CLINICAL MANIFESTATIONS, BLOOD PARAMETERS, RESPONSE TO TREATMENT OF NATURALLY OCCURING CASES OF BOVINE TRYPANOSOMOSIS IN NIGER STATE, NIGERIA

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DECLARATION

I declare that the work in this dissertation entitled "Clinical Manifestations, Blood Parameters, Response to Treatment of Naturally Occurring Cases of Bovine Trypanosomosis in Niger State, Nigeria" is a record of my own work carried out in the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria under the Supervision of Professor L. Allam and Professor A.K.B. Sarkey. 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.

Muhammad Baba, ALIYU _____ _____

Signature Date

CERTIFICATION

This dissertation entitled "CLINICAL MANIFESTATIONS, BLOOD PARAMETERS, RESPONSE TO TREATMENT OF NATURALLY OCCURRING CASES OF BOVINE TRYPANOSOMOSIS IN NIGER STATE, NIGERIA" by Muhammad Baba, ALIYU 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.

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DEDICATION

This thesis is dedicated to the Almighty Allah, the ultimate source of knowledge, to the scientific community who made the world a better place to live and to my late parents who were not alive to rejoice with me in this achievement.

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TABLE OF CONTENTS

CHAPTER TWO

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATION

AAT	African animal trypanosomosis
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
AST	Aspartate amino transferase
CK	Creatinine kinase
HDL	High density lipoprotein
LDL	Low density lipoprotein
DA	Diminazene aceturate
DDT	Dichloro diphenyl trichloroethane
DNA	Deoxyribonucleic acid
EEC	European Economic Community
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme Linked Immuno Sorbent Assay
FAO	Food and Agriculture Organization
Hgb	Hemoglobin
HCT	Haematocrit Centrifugation Technique
IFAT	Indirect Fluorescent Antibody
IFN-γ	Interferon Gamma
Ig	Immunoglobulin
IgM	Immunoglobulin M
IL	Interleukin
IL-10	Interleukin-10
IL-12	Interleukin-12
ILRAD	International Laboratory for Research on Animal Diseases
IM	Intramuscular
LGA	Local Government Area
NGSG	Niger State Government
NGN	Nigerian Naira
ISM	Isomethamidium chloride
OIE	Office International des Epizooties
PAAT	Programme Against African Trypanosomosis
PATTEC	Pan African tsetse and trypanosomosis eradication campaign
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
RBC	Red Blood Cell
SD	Standard deviation
TNF-α	Tumor necrosis factor-alpha
VAT	Variable antigen type
VSG	Variable surface glycoprotein
WBC	White Blood Cell
WHO	World Health Organization

ABSTRACT

A longitudinal study was conducted to assess the clinical manifestation, response to treatment, and biochemical alterations to bovine trypanosomosis in some selected Local Government Areas of Niger State. A total of 343 cattle from thirty nine (39) herds were examined for infection with trypanosomes. Parasitological, haematological and biochemical tests were carried out on their blood and serum samples. Clinical signs, changes in haematological and biochemical values were monitored post treatment with diminazene aceturate and isomethamidium chloride. The most prevalent clinical signs observed were emaciation (75%), weakness (71%) intermittent anorexia (65%), pale mucous membrane (58%), epiphora (45%), and dark/rough hair coat (41%). Three species of trypanosomes were identified in the infected animals, *Trypanosoma vivax* (5.5%), *T. congolense* (5.5%), and *T. brucei* (2.0%). The mean body weights of infected animals (257.94 ± 74.13kg) were significantly lower than that of the control (386.96 ± 62.69kg). The mean rectal temperature of infected animals (39.82 ± 1.79ºC) differed significantly from the control (38.490.84ºC) ($P<0.05$). The mean PCV (23.27 ± 6.82%), platelet (93.23 ± 42.02 $x10^3$ µl), and total leukocyte counts (4.40 ± 1.64$x10^3$ µl) of infected cattle before treatment were significantly lower than those of the control (32.47 ± 8.35%, 209.67 ± 55.75 $x10^3$ µl and 8.14 ± 3.34 $x10^3$ µl respectively). Lymphocyte counts (64.64 ± 12.19%) were significantly higher in the infected cattle when compared to the control (58.19 ± 15.29%). The mean neutrophils count (32.62 ± 12.25%) of the infected cattle was significantly lower than the control (39.46 ± 15.05%). The mean values of Alanine amino transferase (34.62 ± 20.57 IU/L), alkaline phosphatase (105.48 ± 37.97 IU/L), Creatinine kinase (265.71 ± 21.25 IU/L) of the infected cattle were significantly higher than the control (16.60 ± 3.73 IU/L, 65.60 ± 18.90 IU/L, and

254.12 ± 11.32 IU/L respectively). The mean total proteins (51.50 ± 18.28 mg/dL), glucose (31.94 ± 13.68 mg/dL), cholesterol (2.62 ± 1.33 mg/dL) of the infected cattle were significantly lower than the control (77.20 ± 14.46 mg/dL, 46.80±13.59 mg/dL, 3.25 ± 1.66 mg/dL) respectively. The levels of albumin (24.84 ± 8.31 mg/dl) and globulins (29.34 ± 15.31 mg/dl) of the infected cattle were significantly lower than the control (27.60 ± 6.73 mg/dL and 49.80 ± 15.05 mg/dL) respectively. The mean triglycerides levels of the infected cattle (2.32 ± 1.08 mg/dL) were significantly higher than the control (1.90 ± 0.58 mg/dL). The mean levels of sodium (111.82 ± 28.84 mg/dL), chloride (91.76 ± 25.59 mg/dL) and bicarbonates (17.46 ± 6.76 mg/dL) of the infected cattle were significantly lower than the control (127.80 ± 34.95 mg/dL, 98.60 ± 19.48 mg/dL, and 20.6012.58 mg/dL) respectively. The levels of calcium (2.98 ± 0.84mg/dL), iron (1.55 ± 0.60 mg/dL), copper (0.49 ± 0.36 mg/dL) and zinc (2.08±1.42 mg/dL) were significantly lower in infected cattle compared to the values of the control (4.16 ± 0.54 mg/dL, 4.45 ± 2.07 mg/dL, 0.81 ± 0.08 mg/dL, and 7.88 ± 2.52 mg/dL) respectively. All values were held at $P \leq 0.05$ confidence interval. This study has suggested for the first time the clinical signs of natural cases of trypanosomosis in cattle in selected Local Government Areas in Niger State. Diminazene aceturate and isomethamidium chloride were effective in the treatment of naturally occurring trypanosomosis. However, relapse infection was observed using both drugs. The study has shown that the current trend in diagnosis, treatment and management of trypanosomosis is important to avoid treatments that will potentiate drug resistance. Further work should be done to establish the parasitological and pathological bases for each of the clinical signs that were observed in this study.

CHAPTER ONE
INTRODUCTION

1.1 Background to the study

Trypanosomosis is a complex disease caused by trypanosomes, a group of unicellular protozoan parasites found in the blood and other tissues of vertebrates including livestock, wildlife (Tesfaye, 2002) and man (Franco *et al.*, 2014). The disease is commonly known as "Nagana", "Samore" or Tsetse fly disease in the bovines (Radostits *et al.,* 2006), and sleeping sickness in man (Franco *et al.,* 2014).The disease is characterized by anorexia, anaemia, diarrhoea, excessive epiphora, emaciation, weakness and eventual death, in addition to leucopaemia, thrombocytopaenia, serum biochemical changes and lesions in some tissues and organs (Igbokwe, 1989; Esievo and Saror, 1991; Uilenberg, 1999; Rodosttis *et al.*, 2006).

The most important trypanosome species infecting livestock are *Trypanosoma (T) congolense, T. vivax* and *T. brucei*, primarily for ruminants (Igbokwe, 1995; Onyiah, 1997; Takeet *et al.,* 2013) and *T. simiae* which primarily infects pigs (Sarkey, 1998). *Trypanosoma evansi* and *T. equiperdum* infects equidae (Moloo *et al.,* 2000; Getachew, 2005; OIE, 2013) while *Trypanosom brucei rhodesiense* and *T. brucei gambiense* are known to infect man (Dumas *et al.*, 1999; Franco *et al.,* 2014). Trypanosomes are commonly transmitted biologically by tsetse flies (*Glossina* spp), but some cases of mechanical transmission by other haematophagus flies and trans-placental transmission have been reported (Ikede and Losos 1972; Ogwu *et al.*, 1986).

Trypanosomosis is widespread and endemic in Nigeria, occurring in all areas infested by tsetse flies (Onyiah *et al.*, 1983) as well as the tsetse-free arid zones of the north that is infested by other biting flies (Nawathe *et al.*, 1988, 1995; Ahmed *et al.*, 1994).

The tsetse flies are found between latitudes 14ºN and 29ºS covering about 10 Million km² stretching across 37 countries in Africa (Seifert, 1996; WHO, 1998; Mulumba, 2003).

1.2 Statement of the Research Problem

The annual production losses due to Africa Animal Trypanosomosis (AAT) morbidity and mortality are valued at $4.5 billion across Africa while the indirect annual costs of AAT are estimated to be $134 billion (Kristjanson *et al.,* 1999; Fadiga *et al.,* 2013). In the Sub Saharan Africa over 3 million cattle and other domestic livestock are lost annually through deaths caused by trypanosomosis (ILRAD, 1990; Mulumba, 2003; Abenga *et al.,* 2003; FAO, 2005). The disease costs the Nigerian economy $135 million per annum due to its negative effects on weight gain, growth rate, milk yield, reproduction in cattle and discouragement of the use of draught animals in arable farming (Omotainse *et al.,* 2004).

Trypanosomosis has been shown to reduce calving rates by 11-20% and increases calf mortality by 10-20% in susceptible breeds of cattle. Similarly, it is known to reduce milk off take in trypanotolerant breed of cattle by 10-20% and off take for sale or slaughter by 5–30%. The work performance of draught oxen in susceptible cattle can drop by 38% in high risk areas (PAAT, 2000; Shaw, 2004). Generally, trypanosomosis reduces total stock of livestock by 10–60% (Kristjanson *et al.,* 1999; PAAT, 2000; Gilbert *et al.,* 2001) with consequent reduction in meat and milk output by 50% and reduction in total agricultural production by 2–10 per cent (PAAT, 2000). Trypanosomosis is one factor that has constrained the development of specialized dairy enterprises in sub Saharan Africa (Swallow, 2000). The overall impact of the disease extends from restricted access to fertile and cultivable areas, imbalances in land use and exploitation of

natural resources and compromised growth and diversification of crop-livestock production systems (Mattioli *et al.*, 2004).

1.3 Justification of the Study

The management of clinical disease due to trypanosomes through diagnosis and treatment remains difficult under field conditions (Nantulya, 1990; Schlater and van den Bossche, 2004), because of its protean manifestations and lack of pathognomonic signs (Eisler *et al.*, 2004; Rodostits *et al.*, 2006).Simultaneous infections with more than one *Trypanosoma species* (Nyeko *et al.*, 1990) and their ability to co-infect with other parasites (*Babesia* spp., *Theileria* spp., *Anaplasma* spp., *Ehrlichia* spp., and helminths) (Thumbi *et al.*, 2014) further complicate the prognosis and clinical manifestations of the disease (Van Wyk *et al.*, 2014). It has also been estimated that close to 50% of all cases of trypanosomosis are not diagnosed and a large proportion of the infected animals remained untreated (Picozzi *et al.*, 2002). Poor and inadequate tools for the diagnosis of the disease in the field make it difficult to conclude which clinical signs are attributable to a given parasite (Machila, 2004).

There is the need therefore to document clinical signs due to natural *Trypanosoma* infection with the view aiding field personnel and farmers in the recognition of animals suffering from the disease in the field to enable rapid and accurate diagnosis of the disease and subsequent monitoring of the incidence of infection. This would assist in management decisions; provide basis for disease surveillance, monitoring and control programmes. It will also assist in the control and eradication of tsetse and trypanosomosis which would eventually benefit and

promote human and livestock health, diversify agricultural production and allow for optimum exploitation of the abundant fodder and water resources for large scale livestock production.

1.4 Aim of the Study

The aim of the study was to determine the clinical manifestations, blood parameters, response to treatment of naturally occurring cases of bovine trypanosomosis in Niger State, Nigeria

1.5 Objectives of the Study

The objectives of the study were to:

i. Determine the species of trypanosomes infecting cattle in Niger State
ii. Determine the clinical signs naturally infected cattle manifest in Niger State, Nigeria;
iii. Determine the haematological and serum biochemical parameters in the naturally infected cattle in Niger State, Nigeria;
iv. Evaluate the treatment response to selected trypanocides by naturally infected cattle during the study period in Niger State, Nigeria.

1.6 Research Questions

i. What are the species of trypanosomes infecting cattle in Niger State
ii. What are the commonly observed clinical signs that naturally infected cattle manifest in Niger State, Nigeria?
iii. What are the haematological and serum biochemical profiles of natural trypanosomosis in cattle in Niger State, Nigeria?

iv. What is the response of naturally infected cattle in Niger State following treatment with Diminazene aceturate and Isomethamidium chloride?

CHAPTER TWO

LITRATURE REVIEW

2.1 African Animal Trypanosomosis

Trypanosomosis was first discovered by Sir David Bruce in 1894, who found a correlation between "Nagana", a disease affecting cattle, being transmited by tsetse fly along side its causative aents the trypanosomes, while investigating the deaths of many cattle in the Zululand region of South Africa (Duggan, 1977). It is an important parasitic protozoan disease of humans and animals which is prevalent throughout the tropical and sub-tropical parts of the world (Fatihu *et al.,* 2008). The disease is transmitted by arthropod tsetse flies (*Glossina spp*) cyclically and also mechanically by tsetse and other biting flies through the transfer of blood from one animal to another (Songa *et al.,* 1990). The disease occurs widely in the tropics and subtropics and is well recognized and known by various local names such as *Nagana*, *Samore*, or *tsetse fly* disease in sub-Saharan Africa; "Malde Caderas" of equines in central and South America; and "Surra" in draught and transport animals (horses, donkeys and camels) in Asia, Middle East, South America and some part of Africa (Desquesnes *et al.,* 2013). While in man the disease is commonly known as sleeping sickness in Africa (Franco *et al.,* 2014) and Chagas disease in Central and South America **(**Connor, 1992; Kettle, 1995**).**

2.2 Aetiology

The term trypanosoma was derived from two Greek words, *trypano* (borer) and *soma* (body), largely because of their typical corkscrew-like motion (Levine *et al.,* 1980). They are unicellular flagellate microscopic elongated spindle-shaped protozoa **(**Hoare, 1972) and an obligate parasite that lives and multiply in the blood and tissue fluids, but some occupy intracellular habitats of their hosts (Schmidt and Robert, 1989; Masocha *et al.,* 2004).

They are mainly transmitted by hæmatophagous vectors-mammals (vampire-bats) or arthropods, sometimes ticks, but most often by biting insects that transmit cyclically (*Glossina*, reduviid bugs) or mechanically (horseflies, Stomoxys, etc.) (Itard, 1989; Desquesnes, 2004). The major pathogenic tsetse transmitted trypanosome species are *T. congolense, T. vivax and T. brucei* in cattle, sheep and goats and *T. simiae* in pigs (Sackey, 1998).

2.3 Taxonomy

Trypanosomes are a group of monophylectic, unicellular organisms belonging to the phylum Protozoa, of the genus *Trypanosoma*, family Trypanosomastidae and of the order Kinetoplastida, of which a major characteristic of this order is the possession of a mitochondrial genome called kinetoplast (Hamilton *et al.*, 2004, Stevens and Brisse, 2004)**.** The genus *Trypanosoma* is further subdivided into two sections, namely the Stercoraria and Salivaria, based on how the parasites are transmitted from the insect vector to the mammalian host once the parasite has completed its cyclic development (Desquesnes *et al.*, 2013). The important pathogenic trypanosomes causing severe diseases in animals and man belong to the *Salivarian* group of trypanosomes. The parasites develop into the metacyclic stage in the anterior part of the digestive tract of the tsetse fly and they are inoculated via the saliva into the mammalian host (Stevens and Brisse, 2004). The *Salivarian* group is made up of sub-genuses *Nannomonas, Duttonella, Pycnomonas* and the *Trypanozoon*.

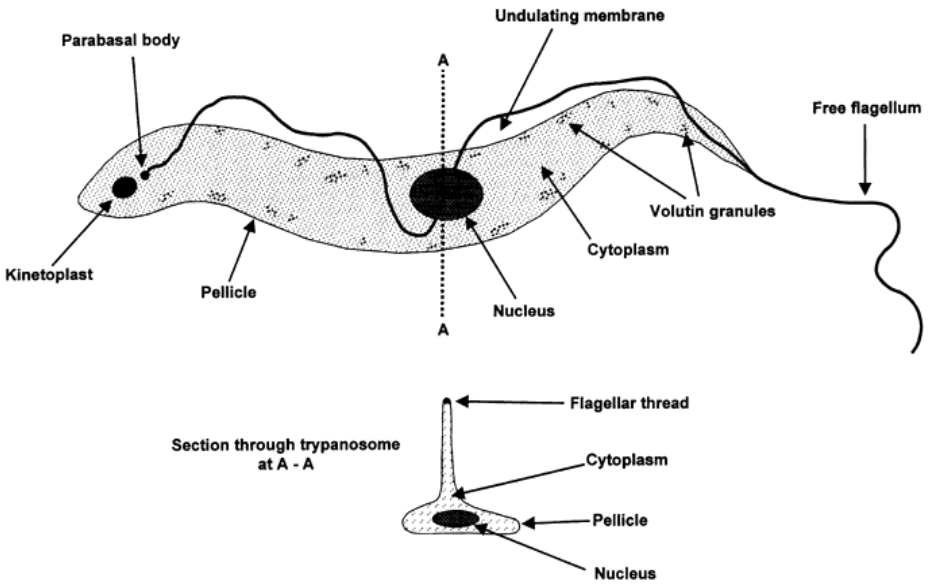

Figure 2.1: Diagrammatic representation of the major anatomical features of a trypanosome

Source: Uilenberg (1999): *A field guide for the treatment and diagnosis of African Animal Trypanosomosis*. http://www.fao.org/DOCREP/oo6/x0413E01.gif

2.3.1 Subgenus Nannomonas

Species belonging to this sub genus are the smallest among the pathogenic trypanosomes, with a length of 9–22 μm, a centrally located nucleus with a medium size kinotoplast (Hoare, 1972). The kinetoplast is usually situated at the margin of the body; just in front of the posterior extremity (marginal and subterminal). The undulating membrane is poorly developed and inconspicuous. Generally two variants are known, a shorter form (9–18 μm), the typical *congolense* type and a longer form up to 25 μm (Hoare, 1972; Uilenberg, 1999).

