COMPARATIVE STUDY OF TRYPANOSOMOSIS EXPERIMENTALLY INDUCED IN SAVANNAH BROWN GOATS USING *Trypanosoma brucei*, *Trypanosoma congolense* AND *Trypanosoma vivax.*

BY

ANTHONY KOJO BEDU SACKEY

B.Sc. HONS - AGRICULTURE, UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI-GHANA, JUNE, 1974

D.V.M., AHMADU BELLO UNIVERSITY, ZARIA NIGERIA, JUNE, 1980

CERTIFICATE IN VETERINARY ECONOMICS AND EPIDEMIOLOGY ABU ZARIA, NIGERIA/UNIVERSITY OF READING, GREAT BRITAIN, 1983

M.Sc. VETERINARY MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA NIGERIA, 1986

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented in any previous application for a higher degree. All quotations are indicated and the sources of informations are specifically acknowledged by means of references.

ANTHONY KOJO BEDU SACKEY

DATE 18-8-98

CERTIFICATION

This thesis titled "COMPARATIVE STUDY OF TRYPANOSOMOSIS EXPERIMENTALLY INDUCED IN SAVANNAH BROWN GOATS USING *Trypanosoma brucei, Trypanosoma congolense* AND *Trypanosoma vivax"* by Anthony Kojo Bedu Sackey meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Professor R. I. S. Agbede — Date
Internal Examiner
Department of Veterinary Parasitology and Entomology
Chairman Supervisory committee

Professor K. A. N. Esievo — 18th August 1998
Internal Examiner — Date
Head of Department of Veterinary Pathology and Microbiology
Member, Supervisory Committe

Professor S.U. Abdullahi — Date
Department of Veterinary Surgery and Medicine,
Member, Supervisory Committe

Dean, Postgraduate School — 28/05/99
Ahmadu Bello University, Zaria — Date

DEDICATION

This Thesis is dedicated to my wife Emelda, Children Kweku, Panyin, Kakra, Ekua; my late dear mother Josephine; my brothers and sisters; all those who contributed towards my academic pursuit; members of the Animal Kingdom who made it possible for me to pursue this highly demanding and respectable profession called VETERINARY MEDICINE and finally to the ALMIGHTY GOD THROUGH HIS SON JESUS CHRIST FOR "HIS LOVE FOR ME ENDURETH FOR EVER" AMEN.

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A B S T R A C T

Trypanosomosis in the Savannah Brown (SB) breed of goat was evaluated in a two phase investigation. The first phase involved the study oi the prevalence of natural trypanosomosis in the SB goat in its natural habitat of the Guinea and Sudan Savannah zones of Northern Nigeria. The study which spanned 28 months involved the random examination of 717 goats of all sexes and aged between 3 months and 5 years and from a total of 42 flocks through physical and haematological evaluations. These goats were kept by nomadic Fulani cattle herdsmen; established private livestock farms; Institutional Livestock farms and household flocks, which were receiving Veterinary Health Services regularly or on request. Twenty of the goats were positive for trypanosome parasites, giving an infection rate of 2.79%. Out of 20 goats sampled in the Southern Guinea Savannah 2 (infection rate of 10.00%) were positive, 11 out of 522 goats (2.1096) in the Northern Guinea Savannah and 7 out of 175 goats (4.00%) in the Sudan Savannah were also positive. Major clinical signs observed included orchitis, pyrexia of up to 41.0°C, abortion, emaciation in those positive for trypanosome parasites and weak kids born to infected does. *T. vivax* was the only trypanosome species isolated. The only potential trypanosome vectors identified were biting flies namely *Stomaxys calcitrans, Tabanus sp., Hipobosca sp.* and *Musca spp.* The study revealed the occurrence of natural trypanosomosis in the Savannah Brown goat in its presumed tsetse-fly free Savannah zone where this breeed of goat is predominantly found: *T. vivax* was the only trypanosome from this zone, a finding which confirm the observation of earlier workers that *T. vivax* is easily transmitted by biting flies. The result goes forth to show the need

for vigorous control measures against trypanosomosis in the small ruminants represented by this breed of goat in an effort to enhance the regular supply of animal protein to the teeming population as this breed of goat is a major source of meat in Nigeria.

A comparative study of trypanosomosis experimentally induced in the Savannah Brown breed of goats by using *T. brucei, T. congolense* and *T. vivax* was carried out, using four groups of 8 Savannah Brown bucks each, aged between 6-12 months.

An aspect of it involved observing clinical and haematologicai manifestations of trypanosomosis due to these three pathogenic African animal irypanosomes. One group was inoculated via the jugular route with *T. vivax* (stabilate 146), another with *T. cungoleme* (stabilate 136), the third group with *T. brucei* (stabilate 135) and the fourth group used as uninfected control. The goats were later bled at regular intervals and level of parasitaemia, rbc and pcv levels, total wbc levels, total protein and body- weights evaluated using approved standard procedures at regular intervals. Prcpatcnt periods observed were 2.3, 4.3 and 4.4 days for the *T. vivax, T. brucei* and *T. congoleme* infected groups respectively. Observed clinical signs included fluctuating pyrexia which in most cases coincided with parasitaemia. Parasitaemic peaks were pronounced in the *T. brucei* group, an indication that this trypanosome species is pathogenic to goats contrary to previous reports. The lowest mean pcv level of 8.0% occured in the *T. congolcnsc* group. The greatest fall in mean rbc level was in the *T. vivax* group whilst the least fall was in the *T. brucei* group. The disease produced three distinct phases namely the acute (0-15 days post infection), subacute (16 - 33 days) and chronic (34 - 74 days). Anaemia was of the normocytic normochromic form in the early stages of the disease but became microcytic normochromic at the

latter stage in all the infected groups. The primary leucocyte response in all the infected groups was lymphocytosis and later neutrophilia in the *T. vivax* group, concurrent lymphocytosis and neutrophilia in the *T. congolense* and *T. brucei* groups, though there occured neutropenia in the *T. hrucei* group in the chronic phase. Bilateral epiphora was very severe in the *T. vivax* and *T. congolense* infected goats. The occular discharges of some of the infected goats were found to he positive for trypanosome parasites especially during period of peak parasitaemia. How the parasite got in to the occular discharges cannot be explained from our study. Central nervous system complications like trembling, paddling movements, limb paralysis, staggering were observed most particularly in the *T. brucei-infected* goats. All the infected groups had progressive fall in mean body weights especially in the chronic phase of the infection. Most of the observations here agree with findings reported for trypanosome infected cattle, thus further indicating that the Savannah Brown goal is highly susceptible to Uypanosomosis in the same vein as it s cattle counterpart. Also that this breed of goat is highly susceptible to all the three species of trypanosomes contrary to earlier reports that *T. brucei* was as pathogenic to small ruminants as *T. vivax* and *T. congolense* arc.

The pathological changes due to experimentally induced trypanomosis due *T. brucei, T. congolense* and *T. vivax* were also investigated. Carcasses of all infected goats were anaemic especially in the subacute and chronic cases. Lymphoid organs (lymph nodes and spleen) were enlarged and hyperplastic, livers of infected goats were enlargement with areas of necrosis as well as fibrous connective tissue proliferation especially in the *T. brucei* and *T. congolense* infected goats. In the *T brucei* and *T. congolense* infected goats, kidney lesions included areas of petechial

and echymotic haemorrhages in the cortex and medulla regions as well as areas of focal and diffuse necrosis in the proximal and distal convoluted tubules. Haemorrhages of tracheal and bronchial mucosal surfaces as well as mononuclear cellular infilteration of the alveoli were evident in the *T. brucei* and *T. congolense* infected goats. All except one of the goats that developed cough were negative for bacterial isolates. Testicles were atrophied; they had also undergone degeneration as shown by the distortated basal layers. Also seminiferous tubules necrosis, fibrous tissue proliferative and aspermia in the chronic stages in all the infected goats. Pericardial space fluid ranged from 20 - 45 mis compared to 5 - 10 mis in the control group. The *T. brucei* and *T. congolense-infected* goats manifested areas of necrosis as well as infilteration of mononuclear cells in the myocardia. Although brain and pituitary gland capillaries were highly congested but had no significant microscopic lesions were observed in all the infected goats.

Impression smears taken at post mortem from jugular and aortic blood vessels were positive for trypanosomes though at very low parasitaemia. However smears from organs at post mortem were negative for trypanosome in all the infected goats. The three trypanosome species produced severe lesions which were closely related. These lesions so produced could adversely affect the performance of the goats affected goats.

The effect of trypanosomosis on the serum biochemical components was studied during this second phase. The experimental goats were bled at specific intervals in the course of the study and serum samples prepared and assayed using the appropriate methods, for chlorine (C1-) sodium (Na+), pottasium (K+), bicarbonate (HC03-), glucose, bilirubin, albumin, serum glutamic oxalo-acetic transminase (SGOT) and Total protein (TP) levels. Differences between the control and the

infected groups were significant ($p<0.05$). The mean albumin levels in the *T. vwax* group was significantly lower ($p<0.05$) than those of *T. brucei* and *T. congolense* groups. Whilst the mean glucose level in the *T. congolense* group was also significantly lower ($p<0.05$) compared to the other infected groups. A significant drop in the values of Na+> CI- and HCG3- levels occured in the *T. vivax* infected goats. Similarly *T. brucei* infected group produced drop in the level of albumin, total protein as well as the greatest rise in K+ and SGOT levels. On the other hand, the lowest mean glucose level and highest bilirubin and blood urea Nitrogen levels were observed in the *T. congolense* group. The study revealed the serious effect of trypanosomosis on the serum biochemical components and therefore an idea of the consequences of the disease on the metabolic activities of affected goats.

The role of trypanosome sialidase enzymes in the pathogenesis of anaemia in animal trypanosomosis using the goal as an animal model was evaluated. Significant differences ($p<0.001$) existed between the mean rbc sialic-acid concentration of the control and infected groups. The greatest fall in rbc sialic-acid concentration or the highest sialidase enzyme cleavage activity occured in the *T. vivax* group (5.82 ± 0.19 -1.88 ± 0.28 mg/dl or 67.7% reduction) followed by *T, brucei* (5.85 ± 0.43 to 2.28 ± 0.36 mg/dl or 61.33% reduction) and *T. congolense* (5.09 ± 0.11 to 3.94 ±0.18 mg'dl or 33.22% reduction). That of the control group was 1.04% reduction (5.78 ± 0.32 to 5.72 ± 0.25 mg/dl). The study further reinforced the reported findings of earlier workers that there is a mammalian blood stream form of sialidase enzyme in *T. vivax.* It also added weight to the suspicion that though mammalian form of sialidases have not been confirmed in *T. brucei* and *T. congolense,* the rbc sialic-acid cleavage activities of these two trypanosome

species suggest the presence of some forms of sialidase, possibly trans-sialidase which undertake the cleavaging. The study also revealed that sialidase enzyme plays a major role in the pathogenesis of trypanosomosis induced anaemia because of the relationship between fall in rbc sialic acids and consequent tall in rbc and pcv levels in all the infected goats. The results obtained also gave a favourable disposition to the future use of small ruminants in further investigations into the relationship between sialidase enzyme and anaemia of animal trypanosomosis

TABLE OF CONTENTS

		PAGE
	Title Page	i
	Declaration	ii
	Certification	iii
	Dedication	iv
	Acknowledgement	v
	Abstract	vii
	Table of Contents	xiii
	List of Tables	xviii
	List of Figures	xix
	List of Plates	xx
	List of Appendices	xxii
CHAPTER ONE		1
	INTRODUCTION	1
CHAPTER TWO		7
	LITERATURE REVIEW	7
2.0.	Trypanosomes of Ruminants	7
2.1.	The Trypanosoma Genus	7
2.1.1.	Sub-genus Trypanosoma	9
2.1.2.	Sub-genus Nannomonas	9
2.1.3.	Sub-genus Duttonela	12
2.2.	Distribution	12
2.3.	Transmission	12
2.3.1.	*T. brucei*	13
2.3.2.	*T. congolense*	13
2.3.3.	*T. vivax*	14
2.4.	Host Range	14
2.4.1.	*T. brucei*	14
2.4.2.	*T. congolense*	15
2.4.3.	*T. vivax*	15
2.5.	Pathogenesis of Trypanosomosis	15
2.5.1.	Host Parasite Relationship	15

2.5.1.1	*T. brucei*	15
2.5.1.2.	*T. congolense*	16
2.5.1.3.	*T. vivax*	16
2.5.2.	Trypanosome distribution in the host	17
2.5.2.1.	*T. brucei*	17
2.5.2.2	*T. congolense* and *T. vivax1*	17
2.6.	Factors involved in the susceptibility of domestic ruminants to trypanosomosis.	18
2.6.1.	Parasite related factors.	18
2.6.2	Host related factors	18
2.6.2. 1.	Age	18
2.6.2.2.	Stress	19
2.6.2.3	Trypanotolerance	20
2.6.2.3.1	Immunological factors	22
2.6.2.3.2	Physiological factors	23
2.6.2.3.3	Red blood cell sialic acid concentration factor.	24
2.6.2.3.4	O-Acetyl group substitution of Acetyl Nuraminic acid factor.	25
2.6.3.	Pathogenesis of trypanosomosis in domesticated ruminants.	26
2.6.3.1	Incubation period	26
2.6.3.2	Parasitaemia	27
2.6.3.3	Pyrexia	28
2.6.3.4	Anaemia	29
2.6.3.4.1	Haemodilution	29
2.6.3.4.2	Haemopoietic-Response	30
2.6.3.4.3	Erythrophagocytosis	31
2.6.3.4.4	Immunoglobulins and Complements	33
2.6.3.4.5	Direct Effect of Trypanosomes	34
2.6.3.4.6	Haemolytic Factors	34
2.6.3.4.7	Reduced RBC Lifespan	36
2.6.4	Morphological Classification of	37
2.6.5	Leucocyte Response in trypanosomosis	38
2.6.6.	Animal trypanosomosis and thrombocyte Response	40
2.6.7.	Trypanosomosis and Serum Biochemistry	40
2.6.7.1.	Plasma Protcins (T.P., Albumin Gammglobulin.....)	40
2.6.7.2.	Bilirubins	42
2.6.7.3	Blood Sugar (Glucose)	42
2.6.7.4	Blood Urea Nitrogen(BUN)	43
2.6.7.5.	SGOT(ALT) Level	44
2.6.7.6	Pottasium ion (K+)	44
2.6.7.7.	Sodium ion (Na+)	45
2.6.7.8.	Chlorine ion (Cl-)	45

2.6.7.9. Bicarbonate (HCO_3) 46
2.6.8. Organ Pathologies 46
2.6.8.1. Edema 46
2.6.8.2. Lymphoid Organs (spleen, lymph nodes, thymus) 46
2.6.8.3. Reproductive Organs 47
2.6.8.4. Liver, Gall-bladder, Kidney 48
2.6.8.5. Other Body Organs: Body Fluids 49
2.7. The Role of Sialidase (Neuraminidase) and Sialic Acid Neuraminic acid) in trypanosomosis 50
2.7.1. Sialidases 50
2.7.2. Sialic Acid 52
2.7.3 Sialidase: Sialic acid and Trypanosomes 55
2.8. Trypanosomosis of Small Ruminants. 57
2.8.1. In Africa in General 57
2.8.2. Small Ruminants Trypanosomosis in Nigeria 62
2.8.2.1. Some Reports on Natural Occurrence 62
2.8.2.2. Use of Small Ruminants in Experimental Trypanosomiasis 63
2.8.2.3. Present Status of Trypanosomosis and Animal Production in Nigeria. 64
2.8.2.3.1. Epidemiology of the Disease and Economic Importance 64
2.8.2.3.2. Diagnostic Procedure 64

CHAPTER THREE

3.0. SURVEY OF THE PREVALENCE OF TRYPANOSOMIASIS IN SAVANNAH BROWN GOATS 68
3.1.0. Introduction 68
3.2.0. Materials and Methods 69
3.3.0. Results 70
3.4.0. Discussion 74
3.5.0. Summary 75

CHAPTER FOUR 77

4.0. THE CLINICAL, HAEMATOLOGICAL AND PATHOLOGICAL MANIFESTATIONS OF EXPERIMENTAL TRYPANOSOMOSIS 77

4.1. Introduction 77
4.2. Materials and Methods 78
4.2.1. Animals 78
4.2.2. Parasite (Stabilates) 80
4.2.3. Inoculations 80
4.2.3.1. Donor Animals 80

4.2.3.2.1. Inoculation of Experimental Animals, Monitoring and Sample Collection 81
4.2.3.2.2 Sample collection 81
4.2.3.2.3. Monitoring 81
4.2.4. Method of statistical analysis 84
4.3. Results 84
4.3.1. Pre-infection Base Line Data 84
4.3.2. Post-infection Results 85
4.3.2.1. Phases of Infection 85
4.3.2.2. Temperature 85
4.3.2.3. Parasitaemia 86
4.3.2.4. Packed Cell Volume 86
4.3.2.5. Haemoglobin Level 87
4.3.2.6. Total RBC Count 87
4.3.2.7. Total and Differential WBC Count 91
4.3.2.8. Observed Clinical Signs 95
4.3.3. Pathology 102
4.3.3.1. Gross Pathogical Findings 102
4.3.3.2. Histopathological Findings 105
4.3.3.2.1. Lymphoid Organs (Spleen and Lymph nodes) 105
4.3.3.2.2. Kidney 106
4.3.3.2.3. Liver 106
4.3.3.2.4. Heart 107
4.3.3.2.5. Lung 107
4.3.3.2.6. Testicles 107
4.3.3.2.7. Other Organs 108
4.4. Discussion 119
4.5. Summary 132

CHAPTER FIVE

5.0. OBSERVATIONS ON SOME SERUM BIOCHEMICAL CHANGES IN THE SAVANNAH BROWN GOAT 137

5.1. Introduction 137
5.2. Materials and Methods 139
5.2.1. Animals 139
5.2.2. Sample Collection 139
5.2.3. Sample Analysis 139
5.3. Results 140
5.4. Discussion 150
5.5. Summary 156

CHAPTER SIX

6.0. THE SAVANNAH BROWN BREED OF GOAT AS A POTENTIAL ANIMAL MODEL IN THE STUDY OF THE ROLE OF ERYTHROCYTE SIALOGLYCOCONJUGATES IN THE DEVELOPMENT OF ANAEMIA IN ANIMAL TRYPANOSOMOSIS 158

6.1.. Introduction 158
6.2.. Materials and Methods 162
6.2.1. Experimental Animals 162
6.2.2. Parasites 162
6.2.3. Experimental Inoculation 162
6.2.3.1. Donor Goats 162
6.2.3.2 . Experimental Groups, Monitoring and Sample Collection 162
6.2.3.2.1. Inoculations 162
6.2.3.2.2. Monitoring and Sample Collection 162
6.2.3.2.3. Sample Analysis 163
6.3. Results 163
6.4. Discussion 166
6.5. Summary 167

CHAPTER SEVEN 169

7.0. GENERAL DISCUSSION 169

REFERENCES 179

LIST OF TABLES

2.1.	Characteristics of the Subgenera of the Genus Trypanosoma	**8**
3.1.	Prevalence of Trypanosomosis in the Savannah Brown Goats (SBG) in Some Parts of Northern Nigeria	72
3.2.	Summary of the Prevalence of trypanosomosis in the Savannah Brown Goat in Different Vegetational Zones of Northern Nigeria	73
4.1.	Pre-Experimental Infection Baseline Values.	99
4.2.	Response of SBG to Trypanosome Infection	100
4.3.	Parasitaemia in Trypanosome infection of SBG.	101
5.1.	Some Serum Biochemistry of Experimental Infection in SBG as Percentage of Control Group's Values.	141
5.2.	Comparison of Degree of Change in the Level of Some Serum Biochemistry in Experimental *T. vivax, T. congolense* and *T. brucei* infection of SBG.	142
6.1.	Comparison of Erythrocyte Sialic acid Component, in Experimental Trypanosoma Infection of Savannah Brown breed of goat	165

LIST OF FIGURES

1.1.	Distribution of Nigerian Breeds of Sheep and Goats	3
2.1.	Nigeria's Vegetational Zones	11
4.1.	Mean Parasitaemia and Packed Cell Volume of Infected and Uninfected Control Savannah Brown bucks.	89
4.2.	Mean- Cell Volume, Mean-Cell Haemoglobin and Mean-Cell Haemoglobin Concentration of Infected and Uninfected Savannah Brown bucks.	90
4.3.	Mean Total Leukocyte Count, Lymphocyte Count, and Neutrophil Count of Infected and Un-infected Control Savannah Brown bucks	94
4.4.	Mean Weekly Body Weights and Weekly Body Weight Gains of Infected and Un-infected Control Savannah Brown bucks	98
5.1.	Mean Parasitaemia, Mean Serum Sodium, and Chlorine of Infected and Un-infected Control Savannah Brown bucks	145
5.2.	Mean Potassium and Bicarbonate Levels of Infected and Uninfected Savannah Brown bucks	146
5.3.	Mean Serum Total Protein, Albumin, and Globulin Levels of Infected and Uninfected Control Savannah Brown bucks	147
5.4.	Mean Serum Urea Nitrogen Levels of Infected and Uninfected Control Savannah Brown bucks	148
5.5.	Mean Serum Glucose and SGOT(ALT) Levels of Infected and Uninfected Control Savannah Brown bucks	149

LIST OF PLATES

Plate 4.1.1. A section of the kidney with no significant findings (Control S.B. bucks). H & E. x400 109

Plate 4.1.2. A section of the kidney. Note non-suppurative necrosis of the glomelurus and the renal tubules. Also note adhesion of the glomerulus to the Bowman's capsule (*T. congolense*-infected S.B. bucks). H & E. x 400. 109

Plate 4.1.3. A section of the kidney. Note necrosis of the glomerulus and the renal tubules. Also note the adhesion of the Bowman's capsule by the right side. (*T. vivax* infected S.B. bucks). H & E. x400. 110

Plate 4.1.4. A section of the kidney. Note necrosis of the renal tubules with some intertubular mononuclear cellular infilteration. Also note the adhesion of the Bowman's capsule by the right side. (*T. vivax* -infected S.B. bucks). H & E. x400. 110

Plate 4.2.1. A section of the liver with no significant findings (Control S.B. bucks). H & E. x400 111

Plate 4.2.2. A Section of the liver. Note diffuse necrosis of the liver at the portal area (*T. vivax* infected S.B. bucks) H & E. x 400 111

Plate 4.2.3. A Section of the liver. Note necrosis of the lobe especially at the middle portion as well as mononuclear cellular infiltration. (*T. congolense* as well as *T. brucei*- infected S.B. bucks). H & E. x 400 112

Plate 4.3.1. A section of the lungs with no significant findings (Control S.B. bucks). H & E x400. 113

Plate 4.3.2. A section of the lung. Note mononuclear cellular infiltration in the interalveolar spaces and some alveoli (interstitial pneumonia) and congested blood vessels at the top right corner (*T. congolense*-infected S.B. bucks) H & E x400. 113

Plate 4.3.3. A section of the lung. Note mononuclear cellular infiltration in the interalveolar spaces (interstitial pneumonia). (*T. brucei*- infected S.B. bucks) H & E x400. 114

Plate 4.4.1. A section of the testis. Note disorganised seminiferous tubules (*T. vivax*- infected S.B. bucks) H & E x400. 115

Plate 4.4.2. A section of the testis. Note the non-suppurative necrosis of the seminiferous tubules (*T. brucei*-infected S.B. buck) H & E x400. 115

Plate 4.4.3. A section of the testis. Necrosis of the seminiferous tubules. Note fibrosis at the intertubular spaces (*T. congolense*-infected S.B. bucks) H & E x400 116

Plate 4.4.4. A section of the testis. Note severe necrosis of the seminiferous tubules and marked intertubular fibrosis (*T. vivax*-infected S.B. bucks) H & E x400. 116

Plate 4.4.5. A section of testis. Note necrosis of the seminiferous tubules (*T. congolense*-infected S.B. bucks) H. & E. x400. 117

Plate 4.5.1. A section of the heart(myocardium) with no significant findings (Control S.B. bucks) 117

Plate 4.5.2. A section of the heart (myocardium). Note necrosis of the myocytes and mononuclear cellular infiltration (myocarditis) (*T. brucei*-infected SB bucks). H & E x 400 118

Plate 4.5.3. A section of the heart (myocardium). Note mild necrosis of the myocytes and few mononuclear cellular infiltration (mild myocarditis) (*T. vivax* as well as *T. congolense*-infected SB bucks). H & E. x 400 118

LIST OF APPENDICES

1. Analyses of Variance for Mean Rectal Temperature, Parasitaemia, Haematological Values, Weekly Body Weights and Weight Gains of Infected and Uninfected Control Savannah Brown Bucks 209

2. Analyses of Variance for Mean Serum Biochemical Components Levels of Infected and Uninfected Control Savannah Brown Bucks 223

3. Summary of Methods Used in the Estimation of Some Serum Biochemical Components Levels and Red Blood Cell Sialic Acid Concentrations 235

CHAPTER ONE

INTRODUCTION

The importance of small ruminants as a source of animal protein has been stressed in many reports. Between 1978 and 1980, small ruminants provided about 250,000 tonnes of meat which accounted for 36.0 percent of the total meat consumption in Nigeria. This is higher than the world average of 6.0 percent during the same period (Adegbola, 1982). Goats and sheep are easy to manage, requiring minimum investment in housing and feeding; additionally, their production is non-labour intensive and are efficient utilisers of roughage and agricultural by-products. Their small size, early maturity, short reproductive cycle and high fecundity make them suitable for farmers with relatively small capital.

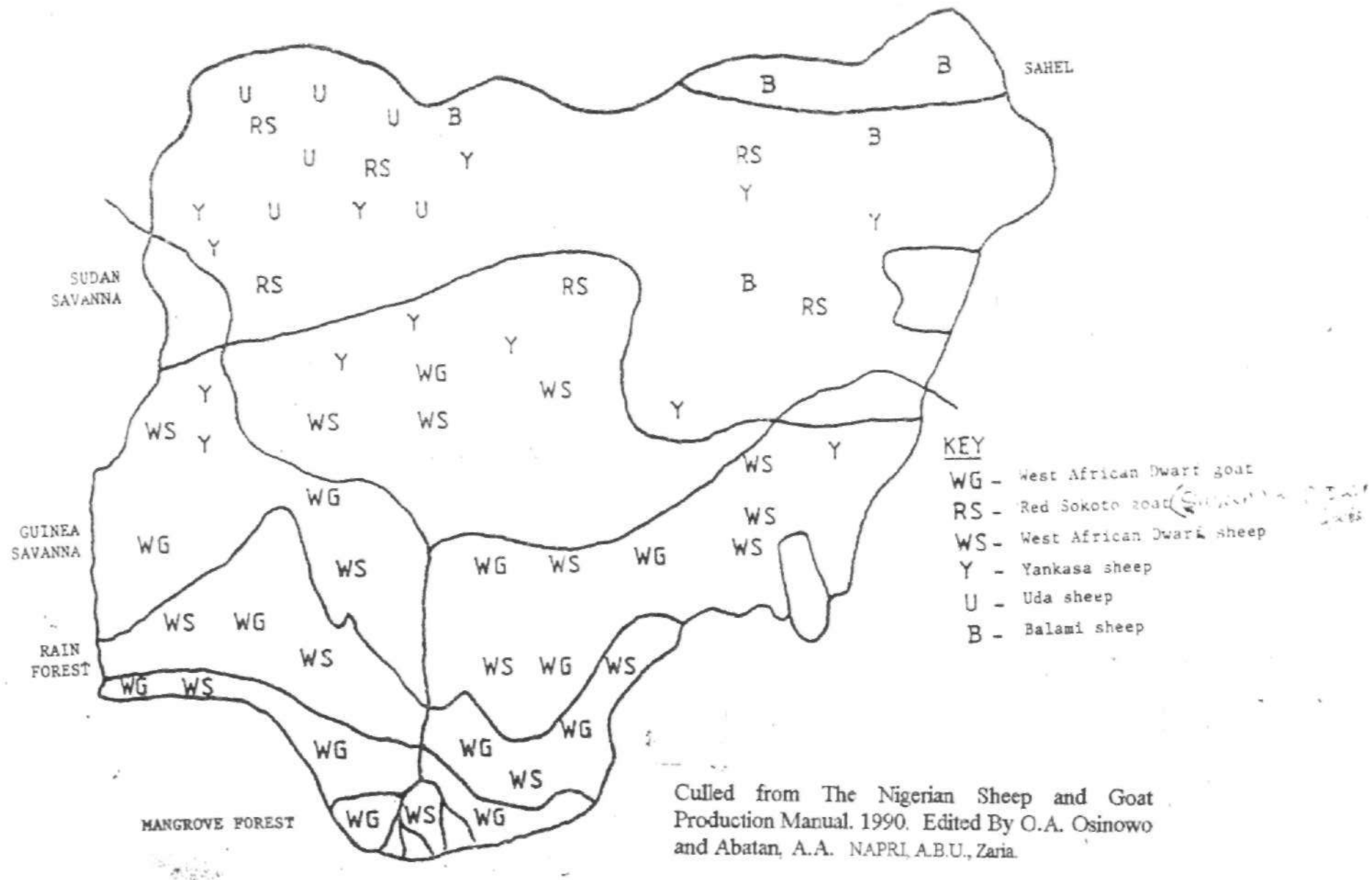
Goats are well distributed over and across the diverse climatic zones, with an average herd size of 4 and 10 per household in the southern and the northern parts of Nigeria respectively and providing ready source of meat, gift, income and security to the house-holds (Olundimu *et al.*, 1990).

Profitable animal production is enhanced by efficient husbandry and healthy animals (Agwuma, 1983). The livestock industry apart from being a source of animal protein also provides occupation for many people and plays a major role in the economic growth of Nigeria(David West, 1990). However, the demand for animal protein far outstrips the supply (Agwuma, 1983).

According to Agwuma (1983), disease is a major limiting factor to profit attainment in large scale holding of goats and sheep. Prominent diseases in this respect include helminthiasis, ectoparasitism, haemoparasitism as well as viral and bacterial diseases

(Agwuma, 1983). One of the major haemoparasitic diseases of livestock is trypanosomosis due to *Trypanosoma (T.) brucei; T.vivax* and *T. congolense* (Leeflang, 1978; Agu,1983). This disease condition occurs in domestic livestock in many areas of Africa especially West, Central, East and Southern Africa all of which are areas of high tsetse-fly infestation. It has also established itself among bovines in The West Indies, Mauritius, Central and South America (Hoare, 1972). *T. vivax* was introduced into the new world as far back as 1930 with the importation of zebu cattle from Senegal into French Guyana, the Antiles-Guadeloupe and Martinique (Currason, 1943) and from where it spread to Panama (Johnson, 1941), Dutch Guyana, British Guyana, Venezuela and Colombia where it is refered to as "Secadera" (Nieshulz, 1939 cited by Hoare, 1972.) In Africa, trypanosomoses is also referred to as "Nagana". In 1904 one Chichester was reported to have diagnosed trypanosomosis in 19 out of 150 heads of cattle brought in from Zaria province in Northern Nigeria to an experimental farm at Asaba (now in Delta state) in Southern Nigeria (Johnson, 1941). According to Johnson(1941), this was the first diagnosis of trypanosomosis in Nigeria and was predominanatly due to *T.vivax*. Garden(1910), also reported trypanosomosis in sheep at Epe. Also Macfie(1913a, b) identified *T. brucei, T. congolense,* and *T. vivax* in sheep slaughtered at Ilorin.

Fig. 1.1. Distribution of Nigerian Breeds of Sheep and Goats.



Culled from The Nigerian Sheep and Goat Production Manual. 1990. Edited By O.A. Osinowo and Abatan, A.A. NAPRI, A.B.U., Zaria.

According to an FAO Report(1962), about 4.5 million square miles of potential grazing land in Africa is infested by tsetse-flies. Only a fifth of Nigeria's total land area of 920,00 square kilometres is free of tsetse flies. This report also observed that there were enormous economic losses due to animal trypanosomosis arising from mortality, abortion, reduced milk yield, damaged hides, condemnation at meat inspection, poor quality meat, reduced body weight and loss of draught power. Under poor husbandry especially in areas with enzootic trypanosomoses, the performance of affected animals is seriously impaired and as such the continuing pressure on animal protein resources in such areas tend to cause sheep and goats to play increasing role in the national economy (Griffin, 1978).

According to the Nigerian Sheep and Goats Production Manual(1990), most of the sheep and goats population of Nigeria are located in the Savanna zones (Fig I.) which are said to be predominantly free of tsetse flies, the major vector of trypanosomes. Over 90% of cattle in Nigeria are herded by the nomadic Fulani (Nuru, 1974) who, during the dry lean period of the year characterised by lack of feed and water, are forced to move into the derived savanna and forest zones in search of feed and water, thereby exposing their herds to tsetse flies and consequently trypanosomosis (Leach *et al.*, 1965; Leeflang, 1978). Apart from cattle, the nomads also keep large numbers of Savanna breeds of goats and sheep which also thus become exposed to trypanosomosis to which they are also less resistant. Trypanosomosis research in Nigeria is mostly centred around cattle with less emphasis on sheep and goats especially the Savanna breeds. It is generally believed that small ruminants only serve as alternative sources of blood meal for the tsetse flies in the epizootiology of trypanosomosis(Leeflang, 1978). According to Fischer *et al.* (1984) there is the belief that

natural occurrence of trypanosomosis is rare as the highly susceptible Sahelian breeds of sheep and goats avoid the tsetse fly infested zones while in the Southern Guinea Savanna (SGS) and Forest (F) zones the local dwarf breeds seem completely resistant. However, Krammer (1966) had reported high infection rates of 13% in goats.

The major breed of goats in the tsetse fly free savanna zone is the Savanna Brown Goat (SBG) which is bigger in size, more prolific and with greater milking potential compared to the trypanotolerant dwarf breed, the West African Dwarf Goat (WADG) found in the Southern Guinea Savanna and Forest zones(Wilson, 1991). The SBG is being gradually introduced into the SGS and F zones of Nigeria, a process being enhanced by a Breeding and Selection Program for litter size and milking potential being carried out presently by the National Animal Production Research Institute (NAPRI) at Shika - Zaria in Kaduna State, Nigeria. As this breed, which is also well known for its high quality hide from which the once famous Morroco leather was made, penetrates the tsetse infested area of the country its exposure to trypanosomoses increases.

There is therefore the need to further investigate the susceptibility of the Savanna Brown Goat to trypanosome infection due to *T. vivax, T. congolense* and *T. brucei* with emphasis on the underlisted features of trypanosomosis due to the above mentioned three speces in the SBG.

1. Prevalence of trypanosomosis in the SBG in some ecological zones of Northern Nigeria.
2. The clinical and Haematological picture of experimental trypanosomosis in the SBG.

3. The pathology of trypanosomosis experimental infection in SBG.
4. Some serum biochemical changes in SBG due to experimental trypanosomosis
5. The potential of the SBG as an animal model in the study of the roles of erythrocyte sialoglycoconjugates in the development of anaemia in "Animal-Trypanosomosis ".

CHAPTER TWO

LITERATURE REVIEW

2.0. TRYPANOSOMAS OF RUMINANTS

The importance of tsetse fly and its associated disease has long been known to African livestockmen. It was not however until the turn of the century that the presence of small, highly motile, flagellated protozoa in the blood of infected cattle were observed.Painstaking observations between 1895 and 1905 established these organisms as the cause of the cattle disease in tsetse fly belts and which became known as "Nagana". Further studies revealed at least three distinct parasites affecting cattle, namely *Trypanosoma (T.) vivax, T. congolense* and *T. brucei* and a fourth one *T. uniforme* which is very similar to *T.vivax* except its smaller size (Hagan and Wilmshurst, 1972). All were placed under the phyllum Protozoa and Genus Trypanosoma.

2.1. THE TRYPANOSOMA GENUS

The Genus Trypanosoma consists of three sub-genera (Table I.) namely Duttonella, Nannomonas and Trypanozoon with members undergoing multiplication in the blood stream of infected domestic and wild animals in the form of flattened spindle-shaped structure known as trypomastigote (Hoare, 1972).The members of these sub-genera are highly pathogenic.They are also referred to as "salivarian" because they develop in the salivary system of the tsetse fly vector where the development forms are called epimastigotes which are non-infective to mammals.

Table 2.1. Characteristics Of The Sub-Genera Of The Genus Trypanosoma

SUB-GENERA	SPECIES	MORPHOLOGY IN MAMMALIAN BLOOD STREAM	FORMS AND LOCALISATION IN TSETSEFLY				MORPHOLOGY IN TSETSEFLY
			PROBOSCIS	SALIVARY GLAND	MIDGUT	HINDGUT	
DUTONELLA	*T. vivax*	Large kinetoplast, free flagellum club shaped type with inconspicious undulating membrane and slender type with conspicious undulating membrane. Rapid motion in wet film, 21-25.4 *u* in length.	Members of subgenus occur as trypomastigotes, epimastigotes and metatrypanosomes.	No development (N.D.)	N.D.	N.D.	Large kinetoplast and free flagellum in all stages
	T. uniforme	Regularly monomorphic, unconspicious undulating membrane, sluggish motions in wet smears, 14.6-16.5*u* in length.					
NANNOMONAS	*T. congolense* *T. simiae;* *T. godfreyi**	Slightly polymorphic, medium sized kinetoplast, no free undulating membrane. Sluggish movement in wet smears, 12.2 - 17.6*u* in length.	"	N.D.	Trypomatigotes	N.D.	Medium kinetoplast no free flagellum at any stage
TRYPANOZOON	*T. brucei* (Also *T. brucei gambiense* and *T. brucei rhodesiense*)	Polymorphic, small kinetoplast, conspicious undulating membrane long and intermediate forms have free flagellae. Stumpy (short) form without free flagellum 23-30 u; 20-25 u; and 17-22 u in length respectively.	N.D.	Trypomastigotes, epimastigotes and metatrypansomes	Trypomastigotes	N.D.	Small kinetoplast; free flagellum in all stages except in metatrypanosomes

* McNamara 1990

Culled from: The Control of Bovine Trypanosomiasis By Agro Chemicals Division The Boots Company Ltd Nottingham, England, 1978. Pg. 10 - 11.

2.1.1. SUB-GENUS TRYPANOZOON

The major trypanosome here is *T. brucei* previously referred to as *T. pecandi* (Laveran, 1909) *T. elephantis* (Bruce *et al.*, 1914). It is clearly related to the human trypanosomes *T. brucei gambiense* which causes human trypanosomosis in West Africa sub region, and *T.brucei rhodesiense* the causative agent of human trypanosomosis in East and Central African sub regions. It was originally observed in the blood of cattle suffering from "Nagana" in Zululand in South Africa and later confirmed as the causative agent of the disease (Bruce, 1914). It was later given the name *T.brucei* after Bruce, who was the first to describe it. Morphologically, *T. brucei* is polymorphic in the mammalian hosts as slender, or intermediate or stumpy form (Table I). *T.brucei* exhibits wriggling or snake-like movement, seldomly moving out of the microscopic field(Hoare, 1972).

2.1.2. SUB-GENUS NANNOMONAS

Here is found one of the most important trypanosomes of cattle, *T. congolense*. It develops cyclically in the proboscis and midgut of tsetse fly vectors. Also under this sub-genus are *T. simiae* which causes acute trypanosomosis in pigs; and the recently described *T. godfreyi* which also causes acute trypanosomosis in sheep and pigs(MacNamara, 1990). The three members bear close resemblance in stained blood films and only differentiated via mesural methods (Hoare, 1972) with length of 12 - 18 units; 17 - 18 units and 13 - 17 units for *T. congolense, T. simiae* and *T. godfreyi respectively*. Members have subterminaly and marginally placed kinetoplast, centrally located nuclei, and lacking free

flagellum (Kreier and Baker, 1987; MacNamara, 1990). *T. congolense* (sometimes also referred to as *T. [Nannomonas] congolense*) was first discovered in domestic ungulates in the former Belgian Congo (formerly Republic of Zaire and presently Democratic Peoples Republic of the Congo). Several strains of it have been reported (Hoare, 1959)

Nigeria's Vegetational Zones



Culled from: Leeflang, P. (1978). Bovine trypanosomiasis in Northern Nigeria. A contribution to the epidemiology, host susceptibility and drug sensitivity of *T. vivax*. Ph D. Thesis University of Utrecht, Utrecht.

2.1.3. SUB-GENUS DUTTONELA

This sub-genus is made up of 2 members namely *T. uniforme* and *T. vivax*. These two are similar in all respects except that the latter is smaller. *T. vivax* also has two forms namely a club-shaped and slender forms. *T. vivax* and *T. uniforme* develop only in the proboscis of the tsetse fly vector. Also *T. vivax* moves extremely rapidly in wet smears whereas *T. uniforme* is more sluggish.

2.2. DISTRIBUTION

All the three major trypanosome species of cattle (and other ruminants) namely *T. brucei*, *T. congolense* and *T. vivax* with their corresponding tsetse vectors are widely distributed in Tropical Africa between latitudes 15° North and 25° South (Davies, 1977). In Nigeria, only about 1/5 of the country is said to be naturally free from tsetse flies and this area is in the far North where water and pasture for cattle are also scarce most time of the year (FAO, 1962).

2.3. TRANSMISSION

The major ruminant trypanosomes are all salivarian and tsetse borne. They are all said to be capable of developing in any species of *Glossina (G.)* though with some specific preference.

2.3.1. *T. brucei*

The prevalent or the main tsetse vectors are those belonging to the morsitans group, namely *G. morsitans* and *G. pallidipes* (Currasson, 1943; Duxton, 1955). Also as well as *G. palpalis, G. longipalis* and *G. tachinoides*. According to Davies (1977), very little *T. brucei* appears to he carried by any of the tsetse fly vectors. The trypanosome undergoes developmental cycle in the tsetse flies before becoming infective to mammals. Mechanical transmission which does not involve any stage of development in the intermediate host does occur through biting flies like *Tabanids spp* and *Stomoxys spp.* (Duke, 1934; Hornby, 1952) with the success of this form of transmission depending on the of survival of the blood trypanosomes in the proboscis of the insect during the short interval between interrupted feeding.

2.3.2. *T. congolense*

T. congolense is capable of developing in any species of *Glossina*, but mostly in *G. morsitans, G. longipalpis* and less in *G. tachinoides, G. palpalis, G. medicorum* and *G. nigrofusca* (Davies, 1977; Leach *et al.*, 1965). Mechanical transmission occurs through biting flies like *Tabanid spp* and *Stomoxys spp.* (Duke, 1934; Hornby, 1952). It should be noted that mechanical transmission is possible through tsetse fly, occurring when the fly is interrupted during feeding on an infected animal and then completes its feeding on another host especially when animls are herded together (Leach *et al.*, 1965; Morrison *et al.*, 1981). On rare occassions, animals are said to come down with trypanosomosis by feeding on fresh carcasses of infected animals and also by drinking infected milk (Leach *et al.*,

1965; Ford, 1971 and Siegmund, 1973). Ticks have also been incriminated in the transmission of *T. congolense* (Leach *et al.*, 1965), *T. vivax* and *T. theileri* (Agbede, 1987).

2.3.3. *T. vivax*

T. vivax can be transmitted by any species of the tsetse fly, especially by *G. longipalpis* and *G. morsitans* having a higher infection rate than the former. Others vectors include *G. tachinoides* and *G. palpalis* (Davies, 1977). Also, transmission of these species has also been attributed to biting flies like *Tabanid* and *Stomoxys spp* all of which are often abundant near cattle sites(Davies, 1977). The rate of transmission in Nigeria is said to be higher than that of *T. congolense* especially in the North than in the South where *T. congolense* is more common(Davies, 1977). *T.vivax* is also well established in parts of Africa at distances from the tsetse fly belts(Davies, 1977).

2.4. HOST RANGE

The three most pathogenic trypanosome spp of ruminants do vary in their host range (Stephen, 1970).

2.4.1. *T. brucei*

T brucei infects cattle, sheep, goats, dogs, pigs, horses, donkeys, camels, cat, and reportedly monkeys (Stephen, 1970; Godfrey *et al.*, 1964; Godwin, 1070; Meltam, 1934; Davies,1977; Baker, 1962) and experimentally in laboratory animals.

2.4.2. *T. congolense*

T. congolense infects cattle, sheep, goats, horses pigs, camels, dogs, cats, rodents (Hoare, 1970; 1972; Godfrey, 1982; Stephen, 1970; Davies, 1977)

2.4.3. *T. vivax*

T. vivax affects cattle, sheep, goats, camels, horses, pigs, cats, dogs and experimentally laboratory animals (Davies, 1977)

2.5. PATHOGENESIS OF TRYPANOSOMOSIS

2.5.1. HOST-PARASITE RELATIONSHIP

The various animal trypanosomes vary in the level of severity of infection caused in their hosts, an indication of their varying degrees of virulence

2.5.1. 1. *T. brucei*

T.brucei is the cause of Nagana and hence of considerable veterinary importance since practically all domestic animals are susceptible to the infection. It is very virulent for horses and other equines; sheep, goats and producing a severe and fatal disease. The pathogenicity in pigs is low with cattle being relatively resistant(Stephen, 1970). According to Godfrey *et al.* (1964) and Godwin (1970), the experimental infection often produces severe disease and high mortality. It is highly pathogenic in laboratory animals with

virulence increasing rapidly with passage until the animal succumbs within a few days (Godwin, 1970).

2.5.1.2. *T. Congolense*

T. congolense is pathogenic to all domestic animals. It causes mild but chronic infection in cattle, severe infection in sheep, goats, camels and less of a problem in horses, pigs, dogs, man and cats(Davies, 1977). *T. congolense* is the most pathogenic and important cattle trypanosome in East and Central Africa and second to *T. vivax* in importance in West Africa (Leach *et. al.*, 1965; Beaton, 1968; Tizard *et. al.*, 1977; Mwambu, 1983).

2.5.1.3. *T. vivax*

According to Macleanan (1970), *T. vivax* causes a more acute form of trypanosomosis in cattle in West Africa and chronic form in East Africa. *T. vivax* is less pathogenic in sheep, goats and camels and horses (Macleanan, 1970). Davies (1977), reported that pigs, dogs and cats are less susceptible to *T. vivax*. *T. vivax* like *T. congolense* causes a very severe anaemia in dwarf goats (Edwards *et. al.*, 1956a). Anosa and Isoun (1974; 1980) observed pronounced anaemia in small ruminants with acute *T. vivax* infection than those with subacute or chronic forms of the infection.

2.5.2. TRYPANOSOME DISTRIBUTION IN THE HOST

The distribution of trypanosomes within the affected animals depends on the species of trypanosome involved.

2.5.2.1. *T. brucei* group

The *T. brucei* group made up of *T. brucie brucei*, *T. brucei gambiense* and *T. brucei rhodesiense* are capable of invading tisuues (Losos and Ikede, 1970; Godwin and Guy, 1973) and multiplying outside the blood vascular systems such as the cerebrospinal fluid, acquous humour, lymph and connective tissue and thus producing more widespread lesions (Banks, 1970; Loso *et. al.*, 1973).

2.5.2.2. *T. congolense and T. vivax*

These two trypanosome species usually do not leave the blood stream (Losos and Ikede, 1972; Losos *et. al.*, 1973) but rather prefer the microcirculation, attaching to the capillary vascular endothelium (Banks, 1970; Losos and Ikede, 1972; Bungener and Muller, 1976) and are therefore more concentrated in capillary beds especially in the jugular veins; thus producing lesions more confined to walls of blood vessels.

However, Luckins and Gray (1979) reported the presence of *T. congolense* in lymph nodes of cattle and sheep, an indication of the tissue penetrating capability of *T. congolense*, hence not a strict plasma parasite although Tizard *et. al.* (1978a) in their earlier work did agree with this observation.

2.6. FACTORS INVOLVED IN THE SUSCEPTIBILITY OF DOMESTIC RUMINANTS TO TRYPANOSOMOSIS

Several reasons have been adduced by investigators in explaining the incidence of trypanosomosis in domestic ruminants. Masake (1980) and Ikede (1981; 1986) reported that the cause of the infection is determined by factors related to the parasite and host.

