PREVALENCE OF BOVINE TRYPANOSOMOSIS IN SELECTED LOCAL GOVERNMENT AREAS OF KOGI STATE, NIGERIA

BY

HALIDU AROKE AHMED

DEPARTMENT OF VETERINARY MEDICINE,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

JANUARY, 2018

PREVALENCE OF BOVINE TRYPANOSOMOSIS IN SELECTED LOCAL GOVERNMENT AREAS OF KOGI STATE, NIGERIA

BY

Halidu Aroke AHMED, DVM (ABU) 2011
P14VTVM8010

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN FOOD ANIMAL MEDICINE

DEPARTMENT OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

JANUARY, 2018

DECLARATION

I declare that the work in this dissertation entitled "**Prevalence of Bovine Trypanosomosis in Selected Local Government Areas of Kogi State, Nigeria**" was performed by me in the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria under the Supervision of Professors A. K. B. Sackey and S. O. Okaiyeto. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation has been presented for another degree or diploma at this or any other Institution.

Halidu Aroke AHMED _____ _____

Signature Date

CERTIFICATION

This dissertation entitled "PREVALENCE OF BOVINE TRYPANOSOMOSIS IN SELECTED LOCAL GOVERNMENT AREAS OF KOGI STATE, NIGERIA" by Halidu Aroke AHMED, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

Professor A.K.B. Sackey, Chairman, Supervisory Committee	_____ Signature	_____ Date
Professor S.O. Okaiyeto, Member Supervisory Committee	_____ Signature	_____ Date
Professor P. A. Abdu Head, Department of Veterinary Medicine, Ahmadu Bello University, Zaria.	_____ Signature	_____ Date
Professor S. Z. Abubakar Dean, School of Postgraduate Studies, Ahmadu Bello University, Zaria.	_____ Signature	_____ Date

DEDICATION

This dissertation is dedicated to the Almighty Allah for his mercy and guidance throughout the research period.

Also to my late father (Ahmed Aninya) and my beloved mother (Aminat Omeneke).

ACKNOWLEDGEMENTS

I will first of all acknowledge my creator, ALLAH who kept me alive and healthy and also gave me the strength, and the wisdom to undertake this work from the beginning to the end.

My gratitude goes to my supervisors' Professors A.K.B. Sackey and S.O. Okaiyeto, for their fatherly guidance, patience, understanding and word of advice throughout the research period.

I wish to appreciate the profound support given to me by my elder Brothers Shahid and Umar for their financial support, encouragement and patience. To my sisters, Huseinat (Late), Zuleikha, Khatumi and Aisha, I say thank you. To my in-laws Rabiyat, Ruqqayya, Kamil, Saeed and Mubaraq thank you for your encouragement. To my mother-in-law Suliyat, thank you Ma.

I would like to appreciate the Head of Department, Veterinary medicine, and the departmental staff for their support. I also appreciate the staff of Protozoology Research Laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria for their support and technical assistance, and the staff of Sauki Medical Laboratory for helping in analyzing the samples. Also my appreciation goes to Dr. Babashani Shettima who helped tremendously during analysis of the result.

To my lecturers at the postgraduate level, Professors A.K.B. Sackey, L.B. Tekdek, C.A. Kudi, P.A. Abdu, I. Lawal, K.A.N. Esievo, and Drs. S.N.A. Saidu, M.M. Sulaiman, M.A. Wakawa, I appreciate you all for what you have taught me and for adding more value to my knowledge. My appreciation also goes to Drs. L. Maruf, Abdurrahman, Rashidah M., U. Bala, A. Bada, M. Tauheed, H. Buhari, (Mrs) H. Buhari, Abdulrazak I. Bello, Edeh E.R., Alhassan B.Y., Jibril Y. J., Aadamin A. A., Kolo, H., Kanti, M., Arotiba, B. and Onoja E. thank you all for your friendly advice.

My appreciation also goes to my friends, Adams Adinoyi O., Abdulrahim Salawudeen, Abdulkareem Salawu, Siyaka Halimat, Hafsat Yahaya and others, I say thank you all.

My sincere gratitude also goes to Dr. Isa Ochepa, Director of Veterinary Services, Kogi State Ministry of Agricultural and Natural Resources, and the Staff of Zonal Veterinary Offices and Abattoir Staff of Kogi State, without you people, my sample collection would not have gone smoothly.

Finally to my lovely wife Maryam and my sons Mutasim and Hammam, I thank you all for your understanding and patience, May Allah with his infinite mercy continue to bless you all. Ameen.

ABSTRACT

A cross sectional study and random sampling was carried out in six selected Local Government Areas of Kogi State in order to determine the prevalence of bovine trypanosomosis in the state. In all, 410 blood samples were collected and examined for presence of trypanosomosis using Geimsa stained, thick blood and thin blood smears, for the detection and identification of the trypanosome parasite. PCV and differential leucocyte count were also determined. Of the 410 blood samples obtained, 116 and 294 were obtained from herds and abattoirs respectively. Among these, 232 cattle were < 3years and 162 cattle were 3 years and above, 212 female and 198 male cattle were sampled respectively. Also, 71 cattle with body condition 2 (poor), 245 cattle with body condition 3 (fair) and 94 cattle with body condition 4 (good) respectively were sampled. The clinical signs observed during the sampling were fever, lethargy, lacrymation, pale mucus membrane, enlarged superficial lymph nodes, loss of appetite, epiphora and emaciation. The mean body temperature for the parasitaemic cattle were 42.5 $\pm$ 0.50 ^{oC} and 38.2 $\pm$ 0.15 ^{oC} for aparasitaemic cattle. Of the 16 cattle sampled 3.9% were positive for trypanosome parasite using the thick blood smear technique, while 2.9% (12) were positive using thin smear technique. The overall prevalence of bovine trypanosomosis in Kogi State was 3.9%. The mean Packed Cell Volume (PCV) of the parasitaemic cattle (30.18 $\pm$ 1.73%) was lower than that of aparasitaemic cattle (40.48 $\pm$ 0.44%), a difference which was significant statiscally (P <0.05). The mean haemoglobin concentration of parasitaemic cattle (10.06 $\pm$ 0.43g/µl) was lower than that of aparasitaemic cattle (13.61 $\pm$ 0.014g/µl), while the mean eosinophil count of parasitaemic cattle (0.39 $\pm$ 1.10(10^9/l)) was higher than that of aparasitaemic cattle (0.17 $\pm$ 0.26(10^9/l)). All the differences were statistically significant (P <0.05). The species of trypanosomes identified were *T. brucei* 10 (2.4%), while 2 (0.5%) had mixed infection of *T.*

Comment [MP1]:

brucei and *T. congolense* and the species could not be identified in 4 (0.98%) of the parasitaemic cattle. The infection rate based on herd samples and abattoir samples were 10 (8.6%) and 6 (2%) of cattle using thick blood smear while 10 (8.6%) and 2 (0.7%) of cattle using thin blood smear technique and the differences were significant (P <0.05). With regard to infection rate based on sex, 4.2% (9) and 3.5% (7) females and males cattle were positive (parasitaemic) via thick blood smear technique respectively while 3.3% (7) and 2.5% (5) were parasitaemic using thin blood smear technique respectively, though the differences were not significant (P >0.05). Based on age, prevalence was highest among the young cattle (< 3years) (4.1%) than adult cattle (3.6%), though not statiscally significant (P >0.05). Emaciated cattle had a high prevalence rate of trypanosomosis (3.7%) than the non-emaciated cattle, which was not significant (P >0.05). There was no significant differences in relation to age, sex, body condition and trypanosomosis in the study area. The finding therefore requires an urgent approach to parasite and vector control to safeguard the cattle population in Kogi State. It is recommended that further study should be carried out to identify other species of trypanosomes present in the Kogi State, and also to employ other sensitive methods like polymerase chain reaction (PCR) and enzyme linked immunosorbent assay (ELISA) to determine the true picture of trypanosomosis in Kogi State. There is also the need to enlighten cattle owners and herdsmen in the state about the effect of trypanosomosis and its economic implications to their cattle.

TABLE OF CONTENTS

Title	Page
Title page	ii
Declaration	iii
Certification	iv
Dedication	v
Acknowledgements	vi
Abstract	vii
Table of contents	ix
List of tables	xiv
List of figures	xv
List of plates	xvi
Lists of appendices	xvii
List of abbreviations	xviii
CHAPTER ONE	1
1.0 INTRODUCTION	1
1.1 Background to the Study	1
1.2 Statement of Research Problem	3
1.3 Justification of the Study	5
1.4 Aim of the Study	6
1.5 Objectives of the Study	6
1.6 Research Questions	6
CHAPTER TWO	8

2.0 LITERATURE REVIEW 8

2.1 Overview 8

2.2 Taxonomy 9

2.2.1 Family *Trypanosomatidae* 10

2.2.2 Genus *Trypanosoma* 10

2.3 Aetiology of African Animal Trypanosomosis 13

2.4 Epidemiology 13

2.4.1 The vector 14

2.4.2 The parasite 15

2.4.3 The host 15

2.5 Life Cycle 16

2.5.1 Life cycle in the mammalian host 17

2.5.2 Life cycle in the tsetse fly 18

2.6 Morphology 18

2.6.1 Subgenus *Trypanozoon* (brucei group) 20

2.6.2 Subgenus *Duttonela* (vivax group) 21

2.6.3 Subgenus *Nannomonas* (congolense group) 21

2.7 The Vector 23

2.8 Species Affected and Host Pathogenicity 24

2.9 Immunology andAntigenic Variation 26

2.10 Transmissions 27

2.10.1 Cyclical transmission 27

2.10.2 Mechanical transmission 28

2.10.3 Transmission by other means 28

2.11 Pathogenesis 30

2.12 Mechanism of Anaemia 32

2.12.1 Phase I: Acute crises 32

2.12.2 Phase II: Chronic 33

2.12.3 Phase III: Recovery 33

2.13 Incubation Period 34

2.14 Clinical Signs 34

2.15 Pathological Signs 36

2.16 Diagnosis 37

2.16.1 Identification of the parasite 38

2.16.2 Direct examination technique 39

2.16.3 Parasite concentration technique 43

2.16.4 Animal inoculation 48

2.16.5 Serological test 49

2.16.6 Molecular test 55

2.17 Economic Impact of African Trypanosomosis 58

2.18 Public Health Significane of Trypanosomosis 61

2.19 Treatment 63

2.19.1 Diminazene aceturate 65

2.19.2 Homidium 65

2.19.3 Isometamidium 66

2.19.4 Pyrithidium bromide 66

2.19.5 Quinapyramine sulphate 67

2.19.6 Suramin 67

2.19.7 Melarsomine 68

2.19.8 Drug resistance 68

2.20 Prevention and Control 70

2.20.1 Use of insecticide 70

2.20.2 Sterile male technique 71

2.20.3 Use of traps or screen 72

2.20.4 Innate resistance to trypanosomosis 73

2.20.5 Integrated control 75

CHAPTER THREE 76

3.0 MATERIALS AND METHODS 76

3.1 Study Area 76

3.2 Study Design 76

3.3 Sample Size 80

3.4 Sample Collection 80

3.5 Sample Processing/Laboratory Procedures 81

3.5.1 Determination of packed cell volume (PCV) 82

3.5.2 Determination of differential leucocyte count 82

3.5.3 Thick blood smear 82

3.5.4 Thin blood smear 83

3.6 Data Analyses 83

CHAPTER FOUR 84

4.0 RESULTS 84

4.1 Distribution of Cattle and Prevalence Rate of Trypanosomosis in the Study Area 84

4.2 Clinical Signs Observed in Trypanosomes Infected Cattle in Kogi State, Nigeria 85

4.3 Mean Haematological Value of infected and unifected Cattle with trypanosomes in Kogi State, Nigeria 85

4.4 Species Identification 90

4.5 Prevalence of Trypanosomosis in Cattle Sampled Between Herds and Abattoir in Kogi State, Nigeria 95

4.6 Prevalence of Trypanosomosis with regards to Sampled Local Government Areas, Using Thick and Thin Blood Smear Techniques in Kogi State, Nigeria 97

4.7 Prevalence of Trypanosomosis Based on Sex of Cattle Sampled, Using Thick and Thin Blood Smear Techniques in Kogi State, Nigeria 97

4.8 Prevalence of Trypanosomosis Based on Age of Cattle Sampled, Using Thick and Thin Blood Smear Techniques in Kogi State, Nigeria 100

4.9 Prevalence of Trypanosomosis Based on Body Condition Score of Cattle Sampled, Using Thick and Thin Blood Smear Techniques in Kogi State, Nigeria 100

CHAPTER FIVE 103

5.0 DISCUSSION 103

CHAPTER SIX 107

6.0 CONCLUSION AND RECOMMENDATIONS 107

6.1 Conclusion 107

6.2 Recommendations 107

6.3 Contribution to Knowledge 108

6.4 Limitation of the Study 108

REFERENCES 109

APPENDICES 129

LIST OF TABLES

Table	Title	Page
3.1	Senatorial Zones and Local Government Areas sampled in Kogi State	77
4.1	Clinical signs observed in trypanosomes infected cattle in Kogi State, Nigeria	87
4.2	Mean haematological values of infected and unifected cattle with trypanosomes in Kogi State, Nigeria	88
4.3	Prevalence of trypanosomosis in cattle sampled at settled herds and abattoirs in Kogi State, Nigeria	96
4.4	Prevalence of trypanosomosis based on sampled Local Government Areas, using thick and thin blood smear techniques in Kogi State, Nigeria	98
4.5	Prevalence of trypanosomosis based on sex of cattle sampled, using thick and thin blood smear techniques in Kogi State, Nigeria	99
4.6	Prevalence of trypanosomosis based on age of cattle sampled, using thick and thin blood smear techniques in Kogi State, Nigeria	101
4.7	Prevalence of trypanosomosis based on body condition score of cattle sampled, using thick and thin blood smear techniques in Kogi State, Nigeria	102

LIST OF FIGURES

Figures	Title	Page
3.1	Map of Nigeria showing location of Kogi State	78
3.2	Map of Kogi State showing Local Government Areas and the sampling areas	79

LIST OF PLATES

Plate	Title	Page
I	One of the sampled cattle showing lacrymation	89
II	Photomicrograph of *Trypanosoma brucei* (arrowed) in the blood of Infected cattle sampled from Kogi State, Nigeria (x100)	91
III	Photomicrograph of *T. congolense* (arrowed) in the blood of Infected cattle sampled from Kogi State, Nigeria (x100)	92
IV	Photomicrograph of *Trypanosoma spp* (arrowed) in the blood of Infected cattle sampled from Kogi State, Nigeria (x100)	93
V	Photomicrograph of *Trypanosoma congolense* and *T. brucei* (arrowed) in the blood of Infected cattle sampled from Kogi State, Nigeria (x100)	94

LIST OF APPENDICES

Appendix	Title	Page
I	Photograph of thick blood smears preparation before staining	129
II	Photograph of thin blood smears preparation before staining	130
III	Photograph of staining procedure	131
IV	Photograph of thick blood smears preparation after staining	132
V	Photograph of thin blood smears preparation after staining	133
VI	Photograph of one of the herds used in the study	134

LIST OF ABBREVIATIONS

AAT	Animal African Trypanosomosis
CAPC	Companion Animal Parasite Council
CFSPH	Center for Food Security and Public Health
CNS	Central Nervous System
DDT	DichloroDiphenylTrichloroethane
DEAE	Diethyl Amino Ethyl
DNA	Deoxyribonucleic Acid
E	East
EDTA	Ethylene Diamine Tetra acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organisation
FCT	Federal Capital Territory
G	Glossina
HAT	Human African Trypanosomosis
Hb	Haemoglobin
IAEA	International Atomic Energy Agency
IFAT	Indirect Fluorescent Antibody Test
IgM	Immunoglobin M
IM	Intramuscular
IV	Intravenous
Kg	Kilogram
km	Kilometer

m-AECT	miniature Anion Exchange Chromatography Technique
mg	Milligram
ml	Milliliter
mm	Millimeter
N	North
NITR	National Institute of Trypanosomiasis Research
NPC	National Population Commission
OIE	Office International des Epizootics
PATTEC	Pan African Tsetse and Trypanosomosis Eradication
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PCV	Parked Cell Volume
PP	Percentage Positive
QBC	Quantitative Buffy Coat Method
RBC	Red Blood Cell
RNA	Ribonucleic Acid
S	South
SC	Subcutaneous
SDS	Sodium Dodecyl Sulphate
SIRS	System Inflammatory Response Syndrome
SIT	Sterile Insect Technique
T	*Trypanosoma*
US	United States

VAT	Variant Antigen Type
VSG	Variable Surface Glycoprotein
WBC	White Blood Cell
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

African animal trypanosomosis is a disease condition affecting all domestic animals including dogs and cats, as well as a wide range of wildlife species that serve as reservoirs of infection for both humans and domestic animals (Anderson *et al*., 2011; Amanuel *et al*., 2015). Trypanosomosis is a complex disease caused by unicellular parasites (trypanosomes) found in the blood and other tissues of vertebrates including cattle and man (Tesfaye, 2002). The *T. brucei* (*T. b*) group of trypanosomes (*T. brucei, T. b. gambiense and T. b. rhodesiense*) mostly invade tissues (humoral) whereas, *T. congolense* and to a lesser extent *T. vivax* and *T. cruzi* predominantly restrict themselves to the blood circulation (haemic) (Sima *et al*., 2011; Mbaya *et al*., 2012; Abenga, 2014).

In cattle, the disease is caused by *Trypanosoma vivax, T. congolense* and *T. brucei* (Magona *et al*., 2008; Bezie *et al*., 2014; Amanuel *et al*., 2015). Trypanosomosis is mainly transmitted cyclically by the genus *Glossina species* (tsetse flies), (Desquesnes and Dia 2003; Kone *et al*., 2011), as well as mechanically by biting flies, among which Tabanids and Stomoxes (Molalegne *et al*., 2011), which are presumed to be the most important vectors especially for *T. vivax* (OIE, 2013; Gamechu *et al*., 2015). Transplacental transmission has also been recorded in cattle (Ogwu *et al*., 1992) and also by *T. equiperdum* in horses known as dourine which is transmitted by coitus (Wanga and Munga, 2011).

In ruminants the major signs of trypanosomosis are anaemia, generalized enlargement of the superficial lymph nodes, lethargy and progressive loss of body condition. Fever and loss of appetite occur intermittently during parasitemic peaks, but these latter become marked in the

terminal stages of the disease. There is also reduction in birth rates, increasing abortion and mortality rates (Mersha *et al*., 2013). The acute disease in bovine is characterized by high fever, emaciation, lacrymation, reduced milk yield, corneal opacity, nervous signs and death may occur within 24 hours of onset of clinical signs (Radostits *et al*., 2007; Munday *et al*., 2015; Amanuel *et al*., 2015). Typically, the disease is chronic, extending over several months and terminates fatally if untreated (Odeyemi *et al*., 2015). As a herd phenomenon, the growth of young is stunted, the adults show decreased fertility, and the pregnant, may abort (Shimelis and Mkamu, 2015). In the terminal stage, animals become extremely weak, the lymph nodes are reduced in size and there is often a jugular pulse (FAO, 2014). Death is also associated with congestive heart failure due to anemia and myocarditis (Radostits *et al*., 2007; Kato *et al*., 2015). Occasionally, with some strains of *T. vivax*, the disease is subacute, with death occurring within 2 - 3 weeks of infection preceded by fever, anemia and wide spread hemorrhages (Radostits *et al*., 2007; Okaiyeto *et al*., 2010).

Parasitaemia due to *T. brucei* in cattle varies substantially according to the phase of infection, being usually high in the acute phase and low in the chronic phase but increases substantially when chronically infected cattle are challenged with *T. congolense* (Van den Bossche *et al*., 2004; Okaiyeto *et al*., 2010). Regarding other ruminants, trypanosomes of major threat to goats include *Trypanosoma vivax, Trypanosoma congolense* and *trypanosome brucei* (Abenga *et al*., 2008; Gamechu *et al*., 2015). *T. evansi* is the cause of trypanosomosis known as surra in camels (*Camelus*) (Mbaya *et al*., 2010). Bovine trypanosomosis is a threat to livestock health and agricultural production, thereby affecting rural development and poverty alleviation in Africa (Machila *et al*., 2003).

The diagnosis of trypanosomosis is based on demonstration of trypanosomes in tissues or blood of hosts. This is done using direct microscope examination of fresh (wet mount) or Giemsa stained thick or thin blood smears, haematocrit centrifugation technique, the anion exchange centrifugation technique and mouse inoculation test (OIE, 2013). Other methods include serology (ELISA and IFAT) (Aslam *et al*., 2010) and polymerase chain reaction (PCR) (OIE 2013).

A variety of control measures have been implemented to combat bovine trypanosomosis in sub-Saharan Africa, though none has been successful at eradicating the disease. Presently, effective control of the disease is largely dependent on vector management using insecticides, tsetse traps and parasite management via the use of trypanocidal drugs (Schofield and Maudlin, 2001). Vaccine development has been hindered by the ability of the parasite to evade the host immune response via an elaborate mechanism combining antigenic variation and immunosuppression (Donelson, 2003). A particularly promising approach to disease control is to understand and exploit naturally evolved trypanotolerance by the ability of certain cattle breeds to remain productive in areas of high tsetse challenge. Certain indigenous cattle populations, particularly in West and Central Africa (e.g. N'Dama), have evolved a relative tolerance to the disease, presumably due to strong natural selection over several millennia (Shimelis and Melkamu, 2015).

1.2 Statement of Research Problem

African animal trypanosomosis (AAT) was described as the commonest and most threatening disease of ruminant livestock in regions where tsetse fly is prevalent (Nyimba *et al*., 2015). It causes serious economic losses in livestock due to anaemia, loss of condition, emaciation and

untreated cases are usually fatal (FAO, 2002; Nyimba *et al*., 2015). Trypanosomosis has been ranked the fourth most important disease of cattle in Nigeria after rinderpest (which has been eradicated), contagious bovine pleuropneumonia and dermatophilosis (FAO, 2014). The last decade witnessed upsurge in the menace of African Trypanosomosis in man and animals in Nigeria (Abenga *et al*., 2005). In Nigeria, eleven of the twenty three species of tsetse flies are known to infest over 80% of the 928,300 km² of landmass, and are widely distributed from latitudes 4ºN and 13ºN in the country (NITR, 1989; Onyiah, 1997; Zubairu *et al*., 2013).

Bovine trypanosomosis is a serious constraint to cattle development in large parts of Nigeria and infections with pathogenic trypanosome species, especially *T. vivax, T. congolense* and *T. brucei* affect various aspects of cattle productivity (Swallow, 2000; Tsegaye *et al*., 2015). It infects animals over an area known as the 'tsetse belt', which extends approximately 10 million km^2 across 37 countries in Africa (Marquardt *et al*., 2000; Parryet *et al*., 2004; Ilemobade, 2009). Some trypanosome species such as *Trypanosoma vivax* can be transmitted by biting flies mechanically and can establish itself even outside the tsetse belt, placing an estimated 160 million cattle at risk (FAO, 2014; Bayisa *et al*., 2015). Also, trypanosomosis in livestock causes great losses in terms of mortality, abortion, reduced fertility, milk and meat production, and ability to work as traction animals (Maudlin *et al*., 2004; Samuel *et al*., 2001; Odeyemi *et al*., 2015). Currently, there are three principal control strategies for tsetse-transmitted trypanosomosis, namely: trypanocidal drugs (chemotherapy and chemoprophylaxis), trypanotolerant cattle and tsetse control (Waiswa *et al*., 2006). Treatment of livestock in sub-Saharan Africa with trypanocidal drugs has been hindered by the problem of drug resistance as well as being expensive for many farmers (Bourn *et al*., 2005). Treatment of human

trypanosomosis (sleeping sickness) is also expensive, normally ranging from US$150 to US$800 per patient (CAPC, 2014).

1.3 Justification of the Study

The current threat of African animal trypanosomosis which is ranked among the four major cattle diseases on sustainable livestock production and mixed farming, coupled with the failure of vector control as well as chemotherapy/chemoprophylaxis to control the present resurge of the disease presents a major constraint in the development of livestock industry in Nigeria (Perry *et al*., 2002; Samdi *et al*., 2010). These constitute major threat to attaining food security in several parts of Nigeria (Samdi *et al*., 2010). The current estimation of about 48 million cattle are at the risk of contracting trypanosomosis from 23 species and 33 subspecies of tsetse flies infesting part of Sub-saharan Africa (Abenga *et al*., 2002a; Samdi *et al*., 2011; Bayisa *et al*., 2015).

Several studies on animal trypanosomosis have been carried out in various parts of Nigeria (Agu *et al*., 1990; Kalu *et al*., 1991; Ahmed and Agbede, 1993; Kalu *et al*., 1996; Kalu and Lawani, 1996; Onyia, 1997; Abenga, *et al*., 2004; Agu and Amadi, 2001; Ameen *et al*., 2008; Zubairu *et al*., 2013).

Among the 23 species of *Glossina spp* capable of transmitting trypanosomes, 11 of which are known to occur in Nigeria infesting 80% of Nigeria surface area including Kogi State (Enwezor *et al*., 2012). There is no available information regarding the prevalence of trypanosomosis as well as the disease status in Kogi State, hence the need to evaluate the disease' status in the state. The annual bulletin of Nigeria Institute of Trypanosomiasis Research (NITR, 1989), equally indicate the presence of *Glossina spp* in many states of the country including Kogi State (Zubairu *et al*., 2013). This shows that the disease may be present in Kogi State as well as the

presence of habitats suitable for the proliferation and survival of the vector for the transmission of the disease (Fasanmi *et al*., 2014). There is therefore need for the study of the presence and prevalence of trypanosomosis in Kogi State which has a large population of cattle and other ruminants susceptible to trypanosomosis.

1.4 Aim of the Study

This study is aimed at determining the prevalence of bovine trypanosomosis in selected local government areas of Kogi State.

1.5 Objectives of the Study

- To determine the prevalence of bovine trypanosomosis selected local government areas of Kogi State using parasitological technique
- To identify trypanosome species associated with bovine trypanosomosis in selected local government areas of Kogi State.
- To determine the age, sex, body condition and susceptibility of cattle to trypanosomosis in selected local government areas of Kogi State.
- To evaluate haematological indices and clinical manifestations associated with bovine trypanosomosis in the sampled area.

1.6 Research Questions

- Is bovine trypanosomosis present in the selected local government areas of Kogi State?
- Which species of trypanosome present in the study area?
- Is there any differences in susceptibility of cattle in relation to age, sex and body condition to bovine trypanosome in the study area?

- What the clinical signs associated with bovine trypanosome in the sampled area?

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview on African Animal Trypanosomosis

African bovine trypanosomosis, caused by the extracellular flagellate protozoan trypanosome parasite (*Trypanosoma* species), is endemic throughout the humid and semi-humid zones of sub-Saharan Africa. The disease is coincident with the distribution of the tsetse fly (*Glossina* species), which acts as a vector for the parasite and infests an area of some 10 million km^2 encompassing 37 countries (Black and Seed, 2002; Jacobs *et al.,* 2011; Nigatu *et al*., 2015). Trypanosomosis is one of major haemoparasitic disease in domestic cattle and is caused by the protozoan parasite *Trypanosoma*. Across sub-Saharan Africa a variety of *Trypanosoma* species transmitted by tsetse flies (*Glossina* species) cause Human and Animal Trypanosomosis (Brun *et al*., 2010; Hargrove *et al*., 2012). Transmission occurs largely among rural populations, where activities such as agriculture, fishing and pouching expose people to the bite of the tsetse fly (Muturi *et al*., 2011).

African trypanosomosis is responsible for about 55,000 human, 3 million livestock deaths annually (Okwelum *et al*., 2011) and hinders mixed farming through reduced work efficiency of draft cattle. Some trypanosome species such as *Trypanosoma vivax* can be transmitted by biting flies mechanically and can establish itself outside the tsetse belt, placing an estimated 160 million cattle (as well as 260 million sheep and goats) at risk (Ilemobade, 2009). In additon to being frequently fatal, the disease is a major constraint to livestock and agricultural production in Africa (Swallow, 2000; Nigatu *et al*., 2015). Accordingly, trypanosomosis is ranked among the top ten global cattle diseases impacting on the poor (Perry *et al*., 2002). Trypanosomosis in

livestock causes great losses in terms of mortality, abortion, reduced fertility, milk and meat production, and ability to work as traction animals (Maudlin *et al*., 2004). In addition to these, the disease is also responsible for an annual loss of millions of dollars in livestock production as a result of the cost related to treatment, prevention and vector control efforts (Samuel *et al*., 2001).

2.2 Taxonomy

Trypanosomosis is also a debilitating, severe and often fatal disease affecting both humans and animals and is also transmitted by biting flies, which have acquired their infection from animals harboring the pathogenic parasites (Kato *et al*., 2015; Nigatu *et al.,* 2015).

The causative agents of African trypanosomosis (HAT and AAT) belong to the following taxonomic classification (Levine *et al*., 1980):

Kingdom: Protista

Subkingdom: Protozoa

Phylum: Sarcomastigophora

Class: Zoomastigophora

Order: Kinetoplastida

Family: Trypanosomtidae

Section: Salivaria

Genus: *Trypanosoma*

Subgenus: Nannomonas (*T. congolense*), Duttonella (*T. vivax*), Trypanozoon (*T. brucei*) and Pycnomonas (*T. suis*).

2.2.1 Family *Trypanosomatidae*

Species within this group have a single nucleus and are either elongated with a protruding flagellum or more rounded with a non-protruding flagellum. Those parasitic in vertebrates generally require an intermediate host (usually a bloodsucking insect); the vertebrate parasites would be referred to as heteroxenous occurs with more than one host during its life cycle. Those parasitic with invertebrates can undergo their entire life cycle within the same host; referred to as monoxenous, since they have only a single host during the entire life cycle. Among the trypanosomatids, members vary in size, body shape, location of the flagellum, location of the kinetoplast, and other structures (Urquhart *et al*., 1996).