Studies have resulted in a subdivision of the *T. congolense* into several "types". These are designated as *T. congolense* savannah type, *T. congolense* Tsavo type, *T. congolense* forest type, *T. congolense* Kilifi type (Majiwa *et al.*, 1985, 1993). One type *T. congolensi godfreyi* has received a separate species name as it is different in its pathogenicity to various hosts. It is pathogenic to pigs and runs a chronic course than disease caused by *T. simiae*. Another member of this sub genus is *T. simiae* which is pathogenic for pigs and morphologically similar to *T. congolense*, with an average length of 9–22 μm. The blood forms are monomorphic and they lack a free flagellum (Hoare, 1972).

2.3.2 Subgenus Duttonella

This sub genus is made up of two species-*Trypanosoma vivax* and *T. Uniforme. Trypanosoma vivax* is a medium size parasite with range of lengths from 18 to 31 μm (including free flagellum 3-6 μm long) (Hoare, 1972). However, *T. uniforme* are smaller (from 12 to 20 μm) and similar to *T. vivax* (Molyneux and Ashford, 1983). The main characteristics of bloodstream forms of *T. vivax* are large terminal kinetoplasts situated at a rounded posterior extremity, a medium devel-

oped undulating membrane, and a free flagellum. The undulating membrane exhibits a medium level of development (more than in *T. congolense* and less than in *T. brucei*) and a free flagellum is always present. The kinetoplast is rounded, ranging in shape from circular to elliptic, and is usually terminally positioned. In some forms, however, it can be subterminal or marginal. Among other characteristics, the size of the parasite, the rounded posterior extremity and the position of the kinotoplast of *T. vivax* distinguishes it from other Salvarian trypanosomes (Desquesnes, 2004).

Trypanosoma vivax is characterized by its capacity to quickly cross the microscope field of vision while other parasites move around. Its intermediate undulating membrane distinguishes it from large membrane ones exhibited by trypanozoan parasites such as *T. brucei* which can trap light thereby creating typical "pockets of light" (Uilenberg, 1999; OIE, 2013).

2.3.3 Pycnomonas

The subgenus Pycnomonas is represented by a single species, *Trypanosoma (Pycnomonas) suis*, which is characterized by stout monomorphic forms with a free flagellum and a small sub terminal kinetoplast. It develops in *Glossina* like the tsetse-borne species of Trypanozoon: the midgut and salivary glands. Pigs are the only domestic mammalian hosts. *Trypanosoma suis* is the least known pathogenic trypanosome (Mulligan, 1970).

2.3.4. Subgenus Trypanozoon

The subgenus Trypanozoon is the most homogeneous group of the Salivarian trypanosomes, represented by species which are morphologically indistinguishable but differ in biological

features. This group comprises five members: *T b. brucei*, *T. b. gambiense*, *T. b. rhodesiense*, *T. evansi* and *T. equiperdum.*

T. brucei is polymorphic, with three main forms, all of which have a small kinetoplast and a conspicuous undulating membrane.

2.3.4.1 Trypanosoma brucei

Trypanosoma brucei is subdivided into three subspecies*: T. brucei brucei,* a primary African trypanosome transmitted by tsetse flies, not infective to humans, *T. brucei gambiense,* the causal agent of classical or Gambian human sleeping sickness, and *T. brucei rhodesiense,* which causes the type of human sleeping sickness common in Zimbabwe. The three sub species of *trypanosoma brucei are* transmitted by tsetse flies (in contrast to *T. evansi and T. equiperdum)* and are similar in morphology. They are polymorphic with a small kinetoplast and a conspicuous undulating membrane**.**

2.3.4.2 Trypanosoma evansi

These species of trypanosome cannot be distinguished morphologically from the long slender forms of *T. brucei*, and it is almost certain that *T. evansi* had developed from *T. brucei* by continual mechanical passage, the vectors being blood-sucking flies, especially those of the Tabanid family (Hoare, 1972). Direct mechanical transmission has resulted in *T. evansi* losing its ability to undergo the cyclical development in tsetse flies. *T. evansi* is typically represented by thin, slender and intermediate form corresponding to those in *T. brucei*. The slender forms have a long free flagellum and a narrow posterior extremity, which may be rounded or truncated, with the kinetoplast situated at some distance from the tip. The intermediate forms have a shorter free

flagellum and a short, frequently pointed, posterior extremity, with the kinetoplast lying near this end (Hoare, 1972).

2.3.4.3 *Trypanosoma equiperdum*

This trypanosome is probably also derived from *T. brucei*. Its morphology is identical to that of *T. evansi* and the long slender forms of *T. brucei*, but it is different because it causes a natural disease only in animals of the equine family (horse, donkey, mule), among which it is transmitted by genital contact. They cause venereal disease called dourine in the equine species. Its transmission is not dependent on insect vectors. The disease has spread as far north as Canada, Russia and other European countries, and as far south as Chile in South America. *Trypanosoma equiperdum* is morphologically indistinguishable from *T. evansi*. It is typically monomorphic, being represented by thin (slender and intermediate) trypomastigotes possessing a free flagellum (Hoare, 1972).

2.4 Host Range to Trypanosoma Species

All species of domestic animals are susceptible to infection by one or more species of the salivarian trypanosomes (Agu and Bajeh, 1987; Anosa, 1988; Kalu *et al.*, 1996; Budd, 1999; Hursey, 2000). The three most pathogenic trypanosoma species infects cattle, sheep, goats, camels, horses, dogs, pigs, cats (Stephen, 1970) and reportedly monkeys, rats, mice, guinea pigs, rabbits are susceptible to trypanosomal infection (Molyneux and Ashford, 1983). Because of the high susceptibility of the small mammals to trypanosome infection they are commonly used for research models for further elucidation of trypanosomal infection. More than 30 species of wild animals, for example, the antelope, monitor lizard, chimpanzee, vervet monkey (Godfrey and

Killick-Kendrick, 1967; Njagu *et al.,* 1999; Jelinek *et al.,* 2002; Abenga and Vuza, 2005) among others, have been found to be susceptible to pathogenic trypanosomes. Wild equidae, lions, leopards, and wild pigs are susceptible and can serve as carriers of trypanosomes (Molyneux and Ashford, 1983). Many of these remain carriers of the organisms and become active reservoirs of infection (Njagu *et al.,* 1999; Welburn *et al.,* 2001). Non mammalian animal species such as fishes (Overath *et al.,* 1999; Nico *et al.,* 2004), amphibians (Christine and David 2007), reptiles (Stephen *et al.,* 1983; Njagu *et al.,* 1999), birds (Baker, 1976; Apanius, 1991; Peirce, 2003) and even plants (Camargo, 1999; Dollet *et al.,* 2000; Jaskowska *et al.,* 2015) are shown to be susceptible to *Trypanosoma* infection. These pathogenic trypanosomes are reported to induce a wide range of disease condition ranging from sub-clinical, acute to chronic syndrome which may terminate in death depending on the *Trypanosoma* species and host susceptibility (Rodostits *et al.,* 2006).

2.5 Pathogenesis of Trypanosomosis

Trypanosomal infection is established when infective metacyclic trypanosomes are introduced into the host either through tsetse bites, by mechanical or iatrogenic inoculation (Jones and Davila, 2001; Desquesnes and Dia, 2004). Once the metacyclic trypanosomes are injected into the host by the vector during feeding, they multiply at the subcutaneous site provoking a local skin reaction called a chancre which is most pronounced in a fully susceptible host and may be slight or absent with some strains or species of trypanosomes (Rodostits *et al.,* 2006). Chancre is a cutaneous reaction due to the multiplication of parasites at wound site due to insect bite where polynuclear cells, lymphocytes and macrophages congregate. It occurs when trypanosomes invade the site locally and is no doubt connected with *Glossina*-borne transmission (Emery and

Moloo, 1981 and Dwinger, 1985). The composition of the cells within the chancre suggests that the reaction consists of an initial inflammatory reaction followed by an immune response. Within a chancre, metacyclic parasites change to trypomastigote form and enter the blood stream directly or through the lymphatics and initiate characteristic intermittent parasitaemia (Rodostits *et. al*., 2006).

Successful inoculation is usually followed by a pre-patent period when the parasites undergo rapid multiplication within the host's blood. Pre-patency has been reported to be variable and depends on factors such as the species of the infective organism and the susceptibility of the host (Omeke and Onuora, 1992; Urquhart *et al.,* 1996; Adeiza *et al.,* 2008). The onset of trypanosomosis is characterized by an initial increase in body temperature (Dargie *et al.,* 1979). However, other reports indicate that in some animals the onset and even development of the pathology may be asymptomatic (Munang'andu *et al.,* 2010). Initial pyrexia according to Uilenberg, (1999) occurs as a response by the host's humoral system to the rapidly multiplying parasites. Peaks of high parasitaemia and accompanying pyrexia persist for several days before a trypanolytic crisis occurs, markedly reducing parasite population and inducing a return to near-to-normal body temperature (Omeke and Onuora, 1992). Authie *et al.* (1993) had observed that trypanolysis occurs when the trypanosome surface glyco-proteins provoke the host into making specific antibodies called immunoglobulin M (IgM) against the glyco-proteins. These immunoglobulins then destroy the parasites and lead to the development of immune complexes. Several studies have however shown that parasite elimination is not total, but that due to the trypanosmes' unique attribute of antigenic variation, a few trypanosomes are able to survive by replacing their surface glycoproteins with new ones against which the IgM is unable to act (Capbern *et al.,* 1977; van Miervene *et al.,* 1977). The surviving trypanosomes subsequently

replicate causing new wave of parasitemia while the host attempts to make new antibody antagonists to the new surface proteins (Cross, 1978; Vickerman, 1978). This situation is perpetuated until the trypanosomes antigenic repertoire is exhausted in which case, self-cure may occur, or the host's ability to respond to all the antigenic variants is diminished and its humoral responses are overwhelmed, thus resulting in debility and death (Stephen, 1986; Mare, 1998; Penchenier *et al.,* 2005).

Their behavior thereafter depends largely on the species of trypanosome and the host. *T. congolense* localizes in the endothelial cells of small blood vessels and capillaries principally causing severe anemia and mild to moderate organ damage. *T. b. brucei* and *T. vivax* on the contrary localize in the heart, brain and connective tissue (Rodostits *et al.,* 2006). It is based on this behavior that tsetse-transmitted pathogenic trypanosomes may be divided into two groups: Those that invade and are confined to the plasma and blood vessels, i.e. the *haematic group* (*T. congolense* and *T. vivax),* and the *humoral group*, (*T. brucei, T. b. rhodesiense* and *T. b. gambiense),* in addition to infecting the plasma, they infect the intercellular tissue fluids, particularly the connective tissues, and fluids of body cavities (Losos and Ikede, 1972; Abubakar *et al.,* 2005; Chretien and Smoak, 2005; Ngure *et al.,* 2008). These different distributions in the host govern the type of lesions responsible for the disease. While the *haematic group* causes more of haematological and immunological injuries with minimal solid tissue and organ changes, the humoral group on the other hand causes extensive degenerative, necrotic, and inflammatory changes, in the various organs and tissues with secondary haematologic changes (Losos and Ikede, 1972; Abebe, 1991; Luckins *et al.,* 1994; Ngure *et al.,* 2008).

The most significant factor in trypanosomosis is the profound immune suppression that occurs following infection by these parasites **(**Rodostits *et al.,* 2006**)**. Immuno-depression was found to compromise the capacity of the immune system to effectively respond to secondary infections which would have been normally resisted by the host. In this regard, Nyeko *et al.,* (1990) asserted that the pathological features of AAT were often complicated by the presence of concurrent infections involving secondary bacterial infection, *Babesia, Theileria and Anaplasma* species

2.5.1. Haematological changes

Haematological changes have been reported in African animal Trypanosomosis (Sekoni *et al.,* 1990; Ogunsanmi *et al.,* 1994; Adeiza *et al.,* 2008). The main changes include anaemia, thrombocytopaenia and leucopaenia, which are intensified as the disease progresses (Robins-Browne *et al.,* 1975; Stephen, 1986). The anaemia in trypanosomosis is characterized by a rapid reduction in RBC count, hemoglobin concentration (Hgb) and packed cell volume (PCV). This had been a consistent finding in trypanosome infected cattle, goats, sheep, dogs and rabbits (Taiwo *et al.,* 2003; Bisalla *et al.,* 2007; Gow *et al.,* 2007; Kobo *et al.,* 2014) under experimental condition. Total white blood cell (WBC) counts also vary from normal range to significant reduction in animal trypanosomosis (Taylor *et al.,* 1996; Osman *et al.,* 2008). The severity of these changes is highly variable and depends on the virulence of the species and strain of trypanosome (Anosa, 1983; Kalu., *et al.,* 1991) and factors associated with the host like age, breed, nutritional status of infected animals (Murray and Dexter, 1988) and acuteness of the infection (Jenkins and Facer, 1985; Stephen, 1986). The mechanism of anaemia in trypanosomosis is said to be complex and multifactorial in origin (Naessens *et al.,* 2005). Widespread phagocytosis of blood cells, such as erythrocytes and platelets, by macrophages

invading bone marrow sinusoids is seen (Murray and Dexter, 1988). This anaemia could also be due to the haemolysins such as proteases, phospholipases, sialidases and neuraminidases produced by the trypanosomes (Esievo, 1983; Nok *et al.,* 2003; Girma *et al.,* 2014) which causes red blood cell (RBC) surface alterations thus, leading to their subsequent phagocytosis (Nok *et al.,* 2003; Buratai *et al.,* 2006).

2.5.2 Serum biochemical changes

Trypanosomes depend on the host's supplies of carbohydrates, proteins, lipids and some micronutrients. The capacity of a parasite to import critical nutrients is central to its ability to be transmitted, to infect a host, and to cause disease (Scott, 2011). During periods of high parasitaemias, the parasite depletes energy supplies of the host and release metabolites which may have adverse effects on the host (Seed and Hall, 1985). The parasite increases the host energy requirement (Verstegen *et al.,* 1991) thereby causing a state of energy deficit (Seed and Hall, 1985). Similarly, there is evidence to show that trypanosomes may have adverse effect on the energy metabolism of the host through its metabolites and alteration of the host's hormonal balance (Mutayoba and Gombe, 1989; Abebe and Eley, 1992; Ogwu *et al.,* 1992).

Trypanosome have been shown to take up and digest protein molecules in their organelles (Brown *et al.,* 1965; Lengreth and Balber, 1975), free amino acids, (Gutterridge and Coombs, 1977), fatty acids (Mellor and Samad, 1989) and a host of other micronutrients such as amino-benzoic acid, vitamins like thiamine, folic acid, riboflavin, cobalamin, ascorbic acid, nicotinamide (Von Brand, 1973) and some nucleotide precursors such as hypoxanthine and thymidine (Baltz *et al.,* 1985).

2.5.2.1 Serum enzymatic changes

Significant elevations of serum enzymes indicate that the integrity of the vital organs such as the liver in the case of elevations in ALP, ALT, or AST and skeletal and cardiac muscles in case of CK were compromised following infection with trypanosomes **(**Ezeokonkwo *et al.,* 2012**)**. Changes in enzyme levels are good markers of soft tissue damage and that damage to body cells results in alteration of membrane permeability and consequent release of enzymes into the extracellular fluid (Obaleye *et al.,* 2007).

A rise in AST activity can be attributed partly to cellular damage caused by the trypanosomes lysis, while the increase in ALT activity probably results from host destruction of trypanosomes (Enwezor and Sackey, 2005). The causes of the elevation of AST levels in the serum of animals are necrosis of the liver, skeletal muscles and kidneys (Lording and Friend, 1991). Raised levels of alkaline phosphatase (ALP) can also be seen in inflammatory conditions of the gut and liver, while active hepatocellular damage is reflected by increases in plasma levels of AST and ALT (Lording and Friend, 1991).

Serum enzymes alteration have been reported in several experimental conditions such as rabbits and rats experimentally infected with *T. brucei* (Orhue *et al.,* 2005) and *T. congolense* (Egbe-Nwiyi *et al.,* 2005; Takeet and Fagbemi. 2009), swine experimentally infected with *T. brucei* (Allam *et al.,* 2011), vervet monkeys experimentally infected with *Trypanosoma brucei rhodesiense* (Ngure *et al.,* 2008) and cattle experimentally infected with *Trypanosoma vivax* (Dagnachew *et al.,* 2014).

2.5.2.2. Serum glucose changes

Trypanosomes have very small polysaccharide store and glucose is the most important exogenous substrate used by the blood stream forms for energy (Gutteridge and Coombs, 1977). Energy is generated primarily during glycolysis in specialized microbodies called glycosomes (Fairlamb and Opperdoes, 1986). The products of this metabolism are mainly pyruvate, glycerol, acetate and carbon dioxide (Gutterridge and Coombs, 1977). However, the adverse effect of infection with trypanosomes is reflected with impaired gastrointestinal absorption of nutrients as result of impaired endocrine imbalances (Ogwu *et al.,* 1992) even though gastrointestinal lesion has not been associated with trypanosomosis. Similarly trypanosomosis causes 15-16% increase in metabolic rate (Blaxter, 1989) and a 25% rise in maintenance requirements (Verstegen *et al.,* 1991) for every degree rise in body temperature. Consequently, there is little synthesis of depot fat and glycogen and stored energy substrates are metabolize for energy because of the great energy demand (Stephen, 1970; Ikede and Losos, 1975).

Hypoglycaemea has been reported in trypanosome infection (Kaushik *et al.,* 1989; Laha *et al.,* 1991). It has been hypothesized that the hypoglycaemia observed in some cases of trypanosomosis may be due to a failure of the endocrine mechanism controlling the mobilization of carbohydrates reserves (Von Brand, 1973) resembling diabetes mellitus (Vooheis, 1969). Recent reports of endocrine dysfunctions in trypanosomosis seem to support these hypotheses. Infection with trypanosomes causes polyglandular failure by local inflammation of the pituitary, thyroid, and adrenal glands (Abebe *et al.,* 1993; Fatihu *et al.,* 2009; Leigh *et al.,* 2015).

