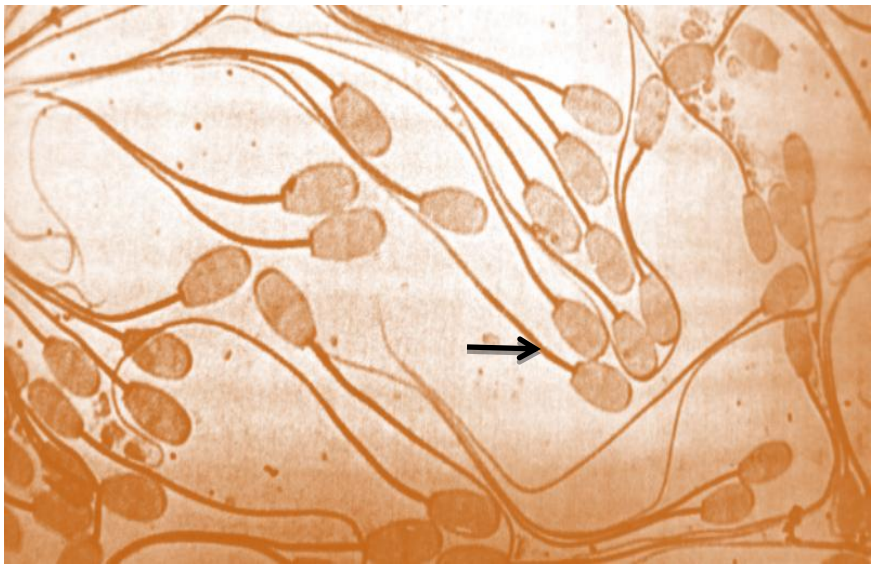


Plate VI: Normal spermatozoa morphology of experimental Yankasa ram (Control)Eosin-Nigrosin stain (X 400)

4.3.0 Mean Weekly Spermatozoan Abnormalities (%)

4.3.1 Incidence of cytoplasmic droplets (MPD) (%)

There was a high rise in the mean weekly percent cytoplasmic droplets (Plate VII) of all the infected Yankasa rams (groups I, II and III) in comparison with the uninfected control (group IV) which had values that remained within the normal range upto the end of the experiment (Figure 4.8). Statistically, there was a significant increase ($P < 0.01$) in percent cytoplasmic abnormality in the infected rams in comparison to that of the control group, being more pronounced in mixed infection (Figure 4.8).

4.3.2 Incidence of detached head (Free head) (DH) (%)

The mean weekly percent incidence of detached head (Plate VIII) of the infected Yankasarams rose dramatically from the pre-infection values of 1.33 ±0.01% (group I), 0.67±0.07 % (group II) and 1.33±0.01% (group III) to higher significant ($P < 0.01$) values of 20.50±2.13%, 13.00±1.38% and 16.25±2.13% by days 98, 84 and 98 post infection respectively (Figure4.9).

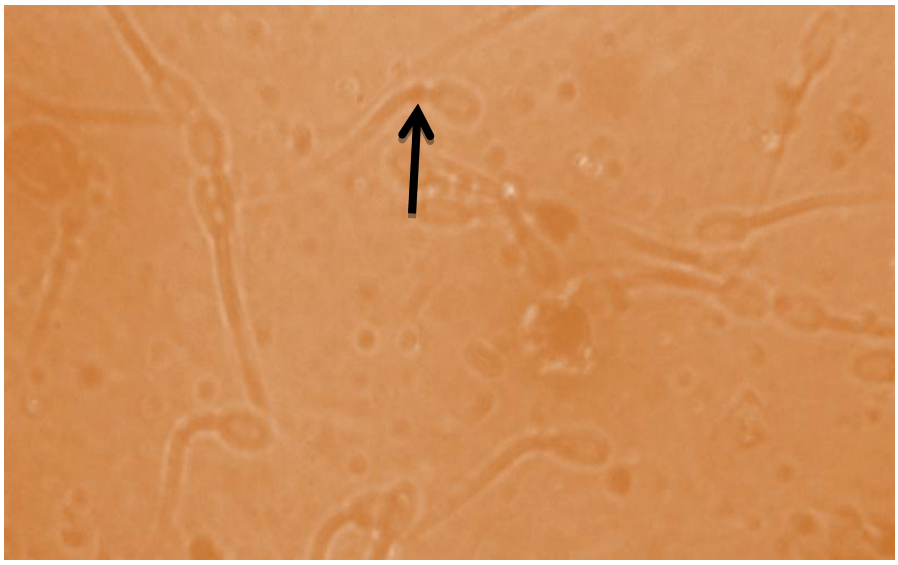


Plate VII:Cytoplasmicdroplets in spermatozoa of experimental Yankasa ram infected with Trypanosomes (X 400 mag)

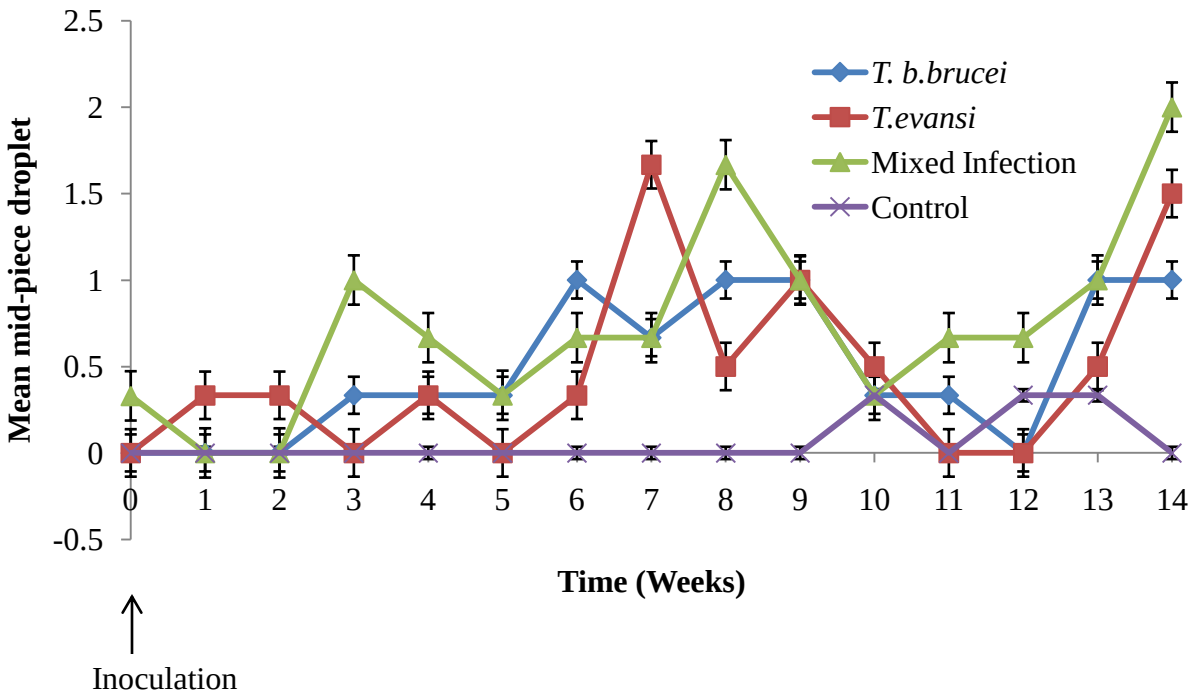


Figure 4.8: Mean weekly incidence of cytoplasmic droplets in spermatozoaof uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III)

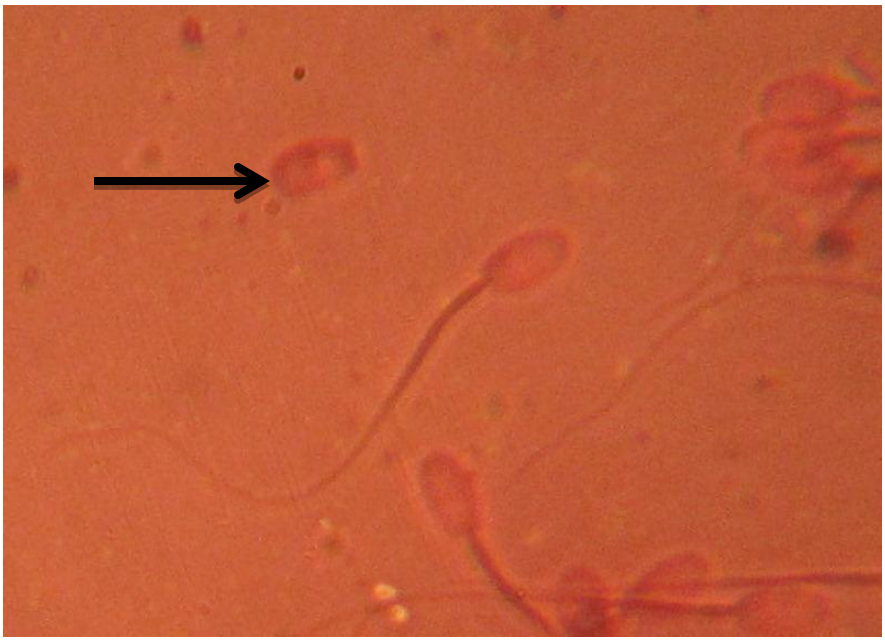


Plate VIII:Detached head in spermatozoa of experimental Yankasa ram infected with *Trypanosoma* Species (X 400).

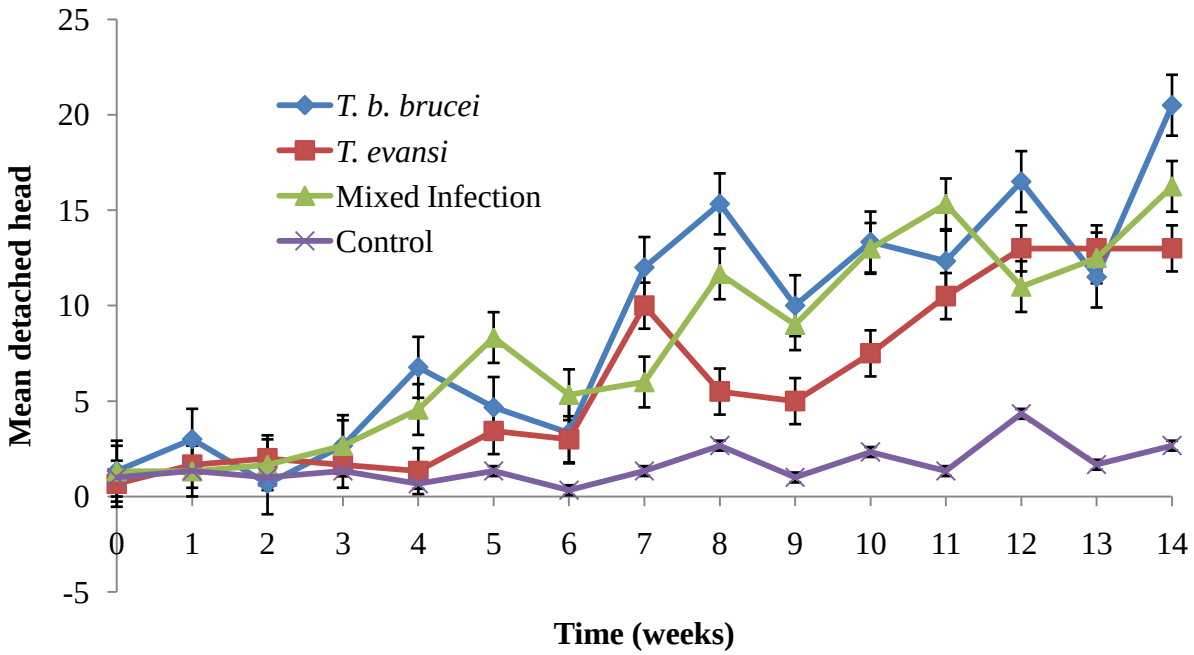
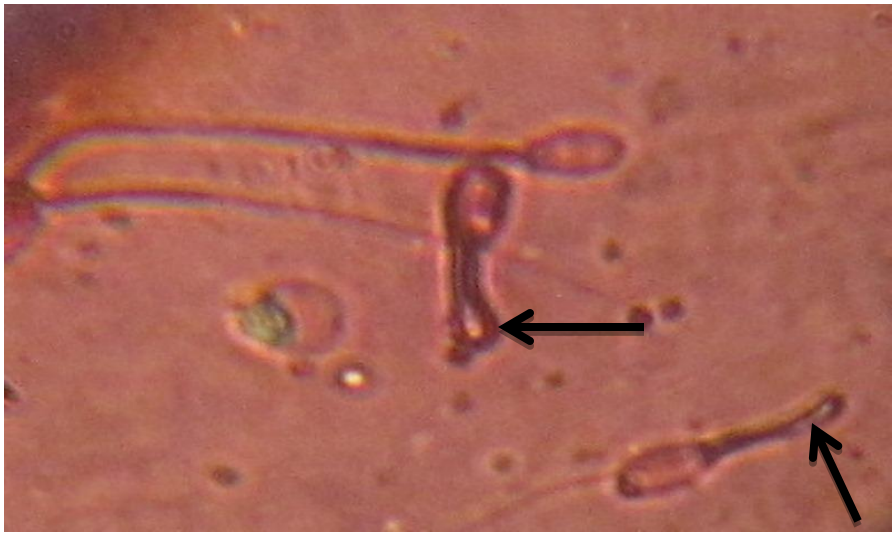


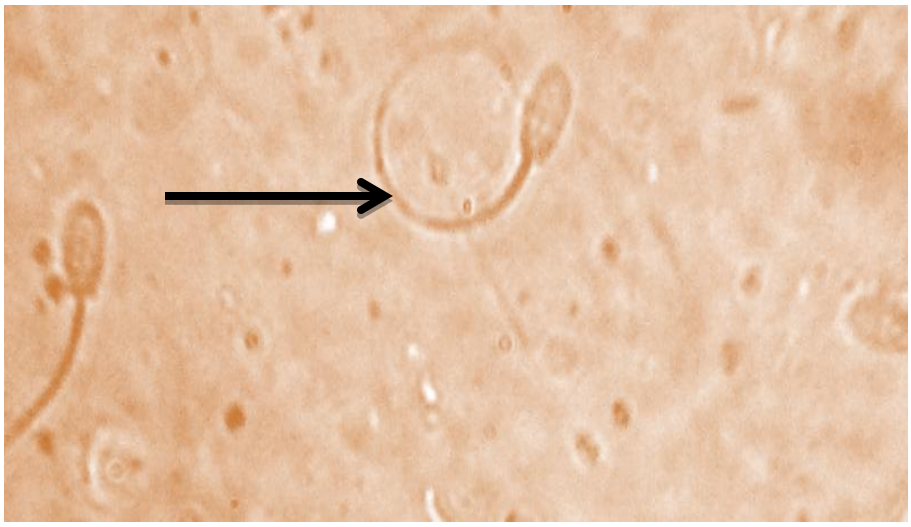
Figure 4.9: Mean weekly incidence of detached head in spermatozoa of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III)

4.3.4 Incidence of tail abnormalities (CT)(%)

Figure 4.10 shows the percent incidence of all tail abnormalities (Plates X) of infected and uninfected control Yankasa rams. At the beginning of the experiment, the mean percent tail abnormality of the infected groups I, II and III were 1.00±0.11%, 1.00±0.02 and 1.17±0.01 respectively.There was a drastic and sharp increase in the mean percent tail abnormalities in all the infected groups I, II and III with values 22.50±2.33%, 17.50±2.14% and 22.33±2.33%, observed at days 91 (week13), 77 (week 11) and 98 (week 14) p.i. respectively. There was significant difference ($P < 0.01$) between the infected groups in comparison to the control group IV (Figure 4.10).



a



b

Plate X: Tail abnormalities in spermatozoa of experimental Yankasa ram infected with *Trypanosoma* Species (a) bent tail (arrow)(b) coiled tail. Eosin-Nigrosin stain (X400)

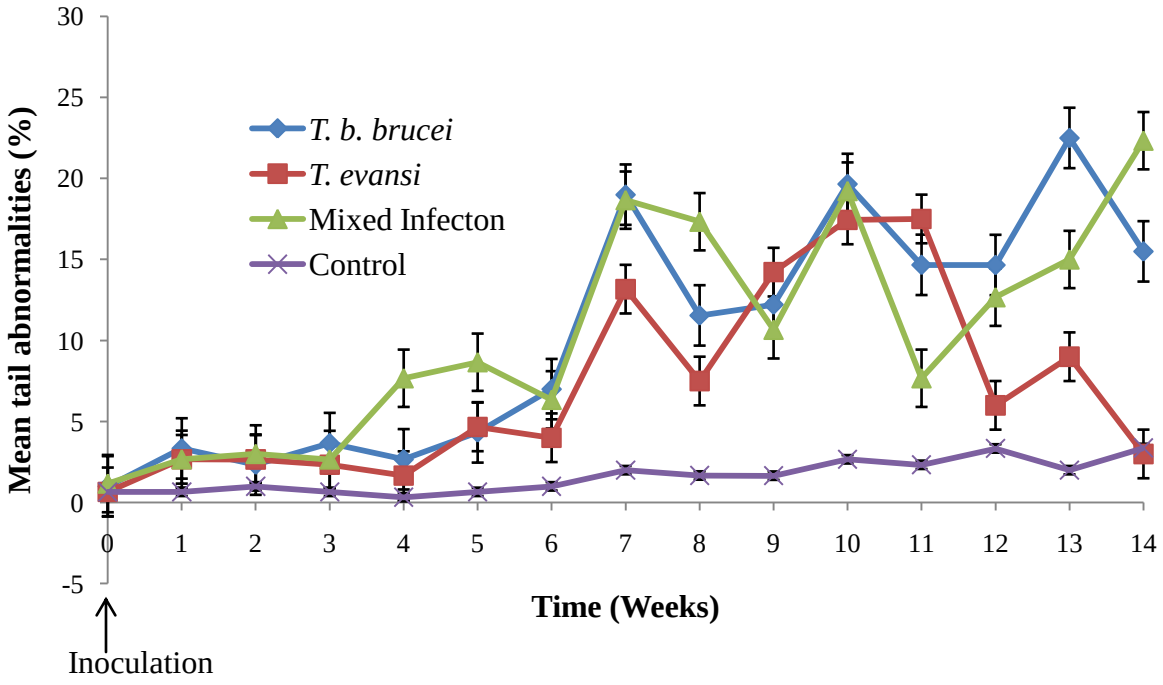


Figure 4.10: Mean weekly incidence of tail abnormality in spermatozoa of uninfected control Yankasa rams and rams experimentally infected with either *T. b. brucei* (Grp I), *T.evansi* (Grp II), or both parasites (Grp III)

4.4.0 Gonadal and Epididymal Sperm Reserve ($X10^6$/gm)

4.4.1 Gonadal sperm / Spermatid reserve ($X10^6$/gm)

The live weight, scrotal circumference, testicular weight, testicular volume and the gonadal sperm reserves of the groups at the end of 98 days post infection period are presented in Table 4.3. There was significant decrease ($P < 0.01$) in the live weights of all the infected groups in comparison to the control group IV. The scrotal circumferences of the infected groups decreasedsignificantly at the end of the experiment when compared with the control group IV (Table4.3). The values of testicular length, volume and weight of all the infected groups were found to be lower ($P < 0.01$) than those of the control (group IV) at the end of the experiment (Table 4.3). There was a drastic decrease in gonadal sperm reserve of the rams in the infected groups I and III which were not significantly different ($P > 0.05$) from each other, but differed significantly ($P < 0.01$) to those of groups II and IV, respectively.The control rams had the highest gonadal sperm reserve at the end of the experiment (Table 4.3).