2.2.2 Genus *Trypanosoma*

One curious feature of some pathogenic species is that they may also be parasitic in other animals in which they are apparently harmless. In the natural hosts, the tissues have presumably become physiologically adapted to the presence of the parasites through long periods of association. Consequently, such animals act as "reservoir" hosts (animals that harbour an infection that can be transmitted to other animals) for these potentially pathogenic species (Urquhart *et al*., 1996).

For most trypanosomes, the life cycle includes both an invertebrate and a vertebrate host. They are usually found in the body fluids of vertebrates, especially the blood and tissue fluids. But some forms (e.g. *T. cruzi*) occur intracellularly and in the digestive tracts of invertebrates, especially arthropods (Radostits *et al*., 2007).

Trypanosomes are divided into 2 main groups or "sections": Salivaria and Stercoraria. The distinctions are primarily based on the precise characteristics of their development in the insect hosts (Radostits *et al*., 2007).

2.2.2.1 Salivaria group

These trypanosomes develop in the anterior part of the digestive tract (e.g. the salivary glands) of their insect vector; they are said to undergo anterior station. Also, these forms typically infect new hosts as a consequence of insect bite. The trypanosomes are widely distributed throughout Africa; their distribution is strongly dictated by the distribution of the insect vectors (e.g. tsetse flies of the genus Glossina) (Radostits *et al*., 2007).

Subgenus Trypanozoon

The major trypanosome here is *T. brucei brucei*. It is related to *T. brucei gambiense* and *T. brucei rhodesiense* causing human trypanosomosis in West Africa and East Africa respectively (Sackey, 1998). *Trypanosoma brucei brucei* is primarily a parasite of native ruminants such as antelopes. However, it is widely distributed in Africa and found mainly in the domestic animals (e.g. dogs) and livestock (e.g. cattle, sheep, goats, horses, mules, donkeys, and camels). It is extremely pathogenic to these animals and causes the disease known as 'nagana' (Sackey, 1998). Cattle suffer mild infections and it is severe in pigs; however, humans are not susceptible to disease. The vector for *T. brucei* is tsetse flies (e.g. *G. morsitans, G. pallidipes* and *G. swynnertoni*). Salivarian parasites evade the adaptive immune system of the host using an antigenic variation strategy (Horn, 2014; Cnops *et al*., 2015). *T. brucei brucei* is polymorphic in the mammalian host as slender, intermediate or stumpy form and the parasite exhibit a wriggling movement (Sackey, 1998).

Subgenus Nannomonas

One of the most important trypanosomes of cattle found in this sub-genus is *T. congolense*. It develops in the midgut and proboscis of the vector (Sackey, 1998). This sub-genus also has *T. simiae* causing an acute trypanosomosis in pigs; and another organism *T. godfreyi* causing an acute trypanosomosis in sheep and pigs. They have marginal and subterminal kinetoplast with centrally located nuclei, lacking free flagellum (McNamara and Snow, 1991).

Subgenus Duttonela

This subgenus include *T. vivax* and T. *uniforme. T. vivax* is smaller and has two forms which are club shaped and slender form. They develop only in the proboscis of the vector. *T. vivax* moves rapidly in wet mount smears (Sackey, 1998).

Subgenus Pycnomonas

This subgenus include only *Trypanosoma suis* which was originally thought to be *Trypanosoma simiae* but later became recognized as a distinct species (Peel and Chardome, 1954). It is monomorphic, small and subterminal kinetoplast. Cyclical development occurs in the midgut and salivary glands. The parasite is pathogenic to pigs (Hutchinson and Gibson, 2015).

2.2.2.2 Stercoraria group

Trypanosoma cruzi

Infective forms develop in the hindgut of the insect vector; they are said to undergo posterior station development. Also, these forms leave insects with the faeces and infect new hosts by penetration through the skin or mucous membranes or by lesions made by vector bites (Radostits *et al*., 2007). The parasite is transmitted to humans through the bite from a reduviid bug; and the

disease caused is called chagas' disease. *T. cruzi* infection is prevalent throughout South America and Latin America, affecting an estimated 12-19 million people. Also, many kinds of animals (e.g. cats, dogs, bats, armadillos, rodent and other mammals) serve as reservoir hosts (Radostits *et al*., 2007).

2.3 Aetiology of African Animal Trypanosomosis

Trypanosoma vivax T. congolense, T. brucei and *T. simiae* are the four main species responsible for African trypanosomosis affecting virtually all domestic mammals while *T. evansi* causes Surra in camels (*Camelus dromedarieus*) (Mbaya *et al*., 2010). The four species are members of the Salivaria group of trypanosomes and are transmitted cyclically via the mouthparts of tsetse flies, hence the name salivarian trypanosomes (Abenga, 2014). *T. vivax, T. congolense* and *T. brucei* are the main trypanosome pathogens of cattle. *T vivax* is usually numerous in bovine bloods, and can be identified by its very fast movement in wet films (Urquhart *et al*., 1996; Radostits *et al*., 2007).

2.4 Epidemiology

In Africa, trypanosomes can be found wherever the tsetse fly vector exists. Tsetse flies are found from the southern edge of the Saharan Desert to Zimbabwe, Angola, and Mozambique. However, some species of trypanosomes have been spread farther by other fly species that serve as mechanical vectors. *T. vivax* is found in South and Central America and the Caribbean, areas free of the tsetse fly, but where other biting flies act as mechanical vectors to spread the disease, though its vector occurs also in Central and South America. Affected countries include Bolivia, Brazil, Colombia, French Guiana, Guyana, Peru, Suriname, and Venezuela where it affects mainly cattle and sheep (CFSPH, 2015). The epidemiology of African trypanosomosis is

determined mainly by the ecology of the tsetse fly which is found only in tropical Africa. *T. congolense* and *T. vivax* are responsible for severe disease in cattle, sheep and goats, *T. brucei* usually causes a subclinical infection in cattle, but a severe disease in sheep, goats, horses and occasionally, pigs. *T. simiae* causes a very acute and highly fatal disease in exotic pigs. It is not pathogenic to cattle, sheep, or goats (Radostits *et al*., 2007).

The epidemiology of trypanosomosis depends on three factors, the distribution of the vectors, the virulence of the parasite and the response of the host (Radostits *et al*., 2007).

2.4.1 The vector

Among the group of *Glossina* flies, the savannah and riverine varieties are the most important since they inhabit areas suitable for grazing and watering. Although the infection rate of *Glossina* with trypanosomes is usually low, ranging from 1 to 20% of the flies, but each is infected for life. Biting flies may act as mechanical vectors but their significance in Africa is still undefined (Leak, 1998). Tsetse fly density is the most variable factor in the transmission of trypanosomosis. Climate affects tsetse abundance via one or more of four demographically important rates namely birth, mortality, immigration and emigration (Rogers, 1991). Tsetse fly species differ in their susceptibility to trypanosomes and their subsequent ability, if infected, to transmit trypanosomes. For example, *G. fuscipes* appears to be a better vector of *T. vivax* to cattle than *G. pallidipes*, which is a better transmitter of *T. congolense* than *G. swynnertoni* which is a better vector for *T. brucei* (Baylis and Nambiro, 1993). Tsetse flies prefer to feed on particular hosts; the bushbuck for example is much preferred to the waterbuck. Cattle inhabit a medium position. There are also differences within one host species in that trypanosome infected animals attract tsetse more than uninfected hosts (Baylis and Nambiro, 1993).

2.4.2 The parasite

Since parasitaemic animals commonly survive for prolonged periods, there are ample opportunities for fly transmission of the parasite. Perhaps the most important aspect of trypanosomosis which accounts for the persistent parasitaemia is the way in which the parasite evades the immune response of the host through antigenic variation, the repeated switching of the glycoprotein coat is now known to depend on a loosely ordered sequential expression of an undefined number of genes, each coding for a different glycoprotein coat (Urquhart *et al*., 1996). This together with finding that metacyclic trypanosomes may be a variation of antigenic types each expressing a different genetic repertoire, explains why domestic animals even if treated successfully, are often immediately susceptible to reinfection (Mansfield *et al*., 2014).

2.4.3 The host

The mechanisms underlying bovine trypanotolerance remains mostly unknown however, immune response to trypanosomes greatly differs between susceptible and resistant cattle. Whereas the immunity has a genetic basis, the intensity of the tsetse challenge has a strong influence on the degree of tolerance (Murray *et al*., 1983). Trypanotolerance has been defined as the relative capacity of an animal to control the development of the parasites and to limit their harmful effects, the most prominent of which is anaemia. Both acquired and innate resistance to African trypanosomosis can occur in cattle (Mony and Mathews, 2015).

The two most important trypanotolerant breeds are the *Bos Taurus* subtypes N´dama and Baoule, while a degree of trypanotolerance has also been shown to occur in some *Bos indicus* zebu breeds example the Orma boran and the Maasai zebu (Mwangi *et al.,* 1993). The effects of trypanotolerant cattle on trypanosomosis transmission have not been investigated. It might be

expected that both the probability of a fly becoming infected from an infected cow, and cows becoming infected from an infected fly would decrease (McDermott and Coleman, 2001).

Herd management is also important. Daily activity patterns of the tsetse and the grazing patterns of the herds are of great influence. If the herds graze on infested sites at the time of day that the flies are most active, transmission will occur more frequently. The risk to susceptible livestock living in comparatively free areas surrounded by tsetse belts varies from year to year. Generally during wet years tsetse populations will increase, spread and persist during the dry season, even in areas from where they disappear in the dry years (OIE, 2013).

2.5 Life Cycle

The life cycle of any one species may include more than one of these configurations;

I. Promastigote: elongated form with antenuclear (in front of nucleus near the anterior end of the body) kinetoplast; flagellum arising near it and emerging from the anterior end of body e.g. *Leptomonas*
II. Epimastigote: elongated form with a juxtanuclear kinetoplast (between nucleus and anterior end); flagellum arising near it and emerging from the site of the body as a short undulating membrane e.g. *Blastocrithidia* and some *Trypanosoma* species
III. Trypomastigote: the "true" trypanosome type; postnuclear kinetoplast; flagellum arising near it to run along a long undulating membrane
IV. Amastigote: rounded or oval forms devoid of external flagellum e.g. *Leishmania* species (Hunt, 2010).

As trypanosomes progress through their life cycle they undergo a series of morphological changes as is typical of trypanosomatids. The life cycle often consists of the trypomastigote form

in the vertebrate host and the trypomastigote or promastigote form in the gut of the invertebrate host. Intracellular lifecycle stages are normally found in the amastigote form. The trypomastigote morphology is unique to species in the genus *Trypanosoma* (FAO, 2006).

2.5.1 Life cycle in the mammalian host

The infective metatrypanosomes undergo development and multiplication at the site of infection where a swelling or chancre may be detected in the skin, and finally the mature blood trypanosomes (or trypomastigotes) are released via lymph vessels and lymph nodes into the blood circulation (Sinshaw *et al*., 2006). During their life cycle in the mammalian host and in the tsetse fly vector, trypanosomes proceed through several morphological stages (Shimelis and Melkamu, 2015). Infection of mammalian host is initiated by the injection of metacyclic trypanosomes present in this vector saliva (Gemeda, 2015). Once introduced into the body of mammalian host; metacyclic trypanosomes become differentiated into rapidly proliferating (generation 4-6 hours); long, slender blood stream form parasites (Desquesnes, 2004). Reproduction in the mammalian host occurs through a process of binary division (FAO, 2006).

Trypanosomes feed by absorbing nutrients, through their outer membrane, from the body fluids of the host. The proteins, carbohydrates and fats are digested by enzyme systems within their protoplasm. Oxygen dissolved in the tissue fluids or blood plasma of their host is absorbed in a similar manner, to generate the energy necessary for the vital processes. Waste products are disposed of by a reverse process, through the outer membrane, into the body fluids of the host. They include carbon dioxide formed during respiration, as well as more complex metabolic products (FAO, 2006).

2.5.2 Life cycle in the tsetse fly

The parasite develops in the proboscis where it undergoes considerable changes in morphology as well as in their metabolism. They change into long slender forms called epimastigote (Batista *et al*., 2009). This is a crucial phase, which multiplies and finally gives rise to the infective metacyclic trypomastigote (FAO, 2006); the only form capable of infecting vertebrate host through fly bites (Shimelis and Melkamu, 2015). The life cycle of trypanosomes in tsetse involves cyclical development for varying length of time, depending on species and ambient temperature. *T. vivax* completes its developmental cycle in the proboscis and pharynx and can be transmitted as metacyclic trypanosomes within a week of initial infective feed (Shimelis and Melkamu, 2015). The cycle of *T. congolense* involves the mid gut and the proboscis and is completed in about two weeks. That of *T. brucei* is more complex; it takes 3 or more weeks and involves the mid gut and salivary glands. Once infected, flies remain alive for 1 to 2 months. For any fly, its vectorial capacity and efficiency are the highest for *T. vivax* (Ikede, 1986).

2.6 Morphology

A sound knowledge of the basic features of the various *Trypanosoma* species enables the identification of each species and exact cause of diseases. The most structure are suspended in the cytoplasm, most prominent being the nucleus (Shimelis and Melkamu, 2015). For specific identification, a number of trypanosomes should be examined systematically for the presence or absence, size and position of a number of features:

I. Presence or absence of trypanosomes of different appearance. If all individual trypanosomes are alike, the infection is called monomorphic (of one form); if there are distinctly different types it can be either a polymorphic (pleiomorphic) species, or a mixed infection of different species.

II. Presence or absence of a free flagellum. In certain species there may be some trypanosomes with, and some without, a free flagellum.

III. Size of the trypanosome (expressed in μm).

IV. The size and position of the kinetoplast. The position is related to proximity to the posterior extremity (rear end) of the organism.

V. The degree of development of the undulating membrane. It may be conspicuous or inconspicuous.

VI. The shape of the parasite, particularly the shape of its posterior part. The posterior extremity may vary from blunt to pointed (FAO, 2006).

The trypanosomes are elongated spindle-shaped protozoa ranging from 8.0 to 39 micrometers in length. All possess a flagellum which arises at the posterior end of the trypanosome from a basal body at the foot of a flagellar pocket. The flagellum runs to the anterior end of the body and is attached along its length to the pellicle to form an undulating membrane (Shimelis and Melkamu, 2015). The flagellum may continue forward as a free flagellum. Within a stained specimen a single centrally placed nucleus can be seen, and adjacent to the flagellar pocket, a small structure, the kinetoplast, which contains the DNA of the single mitochondrion (Urquhart *et al.*, 1996).

2.6.1 Subgenus *Trypanozoon* (brucei group)

This group comprises five members: *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. evansi* and *T. equiperdum*. The three subspecies of *T. brucei* are normally transmitted by tsetse flies (in contrast to *T. evansi* and *T. equiperdum*) and are exactly similar in morphology, but only *T. brucei gambiense* and *T. brucei rhodesiense* are the cause of human sleeping sickness, the former mainly in West and Central Africa and the latter in eastern and southern Africa. *T. brucei brucei* is not infective to humans. *T. brucei* is polymorphic, with three main forms, all of which have a small kinetoplast and a conspicuous undulating membrane (OIE, 2013):

I. Long slender forms (23–30 μm in length) with a free flagellum, which may be up to one half of the length of the organism. The posterior end is pointed and the nucleus is central. The kinetoplast is placed up to 4 μm in front of the posterior extremity (Francois *et al*., 2005).

II. Short stumpy forms (17–22 μm in length) normally without a free flagellum, but in which there may occasionally be individuals with a short free flagellum. The kinetoplast is usually subterminal. The position of the nucleus varies greatly and it is in some cases in the posterior part of the cell, sometimes so far posterior that the kinetoplast is anterior to it (postero-nuclear forms). There is considerable variation in appearance between short stumpy forms, from broad, squat types (which include the postero-nuclear forms) to a form similar to *T. congolense*, although longer. In stained specimens blue volutin granules are often present in the cytoplasm, often arranged in a line along the margin of the cell (OIE, 2013).

III. Intermediate forms, varying in length between the two previously mentioned types. A free flagellum, of varying length, is always present. The nucleus is centrally placed. The posterior end is somewhat variable in shape, but usually bluntly pointed. The kinetoplast is close to the posterior extremity. Volutin granules are occasionally present but neither as common nor as plentiful as in the short, stumpy forms (FAO, 2006).

2.6.2 Subgenus *Duttonella* (vivax group)

This trypanosome as seen in the blood of mammals is also essentially monomorphic, with a free flagellum. Its length, including the free flagellum, varies from 18 to at least 27μm. The following description concerns typical specimens.

I. The kinetoplast is large and terminal or almost so. It is much larger than in any of the other pathogenic species, and this is a distinguishing feature.

II. The nucleus is centrally placed, but the bulk of the cytoplasm is found in the posterior part of the body as this is somewhat swollen.

III. The posterior extremity is swollen and blunt and the undulating membrane is inconspicuous (Urquhart *et al.*, 1996).

2.6.3 Subgenus *Nannomonas* (congolense group)

This is the smallest of the pathogenic trypanosomes, with a length of 9–22 μm. The blood forms are monomorphic, in that they lack a free flagellum (in the longer forms the shape of the anterior extremity may suggest the presence of a very short free flagellum). The use of the term monomorphic is somewhat misleading in this species in that there is a variation in size and

shape between strains (OIE, 2013). Generally two variants are to be seen, a shorter form (9–18μm), the typical *congolense* type and a longer form (up to 25μm), with individuals intermediate in length between the two. The proportion of long and short forms varies in different cases and, it has been said, localities of origin. There is evidence which indicates that strains with the longest forms, the so called "dimorphic" strains, cause a more severe form of trypanosomosis (FAO, 2006).

T. uniforme, 12–20 μm long; monomorphic; flagellum is free, shorter than *T. vivax* affecting domestic ruminants; antelope, *T. evansi* (Surra) 15–34 μm long; usually monomorphic; same as slender form of *T. brucei*; occasional stumpy form, affecting Camels, equines, dogs, water buffalo, *T. equiperdum* (Dourine), 15–34 μm long; same as *T. evansi* affecting majorly Horses (Mary and David, 2009).

T. cruzi measures about 10μm long, slender, thin with an irregular shaped undulating membrane. Its nucleus is centrally positioned and the kinetoplast is posterior (Hunt, 2010). The free flagellum runs through the remainder of the parasite and also extends beyond it. Visualized in stained sample, the parasite assumes a C or U shape (OIE, 2013).

When studying the trypanosomes under the microscope, *T. brucei* and *T. vivax* can be separated on the basis of their movement. In fresh unfixed blood films *T. brucei* moves rapidly within small areas of the microscope field whilst *T. vivax* moves rapidly across the whole field (OIE, 2013).

2.7 The Vector

African trypanosomes can be transmitted by 23 species of tsetse (Glossina) found only in sub-Saharan Africa between latitudes 14°N and 29°S, excluding areas of high altitude, extreme drought or cold temperatures where tsetse cannot survive (Radostits *et al*, 2007). Tsetse flies are classified into one genus *Glossina*, of the family *Glossinidae*, order *Diptera*, the two winged flies.

The three main species of tsetse flies for transmission of trypanosomes are *G. morsitans* which favors the open woodlands of savannah, *G. palpalis* which prefers the shaded habitat immediately adjacent to rivers and lakes and *G. fusca* which favors the high dense forest areas (Leak 1998).

According to Emanuel and Jackson, 2012, the most important subspecies from each group are:

G. Morsitans: G. swynertoni, G. pallidipes and *G. morsitans submorsitans*.

G. Palpalis: *G. fuscipes, G. tachinoides* and *G. palpalis gambiense*

G. Fusca: G. brevipalis and *G. longipennis*

The savannah species pose the greatest threat to livestock because they inhabit grasslands where cattle are traditionally reared. They can easily adapt to other ecological niches and they feed primarily on cattle and pigs, and they are efficient vectors of trypanosomes (Radostits *et al*., 2007). The riverine species are important as vectors of bovine and porcine trypanosomosis, as well as of Gambian sleeping sickness due to *T. brucei gambiense*. On the other hand, the 13 or so forest species are not frequently incriminated vectors of trypanosomes even though their preferred food hosts are ruminants and suids (Kato *et al*., 2015). The morsitans (savannah)

group is of great importance in the transmission of animal trypanosomosis and the palpalis (river) group in the transmission of human sleeping sickness (Rogers, 1991).

Adult *Glossina species* are dull in appearance, varying in colour from a light yellowish brown to a dark blackish brown. In some species the abdomen may have alternate darker and lighter bands. The smallest species is 6-8mm long and the largest 10-14 mm (Jordan, 1986). The adult female produces a single egg, which hatches to a first stage larva in the uterus. After a period of development and moulting a third stage larva is deposited on the ground. Females produce one full grown larva every 9-10 days which then pupates in light or sandy soil. The adult fly will emerge after a puparial period that varies according to temperature but may be around 30 days at 24 degrees Celsius. Consequently tsetse flies have a very low rate of reproduction, closer to that of a small mammal than to most insects. The reproductive method of tsetse flies is known as adenotropic viviparity (FAO, 1998).

2.8 Species Affected and Host Pathogenicity

The effect of infection varies with the host, in that most wild animals and some domestic ones, establish a balance with the parasite and remain as clinically normal carriers for long periods. Specifically, some breeds of cattle indigenous to Africa can tolerate light to moderate challenge with tsetse flies by limiting the multiplication of trypanosomes in their blood and by apparently warding off the infection, especially *T. vivax*. The phenomenon is called trypanotolerance, it is both genetic and environmental in origin, and the level of tolerance varies (Radostits *et al*., 2007). Trypanosomes can infect a wide variety of domestic animals and more than 30 wild animal species. *Trypanosoma congolense* infects cattle, pigs, goats, sheep, and horses. Dogs occasionally become chronically infected carriers of this species. *T. brucei brucei* can infect

cattle, horses, dogs, cats, camels, sheep, goats and pigs. *T. vivax* primarily infects cattle, sheep and goats. Monkeys, rats, mice, guinea pigs and rabbits can also be experimentally infected by trypanosomes (CFSPH, 2015).Various species of trypanosomes affect different species of animals, producing varying disease conditions. Trypanosomes can infect all domesticated animals but in Africa, cattle are mainly affected. More than 30 species of animals including ruminants such as Antelopes (*Addax* species, *Oryx* species and *Hippotragus* species), Buffalos (*Syncercus caffer*) and wild equidae are also susceptible and may serve as reservoirs of trypanosomes (Morrison and Macleod, 2011). These animals suffer syndromes ranging from mild chronic infection to acute fatal disease. Although host preferences of each *Trypanosoma species* may vary, *T. congolense, T. vivax* and *T. brucei* have a wider range of hosts among domesticated animals (Abenga, 2014). There is a range of variation from the very acute disease in pigs caused by *Trypanosoma simiae* to the usually mild conditions and *Trypanosoma brucei (T. brucei*)/ *Trypanosoma evansi* infections in cattle. *Trypanosoma vivax* induces a hyper acute haemorrhagic form of the disease in cattle, sheep and goats (FAO, 2014). In cattle, *T. vivax* generally produces a higher level of parasitaemia than other species. Since its life cycle in the tsetse is also shorter, *T. vivax* is more readily transmitted than the others when animals are newly introduced into a tsetse infested area and the higher parasitaemia also facilitates mechanical transmission (Radostits *et al*., 2007). In dogs, *T. brucei brucei* induces a severe disease condition (Nwoha and Anene, 2011), while *T. brucei brucei induce* a fatal central nervous system disease in cattle compared to that seen in man (Munday *et al*., 2015). *Trypanosoma cruzi* infection in dogs causes chronic myocarditis, dilation of the heart, electrocardiographic abnormalities, cardiac complications and death (CAPC, 2014). The disease in horses is generally characterized

by infiltration of subcutaneous tissues causing swelling of the eyelids, lips and skin beneath the lower jaw as what is seen in dogs (Getachew, 2005; FAO, 2014; Gemeda, 2015).

2.9 Immunology and Antigenic Variation

For some organisms the infection are highly variable for the life of the organism. For example, among guinea pigs, dogs, and rabbits there is repeated high and low levels of parasitaemia. These fluctuations are attributed to the ability of these organisms to change the chemical composition of their surface coat (glycocalyx) (Sengupta *et al*., 2012; Cnops *et al*., 2015a).

They produce a parade of successive variant antigenic types (VATs) in the vertebrate host so there is a period of remission during which time the host produces antibodies that are capable of destroying the parasite. However, the parasite counters this by producing a new surface glycoprotein for which the host has not yet developed antibodies (Liu *et al*., 2015). Once this is accomplished the parasite can begin to multiply rapidly, the host actually recognizes a specific antigen of the parasite; a variant specific surface glycoprotein (VSG) released through the flagellar reservoir of the parasite an eventually covers the organism in a surface coat, this parasite has at least hundred genes that code for VSGs; VSGs are, however, expressed one at a time (Van Nieuwenhove *et al*., 2012). The surface of salivarian trypanosomes is covered with a densely packed layer of dimers of one type of variant surface glycoprotein (VSG). These VSGs are strong immunogens, but the parasite avoids elimination by the host immune system by changing the variant antigen type (VAT) of its VSG coat. Each parasite genome contains a large repertoire of several hundred to thousand VSG genes, supplemented by recombination and gene conversion events; however, only one is predominantly expressed at a time (McCulloch and Horn, 2009; Mansfield *et al*., 2014). Switching the expression of one VSG gene to another

results in a trypanosome bearing a different VAT, that may escape immune destruction as long as the infected host do not possess antibodies against this particular VAT (Barry and McCulloch, 2001; Cnops *et al*., 2015b). This antigenic variation strategy makes it difficult to develop a VSG based vaccine against animal trypanosomosis, and vaccine design strategies have been focused on invariant trypanosome molecules that mediate pathogenesis. Despite the switching strategy of VSG genes, various reports have shown that native and recombinant VSG antigens, VSG peptides and VSG mimotopes can be used for the diagnosis of salivarian trypanosomes (Sengupta *et al*., 2012; Van Nieuwenhove *et al*., 2012, 2013).

2.10 Transmission

2.10.1 Cyclical transmission

When a tsetse fly hatches from its pupal case it is free from trypanosomes. Until its first blood meal, it is called a *teneral* fly. It acquires a trypanosomal infection when feeding on a parasitaemic (having parasites in the circulating blood) mammalian host. The trypanosomes undergo a cycle of development and multiplication in the digestive tract of the fly until the infective metacyclic trypanosomes (metatrypanosomes) are produced, different trypanosome species develop in different regions of the digestive tract of the fly, and the metatrypanosomes occur either in the biting mouthparts or the salivary glands (Sinshaw *et al*., 2006). The period from ingesting infected blood to the appearance of these infective forms varies from one to three weeks; once infective metatrypanosomes are present the fly remains infective for the remainder of its life (Shimelis and Melkamu, 2015). During the act of feeding the fly penetrates the skin with its proboscis. By the rupture of small blood vessels a pool of blood is formed in the tissues

and the fly injects saliva to prevent coagulation. Infection of the host takes place at this stage, with infective metacyclic trypanosomes in the saliva (FAO, 2006; Shimelis and Melkamu, 2015).

2.10.2 Mechanical transmission

A biting insect passes the blood forms from an infected animal to another in the course of interrupted feeding. The time between the two feeds is crucial for effective transmission because the trypanosomes die when the blood dries. The importance of this mode of transmission is variable from place to place, depending on the numbers of hosts and biting insects present, and also on the species of trypanosome. Large biting insects such as tabanids carry more blood and are more likely to act as mechanical vectors than for example stomoxes. (Tsetse flies themselves can of course also act as mechanical vectors.) In non-cyclical transmission, trypanosomes can be transmitted in the absence of tsetse flies (*Glossina*), but *Glossina* species are also capable of transmitting mechanically, in these case the fly feeds on more than one animal before repletion and remain infective for only a short time (Levine, 1973), after trypanosomes have been introduced into a herd. Biting flies are capable of transmitting in their mouthparts if they feed on more than one host in a short interval. Trypanosomes are mechanically transmitted by blood sucking flies chiefly *Tabanus striatus, Stomoxy calcitrans*, *Chrysops* species, *Haematobia irritans*, *Lyperosia* species and *Hippoboscidae. T. vivax* is commonly spread by this mechanism (Paris *et al*., 1982; Desquesne, 2004).

2.10.3 Transmission by other means

It is well known that carnivores may be infected with *T. evansi* and *T. brucei* by ingesting meat or organs from infected animals, as long as these are still sufficiently fresh to contain live

trypanosomes. Infection occurs probably through the mucosa of the mouth (in which moreover bone splinters make wounds through which the parasites penetrate even more easily) (FAO, 2006).

Transmission of *T. evansi* in Latin America by the bites of vampire bats is common. These bats become infected by ingesting blood from infected horses or cattle, the trypanosomes multiply in the bats and these are thereafter able to transmit the disease to healthy animals. The trypanosomes apparently pass readily through the oral mucosa of the bat in both directions (FAO, 2006).

All trypanosome species are occasionally transmitted congenitally, from the mother to the offspring, either through the placenta while the foetus is still in the uterus, or when bleeding occurs during birth. Congenital transmission of *T. vivax*, for example, has been observed in Latin America as well as in Africa, but its real importance is not well known (FAO, 2006). Transplacental transmission has also been recorded in cattle (Ogwu *et al*., 1992).