2.5.2.3 Protein changes

A decrease in blood levels of several amino acids has been reported in trypanosomosis. These amino acids include tryptophan, threonine, arginine, asparagines, serine, valine, isoleusine, and leucine (Newport *et al.,* 1977; Isoun *et al.,* 1978; 1979). Trypanosomes in the blood stream are known to take up and utilize plasma amino acids and proteins (Brown *et al.,* 1965; Langreth and Balber, 1975; Gutterridge and Coombs, 1977) and may contribute to the lowering of the plasma amino acids or protein in the hosts.

However, inconsistent abnormalities in serum protein metabolism have been reported in several experimental animal models with various species of trypanosomes. The serum protein level however, could remain normal, increased or decreased (Anosa, 1988). Hypoproteinemia was also reported in various experimental animals infected with various species of trypanosomosis (Osaer *et al.,* 2000; Biryomumaiso *et al.,* 2003; Bisalla *et al.,* 2007; Sulaiman and Adeyemi 2010; Allam *et al.,* 2011; Nwoha *et al.,* 2013). Total serum protein decreases as result of trypanosomes increased uptake coupled with increase catabolism by the host. However, in contrast to these observations, considering the role protein plays in the course of trypanosome infection (Romney *et al.,* 1994) an increase in the level of the serum protein may be observed. During the acute phase of the disease, protein requirement is increased in response to production of chemical mediators released by macrophages and leukocytes during inflammatory and infectious processes (Kent, 1992). Increase in total protein could be due to elevation in the gamma-globulin, as immunological response against the parasite (Orhue *et al.,* 2005; Hilali *et al.,* 2006; Mahmood *et al.,* 2014). Hyper gamma globulinaemias in African trypanosomosis on the other hand is usually

associated with the increase in immunoglobin M (IgM) which is a consistent finding in trypanosomosis of man and animals (Anosa, 1988).

2.5.2.4. Lipid changes

Lipids constitute 15-20% of trypanosomal dry weight (Venkatessen and Ormerod, 1976). They obtain their cholesterol requirement from the host by uptake and degradation of low density (Coppen *et al.,* 1987; Gillet and Owen, 1987) or high-density lipoproteins (Traore-Leroux *et al.,* 1987). Blood stream forms of trypanosomes which are unable to synthesize cholesterol are known to require it along with phospholipids and total lipids for membrane synthesis and growth (Hue *et al.,* 1990; Nok and Balogun, 2003). The enormous requirement of lipids may lead to its depletion in an infected hosts (Katunguka-Rwakishaya *et al.,* 1991) which could be associated for example with a hypolipidaemia, hypophospholipidaemia and hypocholesterolaemia in experimental infection (Traore-Leroux *et al.,* 1987). Abnormalities of lipid metabolism have been identified in several laboratory and domestic animals infected with various species of trypanosome (Anosa, 1988). Reduced cholesterol level have been reported in cattle infected with *T. vivax* (Dagnachew *et al.,* 2014), *T. congolense* and *T. brucei* infection in small East African goats (Biryomumaisho *et al.,* 2003) in pigs (Eze *et al.,* 2015) in sheep (Taiwo *et al.,* 2003), *T. evansi* in camel (Sazmand *et al.,* 2011), and *T. congolense* in sheep (Adamu *et al.,* 2008).

2.5.2.5 Changes in serum minerals

Serum minerals are inorganic substances essential to maintain the normal function and living status of domestic animals (Sharma *et al.,* 2006; Kurćubić *et al.,* 2010; Soetan *et al.,* 2010). These elements are needed for physiological processes related to health, growth and reproduction, such as permeability of cell membranes, bones and teeth formations, blood

clotting mechanism, the regulation of immune system, hormone production, enzyme reactions, energy production, biomolecule metabolisms, vitamin synthesis, neuromuscular transmission, milk production, genetic transmission of nucleic acids and muscle and heart contraction (Asif *et al.,* 1996; Dhanotiya, 2004; Sharma *et al.,* 2006; Hosnedlova *et al.,* 2007). Maintaining minerals in the form of electrolytes in appropriate size and amounts is essential for normal biochemical and physiological functions of the body. Many diseases including trypanosomosis cause changes in fluids and electrolytes balance (Rodostits *et al.,* 2006; Smith, 2007). Alterations in the concentrations of these minerals results in reduced animal performance, such as impaired reproduction, a high incidence of mastitis, reduced milk yield, impaired immunity, increased degree of lameness due to laminitis and subsceptibility to infections (Dvořák *et al.,* 1980; Ballantine *et al.,* 2002; Dobrzański *et al.,* 2005; Nocek *et al.,* 2006).

Concentration of sodium was found to be increased and that of potassium decreased in sheep infected with *T. congolense* (Tella, 2005) while the levels of potassium in these same species infected with *T. brucei* was reported to be reduced (Ogunsanmi *et al.,* 1994b). However, no changes in these minerals were observed in *T. brucei*-infected boars, when compared to non-infected counterparts (Otesile *et al.,* 1991). Calcium and phosphorus concentrations remained unaltered in cattle infected with *T. vivax* (Schenk *et al.,* 2001) and camels infected by the protozoan (Chaudhary and Iqbal, 2000). However, sheep infected with *T. congolense* (Neils *et al.,* 2006) and rabbits infected with *T. evansi* (Da silva *et al.,* 2011) were shown to have increased levels of calcium and phosphorus.

Some of the clinical signs presented by the *Trypanosoma* infected animals like edema, disorientation, instability and paralysis of hind limbs might be related to the changes in concentrations of sodium and potassium (Marques *et al.,* 2000; Herrera *et al.,* 2004; Da Silva *et al.,* 20011), since these minerals are involved in the regulation of the acid-base balance and in the volume of body fluid, participating also in the transmission of nerve impulses and in muscle contraction (McDowell, 1992). Similarly alteration in calcium level may not be unconnected with some of the manifested clinical sign like incoordination and instability of hind limbs, atrophy of the large muscles of the limbs, difficulty to stand up and muscle weakness. Moreover, low calcium concentrations might be involved in clotting disturbances that are often identified in trypanosomosis as reported in dog infected with *T. evansi* and cat also infected with *T. evansi* (De La Rue *et al.,* 1997; Da silva *et al.,* 2010). More so, calcium is essential for the life of trypanosomes (Clarkson and Amole, 1982), and consequently trypanosomes have adapted a mechanism of calcium storage (Mendoza *et al.,* 2004).The affinity of trypanosomes to the muscle can be easily explained by its need for the calcium that is involved in muscle contraction (Da silva *et al.,* 2011).

Similarly, alterations in the concentrations of micro-minerals like copper, iron and zinc have also been reported in rabbits, rats, cats, and sheep infected with various species of trypanosomes (Mwangi *et al.,* 1995; Neils *et al.,* 2007). Alterations in the concentrations of copper, iron and Zinc have been reported in rabbits and sheep infected with *T. brucei* and *T. congolense*, respectively (Mwangi *et al.,* 1995; Neils *et al.,* 2007). Also, increased levels of copper and decreased levels of iron and zinc were observed in cat and Wister rats infected with *T. cruzi* (Mwangi *et al.,* 1995; Gutierrez *et al.,* 2006; Neils *et al.,* 2006, 2007). Fluctuated levels of

copper have also been reported in bovines and sheep infected with *T. vivax* and *T. congolense*, respectively (Joshua *et al.,* 1994; Neils *et al.,* 2006).

The depletion of iron in trypanosome infection could be attributed to the high demand for regenerative haemopoesis (Saror, 1976; Wolkmer *et al.,* 2007), the need for iron by the lymphoid cells for the process of cell division and iron need by the the parasite itself, since the mineral is used for its own growth and multiplication (Weinberg, 1978). Therefore, the decrease in iron concentrations might cause a reduction in mitotic activity of T lymphocytes, reduction in the production of lymphokynes, decreased production of antibodies and reduced phagocytic activity (Saker, 2006). Studies have also shown a decrease in immunoglobulin level in *Trypanosoma musculi* infected mice (Humphrey *et al.,* 1994). Reduced level of Zink causes immune defects associated with thymic hormone production and activity, impaired lymphocyte, reduction in natural killer cells and reduction in the chemo-taxis of neutrophils and monocytes (Fraker and King, 2004; Saker, 2006). Other signs of long standing Zink deficiency include decreased efficiency of food utilization, impaired growth, diarrhea, poor weight gain severe dermatitis, dry scaly coat, alopecia, hyperkeratosis, conjunctivitis and delayed sexual maturation (Clegg *et al.,* 2006).

2.6 Pathological Changes in Trypanosomosis

The pathological alterations due to trypanosome infection depend on one or more species of trypanosomes that cause infection, on the strains within any one species, and on the species of the infected host. The hematic trypanosomes (*T. congolense* and *T. vivax*) cause injury to the host mainly by the production of severe anaemia, which is accompanied in the early stages of the

disease by leukopenia and thrombocytopenia. These trypanosomes in acute cases causes generalised congestion of the viscera and extensive hemorrhages in all tissues. Chronic cases show cachexia, often complicated with secondary bacterial or other parasitic diseases. In the terminal stage, massive accumulation of parasites in the terminal capillaries of the brain results in ischemia as a results of focal polioencephalomalacia (Losos and Ikede, 1972). Accordin to them no pathognomonic gross lesion has been seen in animals that died of trypanosomosis, but as the infection becomes chronic the carcass become anaemic, emaciated and usually cachexic. The lymph nodes, spleen and the liver have been variously described as enlarged, normal, atrophied or fibrosed (Losos and Ikede, 1972; Morrison *et. al*., 1981; Kaggwa *et. al.,* 1983). Haemorrhages were reported in the lymph nodes, diaphragm, the viscera (intestines and mesentery) and the central nervous system (Morrison *et al.*, 1981). Tissue lesions associated with trypanosoma infection include myositis, myocarditis and pericaditis characterized by degeneration of muscle tissues (Urquhart, 1980, Wellde *et al.*, 1989; Abenga, 2014).

The involvement of the CNS is associated with chronic infection which may manifest grossly as oedema of the brain and meninges. Other parts of the brain commonly involved include the thalamus, hypothalamus, hippocampus and basal ganglia (Ikede and Losos, 1972). Ocular lesions such as unilateral or bilateral conjunctivitis, keratitis, corneal opacity and sometimes blindness have also been reported (Losos and Ikede, 1972; Morrison *et al.*, 1981 and Abenga, 2014). Lesions leading to disturbances in reproduction due to uterine and placental pathology coupled with endocrine crisis in the female and marked testicular degeneration have been reported (Losos and Ikede, 1972; Poltera, 1985; Ikede *et al.*, 1988). These have been supported by observations made by Akpavie *et al.* (1987); Anosa and Isoun (1980); Ikede *et al.* (1988); and Sekoni *et al.* (1990). In female animals, severe genital lesions (Ikede *et al.*, 1988) results in abnormal oestrous

cycles, death during pregnancy, dystocia, abortion, premature birth, low birth weight, stillbirth, transplacental infection, neonatal death and other pathogenic effects on fetuses (Sekoni, 1994; Bawa *et al.,* 2000; Faye *et al.,* 2004) while in the male the severity of testicular and epididymal lesions is reflected in poor quality semen and high percentage of abnormal spermatozoa present in the ejaculate of bulls and rams under experimental infection (Isoun *et al.,* 1975; Akpavie *et al.,* 1987; Wada *et al.,* 2016).

2.7 Histopathological Changes

Several authors have reported histopathological changes in animals infected with trypanosomes (Losos and Ikede, 1972 Maxie *et al.,* 1997; Anosa and Isoun 1983; Olubayo and Mugera, 1985; Biswas *et al.,* 2001; Omotainse and Anosa, 2009; Leigh *et al.,* 2015).The interstitial activities of trypanosomes in tissues attract severe inflammatory reaction in various organs (Losos and Ikede, 1972; Anosa and Kaneko, 1983a, b; Poltera 1985) which are characterized by mononuclear cellular infiltration composed largely of lymphocytes and plasma cells presumably in response to the need to phagocytize parasites and damaged erythrocyte; thickening of the alveolar septa of the lungs; and dessiminated intravascular coagulation, with formation of thrombi, particularly in the brain, kidneys and testicles (Anosa and Isoun, 1983).

2.8 Risk Factors for Trypanosome Infection

The degree of risk to which domestic animals are exposed to trypanosomosis depends on three major factors: the species and distribution of the vectors, the strain and the virulence of the trypanosome, and the response of the host (McLennan, 1970; Van den Bosche *et al.,* 2010).

2.8.1 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 (Prowse, 2005). The repeated switching of the glycoprotein coat is now known to depend on a loosely ordered sequential expression of an undefined number of genes, each coding for a different glycoprotein coat. This together with 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 re-infection (Urquhart *et al.,* 1996).

2.8.2. The vector

Tsetse flies are biological vectors of African trypanosomosis in animals and man. Their distribution and prevalence are mostly influenced by spatial factors such as climate, vegetation and land utilization (Rogers *et al.,* 1996). The occurrence and impact of trypanosomosis, on the other hand, depends on tsetse challenge, host distribution, livestock breeds, farming practices and control practices. Tsetse challenge is determined by the product of relative tsetse density, trypanosome prevalence in tsetse and the proportion of meals obtained by the tsetse from a defined host (Leak, 1988).

Of the group of *Glossina* flies, the savannah and riverine varieties are the most important since they inhabit areas suitable for grazing and watering. While each fly may be infected for life with trypanosome, the infection rate for tsetse ranges from 1-20% of the flies **(**Pollock, 1982**)**. 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 of 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 on the other hand is a better transmitter of *T.congolense* than *G.swynnerton* (Stephens 1986). Tsetse flies prefer to feed on particular hosts. The bushbuck for example is much preferred whilst the waterbuck is not. 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).

2.8.3. The host

A major feature of tsetse transmitted animal trypanosomosis is that it can affect a complete spectrum of domestic animals including cattle, sheep, goats, pigs, equidae, camels, dogs, cat and birds (Murray, 1989). All of these species can develop marked parasitaemias if infected with appropriate trypanosome. However, within each host, several other features have been shown to have a significant effect on the intensity and duration of parasitaemia and severity of the disease. Breed and environment are two factors that can affect host susceptibility to infection with trypanosome (Murray, 1989). It has been recognized that certain breeds of cattle, sheep and goats are more resistant to the effect of trypanosomosis. This trait has been termed trypano-tolerance, and is generally attributed to the indigenous taurine breed of cattle in west and central Africa. This trait has also been reported in the Orman Boran (*Bos indicus*) in Kenya (Njoku *et al.,* 1985), although it has been found that most *Bos Indicus* breeds, and to a greater extent, the European breeds are highly susceptible. A large number of other factors are believed to affect host susceptibility to trypanosomosis possible by influencing parasites kinetics. Factors

incrimated include age, nutrition, sex, stressors, previous exposure, migration and management practice (Murray, 1989).

2.9 Epidemiology of Trypanosomosis

2.9.1 Occurrence of trypanosomosis

The epidemiology of African trypanosomosis is determined mainly by the ecology of the tsetse fly which is found only in tropical Africa occurring in some 37 countries and across 10 million km^2 of tropical Africa. *Trypanosoma vivax* is also transmitted mechanically by biting flies and occurs also in Central and South America where it affects mainly cattle and sheep (Rodostits *et al.,* 2006). *Trypanosoma congolense* and *T. vivax* are responsible for severe disease in cattle, sheep and goats, while *T. brucei brucei* usually causes a subclinical infection in cattle, but a severe disease in sheep, goats, horses and occasionally, pigs. *Trypanosoma simiae* causes a very acute and highly fatal disease in exotic pigs. It is not pathogenic to cattle, sheep, or goats (Rodostits *et al.,* 2006). There are 31 species and subspecies of tsetse flies identified at present (Leak, 1999). Eleven of these known species of tsetse flies are found in Nigeria (Baldry, 1964), infesting approximately 74% (686,488 km^2) of the country's landmass from approximately latitudes 4^oN to latitudes 12^oN covering all the agro-ecological zones of the country (Jawonisi 1988), including the highlands of Jos, Mambilla and Obudu plateux, which were hitherto described as tsetse and trypanosomosis free (Marshal, 1948; Dede *et al.,* 1996, Majekodummi *et al.,* 2013).

2.9.2 Prevalence of trypanosomosis

A number of workers have reported different prevalences using different diagnostic techniques in various parts of Nigeria. In North Central Nigeria, Kalu, (1995c) reported a prevalence of 9.0% prevalence among trypanotolerant breeds of cattle (Muturu, N'Dama, Keteku and their crosses)

in the lower Benue area of Nigeria, while Kalu *et al.* (1991) reported 21.3% prevalence among semi nomadic animals in sleeping sickness endemic area of Benue state Nigeria. Enwezor *et al.* (2012) also reported prevalence of 3.8% among cattle in three Local Government Areas (LGAs) of Benue state, namely, Oju, Guma and Gwer West. In plateau state, Majekodummi *et al.* (2013) reported prevalence rate of 46.8% with *Trypanosome vivax* being the dominant species in the survey accounting for 26.7% of all the identified species of trypanosomes. Adama *et al.* (2007) reported an incidence of 6.3% among the white Fulani breed of cattle In Niger state. In the upper part of Northern Nigeria on the other hand, Abenga *et al.* (2004), reported a prevalence of 9.7% in Lere Local Government area of Kaduna state with *T. vivax* as the dominant species, accounting for 82.9% of identified species of trypanosomes. *T. congolense* and *T. brucei* accounted for 14.9% and 4.3% respectively. In a survey of cattle at slaughter in Kaduna State, Samdi *et al.* (2011) reported 20.2% infection rate among the cattle with *T. congolense* as the dominant species accounting for 46.7.% of all identified species of trypanosomes. While *Trypanosoma vivax* and *T. brucei* accounted for 13.3% and 20.0% respectively. Similarly, in Song local Government area of Adamawa State a prevalence of 26.67% was recorded (Zubairu *et al.*, 2013). *T. vivax* was also the predominant species infecting the surveyed animals.

In the Western part of Nigeria, Ameen *et al.* (2008) in Ogbomosho area of Oyo State reported a prevalence of 3.9% in cattle with *T. congolense* being the only specy identified in the survey while Sam-Wobo *et al.* (2010) recorded 31.62% prevalence in a survey carried out on Bovine trypanosomosis and its impact on cattle in derived savanna areas of Ogun State. They also found *T. vivax* as the dominant trypanosome infecting cattle in Ogun State.