2.6.1. PARASITE RELATED FACTORS

These include antigenic and strain variation and virulence. According to Cross (1975), different strains of trypanosomes succeed in their activities due to their ability to undergo antigenic variation which allows the strains to invade the immune system of the host and thus produce persistent infection. Van Meirenne et al. (1977), reported that all trypanosoma strains have the potential of producing varying stocks of variable antigens.

With regard to strains, West African strains of *T. vivax* are said to be more virulent than those of East Africa, whilst the opposite holds for the strains of *T. congolense* (Griffin and Allonby, 1979c).

2.6.2. HOST RELATED FACTORS

These include variations due to age breed nutritional status and trypano-tolerance (related to breed). Others include degree of challenge and health status.

2.6.2.1. AGE:

Age is said to be a significant factor in the incidence of animal trypanosomosis. In cattle, calves are said to be more resistant to *T. congolense* infection than adults (Fiennes,

1970a). Welde *et. al.* cited by Uzoigwe (1984) had reported that calves are more resistant than adults to *T. vivax* infection. But in sheep and goats, young lambs and kids within the ages of 2 - 64 days were reported to be more susceptible to *T. congolense* than adults (Griffin and Allonby, 1979d). All these reports are indications that age factor and susceptibility vary from species to species

2.6.2.2. STRESS

This is said to compromise the resistance of domestic ruminants to trypanosomosis. This stress results from intercurrent diseases, pregnancy and parturition, lactation, long distance trekking, starvation and exhaustion (Murray and Trail, 1984). Well fed domestic ruminants tend to resist trypanosome infection more than those on poor or low planes of nutrition (Maclcanman, 1970; Anosa, 1983; Murray and Trail, 1984). Maclcanman (1970) also reported that well fed domestic animals, when exposed to high tsetse fly challenge, developed chronic or refractory infections. Maxie *et. al.* (1979) observed relationship between trypanosome infection and infection with haemoparasites, bacterial infection, abscesses in animals and severity of the infection.

According to Katunguka-Rwakishuga *et. al.* (1993a) although sheep on high plane nutrition though developed higher parasitaemia than those on low plane, both groups had similar degrees of anaemia, but erythropoetic activity was more pronounced in animals on higher plane. This animals also showed faster recovery rate post treatment as well as better live weight gain post recovery. Oyejide *et. al.* (1989) observed that WAD rams infected

with *T. vivax* but placed on high plane of nutrition developed subacute infection with minimal pulmonary complication compared to the acute severe disease coupled with bronchopneumonia in those on low plane nutrition. However, it was observed that level of nutrition had no significant effect on packed cell volume (PCV) neither plasma testosterone nor Immunoglobin (Ig) antibodies level. Pregnancy is another stress factor in trypanosomosis. Osborne and Kennedy (1981) reported that in such situations the foetus acts as stress factor and causes production of corticosteroids(from foetal adrenal glands). These steroids then act on the placenta to stop the production of gonadotrophic hormones and progesterone resulting in foetal death and abortion. Late pregnancy stress factor has been incriminated in abortion in buffaloes infected with *T. evansi* (Lohr *et al.*, 1986). Also, Ogwu and Nuhu(1981) and Ogwu(1986) reported that stressful effect of pregnancy led to either embryonic death or stillbirth in *T. vivax* infected pregnant heifers.

2.6.2.3. TRYPANOTOLERANCE

This is a phenomenom whereby certain breeds of domestic ruminants, some wild game species survive and become even productive without the aid of trypanocidal drugs in tsetse fly infested areas where other breeds cannot survive (Murray and Morrison, 1979). This phenomenom according to the authors has been found to be of tremendous economic benefit to livestock producers. The humpless and short horned breed of cattle namely the N'dama, Muturu and Keteku are said to be less susceptible to pathogenic diseases of trypanosomes than the West African Zebu cattle (Roberts and Gray, 1973). This feature is dependent on exposure of dam and new born calf to low grade trypanosome challenge,

continual exposure thereafter to the same antigenic types of trypanosome and the level of husbandry (Chandler, 1952; Desowitz, 1954; Fiennes, 1970a). According to Stewart (1951); Desowitz (1959) trypanotolerance depends on transfer of trypanosomal antibodies from infected dams to new born calves via colostrum, coupled with low prevalence of intercurrent disease and less migration.

Certain indigenous breeds of goats and sheep in East and West Africa, several wild life, some strains of in-bred mice [O57BI/6J and C57BI/IOJ] are also trypanotolerant (Ashroft *et. al.*, 1959; Morrison *et. al.*, 1978; Griffin and Allonby, 1979a, 1979c). Due to the constraint of African animal trypanosomosis to livestock development in Africa in general and Nigeria in particular and also as a result of the failure to prevent the disease and to control the vectors, coupled with the frustration encountered in vaccine production arising from antigenic variation, there is now a considerable interest in the exploitation of trypanotolerance as an alternative design for the control of animal trypanosomosis and livestock improvement in tsetse zones (Griffin and Allonby, 1979b; Trail *et. al.*, 1979). However, the N'dama without previous exposure to trypanosomes is said to become clinically ill on exposure to primary trypanosome infection with severe disease syndrome like stunting, wasting, infertility, abortion and even death (Murray and Trail, 1983).

With regard to sheep and goats and trypanotolerance, it has been established that certain breeds of sheep and goats exhibit this characteristic. The Galla breed of goat; some East African goats; the Blackhead Persian and Red Masai sheep show low grade clinical infection with *T. congolense* compared with the severe clinical signs exhibited by Saanen and Karakul sheep, with the former group even undergoing self cure. (Griffin and Allonby,

1979a,c,d). Also, Toure *et. al.* (1981) observed that Dwarf Djallonke sheep breed exhibited more resistance to experimental *T. congolense* infection whilst Sahelian Fulani sheep were highly susceptible with 60% mortality within 24 weeks of infection.

In Nigeria, the trypanotolerance of the indigenous breeds of small ruminants has not been assessed though the nature of the infection of trypanosomosis has been fairly carried out by Anosa and Isoun (1974; 1976; 1980), Ikede and Losos (1975), Saror (1980), Saror *et. al.* (1981), Kalu (1983) in Nigeria however, the trypanotolerance of the indigenous breeds of small ruminants has not been properly assessed.

Trypanotolerance in animals is determined by several factors. These include the following.

2.6.2.3.1. IMMUNOLOGICAL FACTORS

According to Desowitz (1959) previously exposed trypanotolerant N'dama cattle throw off trypanosome infection than susceptible Zebu cattle during subsequent challenge. This, according to the authors, is an indication that the trypanotolerant character in N'dama is due to its ability to mount a superior secondary immune response to the infection.

Trypanosoma brucei brucei infected Boran and Legune breeds of cattle were shown to be tolerant to their production of An Tat 1.1 specific trypanolytic antibodies which were produced in the first week post experimental infection and persisted in level throughout the period of observation (Doko *et al.*, 1997). Flynn and Sileghem (1993), observed immunosuppression in trypanotolerant N'dama cattle experimentally infected

with *T. congolense* and this they adduce to the presence of T-suppressor cells which acted in prostaglandin independant manner. They showed that the suppressor cells acted by arresting nitrogen-induced T-cell proliferation.

Immunological differences between breeds of cattle with regards to response to trypanosomosis has also been demonstrated. Okech, *et al.* (1996), observed that infection with *T. vivax* disrupted maintenance of preganancy in susceptible Galana Boran breed of cattle but not in the Orma Boran breed of cattle. This is an indication that the latter breed is more tolerant than the former breed to *T. vivax* infection. Pathogenic trypanosomes according to Twinamasiko and Kakaire (1994) may cause a delay in antibody response to rinderpest vaccine. This is possibly as a result of immunosuppression elicited by trypanosome infection.

Lutje *et al.*(1995), demonstrated that three years after trypanotolerant N'dama cattle were experimentally infected with *T. congolense*, high levels of memory T and B-cells were detected in the lymphoid organs though such has not been demonstrated in susceptible breeds. This could be a major factor in the ability of trypanotolerant breed of cattle or livestock to withstand trypanosome infection despite several exposures, a feature which is not found in the trypanosome susceptible livestock.

2.6.2.3.2. PHYSIOLOGICAL FACTORS

The anaemia in trypanotolerant N'dama is less severe in comparison to that of the susceptible Zebu in the course of infection and it is believed that N'dama mounts a

superior and effective erythropoietic response to trypanosome challenge than the Zebu, rapidly replacing destroyed or damaged erythrocytes (Murray *et. al.*, 1977). Dargie *et. al.* (1978) observed in their work on *T. congolense* and *T. brucei* infection in N'dama and Zebu cattle that anaemia in trypanosomosis is related to the level of parasitaemia, and with the N'dama being more able to control parasitaemia and thus more resistance to trypanosomosis. It is also believed that the skin colour and smell of the Zebu is more attractive to tsetse flies than that of the N'dama, whose skin is thought to be tougher for the penetration of tsetse flies proboscis in the course of feeding (Dargie *et. al.*, 1978). It is also believed, that phagocytic and complement systems are very active as to destroy most of the trypanosomes at the site of innoculation in N'dama cattle as it has a significantly higher level of leucocytes than in the Zebu (Murray and Trail, 1984).

2.6.2.3.3. RED BLOOD CELL SIALIC ACID CONCENTRATION

Esievo *et. al.* (1986), had reported that the concentratiom of rbc surface sialic acid in N'dama cattle is seven times that found on the surface of Zebu's erythrocyte.

Esievo *et. al.* (1990) also observed that both N'dama and Zebu cattle have rbc possessing sialic-acid coinciding with N-acetylneuraminic acid during polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate (SDS-PAGE). In addition the authors also observed that the N'dama rbc exhibited an extra band or type of sialic-acid of molecular weight of about 30,000, trailing behind the N-acetylneuraminic acid, though this was not observed in the rbc of Zebu. This extra band of sialic acid may be the reason behind the higher concentration of sialic acid in N'dama rbc and the therefore the relative

on the N'dama rbc surface. Thus with the reported resistance of O - acetylated sialic acid of quick hydrolysis by sialidase (Schauer *et. al.*, 1974; Voh *et. al.*, 1979) coupled with the fact that sialidase activity is related to level of parasitaemia (Esievo, 1983) and the observation that the severity of anaemia in bovine trypanosomosis is associated with the intensity and duration of parasitaemia (Dargie *et. al.*, 1978), it is then very clear that N'dama cattle will require a much higher level of parasitaemia in order to develop the same degree of anaemia as is observed commonly in the Zebu.

2.6.3. PATHOGENESIS OF TRYPANOSOMOSIS IN DOMESTICATED RUMINANTS

This is determined by factors which relate to the parasite and the host as these determine the incubation period (I.P.), level of parasitaemia, pyrexia, anaemia and the duration of the infection).

2.6.3.1. INCUBATION PERIOD

This refers to the period of time the parasite enters the host to the appearance of clinical signs. This according to Macleanan (1970), is determined by (mostly in experimental infection) the dosage or amount of parasite inoculated, the route of inoculation (infection), species of the parasite, mode of incubation of the parasite and also the plane of nutrition of the host.

Edwards *et al.*(1956a), observed an IP of 10 - 17 days in two groups of goats experimentally infected with *T. vivax* and *T. congolense* respectively via the intravenous

route. Saror (1979) reported and IP of 4 - 6 days in cattle experimentally infected with *T. congolense* via the intravenous route whilst an IP of 5 days were observed for both *T. vivax* and *T. congolense* in calves experimentally inoculated subcutaneously. An IP of between 9 - 20 days was obtained in *T. congolense* infected cattle through experimental tsetse fly feeding on the animals (Luckins, 1976).

Unsworth and Briskett (1952), reported an IP of 4 - 6 days in sheep exposed to laboratory bred *Glossina palpalis* infected with *T. vivax*. Also IP of 2 - 3 days was obtained for *T. vivax* in an experimental intrperitoneal infection of sheep (Saror, 1980). Kalu (1983) reported an i.p. of 7 - 8 days in *T congolense* infected West African Dwarf (WAD) sheep intravenously. Esievo *et. al.* (1982) obtained an i.p. of 2 - 3 days in calves intravenously infected with *T. vivax*. An an i.p. of 2 days was observed for *T.vivax* in experimentally and intrvenously infected Uda breeds of ram (Maikaje *et. al.*, 1991). Anosa and Isoun (1980) obtained i.p. of 6days for experimental *T. vivax*. infected splenectomised WAD sheep whilst an IP of 8 - 10 days was observed for *T. vivax* infected intact WAD sheep with both sets of sheep being inocuated via the intravenous. These results show relationship between IP and the species of parasite, route of infection, species and breed of the host and the immune status of the host.

2.6.3.2. PARASITAEMIA

This is considered mostly in terms of number of waves (occurrence), peaks (height), interval between peaks which are in turn are determined by the breed and susceptibility of

the host; species and strain of the trypanosome parasite (Maclennan, 1970; Stephen, 1970).

According to Desowitz (1959), Griffin and Allonby (1979b) and Murray *et. al.* (1977), low levels of parasitaemia are achieved by trypanotolerant domestic ruminants than the susceptible ones. Also, lower peaks of parasitaemia are often exhibited by asymptomatic domestic ruminants compared to the higher peaks shown by those with severe trypanosomosis (Anosa and Isoun, 1974; Maxie *et. al.*, 1979)

Edwards *et. al.* (1956a) and Ikede (1981) reported that *T. vivax* infections give higher parasitaemic peaks than those of *T. congolense*, with the highest *T. vivax* peaks occuring in the early phase of the infection. However, *T. brucei* is said to exhibit barely heavy parasitaemia The rise and fall in parasitaemic waves indicates the destruction of some members of each antigenic variant and the multiplication of each new one, and also, the number and heights of parasitaemic peaks correlate with the number and population sizes of antigenic variants in the course of an infection (Stephen, 1970).

2.6.3.3. PYREXIA

Trypanosomosis in domestic ruminants is characterised by intermittent pyrexia (Fiennes, 1970). Pyrexic peaks observed in *T. vivax*-infected cattle (Maxie *et. al.*, 1979) and sheep and goats (Anosa and Isoun, 1974; 1980) were observed to be related to increase in the number of circulating trypanosomes. Each period of pyrexia being followed by destruction of trypanosomes with consequent fall in body temperature.

However, Anosa and Isoun (1974) reported that the level of pyrexia was not always proportional to that of the parasitaemia. Pyrexia could also possibly be due to the toxic products of the trypanosome parasite to which the host's immune systme reacts to eliminate culminating in increase metabolic acitivities which cause increase in the body temperature of the host.

2.6.3.4. ANAEMIA

Anaemia, a prominent blood picture in trypanosomosis of domestic ruminants is a major discase promoting factor in trypanosomosis, with its pathogcncsis bcing multifactorial. Generally, it is agreed that the anaemia is haemolytic during acute trypanosomosis. According to Fiennes (1954), Mamo *et. al.*(1977), anaemia in trypanosomosis is haemolytic in nature especially in the acute form of trypanosomosis.

Several factors have been incriminated in the cause of anaemia in trypanosomosis this factors include:

2.6.3.4.1. HAEMODILUTION

This is said to be a major cause of trypanosoma anaemia in cattle (Finnes, 1954; Naylor, 1971; Maxie and Valli, 1978; Forsberg, 1979). These authors have also reported increase in blood volume in animal trypanosomosis although Clarkson (1968) did not support this in his earlier work. Anosa and Isoun (1976) also observed haemodilution in *T. vivax* infected sheep and goats. Similarly, Holmes and Jennings (1976), reported haemodilution in *T. brucei* and *T. congolense* infected rabbits. The mechanism of

haemodilution is not well understood presently and as such its part in anaemia development is questionable. Maxie and Valli (1978), opined that this phenomenun could be a factor in the development of anaemia provided plasma volume increases out of proportion to the drop in red blood cell volume. According to Dargie (1978) haemodilution is not a characteristic feature of animal trypanosomosis, he explained that the radioactive Fe^{59} used by other investigators to measure plasma volume did not equilibrate with the plasma space and therefore seriously over estimated the plasma volume of anaemic animals.

2.6.3.4.2. HAEMATOPOETIC RESPONSE

Failure of bone marrow in its red blood cell production activity has been noted as a prominent factor in anaemia of trypanosomosis (Fiennes, 1954; Losos and Ikede, 1972) Although there seems to be indication of increased erythropoeisis evidence by the presence of erythroid hyperplasia in rat bone marrow (Anosa *et al.*, 1970), increase in red bone marrow quantity in sheep (Anosa and Isoun, 1980a) and the formation of erythroblast island in the spleen of mice (Anosa and Kaneko, 1983b) in trypanosomosis the regenerative response is not as much as the rbc destruction taking place (Dargie *et al.*, 1979). According to Murray (1974), and Dargie (1978) blocking of iron transfer to bone marrow by the mononuclear phagocytic system (MPS) causes none availability of iron for further haemoglobin formation post erythrophagocytosis. This therefore could be a contributory factor in the bone marrow inadequacy coupled with its degeneration in the chronic stage of the trypanosomosis. On the other hand, active erythropoeisis have been reported in *T. brucei* infected rabbits (Boreham, 1966) and in *T. congolense* infected

rabbits (Holmes and Jennings, 1976); rats infected with *T. brucei* (Jennings *et. al.*, 1974; Murray *et. al.*, 1974) and with *T. evansi* (Assoku, 1975; *T. congolense*-infected sheep (Mackenzie and Cruick-shank, 1973), *T. vivax* infected goats (Saror, 1980) and cattle (Van den Ingh *et. al.*, 1976). These varying findings tends to show that anaemia in trypanosomosis may or may not be due to depressed bone marrow activity.

2.6.3.4.3. ERYTHROPHAGOCYTOSIS

This is another major contributory factor in the development of the anaemia of animal trypanosomosis. It is well documented in *T. brucei*-infected rabbits (Boycott and Price-Jones, 1913) and rats and mice (Jennings *et. al.*, 1974; Murray *et. al.*, 1974; Anosa and Kaneko, 1983), *T. congolense*-infected sheep (Mackenzie and Cruickshank, 1973), *T. vivax* -infected goats (Saror, 1980) and *T. congolense*-infected cattle (Fiennes, 1954; Naylor, 1971; Losos *et. al.*, 1973; Valli and Forsberg, 1979). The phenomenom is brought about by several causes which include the attachment of trypanosome antigen to red blood cells surface leading to combination with antitrypanosome antibody in the plasma and consequent erythrophagocytosis (Jennings *et. al.*, 1974).

With the help of Indirect Flourescent Antibody Test (IFAT), Mackenzie *et. al.* (1978) were able to show trypanosome antigenes on sheep red cells and indication that the phagocytosis of erythrocytes in sheep infected with *T. congolense* was due to the coating of the surface of red blood cells with trypanosomal antigen.

Trypanosome enzyme sialidase (neuraminidase) produced by *T. vivax* (Esievo, 1979) has been incriminated as a factor in erythrophagocytosis; during bovine trypanosomosis, sialic (neuraminic) acid is cleared off the surface of erythrocytes by the enzyme (Esievo *et. al.*, 1982) thus exposing new antigenic sites on the surface of affected red blood cells. Antibody might be produced against these new antigenic sites on the red cells during trypanosomosis, rendering such cells vulnerable to erythrophagocytosis.

Red cells from *T. vivax* infected cattle did not show any membrane abnormalities via electron microscope scanning (Esievo, Moore and Miller, 1982 Unpublished). Also Jancik *et. al.* (1978) using scanning electron microscope, did not reveal any change in the shape of rabbit red blood cells treated with sialidase. However, Anosa and Kaneko (1983a) using same scanning electron microscopy, showed alterations on red blood cells of deer mice with *T. brucei* infection.

With regard to the uptake (removal) of targeted erythrocytes by the liver and peritoneal macrophages, Kuster and Schauer (1981) observed that rat peritoneal macrophages bound homologous sialidase treated erythrocytes through a b-D-Galactose specific lectin on the macrophage surface for phagocytosis. According to Muller *et. al.* (1983) the amount of sialic acid released decides the binding and phagocytosis of sialidase treated erythrocytes. with a requirement for factors of the complement system and later, according to are phogocytosed as a function of their number and binding strength to the macrophages.

2.6.3.4. IMMUNOGLOBULINS AND COMPLEMENT IN ANAEMIA OF TRYPANOSOMOSIS

The immune response elicited by trypanosomes plays a major role in the cause of anaemia (Balber, 1974). According to Assoku (1975), rats infected with *T. evansi* exhibited significant immune response to the parasites, developing peak agglutinin titres 10 - 12 days post infection and coinciding with destruction of red blood cells.

The view that the development of anaemia following *T. evansi* infection is immunologically mediated is supported by the observation of trypanosome anti-body-complement complexes on the surfaces of red cells of *T. congolense*-infected calves. This leads to the elimination of affected red cells and hence the resultant clinical anaemia (Kobayashi *et. al.*, 1976); increases in levels of IgM during trypanosomosis have been reported by Clarkson *et al.*(1975), Kobayashi and Tizard (1976), Moulton and Sollod (1979), Tabel *et. al.* (1978). Immunoglobulins and complement may be involved in opsonizing desialylated erythrocytes (Jancik *et. al.*, 1978; Kuster and Schauer, 1981; Muller and Schauer, 1983) produced in acute bovine trypanosomosis (Esievo, 1979; Esievo *et. al.*, 1982) with resultant erythrophagocytosis. Also, Woodruff *et al.* (1973), observed the presence of immunoglobulins and complements capable of lysing erythrocytes in some patients infected with *T. brucei rhodensiense*; Similar observations were also recorded in *T. congolense* and *T. brucei* infected mice (Ikede *et al.*, 1976), *T. congolense* infected calves (Kobayashi *et al.*, 1979), *T. vivax* infected bovine (Facer *et al.*, 1982). These immunoglobulins on attaching to erythrocyte membranes tend to render the rbc

AHMADU BELLO UNIVERSITY
KASHIM IBRAHIM

highly susceptible to phagocytosis by macophages, whereas the complements have cytotoxic agents to the rbc.

2.6.3.4.5. DIRECT EFFECT OF TRYPANOSOMES

Jennings *et. al.* (1974) reported anaemia in *T. brucei*-infected mice 4 days p.i. and attributed the anaemia to damage on the erythrocytes due to the direct or indirect effects of the trypanosomes and not due to immunological action which could have been significant only after day 7 p.i., when the host might have mounted an immunoresponse. In situations of very high parasitaemia, injury may be inflicted on the red blood cells due to the lashing action of the trypanosomes (Wintrobe, 1975). Also, the life span of the erythrocytes are said to be reduced when coated with trypanosome material post trypanolysis as observed in *T. congolense* infected sheep (Mackenzie and Cruikshank, 1973; Murray, 1974) and in *T. evantsi* infected rats (Assoku, 1975). Anosa and Kaneko (1973a), observed many tiny foci of discontinuety on rbc membrane at points of contact between trypanosomes and rbc in *T. brucei* infected mice and which tended to expose these cells to easy phagocytosis by macrophages.

2.6.3.4.6. HAEMOLYTIC FACTORS IN ANAEMIA

Fiennes (1954) observed haemolysis of erythrocytes from normal bovine blood when being mixed with plasma from trypanosome-infected cattle, he therefore suggested the presence of haemolytic factors in the blood of trypanosome-infected animals. The factors

were observed to be present intermittently with its highest occurrence (concentration) at the time of trypanolysis.

One factor, a protein with small molecular weight has been isolated, characterised and purified and found to be produced during major haemolytic changes in the course of an experimental *T. brucei*-infection in cattle, though its role in the anaemia was not determined (Murray *et. al.*, 1977). According to Tizard *et. al.* (1978a), *T. congolense* on autolysis produces a lipid, which behaves like a very potent cytotoxic and haemolytic factor. According to the aauthors the activity of this lipid depends on the free fatty acids released by phospholipase A from the host's phospholy-dylcholine. The authors also observed that lysolecithin was also released and enhanced the activity of the haemolytic factor. The haemolytic factor possessed by *T. brucei* is not as effective as that of *T. congolense* due to its low level of phospholipase A and hence the free fatty acids produced (Tizard *et. al.*, 1978a). Thus, it is now generally believed that *T. congolense* and *T. brucei* possess similar mechanisms for the production of free fatty acids from endogenous phosphotidyl choline by phospholipase A, and the haemolytic factor in *T. brucei* observed by Nguyen Huan *et al.* (1975) may possibly be a phospholipase A (Tizard *et. al.*, 1978a). Huan *et al.* (1975(observed that *T. brucei* produced a protein of molecular weight of 10,000 daltons capable of lysing red blood cells. This could possibly take place when red blood cells adhere to trypanosomes and or post trypanosome lysis. This haemolytic activity post trypanosome lysis is also possesed by *T. vivax*, *T. brucei gambiense* and *T.*

brucei rhodensiense ((Murray *et al.*, 1977). Accoding to Tizard *et. al.* (1977; 1978a), *T. lewisi* is non-haemolytic due to its lack of phospholipase A.

2.6.3.4.7. REDUCED ERYTHROCYTE LIFE SPAN

Mamo *et al.* (1977) reported that the half-life of Cr^{51}-labelled erythrocytes was 216 ± 47 days in control Zebu calves and 142 ± 33 days in *T. congolense*-infected calves, with the latter group developing severe anaemia. This is an indication that the reduced life span produced a reduction in the level of erythrocytes in the infected calves thereby giving rise to the anaemia. Similarly, Valli e*t al.* (1979), observed a drop in the half-life of Cr^{51}-labelled erythrocytes in *T. congolense* infected calves from 10 days to 4.7 days after 30 days post infection. This was followed by gradual return to normal half-life 12 days later. This reduction could be due to direct effect of trypanosomes on the erythrocytes.

Though the mechanism of erythrocyte life-span reduction is not well understood. Jancik and Schauer (1974), Jancik *et. al.* (1975) and Burocher *et. al.* (1975) had observed that partial removal of rbc surface sialic acid leads to rapid disappearance of the cells from the circulation in rabbit, rat and man. According to Jancik *et. al.* (1978) sialidase treatment leads to a rapid disappearance ^{51}Cr-labelled bovine rbc from the circulation, with mean half-life of 3 hours for neuraminidase treated and 21 days for control rbc respectively.

The role of erythrocyte sialic acids in the development of anemia in animal trypanosomosis is evidenced by the attachment of *T. congolense* to bovine erythrocytes through sialic acid receptors on the red cells and their consequent injuries (Banks, 1979;

1980). Other evidences include the production of sialidase by *T. vivax* (Esievo, 1979), the loss of rbc surface sialic acids and development of anaemia at the peak of parasitaemia (Esievo *et. al.*, 1982).

2.6.4. MORPHOLOGICAL CLASSIFICATION OF TRYPANOSOMAL ANAEMIA

In terms of the morphology of the erythrocytes in the ensuing anaemia of animal trypanosomosis, Roberts and Gray (1973) reported that single or mixed infection of cattle with *T. vivax, T. congolense* and *T. brucei* produced macrocytic-normochromic type of anaemia in affected cattle. However Saror (1979) observed normocytic normochromic anaemia with tendency towards macrocytic-normochromic in *T. vivax* infected cattle. Anosa and Isoun (1980) and Maxie *et. al.*, (1974) reported macrocytic normochromic anaemia in *T. vivax*-infected sheep and goats which later tended towards normocytic normochromic in the chronic stage of the infection. Whilst *T. vivax* or *T. congolense*-infected cattle on the other hand produced macrocytic-normochromic anaemia (Maxie *et. al.*, 1974). However, Naylor (1971) had earlier reported that the anaemia due to *T. congolense* is initially macrocytic normochromic in the acute stage but later becomes normocytic normochromic. According to Mamo and Holmes (1975) the severe normocytic normochromic anaemia of *T. congolense* infection is due to loss of rbc from circulation and not as a result of decreased erythropoeisis. These various classification are due to the effect of trypanosome on erythrocyte volume and haemoglobin concentration possibly as a result of the infection on the host erythropoeitic activity.

2.6.5. LEUCOCYTE RESPONSE IN TRYPANOSOMOSIS

Cattle infected with *T. congolense* or *T. vivax* exhibit leukopenia which tends to coincide with the onset of anaemia (Fiennes *et. al.*, 1946; Maxie *et. al.*, 1978) which is soon followed by leucocytosis. The early leucopenia is believed to be due to massive leucophagocytosis due to coating of the leucocytes by trypanosomal antigen (Mackenzie *et. al.*, 1978) or due to loss of stem cells as a result of circulating toxic factor from the trypanosome (Kaaya *et. al.*, 1978).

Naylor (1971) and Wellde *et. al.* (1974) observed that *T. congolense*-infected animals remain leucopenic, whereas in *T. vivax* animals, mean leucocyte count increased after transient leucopenia, and this, according to Maxie *et. al.* (1974) may indicate varying response of leucocytes to pathogenesis in *T. congolense* and *T. vivax* infections. Also leucocytes may be attracted to trypanosomes localised in the microcirculation in *T. congolense*-infected animals, thus ceasing to circulate (Maxie *et. al.*, 1974) or that bone marrow erythropoiesis may lay too much emphasis on erythropoeisis at the expense of granulopoeisis thereby resulting in low leucocyte population (Maxie *et. al.*, 1974).

Also in *T. congolense*-infected cattle, there were neutropenia, lymphocytopenia and eosinopenia, no change in the monocyte level, with basophils tending to disappear in the early stage of the infection (Naylor, 1971). Maxie *et. al.* (1979) in a further investigation reported that leucopaenia was more prominent in *T. congolense* than in *T. vivax* infected cattle.

Losos *et. al.* (1973), also observed lymphopenia and neutropenia in *T. congolense* infected cattle and leucopenia in *T. brucei*-infected mice. The authors also observed predominance of immature forms of these granulocytes in the *T.* congolense infected cattle this shows rapid destruction of mature leucocytes which is an indication of severe breakdown in the host defence mechanism. Edwards *et. al.* (1956) had earlier on reported leucopenia in the acute stage but later leucocytosis in trypanosomosis of sheep and goats. This observation points to the depresssion of the host defence mechanism in the early stage of the infection and with attempt at recovery in the chronic stage

Anosa (1977), Anosa and Isoun (1980) observed leucopenia due to lymphopenia and neutropenia during crisis in *T. vivax*-infected sheep and goats. These authors attributed the lymphopenia to depopulation of the lymphoid nodules and transformation of lymphocytes to morula cells, whilst the neutropenia resulted from marrow depression. According to Valli *et. al.* (1979) neutropenia promotes acute death in bovine trypanosomosis. This can be as a result of reduced phagocytic activity against the trypanosomes as part of the host defence mechanims against the infection. Esievo (1983) reported that plasma neuraminidase (sialidase) released by trypanosomes may influence the early leucopenia by removing sialic acid from leucocytes which later get diverted from the lymph nodes and spleen to the liver for destruction . Schauer *et al.* (1984), also observed that lymphocytes rapidly disappear from circulation in animals administered with sialidase. This could have been due to the cleavage of sialic acid from the lymphocytes and their resutant removal from circulation as earlier on explained by Esievo(1983).

attributed to an osmotic effect due to high serum protein. Low serum protein levels have been reported in *T.congolense* infected cattle (Tabel *et. al.*, 1978; Wellde *et. al.*, 1978), *T. rhodesiense*-infected monkeys (Sadun *et. al.*,1973). On the other hand, high serum protein levels were observed in *T. brucei* infected rabbits (Facer,1976), *T. vivax*-infected sheep and goats (Clarkson, 1968), *T. brucei*-infected WAD sheep (Ogunsamin *et. al.*, 1994). On the other hand, *T. brucei*-infected dogs exhibited no significant chang in the total plasma protein level, whilst *T. rhodesiense* infected monkeys did not exhibit changes in their normal values (Smithers and Terry, 1959), and mice (Moon *et. al.*, 1968), *T. evansi* infection of buffalo calves (Singh and Gaur, 1983; Verma and Gautam, 1977), calves (Verma and Gautam, 1983) camels (Jaktar *et. al.*, 1973) and *T. congolense*-infected mice (Whitelaw *et. al.*, 1980). Anosa and Kaneko (1983a) reported that in two groups of deer mice infected with *T. brucei*, one of the groups became anaemic but had normal total protein levels, whereas the other group though did not develop anaemia however produced elevated serum protein levels.

The decrease in albumin levels in the first week post infection (p.i.) in *T. vivax*-infected sheep and goats (Anosa and Isoun, 1976) was suggestive of serum extravasation due to vasoactive substances, increased protein breakdown and loss by proteinuria, and increase during the fifth week post infection manifested the recovery of the infected goats from the infection and also the clearance of the parasite from the host.

Mackenzie *et. al.* (1975) reported increased serum albumin which coincided with the peak of parasitaemia.

2.6.7.2. BILIRUBINS

Saror (1981) observed that jaundice is not a feature of animal trypanosomosis, neither is bilirubinaemia nor bilirubinuria, even in very acute cases. Similarly, normal levels were observed in *T. brucei* infected rabbits (Jenkins *et. al.*, 1980), *T. rhodesiense*-infected mice, monkeys and man (Moon *et. al.*, 1968; Sadun *et. al.*, 1973 and Barret *et. al.*, 1973) respectively, all in the presence of erythrocliasis. Zwamer and Culbertson (1939) observed bilirubinaemia in cattle with acute *T. congolense*, but normal levels in the chronic phase of the infection.

Increase in bilirubin levels have been reported in *T. congolense*-infected cattle (Fiennes, 1954; 1970; Naylor, 1971; Wellde *et. al.*, 1974; 1983), *T. vivax*-infected cattle (Fiennes, 1954; 1970), *T. brucei*-infected rabbits (Jenkins *et. al.*, 1974), horses (Neitz and Macauley, 1971) and dogs (Otnotainse *et. al.*, 1991) and in *T. vivax* infected Savanna Brown (SB) goats (Igbokwe and Mohammed, 1992).

2.6.7.3. BLOOD SUGAR

Measurement of blood sugar levels in trypanosomosis has not been extensively investigated. Edwards *et. al.* (1956) observed no change in blood sugar levels in *T. vivax*-infected goats. Van Den Ingh et. al (1976) reported slight increases in blood sugar levels

and also in lactic and pyruvic acid levels in *T. vivax*-infected cattle and this they attributed most likely to its utilisation by the trypanosome parasites.

Roberts (1975) suggested that changes in glucose level were due to the effect of biochemical reactions at the molecular levels of trypanosomes in the liver of infected animals whilst blood stream trypanosomes are also said to utilise the blood glucose of hosts with resultant fall in plasma glucose levels.

Hypoglycemia has also been reported in acute *T. vivax* and *T. congolense* infection in cattle (Wellde *et. al.,* 1973), in chronic *T. rhodesiense* infection in cattle (Wilde and French, 1945) and man (Barret *et. al.*, 1973), in acute *T. evansi* infection of buffalo calves coupled with fluctuating levels in the chronic stage (Singh and Gaur, 1983) and in peracute *T. simiae* infection in pigs (Singh and Gaur, 1983).

2.6.7.4. UREA

T. congolense-infected cattle produced high level of urea at peak parasitaemia, but levels returned to normal in the chronic phase (stage) of infection (Wellde *et. al.*, 1974). Terminal case of severe *T. vivax* infection in a cow produced high levels of urea in the blood (Van Den Ingh *et. al.*, 1976). Similar high levels of urea were observed in *T. rhodesiense* infected monkeys (Sadun *et. al.*, 1973) and mice (Moon *et. al.*, 1968) and in *T. brucei*-infected dogs (Omotainse *et. al.*, 1994). As urea (blood urea nitrogen or BUN) is a by-product of protein catabolism, the increase in serum urea levels in cases of trypanosomosis indicate excessive protein breakdown due to trypanosome infection.

2.6.7.5. S G O T LEVELS IN TRYPANOSOMOSIS

High levels of SGOT have been observed in *T. rhodesiense*-infected mice (Moon *et. al.*, 1968), man (Barret-Conner *et. al.*, 1973) and monkeys (Sadun *et. al.*, 1973). Wellde *et. al.*, (1983) and Gary (1963) reported slight increase in SGOT levels in *T. vivax*-infected cattle and sheep. Adah *et. al.* (1992) reported increased levels of SGOT in *T. congolense* infected Red Sokoto (RS) and WAD goats with the levels being higher in the former than in the latter.

Specific organ (liver, heart, skeletal muscles) damage and also parasitic metabolic products are said to be responsible for increase in levels of SGOT and other serum enzymes in trypanosomosis. However in situations where marked increase in serum enzymes are observed, especially within the first 8 days of infection the most possible sources of enzymes will be the parasites as the period may be too short for significant organ damage (Moon *et. al.*, 1968; Whitelaw *et. al.*, 1980). However, highly pathogenic and tissue invasive trypanosomes like *T. brucei* may be able to produce tissue (i.e. liver, cardiac and skeletal muscles) damage early in the infection and hence gives rise to high SGOT levels.

2.6.7.6. POTASIUM ION (K^+)

Ikejani (1946a;b), Zwemer and Gulbertson (1939) observed increased levels of K^+ in *T.brucei* and *T. equipardum* infections in rats. Normal levels were reported in *T.*

rhodesiense-infected mice (Moon *et. al.*, 1968) and low levels in *T. evansi*-infected camels (Raisinghani *et. al.*, 1981)

According to Ikejiani (1946a, b) rbc destruction, damaged tissues, kidneys provide the extra increase in K^+ levels, with most increased levels of K^+ correlating with fall in rbc levels.

2.6.7.7. SODIUM ION (Na^+)

T. rhodesiense-infected human (Barret-Conner *et. al.*, 1973) and *T. evansi* infected mice (Raisinghani *et. al.*, 1981) produce low levels of Na^+. Normal Na^+ levels were observed in acute *T. rhodesiense* infection in mice (Moon *et. al.*, 1968). On the other hand, Fiennes *et. al.* (1946) observed high levels of Na^+ in *T. congolense*-infected cattle. The Na^+ decrease suggests its removal possibly by leaving the plasma partly intracellularly among other serum components.

2.6.7.8. CHLORINE ION (Cl^-)

Ogunsammi *et. al.* (1994) observed increased serum chloride concentration in *T. brucei* infected WAD sheep. Movement of serum Na^+ is always followed by a corresponding passive moevement by Cl^- in the same direction. Though Fiennes *et al.* (1946), Moon *et al.*(1968), Barret-Conner *et al.* (1973) and Raisingham *et al.* (1981) did not comment about the Cl^- level in their observations on Na^+ level, it is assumed that

whatever variations serum Na^+ level went through similar fate befell the Cl^- level.

2.6.7.9. BICARBONATE ION (HCO3-)

T. brucei-infected WAD sheep produce high levels of serum HCO3- (Ogunsammi et. al, 1994).

2.6.8. ORGAN PATHOLOGIES (GROSS AND MICROSCOPIC.)

2.6.8.1. EDEMA

Ikede and Losos (1972) observed that affected cattle developed subcutaneous edema of about 2cm thick;serous effusion into the peritoneal and pericardial cavities of 1 litre and 10 millilitres respectively; edematous lymph nodes three times the normal size and complete opacity of the entire right cornea.

2.6.8.2. LYMPHOID ORGANS (SPLEEN, LYMPH NODES AND THYMUS)

According to Moulton and Sollod (1976) the most significant pathological finding in experimental *T. brucei* infection of calves were generalised enlargement (usually ½ to 2 times) of the lymph nodes (except the mesenteric lymph nodes); the spleen where the lymph follicles were prominent and the red pulp very firm.

Fiennes (1954) demonstrated haemosiderosis of the spleen, hyperplasia of the lymph nodes and spleen in *T. congolense* and *T. vivax*-infected cattle. Splenic necrosis was observed in *T. vivax*-infected cattle (Van Den Ingh *et. al.*, 1976). Anosa and Isoun (1974; 1980b), reported haemosiderosis in lymph nodes of sheep and goats suffering from

trypanosomosis. Moulton and Sollod (1976) observed splenic hyperplasia in *T. brucei* infected calves.

2.6.8.3. REPRODUCTIVE ORGANS

Isoun and Anosa (1974) and Anosa and Isoun (1980) observed testicular atrophy in *T. vivax* infected sheep and goats. Similar findings were made in *T. brucei*- infected rams by Ikede (1979). Histollogically, Isoun and Anosa (1974) reported sperm abnormalities, testicular calcification in *T. vivax*-infected sheep and goats. Sekoni (1992) reported severe sperm abnormalities in *T. vivax* infected Yankasa rams. Similarly, Ikede (1979) observed in *T. brucei* infection of WAD rams testicular calcification and sclerosis.

Sekoni (1987) reported that testicular degeneration was more severe in *T. congolense*-infected than *T. vivax*-infected bulls, same for the epididymes, seminal-vesicles and the prostrate glands.

It was observed by El-Hassan (1987) that *T. vivax* infected pregnant WAD ewes died with or without abortion, premature birth, stillbirth and neonatal death whilst non-pregnant but *T. vivax* infected WAD ewes experienced disrupted oestrus cycle. The author attributed the former observation to stress caused by pyrexia, intrauterine infection and anorexia; and the latter to alterations in metabolism body conditions and hypothalamo - pituitary axis which therefore affected the endocrine mechanism regulating ovarian activity.

Ikede and Losos (1972) reported *T. vivax* in a lamb whilst Isoun and Anosa (1974) and Ogwu (1983) observed *T. vivax* in the amniotic fluid of an infected pregnant ewe and in calves of infected heifers respectively. Abortion was also reported in *T. vivax* infected cows (Hornby, 1921; Pigneur, 1932); *T. congolense* infected cows (Kramptz, 1970); in *T. evantsi* infected cows (Paikine and Dhake, 1972) and *T. vivax* infected ewes (Ikede and Losos, 1972). All these observations point to intrauterine infection.

2.6.8.4. LIVER, GALL-BLADDER, KIDNEY

Van Den Ingh *et. al.* (1976) observed significant liver necrosis, granulation formation in the liver in *T. vivax*-infected cattle. Moulton and Sollod (1976) reported that in *T. brucei*-infected calves, the liver showed accumulation of edema fluid and mononuclear cells around portal blood vessels in moderate cases.

Edward *et. al.* (1956) reported distended gall-bladder filled with dark and oily bile fluids in *T. vivax*, *T. brucei* and *T. congolense* infected sheep and goats. However, Moulton and Sollod (1976) did not observe any gall-bladder abnormality in *T. brucei*-infected calves with the exception of slight hyperplasia of bile duct epithelium in severely affected liver. Fiennes (1954) observed mild nephrosis. Haemosiderosis were observed in liver of sheep and goat suffering from trypanosomosis as well as capillary congestion and distension of hepatic sinusoids (Anosa and Isoun, 1974; 1980).

2.6.8.5. OTHER BODY ORGANS; BODY FLUIDS

Fiennes (1954), reported haemosiderosis in *T. vivax* and *T. congolense*-infected cattle. Whilst Mackenzie and Cruickshank (1973) observed reticulosis and increased erythrophagocytosis in the acute phase *T. congolense*- infection in sheep. The erythrophagocytosis arose from increased damage to rbc by either the direct effect of the trypanosomes or their secretions like enzyme sialidase whilst the increase reticulosis was attempt by the host to rapidly replace the damage rbc.

Van Den Ingh *et. al.* (1976) reported that microscopic findings in *T. vivax*-infected cattle included microthrombi in the blood vessels coupled with skeletal and cardiac muscle degeneration, granuloma formation in the heart, lymphoid hyperplasia with localised erythropoiesis.

Ikede and Losos (1975) reported significant microscopic lesions notably interstitial and perivascular mononuclear cell infiltration associated with extravascular localization of trypanosomes in the heart, brain and eyes in *T. brucei*-infected sheeep, with the cardiac changes being more extensive in the arterial walls and made up of diffuse and focal infiltration of lymphocytes, histocytes and trypanosomes (though trypanosomes were not seen in the blood vessels of both the epicardium and endocardiac).

Moulton and Sollod (1976) did not observe microscopic changes in the cervical, thoracic and lumbar spinal cords; small intestines, colons, caecum, forestomach, abomasum, thyroid gland, oesophagus, eyes, tongue, oropharynx, thymus and skin in *T. brucei*-infected calves.

Ikede and Losos (1975), working on *T. brucei*-infected sheep observed trypanosomes in smears of pericardial, peritoneal and cerebrospinal fluids and the aqueous humour of the eye, with other body fluids being infective when inoculated into rats.

2.7.1. THE ROLES OF SIALIDASE (NEURAMINIDASE) AND SIALIC ACID (NEURAMINIC ACID) IN TRYPANOSOMOSIS

2.7.1.1. SIALIDASES

Sialidases (Neuraminidases EC 3.2.1.18) are enzymes belonging to a class of glycerol hydrolases which release N-acylneuraminate residues from glyco-proteins, glyco-lipids and polysaccharides with their efficiency depending on

i enzyme sources

ii nature of the glycosidic linkage

iii type of substrate

These enzymes are common in animals from Echinodermata through mammalian and other micro organisms which exist as commensals or facultative pathogens to animals (Engstler and Schauer, 1993). Gottschalk (1957) cited by Engstler and Schauer (1993) proposed the term "Neuraminidase" for sialidase on observing that N-acetylneuraminic acid results when bacterial and viral extracts cleave submaxillary gland mucins and other glycoprotein substrates.

The investigation of Hist (1941) about the agglutination of red blood cells by influenza-virus led to the understanding of sialic acid structure and the direct interraction between viral haemagglutination and cell associated sialiglycoconjugates. Formerly, the

specific role of sialidases were unknown, but presently the action of trypanosomal sialidases is tending to provide the opportunity of assessing the pathophysiological role of sialidases (Engstler and Schauer, 1993).

Roggentin *et. al.* (1993) reported that though little is known about the sialidases at the molecular level, the rate of sialic acid cleavage from receptive substrate is a property specific to each sialidase and depends on three main factors namely:

a. The type of ketosidic linkage
b. The kind of sialic acid derivative
c. Nature of the penultimate sugar residues

2.7.1.2. SIALIC-ACID

Sialic (Neuraminic) acids are from a family of carboxylated saccharides with nine carbon atoms (Schauer, 1978; Veh, 1979). Sialic-acid is the acylated neuraminic acid which according to Seamon and Uhlenbruck (1963) occurs in appreciable level on the erythrocyte surface in several animal species. Durocher *et. al.* (1975) reported that the rapid clearance of ^{51}Cr labelled erythrocytes (rbc) desialylated with *Vibrio cholera* neuraminidase from the circulation of rats and rabbits with sequestration by the liver led to the opinion that reduction in rbc sialic acid is a mechanism of rbc senescence and destruction. According to Magaji (1975) *T. vivax* and *T. brucei* infected cattle were observed to show elevated serum sialic acid level.

Natural sialidase substrates are generally terminally bound sialic-acids which are derivatives of the 9 carbon acidic sugar neuraminic-acid, and because of the variation of substituents at carbon-4, 5, 7, 8 and 9 positions, more than 30 naturally occuring sialic-

acids have been described (Faillard, 1981). N-acetyl neuraminic-acid (Neu-5-AC) is the biosynthetic precursor for all sialic acids.

Being components of soluble or cell-bound glycoconjugates, sialic acid are involved in a variety of biological events, especially as regulators of molecular and cellular interractions (Engstler and Schauer, 1993).

According to Reuter *et. al.* (1983) and Schauer *et. al.* (1984) the protective nature of rates of hydrolysis of sialic-acids by sialidases depend on the type and concentration of side groups (O-acetyl : glycol).

2.7.2. SIALIDASE : SIALIC ACID AND TRYPANOSOMES

A lot of detailed study has been carried out on the biochemistry, molecular biology and physiological significance of the surface sialidase from *T. cruzi*, the causative agent of American trypanosomosis- "chagas". However, the sialidase of African pathogenic trypanosomes has not received such attention despite the attempts of Esievo (1979; 1980) on *T. vivax* and Engstler and Schauer (1993) on *T. vivax* and *T. brucei* sialidases.