Venereal transmission is the normal means by which dourine of equines, caused by *Trypanosoma equiperdum*, is propagated. Because of its presence in the mucous exudate of penis and sheath of the stallion and the vaginal mucus of the mare, *T. equiperdum* is easily transmitted directly during copulation from an infected to a healthy animal and its geographical distribution is not restricted to specific climatic conditions. This species is essentially a tissue parasite and causes at most very low parasitaemia in the circulating blood of equines (Brun *et al*., 2010). Iatrogenic transmission means that it is caused by the (veterinary) operator. Iatrogenic infections are induced (involuntarily) by the operator using unhygienic procedures, such as contaminated instruments. (FAO, 2006)

2.11 Pathogenesis

The precise pathogenesis of trypanosomosis remains far from clear. Four features: chancre, lymphadenopathy, anaemia, and tissue damages dominate the pathology of trypanosomosis. The trypanosome species affecting man and domestic animals have been subdivided into two groups, the haematinic group (*T. congolense* and *T. vivax*) which remains in the plasma and the tissue invading group (*T. brucei, T. evansi, T. b. gambiense, T. b. rhodesiense* and *T. equiperd*um found in extra and intra vascular spaces (Ngure *et al*., 2008). Because of their presence in the blood, these invading parasites produce numerous changes in the cellular and biochemical constituents of blood (Taiwo *et al*., 2003). Metacyclic trypanosomes are inoculated intradermally as the fly feeds. They multiply at this site provoking a local skin reaction (chancre), which is most pronounced in a fully susceptible host and may be slight or absent with some strains or species of trypanosomes. Within the chancre, metacyclic parasites change to trypomastigote form, enter the bloodstream directly or through the lymphatic, where they reproduce asexually by binary fission (Maudlin *et al*., 2004). *T. vivax* and *T. brucei* invade tissues and result in tissue damage in several organs and initiate characteristic intermittent parasitaemia. Their behavior thereafter depends largely on the species of trypanosome transmitted and the host. *T. vivax* usually multiplies rapidly in the blood of cattle, sheep and goats, and is evenly dispersed throughout the cardiovascular system, whereas *T. congolense* tends to be aggregated in small blood vessels and capillaries of the heart, brain, and skeletal muscle, and rarely causes heavy parasitaemia in ruminants (Radostits *et al*., 2007; Mary and David, 2009). *T. brucei* is also found extravascularly, for example in the myocardium, the central nervous system and the reproductive tract.

When an animal is infected with trypanosomes, antibodies against the surface coat are produced (Shimelis and Melkamu, 2015). The parasite releases toxic substance when destroyed within the circulatory system and hence damages the lining of the blood vessels. Therefore the damage to the host does not depend on the nutrient being depleted by the parasite but rather on the production of toxic substances (Abenga, 2014). The ability of *Trypanosoma spp* to change their surface coat antigen continuously leads to exhaustion of the antibody production by the host leading to immunosuppression (Shimelis and Melkamu, 2015). Lymphoid enlargement and splenomegaly develop associated with plasma cell hyperplasia and hypergammaglobulinaemia, which is primarily due to an increase in IgM. Concurrently there is a variable degree of suppression of immune responses to other antigens such as microbial pathogens or vaccines. Ultimately, in infections of long duration, the lymphoid organs and spleen become shrunken due to exhaustion of their cellular elements (FAO, 2006). Anaemia is a cardinal feature of the disease, particularly in cattle, and initially it is proportional to the degree of parasitaemia. It is hemolytic because the red blood cells are removed from the circulation by the expanding mononuclear phagocytic system. Cell degeneration and inflammatory infiltrates many organs such as skeletal muscle and the central nervous system (CNS), but perhaps most significantly in the myocardium where there is separation and degeneration of the muscle fibers (Urquhart *et al.*, 1996).

The response of antibodies developed to the glycoprotein coat of the trypanosomes kills the parasites and results in the development of immunocomplexes (FAO, 2006; Hamilton *et al.*, 2007). Immunologic lesions are significant in trypanosomosis and it has been suggested that many of the lesions (e.g. anaemia and glomerulonephritis) in this disease may be the result of deposition of immune complexes that interfere with, or prevent, normal organ function. Profound

immunosuppression occurs following infection and this lowers the hosts' resistance to other infections and thus results in secondary disease. Most trypanosomes have to survive within two hosts, mammalian and insect, necessitating adaption to differing nutritional environments, and remodelling of their surface coat (Gadelha *et al*., 2011); and must also live within two specialized environments in their mammalian host. In the bloodstream and lymphatic system the parasites evade both the acquired and innate immune systems, predominantly by antigenic variation, changing the variant surface glycoprotein (VSG) expressed on their surface to avoid antibody mediated responses (Mansfield *et al*., 2014). During the second stage of infection, in the CNS, they are more protected from the immune system, and may exist as a reservoir, able to reinfect the bloodstream (Mogk *et al*., 2014).

2.12 Mechanism of Anaemia

The interplay of several factors acting either individually or synergistically contribute to the development of haemolytic anaemia in human and animal trypanosomosis. Most common among these factors are erythrocyte injury caused by lashing action of trypanosome flagella, undulating pyrexia, platelet aggregation, toxins and metabolites from trypanosomes, lipid peroxidation and malnutrition (Saror, 1982; Igbokwe, 1994). Meanwhile, idiopathic (unknown) serum and tumor necrotizing factors are responsible for dyserythropoieses (Liew & Turner, 1999; Maclean *et al*., 2001). Three phases of anaemia have been reported to occur in trypanosomosis. They are, phase I (acute crises), phase II (chronic) and phase III (recovery) (Anosa, 1988).

2.12.1 Phase I: Acute crises

This phase begins with the initial appearance of trypanosomes in peripheral circulation. The parasitaemia in this case is usually high, fluctuating and evident in most days (Mbaya *et al*.,

2009b, 2010; Mbaya *et al*., 2011). During this phase the anaemia is morphologically classified as macrocytic and normochromic (Maxie and Losos, 1979). At this stage death commonly occurs due to severe pancytopoenia and other pathologies (Anosa, 1988). Subacute cases have been produced experimentally in rodents infected with *T. congolense* and with *T. brucei* (Mbaya *et al*., 2010, 2011).

2.12.2 Phase II: Chronic

This phase follows the acute crises phase and is characterized by low levels of parasitaemia. The low to moderate erythrocyte value at this point persists with minor fluctuations. This period ranges from several weeks to months. With the *T. brucei* groups which mostly invade tissues, this is the aparasitaemic phase when the parasites establish extravascularly and are less numerous in peripheral circulations (Rabo, 1995) or absent (Mbaya *et al*., 2009a, b). In this chronic phase, the morphological classification of the anemia is normochromic and normocytic (Maxie and Losos, 1979).

2.12.3 Phase III: Recovery

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

The mechanism of anaemia in trypanosomosis was caused mainly by extra vascular haemolysis in the expanded active mononuclear phagocytic system of the host. This was followed by a drastic reduction of all red blood cell indices during successive waves of parasitaemia. The pattern of anaemia varied, depending on whether the specie of trypanosome was “humoral” or

"haemic". Although the mechanism of anaemia is complex and multifactorial, it primarily compromised the cellular integrity of erythrocytes leading to either haemolytic anaemia or enhanced erythrophagocytosis. Injuries sustained by red blood cell (RBC) membranes caused by the flagella and microtubule reinforced body of the organisms' greatly enhanced erythrophagocytosis of damaged RBC (Mbaya *et al*., 2012).

2.13 Incubation Period

T. congolense infections usually become apparent 4 to 24 days after infection and *T. vivax* in 4 to 40 days. The incubation period for *T. brucei* is 5 to 10 days (CFSPH, 2015). The prepatent period of trypanosomes for example *T. vivax* is variable depending on the host and parasite isolate. In sheep and goat the incubation period last from 4-12 days, while in cattle is about 9-48 days in infection with less pathogenic isolates (Abenga, 2014). *Trypanosoma spp* may exhibit irregular fluctuations, with some cases occurring at high level in morning and at lower level in the afternoon on the same day (Shimelis and Melkamu, 2015)

2.14 Clinical Signs

Bovine trypanosomosis causes severe anaemia, oedema, immunosuppression, and various neurological disorders, which may eventually produce the death of the affected animals (Gonzatti *et al*., 2014). The basic clinical syndrome appears after an incubation period depending on the species causing the infection. There is fever, which is likely to be intermittent and to last for a long period. Affected animals are dull, anorexic, and apathetic, have a watery ocular discharge, loss of condition and nervous signs (Bastita *et al*., 2009). Superficial lymph nodes become visibly swollen; mucous membranes are pale, diarrhoea occasionally occurs, and some animals have oedema of the throat and the underline (Mary and David, 2009; Chanie *et al*.,

2013), anaemia appears with progressing parasitaemia and there is lysis of large numbers of red blood cell resulting in drop in Packed Cell Volume (PCV) (Gonzatti *et al*., 2014). Estrus cycles become irregular, pregnant animals may abort, and semen quality progressively deteriorates (Radostits *et al*., 2007). The animal becomes very emaciated and cachectic and dies within 2-4 months or longer. In general, *T. congolense* is more pathogenic to cattle in eastern and southern Africa, whereas *T. vivax* produces a more serious disease in West Africa (OIE, 2013). However, severe outbreaks of *T. vivax* involving exotic dairy animals in East Africa occurred where affected animals showed mucosal petechiation, dysentery, and death after an illness of only a few weeks (FAO, 2006).

Mixed infections are common and are usually more severe. Furthermore, concurrent bacterial, viral, or other parasitic infections may mask or complicate the basic clinical syndrome. Immune response to bacterial, and some viral, vaccines is also depressed but is restored if trypanocidal therapy is given at the time of vaccination (Radostits et *al*., 2007; Comini *et al*, 2011). Anaemia usually develops in affected animals and is followed by loss of body condition, reduced productivity and often mortality (OIE, 2013). Trypanosomosis directly constrains the productivity of cattle by reducing birth rates, increasing abortion rates and increasing mortality rates (Mersha *et al*., 2013). The acute disease in bovine is characterized by high fever, lacrymation, corneal opacity, nervous signs and death may occur within 24 hours of onset of clinical signs (Maigari *et al*., 2015). Chronic surra can lead to loss of body condition with impaired reproductive performance (Radostits *et al*., 2007). Acutely infected dogs develop anorexia and a fever; oedema of the face, genitalia, and subcutaneous tissues; purulent nasal and ocular discharge; orchitis in males; and weakness (OIE, 2013; CFSPH, 2015). *T. brucei* causes a subacute to chronic disease, in which subcutaneous oedema and keratoconjunctivitis may be

marked, nervous signs are manifested in horses, pigs, and small ruminants by ataxia, circling, head pressing, and paralysis. Cattle are usually asymptomatic, with few exceptions (Radostits *et al*., 2007). All three species of trypanosomes will eventually cause death in their hosts unless treated (OIE, 2013). Abenga, (2014) showed that the disease can become subclinical in an endemic area, though is still cause's sizeable losses in production.

2.15 Pathological Signs

In cattle infected with *T. vivax*, at post mortem hemorrhages are widespread and extensive. The intestinal tract, from the abomasum to the rectum, contains large amounts of blood (Mary and David, 2009). Signs of bleeding are seen beneath the lining of various organs; the heart, pleural cavity, peritoneum, diaphragm, virtually every organ and tissue (Maigari *et al*., 2015). The disease progresses so quickly to death that there is no loss of condition (FAO 1998). Postmortem signs may include emaciation, enlarged lymph nodes, enlarged liver and splenomegaly associated with the plasma cell hyperplasia and hypergammaglobulinemia, excessive fluid in the body cavities, petechial haemorrhages and mucosal bleeding may also occur (CFSPH, 2015). The heart may be somewhat enlarged and may show a few haemorrhages on the muscle surface. There is also likely to be more fluid than normal in the chest, lungs, abdomen and pericardium (heart sac). The kidneys are pale and swollen (Chanie *et al*., 2013). Subcutaneous oedema may be present particularly in horses and sheep (FAO, 2006). In animals that died during the chronic phase of the disease, the lymphoid organs are usually no longer enlarged and severe myocarditis is a common finding (OIE, 2013).

In the male reproductive tract, pathological disorders attributed to trypanosomosis include testicular degeneration, scrotal inflammation, penile protrusion, prepucial inflammation,

testicular oedema, epididymitis and abnormal spermatogenesis (Adamu *et al*., 2007; Victor *et al*., 2012). In the female, there is abortion, irregular oestrus cycle, cystic degeneration of the ovary, follicular cyst, flaccidity of the uterine horn, decreased conception rate, low birth-weights and neonatal death (Rodrigues *et al*., 2013). In addition, pregnant animals infected by trypanosomes may die before or after parturition (Bawa *et al*., 2005). Postmortem lesions are nonspecific and are usually related to anaemia, lymphoid tissue may be atrophic in the terminal phases as the animal is too debilitated to mount an immune response, and severe myocarditis is common. In chronic trypanosomosis the pathological changes seen at post mortem are more striking. The carcass is emaciated and often dehydrated. The skin may show pressure sores and ulcers, when the animal has been unable to stand up for some time. The fat reserves under the skin have been used up and the skin closely adheres to the underlying muscles and bone (FAO, 2006). In bovine the anaemia is described as normochromic normocytic anaemia with a tendency to be normochromic macrocytic anaemia (Abenga, 2014), the macrocytosis is due to erythrogenesis that takes place two weeks after onset of infection at which time, immature erythrocytes are released into the blood stream. *T. brucei* tends to invade tissues to cause inflammation and degeneration of multiple tissues, in addition to anaemia (OIE, 2013; Abenga, 2014).

2.16 Diagnosis

A variety of diagnostic tests are available and researchers are still trying to improve existing tests and to develop new ones. Current diagnostic tests vary in their sensitivity and specificity, the ease with which they can be applied and their cost (Paris *et al*., 1982). The choice of a particular test will be guided by economic principles and the availability of expertise, but especially by the diagnostic requirement. For example, different degrees of sensitivity and specificity are applied to the confirmation of the infection in an individual animal as compared to the detection of

infection at a herd level. Similarly, the diagnostic test(s) to establish the parasitological prevalence of trypanosomosis are different from those required to establish the presence or absence of the disease in an area (Jamonneau *et al*., 2015). Reliable diagnosis may be achieved by combining appropriate diagnostic tests. Reliable interpretation of results from diagnostic tests will depend on test validity as well as on proper sample selection/collection, the sample size, and the way the diagnostic tests are conducted (OIE, 2013).

2.16.1 Identification of the parasite

Accoeding to OIE (2014), parasite detection techniques are highly specific, but their sensitivity is relatively low (i.e. the proportion of false-negative results recorded is high). Sensitivity is especially low when results are considered at the individual animal level rather than the herd level. Sensitivity is highly variable during the course of the infection:

I. In the early phase, the sensitivity is high as parasites are actively multiplying in the blood in the absence of immunological control.

II. During the chronic phase the sensitivity is low as, due to the immune response of the host, parasites are scanty and rarely seen in the blood;

III. Finally the sensitivity is almost nil in healthy carriers, where parasites are never seen.

At the population level these variations mean that parasite detection techniques are highly sensitive during epizootic outbreaks (when most of the animals are in the early stages of infection), and are of low or very low sensitivity in enzootic areas (most of the animals are in the chronic stages of infection), especially during subclinical phases of the infection (healthy

carriers). Due to this low sensitivity, the apparent parasitological prevalence of trypanosomosis is a little or much lower than the true parasitological prevalence (FAO, 2006).

The low diagnostic sensitivity also makes it difficult to detect trypanosomosis when present at low parasitological prevalence and it is impossible to establish the absence of the disease with a high degree of confidence (Mumba *et al*., 2014). Moreover, in areas where trypanocidal drugs are used extensively, parasites may not be detected. Several parasite detection techniques are available, each with varying sensitivity. The choice will depend on the laboratory facilities available and the aim of the diagnosis (OIE, 2013; Nakayima, 2016).

2.16.2 Direct examination techniques

The simplest techniques are examination of wet mount, thick or thin films of fresh blood, usually obtained from the ear vein, jugular vein or the tail. Amongst the direct examination techniques, stained thin blood films are generally regarded as more specific but less sensitive than the other two. The actual specificity and sensitivity of these techniques is directly dependent on the volume of blood actually examined and the skill and experience of the technician (OIE, 2013).

2.16.2.1 Wet blood films (wet mount)

These are made by placing a droplet of blood (about 2 µl) on a clean microscope slide and covering with a cover-slip (22 × 22 mm). The blood is examined microscopically at ×400 total magnification with condenser aperture, phase-contrast or interference contrast. Approximately 50–100 fields are examined (Kemal, 2014). Trypanosomes can be recognized by their flagellated movement among the red blood cells (RBCs).The method is simple, inexpensive and gives immediate results. Depending on the trypanosome size and movements a presumptive diagnosis can be made of the trypanosome species (Dabo and Maigari, 2017). Final confirmation of the

species is made by the examination of the stained thin blood smear preparation. The diagnostic sensitivity of the method is generally low but depends on the examiner's experience and the level of parasitaemia. Sensitivity can be improved significantly by lysing the RBCs before examination using a haemolytic agent such as sodium dodecyl sulphate (SDS) (FAO, 2006; OIE, 2013).

2.16.2.2 Thick blood smear

These are made by placing a drop of blood (5–10 µl) on a clean microscope slide and spreading it over an area of approximately 2 cm in diameter, using the corner of another slide. The thickness of the resultant film should be such that, when dry, the figures on a wrist watch dial can just be read through it (Nakayima, 2016). The film is dried thoroughly by rapidly waving in the air and, without fixation, is dehaemoglobinised by immersion in distilled water for a few seconds and dried before staining (Kemal, 2014). A dry smear should be kept dry and protected from dust, heat, flies and other insects. It is stained for 30 minutes with 4% diluted Giemsa stain in phosphate buffered saline, pH 7.2. Staining time and stain dilution may vary with stain and individual technique. Therefore, it is important to start with the manufacturer's directives and to vary the staining time and concentration of the stain to obtain optimal result. The stained smear is then washed with buffered water and examined at ×500 to ×1000 total magnification (Norgan *et al*., 2013). The method is simple and relatively inexpensive, but results are delayed because of the staining process; however commercial kits are available for quick staining. Trypanosomes are easily recognized by their general morphology, but may be damaged during the staining process. This may make it difficult to identify the species (OIE, 2013).

2.16.2.3 Thin blood smear

Thin blood smears are made by placing a small drop of blood (about 5 µl), for example from a microhaematocrit capillary tube, on a clean microscope slide approximately 20 mm from one end (allowing for space to apply the thin smear) and spreading with the edge of another slide (Nakayima, 2016). This slide is placed at an angle of approximately 30° to the first slide and drawn back to make contact with the blood droplet. The blood is allowed to run along the edge of the spreader, which is then pushed to the other end of the slide in a fairly rapid but smooth motion (Kemal, 2014). If the correct amount of blood is used, the slide should be covered with a thin film of blood with no surplus before the end of the slide is reached, and the smear should take the shape of a bullet. Ideally, thin films should be prepared so that the RBCs are fairly close to each other but not overlapping. The slide is dried quickly by waving in the air and protected from dust, flies and other insects (OIE, 2013).

The slide is fixed for 3 minutes in methanol, and stained as for thick blood smears. After staining, the slide is washed gently under tap water and allowed to dry (Dabo and Maigari, 2017). A variation of this method is to fix in methanol for 2 minutes, apply May–Grünwald stain for 2 minutes, then add an equal volume of buffered water, pH 7.2, and leave for a further 8 minutes and drain off. Approximately 50–100 fields of the stained thin blood smear are examined, with a ×100 oil immersion objective lens, before the specimen is considered to be negative. Even after a trypanosome has been detected, approximately 20 extra fields are investigated to determine if more than one species is present (Nakayima, 2016). The sharp extremity of the smear must be extensively explored as, because of their capillary properties, trypanosomes may be concentrated at this place (especially true for large species like *T. brucei* and *T. vivax*) (OIE, 2013; Jamonneau *et al*., 2015).

Usually, both thin blood and thick blood smear are made from the same sample. Thick blood smears contain more blood than thin blood smears and, hence, have a higher diagnostic sensitivity than the thin blood smear. Thin blood smears on the other hand allow *Trypanosoma species* identification (OIE, 2013).

Trypanosome species can be identified by the following morphological characteristics:

Trypanosoma vivax: 18–27 µm long, undulating membrane is medium or not obvious, free flagellum present at the anterior end, posterior end rounded, kinetoplast large and terminal.

Trypanosoma brucei is a polymorphic trypanosome species. Two distinctly different forms can be distinguished, i.e. a long slender form and a short stumpy form. Often, intermediate forms, possessing characteristics of both the slender and stumpy forms, are observed. The cytoplasm often contains basophilic granules in stained specimens (Abenga, 2014).

Trypanosoma brucei (long slender form): 23–30 µm long and about 2.8 µm wide, undulating membrane is conspicuous, free flagellum present at the anterior end, posterior end pointed with small kinetoplast which is subterminal (Sima *et al*., 2011).

Trypanosoma brucei (short stumpy form): 17–22 µm long and about 3.5 µm wide, undulating membrane is conspicuous, free flagellum absent, posterior end pointed with small kinetoplast which is subterminal (Bezie *et al*., 2014).

Trypanosoma congolense: 9–18 µm (small species) and a longer form (up to 25 µm), undulating membrane not obvious, free flagellum absent, posterior end rounded, kinetoplast is medium sized and terminal, often laterally positioned (Desquesnes *et al*., 2012). Additionally, sphaeromorph and rosettes have also been described. Within *T. congolense*, different types or

subgroups exist (savannah, forest, kilifi or Kenya coast) that have a different pathogenicity (Bengaly *et al*., 2002); also there is a large variation in pathogenicity within the savannah subgroup. These types can only be distinguished using PCR (Bashir *et al*., 2014).

Trypanosoma theileri: (large species), typically 60–70 µm but individual organisms can range from 19 to 120 µm, undulating membrane is conspicuous, long free flagellum present, posterior end pointed and rigid, kinetoplast is large and positioned near the nucleus and in a marginal position (Mumba *et al*., 2014). *Trypanosoma theileri* is normally nonpathogenic, but its presence can confuse the parasitological diagnosis. In Western Europe, *T. theileri* is the only trypanosome species occurring in cattle (OIE, 2013).

2.16.3 Parasite concentration techniques

The probability of detecting trypanosomes in a sample from an infected animal depends largely on the amount of blood examined and the level of parasitaemia. The amount of blood examined with direct examination techniques is low and often the parasites are very scanty in the blood of an infected animal (Jamonneau *et al*., 2015). Both of these factors contribute to the low sensitivity of direct examination techniques. Sensitivity can be improved by increasing the volume of blood to be examined and by concentrating the trypanosomes (OIE, 2013).

2.16.3.1 Microhaematocrit centrifugation technique (Woo method)

The microhaematocrit centrifugation technique, or the Woo method (1970), is widely used for the diagnosis of animal trypanosomosis. It is based on the separation of the different components of the blood sample depending on their specific gravity. The method is as follows:

I. Fresh, usually ear vein blood (about 70 µl) is collected into heparinised capillary tubes (75 × 1.5 mm).

II. One end of the capillary tube is sealed with crista seal or by heating, ensuring that the column of blood is not charred by the flame.

III. The sealed capillary tubes are placed in a microhaematocrit centrifuge with the sealed ends pointing towards the outside. To ensure good balance, the tubes are loaded symmetrically.

IV. The rotary cover is screwed on and the centrifuge lid is closed.

V. The capillary tubes are centrifuged at 9000 g for 5 minutes.

VI. A tube carrier is made from a slide on which two pieces of glass 25 × 10 × 1.2 mm have been fixed, 1.5 mm apart, to form a groove.

VII. The tube is placed in the groove, a cover-slip is placed on top and the interface is flooded with water.

Alternatively, examination can be done without flooding the interface with water, but in such case, the light condenser must be placed in such a way that cells become refringent.

VIII. The plasma/white blood cell interface (buffy coat) is examined by slowly rotating the tube (OIE, 2013).

Trypanosome movement can first be detected using the ×10 objective lens with reduced condenser aperture; the trypanosomes can be seen more clearly using the ×40 objective lens preferably with a long working distance to allow adequate depth of focus through the capillary tube. The microhaematocrit centrifugation technique is more sensitive than the direct

examination techniques (Abenga, 2014; Kemal, 2014). In the case of *T. vivax* infections, the sensitivity of the Woo methods approaches 100% when the parasitaemia is >700 trypanosomes/ml blood. Sensitivity decreases to 50% when parasitaemia varies between 60 and 300 trypanosomes/ml blood. Trypanosomes become very difficult to detect when the parasitaemia is lower than 60 trypanosomes/ml blood (Desquesnes, 2004). Identification of trypanosome species is difficult. As the specific gravity of *T. congolense* is similar to that of RBCs, parasites are often found below the buffy coat in the RBC layer. To improve the separation of RBCs and parasites, and increase the sensitivity for *T. congolense*, the specific gravity of RBCs can be increased by the addition of glycerol.

A modification of the Woo method is the quantitative buffy coat method (QBC) (Norgan *et al*., 2013). The method has been used for the diagnosis of *T. b. gambiense* infections; it is generally too expensive for the routine large-scale use in animal trypanosomosis surveys.

2.16.3.2 Buffy coat technique (Murray method)

The buffy coat technique or Murray method (Murray *et al*., 1977) represents an improved technique for the detection of trypanosomes and is widely used. It is carried out following steps (I) to (V) above, after which the capillary tube is cut, with a diamond tipped pencil, 0.5 mm below the buffy coat, to include the top layer of RBCs. The buffy coat and the uppermost layer RBCs are extruded on to a clean microscope slide (check that the buffy coat is not sticking to the capillary tube; it should be visible on the slide before covering it with a cover-slip (22 × 22 mm). Approximately 200 fields of the preparation are examined for the presence of motile trypanosomes with a dark-ground or a phase-contrast microscope with a ×40 objective lens (OIE, 2013).

Trypanosome species can be identified by reference to the following criteria:

Trypanosoma vivax: Large, extremely active, traverses the whole field very quickly, pausing occasionally.

Trypanosoma brucei: Various sizes, rapid movement in confined areas; undulating membrane traps the light into 'pockets' moving along the body (Kemal, 2014).

Trypanosoma congolense: Small, sluggish, adheres to RBCs by anterior end.

Trypanosoma theileri: More than twice the size of pathogenic trypanosomes tends to rotate; the posterior end is clearly visible, very long, sharp and rigid (Nakayima, 2016).

As with the microhaematocrit centrifugation technique, the buffy coat technique is more sensitive than direct examination techniques. The sensitivity of the buffy coat method can be improved by using the buffy coat double-centrifugation technique (Norgan *et al*., 2013). A total amount of 1500–2000 µl of blood is centrifuged, after which the buffy coat is aspired into a microhaematocrit capillary tube and centrifuged again. The buffy coat is examined. However, collection of the buffy coat after the initial centrifugation is a delicate step and results may vary from one technician to another (Jamonneau *et al*., 2015).

Compared with the microhaematocrit centrifugation technique, the buffy coat technique has the added advantage that preparations can be fixed and stained for more accurate identification of species and for retention as a permanent record. Both the microhaematocrit centrifugation and buffy coat techniques give direct results and can be used for screening large numbers of animals. They require specialized equipment and an electricity supply making the test more expensive compared with the examination of the wet blood film (Mumba *et al*., 2014). However, this is

compensated for by increased sensitivity. Both parasite concentration techniques rely on the detection of motile, live, trypanosomes. Because trypanosomes can lose their vigor and die rather quickly once the blood sample is drawn, samples collected in capillary tubes should be cooled immediately and not be allowed to overheat in the microhaematocrit centrifuge or on the microscope stage. Samples should be examined as soon as possible after collection, preferably within a couple of hours. The microhaematocrit centrifugation and buffy coat techniques are particularly useful in that the packed cell volume (PCV) can be assessed at the same time.

To determine the PCV after centrifugation, the microhaematocrit capillary tube (containing ear vein or jugular vein blood) is placed in a haematocrit reader. The length of the packed RBC column is expressed as a percentage of the total volume of blood (Kemal, 2014). Measuring the PCV is useful for determining the degree of anemia. Anemia can be caused by factors other than tsetse-transmitted trypanosomosis (Maigari *et al*., 2015). It remains, however, one of the most important indicators of trypanosomosis in cattle. As trypanosomosis is a herd problem, the PCV-profile of a herd is influenced by the number of trypanosome-infected animals and can be used to indicate differences in disease challenge. The average PCV is also influenced by the age and level of genetic susceptibility of cattle (OIE, 2013).

2.16.3.3 Anion exchange

The miniature anion-exchange chromatography technique (m-AECT) is widely used for the diagnosis of human sleeping sickness caused by *T. b. gambiense* (Sima *et al*., 2011). Blood is passed through a diethyl amino-ethyl (DEAE)-cellulose column equilibrated with a phosphate buffered saline (PBS) solution of an ionic strength suited to the blood of the animal species under examination (Nakayima, 2016). As the RBCs are more negatively charged than the

trypanosomes, they are held in the column and the trypanosomes pass through with the eluate, which is collected, centrifuged to concentrate the trypanosomes and examined under the microscope (Dabo and Maigari, 2017). Large volumes of blood can be examined from each animal and, therefore, the method has high sensitivity (Sanni *et al*., 2013). However, the technique is cumbersome and is not suitable for the examination of a large number of animals because it is very expensive and time consuming (Sima *et al*., 2011).

2.16.3.4 In-vitro cultivation

A procedure for the in-vitro cultivation of *T. brucei* has been described, but success has been irregular over many years (Nakayima, 2016). Moreover, the method needs sophisticated equipment, produces results after a considerable delay and is certainly not suitable for large-scale use. A kit for in-vitro isolation of trypanosomes has proven to be promising in isolating and amplifying all species of T. brucei in humans, domestic and game animals (Truc *et al*., 1992). The test value in isolating *T. congolense* and *T. vivax* is still unknown. As it is based on the cultivation of procyclic forms of trypanosomes, species differentiation is not possible; however a recent method has been described for a complete in-vitro life-cycle of *T. congolense* (Coustou *et al*., 2010). It should be noted that cultivation is a highly efficient and sensitive method for the detection of tabanid-transmitted *T. theileri*, the prevalence of which is often found to be close to 100% using this technique. In the case of mixed infections, *T. theileri* easily overgrows *T. b. brucei* (Verloo *et al*., 2000).

2.16.4 Animal inoculation

Rodent inoculation is expensive, diagnosis is not immediate, and the method should be avoided as much as possible as it raises serious animal welfare concerns (Salim *et al*., 2011). However,

the inoculation of blood into rodents, usually mice or rats, is more sensitive than the Haematocrit centrifuge technique and sometimes PCR as well, thus it is particularly useful in revealing subpatent infections, which may be especially important in non-endemic areas (FAO, 2014).