In a study of trypanosomosis in ruminants in parts of Abia state, Ohaeri, (2010) recorded 3.7% prevalence with *T. vivax* and *T. congolense* as the major trypanosomes infecting cattle each accounting for 80% and 20% respectively. In a study across all agro-ecological zones in Nigeria, Onyia, (1997) reported a prevalence of 8.6% in sheep, 8.1% in goat and 10% in cattle while survey by EEC- trypanosomosis control project reported 4.3% in cattle, 1.6% in sheep and 1.0% in goats. A lower rate of 5% was obtained on the high Plateau of Jos, Mambilla and Obudu, areas previously reported to be tsetse-free (Anene *et al.,* 1991, Anosa, *et al.,* 1993, Dede *et al.,* 1996).

2.9.3 Transmission of trypanosomosis

Trypanosomes are transmitted by tsetse flies of the genus *Glossina* and to a lesser extent by other biting flies (Tabanids and Stomoxys). Thirty-one species and subspecies of tsetse flies inhabit approximately 10 million km^2 between 15°N and 29°S (FAO, 1992; Phelps and Lovemore, 1994; Kettle, 1995). Of the 31 specie of *Glossina*, 23 species are found within the sub Saharan Africa and are adapted to a particular habitat and range of hosts (FAO, 1992; Kettle 1995). Tsetse flies are grouped into three main subgroups depending on the environment they inhabit: thus, riverine or *palpalis* group, savannah or *morsitans* group, and forest-dwelling tsetse or *fusca* group (FAO, 1992; Phelps and Lovemore, 1994; Kettle 1995)

In Nigeria, the major species of tsetse are the *Glossina morsitans* and *G. longipalpis* of the morsitans (Savanna) group species and *Glossina palpalis* and *G. tachinoides* of the palpalis (Riverine) group. Although tsetse of the fusca (forest) group are found in the southern part of Nigeria, they rarely come into contact with either human or livestock populations and are therefore of minimal importance as vectors of trypanosomosis **(**Putt and Shaw, 1982**)**. Morsitans group of tsetses have never been directly implicated in the transmission of human

trypanosomosis in Nigeria, they are however the most efficient vectors of trypanosomes pathogenic to livestock while species of tsetse belonging to the Palpalis group are the vectors of sleeping sickness but are less important as vectors of livestock trypanosomes. As such they have acted mainly as a constraint on activities which have brought humans into close contact with them, particularly when such activities have been associated with the development or exploitation of riverine areas **(**Cardonet and Mailard, 2002; Otto *et al.*, 2003**).**

2.9.3.1. Biological or cyclical transmission

When a tsetse fly hatches from its pupal case it is free from trypanosomes until its first bloodmeal when it will acquire trypanosomal infection as it feeds on an infected mammalian host (Uilemberg, 1999). The trypanosomes undergo a cycle of development and multiplication in the digestive tract of the fly until the infective metacyclic trypanosomes (meta-trypanosomes) are produced. Different trypanosome species develop in different parts of the digestive tract of the fly, and the metatrypanosomes occur either in the biting mouthparts or the salivary glands. Once infective metatrypanosomes are produced, the fly remains infective for the remainder of its life. 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 (Uilenberg, 1999).

2.9.3.2. Mechanical transmission

A mechanical insect vector is defined as any haematophagous insect that is able to bite several hosts in succession within a few minutes or hours. The residual blood and/or lymph that remains in the mouthpart possibly contains pathogenic agents (although these do not develop or multiply in the vector) and is inoculated through the saliva (Rodhain and Perez, 1985). It would be difficult to compile a complete list of the potential mechanical vectors for trypanosomes, especially in view of the fact that local factors of over abundance may turn a specy into a vector at a given point in time and space (Desquesnes, 2004). By way of example, Noirtin *et al.,* (1981) reported a record of 60,000 bites by blackflies in one day on one cow. In a situation like this, Simuliidae could very well become vectors of diseases. Any hæmatophagous insect that is able to pullulate in a stock farming area is therefore a potential mechanical vector for livestock trypanosomes; however, epidemiological and experimental observations indicate that the most important are some of the members of the Stomoxyinae, Hippoboscidae, Culicidae and especially Tabanidae families.

In Africa, mechanical transmission of *T. vivax* had been observed long ago (Bouet and Roubaud, 1912) and more recently, was confirmed in various instances under experimental conditions (Mihok *et al.,* 1995; D'Amico *et al.,* 1996; Sumba *et al.,* 1998; Desquesnes and Dia, 2004; Mohammed *et al.,* 2010; Baldacchino *et al.,* 2013). It is principally due to tabanids and *Stomoxys* spp., but other biting insects, including tsetse flies themselves, are involved as mechanical vectors (Moloo *et al.,* 2000).

2.9.3.3. Congenital transmission

Congenital transmission of various species of Trypanosomes had been reported by various workers **(**Ogwu *et al.,* 1986; Ikede *et al.*, 1988; Gardiner and Mahmoud, 1990, Elhassan *et al.*, 1995 and Okech *et al.,* 1996). It has not been clearly established whether the transmission of the parasite occurs by the transplacental and or the intravenous route when there is vascular breach during parturition. High parasitaemia observed immediately after birth argues in favour of infection before parturition (transplacental route) (Betancourt, 1978). Congenital transmission may be very important factor in the epidemiology of trypanosomosis (Gardiner and Mahmoud, 1990).

2.9.3.4. Iatrogenic Transmission

Experimentally, most of the trypanosome species including *T. vivax and T. evansi* may also be transmitted by syringe passage of infective blood (Van den Bossche *et al.,* 2000). Trypanosomosis may still be artificially transmitted through the shared use of a needle for several animals during application of medications or vaccinations (Desquesnes. 2004).

2.10 Clinical Signs of Trypanosomosis

At present, the information available on clinical manifestation of bovine trypanosomosis were drawn from several controlled studies which make it difficult for a “typical” clinical response to be drawn. What follows as clinical signs of trypanosomosis in cattle are summation of syndromes observed in experimental trypanosomosis caused by various species of trypanosomes (Maré, 2004). The cardinal clinical sign observed in AAT is anaemia (Murray and Dexter, 1988;

Biryomumaisho *et al.*, 2007). Within a week of infection with *T. congolense* or *T. vivax* there is usually a pronounced decrease in packed cell volume (PCV), haemoglobin and red blood cells, and within 2 – 3 months the PCVs may drop to below 30 percent of their pre-infection values. Also, invariably, present are intermittent fever, oedema and loss of condition. Abortion (Bawa *et al.,* 2000) and infertility of males and females may be a sequel (Sekoni *et al.,* 1990; Desquesnes, 2004; Kabir., 2014; Okubanjo *et al.,* 2014). The severity of the clinical response is dependent on the species and the breed of affected animals and the dose and virulence of the infecting trypanosome. Stress, such as poor nutrition or concurrent disease, plays a prominent role in the disease process (Taylor and Authie, 2004).

Trypanosoma vivax sometimes causes acute disease characterised by fever, high parasitaemia, severe anaemia and generalized visceral and mucosal haemorrhages particularly of the gastrointestinal tract. Cattle may die within 2 weeks or, under favourable conditions, rapidly self-cure occur after 2 months (Taylor and Authie, 2004). *Trypanosoma vivax* has a variable incubation period, ranges from 4-12 days in cattle with virulent isolates and 9-59 days in less pathogenic isolates (Hoare, 1972; Stephen, 1986). Although it is considered to be less virulent for cattle than *T. congolense*, mortality rate of over 50% can occur. There seems to be a marked variation in the virulence of different strains of *T. vivax* but it remains the most important cause of the AAT of cattle in West Africa. Certain African isolates of *T. vivax* can cause acute disease accompanied by haemorrhagic syndrome (Mwongela *et al.,* 1981; Roeder *et al.,* 1984).

In the field, the disease affecting adult cattle can be severe enough to lead to death or abortion even before diagnosis is reached and treatment can be started (Mwongela *et al.,* 1981). In the

aparasitemic phases, trypanosomes can be found extravascularly in lymph nodes (Hoare, 1972), eyes (in the choroid plexus and aqueous humor) (Whitelaw *et al.,* 1988) and cerebrospinal fluid (Tuntasuvan *et al.,* 1997). Affected animals exhibited fever, anaemia, weight loss, and hypoglycaemia, increased serum levels of aspartate aminotransferase, nervous signs (Batista *et al.,* 2007) and serious losses in production (Otte *et al.,* 1994).

Trypanosoma congolense is a hematic trypanosome found only in the blood vessels of the animals it infects. It does not localize and multiply outside blood vessels (Losos and Ikede, 1972). The clinical manifestations are insidious in onset without the development of any pathognomonic signs**.** Infection with *T. congolense* may result in peracute, acute, or chronic disease in cattle and the number of red blood cells tends to vary directly with the degree of parasitaemia (Valli *et al.,* 1978). The incubation period is followed by intermittent febrile episodes, depression, lethargy, weakness, loss of condition, anemia, salivation, lacrimation, and nasal discharge. Also, loss of condition and hair colour changes from black to metallic brown. The back is often arched and the abdomen "tucked up." Accelerated pulse and jugular pulsation occur and breathing is difficult. A peracute form of the disease has also been described (Parkin, 1935) which also appeared through the chronic course of infection as consisting of marked depression, high temperature, lacrimation, photophobia, nasal discharge and subcutaneous oedema. Signs of eye involvement such as epiphora, conjunctivitis, corneal opacity and sometimes blindness have also been reported (Ikede and losos, 1972; Whitelaw *et al.,* 1988). In the acute form, the drop in the red blood cell level was rapid and quickly became incompatible with life. The severity of anaemia at any time throughout the course of infection determines the severity of clinical signs (French and Hornby, 1938).

It has been reported that up to 30% of cattle in some regions of Nigeria may be infected with *T. brucei* (Goufrey and Killick-Kendrirc, 1961; Godfrey *et al.,* 1964; Gray, I970). This specie was considered to be non pathogenic, or at most only mildly pathogenic for cattle (Hornby, 1952; Henning, 1956; Stephen, 1970). On one occasion, however, it has been reported that severe disease and high mortality can occur in experimental infections in cattle (Mettam 1933; 1934). Infections are usually mixed with *T. congolense* and *T. vivax,* which are present in larger numbers than *T. brucei;* the latter can be detected with reasonable certainty only by injecting blood into laboratory rodents. Recent studies have emphasized that T. *brucei* is essentially a connective tissue parasite in several species of laboratory (Goodwin, 1970) and domestic animals (Losos and Ikede, 1970).

2.11 Diagnosis of Trypanosomosis

Diagnosis as it relates to trypanosomosis refers to methods for detecting infection, either by identifying the parasites themselves or by interpretation based on the results of immunological tests (Luckins, 1989). A variety of diagnostic tests are available (Toure, 1976) and efforts are still ongoing to improve the 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).

2.11.1 Clinical diagnosis of trypanosomosis

Despite the availability of various tests for the diagnosis of animal trypanosomosis, there are no simple pen side diagnostic tests for 'on-spot' diagnosis of individual cases of animal trypanosomosis. Under such circumstances clinicians must make a clinical diagnosis before treating disease cases. The sensitivity of a clinical diagnosis based on manifested clinical signs is

not known and has been a subject of comparison with other parasitological and molecular diagnoses (Nantulya, 1990; Magona *et al.,* 2003).

Many clinical signs of animal trypanosomosis are similar to those of other parasitic infections, such as, anaplasmosis, babesiosis, theileriosis, fasciolosis and gastrointestinal nematodosis (Molyneux and Ashford, 1983). Thus, many false positive diagnoses may be made which may diminish its specificity. Under field conditions, in the absence of reliable diagnostic tools or access to laboratory facilities to rely on, clinical diagnosis could be employed to screen and treat cases of trypanosomosis before confirmatory diagnosis in diagnostic centres are sought. The clinical signs of the disease are varied and the ecological conditions under which trypanosomosis occurs are also so diverse that, in terms of identifying animals with active infections, clinical diagnosis is too imprecise a procedure to use as a basis for the control of trypanosomosis, and other means of diagnosis must therefore be employed (Luckins, 1980).

2.11.2 Parasitological diagnosis of trypanosomosis

This is the process of examining the blood by light microscopy. It is the most readily applied method for the diagnosis of trypanosomosis and, more importantly, is a technique which can be easily applied in the field (Lukins, 1989). A number of techniques have been reviewed extensively (Nantulya, 1990; OIE, 2013). The basic techniques are examination of wet, thick or thin stained blood films of fresh blood. 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 examined and the skill and experience of the technician (OIE, 2013).

The basic parasitological techniques (thin and thick smear) have been modified to improve diagnostic sensitivity by concentrating the blood through centrifugation in a haematocrit tube (Woo, 1970) and the dark ground buffy coat technique (DG) (Murray *et al.,* 1977; Paris *et al.,* 1982). Other modified but not widely used methods, include the separation or removal of blood cells prior to centrifugation by anion exchange chromatography or hypotonic lysis (Nantulya, 1990) and laboratory rodents inoculation method which can then be examined for periods of 30 to 60 days to determine if they have developed trypanosome infections (Uilenberg, 1999; OIE, 2013).

These parasitological procedures differ significantly in their ability to detect a parasite (Nantulya, 1990; Paris *et al.,* 1982). The use of some of these techniques under experimental conditions has given an indication of their detection limits in relation to the numbers of different species of trypanosomes in a blood sample. In order of decreasing sensitivity, the results were as follows: DG>HCT>thick film>thin film>wet film (Paris *et al.,* 1982). Consequently, varying degree of sensitivity of the parasitological tests and their failure to detect trypanosomes if the number of parasites is too low illustrate the limitations of parasitological diagnosis, as is the case with chronic infections (Masake and Nantulya, 1991).

2.11.3 Immunological diagnosis of trypanosomosis

Although parasitological diagnosis gives a conclusive proof of infection, their limitations have been the driving force for alternative techniques that provide indirect evidence of infection which is refered to as immunodiagnostic techniques. Several immunodiagnostic techniques have been developed to detect trypanosomal antibodies or antigens for the diagnosis of animal trypanosomosis, with variable sensitivity and specificity. The methods of choice is the indirect

fluorescent antibody test (IFAT) (Katende *et al.,* 1987) and the trypanosomal antibody detection ELISA (Luckins, 1977; Hopkins *et al.,* 1998).

There are many reports of the use of immunodiagnostic techniques for diagnosis of trypanosomosis but, invariably, most of them have been retrospective surveys, intended to add further information on epidemiology of the disease. However, immunodiagnostic tests are associated with several shortcomings which have made them not applicable under field conditions. The persistence of trypanosomal antibodies following curative treatment or self cure for 3-4 months or sometimes up to 13 months (Desquesnes, 2004); before it disappeared in some animals (Van den Bossche *et al.,* 2000) make standardisation difficult in terms of sensitivity and specificity of immunodiagnostic test. More so, in terms of equipment, immunodiagnosis needs expensive, sophisticated equipment and expertise, which are not always available. The tests have to be performed in specialised laboratories and there is a substantial delay between the actual sampling and the availability of the results (Desquesnes, 2004).

2.11.4 Molecular diagnosis of trypanosomosis

Molecular diagnostic tools have been developed with high sensitivity and specificity (Cox *et al.,* 2005) to provide a species-specific diagnosis of active infections by trypanosomes; even in cases of low parasitaemia. Up till now molecular diagnostic techniques have not been easily applied in the field conditions owing to the amount of time required to process samples by conventional methods prior to analysis and the equipment needed. However, these have been overcome by improved extraction kits which make sample collection in the field easier (Picozzi *et al.,* 2002). The main benefit of PCR is its species-specificity. It is superior to any other technique and it is

an essential epidemiological tool capable of identifying various species that are present in the populations surveyed whether in the hosts or vectors.

The PCR techniques has a high sensitivity (1 to 10 trypanosomes/ml of blood) making it more sensitive than the parasitological diagnostic techniques (100 to 1,000 trypanosomes/ml of blood) which translates under field condition the detection of twice as many positive samples compared to parasitological methods (Clausen *et al.,* 1998; Solano *et al.,* 1999). However, the molecular techniques have limited application when the parasite do not circulate in the bloodstream or it is circulating at a rate less than one per volume of treated sample material. Infact, aparasitæmic periods in trypanosomosis are a biological limitation to the application of PCR on blood and its derivatives (Desquesnes and Dávila, 2002). PCR techniques have been used in detecting *T. congolense*, *T. brucei* and *T. vivax* infections in various experimentally infected animals models and livestock under natural infection (Masiga *et al.,* 1992; Majiwa *et al.,* 1994; Masake *et al.,* 1997). False-positive results may occur as a result of contamination of the samples with other DNA while false-negative results are common especially in low parasitaemia (<1 trypanosome/ml of blood), which occurs frequently in chronic trypanosome infections (OIE, 2013).

2.12 Differential Diagnosis of Trypanosomosis

In its various stages, trypanosomosis resembles a number of other disease conditions, and it frequently occurs at the same time as other infections. In the acute febrile stage, trypanosomosis must be differentiated from redwater (babesiosis), anaplasmosis, and even East Coast fever in Africa (Rodostits *et al.,* 2006). Acute trypanosomosis does not produce icterus and is not

accompanied by haemoglobinuria. These features and parasitological findings differentiate trypanosomosis from redwater. These two infections, however, may occur concurrently. Similarly, anaplasmosis can occur in trypanosome-infected cattle. On its own, anaplasmosis often produces icterus, as well as fever and anemia; there may also be enlargement of lymph glands. Differentiation relies on the detection of parasites in blood smears (OIE, 2013). Carcasses of animals that have succumbed to trypanosomosis are often edematous and anemic. Chronic trypanosomosis is an afebrile disease of which anemia, emaciation, and lymph node enlargement are common symptoms. It is also important to differentiate this disease from malnutrition and helminthosis. In neither of these conditions is lymph node enlargement found. The detection of helminth eggs in feces is a useful adjunct to the diagnosis of helminthosis, but the absence of eggs in fecal samples taken from cattle with chronic fasciolosis, and the occurrence of anaemia and subcutaneous edema, can make differential diagnosis difficult. In cases of malnutrition, the degree of anemia is rarely as extensive or severe as it is with trypanosomosis, although marked individual variations characterised by emaciation and lymph node enlargement, but unlike animals suffering from chronic trypanosomosis, appetite is diminished and anemia is rarely severe (Connor and Van den Bossche, 2005).