4.4.2 Epipididymal sperm reserve ($X10^6$/gm)

The epididymal length, epididymal weight and epididymal sperm reserve of experimental Yankasa rams are as shown in Table 4.4 below. Animals in the control group had the highest mean epididymal length of 13.98±0.24cm, which was statistically different ($P<0.01$) from those of the infected groups III (9.88±0.15cm), II (13.14±0.17cm) and I (11.40±0.21cm) respectively. The same trend was observed for the epididymal weights, with those of the control significantly higher ($p< 0.01$) than those of the infected groups (Table 4.4). The overall epididymal sperm reserve was highest in the control group I

(1689.50±71.71 x10^6 /ml). Rams in groups I (494.50 ± 39.57 x10^6 /ml) and III (327.50 ± 28.40 x10^6 /ml) had the lowest values of epididymal sperm reserve which were significantly lower ($P < 0.01$) when compared to those of groups II (776.00 ± 44.15x10^6 /ml) and IV (1689.50±71.71 x10^6 /ml) respectively (Table 4.4).

4.5.0 Gross Pathological Findings

4.5.1 Post mortem

Post mortem examination of rams that served as the uninfected control revealed no significant post mortem findings. There were normal tissues and organ architecture (Plate XII). The rams that had mixed infections (group III) revealed the presence of a very paled and anaemic carcass composition (Plate XIII) with watery blood and severe atrophy of body fats. There was also severe testicular degeneration (Plate XIV) and splenomegaly.

For *T. b brucei* infected rams (group I), both the one (R8) that died and was necropsied at day 77 p.i., and the additional member of the group (R7) that was sacrificed at the end of the experiment (day 98 p.i), revealed the presence of congestedand pneumonic lungs with severe haemorragic points (Plate XV), pale carcass composition, serous atrophy of body fats. There was severe lymphomegaly (Plate XVI), swollen liver (hepatomegaly) and spleen (Plate XVII), with severely degenerated testes.

Table 4.3: Testicular length, Volume, Weight and Gonadal Sperm Reserves of Yankasa rams infected with Trypanosomes, 98 days P.I (Mean ± SEM)

Parameter	GI	G II	G III	G IV (Control)	Level of sig.
Live weight (kg)	21.43 ± 1.69^{bc}	21.32 ± 1.47^{c}	21.70 ± 1.63^{b}	25.61 ± 0.37^{a}	< 0.001**
Scrotal circumference (cm)	23.99 ± 1.19^{b}	23.53 ± 1.56^{c}	23.79 ± 1.08^{bc}	24.59 ± 1.47^{a}	< 0.002**
Testis weight (g) – Right	36.79 ± 3.43^{bc}	47.51 ± 3.94^{b}	30.68 ± 1.88^{c}	90.75 ± 3.35^{a}	< 0.001**
Testis weight (g) – Left	37.20 ± 3.00^{bc}	47.50 ± 3.48^{b}	30.26 ± 1.59^{c}	90.65 ± 2.55^{a}	<0.001**
Paired Testis weight (g)	36.99 ± 2.12^{c}	47.51 ± 2.12^{b}	30.47 ± 2.12^{c}	90.70 ± 2.12^{a}	<0.001**
Testis length (cm) – Right	6.65 ± 0.15^{bc}	7.25 ± 0.25^{b}	6.10 ± 0.30^{c}	10.05 ± 0.35^{a}	< 0.002**
Testis length (cm) – Left	6.45 ± 0.25^{bc}	7.15 ± 0.25^{b}	5.75 ± 0.25^{c}	9.85 ± 0.35^{a}	< 0.002**
Paired testes length (cm)	6.55 ± 0.20^{c}	7.20 ± 0.20^{b}	5.93 ± 0.20^{c}	9.95 ± 0.20^{a}	< 0.001**
Testis volume (ml) – Right	23.50 ± 1.50^{c}	40.50 ± 0.50^{b}	31.50 ± 0.50^{bc}	75.00 ± 5.00^{a}	< 0.001**
Testis volume (ml) – Left	24.00 ± 4.00^{c}	38.00 ± 0.50^{b}	25.50 ± 3.50^{c}	68.50 ± 3.50^{a}	< 0.002**
Gonadal sperm reserves ($x10^6$/gm)	40.00 ± 2.12^{c}	86.00 ± 13.50^{b}	32.00 ± 8.82^{c}	112.50 ± 21.77^{a}	< 0.001**

Means with different superscripts across the same row differed significantly. ** = significant difference exists at $P \leq 0.01$
GI (*T.b. brucei*); GII (*T. evansi*); GIII (*T. b. brucei* and *T. evansi* Mixed); GIV (Uninfected Control)

Table 4.4: Epididymal length, Weight and Sperm reserve of Yankasa rams infected with Trypanosomes, 98 days P.I (Mean ± SEM)

Parameter	GI	G II	G III	G IV (Control)	Level of sig.
Paired epididymal length (cm)	11.40 ± 0.21^{c}	13.14 ± 0.17^{b}	9.88 ± 0.15^{d}	13.98 ± 0.24^{a}	< 0.001**
Weight of epididymis(g)- Right	8.43 ± 0.19^{bc}	10.02 ± 0.75^{b}	6.57 ± 0.22^{c}	20.60 ± 0.80^{a}	< 0.001**
Weight of epididymis (g)- left	8.14 ± 0.14^{c}	11.08 ± 0.74^{b}	6.76 ± 0.05^{c}	20.05 ± 0.75^{a}	< 0.001**
Paired epididymal weight (g)	8.28 ± 0.39^{c}	10.55 ± 0.39^{b}	6.64 ± 0.39^{d}	20.33 ± 0.39^{a}	< 0.001**
Epididymal Sperm Reserve ($X10^{6}$/gm)					
Epididymal head`(Caput)($x10^{6}$/gm)	109.50 ± 16.29^{b}	116.50 ± 4.59^{b}	95.00 ± 22.42^{b}	201.50 ± 19.97^{a}	< 0.001**
Epididymal body (Corpus) ($x10^{6}$/gm)	35.00 ± 3.50^{b}	72.00 ± 4.81^{b}	18.00 ± 3.54^{b}	110.25 ± 19.32^{a}	< 0.001**
Epididymal tail (Cauda) ($x10^{6}$/gm)	350.50 ± 37.73^{bc}	587.50 ± 47.10^{b}	214.00 ± 23.78^{c}	1378.50 ± 60.80^{a}	< 0.001**
Total	494.50 ± 39.57^{bc}	776.00 ± 44.15^{b}	327.50 ± 28.40^{c}	1689.50 ± 71.71^{a}	< 0.001**

Means with different superscripts across the same row differed significantly. ** = Significant difference exists at $P \leq 0.01$
GI (*T.b. brucei*); GII (*T. evansi*); GIII (*T. b. brucei* and *T. evansi* Mixed); GIV (Control)

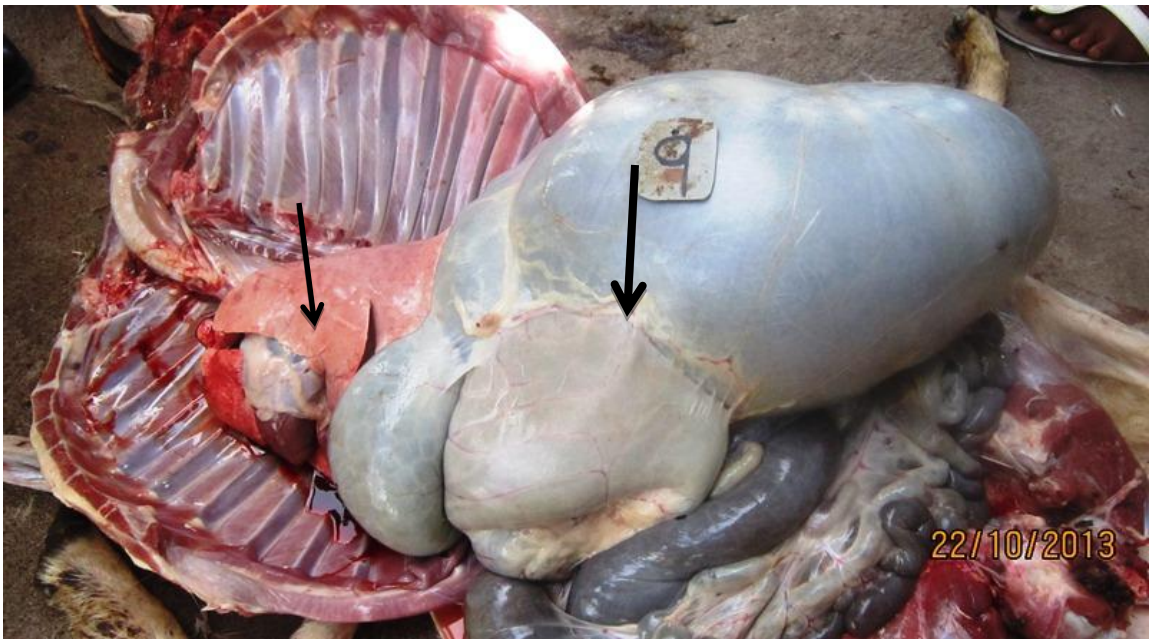


Plate XII: A normal tissue and organ architecture of control Yankasa ram with fat deposits (arrow) on most of the tissues and organs with red and normal carcass composition.

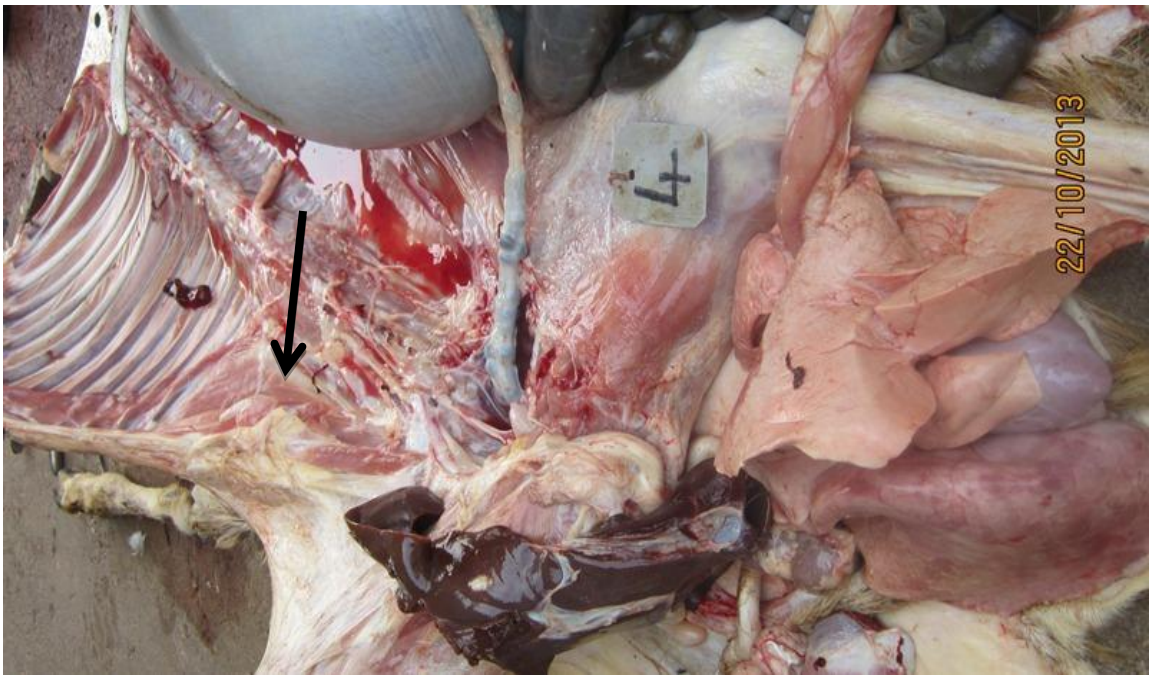


Plate XIII: A very pale and anaemic carcass (arrow) composition, with watery blood and severe atrophy of body fats inYankasa ram with mixed infection (*T. brucei brucei* and *T. evansi*).



Plate XIV: Testicular atrophy inYankasa ram with mixed infection (*T. brucei brucei* and *T. evansi*).

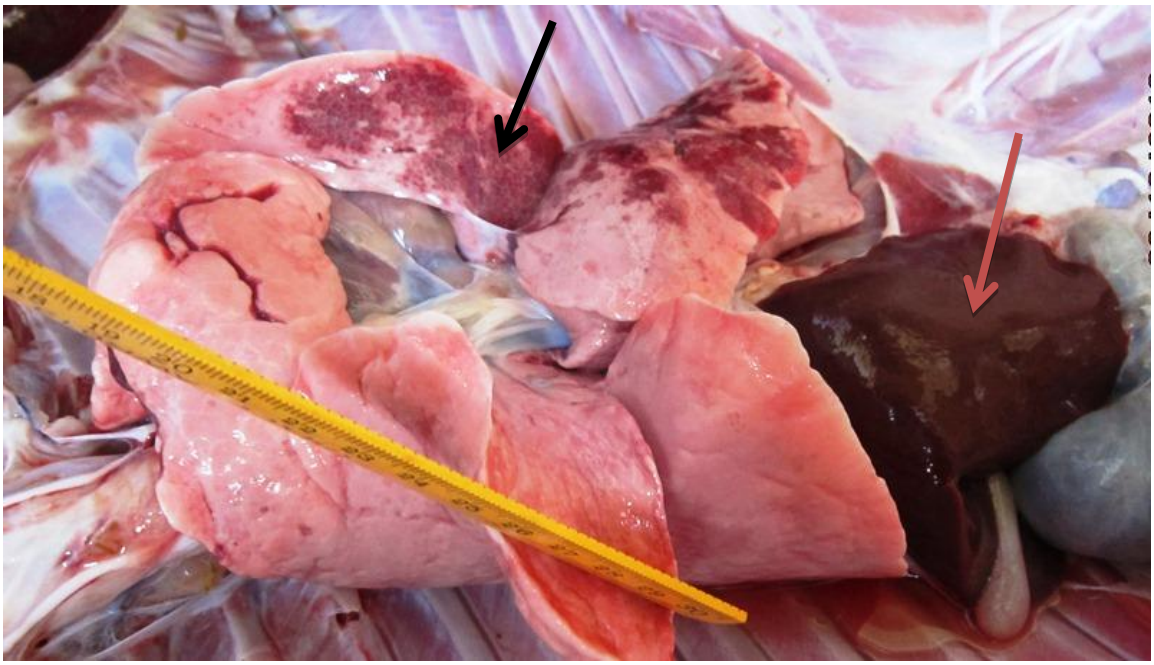


Plate XV: A congested and pneumonic lungs with severe haemorragic points (black arrow) and an enlarged liver (red arrow) inYankasa ram infected with *T. brucei brucei* (group I).



Plate XVI: An enlarged lymph node (black arrow) and a normal lymph node (red arrow) of infected and control Yankasa rams respectively.

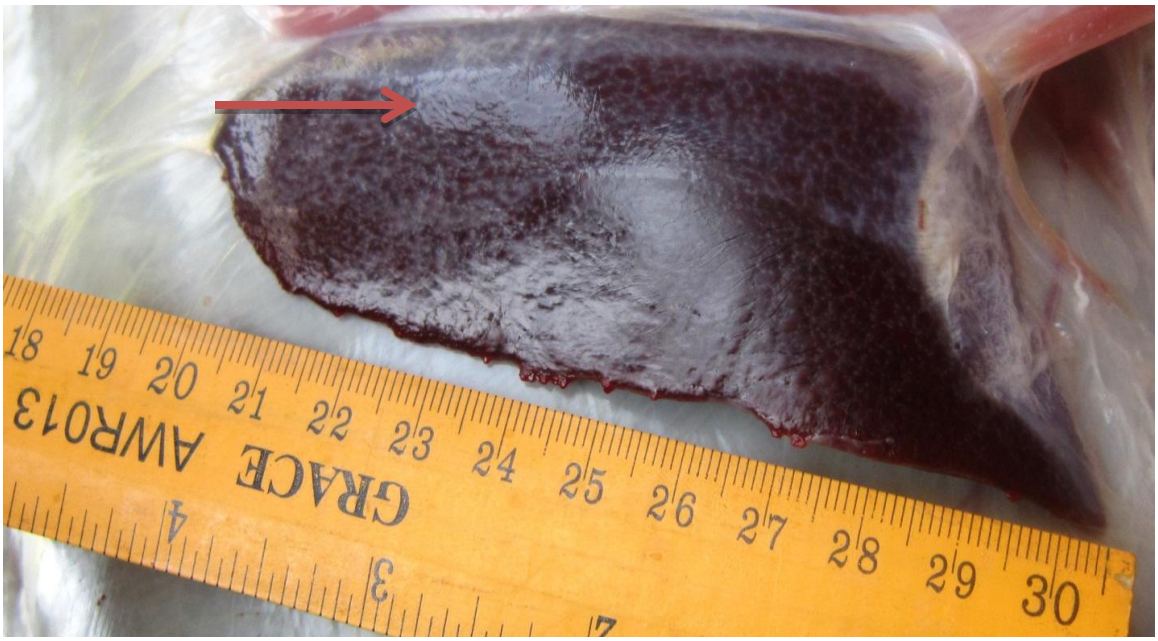


Plate XVII: An enlarged spleen ofYankasa ram infected with *T. brucei brucei*.

For rams in group II (*T. evansi*) that died by day 56 p.i, post mortem analysis revealed the presence of severe atrophy of body and intestinal fats, loss of tissue architecture, and severe paled and anaemic carcass composition with severe watery blood (Plate XVIII). However, the other member of the group that was sacrificed at the end of the experiment (day 98 p.i.) did not reveal significant ($P > 0.01$) post mortem finding.

4.5.2 Histological findings of tissues and organs

4.5.2.1 *Testes*

Histological sections of the testes of the control Yankasa rams (group IV) showed normal tissue architecture with well defined basement membrane, spermatogonic cell layers, spermatocytes with full sperm reserve in the lumen of the seminiferous tubule (Plate XIX-a), while those of the infected Yankasa rams (groups I, II and III) showed moderate (*T. evansi*-infected group) (Plate XIX-b) to severe (Mixed and *T. b. brucei*-infected groups) testicular degenerations with reduction in number of spermatogonic cell layers, degenerated seminiferous tubules and congested interlobular spaces with loss of tissue architecture and loss of gonadal sperm reserve in *T brucei brucei*-infected group (Plate XIX-c) as well as in those infected with mixed infection (Plate XIX-d).