According to Esievo (1979; 1980) *T. vivax* hydrolyses sialic-acid *in vitro*, and that neuraminidase activity was proportional to the concentration of trypanosomes. He therefore postulated that if this could occur *in vitro*, then sialic acid could be cleared off erythrocytes, resulting in their being removed from circulation and leading to anaemia.

T. cruzi is highly cell invasive, and is also able to escape the host's immune response and effected therapeutic treatment. On the other hand, *T. brucei, T. congolense* and *T.*

vivax use intermittent antigenic variants which are able to invade or penetrate cell layer of vectors and final hosts and also use limitless antigenic variation to evade the mammalian host's immune response (Engstler and Schauer, 1993) and that early anaemia observed was possibly due to the circulating trypanosomes producing sialidase to cleave off surface sialic acids, thus rendering the rbc more vulnerable to phagocytosis by the reticulo-endothelial system.

In *T. brucei*, sialidase has been described in procyclic trypamastigotes forms but the mammalian blood stream forms show no enzyme activity. This sialidase is reported to be a cell surface protein linked to the membrane via a modified glycosylthosphatidyl inositol (C.P.I.) anchor which probably is identical to the anchor of the procyclic acidic repetative protein (PARP) which coats the surface of procyclic trypanosomes (Engstler and Schauer, 1993). It was also observed that conventional sialidase inhibitors like Neu 5 AC 2 en and

N. (4-trophonyl) oxalic acid lack significant influence over this enzyme which is also said to be activated by di and tri carboxylic acids and some saccharides especially lactose. The enzyme is reported to prefer b(2-)-glycosidic linkages than a(2-6) and (2-8) linked terminal sialic acids, with oligosaccharides acting as better substrates than either glycoprotein or gangliosides (Engstler and Schauer, 1993).

T. brucei, compared to other species of African trypanosomes stay in the tsetsefly vector for more than 5 weeks undergoing division as large procyclic trypamastigotes, then penetrate the peritrophic membrane and later migrate via the proventriculus to the salivary glands; whereas *T. vivax* completely by passes the tsetsefly's midgut region and leaving the insect only after ten days (Vickerman, 1978). It may be said that too much of the parasite's

sialidase is used up in its insect stage form thus having insufficient amount ot elicit any activity in the blood stream form of *T. brucei.* The parasite's insect stage sialidase is essential for the cleavage of sialic acid from host blood ingested by the vector because glycoproteins are easily digested when decoid of sialic acid.

According to Engstler *et. al.* (1993) transialidase of *T. brucei* bears close resemblence to that of *T. cruzi* which is capable of transfering sialic-acids from a variety of sialo-glycoconjugates, to a sialo-acceptor molecule, but that of *T. brucei* is found to be lacking the key enzymes of sialic-acid metabolism. However, it is believed that the major functions of *T. brucei* trans-sialidase is to enable the procyclic *T. brucei* to sialylate parasite glycoconjugates in order to protect surface molecules from glycosidases in the digestive tract of the tsetsefly (Engstler and Schauer,1993).

It is not yet established that *T. congolense*, another highly pathogenic trypanosome has sialidase enzyme either in the insect form (as it occurs in *T. brucei*) or the mammalian blood stream form (as it occurs in *T. vivax*). Sialic acid has been observed on the surface of *T. congolense* is in the mammalian blood stream (Rautenberg *et. al.*, 1981). This is similar to reported presence of sialic acid of the American human trypanosome *T. cruzi*, the cause of Chagas disease (Schauer *et. al.*, 1983). It has also been shown that *T. congolense* binds to bovine erythrocyte *in vitro*, via neuraminic (sialic) acid receptors on the rbc (Bank, 1979) with resultant damage to the erythrocytes (Bank, 1980). Mamo and Holmes (1975) had earlier reported that the half-life of ^{51}Cr-labelled rbc in *T. congolense* infected calves dropped from initial value of 10 days to 4.7 days at about 30 days post

infection. All these findings are indications that *T. congolense* may be having enzymatic activity against erythrocytes, as observed in *T. vivax* blood stream forms or *T. brucei* insect vector form but this is yet to be confirmed. Thus it could be seen that removal of erythrocyte sialic-acid by sialidase enhances the complement fragment opsonization of rbc or exposes the β-galactose on rbc surface with consequent removal of the rbc by erythrophagocytosis.

It is believed that the sialidase of trypanosome plays a role in antigenic variation by cleaving the trypanosome of its surface sialic acid, thereby rendering the new trypanosome with its new act of incorporated or metabolised sialic acids (Esievo and Saror, 1991) and that sialidase from the trypanosomes during different parasitaemic waves may have a slightly different biochemical characterics. This antigenic-variation (AV) is described as the method whereby trypanosomes develop sequentially different antigens (ag) on their surface coats, thus evading the immune response of the host (Cross, 1978; Boyle *et. al.*, 1978; Vickerman and Taetly, 1978; Williams, 1978). AV is said to be very important to the trypanosomes for their survival in the mammalian host.

2.7.3. OTHER FACTORS AFFECTING TRYPANOSOMOSIS - THE ROLE OF IMMUNOSUPPRESION IN TRYPANOSOMOSIS

Immunosuppresion is the suppresion of immune response to heterelogous antigenes introduced after established infections of pathogenic African trypanosomes (Godwin *et. al.*, 1972); Ilemobade *et. al.*, 1982; Mackenzie *et. al.*, 1975. This condition is said to be due to several factors, including

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i. Breakdown in the co-operation between the cellular components of the humoral response(Terry *et. al.*, 1973).

ii. Non-specific activation of the immunoglobin producing cell causing thus an inhibition of new responses to further infection (Urquhuart *et. al.*, 1973)

iii. Polyclonal stimulation of B-cells with resultant excess immunoglobulin production and thereby causing immunosuppression by clonal exhaustion (Greenwood, 1975a,b; Assoku, 1974; Askommas *et. al.*, 1979)

iv. Impairment in suppressor T. cell control causing hypergamma globulinaemia M (Jayawardena and Wakaman, 1977; Manafield, 1978).

Immunosuppression is also reported in human-influenza virus infections (Read *et. al.*, 1972) where the virus diverts the B-lymphocytes from lymph nodes and spleen to the liver (Woodruff and Woodruff, 1976), and this alteration in lymphocytes homing was mediated by viral sialidase. Thus the inhibitation of *T. vivax* sialidase activity by influenza virus antiserum suggests some kind of similarities between the sialidase of *T. vivax* and the influenza virus (Esievo, 1983).

It is being suggested that immunosuppression in animal trypanosomosis is mediated through trypanosome production of sialidase or attachment and subsequent hydrolysis of leukocyte sialic acids pimarily of lymphocytes resulting in the diversion of the lymphocytes to the reticuloendothelial system (RES) for elimination. This is suppoted by :

a. The lymphopenia seen early in bovine trypanosomosis when there is high parasitaemia (Fiennes *et. al.*, 1946; Maxie *et. al.*, 1979).

b. The high sialidase activity and the direct relationship between level of parasitaemia and degree of immune suppression (Baltz *et. al.*, 1981).

Immunosuppression seems to be severe during acute infection. According to Sacks *et. al.* (1980) and Askommas *et. al.* (1980a), trypanosome-induced suppression of of host response to parasite was prominent in the acute phase of *T. brucei* infection in mice. This suppression was associated with the failure to control the initial relapse variant of the acute clone. There is less suppression in the chronic phase. Though IgG responses were elimimnated early. Its responses persisted at low level to mediate continued control of parasitic variants (Sack *et. al.*, 1980)

2.8. TRYPANOSOMOSIS OF SMALL RUMINANTS

2.8.1 IN AFRICA IN GENERAL

According to Jasiorowski (1972) one of the greatest hindrances to increased productivity and steady marketing of livestock in Africa is the problem of diseases in animals which account for between 30 - 60% of production cost compared to 15% in developed nations

Urguhart (1974) reported that of all the livestock diseases endemic to the African continent, trypanosomosis has been regarded as the biggest single factor limiting the number of livestock population. Hornby (1952) described trypanosomosis as being unique among diseases because it is the only one which by itself has denied vast areas of land to all domestic animals except poultry. Africa, according to Finelle (1974) is said to

have seven million square kilometres of tsetse-infected land capable of supporting livestock. Jasiorowski (1972), estimated the tsetse-infected area in Africa as 10 million square kilometres, including 3 square kilometres of equatorial rainforest which according to Finelle (1974) is unsuitable for livestock production. Within this area, is an estimated population of 30 million sheep and 50 million goats (F.A.O., 1975; Finelle, 1974; Griffin, 1978) and also 52.2 million cattle (F.A.O., 1975). According to Griffin (1978) eradication of trypanosomosis from this area can lead to increased goat population to 250 million, that of sheep to 157 million and also cattle population could be raised by a further 125 million.

Finelle (1974) reported that the benefits resulting from effective trypanosomosis control lie not only in increased livestock numbers and reduction in mortality rate due to the diseases, but also in improved carcass quality and increases in growth rate, milk production and fertility.

In trying to formulate new livestock policy for East Africa, F.A.O. (1962) had suggested that it would be advisable to explore fully sheep production in the range areas of East Africa instead of concentrating on cattle breeding, due to the enormous advantages of small ruminants over cattle. These according to F.A.O. (1962) include the following:

- Cattle requires, in the drought stricken range-grazing areas of Africa, to be watered everyday, needing 30 - 40 litres of water per day. Thus, cattle cannot graze too far from watering points whereas sheep and goats can roam further
- Cattle are poor browsers in the absence of adequate pasture for grazing weree as sheep and goats in similar situations survive efectively through browsing.

- Small ruminants have lower per capital value than cattle, thus owners are more willing to sell.
- Small ruminants have a higher annual offtake; a higher reproductive efficiency, producing most often twins and triplets.
- Small ruminants produce skins of considerable economic value as examplified by the famous Moroccan -leather derived from Kano Brown goats.

Inspite of the fact that trypanosomosis has been recognised for years to be the most important obstacle to increased production of livestock in Africa until recently, not much investigation has been carried out on trypanosomosis in sheep and goats.

Kramer (1966) in a limited survey indicated that small ruminants trypanosomosis in Nsukka area in Eastern Nigeria was of little importance though incidence was between 11 - 15%.

Stephen (1970) reported that sheep and goats are not often infected under natural conditions, and Maclennan (1970) dismissed trypanosomosis as an unimportant disease in small ruminants.

Even a joint FAO/WHO expert committe on trypanosomosis (1963) laid emphasis on the importance of the disease in cattle, camels and pigs, but without any mention on sheep and goats. This could be due to the fact that until recently, only few studies on the natural occurrence of the disease in small, ruminants had been carried out and with the emphasis on cattle production; most earlier observations on the disease in small ruminants have been subjective and anecdoted from stock owners whose major concern was with cattle.

However, surveys and epidemiological studies indicate that small ruminants are and can be seriously affected by trypanosomosis. Hornby (1952) in an extensive survey of the endemic Lambwe Valley of Kenya reported that pathogenic trypanosomosis affected 5% and 2.01% of the sheep and goats population respectively and also observed that *T.congolense* was the most common species, followed by *T. vivax*, with *T. brucei* being the least common.

Zwart *et. al.* (1973) in a survey in the Kiboko area of Kenya reported that 42% of sheep and 44% of goats had antitrypanosomal antibodies (detected via Indirect Flourescent Antibody Test IFAT) or showed parasitaemia by standard detection methods.

Mahmoud and Elmalik (1977) have emphsized the importance of sheep and goats in transmission of trypanosomosis to other domestic stock in Sudan; repoting the case with which a *T. congolense* isolate may be transfered between calves and goats, producing an acute fatal disease in calves but a milder chronic form of about 6 months duration in the goats. This isolate was observed not to loose its virulence for calves during infection in goat, an indication that goats may act as ready source of infections under field conditions where they are grazed along-side cattle.

Leach (1973) observed that heavy losses among sheep and goats due to the disease do occur when exposed to the Savanna speces of *Glossina*, However Leach (1973) also reported that small ruminants are rarely fed upon by riverine speces of *Glossina* as indcated by a survey carried out in Nigeria which revealed an incidence of only 2% in sheep and goats exposed to riverine tsetse species.

Griffin (1978) reported an infection rate with *T. congolense* of up to 80% in Karakuul sheep and 90% in Saanan and Galla cross-bred goats these animals having been grazed on range land infested with *G. Pallidipes*, a Savannah species of tsetsefly.

Mackenzie and Cruicshank (1973) observed that black persian sheep grazed in an endemic area of Rhodesia (Now Zimbabwe) easily got infected with *T. congolense*, though *T. vivax* infection was intermittent. They also observed 3-phases (stages) of the disease syndrome namely an acute fatal disease lasting 43 days after appearance of trypanosomes in the general circulation; a protracted form of eleven to twelve months. Similarly, Griffin (1978) observed 3 phases of the disease in *T. congolense*-infected sheep and goats in Kenya, namely an acute form lasting up to 6 months with a rapid fall in pcv, fluctuating temperature during the period of parasitaemia and with death in some cases and self cure in others; the chronic form in which the host makes a complete recovery with normal pcv level and temperature and the disappearance of parasites from the blood stream; and the sub-acute form lasting up to 12 weeks (3 months) with slow but steady fall in pcv and weight with fluctuating temperature. However, no acute episode just before death was observed as reported by Mackenzie and Cruicshank (1973).

According to Griffin (1978), most accounts of naturally occurring trypanosomosis in sheep and goats occurs in East Africa and is due to infection with *T. congolense* which is the most widespread and economically important trypanosome in East Africa., but with fewer reports from West Africa where *T. vivax* is the most important and widespread trypanosome. Natural *T. brucei* infections in sheep and goats are scarce because they are

nor the preferred hosts for the tsetse flies. It is believed that when sheep and goats are infected they may act as reservoirs of the sub-species of *T.brucei* that can be transitted to man. The few reports on the incidence of natural *T.brucei* infection could be due to limited interest in its occurrence in small ruminants in Nigeria. Sheep and goats like cattle, are also believed to be reservoirs for *T.brucei* infection in man because, *T. brucei* isolated from sheep and goats in Kenya was found to be infective to man (Hornby, 1952). Rickman (1974) reported that 33 goats within a sleeping sickness focus in Zambia, were found to be positive for *T. brucei* and that these goats possibly acted as reservoirs of infection in the course of the outbreak. However, these *T.brucei* isolates may be that of the *T.brucei rhodesiense* which is the cause of sleeping sickness in East and Central parts of Africa where Kenya and Zambia are respectively located.

Natural *T. simiae* infection in small ruminants had been reported by Mackenzie *et. al.* (1972) in which one case was fatal and another one mild, isolates from the latter case produced a mild disease when introduced into a pig. However, Godfrey (1972) observed that due to variation of trypanosome from different hosts or stages of development, *T. simiae* may be confused with *T. congolense*.

2.8.2. SMALL RUMINANTS TRYPANOSOMOSIS IN NIGERIA

2.8.2.1. SOME REPORTS ON NATURAL OCCURRENCE

Most of the work on animal trypanosomosis in Nigeria had centered on cattle with very little emphasis on sheep and goats. Not much information is available on natural incidence of small ruminant trypanosomosis As stated earlier in this work, trypanosomosis

was reported in sheep at Epe by Gordon (1910). Also *T. congolense, T. vivax* and *T. brucei* were identified in sheep slaughtered at Ilorin (Macfie, 1913a,b). Connal and Coghil (1916, cited by Kramer, 1966) observed *T. vivax* in one out of 44 sheep and one out 16 goats in Southern Nigeria. Kramer (1966) made observation on trypanosomosis and trypanosome infection rates of between 11 - 15% in the WADS. Kalu and Agu (1984) reported on natural infections of trypanosomosis in WAD sheep and goats in Jos - Plateau area of Nigeria and and Ademosun (1990) reported on natural infection of trypanosomosis in WAD sheep and goats with the latter observing that only goats under stress succumb to the infection. Nawathe *et. al.* (1994) reported an infection rate of 1.10% in the SB goats. Daniel et. al (1994) reported a prevalence rate of 7.4% in sheep and 5.0% in goats in Alkaleri and Gombe areas of Bauchi State, due to *T. vivax*. Fallae and Chiejina (1993) observed an infection rate of 13.64% in WAD goats with *T. brucei, T. congolense* and *T. vivax* accounting for 50%, 43.0% and 36.0% infection respectively. This finding contradicts the earlier opinion that *T. vivax* is the most important trypanosome in Nigeria. However, this finding may be an isolated finding with regard to level of infection due to this 3 pathogenic ruminant trypanosomes.

2.8.2.2. USE OF SMALL RUMINANTS IN EXPERIMENTAL TRYPANOSOMOSIS

Sheep and goats are said to have greater advantage over cattle as research models. The advantages of small ruminants in experimental trypanosomosis as listed below have led to their being used in several areas. Trypanocidal activity of isometamidium chloride

administered intravenously (Tour *et. al,* 1981); antigens of various trypanosomes (Gray, 1961); resistance to drugs (Ilemobade *et al.*, 1985).

2.8.2.3. PRESENT STATUS OF TRYPANOSOMOSIS AND ANIMAL PRODUCTION IN NIGERIA

Vegetationally, Nigeria is divided into mangrove; Forest; Derived-Savannah; Southern-Guinea Savannah; Northern-Guinea Savannah; Sudan-Savannah; Sahel and Montane (Fig. 1). The Derived-Savannah up to the Northern-Guinea Savannah areas are also often referred to as the Sub-Humid zone; and the Sudan-Savannah and the Sahel as Semi-Arid zone consisting of the northern fringes of Nigeria.

According to Bourn (1983), aerial surveys in 1982 showed that the Sub-Humid zone of Central part of the country supported about 4.5 million zebu cattle (40% of the national herd) with no significant change with season.

2.8.2.3.1. EPIDEMIOLOGY OF THE DISEASE AND ECOMOMIC IMPORTANCE

Akinwumi and Ikpi (1985) showed that 76% of cattle permanently residing in the Humid-zone were of the zebu breed. Takken *et. al.* (1986) observed that tsetse fly catches in the humid zone are low (0.01 to 5.50 tsetse per trap per day for *G. tachinoides* and 0.10 to 19-30 for *G. palpalis*), but, lower catches were obtained by Madubunyi (1986), Omogun (1987) and Ikede (1987) with *G. morsitans* subsp *morsitans* confined mostly to game reserves Kalu (1984) reported detection of tsetse and trypanosomosis in the high altitude Jos Plateau, formerly thought to be too high for tsetse fly survival. And also an

acute trypanosomosis outbreak in a sedentary herd of cattle on the Jos Plateau (Kalu, 1996). Infact, Nawathe *et. al.* (1994) reported an outbreak of *T. vivax* infection in cattle near Lake Chad which was said to be tsetse-free.

Bourn (1983) observed that both organised and unorganised control measures have brought about the decline in prevalence, of trypanosomosis, and these include improved road network allowing for economical and profitable transportation of trade cattle to abbatoirs in vehicles thereby greatly reducing or totally eliminating the possibility of animals coming down with trypwnosomiasis in transit through high tsetse challenge areas.

According to Ikede (1987) despite the observed fall in prevalence of tsetse and infection rate of trypanosomes in the supposedly tsetse infested areas of the country, there is still deficit in meat and milk supply due to the fact that industrialists find it not easy to obtain credit facilities for commercial cattle production in the humid and submid zones due to the old belief that these zones are heavily infested with tsetse and hence are credit risk areas for such investments.Maclennan (1983) reported that 86% of the cattle population in the country's northern area are found in the tsetse-free/lightly infected areas during the rainy season. According to Ikede (1987), between 1955 and 1978, nearly 200,000 square kilometres of land in North-Eastern and North-Central Nigeria got reclaimed from tsetse flies through the use of insecticides and the impact of the measure is now being felt. Bincan (1990) observed that the majority of livestock especially ruminants are found in the semi-arid and sub humid zone, with the two zones containing 90% of Nigeria's cattle herd; and with the semi-arid area characterised by a short growing season, limited rainfall but relatively free of tsetse flies but holding nearly 80% of Nigeria's cattle population during

the short wet season. These cattle however move to the helminth and tsetse infested sub-humid zone as a result of lack of grazing land and water for them.

The Semi-Humid zones contain nearly 26 million hectares of savannah range-land highly favourable for ruminant production, however the range land has the constraint of not being able to sustain livestock year round due to seasonal scarcity of grazing resources and water despite the fact that these zones harbour most of Nigeria's pastoralists (Bincan, 1990). On the other hand, though the Humid zone favours livestock production as a result of short dry season and all year round supply of herbage and water, it is also hampered by high tsetse-fly and helminth problem and also most of the inhabitants of this zone are mostly arable farmers lacking experience in livestock rearing

Bincan (1990), have observed that there is ample evidence that lots of zebu animals, predominantly raised by the pastoralists and best suited to the tsetse-free but feed and water deficient for most part of the year Semi-Arid zones are now being raised successfully in tsetse infested areas. This therefore is an indication that the small ruminants of the semi-Arid areas can also thrive in this tsetse infested zones.

In a recent study carried out by Anosa (1991), under an EEC sponsored program on the epidemiology of trypanosomosis in Nigerian livestock, the following observations were made

i. that there is generally low prevalence of trypanosomosis in cattle, sheep and goats compared to earlier years due possibly to forest degradation, increasing human population which is encroaching on fly habitats coupled with the use of trypanocides by veterinarians and farmers.

ii. That the highest incidence rates of trypanosomosis in cattle, sheep and goats occured in the Southern-Guinea Savannah, followed by the Derived-Savannah. Slightly lower rates in the forest, Northern-Guinea Savannah, Sudan-Savannah and the Montane zones, whilst the Sahel-zone has the lowest incidence

iii. That *T. vivax* is the most common trypanosome followed by *T. congolense* and lastly *T. brucei*

iv. That, there is relatively high rates of trypanosomosis in the Jos, Obudu and Mambila plateaux, with Jos plateaux. Tsetse flies notably *G. tachinoides* species were also found in the previously tsetse-free Jos plateau.

2.8.2.3.2 DIAGNOSTIC PROCEDURE

According to a study carried out by Anosa (1991), with regard to epidemiology of trypanosomosis in livestock in Nigeria, ELISA technique developed by Nantulya and Lundquist (1989), for detecting trypanosome antigens in sera, is more sensititve and efficient than normal parasitological methods. According to Anosa (1991), the ELISA tchnoque gave a positive result of 357 out of 850 cattle compared to 54 out of the same 850 cattle by the normal parasitological techniques. Nantulya and Lundquist (1989) had indicated that animals infected with trypanosomes had antigens in their sera for a while post treatment with Diminazene-aceturate (Berenil®) hence some of those tested positive might have been recently treated or had self-cure.

CHAPTER THREE

SURVEY OF THE PREVALENCE OF TRYPANOSOMOSIS (TRYPANOSOMOSIS) IN THE SAVANNAH BROWN GOAT (SBG) IN NORTHERN NIGERIA

3.1. INTRODUCTION

Caprine trypanosomosis (trypanasomosis) has not been regarded as important as that of cattle (Maclennan, 1970). Infact, goats and sheep have long been seen as alternate source of blood meal for tsetseflies in the absence of cattle, the primary target of these flies. Maclennan (1983) reported that dwarf breeds of goats survive in areas which cannot support cattle due to the presence of tsetseflies. However, reports from East Africa (Griffin and Allonby, 1979a, b); Central Africa (Mahmoud and El-malik, 1977) and West Africa (Kalu and Agu, 1984) have stressed the importance of trypanosomosis in goats and sheep and regarded these small ruminants as reservoirs of trypanosomosis for cattle and possibly man. In Nigeria, natural infections in goats have been described mostly in the dwarf breed found in the Forest, Derived Savannah and the Southern Guinea Savannah zones (Kalu and Agu, 1984; Ademosun *et al.*, 1985). Falae and Chiejina (1993) reported an infection rate of 13.6% in the dwarf goats with *T.brucei*, *T.congolense* and *T.vivax* accounting for 50.0%, 43.0% and 36.0% of the infection respectively.

The Savannah Brown Goats (SBG) are found in Southern Nigeria and Northern Nigeria between latitudes 12° North and 14° N and longitudes 4°N and 10° E. In Nigeria, its main areas of distribution are Sokoto Emirate where the breed is commonly known as "Sokoto Red" and in Kano Emirate where it is referred to as "Kano Brown", with the variant around the Bornu area referred to as "Borno White" and even the "Sokoto Red" also often referred to as "Maradi"

(Wilson, 1991). The SBG is kept primarily by the sedentary Hausas, though large flocks are also raised by the nomadic Fulanis as part of their herd of cattle and sheep..

Information on natural incidence of trypanosomosis in the Savannah Brown Goat is limited. Daniel *et al.* (1994), Krammer (1966), Nawathe *et al.* (1994), and Ahmed and Agbede (1993) reported infection rates of 5.0%; 13.9%; 1.1% and 0.7% respectively in SB-goats. In order to further assess the level of the prevalence of trypanosomosis in the SB-goats, a survey was undertaken to ascertain the situation in the Sudan and the Guinea Savannah vegetational zones of Northern Nigeria.

3.2. MATERIALS AND METHODS

The survey was carried out between January, 1989 and December, 1993. It involved goat flocks kept by nomadic Fulani herdsmen, private established farms and institutional (Government) farms where Veterinary Health Services were provided either regularly or on request. Prior to sampling each flock, flock health history was obtained from the owners. Randomly selected goats were then physically examined. Then, prominent ear veins were picked up, disinfected and pinpricked to obtain blood sample for a wet mount, which was then examined under a field-microscope. Where wet mounts were positive for trypanosomes, about 2 millilitres of blood was then obtained via the jugular vein into blood sample bottles containing ethylene diethyltetra-acetic acid (EDTA) as anti-coagulant and analysed in the laboratory using hematocrit centrifuge technique (HCT) and thin blood smear methods. Only trypanosome positive goats were later bled, and this was done to cut down the number of animals to be bled to the satisfaction of the goat blood owners. Attempts were also made to trap flies observed within the environment of the flock

being sampled. The type of vegetational zone in which the sampled flocks were located were noted. 42 flocks of Savannah Brown goats located in some parts of the Southern Guinea Savannah, Northern Guinea Savannah and Sudan Savannah zones were sampled, and 717 goats were bled.

3.3 RESULTS

A total of 717 Savannah Brown goats were sampled, this was made up of 570 adults aged 10 months and above; 115 growers aged between 5 months and 9 months and 32 young goats (kids), aged between 1 month and 4 months.

Among the total number were 70 bucks out of which 4 (5.7%) were positive for trypanosomes and 15 male kids with none being positive; 500 does with 13 (2.6%) positive, 115 young females with 3(2.6%) positive and 17 female kids with none positive.

Trypanosome infection in the SBG was detected in all the three vegetational zones namely the Southern Guinea Savannah, Northern Guinea Savannah and the Sudan Savannah zones (Tables 3.1. and 3.2.). The prevalence rate was highest in the Southern Guinea Savannah (10%) followed by the Sudan Savannah (4%) and then the Northern Guinea Savannah (2.1%) (Table 3.2.).

The prevalence rate was observed to be higher in the dry season (3.3%) than in the wet season (1.69%). In all, 20(2.79%) out of the total of 717 SBG sampled were positive for trypanosomes as determined by the combined Wet Mount, HCT (Buffy-coat) and stained Thin Smear methods.

The level of parasitaemia ranged from 3 - 20 trypanosome parasite per microscopic field. *T. vivax* accounted for 100% of the observed infection (Table 3.2.).

Observed clinical signs included abortion in 8 of the positive does, whilst 2 other such does gave rise to very weak kids; orchitis and lymphadenitis of superficial lymph nodes (prescapular and prefemoral), reduced libido and inappetance in 2 of the positive bucks. Rectal temperature of the tryparosome infected goats ranged between 38.5 - 39.2°C.

No tsetse fly was trapped throughout the survey period, though some of the herdsmen in the Southern Guinea Savannah zone reported seeing tsetse flies. The biting flies trapped were *Musca domestica, M. autumnalis, Stomoxys calcitrans, Tabanids species* and *Hipobosca species*. These flies were predominant during the rainy season (May to October) than during the dry season (November to April).

TABLE 3.1. PREVALENCE OF TRYPANOSOMOSIS IN THE SAVANNAH BROWN GOATS IN SOME PARTS OF NORTHERN NIGERIA.

Year	Month	Vegetational zone	Sample size	Number +ve	% +ve
1989	Jan.	NGS	30	0	0.00
	Feb.	NGS	45	2	4.44
	Apr.	NGS	20	3	15.00
	May	SS	30	0	0.00
	Jun.	NGS	20	0	0.00
	Jul.	NGS	10	0	0.00
	Oct.	NGS	15	0	0.00
	Dec.	NGS	15	1	6.67
1990	Feb.	SS	30	1	3.33
	Mar.	SS	15	1	6.67
	Mar.	NGS	25	2	8.00
	Apr.	NGS	10	1	10.00
1991	Feb.	NGS	25	0	0.00
	Mar.	NGS	15	0	0.00
	Mar.	SS	30	2	6.67
	Apr.	NGS	10	1	10.00
	Jun.	NGS	20	1	5.00
	Oct.	SS	20	1	5.00
	Nov.	NGS	25	0	0.00
	Dec.	NGS	10	0	0.00
1992	Oct.	SS	25	0	0.00
	Nov.	NGS	15	0	0.00
1993	Jan.	SGS	20	2	10.00
	Jan.	NGS	20	0	0.00
	Feb.	NGS	35	0	0.00
	Mar.	NGS	35	0	0.00
	Apr.	NGS	10	0	0.00
	May	SS	25	1	4.00
	Oct.	SGS	15	1	6.67
	Nov.	NGS	20	0	0.00
	Dec.	SS	10	0	0.00
Total			717	20	2.79

TABLE. 3.2. SUMMARY OF THE OCCURRENCE OF TRYPANOSOMOSIS IN THE SAVANNAH BROWN GOAT IN DIFFERENT VEGETATIONAL ZONES OF NORTHERN NIGERIA

Vegetational zone	Sample size	No. positive	% positive	Species of trypanosomes isolated
SGS	20	2	10.00	*T. vivax*
NGS	522	11	2.10	*T. vivax*
SS	175	7	4.00	*T. vivax*
Total	717	20	2.79	

Total number of flocks sampled - 42

SGS - Southern Guinea savannah
NGS - Northern Guinea savannah
SS - Sudan savannah

3.4. DISCUSSION

This study shows that natural incidence of trypanosomosis in the savannah Brown goats does occur, as reported by other workers (Krammer, 1966; Daniel *et al.*, 1944; Nawathe *et al.*, 1994). The finding that only biting flies were trapped in this study is a further indication that these flies play a major role in the transmission of trypanosomosis as reported by Anosa (1983), Nawathe *et al.* (1994). Also, the presence of only *T. vivax* in this natural infection further confirms the observations of Davies (1977) that biting flies play major role in *T. vivax* transmission and also that *T. vivax* transmission is higher than that of *T. congolense* in the Northern part of Nigeria. Similarly, the presence of only *T. vivax* in these three ecological zones in the North confirms the observations of Davies (1977) and Anosa (1983) that *T. vivax* is often found well established at distances from the tsetsefly belts. The observation that highest prevalence of trypanosomosis occured in the Southern Guinea Savannah is in agreement with the findings of Anosa (1991).

A noteworthy finding in this study is the observation of abortion and orchitis in naturally infected does and bucks. This agrees with similar findings in East Africa made by Griffin (1978) and Mackenzie and Cruickshank (1973), but not yet reported in Nigeria. Though there other causes of abortion and orchitis notably brucellosis, babesiosis and leptospirosis the major disease condition in diagnosed in this study was trypanosomosis which also has these as clinical signs. The infection rate of 2.79% observed in this study is much higher than the value of 1.1% reported by Nawathe *et al.* (1994) and 0.7% observed by Ahmed and Agbede (1993) but lower than values of 5.0%; and 13.9% reported by Daniel *et al.* (1994) and Krammer (1966 respectively). However, these differences could possibly be due to variations in sample sizes and the type vegetational zones involved, though methods of sampling were mostly that of random sampling technique. The

high infection rate observed in the goats in the Sudan Savannah zone compared to those in the Northern Guinea Savannah zone could be due to the fact that most of the herds in the former had migrated to the Southern Guinea Savannah zone during the dry season of the North and might have contacted the infection whereas most of the herds in the latter zone we were told had not migrated for some years.

There is no plausible explanation for the observed higher infection rate during the dry season compared to the wet season. It could be due to the fact that more animals were sampled during the dry season (460) than in the wet season (257).

3.5.0. SUMMARY

The prevalence of natural trypanosomosis in the SB-goat in the Sudan and Guinea Savannah zones of Northern Nigeria was determined in a survey lasting 28 months. Of the 717 goats examined, 20 were positive, giving an infection rate of 2.79%. *T. vivax* was the only trypanosome parasite observed in the study. No tsetsefly was trapped, only biting flies notably *Stomoxys sp., Tabanus sp., Hipobosca sp., Musca domestica* and *Musca autumnalis.*

The highest infection rate was in the Southern Guinea Savannah (10.0%) followed by the Sudan Savannah (4.0%) and the Northern Guinea Savannah (2.1%). The infection rate was higher in the dry season (3.37%) than in the wet season (1.69%). Of the 717 goats examined, 70 were bucks, with 4 (5.7%) positive out of which 2 of them had severe orchitis. 500 of the goats were does of which 13 (2.60%) were positive with 8 out of this aborting prior to being examined.

The study confirms the presence of natural trypanosomosis infection in Savannah Brown Goats even in their natural habitats which previously were thought to be trypanosome free due to

absence of tsetseflies, the primary vectors. Biting flies play major role in the transmission of trypanosomosis. There is need to pay attention to control of trypanosomosis in goats so as to improve their performance, as indicated by the orchitis and reduced libido, abortion and weak kids observed in the study. The observed abortion is a further confirmation of the report of Ogan *et al.* (1993) of this episode in goats experimentally-infected with *T. congolense.*

CHAPTER FOUR

4.0. THE CLINICAL, HAEMATOLOGICAL, AND PATHOLOGICAL MANIFESTATION OF EXPERIMENTAL TRYPANOSOMOSIS IN THE SAVANNAH BROWN GOATS DUE TO *T. vivax*, *T. congolense* and *T. brucei* INFECTIONS

4.1. INTRODUCTION

Goats are well distributed over Nigeria, across the diverse vegetational zones, with an average of 4 per house-hold in the southern and 10 in the northern parts of the country respectively (Wosu and Nwanta, 1992; Oludima *et. al.*, 1990).

Most of the goat population is located in the Northern Guinea-Savannah and the sudan-savannah. In these zones, the major breed of goats encountered is the savannah Brown Goat (SBG) with its two subspecies of Kano Brown (KB) and Sokoto Red (SR) which are light brown and deep brown in colour respectively. These goats, like the cattle found in these ecological zones are believed to be highly susceptible to trypanosomosis when exposed to highly tsetsefly-infected zones of the country.

Not much work has been done on trypanosomosis of goats in Nigeria. The works of Akinbamijo *et. al.* (1990); Bealby *et. al.* (1993), involved the use of the West African dwarf (WAD) breed of goat which is believed to be less susceptible to trypanosomosis.

The SBG is much bigger in size, more prolific, has greater milking potential compared to the WADG. On the account of the last two faetures, the National Animal Production Research Institute (NAPRI), which is a Federal Nigerian Research station in Zaria is gradually introducing the SBG into the suspected high tsetse zones of Nigeria through the breeding and selection

programs for litter size and milk production. Thus as the SBG penetrates the tsetsefly infected areas of the country there is the high probability of it getting more exposed to trypanosomosis.

This study was carried out to obtain more information on trypanosomosis in the SBG due to experimental infection with *T. vivax, T. congolense* and *T. brucei* with emphasis on clinical, haematological and pathological manifestations.

4.2. MATERIALS AND METHODS

4.2.1. ANIMALS:

a). Forty (40) Savannah Brown intact male goats aged between 6 - 12 months, were procured from an area on the Kaduna/Katsina States border in the Northern Guinea Savannah Zone which is believed to be tsetse free. Future research would be done using only females.

b). Pre-infection preparation.

(i) Housing: The goats were housed in fly-and tick-proof pens

(ii) Feeding: The goats were placed on groundnut and beans crop residue(harawa); cotton seed cake/cotton seed meal; wheat and corn bran; *Andrpogon gayanus* hay. Salt-lick and water were also provided *ad libitum*.

(iii) Treatment: The goats were screened for haemo and intestinal parasites and later dewormed with broadspectrum anthelminthic. Also, the goats were prophylactically treated against coccidiosis using Amprolium. Goats positive for *Anaplasma ovis* were treated with Terramycin Long Acting® (Pfizer Products Ltd., Ikeja, Nigeria). The goats were also vaccinated against Pestes de Petit Ruminantes (PPR), and Anthrax using Tissue Culture Rinderpest Vaccine (TCRV) and Anthrax Spore Vaccine (ASV)

respectively. Ectoparasite control was carried out by spraying the goats with solution of Asuntol (Bayer AG., Germany) fortnightly. The goats were routinely dewormed every 6 weeks. All these were done in order to ensure that the animals were free from bothe ecto and intestinal parasites completely to eliminate their possible effects on haematocrit values.

(iv) Evaluations: Daily body (rectal) temperature were evaluated in the mornings and evenings (8.00 am and 5.00 pm respectively).

Blood samples were collected from the jugular vein from each goat once a week, the packed cell volume (pcv), haemoglobin concentration (Hb), total serum protein (TP) concentration were determined for each blood sample using the micro-haematocrit technique (HCT), Electronic haemoglobinometer (Coulter Electronics, Luton, U.K.) and the refractometric method respectively, as described by Coles (1967). Total erythrocyte (RBC) and total leucocytes (WBC) counts were estimated using a coulter counter (Coulter Electronics Model ZBI: Hialeash Florida, U. S.A) whilst the differential leucocyte counts made according to Schalm *et. al.* (1975).

The goats were also weighed once a week. All these evaluations were carried out for nine weeks. The goats were later randomly divided into four groups of eight goats each. Thereafter, the evaluations were carried out for a further 4 weeks prior to experimental infection, to obtain base-line data, including those for serum electrolytes.

4.2.2. PARASITES:

a) Trypanosome stabilates:

(i) *T. vivax* stabilate 146 (bovine isolate from Zangon Aya in Northern part of Kaduna State)

(ii) *T. congolense* stabilate 136 (bovine isolate from Kachia in Southern part of Kaduna State

(iii) *T. brucei* stabilate 155 (bovine isolate from Kaduna municipality and received from the National Institute of Trypanosomosis Research (NITR) in Kaduna State).

The stabilates were stored in liquid nitrogen. Before being used, they were inoculated into white mice and when these became positive for trypanosomes, the infection was transferred into donor goats.

4.2.3. INFECTIONS (INOCULATIONS):

4.2.3.1. Donor Animals: 3 of the goats were used as donors.

(i) One goat was infected with 2 millilitres (mls) of *T. vivax* stabilate 146 at level of parasitaemia of 10 - 20 parasite per microscopic field (+++) via the jugular vein.

(ii) A second goat was infected with *T. congolense* stabilate 136 also at level of parasitaemia of +++

(iii) A third goat received the *T. brucei* stabilate 155 as described above, with parasitaemia of +++ (20 trypanosomes per field)

All the donor goats were then monitored daily to establish thier levels of parasitaemia.

4.2.3.2. Inoculation of Experimental Animals, Monitoring and Sample collection.

4.2.3.2.1. Inoculation:

When the *T. vivax* donor goat on attaining parasitaemic level of ++++ (> 20 trypanosomes per microscope field for two consecutive days, 2 mls of its blood was obtained via the jugular vein and inoculated also via the jugular vein into each of the eight goats in the *T. vivax* group. The same procedure was repeated for the *T. congolense* and *T. brucei* groups using their respective donor goats.

4.2.3.2.2. Sample Collection:

From day 2 post infection,(pi), 15 mls of blood was collected via the jugular vein of each goat three times a week at 2 days interval for 2 weeks, followed by twice a week for another 3 weeks and then once a week for the rest of the experimental period. Seven mls of the collected blood was placed in vacutainers containing EDTA as anticoagulant, and the remaining eight mls placed in bijou bottles without anticoagulant for serum preparation. On other days, blood was obtained from each goat via ear venapuncture for the determination of level of parasitaemia using HCT, wet film (Wet mount) and Thin smear methods.

4.2.3.2.3. Monitoring:

(a). Haematology:

Packed Cell Volume,(pcv %), haemoglobin (Hb) concentration (g %), total plasma protein (TP, g/100ml), total erythrocyte count(rbc, 10^{12}/l). Total leucocyte count (WBC, 10^{9}/l) and differential leucocyte count (10^{9}/l) were evaluated as earlier described.

(b). Parasitaemia:

This was determined using three major parasitological methods namely

-Wet film (Wet mount): About 5 microlitres (or a drop) of blood was placed on the centre of a clean, grease-free microscope slide. This was then covered with a clean coverslip and then viewed under the light-microscope at x40 magnification.

-Hematocrit Centrifuge Techniques (HCT): A microhaematocrit capillary tube containing about 70 ml of blood was centrifuged for 5 minutes (just as for estimation of pcv). The tube was then placed on a clean glass slide,then a drop of immersion oil was put on top of the tube, after which the buffy coat/plasma junction was then examined under the light microscope using x 100 magnification (Woo, 1969).

-Thin blood smear method: A drop (about 5 microlitres) of blood was placed at one end of a clean, grease-free microscope slide. With the help of another microscope slide, a thin smear was made. The slide was quickly air-dried, then fixed in methanol for 3 minutes, and then stained with 10% Giemsa and rinsed under the tap and then dried. Oil-immersion was then placed on it and viewed under the light microscope at x100 magnification.

The level of parasitemia was rated +; ++; +++; ++++; and massive representing 1-5, 6-10, 10-20, 20 and >20 trypanosomes per microscopic field respectively. On other days, blood was obtained via ear venapuncture from each of the infected goats for the determination of parasitaemic level using the techniques described above.

(c). Temperature:

The daily body (rectal) temperature in degrees centigrade (^{o}C) of both infected and control goats were determined every morning (and evenings) using a clinical thermometer.

d). Clinical-Signs:

Both the infected and control goats observed for any abnormal behaviour from morning to evening each day. Their occular and buccal mucous membranes were examined, also were their appetites, physical appearance, nature of faeces, occular/nasal/oral secretions, gait, posture, appearance of superficial lymph nodes notably the pre-scapula and pre-femoral, scrotal sac (and also reaction to palpation of testicles), nature of urine (if possible), cutaneous swellings and appearance of hair-coat.

e). Body Weights:

All the experimental goats were weighed once weekly using the foot weigh-balance, to determine body weight changes in kilogram (Kg).

f). Pathology:

i). All goats which died in the course of the experimental infection were immediately necropsied. Some control goats were similarly euthanised. Gross pathological examination were performed on the carcasses and their organs. Also, impression smears were made from the liver spleen, lungs, heart, kidney, bone marrow, acqueous-humour, lymph nodes, brain, and also pericardial, peritoneal and cerebrospinal fluids together with blood from aorta and jugular vessels of each necropsied goat. These were then stained with Giemsa and examined for trypanosomes.

ii). Pericardial sac content of each of these goats were aspirated using syringes into measuring jars and volume estimated.

iii). Samples of lungs from all the goats which exhibitd clinical signs like nasal discharges and coughing were subjected to microbiological examination.

iv). Similarly, samples were taken from heart, liver, kidney, spleen, lymph nodes, lung, bone marrow, skeletal muscles, brain, eyes, pituitary gland, spinal cord from each necropsied goat and fixed in 10% formalin solution and later transferred to buffered formalin after 24 hours. Samples from testicles, spermatic cord and vas deferens were fixed in Bouins solution. These fixed samples were later processed first by being embedded in paraffin wax, then sectioned into 6 mm thickness and stained first with Haematoxylin and Eosin(H&E) and later Giemsa. The prepared slides were later examined under the light microscope at x 40 magnification. In tissues showing haemosiderosis during examination of stained samples, fresh preserved samples of such were stained with Pearl's Prussian Blue Stain (PPBS) and then examined to confirm the suspected haemosiderosis.

4..2.4. Method of Statistical Analysis:

Statistical analysis of the variable data were carried out by the use of ANOVA via the Duncan's Multiple Range Test for variables. Means of the various parameters were compared and those that had a probability of 0.05 or less of being from the same population were reported as significantly different.

4.3. RESULTS

4.3.1. PRE-INFECTION BASELINE DATA:

All the goats were clinically in apparent good health prior to the experimental infection. They were negative for blood parasites/intestinal parasites and with normal rectal temperatures.

Haematological parameters notably PCV, Hb, RBC, WBC (total and differential counts) and total plasma proteins (TP) were all within normal ranges (Table 4A.)

4.3.2. POST-INFECTION RESULTS

4.3.2.1. Phases (stages) of Infection:

In all the infected groups, three phases of infection developed, namely an caute, phase which lasted for 13 days p.i.; a sub-acute phase lasting up to 23 days p.i. and a chronic phase which persisted up to day 58 p.i. in the *T. brucei* infected goats and to 74 days p.i. in the *T. vivax* and *T. congolense* infected goats (Table 4.b.)

4.3.2.2. Temperature:

Temperature increases were observed on the day of patent parasitaemia and the next 3 to 4 days after, in all the infected groups. Thereafter, temperature increases became intermittent. Though *T. vivax*-infected goats which died in the course of the infection still showed high temperature, those of the *T. congolense* and *T. brucei* groups died with subnormal temperatures. On the whole, increases were highest in the *T. vivax* group than in the other infected groups. Also, peaks of pyrexia did not always correspond to peak of parasitaemia or fall in PCV in the infected groups (Fig.1). A highly significant difference between the temperature of the control groups ($p<0.001$) was observed, also the temperature increase in *T. vivax* group was higher than that in both the *T. congolense* and *T. brucei* group ($p<0.01$) (Appendix 1. Table 1.)

4.3.2.3. Parasitaemia:

Seven of the *T. vivax* infected goats were patent by day 4 p.i.. All but one of the goats in *T.congolense* infected group were patent by day 4 p.i., with the last being patent day 7 p.i.. In the *T. brucei* infected group, one goat patent by day 2 p.i., 6 others by day 4 p.i. and the remaining goat 8th day p.i. (Table 4B). The study therefore showed an average pre-patent period of 2.3 days for *T. vivax*, 4.4 days for *T. congolense* and 4.3 days for *T. brucei*. These differences in patency could be due to trypanosome species variation and host response to infection due to different trypanosome species.

T. vivax produced parasitaemic waves at an average of 9.3 days interval (day 4, 8,16 and 32 post inoculation); an average of 8.0 days interval (4, 10, 21, 28 days p.i.) for *T. brucei* and 6.7 days (day 8, 14, 18 and 28 p.i.) for *T. congolense* (Table 4.4. , Fig. 4.1)

All the *T. brucei* infected goats died within 58 days post infection, whereas *T. vivax* and *T.congolense* infected goats died within 74 days post infection (8th and 10th week of infection respectively). (Tables 4.2.). The infected goats also died with varying levels of parasitaemia (Table 4.2) The differences in the level of parasitaemia between *T. brucei* group and those of *T. vivax* and *T.congolense* groups was significant ($P<0.05$) but not so between *T. vivax* and *T. congolense* infected groups ($p>0.05$) (Appendix 1, Table 2).

4.3.2. 4. Packed Cell Volume:

The onset of patency triggered off rapid fall in PCV in all the infected groups. PCV fell faster in *T.vivax* and *T. brucei* groups than in *T. congolense* group in the first six days p.i., but there after the PCV fall was faster in the *T. congolense* group than in *T. vivax* and *T. brucei* groups.