2.13 Prevention and Control of Trypanosomosis

Control of animal trypanosomosis is at present largely achieved through application of insecticides to eliminate or control the tsetse populations and the administration of chemo-prophylactic or chemotherapeutic drugs to control infections in domestic livestock (Doyle *et al.*, 1984). Each of these control approaches may have useful application but they are not without

important limitations, such as high cost of implementation, environmental unfriendliness, drug resistance and availability.

2.13.1 Vector control

Early methods to control the tsetse fly such as the removal of preferred vegetation or the destruction of host game animals were very effective but have become unacceptable on environmental grounds (Dransfield *et al.,* 1991; Vreysen *et al.,* 2013). Following the discovery of DDT in the 1940s, it was widely believed that through the use of insecticide tsetse flies could be eradicated without any negative impact to the environment. The following are the established methods of tsetse control by application of insecticides: ground spraying, aerial spraying, odour-baited traps and targets and applications of insecticides to cattle by dipping or pour-on formulations. The other widely used tsetse control is the Sterile-insect techniques. Of these methods, ground spraying has probably cleared more areas of tsetse infestation than any other technique, and aerial spraying has the proven capability of reducing tsetse densities over huge areas more rapidly than by any other method (Chadenga, 1990). No single method of insecticide control has however, proved to be the method of choice in all circumstances. All the available techniques have their advantages and disadvantages, and often greater gains can be obtained by using a suite of control techniques than by relying on a single one (Vreysen *et al.,* 2013).

2.13.2 Trypanotolorent breed of cattle

Trypanotolerance has been defined as the relative capacity of an animal to control the development of the parasites and to limit their pathological effects, the most prominent of which is anaemia (Murray and Dexter, 1988; Igbokwe, 1989). These traits have been reported in cattle

(Murray *et al.,* 1990), buffaloes (Black *et al.,* 2001), sheep and goats (Murray *et al.,* 1981), horses and dogs (Abenga *et al.,* 2005a, b). Early studies described that certain taurine breeds in West Africa could cope better in tsetse-infested areas (Murray *et al.,* 1982; Murray and Dexter, 1988). Under natural conditions of tsetse challenge, trypanotolerant cattle had lower mortality, lower trypanosome levels, less severe anaemia, superior weight gain and better reproductive performance than the more susceptible *Bos indicus* breeds. The breeds were tolerant to both *T. vivax* and *T. congolense*, with a higher degree of resistance to *T. vivax* (Murray *et al.,* 1981; 1982; Mattioli *et al.,* 1999).

Trypanotolerance is a relative phenomenon rather than an absolute trait, and may be affected by nutritional status and stress of work as well as concurrent infections (Seck *et al.,* 2002). Trypanotolerance is true among the West African short-horned breed of cattle like Muturu, Baoule, Laguna, Samba and the long-horned breed the N'Dama. However, susceptibility studies have shown the N'Dama to be the most resistant breed of these breeds followed by the smaller West African short-horned cattle, while the Zebu is the most susceptible (Murray *et al.,* 1979). In Nigeria, the N'dama and Muturu are the notable trypanotolerant breeds of cattle (Enwezor and Lawal, 2003). This constitutes a significant minority of the 10-14 million susceptible cattle breeds and numbers at risk of trypanosomosis in Nigeria (Adeniji, 1993).

2.13.3 Immunologic control of trypanosomes

Several reviews on the immunobiology of African trypanosomes have been published (Tabel *et al.,* 2000; de Baetselier *et al.,* 2001; Mansfield and Paulnock, 2005; Tabel *et al.,* 2008; Paulnock *et al.,* 2010; Musa *et al.,* 2015; Cnops *et al.,* 2016). One of the major characteristics of trypanosomes is the presence of the Variant Surface Glycoprotein (VSG) which covers nearly all

the membrane of trypanosomes and is the pre-dominant surface antigen of African trypanosomes (Barry and McCulloch., 2001; Musa *et al.,* 2015). The parasite has a repertoire of more than 1000 inactive VSG genes and a single active transcription site which result is a continuous switching of the VSG genes (Turner and Barry, 1989). This mechanism enables the parasite to manipulate and evade the host immune response, evading effective elimination and establishing a chronic infection (Cnops *et al.,* 2016). Hence the bloodstream no longer poses a hostile threat, but has become a niche where trypanosomes thrive and await transmission through the bites of tsetse flies, ideally without causing severe infection associated pathology to their host. However, as the infection progress, the immune system becomes increasingly exhausted (Mansfield and Wallace, 1974; Darji *et al.,* 1993), leading to loss of parasitemia control, severe inflammation associated pathology and ultimately death of the infected host. These pathologies weaken the host but ensure parasite survival by enhancing infection chronicity and transmission potential (Cnops *et al.,* 2016).

2.14. Chemotherapy and Chemoprophylaxis

Chemotherapy and chemoprophylaxis represent the mainstay of animal trypanosomiases control, ensuring animal health and production in enzootic countries (Peregrine and Mamman, 1993; Giordani *et al.,* 2016). Treatment and prophylaxis of the disease in animals caused by trypanosomes is dependent upon the salts of three compounds; homidium, isometamidium, and diminazene, (Williamson, 1970; Leach and Roberts, 1981). In contrast, the salts of three other compounds are generally used for therapeutic and prophylactic purposes in camels, equidae and buffaloes; suramin, quinapyramine, (Leach and Roberts, 1981); and melarsomine (Cymelarsan^{R}) (Raynaud *et al.,* 1989). It has been estimated that annually about 35 million doses of these

compounds are currently used in Africa (Geerts and Holmes, 1998; Holmes, 2013) with isomethamidium chloride, diminazene aceturate and ethidium bromide estimated to represent 40%, 39% and 26% respectevely of the total trypanocidal drug market by value (Sones, 2001).

2.14.1 Diminazene aceturate

Diminazene aceturate is currently marketed in Nigeria under the trade names Berenil^{R} (Intervet), and Veriben^{R} (Sanofi AH) and has historically been marketed as Azidine and Ganasang elsewhere in the world as both a trypanocide and babesiacide for domestic livestock. For all animals the general intra-muscular dose is 3.5 mg/kg b.w. but twice this amount is recommended for *T. brucei* infections. The compound was introduced into the market in 1955 (Jensch, 1955) and has become the most commonly used compound for the treatment of trypanosomosis in livestock. More so, since it also has anti-babesial activity, it has also been used as a babesiacide (Kuttler, 1988; Mosqueda *et al.*, 2012), thereby enhancing its field applicability.

Diminazene aceturate is recommended only for use as a therapeutic agent since it is rapidly excreted and therefore thought to have little prophylactic activity (Fussgänger and Bauer, 1958). However, in contrast to these findings biological assays of trypanocidal activity in cattle blood (Van Hoeve and Cunningham, 1964; Peregrine and Mamman, 1993) and rabbit blood (Goodwin and Tierney, 1977) suggest that some trypanocidal activity is retained for 2-3 weeks following intramuscular dosage of 7 mg/kg^{-1} (cattle) or 15 mg/kg-^{1} (rabbits)(Gilbert, 1983).

2.14.2 Isomethamidium chloride

Isometamidium chloride (Samorin®, May and Baker Ltd, England; Trypamidium®, Rhône Mérieux, France) has been in the market since 1961 as a prophylactic and therapeutic drug (Berg *et al.,* 1961). Isomethamidium chloride can be used at dose rate of 0.25 to 0.5 mg/kg (Samorin®) or 0.5 to 1 mg/kg (Trypamidium®), by deep intramuscular route as preventive and therapeutic drug. Both isomethamidium and homidium are active against *T. congolense* and *T. vivax* in ruminants and in addition, it is of value against *T. brucei* and *T. evansi* infections in donkeys, horses and camels. The compound has been used successfully to maintain the productivity of Zebu cattle exposed to tsetse challenge in various management systems in East Africa (Trail *et al.,* 1985; Moloo *et al*. 1987). However, considerable variation in prophylactic activity has been observed in that a dose of 1.0 mg kg^{-1}bw has been shown to confer prophylaxis to cattle for 2-22 weeks (Pinder and Authié, 1984; Whitelaw *et al.,* 1986; Peregrine *et al.,* 1991).

2.15 Drug Resistance in Trypanosomosis

Drug resistance, may be defined as a loss of sensitivity by a strain of an organism to a compound to which it had previously been susceptible. It implies failure of treatment and prevention and if no other active drugs are available the animal has to rely on its immune defenses alone to combat the disease (Uilenberg, 1999). Resistance to trypanocidal drugs have been reported in trypanosome populations in about 17 countries in Sub-Saharan Africa including Nigeria (Peregrine *et al.,* 1997; Holmes *et al.,* 2004).

Until recently, diminazene aceturate and isometamidium chloride were considered the best therapeutic and prophylactic trypanocides, respectively. The former was reputed as the only drug to which trypanosomes do not easily develop resistance because of its rapid elimination from the system when compared with the most persistent prophylactic drugs such as isomethamidium

(Aliu *et al.,* 1984; Rushigajiki *et al.,* 1986). Unfortunately, this view is no longer accepted, as trypanocide resistance to these drugs has been demonstrated under laboratory conditions (Peregrine *et al.,* 1991; Peregrine, 1994).

Resistance to diminazene aceturate by trypanosomes has been reported (Chitamo and Arakawa, 1992; Peregrine and Mamman, 1993) while isometamidium treatment failures and shortened prophylactic intervals have been attributed to infections with drug-resistant trypanosome species (Peregrine *et al.,* 1991; Sutherland *et al.,* 1991). Similarly, widespread resistance to homidium, another trypanocidal drug has also been reported from East and West Africa (Clausen *et al.,* 1992; Codjia *et al.,* 1993; Mulugeta *et al.,* 1997).

More worrisome, however, are the reported incidences of field stocks that have developed multiple resistances to these trypanocidal drugs (Ainanshe *et al.,* 1992; Mohammed-Ahmed, *et al.,* 1992; Mulugeta *et al.,* 1997; Afewerk *et al.,* 2000). For instance in Nigeria resistance to the common trypanosomes have been reported on *T. congolense* (Jones-Davies and Folkers, 1966; MacLennan and Jones-Davies, 1967; Na'Isa, 1967; Jones-Davies, 1968; Gray and Roberts, 1971); *T. vivax* (Jones-Davies, 1967; Ilemobade, 1979) and *T. brucei* (Kalu, 1995a)

Drug resistance in trypanosomes poses a serious problem to livestock productivity in countries where it has been reported, unless checked and brought under control. The development and spread of drug resistance to the point where drugs become ineffective over large areas of Africa is probably the greatest risk to the future use of the existing three trypanocides (Holmes *et al.,* 2004).

2.16. Economic Implication of Trypanosomosis

It is estimated that tsetse flies are distributed over approximately 11 million km^2 of Africa (Jordan, 1996), which is about 37% of the continent (FAO/WHO/OIE, 1982). Out of the estimated 172 million heads of cattle, approximately 44.7 million are at risk of trypanosomosis (Gilbert and Vance, 2001). The economic losses attributed to AAT are due to decreased meat and milk production as a result of infertility, morbidity and mortality. When the impact on crop productivity due to reduced animal draught power and manure is considered, the true economic losses could be much higher (Budd, 1999).

In Nigeria, animal trypanosomosis still constitutes a major obstacle to food security inspite of previous attempts towards chemotherapeutic and tsetse control (Onyiah, 1997; Abenga *et. al;* 2004). Like other parts of Sub-Saharan Africa, the disease is most devastating in terms of poverty and loss of agricultural production (Hursey, 2000). These losses include; reduction in herd sizes as a result of livestock deaths and drop in calving rate, reduced market value of animals as a result of loss in condition, drop in milk production, reduced work efficiency of draught animals and prevention of mixed farming (Swallow, 2000). Direct losses due to trypanosomosis are estimated to amount to between US$ 1-1.2 billion each year. A pondered evaluation extrapolated for the total tsetse-infested lands values the total losses, in terms of agricultural Gross Domestic Product, at US$ 4.75 billion per year (FAO, 2004). An indirect loss stem from farmers' responses to the perceived risk of the disease, including the reduction and in some cases the exclusion of livestock from tsetse-infested grazing lands and reduced crop production due to insufficient animal draught power (ILRAD, 1993). Estimated physical loss caused by trypanosomosis in cattle (death, beef, milk and loss of draught power) were estimated to be worth 865 million NGN with about 85% of the loss being in the Northern Guinea Savannah (Fadiga *et al.*, 2013). In Nigeria, the cost of control intervention (treatments, vector control,

surveillance, and disease burden) of the disease was estimated to be worth 976 million NGN with 90% of all the interventions in the Northern Guinea Savanna of Nigeria (Fadiga *et al.,* 2013).

According to MacLennan (1980) about 7 million km^2 of tsetse infested areas of Africa would otherwise be suitable for livestock and mixed agriculture if trypanosomosis could be controlled. Heavily infected areas are devoid of cattle and other domestic livestock. Of the estimated 165 million cattle found in Africa, only about 50 million are kept in the infested areas and they are mostly of low producing breeds. Those areas, had they been free of tsetse, could support another 140 million cattle and an equivalent number of sheep and goats (FAO, 1984). Estimates indicate increases of 16% and 14% in meat and milk production, respectively, following effective trypanosomosis control (Jahnke *et al.,* 1988).

2.17 Public Health Significance of Bovine Trypanosomosis

The typical human trypanosomes are *T. brucei gambiense* and *T. b. rhodesiense* the causative agent for sleeping sickness in sub-Saharan Africa (Hoare, 1972) and the *T. cruzi* the cause of Chagas disease which is transmitted by triatomine bugs (Maraghi *et al.,* 1995; Rassi *et al.,* 2010) and *T. rangeli* infection (Hoare, 1972). Human infection by animal species of *Trypanosoma* is usually impossible because of a trypanolytic factor in human serum (Hawking, 1978; Xong *et al.,* 2002). However, it has been demonstrated that any of the salivarian trypanosomes (*T. vivax, T. Congolense, T. brucei and T. evansi)* are capable of resisting human plasma in certain circumstances **(**Truc *et al.,* 2013**)**. The degree to which trypanosome species that are not usually associated with human infection actually infect humans is difficult to ascertain since such infections may be short-lived and pass undiagnosed. Human innate

immunity against African animal trypanosomes stems from the trypanolytic activity of the human-specific serum protein called apolipoprotein L-I (apoL-I), which is partially associated with high-density-lipoprotein (HDL) (Vanhamme *et al.,* 2003; Vanhamme and Pays 2004; Pays *et al.,* 2006; Vanhollebeke *et al.,* 2007).

The possible existence of human infection with typical animal trypanosomes has been reviewed (Truc *et al.,* 2013). The earliest observation which shows human infection with *T. vivax*, a typical animal trypanosome was by Macfie, 1917 in Ghana (Hoare, 1972). Thereafter, reports abound on human cases of infection with various livestock trypanosomes. Some of these infections in humans are transient in nature, while others can be serious requiring treatments that were often successful in most cases, although fatal cases have also been reported (Touratier and Das, 2006; Doke and Kar, 2011). Livestock trypanosome caused by *T. b. brucei* (Hoare, 1972; Abebe *et al.,* 1988; Deborggraeve *et al.,* 2008); *T. vivax*, (Hoare, 1972) and *T. congolense* (Truc *et al.,* 1998) have been reported in humans in several geographic locations. Similarly, *T. evansi* the cause of *''surra''* in domestic and wild animals have also been reported to infect and establish disease in humans (Gill, 1977; Joshi *et al.,* 2005; Touratier and Das, 2006; Haridy *et al.,* 2011; Chau *et al.,* 2016) while *T. lewisie* which are primary trypanosomes of rats (*rattu rattus*) was also reported to infects humans (Shrivastava and Shrivastava, 1974; Kaur *et al.,* 2007; Shah *et al.,* 2011; Doke and Kar, 2011 and Verma *et al.,* 2011).

Due to recent changes in tsetse ecology as a result of deforestation, climate change, and loss of native blood meal species, domestic hosts (livestock and farmers), are becoming an important source of food for tsetse flies (Van den Bossche *et al.,* 2010). In areas where there is a high

density of human, cattle, and tsetse fly cohabitation, the risk of disease transmission across different species is high, as it is estimated that trypanosomosis is more likely to be transmitted by a cattle-fly-human transmission cycle (Hide, 1999). It is therefore perfectly justified to re-evaluate the multi species interaction of trypanosomosis whether human infection with livestock trypanosomes could be an emerging and neglected threat to public health **(**Truc *et al.,* 2013**)**.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Area

The study was carried out in Niger State which lies on latitude 08^{o} to 11^{o}:30' North and Longitude 03^{o} 30' to 07^{o} 40' East within the North-central zone of Nigeria with a total land area of 76,363 square kilometers and an estimated livestock population of 1.814 million cattle, 1.5 million sheep, and 1.89 million goats as at 2004 (Ezeokafor, 2006). It shares a border with the Republic of Benin (West), Zamfara State (North), Kebbi State (North West), Kaduna state (North East), Kogi State (South), Kwara State (South West), and the Federal Capital Territory (FCT) (South East) (NGSG 2015). The state has 25 local government areas spread across three (3) Agro-ecological zones (Fig. 3.1).

Niger State experiences distinct dry (November to April) and wet (May to October) seasons with annual rain fall varying from 1,100 mm in the Northern part to 1,600 mm in the Southern parts. The main economic activities in the state are crop farming, fishing, and livestock production (NGSG 2015). The White Fulani breed of cattle is the dominant breed in Niger state, with the remainder composed of Sokoto Gudali and some exotic breeds (Freisians, Bonsmara, Ayshire, and Simentals) and their crosses found in private ranches. Cattle are extensively grazed on communal pastures with minimal or occasional feed supplement. During the cropping season (rainy season), cattle are grazed away from farms but allowed to graze on crop residues after harvesting (during dry season) with their dung directly fertilizing fields in readiness for the next cropping season (Grace *et al.*, 2009). They are watered in rivers and other water points when seasonal rivers dry out.