The laboratory animals are injected intraperitoneally with 0.1–0.5 ml (depending on the size of the rodent) of freshly collected blood (Yusuf *et al*., 2015). Artificial immunosuppression of recipient animals by irradiation or drug treatment (cyclophosphamide 200 mg/kg) will greatly increase the chances of isolating the parasite (Maigari *et al*., 2015). A drop of blood is collected from the tip of the rodent's tail three times a week. The blood is examined using the wet film method. If an infection occurs, it generally shows after 3–10 days; however the rodents must be followed for at least 1 month (OIE, 2013; Nakayima, 2016).

In cases of suspected trypanosome infection, the success rate of this method depends on the *Trypanosoma species* involved: it is highly sensitive for detection of Trypanozoon infections, of medium sensitivity for *T. congolense* strains, and generally poor but in rare cases effective for *T. vivax* (Mumba *et al*., 2014)

The modern use of rodent inoculation should therefore be restricted to

- Massive parasitic antigen-production for serological diagnosis
- Attempts at parasite demonstration and isolation when a trypanosome infection is suspected in animals living in or travelling towards a non-endemic area.

2.16.5 Serological tests

Antigen detection enzyme-linked immunosorbent assays (ELISA) for trypanosomosis have been described (Nyimba *et al*., 2015). Field evaluations of the tests have given inconsistent results

(International Atomic Energy Agency [IAEA], 1993). Additional works have been done under controlled conditions, which led to the conclusion that the sensitivity and specificity of these tests are not suitable for the diagnosis of trypanosomosis (Desquesnes, 2004; Eisler *et al*., 1998).

Serological assays, however, are useful tools for large scale epidemiological surveillance (Verloo *et al*., 2000). According to the World Organization for Animal Health (OIE 2012), antibody detection ELISA using trypanosome crude antigen is regarded as a conventional and standard method for the diagnosis of animal trypanosomosis. In addition to the conventional tests, the development of recombinant technology has led to the introduction of a number of new recombinant antigens, including T. evansi GM6 which consisted of 4 repeat domains (TeGM6-4r) for use in disease diagnostic surveillance (Goto *et al*., 2011; Thuy *et al*., 2012). Several antibody detection techniques have been developed to detect trypanosomal antibodies for the diagnosis of animal trypanosomosis, with variable sensitivity and specificity. The best methods of choices are the indirect fluorescent antibody test (IFAT) (Katende *et al*., 1987; Ezeani *et al*., 2008) and the trypanosomal antibody detection ELISA (Hopkins *et al*., 1998). The identification of major antigens of trypanosomes, and their production as recombinant molecules or synthetic peptides, should hopefully lead to the development of new tests based on the use of defined molecules. Thus, in the future, it may be possible to improve the specificity of serological tests to allow the detection of species specific antibodies, and to reach a high level of standardization that is currently not achieved by the use of total parasite extracts (Dabo and Maigari, 2017).

2.16.5.1 Indirect fluorescent antibody test

The technique for the preparation of trypanosomal antigens (Katende *et al*., 1987) involves fixation of live trypanosomes using a mixture of 80% cold acetone and 0.25% formalin in normal saline.

Test procedure

I. Prepare thin smears from heavily parasitaemic blood or from a trypanosome suspension. Air-dry and fix in acetone for 5 minutes.

II. Mark circles of 5 mm diameter on glass slides using nail varnish.

III. Using a pipette, place a test serum, diluted 1/40, in each circle, ensuring that the area in each circle is completely covered.

IV. Incubate the antigen/test serum preparation at 37°C for 30 minutes in a humid chamber.

V. Wash the preparation three times in PBS for 5 minutes each time at4°C, with gentle agitation. Air-dry the slides.

VI. Apply conjugate: rabbit or goat anti-bovine IgG (for tests on bovine sera) conjugated to fluorescein isothiocyanate.

VII. Incubate and wash as above. Rinse in distilled water. Air-dry the slides.

VIII. Mount the slides in PBS or buffered glycerol and examine for fluorescence (FAO, 2006; OIE, 2013; Nakayima, 2016).

2.16.5.2 Antibody-detection enzyme linked immunosorbent assay

The antibody ELISA has recently been further developed for use in large-scale surveys of bovine trypanosomosis (Desquesnes, 1997; Hopkins *et al*., 1998). Recommendations have been made that allow antigen production and standardization of the test on a local basis (Desquesnes, 1997; 2004; Greiner *et al*., 1997). The standard antigen for trypanosomosis antibody tests is derived from bloodstream form trypanosomes.

Trypanosomes are purified by DEAE anion-exchange chromatography of parasites from whole blood of infected rats. Antigens are prepared as a soluble fraction with blood (with the addition of anti-enzyme) using seven freeze thaw cycles and centrifugation at 10,000g for 10 minutes (Greiner *et al*., 1997; Nakayima, 2016). Soluble antigens must be added with a protease inhibitor cocktail and be stored at –80°C or –20°C for long and short periods, respectively, but they may also be lyophilised for conservation at room temperature. *T. congolense* or *T. vivax* precoated microtitre plates have been developed that have the advantage of denaturing standardised antigen is used at room temperature (Rebeski *et al*., 2000).

Both the IFAT and antibody detection ELISA have been adapted for the analysis of blood samples collected on filter paper. Blood contained in one heparinised microhaematocrit centrifuge capillary tube is extruded on to a filter paper (Dabo and Maigari, 2017). Samples are air dried out on direct sunlight and placed in a plastic bag with self-indicating silica gel desiccant. The bag is sealed and should be kept as cool as possible until specimens are refrigerated or frozen (Sumbria *et al*., 2014).

Each ELISA microplate is run with strong positive, weak positive and negative reference sera, which are required to comply with pre-set values for quality assurance (Salim *et al*., 2011). The

absorbance of each ELISA sample tested is expressed as a percentage (percentage positivity: PP) of the strong positive reference standard, or the positive and negative reference standards (Desquesnes, 1997); results are, therefore, quantifiable. The cut off value is determined using known positive and negative field or experimental samples (Desquesnes, 1997; 2004).

Both antibody detection tests have high sensitivity and genus specificity. Their species specificity is generally low, but may be improved by using a standardized set of the three species specific tests (Desquesnes, 2004). They detect immune responses to current and past infections and can, therefore, only provide a presumptive diagnosis of active infection. However, persistence of antibodies after a curative treatment or a self-cure is estimated to be on the average of 3–4 months in young and adult cattle infections (Nahla *et al*., 2011); although it might take up to 13 months before all antibodies have disappeared in some animals (Van den Bossche *et al*., 2000) consequently, proper sampling and knowledge of trypanocidal use will give more acute information. Immunodiagnosis needs expensive, sophisticated equipment and expertise, which is not always available. It has to be performed in specialised laboratories and there is a substantial delay between the actual sampling and the availability of the results. Nevertheless, the antibody ELISA lends itself to a high degree of automation and standardisation (Nguyen *et al*., 2014). Sample collection and storage is made easy through the use of filter papers. All of these factors make the antibody ELISA a very useful test for large-scale surveys to determine the distribution of tsetse-transmitted trypanosomosis (OIE, 2013).

Card agglutination test: this has been developed from a commercial test for the diagnosis of human sleeping sickness (the Testryp® CATT), into a commercial kit for *T. evansi*, CATT test *T. evansi*®. For the detection of antibodies to surra (*T. evansi* infection) serum samples are mixed on a plastic card with fixed and stained trypanosomes as antigen and the test is positive

when the antigen agglutinates (Takeet *et al*., 2013). A titre can be determined by serial dilutions of the serum (Nakayima, 2016).

The great advantage of this test is that in principle it is easy to carry out even in the field. Its specificity and sensitivity appear to need further evaluation, and in the experience of the author reading the test results is not always easy. (OIE, 2013),

Antigen-detecting tests (Ag-ELISA): these tests have been developed for the detection of circulating trypanosomal antigens. The surface antigens of trypanosomes are variable; only one or two of the many different variants are present in the blood at any one time and, unless one would possess mixtures of antibodies to all the possible variants, their detection is not reliable. Therefore, the tests that have been developed are based on so-called monoclonal antibodies against invariable (internal) antigens (Takeet *et al*., 2013). This needs some explanation.

Among the constituents of the immune system are various types of lymphocytes. Each of the lymphocytes that manufacture antibodies in response to an infection only makes one type of antibody, directed against only one antigen of the infectious organism (Mumba *et al*., 2014). In fact, antibodies are so specific that they only fit on a particular site of an antigen (a so-called *epitope*), like a key in a lock (Aslam *et al*., 2010). By genetic engineering it is possible to fuse single lymphocytes with cancer cells of a mouse; single-fused (hybridized) cells will grow (because of their cancerous component) in cell culture or in mice, and produce a single type of antibody, a so-called monoclonal antibody. Such monoclonal antibodies in principle react very specifically with one antigen only, or rather fit in one particular shape of epitope only. (FAO, 2006).

2.16.6 Molecular tests

The principle of molecular tests is the demonstration of the occurrence of sequences of nucleotides, which are specific for a trypanosome subgenus, species or even type or strain. Nucleotides are the constituents of DNA (deoxyribonucleic acid), the molecules which constitute the genes on the chromosomes in the cell nucleus (Sanni *et al*., 2013; Nakayima, 2016). A positive result indicates active infection with the trypanosome for which the sequences are specific, as parasite DNA will not persist for long in the host after all live parasites have been eliminated. These tests are not only suitable for detecting parasites in the mammalian host, but also in the insect vector. The general principle of these tests will be shortly explained here, but as they can only be carried out reliably in well-equipped laboratories by specifically trained staff, and are still mainly research tools, no technical details will be given (Sumbria *et al*., 2014).

It seems appropriate to recall first the general structure of DNA, which contains the basic genetic information for all living organisms (except for some viruses which possess only RNA, another form of nucleic acid) (Ezeanai *et al*., 2008). DNA occurs as a double helix (= screw-shaped coil, or a spiral staircase), made up of two strands of nucleotides or bases, which are linked together by hydrogen bonds. There are only four different nucleotides, adenine (A), thymine (T), cytosine (C) and guanine (G) (Takeet *et al*., 2013). All genetic information (the genetic code) depends on the linear sequence of these four bases. The bonds between the two strands either connect A to T or C to G, i.e. the only possible base pairs are A-T and C-G. The hydrogen bonds between base pairs can be broken by heating DNA, resulting in separation of the strands (Shahzad *et al*., 2012). The bonds will be restored when the sample is cooled down (FAO, 2006).

This basic knowledge will make it easy to understand the first of the two main molecular methods for the diagnosis of disease.

2.16.6.1 DNA probes (nucleic acid probes)

The sample to be examined is heated to separate the two strands of DNA (this is also called denaturing of DNA), and these are fixed to a membrane, so that they cannot recombine again on cooling. A probe is then added (Bashir *et al*., 2014). A probe consists of a linear sequence of nucleotides of a certain length, which has been prepared to correspond with a similar sequence of nucleotides in one of the strands of the parasite which the test is meant to detect. The probe will link (hybridize) with that part of the parasite DNA strand which is the mirror image of the base sequence of the probe (Aslam *et al*., 2010). Depending on the sequence of DNA that has been selected for the probe, the test can be more or less specific, certain sequences are common to all species of a subgenus (and thus will for example not allow to distinguish between *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. evansi* and *T. equiperdum*, but indicate the presence of trypanosomes of the subgenus *Trypanozoori*), while other sequences are so specific that they only occur in each species, or subspecies, or even type (Takeet *et al*., 2013; Yusuf *et al*., 2015). Whether hybridization has occurred or not is demonstrated by showing that the probe remains fixed to the sample after washing. For this it is of course necessary to "label" the probe and this can be done by incorporating radioactive isotopes in the probe molecule, and showing that the radioactivity persists. The method is suitable for simultaneously processing large numbers of samples. (OIE, 2013).

2.16.6.2 Polymerase chain reaction (PCR)

A PCR method has been developed as a tool for the diagnosis of infections with African trypanosomes in humans and animals, as well as tsetse flies. Specific repetitive nuclear DNA sequences can be amplified for *T. vivax* and three types of *T. congolense* (Desquesnes and Davila, 2002; Masiga *et al*., 1992). A common primer set is available for detection of the three T. brucei subspecies. The primer sets available for different trypanosome subgenus, species and types are referred to as follows: Trypanozoon subgenus– TBR1 and TBR2; *T. congolense* (savannah type) –TCN1 and TCN2; *T. congolense* (forest type) –TCF1 and TCF2; *T. congolense* (Kenya Coast type) –TCK1 and TCK2; and *T. vivax* –TVW1 and TVW2. Due to the multiplicity of these taxon-specific primers in tsetse flies or cattle, a full trypanosome species identification requires that five PCR test be carried out per sample, which considerably increases the cost of diagnosis (Dabo and Maigari, 2017). Recently PCR restriction fragment length polymorphism (RFLP) assays and ITS1 of ribosomal DNA amplification have been developed that allow the identification of all *Trypanosoma* species as single or mixed infections using one single test (Desquesnes and Davila, 2002; Desquesnes *et al*., 2001); however, these tests are not yet suitable for routine diagnosis. Loop mediated isothermal amplification is also under development for trypanosome diagnosis (Kuboki *et al*., 2003).

Standard monovalent PCR amplifications are carried out in a reaction mixture containing Tris/HCl, $MgCl_2$, KCl, each of the four deoxyribonucleotide triphosphates, primers, DNA template and Taq DNA polymerase. Samples are incubated during several cycles at varying temperatures (Shahzad *et al*., 2012). The PCR products are electrophoresed through agarose. Gels are stained with Ethidium bromide and visualised under UV light for the presence of specific weight products (Takeet *et al*., 2013). The procedure is extremely sensitive, but false-

positive results may occur as a result of contamination of samples with other DNA. The test requires specialised equipment and highly trained personnel, so it is not suitable for use in many laboratories (Yusuf *et al*., 2015). False-negative results may occur when the parasitaemia is very low (< 1 trypanosome/ml of blood), which occurs frequently in chronic infections; they may also occur when the specificity of the primers is too high, so that not all isolates of a particular trypanosome species are recognised. Sample collection has been simplified by adapting the test using blood or buffy coats spotted on to filter paper (Sumbria *et al*., 2014). A large number of samples can be processed at one time, making it potentially suitable for large-scale surveys. However, at the moment, the cost of PCR analyses is prohibitive for the routine use of the test (Geysen *et al*., 2003; Sanni *et al*., 2013).

2.17 Economic Impact of African Trypanosomosis

African Animal trypanosomosis continues to be a serious health concern across large areas of sub-Saharan Africa despite several decades of research (Kubi *et al*., 2006). It causes economic losses in livestock with many untreated cases being fatal. Globally, trypanosomosis causes economic loss of approximately 1.5 billion dollars with African farmers spending 35 million dollars on treatment (Bitew *et al*., 2011). It vastly affects meat and dairy production (Wille *et al*., 1996). Nagana has restricted agricultural development and nutritional resources in Sub-Saharan Africa, profoundly impacting the economy of much of the continent (Brian *et al*., 2007). This disease decreases animal production on about 7 million km squares of land and leads to considerable financial losses due to high morbidity and mortality rates in susceptible but reproductive breeds as well as in the cost of trypanocide treatments (Kone *et al*., 2011). Moreover, the use of trypanocide and trypanocidal drugs is massive with nearly 50% of veterinary products sales in Africa thereby incurring risk of developing drug resistance.

Livestock farmers continue to suffer losses resulting from reductions in fertility (due to abortion and testicular damage affecting sperm viability) and weight loss and mortality. It has been estimated that direct production losses in cattle alone are between US$ 1200 -6000 million per year in 36 tsetse infested countries of Sub-Saharan Africa. These estimates do not include indirect losses due to inavailability of livestock, manure and draught power (Nigatu *et al*., 2015). It is probably the only disease which has profoundly affected the settlement and economic development of a major part of the continent and thus limits rural development in vast areas of tropical Africa (Cecchi *et al*., 2008).

In high challenge areas, and in the absence of trypanotolerant breeds, tsetse flies and AAT prevent the keeping of livestock, at least of those species that are affected by AAT. In that case, the economic impact on livestock production is most pronounced. In several African countries livestock (draught oxen) and their products (manure) play an important role in crop production, and the integration of livestock in crop agriculture is therefore severely affected (OIE, 2013).

All kinds of situations exist between this extreme case and the absence of trypanosomosis; the economic impact varies accordingly. Many factors are involved when economic aspects are considered, such as:

I. Species, type, productivity, value and susceptibility of livestock. For instance: exotic dairy cattle with a high production are both very valuable and very susceptible; exotic breeds in general are of high monetary value and more susceptible than indigenous ones having been exposed to the disease since many generations. Nevertheless, indigenous draught oxen are valuable and, when worked hard, have an increased susceptibility to the effects of the infection. Horses are particularly susceptible to trypanosomes of the subgenus *Trypanozoon*, cattle much

less so. Trypanotolerant breeds can survive and even produce where other breeds can only be maintained under intensive and expensive chemoprophylactic or chemotherapeutic regimens.

II. Challenge, which depends on species of fly present (vectorial ability, host preferences), density of the fly, their daily activity patterns and the grazing patterns of the livestock, and wild reservoir hosts).

III. Presence of drug resistance. (As we shall see further on, very few drugs against trypanosomosis remain commercially available.)

IV. Type of production (commercial, subsistence, transhuman).

V. Economic situation and management of the country (part of national budget and foreign exchange allotted to livestock resources).

VI. Commercial factors, such as "dumping" of surplus meat by the European Union (which decreases the price of locally produced animals), or an unrealistic exchange rate, may bias cost-benefit aspects of trypanosomosis control (FAO, 2006).

The economic impact is made up of direct losses (consisting of loss of production, mortality, abortion), as well as the cost of control (which includes the cost of drugs, their transport to the field site, the salaries of the operators, etc.) (Nigatu *et al*., 2015).

The loss of potential production (i.e. the production that could be achieved if trypanosomosis did not occur) is indirect losses. At present unused grazing areas in many of the tsetse-infested areas of Africa could support a large ruminant livestock population. However, the control of the fly should only be envisaged when really needed, and then only when proper and sustainable land

use plans have been elaborated, and when the political will and legislative means to carry such plans through are assured. If not, overgrazing is bound to occur, followed by erosion and, depending on the climatic zone, by desertification, leading to permanent loss of the land. In the meantime, the presence of tsetse fly preserves these areas (FAO, 2006). Because livestock keepers avoid certain tsetse infested areas, cattle distribution is often imbalanced or even distorted. From the continental cattle distribution we know that this indirect effect of AAT is very important; only 10 million out of 165 million head of cattle in the tsetse-infested countries of sub-Saharan Africa are distributed within the limits of the continental fly belt while most of the remainder is distributed at the perimeter of the fly distribution. At the local level, it is extremely difficult to clarify this point because nobody is sure about the magnitude of these indirect losses. Still, the collective, indirect AAT losses are estimated by FAO to be in excess of the 0.6 to 1.2 hundred thousand US dollar direct losses incurred by trypanosomosis-affected cattle (Nigatu *et al*., 2015). Increasingly, tsetse and trypanosomosis control schemes become concentrated in selected areas of high priority. These are areas where control is technically feasible, where the economic returns are considerable and where the transformation of the landscape, from bush to farmland, already occurs because of demographic pressure. It is in such dynamic environments, which become progressively less suited for tsetse survival, that it is economically attractive to intervene (OIE, 2013).

2.18 Public Health Significance of Trypanosomosis

Human African Trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease. The parasites concerned are protozoa belonging to the *Trypanosoma*. They are transmitted to humans by tsetse fly bites which have acquired their infection from human beings

or from animals harbouring the human pathogenic parasites (Munday *et al*., 2015; Kato *et al*., 2015).

Tsetse flies are found in sub Saharan Africa. Only certain species transmit the disease. Different species have different habitats. They are mainly found in vegetation by rivers and lakes, in gallery forests and in vast stretches of wooded savannah (Brun *et al*., 2010; Steverding, 2015).

Sleeping sickness occurs only in sub Saharan Africa in regions where there are tsetse flies that can transmit the disease. For reasons that are so far unexplained, there are many regions where tsetse flies are found, but sleeping sickness is not (Pelloux *et al*., 2006; van Coller *et al*., 2007; Jha *et al*., 2015). The rural populations living in regions where transmission occurs and which depend on agriculture, fishing, animal husbandry or hunting are the most exposed to the bite of the tsetse fly and therefore to the disease. Sleeping sickness generally occurs in remote rural areas where health systems are weak or non-existent. The disease spreads in poor settings. Displacement of populations, war and poverty are important factors leading to increased transmission. Within a given area, the intensity of the disease can vary from one village to the next (Kabasa, 2007; Mwai *et al*., 2015).

Human African Trypanosomiasis takes two forms, depending on the parasite involved: *Trypanosoma brucei gambiense* is found in west and central Africa. This form represents more than 90 percent of reported cases of sleeping sickness and causes a chronic infection. A person can be infected for months or even years without major signs or symptoms of the disease. When symptoms do emerge, the patient is often already in an advanced disease stage when the central nervous system is affected (Sima *et al*., 2011; Aksoy *et al*., 2017).

Trypanosoma brucei rhodesiense is found in eastern and southern Africa. This form represents less than 10 percent of reported cases and causes an acute infection. First signs and symptoms are observed after a few months or weeks. The disease develops rapidly and invades the central nervous system (Croft *et al*., 2006; Kato *et al*., 2015).

Another form of trypanosomiasis occurs in 15 Central and South American countries. It is known as American trypanosomiasis or Chagas disease. The causal organism is a different species from those causing the African form of the disease (Lutumba *et al*., 2007).

Mother to child infection: the trypanosome can cross the placenta and infect the fetus. Mechanical transmission is possible. However, it is difficult to assess the epidemiological impact of transmission through other blood-sucking insects. Accidental infections have occurred in laboratories due to pricks from contaminated needles (Steverding, 2015).

Achieving disease control in the mammalian host has been difficult given the lack of mammalian vaccines due to a process of antigenic variation the parasite displays in its mammalian host. Hence, accurate diagnosis of the parasite and staging of the disease are important, particularly because of the toxicity of current drugs. (Aksoy *et al*, 2017)

2.19 Treatment

Over most of sub-Saharan Africa, bovine trypanosomosis continues to be controlled primarily by trypanocides (Holmes *et al*., 2004). Trypanocidal drugs remain widely available and also affordable for farmers (at approximately US$1 per treatment). They are often the first drugs tried by farmers when their cattle develop (any) symptoms of the disease (Geerts *et al*., 2001). Consequently, treatment given by livestock owners is not without serious drawbacks because most farmers do not have adequate knowledge on diagnosis and the appropriate drug to use even

in areas of high prevalence of trypanosomosis; and because trypanocides are frequently used in the absence of diagnosis or used to treat conditions for which they are not effective (Holmes *et al*., 2004). However, when used properly, trypanocidal drugs permit higher levels of production, improve animal welfare and safeguard the livelihood assets on which 700 million poor farmers in developing countries rely. Used improperly, such drugs waste scarce resources, result in occasional avoidable sickness and death, mask poor production and promote drug resistance leading to exacerbated disease in animals and humans (Onono *et al*., 2013).

Trypanocidal drugs are the most widely applied method that farmers use to treat and prevent trypanosomosis in sub-Saharan Africa. It has been estimated that about 35 million doses of trypanocides are administered each year to an approximately 45 - 60 million cattle at-risk of trypanosomosis. Trypanocides are popular because farmers can directly treat and, if successful, cure their own animals without relying on the efforts of others (Holmes *et al*., 2004). Despite livestock keepers' dependence on trypanocides only three compounds namely isometamidium chloride, homidium (bromide and chloride) and diminazene aceturate, are currently available for treating cattle. All these drugs have been on the market for over 40 years and several generic forms of them from a wide range of companies have become available on the African market (Dabo and Maigari, 2017). Isometamidium is principally used as a prophylactic drug and can provide up to 6 months protection against trypanosomosis, homidium has limited prophylactic properties, but it is primarily used as a therapeutic agent. Whilst diminazene provides also a short term protection of 2 to 3 weeks, it is mainly used for therapeutic purposes (Onono *et al*., 2013). The specific dose rates vary with animal species, the specific trypanosome, and the specific purpose (curative, prophylactic or enhancer).

2.19.1 Diminazene aceturate (Berenil) (7%, IM)

This is a yellow powder that produces a clear yellow solution in water. It is normally injected intramuscularly, as some swelling may occur after subcutaneous administration. It is not normally used in horses, because even after IM injection the reaction is sometimes severe. The tissues at the site of treatment are stained by the drug and 14 days should elapse after (withdrawal period) treatment before slaughtering animals. Diminazene aceturate is less effective against trypanosomes of the subgenus *Trypanozoon* (such as *T. evansi* and *T. brucei*) than against *T. congolense* and *T. vivax.* It has given fatal reactions in camels, horses, donkeys and dogs at doses which are considered to be normal and harmless in cattle (FAO, 2006). Diminazene aceturate is used widely against T. *vivax* and *T. congolense* as a curative and sanative drug at 3.5/7.0 mg/kg (1–2 ml of prepared solution /20 kg/bw). It is well tolerated by ruminants and it is one of the two recommended drugs for bovine trypanosomosis. It is not well tolerated by horses (Radostits *et al*., 2007; OIE, 2013).

2.19.2 Homidium

Homidium bromide (ethidium) (2.5%, IM) and homidium chloride (novidium) (2.5%, IM), are both crimson powders dispensed as tablets that produce a deep red solution. Contrary to the chloride, the bromide requires warm water to ensure that it is completely dissolved. At normal dosage rates no toxicity problems have been reported. Deep intramuscular injection is recommended as both drugs causes irritation at the site of injection. Horses are the most susceptible to the irritant effect and should be treated only by the intravenous route, taking great care to avoid leakage into the tissues surrounding the jugular vein. They are also widely used against *T. congolense* and T. *vivax* as curative and sanative drugs at 1 mg/kg (1 ml prepared

solution/25 kg) bw. They are mainly used in cattle and small ruminants, should be dissolved in hot water. This bromide salt is potentially carcinogenic (FAO, 2006; Onono *et al*., 2013).

2.19.3 Isometamidium (Samorin or Trypamidium) (1% or 2%, IM)

(Samorin or Trypamidium) is the other preferred drug against *T. vivax* and *T. congolense* in ruminants. It is used as a curative and prophylactic drug at 0.25–0.5 mg/kg (1.25–2.5 ml/50 kg), 1 .0 mg/kg (2.5 ml/50 kg) bw (FAO, 2006). At much higher doses (12. 5-35 mg/kg BW), it can be used prophylactically against T. simiae in pigs but not without the risk of death from acute cardiovascular collapse. (Radostits *et al*., 2007). It is a dark red powder, producing a brownish red solution in water. It is normally administered by deep intramuscular injection as it causes irritation at the site of injection. Preferably, the neck should be used to avoid damage to the more valuable areas of the carcass. Pressure should be applied to the site immediately after the needle is withdrawn to prevent leakage into the subcutaneous tissue. It should not be administered by the subcutaneous route. As far as systemic toxicity is concerned, isometamidium has a wide margin of safety (FAO, 2006). In camels, intramuscular administration may give a severe local reaction, especially at the high dosage rate required for the cure of *T. evansi* infection (1–2 mg/kg). In horses it is not advisable to exceed a dosage of 0.5 mg/kg. This drug was shown to produce signs of shock or death in goats if given intravenously at doses greater than or equal to 0.5 mg/kg (Schillinger *et al*. 1985).

2.19.4 Pyrithidium bromide (prothridium)

Pyrithidium bromide (prothridium) is less widely used against *T. congolense* and *T. vivax* as prophylaxis at 2 mg/kg bw (Radostits *et al*., 2007).

2.19.5 Quinapyramine sulphate

Quinapyramine sulphate (Antrycide) (10%, SC) is no longer used extensively in cattle, but it is the preferred curative drug (at 5 mg/kg BW) against *T. brucei* in horses. Quinapyramine sulphate and chloride (Antrycide prosalt) are used prophylactically at 7.4 mg/kg bw (FAO, 2006). Its use in cattle is now strongly advised against, because drug resistance to it develops readily and can be associated with cross resistance to all the other trypanocidal drugs in use. Moreover, there are toxicity problems in cattle. Quinapyramine is now produced mainly for the treatment of surra in camels and horses, in particular where there is resistance of *T. evansi* to suramin. It is dispensed as a pale cream powder, producing a clear solution in water. It is administered by subcutaneous injection (FAO, 2006).

Under certain conditions, which have never been fully explained, the drug causes systemic toxic effects in cattle and this is aggravated by heat, fatigue, fear, etc (Radostits *et al*., 2007). Toxicity in horses and dogs is also well known. Acute toxicity in dogs may be avoided if the drug is preceded by a dose of a tranquillizing drug some 20 minutes before treatment and dogs should only be treated when cool and rested.

2.19.6 Suramin

Suramin (naganol) (10%, IV): This is effective against trypanosomes of the subgenus *Trypanozoon* (*T. evansi*, *T. brucei*), not against *T. congolense* and *T. vivax*. It causes irritation at the site of injection and is normally given intravenously. Toxicity may sometimes be evident in horses, even at recommended dosage rates, whilst other animal species are much more

tolerant. Resistance to suramin of *T. evansi* is widespread, may also be used against *T. brucei* as a curative and prophylactic drug at 10 mg/kg BW in horses and camels (Onono *et al*., 2013).

2.19.7 Melarsomine (Cymelarsan)

Melarsomine (0.5%, IM or SC): is administered preferably intramuscularly, but the subcutaneous route is also possible. There are often transient local and systemic reactions. It has also been tested against *T. evansi* in domestic buffaloes and *T. evansi* and *T. brucei* in other animals. The dosage rate originally recommended by the manufacturer for camel surra (0.25 mg/kg) proved to be too low in these other animals and even for camels a dose of 0.5 mg/kg may be more realistic (FAO, 2006).