4.5.2.2 *Spleen*

Histological sections of the spleen of control Yankasa rams showed normal tissue architecture (Plate XX-a), while those of the infected groups I, II and III showed congested spleens with severe lymphocytic proliferations at their germinal centres (Plate XX-b). These features were more prominent in *T brucei brucei*-infected rams (group I) than those with mixed and *T. evansi* infections (groups II and III respectively).

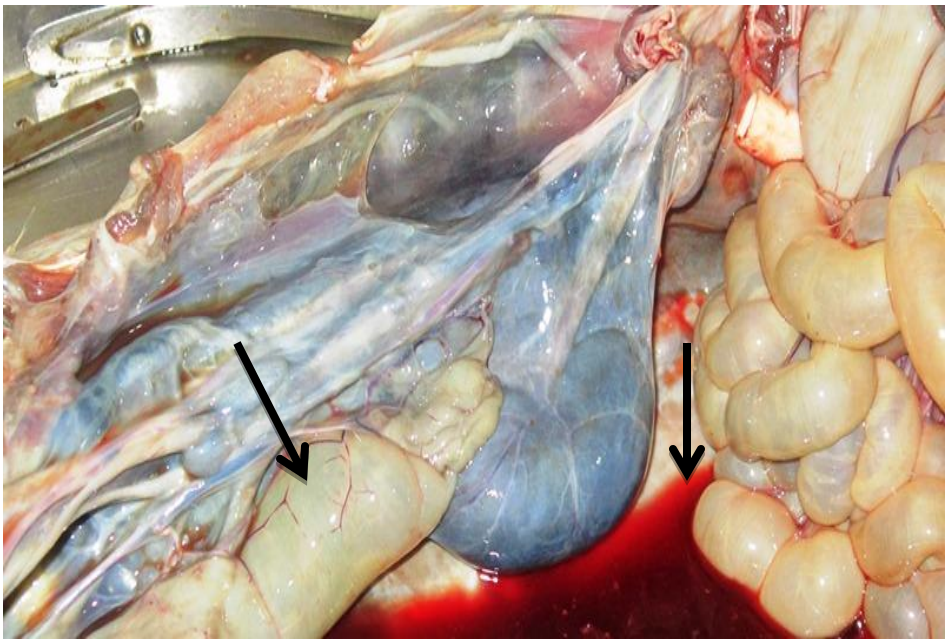
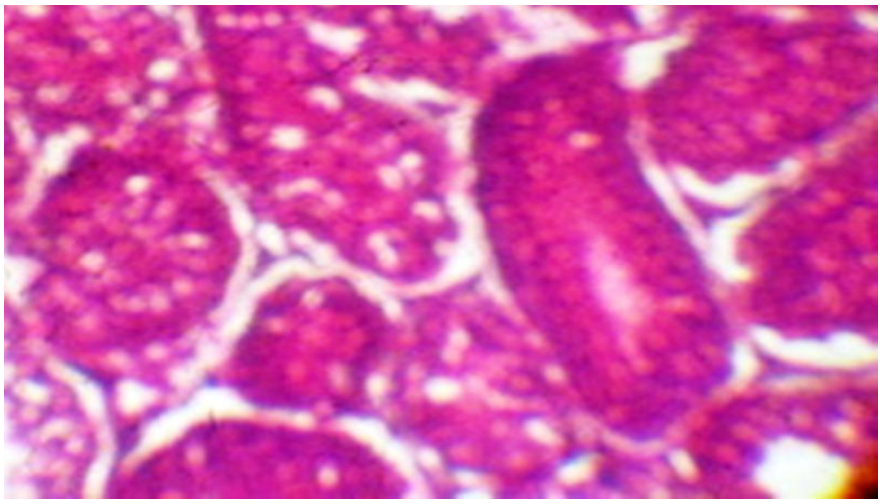
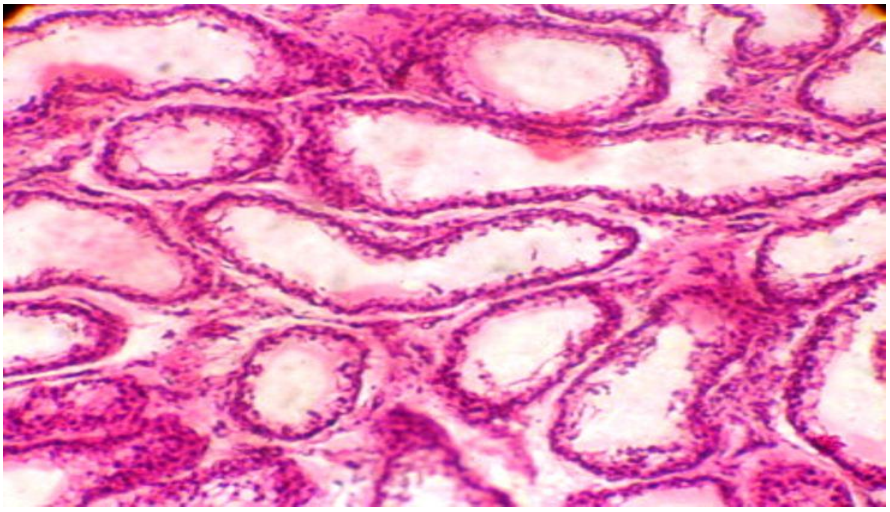


Plate XVIII: Serous atrophy of intestinal (arrow) and body fats with watery blood (arrow) of Yankasa ram infected with *T. evansi* (group II).

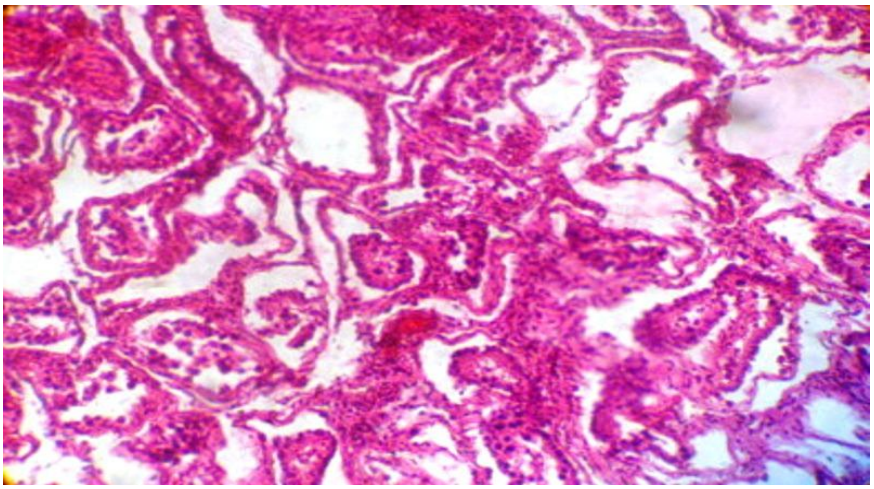


a

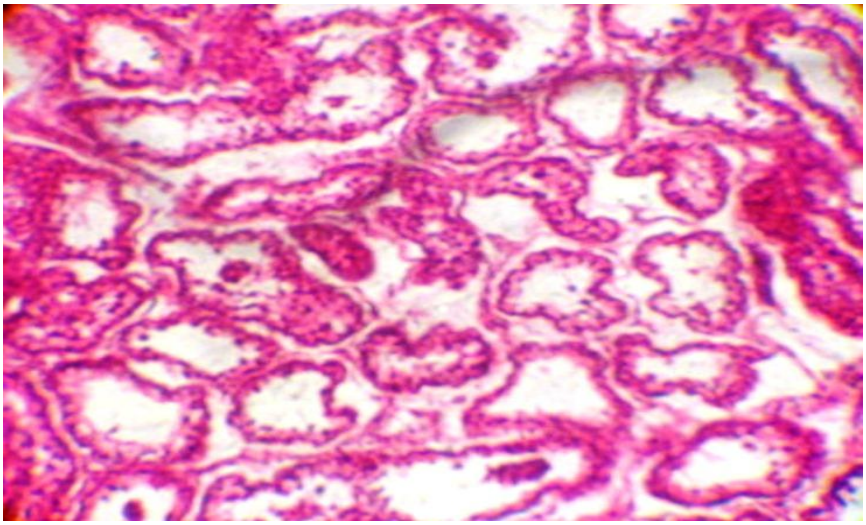


b

Plate XIXa-b: A typically normal testes (a) of control Yankasa ram showing full tissue architecture with well defined basement membrane, spermatogonic cell layers, 1^{o} spermatocytes, 2^{o} spermatocytes, spermatids and full sperm reserve in the lumen of the seminiferous tubules and a moderately degenerated testes (b) of*T. evansi*-infected Yankasa ram with reduction in the spermatogonic cell layers, loss of spermatids and less commonly spermatocytes with absence of gonadal sperm reserve in most of the lumen of the seminiferous tubules (Haematoxylin and Eosin, X400)

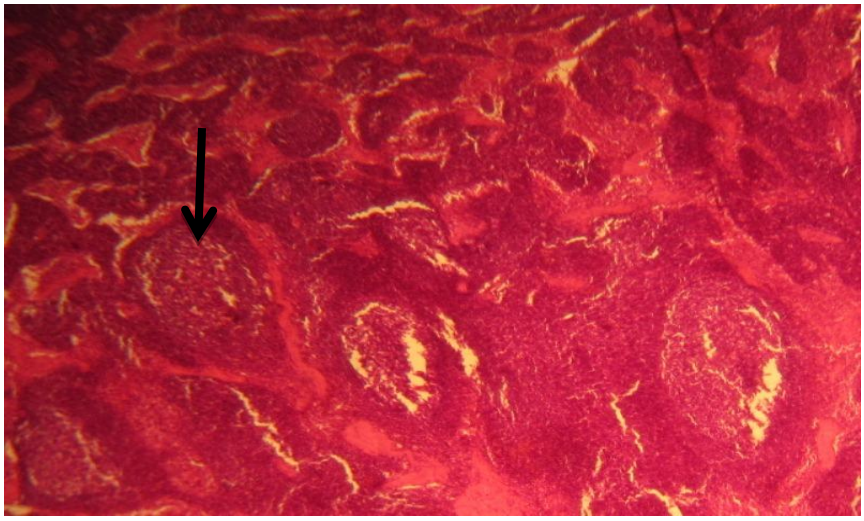


c

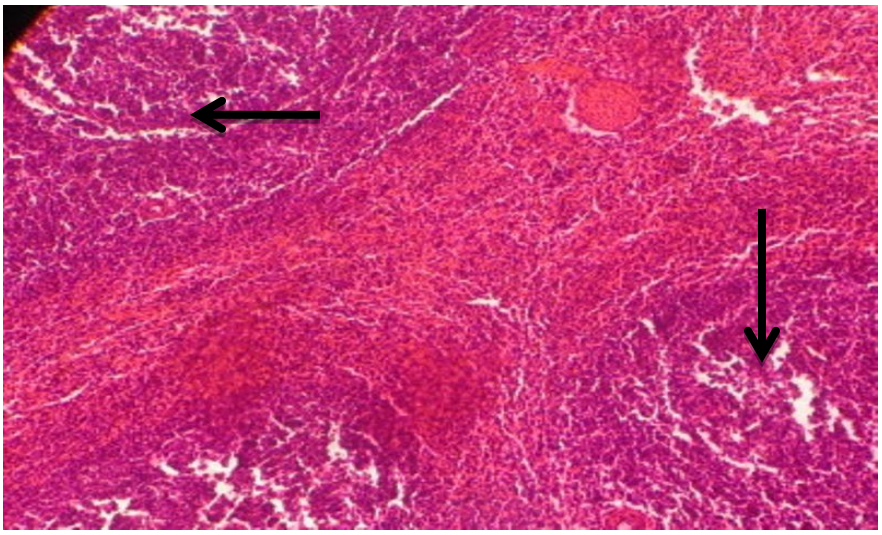


d

Plate XIXc-d: Severe testicular degeneration (c) of *T. b. brucei*-infected Yankasa ram showing reduction in number of spermatogenic cell layers, degenerated leydig and sertoli cells with mononuclear cell infiltration in the seminiferous lumen with almost lost of sperm reserveand a section of testes (d) of Yankasa ram with mixed infection showing diffused degeneration of the basement membrane and sertoli cells with severe depletion of epididymal sperm reserve (Haematoxylin and Eosin, X400)



a



b

Plate XXa-b: A Normal spleen architecture (a) of a control Yankasa ram showing a normal germinal centre (arrow)and proliferation of lymphocytes at the germinal centres of the spleen (b)of *T. b brucei*-infected Yankasa ram(Haematoxylin and Eosin, X400)

4.5.2.3 *Liver*

All the infected rams showed congested liver, with mononuclear cell infiltrations. There was in addition, observed sinusoidal spaces (Plate XXI) in those rams with mixed infections (*T.evansi* and*T brucei brucei*). There were no significant histhological findings in those of the control rams.

4.5.1.4 *Lungs*

No significant histhopathological findings occurred in the control rams (Plate XXIIa). Histhological sections of the lungs of all the infected groups revealed lungs with alveolar degeneration, congestion, diffuse macrophage infiltration into the lung parenchyma and pulmonary oedema in both mixed infections and *T brucei brucei*-infected group, which was moderate in the *T. evansi* infected group (Plate XXIIb).

4.5.2.5 *Kidney*

No significant histhopathological findings were observed in the kidney sections of the control rams as well as those of mixed infection and T.evansi infected rams (Plate XXIIIa) However, significant changes were observed in the kidney sections of *T. brucei brucei*-infected rams characterized by renal tubular degeneration, with mononuclear cell infiltration (Plate XXIIIb).

4.5.2.6 *Lymph nodes*

There was proliferation of lymphocytes of tissue sections of the lymph nodes of all the infected rams (Plate XXIV). The control rams showed normal lymph nodes with no significant histopathological changes.

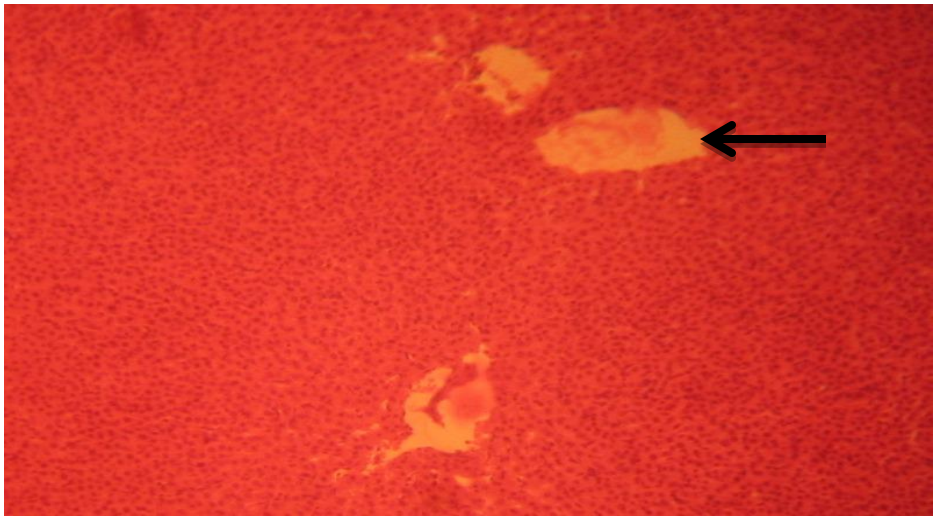
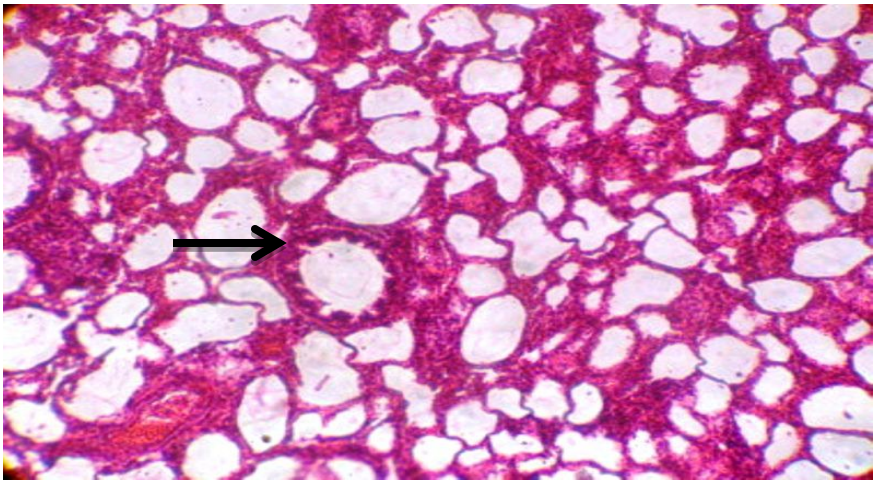
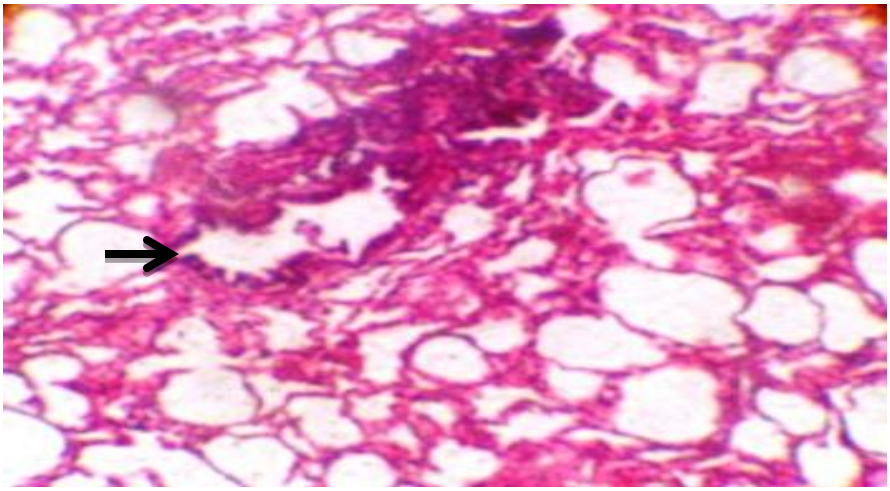


Plate XXI: Congested liver with mononuclear cell infiltration with sinusoidal spaces (arrow) in the liver of Yankasa ram with mixed infection (group III) (Haematoxylin and Eosin, X400).