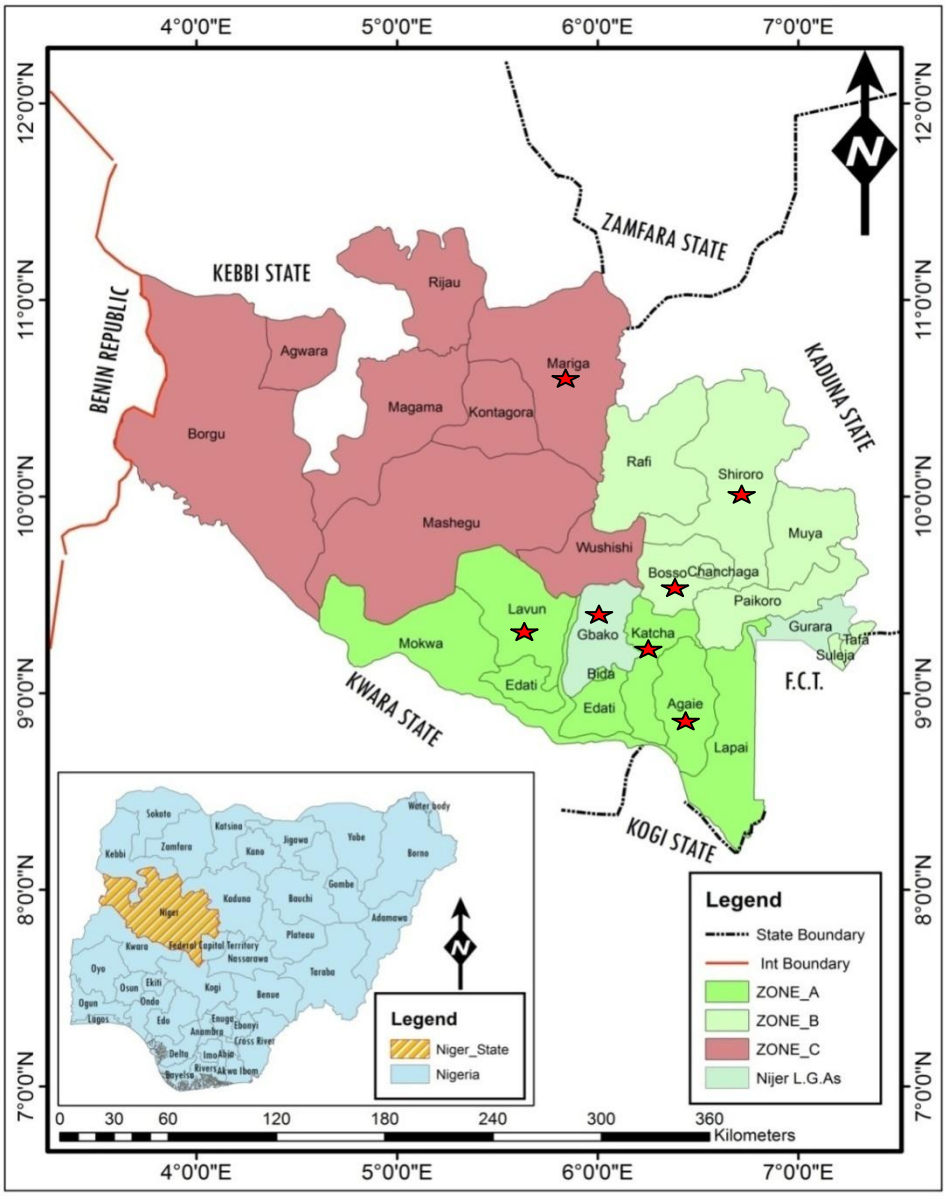

Figure 3.1: Map of Niger State showing the location of the sampled LGAs marked with ★ icon. Inset: Map of Nigeria showing the location of Niger State.

Source: Modified From Administrative Map of Niger State (NGSG, 2015).

3.2 Study Design

A longitudinal study design was used to assess the clinical manifestations, blood parameters, response to treatment of bovine trypanosomosis in Niger state. The study was carried out from January to May, 2016.

3.3 Study Population

The study population was the indigenous cattle and few exotic breeds of cattle with their crosses found in the state at the time of the study. Animals for the study were drawn from cases reported to the Niger State Veterinary Hospital Minna and Area Veterinary Clinics in the sampled LGAs in the state by herd owners with health problems in which haematological examination revealed the presence of trypanosomes in their circulation. All the cattle were presented by the farmers with major complaint suggestive of bovine trypanosomosis and were screened irrespective of their age or sexes. Ten percent of presented animals comprising both clinically sick and apparently healthy animals were sampled (Rodostits *et al.*, 2006). The uninfected animals served as control. The sampled animals were raised under extensive or semi intensive production systems.

3.4 Sample Size

Thirty Nine (39) herds comprising 343 cattle were purposively selected (Putt *et al.,* 1987) based on the herd owners' consent, willingness to be available throughout the study period and acceptance of research protocol. Relevant information and samples were collected for laboratory investigation from these animals. Among the 343 sampled cattle, 262 were diagnosed of clinical trypanosomosis, 81 others living under the same conditions were uninfected and served as control.

3.5 Ethical Approval

A verbal consent was sought from the herdsmen and cattle owners, with care being taken during blood collection in order not to harm the animals. Motivations were made, which included treatment of other animals suffering from other diseases like helminthosis, ecto-parasitism during and after the study.

3.6 Clinical Examination of the Animals

The animals presented for this study were examined and screened for the presence of trypanosome parasites between January and May, 2016. Herd size, breed, sex, age, history of trypanocidal usage, production system and farm/herd location were recorded. The rectal temperatures of these animals were taken using a digital clinical thermometer while their body weights were also determined using a weighing band. The animals were observed for abnormal signs which were then recorded.

3.7 Trypanocidal Drugs Treatment of Infected Animals

Based on history of trypanocidal usage a total of 136 cattle from 15 herds were treated with diminazene aceturate (Batchno.A189A01, exp.03/2018 South Africa) at a dose rate of 3.5 mg/kg body weight IM while 126 cattle from 24 herds were treated with isomethamidium chloride (Batchno.199A1, fab. 07/2010,exp.07/2018 France) at a dose rate of 0.5 mg/kg body weight deep IM. The uninfected animals that were sampled (control group) were not treated with any of the trypanocidal drugs during the period of the study.

3.8 Clinical and Laboratory Investigation

3.8.1 Haematological samples collection

All the sampled animals in each of the herds were bled from the jugular vein using a 10ml syringe and an 18G needle. About 10ml of blood was taken with 5 ml of the blood being transferred into commercially prepared sample bottles containing Di-sodium salt of ethylene diamine tetra-acetate (EDTA, 1 mg/ml) as anti-coagulant and transported on ice for hematological and parasitological analysis (Waiser, 2012; Jelalu, 2014). The remaining 5 ml of the blood was transferred into anticoagulant free vacutainer tubes and allowed to stand in a rack for 30 minutes to clot. Resultant sera that separated were harvested from each of the sample into labeled vials and were used for serum biochemical examination.

3.8.2 Parasitological evaluation

3.8.2.1 Thin blood smear, staining and examination for trypanosomes

Blood samples were examined for the presence of trypanosomes using Standard Trypanosome Detection Methods (STDM) (OIE, 2013) on day 0, before treatment and on day 14, and day 28 post treatment. Thin blood smears were made using glass slides immediately after collection of blood. All the smears were air dried, fixed with absolute methanol for 5 minutes and stained with Giemsa stain for 45 minutes. Excess stain was washed off using distilled water and allowed to air dry for examination (Murray, *et al*., 1983; Uilenberg, 1999). The slides were then examined under the light microscope at ×100 magnification under oil immersion. About fifty random fields of each slide were examined for detection of trypanosomes. For the positive smears, the species of trypanosomes were identified to species level using morphological appearances and characteristics according to the methods of Murray *et al*. (1983), and the OIE guidelines (OIE, 2013).

3.9 Haematological Evaluations

Total Red Blood Cell counts (TRBC), Total White Blood cells counts (TWBC), Hemoglobin Concentration (Hb), and differential white blood cell counts were estimated according to the methods described by Waiser (2012) and Jelalu (2014). Packed Cell Volume (PCV) was also measured by the haematocrit centrifugation technique using a Hawksley microhaematocrit reader. The differential WBC counts were determined based on 100 cells per slide according to their staining reactions; shape of the nucleus, and presence or absence of granules in their cytoplasm (Cole, 1986). The absolute numbers of lymphocytes, neutrophils, eosinophils basophils and monocytes per millilitre of blood were obtained using the differential count percentages.

3.10 Serum Biochemical Evaluation

3.10.1 Measurement of some serum enzymes levels

Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatine kinase (CK) were determined according to the method described by Coles, (1986).

3.10.2 Estimation of serum glucose, total serum protein, globulin and albumin levels

Serum glucose concentration was estimated by the glucose hexokinase method as described by Duxbury (2004). Total serum protein level was determined by the Biuret method as described by Amole *et al.,* (1990). Total albumin concentration was estimated by the bromo cresol-green

(BCG) method as also described by Amole *et al.*, (1990). Globulin concentration was however calculated as the difference of the total protein and the albumin levels.

3.10.3 Determination of total serum cholesterol, triglycerides, high and low density lipo-protein- cholesterol levels

The total serum levels of cholesterol and triglycerides were analysed by enzymatic methods while HDL-cholesterol was analysed using precipitant method as all described by Adamu *et al.*, (2008). Low desity Lipoprotein-cholesterol however was calculated from the values of total cholesterol, triglycerides and HDL-cholesterol using method described by Adamu *et al.*, (2008).

3.10.4 Measurement of serum calcium, iron, zinc and copper

Serum calcium concentration was measured by the cresolphtalein complexone technique as described by Ogunsamni *et al*. (1994b) while serum iron level was determined spectrophotometrically as described by Isaac *et al*. (2011). Serum level of copper and Zinc were determined as described by Smith *et al.* (2000) and da Silva *et al.* (2009b) respecively.

3.10.5 Measurement of total serum sodium, potassium, chloride, and bicarbonates Levels

Sodium and potassium concentration were determined by flame photometer according to the method described by Skoog *et al.* (2005) while those of chloride and bicarbonate were measured according to method described by Toro and Ackerman (1975).

3.11 Data Management and Analyses

Descriptive statistics: proportions, means, standard deviation and tables were used to present the data. Z test was used to compare the mean haemotological and serum biochemical values of cattle with clinical trypanosomosis and those that served as control. Repeated measure ANOVA was used to compare the changes in haematological response to trypanocidal treatments. All analyses were performed using Microsoft excel 7 (Microsoft corporation, USA). The analyses were conducted at 95% confidence interval and $P \leq 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 Proportion of Trypanosome Infections in the Sampled Cattle

Out of the total of 343 cattle that were examined for infection with species of trypanosomes 45 (13%) cattle were positive for trypanosoma infection as presented in Table 4.1. *Trypanosoma congolense* and *T. vivax* accounted for 5.5% each while *T. brucei* accounted for 2.0%. The proportion of cattle in each of the sampled Local Government Areas (LGAs) which were positive for trypanosome infection were Agaie, 15.7%; Bosso, 20%; Gbako, 11.8%; Katcha, 9.7%; Lavun 9.8%; Mariga, 60% and Shiroro with 11.6%. In Agaie LGA the percentage of animals infected with *T. vivax* was 5.2% and *T. congolense* was 10.5%.

In Bosso LGA 2 (20%) and Gbako LGA 2 (11.8%) of the animals were positive for *T. vivax* respectively. While in Katcha LGA the animals that were positive for *T. congolense* were (4.9%) and *T. vivax* were (4.9%). In Lavun LGA, of the 5 sampled cattle that tested positive, 2 were positive for *T. congolense* (3.9%) while 3 were positive for *T. brucei* (5.9%) (Table 4.1). In Mariga LGA, 6 (60%) of the sampled animals were positive for trypanosomes. *T. vivax, T. congolense* and *T. brucei* accounting for 10%, 30% and 20% respectively. In Shiroro LGA 11 (11.6%) of the sampled animals were positive for trypanosomes with *T. vivax* accounting for 6 (6.3%), *T. congolense* for 3 (3.1%) and *T. brucei* for 2 (2.1%). No mix infection was encountered in any of the LGA. With respect to the three different species of trypanosomes, only *T. vivax* was found in Bosso and Gbako LGAs, and *T. vivax* and *T. congolense* from Agaie and Katcha LGAs, while all the three species of trypanosomes were recovered from Lavun, Mariga and Shiroro LGAs.

Table 4.1: Proportion of trypanosome infection in cattle sampled in selected Local Government Areas of Niger State, Nigeria

[1]LGAs	Number of Animals Examined	Number of Animals Positive	Proportion of Animals Positive (%)	Species of Trypanosomes Identified		
				[2]TV	*[3]TC*	*[4]TB*
				No (%)	*No (%)*	*No (%)*
Agaie	57	9	15.7	3 (5.2)	6 (10.5)	0 (0.0)
Bosso	10	2	20.0	2 (20.0)	0 (0.0)	0 (0.0)
Gbako	17	2	11.8	2 (11.8)	0 (0.0)	0 (0.0)
Katcha	103	10	9.7	5 (4.9)	5 (4.9)	0 (0.0)
Lavun	51	5	9.8	0 (0.0)	2 (3.9)	3 (5.9)
Mariga	10	6	60.0	1 (10.0)	3 (30.0)	2 (20.0)
Shiroro	95	11	11.6	6 (6.3)	3 (3.1)	2 (2.1)
Total	343	45	13.0	19 (5.5)	19 (5.5)	7 (2.0)

[1] *LGA= Local governmemt areas*
[2] *TC = Trypanosoma congolense*
[3] *TV = T. vivax*
[4] *TB = T. brucei*

4.2 Clinical Manifestation and Parasitological Analysis

Of the total number of 343 cattle that were sampled in the selected 39 herds, 262 cattle manifested various clinical signs suggestive of trypanosomosis and 81 uninfected cattle served as control during the survey. Clinical signs observed in the infected cattle during the survey are presented in Table 4.2. The most frequent clinical signs were emaciation (78%), weakness (71%), anorexia (65%), pale mucous membrane (58%), Lacrimation (45%), and dark or rough hair coat (41%) accordingly. Other clinical signs were alopecia (40%), pica (39%), pyrexia (37%), nasal discharge (34%), cough (30%), corneal opacity (25%), agalactia (22%) and teeth grinding (19%). In addition, the cattle also showed cutaneous haemorrhages (14%), shade seeking (12%), pelleted stool (10%), retained placenta (8%), weak calving (6%), abortion (4%), nervous signs like muscle tremors and ataxia (2%) and swollen lymph nodes (1%).

The mean body weight of infected cattle was 257.94 ± 74.13 kg and was significantly ($P \leq 0.05$) lowr when compared to the control (386.96 ± 62.69 kg). The animals in the control group showed a 14% higher body weight than the animals with trypanosome infection. The mean rectal temperature of cattle infected with trypanosomes was 40.21 ± 0.45^0C which is significantly ($P \leq 0.05$) higher than those of the control (38.28 ± 1.31^0C) as show in table 4.3.

Table 4.2: Proportion of observed clinical signs in bovine trypanosomosis in selected

Local Government Areas in Niger State

S/No	Observed Clinical Sign	No of clinically sick cattle
1.	Weight Loss/Emaciation	204(78)*
2.	Weakness	186(71)*
3.	Anorexia	162(65)*
4.	Pale Mucous Membrane	151(58)*
5.	Epiphora	117(45)*
6.	Dark/Rough Hair Coat	107(41)*
7.	Alopecia	104(40)*
8.	Pica	103(39)*
9.	Pyrexia (Temperature over 38.5^0c)	97(37)*
10.	Nasal Discharge	89(34)*
11.	Cough	77(30)*
12.	Corneal Opacity	66(25)*
13.	Agalactia	58(22)*
14.	Teeth Grinding	50(19)*
15.	Cutaneous Haemorrhages	37(14)*
16.	Shade Seeking	31(12)*
17.	Pelleted stool	26(10)*
18.	Retained Placenta	20(8)**
19.	Weak Calving	16(6)**
20.	Abortion	11(4)**
21.	Nervous Signs (Muscle tremor, Ataxia)	5 (2)*
22.	Swollen Lymph nodes	3(1)*

N* (Overal total sampled cattle) N** (Total of sampled Females cattle)*

Table 4.3: Mean weights, rectal temperatures and pack cell volume of

infected and uninfected cattle in selected Local Government Areas in Niger State

LGA	**Uninfected cattle**	**Infected cattle**	**P-value**
Mean body weight	386.96 ± 62.69 kg	257.94 ± 74.13 kg	0.001*
Mean rectal - temperature	38.2 ± 81.31ºC	40.2 ± 10.48ºC	0.001*
Pack Cell Volume	32.47 ± 8.35%	23.27 ± 6.82%	0.001*

***Statistically Significant at P ≤0.05**

4.3. Haematological Findings on day 0 Before Treatments

The mean haematological parameters such as packed cell volume (PCV %), total erythrocytes count (RBCs), hemoglobin concentration (Hgb), total leucocyte (absolute and differential) counts observed in the naturally infected cattle are presented in Table 4.4.

The mean values of PCV, total erythrocyte counts, haemoglobin concentration of the infected cattle differed significantly ($P \leq 0.05$) when compared to the control. The mean PCV for the infected cattle on day zero was 23.27 ± 6.82% which was significantly ($P \leq 0.05$) lower than that of the control (32.47 ± 8.35%).

The total erythrocyte count and the haemoglobin concentration were statistically different ($P \leq 0.05$) on day zero when compared to the control. The mean total erythrocyte count was 4.28 ± 1.53 $x10^6$ µl and that of the control was 5.53 ± 1.55 $x10^6$ µl. Similarly the haemoglobin concentration of the infected animals was 7.892.32 gm/dl while that of the control was 11.356.79 gm/dl.

Thrombocytopenia was present on day zero before treatments of the infected cattle with a mean platlet count value of 93.23 ± 42.02 $x10^3$ µl while the platelet count for the uninfected cattle on same day was 209.67 ± 55.75 $x10^3$ µl. There was significant ($P \leq 0.05$) difference in the mean value of platelets counts between the infected cattle when compared to the control (Table 4.3).

The mean total leukocyte count for the infected cattle was 4.40 ± 1.64$x10^3$ µl and it was significantly ($P \leq 0.05$) lower than that of the control (8.14 ± 3.34 $x10^3$µl). The mean differential

WBC count indicated that lymphocyte levels (64.64 ± 12.19%) was significantly (P ≤0.05) higher in the infected animals compared to the control while monocytes (1.53 ± 1.97%) were in-significantly (P ≤0.005) higher in the infected animals when compared to the control (Table 4.4). However, the mean neutriphils (32.62 ± 12.25%), eosinophil (1.02 ± 0.75%), and basophil (0.23 ± 0.52%) counts were significantly (P ≤0.05) lower in infected cattle when compared to the control as shown in Table 4.4.