(Fig.1; Appendix 1, Table 3). The PCV of the infected groups between day zero of infection and death of the last member of the group ranged from a mean of 30.11 - 9.0%; 30.6 - 8.0% and 30.3 - 10.0% for *T. vivax, T. congolense* and *T. brucei* groups respectively. That of the control group had a mean of 30.00%. Despite the high mean parasitaemia of *T. congolense* and *T. brucei* animals, these two groups exhibited high levels of PCV, an indication of much early recovery. The mean PCV of all the infected groups after the onset of patency were highly significantly less than their initial mean values and that of the control group ($p<0.001$) (Appendix 1, Table 3). Also, there were significant differences ($p<0.05$) between the PCV of the *T. brucei* group on one hand, and the *T. vivax* and *T. congolense* groups on the other hand (Appendix 1, Table 3). Infected animals which died acutely exhibited a more rapid fall in PCV, compared to those which survived much longer.

4.3.2.5. Haemoglobin (Hb) level:

The variation in all the infected groups were similar to that of PCV described above.

4.3.2. 6. Total Red Blood Cell (RBC) Count:

The mean total rbc level of the *T. vivax* and *T. congolense* infected groups fell much faster than that of the *T. brucei* infected group within the first 16 days p.i. (Fig.1; Appendix 1, Table 5). Mean PCV values increased moderately between day 18 and 40 p.i. in the *T. congolense* group; between day 18 and 35 in the *T. vivax* group; and from day 35 p.i. in the *T. brucei* group. All these increases were indications of attempts at revovery by the infected groups. The mean total rbc levels in all the infected groups bore relationship to their corresponding PVC and Hb levels.

The differences between the infected and the control groups were highly significant ($p<0.05$) (Appendix 1, Table 5). Anaemia was progressive in all the infected groups. Anaemia was

initially that of normocytic normochromic but had become microcytic normochromic by day 16 p.i. in the *T. vivax* and *T. congolense* infected groups and day 32 p.i. in the *T. brucei* infected group, as indicated by the Mean Cell Volume (MCV), Mean Cell Haemoglobin Concentration (MCHC) values (Fig 2; Appendix 1, Table 6). Also, the *T. vivax* and *T. congolense* infected goats which survived up to day 74 p.i. developed microcytic microchromic anaemia between days 67 and 74 p.i.). The *T. brucei* infected goats which went through the chronic phase of the infection exhibited anisocytosis but this was not observed in the chronic cases of the *T. vivax* and *T. congolense* groups.

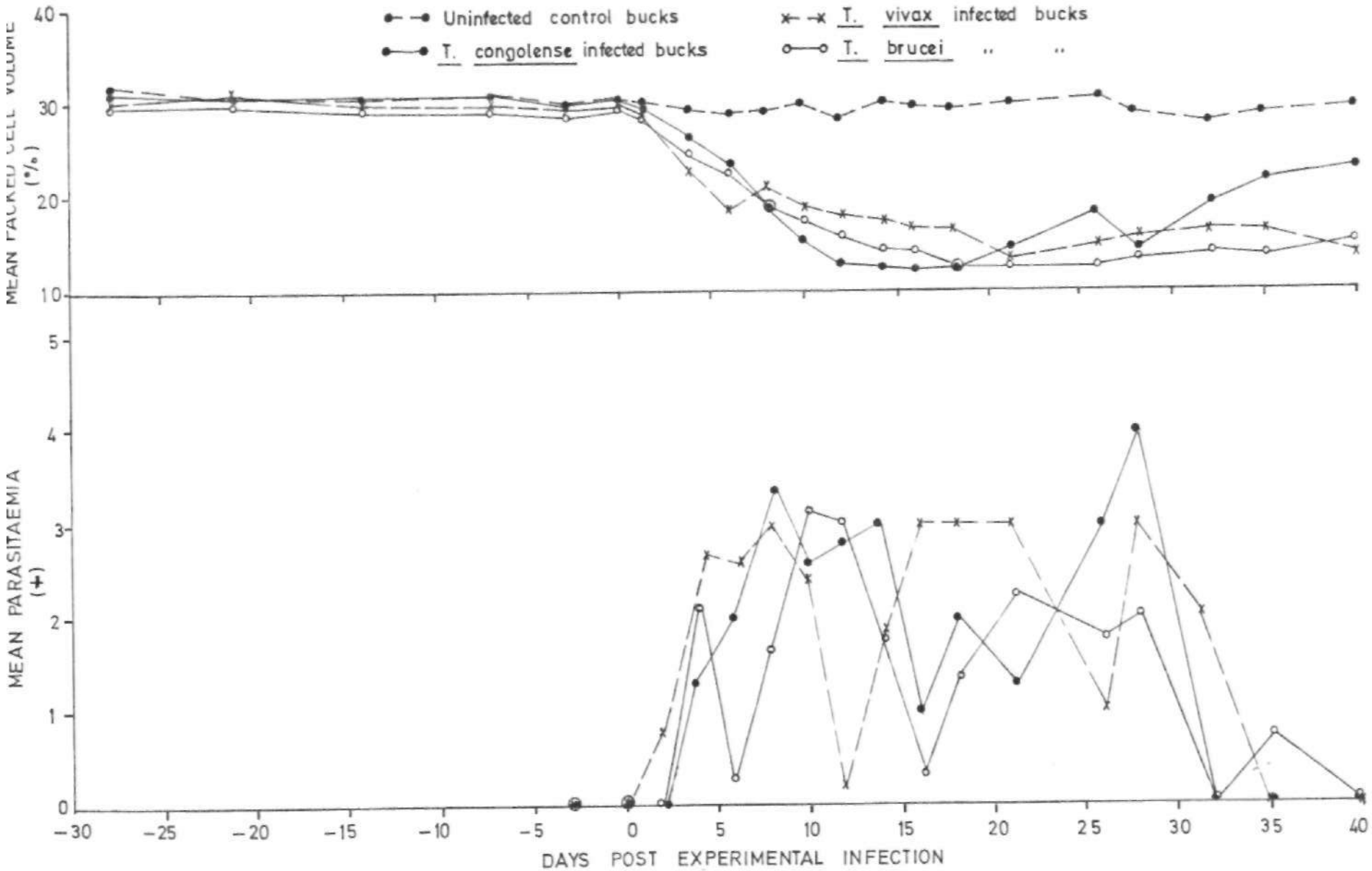


FIG. 4.1. Mean-parasitaemia and packed cell volume of infection and uninfected control savannah brown bucks

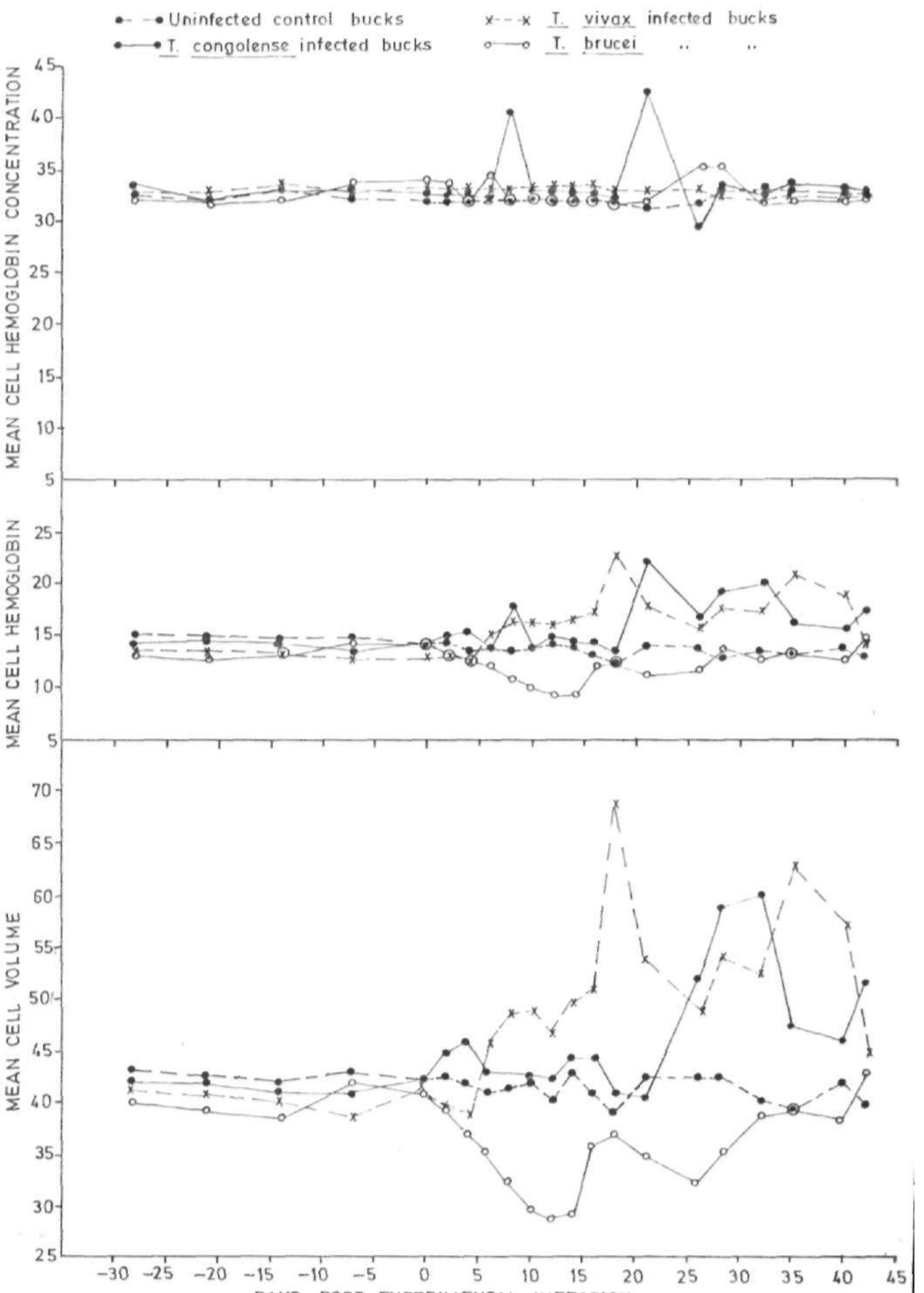


FIG. 4.2 Mean-cell volume, mean cell hemoglobin and mean cell hemoglobin concentration of infected and uninfected savannah brown bucks

4.3.2.7. Total and Differential White Blood Cell (WBC) Counts:

In the *T. vivax* infected goats, mean total wbc level rose gradually from 15.4 x 10^9/l on day 12 p.i. to 16.9 x 10^9/l by day 26 p.i. (Slight leucocytosis), then dropped to 15.93 x 10^9/l on day 28 p.i and increasing again to 17.5 x 10^9/l by day 35 p.i. (Moderate leucocytosis). It came down to 16.3 x 10^9/l by day 40 p.i. (Fig 4.3.; Appendix 1, Table 7). During the same period the mean lymphocyte level was elevated from 12.94 x 10^9/l on day 12 p.i. to 13.12 x 10^9/l by day 26 p.i. (Moderate lymphocytosis), then dropped to 11.96 x 10^9/l by day 28 p.i., and again rising to 12.34 x 10^9/l by day 35 p.i. (Slight lymphocytosis). All these are indications of the level of immune response of the infected groups and different degrees of recovery.

The mean neutrophil level fell from 3.6 x 10^9/l by day 8 p.i. to 2.18 x 10^9/l by day 12 p.i. (Moderate neutropenia), there after it increased to 3.19 x 10^9/l by day 28 p.i. and later up to 4.64 x 10^9/l by day 35 p.i. (Fairly high neutrophilia) and slightly down to 3.91 x 10^9/l by day 40 p.i. On the other hand, the mean eosinophil level rose sharply from day 12 p.i. and continued in this trend up to day 40 p.i. (117 - 139 respectively) with a peak mean of 255.5 on day 26 p.i. This is an indication of progressive eosinophilia.

The mean monocyte levels also rose from 150 on day 4 p.i. to 326 by day 40 p.i. with the exception of days 12 and 21 p.i. where mean values were 51 and 68 respectively. The monocyte response is that of progressive monocytosis.

The *T. congolense* infected group exhibited increase the mean total leucocyte level from 15.02 x 10^9/l on day 12 p.i. to 17.1 x 10^9/l by day 28 p.i (moderate leucocytosis), falling there after to 13.9 x 10^9/l by day 40 p.i. (Leucocytopenia) (Fig 3; Appendix 1, Table 7). The mean leucocyte level rose slightly, from 11.74 x 10^9/l on day 8 p.i. to 12.69 x 10^9/l by day 10 p.i., but plummeted

to 9.54 x 10^9/l by day 18 p.i. (Severe leucopenia). It then started to rise, reaching 11.1 x 10^9/l by day 21 p.i. and continued up to 13 .5 x 10^9/l by day 35 p.i. (Moderate leucocytosis). The level started to fall down again and by day 40 p.i. was at 10.98 x 10^9/l. The mean neutrophil level fell from 3.6 x 10^9/l on day 2 p.i. to 2.2 x 10^9/l by day 10 p.i (moderate neutropenia). The level later started increasing, reaching 5.01 x 10^9/l by day 21 p.i. (High neutrophilia) and again fell to 2.50 x 10^9/l by day 40 p.i. (Severe neutropenia). There was increase in the mean eosiniphil level between days 14 and 40 p.i. (117 - 139 eosinophils), an indication of slight eosinophilia. Similar increases in mean monocyte levels were observed between days 16 and 40 p.i. (121 - 278), indicating moderate monocytosis.

In the *T. brucei* infected group, the mean leucocyte level rose from 15.03 on day 10 p.i. to 17.24 x 10^9/l by day 28 p.i. (Moderate leucocytosis) and this persisted till day 40 p.i. with mean value of 17.0 x 10^9/l (Fig 3; Appendix 1, Table 7). The mean lymphocyte level rose from 11.95 x 10^9/l on day 18 p.i. to 13.43 x10^9/l by day 40 p.i. (Moderate lymphocytosis). There was a slight fall in neutrophil level from 3.57 - 3.0 x 10^9/l between days 2 and 6 p.i., followed by a rise up to 5.21 x 10^9/l by day 21 p.i. (neutrophilia). By day 40 p.i., the level had fallen to 3.17 x 10^9/6. Mean eosinophil level increased consistently between day 26 to 40 p.i (172 - 291) . The mean monocyte levels (with the exception of high levels on days 14 and 40 p.i. (308 and 226 respectively) and a very low level of 34 on day 28 p.i.) were within the mean for the pre-infection values (Fig 3; Appendix 1, Tables 8 - 11).

There were significant differences between the infected and control groups; the *T. vivax* and *T. brucei* groups and the *T. brucei* and *T. congolense* groups ($p<0.05$ in all cases) but not between the *T. vivax* and *T. congolense* groups ($p>0.05$) with regard to mean leucocyte levels post

infection. The differences in the mean lymphocyte levels of the infected and control groups; *T. vivax* and *T. brucei*; *T. vivax* and *T. congolense* infected groups were highly significant ($p<0.001$ in all cases) but not between *T. brucei* and *T. congolense* groups ($P>0.05$). Similar patterns were obtained for neutrophil values and the mean monocyte level ($p<0.001$) and ($p>0.05$). The differences in the mean eosinophil levels between the infected and control groups were significant ($p<0.001$); that between the *T. vivax* and *T. brucei*; *T. brucei* and *T. congolense* groups were also significant ($p<0.001$ and $p<0.01$ respectively) but not between the *T. vivax* and *T. congolense* groups ($P>0.05$).

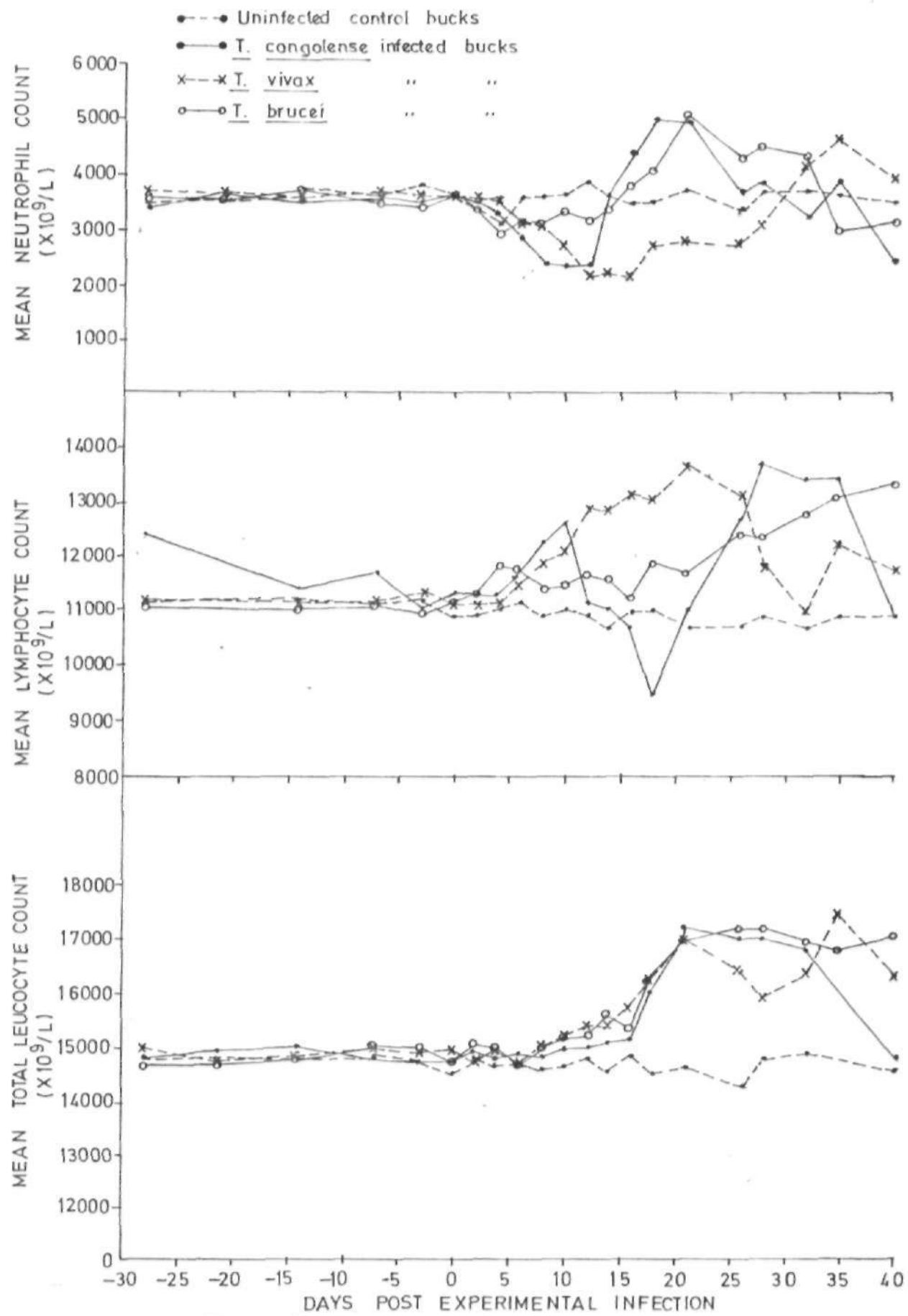


FIG. 4.3. Mean-total leucocyte count, lymphocyte count and neutrophil count of infected and uninfected control savannah brown bucks

4.3.2.8. Observed Clinical Signs:

With the onset of parasitaemia, clinical signs began to manifest, initially mild but increasing in severity as the infection progressed, intermixed with fluctuating features. Initial clinical signs were dullness, reduced feed intake, and later lethargy, anorexia and recumbency in the first week of patency. The mucous membranes initially were highly congested and later became pale after 2 weeks. Animals later became very weak and died. Bilateral epiphora was very much in evidence, especially in the *T. vivax*-infected goats where lacrimation was very pronounced. Wet mounts of tear samples of goats No. 5256 and 5255 (*T. vivax* infected) and goat No. 562 (*T. congolense* infected) were positive for trypanosome parasites. Surprisingly, this phenomena was not observed in any of the *T. brucei* infected groups.

Animals with severe epiphora exhibited evidence of blindness manifested by their bumping into other animals, feeding troughs and the walls of the pens. Most goats, especially in the *T. brucei*-infected groups had their eye-lids stuck together by sero-mucoid ocular discharges. Such goats developed corneal opacity and conjunctivitis, with resultant drastic reduction in feed intake leading to emaciation. These eye lesions could be due to secondary bacterial infection resulting from immunosuppression arising from the trypanosomal infection of the goats.

Edema later developed, affecting mostly the face, knee and hock joits, the lower jaws, scrotal sacs which on palpation elicited sign of pain. These features were severest in the *T. brucei*-infected goats; moderate in the *T. congolense*-infected group and mild in the *T. vivax*-infected group.

All the infected animals developed diarrhoea characterised by watery but non mucoid nor blood stained feces. The diarrhoea was severe in *T. vivax* infected goats and moderate in the *T. congolense* and *T. brucei* infected goats.

The superficial lymph nodes especially the pre-femoral and pre-scapular nodes were prominently enlarged in the infected animals notably those in the *T. brucei* and *T. congolense*-infected group of goats. Wet mounts from lymph nodes aspirates of some of the infected animals were positive for trypanosomes. Goat number 5255 from the *T. vivax*-infected group produced 10 trypanosomes from 20 microscopic fields and dying later in the day with a parasitaemic level of 4+. Also, goat number 5259 and 564 had positive lymph aspirates on days 30, 34 and 21, 23 p.i. respectively (goat 5255 had positive aspirate on day 74 p.i.). in the *T. congolense*-infected group, goats number 562, 551 and 557 had positive lymph node aspirates of an average of 1+ on day 9, 12, 14, 17, 18 and 20, 22 p.i. respectively. Goats number 565, 570 and 563 had positive lymph node aspirates of 1+, 1+ and 2+ on day 20, 38, 39; 21, 22, 45 and 46 p.i. respectively. All the positive lymph aspirates were obtained on days coinciding with high parasitaemia of 4+ or more in the animals involved.

Observed respiratory problems were dyspnea, cough, serous nasal discharges in all the infected goats, being most severe in goat number 5259 (*T. vivax*-infected); 559 and 557 (*T. congolense*-infected) and A_1 and 570 (*T. brucei*-infected).

Signs of central nervous system disorder were observed from day 30 p.i. onwards, being more pronounced in the *T. brucei*-infected goats. These signs included limb paralysis especially the hind limbs; twitching of the eye-lids and ears; trembling, staggering, torticollis.

Prior to death, the goats involved became recumbent and hypersensitive as shown by their padding movements when excited, a situation which can make one to confuse thier condition with cowdriosis.

Mean weekly body weights in both the control and infected groups increased, though more signicantly in the control group, up to the 5th week p.i., and thereafter, a progressive weight loss follwed though feed consumption was not drastically reduced in the infected groups. The mean weight loss was more pronounced in *T. vivax*-infected group; moderate in the *T. congolense*-infected group and mild in the *T. brucei* (Fig. 4.4.; Appendix 1, Tables 12a,b). The mean weekly body weights and mean weight gains of the infected animals were significantly different from the control animals ($p<0.05$). However, the differences among the infected groups were not significant ($p>0.05$).

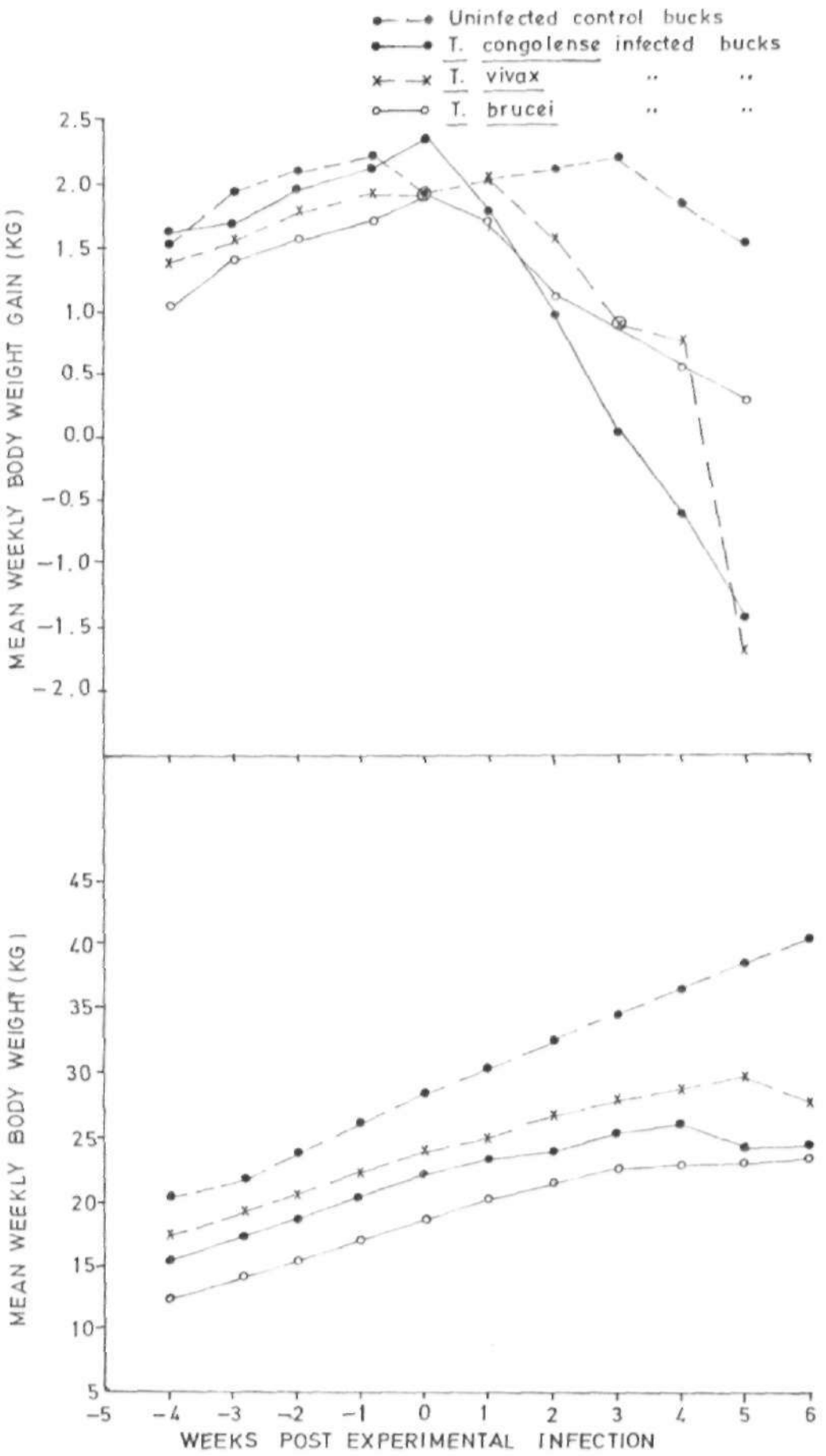


FIG. 4.4 Mean-weekly body weights and weekly body weight gains of infected and un-infected control savannah brown bucks

TABLE. 4.1. PRE-EXPERIMENTAL INFECTION BASELINE VALUES FOR SAVANNAH BROWN GOATS (BUCKS)

PARAMETER		RANGE	MEAN	SD ±
I.				
Rectal Temp. °C		38.20 - 38.41	38.24	0.22
PCV %		28.54 - 32.00	31.45	1.36
Haemoglobin gm/dl		9.50 - 10.26	10.05	0.65
Total Plasma Protein gm %		7.80 - 8.82	8.10	0.30
Total RBC Count 10^{12}/l		6.99 - 7.89	7.65	0.81
" WBC "	10^9/l	14.80 - 15.03	14.95	250.05
Lymphocyte	10^9/l	10.91 - 11.31	11.05	280.00
Neutrophil	10^9/l	3.23 - 3.76	3.45	275.40
Monocyte	10^9/l	0.00 - 0.15	0.069	80.20
Eosinophil	10^9/l	0.00 - 0.13	0.074	56.87
Basophil	10^9/l	0.00 - 0.00	0.00	-
Band Cells	10^9/l	0.00 - 0.00	0.00	-

I. - Mean values for data collected for 4 weeks prior to experimental infection.

TABLE. 4.2. RESPONSE OF SAVANNAH BROWN GOATS TO TRYPANOSOME INFECTION.

Experimental Group	Animal Number	Pre-patent Period(Days)	Duration of Patency(Days)	Duration of Survival Post Infection(days)	Level of Parasitemia at Time of Death	Phase of infection at time of death
T. vivax	571	2	5	7	3+	Acute
	567	2	6	8	-	"
	566	2	13	15	-	"
	5256	2	16	18	4+	Sub-acute
	5255	2	18	20	4+	" "
	560	2	31	33	3+	" "
	564	4	37	41	-	Chronic
	5259	2	72	74	2+	"
		(2.25)				
T. congolense	A_2	4	5	9	4+	Acute
	558	4	5	9	4+	"
	563	4	5	9	>4+	"
	562	4	9	13	4+	"
	551	4	15	19	4+	Sub-acute
	557	4	19	23	3+	" "
	573	4	31	35	-	Chronic
	559	7	67	74	-	"
		(4.38)				
T. brucei	A_1	8	3	11	3+	Acute
	5253	2	13	15	3+	"
	565	4	17	21	4+	Sub-acute
	5258	4	25	29	4+	"
	570	4	36	40	4+	"
	5252	4	41	45	-	Chronic
	569	4	43	47	4+	"
	5251	4	54	58	-	"
		(4.25)				

TABLE 4.3. PARASITAEMIA IN TRYPANOSOME INFECTED SAVANNAH BROWN GOATS.

T. vivax			T. brucei			T. congolense		
DPP	HPP (+)	NPW	DPP	HPP (+)	NPW	DPP	HPP (+)	NPW
4	2.6		4	2.0		8	3.4	
8	3.0		10	3.0		14	3.0	
16	3.0		21	2.2		18	2.0	
32	2.0	4	28	2.0	4	28	4.0	4

4.3.3. PATHOLOGY

Goats in all the infected groups died either in the acute, or subacute or chronic stage of the infection whilst goats from the uninfected control group were euthanised at specific periods(weeks 2, 4, 6, 8 and 11 p.i.) for comparison with the dead infected goats.

4.3.3.1. Gross-Pathological Findings:

Some similarities were observed between lesions in acute and subacute phases whereas pronounced differences occured between them and the chronic cases in virtually all the infected goats. In acute and subacute cases, carcasses appeared normal to slightly cachetic and anaemic with no or minimum serous atrophy of adipose tissue whilst chronic cases were cachetic with severe serous atrophy of adipose tissue. In the acute and subacute cases there was slight lymphadenopathy especially of the superficial lymph nodes in the *T. vivax* and *T. congolense* infected goats but moderate in the *T. brucei* infected goat. In the chronic phase, lymphadenopathy was pronounced in the *T. vivax* group and more so in the *T. brucei* and *T. congolense* infected goats. Suprisingly, none of the lymph node impression smears was positive for trypanosomes, despite the fact that some of the infected goats had produced lymph node aspirates which were trypanosome positive.

Petechial to echymotic haemorrages were seen in the intestinal mucosae especially in the acute cases in *T. vivax* infected goats, and also some *T. congolense* infected goats (# 558 and 563), and *T. brucei* infected goat (# A1) which in addition had blood tinged ingesta in the abomasum. Similar haemorragic lesions were observed in the messenteric fat which also had undergone severe serous atrophy in the chronic cases.

The liver showed no abnormal lesions in the acute cases in all the infected goats but congested in the subacute cases and enlarged , firm with petechial to echymotic haemorragic in the chronic cases in the *T. congolense* and *T. brucei*-infected goats but not in the *T. vivax*-infected goats. Gall-bladders were normal to slightly enlarged in the acute and subacute cases in all infected goats and highly distended with tenacious bile in the chronic cases. However, *T. brucei*-infected goat #5251 showed no gross lesions in the gall bladder even though it died in the chronic-stage.

There were no observable gross kidney lesions in the *T. vivax*-infected goats in both the acute and subacute cases whereas those of *T. congolense* and *T. brucei* infected goats showed petechial to echymotic haemorrages in the cortex and medulla respectively. However, the chronic cases in all the infected groups had echymotic and petechial haemorrages of the cortex and medulla regions respectively .

Spleens were enlarged and highly congested in the acute and subacute cases in all the infected goats with the exception of animal # 5251 from *T.* brucei-infected group. In the chronic cases, the spleens were also enlarged with prominent follicles and congested pulps which were prominent particularly in the *T. brucei* and *T. congolense*-infected goats.

Lungs were highly congested and filled with frothy exudates; whilst the bronchial and tracheal mucosae showed echymotic haemorrhages in the acute, subacute and chronic cases in *T. congolense*-infected goats, in the acute and subacute cases in *T. brucei*-infected goats with *T. vivax*-infected goats exhibiting similar but milder lesions in all the stages. Also, with the exception of goat # 5259 from the *T. vivax* group which was positive for *Pasteurella haemolytica*, all the other lung samples submitted for microbiological investigation were negative.

The volume of fluid aspirated from the pericardial sac in the acute and subacute cases in all the infected - goats was between 20 - 25 millilitres, compared to 5 - 10 mls in the uninfected control groups, with the volume rising to as much as 45 mls in the chronic cases especially in the *T. congolense* and *T. brucei*-infected goats. Serous atrophy of the coronary fat was severe in the subacute and chronic cases of *T. brucei*-infected goats, moderate in the *T. congolense* and slight in the *T. vivax*-infected goats respectively. Also, there were petechial to echymotic haemorrhages in the heart muscles in the acute and subacute cases in all the infected goats most prominently in the *T. congolense* and *T. brucei*-infected goats. Haemorrhagic foci were observed in the ventricles in *T. brucei* and *T. congolense*-infected goats. There were also diffused haemorrhages in the auricles in the chronic cases of all the infected goats.

The blood capillaries in the brain and pituitary glands in the acute and subacute cases were congested in all the infected goats most especially in the *T. congolense*-group. There were petechial and echymotic haemorrages especially in the cerebral cortex and cerebellum in the chronic cases in the *T. congolense* and *T. brucei*-infected goats. The meninges in *T. brucei*-infected goat # 5251 was enlarged and tough.

Scrotal sacs and testicles were prominently thickened and enlarged due to edema in all the infected goats in the subacute cases. Testicular atrophy evidenced by decrease in size of the testicles of the infected goats compared to those of uninfected control goats was clearly evident in the *T. vivax* and *T. congolense*-infected goats in the chronic cases. There were areas of calcification in the testicles of goat # 5251 (*T. brucei*-infected) and # 559 (*T. congolense*-infected) as indicated by the gritty sound produced when cut through in the chronic cases.

Conjuctivitis was observed in five goats namely # 5255, 5256 (*T. vivax*-infected), 551 (*T. congolense*-infected), 565 and 5258 (*T. brucei*-infected) all of which died in the subacute stage of infection. Corneal opacity developed in the chronic cases in all the infected goats.

The bone marrow (from the long bones notably humerus and femur) in all the infected goats in the acute and subacute cases appeared congested, especially in the *T. vivax*-infected goats. In the chronic cases, the bone marrows were pale yellow in appearance especially in goat # 559 (*T. congolense*-infected) and # 5259 (*T. vivax*-infected) though there were some focis of haemorrage.

Blood smears from goats necropsied about an hour after death (# 5256, 560 - *T. vivax;* 551, 557 - *T. congolense* and 570, 5252, 569 - *T. brucei*-infected respectively) were positive for trypanosome parasites but at very low parasitaemic level of between 1 to 5 trypanosomes per 20 microscopic fields. The blood smears were made from heart, aorta, and jugular vessels of infected goats. All the other impression smears were negative for trypanosome parasites.

4.3.3.2. Histopathological Findings:

The prominent organs/tissues, lesions observed and the trypanosome species associated with are summarised below. The observed histopathological changes which bore some degree of similarity in all the infected goats and were mostly observed in the chronic phases of the infection.

4.3.3.2.1. Lymphoid Organs (Spleen and Lymh nodes)

The characteristics lesions observed in these organs were lymphoid hyperplasia (lyymphocyte proliferation) in thr chronic cases of all the infected groups.

4.3.3.2.2. Kidney.

In the *T. vivax* infected goats the most prominent kidney lesions were non-suppurative focal and diffuse areas of necrosis of the glomeruli, proximal and distal convoluted tubules and also the collecting tubules (Plate 4.1.3.).

The *T. brucei* infected goats exhibited slight adhesion of the parietal layer of the glomeruli to the Bowman's capsule, slight proliferation of fibrous connective tissue coupled with non-suppurative focal necrosis of the proximal and distal convoluted tubules, periglomerular and inter tubular mononuclear cellular infiltration (neutrophils and monocytes)(Plate 4.1.4.).

In most of the *T. congolense* infected goats there were areas of diffuse non-suppurative necrosis of the proximal and distal convoluted tubules, proliferation of fibrous connective tissue at the cortico-medullary junction especially in animal # 559 and periglomerular and intertubular mononuclear cellular infiltration (Plate 4.1.2.).

4.3.3.2.3. Liver.

There were areas of diffuse suppurative necrosis of the hepatocytes and also dilated sinusoids in the *T. vivax* infected goats (Plate 4.2.2.).

Focal areas of non-suppurative necrosis of the hepatocytes were prominent in the *T. brucei* infected goats (Plate 4.2.3.).

In the *T. congolense* infected goats significant lesions were diffues necrosis of the hepatocytes, dilated sinusiods, mononuclear cellular infiltration (neutrophils and monocytes) and fibrous connective tissue proliferation in animal #559 (Plate 4.2.3.).

4.3.3.2.4. Heart.

The *T. vivax* infected goats exhibited areas of focal necrosis in the myocardium with mononuclear cellular infiltration into the necrotic areas and the inter-fiber spaces (Plate 4.5.3.). These were prominent in goats #5255.

In the *T. brucei* infected goats there were areas of focalised necrosis in the myocardium, mononuclear cells infiltration of the inter-fiber spaces. There were also congested blood vessels in goat #5251 (Plate 4.5.2.).

The major microscopic lesion observed in the *T. congolense* infected goats were necrosis of myocardial fibers, mononuclear cellular infiltration into the necrotised areas and also the inter-fiber spaces (Plate 4.5.3.).

4.3.3.2.5. Lung.

No significant lesion were observed in the lungs of *T. vivax* infected goats.

The major lesion in the *T. brucei* infected goats were oedema in the alveoli prominently in goat #5252; mononuclear cellular infiltration into the alveoli and inter-alveoli spaces especially in goat #5251 (Plate 4.3.3.).

In the *T. congolense* infected goats there were mononuclear cellular infiltration into the alveoli and inter-alveolar septae (Plate 4.3.2.).

4.3.3.2.6. Testicles.

There was disorganisation and distortion of the basal lamina of the seminiferous tubules in the *T. vivax* infected goats (Plate 4.4.1. and 4.4.4.).

In the *T. brucei* infected goat there areas of non-suppurative necrosis in the seminiferous tubules coupled with proliferation of fibrous connective tissue into the necrotised area (Plate 4.4.2.).

The *T. congolense* infected goats exhibited non-suppurative necrosis of the seminiferous tubules as well as proliferation of fibrous connective tissue into the necrostised areas to replace the seminiferous tubules (Plate 4.4.3. and 4.4.5.).

4.3.3.2.7. Other organs.

In all the infected goats there were no significant findings in the brain pituitary bodies, bone marrow, skeletal muscles, eyes and intestines.

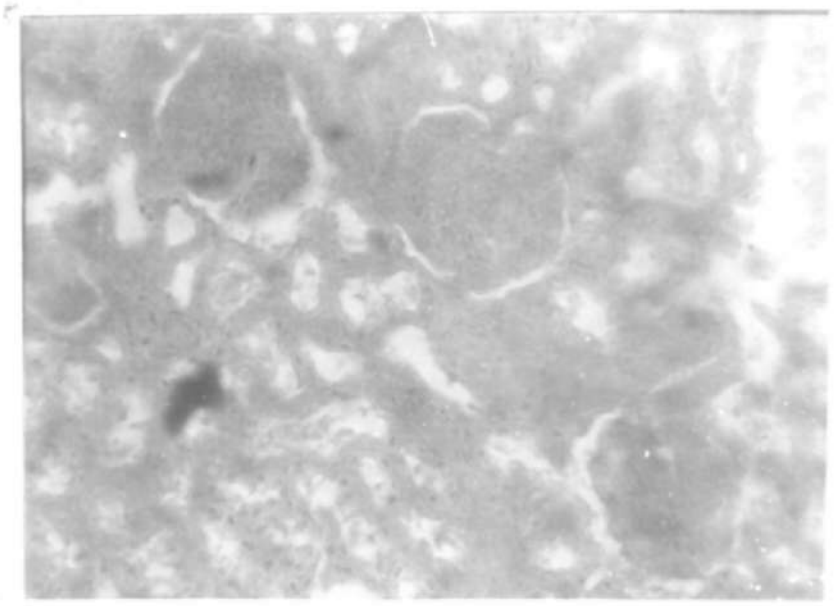


Plate 4.1.1. A section of the kidney with no significant findings (Control S.B. bucks). H & E. x400

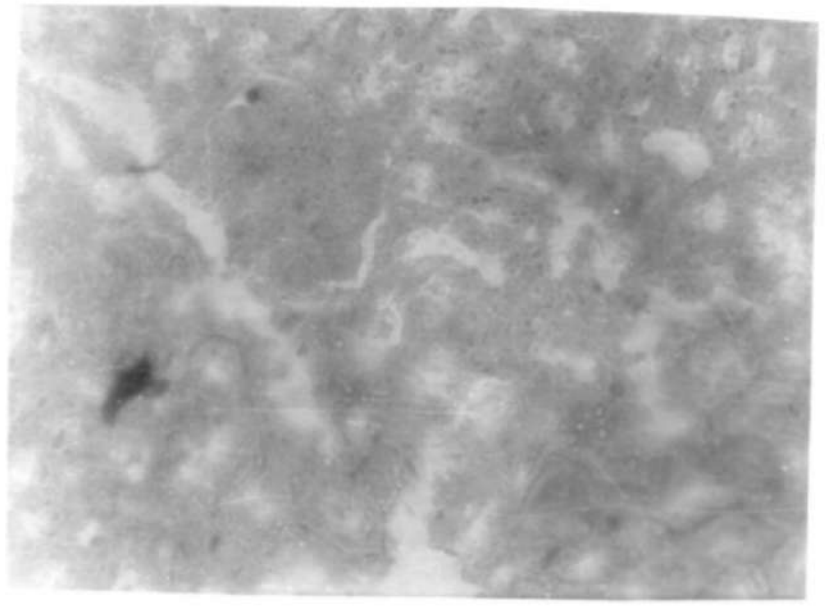


Plate 4.1.2. A section of the kidney. Note non-suppurative necrosis of the glomelurus and the renal tubules. Also note adhesion of the glomerulus to the Bowman's capsule (*T. congolense*-infected S.B. bucks). H & E. x 400.

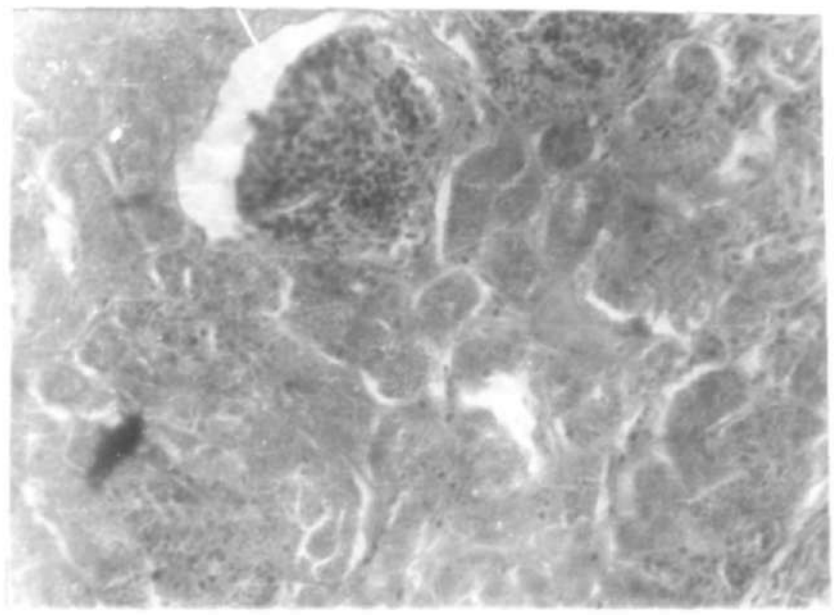


Plate 4.1.3. A section of the kidney. Note necrosis of the glomerulus and the renal tubules. Also note the adhesion of the Bowman's capsule by the right side. (*T. vivax* infected S.B. bucks). H & E. x400.

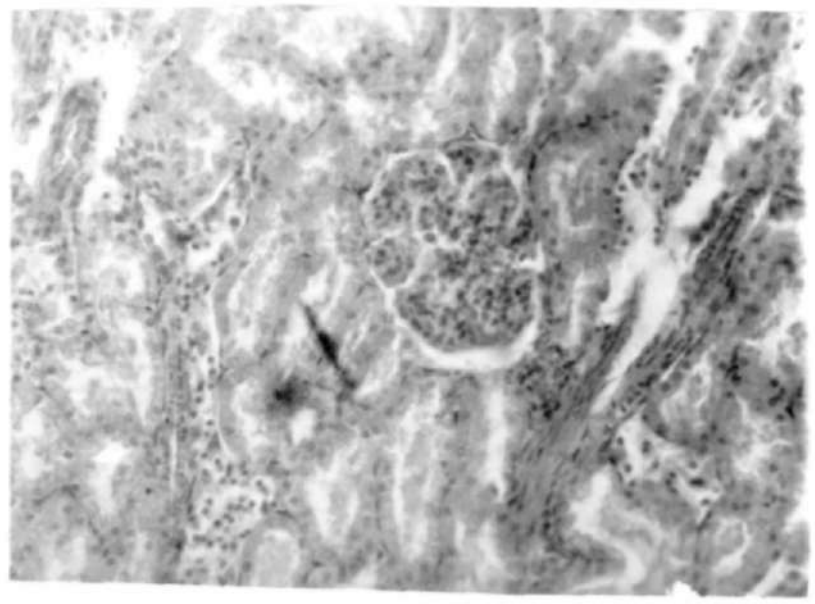


Plate 4.1.4. A section of the kidney. Note necrosis of the renal tubules with some intertubular mononuclear cellular infilteration. Also note the adhesion of the Bowman's capsule by the right side. (*T. vivax* -infected S.B. bucks). H & E. x400.

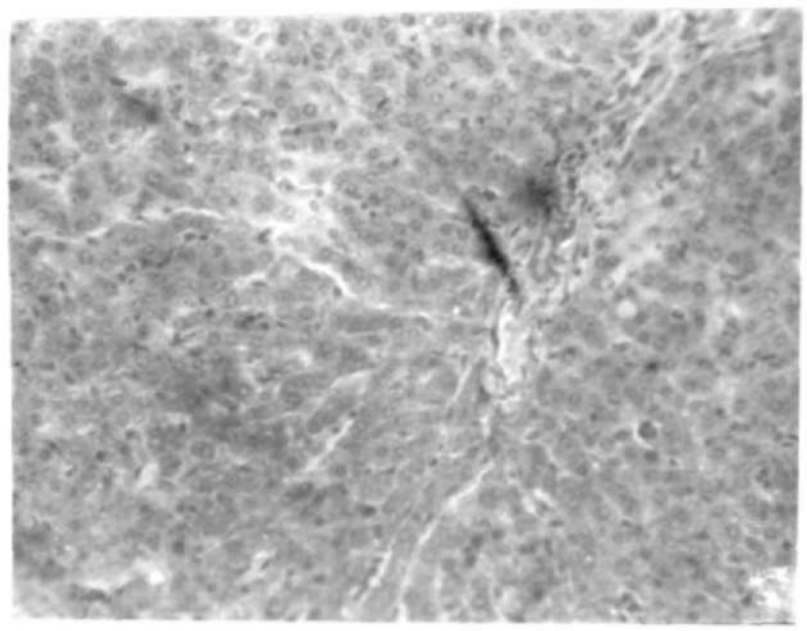


Plate 4.2.1. A section of the liver with no significant findings (Control S.B. bucks). H & E. x400.