2.19.8 Drug resistance

Drug resistance is the heritable loss of sensitivity of a micro-organism to a drug to which it was before sensitive. Information on the extent and significance of the problem of drug resistance is still scant (Sinyangwe *et al*., 2004). The exposure of trypanosomes to sub-therapeutic concentrations of trypanocidal drugs, the treatment frequency and the degree of drug exposure of the parasite population are important factors influencing the development of drug resistance. Furthermore, some trypanocidal drugs such as ethidium are well-known mutagenic compounds and might induce mutations, the most resistant of which might be selected under drug pressure (Holmes *et al*., 2004). The phenomenon of cross-resistance has now been clearly demonstrated. Quinapyramine usage has been shown to induce resistance to isometamidium, homidium and diminazene (Onono *et al*., 2013).

Generally, it has been argued that due to the privatization of veterinary services, farmers have had easy access to these trypanocides and this has tended to give rise to rampant misuse and

under dosage of the medications, practices that have been considered as major factors in development of trypanocidal drug resistance (Chitanga *et al*., 2011, Onono *et al*., 2013). It has been documented that the repeated use of pesticides or chemotherapeutic agents has shown to inevitably lead to the development of drug resistance in target organisms within 10 years of the introduction of the antimicrobials, insecticides, trypanocides and anthelmintics to the market (FAO, 1998). However, the major factor identified as having an influence on development of trypanocide resistance is the nature of drug use practice. With the World Health Organisation (WHO) advocating on focusing on the causes and in an attempt to delay development of trypanocide resistance the Food and Agricultural Organisation (FAO).

(FAO, 1998) has recommended guidelines on trypanocide usage practice as follows:

I. Avoiding under-dosing which results from incorrect weight estimation, using the wrong amount of drug when making up the injectable solution or when the drug has not completely dissolved during preparation.

II. Reducing the frequency of application of trypanocides by integrating their use with other control methods such as vector control and using insecticide treated cattle. Prophylactic treatment should be limited to a maximum of 4 treatments in a year.

III. Limiting curative treatments to individual sick animals as systematic mass treatments exert a strong selection pressure for resistance.

IV. Avoiding the use of quinapyramine in cattle as resistance to this drug induces cross-resistance to isometamidium, homidium and diminazene (FAO, 2006).

It is assumed that adherence by trypanocide users to these recommended trypanocide usage practices would reduce selection pressure of the drug resistant trypanosomes and thereby delaying development of trypanocidal drug resistance (Dia and Desquesnes, 2005).

Trypanocidal drug resistance is more likely to develop in environments which provide conditions where susceptible host and infectious vector can come together for long enough periods for transmission to occur. The drug pressure then encourages such strains to established and spread rapidly (Chitanga *et al*., 2011).

2.20 Prevention and Control

Tsetse and trypanosomosis control and eradication would be of benefit by promoting human and livestock health, diversified agricultural systems, food production and security, and livelihood of the community and utilization of available natural resources.(Maudlin *et al*., 2004) Several control approaches are available to eradicate trypanosomosis and its biological vector, the tsetse fly, from the area (PATTEC, 2000). For the success of these control strategies greater involvement of farmers and communities in decision making, program designing, program implementing, program evaluating and creating awareness are crucial (Sindato *et al*., 2008). Understanding of farmers' knowledge and perceptions on the impacts of trypanosomosis and tsetse fly and their participation in developing intervention strategies are prerequisites for effective implementation (Machila *et al*., 2003).

2.20.1 Use of insecticides

Tsetse flies are highly susceptible to the action of insecticides, and many different products, starting with DDT and dieldrin up to the more recently introduced and less harmful pyrethroids,

have been used over the past 50 years to control and eradicate tsetse. Initially the insecticide, mostly DDT, was applied by hand spraying on the vegetation, in particular on the favourite resting sites on tree stems, and also under fallen logs and other potential pupae sites. This ground spraying has historically been the most prominent weapon against tsetse. Today, with DDT banned in most countries and the general shift towards a more sophisticated use of insecticide, ground spraying has practically been abolished (FAO, 2006). A different way of using insecticides is to apply these on domestic animals, so that flies settling on such animals are killed. Synthetic pyrethroid formulations are applied by spraying, dipping or used as a "pour-on" formulation, which is more expensive but does not need any pump, spray-race or dip. When such live bait animals are used without any other form of tsetse control, difficulties arise with the persistence of flies in areas where the treated animals do not go (PATTEC, 2000). Nevertheless, locally the flies may be reduced in density to a tolerable level. The frequency of application depends on how long the insecticide remains active on the animal (the length of its residual activity); the effect is shorter during the rains (FAO, 2006).

2.20.2 Sterile male technique

It has been known for a long time that male tsetse flies that have been rendered sterile by gamma irradiation or by certain chemical compounds will mate with females, but these will not produce offspring, as females normally mate once only after hatching, contrary to males. With the continuous release of sterile males in large numbers it is possible to eradicate tsetse flies from a particular area. For the recent successful campaign on Zanzibar where a ratio of more than 100 sterile males against one wild one was used at some stage (PATTEC, 2000).

The method is very specific and not polluting in itself, but the effect on the population only becomes apparent after a period, as opposed to control by instantly killing insecticides. Because of this and to allow the sterile males to be competitive, a substantial fly suppression has to precede the application of Sterile Insect Technique (SIT), which is reserved for the final "mopping up" of the remaining population. The released males will seek out the females even in places where the insecticide cannot penetrate. The males to be sterilized have to be mass reared in the laboratory. Also, the numbers of males that can be obtained are limited by the low rate of reproduction of tsetse flies and the fact that they have to be fed at least twice a week on blood. Where tsetse eradication is envisaged in places where several tsetse species occur in association, as is often the case, mass rearing has to be carried out for each individual species separately (FAO, 2006; OIE, 2013).

2.20.3 Use of traps or screen

Another approach is the use of traps or insecticide-impregnated targets (screens). The attraction of traps and targets for tsetse flies depends on their shape, size, colour and colour pattern, and this differs from species to species. The catch can be increased, in many cases considerably, by certain substances which have an attractive odour for the flies, e.g. acetone, phenolic molecules, bovine or buffalo urine. This type of attraction is also called *olfactory* or *olfactive*. The choice for the best substance depends very much on the species of tsetse fly one is dealing with, and can even be seasonally different. Savannah tsetse flies are generally more attracted by the odours than the riverine species (PATTEC, 2000).

Some of the drawbacks of traps and screens are:

I. Community-based fly suppression efforts are difficult to sustain. The motivation of the owners may diminish after the tsetse population has gone down.

II. Traps and screens may be stolen for the cloth they contain. This is particularly serious when they are part of a barrier against reinvasion.

III. During the rainy season the rapidly growing vegetation may camouflage the trap or screen, which thus loses its visual attraction for the flies.

Preliminary observations indicate that certain types of traps and odour substances will also attract mechanical vectors of trypanosomosis, such as tabanids and stable flies. This may be of help in reducing mechanical transmission of the disease (FAO, 2006; OIE, 2013).

2.20.4 Innate resistance to trypanosomosis

It is well known that genetically determined innate resistance to diseases occurs in animal populations that have been subject to natural selection by exposure to disease pressure over many generations. This is also true for trypanosomosis (Mony and Mathews, 2015). Due to the high costs and practical difficulties associated with bovine-challenge experiments, mouse models have become an alternative to study the mechanisms underlying resistance and tolerance to trypanosome infection. Most studies have been performed with *T. congolense* or *T. brucei* delivered to mice by intraperitoneal injection. The two parasite models share common features, including the critical role of CD11b+ myeloid cells to control parasite growth or to induce tissue pathogenicity. More than 80% of the parasites are eliminated by liver myeloid cells and infected mice die from a systemic inflammatory response syndrome (SIRS) that is initiated by

phagocytosis of parasites by liver myeloid cells and culminates in apoptosis/necrosis of liver immune and parenchymal cells (Beschin *et al*., 2014).

The trypanotolerant breeds incude the Taurine (humpless) breeds of cattle were the first to be introduced into Africa. They populated what is now the Sahara, but were pushed back further south when this area became a desert thousands of years ago. At present, they persist in the subhumid and humid northern parts of sub-Saharan Africa where they live and produce in tsetse areas. Such taurine breeds are now mainly confined to West Africa, from Senegal to Nigeria, but they used to occur as far to the east as the central Sudan (Nuba Mountains) and even western Ethiopia. N'Dama cattle (which originate from Guinea) have rather long horns, while breeds with short horns comprise, for example, the Baoulé (Burkina Faso and northern Côte d'lvoire) and the Muturu (Nigeria). They are "dwarf" cattle (although N'Dama cow can weigh as much as 200 kg, similar to the size of many of the smaller zebu breeds) (Tsegaye *et al*., 2015).

It should be added that trypanotolerance is not limited to taurine cattle, as it has been found that some of the zebu breeds in East Africa, such as the Orma Boran and the Masai zebu, also have a higher resistance to trypanosomosis than other breeds such as the "normal" Boran zebu, Indian breeds of zebu and other "exotic" (i.e. introduced) breeds such as European taurine breeds. However, the resistance of West African taurine breeds appears to be considerably more pronounced. Apart from cattle, breeds of sheep and goats (and even of horses) living in tsetse areas are also relatively trypanotolerant. This is particularly true of the Djallonke sheep and dwarf goats in West Africa. Knowledge of the resistance of small ruminants is still fragmentary and far more is known about resistance in cattle (FAO, 2006; OIE, 2013).

2.20.5 Integrated control

Drug prices and drug and pesticide resistance are on the increase, and there is the need to use all available methods that can be applied in each particular situation and are cost-effective. Combining different control methods against a parasitic disease is called integrated disease control or integrated disease management and is generally not intended to achieve eradication of the parasite in question (Nakayima, 2016). Such a cost effective combination of techniques, adapted to each particular set of circumstances, is very relevant for the control of African animal trypanosomosis. It cannot be sufficiently stressed that the cost benefit principle is an essential aspect of all methods. Apart from this crucial point, all of the available methods have advantages and disadvantages and the various techniques act in a complementary way; an advantage of one may offset a disadvantage of another (FAO, 2006). A combination of chemoprophylaxis against the disease and insecticidal application on the cattle against the vector may greatly improve the trypanosomosis situation (PATTEC, 2000). Where drug resistance is a problem, the accent will be put more on vector control and/or on the use of trypanotolerant livestock (FAO, 2006; OIE, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Kogi State, Nigeria (Fig 3.1). The State lies between latitude 7˚30`N and longitude 6˚42'E with a total land area of 29,833 km^2 and a population of about 3.5 million people (NPC, 2005). Kogi state share a common boundary with FCT and nine states, namely: Nasarawa and Niger to the north, Benue to the east, Ondo and Ekiti to the west, Kwara to the north west, Edo to the south west, Anambra to the south and Enugu to the south east (Ibitoye, 2006). The study covered six Local Government Areas (Adavi, Ankpa, Okene, Lokoja, Kabba/Bunu and Dekina) cutting across the three Senatorial Zones of Kogi State namely East, West and Central (Fig. 3.2). The sampling areas were selected based on their cattle population and across the three Senatorial zones.

3.2 Study Design

A cross sectional study and random sampling of cattle was carried out in the six randomly selected areas of the three senatorial zones in the State (East, Central and West) and made up of two Local Governments Areas randomly selected from each zone. From Kogi East, Ankpa and Dekina LGAs were selected, Adavi and Okene LGAs were selected from Kogi Central, Lokoja and Kabba/Bunu LGAs were selected from Kogi West senatorial district respectively (Fig. 3.2). Sampling of the cattle was carried out in settled Fulani herds as well as abattoirs. These were based on advice from the Director of Veterinary Services, Kogi State Ministry of Agriculture, Lokoja and availability of cattle in the State and also the Zonal Offices in the State. Samples from herds were collected from Adavi and Dekina LGAs and the abattoirs sample were collected from Okene, Lokoja, Ankpa, and Kabba LGAs (Table 3.1)

Table 3.1: Senatorial Zones and the Local Government Areas sampled in Kogi State

Cattle group sampled	LGA sampled	Senatorial Zones sampled
Settled cattle herds	Adavi	Central
	Ankpa	East
Abattoir	Okene	Central
	Lokoja	West
	Kabba	West
	Dekina	East



Figure 3.1: Map of Nigeria showing location of Kogi State, Source: Kogi State Ministry of Agriculture and Natural Resources, 2010.

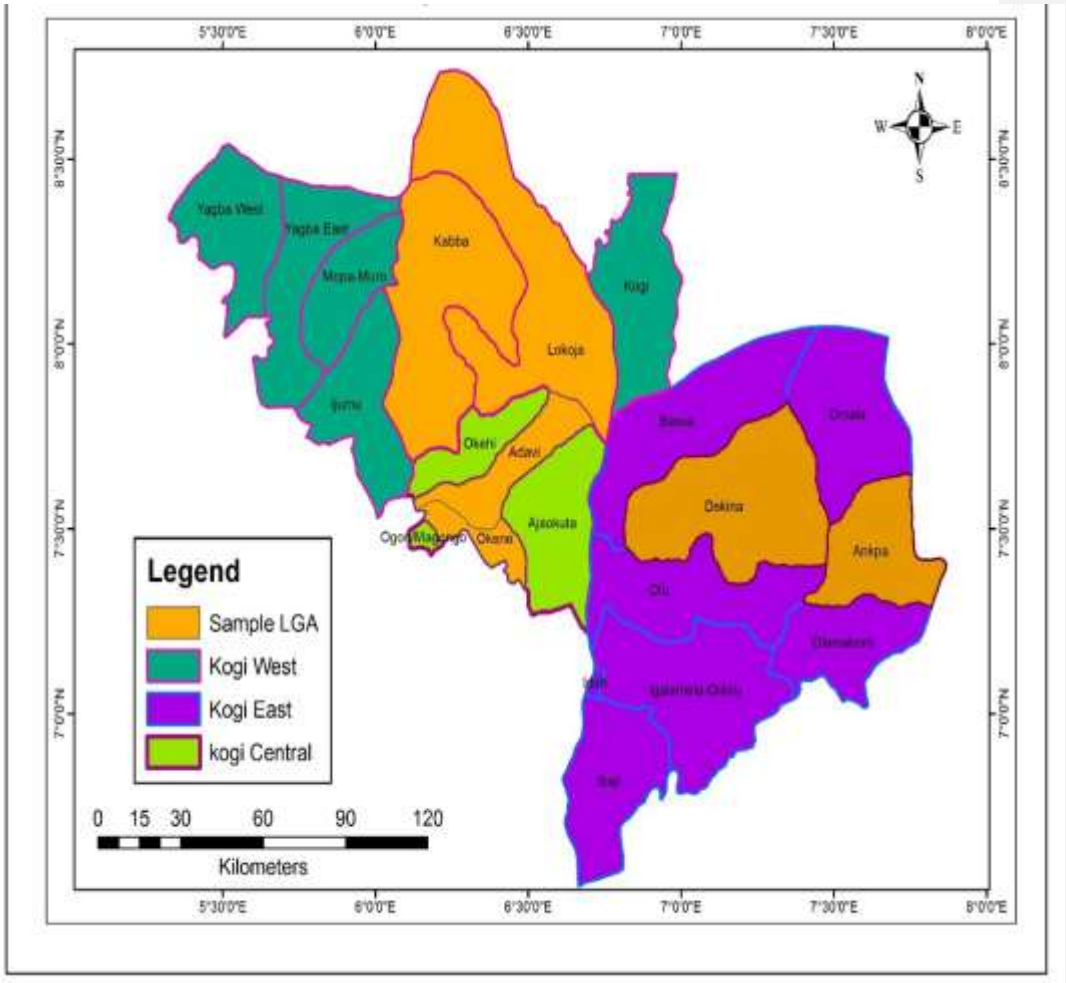


Figure 3.2: Map of Kogi State showing Local Government Areas and the sampling area, Source: Kogi State Ministry of Agriculture and Natural Resources, 2010.

3.3 Sample Size

The sample size was determined using the formula described by Thrusfield (2005), using 95% confidence interval, 50% as expected prevalence due to lack of information regarding the prevalence of the disease in the study area and 5% desired precision.

Using the formula $n = \frac{Z^2pq}{d^2}$

Where

n = sample size

p = prevalence (50%)

d = desired absolute precision (0.05)

Z = Standard normal deviate for 95% confidence level (1.96)

q = 1 – p (1- 0.5)

$$\frac{1.96^2 \times 0.5 \times (1- 0.5)}{0.05^2} \quad n = \frac{3.84 \times 0.5 \times 0.5}{0.0025}$$

$$\frac{0.96}{0.0025} = 384$$

Thus, a total of 384 heads of cattle were needed for the study, however to increase precision, samples size was raised to 410.

3.4 Sample Collection

A total of 410 blood samples were collected made up of 116 from cattle herds while 294 were collected from cattle at abattoirs from six Local Government Areas during the period May, 2016 to October, 2016. Prior to blood sampling of selected cattle, information on: age, sex, and body

condition score and also any clinical signs of trypanosomosis for each animal were recorded (Nicholson and Butterworth, 1986). Regarding age, the selected cattle were categorized into age groups of young (less than three years of age) and old (three years and above) (Canario *et al*., 2013; Friedrich *et al*., 2015). Aging was estimated using dentition (Canario *et al*., 2013; Friedrich *et al*., 2015). Body condition score was evaluated as described by Lawton and Ted, (2006), as poor, fair and good based on the appearance of ribs and dorsal spines as applied for Zebu cattle (Maigari *et al*., 2015). Two millilitres of blood sample was taken from the randomly selected cattle through the jugular venipuncture using 18G needles and transferred into a sample bottle containing EDTA as anticoagulant, properly labeled according to the locations (AD- Adavi, KB- Kabba, OK- Okene, DE- Dekina, AN- Ankpa and LK- Lokoja) and placed in ice-packs for further processing. Thick and thin blood smears were prepared and the rest of the samples were quickly transported to Sauki Medical Laboratory in Okene, Kogi State for analysis.

3.5 Sample Processing and Laboratory Procedure

The blood samples collected were processed for evaluation of PCV, absolute and differential leucocyte count, and to observe and identify the trypanosome. Thick and thin blood smears were prepared, air dried and properly labeled according to their locations and labeled using blue and green colours to distinguish them from others. The smears were air dried and wrapped with tissue paper until they were brought to the Protozoology Laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria for parasites identification. Evaluation of Packed Cell Volume (PCV) and the differential leucocyte count were carried out at the Sauki medical laboratory, Okene, using the remaining blood samples.

3.5.1 Determination of packed cell volume (PCV)

A haematocrit centrifugation technique was used to determine the PCV. A microhaematocrit tube was filled with blood to about ¾ and sealed with a sealer, centrifuged at 200G for 5 minutes. The PCV value was determined using haematocrit reader (Kemal, 2014).

3.5.2 Determination of absolute and differential leucocyte count

A small drop of blood was placed near an end of a microscope slide and spread at an angle of 30-40 degree with another slide as a spreader, air dried and fixed with 95% methyl alcohol for 3-5 minutes. The slide was then stained using Geimsa stain for 16-60 minutes and excess stain washed off using running water, then air dried. A drop of Canada balsam was placed on the smear and covered with cover slip, observed under the dry objective lens or oil immersion (×100) of the microscope and viewed for individual leucocyte (Kemal, 2014) and observed for WBC absolute and differential values.

3.5.3 Thick blood smears technique

This procedure was carried out at the Protozoology Laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. A drop of blood (10 µl) was placed at the middle of a clean microscope slide and spread over an area of approximately 2 cm in diameter, using the corner of another slide. The film waere air dried, and stained in a 10% Geimsa stain for 30 seconds, followed by washing with buffered water and then examined at ×100 oil immersion objective lens for trypanosomes (OIE, 2013; Norgan *et al*., 2013).

3.5.4 Thin blood smears technique

This procedure was carried out at the Protozoology Laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. Thin blood smears were made by placing a small drop of blood at one end (about 5 µl), and spread with the edge of another slide. The slide was placed at an angle of approximately 30° to the first slide and drawn back to make contact with the blood droplet. The blood was allowed to run along the edge of the spreader, which was then pushed to the other end of the slide in a fairly rapid but smooth motion. The slide was then dried quickly by waving in the air and thereafter fixed for 3 minutes in methanol, then stained for 15-30 minutes with Geimsa stain. Thereafter approximately 50–100 fields of the stained thin smear were examined, with ×100 oil immersion objective lens to obtain the morphological characteristic and hence identify the specific trypanosome species observed (OIE, 2013).

3.6 Data Analyses

All the data collected were entered into a Microsoft excel spread sheet program and then transferred into SPSS version 20.0. Level of Prevalence was analyzed by determining total positive cases out of the total number of animals sampled. Prevalence on the basis of location, herds and abattoir, sex, age, and body condition was compared using $\chi 2$ test (chi-square). Mean PCV in parasitaemic and aparasitaemic animals were compared using t- test. Significance t- test Level was set at 5% alpha and 95% confidence interval.

CHAPTER FOUR

RESULTS

4.1 Distribution of Sampled Cattle and Prevalence rate for Trypanosomosis in the Study Area

A total of 410 heads of cattle were sampled from the study area, made up of 116 cattle from settled Herds in Adavi and Ankpa LGAs and 294 cattle from Abattoirs in Lokoja, Okene, Kabba and Dekina LGAs. Of the 410 cattle sampled, 16 (3.9%) were positive for trypanosomes using thick blood smear, while 12 (2.9%) were positive via thin blood smear (Table 4.3). The overall prevalence rate for trypanosome for the study area was 3.9%.

Based on age, 242 cattle sampled were <3years old, with 80 from settled cattle Herds, 6 of which were positive, while 162 cattle were sampled from abattoir, 4 of which were positive for trypanosomes. Also, 168 cattle aged 3years and above were sampled, with 36 from settled cattle Herds 4 of which were positive for trypanosomes and 132 cattle from abattoir, 2 of which were positive (Table 4.6).

Based on sex, out of 410 cattle sampled, 212 were females, out of which, 86 were from settled cattle Herds, 7 of which were positive while 126 were from abattoir, with 2 of which were positive. Of the 198 males cattle sampled with 30 were from settled cattle Herds with 3 positive cases and 168 were from abattoir, with 2 positive cases (Table 4.5).

Based on body condition score, out of the 410 cattle sampled, out of 71 cases with 2.0 (poor) body condition score, of which, 40 were from settled cattle Herds, with 2 positive cases, while 31 came from abattoir with 3 positive cases. Out of 245 cattle with 3.0 (fair) body condition score, 32 were from settled cattle Herds, of which 7 were positive, while 213 were from abattoir with 2

positive cases. Among the 94 cattle with 4.0 (good) body condition score, 44 were from settled cattle Herds, with 1 positive cases while 50 were from abattoir with 1 positive case (Table 4.7).

4.2 Clinical Signs observed in Trypanosomes Infected Cattle in Kogi State, Nigeria

The clinical signs observed during the sampling were fever, lethargy, lacrymation (Plate 1), loss of appetite, pale mucus membrane, enlarged superficial lymph nodes and emaciation. These sign were seen mainly in cattle sampled from the settled Fulani herds (Table 4.1).

4.3 Mean Haematological Values of infected and uninfected Cattle with trypanosomes in Kogi State, Nigeria

The mean PCV value for parasitaemic cattle sampled was $30.18 \pm 1.73\%$ while that for aparasitaemic cattle sampled was $40.80 \pm 0.44\%$. There was significant difference ($p = 0.000$) in the PCV between the infected and non-infected sampled cattle (Table 4.2).

The mean haemoglobin concentration for the parasitaemic cattle was 10.06 ± 0.43g/µl while that of the aparasitaemic cattle was 13.61 ± 0.014g/µl. There was statistical difference ($p = 0.000$) between the parasitised and aparasitised sampled cattle (Table 4.2).

The mean WBC count for the parasitaemic cattle was $3.26 \pm 0.22(10^9/l)$ while that of the aparasitaemic cattle was $2.49 \pm 0.05(10^9/l)$. There was no statistical difference ($p < 0.05$) between the parasitised and aparasitised sampled cattle (Table 4.2).

The mean neutrophil count of the infected cattle was $1.10 \pm 2.56(10^9/l)$ and for the non-infected cattle was $0.84 \pm 0.54(10^9/l)$. There was no significant difference ($p < 0.05$) between the infected and non-infected cattle (Table 4.2).

The mean eosinophil count of the infected cattle was 0.39 $\pm$ 1.10 and for the non-infected cattle was 0.17 $\pm$ 0.26. The difference between the two values were statistically significant ($p = 0.000$) (Table 4.2).

The mean lymphocyte count of the infected cattle was 1.65 $\pm$ 0.53(10^9/l) and for the non-infected cattle was 1.32 $\pm$ 2.73(10^9/l). There was no significant difference ($p < 0.05$) between the infected and non-infected cattle (Table 4.2).

Table 4.1: Clinical signs observed in Trypanosomes infected cattle in Kogi State, Nigeria

Clinical signs	Parasitaemic cattle	Aparasitaemic cattle
Mean temperature(ºC)	42.5 ± 0.50	38.2 ± 0.15
Fever	+	-
Lethargy	+	-
Loss of appetite	+	+
Lacrymation	+	-
Pale mucus membrane	+	-
Enlarged superficial lymph nodes	+	+
Emaciation	+	-

+ PRESENT
- ABSENT

Table 4.2: Mean haematological values of uninfected and infected cattle with trypanosomosis in Kogi State, Nigeria

Haematological Indices evaluated	Positive (parasitaemic cattle) (Mean values)	Negative (aparasitaemic cattle) (Mean values)	p- value
PCV (%)	30.18 ± 1.73	40.80 ± 0.44	0.000*
Hb (g/µl)	10.06 ± 0.43	13.61 ± 0.14	0.000*
WBC (10^9/l)	3.26 ± 0.22	2.49 ± 0.05	0.53
Neutrophils (10^9/l)	1.10 ± 2.56	0.84 ± 0.54	0.223
Lymphocytes (10^9/l)	1.65 ± 0.53	1.32 ± 2.73	0.387
Eosinophils (10^9/l)	0.39 ± 1.10	0.17 ± 0.26	0.000*
Monocytes (10^9/l)	0.21 ± 0.75	0.16 ± 0.15	0.606

***** Statistically significant

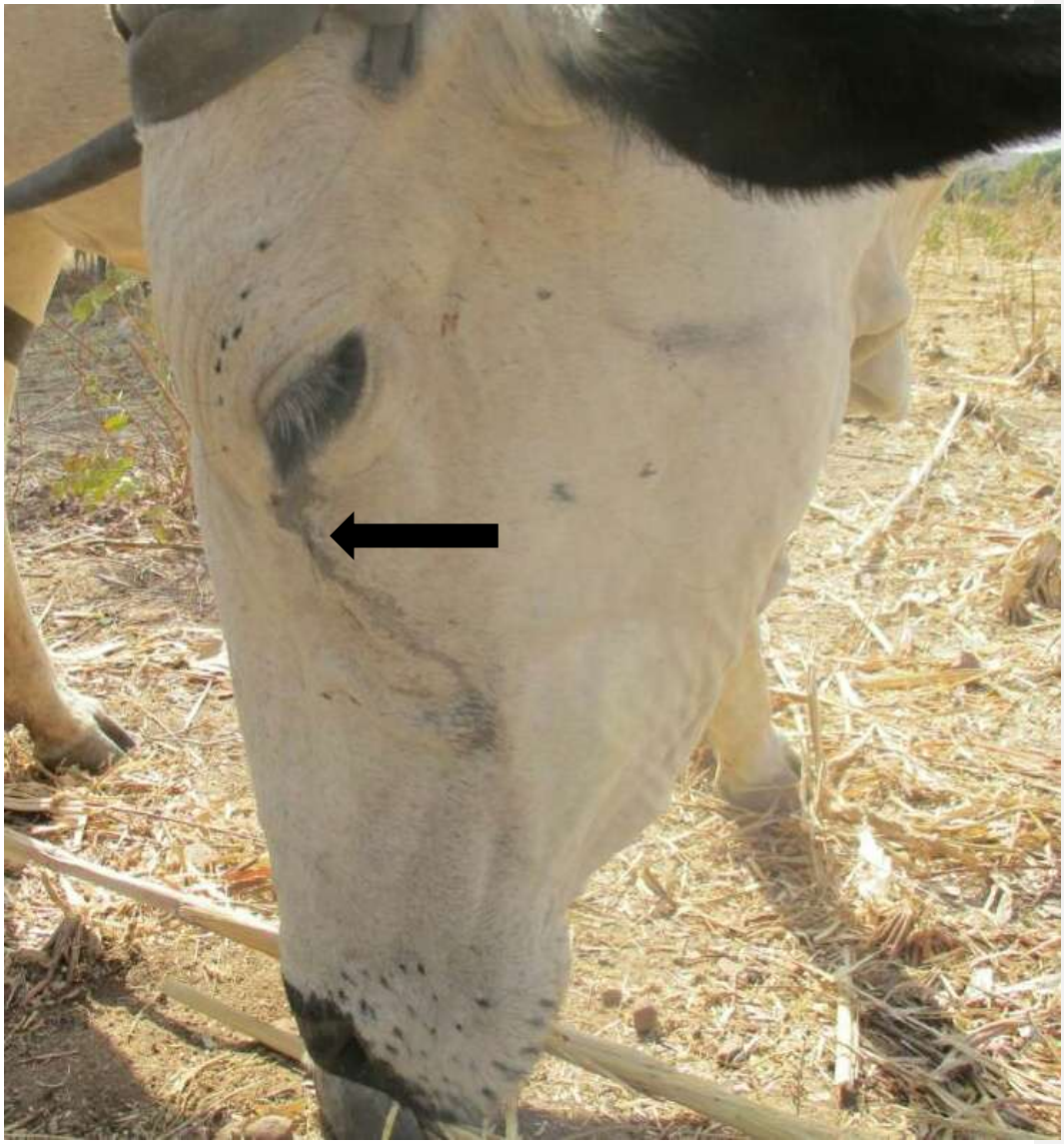


Plate I: Lacrymation from one of the sampled cattle

4.4 Species Identification

The trypanosome species identified in this study were *T. brucei* (Plate II) occurring in 10 samples as single infection, with a prevalence rate of 2.4%, while mix infection of *T. brucei* and *T. congolense* (Plate III) occurring in 2 samples with prevalence rate of 0.49%. The trypanosome species could not be identified in 4 other positive samples (Plate V) representing 0.98% of the total positive cases recorded.