Table 4.4: Haematological parameters of infected and uninfected cattle in selected Local Governments Areas in Niger State, Nigeria.

Parameters (Units)	Uninfected Cattle	Infected Cattle	Mean Difference	P-value
Pack Cell Volume (%)	32.47 ± 8.35	23.27 ± 6.82	9.2	<0.001*
Total Erythrocyte Counts ($x10^6 \mu l$)	5.53 ± 1.55	4.28 ± 1.53	1.2	<0.001*
Total Leuccocyte Counts ($x10^3 \mu l$)	8.14 ± 3.34	4.40 ± 1.64	3.7	<0.001*
Haemoglobin Concentration (gm/dl)	11.35 ± 6.79	7.89 ± 2.32	3.5	<0.001*
Thronbocyte Counts ($x10^3 \mu l$)	209.67 ± 55.75	93.23 ± 42.02	116.4	<0.001*
Lymphocyte Counts (%)	58.19 ± 15.29	64.64 ± 12.19	-6.45	<0.001*
Neutrophil Counts (%)	39.46 ± 15.05	32.62±12.25	6.8	0.001*
Eosinophil Counts (%)	1.04 ± 0.89	1.02 ± 0.75	-0.02	0.855
Basophil Counts (%)	0.27 ± 0.54	0.23 ± 0.52	-0.04	0.557
Monocyte Counts (%)	1.33 ± 1.23	1.53 ± 1.97	-0.2	0.388

*Statistically significant at $P \leq 0.05$

4.4 Biochemical Analysis of Trypanosome Infected Cattle on day 0.

Serum biochemical values of infected cattle and those of the control are presented in Tables 4.5, 4.6 and 4.7.

4.4.1 Serum enzymes

Mean aspartate amino transferase (AST) concentration of infected cattle was 30.40 ± 9.45 IU/L on day zero and was insignificantly ($P \geq 0.05$) higher than those of the control (28.82 ± 10.50 IU/L) as shown in Table 4.4. Also, serum levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) level for the infected animals were 34.62 ± 20.57 IU/L and 105.48 ± 37.97 IU/L respectively on day zero. These serum values were significantly ($P \leq 0.005$) higher than the respective values of the control. The ALT and ALP values for the control were 16.60 ± 6.75 IU/L and 65.60 ± 18.90 IU/L respectively. The mean serum level of creatine kinase (CK) was significantly higher in the infected cattle in comparison to the control. The mean serum level of creatine kinase (CK) was 265.71 ± 21.25 IU/L in the infected animals while that of control was 254.12 ± 11.32 IU/L (Table 4.5).

4.4.2 Serum biomolecules

The values of serum biomolecules (glucose, total protein, globulins, albumin, total cholesterol, high and low density lipo-proteins, and triglycerides) of the infected cattle were different from those of the control as shown in Table 4.6.

Table 4.5 The mean serum enzymes levels of infected and un-infected cattle in some Local Governent Areas in Niger State.

Parameters (Units)	Uninfected Cattle (control)	Infected cattle	Mean difference	P-value
Aspartate aminotransferase **(IU/L)**	28.82 ± 10.50	30.40 ± 9.45	-1.6	0.201
Alanine aminotransferase **(IU/L)**	16.60 ± 6.73	34.62 ± 20.57	-18.0	0.001*
Alkaline phosphatase **(IU/L)**	65.60 ± 18.90	105.48 ± 37.97	-39.9	0.001*
Creatine kinase **(IU/L)**	254.12 ± 11.32	265.71 ± 21.25	11.59	0.001*

*statistically significant at P ≤0.05

The mean values of serum glucose for the infected cattle was (31.94 ± 13.68 mg/dL) and was significantly (P ≤0.05) lower than the mean glucose value (46.80 ± 13.59 mg/dL) of the control.

Total protein level of the infected cattle (51.50 ± 18.28 mg/dL) was also significantly (P ≤0.05) lower than that of the control (77.20 ± 14.46 mg/dL).

The mean albumin and globulins concentration in the infected animals were 24.84 ± 8.31 mg/dL and 29.34 ± 15.31 mg/dL respectively. These values were significantly (P ≤0.05) lower than the levels of the controls. The serum levels of the globulins and albumin concentration of the control were 49.80 ± 15.05 mg/dL and 27.60 ± 6.73 mg/dL, respectively. Similarly, the mean total serum cholesterol levels in the infected animals (2.62 ± 1.33 mg/dL) when compared to the control (3.25 ± 1.66 mg/dL) was significantly (P ≤0.05) lower as shown in Table 4.6. High Density Lipo-proteins (HDL) was in-significantly (P ≥0.05) lower in the infected cattle while Low Density Lipo-proteins (LDL) was significantly (P ≤0.05) lower in the infected cattle when compare to their controls. The mean values of HDL for the infected cattle (1.40 ± 0.66 mg/dl) was in-significantly lower than the level for the control (1.52 ± 0.64 mg/dl) while the mean serum values of LDL (2.57 ± 0.78 mg/dl) for the infected animals was significantly lower than the level for the control (2.76 ± 0.44 mg/dl). However, mean values of the serum triglycerides (2.32 ± 1.08 mg/dL) in the infected cattle was significantly (P ≤0.05) higher when compared to that of the control (1.90 ± 0.58 mg.dL).

Table 4.6 The Mean serum biomolecules of infected and un-infected cattle in Niger State, Nigeria (measured in mg/dL).

Variable	Uninfected cattle (control)	Infected cattle	Mean difference	P-value
Glucose	46.80 ± 13.59	31.94 ± 13.65	14.9	0.001*
Total Protein	77.20 ± 14.46	51.50 ± 18.28	62.7	0.001*
Albumin	27.60 ± 6.73	24.84 ± 8.31	2.8	0.006*
Globulins	49.80 ± 15.05	29.34 ± 15.31	34.8	0.001*
Total Cholesterols	3.25 ± 1.66	2.62 ± 1.33	0.6	0.001*
Triglycerides	1.90 ± 0.58	2.32 ± 1.08	-0.4	0.001*
High Density lipoproteins	1.52 ± 0.64	1.40 ± 0.66	0.1	0.150
Low Density Lipoproteins	2.76 ± 0.44	2.57 ± 0.78	0.2	0.030*

*statistically significant at $P \leq 0.05$

4.4.3 Serum minerals

The mean serum sodium level for the infected cattle was 111.82 ± 28.84 mg/dL while that of the control was 127.80 ± 34.95 mg/dL. This difference was statistically significant (P ≤0.05). The mean serum level of potassium in the infected animals was 7.55 ± 11.87 mg/dL while the value for the control was 5.64 ± 2.15 mg/dL. This difference of potassium levels between the two groups were however not statistically significant (P ≥0.05) as shown in Table 4.7.

The mean serum values of calcium in the infected animals were lower than the control. The serum calcium level of the infected animals was 2.98 ± 0.85 mg/dL while the control was 4.16 ± 0.54mg.dL. The levels in the infected animals were statistically (P ≤0.05) lower than the level of the control. The mean levels of chloride and bicarbonates were low in infected animal. The mean serum chloride and bicarbonates values for the infected animals were 91.76 ± 25.59 mg/dL and 17.46 ± 6.76 mg/dL respectively, while those of the control were 98.60 ± 19.48 mg/dL and 20.60 ± 12.54 mg/dL respectively. These mean serum chloride and bicarbonates levels in the infected cattle were statistically (P ≤0.05) different from those of the control animals as shown in Table 4.7.

The mean serum Iron level of infected animals (1.55 ± 0.60 mg/dL) was significantly (P ≤0.05) lower than the control (4.45 ± 2.07 mg/dL) while the serum copper level for the infected animals (0.49 ± 0.36 mg/dL) was also significantly (P ≤0.05) lower than the control (0.81 ± 0.08 mg/dL). The serum level of zinc was 2.08 ± 1.42 mg/dL in infected cattle while that of the control was 7.88 ± 2.52 mg/dL. These values were significantly (P ≤0.05) different as shown in Table 4.7.

Table 4.7: The mean of serum micro-nutrients of infected and un-infected cattle in selected Local Government Areas of Niger State, Nigeria.
(measured in mg/dL)

Variable	Uninfected cattle (control)	Infected cattle	Mean difference	P-value
Sodium	127.80 ± 34.95	111.82 ± 28.84	16.0	0.001*
Potassium	5.64 ± 2.15	7.55 ± 11.87	-1.9	0.150
Chloride	98.60 ± 19.48	91.76 ± 25.59	6.8	0.020*
Bicarbonates	20.60 ± 12.54	17.46 ± 6.76	3.2	0.003*
Calcium	4.16 ± 0.54	2.98 ± 0.85	1.18	0.001*
Iron	4.45 ± 2.07	1.55 ± 0.60	2.9	0.001*
Copper	0.81 ± 0.08	0.49 ± 0.36	0.3	0.001*
Zinc	7.88 ± 2.52	2.08 ± 1.42	5.8	0.001*

*Statistically significant at P ≤0.05

4.5 Response to Diminazene Aceturate and Isomethamidium Therapy

4.5.1 Haematological response to diminazene aceturate therapy

There was a significant reduction in erythrocyte parameters in the infected cattle on day zero before treatment. However following treatment with diminazene aceturate all the infected animals had improvements in their PCV, the total erythrocyte counts, the haemoglobin concentration, the thrombocytes and the total leucocyte counts. The PCV and the total erythrocyte counts appreciated from 23.27 ± 6.82% and 4.23 ± 1.53 $x10^6$ µl respectively on day zero to 28.28 ± 4.52% and 4.76 ± 0.83 $x10^6$ µl respectively by day 14 post treatment (Table 4.8) and further to 29.50 ± 0.71% and 4.85 ± 0.71 $x10^6$ µl respectively by day 28 post treatment.

Similarly level of the thrombocytes observed on day zero in the infected animals was 93.23 ± 42.02 $x10^3$ µl. The mean thrombocytes counts following treatment with diminazene aceturate by day 14 post treatment rose to 139.18 ± 32.89 $x10^3$ µl and then to 198.00 ± 12.73 $x10^3$ µl on day 28 post treatment (Table 4.8).

More so, the mean total lenukocyte count on day zero of the animals before they were treated with diminazene aceturate was 4.40 ± 1.64 $x10^3$ µl. By day 14 following treatment there was an increase in the values of the total leukocyte counts to 5.35 ± 1.63 $x10^3$ µl and further to 6.75 ± 2.73 $x10^3$ µl by day 28 post treatment. On day zero lymphocytes and monocytes were higher in the infected cattle. However, following treatment with Diminazene aceturate, their values declined as noted on day 14 post treatment. Lymphocyte counts depreciated from 64.64 ± 12.19% on day zero before treatments to 59.16 ± 15.31% following treatment with Diminazene aceturate. These values depreciated further to 53.00 ± 2.83% on day 28 post treatment. The vaues of monocyte by day zero before the treatment with Diminazene aceturate was 1.53 ± 1.97%, this decreased to 1.13 ± 1.43% on day 14 and on day 28 post treatment, the monocyte levels further declined to 1.00 ± 0.00%. However, the monocyte counts for both the infected and the control groups were within normal ranges.

The values of neutrophils, eosinophils and basophils increased after treatment with Diminazene aceturate. The mean neutrophil counts increased from 32.62 ± 12.25% on day zero to 38.52 ± 15.16% on day 14 and then to 44.00 ± 2.83% on day 28 post treatments. The mean eosinophil counts rose from 1.02 ± 0.75% on day zero to 1.42 ± 1.78% on day 14 and then to 1.50 ± 0.71% on day 28 following treatment with Diminazene aceturate. The basophil counts increased from 0.23 ± 0.52% on day zero to 0.32 ± 0.65% on day 14 and then to 0.50 ± 0.71% on day 28 post treatment.

Table 4.8 Mean of haematological response to treatment with diminazene aceturate of cattle with trypanosomosis

Variable (unit)	Day 0	Day 14	Day 28	P-Value
Packed Cell Volume (%)	23.27 ± 6.82	28.28 ± 4.52	29.50 ± 0.71	0.6162*
Total Erythrocyte Counts($x10^6$µl)	4.28 ± 1.53	4.76 ± 0.83	4.85 ± 0.71	0.9234*
Haemoglobin Concentration (gm/dl)	7.89 ± 2.32	9.60 ± 0.28	10.28 ± 6.28	0.9037*
Thrombocyte Counts ($x10^3$µl)	93.23 ± 42.02	139.18 ± 32.89	198.00 ± 12.73	0.0653*
Total Leuccocyte Counts($x10^3$µl)	4.40 ± 1.64	5.35 ± 1.63	6.75 ± 2.73	0.7209*
Lymphocyte Counts (%)	64.64 ± 12.19	59.16±15.31	53.00±2.83	0.7710*
Neutrophil Counts (%)	32.62 ± 12.25	38.52±15.16	44.00±2.83	0.7785*
Eosinophil Counts (%)	1.02 ± 0.75	1.42 ± 1.78	1.50 ± 0.71	0.9542*
Basophil Counts (%)	0.23 ± 0.52	0.32 ± 0.65	0.50 ± 0.71	0.9537*
Monocyte Counts (%)	1.53 ± 1.97	1.13 ± 1.43	1.00 ± 0.00	0.9621*

*statistically not significant at $P \geq 0.05$

4.5.2 Haematological response to isomethamidium chloride therapy

There was reduction in the values of haematological parameters on day zero in the infected cattle before they were treated with Isomethamidium chloride on that day. The PCV and total erythrocyte counts of the infected animals before treatment with isomethamidium chloride on day 0 were 23.27 ± 6.82% and 4.28 ± 1.53 $x10^6$µl which increased to levels of 30.50 ± 5.73% and 5.12 ± 0.99 $x10^6$ µl respectively on day 14 post treatment as presented in Table 4.9. The PCV and total erythrocyte counts of these animals appreciated further to 32.41 ± 7.24% and 5.78 ± 1.65$x10^6$ µl respectively on day 28. Similarly the thrombocytes level on day zero (93.23 ± 42.02 $x10^3$ µl) got increased to 128.37 ± 34.10 $x10^3$ µl on day 14 following treatment. On day 28 post treatments the mean value of thrombocytes count further increased to 140.84 ± 50.46 $x10^3$ µl.

The leucopenia that was observed in the infected cattle got increased when they were treated with isomethamidium chloride. On day zero the total leukocytes counts was 4.40 ± 1.64 $x10^3$ µl. There was an increase in the values of the total leukocyte counts on day zero from 4.40 ± 1.64 $x10^3$ µl to 7.23 ± 3.46 $x10^3$ µl on day day 14 and then to 9.44 ± 4.58 $x10^3$ µl on day 28 post treatment with isomethamidium chloride.

The lymphocyte and the monocytes were higher in the infected animals on day zero, when compared to their controls. But following treatment with isomethamidium chloride these values declined as noted on day 14. Lymphocyte counts got depreciated from 64.64 ± 12.19 on day zero to 59 ±14.83% on day 14 and further to 55.69 ± 18.48 on day 28 post treatment. Similarly, monocyte level declined from 1.53 ± 1.97% on day zero to 1.38 ± 1.62% on day 14 but then rose to 1.81 ± 1.89% on day 28 post treatment. However, the monocyte counts for the infected group and the controls were within normal ranges for cattle.

The neutrophils, eosinophils and basophils levels increased after treatment with isomethamidium chloride. The mean neutrophil counts for infected cattle appreciated from 32.62 ± 12.25% on day zero to 35.59 ± 16.05% on day 14 and to 40.80 ± 14.13% on day 28 post treatment. The mean eosinophils was lower in infected cattle on day zero but following treatment with isomethamidium chloride, their mean values increased from 1.02 ± 0.75% on day zero to 1.05 ± 0.82% on day 14 and then to 1.30 ± 1.94% on day 28 post treatment. The basophil counts increased from 0.23 ± 0.52% on day zero to 0.25 ± 0.58% on day 14 but then the level declined to 0.07 ± 0.25% on day 28 post treatment.

Table 4.9 Mean of heamatological response to treatment with isomethamedium chloride of cattle with trypanosomosis

Variable (units)	Day 0	Day 14	Day 28	P-Value
Packed Cell Volume (%)	23.27 ± 6.82	30.50 ± 5.73	32.41 ± 7.24	0.5895*
Total Erythrocyte Counts ($x10^6\mu l$)	4.28 ± 1.53	5.12 ± 0.99	5.78 ± 1.65	0.7555*
Haemoglobin Concentration (gm/dl)	7.89 ± 2.32	9.94 ± 2.03	10.60 ± 2.35	0.6715*
Thronbocyte Counts ($x10^3\mu l$)	93.23 ± 42.02	128.37 ± 34.10	140.84 ± 50.46	0.7163*
Total Leuccocyte Counts ($x10^3\mu l$)	4.40 ± 1.64	7.23 ± 3.46	9.44 ± 4.58	0.5848*
Lymphocyte Counts (%)	64.64 ± 12.19	59.50 ± 14.83	55.69 ± 18.48	0.9183*
Neutrophil Counts (%)	32.62±12.25	35.59 ± 16.05	40.80 ± 14.13	0.8938*
Eosinophil Counts (%)	1.02 ± 0.75	1.05 ± 0.82	1.30±1.94	0.9859*
Basophil Counts (%)	0.23 ± 0.52	0.25 ± 0.58	0.07 ± 0.25	0.9573*
Monocyte Counts (%)	1.53 ± 1.97	1.38 ± 1.62	1.81 ± 1.89	0.9859*

*Statistically not significant at $P > 0.05$

4.5.3. Effect of treatment with diminazene aceturate therapy on clinical signs

The number of clinical signs observed on the infected cattle on day zero was 19 as presented in Table 4.9. Following treatment with diminazene aceturate, 26.3% of these clinical signs-anorexia, dark/rough coat, pyrexia, corneal opacity, cutaneous haemorrhages and retained placenta were absent by day 14 while on day 28 anaemia, weakness, epiphora, alopecia, nasal discharge coughing, agalactia, shade seeking had further disappeared. However, emaciation, pica, teeth grinding and pelleted stool were still manifested on day 28 post treatment.