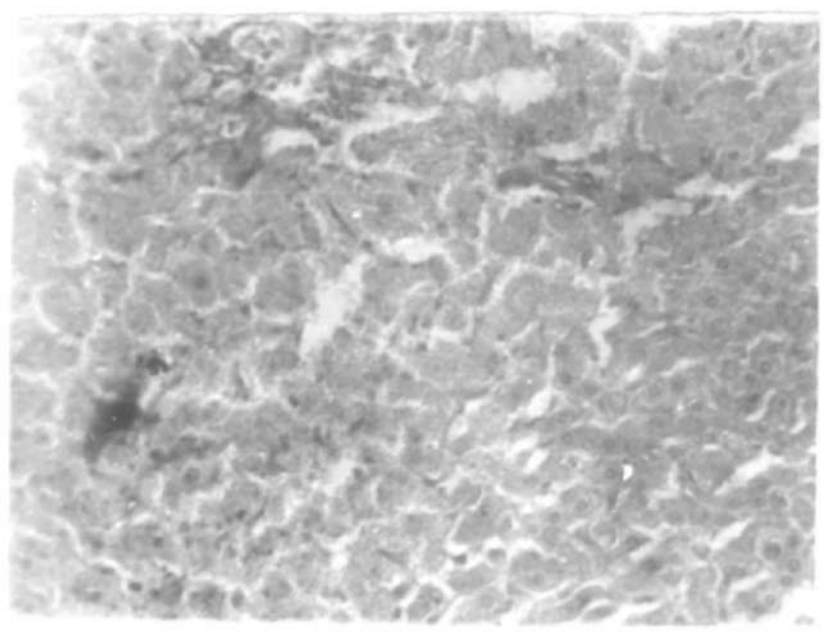


Plate 4.2.2. A Section of the liver. Note diffuse necrosis of the liver at the portal area (*T. vivax* infected S.B. bucks) H & E. x 400

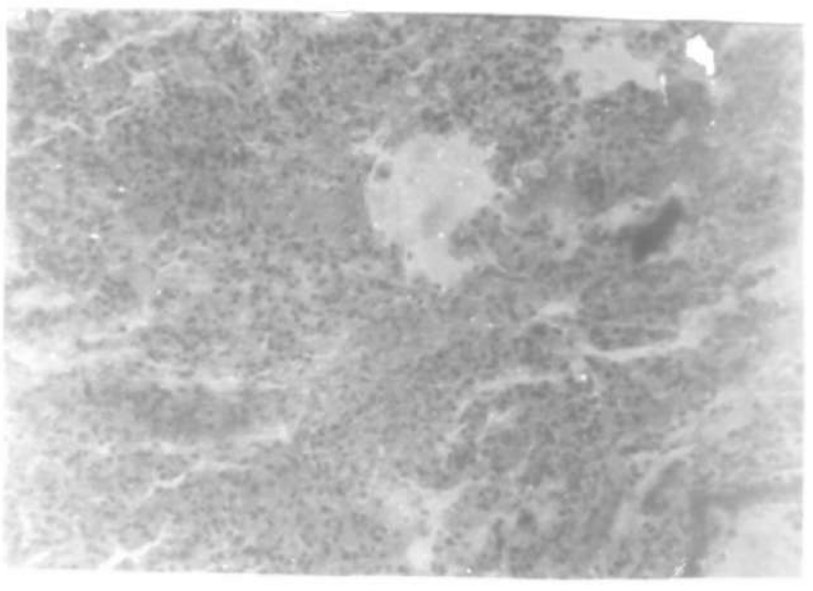


Plate 4.2.3. A Section of the liver. Note necrosis of the lobe especially at the middle portion as well as mononuclear cellular infiltration. (*T. congolense* as well as *T. brucei*- infected S.B. bucks). H & E. x 400.

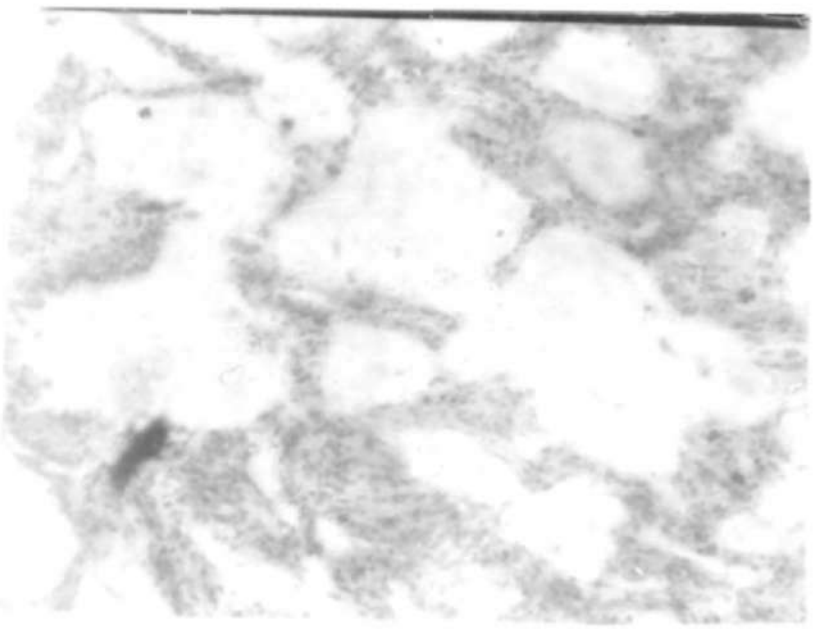


Plate 4.3.1. A section of the lungs with no significant findings (Control S.B. bucks). H & E x400.

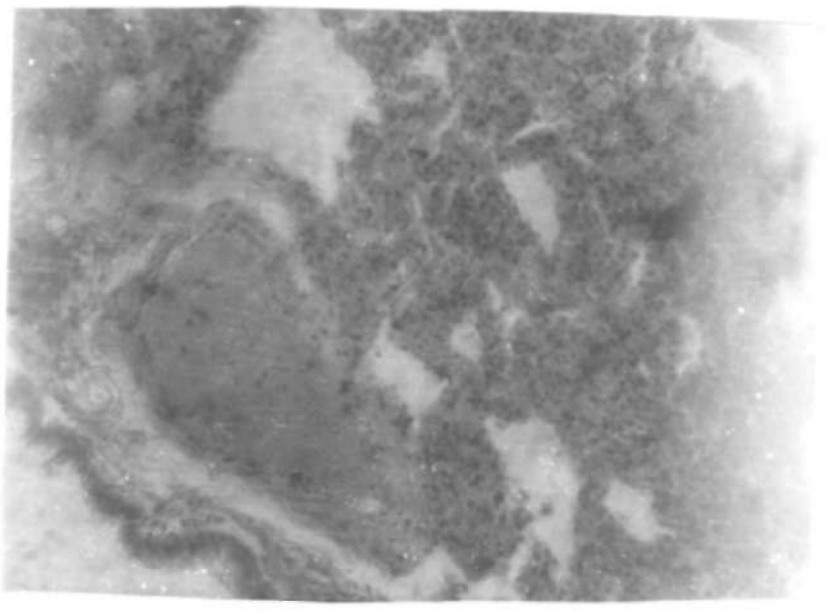


Plate 4.3.2. A section of the lung. Note mononuclear cellular infiltration in the interalveolar spaces and some alveoli (interstitial pneumonia) and congested blood vessels at the top right corner (*T. congolense*-infected S.B. bucks) H & E x400.

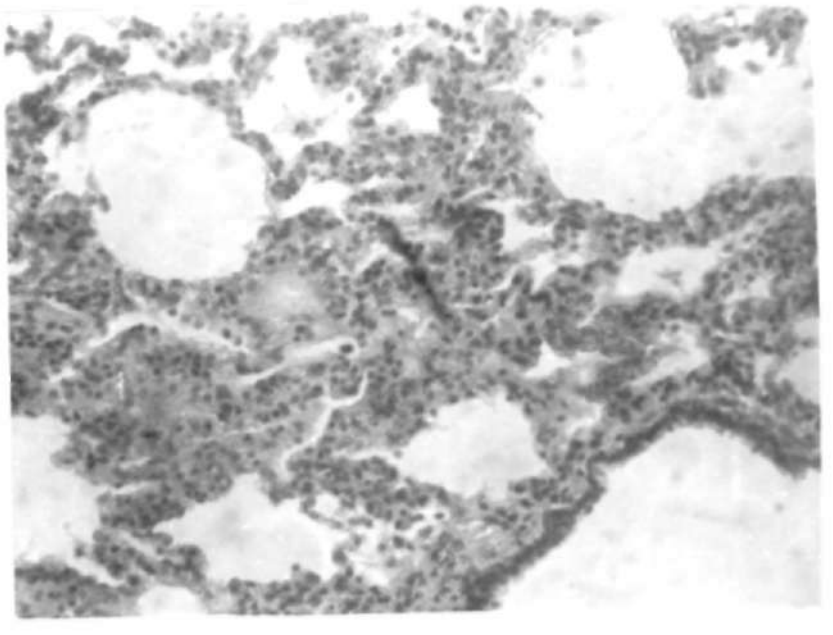


Plate 4.3.3. A section of the lung. Note mononuclear cellular infiltration in the interalveolar spaces (interstitial pneumonia). (*T. brucei*- infected S.B. bucks) H & E x400.

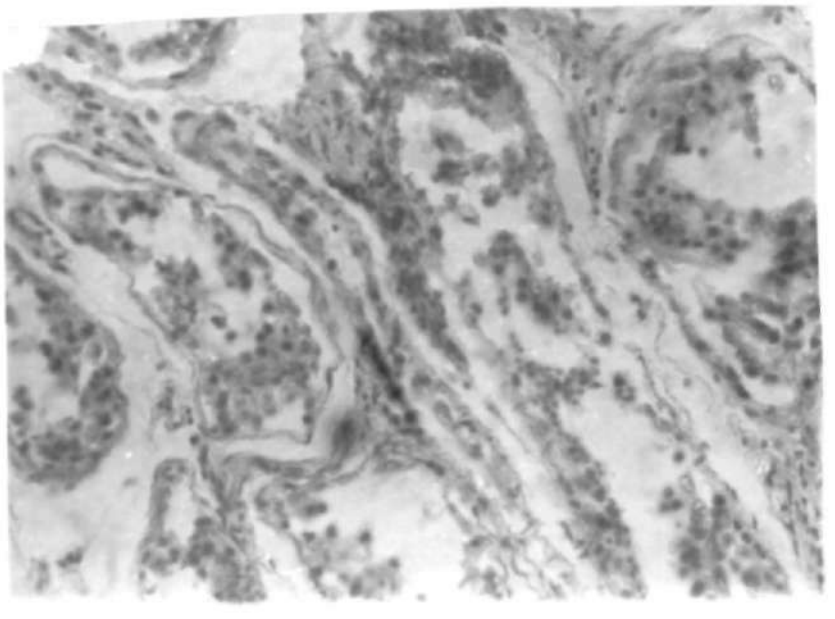


Plate 4.4.1. A section of the testis. Note disorganised seminiferous tubules (*T. vivax*- infected S.B. bucks) H & E x400.

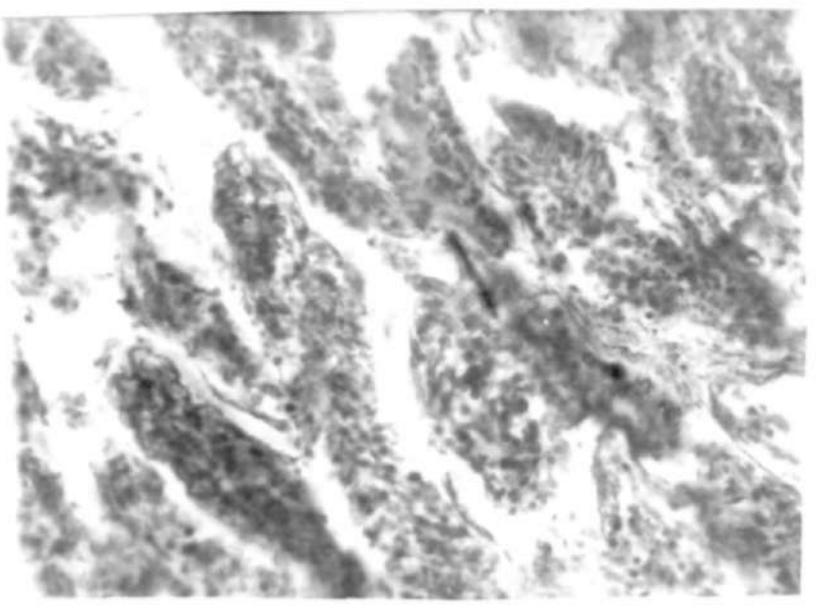


Plate 4.4.2. A section of the testis. Note the non-suppurative necrosis of the seminiferous tubules (*T. brucei*-infected S.B. buck) H & E x400.

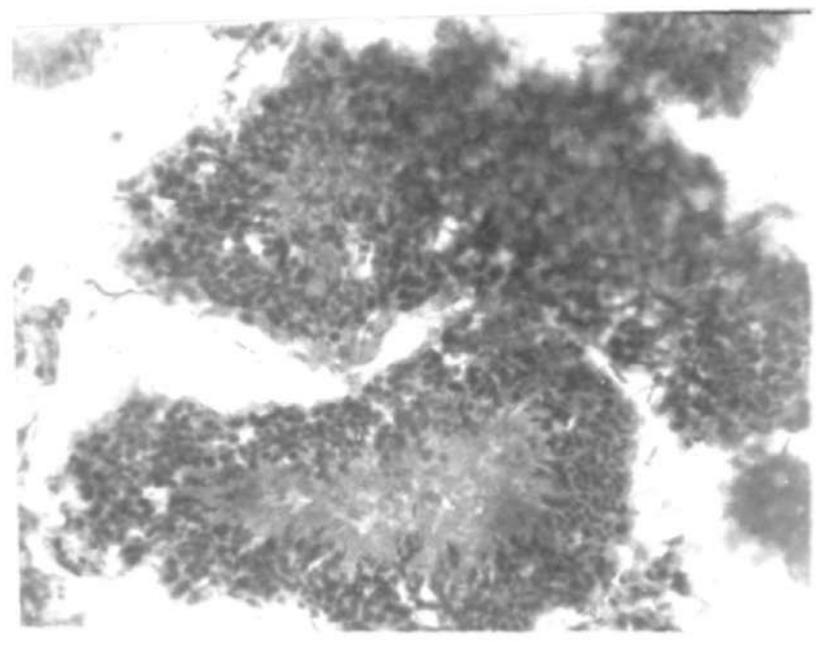


Plate 4.4.3. A section of the testis. Necrosis of the seminiferous tubules. Note fibrosis at the intertubular spaces (*T. congolense*-infected S.B. bucks) H & E x400.

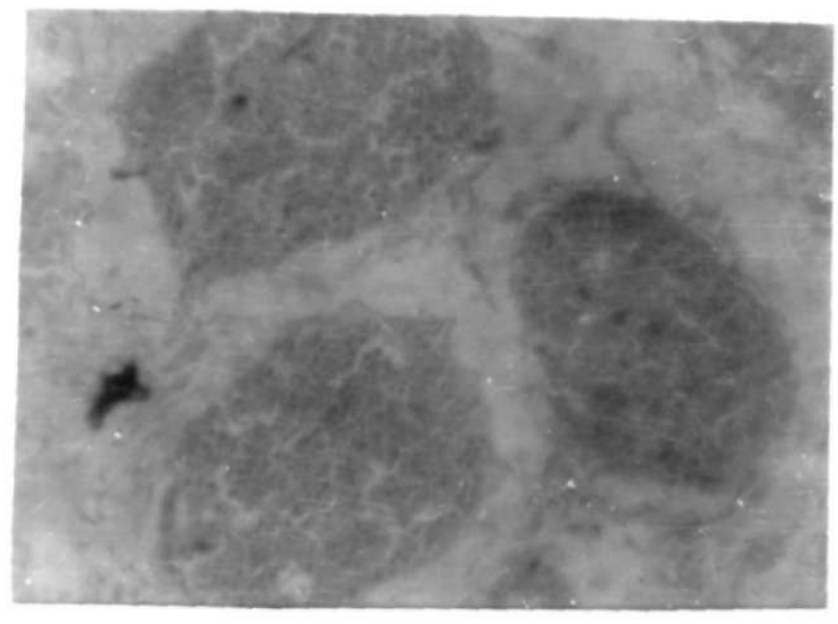


Plate 4.4.4. A section of the testis. Note severe necrosis of the seminiferous tubules and marked intertubular fibrosis (*T. vivax*-infected S.B. bucks) H & E x400.

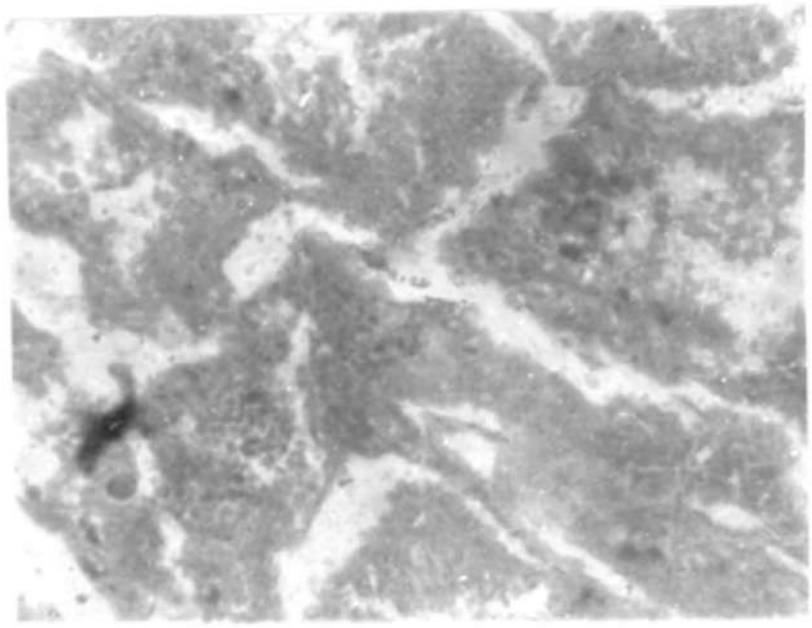


Plate 4.4.5. A section of testis. Note necrosis of the seminiferous tubules (*T. congolense*-infected S.B. bucks) H. & E. x400.

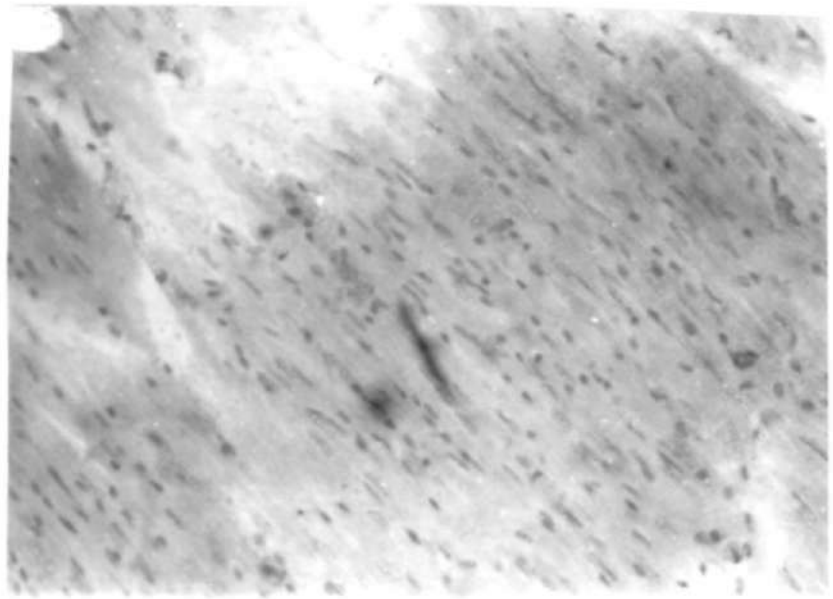


Plate 4.5.1. A section of the heart(myocardium) with no significant findings (Control S.B. bucks)

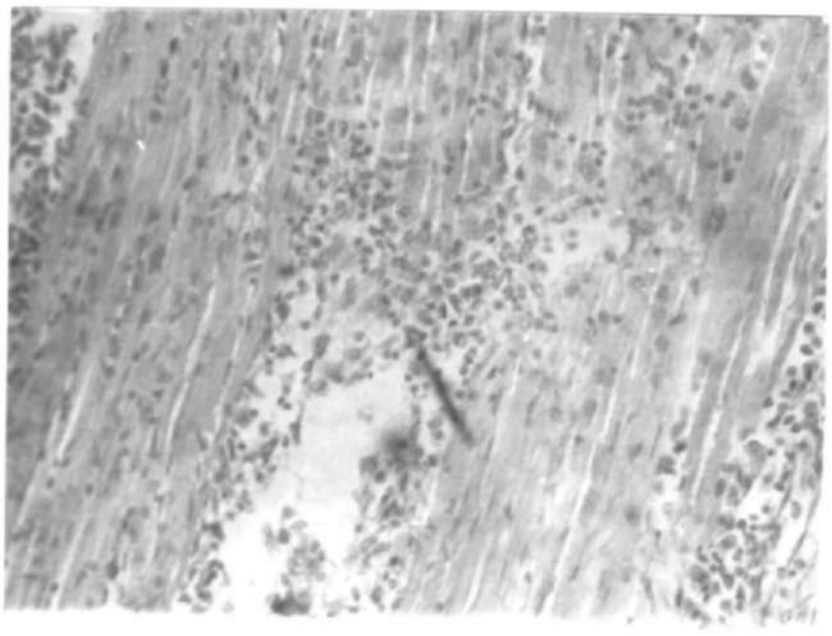


Plate 4.5.2. A section of the heart (myocardium). Note necrosis of the myocytes and mononuclear cellular infiltration (myocarditis) (*T. brucei*-infected SB bucks). H & E x 400

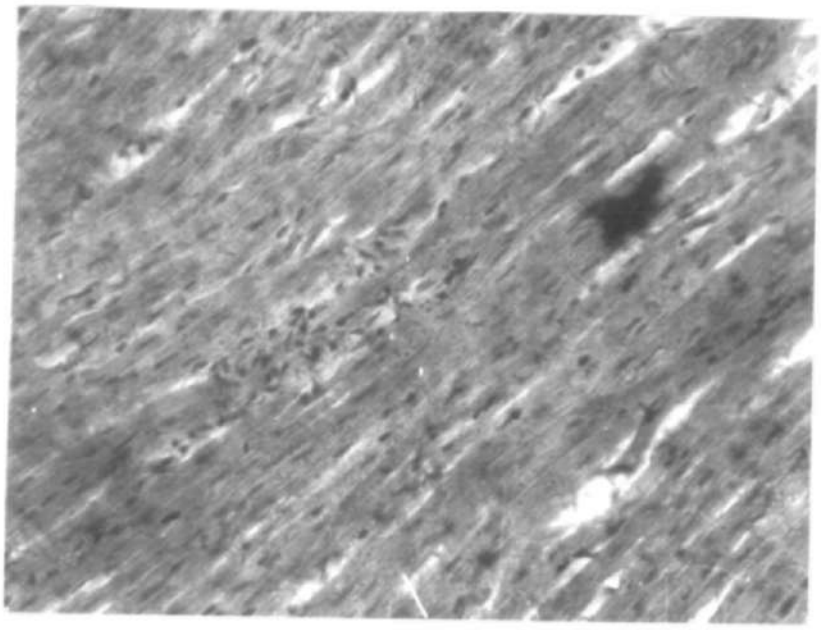


Plate 4.5.3. A section of the heart (myocardium). Note mild necrosis of the myocytes and few mononuclear cellular infiltration (mild myocarditis) (*T. vivax* as well as *T. congolense*-infected SB bucks). H & E. x 400

4.4. DISCUSSION

Increase in body temperature (pyrexia) indicates metabolic disorder due to activities of infectious agents which in this case were trypanosomes. Pyrexia in this study (which was due to circulating trypanosomes and their by-products) mostly coincided with high levels of parasitaemia in all the infected groups, in agreement to similar observations made by Anosa and Isoun (1974; 1980). In other situations pyrexia was not concurrent to high parasitaemia as also reported by Anosa and Isoun (1974). *T. vivax* elicited the highest peaks of pyrexia among the three species of parasites and this may indicate the level of virulence of *T. vivax* in the Savannah Brown goat.

The mean prepatent period (pp) of 2.25 days in the *T. vivax*-infected goats agrees with similar findings made in *T. vivax*-infected Uda rams (Maikaje *et. al.*, 1991), *T. vivax*-infected yankassa sheep (Saror, 1980), *T. vivax*-infected calves (Esievo *et. al.*, 1982); but at variance with 10.0 days reported by Edwards et. al. (1956a) in *T. vivax*-infected goats and 16 days in *T.vivax*-infected sheep and goats (Stephen, 1970) and 5 days in *T. vivax*-infected calves (Maxie *et. al.*, 1979).

Maxie *et. al.* (1979) observed a pp of 5 days for *T. congolense*-infected calves, which agrees with the observed value of 4.38 days in this study, which however contrast the value of 17 days reported by Edward *et. al.* (1956a) in *T. congolense*-infected goats, and 7.8 days in WAG sheep as reported by Kalu (1983). According to Maclennan (1970), the pp depends on the nature and species of the trypanosome, dose of the infective organism, route of infection, and the plane of nutrition provided for the host. In this study, the only variation was in the species of the trypanosome. Thus this study confirms the observations of most investigators that *T.vivax* has the shortest pp compared to *T.congolense*. Though information on PP of *T. brucei* is scarce, the

result of the study indicates that it is longer than that of *T. vivax* but comparable to that of *T. congolense*.

Edward *et. al.* (1956a) and Ikede (1981) reported higher parasitaemic peaks for *T. vivax* than *T.congolense*, with minimum peak for *T. brucei* in the early phase of infection, but in this present study, similar levels of parasitaemic peaks were produced by both *T. vivax* and *T. congolense* during the early phase of infection. Also, *T.brucei* produced pronounced peaks, indicating that the SBG succumbed fairly equally to all the three species of trypanosomes used in this study. The shorter interval between parasitaemic waves exhibited by all the species in the early phase of the infection is an indication of much faster multiplication during this period.

Although the infection became patent earlier in the *T. vivax* group, followed by both the *T. congolense* and *T. brucei* groups, *T. brucei* produced a shorter course of the disease (58 days). This is an indication that *T. brucei* could be economically important to goats in the same way that *T. vivax* and *T. congolense* were previously regarded. The observed course of 10.5 weeks for the *T. vivax*-infected goats in this study is shorter than the 12 weeks reported for *T. vivax*-infected Uda rams by Maikaje *et. al.* (1991), further showing that SBG succumbs to *T. vivax* much more easily than Uda sheep, both of which are found predominantly in the Northern Guinea Savanna vegetational zone. The rapid death within 10.5 week of all the infected goats in this study is a pointer to the fact that all the three species of trypanosomes are of equal economic importance in the SBG, an observation which is at variance with the reports of Griffin (1978), Leeflang (1978) and Anosa (1983) all of whom stated that *T. vivax* is the most pathogenic trypanosome in West Africa, followed by *T. congolense*; with *T. brucei* as the least, if any at all.

The observed acute, sub-acute and chronic phases within a period of 74 days agrees with similar observations by Edward *et. al.* (1956a); Anosa and Isoun (1974); Griffin and Allonby (1979d); Saror (1980) and Maikaje *et. al.* (1991). The fact that all *T. brucei*-infected goats died within 56 days, compared to 74 days in the *T. vivax* and *T. congolense*-infected goats further shows the susceptibility of the SBG to *T. brucei*, a fact usually overlooked or discounted by other investigators. This is also an indication that SBG is highly susceptible to trypanosomosis due to these three pathogenic specie and therefore needs to be protected from the disease like the zebu cattle found in the same geographical or vegetational zone as the SBG in Nigeria.

The faster fall in the pcv of *T. vivax* and *T. brucei*-infected goats may be due to the appearance of these two parasites in the peripheral circulation earlier than *T. congolense*. The anaemia in animal trypanosomosis has long been shown to depend on the magnitude and duration of parasitaemia as indicated during the first 8 days pi in this work. Further support to this is shown by the marked drop in pcv between days 8 and 18 pi following the first and higher peak of *T. congolense*. But despite the highest fall in pcv of the *T. congolense*-infected animals in the initial stage, the pcv recovery seems to be highest in this group than the other infected groups. The only plausible explanation for this could be that the trypanosomes appearing in the peripheral circulation after successive parasitaemic waves may not be as virulent as the initial infecting trypanosomes since it is expected that new variants of trypanosomes follow successive, parasitaemic waves which is a characteristic of the phenomenom of antigenic variation. The variation in the mean pcv and RBC values was similar in all the infected groups. However, the variation in the RBC level in the *T. brucei* infected group was low compared to the other two infected groups and this could be due to low sialidase activity in *T. brucei* and hence slower rate of RBC removal in the *T. brucei*

infected group. This tends to agree with the observations of Muller *et. al.* (1983b) that the amount of sialic-acids released from the RBC surface decided the binding and phagocytosis of sialidase treated erythrocytes. However, this is not strongly supported by the sharp fall in pcv. Pyrexia as observed in this study may be due to the circulating trypanosomes and their by-products and this could play a significant role in the process of haemolysis and consequently the pcv level.

The severe anaemia exhibited in the acute phase of the infection in all the infected groups agrees with observations of Anosa and Isoun (1974; 1980) and Maikaje *et. al.* (1991) in *T. vivax*-infected small ruminants. The anaemia in this study was classified based on mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) values. All the infected groups developed normocytic-normochromic anaemia in the first 14 days p.i.. This later became microcytic-normochromic by day 18 pi in the *T. vivax* and *T. congolense*-infected groups and by 32 pi in the *T. brucei* group. Those surviving up to and beyond 67 day pi developed microcytic microchromic anaemia. These observations do not agree with the observations of Maxie *et. al.* (1974) and Anosa and Isoun (1980) who reported macrocytic-normochromic and normocytic-normochromic anaemia in the acute and chronic phases respectively in *T. vivax*-infected goats and sheep. However the type of anaemia observed in the acute phase agrees with the findings of Saror (1979) who also reported an initial normocytic-normochromic anaemia in *T. vivax*-infected cattle but which later developed macrocytic-normochromic anaemia. Also the observed form of anaemia in the *T. congolense* and *T. brucei*-infected goats is not in agreement with the macrocytic-normochromic anaemia observed in *T. congolense* and *T. brucei*-infected cattle (Roberts and Gray, 1973) and Scottish Blackhead sheep (Katunguka-Rwakishaya *et. al.*, 1992) and early macrocytic-normochromic which later became normocytic-normochromic in *T. congolense*-

infected cattle (Fiennes, 1950; 1954; Naylor, 1971). The reported macrocytic-normochromic anaemia in *T.congolense*-infected WAD and Red Sokoto goats also do not agree with the observed findings in this study. This is an indication that recovery was slower in the present study compared to the previous ones.

The observed anisocytosis in the *T. brucei*-infected goats and the basophilic stipplings (indicating degenerative erythrocyte changes) in the *T. vivax* and *T. congolense* infected goats in the chronic stage of the infection are not common occurrences in trypanosomosis. All these point to severe anaemia as indicated by the very low pcv of 9 - 10% in such animals.

The leucocytosis in the *T. congolense*-infected goats observed in this study agrees with similar findings in *T. congolense*-infected WAD goats (Adah *et. al.*, 1993) and Scottish-Blackhead sheep (Katunguka-Rwakishaya *et. al.*, 1992); but, whereas it was due to neutrophilia and later lymphocytosis in the present study, those of Adah *et. al.* (1993) and Katunguka-Rwakishaya *et. al.* (1992) arose from lymphocytosis only. The observed lymphocytosis is however in agreement with the nature of leucocytosis observed in the *T. vivax*-infected goats in the present study, but contrary to that of the *T. brucei*-infected goats which was as a result of both lymphocytosis and neutrophilia. The observed leucocytosis in this study in all the infected animals does not agree with the reported leucopenia due to lymphopenia and neutropenia in *T. vivax*-infected goats and sheep (Anosa, 1977; Anosa and Isoun, 1984), *T. congolense*-infected animals (Naylor, 1971; Volhradsky, 1971; Wellde *et. al.*, 1974; Maxie *et. al.*, 1974), and in *T. vivax*-infected cattle (Esievo and Saror, 1983). The variation could be possibly to strain differences. The neutrophilia could be attempts by the infected animals to eliminate the trypanosomes from circulation and also as an attempt at recovery as indicated by the lymphocytosis. The observed neutropenia in the *T. vivax*-

infected goats agrees with similar findings in *T. vivax*-infected goats and sheep, coupled with lymphopenia (Anosa and Isoun, 1984) and the neutropenia, according to the authors, was possibly due to bone marrow depression though microscopic examination did not reveal any significant bone marrow lesions. Valli *et. al.* (1979) and Saror *et. al.* (1979) suggested that neutropenia enhances acute death in bovine trypanosomosis, and this could also explain the high death rate of 5 goats within the first three weeks p.i. in the *T. vivax* group, the very period when neutropenia was very pronounced. (But this could not be said of the *T. congolense* group which also had similar high death rate during the same period but without pronounced neutropenia).

The observed leucocytosis in the *T. brucei*-infected goats is in contrast to leucocytopenia reported for *T. brucei*-infected mice by Fiennes *et. al.* (1946). The eosinophilia and monocytosis observed in all the infected animals in this work does not agree with the reported eosinipaenia and normal monocyte level seen in *T. congolense*-infected cattle by Naylor (1971b). This may be due to difference in species response to these types of wbc.

This study also revealed that red cell destruction by trypanosomes did not correspond to white-cell destruction. The variation in bilirubin level which increased soon after the fall in rbc and pcv, Hb levels supports the haemolytic nature of the anaemia in trypanosomosis. Infact, Esievo *et. al.* (1984) had reported depleted serum haemoglobin binding protein-haptoglobin during acute bovine trypanosomosis at the time of high parasitaemia, an indication of early intravascular haemolysis.

The observed trypanosome remnants in neutrophils of some of the infected goats in the chronic syndrome has not been reported anywhere before. The reported erythrophagocytosis in the chronic phase, especially in animals surviving beyond day 65 p.i. in the *T. vivax* and *T.*

congolense groups supports similar findings by Boycott and Prince-Jones (1913), Jennings *et. al.* (1974), Murray *et. al.* (1974) and Anosa and Kaneko (1983a) in *T. brucei* infected rabbits and mice/rats; in *T.congolense*-infected sheep (Mackenzie and Cruikshank, 1973); cattle (Naylor, 1971; Losos *et. al.*, 1973; Valli and Forsberg, 1979) and in *T. vivax*-infected goats (Saror, 1980). This phenomenom, according to Jennings *et. al.* (1974) is due to several factors including attachment of trypanosome antigens (a.g.) to red cells surfaces and combination with plasma anti-trypanosome a.b. and consequent sequestration and phagocytosis. Kimeto (1989) had also reported erythrophagocytosis in the spleen, lymph-nodes, bone-marrow, myocardium and peripheral leucocytes in experimental *T.vivax* infection in cattle. The author reported that erythrophagocytosis was mostly seen in the spleen. Affected spleen was enlarged and iron was not release erythropoiesis which led to low pcv and hence the anaemia in the infected animals. Similar observations were reported in humans suffering from "sleeping sickness" (Human Trypanosomosis) as reported by Eisen (1974).

The observed fall in the value of total plasma (T.P.) concentration in all the infected animals in this study is not in agreement with the reported normal values seen in *T. vivax, T. brucei* and *T. congolense*-infected goats and sheep by Edwards *et. al.* (1956). Anosa and Isoun explained that decrease in albumin and gammaglobulin (and consequently T.P.) were suggestive of increased protein breakdown, protein or urea loss, haemodilution and serum extravasation., the latter of which could be the cause of the observed facial, lower limbs and scrotal edema in the infected goats. This observation is in agreement with similar reports by Losos and Ikede (1972) and Ikede and Losos (1975) in *T. vivax*-infected WAD sheep; Maikaje *et. al.* (1991) in *T. vivax*-infected Uda rams. It is also a general belief that hypoproteinaemia (coupled with hypoalbuminaemia) is a

pointer to liver damage which usually also evidenced possibly by rise in SGOT level (as indicated in Chapter 5 of this work).

All the 3 species of trypanosomes used in this study tended to show similar levels of pathogenecity. The survival period was 7 - 74, 9 - 74 and 12 - 58 days for *T. vivax, T. congolense* and *T. brucei*-infected goats respectively, whereas Adah *et. al.* (1993) and Josuah *et. al.* (1994) reported a duration of 11 days in *T. congolense*-infected red sokoto goats, Maikaje *et. al.*, (1991) reported a duration of 18 - 85 days in *T. vivax*-infected Uda rams. The longer duration in this study compared to the very short period of 11 days in the cases of Adah *et. al.* (1993) and Joshua *et. al.* (1994) could be due to the high plane of nutrition which was higher in this study (as the present work and those of the authors involved the same species and breed of animals) though the plane of nutrition in the other works was not indicated. This is also supported by the findings of Katunguka-Rwakishaya *et. al.* (1995) that adequate energy and protein nutrition promotes the ability of infected animals to withstand adverse effects of infection by moderating the severity of the pathophysiological changes of the disease. The fact that all the *T. brucei*-infected goats died much earlier than the *T. vivax* and *T. congolense*-infected goats tend to disagree with the belief of several workers including Unsworth (1953) and Fiarharn (1962) that *T. vivax* is the most pathogenic trypanosome in West Africa, followed by *T. congolense*, whilst *T. brucei* is considered less pathogenic. The disparity could be due to the fact that most of the earlier works involved species and breeds of domestic animals in the tsetsefly infested areas, with some degree of trypanotolerance; whereas the breed of goats used in the present work thrives in the tsetse free zone and hence could easily succumb, to infections with fulminating clinical signs. The findings in the present study is an indication that all three species of trypanosome are of great economic

The presence of *T. vivax* and *T. congolense* in the lymph node smears of some of the infected goats confirm the findings of Luckins and Gray (1979) and Mwangi *et. al.* (1993) about the presence of these parasites in the lymph of *T. congolense*-infected sheep and cattle, and in both the afferent and efferent lymph of *T. vivax* and *T. congolense* infected cattle. However, the finding in this study contradicts the observation of Tizard *et. al.* (19) that no trypanosome parasite was observed in the lymph of *T.congolense*-infected sheep. Though there is no report on the presence of *T. brucei* in the lymph of domestic animals, the observation made in this study further confirms the tissue invasiveness of *T. brucei*. Hagan and Wilmshurst (1978) had earlier repoted that trypanosomes can be detected in wet preparations of lymph obtained from superficial lymph nodes smears and also in Giemsa stained lymph node smears, an observation contradicting the commonly held view that *T. vivax* and *T.congolense* are strictly confined to the vascular system.

The observed broncho-pneumonia (as both clinical signs and postmortem lesions) in some of the infected goats agrees with similar findings made by Krammer (1966) in natural infections of *T. brucei, T. congolense* and *T. vivax* in WAD sheep and goats in Eastern part of Nigeria. This could be due to the resultant stress from the infection leading to lowered body immunity against other infectious agents like those along the respiratory tract.

The observation in this study, of limb paralysis, lethargy, trembling, staggering and paddling movements in all affected groups (all in the chronic stages of the infection) shows that trypanosomosis causes CNS derangement. Cowdriosis can produce similar manifestations but they were negative for cowdriosis. Mamman *et. al.* (1994) observed that relapses in treated *T. congolense*-infected goats was not due to CNS involvement because the cerebrospinal fluid (CSF) was negative for parasites or its antigen, an observation contrary to the findings of Ferdinand *et. al.*

(1974) that trypanosomes multiply in CSF, thus indicating that the parasites could elicit CNS derangements, a situation usually observed in trypanosomosis of humans with features like lethargy, anorexia, inability to stand and walk unaided, trembling and ataxia (Triolo *et. al.*, 1992; Villannueva, 1993). Some of these signs were observed in the infected goats in this study, thus indicating CNS derangement.

Fall in weekly body weights and body weight gains are indications of the wasting nature of trypanosomosis. The observed muscle wastage in this study in the infected animals was more pronounced in those which survived longer, this is in agreement with the observations of minimum loss in weight in *T. vivax*-infected Uda ram which died in the acute stage of the infection (Maikaje *et. al.*, 1991) and similar findings in acute animal trypanosomosis (Griffin and Allonby, 1979c,d). The pronounced wastage in the *T. vivax*-infected goats in comparison to the other infected groups appeared to suggested that *T. vivax* produced a more chronic and wasting disease in the SBG.

The fall in the body weight of the un-infected control goats is an indication that goats do not do well in confinement despite proper feeding under such condition as observed in this study.

The study also reveals the very high susceptibility of SBG to trypanosomosis. It is therefore pertinent that for this breed to thrive in the tsetse and trypanosomosis endemic zones, there is need for effective tsetse control and prophylactic therapeutic cover.

Also, the general belief that *T. brucei* is not very pathogenic to small ruminants especially goats, compared to *T. vivax* and *T. congolense* should be reconsidered in line with the findings of this study.

Pathologically the severity of the anaemia in trypanosomosis in Savannah Brown goats due to *T. brucei. T. congolense* and *T. vivax* was clearly evident in this study as shown by the paleness of the carcasses of affected goats especially those in the subacute and chronic stages of the infection.

The observed lymphadenopathy, splenomegally and lymphoid hyperplasia (lymphocytes proliferation) in this study agree with the findings of Moulton and Sollod (1979) in *T. brucei*-infected calves and Ikede and Losos (1979) in cattle trypanosomosis, though reports on *T. vivax* and *T. congolense*-infected livestock regarding lymphoid hyperplasia are rare.

The liver necrosis in the chronic stage in all the infected goats agrees with similar findings by Van Den Ingh *et. al.* (1976) in *T. vivax*-infection of cattle. The observed mononuclear cell infiltration in the liver of all infected goats was similarly reported by Moulton and Sollod (1979) in *T. brucei* infected calves. There is no previous report of concerning fibrous connective tissue proliferation in the liver due to trypanosomosis. The observed gall-bladder distension in this work agrees with similar findings by Edward *et. al.* (1956) in *T. vivax., T. congolense* and *T. brucei* infected sheep and goats but not in *T. brucei*-infected calves (Moulton and Sollod, 1979).

Though *T. brucei* and *T. congolense*-infected goats produced haemorrhagic lesions in the kidney, the kidney nephrosis reported by Fiennes (1954) in *T. congolense* and Naylor (1971) in *T. vivax*-infected cattle were not observed in this study.

The cardiac mononuclear cell infiltration, myocarditis and necrosis of cardiac muscles observed in this work agree with the reports of Ikede and Losos (1975) in *T. brucei*-infected sheep and Van Den Ingh *et. al.* (1976) in *T. vivax*-infected cattle. The cardiac muscle necrosis observed in this study could explain the cause of the increased volume of pericardial sac fluid in all the affected goats especially in the chronic cases.

The pulmonary haemorrhage in all the infected goats, alveoli edema in the *T. brucei* infected goats and the mononuclear cell infiltration into the alveoli and interalveolar spaces in the *T. brucei* and *T.congolense* infected goats confirms the tissue invasiveness of *T. brucei* though there is no similar picture reported for *T. congolense* yet. The absence of bacterial isolate from lung samples of infected goats which exhibited respiratory distress signs like coughing may indicate a possible relationship between trypanosomosis and the observed pneumonia and lung necrosis in some of the infected animals.

The absence of any significant histopathological lesions in the brain and pituitary bodies (though were observed to be grossly congested) is an indication that *T. vivax* and *T. congolense* rarely find their way into such areas of the body of affected animals to cause damages. However *T. brucei* has been reportedly observed in brain tissue.

The testicular atrophy in the *T. brucei* and *T. vivax* infected goats agree with similar findings by Isoun and Anosa (1974), Anosa and Isoun (1980) in *T. vivax*-infected bucks and Ikede (1979) in *T. brucei* infected rams. Similarly the observed necrosis of the seminiferous tubules and fibrous connective tissue proliferation in the *T. congolense* and *T. brucei* infected goats agree with the reports of Isoun and Anosa (1974) in *T. vivax* infected rams and bucks, Ikede (1979) in *T. brucei* infected WAD rams and Sekoni (1987) in *T. vivax* and *T. congolense* infected bulls and Anosa and Kaneko (1983) in *T. brucei*-infected deer mice.

The corneal opacity observed in all the infected groups has been reported in cattle trypanosomosis by Ikede and Losos (1975).

Despite the observed congestion of the bone marrow in the acute and subacute cases and the haemorrhagic foci with pale yellow background in the infected group, similar reports were not

found in the literature. The haemorrhagic foci could have been areas of active erythropoiesis amidst depleted cellular active areas as represented by the pale yellowish areas.

Aortic and jugular vein impression smears were all positive for trypanosomes though at very low level of parasitaemia but similar smears from other body fluids and organs were however negative although Ikede and Losos (1975) had observed trypanosomes in fluids from peritoneal cavity, pericardial sac, aqueous humour of eyes and also C.S.F.

The observed clinical, haematological and pathological findings in this study are indicative of the fact that the Savannah Brown breed of goat is highly susceptible to these three pathogenic species of African trypanosomes though with slight variations in the lesions produced by them.

4.5. SUMMARY

Four groups of eight SBG each were used. 3 of the groups were infected via the jugular vein with stabilate 146 of *T. vivax*, stabilate 136 of *T. congolense* and stabilate 155 of *T. brucei* respectively, all of which were isolates from field cases of bovine trypanosomosis. The other group was used as un-infected control. The observed mean prepatent periods were 2.25 days, 4.25 days and 4.38 days for *T. vivax*, *T. brucei* and *T. congolense* respectively. Clinical trypanosomosis which developed in all the infected groups within seven days p.i. was characterised by fluctuating pyrexia and parasitaemia especially in the *T. vivax* group. Although pyrexia did not always coincide with peak of parasitaemia. *T. brucei* exhibited pronounced peaks, a very rare occurence and an indication of high susceptibility to it by SBG. Anaemia (pcv level) was as low as 8.0%, 9.0% and 10.2% in *T. congolense*, *T. vivax* and *T. brucei* groups respectively.

There was a more rapid fall in RBC level in *T. vivax* and *T. congolense* than in *T. brucei*-infected groups with that of the latter being at constant level up to day 42 p.i. Anaemia initially was normocytic normochromic in all infected groups, becoming later microcytic normochromic by day 18 p.i in the *T. vivax* and *T. congolense* groups, and by day 32 p.i in the *T. brucei* group. Goats in the *T. vivax* and *T. congolense* groups which survived beyond 60 days p.i. produced microcytic microchromic anaemia.

All the three infected groups exhibited anisocytosis and basophilic stipplings in the chronic syndrome of the disease. Leucocytosis was the leucocyte response in all the infected groups and was due to an initial lymphocytosis and later neutrophilia in the *T. vivax* group, concurrent lymphocytosis and neutrophilia in the *T. congolense* and *T. brucei* groups with the later developing neutropenia. There were monocytosis and eosinophilia in all the infected groups from day 15 p.i. onwards.

Remnants of trypanosomes were found in neutrophils in some of the infected *T. vivax* and *T.congolense* animals that survived beyond day 60 p.i.

Moderate hypoproteinemia occured in all the infected groups.

Pronounced bilateral epiphora occured especially in the *T. vivax* and *T. congolense* infected goats with some showing presence of trypanosomes in the occular discharges. Conjunctivitis and keratitis developed in most of the infected animals.

Edema of the lower jaws, face, knee and hock joints and limbs and scrotal sacs was more pronounced in the *T. brucei* groups. There was lymphadenitis especially of the pre-scapular and pre-femoral nodes with aspirates from some of them being positive for trypanosomes especially during high level of parasitaemia.

Respiartory problems characterised by nasal discharges and coughing were exhibited by all infected goats. Central nervous system degeneration manifested by staggering; uncoordinated movements; paddling when recumbent, body tremors, uncoordinated chewing movements and twitching of eye-lids, limb paralysis were observed in all the infected goats, most prominent in the *T. brucei*-infected goats.