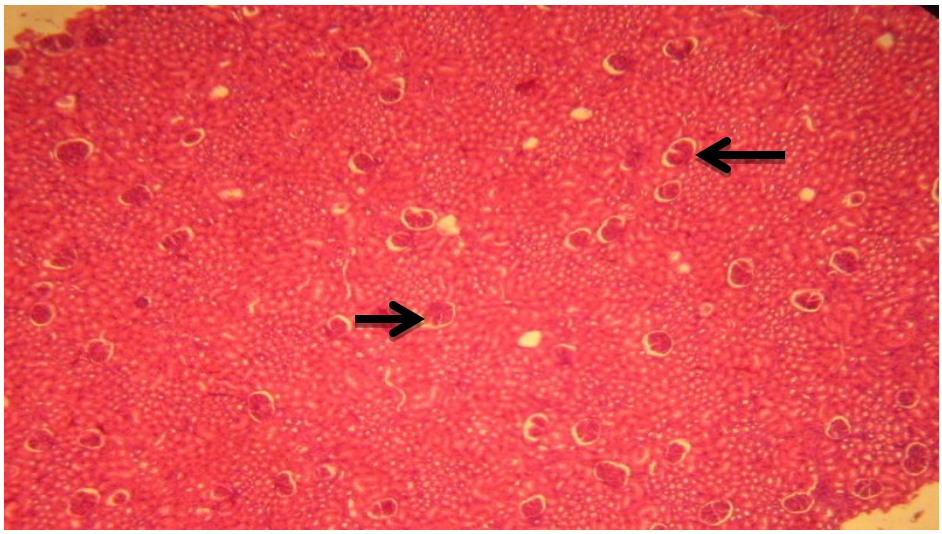


a

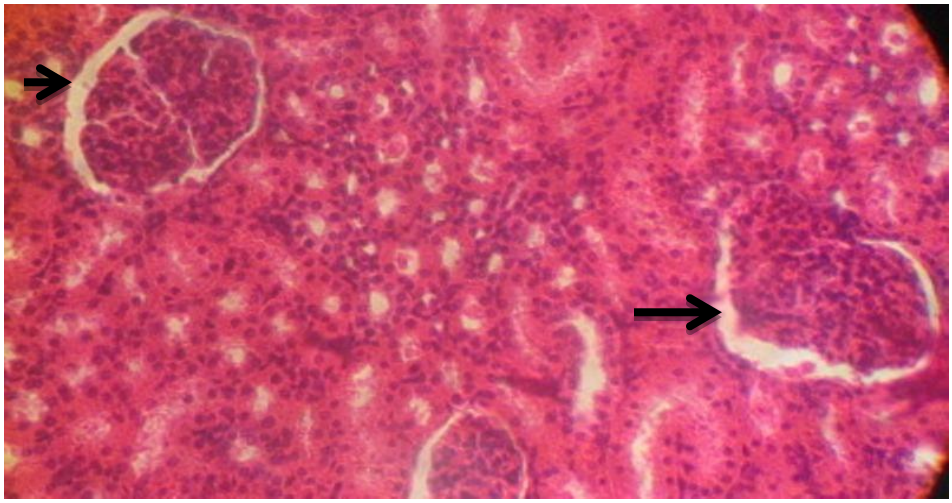


b

Plate XXIIa: A normal lung architecture (a) with normal alveoli and complete folds (arrow) of control ram andalveolar degeneration with loss of folds in the lumen (b)of the lungs of Yankasa ram with mixed infection (Haematoxylin and Eosin, X400)



a



b

Plate XXIIIa-b: A normal kidney architecture(a) showing normal renal tubules (arrow) of Control Yankasa rams andrenal tubular degeneration (arrow) with mononuclear cell infiltration(b)in the kidney of Yankasa ram with *T .b. brucei* infection (Haematoxylin and Eosin, X400)

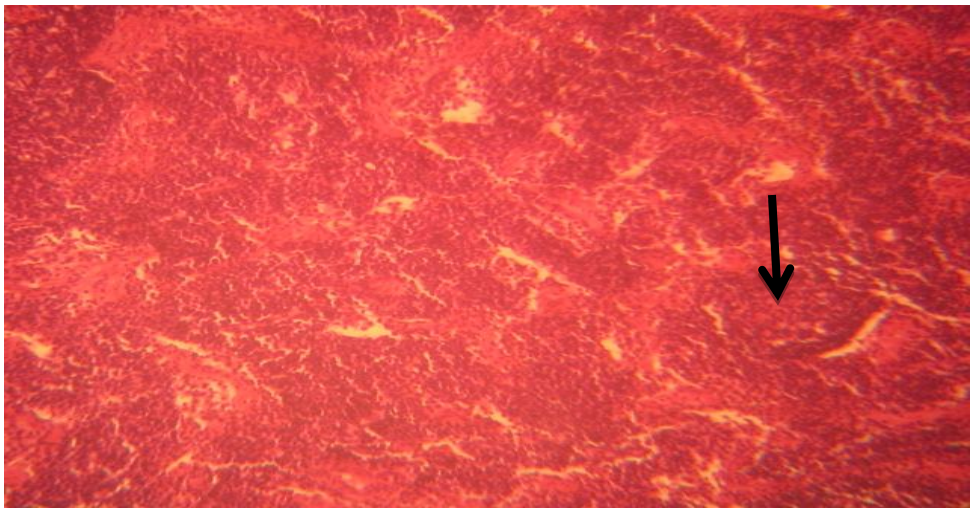


Plate XIV: Proliferation of lymphocytes in the lymph node of Yankasa ram infected with *T.evansi* (group II) (Haematoxylin and Eosin, X400)

4.5.2.7 *Heart*

There was mononuclear cell proliferation of tissue sections of the hearts of all the infected rams (Plate XV). The control rams showed normal heart architecture with no significant histopathological changes.

4.5.2.8 *Brain*

There was mononuclear cell proliferation of tissue sections of the brain of infected rams (Plate XVI). The control rams showed normal brain architecture with no significant histopathological changes.

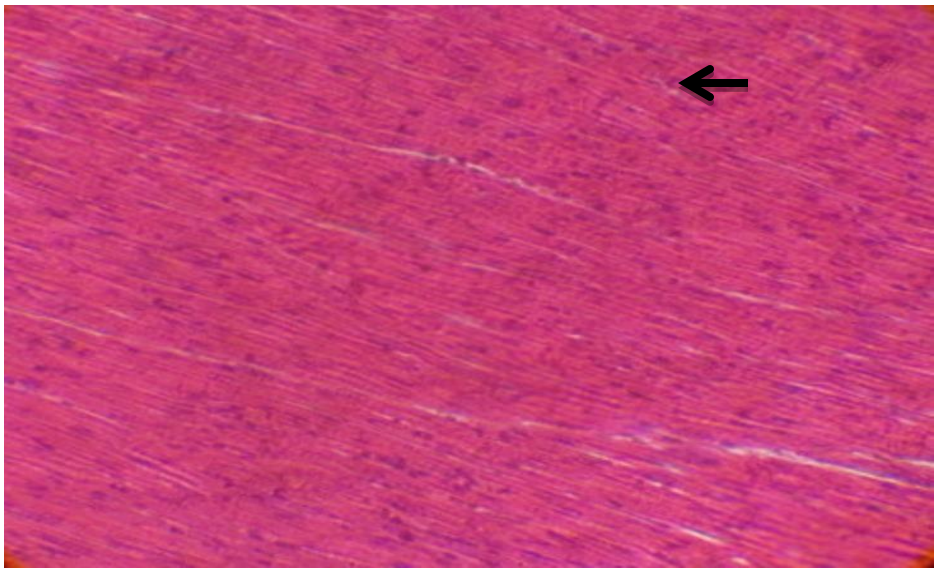


Plate XV: Mononuclear cell infiltrations (arrow) in the Heart of Yankasa ram infected with *T.evansi* (group II) (Haematoxylin and Eosin, X400)

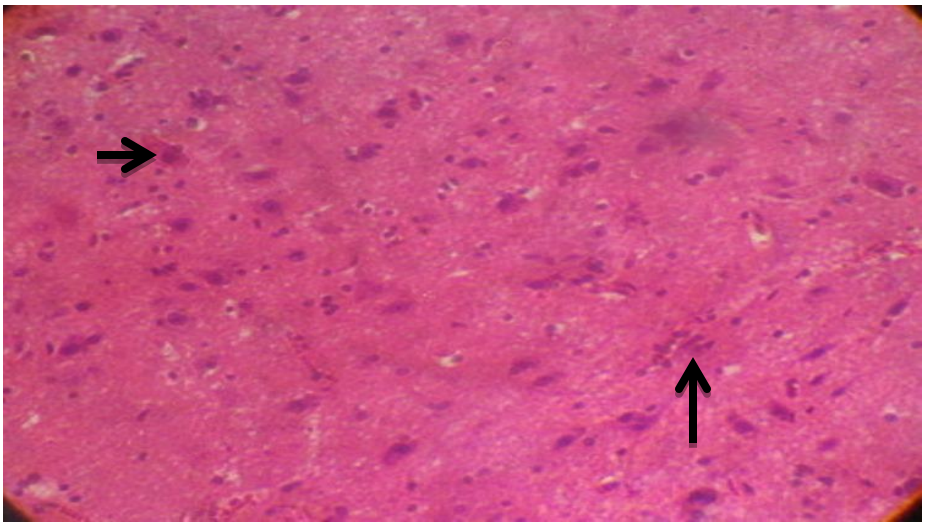


Plate XVI: Mononuclear cell infiltrations on the tissue section of the brain of Yankasa ram infected with *T. b.brucei*(Haematoxylin and Eosin, X400).

CHAPTER FIVE

5.0 DISCUSSION

The prepatent periods in the present study were found to be shorter in *T. brucei brucei*-infected rams and those with mixed infection (*T. brucei brucei and T. evansi*), but longer in *T. evansi* infected rams. For those with mixed infections, it implies that *T. brucei brucei* would appear earlier in blood circulation than *T. evansi* as evident in Giemsa-stained thin blood films. This may be due to the dominant competitive ability of *T. b. brucei* to suppress *T. evansi*in mixed infections. The shorter prepatent period observed for *T.b.brucei*-infected rams in the present study agrees with similar findings by Allam *et al*.(2006), Adeiza *et al*.(2008) and Mbaya *et al*.(2009b) that *T. brucei brucei* had a very short prepatent period.

However, the longer prepatent period of 19-21 days observed for *T. evansi* infected rams in the present studydeviates from earlier reports by Audu *et al*. (1999), Shehu *et al*. (2010) and Ogbaje *et al*. (2011); they reported prepatent periods of 3-6 days (in Yankasa sheep), 7-11 days (in Savanna brown bucks) and 3 days (in WAD goats) with *T. evansi* infections, respectively. These discrepancies in the prepatent periods with respect to *T. evansi* may be attributed to such factors asstrains and virulence of the isolates, the immune, nutritional and degree of susceptibility of the hosts to the isolates.Audu *et al*. (1999) reported *T. evansi* (Kano isolate) to be very pathogenic to Yankasa sheep, while Mohammed (2000) reported *T.evansi* (Sokoto isolate) to be very pathogenic to Savanna brown goats, mice and rats. However, Ogbaje*et al*. (2011) in a study of infectivity and pathogenicity of the Sokoto isolate of *T. evansi* in WAD goats reported the isolate to be

infective but mildly pathogenic. Therefore, the longer prepatent period of *T.evansi*(Sokoto isolate) in Yankasa rams in the present study as compared to other reports may be due to the fact that the Sokoto isolate used in the present study may not be as virulent and pathogenic as the Kano isolates. In addition, the good nutrition that was supplied during the study may have confered high level of immunity on the host against the parasites.

Parasitaemic waves were observed to concide with rise in rectal temperature and a corresponding decline in PCV at some points during the study. The characteristic nature of the parasitaemia may be attributed to the ability of the parasites to evade the immune response of the host through the phenomenon of antigenic variation. Trypanosomes are covered by a dense coat of variant surface glycoproteins that stimulate antibody production in the hostas has been reported by Morrison *et al*. (2009). The absence of parasites from the peripheral blood circulation in *T. evansi*-infected rams towards the end of the experiment might be as a result of the extravascular nature of the parasites, thus, they completely invade the tissues of the host thereby affecting the tissues and organs. Another possible reason may be due to the inherent ability of the rams to manifest self cure as has been reported by Ogbaje *et al.* (2011) in west African dwarf goat infected with *T. evansi* - i.e,ability of the animalsto undergo spontaneous recovery without being treated- against *T. evansi* (due to mild nature of the parasite on the host) thereby, limiting the parasitaemia and possible anaemia evidenced by the gradual recovery in weight gain and haematological indices (PCV and Hb) towards the end of the experiment. Another reason may be the good nutrition supplied during the experiment, which enabled the rams

to mount effective immunity in limiting and reducing the parasites. The ram that died of *T.evansi* infection at day 49 p.i.could be attributed to the fact that the animal's immune response was weak to mount effective immunity against the parasite, thereby predisposing it to other secondary infections as evidenced by congested andpneumonic lungs at necropsy. Greenwood and Whittle (1980)were of the opinion that impairment of the immunity may play some part in predisposing animals to secondary infections. The observed pneumonic lungs in the ram that died of *T. evansi*agrees with the observation of Audu *et al*. (1999) in *T.evansi*-infected sheep in Zaria (Northern Nigeria) and Krammer (1986) in natural trypanosomosis of WAD sheep and goats in Eastern Nigeria. The nature of parasitaemia observed ingroups I(*T.brucei brucei*) and III (mixed infection) were suggestive that *T. b. brucei* was more virulent and pathogenic than *T. evansi* for Yankasa rams in the present study.

Increase in rectal temperature could lead to metabolic disorder, which could possibly play a significant role in the process of haemolysis and consequent decrease in PCV (anaemia). Red blood cells exposed to temperature a few degrees above normal body temperature exhibit increase osmotic fragility, shortened lifespan and undergo accelerated haemolysis (Zwart *et al*., 1991).

Despite the apparent good diet supplied to the experimental animals, there was reduction in feed intake and loss in body weights. The loss in body weight might be associated with fever. Fever during infection is associated with increased heat production and increased metabolizable energy for maintenance so that the proportion of protein used for growth

was reduced, as it is metabolized by infected animals to provide the extra energy required. This increased synthesis of protein, occured at the expense of muscle protein catabolism, hence, loss in body weight, resulting in the wasting nature of the disease. Zwart *et al*. (1991) reported a relationship between dry matter and rectal temperature; in that the dry matter intake by goats decreased with increase in rectal temperature resulting in weight loss.

Most of the clinical observations made during the course of the disease may be directly attributed to the extravascular invasion by the parasites and resultant tissue lesions. Such lesions in the skin, skeletal muscles and testiclesgave rise to severe oedema, which on palpationelicited painful reactions in affected animals as was reported by Okubanjo *et al*. (2014). Similarly, the various nervous signs (i.e. stiffness of the neck and reduced libido) may be attributed to lesions on the nervous system,though not investigated in the present study.

The swelling of the scrotum (scrotal oedema) at the early stage of the experiment may be associated with inflammation process (orchitis) of the testes due to invasion by trypanosomes, thereby resulting in the increase in scrotal circumference as well as increase in body temperature (Bandyopaghy *et al*., 2007). Such inflammatory processes within the testes or scrotum incited by the trypanosomes also resulted in degeneration of the testicular and scrotal tissues leading to decrease in scrotal circumference(Bandyopaghy *et al*., 2007). Okubanjo *et al*. (2008) also reported variation in scrotal sizes of rams infected with *T. congolense*.

Anaemia sequelae to trypanosomosis in *T.b. brucei*-infected rams and those with mixed infections compared to those of *T. evansi* infected rams, suggests that the *T. b. brucei* strainused in this study was more virulent than the*T. evansi*strain(Sokoto isolate). Similarly, Ogbaje *et al*. (2010) reported that *T. evansi* isolate was less pathogenic to West African dwarf goats with mild clinical observations. The severity of the infection was more in rams infected with *T. b. brucei* and those with mixed infections in this study.

In the present study, the *T. brucei brucei*-infected rams had more severe form of anaemia (as evident by significant decrease in PCV), followed by those with mixed infections. The fact that PCV and Hb values decreased sharply in periods of high parasitaemias, but maintained a gradual decrease during the periods of low parasitaemias, showed a direct relationship between anaemia and parasitaemia.Deficiency of haemoglobin in red blood cells decreases their oxygen carrying capacities leading to symptoms of anaemia. Low value of PCV is an indication of low mean red blood cells count that are responsible for carrying oxygen to body tissueswhich aid in the characterization of anaemia. Fluctuations in PCV and haemoglobin values have been reported in sheep and goatsduring trypanosomosis (Audu *et al*., 1999; Shehu *et al*., 2010; Ogbaje *et al*., 2011; Silva *et al*., 2013).Possible explanation for the observed anaemia could be the complexity of the mechanism of the red cell injury in trypanosomosis. Anaemia caused by mechanical injury to erythrocyte occurs by the lashing action of the powerful locomotory flagella and microtubules reinforced bodies of the millions of the trypanosomes during parasitaemia (Vickerman and Tetley, 1978). The development of anaemia in animal trypanosomosis in general is haemolytic in nature with the etiology complex and multifactorial. Among the

complex and multifactorial etiologies associated with the anaemia is haemolysin, a sensory or excretory product of living trypanosome(Dargie, 1980), which is known to lyse red blood cells in the absence of antibodies (*in-vitro*) and haemodilution (*in-vivo*). Initially, the anaemia is haemolytic with a shortened half life time of the red blood cells (Dargie, 1980).The bone marrow remains active during the first period of infection reflected by the presence of enzymes characteristics of young erythrocytes. Later during the chronic stage of disease, the anaemia may not be related to the presence of the parasites but due to a defect in iron metabolism, resulting in iron being trapped in the liver (Murray, 1979). Richardson and Kendall (1963) observed anaemia with reduction in the number of RBC and haemoglobin to the level of 25% of the normal value. Furthermore, at an early stage there was phagocytosis of red cells by the white cells of the host. The red cells apparently became coated with materials from the lysed trypanosomes which tricked the phagocytes into mistaking them for foreign invaders and removed them (autoimmunity). It is possible that the anaemia caused by phagocytosis was increased by toxic substances emanating from the trypanosomes, which destroyed cells directly by lysis (Richardson and Kendall, 1963).

Decreases in plasma protein during trypanosomosis are suggestive of increased protein breakdown or urea loss, haemodilution and serum extravasation (Anosa and Isoun, 1974); which could cause oedema in the infected sheep. For instance, albumins constitute approximately 60% of the plasma proteins that help to maintain normal blood volume and blood pressure(Anosa and Isoun, 1974). The abundance of albuminattracts water into the vessels through the capillaries by osmosis. When this happens, the balance between

the fluid in the blood and the fluid in the interstitial tissues is maintained, that is, the fluid will remain in the blood vessels as it should and will not leak out into the surrounding tissues. If this balance (osmotic pressure) is upset as a result of infection, the fluid will leave the blood vessels, seep into the surrounding tissue spaces and result in swelling of the tissues (oedema). Oedema of the face, lower jaw, limbs and scrotum has been reported in trypanosome-infected rams (Maikaje *et al*., 1991; Okubanjo *et al*., 2008). The decrease in plasma protein has been shown to affect nutritional efficiency of infected animals by decreasing the feed conversion efficiency, increasing the calculated maintenance energy requirement and causing a state of energy deficit (Seed and Hall, 1985). These reports are consistent with the progressive weight loss, weakness and recumbency observed in the present study. Seed and Hall (1985) reported that trypanosomes depend on the host's supplies of carbohydrate, proteins, lipids and some micronutrients, andduring the period of high parasitaemia, parasites may deplete these supplies and release metabolites which may have adverse effects on the host.