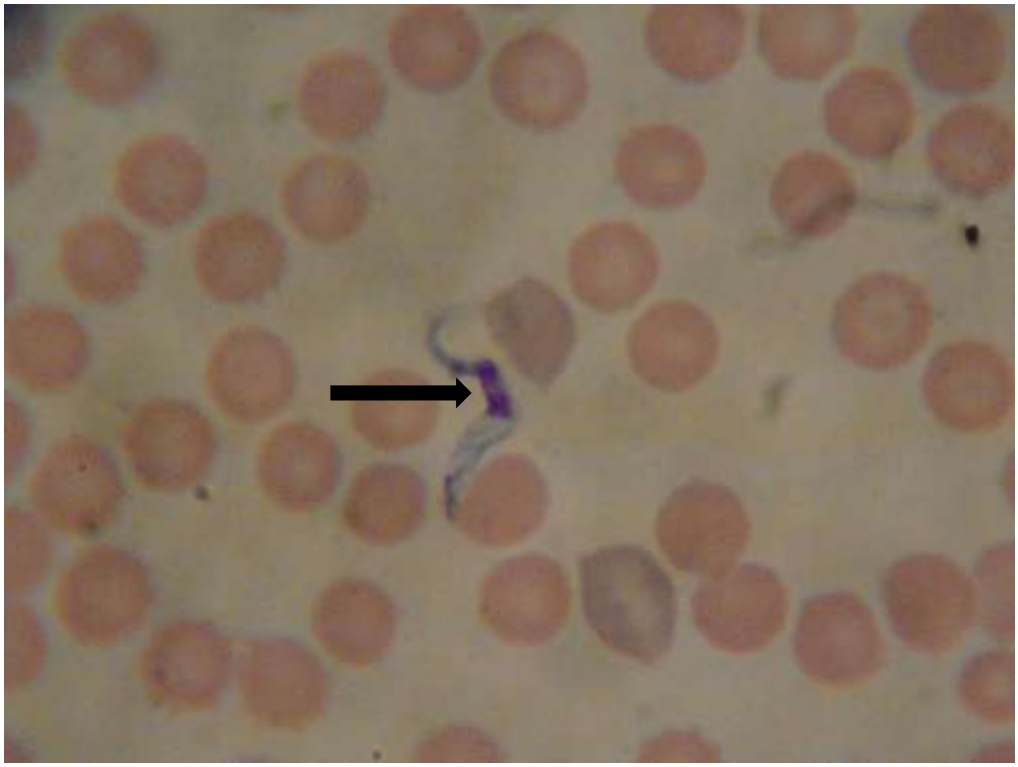


Plate II: Photomicrograph of *Trypanosoma brucei* parasite (arrowed) in the blood of an infected cattle (thin blood smear) sampled from Kogi State, Nigeria (x100).

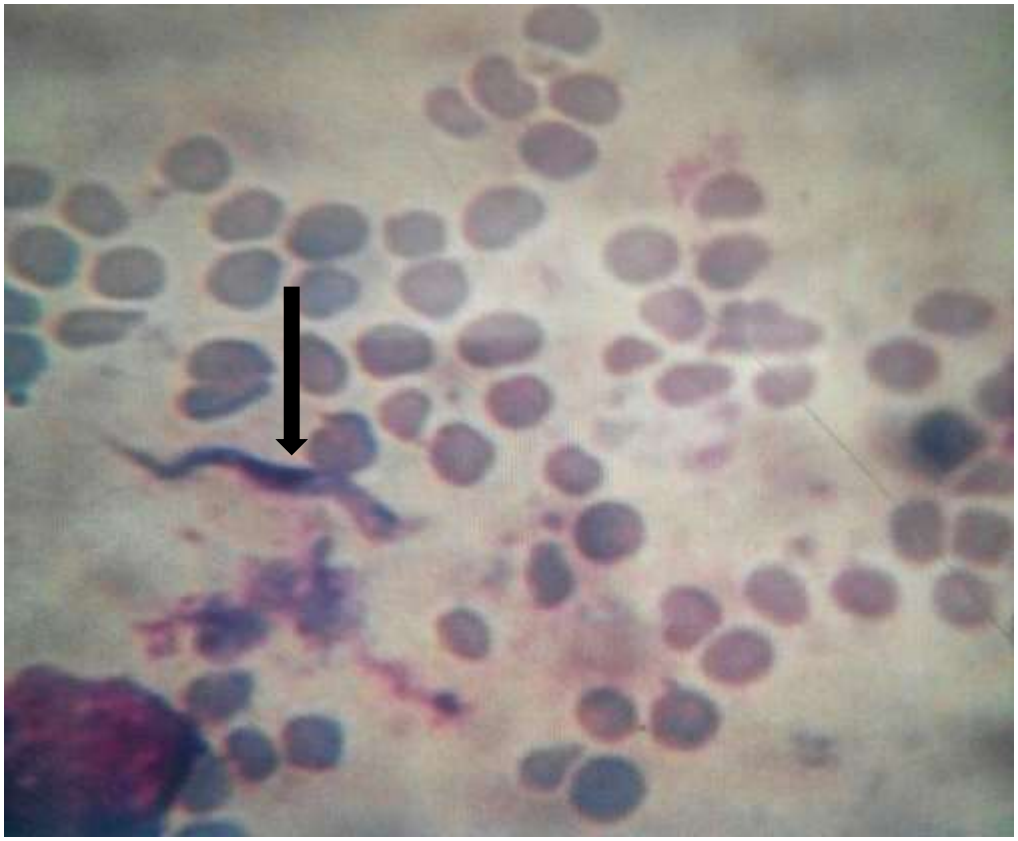


Plate III: Photomicrograph of *Trypanosoma congolense* parasite (arrowed) in the blood of an infected cattle (thin blood smear) sampled from Kogi State, Nigeria (x100).

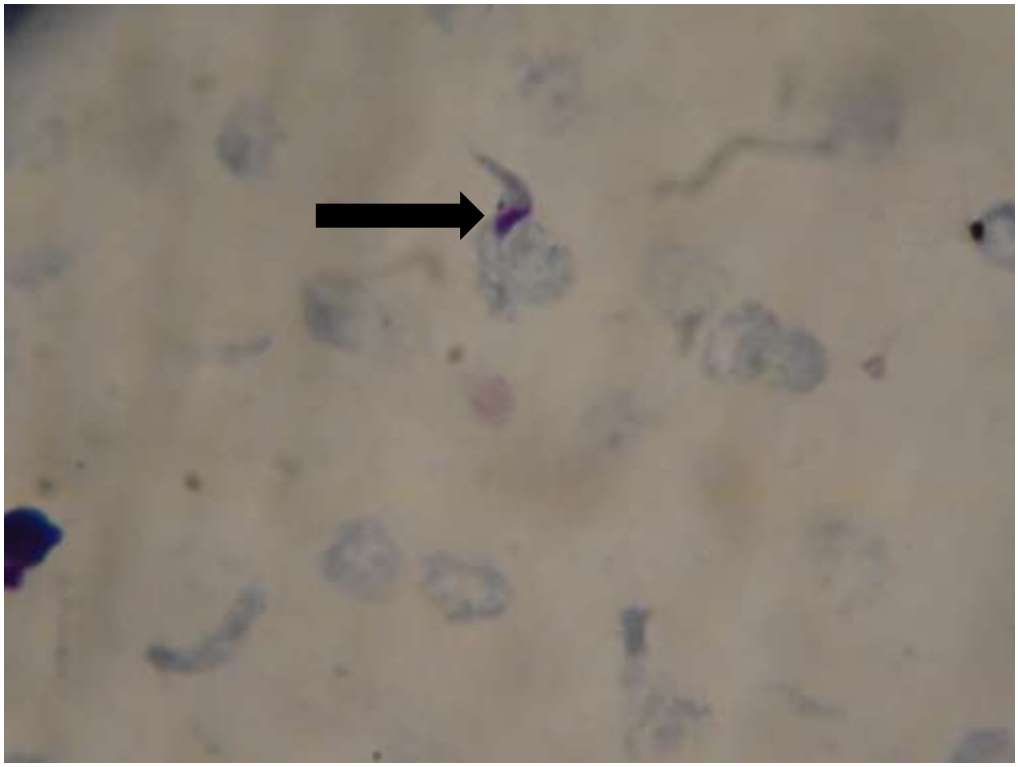


Plate VI: Photomicrograph of *Trypanosoma spp* parasite (arrowed) in the blood of an infected cattle (thick blood smear) sampled from Kogi State, Nigeria (x100).

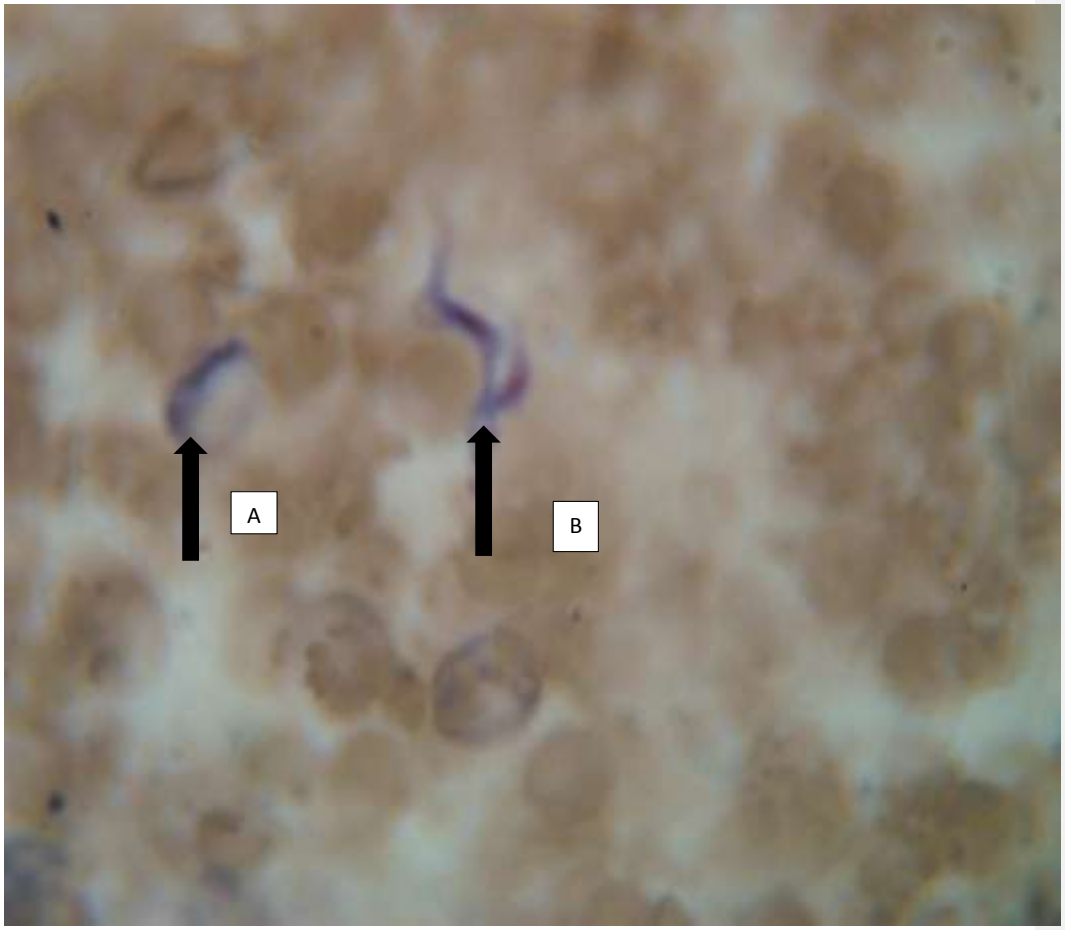


Plate V: Photomicrograph of mix infection of *Trypanosoma brucei* (A) and *Trypanosoma congolense* (B) parasite (arrowed) in the blood of an infected cattle (Thin blood smear) sampled from Kogi State, Nigeria (x100).

4.5 Prevalence of Trypanosomosis in Cattle Sampled in Settled Herds and Abattoirs in Kogi State, Nigeria

Of the116 samples collected from settled Fulani herds, 10 (8.6%) were positive for trypanosomes for both thick and thin blood smears and all positive cases were from Adavi LGA. From the 294 samples collected from abattoirs, 6 (2%) and 2 (0.7%) were positive for trypanosomes using thick and thin blood smears respectively (Table 4.3) and Okene LGA had 1 positive case, Lokoja LGA has 2 positive cases while Kabba with 3 positive cases. There was a significant difference ($p = 0.002$; $p = 0.000$) observed in the prevalence rate for trypanosomosis between herds and abattoir for both thick and thin blood smears.

Table 4.3: Prevalence of trypanosomosis in cattle sampled in settled herds and abattoirs using thick and thin blood smear techniques in Kogi State, Nigeria

Sources of Sampled cattle	Number of cattle sampled	Thick blood smear technique		Thin blood smear technique	
		Positive (%)	**Negative (%)**	**Positive (%)**	**Negative (%)**
Settled herds	116	10 (8.6)	106 (91.4%)	10 (8.6)	106 (91.4%)
Abattoir	294	6 (2%)	288 (98.0%)	2 (0.7%)	292 (99.3%)
Total	**410**	**16 (3.9)**	**394 (96.1)**	**12 (2.9)**	**398 (97.1)**

Thick Blood; $X^2 = 9.603$; df = 1; p = 0.002
Thin Blood; $X^2 = 15.650$; df = 1; p = 0.000

4.6 Prevalence of Trypanosomosis with Regards to Sampled Local Government Areas, Using Thick and Thin Blood Smear Techniques in Kogi State, Nigeria

Out of the 410 samples collected, 76 were collected from Okene, out of which 1(1.3%) was positive for trypanosomes, 90 samples were collected from Lokoja, with 2 (2.2%) being positive. From Adavi 50 samples were collected and 10 (20%) were positive, from Kabba, 72 samples were collected, out of which 3 (4.2%) were positive. From Ankpa and Dekina, 66 and 56 samples were collected respectively and none were positive for trypanosomes. Thus Adavi had the highest positive cases while Ankpa and Dekina recorded the lowest cases (0%), (Table 4.4). The differences were significant ($p < 0.05$) between the sampled locations.

4.7 Prevalence of Trypanosomosis Based on the Sex of the Cattle Sampled, Using Thick and Thin Blood Smear Techniques in Kogi State, Nigeria

A total of 212 female and 198 male cattle were sampled, out of which 9 (4.2%) and 7 (3.3%) of the females were positive for both thick and thin blood smears respectively, while 7 (3.5%) and 5 (2.5%) of the males were positive for thick and thin blood smear respectively (Table 4.5). There was no significant difference ($p = 0.711$; $p = 0.471$) between the sexes.

Table 4.4: Prevalence of trypanosomosis with regards to sampled Local Government Areas using thick and thin blood smear techniques in Kogi State, Nigeria.

Location (LGA)	Number of cattle sampled	Thick blood smear technique		Thin blood smear technique	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)
Okene	76	1 (1.3%)	75 (98.7%)	0 (0.0%)	76 (100%)
Lokoja	90	2 (2.2%)	88 (97.8%)	2 (2.2%)	88 (97.8%)
Kabba	72	3 (4.2%)	69 (95.8%)	0 (0.0%)	72 (100%)
Ankpa	66	0 (0.0%)	66 (100%)	0 (0.0%)	66 (100%)
Adavi	50	10 (20%)	40 (80%)	10 (20%)	40 (80%)
Dekina	56	0 (0.0%)	56 (100%)	0 (0.0%)	56 (100%)
Total	**410**	**16 (3.9)**	**394 (96.1)**	**12 (2.9)**	**398 (97.1)**

Thick Blood; $X^2 = 41.551$; df = 5; p = 0.000
Thin Blood; $X^2 = 53.593$; df = 5; p = 0.000

Table 4.5: Prevalence of trypanosomosis based on sex of the cattle using thick and thin blood smear techniques in Kogi State, Nigeria.

Sex	Number of cattle sampled	Thick blood smear technique		Thin blood smear technique	
		Positive (%)	**Negative (%)**	**Positive (%)**	**Negative (%)**
Female	212	9 (4.2)	203 (95.8)	7 (3.3)	205 (96.7)
Male	198	7 (3.5)	191 (96.5)	5 (2.5)	193 (97.5)
Total	**410**	**16 (3.9)**	**394 (96.1)**	**12 (2.9)**	**398 (97.1)**

Thick Blood; $X^2 = 0.138$; df = 1; p = 0.711
Thin Blood; $X^2 = 0.520$ df = 1; p = 0.471

4.8 Prevalence of Trypanosomosis based on Age of Cattle Sampled, Using Thick and Thin Blood Smear Techniques in Kogi State, Nigeria

All the sampled cattle were classified into two groups of three years and above, (old) and less than three years (young). 242 young cattle and 168 old cattle were sampled respectively. The young cattle showed prevalence rate of 10 (4.1%) each for thick and thin blood smears techniques, while adult cattle showed prevalence rate of 6 (3.6%) and 2 (2.1%) for thick and thin blood smear respectively (Table 4.6). There was no statistical difference ($p = 0.773$; $p = 0.57$) in the prevalence rate according to age (Table 4.6).

4.9 Prevalence of Trypanosomosis based on Body Condition Score of Cattle Sampled, Using Thick and Thin Blood Smear Techniques in Kogi State, Nigeria

Based on the body condition score of 2 (poor), 3 (fair) and 4 (good), the body condition 2 gave prevalence rate of 5 (7.0%) for both thick and thin smear, body condition 3 had prevalence rate of 9 (3.7%) and 7 (2.9%) for thick and thin blood smear respectively while body condition 4 showed prevalence rate of 2 (2.1%) and 0 (0.0%) for thick and thin blood smear respectively (Table 4.7). The differences in each and between the groups were not statistically significant ($p = 0.261$; $p = 0.86$).

Table 4.6: Prevalence of trypanosomosis based on age of the cattle sampled, using thick and thin blood smear techniques in Kogi State, Nigeria.

Age group	Number of cattle sampled	Thick blood smear technique		Thin blood smear technique	
		Positive (%)	**Negative (%)**	**Positive (%)**	**Negative (%)**
< 3years (young)	242	10 (4.1)	232 (95.9)	10 (4.1)	232 (95.9)
3 years and above (old)	168	6 (3.6)	162 (96.4)	2 (1.2)	166 (98.8)
Total	**410**	**16 (3.9)**	**394 (96.1)**	**12 (2.9)**	**398 (97.1)**

Thick Blood; $X^2 = 0.083$; df = 1; p = 0.773
Thin Blood; $X^2 = 3.635$; df = 1; p = 0.57

Table 4.7: Prevalence of trypanosomosis based on body condition score of cattle sampled, using thick and thin blood smear techniques in Kogi State, Nigeria

Body condition Score	Number of Cattle sampled	Thick blood smear technique		Thin blood smear technique	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)
2 (poor)	71	5 (7.0)	66 (93.0)	5 (7.0)	66 (93.0)
3 (fair)	245	9 (3.7)	236 (96.3)	7 (2.9)	238 (97.1)
4 (good)	94	2 (2.1)	92 (97.9)	0 (0.0)	94 (100)
Total	**410**	**16 (3.9)**	**394 (96.1)**	**12 (2.9)**	**398 (97.1)**

Thick Blood; $X^2 = 2.690$; df = 2; p = 0.261
Thin Blood; $X^2 = 4904$; df = 2; p = 0.86

CHAPTER FIVE

DISCUSSION

The study revealed an overall prevalence of 3.9% of bovine trypanosomosis. The finding though slightly higher but is similar to those of Abenga *et al*. (2004), Ohaeri (2010) and Enwezor *et al.* (2012) whereby prevalence rates of 2.2% in Lere, Kaduna State, 3.7% in Abia State and 3.8% in Benue State were reported respectively. However result of this work is lower than the findings of Adama *et al*. (2010), Andrew *et al*. (2014) and Fasanmi *et al*. (2014) who reported 6.3% prevalence rate in Niger State, 15.6% prevalence rate in Tudun Wada area of Kaduna State, 4.6% prevalence rate in Iddo LGA, Oyo respectively. These differences in prevalence may be attributed to the differences in geographical location (Savannah region) of the study area which is endemic for the vector causing trypanosomosis and also the sensitivity of the methods of analysis of the sampled materials in the various works.

The clinical signs such as lacrymation, lethargy, pale mucus membrane, emaciation, loss of appetite and intermittent fever was observed in the parasitaemic cattle which were similar to that reported by Andrew *et al*. (2014) in Tudun Wada area of Kaduna State and Hassan *et al*. (2016) in Lafia Abattoir, Nassarawa State.

The observed higher PCV in the non-infected compared to the infected cattle was similarly reported by Samdi *et al*. (2011), Sam-Wobo *et al*. (2010) and Andrew *et al*. (2014) in sampled cattle in Kaduna State, Ogun State and Tudun Wada area of Kaduna State respectively. The difference in mean PCV between parasitaemic animals and aparasitaemic animals indicated that trypanosomosis was adversely involved in lowering the PCV values (resulting from destruction of RBC) of infected animals.

The observed low haemoglobin concentration level of the infected cattle compared to the non-infected cattle followed the trend for the PCV values and is in agreement with the report of Abenga *et al*. (2002b) who observed a similar finding in experimentally infected sheep in Kaduna.

There was an increase in mean white blood cells count for the parasitaemic cattle which was due to neutrophilia and eosinophilia. The eosinophilia implied a response by the host immune system to the parasitic infection and this agrees with the finding of Karaye (2012) who reported eosinophilia in *T. brucei* and *T. congolense* infected Sokoto Red Bucks. The observed lymphocytosis in this study is in contrast to the finding of Adah *et al*. (1993) who reported lymphopenia as a result of excessive lymphoid tissue damage during the course of the infection in Sokoto Red Goats experimentally infected with *T. congolense*. These may be due to an active infection possibly due to virus, bacteria, fungus and protista affecting the sampled cattle.

The presence of *T. brucei* in the study area with the prevalence of 2.4%, agrees with the findings of Samdi *et al*. (2011) and Majekodumi *et al*. (2013), who also reported high prevalence of 21.4% in Kaduna Central Abattoir and 38.2% in Jos respectively. The occurrence of *T. brucei* and *T. congolense* as mixed infection with prevalence rate of 0.48%, was similarly reported by Samdi *et al*. (2011) but with a prevalence of 7.1% in Kaduna Central Abattoir. The fact that *T. brucei* in this study had the highest prevalence rate, though this is contrary to the findings of Fasanmi *et al*. (2014) who reported a higher prevalence rate for *T. congolense* infection in Iddo LGA, Oyo State, while Enwezor *et al*. (2012) reported a higher prevalence rate for *T. vivax* infection in cattle in Benue State. The differences in species may be due to geographical locations of the sampled areas.

The finding of a prevalence rate of 8.6% of *T. brucei* and *T. congolense* in herds in this study is higher than the finding of Fasanmi *et al*. (2014), who reported a prevalence rate of 4.6% in farms in Iddo LGA, Oyo State. This may be due to the management system being practiced by the farms which is semi-intensive, compared to that of the herds in this study which were extensively managed.

The prevalence rate of 2% for *T. brucei* and *T. congolense* in cattle sampled at the abattoir in this study was far lower than that reported by Abenga *et al*. (2002a) and Andrew *et al*. (2014) where they reported 3.9% prevalence rate in Kaduna Central Abattoir and 15.6% prevalence rate in Tudun Wada abattoir, Kaduna State respectively.

In This study, Adavi LGA had the highest prevalence rate of 20%, followed by Kabba LGA with 4.2%, Lokoja LGA and Okene LGA 2.2% and 1.3% respectively. There was a statistically significant between prevalence of trypanosomosis and the sampling locations in this study. This agrees with the findings of Ohaeri (2010) who reported a similar finding in Abia but contrary to that of Abenga *et al*. (2004) who reported no significant difference between prevalence of trypanosomosis and sampling locations in Kaduna State. The observed highest infection rate in Adavi LGA in this study can be attributed to presence of conditions suitable for the proliferation of the vector (*Glossina* species and the biting flies) in the area which could give rise to the high prevalence of trypanosomes in the area. Of interest in this study is the fact that Ankpa LGA and Dekina LGA recorded no infection.

The differences though insignificant in prevalence rate among sexes observed in this study agrees with the reports of Ohaeri (2010) and Samdi *et al*. (2011) who observed similar trends in the susceptibility of male and female cattle to trypanosomal infection in Abia and Kaduna States.

However Sam- Wobo *et al*. (2010) and Fasanmi *et al*. (2014) reported difference trends in the susceptibility between sexes, which were not statiscally significant. This shows that both sexes are more or less equally susceptible to the infection but higher prevalence rate found in female in this study may be due to their longer stay in the herds compared to their male counterpart which are sold off for slaughter, thereby staying shorter period in the herd.

The higher prevalence rate in the age group less than three years old and the observed non-significant difference in the prevalence of bovine trypanosomosis among the studied age groups is in contrast to the report by Abenga *et al*. (2004), Ohaeri (2010) and Zubairu *et al*. (2013) who found a higher prevalence among the older animals though not significant. The result of the present study may be due to the fact that the younger animals have not developed the necessary defence mechanism in response to trypanosomal infection.

The difference in prevalence rate of sampled cattle based on body condition scores though not significant was higher in animals with (2) poor body condition score (7%). This finding is similar to that reported by Abenga *et al.* (2002a) who observed a prevalence rate of trypanosomosis (7.6%) among severely emaciated cattle and also Samdi *et al*. (2011) who also reported a high prevalence rate of trypanosomosis in emaciated cattle. This may be attributed to the fact that trypanosomosis is a debilitating disease causing emaciation and resulting in immunosuppression thereby causing the affected animals to easily succumb to infection.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. The overall prevalence rate of bovine trypanosomosis in Kogi State based on this study was 3.9%.
2. Clinical signs such as lacrymation, lethargy, pale mucus membrane, emaciation, loss of appetite and intermittent fever was observed were similar to those reported for experimental infections.
3. From this study 2.4% of *T. brucei* and 0.49% of *T. congolense* were identified as the species of trypanosome affecting cattle in the study area
4. There was no significant differences in relation to Age, sex, body condition and occurrence of trypanosomosis in the study area.
5. The study has shown that trypanosomosis do exist in Kogi State and this poses a potential threat to cattle productivity, hence requiring urgent integrated parasite and vector control to safeguard cattle production in the study area.

6.2 Recommendations

1. There is need to employ other sensitive methods like PCR and ELISA to determine the true picture of trypanosomosis in Kogi State.
2. There is need for further studies to know if trypanosomes do exist in the other unsampled LGAs of the state to get an overall prevalence level for Kogi State.
3. There is need to enlighten the cattle owners and herdsmen about the effect of the disease on their animals and its economic significance.

4. There is need for Kogi State in collaboration with Federal Government to intensify tsetse control to rid the state of this vector and other biting flies.

6.3 Contribution to Knowledge

1. Trypanosomosis exist in the study at prevalence level of 3.9% in cattle.
2. *T. vivax* which is conventionally believe to be present where trypanosome is found is consipicuously absent in this study.
3. *Trypanosoma brucei* and *T. congolense* are the main pathogenic species found in cattle in the study area.
4. Some cattle (0.49%) had mixed infection of *T. brucei* and *T. congolense* which boils down to potential serious form of the disease and drastic effect on the performance of the affected cattle

6.4 Limitation of the Study

1. Lack of accessible road limit our access to cattle because most herdsmen lives deep in villages.
2. Getting blood samples was challenging because most cattle owners are uneducated.
3. Incessant fight between herdsmen and farmers also limit our access to the cattle.
4. ELISA and PCR kits were expensive this limit our access to this method of determing the true picture prevalence of trypanosomosis.

REFERENCES

Abenga, J. N. (2014). Review: A comparative pathology of *Trypanosoma brucei* infections. *Global Advanced Research Journal of Medicine and Medical Science*, 3(12): 390-399.

Abenga, J. N. and Anosa, V. O. (2006). Clinical studies on experimental Gambian trypanosomosis in vervet monkeys, *Veternarski Arhiv*, 76(1): 11-18, ISSN 0372-5480.

Abenga, J. N., David, K. M., Ezebuiro, C. O. G. and Lawani, F. A. G. (2005). Observation on the tolerance of young dogs to infection with *Trypanosoma congolense. African Journal of Clinical Experimental Microbiology*, 6(1): 28 – 33.

Abenga, J. N., Enwezor, F. N. C., Lawani, F. A. G., Osue, H. O. and Ikemereh, E. C. (2004). Trypanosome prevalence in cattle in Lere area of Kaduna State, North Eastern Nigeria. *Journal of Veterinary Research*, 57 (1-2): 45-48.

Abenga, J. N., Fajinmi, A., Idowu, T., Kalgo, A., Lawani, F. A. G. and Samdi, S. (2008). Seasonal variation of Trypanosomosis rates in small ruminants at Kaduna abattoir, Nigeria. *African Journal of Biomedical Research*, 11(2): 229 -232.

Abenga, J. N., Sanda, S. A., Idowu, T. B. and Lawani, F. A. G. (2002b). Effect of acute caprine trypanosomosis on haemoglobin, urea and serum electrolytes. *African Journal of Clinical and Experimental Microbiology*, 3(1): 45-47.

Adah, M. I., Otesile, E. B. and Joshua, R. A. (1993). Susceptibility of Nigerian West Africa Dwarf and Sokoto Red Goats to a strain of *Trypanosoma congolense*. *Veterinary Parasitology*, 47 (3-4): 177-188.

Adama, J. Y., Usman, A., Maigida, R. and Adeyemi, R. A. (2010). Incidence of trypanosome among White Fulani and Sokoto Gudali breeds of Cattle in Niger state, Nigeria. *Sokoto Journal of Veterinary Sciences*, 8(1): 22 – 25.