With regard to the observed diarrhoea, the fact that it was not associated with helminthiasis nor coccidiosis (protozoa) lends credence to field observations by the investigator with regard to diarrhoea in diagnosed cases of trypanosomosis in cattle.

Mean weekly body weights and weight gains started falling progressively from day 35 p.i. onwards. There was observed mean weights loss in the control group also.

The SBG was shown to be highly susceptible to all the three major trypanosome species of ruminants.

With regard to differences between infections of the different species of trypanososmes, the following were observed. Pre-patent period was shortest in the *T. vivax*-infected group with the two other groups showing similar periods. Fall in red cell level was least in the *T. brucei* group. *T. vivax* and *T. congolense*-infected goats developed microcytic microchromic anaemia in the chronic syndrome. The *T. brucei* group exhibited neutropenia after an initial neutrophilia. Trypanosome remnants were observed in neutrophils of *T. vivax* and *T. congolense*-infected goats. The observed CNS degenerative changes were most pronounced in the *T. brucei*-infected goats. The fall in mean weekly body weight and weight gain was most severe in the *T. vivax*-infected group.

Pathological changes resulting from experimental infection of the Savannah-Brown goats with *T.brucei, T. congolense* and *T. vivax* were investigated. Gross and microscopic lesions were observed among nearly all the major organs in all the infected goats.

Carcasses were anaemic especially in the chronic cases, adipose tissue degeneration were prominent.

There were enlarged lymph nodes and spleen coupled with lymphoid hyperplasia.

Subacute and chronic cases produced hepatomegaly and congestion of liver with focal and diffuse areas of necrosis characterised by connective tissue proliferation especially in the chronic cases of the *T. congolense*-infected goats, and areas of petechial to echymotic haemorrhages in the *T. brucei* and *T. congolense*-infected goats.

Gross kidney lesions of petechial and echymotic haemorrhages in the cortex and medulla regions were observed in the *T. brucei* and *T. congolense*-infected goats but focal and diffuse non suppurative necrosis in the proximal and distal convoluted tubules in all the infected goats. Also fibrous connective tissue proliferation was observed in the *T. brucei* and *T. congolense*-infected goats.

Though lungs were highly congested with haemorrhages on the tracheal and bronchial mucosal surfaces in the infected goats, mononuclear cellular infiltration into the alveoli occured only in the *T. brucei* and *T. congolense*-infected goats. *Pasteurella haemolytica* was isolated only in one goat among all those coughing in the course of the study.

The major lesions that were observed in the testicles were atrophy; degeneration of seminiferous tubules characterised by distortion, necrosis and fibrous connective tissue proliferation.

Pericardial sacs contained significantly higher volumes of fluid compared to those of the uninfected control animals though none of the aspirated fluids was positive for trypansomes. Focal necrosis, mononuclear cellular infiltrations were observed in the myocardia in all the infected goats in the chronic phase, coupled with coronary fat atrophy, and petechial to echymotic haemorrages which were prominent in the *T. brucei* and *T. congolense*-infected goats especially in the subacute and chronic cases.

Brain and pituitary gland capillaries were congested grossly but there were no significant microscopic lesions.

Impression smears from the aorta and jugular vein were positive for trypanosomes in all the infected groups, however, smears from the other organs were negative.

The three pathogenic African trypanosomes produced lesions in the Savannah Brown goat indicating the susceptibility of these breed to these trypanosomes.

CHAPTER FIVE

OBSERVATIONS ON SOME SERUM BIOCHEMICAL CHANGES IN SAVANNAH BROWN GOATS (SBG) EXPERIMENTALY INFECTED WITH *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei*

5.1. INTRODUCTION

Not much data is available on serum biochemical changes due to animals trypanosomosis in ruminants. Of the available information, most of it is centred on cattle, with goats and sheep providing very little.

Edwards *et. al.* (1956) did not observe any change in total serum protein (T.P) level of *T. vivax*, *T. congolense* and *T. brucei*-infected goats and sheep. Anosa and Isoun (1976) on the other hand reported increased level of T.P and also gamma-globulins and decrease in albumin in *T. vivax*-infected goats and sheep. Similar fall in albumin level were reported in *T. vivax*-infected sheep (Clarkson, 1978), *T. evansi*-infected buffalo calves (Singh and Geur, 1983), calves (Verma and Gautam, 1983); *T. congolense*-infected cattle (Wellde *et. al.*, 1974); *T. brucei*-infected WAD sheep (Ogunsanmi *et. al.*, 1994) and *T. brucei*-infected Red Sokoto goats (Igbokwe and Mohammed, 1992).

Saror (1981) reported that despite acute cases of trypanosomosis in cattle, there were neither bilirubinaemia nor bilirubinuria. However, high levels of bilirubin in serum have been reported in acute *T. congolense* infection in cattle (Zwemer and Culbertson, 1939; Fiennes, 1954, 1970; Naylor, 1971; Wellde *et. al.*, 1974, 1983); in *T. vivax*-infected cattle (Fiennes, 1954, 1970). *T. vivax*-infected goats did not produce change in blood sugar

levels (Edwards *et. al.*, 1956). Van Den Ingh *et. al.* (1976) observed slight fall in blood sugar, lactic and pyruvic acid levels in which they attributed to metabolism of trypanosomes *T. vivax*-infected cattle. Wellde *et. al.* (1973) had reported hypoglycemia in acute *T. vivax, T. congolense* in cattle. Similar findings were also made in chronic *T. rhodesiense*-infection in cattle (Wilde and French, 1945) and acute *T. evansi*-infection of buffalo calves with fluctuating levels in the chronic stage (Singh and Gaur, 1983).

At peak of parasitaemia, there was high level of urea; which however returned to normal in the chronic phase during *T. congolense*-infection in cattle (Wellde *et. al.*, 1974). A terminal case of severe *T.vivax* infection in a cow revealed pronounced ureamia (Van Den Ingh *et. al.*, 1976).

Slight increases in SGOT levels in *T. vivax*-infected cattle and sheep have been reported (Gary, 1963; Wellde *et. al.*, 1983). Adah *et al.* (1992) observed increased levels in *T. congolense*-infected Red Sokoto and WAD goats. These levels were higher in Red Sokoto goats.

Fiennes *et. al.* (1946) reported high levels of sodium ions (Na+) in *T. congolense*-infected cattle. Increased serum chloride (Cl-) and bicarbonate (HCO3-) levels have been observed in *T. brucei*-infected WADsheep (Ogunsanmi *et. al.*, 1994).

The objective of this study was to further obtain more information on the effect of trypanosomosis due to *T. vivax, T. brucei* and *T. Congolense* on serum biochemical components and constituents particularly in the Savannah Brown goats.

5.2. MATERIALS AND METHODS

5.2.1. Animals:

The same sets of goats used for the previous studies (Chapter 4) were used.

5.2.2. Sample Collection:

Samples were taken once a week for 3 weeks and then 2 days prior to experimental infection, and thereafter thrice a week for 4 weeks p.i. to measure the serum biochemical changes in acute, subacute and early chronic stages of the infection. Based on this, collections were made on days 2, 4, 6, 8, 12, 14, 16, 18, 21, 26 and 28 p.i. On such days 20 millilitres of blood was collected from each of the experimental goats by jugular venapuncture using disposable syringes and sterile 18 guage by 1½ inches needle and placed into anticoagulant free vacutainers. The clotted blood samples were later centrifuged at 2000 rpm for 15 minutes. The resultant serum was decanted into small clean sterile polythene bags which were sealed and stored at 20° C until required for analysis.

5.2.3. Sample-Analysis:

The serum samples were analysed for the following components using the appropriate method: Sodium (Na+) and Pottasium (K+) were analysed using Flame Emission Spectrometry (Cheesbrough, 1991); Chloride (Cl-) by the method of Schales and Schales (1941) as outlined by Coles (1974); Bicaronate (HCO3-) by Titrimetric method (Cheesbrough, 1991) modified from Van Slyke (1956); Blood Urea Nitrogen (BUN) by the method of Stroev and Makarova (1989); Bilirubin by the method of Iendrassik *et. al.* (1958) as cited by Cheesbrough (1991); Glucose by the Glucose oxidase-peroxidase method (Cheesbrough, 1991); Total protein by the Biuret method of Doumas (1975) as

cited by Cheesbrough (1991); Albumin by the method of Cheesbrough (1991) and SGOT (AST) by the Reitman-Frankel AST method as described by Cheesbrough (1991).

Summaries of all the listed methods as described (Appendix 3A).

5.3. RESULTS

There were fluctuating levels in the serum biochemical components of all the infected goats with varying degrees in relation to the species of trypanosome used when compared to the levels in the control uninfected goats. Some components experienced increase, whilst others exhibited fall or decrease in levels (Figures 5.1. - 5.5; Appendix 2, Table 1 - 13).

The establishment of the disease was indicated by the devlopment of anaemia. Two parasitaemic waves within the first 14 days preceeded changes in the levels of all the serum biochemical components under investigation.

Significant decreases were observed in the levels of Na+ (hyponatremia), Cl-, and HCO3- ($P<0.05$) and increase in that of K+ (hyperkalemia) ($P<0.05$) Compared to those of the control group, however, the differences between the infected groups in the levels of these components were not significant ($p>0.05$). There were highly significant differences between the levels of bilirubin in the infected groups (with an increased hyperbilirubinaemia) and those of the control group

TABLE. 5.1. SOME SERUM BIOCHEMISTRY OF EXPERIMENTALY INFECTED SBG AS PERCENTAGE OF CONTROL GROUP'S VALUES

SERUM COMPONENT/ CONSTITUENT	CONTROL GROUP VALUES	T. vivax GROUP	T. congolense GROUP	T. brucei GROUP
Pottasium K+ (mmol/l)	5.61 ± 0.35	105.86 %	107.01 %	107.46 %
Bilirubin (mmol/l)	2.59 ± 0.56	165.60 "	205.74 "	157.78 "
BUN (mmol/l)	6.10 ± 0.22	115.56 "	117.01 "	115.89 "
SGOT (IU)	60.77 ± 1.56	138.01 "	139.89 "	140.97 "
Sodium Na+ (Mmol/l)	149.69 ± 1.82	90.20 "	90.89 "	91.01 "
Chlorine Cl- (Mmol/l)	100.54 ± 3.86	91.12 "	91.98 "	91.41 "
Bicarbonate HCO3-(Mmol/l)	20.94 ± 1.27	82.45 "	85.05 "	83.51 "
Albumin (gm/dl)	3.07 ± 0.72	83.51 "	79.22 "	78.45 "
Total Protein (gm/dl)	7.93 ± 0.30	90.80 "	89.38 "	89.02 "
Glucose (mmol/l)	5.94 ± 0.35	58.51 "	47.22 "	55.62 "

The mean values - All data collected from the day of the first peak of parasitaemia to the last day of sampling post experimental infection

This was between day 4 - 30 pi in the *T. vivax* and *T. brucei* infected group

" 8 - 30 pi in the *T. congolense* group

TABLE. 5.2. COMPARISON OF DEGREE OF CHANGE IN THE LEVELS OF SOME SERUM BIOCHEMICAL COMPONENTS IN EXPERIMENTAL *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei* INFECTION OF SAVANNAH BROWN GOATS.

I. Changes characterised by increase in the level of serum component/constituent

COMPONENT	HIGHEST IN	HIGHER IN	HIGH IN
K+ (mmol/l)	*T. brucei*	*T. congolense*	*T. vivax*
Bilirubin (mmol/l)	*T. congolense*	*T. vivax*	*T. brucei*
BUN (mmol/l)	*T. congolense*	*T. brucei*	*T. vivax*

II. Changes characterised by fall in the level of serum component/constituent

COMPONENT	MOST DECREASE IN	MODERATE DECREASE IN	LEAST DECREASE IN
N+ (mmol/l)	*T. vivax*	*T. congolense*	*T. brucei*
Cl- (mmol/l)	*T. vivax*	*T. brucei*	*T. congolense*
HCO3- (mmol/l)	*T. vivax*	*T. brucei*	*T. congolense*
Albumin (g/dl)	*T. brucei*	*T. congolense*	*T. vivax*
Total Protein (g/dl)	*T. brucei*	*T. congolense*	*T. vivax*
Glucose (mmol/l)	*T. congolense*	*T. brucei*	*T. vivax*

($p<0.001$) and this corresponded to fall in RBC levels in the infected groups from day 8 p.i. onwards. Though differences emerged between the *T. congolense* group and the other infected groups from day 16 p.i onwards, these were however not significant ($p>0.05$).

Significant falls ($p<0.001$) in the levels of total protein (hypoproteinemia) and albumin (hypoalbuminaemia) in the infected groups became noticeable from day 10 p.i. There were also significant differences ($p<0.001$) among the infected groups with regard to the total protein level. Differences occured in the albumin level between the *T. vivax* and *T. brucei* groups was significant ($p<0.01$) and between *T. congolense* and *T. brucei* ($p<0.05$).

There was significant difference ($p<0.05$) between the blood urea nitrogen (BUN) levels of the infected and control animals. The difference between the levels in the *T. vivax* and *T. congolense* group was also significant ($p<0.05$).

Fall in the glucose levels (hypoglycemia) of the infected groups became evident by day 6 p.i., with the difference between them and the control group being highly significant ($p<0.001$). The *T. vivax* and *T. brucei* groups had differences which were also significant ($p<0.05$).

The increases in the levels of the enzyme serum glutamic oxaloacetic transaminase (SGOT) also known as Aspartate Aminotransferase (AST) in the infected groups was significant ($p<0.01$) when compared to that of the control group. Also among the infected groups, there were significant differences ($p<0.01$) between *T. vivax* and *T. congolense* groups, and *T. congolense* and *T. brucei* groups but not between *T. vivax* and T. *brucei* groups ($p>0.05$).

Thus the following serum biochemical components or constituents K+, bilirubin, BUNand SGOT exhibited increased levels, whilst Na+, Cl-, HCO3-, albumin, glucose and total protein had fall in their levels in the infected groups.

The *T. brucei* group exhibited the highest increase in levels of K+ and SGOT and the lowest increase in bilirubin level; *T. congolense* group exhibited the highest increase in levels of bilirubin and BUN; *T. vivax* group showed the lowest increase in levels of K+, BUN and SGOT (Appendix 2, Table 12).

The *T. vivax* group exhibited the greatest fall in Na+, Cl- and HCO3- but the least fall in albumin, total protein and glucose levels; *T. brucei* group produced the highest drop in albumin and total protein levels and the least fall in Na+ level, whilst *T. congolense* group had the greatest fall in glucose and the least in Cl- and HCO3- levels (Appendix 2, Table 12).

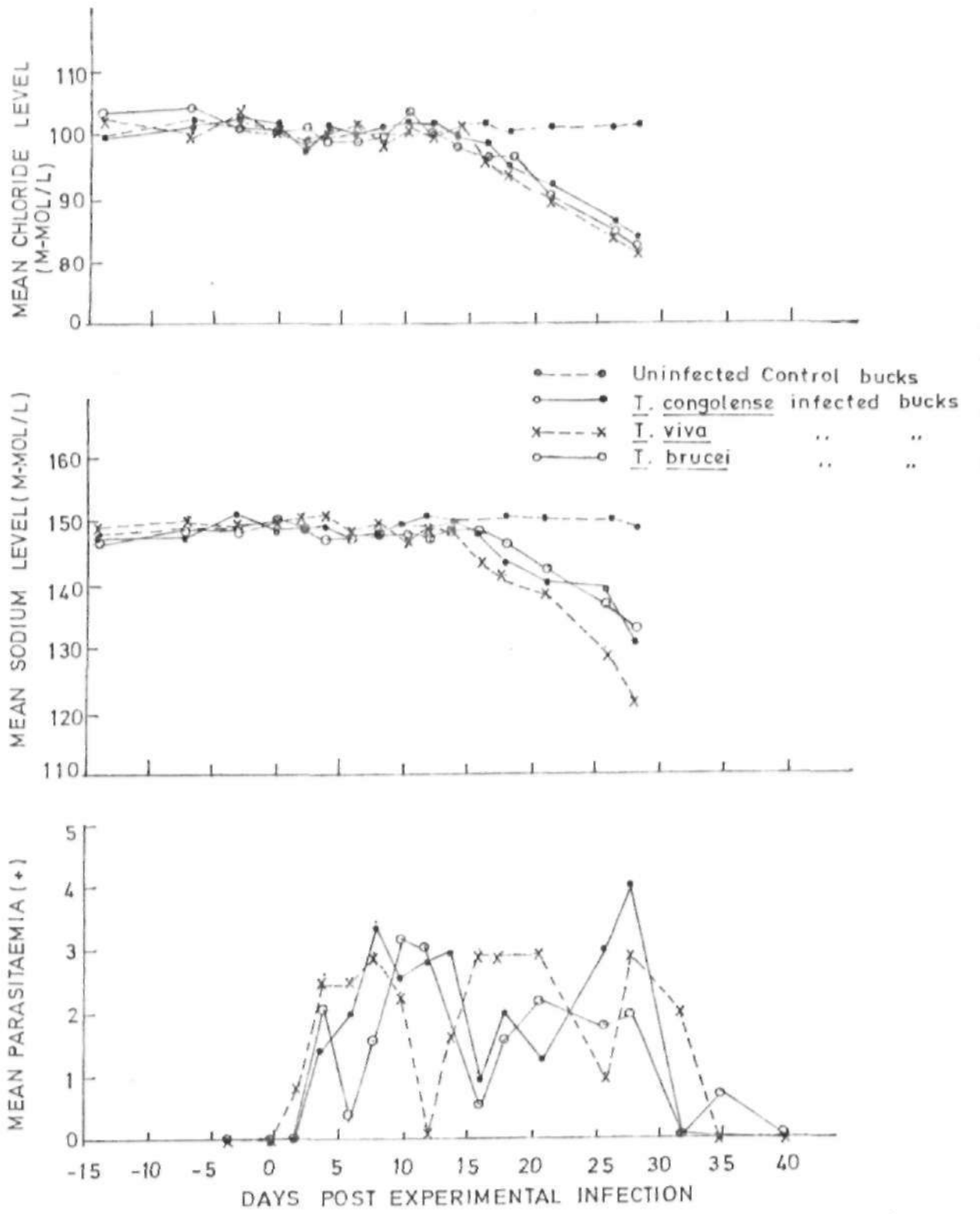


Fig. 5.1 Mean Parasitaemia, Sodium and Chloride levels of Infected and Uninfected Control Savannah Brown Bucks

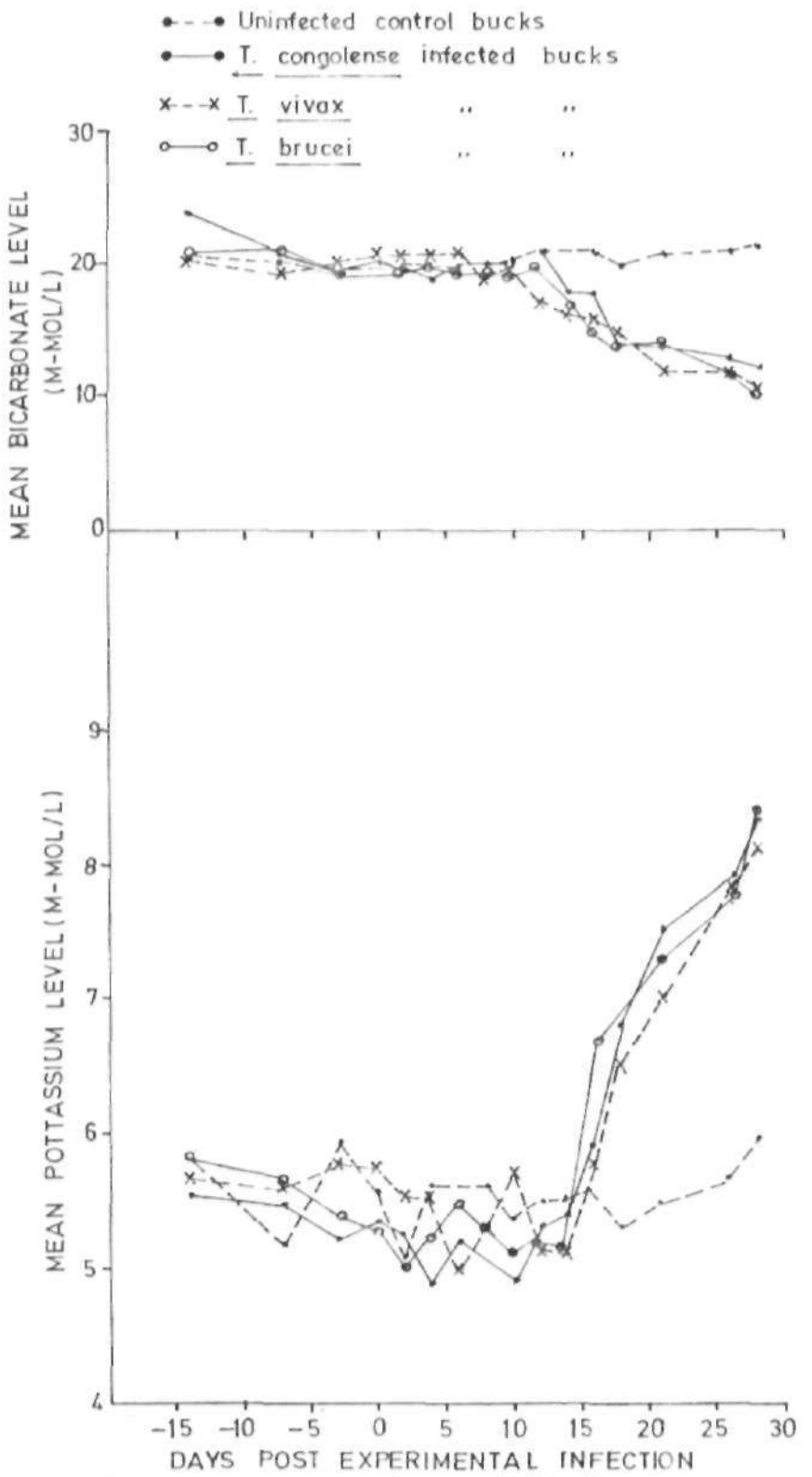


FIG. 5.2. Mean-pottassium and bicarbonate levels of infected and un-infected control savannah brown bucks

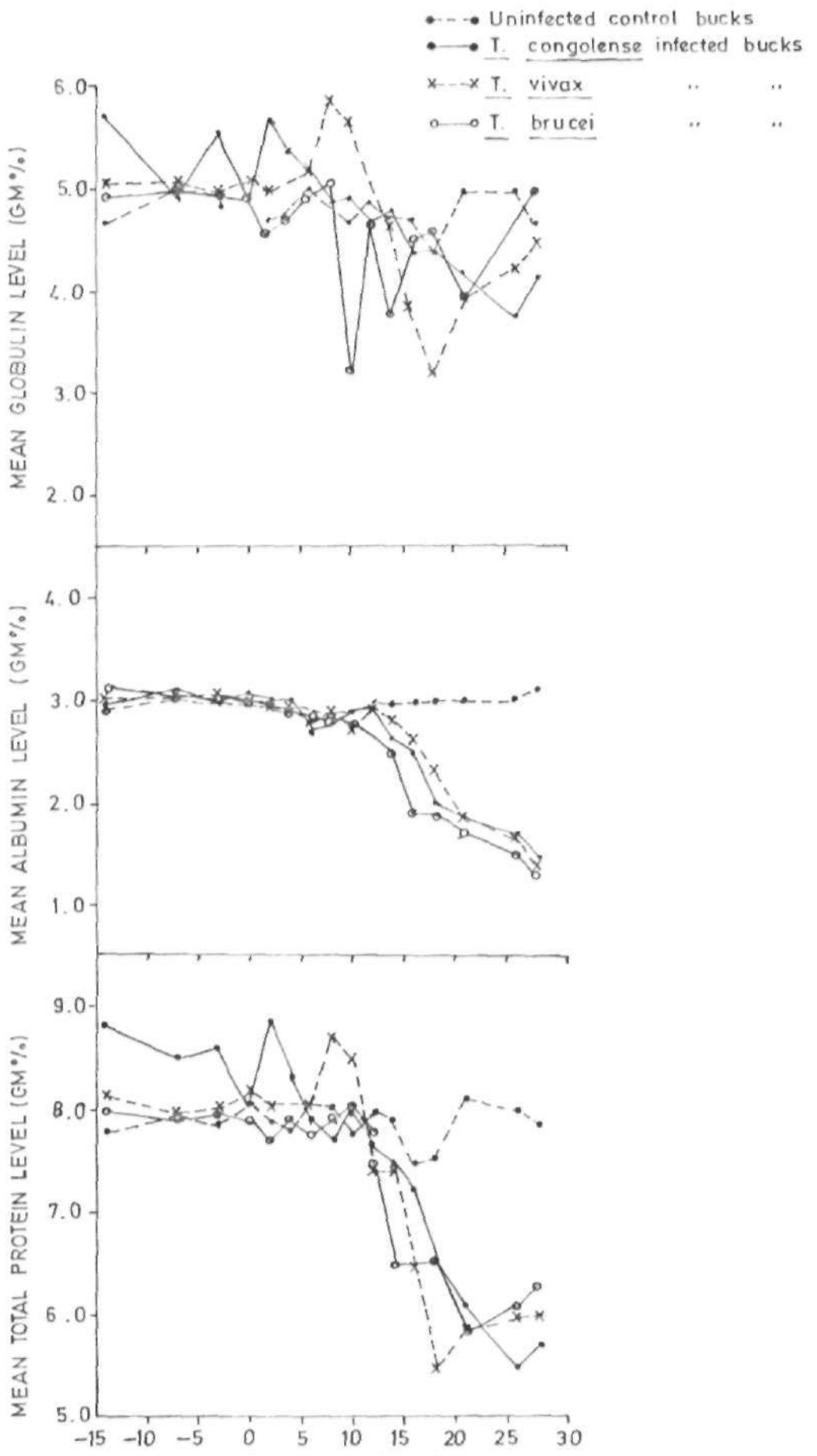


FIG.5.3. Mean serum-total protein, albumin and globulins levels of infected and uninfected control savanna brown bucks

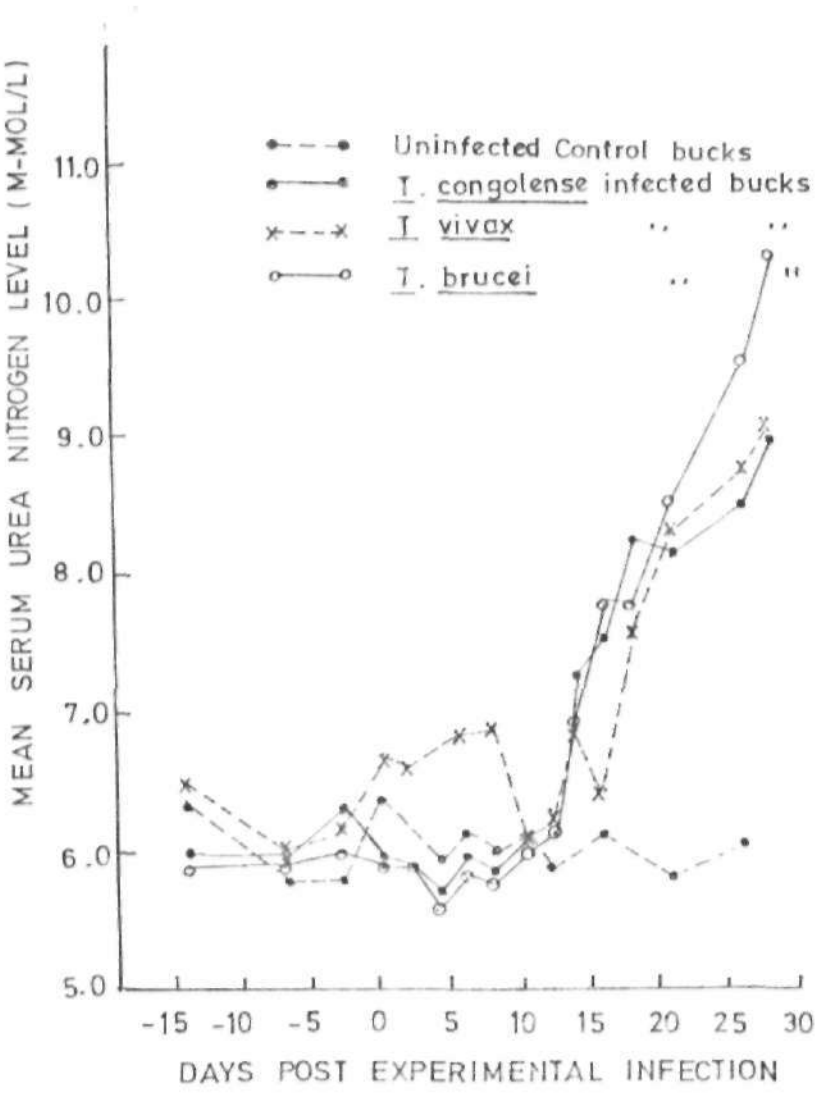


Fig. 5.4 Mean Serum Urea Nitrogen level of Infected and Uninfected Control Savannah Brown Bucks.

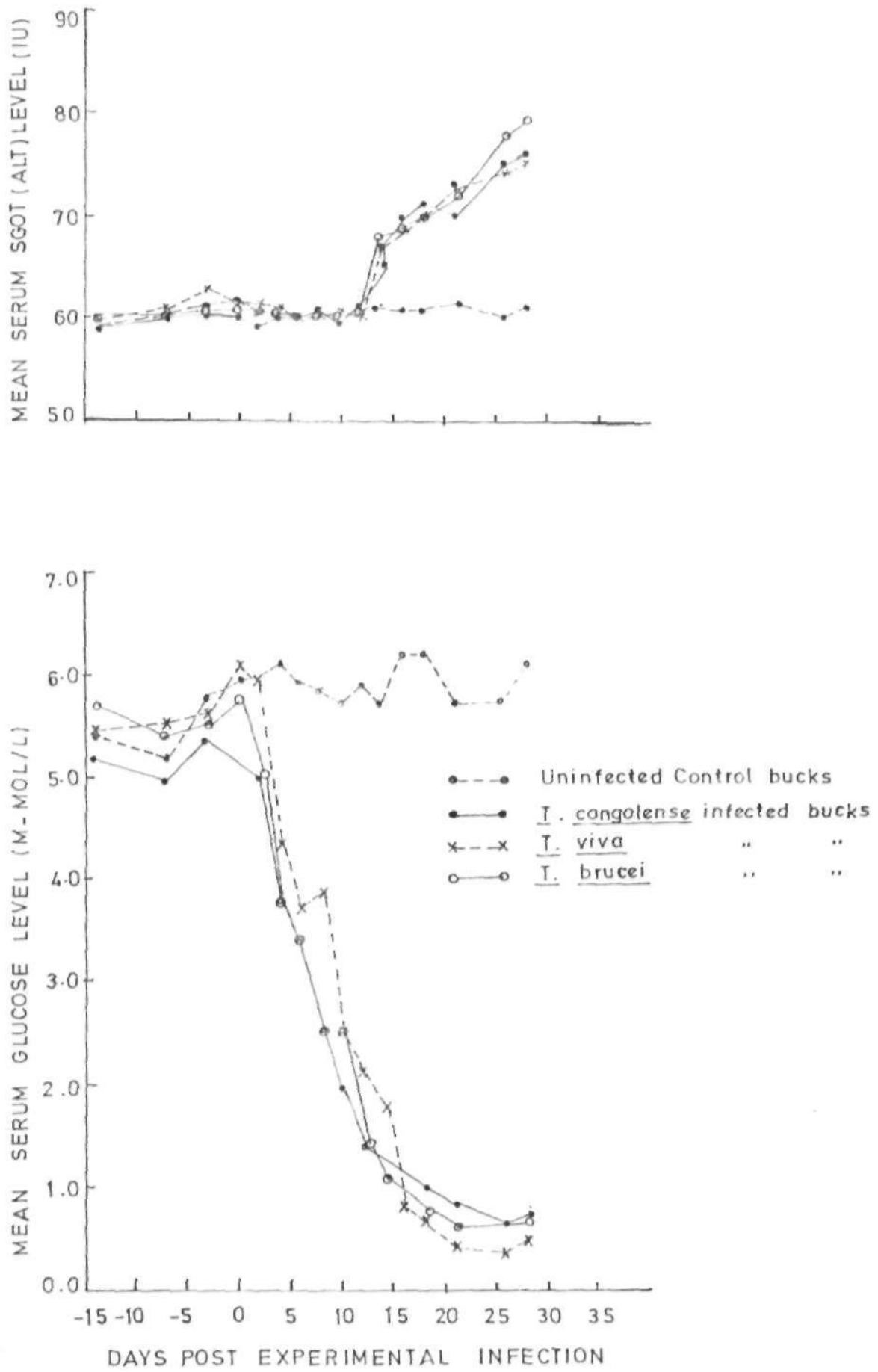


Fig. 5.5 Mean Serum Glucose and SGOT (ALT) Levels of Infected and Uninfected Control Savannah Brown Bucks

5.4. DISCUSSION

The observed increase in bilirubin levels in all the infected groups in this study agrees with similar findings made by Igbokwe and Mohammed (1992) in *T. brucei*-infected Red Sokoto goats; in *T.congolense* infected cattle (Fiennes, 1954, 1970; Naylor, 1971; Wellde *et. al.*, 1974, 1983) and in *T.vivax*-infected cattle (Fiennes, 1974,1980). Other animals in which similar reports have been made include *T. brucei*-infected rabbits (Jenkins *et. al.*, 1974) , horses (Neitz and Macauley, 1971) and dogs (Omtainse *et. al.*, 1994). This, they explained may be due to increased red cell destruction or increased haemolytic crisis (erythrocliasis), and this is supported by the observation in this study that the level of bilirubin increased soon after the fall in RBC, PCV and Hb became prominent, thus supporting the haemolytic nature of the anaemia of trypanosomosis which may partly be due to very early intravascular and mostly extravascular due to destruction of damaged erythrocytes in the liver and spleen of affected host. The increase in bilirubin is similar to the observation of Zwenner and Culbertson (1939) of initial bilirubinaemia but later normal levels in the chronic stage in *T. congolense*-infected cattle. However, the increase in bilirubin levels observed in this study is in contrast to the observations of Saror (1981) who observed normal bilirubin levels in cattle with acute trypanosomosis. Similar observation were made in *T. brucei*-infected rabbits (Jenkins *et. al.*, 1980), *T. rhodesiense*-infected mice, monkeys and man (Moon *et. al.*, 1968; Sadun *et. al.*, 1973 and Barret *et. al.*, 1973) respectively, all in the presence of erythrocliasis. This could be explained by the fact that liver functional reserve could be mobilised even in haemolytic disease condition to

conjugate and excrete excess bilirubin, thus giving rise to normal bilirubin levels (Zwenner and Culbertson, 1939).

The observed increase in BUN levels reported in this work is in agreement with similar reports by Wellde *et. al.* (1974) in *T. congolense*-infected cattle at peak parasitaemia but which returned to normal in the chronic stage; in a severe case of *T. vivax* infection in a cow (Van Den Ingh *et. al.*, 1976); in *T. brucei*-infected WAD ewes (Ogunsanmi *et. al.* , 1994) and in *T. congolense*-infected cattle (Isoun *et. al.*, 1979). Others include *T. rhodesiense*-infected monkeys (Saun *et. al.*, 1973), mice (Moon *et. al.*, 1968) and in *T. brucei*-infected dogs (Omotainse *et. al.*, 1994). Most of the reported increase in BUN, as also observed in this study coincided with periods of peak pyrexia and parasitaemia. Glomerulonephritis is often associated with pyrexia in trypanosomosis and results in increased levels of BUN which also could be due to damage to the liver, heart muscles coupled with increased protein breakdown (as observed in this study). The glomerulonephritis enhances leads to reduced excretion of BUN which therefore increases in level.

The increased levels of SGOT observed in this study agree with similar observations by Adah *et. al.* (1992) in *T. congolense*-infected Red Sokoto and WAD goats, Wellde *et. al.* (1983) and Gary (1963) in *T. vivax*-infected cattle and sheep and also in *T. rhodesiense*-infected mice (Moon *et. al.*, 1968), man (Barret-Connor *et. al.*, 1973) and monkeys (Saun *et. al.*, 1973) but in contrast to fall in SGOT level reported in *T. congolense*-infected cattle (Wellde *et. al.*, 1974) in the first 8 weeks of infection but later stabilized. Increase in levels of SGOT and other serum enzymes ie Apase, OCT, IDH., are

said to be as a result of damage to liver, heart and skeletal muscles and also from metabolic products of parasites (Moon *et. al.*, 1968; Whitelaw *et. al.*, 1980; Gumbmann and Williams, 1969; Cysewski *et. al.*, 1968; Sisk *et. al.*, 1968). However, in situations where marked increase in serum enzymes are observed especially within the first few days of infection, the most possible sources of the enzymes will be the parasites as the period may be too short for significant organ damage (Moon *et. al.*, 1968; Whitelaw, 1980). However in this study, the increase in SGOT levels in all the infected animals was from day 20 p.i. onward, indicating damage to organs as the potential sources of the enzyme.

The observed increase in levels of K+ in all the infected animals agree with similar reports by Ikejani (1946a, b) and Zwemer and Culbertson (1939) in *T. brucei* and *T. congolense* infected rats, but in contrast to normal levels observed in *T. rhodesiense*-infected mice (Moon *et. al.*, 1968) and *T. evansi*-infected camels (Raisinghani *et. al.*, 1981). RBC destruction, damaged tissues and the kidney are said to be the sources of extra increase in K+ level. The notion that rbc destruction is a source of extra K+ may agree with observations in this study because increased K+ levels coincided with fall in rbc levels. However, the increase in K+ levels corresponded with the fall in HCO3- level which is the normal occurrence physiologically in mammals in case of metabolic acidosis. The observations on K+ levels made in this study could lead to significant opening into what the effect is of trypanosomosis is on K+ levels in ruminants. The fall in the levels of Na+, Cl- and albumin agrees with the known physiological observations that increase in plasma K+ level is always accompanied with subsequent fall in plasma levels of the latter ions due possibly to some metabolic malfunctioning (Breazile, 1971). The observed fall in Na+

which occured in the infected groups agrees with the report of Barret-Connor *et. al.* (1973) in *T.rhodesiense*-infected humans and that of Raisinghani et. al (1981) in *T. evansi*-infected mice but is contrary to the observed normal Na+ levels in acute *T.rhodesiense* infection in mice and the high levels reported in *T. congolense*-infected cattle by Fiennes *et. al.* (1946). The Na+ decrease suggests its removal possibly by leaving the plasma partly intracellularly among other serum components. This movement is supported by the significant increase in K+ in the plasma since any movement of Na+ intracellularly is accompanied by displacement of K+ from intracellular compartments into the plasma. Also, K+ movement out of the cell may lead to the retention of Na+ intracellularly hence the observed fall in extracellular concentration.

Fall in Cl- levels was reported in all the infected groups in this work, but this is not in agreement with the high levels reported by Fiennes *et. al.* (1946) in *T. congolense*-infected cattle and Ogunsanmi *et. al.* (1994) in *T. brucei*-infected WAD sheep, though no mention was made of the Na+ levels. However, it is the general belief that a corresponding fall in Cl- level always follows a fall in Na+ level, which was observed in this study due to its movement in the same gradient with Na+.

The observed fall in HCO3- levels in this study is in contrast to the reported increase in its levels in *T. brucei*-infected WAD sheep by Ogunsanmi *et. al.* (1994) HCO3- is physiologically said to move in opposite direction (gradient) to K+ ions (Breazile, 1971). As such, any movement of K+ ions extracellularly and hence increase in plasma level, leads automatically to movement of HCO3- ions intracellularly and as such fall in the plasma level, a phenomenom observed in this study. Also, the fall in the plasma Na+ level together

with that of Cl- especially during the acute phase of the infection especially after 14 days p.i. and the increase in K+ level suggests a level of metabolic acidosis especially in cases of diarrhoea which was a very prominent symptom in the infected goats in this study.

The significant low level of total serum protein in this study agrees with findings made by Tabel *et. al.* (1978) in *T. congolense*-infected cattle; Wellde *et. al.* (1978) also in *T. congolense* infection in cattle and Sadun *et. al.* (1973) in *T. rhodesiense* infected monkeys but in contrast to the high levels reported by Facer (1976) in *T. brucei*-infected rabbits; Anosa and Isoun (1976) and Clarkson (1968) in *T. vivax*-infected goats and sheep; and Ogunsanmi *et. al.* (1994) in *T. brucei* infected WAD sheep and the normal values reported in *T. brucei* infected dogs, *T. rhodesiense* infection in monkeys (Smithers and Terry, 1959) and mice (Moon *et. al.*, 1968); in *T. evansi*-infected buffalo calves (Singh and Gaur, 1983; Verma and Gautam, 1977), calves (Verma and Gautam, 1983) camels (Jaktar *et. al.*, 1973) and *T. congolense* infection in mice (Whitelaw *et. al.*, 1980). Albumin, which together with globulins determine the total protein level of plasma, was significantly low in all the infected animals as was previously reported by Clarkson (1968) in *T. vivax*-infected sheep, *T. congolense* infected cattle (Wellde *et. al.*, 1974), in *T. brucei* infected Red Sokoto goats (Igbokwe and Mohammed, 1992) and WAD sheep (Ogunsanmi *et. al.*, 1994), though the last two groups of investigators observed in addition, increase in the levels of globulins. The fall in total serum protein level may be due to reduced protein synthesis arising from damaged liver which was somewhat amplified by the high SGOT level or as a result of excessive protein breakdown arising from reduced feed-intake as observed in all the infected animals. It could also arise from low level of albumin which is a

contributory factor to plasma total protein level. Low plasma total protein level could also be due to protein deficient diet. However, this counteracted by the fact that these animals received high protein diet. The fall in albumin level in all the infected groups could not had therefore come from deficient protein intake. Hepatocytes, the sources of albumin are said to withstand severe pathology, but in this work, it is possible that these were overwhelmed by the pathological conditions. However, it is an accepted fact that hypoproteinemia and hypoalbuminemia are indications of liver damage. Hypoalbuminemia has been reported in concurrent *T. congolense* and *Haemonchus contortus* infection in N'dama calves; but in this study, all the experimental animals were routinely dewormed so the hypoalbuminemia could not have been due to concurrent helminthiasis.

The observed hypoglycaemia reflected in all the infected goats tally with similar findings in cattle with acute *T. vivax* and *T. congolense* infections but not in the chronic phases (Wellde et al., 1974); chronic *T. rhodesiense* infection in cattle (Wilde and French, 1945), man (Barret *et. al.*, 1973) and monkeys (Wintrobe, 1974), acute *T. evansi* infection of buffalo calves (Singh and Gaur, 1983) and peracute *T. simiae* infection of pigs. Thus, fall in glucose level evidenced in trypanosomosis is possibly due to its utilization by the large parasite level in circulation which may be using it for their metabolic activities. Ruminants in particular have low levels of glucose in their body. Hence, the rapid fall in their glucose levels due to trypanosome activity may account for the generally observed lethargy in such animals. In other situations, especially in the chronic stages of infection lethargic conditions could lead to impairement of gluconeogenesis.

5.5. SUMMARY

An investigation was carried out to establish any changes in some serum biochemical components, in three groups each of 8 Savannah Brown goats experimentally infected with *Trypanosoma vivax*, *T. brucei* and *T. congolense* respectively, and 8 uninfected goats (SBG) serving as control group.

A mean prepatent period of 2.3; 4.3 and 4.4 days emerged for the *T. vivax, T. brucei* and *T. congolense*-infected groups respectively, followed by the establishment of the disease (trypanosomosis) as manifested by the anaemia. Within the first 14 days (2 weeks) post infection 2 parasitaemic waves occurred in all the infected groups, followed by significant changes ($p<0.05$) in the activity of SGOT and the concentrations of glucose, blood urea nitrogen (BUN) bilirubin, albumin, total protein, HCO3-, Cl-, Na+ and K+ of the infected groups in comparison to the control group. Though there were differences between the infected groups these were not significant ($p>0.05$) with the exception of albumin and glucose. The level of albumin in the *T. vivax*-infected group was significantly different ($p<0.01$) from those of the *T. brucei* and *T. congolense*-infected groups, whilst the level of glucose in the *T. congolense*-infected groups was significantly different ($p<0.05$) from those of the *T. vivax* and *T. brucei*-infected group.

The 3 species of trypanosomes used in this study were observed to affect these serum components or constituents similarly in the SBG though with different degrees of influence, for instance *T. vivax* caused the most severe fall in Na+, Cl- and HCO3-; whilst *T. brucei* did cause same in albumin and total protein and the greatest rise in K+ level and

SGOT activity with *T. congolense* causing the most severe fall in glucose and rise in bilirubin and blood urea nitrogen (BUN) concentrations.

The fall in plasma Cl- and HCO3- suggest some level of metabolic alterations. Also the changes in bilirubin, BUN, SGOT and albumin levels are indication that organ damages do occur very early in trypanosomoses. Therefore, there is need for early detection of the disease condition in infected animals for quick intervention in the form of treatment to be effected. From the findings observed in this srudy, caprine trypanosomosis is as important as those of cattle and as such greater emphasis should be laid on it's elimination as is being done for cattle. Also, acidosis due to low levels of Cl-, Na+ and HCO3- and high level of K+ and BUN may be a primary feature of trypanosomosis in small ruminants as exemplified by the reported finding in the Savannah Brown goats in this study.

CHAPTER SIX

THE SAVANNAH BROWN BREED OF GOAT AS A POTENTIAL ANIMAL MODEL IN THE STUDY OF THE ROLE OF ERYTHROCYTE SIALO GLYCOCONJUGATES IN THE DEVELOPMENT OF ANAEMIA IN ANIMAL TRYPANOSOMOSIS

6.1. INTRODUCTION

Anaemia, a prominent blood picture in trypanosomosis of domestic animals, is regarded as the major pathological finding in animal trypanosomosis. Several factors have been incriminated in the pathogenesis of anaemia. These are haemodilution, reduced haematopoetic response, immunological response, erythrophagocytosis, reduced rbc life span and of late, the role of sialidase enzyme in the cleavage of sialic acid off the surface of erythrocytes, making them thus highly vulnerable to erythrophagocytosis.

Sialidase enzymes (also known as Neuraminidases EC 3.2.1.18) are enzymes of the glycerol hydrolases group. They act on substrates like the glyco-proteins and glyco-lipids with the release of N-acylneuraminate residues. They are present in lower animals like Echinodermata, micro organism which exist as commensals or facultative pathogens to animals (Engstler and Schauer, 1993). Though originally called sialidases, these enzymes later became known as Neuraminidase when it was observed by Gottachalk (1957) that bacterial and viral extracts of sialidases on cleaving submaxillary gland mucins and glycoprotein substrates produce N-acetylneuraminic acid.

The action of trypanosomal sialidases is providing the opportunity of assessing the pathophysiological role of sialidases (Engstler and Schauer, 1993). Each enzyme cleaves off sialic acid from receptive substrate, an action determined by factors which include:

i. the type of ketosidic linkage

ii. the kind of sialic acid derivative

iii. the nature of the penultative sugar residue

Sialic acid (also known as acylated Neuraminic acid) is from a family of carboxylated saccharides with nine carbone atoms (Schauer, 1978; Veh, 1979). It occurs in appreciable level on rbc surfaces in several animal species (Seamon and Uhlenbruck, 1963). The observed rapid clearance of ^{51}Cr-labelled rbc from rats and rabbits circulation desialylated with *Vibrio cholera* neuraminidase with resultant sequestration by the liver led to the opinion that reduction in rbc sialic acid is a mechanism of rbc senescene and reduction. Being a derivative of the 9 carbon acidic sugar - neuraminic acid, sialic acid is said to offer protective cover for the sugar moiety b-galactose on the surface of rbc to avoid their being picked up by the lectins in the reticulo-endothelial system (res) of the liver and spleen. When it happens, the removal of such rbc from the circulation via phagocytosis is enhanced.