Depression in the reproductive performance of all infected rams as observed by increased semen collection reaction time and decreased libido in the present study is in agreement with previous findings (Sekoni *et al*., 2004; Okubanjo *et al*.,2008; Raheem *et al*., 2009). Though, the mechanism of increasedsemen collection reaction time or declined libido was not investigated the present study, it may be attributed to decline in semen testosterone levelas a result of damage to the Leydig cells (responsible for testosterone production) within the testes by the parasites. Testosterone plays an important role in optimal functioning of the testes and initiation of sex drive (MacDonald and Pineda,

1989). Though, testosterone assay was not done in the present study, there are reports of reduced testosterone levels associated with trypanosomosis in bulls (Adamu *et al*., 2004), rams (Adeyemo *et al*., 1990; Okubanjo *et al*., 2008) and goats (Waindi *et al*., 1986). High levels of testosterone are necessary for normal testicular and epididymal functions. Pituitary Luteinizing Hormone (LH) stimulates the secretion of testosterone by the Leydig cells and Follicle Stimulating Hormone (FSH) optimizes testosterone concentration in the seminiferous tubules by increasing the product of androgen-binding protein in the sertoli cells (Sharpe, 1982). Impairment of the Leydig cells (as evidenced in histology) by the trypanosomes in the present study may be responsible for alteration in testosterone level, hence the observed decline in libido and increase in semen collection reaction time. This is capable of rendering the rams infertile. This is in agreement with the findings of Sekoni *et al*. (1990b) in the bovine.

Decrease in sperm outputs accompanied with increased semen abnormalities showedthat the trypanosomes have adversely impaired the ultrastructure of the spermatogenic cells resulting in degeneration of the seminiferous tubules as evidenced in the histology of the testes. The consequence is an impairment of the process of spermatogenesis manifested by poor semen quality and increased spermatozoa abnormalities, degeneration in testicular size, decreased testicular parenchyma and decrease in the volume of seminiferous epithelium and diameter of the seminiferous tubules where spermatogenesis occurs.The percentage of spermatozoa abnormalities in all infected rams far exceeds the upper limit of 20% recommended for good reproductive potential and fertility in either normal mating and or artificial insemination for rams (Zemjanis, 1977; WHO, 1992;

Oyeyemi and Okediran, 2007). This implies that the rams may be rendered infertile and unfit for breeding inspite of the fact that, the values of spermatozoa concentrations of all the experimental rams fell within the acceptable limits of 20 X 10^6 /ml for fertile rams as recommended by WHO(1992).

Decrease in the gonadal and epididymal sperm reserve of the infected groups as compared to the uninfected control group in this study may beattributed to the inflammatory lesions on the reproductive organs by the trypanosomes which resulted in the degeneration of the seminiferous tubules with consequence disappearance of spermatozoa, spermatids and less commonly spermatocytes. The latterresults in a reduction of the mean seminiferous tubules diameter and epithelial thickness which resulted to the decreased semen volume, decreased spermatozoa concentration and motility and increased number of abnormal and dead spermatozoa, leading to reduction or absence of the gonadal and epididymal sperm reserve. The increase in dead spermatozoa in all the infected groups of rams may also be due to depletion of cellular nutrients and oxygen, because trypanosomes require these for their movement, multiplication and growth thereby denying the sperm cells access.Similarly, Okubanjo *et al*. (2014) reported that *T.congolense*infection inYankasa rams resulted in the loss of epididymal sperm reserves. According to Anosa and Isoun (1980), spermatozoa were absent or very scanty in the epididymis of sheep and goats with severe testicular degeneration.

All the infected rams showed moderately to severely pale carcasses, degenerated testes, enlarged spleen, enlarged liver, enlarged lymph nodes, serous atrophy of body fats and pneumonic lungs with watery and less viscous blood. The paleness of the carcasses indicates anaemia which was evidenced by decreased PCV and haemoglobin concentration. Due to the fact that both parasites are tissue-invasive, they cause sequestration and destruction of red blood cells in the reticuloendothelial system as reported in *T. congolense*-infected calves (Kobayashi *et al.*, 1976). The watery and less viscous blood observed in the present study was a reflection of low haemoglobin which reacts with oxygen to form oxyhaemaglobin that is responsible for the brightness (redness) and viscousity of blood. The spleen and lymph node swellings might be due to widespread proliferation in the cortices which resulted in the swelling of the draining lymphnodes.According to Woodruff (1973) a progressive enlargement of spleen may occur when small amounts of antigens are released successively over a prolonged period, leading to a slight and continuous haemolysis. If red blood cells are coated with immune complexes or sensitized in some way, they are likely to be removed, at least in some measure, by the splenic reticuloendothelial tissues.The lymph node is also known to produce antibodies and lymphocytes, which are important in immunity. This finding is in agreement with previous findings of Audu *et al*. (1999).

The observed lesions seen inthe epididymis and testesmay be due to inflammation of the scrotal skin which elevates the temperature of the testes and cause testicular degeneration. Higher temperature causes degeneration of the cells lining the wall of the seminiferous tubule and severity of testicular and epididymal lesion is reflected in the

poor quality of semen and high percentages of abnormal spermatozoa observed. Impairment of the seminiferous tubule together with germ and Sertoli cells will consequently alter the process of spermatogenesis which may render the rams infertile or sterile (if untreated) and the overall effect will be a reduction or complete absence of gonadal sperm reserve.This agrees with earlier reports in boar (Omeke and Igboeli, 2000), bucks (Shehu *et al*., 2006), bulls (Adamu *et al*., 2007), gazelles (Mbaya *et al*., 2011),and rams (Sekoni, 1994; Okubanjo *et al*., 2014).

The histological lesions (cell proliferations and infiltration by lymphocytes, mononuclear infiltration of interstitial tissues) seen in other tissues and organs could beimmunological in nature. Greenwood and Whittle (1980), considered trypanosomosisto cause immunoproliferative disorder of B-lymphocytes and plasma cells. Disorder in these cells may either directly or indirectly be responsible for the impaired functions of various organs.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

It is concluded that:

- *Trypanosoma b. brucei*, *T. evansi* and mixed populations of both species are clinically pathogenic to Yankasa rams as evidenced by development of parasitaemia, anaemia, with severe tissue and organ damage. The severity of the diseases was more with *T. b. brucei* and mixed infections than with *T. evansi*.
- Trypanosomosis due to*T. b. brucei*, *T. evansi* and mixed infections resulted in marked increase in semen collection reaction time and spermatozoamorphological abnormalities ofYankasarams which may render the rams infertile and unfit for breeding if untreated.
- The gonadal and epididymal sperm reserves of Yankasa rams were significantly depleted following trypanosome infection. The effect being moderate in *T. evansi* infection and severe in *T. b. brucei* and mixed infections.
- Gross and histopathological examination of the testes, spleen, lymph nodes, lungs, liver, brain, heart and kidney revealed that trypanosomosis due to *T. b. brucei*, *T. evansi* and mixed infections (of both parasites)are very debilitating to Yankasa rams especially as they severely affects the spermiogram via degeneration of the seminiferous tubules.

6.2 Recommendations

Based on the findings of the present study, it is recommended that:

- Infertility is imminent in *T. b. brucei, T.evansi* and mixed infections in Yankasa rams. Therefore screening for trypanosomosis in the course of investigating infertility in farm and migratory or roaming animals should be a welcome initiative, especially in tse-tse infested areas.
- Livestock,especially camels and cattle, that are imported from neighbouring countries such as Niger Republic and Chad into northern part of Nigeria should be properly quarantined and screened for trypanosomosis. This will prevent the possibility of cross infection to other animals such as sheep and goats.
- Studies on the pathogenicity of *T. b. brucei,T.evansi* and mixed infections on the semen biochemical profile and testosterone production inYankasa rams as well as histology of the hypothalamus, adrenal, thyroid andpituitary glands, are areas in which information is very scanty and are suggested as a follow up to this study.

REFERENCES

Abebe, G., Eley, R.M. and Olemoiyoi, O.K. (1993). Reduced responsiveness of the hypothalamic-pituitary adrenal axis in Boran (*Bos indicus*) cattle infected with *Trypanosoma congolense*. *Acta Endocrinologica*, 129: 75-80.

Abenga, J.N. and Anosa, V.O. (2006). Clinical studies on experimental Gambian trypanosomosis in vervet monkeys.*Veterinarski Arhiv*, 76(1): 11-18.

Adamu, S., Fatihu, M. Y., Useh, N. M., Mamman, M., Sekoni, V. O. and Esievo, K. A. N. (2004). Effect of Experimental. *T.vivax* infection on serum testosterone levels in zebu bulls:a preliminary report. *Journal of Veterinary Science,* 6: 14-17.

Adamu, S., Fatihu, M.Y., Useh, N.M., Mamman, M., Sekoni, V.O. and Esievo, K.A.N. (2007). Sequential testicular and epididymal damage in Zebu bulls experimentally infected with *Trypanosoma vivax*. *Veterinary Parasitology*, 43: 29–34.

Adamu, S., Useh, N.M., Ibrahim, D.N., Nok, A.J. and Esievo, K.A.N. (2009). Erythrocyte surface sialic acid depletion as predisposing factor to erythrocyte destruction in sheep experimental model of African trypanosomosis: A preliminary Report.*Slovenian Veterinary Research*, 46 (1): 19-28.

Adeiza, A.A., Maikai, V.A. and Lawal, A.I. (2008). Comparative haematological changes in experimentally infected savannah brown goats with *Trypanosoma brucei* and *Trypanosoma vivax*. *African Journal of Biotechnology*, 7: 2295-2298.

Ademosun, A.A. (1988). Trends in small ruminant production for the last two decades and its future in West and Central Africa. Keynote address. In: *Improvement of small ruminants*. Adeniji, K.O. (ed). O.A.U. (Organization of African Unity), Nairobi, Kenya.

Adeyemo, O., Oyejide, A. and Agbedana, O. (1990). Plasma testosterone in *Trypanosoma congolense* and *Trypanosome brucei* infected West Africandwarf rams. *Animal Reproduction Science*, 22: 21-26.

Adu, I.F. and Ngere, L.O. (1979). The indigenous sheep of Nigeria. *World Review of Animal Production*, 15(3): 51-62.

Aganga, A.A., Umunna, N.N., Oyedipe, E.O. and Okoh, P.N. (1988). Seasonal variations in water requirement and influence of intermittent watering on grazing Yankasa sheep.*SmallRuminant Research,* 1: 381–386.

Agu, W.E., Ige, K. and Olatunde, D.S. (1986). Evaluation of semen quality of rams infected with *Trypanosoma vivax*. *Animal Reproduction Science*, 22: 123-127.

Agwuma, E.N. (1983). An overview of the livestock industry in Nigeria. *A.C.I.A.R.Proceedings,* 41:55-60.

Alabi, J.F. (2005). *Effects of energy supplementation on growth and reproductive function of Bunaji and Friesian X Bunaji bulls*. Ph.D. Dissertation, Ahmadu Bello University Zaria.

Albert, A. (1961). The mammalian testis. In: *Sex and Internal Secretions* (3rd Ed.). Young, W.C. and Corner, G. W. (Ed.). The Williams and Wilkins Co. Baltimore, MD. p 305.

Allam, L., Ogwu, D., Agbede, R.I.S. and Sackey, A.K.B. (2006). Clinical and mating problems in gilts experimentally infected with *Trypanosoma brucei*. *Journal ofTropical Biosciences*, 6: 28-31.

Allison, A.C. (1978). Mechanisms by which activated macrophages inhibit lymphocyte responses.*Immunology Review*, 40: 23-27.

Al-Mohammed, H. I. (2006). Parasitological and immunological response of experimental infection with *Trypanosoma evansi* in rats. *Journal of the Egyptian Society of Parasitology*, 36 (2): 363- 371.

Amann, R. P., Kavanaugh,J.F, Griel,L.C., Jr. and Voglmayr, J.K. (1976). Sperm production of Holstein bulls determined from testicular spermatid reserves, after cannulation of rete testis or vas deferens and by daily ejaculation. *Journal of Dairy Science,* 57: 91-93.

Amann, R.P. and Schanbacher, B.D. (1983). Endocrine changes associated with onset of spermatogenesis in Holstein bulls. *Journal of Dairy Science,* 66: 87-89.

Ameh, D.A. (1984). Depletion of reduced glutathione and the susceptibility of erythrocytes to oxidative haemolysis in rats infected with *Trypanosoma brucei gambiense*.*Parasitology and Infectious Disease*, 12: 130-139.

Anene, B.M., Onah, D.N. and Nawa, Y. (2001). Drug resistance in pathogenic African trypanosomes: what hopes for the future? *Veterinary Parasitology*, 96: 83-100.

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

Anosa, V.O. (1988). Haematological and biochemical changes in human and animal trypanosomosis, Parts I and II, *Revue d' Elevage et de Medicine Ve'te'rinaire des pays Tropicaux*, 41(2): 65-78.

Anosa, V.O. (1991a). Control of animal trypanosomiasis in Nigeria as a strategy for increased livestock production edited by B.O Ikede. *Proceedings of a preparatory Workshop held in Vom, Plateau State, Nigeria.* 5-9 June, 1989. Pp 71-89.

Anosa, V.O. (1991b). Prevalence, pathogenesis and pathology of African trypanosomiasis. In: Control of animal trypanosomiasis in Nigeria as a strategy for increased livestock production edited by B.O Ikede. *Proceedings of a preparatory Workshop held in Vom, Plateau State, Nigeria,* 5-9 June, 1989. Pp. 71-89.

Anosa, V.O. and Isoun, T.T. (1974). Pathology of experimental *Trypanosoma vivax* infection in sheep and goats.*Zentraiblatt fur Veterinarmedizin,* 30 (1): 685-700.

Anosa, V.O. and Isoun T.T. (1980). Further observations of the testicular pathology in *Trypanosoma vivax* infection in sheep and goats. *Research inVeterinary Science*, 28: 151-160.

Anosa, V.O. and Isoun, T.T. (1983). Pathology of experimental *Trypanosoma vivax* infection in sheep and goats.*Zentraiblatt fur veterinarmedizin* 30 (1):685-700.

Anosa, V.O. and Kaneko, J.J. (1983). Pathogenesis of *Trypanosoma brucei* infection in deer mice (*Peromyscus maniculatus*), light and electron microscopic studies on erythrocyte pathologic changes and phagocytosis.*American Journal of Veterinary Research*, 44 (4): 645-651.

Apted, F.J.C. (1970). Clinical manisfestation and diagnoses of sleeping sickness. In: *The African trypanosomiases*, Mulligan, H.W. (Ed), Allan and Unwin Ltd., London, Pp. 661-683.

Aquino, L.P.C.T., Machado, R.Z. and Alessi, A.C. (2002). Haematological, biochemical and anatomopathological aspects of experimental infection with *Trypanosoma evansi* in dogs.*Arquivo Brasileiro de Medicina Veterinária e Zootecnia (ABMVZ)*, 54: 255-260.

Asfaw, N., Shahidur R. and Berhanu, G. (2011). Livestock production and marketing, development strategy and governance division. *International Food Policy ResearchInstitute* – Ethiopia Strategy Support Program II, Ethiopia. PP 1 – 40.

Assoku, R.K.G. and Gardiner, P.R. (1992). Detection of antibodies to platelets and erythrocytes during haemorrhagic *Trypanosoma vivax* infection of Ayrshire cattle.*Veterinary Parasitology*, 31(2): 199-216.

Audu, P.A., Esievo, K.A.N., Mohammed, G. and Ajanusi, O.J. (1999). Studies of infectivity and pathogenicity of an isolate of *Trypanosoma evansi* in Yankasa sheep.*Veterinary Parasitology*,86 (4): 185-190.

Awobode, H.O. (2006). The biochemical changes induced by natural human African trypanosome infections. *African Journal of Biotechnology*, 5(9): 738-742.

Ayo, J.O., Oladele,S.B. Fayomi,A., Jumbo, S.D. and Hambolu,J.O. (1998). Body temperature, respiration and heart rate in Red Sokoto goat during harmattan season. *Bulletin of Animal Health and Production in Africa*, 46: 161-166.

Bandyopaghy, S.K., Bhattacharyya, B., Choudhury, R.R. and Basu, S. (2007). *Textbook of Veterinary gynaecology, artificial insemination, obstetrics and assisted reproduction*. Kalyani Publishers, New Delhi, Second Edition. 125 pp.

Bangladesh Agricultural Research Council (BARC) (2010). *Agricultural Research Priority: Vision 2030 and Beyond*. Farmgate, Dhaka.Pp 1-8.

Banks, K.L. (1980). Injury induced by *Trypanosoma congolense* adhesions to cell membranes.*Journal of Parasitology,* 6 (1): 34-37.

Barret-Connor, E., Ugoretz, J.R. and Braude, A.I. (1973). Disseminated intravascular coagulation in trypanosomiasis.*Archives of Internal Medicine,* 131 (4): 574-577.

Batista, J.S., Oliveira, A.F., Rodrigues, C.M.F., Damasceno, C.A.R., Oliveira, I.R.S., Alves, H.M., Paiva, E.S., Brito, P.D., Medeiros, J.M.F., Rodrigues, A.C. and Teixeira, M.M.G. (2009). Infection by *Trypanosoma vivax* in goats and sheep in the Brazilian semiarid region: from acute disease outbreak to chronic cryptic infection. *Veterinary Parasitology*, 165: 131–135.

Batista, J.S., Riet-Correa, F., Teixeira, M.M.G., Madruga, C.R., Simões, S.D.V. and Maia, T.F. (2007). Trypanosomosis by *Trypanosoma vivax* in cattle in the Brazilian semiarid region: description of an outbreak and lesions in the nervous system. *Veterinary Parasitology*, 143: 174–181.

Batista, J.S., Rodrigues, C.M.F., Garcia, H.A., Bezerra, F.S.B., Olinda, R.G., Teixeira, M.M.G. and Soto-Blanco, B. (2011). Association of *Trypanosoma vivax* in extracellular sites with central nervous system lesions and changes in cerebrospinal fluid in experimentally infected goats. *Veterinary Research*, 42: 63-68.

Batista, J.S., Rodrigues, C.M.F., Olinda, R.G., Silva, T.M., Vale, R.G., Câmara, A.C., Reboucas, R.E., Bezerra, F.S., García, H.A. and Teixeira, M.M.G. (2012). Highly debilitating natural *Trypanosoma vivax* infections in Brazilian calves:

epidemiology, pathology and probable transplacental transmission. *Parasitology Research*, 110: 73–80.