Adamu, S., Fatihu, M. Y., Useh, N. M., Mamman, M., Sekoni, V. O. and Esievo, K. A. N. (2007). Sequential testicular and epididymal damage in Zebu bulls experimentally infected with *Trypanosoma vivax*. *Veterinary Parasitology*, 143(1): 29-34.

Agu, W. E. and Amadi, I. N. (2001). Trypanosomosis of small ruminants (Sheep and Goats) in Abakaliki area of Ebonyi State, Nigeria. *Tropical Veterinarian*, 19(2): 1−8.

Agu, W. E., Kalejaiye, J. O. and Olatunde, A. O. (1990). Prevalence of bovine trypanosomosis in some parts of Kaduna and Plateau State, Nigeria. *Bulletin of Animal Health and Production in Africa*, 37(2): 161-166.

Ahmed, M. I. and Agbede, R. I. S. (1993). A three-year retrospective study of the prevalence of trypanosomes infection in cattle, sheep and goats in Zaria, Northern Nigeria. *OAU/STRC Publication*. 116, Nairobi, Kenya.

Aksoy, S., Buscher, P., Lehane, M., Solano, P. and Van Den Abbeele, J. (2017). Human African trypanosomiasis control: Achievements and challenges. *PLoS Neglected Tropical Diseases* 11(4): e0005454.

Amanuel, D., Tadele, A., Kassahun, B., Tilahun, Z., Girma, K. and Tadele, K. (2015). Prevalence of Bovine Trypanosomosis, and it's Associated Risk Factors in Abaya District, Borena Zone, Ethiopia. *Nature and Science*, 13(10): 64-70.

Ameen, S. A., Joshua, R. A., Adedeji, O. S., Raheem, A. K., Akingbade, A. A. and Leigh, O. O. (2008). Preliminary studies on prevalence of Ruminant trypanosomosis in Ogbomoso area of Oyo State, Nigeria. *Middle East Journal of Scientific Research*, 3 (4): 214−218.

Anderson, N. E., Mubanga, J., Fevre, E. M., Picozzi, K., Eisler, M. C. and Thomas, R. (2011). Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia', PLoS *Neglected Tropical Diseases 5*.

Andrew, T., Ibrahim, J. L., Maikai, B. V., Baraya, K., Daniel, J., Timothy, T., Kingo, J. L. and Ogbole, M. (2014). Prevalence of *Trypanosoma species* found in cattle slaughtered in Tudun Wada abattoir, Kaduna, Nigeria. *Nigeria Journal of Biotechnology*, 28: 60 – 64.

Anosa, V. O. (1988). Haematological and biochemical changes in human and animal trypanosomosis, *Revue d' Elevage et de Medicine Ve'te'rinaire des Pays Tropicaux* 41(2): 65-78. ISSN 151-164.

Aslam, A., Chaudhary, Z.I., Rehman, H. Ashraf, K., Ahmad, N., Yaqub, T., Maqbool, A and Shakoori (2010). Comparative Evaluation of Parasitological, serological and DNA Amplification Methods for Diagnosis of Natural Trypanosomal Infection in Equines. *Pakistani Journal of Zoology*, 42(4):371-376.

Barry, J. D. and McCulloch, R. (2001). Antigenic variation in trypanosomes: enhanced phenotypic variation in a eukaryotic parasite. *Advance Parasitology*, 49: 2–70.

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

Bawa, E. K., Sekoni. V. O., Olorunju. S. A. S., Uza, D. V., Ogwu, D. and Oyedipe, E. O. (2005). Comparative clinical observations on *Trypanosoma vivax* infected pregnant Yankasa and West African Dwarf ewes. *Journal of Animal and Veterinary Advances*, 4(7): 630-636.

Bayisa, K., Getachew, D. and Tadele, T. (2015). Bovine Trypanosomosis in Asossa District, Benishangul Gumuz Regional State, Western Ethiopia; prevalence and associated risk factors. *European Journal of Applied Sciences*, 7(4): 171-175.

Baylis, M. and Nambiro, C. O. (1993). The effect of cattle infection by *Trypanosoma congolense* on the attraction and feeding success of the tsetse fly *Glossina pallidipes*. *Parasitology Today*, 106: 357-361.

Bengaly, Z., Sidibe, I., Ganaba, R., Desquesnes, M., Boly, H. and Sawadogo, L. (2002). Comparative pathogenicity of three genetically distinct types of *Trypanosoma congolense* in cattle: clinical observations and haematological changes. *Veterinary Parasitology*, 108: 1–19.

Beschin, A., Abbeele, J. V. D., Baetselier, P. D. and Pays, E. (2014). African trypanosome control in the insect vector and mammalian host. *Trends in Parasitology*, 30(11): 538-547.

Bezie, M. G., Dagnachew, M., Tadesse, S. and Tadesse, D. (2014). African Trypanosomes: Virulence Factors, Pathogenicity and Host Responses. *Journal of Veterinary Advances*, 4: 732-745.

Bitew, M., Amide, Y., Zenebe, T. and Degefu, H. (2011). Trypanosomes infection Rate in *G. pallidipes* and *G. fuscipes* in Gojeb Valley, Southwest Ethiopia. *Global Veterinaria*, 6(2): 131-135.

Black, S. J. and Seed, J. R. (2002). *The African Trypanosomes*. Kluwer Academic Publishers, London, United Kingdom.

Bourn, D., Grant, I., Shaw, A. and Torr, S. (2005). Cheap and safe tsetse control for livestock production and mixed farming in Africa, *Aspects of Applied Biology*, 75: 50-54.

Brian, L. W., Geoffrey, M. A., Roshen, P., Jingwen, W. and Serap, A. (2007). Novel strategies targeting pathogen transmission reduction in insect vectors: Tsetse transmitted trypanosomiasis control. *Entomological Research*, 37: 231-237.

Brun, R., Blum, J., Chappuis, F. and Burri, C. (2010). Human African Trypanosomiasis. *The Lancet*, 375: 148-159.

Canario, L., Mignon-Grasteau, S., Dupont-Nivet, M. and Phocas, F. (2013). "Genetics of behavioural adaptation of livestock to farming conditions". *Animal*, 7(3): 357–377.

Cecchi, G., Mattioli, R. C., Slingenbergh, J. and Dela Rocque, S. (2008). Land cover and tsetse distribution in Sub- Saharan Africa. *Medical and Veterinary Entomology*, 22: 364-373.

Center for Food Security and Public Health (CFSPH), (2015). *Diseases brief; African Animal Trypanosomiasis*, Iowa State University.

Chanie, M., Dulecha, A., and Basaznew, B. (2013). Socio-Economic Assessment of the Impacts of Trypanosomiasis on Cattle in Girja District, Southern Oromia Region, Southern Ethiopia. *Acta Parasitologica Globalis* 4 (3): 80-85.

Chitanga, S., Marcotty, T., Namangala, B., Van Den Bossche, P., Van Den Abbeele, J. and Al, E. (2011). High Prevalence of Drug Resistance in Animal Trypanosomes without a history of Drug Exposure. PLoS *Neglected Tropical Diseases*, 5: 21-27.

Cnops, J., De Trez, C., Bulte, D., Radwanska, M., Ryffel, B. and Magez, S. (2015a). IFNγ mediates B cell loss in experimental African trypanosomosis. *Parasite Immunology*, in press.

Cnops, J., Magez, S. and De Trez, C. (2015b). Escape mechanisms of African trypanosomes: why trypanosomosis is keeping us awake. *Parasitology*, 142: 417– 427.

Comini, M. A., Medeiros, A. M. and Manta, B. (2011). Stress response in the infective stage of *Trypanosoma brucei*. In: *Stress response in microbiology*. Ed. José M. Requena. Horizon Scientific Press, Norwich, UK. In press.

Companion Animal Parasite Council (CAPC), (2014). *Current advice on parasite control: vector-borne diseases-Trypanosomiasis.Species*: *Trypanosoma cruzi*. Salem: Companion Animal Parasite Council; 2014. [Online] Available from: http://www.capcvet.org/capc-recommendations/trypanosomias is [Accessed on 9th June, 2013].

Coustou, V., Guegan, F., Plazolles, N. and Baltz, T. (2010). Complete *in vitro* life cycle of *Trypanosoma congolense*: development of genetic tools. *PLoS Neglected Tropical Diseases*, 4: e618.

Croft, A. M., Jackson, C. J., Friend, H. M. and Minton, E. J. (2006). African trypanosomiasis in a British soldier. *Journal of the Royal Army Medical Corps*. 152(3): 156-160.

Dabo, N. T. and Maigari A. K. (2017). Soft Options for Effective Diagnosis of African Animal Trypanosomiasis: A Review. *International Journal of Medical Evaluation and Physical Report*, 2(2):1-9.

Delespaux, V., Ayral, F., Geysen D. and Geerts S. (2003). PCR-RFLP using Ssu-rDNAamplification: applicability for the diagnosis of mixed infections with different trypanosome species in cattle. *Veterinary Parasitology*, 117: 185–193.

Desquesnes M. (2004). *Livestock Trypanosomoses and their Vectors in Latin America*. OIE and CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement). World Organisation for Animal Health (OIE), Paris, France, 192, ISBN 92-9044-634-X.

Desquesnes M., McLaughlin G., Zoungrana A. and Davila A. M. R. (2001). Detection and identification of *Trypanosoma* of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. *International Journal of Parasitol*ogy, 31: 610–614.

Desquesnes, M. (1997). Standardisation internationale etrégionale des épreuves immuno-enzymatiques: méthode, intérêts et limites. *Rev. Sci. Tech. Office Internationale des Epizootic*, 16: 809–823.

Desquesnes, M. and Davila A. M. R. (2002). Applications of PCR-based tools for detection and identification of animal trypanosomes; a review and perspectives. *Veterinary Parasitology,* 109: 213–231.

Desquesnes, M. and Dia, M.L. (2003). *Trypanosoma vivax*: Mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis*, *Experimental Parasitology*, 103: 35–43.

Desquesnes, M., Ravel, S., Deschamps, J. Y., Polack, B. and Roux, F. (2012). Atypical hyperpachymorph *Trypanosoma (Nannomonas) congolense* forest type in a dog returning from Senegal. *Parasite*, 19: 239–247.

Dia, M. L. and Desquesnes, M. (2005). *Rational use of trypanocides*. Animal health in West Africa.

Donelson, J.E. (2003). Antigenic variation and the African trypanosome genome. *Acta Tropica*, 85: 391-404.

Dransfield, R. D. and Brightwell, R. (2004). Community participation in Tsetse control: the principles, potential and practice. *In The Trypanosomiasis*. Edited by Maudlin I, *et al*. Wallingford, UK: CAB International Publishing, pp. 533–546.

Eisler, M. C., Lessard, P., Masake, R. A., Moloo, S. K. and Peregrine, A. S. (1998). Sensitivity and specificity of antigen-capture ELISAs for diagnosis of *Trypanosoma congolense* and *Trypanosoma vivax* infections in Cattle. *Veterinary Parasitology*, 79(3): 187–201.

Emanuel, S. S. and Jackson, E. K. (2012). A parasitological survey for bovine trypanosomosis in the livestock/wildlife ecozone of Northern Tanzania. *Veterinary World*, 5(8): 459-464.

Enwezor, F. N. C., Samdi, S. M., Ijabor, O. and Abenga, J. N. (2012). The prevalence of bovine trypanosomes in parts of Benue state, North Central, Nigeria. *Journal of Vector Borne Diseases*, 49: 188-190.

Ezeani, M. C., Okoro H., Anosa V.O, Onyenekwe C. C., Meludu S. C., Dioka C. E. and Azikiwe, C. C. (2008). Immunodiagnosis of bovine trypanosomiasis in Anambra andImo states, Nigeria, using enzyme-linked immunosorbent assay: Zoonotic implications to human health. *Journal Vector Borne Dis*eaes, 45:292-300

Fasanmi, O. G., Okoroafor, U. P., Nwufoh, O. C., Bukola, O. M. and Ajibola, E. S. (2014). Survey for *Trypanosoma species* in Cattle from three farms in Iddo Local Government Area, Oyo state. *Sokoto Journal of Veterinary Sciences*, 12(1): 57-61.

Food and Agricultural Organization (FAO), (2002). Food, Agriculture and food Security: The Global Dimension, *WFS02/Tech/ Advance Unedited Version*. FAO. Rome. Pp, 19-28.

Food and Agricultural Organization (FAO), (1998). *Drug management and parasite resistance in bovine Trypanosomiasis in Africa*, Rome, Italy.

Food and Agricultural Organization (FAO), (2006). *A field guide for diagnosis, treatment and prevention of African Animal Trypanosomosis*. Corporate document repository. Food and Agriculture Organization of the United Nations.

Food and Agricultural Organization (FAO), (2014). *Clinical signs*. Italy: Food and Agriculture Organization. [Online] Available from: www.fao.org/ag/againfo/programmes.

Francçois, C., Louis, L., Pere, S., Veerle, L. and Philippe, B. (2005). Options for Field Diagnosis of Human African Trypanosomosis. *American Society of Microbiologist*, 18(1): 133-146.

Friedrich, J., Brand, B. and Schwerin, M. (2015). "Genetics of cattle temperament and its impact on livestock production and breeding - A Review" *(PDF). Archiv Fur Tierzucht-Archives of Animal Breeding, 58: 13–21*.

Gadelha, C., Holden, J. M., Allison, H. C., and Field, M. C. (2011).Specializations in a successful parasite: what makes the bloodstream-form African trypanosome so deadly? *Molecular Biochemistry and Parasitology*, 179: 51–58.

Gamechu, F., Aynalem, M., Birhanu, H., Gemechu, C. and Gezahegn, A. (2015). Epidemiological Status and Vector Identification of Bovine Trypanosomiosis in didesa District of Oromia Regional State, Ethiopia. *International Journal of Nutrition and Food Sciences*, 4 (3): 373-380.

Geerts, S., Holmes, P. H., Diall, O. and Eisler, M. C. (2001). African bovine trypanosomiasis: the problem of drug resistance. *Trends in Parasitology*, 17: 25-28.

Gemeda, F. (2015). Prevalence of Bovine Trypanosomosis in and around Nekemte Areas, East Wollega Zone, Ethiopia. *Open Access Library Journal*, 2: e1521. http://dx.doi.org/10.4236/oalib.1101521.

Getachew, A. (2005). Trypanosomosis in Ethiopia, Addis Ababa University, Faculty of Veterinary Medicine. Debre Zeit, *Ethiopian Journal of Biological Sciences*, 4(1): 75-121.

Geysen, D., Delespaux, V. and Geerts, S. (2003). PCR-RFLP using Ssu-Rdna amplification as an easy method for species-specific diagnosis of *Trypanosoma species* in Cattle. *Veterinary Parasitology*, 110: 171–180.

Gonzatti, M. I., González-Baradat, B., Aso, P. M. and Reyna-Bello, A. (2014). *Trypanosoma (Duttonella) vivax* and Typanosomosis in Latin America: secadera/huequera/cacho hueco. In: Magez, S., Radwanska, M. (Eds.), *Trypanosomes and Trypanosomiasis*. *Springer*, 261–285.

Goto, Y., duthie, M. S., Nguyen, T. T., Asada, M., Kawazu, S., Carter, D. and Inoue, N. (2011). Serological characterizations of tandem repeat proteins for detection of African trypanosome infection in Cattle. *Parasitology International*, 60: 538–*540*.

Greiner, M., Kumar, S. and Kyeswa, C. (1997). Evaluation and comparison of antibody ELISAs for serodiagnosis of bovine trypanosomosis. *Veterinary Parasitology*, 73: 197–205.

Hamilton, P. B., Gibson, W. C. and Stevens, J. R. (2007). Patterns of co-evolution between trypanosomes and their hosts deduced from ribosomal RNA and protein-coding gene phylogenies. *Molecular and Phylogenetics Evolution*, 44(1): 15–25.

Hanotte, O., Bradley, D.G., Ochieng, J.W., Verjee, Y., Hill, E.W. and Rege, J.E. (2002). African pastoralism: genetic imprints of origins and migrations. *Science*, 296: 336-339.

Hargrove, J. W., Ouifki, R., Kajunguri, D., Vale, G. A. and Torr, S. J. (2012). Modeling the control of trypanosomiasis using trypanocides or insecticide-treated livestock. PLoS *Neglected Tropical Diseases*, 6(5): e1615. DOI: 10:1371/journal.pntd.0001615.

Hassan, D. I., Musa, S. I., Yusuf, N. D. and Adua, M. M. (2016). Prevalence of trypanosomosis in Lafia Abattoir, Lafia, Nassarawa State. *Production and Agriculture Technology*, 12(1): 204-210.

Holmes, P. H., Eisler, M. C. and Geerts, S. (2004). Current chemotherapy of animal trypanosomiasis. In The *Trypanosomiases*. In: Maudlin, I., Holmes, P. H. and Miles, M. A. (eds.). Wallingford, UK: CABI International.

Hopkins, J. S., Chitambo, H., Machila, N., Luckins, A. G., Rae, P. F., Van Den Bossche, P. and Eisler, M. C. (1998). Adaptation and validation of the antibody trapping ELISA using dried blood spots on filter paper, for epidemiological surveys of tsetse transmitted trypanosomosis in cattle. *Preventive Veterinary Medicine*, 37: 91–99.

Horn, D. (2014). Antigenic variation in African trypanosomes. *Molecular Biochemistry and Parasitology*. 195: 123–129.

Hunt, R. C. (2010). Molecular parasitology: trypanosomes eucaryotic cells with a different way of doing things. *Microbiology and Immunology*. Online.

Hutchinson, R. and Gibson, W. (2015). Rediscovery of *Trypanosoma suis*, a tsetse transmitted trypanosome closely related to *T. brucei*. *Infection, Genetics and Evolution*, 36: 381 – 388.

Ibitoye, S. J. (2006). The Impact of Cooperative Societies on Rural Development in Ankpa LGA of Kogi State, Nigeria. *National Association of Science, Humanities and Education Research Journal* (NASHERJ), 4 (2): 193-198.

Igbokwe, I. O. (1994). Mechanisms of cellular injury in African trypanosomiasis, *Veterinary Bulletin*, 64(7): 611-620. ISSN 1684-5315.

Ikede, B. O. (1986). African Trypanosomes. In: Honigberg, B.M., Ed., *Mechanisms of Pathogenicity among Protozoa*, Bailiere Tindall, London, 363-378.

Ilemobade, A. A. (2009). 'Tsetse and trypanosomosis in Africa: The challenges, the opportunities', *Onderstepoort Journal of Veterinary Research*, 76: 35–40.

International Atomic Energy Agency (IAEA) (1993*). Improving the Diagnosis and Control of Trypanosomiasis and Other Vector-borne Diseases of African Livestock Using Immunoassay Methods*. IAEA-TECDOC 707, IAEA, Vienna, Austria.

International Atomic Energy Agency (IAEA) (2007). *Developing Methodologies for the Use of PCR in the Diagnosis and Monitoring of Trypanosomosis*. IAEA-TECDOC–1559. IAEA,Vienna, Austria.

Jacobs, R. T., Nare, B. and Phillips, M. A. (2011). State of the art in African trypanosome drug discovery. *Current Topical Medicinal Chemistry*, 11(10): 1255–1274.

Jamonneau, V., Camara, O., Ilboudo, H., Peylhard, M., Koffi, M., Sakande, H., N'Dri, L., Sanou, D., Dama, E. and Camara, M. (2015). Accuracy of individual rapid tests for serodiagnosis of gambiense sleeping sickness in West Africa. *PLoS Neglected Tropical Diseases* 9, e0003480

Jha, B. A., Gazestani, V. H., Yip, C. W. and Salavati, R. (2015). The DRBD13 RNA binding protein is involvedin the insect-stage differentiation process of *Trypanosoma brucei*. *Science Direct* FEBS Letters 589, 1966–1974

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

Kabasa, J. D. (2007). Public-private partnership works to stamp out sleeping sickness in Uganda. *Trends in Parasitology*, 23 **(**5):191-192.

Kalu, A. U. and Lawani, F. A. (1996). Observations on the epidemiology of ruminant trypanosomosis in Kano State, Nigeria. *Reveu d'Elevage de Medicine Veterinare des Pays Tropicaux*, 49 (3): 213−217.

Kalu, A.U., Uzoukwu, M. and Ikeme, M.M. (1996). Prevalence of tsetse fly and ruminant trypanosomosis in Katsina-Ala Local Government Area, Benue State, Nigeria. *Romanian Archives of Microbiology and Immunology*, 55(4): 341-352.

Kalu, A.U., Uzoukwu, M., Ikeme, M. M. and Magaji, Y. (1991). Trypanosomosis in Nigeria: High prevalence among ruminants in Gboko Local Government Area. *Bulletin of Animal Production in Africa*, 39(1): 3-8.

Karaye, G. P. (2012). The efficacy of isomethamidium chloride in the treatment of Trypanosomosis in Red Sokoto Bucks experimentally infected with *T. congolense* and *T. brucei* single and mixed infection of the two. *An M.Sc thesis*, Ahmadu Bello University, Zaria. Nigeria

Katende, J. M., Musoke, A. J., Nantulya, V. M. and Goddeeris, B. M. (1987). A new method for fixation and preservation of trypanosomal antigens for use in the indirect immunofluorescence antibody test for diagnosis of bovine trypanosomiasis. *Tropical Medicine and Parasitology*, 38: 41–44.

Kato, C.D., Nanteza, A., Mugasa, C., Edyelu, A., Matovu, E. and Alibu, V.P. (2015). Clinical Profiles, Disease Outcome and Co-Morbidities among *T. b. rhodesiense* Sleeping Sickness Patients in Uganda. *PLoS Neglected Tropical Diseases* 10, e0118370.

Kemal, J. (2014). *Laboratory Manual and Review on Clinical Pathology*. OMICS Groups eBooks. Foster City, USA.

Kone, N., N'Goran, E.K., Sidibe, I., Kombassere, A.W. and Bouyer, J. (2011). Spatio-temporal distribution of tsetse and other biting flies in the Mouhoun river basin, Burkina Faso', *Medical and Veterinary Entomology*, 25: 156–168.

Kubi, C., Vandenabbeele, J., Dedeken, R., Marcotty,T., Dorny, P. and Van Den Bossche, P. (2006). The effects of starvation on the susceptibility of teneral and non teneral tsetse to trypanosome infection. *Medical and Veterinary Entomology*, 20: 388-392.

Kuboki, N., Inoue, N., Sakurai, T., Di Cello, F., Grab, D. J., Suzuki, H., Sugimoto, C. and Igarashi, I. (2003). Loop-mediated isothermal amplification for detection of African trypanosomes. *Journal of Clinical Microbiology*, 41(12): 5517–5524.

Lawton, S. and Ted, D. (2006). *Body condition scoring in beef cows*. Cooperative Extension. The University of Georgia.

Leak, S. G. A. (1998) Tsetse Biology and Ecology. *Their role in the Epidemiology and Control of Trypanosomiasis*. Wallingford: CABI publishing.

Levine, N. D. (1973). The haemoflagellate. In Levine nd, *protozoa parasites of domestic animal and man* 2nd Ed. Burgess Publishing, Minneapolis, 36-78.

Levine, N. D. Carliss, J. O., Cox, F. E. G., Deroux, G., Grain, J., Honigberg, B. M., Leedale, G. F., Loeblich, A. R., Lom, J., Lynn, D., Merinfeld, F. G., Page, F. C., Poljansky, G. S., Praque, V., Vavra, J. and Wallace, F. G. (1980). A newly revised classification of the protozoa. *Journal of Protozoology*, 27: 37-58.

Liew, F. Y. and Turner, C. M. R. (1999). T- Cell responses during *Trypanosoma brucei* infections in mice deficient in inducible nitric oxide synthase, *Infection and Immunology*, 67(1): 3334- 3338, ISSN 0022-1767.

Liu, G., Sun, D., Wu, H., Zhang, M., Huan, H., Xu, J., Zhang, X., Zhou, H. and Shi, M. (2015). *Trypanosoma brucei* infections: distinct contributions of CD4+ and CD8+ T cells to the pathogenesis in the context of IFN-γ and IL-10. *Infectious Immunology*, 83 (7):2785-2795.

Lutumba, P., Makieya, E., Shaw, A., Meheus, F. and Boelaert, M. (2007). Human African trypanosomiasis in a rural community, Democratic Republic of Congo. *Emerging Infectious Diseases*, 13(2): 248-252.

Machila, N., Wanyangu, S. W., McDermott, J., Welburn, S. C., Maudlin, I. and Eisler, M. C. (2003). Cattle owners' perceptions of African bovine trypanosomosis and its control in Busia and Kwale districts of Kenya. *Acta Tropica*, 86: 25–34.

MacHugh, D. E., Shriver, M. D., Loftus, R. T., Cunningham, P. and Bradley, D. G. (1997). Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos Taurus* and *Bos indicus*). *Genetics*, 146: 1071-1086.

MacLean, L., Odiit, M., and Sternberg, J. M. (2001). Nitric oxide and cytokine synthesis in human African trypanosomiasis, *The Journal of Infectious Disease* 184(4): 1086-1090, ISSN 0022-1899.

Magona, J., Walubengo, J. and Odimin, J. (2008). Acute haemorrhagic syndrome of bovine trypanosomosis in Uganda. *Acta Tropica*, 107: 186–191.

Maigari A. K., Bichi A. H., Sani, H. H., Malami, A. I., Musa, A. M., Jega, Z. H., Abubakar S., Liman, S. B., Sani, A., Jarmai, K. Y., Gide, A. (2015). Body Condition Scores as Putative Diagnostic tool for African Animal Trypanosomosis among Ruminants Slaughtered at Kano Central Abattoir. *Greener Journal of Cell and Animal Biology*, 2 (1): 001-007.

Majekodumi, A. O., Fajinmi, A., Dongkun, C., Picozzi, K. and Thrusfield, M. V. (2013). A longiturdinal survey of African animal trypanosomosis in domestic Cattle on the Jos Plateau, Nigeria! Prevalence, distribution and risk factors. *Parasites and Vectors*, 6: 239-249.

Mansfield, J.M., Paulnock, D.M. and Hedberg, G.M. (2014). Bridging innate and adaptive Immunity in African Trypanosomiasis, In: *Trypanosomes and Trypanosomiasis*. *Springer*, 89-114.

Marquardt, W. C., Demaree, R. C. and Grieue, R. B. (2000). *Parasitology and vector biology*, 2nd edn, Academic Press, London.

Mary, C. S. and David, M. S. (2009). *Goat medicine*, 2nd Ed. Wiley-Blackwell, pp. 296-302

Masiga, D. K., Smyth, A. J., Hayes, P., Bromidge, T. J. and Gibson, W. C. (1992). Sensitive detection of trypanosomes in tsetse flies by DNA amplification. *International Journal of Parasitol*ogy, 22: 909–918Abenga, J. N., Enwezor, F. N. C., Lawani, F .A. G., Ezebuiro, C., Sule, J. and David, K. M. (2002a). Prevalence of trypanomosis in trade cattle at slaughter in Kaduna State, Nigeria. *Journal of Parasitology*, 23: 107-110.

Maudlin, I., Holmes, P. H. and Miles, M. A. (2004). *The Trypanosomiasis*. Wallingford, UK: CABInternational Publishing; 1–634.

Maxie, M. G. and Losos, G. J. (1979). Release of *Trypanosoma vivax* from the microcirculation of cattle by Berenil®, *Veterinary Parasitology* 3: 277-281, ISSN 0019-9867.

Mbaya, A. W. and Ibrahim, U. I. (2011). In-vivo and in-vitro activities of medicinal plants on haemic and humoral trypanosomes: A review, *International Journal of Pharmacology*, 7(1): 1- 11, ISSN 1812-5700.

Mbaya, A. W., Aliyu, M. M., Nwosu, C. O. and Egbe-Nwiyi, T. N. C. (2009a). The relationship between parasitaemia and anaemia in a concurrent *Trypanosoma brucei* and *Haemonchus contortus* infection in red fronted gazelles (*Gazella rufifrons*), *Veterenarski Arhiv*, 79 (5): 451-460, ISSN 03720-5480.

Mbaya, A. W., Aliyu, M. M., Nwosu, C. O., Taiwo, V. O. and Ibrahim, U.I. (2009b). Effects of melarsamine hydrochloride (Cymelarsan®) and diaminazene aceturate (Berenil®) on the pathology of experimental *Trypanosoma brucei* infection in red fronted gazelles (*Gazella rufifrons*), *Veterinary Parasitology*, 163 (1-2): 140-143, ISSN 0304-4071.

Mbaya, A. W., Ibrahim, U. I. and Apagu, S. T. (2010). Trypanosomosis of the dromedary camel (*Camelus dromedarius*) and its vectors in the tsetse-free arid zone of northeastern, Nigeria, *Nigerian Veterinary Journal*, 31(3): 195-200, ISSN 0331-3026.

Mbaya, A. W., Nwosu, C. O. and Onyeyili, P. A. (2007). Toxicity and anti-trypanosomal effects of ethanolic extract of *Butyrospermum paradoxum* (Sapotacea) stem bark in rats infected with *Trypanosoma brucei* and *T. congolense*, *Journal of Ethnopharmacology*, 111: 536-530, ISSN 0378-8741.

Mbaya, A., Kumshe, H. and Nwosu, C. O. (2012). The Mechanisms of Anaemia in Trypanosomosis: *A Review*, *Anemia*, Dr. Donald Silverberg (Ed.), ISBN: 978-953-51-0138-3, InTech, Available from: http://www.intechopen.com/books/anemia/the-mechanisms-of-anaemia-in-trypanosomosis-a-review.

McCulloch, R. and Horn, D. (2009). What has DNA sequencing revealed about the VSG expression sites of African trypanosomes? *Trends in Parasitology*, 25: 359–363.

McDermott, J. J. and Coleman, P. G. (2001). Comparing apples and oranges-model-based assessment of different tsetse transmitted trypanosomosis control strategies. *International Journal for Parasitology*, 31: 603-609.