The degree of sialidase enzyme activity, if any during infection with pathogenic African trypanosomes namely *T. vivax, T. brucei and T. congolense* did not receive much attention until Esievo (1970, 1980) observed the hydrolyses of sialic acid *in vitro* with sialidase enzyme of *T. vivax.* He also observed that the enzyme's activity was proportional to trypanosomal concentration. Esievo (1979) therefore postulated that if this

hydrolysis of sialic acid can occur *in vitro*, then sialic acid could be cleared off erythrocytes, leading to their being removed from circulation and with resultant anaemia. Esievo (1980) also observed that there seems to be in existence a relationship between increased parasitaemia, hence increased sialidase activity at peak parasitaemia, and significant removal of erythrocyte sialic acid with the development of anaemia signified by low rbc and pcv levels. Magaji (1975) had earlier reported a significant increase in serum sialic acid concentration in relationship to parasitaemic level in *T. vivax*-infected Mututu and N'dama cattle and this he attributed to inflammatory response and organ invasion by trypanosomes.

In *T. brucei*, the mammalian blood stream form is not known to show sialidase enzyme activity but it is the procyclic trypomastigotes form in the insect stage that show sialidase enzyme activity, as a cell surface protein linked to the membrane (Engstler and Schauer, 1993). *T. brucei* stays in the tsetse vector for more than 5 weeks, undergoing division as large procyclic trypomastigotes, and later penetrates the peritrophic membrane to the salivary glands via the proventriculus, whereas *T. vivax* by-passes the vector's midgut region and leaves the vector after only 10 days (Vickerman, 1978). Thus it is believed that *T. brucei*'s sialidase enzyme becomes deficient or insufficient to elicit any activity in the mammalian blood stream forms because the enzyme is used to cleave sialic acid from ingested blood cells for easy digestion by the fly becuase proteases and glycosidases act more efficiently on glycoproteins after the removal of sialic acids (Engstler and Schauer, 1993). According to these authors, the transialidase of *T. brucei* functions

primarily to sialylate the parasite's glycoconjugates in order to protect surface molecules from glycosides in the tsetse vector's digestive tract.

The presence of sialidase enzyme on the tsetse-fly vector or the mammalian blood stream form of *T.congolense* has not been established. There have been several reports regarding the possible presence of sialidase enzyme on *T.congolense*. Mamo *et al.* (1975) reported that the half-life of ^{51}Cr-labelled rbc in *T.congolense*-infected calves had its value reduced from 10 days to 4.7 days at about 30 days p.i. Bank (1979) observed the binding of *T.congolense* to bovine rbc *in vitro* via the sialic acid receptors on rbc. Rautenberg *et. al.* (1981) reported the presence of sialic acid on the surface of *T. congolense* an observation similar to that seen in *T. cruzi* a related trypanosome and the cause of Chagas disease in humans in the Americas. However, the presence of sialidase enzyme in either the tsetse fly vector or the mammalian blood stream form of *T.* congolense is yet to be established.

In all these reported studies on trypanosome sialidase enzyme activity, cattle have been the animal model. Small ruminants are the other major links in food animal trypanosomosis. They have also been reported to be highly susceptible to *T. brucei, T. congolense and T. vivax*. Small ruminants are also more economical with regard to feeding, maintenance, and being easier to handle for experimental and research purposes.

This work was aimed at further investigation in to the role of trypanosome sialidase enzymes in the pathogenesis of anaemia in animals trypanosomosis and the potential of small ruminants, in this aspect, the Savannah Brown breed of goat as a potential animal model in this area of investigation.

6.2. MATERIALS AND METHODS

6.2.1. Experimental-animals:-

All as described under Materials and Methods under chapter 4.

i - Feeding

ii - Housing

iii - Screening

iv - Evaluation

6.2.2. Parasites:-

Also as described under materials and methods - chapter 4.

6.2.3. Experimental Inoculations:-

6.2.3.1. Donor-goats:

As described under Materials and Methods chapter 4.

6.2.3.2. Experimental groups; monitoring; sample collection

6.2.3.2.1. Inoculation:

Prior to being inoculated each of the experimental goats had 10 mls of blood taken via jugular venapuncture into sterile screw-capped tubes containing 1.5 mls of acid citrate dextrose (ACD) and assayed for rbc sialic acid concentration.

6.2.3.2.2. Monitoring and Sample Collection:

At first peaks of parasitaemia in each infected group the presumed period of peak sialidase activity 10 mls of blood was collected as earlier described from both infected and non-infected control goats.

6.2.3.2.3. Sample - analysis:

i). PCV (%), RBC (x 10^{12}/L) levels: as earlier described under Materials and Methods chapter 4.

ii). Erythrocyte sialic-acid concentration assaying: The 10 mls of blood samples collected in duplicate into ACD containing screw-cap tubes were assayed for erythrocyte sialic-acid concentrations using the Thiobarbituric acid Assay method for sialic-acid as described by Warren (1959, 1963), Aminoff (1961) and Esievo *et. al.* (1982). Means of values for pcv (%) RBC level (x 10^{12}/L) and sialic-acid concentration (mg/dl) were estimated for the first peak parasitaemia in each infected group and the uninfected control group.

6.3. RESULT

T. vivax-infected group attained first peak of parasitaemia at day 4 p.i. same as *T. brucei*-infected group whilst the *T. congolense* group attained mean first peak of parasitaemia by day 8 p.i. The highest rbc sialic-acid cleavage occured in the *T. vivax* group and therefore the lowest estimated rbc sialic acid concentration of 1.88 mg/dl at first peak of parasitaemia, followed by that of *T. brucei* (2.28 mg/dl) and that of *T. congolense* (3.94 mg/dl)(Table 6.1). The mean value for the control group was down to 5.72 ± 0.25 mg/dl from an initial value of 5.78 ± 0.32 mg/dl (Table 6.1). The difference between the control and infected groups with regard to sialic acid cleavage was highly significant ($p<0.001$). The difference between the *T.vivax* and *T. brucei, T. congolense* groups were significant ($p<0.05$ and $p<0.01$ respectively). The difference between *T. brucei* and

T.congolense groups was also significant ($p<0.01$) (Table 6.1). The fall in rbc sialic-acid concentrations were 1.04%, 33.22%, 61.37% and 67.70% in the control, *T. congolense, T. brucei* and *T. vivax* groups respectively (Table 6.1.). The fall in the infected groups coincided with fall in the rbc and pcv levels and hence the onset of anaemia. It is presumed that fall in rbc sialic acid concetration corresponds to increase in serum sialic acid level.

TABLE 6.1. COMPARISON OF ERYTHROCYTE SIALIC-ACID COMPONENT IN EXPERIMENTAL TRYPANOSOMAL INFECTION OF SAVANNAH BROWN BREED OF GOATS.

Experimental Group	T. brucei	T. congolense	T. vivax	Uninfected Control
No. of goats in group	8	8	8	8
Day (p.e.i. of FPP)	4	8	4	-
Level of FPP (+)	2.13 ±1.55	3.43±0.79	2.63 ±0.92	-
Mean rbc level (x 10^{12}/L)				
On day zero (0)	7.28±0.23	7.28±0.25	7.05±0.25	7.29±0.32
On day of FPP	6.61±0.61	4.36±0.48	5.91±0.21	7.13±0.22
Mean pcv % level				
On day zero		30.57±0.79	30.11±1.06	27.73±0.92
On day FPP	30.30±0.85	18.74±2.06	22.48±0.25	
Mean rbc S-acid conc.	24.56±1.40			29.98±1.44
On day zero (mg/dl)		5.90±0.11	5.82±0.19	
On day FPP "	5.85±0.43	3.94±0.18	1.88±0.23	5.78±0.32
% fall in mean S-acid conc.	2.28±0.36	33.22%	67.70%	5.72±0.25
	61.33%			1.04%

p.e.i. = Post experimental infection
FPP = First Peak of Parasitaemia
Level of significance of difference between

Control	vrs	Trt	P<0.001	Highly significant
Tv	"	Tc	P<0.01	" "
Tb	"	Tc	P<0.01	" "
Tv	"	Tb	P<0.05	Significant

6.4. DISCUSSION

Fall in erythrocyt sialic-acid concentration occured in all the infected groups, coinciding with first peak of parasitaemia and decrease in rbc and pcv values, confirming the observation of Esievo (1979, 1980) of the relationship between increased level of parasitaemia and hence elevated sialidase enzyme activity and development of anaemia as indicated by low rbc and pcv values. It also agrees with the observed relationship between parasitaemia and significant increase in serum sialic-acid conventration in *T. vivax* infected Muturu and N'dama cattle by Magaji (1975) who had attributed the observation to inflammatory response and organ invasion by trypanosomes. The high sialic acid cleavage of *T. vivax*-infected goats in this study agrees with the reports by Esievo (1979, 1980) in cattle infected with *T. vivax*, it could be said that the development of anaemia in *T. vivax* infected goats is similar to that obtained in *T. vivax*-infected cattle. This is a favourable indication that goats can be used as animal model in further investigation into sialidase enzyme activity in animal trypanosomosis.

The pronounced fall in rbc sialic acid concentration in the *T. brucei* group at peak of parasitaemia tends to agree with similar finding made by Magaji (1975) in *T. brucei*-infected cattle. It also gives an indication of the presence of a form of sialidase enzyme in the mammalian blood stream form of *T. brucei*. Engstler *et. al.* (1993) reported that there is a common nature between sialidase and trans-sialidase enzymes because highly enriched trans-sialidase fractions display sialidase activity in *T. brucei* and therefore it could be postulated that trans-sialidase carried over from the tsetse stage of the trypanosome into the

mammalian blood stream stage may be behaving as sialidase in the mammalian host though trans-sialidase activity was not measured in this work.

The significant fall in the rbc sialidase-acid concentration in the *T. congolense*-infected group is a further indication that the sialidase enzyme may be present in this species of trypanosome as was postulated by Rautenberg *et. al.* (1981).

This study has confirmed the earlier reports of sialidase being present on *T. vivax* and with its activity related to the level trypanosomes. It has also revealed that there is sialidase enzyme activity in the blood stream forms of *T. brucei* and *T. congolense* despite their reported absence. However from this study these enzymes, is less potent in its activity when compared to that of *T. vivax* although their activity bears a relationship with the level of parasitaemia.

The study has also confirmed the potential use of small ruminants as animal models in further investigating the sialidase enzyme activity in the pathogenesis of anaemia due African animal trypanosomes namely *T. brucei, T. congolense* and *T. vivax.*

7.5. SUMMARY

The potential of the Savannah Brown breed of goat as an animal model for futher investigations into sialidase enzyme activity in the pathogenesis of anaemia in African animal trypanosomosis due to *T. brucei, T. congolense* and *T. vivax* in relationship to rbc sialic-acid cleaved was studied. Four groups of 8 Savannah Brown-bucks aged between 6 - 12 months were used. Three groups of were inoculated with *T. vivax* stabilate intravenously via the jugular route, *T. brucei*, and *T. congolense* respectively. Whilst the

fourth group was used as uninfected control. The rbc, pcv levels and erythrocyte sialic-acid concentration were evaluated on day zero of experimental inoculation and on the day of first peak of parasitaemia. The first peak of parasitaemia in all the infected groups coincided with fall in rbc sialic-acid concentration and consequent fall in rbc and pcv levels with the resultant development of anaemia. The rbc sialic-acid cleavage was highest in the *T. vivax* group, with a 67.7% fall in rbc sialic-acid concentration, followed by *T. brucei* group (61.37%) and *T. congolense* group (33.22%). The difference between the control and infected groups sialic-acid concentration at first peak of parasitaemia was highly significant ($p<0.001$). The observed sialidase activity in *T. vivax* in this study confirms the enzyme's presence in the mammalian blood stream form of this species of trypanosome. The observed cleavage of sialic-acids off rbc of *T. brucei* and *T. congolense*-infected goats confirmed the possible presence of a form of sialidase enzyme on the surface of these two species of trypanosome which are yet to be confirmed to possess this enzyme on their surfaces in the mammalian blood stream forms.

The study has shown the favourable potential of the Savannah Brown breed of goat as an animal model in further investigating the role of sialidase enzyme in the development of anaemia in animal trypanosomosis due to *T. vivax, T. brucei* and *T. congolense*.

CHAPTER SEVEN

GENERAL DISCUSSION

A study was carried out to determine the level of occurrence of natural trypanosomosis in the Savannah Brown breed of goat (SBG) within the Sudan and Guinea Savannah vegetational zones of Northern Nigeria from January 1989 to December 1993.

Results from this study showed a trypanosomosis prevalence level of 2.8%. All the other observed infections were due to *T. vivax* trypanosome. Also the prevalence was not higher in the dry season (3.37%) than in the wet season (1.69%); highest in the Southern Guinea Savannah (10.0%) followed by Sudan Savannah (4.0%) and the Northern Guinea Savannah (2.1%). Eight pregnant but trypanosome positive goats aborted whilst 2 others kidded very weak kids which died later. Also, affected bucks developed orchitis and reduced libido. The major vectors identified were biting flies namely *Musca spp, Stomoxys calcitrans, Tabanus sp, Hypobosca sp.*

The findings agree with the reports of Krammer (1966), Daniel *et. al.* (1994) and Nawathe *et. al.* (1994) who previously reported natural occurrence of trypanososmosis in the SBG. The importance of biting flies in the transmission of trypanosomes as observed in this study confirms the earlier observations of Anosa (1983) and Nawathe *et. al.* (1994). However, the level of prevalence of 2.8% recorded in this study is much higher than the value of 1.1% reported by Nawathe *et. al.* (1994) and 0.7% by Ahmed and Agbede (1993) though lower than the values of 5.0% and 13.9% reported by Daniel *et. al.* (1994) and Krammer (1966) respectively. The history of the observed abortion and orchitis agree with

similar reports by Griffin (1978); Mackenzie and Cruickshank (1973) in goats with natural infections in East Africa. This observation is being reported for the first time for West African goats. The findings of this study are indications that natural trypanosomosis do occur in the SBG and hence is of economic importance, so efforts should be made towards its control as it is being done in cattle.

The clinical and haematological manifestations of experimental trypanosomosis due to *T. vivax, T congolense* and *T. Brucei* in the Savannah Brown goats were investigated. The shortest prepatent period of 2.4 days occured in *T. vivax*, followed closely by those of *T. brucei* and *T. congolense* (4.3 and 4.4 days respectively). All the three infected groups of 8 SBG each developed clinical trypanosomosis, characterised by fluctuating pyrexia and parasitaemia; anaemia, lethargy, CNS degenerations, enlarged superficial lymph nodes, epiphora, diarrhoea and weight loss. Some of the *T. vivax* and *T. congolense*-infected goats were positive for trypanosomes in their tears (ocular discharges).

The prepatent period of 2.4 days observed for *T. vivax* agrees with the 2.0 days reported by Maikaje *et. al.* (1991) in *T. vivax*-infected Uda rams, Yankassa sheep (Saror, 1980) and calves (Esievo *et. al.*, 1982) whilst the value for *T. congolense* agrees with the reports by Maxie *et. al.* (1974) in calves. Reports on the prepatent period of *T. brucei* in small ruminants is scarce.

The course of infection was shortest in the *T. brucei* infected group (58 days) compared to the other 2 infected groups (74 days), an indication that this spece is very virulent in SBG hence of economic importance as the other two species. It could also be said that SBG seems to succumbs to *T.vivax* much earlier than Uda ram which according

to Maikaje *et. al.* (1991) produced a course of infection of 84 days, compared to the 74 days observed for SBG in this study.

PCV fell faster in the *T. vivax* infected group followed by *T. brucei* and *T. congolense* groups. The anaemia was of the normocytic-normochromic type in the acute phase in all the infected groups which later became microcytic macrochromic. The acute phase anaemia agrees with similar observation by Saror (1979).

Leucocyte response in all the infected groups was that of leucocytosis due primarily to lymphocytosis and neutrophilia in *T. congolense*, and *T. brucei* groups and lymphocytosis in the *T. vivax* group. Previous reports were that of leucopenia (Anosa, 1977; Anosa and Isoun, 1984; Wellde *et. al.*, 1974; Maxie *et. al.*, 1974; Esievo and Saror, 1983). Eosinophilia and monocytosis observed in this study also contradicts the reported eosinopenia and normal monocyte levels (Naylor, 1971b) in *T.congolense* infected cattle. These findings are indications of good immune response by the infected goats to the infections.

There were increase in bilirubin levels in the infected goats and these coincided with fall in rbc and pcv levels and seem to support the haemolytic nature of anaemia in trypanosomosis as reported by Esievo *et. al.* (1984).

There was fall in the total protein level in all the infected groups. This fall could have been due to the decrease in albumin levels observed in this study. Other fators could be increased protein breakdown, protein loss in urine, and haemodilution. Previous reports have noted normal values in *T. vivax, T. congolense* and *T. brucei*-infected goats and sheep (Edwards *et. al.*, 1956).

The facial, limb and scrotal edema observed in the infected goats may be due to hypoproteinaemia and hypoalbuminaemia observed in this study as well as liver damage as indicated by the high SGOT (ALT) levels. Edema incidence in this study agrees with similar findings in *T. vivax*-infected WAD sheep (Losos and Ikede, 1972; Ikede and Losos, 1975) and Uda rams (Maikaje *et. al.*, 1991).

The occurrence of trypanosomes in the occular discharges of some goats in the *T. vivax* and *T.congolense* groups agrees with or confirms the belief of Luckins and Gray (1979) that *T. vivax* and *T. congolense* are capable of tissue penetration. There had been a report of occurrence of *T. vivax* in occular discharges in a calf (Ilemobade and Schilhorn, 1974).

Lymph node smears from some *T. vivax, T. brucei* and *T. congolense*-infected goats were positive for trypanosomes. This observations agrees with those of Luckins and Gray (1979) in *T. congolense*-infected sheep and cattle and *T. vivax* and *T. congolense*-infected cattle (Mwangi *et. al.*, 1993). However, there is no report yet of the presence of *T. brucei* in the lymph node smears of infected domestic animals. The observations further confirm the belief that *T. vivax* and *T. congolense* are not found only in the blood stream.

The observed CNS degenerative signs particularly trembling, staggering, uncoordinated movements, paddling when recumbent, eye lids twitching, limb paralysis, body tremors and uncoordinated chewing movement are similar in human trypanosomosis as reported by Triolo *et. al.* (1992), Villannueva (1993). The fall in the values of weekly body weight and weight gains in the all the infected goats agree with similar observations in *T. vivax* infected Uda rams (Maikaje *et al.*, 1991) cattle and goats (Griffin and Allonby,

1979c,d). The fall in body weights shows the wasting nature of the infection, which was more pronounced in the *T. vivax*-infected group than the other two species. The observed fall in the mean body weight and weight gains in the control group is an indication that goats possibly do not perform well under prolonged restriction or confinement.

The pathology of trypanosomiasis due to *T. brucei, T. congolense* and *T. vivax* in the Savannah Brown breed of goat was investigated.

Carcasses were very anaemic especially in the subacute and chronic stages. The observed lymphoid organ changes like lymphadenopathy, splenomegally and lymphoid hyperplasia agree with similar findings by Moulton and Sollod (1979), Ikede and Loso (1979) in *T. brucei* infected calves and cattle respectively.

Liver necrosis and mononuclear cell infiltration agree with the reports of Van Den Ingh *et. al.* (1976) in *T. vivax* infected cattle and Moulton and Sollod (1979) in *T. brucei*-infected calves. The observed fibrous connective tissue proliferation in the liver is a rare occurrence. The observed gall-bladder distension with tenacious content especially in the chronic form of the infection agree with similar finding by Edward *et. al.* (1956) in *T. vivax, T. congolense* and *T. brucei*-infected sheep and goats.

Changes in the kidney and the heart were evident in all the infected goats and agree with similar findings by Fiennes (1954) in *T. congolense* infected cattle, and *T. vivax*-infected cattle also by Naylor (1971); Ikede and Losos (1975) in *T. brucei*-infected sheep, and Van Den Ingh *et. al.* (1976) in *T. vivax*-infected cattle. Kidney changes include haemorrhagic lesions whilst those of the heart were muscle necrosis and mononuclear cells infiltration.

The absence of bacterial isolates from lung samples of infected goats which were coughing in the course of the study is a further indication of the possibility of trypanosomosis eliciting respiratory tract reactions. Field cases in cattle have been observed to show signs like coughing, though no such report has been cited in the literature so far pulmonary oedema could be the cause of the observed coughing but this was not seen in any of the affected goats in this study.

The observed testicular degeneration coupled with seminiferous tubules necrosis and fibrous connective tissue proliferation agree with similar findings by Isoun and Anosa (1974), Anosa and Isoun (1980) in *T. vivax* infected bucks, Ikede (1979) in *T. brucei*-infected WAD rams, Sekoni (1987) in *T. vivax* and *T. congolense*-infected bulls respectively. Also, the observed corneal opacity in some of the infected goats have been reoprted by Ikede and Losos (1975) in ovine trypanosomiasis.

Investigations were carried out to assess the possible effects of experimental trypanosomosis due to *T. vivax*, *T. congolense* and *T. brucei* on some serum biochemical components/constituents of the Savannah Brown goats.

Increase in serum bilirubin levels were observed in all the infected groups. The increases were found to have coincided with fall in mean total rbc and pcv levels, indicating the possible source of the increase to higher rbc destructions in the general circulation and further confirming the haemolytic nature of the anaemia of trypanosomosis. Similar rise in bilirubin levels have been reported in *T. brucei*-infected Red Sokoto goats (Igbokwe and Mohammed, 1992); *T. congolense*-infected cattle (Wellde *et. al.*, 1974, 1983; Naylor, 1971) and *T. vivax*-infected cattle Fiennes, 1974, 1980

There were significant increases in the levels of BUN in all the infected goats and these increases coincided with peak pyrexia and parasitaemia which could have resulted in kidney damage and consequent reduction in excretion of BUN by the kidney, resulting in high levels in the blood and serum. The increased levels of BUN reported in this study tally with observations of Van Den Ingh *et. al.* (1976) in a *T. vivax*-infected cow; *T. brucei*-infected WAD sheep (Ogunsanmi *et. al.*, 1994) and *T. congolense*-infected cattle (Isoun *et. al.*, 1979).

The possibility of damages to the liver, cardiac and skeletal muscles could have caused the drastic increases in the levels of SGOT (ALT) in all the infected groups in this study. These findings agree with the reports of Wellde *et. al.* (1983) in *T. vivax*-infected cattle; Adah *et. al.* (1992) in *T. congolense*-infected Red Sokoto and WAD goats and also by Barret-Connor *et al.*, (1973). Other sources of SGOT, according to Moon *et. al.* (1968) are the trypanosome parasites themselves. The pathological findings confirmed the suspected liver, kidney and cardiac muscles damages.

There were increase in the levels of K+ with concurrent fall in HCO3-, Na+, Cl- and albumin levels in all the infected groups in this study. The rise in levels of K+ coincided with fall in HCO3- levels, this is the normal physiological gradient movements of these two ions. The increase K+ levels could have been brought about by leakage of K+ from damaged rbc, kidney and other tissues. The increase in K+ levels agrees with similar findings in *T. congolense*-infected rats (Zwener and Culbertson, 1939) and also *T. brucei* infected rats (Ikejani, 1946a,b).

The fall in levels of Na+, Cl- and albumin which also coincided with rise in K+ levels agree with reports of Breazile (1971) who edxplained that fall in the level of these serum components could be due to metabolic malfunctioning. Also the fall in Na+ corraborates the findings of Barret-Connor *et. al.* (1973) in *T. rhodesiense* infection in man and that of Risinghani *et. al.* (1981) in *T. evansi*-infected mice. The fall in Cl- is expected as this component always moves passively along the same gradient with Na+. The variation in the serum ions observed in this study point to a possible acidosis due to the trypanosome infections.

There were significant falls in the levels of total protein and albumin in all the infected groups. The fall in total protein could be due to excessive protein breakdown, reduced feed intake, as observed in this study; fall in albumin level and reduced protein synthesis due to liver damage. The fall in total protein agreed with similar findings in *T. congolense*-infected cattle (Tabel *et. al.*, 1978; Wellde *et. al.*, 1978) and *T. rhodesiense*-infected monkeys (Sadun *et. al.*, 1973).

There was significant hypoglycemia manifested by all the infected groups. The observation is similar to that of Wellde *et. al.* (1974) in acute *T. vivax* and *T. congolense*-infected cattle; Singh and Gaur (1983) in acute *T. evansi* infection in buffalo calves and paracute *T. simiae* infection in pigs. Causes of hypoglycemia include liver damages and resultant fall in gluconeogenesis, and also the utilization of the blood glucose by the trypanosomes in their metabolic activities.

The possible use of small ruminants as potential models in the study of trypanosomal sialidase and erythrocyte sialoglycoconjugates activity in ruminants was investigated using

Savannah Brown goats. The rbc sialic acid cleavage was highest in the *T. vivax*-infected group, followed by that of the *T. brucei* group and then the *T. congolense* group. This shows that sialidase enzyme activity is highest in *T. vivax* and lowest in the *T. congolense* in the mammalian blood stream forms of these three pathogenic species in ruminants. The degree of cleavage of rbc sialic-acid coincided with the level of parasitaemia and the incidence of severe anaemia, thus confirming the reported relationship between increased parasitaemia, and increased sialidase enzyme activity with significant sialic-acid cleavage and development of anaemia as reported by Esievo (1979, 1980). Also, despite the reported absence of the sialidase enzyme from the mammalian blood stream forms of *T. brucei* and *T. congolense*, significant sialic-acid cleavage was observed in goats infected with these trypanosome speces, indicating the possible presence of a form of the enzyme in the mammalian forms of these parasites, as was suggested by the findings of Magaji (1975) and Rautenberg *et. al.* (1981), and the potential role of trans-sialidase in sialic-acid cleavage activity in *T. congolense* as reported by Engstler and Schauer (1994).

This investigation has shown that this breed of goat is a very good animal model for use in further studies concerning sialidase enzyme and development of anaemia in African animal trypanosomosis.

With regards to the menace of trypanosomosis in Nigeria, there seems to have been a serious decline in activities geared towards the control of the disease. Now that this study has shown that the Savannah Brown breed of goat is highly susceptible to the disease, intensive efforts should be made towards its control in order to forestall severe shortage in meat production.

Also, further work is needed in the area of sialic acid cleavage from the surface of rbc of affected hosts. In this regard, preparations (medications) capable of preventing this activity by the trypanosome's sialidase enzyme will go a long way in drastically reducing the anaemia which is the major effect of the disease and as such minimise the destructive nature of the disease in livestock.

The serum biochemistry revealed the serious effect of the disease on electrolytes like HCO_3, Na^+ and Cl^- and other components like glucose which gives rise to acidosis and hypoglycaemia respectively, all of which lead to lethargy which is commonly observed in the infected animals. This therefore calls for the need to minimise this effect by incorporating these electrolytes and components in the medication.

There is need for further investigation into the potential tissue invasiveness of *T. vivax* and *T. congolense* as evidenced by their presence in ocular discharges in some of the infected goats used in this study.

There is also the need to further investigate the effect of these three pathogenic African trypanosomes on the reproductive performance of the female SBG as the bucks used in this study exhibited severe testicular damage due to all the three parasites.

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APPENDIX 1

ANALYSIS OF VARIANCE FOR MEAN RECTAL TEMPERATURE, MEAN PARASITAEMIA, MEAN HAEMATOLOGICAL VALUES AND MEAN WEEKLY BODY WEIGHTS AND BODY WEIGHT GAINS

Table 1. Mean Rectal Temperature(°C) in Groups of Trypanosome Infected Savanna Brown Goats (SBG) and Non-Infected Control.

Days Post Infection	T. vivax		T. congolense		T. brucei		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-28	38.16	0.20	38.33	0.31	38.40	0.39	38.06	0.18
-21	38.36	0.31	38.29	0.29	38.30	0.23	38.34	0.27
-14	38.36	0.39	38.24	0.22	38.34	0.37	38.11	0.27
- 7	38.33	0.27	38.10	0.34	38.40	0.43	38.13	0.30
- 3	38.10	0.21	38.20	0.37	38.42	0.20	38.13	0.29
0	38.28	0.22	38.20	0.41	38.10	0.33	38.31	0.48
2	39.73	0.42	38.79	0.36	39.23	0.88	38.39	0.38
4	40.73	0.45	38.83	0.48	39.86	0.69	38.45	0.17
6	39.30	0.64	39.47	0.62	39.89	0.84	38.54	0.20
8	40.25	0.77	39.19	0.66	39.13	0.78	38.30	0.19
10	39.85	0.55	39.36	0.77	39.54	0.88	38.20	0.28
12	39.43	1.08	38.88	0.47	39.33	1.05	38.30	0.38
14	39.88	0.94	38.95	0.62	38.72	0.91	38.12	0.26
16	39.92	0.89	38.65	0.24	38.95	0.58	38.27	0.33
18	39.83	0.63	38.73	1.21	38.80	0.47	38.50	0.37
21	39.73	0.44	39.10	0.32	38.86	0.40	38.17	0.43
26	39.13	0.49	38.35	1.20	37.88	0.41	38.50	0.41
28	39.41	1.07	39.40	0.20	38.18	1.10	38.34	0.14
32	38.57	0.73	38.70	0.30	37.80	0.50	38.20	0.40
35	39.40	0.90	39.00	-	38.35	0.41	38.02	0.23
40	38.05	0.60	38.00	-	37.83	0.20	38.30	0.29
Group Mean	39.53	0.53	38.89	0.45	38.82	0.32	38.27	0.18

Analysis of Variance

Source of Variation	S S	D F	MS	F	Pr>F	Level of Significance (%)
period	61.375 8	23	2.6685	7.91	0.0001	0.001
Treatment	29.1337	3	9.7112	26.78	0.0001	0.001
Residual	113.3605	336	0.3374			
Total	203.8760	362				
Tv Vs Tb	4.3842	1	4.3842	12.99	0.0004	0.001
Tv Vs Tc	3.3171	1	3.3171	9.83	0.0019	0.01
Tc Vs Tb	0. 0143	1	0.0143	0.04	0.8373	Not significant

T_V - *T. vivax* T_b- *T. brucei* T_C- *T. congolense*

SS - Sum of squares
DF- Degree of freedom
MS- Mean of squares
F- F-value.

Table. 2. Mean Parasitaemic Level of Groups of Trypanosome Infected Savanna Brown Goats.

Days Post Infection	T. vivax		T. congolense		T. brucei	
	Mean	SD	Mean	SD	Mean	SD
-3	0	-	0	-	0	-
0	0	-	0	-	0	-
2	0.88	0.55	0.0	0.0	0.0	0.0
4	2.63	0.92	1.29	0.77	2.13	1.55
6	2.57	0.98	2.00	1.00	0.25	0.46
8	3.00	1.27	3.43	0.79	1.63	1.41
10	2.33	2.07	2.60	0.55	3.13	1.13
12	0.19	0.41	2.80	1.79	3.00	0.82
14	1.83	1.94	3.00	0.62	1.72	0.76
16	3.00	1.93	1.00	1.42	0.50	0.55
18	3.00	1.73	2.00	1.33	1.50	0.84
21	3.00	1.42	1.33	2.70	2.20	1.31
26	1.00	2.05	3.00	0.01	1.80	1.31
28	3.00	2.70	4.00	0.10	2.00	1.67
32	2.00	1.67	0.00	0.00	0.00	0.00
35	0.00	0	0.00	0.00	0.75	0.96
40	0.00	0	0.00	0.00	0.00	0
Group mean	1.65	1.16	1.76	0.65	1.39	0.75

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance (%)
period	211.4666	23	9.1942	10.31	0.0001	0.001
Treatment	78.4136	3	26.1379	29.30	0.0001	0.001
Residual	299.7617	336	0.8922			
Total	589.6419	362				
Tv Vs Tb	74.2421	1	74.2421	5.85	0.0161	0.05
Tv Vs Tc	0.2415	1	0.2415	0.02	0.8904	Not significant
Tc Vs Tb	76.3185	1	76.3185	6.02	0.0147	0.05

Table.3. Mean Packed Cell Volume (%) of Groups of Trypanosome Infected Savanna Brown Goats and Non Infected Control SBG

Days Post Infection	T. vivax		T. congolense		T. brucei		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-28	30.31	1.10	30.43	1.27	30.38	0.92	30.18	0.57
-21	30.33	1.05	30.79	0.81	30.85	0.65	30.48	1.02
-14	29.88	0.85	30.57	0.79	29.63	0.92	30.07	10.42
- 7	30.28	0.66	30.00	0.82	30.28	0.92	29.50	1.23
- 3	29.78	1.08	30.43	0.98	29.63	0.92	28.54	1.07
0	30.11	1.08	30.57	0.79	30.30	0.95	29.79	0.92
2	27.63	0.75	29.14	0.90	28.69	1.34	30.13	1.64
4	22.48	1.38	26.14	0.90	24.56	1.40	29.98	1.44
6	18.43	2.30	23.14	2.79	22.56	0.87	29.13	0.84
8	20.47	0.81	18.72	2.06	19.69	1.63	29.62	1.43
10	18.58	0.59	15.30	2.39	17.44	1.45	30.03	1.29
12	17.58	0.92	13.20	2.17	15.67	0.51	28.74	0.75
14	17.38	0.53	12.50	0.82	14.17	0.63	30.40	1.41
16	16.70	0.45	12.25	2.63	14.15	0.20	30.33	1.02
18	16.00	0.41	13.50	4.66	13.50	0.45	29.55	1.35
21	12.67	0.35	14.33	6.35	13.20	0.28	30.00	1.98
26	14.27	0.65	18.50	0.50	12.40	0.42	30.30	1.21
28	15.17	0.60	14.50	0.50	13.40	0.42	29.36	0.87
32	16.50	0.50	19.50	0.50	13.75	0.29	28.76	0.83
35	16.00	0.10	21.50	-	13.50	0.41	29.24	0.66
40	14.00	0.85	23.00	-	15.00	0.65	29.66	1.09
Group Mean	21.07	0.53	21.86	0.87	20.60	0.52	30.19	1.11

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance (%)
Period	7742.6225	23	336.6358	26.53	0.0001	0.001
Treatment	2435.1286	3	811.7095	63.98	0.0001	0.001
Residual	4263.0878	336	12.6878			
Total	14440.8387	362				
Tv Vs Tb	74.2421	1	74.2421	5.85	0.0161	0.05
Tv Vs Tc	0.2415	1	0.2415	0.02	0.8904	Not significant
Tc Vs Tb	76.3185	1	76.3185	6.02	0.0147	0.05

Table.4. Mean Haemoglobin Level (Gm/dl) of Groups of Trypanosome Infected Savanna Brown Goats and Non Infected Control SBG

Days Post Infection	T. vivax Mean	SD	T. congolense Mean	SD	T. brucei Mean	SD	Control Mean	SD
-28	10.11	0.38	10.16	0.43	10.13	0.30	10.05	0.19
-21	10.11	0.36	10.26	0.28	10.03	0.34	10.16	0.34
-14	9.96	0.28	10.20	0.27	9.88	0.59	10.02	0.48
- 7	10.09	0.24	10.00	0.25	10.13	0.23	9.84	0.42
- 3	9.91	0.35	10.14	0.32	9.88	0.30	9.50	0.56
0	10.06	0.36	10.19	0.29	10.16	0.32	9.93	0.31
2	9.20	0.25	9.72	0.31	9.60	0.36	10.09	0.55
4	7.50	0.46	8.72	0.31	8.20	0.48	9.67	0.50
6	6.14	0.78	7.72	0.91	7.91	0.30	9.80	0.45
8	6.82	0.20	7.67	3.41	6.56	0.53	9.87	0.50
10	6.20	0.19	5.12	0.79	5.81	0.51	10.02	0.44
12	5.87	0.32	4.40	0.74	5.29	0.16	9.97	0.28
14	5.80	0.17	4.18	1.03	4.72	0.21	10.13	0.47
16	5.58	0.18	4.08	0.90	4.72	0.41	10.00	0.41
18	5.33	0.13	4.50	1.80	4.50	0.18	9.78	0.52
21	4.20	0.27	6.10	0.20	4.38	0.11	9.98	0.52
26	4.77	0.33	5.50	0.50	4.46	0.17	10.10	0.41
28	4.93	0.35	4.85	0.25	4.80	0.17	9.78	0.28
32	5.50	0.29	6.50	0.20	4.50	0.12	9.58	0.30
35	5.30	0.10	7.20	-	4.50	0.16	9.76	0.20
40	4.65	0.25	7.70	-	4.97	0.33	9.86	0.50
Group Mean	7.25	0.30	21.86	0.87	6.86	0.30	9.90	0.41

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance (%)
period	790.8770	23	34.3860	22.77	0.0001	0.001
Treatment	290.6075	3	96.8692	64.14	0.0001	0.001
Residual	507.4273	336	1.5102			
Total	1588.9118	562				
Tv Vs Tb	0.9220	1	0.9220	0.61	0.4352	Not significant
Tv Vs Tc	8.0113	1	8.0113	5.30	0.0219	0.05
Tc Vs Tb	13.6484	1	13.6484	9.04	0.0028	0.01

Table.5. Mean Erythrocyte Count (x 10^{12}/L) of Groups of Infected Savanna Brown Goats and Non Infected Control SBG

Days Post Infection	T. vivax		T. congolense		T. brucei		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-28	7.25	0.19	7.33	0.29	7.49	0.54	6.99	0.17
-21	7.41	0.40	7.22	0.32	7.89	0.32	7.10	0.14
-14	7.39	0.35	7.30	0.29	7.54	0.14	7.08	0.24
- 7	7.38	0.20	7.29	0.27	7.12	0.64	7.02	0.51
- 3	7.08	0.19	7.26	0.24	7.20	0.31	7.89	0.17
0	7.29	0.37	7.24	0.25	7.25	0.33	7.08	0.25
2	7.12	0.24	6.43	0.35	7.33	0.59	7.08	0.24
4	5.91	0.21	5.64	0.48	6.61	0.61	7.13	0.29
6	4.03	0.30	5.29	0.99	6.35	0.44	7.00	0.25
8	4.22	0.35	4.36	0.48	6.09	0.30	7.13	0.22
10	3.80	0.25	3.60	0.48	5.74	0.30	7.09	0.16
12	3.77	0.29	2.90	0.42	5.32	0.30	7.02	0.20
14	3.50	0.35	2.80	0.23	4.73	0.39	7.05	0.14
16	3.26	0.20	2.75	0.65	3.88	0.24	7.23	0.24
18	2.33	0.28	3.33	1.36	3.63	0.38	7.53	0.34
21	2.33	0.17	2.73	0.55	3.74	0.26	7.05	0.15
26	3.00	0.30	3.55	0.15	3.76	0.22	7.06	0.29
28	2.77	0.22	2.45	0.05	3.48	0.19	7.06	0.20
32	3.14	0.26	3.25	0.25	3.55	0.06	7.15	0.24
35	2.55	0.05	4.50	-	3.45	0.42	7.34	0.23
40	2.45	0.15	5.00	-	3.93	0.17	7.02	0.34
Group Means	4.66	0.26	4.96	0.34	5.33	0.34	7.14	0.21

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance (%)
period	350.0693	23	15.2204	17.61	0.0001	0.001
Treatment	12.6195	3	4.2065	4.87	0.0025	0.01
Residual	290.4031	336	0.8643			
Total	653.0918	362				
Tv Vs Tb	0.0427	1	0.0427	0.05	0.8243	Not significant
Tv Vs Tc	1.8493	1	1.8493	2.14	0.1445	Not significant
Tc Vs Tb	1.1783	1	1.1783	1.36	0.0438	Not significant

Table. 6. Mean Corpuscular Volume (M.C.V.) Mean Cell Haemoglobin (M.C.H.) And Mean Cell Haemoglobin Concentration (M.C.H.C.) of Groups of Trypanosome Infected Savanna Brown Goats and Non-infected Control SBG

DPI	M. C. V.				M. C. H				M. C. H. C.			
	TV	TC	TB	C	TV	TC	TB	C	TV	TC	TB	C
-28	41.51	41.65	40.56	43.21	13.84	13.97	13.53	14.45	33.37	33.55	33.24	33.44
-21	40.99	42.77	39.05	42.93	13.66	14.31	12.70	14.31	33.34	33.25	32.50	33.33
-14	40.43	41.88	38.83	42.47	13.49	13.97	13. 1	14.16	33.34	33.53	33.73	33.33
-07	38.92	41.15	42.58	42.88	12.97	13.72	14.24	14.02	33.31	33.33	33.44	33.36
-03	41.1	40.2	41.2	42.1	13.1	13.8	14.1	14.2	33.0	331	33.0	33.3
0	41.38	42.23	41.81	42.08	13.08	14.01	14.02	14.02	33.40	33.32	33.53	33.32
2	38.85	45.33	39.17	42.53	12.94	15.11	13.11	14.25	33.30	33.33	33.46	33.49
4	38.03	46.33	37.15	42.06	12.68	15.44	12.40	13.57	33.35	33.33	33.38	32.24
6	45.74	43.78	35.53	41.61	15.25	14.59	12.46	14.00	33.33	33.34	35.07	33.65
8	48.47	42.95	32.34	41.54	16.14	17.60	10.78	13.85	33.31	40.99	33.34	33.33
10	48.90	42. 5	30.39	42.38	16.32	14.22	10.13	14.13	33.36	33.47	33.33	33.35
12	46.68	45.52	29.49	40.93	15.58	15.17	9.95	14.20	33.37	33.33	33.73	34.65
14	49.67	44.64	29.97	43.16	16.57	14.91	9.97	14.38	33.37	33. 4	33.27	33.32
16	51.23	44.55	36.44	41.93	17.12	14.82	12.15	13.83	33.41	33.27	33.34	32.97
18	68.82	40.60	37.16	39.23	22.90	13.54	12.39	12.99	33.28	33.33	33.33	33.11
21	54.30	40.38	35.30	42.49	18.00	22.32	11.71	14.16	33.16	42.56	33.19	33.28
26	47.56	52.11	32.98	42.92	15.89	15.49	11.86	14.31	33.41	29.73	35.97	33.33
28	54.14	59.19	35.63	42.92	17.83	19.80	13.79	13.85	32.53	33.45	35.82	33.31
32	52.67	60.00	38.73	40.22	17.56	20.00	12.68	13.40	33.33	33.33	32.73	33.32
35	62.75	47.78	39.13	39.84	20.78	16.00	13.04	13.32	33.13	33.49	33.33	33.38
40	57.14	46. 0	38.14	42.25	18.98	15.40	12.63	14.05	33.21	33.47	33.10	33.24
42	42.19	52. 5	43.56	40.67	14.06	17.50	14.58	13.56	33.33	33.33	33.46	33.33

D.P.I. - Day Post Infection

T.V. - *T. vivax* Infected Group

T.C. - *T. congolense* Infected Group

T.B. - *T. brucei* Infected Group

C - Control non - Infected Group

Table.7. Mean Total Leucocyte Count (x $10^{9}/6$) of Groups of Trypanosome Infected Savanna Brown Goats and Non Infected

Days Post Infection	T. vivax Mean	T. vivax SD	T. congolense Mean	T. congolense SD	T. brucei Mean	T. brucei SD	Control Mean	Control SD
-28	15,020.0	140.6	14,993.0	381.0	14,725.0	138.5	14,970.5	185.4
-21	14,883.8	190.1	15,029.0	160.0	14,887.5	150.0	14,921.3	204.0
-14	14,937.5	140.8	15,071.4	131.3	14,900.0	130.0	14,820.0	171.1
- 7	15,037.5	226.4	14,928.6	149.6	15,012.5	124.6	14,818.7	188.9
- 3	14,981.2	123.5	14,828.5	170.4	15,000.0	130.9	14,987.0	391.0
0	14,993.7	120.5	14,971.4	98.1	14,975.0	116.5	14,860.0	207.8
2	14,843.7	159.1	15,014.3	226.8	15,187.5	135.6	14,800.0	310.0
4	15,008.3	165.7	14,842.5	127.2	15,081.2	119.3	14,850.0	239.0
6	14,740.3	34.1	14,889.7	90.0	14,888.0	387.0	14,869.7	190.7
8	15,133.3	206.5	14,871.4	111.3	15,075.0	183.0	14,621.0	271.0
10	15,166.7	216.0	15,040.0	114.0	15,037.3	128.0	14,742.9	221.9
12	15,400.0	37.4	15,020.0	109.0	15,221.4	135.0	14,822.9	154.4
14	15,417.0	44.9	15,829.0	33.0	15,400.0	115.5	14,643.0	412.0
16	15,800.0	158.1	15,925.0	500.0	15,283.3	240.1	14,917.0	319.0
18	16,150.0	265.0	16,100.0	100.0	16,117.0	264.0	14,667.0	280.0
21	16,933.0	120.7	17,200.0	120.0	17,100.0	235.0	14,750.0	205.0
26	16,400.0	400.0	17,050.0	500.0	17,230.0	311.0	14,340.0	297.0
28	15,933.0	210.0	17,100.0	100.0	17,140.0	167.3	14,810.0	143.0
32	16,400.0	200.0	16,900.0	110.0	16,800.0	141.4	14,960.0	151.7
35	17,500.0	100.0	16,000.0	-	16,700.0	258.0	14,810.0	188.4
40	16,300.0	-	13,900.0	-	17,000.0	100.0	14,670.4	249.0
Group Means								

Analysis of Variance

Source of Variation	S S	DF	M S	F	Pr>F	Level of Significance %
Period	894162186.1	23	38876616.8	0.41	0.9940	Not significant
Treatment	699237106.2	3	233079035.4	2.43	0.0649	0.05
Residual	32186487458	336	95793117			
Total	33779886750	362				
Tv Vs Tb	382123824.3	1	382123824.3	3.99	0.0466	0.05
Tv Vs Tc	5629.9	1	5629.9	0.00	0.9807	Not significant
Tc Vs Tb	357720606.2	1	357720606.2	3.73	0.0541	0.05

Table.8. Mean Lymphocyte Count (x 10^{9}/L) of Groups of Trypanosome Infected Savanna Brown Goats and Non Infected Control SBG

Days Post Infection	T. vivax Mean	T. vivax SD	T. congolense Mean	T. congolense SD	T. brucei Mean	T. brucei SD	Control Mean	Control SD
-28								
-14	11,264.6	119.5	112,430.0	402.0	11,006.8	103.0	11,171.9	222.4
- 7	11,296.6	144.9	11,260.7	159.2	10,969.8	86.4	11,044.8	182.9
- 3	11,240.1	170.7	11,749.9	129.5	11,127.6	88.6	11,076.7	175.1
0	11,306.4	169.3	11,079.4	185.4	10,988.2	228.8	11,015.0	285.0
2	11,245,0	114.1	11,250.0	129.9	11,100.4	164.3	10,958.0	248.1
4	11,132.6	148.5	11,240.1	252.0	11,305.0	173.4	10,905.0	352.0
6	11,141.9	155.8	11,132.1	127.7	11,857.0	192.4	11,044.4	189.0
8	11,499.0	292.0	11,738.7	178.7	11,728.2	215.0	11,114.1	152.4
10	11,931.0	281.0	12,302.0	269.0	11,439.2	265.9	10,937.0	282.0
12	12,133.0	230.4	12,693.6	104.3	11,493.5	123.5	11,015.1	242.0
14	12,936.0	313.0	11,144.8	99.8	11,655.3	202.8	10,962.2	188.7
16	12,926.0	605.0	11,078.0	391.0	11,605.0	163.4	10,711.0	382.0
18	13,208.8	158.3	10,786.0	241.0	11,282.5	190.0	11,087.0	245
21	13,119.3	112.4	9,538.8	116.1	11,952.5	161.0	10,999.5	199.2
26	13,772.0	153.5	11,085.0	209.0	11,764.4	201.5	10,768.0	251.0
28	13,120.0	320.0	12,702.5	250.0	12,470.6	200.7	10,755.0	234.0
32	11,961.0	1,147.0	13,730.0	460.0	12,409.6	176.4	10,960.2	203.4
35	11,016.0	236.0	13,521.0	265.0	12,849.0	79.8	10,772.0	154.6
40	12,337.0	17.0	13,500.0	-	13,193.0	279.0	10,979.0	157.0
Group Means	11,899.0	120.0	10,981.0	-	13,430.0	221.0	10,972.0	133.3