Bawa, E.K., Sekoni, V.O., Ogwu, D., Esievo, K.A.N. and Uza, D.V. (2005). Results of Novidium (Homidium chloride) chemotherapy on clinicalmanifestation of*Trypanosoma vivax* infected pregnant Yankasa and West African Dwarf (WAD) ewes. *Journal of Animal Veterinary Advances.* 4(7): 637-641.

Baylis, M. and Stevenson, P. (1997). Trypanosomiasis and tsetse control: Fact and fiction? *24th meeting of the International Scientific Council for Trypanosomiasis Researchand Control, Maputo, Mozambique,* pp. 64-68.

Bedford, J.M. (1975). Maturation, transport and fate of spermatozoa in the epididymis.In:*Handbook of Physiology*: *Male Reproductive System*. Hamilton, D.W. and Creep, R.O. (Ed). America Physiological society, Washington, DC, p.303.

Bedford, J.M. (1978). Anatomical evidence for the epididymis as the prime mover in the evolution of the scrotum. *American Journal of Anatomy*,152:483-487.

Bezerre, F.S.B., Garcia, H.A., Alves, H.M., Oliveira, I.R.S., Silva, A.E., Teixeira, M.M.G. and Batista, J.S. (2008). *Trypanosoma vivax* in testicular and epidydimal tissues of experimentally infected sheep. *Pesquisa Veterinária Brasileira*. 28: 575–582.

Biryomumaisho, S., Katunguka-Rwakishaya, E. and Rubaire-Akiiki, C.M. (2003). Serum biochemical changes in experimental *Trypanosoma congolense* and *Trypanosoma brucei* infection in small East African goats. *Veterinary Archive*, 73 (3): 167-180.

Biryomumaisho, S., Rwakishaya, E. K., Melville, S. E., Cailleau, A. and Lubega, G.W. (2013). Livestock trypanosomosis in Uganda: parasite heterogeneity and anaemiastatus of naturally infected cattle, goats and pigs. *Parasitology Research*, 112 (4): 1443-1450.

Bitto, I. I., Akusu, M.O., Egbunike, G.N. and Akpokodje, J.U. (1988). Comparative studiesof the physical and micrometric characteristics of ovine and caprine semen

in the humid tropics.*Proceedings of the 11th Congress on Animal Reproduction and Artificial Insemination, Dublin, Ireland, 3*(230): 3-7.

Bitto, I. I., Akusu, M. O., Egbunike, G. N. and Akpokodje, J. U. (2000). The physical characteristics of ovine-caprine ejaculate mixtures and the survival of their spermatozoa in cow's milk extender in the humid tropics. *Tropical Journal of Animal Science, 3*(1): 147-152.

Blom, E. (1972). The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermiogram. *Atti del VIII. Simposio Internationale di Zootechnia, Milan*. Pp 125-139.

Blum, J.A., Zellweger, M.J., Burri, C. and Hatz, C. (2008). Cardiac involvement in African and American trypanosomiasis. *Lancet Infectious Dieases,* 8 (10): 631-641.

Boersman, A., Hublart, M., Boutignon, F., Noireau, F., Lemesre, J.C. and D'Herbomez, M. (1989). Alterations in thyroid function in patients with *Trypanosome brucei gambiense* infections.*Transactions of the Royal Society of Tropical Medicine and Hygiene,* 83:208-209.

Brun, R., Hecker, H. and Lun, Z.R. (1998). *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). *Veterinary Parasitology*, 79: 95-107.

Budd, L.T. (1999). DFID-funded tsetse fly and trypanosomosis research and development since 1980. An economic analysis.Department for International Development. *Natural Resources International Ltd*., Chatham, UK. Volume 2.

Bungener, W. and Muller, G. (1976).Adharenz phanomene bei *Trypanosoma congolense* [Adgerence phenomena in *Trypanosoma congolense*].*Tropenmedizin und Parasitologie*, 27 (2): 370-371.

Chamond, N., Cosson, A., Blom-Potar, M.C., Jouvion, G., D'Archivio, S., Medina, M., Droin Bergère, S., Huerre, M., Goyard, S. and Minoprio, P. (2010). *Trypanosoma vivax* infections: pushing ahead with mouse models for the study of nagana. I. Parasitological, haematological and pathological parameters. *PLoS Neglected Tropical Diseases*, 4: 1–10.

Coffin, D.L. (1953). *Enumeration of mammalian spermatozoa*. Manual of Veterinary clinical pathology, 3rd Edition, Comstock Publication Company, Ithaca, New York.

Coleman, R.M.and Von, B.T. (1957). Blood pyruvate levels of rats during haematophagus infections, *Journal of Parasitology* 43 (1): 263-270.

Coles, E.H.(1974). *Veterinary clinical pathology*. Saunders, Philadephia, 2nd Ed., Pp. 31-131.

Coles, E.H. (1986). *Veterinary Clinical Pathology.*4thEdition Published by W.B. Sounders Company Philadelphia, London, Toronto, Mexico City Rio de Janeiro, Sydney, Tokyo Hong Kong. 486 pp.

Colley, C.M., Zwaal, R.F.A., Roeofsen, B. and Decssen, L.L.M. (1973). Lytic and non-lytic degradation of phospholipids in mammalian erythrocytes by pure phospholipase, *Biochimica et Biophsica Acta*, 307 (1): 74-82.

Cook, G.M.W., Heard, D.H. and Seaman, G.V.F. (1966). A sialomuscopeptide liberated by trypsin from the human erythrocyte, *Nature* (London) 188 (1): 1011-1012.

Courot, M., Hochereau-de Reviers, M..T. andOrtavant R. (1970). Spermatogenesis. In: *The Testis,* Johnson, A. D., Gomes, W. R. and Van Demark N. L. (Ed.). Academicsearch Press, New York.Pp 339.

Dargantes, A.P., Reid S.A. and Copeman, D.B. (2005a). Experimental *Trypanosoma evansi* infection in the goat. I. Clinical signs and pathology. *Journal of Comparative Pathology*, 133: 261–266.

Dargantes, A.P., Reid, S.A. and Copeman, D.B. (2005b). Experimental *Trypanosoma evansi* infection in the goat. II. Pathology. *Journal of Comparative Pathology*, 133: 267–276.

Dargie, J.D. (1980). Immunoglobulin metabolism in trypanosomiasis. *Proceedings of the 5th International Symposium on Ruminant Physiology*, MTP Press, Lancaster, Pp.349-371.

Davies, C.E., Robins, R.S., Weller, R.D. and Broude, A.I. (1974). Thrombocytopenia in experimental trypanosomiasis.*Journal of Clinical Investigation,* 53(1): 1359-1367.

Delgado, C. (2005). Rising demand for meat and milk in developing countries: Implications for grasslands-based livestock production. In: *Grassland: a global resource* (ed. McGilloway, D.A.), The Netherlands: Wageningen Academic Publishers pp. 29-33.

Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S. and Courbois, C. (1999). *Livestock to 2020: The next food revolution. Food, Agriculture and the Environment*. Discussion Paper 28.

Desquesnes, M. (2004). *Livestock Trypanosomoses and their Vectors in Latin America*. OIE and CIRAD, Paris, 190 pp.

Devendra, C. and McLeroy, C.B. (1982). Goat and sheep production in the tropics: In *Small ruminant production in Nigeria*. *Proceedings of National conference on small ruminants production.* London and New York. Longman. p 271.

Edeghere, H., Igweh, A.C. and Falope, O.O. (1985). Comparative study of experimental *Trypanosoma brucei brucei*, *Trypanosoma congolense* and *Trypanosoma vivax* infections in Yankasa rams. *Paper presented at the 11th Conference of the Nigerian Society for Animal Production,* 23-27 March, 1985.

Egbuka, D.O. (1995). *Effects of male presence and mounting on the induction of ovulation and success rate of artificial insemination in rabbits*. Bachelor of Agricultural Technology Project Report, Federal University of Technology, Owerri.

Elhassan, E., Ikede, B.O. and Adeyemo, O. (1991). Commencement of cyclicity in suckling ewes infected with *Trypanosoma vivax*Abstract.*OAU/STRC*, 70: 82-511.

Esievo, K.A.N. (1979). *In-vitro* production of neuraminidase (sialidase) by *Trypanosoma vivax*.*Proceedings of the 16th meeting of the International Scientific Council for Trypanosomiasis. Nairobi, Kenya: Organization of African Unity, Scientific, Technical and Research and Control*, pp. 205-210.

Esievo, K.A.N. (1983). *Trypanosoma vivax*, stock V 953: inhibitory effect of type A influenza virus anti HAV8 serum on *in vitro* neuraminidase (sialidase) activity.*Journal of Parasitology*, 69(1): 491-495.

Esievo, K.A.N., Saror, D.I., Ilemobade, A.A. and Hallaway, M.H. (1982). Variation in erythrocyte surface and free serum sialic acid concentrations during experimental *Trypanosoma vivax* infection in cattle, *Research in Veterinary Science*,32 (2): 1-5.

Facer, C.A., Crosskey, J.M., Clarkson, M.J. and Jenkins, G.C. (1982). Immune haemolytic anaemia in bovine trypanosomiasis.*Journal of Comparative Pathology*, 92(1): 293-301.

Food and Agricultural Organization of the United Nations (FAO).(1998). *A field guide for the diagnosis, treatment and prevention of AAT*. Uilenberg,G. FAO, Rome, Italy. Pp. 1-5.

Food and Agricultural Organization of the United Nations (FAO) (2000). *A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis*, 2nd edition. FAO, Rome, Italy.

Food and Agricultural Organization of the United Nations (FAO).(2001). Integrating the sterile insect technique as a component of area-wide tsetse and trypanosomosis intervention. *PAAT Technical and Scientific series* by Feldman,U. and Hendricks,J. FAO, Rome.

Food and Agricultural Organization of the United Nations (FAO). **(**2004). The state of food insecurity in the world: *Mornitoring progress towards the world food Summit and Millenium development goals*,Rome, Italy. Pp 1-43.

Food and Agricultural Organization of the United Nations Statistics (FAOSTAT) (2008). World livestock 2008. *http://faostat.fao.org/default.aspx*.

Food and Agricultural Organization of the United Nations (FAO). **(**2011). World livestock 2011– *Livestock in food security.* Rome, Italy.

Fernández, D., González-Baradat, B., Eleizalde, M., González-Marcano, E., Perrone, T. and Mendoza, M. (2009). *Trypanosoma evansi*: A comparison of PCR and parasitological diagnostic tests in experimentally infected mice. *Experimental Parasitology*, 121: 1-7.

Fiennes, R.N.T.W. (1970). Pathogenesis and Pathology of Animal Trypanosomiasis. In: *The African Trypanosomiases*, Mullingan, H.W. (Ed.). Allen and Unwin Ltd., London.Pp. 729-950.

Firew,T. (1999). *Livestock Production, Management and Utilization*. Unpublished report, Mekelle University, Mekelle, Ethiopia.

Foldesy, R.G. and Bedford, J.M. (1982).Biology of the scrotum I. Temperature and androgen as determinants of the sperm storage capacity of the rat cauda epididymidis. *Biology of Reproduction Science,*26: 673-678.

Folkers, C. and Jones-Davies, W.J. (1966). The incidence of trypanosomiasis in blood smears of cattle presented for trypanosomiasis treatment in northern Nigeria. *Bulletin of Epizootic Diseases in Africa,* 14: 409-421.

Fournier-Delpech, S., Colas, G., Courot, M., Ortavant, R. and Brice, G. (1979). Epididymal sperm maturation in the ram: motility, fertilizing ability and embryonic survival after uterine artificial insemination in the ewe. *Annales De Biologie Animale, Biochimie, Biophysique,* 19: 597-561.

Gardiner, P.R., Assoku, R.K.G., Whitelaw, D.D. and Murray, M.(1989). Haemorrhagic lesions resulting from *Trypanosoma vivax* infection in Ayrshire cattle. *Veterinary Parasitology,* 31: 187–197.

Glover, P.E. (1961).The tsetse problem in northern Nigeria, *Patwa News Agency*, Nairobi, Kenya, pp 383-385.

Godfrey,D.G. andKillick-Kendrick, R. (1961). Bovine trypanosomiasis in Nigeria. I: the inoculation of blood into rats as a method of survey in the Donga valley, Benue province. *Annals of Tropical Medicine and Parasitology*, 55: 287-297.

Godfrey,D.G. and Kellick-Kendrick, R and Ferguson, W. (1965). Bovine trypanosomiasis in Nigeria. IV: observations on cattle trekked along a trade-cattle route through areas infested with tsetse fly. *Annals of Tropical Medicine and Parasitology*, 59: 255-269.

Goodwin, L.G. and Guy, M.W. (1973). Tissue fluids in rabbits infected with *Trypanosoma* (*Trypanozoon*) *brucei*.*Parasitology,* 66 (1): 499-513.

Grant, P.T. and Fulton, J.D. (1957). The catabolism of glucose by strains of *Trypanosoma rhodesiense*.*Biochemical Journal,*66, (2): 242-243.

Greenwood, B.M., and H.C. Whittle. (1980). Coagulation studies in Gambian trypanosomiasis. *American Journal of Tropical Medicine and Hygiene*, 25: 390-394.

Greenwood, B.M., Whittle, H.C. and Molyneux, D.H. (1973). Immunosuppression in Gambian trypanosomiasis. *Transactions of the Royal Society of TropicalMedicine and Hygiene*, 67: 846-850.

Griffin, L. (1978). African trypanosomiasis in sheep and goats: a review. *Commonwealth Bureau of Animal Health*, 48(10): 819-825.

Grundler, G. and Djabakon, K. (1985). The impact of trypanosomiasis on the quality of sperm, *Trypanosome etProduction Animale,* 4: 9-14.

Grundler, G., Djabakon, K., Hanichan, T. andAdemofa, K.(1988). Testicular lesion in bulls infected with *Trypanosoma congolense*. *Trypanosome etProduction Animale*, 4: 17-24.

Guegan, F., Plazolles, N., Baltz, T. and Coustou, V.(2013). Erythrophagocytosis ofdesialylated red blood cells is responsible for anaemia during *Trypanosoma vivax* infection. *Cell Microbiology,* 15 (8): 1285-1303.

Hafez, E.S.E. (1980). Physiology of male reproduction. In: *Reproduction in farm animals*. 4th ed. Lea and Fabiger, Philadelphia. Pp.456-458.

Hambrey, P.N., Tizard, I.R. and Mellors, A. (1980). Accumulation of phospholipase A1 in tissue fluid of rabbits infected with *Trypanosoma brucei*.*Tropenmedizin und Parasitologie*,31(1): 439-443.

Hammerstedt, R.H., Keith, A.D., Hay, S.R. and Amann, R.P. (1979). Changes in ram sperm membranes during epididymal transition. *Archives of Biochemistry and Biophysics*, 196: 7-12.

Haynes, N.B. and Schanbacher, B.D. (1983).The control of reproductive activity in the ram. In: *Sheep Production*.Haresign,W. (Ed.).Butterworths, London.Pp 441.

Herbert, W.J. and Lumsden, W.H.R. (1976). *Trypanosoma brucei*: a rapid "matching" method for estimation of the host parasitaemia. *Experimental Parasitology*, 40(3):427-431.

Herrera, H.M., Aquino, L.P.C.T. and Menezes, R.F.(2002). *Trypanosoma evansi* experimental infection in the South American coati (*Nasua nasua*): haematological, biochemical and histopatological changes. *Acta Tropica*, 81: 203-210.

Hoare, C. A. (1972). *Trypanosomes of mammals*: *a zoological monograph*.Blackwell Scientific Publications Oxford and Edinburgh.Pp1-3.

Holland, W.G., Claes, F., My, L.N., Thanh, N.G., Tam, P.T., Verloo, D., Buscher, P., Goddeeris, B. and Vercruysse, J. (2001). A comparative evaluation of parasitological tests and a PCR for *Trypanosoma evansi* diagnosis in experimentally infected water buffaloes. *Veterinary Parasitology*, 97 (1): 23–33.

Holmes, P.H., Eisler, M.C. and Geerts, S. (2004). Current chemotherapy of animal trypanosomiasis. In: *TheTrypanosomiases*.Maudlin, I., Holmes, P.H. and Miles, M.A. (Eds.). CAB International.Wallingford, UK:Pp. 431-444.

Holmes, P.H. and Jennings, F.W. (1976). *Pathogenicity of parasitic infections*, Academic Press, New York, USA.

Hornby, H.E.(1952). *Animal trypanosomiasis in Eastern Africa*. 1949, H.A. Statistics Office, London.

Hublart, M., Tetaert, D., Croix, D., Boutignon, F., Degard, P. and Boersma,A. (1990). Gonadotropic dysfunction produced by *Trypanosoma brucei brucei* in the rat. *Acta Tropica*, 47: 177- 184.

Ifeanyi, A.O. and Olayode, G.O. (2008). Analysis of trends in livestock production in Nigeria: 1970-2005. *Journal of Agriculture and Social Research*,8 (1):78 – 82.

Igbokwe, I.O. (1994). Mechanisms of cellular injury in African trypanosomiasis.*Veterinary Bulletin*,64(7): 611-620.

Igbokwe, I.O., Obagaiye, I.K., Esievo, K.A.N. and Saror,D.I. (1989). Dyserythropoesis in animal trypanosomiasis.*Revue d'Elavage et de Medicine Veterinaire des Pays Tropicaux*,42(4): 423-429.

Ige, K.and Amodu, A.A. (1975).Studies on the pathogenesis of trypanosomiasis in N'dama. *International Scientific Council for Trypanosomiasis Research and Control*, 105: 151-156.

Ijagbone, I.F. and Agbede, S.A. (2000). A case of congenital transmission of *Trypanosoma brucei* in mice. *Tropical Veterinarian*, 18: 37-38.

Ijaz, M. K., Nur-e-Kamal, M. S., Mohamed, A. I. and Dar, F.K. (1998). Comparative studies on the sensitivity of polymerase chain reaction and microscopic examination for the detection of *Trypanosoma evansi* in experimentally infected mice. *Comparative Immunological and. Microbiological Infectious Diseases*, 21: 215-223.

Ikede, B.O. (1979). Genital lesions in experimental chronic *Trypanosoma brucei* infection in rams. *Research in Veterinary Science*, 26: 145 – 151.

Ikede, B.O. and Akpavie, S.O. (1982). Delay in resolution of trypanosome induced genital lesions in male rabbit infected with *Trypanosoma brucei* and treated with Diminazene aceturate. *Research in Veterinary Science*, 32: 374-376.

Ikede, B.O. and Losos, G.J. (1972). Pathology of the disease in sheep produced experimentally by *Trypanosoma brucei*.*Veterinary Pathology*, 9(2): 278-289.

Ikede,B.O. and Losos,G.J. (1975).Pathogenesis of *Trypanosoma brucei* in sheep. III. Hypophysial and other endocrine lesions. *Journal of Comparative Pathology*, 85: 37-44.

Ilemobade, A. A. and Balogun, T. F. (1981). Pig trypanosomiasis: effects of infection on feed intake, live weight gain and carcass traits. *Tropical Animal Health and Production*, 13: 128-136.