McNamara, J. J. and Snow, W. F. (1991). Improved identification of Nannomonas infection in tsetse flies from the Gambia. *Acta Tropica*, 127-136.

Mersha, C., Adula D. and Bogale, B. (2013). Socio-Economic Assessment of the Impacts of Trypanosomiasis on Cattle in Girja District, Southern Oromia Region, Southern Ethiopia. *Acta Parasitologica Globalis*, 4: 80-85.

Mogk, S., Meiwes, A., Shtopel, S., Schraermeyer, U., Lazarus, M. and Kubata, B. (2014). Cyclical appearance of African trypanosomes in the cerebrospinal fluid: new insights in how trypanosomes enter the CNS.*PLoS ONE* 9:e91372. doi: 10.1371/journal.pone.0091372.

Molalegne, B., Yeshitla, A. Tilahun, Z and Hailu, D (2011). Trypanosome Infection Rate in *Glossina pallidipes* and *Glossina fuscipes fuscipes* in Gojeb Valley, Southwest Ethiopia. *Global Veterinaria*. 6 (2): 131-135.

Mony, B.M. and Matthews, K.R. (2015). Assembling the components of the quorum sensing pathway in African trypanosomes. *Molecular Microbiology*, 96: 220-232.

Morrison, L. J. and Macleod, A. (2011). African trypanosomiasis. *Parasite Immunology*, 33: 421-422.

Mumba, N. D., Ali, E. R., Mumvemba, K. M. F., Pyana, P. P., Balharbi, F., Decq, M., Kande, B. V., Van der, V. W., Sese, C. and Menten, J. (2014). Performance of parasitological and molecular techniques for the diagnosis and surveillance of gambiense sleeping sickness. *PLoS Neglected Tropical Diseases* 8, e2954

Munday, J. C., Settimo, L. and de Koning, H. P. (2015). Transport proteins determine drug sensitivity and resistance in a protozoan parasite, *Trypanosoma brucei*. *Frontier Pharmacology*, 6, 32

Murray, M., Trail, J. C. M., Turner, D. A. and Wissocq, Y. (1983). *Livestock Productivity and Trypanotolerance*. Network Training Manual. International Livestock Centre for Africa, Adis Ababa, 198.

Muturi, C. N., Ouma, J. O., Malele, I. I., Ngure, R. M., Rutto, J. J., Mithofer, K. M., Enyaru, J. and Masiga, D. K. (2011). Tracking the feeding patterns of tsetse flies (Glossina Genus) by analysis of bloodmeals using mitochondrial cytochromes genes. *PLoS ONE*, 6(2): e17284, DOI:10.1371/journal.pone.001728.

Mwai, O., Hanotte, O., Kwon, Y. J. and Cho, S. (2015). Invited Review; African indigenous Cattle: Unique Genetic Resources in a Rapidly Changing World. *Journal of Animal Science* 28, 911-921.

Mwangi, E., Stevenson, P., Gettinby, G. and Murray, M. (1993) Variation in susceptibility to tsetse-borne trypanosomiasis among *Bos indicus* cattle breeds in east Africa. In Sones,K (ed)Proceedings of the 22nd meeting of the *International Scientific Council for Trypanosomiasis Research and Control*. Publication, 117: 125-128.

Nahla, O. M., Ali, Hamid, I. M., Croof N. and Hamid, S. (2011) Molecular diagnosis of *Trypanosoma evansi* infection in *Camelus dromedaries* from Eastern and Western regions of the Sudan. *Emirate J*ournal of *Food Agriculture*, 23 (4): 320-329.

Nakayima, J. (2016). *Diagnostic Methods for African Trypanosomiasis*. Retrieved from: https://www.researchgate.net/publication/292616299, September, 2016.

National Population Commission (NPC), (2005). *National Population Commission Census Figure*, Abuja, Federal Government of Nigeria.

Ngure, R.M, Ndungu, J.M., Ngotho, J.M., Nancy, M.K., Maathai, R.G. and Gateri, L.M. (2008). Biochemical changes in the plasma of vervet monkeys (*Chlorocebus aethiops*) experimentally infected with *Trypanosoma brucei rhodesiense*. *Journal of Cell and Animal Biology*, 2: 150-157.

Nguyen, T. T., Zhou, M., Ruttayaporn, N., Nguyen, Q. d., Nguyen, V. K., Goto, Y., Suzuki, Y., Kawazu, S. and Inoue, N. (2014). Diagnostic value of the recombinant tandem repeat anti-gen TeGM6-4r for surra in water buffaloes. *Veterinary Parasitology*, 201: 18–23.

Nicholson, M. J. and Butterworth, M .H. (1986). *A guide to scoring of zebu cattle*, International Livestock Centre for Africa, Addis Ababa.

Nigatu, S. D., Bezie, M., Terefe, G., Abebe, G., Barry, J. D. and Goddeeris, B. M. (2015). Comparative clinico-haematological analysis in young Zebu cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. *Acta Veterinaria Scandinavia*. 57, 24.

Nigerian Institute for Trypanosomiasis Research (NITR), (1989), *Annual report*.

Norgan, A. P., Arguello, H. E., Sloan, L. M., Fernholz, E. C. and Pritt, B. S. (2013). Preparing blood smears; thick blood smears. *Malaria Journal*, 12: 231.

Nwoha, R. I. O. and Anene, B. M. (2011). Clinical signs and pathological changes in dogs with single and conjunct experimental infections of *Trypanosoma brucei brucei* and *Ancylostoma caninum*. *Journal of Veterinary Parasitology*, 25(2): 97-102.

Nyimba, P. H., Komba, E. V. G., Sugimoto, C. and Namangala, B. (2015). Prevalence and species distribution of caprine trypanosomosis in Sinazongwe and Kalomo districts of Zambia. *Veterinary Parasitology* 210:125-130.

Odeyemi S .O., Iliyasu D., Omotosho K., Omolade L. O., Ajani J. A. and Auwal, U. (2015). Hitches of Trypanosomosis in Nigeria from Livestock Production Perspective. *Journal of Animal Production Advances*, 5(9): 747-756.

Odiit M., Coleman P. G., McDermott J. J., F`evre E. M., Welburn S. C.and Woolhouse M. E. J. (2004). Spatial and temporal risk factors for the early detection of *Trypanosoma brucei rhodesiense* sleeping sickness patients in Tororo and Busia districts, Uganda, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98: 569-576.

Office International des Epizootics (OIE), (2012). Trypanosomosis. pp. 809–818. In: *Manual of diag-nostic Tests and Vaccines for Terrestrial Animals*, 7th ed., OIE, Paris

Office International des Epizootics (OIE), (2013), *trypanosomosis (tsetse transmitted*). OIE Terrestial Manual, 2, 4-18.

Ogwu, D., Njoku, C.O. and Ogbogu, V.C. (1992). Adrenal and thyroid dysfunction in experimental *Trypanosoma congolense* infection in cattle, *Veterinary Parasitology, 42*: 15-26.

Ohaeri, C. C. (2010) Prevalence of trypanosomosis in Ruminants in parts of Abia State, Nigeria. *Journal of Animal and Veterinary Advances*, 9(8): 2422-2426.

Okaiyeto, S. O., Ajanusi, O. J., Sackey, A. K. B. and Tekdek. L. B. (2010) Changes in some haematological values associated with mixed *Trypanosoma congolense* and *Haemonchus contortus* infection in yankassa sheep. *Journal of Veterinary Research*, 3(2): 9-13.

Okwelum, N., Iposu, S. O., Sowande, O. S., Smith, O. F., Takeet, M. I., Olugbogi,E. I., Rahman, S. A. (2010). Prevalence of Trypanosome Infection in Trade cattle at Slaughter in Lafenwa abattoir, Abeokuta, Nigeria. *Journal of Commonwealth Veterinary Association* 27(2): 239-241.

Onono, J. O., Wieland, B. and Rushton, J. (2013). Factors influencing choice of veterinary service provider by pastoralist in Kenya. *Tropical Animal Health Production*

Onyia, J.A. (1997). African Animal Trypanosomosis: An overview of the current status in Nigeria. *Tropical Veterinarian*, 15(3): 111−116.

Pan African Tsetse and trypanosomosis Eradication Campaign (PATTEC), (2000). *A continental plan of action for the eradication of Tsetse and trypanosomosis.* The Organization of African Unity pathway for the PATTEC initiative 7–16 December.Nairobi: OAU/STRC Publication; 2000.

Paris, J., Murray, M. and McOdimba, F. (1982). A Comparative Evaluation of the Parasitological Techniques Currently Available for the Diagnosis of the African Trypanosomes in the Cattle. *Acta Tropica*, 39: 307-316.

Parryet, G. F., Mabey, R. D. and Gill, F. (2004). *Principles of Medicine in Africa*, Cambridge University Press, London.

Peel, W. J. A. and Chardome, M. (1954). *Trypanosoma suis* ochmann, 1905-trypanosome monomorph de mammiferes evoluant dans les glandes salivaires de *Glosina brevipalpis*, Newst; Mosso (Urundi). *Animal Soc. Belgeium Medicine Tropical*, 34: 277 – 295.

Pelloux, H., Aznar, C. and Bouteille, B. (2006). Trypanosomiases. *La Revue du Praticien*, 56 (20): 2209-2216.

Perry, B. D., Randolph, T. F., McDermott, J. J., Sones, K. R. and Thornton, P. K. (2002). *Investing in animal health research to alleviate poverty*. ILRI (International Livestock Research Institute), Nairobi, Kenya, 148.

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

Radostits, O. M., Gay, C. C., Hinchcliff, K. W. and Constable, P. D. (2007). *Veterinary Medicine. Text book of the diseases of cattle, sheep, pig and goats*, 10th Edn. Saunders Elsevier publication, Philadelphia, USA, 1531-1540.

Rebeski, D. E., Winger, E. M., Okoro, H., Kowalik, S., Burger, H. J., Walters, D. E., Robinson, M. M., Dwinger, R. H. and Crowther, J. R. (2000). Detection of *Trypanosoma congolense* antibodies with indirect ELISAs using antigen precoated microtitre plates. *Veterinary Parasitol*ogy, 89: 187–198.

Rodrigues, C. M. F., Olinda, R. G., Silvaa, T. M. F., Valea, R. G., Da Silvaa, A. E., Limaa, G. L., Garciab, H. A., Teixeirab, M. M. G. and Batista, J. S. (2013). Follicular degeneration in the ovaries of goats experimentally infected with *Trypanosoma vivax* from the Brazilian semi-arid region. *Veterinary Parasitology*, 191: 146–15.

Rogers, D. J. (1991). Satellite imagery, tsetse and trypanosomiasis in Africa. *Preventive Veterinary Medicine*, 11: 201-220.

Rowlands G.J., Leak S.G.A., Woudyalew M., Nagda S. M., Wilson A. and d'ieteren G.D.M. (2000). Use of deltamethrin 'pour-on' insecticide for the control of cattle trypanosomosis in the presence of high tsetse invasion, *Medical and Veterinary Entomology*, 15: 87-96.

Sackey, A. K. B. (1998). Comparative study of trypanosomosis experimentally induced in savannah brown goats by *Trypanosoma brucei*, *T. congolense* and *T. vivax*. *PhD thesis*, Ahmadu Bello University, Zaria, Nigeria.

Salim, B., Bakheit, M. A., Salih, E., Kamau, J., Nakamura, I., Nakao, R. and Sugimoto, C. (2011) an Outbreak of Bovine Trypanosomiasis in the Blue Nile State, Sudan. Short report. *Parasites and Vectors*, 4:74

Samdi, S. M., Abenga, J. N., Atahir, A., Wayo, B. M. and Sumain, H. M. (2010). Constrains in the control of Africa trypanosomosis: the prevailing factors in Kurmin, Kaduna. *International Journal Animal Veterinary Advances*, 2: 31-36.

Samdi, S. M., Fajinmi, A. O., Kalejaye, J. O., Wayo, B., Haruna, M. K., Yarnap, J. E., Mshelia, W. P., Usman, A. U., Haruna, S. M., Jijtar, A., Ogunwole, R., Ogbagbedia, R. P. and Bizi, R. (2011). Prevalence of trypanosomosis in cattle slaughter at Kaduna Central abattoir. *Asian Journal of Animal Sciences*, 5(2): 162-165.

Samuel, W. M., Margo, J. P. and Kocan, A. A. (2001). *Parasitic diseases of wild mammals*, 4th edn. Blackwell Publishing Company, Malden.

Sam-Wobo, S. O., Igenzoa, A. J., Idowu, O. A., Otesile, E. B., Ekpo, U. F. and Kehinde, O. O. (2010). Bovine trypanosomiasis and its impact on cattle in derived Savannah areas of Ogun State, Nigeria. *Journal of Public Health and Epidemiology*, 2(3): 43-47.

Sanni, M. T., Gbolabo, O. O., Mufliat, A. A., Abdulmojeed, Y., Christian, O. N. I., Olufunmilayo, A. A., Adewale, O. T., Michael, O. O., Mathew, W., Michael, I. T., Sunday, O. P., Marcos De, D., Bolaji, N. T. and Ikhide, G. I. (2013). Molecular Diagnosis of Subclinical African *Trypanosoma vivax* Infection and Association with Physiological Indices and Serum Metabolites in Extensively Managed Goats in the Tropics. *Open Journal of Veterinary Medicine,* 03: 39-39.

Saror, D. I. (1982). *Aspects of the anaemia of acute bovine trypanosomiasis*, Proceedings of the first National Conference on Tsetse and Trypanosomiasis Research, pp. 12-14, ISSN 0049-4747.

Schillinger, D., Maloo, S. H. and Rottcher, D. (1985). The toxic effect of intravenous application of the trypanocide isometamidium (samorin). Zbl. *Veterinary Medicine Article*, 32: 234–239.

Schofield, C. J. and Maudlin, I. (2001). Trypanosomiasis control. *International Journal of Parasitology*, 31: 614-619.

Sengupta, P. P., Balumahendiran, M., Balamurugan, V., Rudramurthy, G. R. and Prabhudas, K. (2012). Expressed truncated N-terminal variable surface glycoprotein (VSG) of *Trypanosoma evansi* in *E. coli* exhibits immuno-reactivity. *Veterinary Parasitology*, 187: 1–8.

Shahzad, W., Munir, R., Khan, M.S., Ahmad, M.U.D and Iqbal, M (2012) Molecular Diagnosis and Chemotherapy of *Trypanosoma evansi i*n Nili-Ravi Buffaloes at District Okara (Pakistan) *The Journal of Animal and Plant Sciences*, 22(3): 212-216.

Shimelis, D. and Melkamu, B. (2015). Review on *Trypanosoma vivax*. *African Journal of Basic and Applied Science,* 7(1): 41-64.

Sima, M., Havelková, H., Quan, L., Svobodová, M., Jarošíková, T., Vojtíšková, J., Stassen, A. P. M., Demant, P. and Lipoldová, M. (2011). Genetic Control of Resistance to *Trypanosoma brucei brucei* Infection in Mice. *PLoS Neglected Tropical Diseases* 5, e1173.

Sindato, C., Kimbita, E. N. and Kibona, S. N. (2008). Factors influencing individual and community participation in the control of tsetse flies and human African trypanosomosis in Urambo district, Tanzania. *Tanzania Journal of Health Research*, 10(1): 20–27.

Sinshaw, A., Abebe, G., Desquesnes, M. and Yon, W. (2006). Biting flies and *T. vivax* infection in three highland district bordering Lake Tana, Ethiopia. *Veterinary Parasitology*, 142: 35-46.

Sinyangwe, L., Delespaux, V., Brandt, J., Geerts, S., Mubanga, J. and Al, E. (2004). Trypanocidal drug resistance in Eastern province of Zambia. *Veterinary Parasitology*, 119: 125-135.

Solano, P., Dela, R. S. and Duvalet, G. (2003). Biodiversity of trypanosomes pathogenic for cattle and their epidemiological importance. *Animals of Society*, 68: 169 – 171.

Soulsby, E.J.L. (1982). *Helminthes, Arthropods and Protozoa parasites of domesticated Animals*, Bailere Tindal, London

Steverding, D. (2015). Evaluation of trypanocidal activity of combinations of anti-sleeping sickness drugs with cysteine protease inhibitors. *Experimental Parasitology*. 151–152: 28-33.

Sumbria, D., Singla L. D., Amrita S., Aman D. M. and Bal, M. S. (2014). Equine trypanosomosis in central and western Punjab: Prevalence, haemato-biochemical response and associated risk factors, *Acta Tropica*, 138:44-50.

Swallow, B. M., (2000). *Impacts of trypanosomiasis on African agriculture*. FAO (Food and Agriculture Organization of the United Nations), Rome, Italy, 52.

Taiwo, V.O., Olaniyi, M.O. and Ogunsanmi, A.O. (2003). Comparative plasma biochemical changes and susceptibility of erythrocytes to *in vitro* peroxidation during experimental *Trypanosoma congolense* and *Trypanosoma brucei* infections in sheep. *Journal of Isreal Veterinary Medicine*, 58: 435-443.

Takeet, M. I., Fagbemi, B. O., Donato, M. D., Yakubu, A., Roduffo, H. E., Peters, S. O., Wheto, M. and Imumorin, I. G. (2013). Molecular survey of pathogenic trypanosomes in naturally infected Nigeria Cattle. *Research in Veterinary Science*, 94: 555-561.

Tesfaye, M. (2002). Report of trypanosome infection rate, in *G. m. submoristans* and *G.tachnoides* in Didessa valley from July 29 to September 26, 2002. Bedele, Ethiopia.

Thrusfield, M. V., (2005): *Veterinary Epidemiology*.3rd edition, Blackwell Publishing, Garsington road, Oxford, UK, PP: 233-250.

Thuy, N. T., Goto, Y., Lun, Z. R., Kawazu, S. and Inoue, N. (2012). Tandem repeat protein as potential diagnostic antigen for *Trypanosoma evansi* infection. *Parasitology Research*, 110: 733–739.

Tsegaye, B., Dagnachew, S. and Terefe, G. (2015). Review on Drug Resistant Animal Trypanosomes in Africa and Overseas. *African Journal of Basic and Applied Science*. 7, 73-83

Urquhart, G. M., Armour, J., Dunkan, J. L., Dunn, A. M. and Jennings, F. W. (1996). *Veterinary Parasitology*. 2nd Edition, Long English Language Society, 212-216.

Van Coller, R., van Rensburg, E., Schutte, C., Brink, D., Welthagen, G. and Dove, M.G. (2007). Awaking a sleeping epidemic. *South African Medical Journal,* 97 (4): 250-251.

Van Den Bossche P., Chigoma D. and Shumba W. (2000). The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs and in the absence of tsetse challenge. *Acta Tropica*, **77**: 263–270.

Van den Bossche, P., De Deken, R., Brandt, J., Seibou, B. and Geerts, S. (2004). Recirculation of *Trypanosoma brucei brucei* in cattle after *T. congolense* challenge by tsetse flies. *Veterinary Parasitology*, 121: 79–85.

Van Nieuwenhove, L., Büscher, P., Balharbi, F., Humbert, M., Dieltjens, T., Guisez, Y. and Lejon, V. (2012). Identification of mimotopes with diagnostic potential for *Trypanosoma brucei gambiense* variant surface glycoproteins using human antibody fractions. PLoS *Neglected Tropical Diseases*, 6, e1682.

Van Nieuwenhove, L., Büscher, P., Balharbi, F., Humbert, M., Guisez, Y. and Lejon, V. (2013). A LiTat 1.5 variant surface glycoprotein-derived peptide with diagnostic potential for *Trypanosoma brucei gambiense*. *Tropical Medicine and International Health*, 18: 461–465.

Verloo, d., Holland, W., My, L. N., Thanh, N. G., Tam, P. T., Goddeeris, B., Vercruysse, J. and Büscher, P. (2000). Comparison of serological tests for *Trypanosoma evansi* natural infections in water buffaloes from North Vietnam. *Veterinary parasitology*, 92: 87–96.

Victor, I., Sackey, A. K. B. and Natala, A. J. (2012). Penile protrusion with hemorrhages and prepucial inflammation in pigs experimentally infected with *Trypanosoma congolense. Journal of Animal Production Advance*s, 2(6): 297-302.

Waiswa, C., Picozzi, K., Katunguka, R. E., Olaho, M. W., Musoke R. A. and Welburn S. C. (2006). *Glossina fuscipes fuscipes* in the trypanosomiasis endemic areas of south eastern Uganda: Apparent density, trypanosome infection rates and host feeding preferences, *Acta Tropica*, 99: 23–29.

Wanga, C. H. and Munga, L. (2011). The effective eradication and control strategies for tsetse and trypanosomiasis the Kenyan experience. *Journal of Commonwealth Veterinary Association* 27(2): 93-100.

World Health Organisation (WHO) (2011), The Control of Neglected Zoonotic Diseases, http: //www.who.int/neglected diseases/zoonoses/en/

Wille, U., Seyfang, A. and Duszenko, M. (1996). Glucose uptake occurs by facilitated diffusion in procyclic forms of *Trypanosoma brucei*. *European Journal of Biochemistry*, 236: 228-233.

Woo, P. T. K. (1970). The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Tropica*, 27: 384–386.

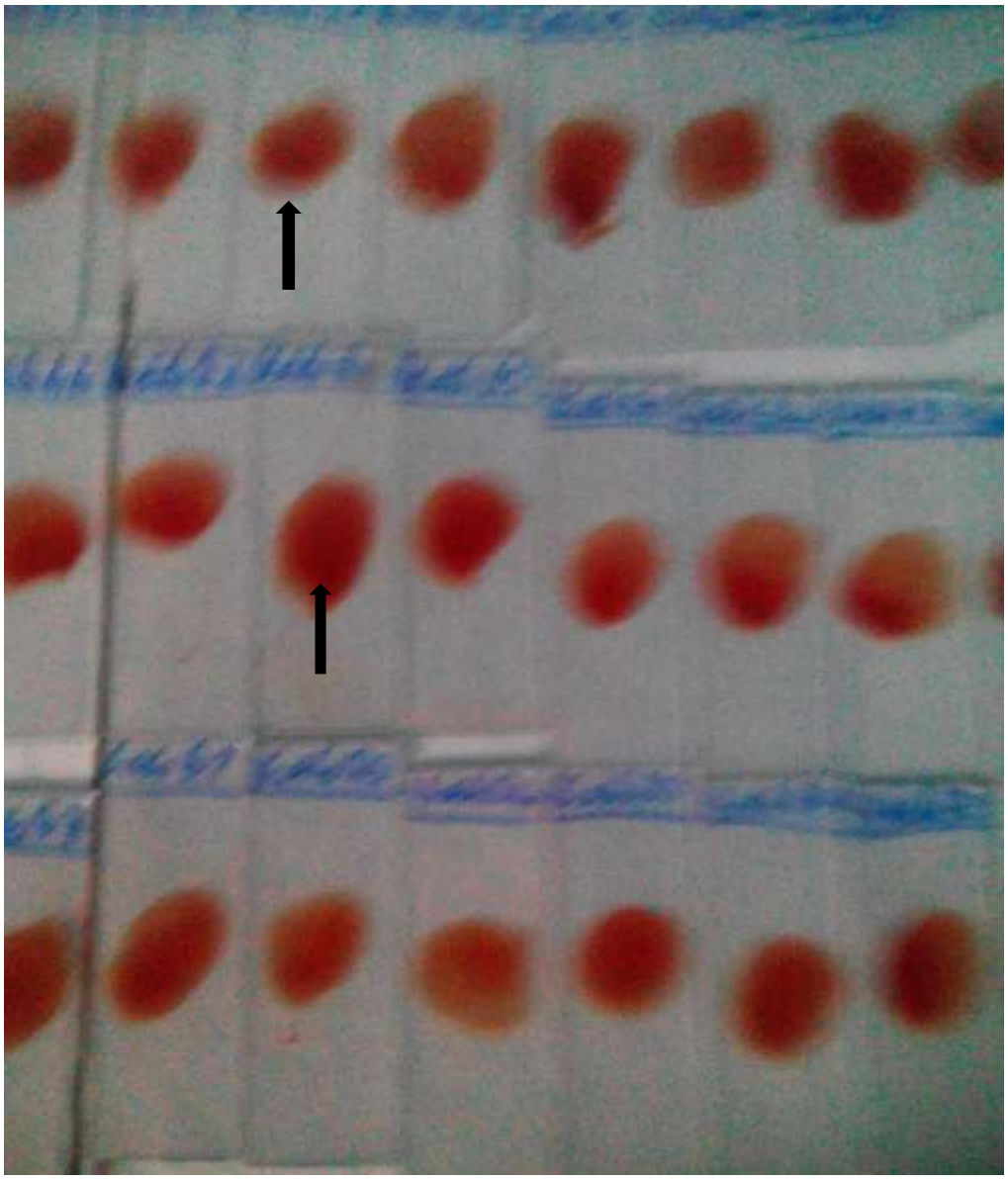
World Organization for Animal Health (OIE), (2009). African Animal Trypanosomiasis, http: //www.who.int/neglected diseases.

Yusuf, A. B., Abubakar, A., Musa U. B., Haruna, M. K., Garba, H. A., Maigari, A. K., Zubair, A. I., Machina, I. B., Shehu, A. A., Galadima, U. S., Adamu, A., Chechet, G. D. and Diggi, S. (2015). Surveillance for tsetse and trypanosomosis in Bagudo Local Government Area North Western Nigeria. *IOSR Journal of Agriculture and Veterinary Science,* 8(7): 43-48

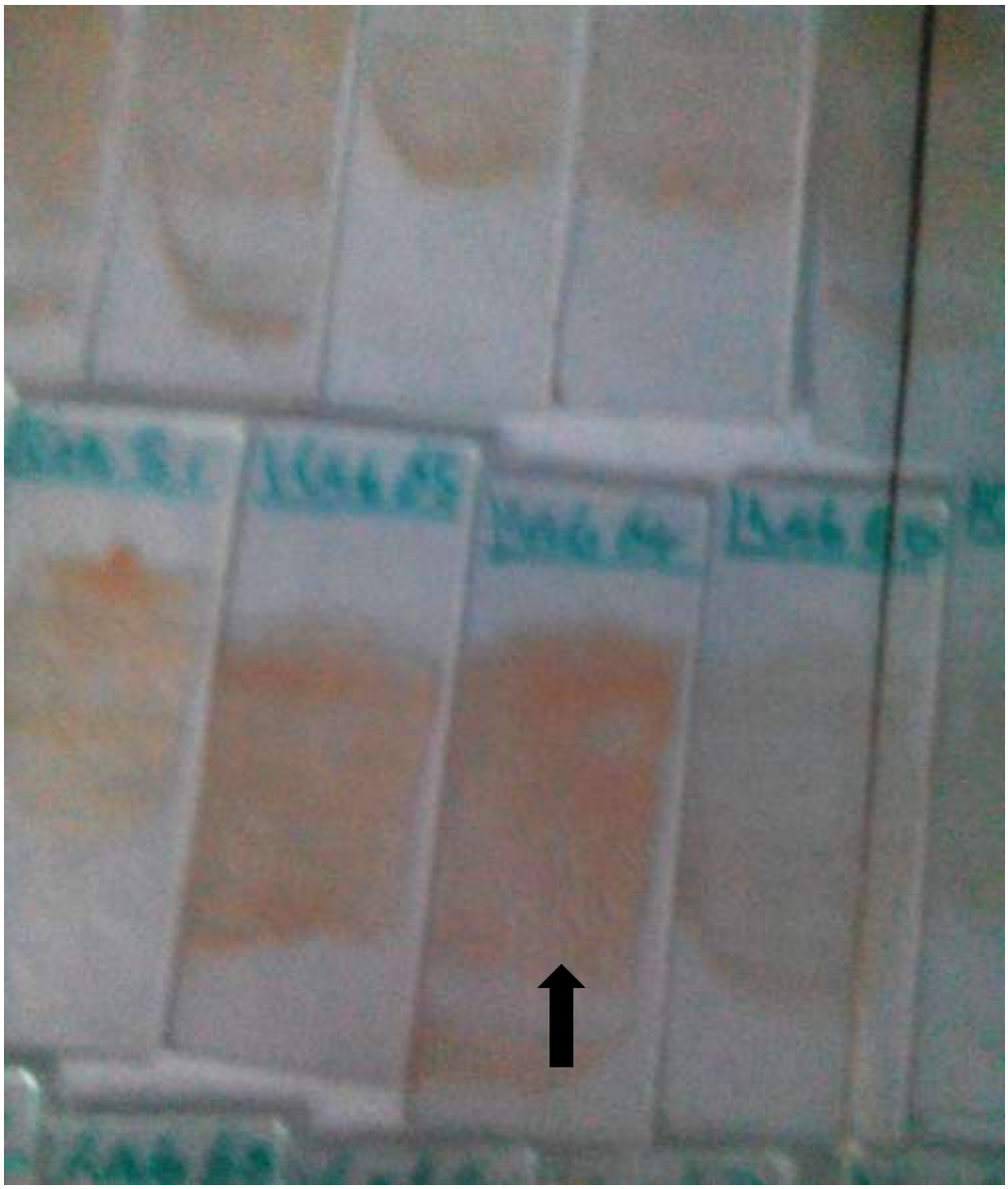
Zubairu, A., Midau, A., Dazala, I.U., Yahya, M.M. and Buba, Z.M. (2013). The prevalence of bovine trypanosomiasis in Song Local Government Area of Adamawa State, Nigeria. *Global Veterinaria*, 11(3): 310-313.

APPENDICES

Appendix I: Photograph of some of the thick smear preparations on slides before staining.



Appendix II: Photograph of some of the thin smear preparations on slide before staining



Appendix III: Photograph of Geimsa staining procedure



A and B: staining of the prepared slides using Geimsa stain

C: washing of the stained slides with normal water

Appendix IV: Photograph of some of the thick smear on slide after staining.



Appendix V: Photograph of some of the thin smears on slide after staining.



Appendix VI: Photograph of one of the settled herds used in the study