Analysis of Variance

Sources of Variation	S S	DF	M S	F	Pr>F	Level of Significance %
Period	842.7941	23	36.6432	5.95	0.0001	0.001
Treatment	327.8069	3	109.2690	17.76	0.0001	0.001
Residual	2,067.8122	336	6.1542			
Total	3,238.4132	362				
Tv Vs Tb	225.7073	1	225.7073	36.68	0.0001	0.001
Tv Vs Tc	141.4706	1	141.4706	22.99	0.0001	0.001
Tc Vs Tb	4.2779	1	4.2779	0.70	0.4050	Not significant

Table.9. Mean Neutrophil Count (x 10^9/l) of Groups of Trypanosome Infected Savanna Brown Goats and Non Infected Control SBG

Days Post Infection	T. vivax Mean	T. vivax SD	T. congolense Mean	T. congolense SD	T. brucei Mean	T. brucei SD	Control Mean	Control SD
-28								
-21	3755.4	131.4	3491.3	156.7	3620.2	81.9	3649.0	89.6
-14	3627.2	92.9	3713.6	38.9	3629.4	139.2	3599.9	105.9
- 7	3548.1	92.4	3681.0	170.4	3669.3	86.6	3625.4	57.9
- 3	3721.9	123.1	3625.7	124.6	3698.8	19.5	3520.1	223.5
0	3650.0	75.1	3522.7	97.9	3693.4	62.7	3761.0	181.8
2	3673.7	126.0	3614.9	117.6	3588.1	91.5	3653.5	154.7
4	3636.9	121.9	3624.0	19.8	3572.4	223.6	3548.0	116.5
6	3595.7	124.6	3392.5	92.5	2941.0	87.5	3676.8	127.3
8	3165.9	125.0	2913.3	78.9	3015.4	174.5	3624.5	128.6
10	3026.8	218.5	2442.4	245.5	3296.6	189.1	3612.2	75.7
12	2783.0	176.0	2196.2	94.9	3305.4	78.2	3727.7	160.5
14	2182.2	138.8	3695.2	100.3	3282.6	154.4	3830.6	120.0
16	2233.8	112.6	4471.0	220.0	3388.1	98.7	3639.4	213.2
18	2212.2	117.6	4899.0	237.0	3898.0	183.3	3555.7	117.7
21	2706.2	181.2	5018.2	149.8	4164.2	150.8	3520.5	131.6
26	2879.0	186.0	5007.0	361.0	5164.0	160.3	3712.2	154.6
28	2853.0	212.0	3751.0	11.0	4342.0	26.3	3499.2	187.6
32	3186.7	41.6	3890.0	160.0	4561.0	109.9	3701.4	114.2
35	4221.0	888.0	3211.0	20.0	4242.0	220.0	3743.2	199.2
40	4637.0	140.0	3960.0	-	3048.0	100.0	3702.0	92.3
Group Means	3912.0	65.0	2502.0	-	3169.7	79.5	3551.0	193.5

Analysis of Variance

Source of Variation	S S	DF	M S	F	Pr>F	Level of Significance %
Period	1072.0938	23	48.6128	3.46	0.0001	0.001
Treatment	302.3628	3	100.7876	7.48	0.0001	0.001
Residual	4530.1131	336	13.4829			
Total	5904.5698	362				
Tv Vs Tb	199.3563	1	199.3563	14.79	0.0001	0.001
Tv Vs Tc	101.4545	1	101.4545	7.52	0.0064	0. 01
Tc Vs Tb	9.5201	1	9.5201	0.71	0.4017	Not significant

Table.10. Mean Monocyte Count (x 10 ^{9}/L) of Groups of Trypanosome Infected Savanna Brown Goats and Non Infected Control SBG

Days Post Infection	T. vivax Mean	T. vivax SD	T. congolense Mean	T. congolense SD	T. brucei Mean	T. brucei SD	Control Mean	Control SD
-28								
-21	110.3	50.0	128.3	56.9	110.1	68.0	112.1	69.2
-14	111.8	69.0	150.6	87.8	129.6	93.6	106.7	38.1
- 7	74.2	79.4	129.3	103.0	149.2	80.2	111.5	105.7
- 3	56.9	78.5	107.0	113.8	112.8	107.0	129.5	94.4
0	37.5	69.4	148.4	84.9	187.4	68.9	130.9	53.9
2	75.0	80.2	128.0	102.9	149.9	113.0	148.0	78.1
4	74.4	79.5	150.1	2.3	151.5	80.7	110.0	68.0
6	150.4	81.8	127.4	56.2	169.7	125.9	111.4	68.8
8	105.0	71.8	127.4	50.5	131.2	53.5	125.7	95.5
10	125.7	112.6	120.3	56.3	169.6	53.5	125.7	50.5
12	126.2	112.7	150.2	105.4	188.4	134.0	130.5	50.1
14	51.0	79.0	150.0	105.4	174.4	137.7	21.1	58.7
16	129.7	119.5	159.3	3.3	308.0	2.3	147.6	85.5
18	157.8	112.4	121.3	80.9	127.8	115.8	149.3	93.7
21	121.7	81.2	281.5	79.7	138.5	50.0	122.0	59.8
26	168.7	108.0	172.0	10.0	137.2	76.7	147.5	18.7
28	162.7	164.0	341.0	1.0	172.2	3.4	29.2	65.3
32	159.0	160.0	190.0	10.0	34.0	26.0	118.2	66.3
35	217.3	88.9	162.0	162.0	100.0	56.0	119.2	66.6
40	350.0	2.0	180.0	-	166.5	135.8	149.0	106.8
	326.0	6.0	278.0	-	226.7	98.2	117.0	65.4

Analysis of Variance

Source of Variation	SS	D F	M S	F	Pr>F	Level of Significance %
Period	8.4760	23	0.3685	1.00	0.4647	Not significant
Treatment	6.6120	3	2.2040	5.98	0.0006	0.001
Residual	123.8267	336	0.3685			
Total	138.9146	362				
Tv Vs Tb	5.6438	1	5.6438	15.31	0.0001	0.001
Tv Vs Tc	2.6035	1	2.6035	7.06	0.0082	0. 01
Tc Vs Tb	0.3636	1	0.3636	0.99	0.3213	Not significant

Table.11. Mean Eosinophil Count (x 10^9/l) of Groups of Trypanosome Infected Savanna Brown Goats and Non Infected Control SBG

Day Post Infection	T. vivax		T. congolense		T. brucei		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-28	0	0	129.9	57.4	0	0	37.6	69.7
-21	93.3	77.2	0	0	129.6	93.6	0	0
-14	18.6	58.7	21.6	57.1	111.9	89.0	89.0	52.3
- 7	21.6	52.7	0	0	75.4	80.6	74.2	79.4
- 3	0	0	106.6	72.5	131.0	96.1	74.6	79.8
0	0	0	0	0	130.9	95.6	111.1	104.7
2	0	0	0	0	152.0	80.7	0	0
4	93.5	111.1	106.1	72.9	113.0	134.6	18.7	53.0
6	0	0	108.3	72.6	122.8	69.6	0	0
8	0	0	106.3	72.6	169.8	76.0	148.0	86.6
10	126.6	61.8	0	0	37.8	69.3	0	0
12	231.2	85.0	30.0	57.1	109.1	116.0	0	0
14	126.8	113.5	117.7	78.5	109.7	75.0	148.0	80.9
16	221.2	88.5	119.2	79.7	127.8	115.8	124.3	111.2
18	162.3	132.5	201.5	81.7	0	0	24.7	60.4
21	112.3	98.1	115.0	99.6	34.2	76.5	122.5	60.0
26	164.0	4.0	255.5	82.7	172.2	30.1	28.4	63.5
28	105.7	91.5	190.0	10.0	132.0	143.7	29.8	65.6
32	212.7	72.6	0	0	126.0	160.2	89.4	81.6
35	175.0	1.0	180.0	-	291.2	84.3	0	0
40	163.0	3.0	139.0	-	170.0	1.0	30.0	67.2
Group Mean								

Analysis of Variance

Source of Variation	S S	DF	MS	F	Pr>F	Level of Significance %
Period	18.2858	23	0.7950	2.57	0.0001	0.001
Treatment	8.2952	3	2.7651	8.94	0.0001	0.001
Residual	103.8653	336	0.3091			
Total	130.4413	362				
Tv Vs Tb	5.0977	1	5.0977	16.49	0.0001	0.001
Tv Vs Tc	0.1856	1	0.1856	0.60	0.4589	Not significant
Tc Vs Tb	2.9290	1	2.9290	9.48	0.0023	0.0 1

Table.12a. Mean Weekly Body Weights (Kg) of Groups of Trypanosome Infected Savanna Brown Goats and Non Infected Control SBG

Week (Days) Post Infection	T. vivax Mean	SD	T. congolense Mean	SD	T. brucei Mean	SD	Control Mean	SD
-4(28)	17.46	0.40	15.86	0.43	12.86	0.36	20.61	0.69
-3(21)	19.23	0.43	17.47	0.39	14.26	0.36	22.16	0.32
-2(14)	20.79	0.60	18.87	0.39	15.66	0.36	24.13	0.43
-1(-7)	22.65	0.65	20.32	0.39	17.16	0.35	26.23	0.42
0(0)	23.98	0.63	22.04	0.48	18.86	0.35	28.43	0.79
1(7)	25.44	0.61	23.52	0.47	20.34	0.34	30.41	1.01
2(14)	26.64	0.73	24.33	0.50	21.43	0.41	32.46	1.01
3(21)	27.70	0.35	25.33	0.48	22.54	0.43	34.59	1.34
4(28)	28.67	0.36	26.10	0.10	23.14	0.44	36.81	0.81
5(35)	29.55	0.56	24.40	-	23.35	0.72	38.67	0.57
6(42)	27.80	-	24.50		23.67	0.33	40.22	0.53
Group Mean								

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance %
Period	17984.6428	23	781.9410	86.65	0.0001	0.001
Treatment	1767.5432	3	589.1811	65.29	0.0001	0.001
Residual	3032.0975	336	9.0241			
Total	2279.2835	362				
Tv Vs Tb	406.2246	1	406.2246	45.02	0.0001	0.001
Tv Vs Tc	43.0932	1	43.0932	4.78	0.0296	0. 05
Tc Vs Tb	155 .5290	1	155.5290	17.23	0.0001	0.001

Table.12b. Mean Weekly Body Weight Gains (Kg) of Groups of Trypanosome Infected Savanna Brown Goats and Un Infected Control Group of SBG

Weeks (Days) Post Infection	T. vivax Mean	SD	T. congolense Mean	SD	T. brucei Mean	SD	Control Mean	SD
-4(-28)	1.46		1.62		1.01		1.55	
-3(-21)	1.56		1.70		1.40		1.96	
-2(-14)	1.76		1.95		1.58		2.10	
-1(-)	1.93		2.15		1.70		2.20	
0(0)	1.97		2.37		1.96		1.98	
1(7)	2.00		1.81		1.72		2.05	
2(14)	1.67		1.01		1.11		2.13	
3(21)	0.97		0.77		0.94		2.22	
4(28)	0.88		-0.40		0.56		1.86	
5(35)	-1.78		-1.40		0.32		1.55	
6(42)								
Group Mean								

APPENDIX 2

ANALYSIS OF VARIANCE FOR MEAN VALUES OF SOME SERUM BIOCHEMICAL PARAMETERS

Table.1. Mean Serum Sodium Levels (M Mol/L) of Trypanosome Infected Groups of Savannah Brown Goats and Un-Infected Control SBG

Days Post Infection	T. vivax		T. congolense		T. brucei		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-14	149.17	1.60	147.33	2.16	149.50	1.05	149.88	9.25
- 7	150.50	1.05	148.83	1.47	150.00	0.63	150.25	3.37
- 3	150.33	0.52	150.67	1.21	150.00	1.41	149.63	6.50
0	149.67	1.63	149.50	0.84	150.17	0.75	150.00	3.76
2	150.50	1.76	149.17	1.17	150.33	0.52	150.37	1.60
4	150.67	1.97	150.17	1.47	149.50	0.84	149.75	2.05
6	149.63	1.37	149.33	1.63	149.60	0.55	150.00	2.56
8	150.20	0.84	149.83	1.83	149.20	0.45	148.20	3.65
10	149.80	0.84	150.40	0.55	150.00	0.21	149.20	2.59
12	149.40	1.52	150.60	0.55	149.80	0.94	149.83	1.72
14	149.40	0.89	150.00	0.82	150.20	0.84	149.67	0.52
16	143.00	2.35	149.50	0.58	149.60	1.52	150.17	2.93
18	140.50	0.58	143.50	0.58	146.00	2.97	150.60	1.34
21	139.33	1.25	141.67	0.34	142.40	3.13	150.20	0.49
26	130.00	4.98	139.50	0.40	138.40	5.23	150.20	0.61
28	121.67	12.25	131.27	1.54	133.35	3.27	149.81	2.36
Group Mean	143.73		146.96		146.75		149.74	

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance %
Period	693.7545	15	49.5539	5.44	0.0001	0.001
Treatment	45.2700	3	15.0900	1.66	0.1763	0.05
Residual	2758.8632	303	9.1052			
Total	3497.8879	320				
Tv Vs Tb	0.3592	1	0.3592	0.04	0.8427	Not significant
Tv Vs Tc	1.8254	1	1.8254	0.20	0.6547	Not significant
Tc Vs Tb	0.6011	1	0.6011	0.07	0.7974	"

Table.2. Mean Serum Pottasium Levels (M Mol/L) of Trypanosome Infected Groups of Savanna Brown Goats and Un-Infected Control Group of SBG

Days Post Infection	T. vivax		T. congolense		T. brucei		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-14	5.56	0.21	5.60	0.46	5.83	0.75	5.83	0.71
- 7	5.63	0.28	5.52	0.40	5.65	0.21	5.20	0.67
- 3	5.77	0.23	5.27	0.61	5.43	0.50	5.94	0.50
0	5.78	0.23	5.35	0.45	5.28	0.37	5.63	0.52
2	5.55	0.30	5.28	0.32	5.03	0.52	5.14	0.57
4	5.55	0.49	4.92	0.31	5.25	0.36	5.60	0.52
6	5.05	0.26	5.20	0.34	5.50	0.47	5.69	0.41
8	5.30	2.30	5.10	0.16	5.38	0.57	5.68	0.13
10	5.70	0.29	4.90	0.10	5.19	0.47	5.64	0.20
12	5.18	0.22	5.30	0.45	5.20	0.48	5.50	0.49
14	5.12	0.13	5.43	0.44	5.20	0.45	5.50	0.28
16	5.82	0.15	5.95	0.42	6.67	0.42	5.58	0.49
18	6.50	0.41	6.85	0.37	6.80	0.28	5.32	0.24
21	7.03	0.17	7.50	0.34	7.26	0.34	5.54	0.49
26	7.83	0.24	7.85	0.35	7.66	0.42	5.66	0.24
28	8.13	0.87	8.37	0.41	8.48	0.38	5.96	0.49
Group Mean	6.05		6.09		5.99		5.61	

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance %
Period	106.5264	15	7.6090	0.93	0.5239	Not significant
Treatment	15.1457	3	5.0486	0.62	0.6033	0.05
Residual	2472.3637	303	8.1596			
Total	2594.0350	320				
Tv Vs Tb	1.4552	1	1.4552	0.18	0.6731	Not significant
Tv Vs Tc	0.3748	1	0.3748	0.05	0.8304	Not significant
Tc Vs Tb	3.3231	1	3.3231	0.41	0.5238	"

Table.3. Mean Serum Chloride Levels (M Mol/L) of Trypanosome Infected Groups of Savanna Brown Goats and Un-Infected Control SBG

Days Post Infection	T. vivax Mean	T. vivax SD	T. congolense Mean	T. congolense SD	T. brucei Mean	T. brucei SD	Control Mean	Control SD
-14	102.17	7.03	99.83	5.56	103.57	9.32	103.63	2.92
- 7	100.67	4.27	101.50	6.86	104.17	2.99	101.62	7.15
- 3	104.83	19.77	103.00	3.35	100.53	4.89	100.63	6.25
0	100.00	6.32	101.83	6.24	100.00	3.69	101.02	3.92
2	99.33	1.75	99.00	5.59	101.67	1.97	100.63	6.76
4	100.33	3.61	101.50	6.09	99.00	4.65	101.87	2.51
6	102.33	6.22	100.33	6.41	99.60	5.27	100.50	6.63
8	98.60	2.83	101.00	6.26	99.00	0.16	100.00	2.91
10	101.60	3.36	102.20	1.92	102.40	2.79	99.40	5.32
12	100.00	5.83	101.60	1.52	100.20	3.36	99.83	4.45
14	100.40	8.50	100.75	1.89	99.80	3.35	99.98	4.03
16	95.80	1.64	98.75	0.96	96.80	5.28	101.33	7.99
18	93.00	0.82	95.25	1.50	96.60	5.37	100.00	1.22
21	90.33	0.67	92.67	0.82	90.60	3.63	100.40	0.55
26	82.67	2.64	87.50	1.50	86.00	7.52	101.00	3.94
28	82.00	1.75	84.52	0.95	82.56	2.63	102.35	1.85
Group Mean	97.004		100.61		97.60		100.89	

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance %
Period	2862.7483	15	204.4820	1.49	0.1142	Not significant
Treatment	423.8348	3	141.2783	1.03	0.3806	0.05
Residual	41657.0792	303	137.4821			
Total	44943.6623	320				
Tv Vs Tb	246.9581	1	246.9581	1.80	0.1812	Not significant
Tv Vs Tc	46.4496	1	46.4496	0.34	0.5612	Not significant
Tc Vs Tb	76. 1666	1	76.1666	0.55	0.4573	"

Table.4. Mean Serum Bicarbonate Levels (m Mol/L) of Trypanosome Infected Groups of Savanna Brown Goats and Un-Infected Control SBG

Days Post Infection	T. vivax		T. congolense		T. brucei		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-14	20.33	0.82	24.33	0.82	20.50	0.55	20.50	1.20
- 7	19.50	0.84	21.17	1.17	21.83	0.75	21.00	1.31
- 3	20.33	0.82	20.83	0.75	20.00	0.89	21.00	1.85
0	21.00	1.27	21.17	0.75	20.33	0.52	21.00	2.00
2	21.00	0.63	20.17	0.75	20.00	0.90	20.63	1.92
4	21.00	1.10	19.83	0.93	20.17	0.75	21.38	1.51
6	21.00	0.63	20.00	0.89	20.20	0.45	21.00	1.51
8	19.00	2.10	20.17	0.75	20.00	0.71	20.40	1.82
10	20.00	0.71	20.80	0.84	20.20	0.45	21.00	1.00
12	17.60	0.90	21.40	0.55	20.20	0.45	20.33	1.03
14	16.20	0.84	18.25	0.50	17.20	0.45	20.50	1.05
16	16.20	0.45	17.50	0.57	15.20	0.84	21.00	1.27
18	15.25	0.50	14.50	0.58	14.60	0.55	20.60	1.14
21	13.67	1.33	14.00	1.53	14.00	1.00	21.00	1.00
26	12.33	0.71	13.00	0.76	12.80	1.30	21.20	0.84
28	11.33	1.68	12.08	1.33	10.83	0.84	21.76	0.64

Group Mean

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance %
Period	1188.1232	15	84.8660	2.64	0.0012	0.01
Treatment	524.2903	3	174.7634	5.44	0.0012	0.01
Residual	9226.0911	303	32.0993			
Total	11438.5047	320				
Tv Vs Tb	1.6700	1	1.6700	0.05	0.8197	Not significant
Tv Vs Tc	27.4656	1	27.4656	0.86	0.3557	Not significant
Tc Vs Tb	43.6790	1	43.6790	1.36	0.2443	"

Table.5. Mean Serum (Blood) Urea Nitrogen Levels (m Mol/L) of Trypanosome Infected Groups of Savanna Brown Goats and Un-Infected Control SBG

Days Post Infection	*T. vivax*		*T. congolense*		*T. brucei*		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-14	6.50	0.71	6.05	0.34	5.88	0.10	6.40	0.68
- 7	6.05	0.61	5.92	0.21	5.92	0.10	5.84	0.82
- 3	6.18	0.29	6.13	0.19	6.07	0.25	5.84	2.06
0	6.75	0.30	6.07	0.12	5.89	0.12	6.41	0.42
2	6.68	0.31	5.85	0.23	5.88	0.12	6.20	0.29
4	6.83	0.19	5.68	0.22	5.57	0.16	5.95	0.52
6	6.87	0.16	6.05	0.23	5.98	0.05	6.21	0.30
8	6.80	0.14	5.93	0.12	5.78	0.21	6.00	0.14
10	6.10	0.16	6.12	0.11	6.00	0.36	6.12	0.13
12	6.32	0.25	6.24	0.25	6.20	0.45	5.92	0.24
14	6.80	0.32	7.25	0.50	6.90	0.23	6.00	0.28
16	6.46	0.75	7.55	0.53	7.70	0.54	6.20	0.18
18	7.55	0.42	8.25	0.29	7.76	0.55	6.04	0.23
21	8.27	0.35	8.17	0.42	8.50	0.50	5.86	0.23
26	8.73	0.29	8.50	0.64	9.56	0.14	6.16	0.15
28	9.10	0.67	8.97	0.48	10.31	0.12	6.61	0.14

Group Mean

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance %
Period	240.6557	15	17.1897	0.60	0.8644	Not significant
Treatment	103.6717	3	34.5339	5.21	0.0307	0.05
Residual	8676.6206	303	28.6357			
Total	9020.8790	320				
Tv Vs Tb	62.2120	1	62.2120	2.17	0.1415	Not significant
Tv Vs Tc	78.6277	1	78.6277	2.75	0.0983	0.05
Tc Vs Tb	1.3245	1	1.3245	0.05	0.8299	"

Table.6. Mean Serum Bilirubin Levels (μ Mol/L) of Trypanosome Infected Groups of Savanna Brown Goats and Un-Infected Control SBG

Days Post Infection	T. vivax		T. congolense		T. brucei		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-14	2.33	0.52	2.33	0.52	2.50	0.55	2.25	0.46
- 7	2.33	0.51	2.33	0.52	2.50	0.55	2.50	0.54
- 3	2.50	0.55	2.50	0.55	2.33	0.52	2.50	0.46
0	2.33	0.52	2.33	0.52	2.17	0.41	2.38	0.52
2	2.33	0.62	2.33	0.95	2.17	0.41	2.38	0.52
4	2.50	0.55	2.50	0.55	2.67	0.82	2.38	0.74
6	2.17	0.41	2.67	0.52	2.80	0.44	2.48	0.52
8	3.82	0.18	3.17	0.41	2.40	0.55	2.60	0.52
10	4.20	0.45	4.40	0.55	3.60	0.55	2.60	0.85
12	4.40	0.55	4.60	0.55	4.00	0.50	2.33	0.52
14	4.80	0.45	5.25	0.50	4.10	0.65	2.67	0.82
16	4.40	0.55	4.50	0.56	4.40	0.42	2.67	0.52
18	4.75	0.50	5.53	0.61	4.60	0.55	2.80	0.45
21	5.00	0.65	6.00	0.54	5.40	0.55	2.60	0.55
26	6.33	0.55	7.50	0.50	6.40	0.74	2.60	0.56
28	7.00	0.60	8.13	0.41	7.250	0.62	2.58	0.66

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance %
Period	191.2048	15	13.6575	24.03	0.0001	0.001
Treatment	48.0083	3	16.0228	28.19	0.0001	0.001
Residual	172.2203	303	32.0993			
Total	411.4933	320				
Tv Vs Tb	0.0000	1	0.00000	0.00	0.9993	Not significant
Tv Vs Tc	0.2811	1	0. 2811	0.49	0.4824	Not significant
Tc Vs Tb	0.2892	1	0.2892	0.51	0.443	"

Table 7. Mean Plasma Total Protein Levels (Gm/100mls) of Trypanosome Infected Groups of Savannah Brown Goats and Uninfected Control SBG.

Days Post Infection	T. vivax		T. congolense		T. brucei		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-14	8.16	0.31	8.82	0.22	8.08	0.28	7.80	0.53
-7	8.13	0.33	8.05	0.50	8.08	0.28	8.05	0.31
-3	8.16	0.52	8.63	0.73	8.00	0.29	7.88	0..46
0	8.23	0.33	8.00	0.64	7.96	0.13	8.11	0.23
2	8.03	0.33	8.86	0.90	7.70	0.46	7.93	0.18
4	8.08	0.41	8.36	0.48	7.93	0.27	7.87	0.21
6	8.03	0.18	7.93	0.16	7.84	0.36	8.13	0.22
8	8.72	0.17	7.72	0.49	7.90	0.16	8.00	0.41
10	8.50	0.45	7.96	0.89	8.08	0.36	7.83	0.41
12	7.43	0.38	7.60	0.42	7.84	0.22	8.02	0.37
14	7.47	0.33	7.50	0.41	6.47	0.38	7.96	0.30
16	6.46	0.75	7.20	0.40	6.52	0.22	7.83	0.34
18	5.50	0.58	6.50	1.04	6.57	0.35	7.55	0.33
21	5.93	0.17	6.10	0.27	5.90	0.23	8.13	0.24
26	6.03	0.22	5.50	0.50	6.10	0.14	8.08	0.13
28	6.00	0.20	5.70	0.25	6.32	0.24	7.88	0.23

Analysis of Variance

Source of Variatio	S S	D F	M S	F	Pr>F	Level of Significance %
Period	116.9000	15	8.3500	23.23	0.0001	0.001
Treatment	25.5103	3	8.5034	23.66	0.0001	0.001
Residual	108.9076	303	0.3594			
Total	251.3180	320				
Tv Vs Tb	1.5330	1	1.5330	4.27	0.0398	0.05
Tv Vs Tc	12.7705	1	12.7705	35.53	0.0001	0.001
Tc Vs Tb	23.6401	1	23.6401	65.77	0.0001	0.001

Table.8. Mean Serum Albumin Levels (Gm/100mls) of Trypanosome Infected Groups of Savanna Brown Goats and Un-Infected Control SBG

Days Post Infection	*T. vivax*		*T. congolense*		*T. brucei*		*Control*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-14	3.03	0.52	3.05	0.55	3.08	0.75	3.01	1.25
- 7	3.00	1.42	3.15	1.05	3.03	0.52	3.00	1.61
- 3	3.12	0.75	3.05	0.55	3.00	0.41	3.06	1.41
0	3.08	0.79	3.10	0.89	3.03	0.52	3.04	1.51
2	2.97	0.52	3.02	0.41	3.02	0.41	3.10	0.86
4	2.90	0.32	2.95	0.55	3.02	0.41	3.09	0.75
6	2.82	0.12	2.72	0.01	2.88	0.45	3.09	1.89
8	2.88	0.84	2.80	0.10	2.80	0.71	3.04	0.90
10	2.72	0.84	2.90	1.00	2.76	0.55	3.08	0.84
12	2.86	0.55	2.92	0.45	2.92	0.84	3.05	0.55
14	2.82	0.84	2.65	0.58	2.54	0.55	3.08	0.98
16	2.60	1.00	2.57	0.50	1.94	0.55	3.07	0.52
18	2.30	0.82	2.03	0.50	1.90	0.56	3.04	1.14
21	1.90	1.42	1.83	1.67	1.82	0.45	3.04	0.55
26	1.73	1.33	1.70	0.13	1.50	0.71	3.08	0.84
28	1.47	0.56	1.50	0.52	1.28	0.82	3.10	0.44

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance %
Period	158.5045	15	11.3217	9.13	0.0001	0.001
Treatment	50.2294	3	16.7451	13.50	0.0001	0.001
Residual	375.8521	303	1.2404			
Total	584.5860	320				
Tv Vs Tb	9.3227	1	9.3227	7.52	0.0065	0.01
Tv Vs Tc	0.8088	1	0.8088	0.65	0.4200	Not significant
Tc Vs Tb	4.5246	1	4.5246	3.65	0.0571	0.05

Table.9. Mean Serum Glucose Level (M Mol/L) of Trypanosome Infected Groups of Savanna Brown Goats and Un-Infected Control SBG

Days Post Infection	*T. vivax*		*T. congolense*		*T. brucei*		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-14	5.50	0.45	5.22	0.45	5.75	0.53	5.48	0.66
- 7	5.55	0.43	5.00	0.14	5.42	0.49	5.25	0.55
- 3	5.68	0.46	5.42	0.24	5.50	0.45	5.70	0.53
0	6.10	0.24	5.18	0.27	5.83	0.52	5.98	0.38
2	5.98	1.02	5.05	0.14	5.17	0.41	5.95	0.49
4	4.48	0.29	4.22	0.23	3.78	0.72	6.14	0.50
6	3.67	0.27	3.50	0.32	3.40	0.65	5.95	0.47
8	3.86	0.26	2.62	0.63	2.60	0.42	5.88	0.24
10	2.64	0.26	2.00	0.35	2.50	0.35	5.70	0.22
12	2.14	0.14	1.40	0.42	1.50	1.00	5.90	0.24
14	1.84	0.17	1.33	0.54	1.10	0.55	5.75	0.25
16	0.82	0.29	1.00	0.71	0.86	0.22	6.20	0.25
18	0.60	0.82	1.00	0.25	0.78	0.23	6.20	0.11
21	0.43	0.11	0.87	0.52	0.64	0.42	5.74	0.44
26	0.33	0.07	0.65	0.04	0.30	0.27	5.78	0.30
28	0.50	0.10	0.84	0.06	0.71	0.10	6.10	0.42

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance %
Period	482.1705	15	34.4407	28.08	0.0001	0.001
Treatment	350.9923	3	116.9974	95.39	0.0001	0.001
Residual	371.6196	303				
Total		320				
Tv Vs Tb	3.9607	1	3.9607	3.25	0.0733	0.05
Tv Vs Tc	3.0389	1	3.0389	2.48	0.1165	Not significant
Tc Vs Tb	0.0447	1	0.0447	0.04	0.8487	"

Table.10. Mean SGOT Levels (I.U) of Trypanosome Infected Groups of Savanna Brown Goats and Un-Infected Control SBG

Days Post Infection	*T. vivax*		*T. congolense*		*T. brucei*		*Control*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-14	60.33	0.52	59.50	0.55	60.00	0.90	60.25	0.71
- 7	61.50	1.05	60.33	0.52	60.50	1.38	62.00	1.20
- 3	63.00	0.90	60.67	0.52	60.50	0.72	61.88	1.46
0	61.50	1.05	60.17	0.52	60.67	0.52	61.00	1.20
2	61.00	0.63	59.50	1.05	61.17	0.98	61.00	0.96
4	60.33	0.52	60.33	0.52	61.67	0.52	61.30	1.70
6	60.50	1.05	60.33	0.52	61.00	0.45	60.88	1.13
8	60.00	1.42	60.67	0.52	61.00	0.21	60.00	1.14
10	60.60	0.55	60.40	0.55	60.00	0.55	60.40	1.14
12	60.40	0.55	61.00	0.71	60.60	0.55	60.67	1.03
14	67.20	1.10	65.25	0.73	67.80	0.84	61.17	1.17
16	68.60	1.52	70.50	1.29	69.60	1.14	60.67	0.82
18	70.25	2.06	71.06	1.29	70.20	1.31	60.40	1.14
21	73.00	1.05	70.33	0.51	72.20	2.05	61.00	1.00
26	74.67	2.50	75.50	0.50	77.80	2.29	61.00	2.90
28	75.33	0.67	75.97	2.31	79.03	1.41	61.35	1.99

Analysis of Variance

Source of Variation	S S	D F	M S	F	Pr>F	Level of Significance %
Period	3496.7897	15	249.7707	3.48	0.0001	0.001
Treatment	978.8059	3	326.2686	4.54	0.0039	0.01
Residual	21764.3437	303	71.8298			
Total	26239.9492	320				
Tv Vs Tb	0.1561	1	0.1561	0.00	0.9629	Not significant
Tv Vs Tc	486.7530	1	486.7530	66.78	0.0077	0.01
Tc Vs Tb	519.7969	1	519.7969	7.24	0.0075	0.01

Table 11. Mean Estimated Serum Globulin Levels (Gm/100mls) of Groups of Trypanosome Infected and Control Savannah Brown Goats

Days Post Infection	*T. vivax* Mean	*T. congolense* Mean	*T. brucei* Mean	Control Mean
-14	5.13	**5.77**	5.00	4.74
-7	5.13	**4.90**	5.00	5.05
-3	5.04	**5.58**	5.00	4.82
0	5.15	**4.90**	4.93	5.09
2	5.06	**5.74**	4.68	4.73
4	5.18	**5.41**	4.71	4.78
6	5.21	**5.21**	4.96	5.04
8	5.94	**4.92**	5.10	4.96
10	5.78	**4.96**	3.32	4.75
12	4.62	**4.68**	4.92	4.97
14	4.65	**4.85**	3.93	4.88
16	3.86	**4.43**	4.58	4.76
18	3.20	**4.47**	4.67	4.41
21	4.03	**4.27**	4.08	5.09
26	4.30	**3.80**	4.60	5.00
28	4.53	4.20	5.04	4.70

APPENDIX 3

SUMMARY OF METHODS USED IN THE ESTIMATION OF:

$A_{i\text{-}viii}$. SOME SERUM BIOCHEMICAL COMPONENTS/ CONSTITUENT LEVELS

B. RBC SIALIC ACIDS CONCENTRATIONS

SERUM ELECTROLYTES ESTIMATION PROCEDURES

Ai. Na+ & K+ determination by Flame Emission Spectrometry (Cheesbrough, 1991)

Using compressed air, diluted serum is sprayed as a fine mist of droplets into a non-luminous gas flame. These then give the colour of emission characteristics of Na+ or K+ metallic ions.

A light filter selects the light of a wavelength corresponding to the metal being measured.

This is then allowed to fall on a photosensitive detector system which then emits lights, the amount of which depends on the concentration of metallic ions. The value is then evaluated in mmol/l

Aii. Serum Bicarbonate Estimation by Titrimetric Method (Cheesbrough, 1991)

Principle: A known amount of strong Hcl is added to a sample of serum. The reaction forms CO2 which is expelled by shaking. The H+ remaining are titrated against NaOH, with phenol red as the indicator.

Summary:

I. Preparation of comparison solution.

The following are pipetted into a conical flask or beaker

NaCl 10g/l	6.0ml
Serum sample (treated with antifoaming agent)	0.1ml
Phenol red indicator	0.1ml

These are then mixed well.

II. HCO3- measurement

The following are pipetted into flasks or beakers

	STANDARD		TEST	
	S1	S2	T1	T2
NaCl 10g/l	4ml		4ml	
HCO3- standard 25mmol/l	0.1ml (100ml)		-	
Serum sample (treated with antifoaming agent)	-		0.1ml	
HCl - 10mmol/l	1ml		1ml	

The content of flask is then swirled to expel CO_2 and then left for 1 minute.

0.1ml phenol red indicator then added to each flask. Then titrated with NaOH 10mmol/l until the colour matches the comparison flask.

Concentration of serum HCO3- = A x 100 mmol/l

Where A = 100 - vol of NaOH used

Then the mean of duplicate tests estimated

1 mEq = 1mmol

(Monovalent ion)

Aiii. Serum (plasma) Total Bilirubin Estimation by Cheesbrough (1991) modified from Jendrassik and Grof (1958)

Principle: Reaction between NaNO3 (Sod. Nitrate) and Hcl (Hydrochloric acid) produces nitrous acid which is then used to diazottize sulphuric acid. This is then used to react with the bilirubin in the serum (plasma) sample to form a pink azobilirubin acid. This is later converted to a blue coloured azobilirubin acid via aikaline tartrate reagent. Then the absorbance of the blue-green solution is read in a colorimeter via an orange filter or in a spectrophotometer at wavelength 600 nm

Summary:

Titration into tubes	Standard		Control		Tests	
	S	S.Blank	C	C.Blank	1,2 etc.	Blank 1,2 etc.
Caffeine Benzoate reagent	1ml	1ml	1ml	1ml	1ml	1ml
Standard serum	0.1ml	0.1ml	-	-	-	-
Control serum	-	-	0.1ml	0.1ml	-	-
Serum sample	-	-	-	-	0.1ml	0.1ml
Diazzo reagent	0.5ml	-	0.5ml	-	0.5ml	-
Sulphanilic acid 5g/l	-	0.5ml	-	0.5ml	-	0.5ml

These are then thoroughly mixed, and then left at room temperature for 5 minutes.

Alkaline tartrate reagent						
added	1ml	1ml	1ml	1ml	1ml	1ml

And mix well.

Then absorbances are then read using spectrophotometer at 600 nm or colorimeter with ora

Principle: Protein molecules made up of amino acids in long chains (peptide cahins). The links between the amino acids are the peptide bonds. In the Biuret reaction, the cupric ions in the reagent join with the peptide bonds to form a blue-violet coloured complex. The absorbance of the colour produced is measured in a clorimeter using a yellow-green filter or in a spectrophotometer at 540 nm wavelength. Blank solutions used to avoid errors due to turbidity

Summary:

Pipette into tubes	Standard		Control		Test	
B.	S.	C.	CB	1,2,etc., Blanks, 1B,2B,etc.		
Biuret reagent	2.5ml	2.5ml	2.5ml	-	2.5ml	-
Biuret Blank reagent	-	-	-	2.5ml	-	2.5ml
Distilled water	50ml	-	-	-	-	-
Standard 60g/l	-	50ml	-	-	-	-
Control Serum	-	-	50ml	50ml	-	-
Serum Sample	-	-	-	-	50ml	50ml

These are then well mixed and left at 37°C for 10 minutes or at room temperature for 30 minutes. Absorbances are then read using either colorimeter (Yellow-Green filter) or Spectrophotometer at 540 nm. These instruments are zeroed with blank solutions. The control and test blanks are read. The blank readings are then substracted.

$$\text{Total protein g/l} = \frac{\text{Absorbance of test or control}}{\text{Absorbance of 60ug/l standard}} \times 60$$

Av. Estimation of Serum Albumin by the BCG Method as of Cheesbrough (1991)

Principle: Bromocresol green (BCG) albumin method is used. BCG is an indicator. It is yellow between pH 3.5 - 4.2. On binding to albumin, the colour changes from yellow to blue-green. Albumin + BCG pH 4.2 _ Albumin.BCG complex (Blue-Green).

Summary:

Pipette into tubes	Blank	Standard	Control	Test
	B	S.	C.	1,2,etc.
BCG reagent	4ml	4ml	4ml	4ml
Distilled Water	20ml	-	-	-
Standard 30g/l	-	20ml	-	-
Control scrum	-	-	20ml	-
Serum sample	-	-	-	20ml

The test tubes are then shaken to mix well (in such a way as to avoid frothing)

Absorbance is read immediately using either colorimeter (orange filter) or spectrophotometer (at 632 nm)

The instrument is zeroed with blank solution in tube B.

$$\text{Albumin} = \frac{\text{Absorbance of test or control}}{\text{Absorbance of 30ug/l standard}} \times 30$$

Avi. Determination of AST Enzyme Activity Level Using the Reitman-Frankel AST Method as Described by Cheesbrough (1991).

Principle: AST is incubated at 37°C for 1 hour (60 minutes) in a pH 7.5 buffered substrate containing aspartate and ketoglutarate. AST catalyses the transfer of the amino group from aspartate to ketoglutarate to form oxaloacetate and glutamate. The former then reacts with 2,4-dinitrophenylhydrazone which in an alkaline medium gives a red-brown colour. The absorbance of this colour is measured using either colorimeter with a green filter or a spectrophotometer at 505 nm wavelength.

$$\text{Aspartate + keto-glutamate} \xrightleftharpoons[37^{0}\text{C } 60 \text{ mins.}]{\text{AST}} \text{Glutamate + Oxaloacetate} \xrightarrow[\text{Alkaline pH}]{\text{DNPH}} \text{Red Brown}$$

Summary:

Pipette into tubes	Blank	Standard	Control	Test
	B	S	C	1, 2 etc
Reagent 1 (substrate)	-	-	0.5 ml	0.5 ml
Substrates in tube C and test 1,2 etc are warmed at 37°C for 5 minutes.				
Control serum	-	-	0.1 ml	-
Serum sample	-	-	-	0.1 ml
Incubate tubes C, test 1,2 etc at 37°C for 60 mins. Then just before 60 mins is due, pipette				
distilled water	0.1 ml	0.1 ml	-	-
Reagent 1 (substrate)	0.5 ml	0.3 ml	-	-
Reagent 4(pyruvate)	-	0.2 ml	-	-
At exactly 60 mins pipette as follows:				
Reagent 3 (color reagent)	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Mix well and leave at room temperature for 30 minutes.				
NaOH 0.4 mol/l	5 ml	5 ml	5 ml	5 ml

Mix well and leave at room temperature for 5 minutes. Then read absorbance using either colorimeter with green filter or spectrophotometer at 505 nm. (Instrument is zeroed with blank solution in tube B). Values obtained in U/l from the calibration graph

Avii. Determination of Serum Glucose level by the Glucose Oxidase - Peroxidase Method (Cheesbrough, 1991).

Principle: Glucose oxidase (GoD) catalyses the oxidation of glucose to give hydrogen peroxide (H_2O_2) and gluconic acid. In the presence of enzyme peroxidase (PoD) the H_2O_2 is broken down and the oxygen released reacts with 4-aminophenazone (4 - aminoantipyrine) and phenol to give a pink colour.

The absorbance of the colour produced is measured via a colorimeter using a green filter or a spectrophotometer at 515nm

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + \text{Hydrogen peroxide}$$

$$\text{H2O2} + \text{Amino phenazone} + \text{Phenol} \xrightarrow{\text{POD}} \text{Coloured complex (pink).}$$

Summary:

Pipette into tubes	Blank	Standard	Control	Test
	B	S	C	1, 2 etc
Protein precipitant				
Reagent	1.5 ml	1.5 ml	1.5 ml	1.5 ml
Distilled water	50 ml	-	-	-
Standard 10.0 mmol/l	-	50 ml	-	-
Control Serum	-	-	50 ml	-
Serum sample	-	-	-	50 ml

These samples are then mixed well. Tubes C and test 1,2 etc are centrifuged to obtain clear supernant fluids.

Take a second set of tubes and label B,S, C, 12 etc.

Transfer from first to				
second set of tubes	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Add colour reagent to				
second set of tubes	1.5 ml	1.5 ml	1.5 ml	1.5 ml

Mix well. Incubate at 37°C for 10 minutes, shaking occasionally.

Read absorbance using either colorimeter with green filter or spectrophotometer at 515 nm wavelength.

Instrument zeroed with blank solution in tube 5. The resulting values are obtained from calibration graph (if the result of 10 mmol standard agrees with the calibration) or with the formula

$$\text{Glucose mmol./l} = \frac{\text{Absorbance of test or control}}{\text{Absorbance of 10.0 mmol/l standard}} \times 10$$

Aviii. Determination of Urea in Blood, Serum by the Method of Stroev and Makarova (1989)

Reagents: Color reagent; a 1g/l (16.65 mmol/l)

Standard solution of "Urea test diagnostic Kit"; Urea.

Material: Blood or serum samples

Apparatus: Set of test tubes in a stand, pipettes of 0, 1, 2 and 5 ml capacity,

aluminium foil, water bath, photocolorimeter

Principle: Urea forms a red coloured compound when reacted with diacetyl monoxime in the presence of thiosemicarbazide and Iron Salts in a strongly acid medium, with the intensity of the red colouration being proportional to the concentration of urea the analysed sample.

Summary: A proportion of the serum is diluted 10 times

Pipette into tubes	Standard	Control	Test
Serum sample (portion)			
diluted 10 times	-	-	0.1 ml
Standard Urea solution	0.1 ml	-	-
Distilled water	-	0.2 ml	-
Colour reagent	2 ml	4 ml	2 ml

Mix contents well with shaking. Cover the tubes with aluminium foil and place them in a boiling water bath for about 10 minutes. Remove them and cool under stream of running water. Then measure the absorbance of the standard and sample solutions against the control solution on a photocolorimeter with green light filter at 540 - 560nm.

$$\text{Blood Serum Urea Concentration mmol/l} = \frac{\text{E sample } 16.65}{\text{E standard.}}$$

E sample = Absorbance of sample solution measured against control solution.

E st = Absorbance of standard solution measured against control solution

16.65 = Concentration of Urea in Standard Solution in mmol/l

$$\text{The Urea nitrogen (in mmol/l)} = \frac{\text{Blood Serum Urea Concentration}}{2.14}$$

$$214 = \frac{\text{Urea Molecular Mass (70)}}{\text{Urea nitrogen molecular mass (28)}}$$

B. THE THIOBARBITURIC ACID ASSAY OF SIALIC ACIDS METHOD

(Warren, 1958, 1963; Aminoff, 1961; Esievo et. al., 1982)

Preparation of Haemoglobin free erythrocyte membrane (Ghosts)

Step1.

Reagents: Isotonic buffer, prepared from NaH_2PO_4 and Na_2HPO_4.

: Hypotonic buffer made by adding 64.5 mls of isotonic buffer to 935.5 mls of distilled water and pH corrected to 7.4

Step 11.

Ghosts Preparation by the Method of Dodge et. al. (1963).

a). RBC-washing:

10 mls of the collected blood in ACD centrifuged at 2000 rpm for 15 minutes. Plasma is then decanted to buffy coat level. Then the isotonic phosphate buffer solution (pH 7.4) is added up to the iniyial plasma level and centrifuged at 2,000 rpm for 15 minutes.

Then supernant is decanted and the process repeated five more times. This is to achieve proper washing of the erythrocytes.

b). RBC-haemolysis:

The hypotonic phosphate buffer (pH 7.4) is added up to the initial level of the plasma and then centrifuged at 2,000 rpm for is minutes. The haemoglobin solution is decanted. The process is repeated 6 times until the Ghosts become pink in colour. After the final decanting, a tiny drop of the hypotonic buffer is then used to resuspend the Ghosts cells.

Hydrolysis and Assay for Erythrocyte Surface Sialic acid

Step1. Hydrolysis:

0.25 ml (250 ml) of Ghosts erythrocyte is incubated with 0.1ml (100 ml) of dilute 0.125 N H_2SO_4 in a water bath at 80°C for one hour to liberate bound erthrocyte surface sialic acid.

Step 11. Assay:

0.125 ml periodate solution is added to hydrolyse sialica-acids from the ghosts cells in a screw cap tubes and mixed vigorously. The mixture is then incubated in water bath at 37°C for 30 minutes to oxidise the sialic acid.

Then 0.1 ml (100 ml) of Arsenite solution is later added (to each tube) and allowed to stay until the mixture turns yellowish to colourless. Thereafter, 1 ml of thiobarbituric acid is added and mixed. The resulting solution is then kept in boiling water bath for 7.5 minutes. A colour reaction is initiated, with the sialic acid producing pink coloured solution. This is then cooled in ice-water. The pink-colour is then extracted with 5 ml of acid butanol by centrifuging at 1000 rpm for 5 minutes.

The aqueous solution is then withdrawn from the tubes into glass curvettes and placed in a spectrophotometer where the absorbance is read at 549 nm.

GLOSSARY ON THESIS COSTING

1. Experimental Animals (37)	18,770:00
2. Feeds	11,795:00
3. Feeders and Waterers	1,025:00
4. Chemicals	4,165:00
5. Drugs, Needles and Syringes	3,450:00
6. Stationaries	5,620:00
7. Analysis (Chemical and Statistical)	14,500:00
8. Photocopying	4,000:00
9. Typing	2,000:00
10. Graphs	2,900:00
11. Binding	1,500:00
Total Expenses	**69,725:00**
Grant Approved by UBR	**7,000:00**
Amount Released by UBR	**4,200:00**