Ileweyn, C.A., Luckins, A.G., Munro, C.D. and Perrie, J.(1987). The effect of *Trypanosome congolense* infection on the oestrous cycle of the goat. *British Veterinary Journal*, 143, 423- 426.

Isoun, T.T. and Anosa, V.O. (1974). Lesions in the reproductive organs of sheep and goats experimentally infected with *Trpanosoma vivax*. *Trypenmedizin und Parasitologie*, 25: 456-476.

Isoun, T.T. and Esuruoso, G.O. (1972). Pathology of natural infection of *Trypanosoma vivax* in cattle.*Nigerian Veterinary Journal*, 1(2): 42-45.

Jelkmann, W. (1998). Proinflamatory cytokines lowering erythropoietin production. *Journal of Interferon and Cytokine Research* 18(2): 555-559.

Jennings, F.W. (1976). The anaemia of parasitic infections.*Proceedings of the 7th International Conference of the World Association for the Advancement of Veterinary Parasitology*, pp. 41-67.

Jensen, R.E., Simpson, L. and Englund, P.T. (2008). What happens when *Trypanosoma brucei* leaves Africa? *Trends in Parasitology*, 24: 428-431.

Jordan, A.M. (1986). *Trypanosomiasis control and African rural development*. Longman,New York.

Kaaya, G.P. and Odour-Okelo, D. (1980). The effect of *Trypanosoma congolense* infection on testis and epididymis of the goat. *Bulletin of Animal Health and production in Afirca, 28: 1-5.*

Kaaya, G.P., Tizard, I.R., Maxie, M.G. and Vall, V.O. (1979). Inhibition of leucopoiesis by sera from *Trypanosoma congolense* infected calves, partial characterization of the inhibitory factor.*Tropenmedizin und Parasitologie*, 30(1): 230-235.

Kalejaiye, J.O., Ayanwale, F.O., Ocholi, R.A. and Daniel, A.D. (1995). The prevalence of trypanosome in sheep and goats at slaughter. *Israel Journal of Veterinary Medicine*, 50(2): 57-59.

Kalu, A.U. (1991). An outbreak of trypanosomosis on the Jos Plateau, Nigeria. *Tropical Animal Health and Production*, 23(4): 215-216.

Kanyari, P.W.N., Allonby,E.W., Wilson, A.J. and Munya, W.K. (1983).Some economic effects of trypanosomiasis in goats. *Tropical Animal Health and Production*, 15: 153-160.

Karle, H. (1974). The pathogenesis of the anaemia of chronic disorders and the role of fever in erythrogenesis, *Scandinavian Journal of Haematology*, 13 (1): 81-86.

Katunguka-Rwakishaya, E., Parkins, J.J., Fishwick, G., Murray, M. and Holmes, P. (1993). The pathophysiology of *Trypanosomacongolense* infection in Scottish Blackface sheep. Influence of dietary protein. *Veterinary Parasitology*, 47: 189–204.

Katunguka-Rwakishaya, E., Parkins, J.J., Fishwick, G., Murray, M. and Holmes, P. (1995). The influence of energy intake on the pathophysiology of *Trypanosoma congolense* infection in Scottisch Blackface sheep. *Veterinary Parasitology*, 59: 207–218.

Killick-Kendrick, R. and Godfrey, D.G. (1963). Bovine trypanosomiasis in Nigeria. II: the incidence among some migrating cattle, with observations on the examination of wet blood preparations as a method of survey. *Annals of Tropical Medicine and Parasitology*,57: 117-126.

Kiier, H. T. and Marion, G. B. (1970). Development of the mammalian testis. In: *The Testis*.Johnson, A. D., Gomes, W. R. and Vandemark, N. L. (Ed.). Academic Press, New York.Pp 1- 5.

Kobayashi, A., Tizard, I.R. and Woo, P.T.K. (1976). Studies on the anaemia in experimental African trypanosomiasis II. The pathogenesis of the anaemia in calves infected with *Trypanosoma congolense*.*American Journal of Tropical Medicine and Hygiene*,25(1): 401-406.

Koefoed-Johnsen, H. H. (1964). Undersgelser over saeddannelsen. Aarsberetning, Kgl. Veter-LandbohSjskole, Inst. Sterilitetsforskning, Copenhagen. p 18.

Krammer, J.W. (1996). Incidence of trypnosomiasis in the West African dwaft sheep and goats in Nsukka, Eastern Nigeria. *Bulletin of Epizootic Diseases in Africa*, 14: 423-428.

Lai, D.H., Hashimi, H., Lun, Z.R., Ayala, F.J. and Lukes, J. (2008). Adaptations of *Trypanosoma brucei* to gradual loss of kinetoplast DNA: *Trypanosoma equiperdum* and *Trypanosoma evansi* are petite mutants of *T. brucei*. *Proceedings of the National Academy of Science*, USA, 105: 1999-2004.

Leak, S.G.A.(1998). *Tsetse Biology and Ecology*. Their role in the Epidemiology andControl of Trypanosomiasis.*Bulletin of Entomology Resource*, 85: 241-251.

Lonsdale-Eccles, J.D. and Grab, D.J.(1986). Proteases in African trypanosomes. In: *Cytokine proteinases and their inhibitors*, V.J. Turk, (Ed.), Walter de Grayter, Berlin, Germany. 189-197.

Losos, G. J. (1980). Diseases caused by *Trypanosoma evansi*, a review. *Veterinary ResearchCommunications*, 4: 165-181.

Losos, G.J. (1986) Trypanosomiases. In : *Infectious Tropical Diseases of Domestic Animals*. 1st Edn. Longman Scientific and Technical, Harlow, pp 183-263.

Luckins, A.G., Llewelyn, C., Munro, C.D. and Murray, M. (1986). Effects of pathogenic trypanosomes on the mammalian reproductive system. *Proceedings of an International Symposium on the Use of Nuclear Techniques in Studies of Animal*

Production and Health in Different Environments. Vienna, IAEA/FAO, Vienna – Austria. Pp. 351-363.

Luna, L.G. (1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd edn. McGraw-Hill Book Company, New York.

Mabbot, N. and Sternberg, J. (1995). Bone marrow nitric oxide production and development of anaemia in *Trypanosoma brucei* infected mice.*Infection and Immunology,* 63(4): 1563-1566.

MacDonald, L.E. and Pineda, M.H. (1989).*Veterinary Endocrinology and Reproduction*. Lea and Febriger, Philadelphia,London.

Mackenzie, P.K.I., Boyt, W.P., Nesham, V.W. and Pirie, E. (1978). The etiology and significance of the phagocytosis of erythrocytes in sheep infected with *Trypanosomacongolense*.*Research in Veterinary Sciences,* 24 (1): 4-7.

Mackenzie, P.K.I. and Cruickshank, J.G. (1973). Phagocytosis of erythrocytes and leucocytes in sheep infected with *Trypanosoma congolense*.*Research in Veterinary Sciences,*15(1): 256-262.

MacLean, L., Odiit, M. and Sternberg, J.M. (2001). Nitric oxide and cytokine synthesis in human African trypanosomiasis.*The Journal of Infectious Disease,*184(4): 1086-1090.

Magez, S., Radwanaska, M., Beschin, A., Sekikawa, K. and Debaetselier, P. (1999). Tumor necrosis factor alpha is a key mediator in the regulation of experimental *Trypanosoma brucei* infections. *Infection and Immunology,* 67(5): 3128-3132.

Maikaje, D.B., Sannusi, A., Kyewalabye, E.K. and Saror, D.I. (1991). The course of experimental *Trypanosoma vivax* infection in Uda sheep. *Veterinary Parasitology,* 38 (4): 267-274.

Maxie, M.G. and Losos, G.J. (1979). Release of *Trypanosoma vivax* from the microcirculation of cattle by Berenil®.*Veterinary Parasitology,* 3: 277 281.

Mbaya, A.W., Aliyu, M.M., Nwosu, C.O. and Taiwo, V.O. (2009a). An assessment of the efficacy of DFMO in baboons (*Papio anubis*) infected with *Trypanosoma brucei gambiense*.*Global Journal of Pure and Applied Sciences,* 15 (1): 69- 78.

Mbaya, A.W., Aliyu, M.M., Nwosu, C.O., Taiwo, V.O. and Ibrahim, U.I. (2009b). Effects of melarsamine hydrochloride (Cymelarsan®) and diaminazene aceturate (Berenil®) on the pathology of experimental *Trypanosoma brucei* infection in red fronted gazelles (*Gazella rufifrons*).*Veterinary Parasitology*, 163 (1-2): 140-143.

Mbaya, A.W., Ibrahim, U.I. and Apagu, S.T. (2010). Trypanosomosis of the dromedary camel (*Camelus dromedarius)* and its vectors in the tsetse-free arid zone of northeastern, Nigeria.*Nigerian Veterinary Journal*, 31(3): 195-200.

Mbaya, A.W., Nwosu, C.O. and Kumshe, H.A. (2011). Genital lesions in male red fronted gazelles (*Gazella rufifrons*) experimentally infected with *Trypanosoma brucei* and the effect of melarsamine hydrochloride (Cymelarsan®) and diminazene aceturate (Berenil®) in their treatment. *Theriogenology*, 16: 721-728.

Mbaya, A.W.,Nwosu, C.O. and Onyeyili, P.A. (2007). Toxicity and anti-trypanosomal effects of ethanolic extract of *Butyrospermum paradoxum* (Sapotacea) stem bark in rats infected with *Trypanosoma brucei* and *T. congolence*.*Journal of Ethnopharmacology*, 111: 526-530.

McCay, P.B. and King, M.M. (1980). Endogenous sources of free radicals. In: *Basic and Clinical Nutrition and development of anaemia in Trypanosoma brucei infected mice*. Machlin,L.J. (Ed.), Marcel Decker Inc, New York, USA. Pp. 269-303.

Meteorological Unit.(2009). Institute for Agricultural Research, Ahmadu Bello University, Zaria.

Micheal, A.J., Alexopoulos, C., Pontiki, E.A., Hadjipavlou-Litina, D.J., Saratsis, P., Ververidis, H.N. and Boscos, C.M. (2009). Effect of antioxidant supplementation in semen extenders on semen quality and reactive oxygen species of chilled canine spermatozoa. *Animal Reproduction Science*, 112:119-135.

Microsoft Excel Chart Wizard, (2010). Microsoft Coporation.

Mohammed, A.A. (2000). *Comparative pathogenicity studies on two isolates of Trypanosoma evansi from the North-West and North-Central Zones of Nigeria.* Ph.D. Dessertation. Ahmadu Bello University, Zaria, Nigeria.

Mohammed, P.W. (2007). Trends and growth in Livestock population in Sindh: A Comparison of Different Censuses. *Indus Journal of Management and SocialSciences*, 1 (1):53-69.

Molyneux, D.H. and Ashford, R.W. (1983). The parasites. In:*The biology of Trypanosoma and Leishmania, parasites of man and domestic animals*. Molyneux, D.H. and Ashford,R.W.(Ed.), 1st ed., Taylor and Francis, London, Pp. 3-62.

Morar, I.A., Groza, I.S., Borzan, M., Emike, P., Neagu, V.R., Pop A.L.R., Mate C. and Groza, E. (2010).Studies regarding collection, evaluation and conservation of European Mouflon (*Ovis ammon musimon*) semen.*Lucrări Stiinłifice Medicină Veterinară*,2: 15-24.

Morrison, L.J., Marcello, L. and McCulloch, R. (2009). Antigenic variation in the African trypanosome: molecular mechanisms and phenotypic complexity. *Cell Microbiology*, 11 (12): 1724-1734.

Morrison, W.I., Max, M., Sayer, P.D. and Preston, J.M. (1981). The pathogenesis of experimentally induced *Trypanosoma brucei* infection in the dog, tissue and organ damage, *American Journal of Pathology,* 102:168-181.

Morrow, D.A.(1980). *Current therapy in Theriogenology.*W.B.Saunders Co. Philadelphia Pp. 878-950.

Moulton, J.E. and Sollod, A.E. (1976). Clinical, serologic and pathogenic changes in calves with experimentally induced *Trypanosoma brucei* infection. American*Journal of Veterinary Research*, 37: 791-802.

Mulligan, H.W., Potts, W.H. and Kershaw, W.E. (1970). *The African Trypanosomiasis*. London George Allen and Unwin Ltd. pp 661 -794.

Murray, M. (1979). *Anaemia of bovine African trypanosomiases: An overview: In: Pathogenicity of trypanosomes*. [Losos, G. and Chouinard, A. Ed.] *Proceedings of a workshop held at Nairobi, Kenya*, 20-23, November, pp 121-127.

Murray,M. and Dexter, T.M. (1988). Anaemia in Bovine African trypanosomiasis: A review.*Acta Tropica*, 45: 389-432.

Murray, M. and Morrison, W.I. (1978). Parasitaemia and host susceptibility in African trypanosomiasis, *Proceedings of a workshop inNairobi, Kenya*.pp.71`-81.

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

Mutayoba, B.M. Eckersall, P.D., Jeffcoate, I.A., Cestnik, V. and Holmes, P.H. (1994). Effects of *Trypanosoma congolense* infection in rams on the pulsatile secretion of LH and testosterone and responses to injection of GnRH. *Journal of Reproduction and Fertility*, 102: 425- 431.

Mutayoba, B.M., Eckersall, P.D., Jeffcoate I.A., Harvey M.J.A. Cestnic, V. and Holmes, P.H. (1996). Effects of castration on luteinizing hormone secretion and response to gonadotrophin-releasing hormone in sheep infected with *Trypanosoma congolense*. *European Journal of Endocrinology*, 134:115-122.

Mutayoba, B.M. and Gombe,S.(1989).Effect of Africa trypanosomiasis on plasma cortisol and thyroxin concentration in goats. *Research in Veterinary Science*, 47: 315-318.

Mutayoba, B.M., Gombe, S., Kaaya, G.P. and Waindi, E.N.(1988a). Effect of chronic experimental *Trypanosoma congolense* infection on the ovaries, pituitary, thyroid and adrenal glands in female goats. *Research in Veterinary Science*, 44: 140-146

Mutayoba, B.M., Gombe, S., Waindi, E.N. and Kaaya, G.P. (1988b). Depression of ovarian function and plasma progesterone and estradiol-17 in female goats chronically infected with *Trypanosoma congolense*. *Acta Endocrinologica*, 117: 477-484.

Naessens, J., Kitani, H., Yagi, Y., Sekikawa, K. and Iraqqi, F. (2005). TNF-a mediates the development of anaemia in a murine *Trypanosoma brucei rhodesiense* infection, but not the anaemia associated with a murine *T. congolense* infection. *Clinical and Experimental Immunology,* 139(3): 403-410.

Nantulya, V.M., Musoke, A.J., Rurangirwa, F.R., Barbet, A.F., Ngaira, J. and Katende, J.M.(1982). Immune depression in African trypanosomiasis: the role of antigenic competition. *Clinical Experimental Immunology*,47: 234-241.

Nazrul-Islam, A.K.M. and Woo, P.T.K. (1991). Anaemia and its mechanism in goldfish (*Carassius auratus*) infected with *Trypanosoma danilewsky.Disease of AquaticOrganisms* (11: 37- 43.

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B.K. and Ganz, T. (2004). IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin.*Journal of Clinical Investigation*,113: 1271-1276.

Newton, B.A. (1978). The metabolism of African trypanosomiasis in relation to pathogenic mechanisms, In: *Pathogenicity of trypanosomes*, G. Losos and A. Chouinard, (Eds.), Pp. 17-22.

Nielsen, K., Sheppard, J., Holmes, W. and Tizard, I.(1978). Experimental bovine trypanosomiasis. Changes in serum immunoglobulins complement and complement components in infected animals. *Immunology*,(35): 817-826.

Njombe, A.P. and Msanga, Y.N. (2009). Livestock and Dairy Industry Development in Tanzania. *http://www.mifugo. go.tz* (accesses 06/04/10).

Nok, A.J. and Balogun, E.O. (2003). A blood stream *Trypanosoma congolense* sialidase could be involved in anaemia during experimental trypanosomiasis.*The Journal of Biochemistry,* 133(6): 725-730.

Nok, A.J., Esievo, K.A.N., Ajibike, M.O., Achoba, I.I., Tekdek, K., Gimba, C.E., Kagbu, J.A. and Ndams, I.S. (1992a). Modulation of the calcium pump of the kidney and testes of rats infected with *Trypanosoma congolense*.*Journal of Comparative Pathology*, 107: 119-123.

Nok, A.J., Esievo, K.A.N., Ukoha, A.I., Ikediobi, C.O., Baba, J., Tekdek, B. and Ndams, I.S. (1992b). Kidney NaK-ATPase: kinetic study of rats during chronic infection with *Trypanosoma congolense*.*Journal of Clinical Biochemistry and Nutrition* 13: 73-79.

Nwosu, C.O. and Ikeme, M.M. (1992). Parasitaemia and clinical manifestations in *Trypanosoma brucei* infected dogs.*Revue d'Elavage et de Medicine veterinaire des Pays Tropicaux,* 45: 273-277.

Nyenko,J.H.P., Ole-Moiyoi,O.K., Majiwa,P.A.O., Otieno,L.H. and Ociba,P.M. (1990). Characterization of trypanosomes isolated from cattle in Uganda with species specific DNA probes reveals predominance of mixed infections. *Insect Science andits Application,* 11: 271-280.

Ogbaje, C.I., Lawal, I.A. and Ajanusi, O.J. (2011). Infectivity and pathogenicity of Sokoto (northern Nigeria) isolate of *Trypanosoma evansi* in West African Dwarf Goats. *International Journal of Animal and Veterinary Advances*, 3(3): 117-124.

Ogbaje, C.I., Lawal, I.A. and Fatihu, M.Y. (2010). Performance of West African Dwarf (WAD) goats infected with the Sokoto (Northern Nigeria) strain of *Trypanosoma evansi. Journal of Animal and Plant Sciences*, 6(3): 709-714.

Ogunlade, J. T., Ewuola, E. O., Gbore, F. A., Bandyopadhyay, R., Niezen, J. and Egbunike G. N. (2006). Testicular and epididymal sperm reserves of rabbits fed fumonisin contaminated diets. *World Applied Science Journal*, 1(1): 35-38.

Ogwu, D. Njoku, C.O. and Ogbogu, V.C. (1992). Adrenal and thyroid dysfunctions in experimental *Trypanosoma congolense* infection in cattle. *Veterinary Parasitology*, 42 :115-126.

Ogwu, D., Njoku, C.O., Osori, D.I.K., Ezeokoli, C.D.and Kumi-Diaka, J. (1984). Effects of experimental *Trypanosoma vivax* infection on fertility of heifers. *Theriogenology*, 22: 625-633.

Okubanjo, O.O., Sekoni, V.O., Ajanusi, O.J., Lawal, I.A. and Jatau, I.D. (2008). Effects of *Trypanosoma congolense* infection on some reproductive parameters in Yankasarams. *Proceedings of Annual Congress of the Nigerian Veterinary Medical Association*. 20th-24th October, 2008, Owerri, Imo State, Nigeria, Pp: 125-127.

Okubanjo, O.O., Sekoni, V.O., Ajanusi, O.J., Nok, A.J. and Adeyeye, A.A. (2014).Testicular and epididymal pathology in Yankasa rams experimentally infected with *Trypanosoma congolense*.*Asian Pacific Journal of Tropical Diseases*, 4(3): 185–189.

Omalaka, E.E.O. (1992). *The effect of time and frequency of collection on the quantity and quality of boar semen*. Bachelor of Agricultural TechnologyProject Report, Department of Animal Science and Technology, Federal University of Technology, Owerri.

Omeke, B.C.O. and Igboeli, G. (2000).Disruption of spermatogenesis in boars sub-clinically infected with *Trypanosoma brucei brucei*.*Animal Reproduction Science*, 63: 197–204.

Osaer, S., Goossens, B., Jeffcoate, I. and Holmes, P. (1998). Effects of *Trypanosoma congolense* and nutritional supplements in Djallonke´ ewes on live weight during pregnancy, post partum weight, haematology parameters and lamb performance. *Research in Veterinary Science*, 65: 65–69.

Osaer, S., Goossens, B., Sauveroche, B.and Dempfle, L. (1997). Evaluation of the semen quality and reproductive performance of Trypano-tolerant Djallonke rams following an artificial infection with *Trypanosoma congolense*. *Small Ruminant Research*, 24:213-222.

Oyeyemi, M.O. and Okediran, B.S. (2007). Testicular parameters and sperm morphology of chinchilla rabbits fed with different planes of soybean meal. *International Journal of Morphology*, 25(1): 139-144.

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

Payne, R.C., Sukanto, I.P., Djauhari, D., Partoutomo, S., Wilson, A.J., Jones, T.W., Boid, R. and Luckins, A.G. (1993). *Trypanosoma evansi* infection in cattle, buffalos and horses in Indonesia. *Veterinary Parasitology*, 38 (2–3): 109–119.

Peregrine, A.S. (1994).Chemotheray and delivery systems: haemoparasites. *Veterinary Parasitology*, 54: 223-248.

Rabo, J.S. (1995). *Toxicity studies and trypanosuppressive effects of stem bark extract of Butyrospermum paradoxum in laboratory animals*, Ph.D. Thesis, University of Maiduguri, Maiduguri, Nigeria.

Raheem, A. K., Fayemi, E. O., Leigh, O. O.and Ameen, S. A.(2009). Selected fertility parameters of West African dwarf bucks experimentally infected with *Trypanosoma congolense. Folia Veterinaria*, 53 (2): 68—71.

Raina A.K., Kumar R., Rajora V.S. and Sridhar, S.R.P. (1985). Oral transmission of *Trypanosoma evansi* infection in dogs and mice. *Veterinary Parasitology*, 18(1): 67-69.

Rao, A.R. (1971).*Changes in the morphology of sperm during their passage through genital in bull abnormal and impaired spermatogenesis*. Unpublished Ph.D. Thesis. Stockholm, Sweden.

Rautenberg, P., Schedler, R., Reinwalde, E. and Risse, H.J. (1982). Study on a proteolytic enzyme from *Trypanosoma congolense*. Purification, molecular and some biochemical properties.*Molecular and Cellular Biochemistry*, 47: 151-159.

Reid, R.S., Kruska, R.L., Wilson, C.J. and Perry, B.D. (1999). The impacts of controlling the tsetse fly on land-use and the environment. In: *Spatial and Temporal Dynamics of African Farming Systems.* Lynam, J., Carter, S. and Reid, R.S. (Eds.).

Reid, S.A., Husein, A. and Copeman, D.B. (2001). Evaluation and improvement of parasitological tests for *Trypanosoma evansi* infection. *Veterinary Parasitology*,102: 291–297.

Rekwot, P.I., Oyedipe, E.O. and Ehoche,G.W. (1994). The effects of feed restriction and realimentation on the growth and reproductive function of Bokoloji bulls. *Theriogenology*, 42: 287-295.

Richardson, U.F. and Kendall, S.B. (1963). *Veterinary Protozoology*. (3rd ed). Oliver and Boyd, Edinburgh and London, 312pp.

Rifkin, M.R. and Landsberger, F.R. (1990). Trypanosome variant surface glycoprotein transfer to target membranes: A model for the pathogenesis of trypanosomiasis, *Proceedings of the National Academy of Science,* pp. 801-806.

Robson, J. and Ashkar, T.S. (1972). Trypanosomiasis in domestic livestock in the Lambwe Valley area and a field evaluation of various diagnostic techniques. *Bulletin of World the Health Organisation,* 47: 727-734.

Rodrigues, C.M.F., Olinda, R.G., Silva, T.M.F., Vale, R.G., Silva, A.E., Lima, G.L., Garcia, H.Á., Teixeira, M.and Batista, J.S. (2013).**Follicular degeneration in the ovaries of goats experimentally infected with Trypanosoma vivax from the Brazilian semi-arid region.** *Veterinary Parasitology*, **191:** 146-153.

Saror, D.I. (1982). Aspects of the anaemia of acute bovine trypanosomiasis, *Proceedings of the first National Conference on Tsetse and Trypanosomiasis Research*, August 10-12, 1981. Kaduna, Nigeria. Pp. 12-14.

Schauer, R. and Kamerling, J.P. (1997). Chemistry, biochemistry and biology of sialic acid. In: *Glycoproteins*. Montrevil, J., Vigenther, .J.F.G. and Shachter, H. (Eds.). Amsterdam, The Netherlands. Pp. 241-400.

Seed, J.R. and Hall, J.E. (1985). Pathophysiology of African trypanosomiasis. In: *Immunology and pathogenesis of trypanosomiasis.*Tizard, I.J. (Ed.), CRC Press, Boca Raton, Florida, USA. Pp. 1-11.

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

Sekoni, V.O. (1993). Elevated sperm morphological abnormalities of Yankasa ram consequent to *Trypanosoma vivax* infection. *Animal Reproductive Science,* 31: 243-248.

Sekoni, V.O. (1994). Reproductive disorders caused by animal trypanosomiasis: A review. *Theriogenology.* 42: 557-570.

Sekoni, V.O., Gustafsson, B.K. and Mather, E.C. (1981). Influence of wet fixation, staining techniques and storage time on bulls sperm morphology. *Nordisk Veterinaermedicin*, 33: 161-166.

Sekoni, V.O., Kumi-Diaka, J., Saror, D.I. and Njoku,C.O. (1988). The effect of *Trypanosoma vivax* and *Trypanosoma congolense* infections on the reaction time and semen characteristics in the Zebu Bull. *British Veterinary Journal*, 144: 388-391.

Sekoni, V.O., Njoku, CO., Kumi-Diaka, J. and Saror, D.I.(1990b). Pathological changes in male genitals of cattle infected with *Trypanosoma vivax* and *Trypanoosoma congolense*.*British Veterinary Journal*, 146-175.

Sekoni, V.O., Rekwot, P.I. and Bawa, E.K. (2004). The effect of trypanosomosis on sperm morphology in Zebu x Friesian crossbred bulls. *Tropical Animal Health andProduction*. 36: 55 – 64.

Sekoni, V.O., Saror, D.I., Njoku, C.O., Kumi-Diaka, J. and Opaluwa, G.I. (1990a). Comparative haematological changes following T*rypanosoma vivax* and *Trypanosoma congolense* infections in the Zebu Bull. *Veterinary Parasitology*, 35:11-19.

Sharpe, R. M. (1982). Cellular aspects of the inhibitory actions of LHRH on the ovary and testis. *Journal of Reproduction and Fertility*,64:517-527.

Shehu, S.A., Ibrahim, N.D.G., Esievo, K.A.N. and Mohammed, G. (2006). Role of erythrocyte surface sialic acid inducing anaemia in Savannah Brown bucks experimentally infected with *Trypanosoma evansi. Veterinarski Arhiv*,26(6): 521-530.

Shehu, S.A., Ibrahim, N.D.G., Esievo, K.A.N. and Mohammed, G. (2010).Study on the infectivity of *Trypanosoma evansi* isolate in Savannah Brown Bucks. *Journal of Veterinary Sciences*, 8(1 and 2): 1-3.

Sileghem, M., Flynn, J.N., Darji, A., De Baetselier, P. and Naessens, J.(1994a). African trypanosomiasis. ln: *Parasite infections and the lmmune System*. Kierszenbaum, F. (ed.). Academic Press, San Diego.Pp. 1-51.

Sileghem, M., Flynn, J.N., Logan-Henfrey, L. and Ellis, J. (1994b). Tumour necrosis factor production by monocytes from cattle infected with *Trypanosoma* (*Dutonella*) *vivax* and *T*. (*Nannomonas*) *congolense*, possible association with severity of anaemia associated with the disease.*Parasite Immunoassay*, 16(1): 51-54.

Silva, T.M., Olinda, R.G., Rodrigues, C.M., Camara, A.C., Lopes, F.C., Coelho, W.A., Ribeiro, M.F., Freitas, C., Teixeira, M.M. and Batista, J.S. (2013). Pathogenesis of reproductive failure induced by *Trypanosoma vivax* in experimentallyinfected pregnant ewes. *Veterinary Research*, 44 (1): 1-5.

Slater, T.F. (1984). Free radical mechanism in tissue injury.*Biochemistry Journal*,222: 1-15.

Snow, W., Wachand, T. and Rawlings, P. (1996). Observations on the prevalence of trypanosomosis in small ruminants, equineand cattle in relation to tsetse challenge, in the Gambia. *Veterinary Parasitology*, 66: 1-11.

Soudre, A., Ouedraogo-Kone, S., Wurzinger, M., Muller, S., Hanotte, O., Ouedraogo, A. G. and Solkner, J. (2013). Trypanosomosis: a priority disease in tsetsechallengedareas of Burkina Faso. *Tropical Animal Health and Production*, 45 (2): 497-503.

Steel, R.G. and Torrie, J.H. (1980). *Principles and Procedures of Statistics*. An Approach. 2nd ed. McGraw-Hill Book Co. Inc. New York.

Stephen, L.E. (1986). *Trypanosomiasis*: *A veterinary perspective*. Pergamon,Oxford. 511 pp.

Stevenson,P., Munga,L., Baylis,M. and Alushula,L.(1991). The control of tsetse and trypanosomiasis by delthamethrine treatment of ranch animals in Kenya. *Proceedingsof 21st ISCTRC Meeting at Yamoussoukro,Ivory Coast*.pp.201.

Superior Performing Statistical Software. (2011). for windows, version 20. IBM Coporation.

Swallow, B.M. (2000). *Impacts of trypanosomosis in African agriculture, programme against African trypanosomosis.*Technical and Scientific series, Food Agriculture Organization (FAO), 2: 45 – 46.

Tafese, W., Melaku, A. and Fentahun, T. (2012). Prevalence of bovine trypanosomosis and its vectors in two districts of East Wollega Zone, Ethiopia. *Onderstepoort Journalof Veterinary Research*, 79 (1): 1-4.

Taylor, K.A., Lutje, V. and Mertens, B.(1996). Nitric oxide synthesis is depressed in *Bos indicus* cattle infected with *Trypanosoma congolense* and *T. vivax* and does not mediate T cell suppression. *Infection and Immunity*, 64: 4115-4122.

Taylor, K.A. and Mertens, B.(1999). Immune response of cattle infected with African trypanosomes. *Mem Inst Oswaldo Cruz 94*: 239-244.

Thompson,J.W., Mitchel,M., Rees,R.B., Shereni,W., Schoenfeld, A.H. and Wilson,A. (1991). Studies on the efficacy of deltamethrine applied to cattle for the control of tsetse flies (*Glossina* spp.) in Southern Africa. *Tropical Animal Health and Production*, 23: 221-226.

Tizard, I.R. and Holmes, W.L. (1977). The release of suitable vasoactive material from *Trypanosoma congolense* intraperitoneal diffusion chambers, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 71: 52-55.

Tizard, I.R., Nielsen, K. H., Mellors, A. and Assoku, R.K.G. (1978a). Biologically active lipids generated by autolysis of *Trypanosoma congolense*. In: *Pathogenecity of Trypanosomes.Proceedings of a workshop*.Nairobi, Kenya. pp. 103-110.

Tizard, I.R., Sheppard, J. and Nielsen, K. (1978b). The characterization of a second class of haemolysin from *Trypanosoma brucei*, *Transactions of the Royal Society of Tropical Medicine and Hygiene*,72: 198-200.

Turner, T.T., Addario, D.D. and Howards, S. S. (1978).Further observations on the initiation of sperm motility. *Biology of Reproduction*, 19:1095- 1098.

Turner, T.T. and Cesarini, D.M. (1981). The effect of an aldosterone antagonist on the sperm concentrating ability of the rat epididymis,*Biology of Reproduction*, 24 (1): 45.

Umar, I.A., Ogenyi, E., Okodaso, D., Kimeng, E., Stanecheva, G.I., Omage, J.J., Isah, S.and Ibrahim, M.A. (2007). Amelioration of anaemia and organ damage by combined intraperitoneal administration of vitamin A and C to *Trypanosoma brucei brucei* infected rats.*African Journal of Biotechnology*,6(18): 2083-2086.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M. and Jennings, F.W. (1996). *Veterinary Parasitology*, 2nd edition, The University of Glasgow Scotland Blackwell Science Ltd. Pp. 212 -230.

Van den Bossche, P. and Doran, M. (2004). *Trypanocidal drugs: use and misuse?* Harare, Zimbabwe:WHO/Regional Tsetse and Trypanosomiasis ControlProgramme.

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

Varki, A. (1992). Diversity in the sialic acids.*Glycobiology*,2: 25-40.

Ventura, R.M., Takeda, G.F., Silva, R.A., Nunes, V.L., Buck, G.A. and Teixeira, M.M. (2000). Genetic relatedness among *Trypanosoma evansi* stocks by random amplification of polymorphic DNA and evaluation of a synapomorphic DNA fragment for species-specific diagnosis. *International Journal of Parasitology*, 32: 53-63.

Verma, B.B. and Gautam, O.P. (1978). Studies on experimental surra (*T. evansi* infection) in buffalo and cow calves.*Indian Veterinary Journal*,55: 648-653.

Vickerman, K. and Tetley, L. (1978). Biology and ultrastructure of trypanosomes in relation to pathogenesis. In: *Pathogenecity of trypanosomes*, *Proceedings of a workshop,* Nairobi, Kenya. pp. 231-236.

Vohradsky, F.(1971). Clinical signs, daily rate of infection, physical changes in catlle artificially infected with *Trypanosome vivax*. *Revue d'Elevage Medecine. Veterinaire des pays Tropicaux*, 24: 251-263.

Waindi, E.N., Gombe, S. and Oduor-okelo, D.(1986). Plasma testosterone in *Trypanosoma congolense*infected Toggenburg goats. *Archives of Andrology*, 17: 7-17.

Wainwright, M. (2010). Dyes, trypanosomiasis and DNA: a historical and critical review. *Biotechnic and Histochemistry*, 85 (6): 341-354.

Weidhaas,D.E. and Haile,D.G. (1978). A theoretical model to determine the degree of trapping required for insect population control. *Bulletin of the Entomological Societyof America (ESA Bulletin)*, 24: 18-20.

Wernery, U., Zachariah, R., Mumford, J.A. and Luckins, T. (2001). Preliminary evaluation of diagnostic tests using horses experimentally infected with *Trypanosoma evansi. Veterinary Journal*, 161: 287–300.

Williams, D.J., Taylor, K.A., Newson, J. and Gichuki, B.(1996). The role of anti-variable surface glycoprotein antibody responses in bovine trypanotolerance. *Parasite lmmunology*, 18: 209- 218.

Wilson, R.T. and Durkin, J.W. (1984). Age at permanent incisor eruption in indigenous goats and sheep in semi-arid Africa. *Small Ruminant and Camel Group*. Group Document No. SR C3, pp. 451-455.

Wolkmer, P., Shafer da Silva, A., Treasel, C.K., Paim, F.C., Cargncutti., J.F., Pagononcelli, M., Picada, M.E., Monterro, S.G. and Anjus Lopes, S.T. (2009). Lipid peroxidation associated with anaemia in rats experimentally infected with *Trypanosoma evansi*.*Veterinary Parasitology*,165: (1-2): 41-46.

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

Woo, P.T.K. and Kobayashi, A. (1975). Studies on the anaemia in experimental African trypanosomiasis, Preliminary communication on the mechanism of the anaemia. *Annals Society Belge Medicine Tropicale*,53(1): 37-45.

Woodruff, A.W. (1973). Mechanisms involved in anaemia associated with infection and splenomegaly in the tropics. *Tropical Medicine and Hygiene*, 67: 313-325.

Woodruff, A.W., Topley, E., Knight, R. and Downie, C.G.B. (1972). The anaemias of kalaazar. *British Journal of Haematology*,22: 319-329.

World Health Organization (WHO) (1992). *Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction*.3rd edn. Cambridge University Press,Cambridge. Pp. 1-5.

World Health Organisation (WHO). (1998). Control and surveillance of African trypanosomiasis. A report of WHO expert committee. *WHO technical report series* no. 781 WHO, Geneva.pp221.

World Health Organization (WHO). (2003). The World Health Report 2002-*Reducing risks, promoting healthy life*, Geneva.pp 1-2.

World Health Organisation (2005). Control and surveillance of African trypanosomosis. *Technical Report Series*, No. 881, Geneva, Switzerland, Pp. 284.

World Organization for Animal Health (OIE). (2009). *African Animal Trypanosomiasis*. Pp. 1-4.

Yesufu, H.M. and Mshelbwala, A.S. (1973). Trypanosomiasis survey in cattle and tsetse flies along a trade cattle route in South Western Nigeria. *Annals of Tropical Medicine and Parasitology*, 67: 3-7.

Young, W.C. (1931). A study of the function of the epididymis. III. Functional changes undergone by spermatozoa during their passage through the epididymis and vas deferens in the guinea pig. *Journal of Experimental Biology*, 8:151.

Zemjanis, R. (1970). Collection and evaluation of semen. In: *Diagnostic and Therapeutic Techniques in AnimalReproduction* (2nd edn). Wilkins Co.Baltimore, USA.pp. 139-156.

Zemjanis, R. (1977). Collection and evaluation of semen. In:*Diagnostic and Therapeutic Techniques in Animal Reproduction* (3rd edn). The Williams and Wilkins Co. Baltimore, pp 242.

Zwart, D., Brouwer, B.C., VanderHel, W., Van den Akker, H.N. and Versegen, M.W.A. (1991). Effect of *Trypanosoma vivax* infection on body temperature, feed intake and metabolic rate of West African Dwarf goat. *Journal of Animal Science,* 69: 3780-3788.

Zwart, D. and Veenendal, G.H. (1978). Pharmacologically active substances in *Trypanosoma vivax* infections. In: *Pathogenecity of trypanosomes*. *Proceedings of a workshop,* Nairobi, Kenya.Pp. 111-113.