4.5.4. Effect of treatment with isomethamidium chloride therapy on clinical signs

Of the 19 clinical signs manifested by the infected cattle on day zero 21% of these clinical signs-anorexia, dark/rough coat, pyrexia, and cutaneous haemorrhages were not observed by day 14 as presented in Table 4.10. By day 28 post treatment anaemia, weakness, pale mucous membrane, epiphora, corneal opacity, alopecia, nasal discharge, agalactia and shade seeking disappeared. However, emaciation, pica, teeth grinding, and pellated stool were still manifested on day 28 post treatment.

Table 4.10 Changes in clinical signs of cattle with trypanosomosis after treatment with diminazene aceturate in selected Local Government Areas in Niger State

S/No	Observed Clinical Sign	Disappeance of Clinical Signs		
		DAY 0	DAY 14	DAY 28
1	Emaciation	++	++	+
2	Anaemia (PCV Less than 24%)	++	+	-
3	Weakness	++	+	-
4	Anorexia	++	-	-
5	Pale mucous membrane	++	+	-
6	Epiphora	++	+	-
7	Dark/rough coat	++	-	-
8	Alopecia	++	+	-
9	Pica	++	+	+
10	Pyrexia (Temperature over 38.5^0c)	++	-	-
11	Nasal discharge	++	+	-
12	Cough	++	+	-
13	Corneal opacity	++	-	-
14	Agalactia	++	+	-
15	Teeth Grinding	++	+	+
16	Cutaneous haemorrhages	++	-	-
17	Shade seeking	++	+	-
18	Pelleted stool	++	+	+
19	Retained placenta	++	-	-

KEY:
(++): Severe clinical signs
(+): Mild clinical signs
(-): Absence of clinical signs

Table 4.11 Changes in clinical signs of cattle with trypanosomosis after treatment with isomethamidium chloride in some selected Local Government Areas of Niger State

S/No	Observed Clinical Sign	Disappeance of Clinical Signs		
		DAY 0	DAY 14	DAY 28
1	Emaciation	++	++	+
2	Anaemia (PCV Less than 24%)	++	+	-
3	Weakness	++	+	-
4	Anorexia	++	-	-
5	Pale mucous membrane	++	+	-
6	Epiphora	++	+	-
7	Dark/rough coat	++	-	-
8	Alopecia	++	+	-
9	Pica	++	+	+
10	Pyrexia (Temperature over 38.5^0c)	++	-	-
11	Nasal discharge	++	+	-
12	Cough	++	+	-
13	Corneal opacity	++	+	-
14	Agalactia	++	+	-
15	Teeth grinding	++	+	+
16	Cutaneous haemorrhages	++	+	-
17	Shade seeking	++	+	-
18	Pelleted stool	++	+	+
19	Retained Placenta	++	-	-

KEY:
(++): Severe clinical signs
(+): Mild clinical signs
(-): Absence of clinical signs

4.5.5 Trypanosome clearance following trypanocides therapy

4.5.5.1 Trypanasome clearance following Diminazene aceturate treatment

Of the 126 cattle that were treated with Diminazene aceturate, 3 cattle (2.3%) had a persistent infection with various species of trypanosomes on day 14 post treatment. These cattle were infected with *Trypanosoma vivax* 2(66.7%) and *T. brucei* 1(33.3%). While on day 28 post treatment, 3 (2.3%) of the animals had persistent infection with three species of trypanosomes: *T. congolense* 1(33.3%), *T. vivax* 1(33.3%), and *T. brucei* 1(33.3%). Two animals in Katcha LGA and one animal in Mariga LGA were found to have persistent infection with *T. vivax* and *T. brucei* each on day 14 post treatment while on day 28, one animal each in Katcha, Mariga and Shiroro LGAs had persistent infection with *Trypanosoma congolense, T. vivax* and *T. brucei* respectively. However, no parasite was detected in Agaie LGA following treatment with diminazene aceturate on days 14 and 28 post treatment (Table 4.12).

4.5.3.2 Trypanosome clearance following to isomethamidium chloride treatment

Of the 136 cattle that were treated with isomethamidium chloride 4 (2.9%) still had persistent infections with *T. congolense* accounting for 2 (50%), *T. vivax* for 1 (25%) and *T. brucei* for 1 (25%) on day 14 post treatment. However on day 28 post treatment, 5 (3.6%) were positive for trypanosome infection. *Trypanosoma congolense* accounted for 3 (60%), *T. vivax* 1 (20%) and *T. brucei* 1 (20%) of the relapse infections on day 28 post treatment as indicated in Table 4.13

Table 4.12 Trypanosome clearance following diminazene aceturate therapy

[4]LGA		DAY 14				DAY 28			
	Number of animal sampled	Number of animal positive	Trypanosome species identified			Number of animal positive	Trypanosome species identified		
			TC	*TV*	*TB*		[1]*TC*	[2]*TV*	[3]*TB*
Agaie	46	0	0	0	0	0	0	0	0
Katcha	16	2	0	2	0	1	0	1	0
Mariga	13	1	0	0	1	1	0	0	1
Shiroro	49	0	0	0	0	1	1	0	0
Total	**126**	**3 (2.3%)**	**0**	**2**	**1**	**3 (2.3%)**	**1**	**1**	**1**

[1]*TC = Trypanosoma congolensi.*
[2]*TV = T. vivax.*
[3] *TB = T. brucei.*
[4] LGA= Local Government Area

Table 4.13: Trypnosome clearance following isomethamidium chloride therapy

[4]LGA	DAY 14					DAY 28			
	Number of animal sampled	Number of animal positive	Trypanosome species identified			Number of animal positive	Trypanosome species identified		
			[1]*TC*	[2]*TV*	[3]*TB*		[1]*TC*	[2]*TV*	[3]*TB*
Agaie	22	2	2	0	0	1	0	1	0
Bosso	10	0	0	0	0	0	0	0	0
Gbako	11	0	0	0	0	0	0	0	0
Katcha	22	1	0	1	0	2	2	0	0
Lavun	32	1	0	0	0	2	1	0	1
Shiroro	39	0	0	0	0	0	0	0	0
Total	**136**	**4 (2.9%)**	**2**	**1**	**1**	**5 (3.6%)**	**3**	**1**	**1**

[1]*TC* = *Trypanosoma congolense.*
[2]*TV* = *T. vivax.*
[3] *TB* = *T. brucei*.
[4] LGA= Local Government Area.

CHAPTER FIVE

DISCUSSION

The results of parasitological screening of the cattle with clinical trypanosomosis in this study was 13%, which falls within the range of 9.0% – 46.8% reported in other surveys in Nigeria (Kalu 1995c; Kalu *et al.,* 1991; Abenga *et al.,* 2004; Oluwafemi 2008; Samdi *et al.,* 2011; Majekodumni, *et al*. 2013; Takeet *et al.,* 2013) and elsewhere in Africa (Mamoudou *et al.,* 2006; Merkuria and Gadissa, 2011). A significantly higher typanosoma infections were reported in Ogun State in Southern Nigeria (Sam-Wobo *et al.,* 2010); Benue, Kaduna and Plateau States in the North Central States of Nigeria (Kalu *et al*. 1991; Samdi *et al*. 2011; Majekodumni *et al.,* 2013) and in a national survey of infection with pathogenic trypanosomes by Takeet *et al.,* (2013). The high infection rate coincided with heavy infestation of both obligate (*Glossina spp*) and mechanical (*Tabanids*) vectors of trypanosomes (Ahmed, 2004). Similarly, this may not be unconnected with the choice of diagnostic technique. The molecular techniques they used in some of the studies have been reported to be more sensitive when compared with parasitological test used in this study (Paris *et al.,* 1982; Geysen *et al.,* 2003).

The most dominant trypanosome species found in this study were *T. congolense and T. vivax*. This is similar with the reports of Abenga *et al.,* (2004); Ohaere, (2010) and Majekodummi *et al.,* (2013) who reported *T. congolense* and *T. vivax* as the dominant species of trypanosomes in their survey. *Trypanosoma congolense* is the most widespread species of trypanosome found over much of sub-Saharan Africa representing over 60% of all identified Trypanosoma infections (Takeet *et al.,* 2013; Auty *et al*., 2015).

The low infection with *T. brucei* is consistent with some other surveys carried out across Nigeria (Kalu, 1996a, b; Kalu *et al.,* 2001; Anosike *et al.,* 2003) and elsewhere in Africa in cattle (Leak *et al.,* 1993; Mahama, 2005; Mamoudou *et al.,* 2006).The relative resistance to infection with *T. brucei* by the West African indigenous breed of cattle (Kalu, 1996a) coupled with low density and vectoral capacity of *G. tachinoides* and *G. p. palpalis* (Majekodummi 2006) are some of the reasons why *T. brucei* is transmited at a lower frequency (Welburn and Maudlin , 1999).

This study observed some clinical signs in the infected animals which have been reported in previous studies on bovine trypanosomosis under experimental conditions (Cadioli *et al.,* 2012; Dagnachew *et al.,* 2015; Fidelis *et al.,* 2016). These clinical signs were emaciation, weakness, anorexia, pale mucous membrane, epiphora, dark hair coat, alopecia, pica, pyrexia, nasal discharge, coughing and corneal opacity. Other signs observed were cutaneous haemorrhage, shade seeking, and nervous signs like muscle tremors and ataxia. In pregnant cattle with trypanosomosis, additional signs observed were agalactia, retained placenta, weak calves, and abortion. The most prominent of these clinical signs observed was emaciation. Emaciation due to trypanosoma infection have been reported in several experimental animal models such as: cattle experimentally infected with *T. vivax* (Fidelis *et al.,* 2016), sheep and goats experimentally infected with *T. brucei* (Mutayoba *et al.,* 1989; Katunguka-Rwakishaya *et al.,* 1993), goats experimentally infected with *T. evansi*, *T. brucei* and *T. vivax* (Dargantes *et al.,* 2005; Adeiza *et al.,* 2008) and rats experimentally infected with *T. brucei* (Eghianruwa, 2012).

The reported emaciation observed in this study could be due to decrease feed intake (Verstegen *et al.,* 1991), generalize gluconeogenesis of energy store (Igbokwe, 1995; Pathak, 2009) to

support the high energy demand by the parasite and by the host's immune response (Menezes *et al.,* 2004) as well as the negative effect of pro-inflammatory cytokines (Yasameen *et al.,* 2015). The pyrexia observed reflects the response to successive waves of parasitaemia and metabolism as previously reported by Stephen (1986) and Holmes *et al.,* (2000). This has been reported by previous workers on different experimental animal models (Adamu *et al.,* 2009; Allam *et al.,* 2012; Osuagwuh, 2014 and Yasameen *et al.,* 2015).

Corneal opacity has been reported in cattle (Losos and Ikede, 1972; Ilemobade and Schilhorn van Veen, 1974); goats experimentally infected with *T. vivax* (Whitelaw *et al.,* 1988) and *T. evansi* (Morales *et al.,* 2006) and dogs experimentally infected with *T. brucei* (Morrison *et al.,* 1981). Corneal opacity has been associated with the presence of trypanosomes extravascularly in the aqueous and vitreous humours, where they cause varying grades of tissue reaction within the eye structures (Ikede, 1974). Although corneal opacity was observed in this study, trypanosomes were not observed in the eye structure.

The alopecia observed in the infected animals during this study may be due to profound nutritional imbalances that characterized infection with trypanosomes. Trypanosomes have been previously reported to cause decrease in feed intake (Verstegen *et al.,* 1991; Zwart *et al.,* 1991), hypoglyceamia (Kadima *et al.,* 2000; Cadioli *et al.,* 2006; Dagnachew *et al.,* 2014); hypoproteinaemia (Sadique *et al.,* 2001; Sivajothi *et al.,* 2013; Moolchandani and Sareen. 2016), hypolipaemia (Adamu *et al.,* 2008; Eze *et al.,* 2015), profound hormonal imbalances (Fatihu *et al.,* 2009; Sivajothi *et al.,* 2015) and alteration in serum mineral constituents (Da Silva *et al.,*

2009b; Wolkmer *et al.,* 2013). The overall consequences of these derangements are dermatological defects like dry scaly coat, alopecia, dermatitis and hyperkeratosis.

Cutaneous haemorrhages observed in this study could have been due to thrombocytopenia which has been reported in cattle experimentally infected with *T.vivax* (Fidelis *et al.,* 2016) in addition to hypocalcaemia that is also reported in sheep experimentally infected with *T. brucei* (Ogunsanmi *et al.,* 1994) and Kamal (2008) in clinically sick dromedary camel. Thrombocytopenia may also be due to thrombocyte phagocytosis as earlier observed in the bone marrow of infected animals (Williams *et al.,* 1991; Anosa 1983; Anosa *et al*. 1992; 1997). Disseminated intravascular coagulation leads to partial blockage of blood capillaries by thrombi and aggregation of platelets which leads to their rupture (Dirie *et al.,* 1988). These coagulopathies in the presence of bites from tsetse flies and biting flies initiates haemorrhages from the intact skin.

Reproductive disorders such as abortions, retained placenta and weak calves were observed in infected animals during this study. Previously, several reports have recognized reproductive disorders as direct consequence of experimental trypanosome infection in animal (Ogwu *et el*., 1986; Bawa *et al.,* 2000; Desquesnes, 2004; Allam *et al.,* 2011; Batista, *et al.,* 2012; Silva *et al.,* 2013). The negative effect of trypanosomosis in pregnant animals is probably due to hematological, biochemical and pathological alterations that the disease is known for. The combined effect of increased energy demands in pregnancy and high energy demand by the trypanosomes in addition to degenerative changes in the hypothalamus, pituitary, and the gonads promotes increased metabolic needs of the animals and diminished plasma hormones, which are

fundamental to reproductive processes (Sekoni, 1994). These changes in an infected animal are normally incompatible with the maintenance of fetal development during pregnancy. In addition, the retained placenta, abortion or birth of weak calves and other reproductive problems may be related to inadequate nutrition, fetal oxygenation and tissue anoxia (Silva *et al*. 2013).

A significant decrease in mean PCV, Hgb concentration, total RBC and leukocyte counts were observed in infected animals when compared to the control. This is in agreement with various experimental studies in cattle conducted much earlier (Cuglovici *et al.,* 2010; Cadioli *et al.,* 2012; Dagnachew *et al.,* 2015). The low PCV observed in the infected group may be as a result of acute haemolysis due to growing infection as was observed by Adenike and Stephen (2010). Also Infection with trypanosomes had been shown to cause haemo-dilution, trace element deficiency, haemolysis of erythrocytes and direct trauma to the red blood cells by the lashing of the trypanosomes and bone marrow failure (Saror, 1980). Similarly, Esievo (1980) had reported that trypanosomes produces neuraminidase which in turn cleaves off erythrocyte surface sialic acid, thus causing physicochemical damage to erythrocyte surface and renders them more prone to phagocytosis by the reticuloendothelial system. It is possible that during Trypanosoma infection, failure of iron incorporation into red cell precursors even in presence of adequate iron storage precipitates the occurrence of anaemia (Dargie *et al.,* 1979).

In this study, a significant decrease in the level of total leukocytes was observed in cattle infected with trypanosomes which may be attributed to the immunosuppressive actions of trypanosome infection (Abubakar *et al.,* 2005; Ekanem and Yusuf, 2008). Maxie *et al.,* (1997) described pancytopenia associated with *T. vivax and T. congolense* infections in cattle; Osman *et al.,*

(2008) in goats infected with *T. vivax* and Allam *et al.,* (2011) with *T. brucei* in pigs. In this study, the increased lymphocyte count could be as a result of the relative depression of neutrophils during the infection or as a result of the immune response by the animals during the chronic phase of the infection.

Decreased leucocyte counts with increased lymphocyte counts as observed in this study seems to be the major characteristic of WBC in the chronic phase of the disease (Espinoza and Aso, 1992; Batista *et al.,* 2006; 2008), and leucopenia is an important event in *T. vivax* infections in cattle, sheep and goats (Espinoza and Aso, 1992). Similarly, Anosa *et al.,* (1992) and Paiva *et al.,* (2000) had reported leukocytosis by lymphocytosis in goats, sheep and cattle during the chronic phase of infection by *T. vivax* while Anosa and Kaneko, (1983a) had reported leukopenia, as well as monocytosis and eosinopenia in *T. brucei* infected mice and *T. vivax* infected sheep as it was the case in this study.

The leukopenia in the course trypanosomosis in cattle may be as a result of the wax and wear syndrome in the immune system caused by the ever changing variable surface glycoprotein of the infecting trypanosomes (Abubakar *et al.,* 2005). The eosinopenia and basopenia as seen in this study were also observed in goats and sheep infected with *T. vivax* (Anosa and Isoun 1980) and in mice infected with *T. brucei* (Anosa, 1975). The eosinopenia and basopenia could have resulted from the chronic nature of trypanosome infection in cattle (Lording and Friend, 1991).

Biochemical changes observed in the infected animals is an indication of pathological and functional disturbances in cattle infected with trypanosomes. Increased serum level of AST,

ALT, ALP, and CK were observed in the infected animals which could be due to the hepato-cellular damage. The increase in the levels of transminases (AST and ALT) as noticed in this study agrees with the results obtained in experimental animal models. These enzymes were reported to be low in cattle experimentally infected with *T. vivax* (Kadima *et al.,* 2000; Dagnachew *et al.,* 2014) and so also in sheep (Gray, 1963). Similar resuts were reported in goats experimentally infected with *T. congolense* (Adah *et al.,* 1992); dogs experimentally infected with *T, brucei* (Omotainse *et al.,* 1994), sheep experimentally infected with *Trypanosoma brucei* (Taiwo *et al.,* 2003), pigs experimentally infected with *T. brucei* (Allam *et al.,* 2011) and camels experimentally infected with *T. evansi* (De La Rue *et al.,* 1997; Sazmand *et al.,* 2011). Significant increase in serum emzymes indicates celular damage of the vital organs like the liver in the case of ALP, ALT, and AST, or inflammatory responses and necrosis of the osteocytes, skeletal and myocardial muscle cells in the case of CK, (Ezeokonkwo *et al.,* 2012).

Hypoglycaemia as reported in this study could have been responsible for the muscle tremors and ataxia that were observed in the infected animals. Reduction in serum glucose had previosly been reported in sheep experimentally infected with *T. congolense* (Taiwo *et al.,* 2003), in West African Dwarf goats infected with *T. congolense* and *T. brucei* (Faye *et al.,* 2005), in cattle infected with *T. vivax* (Kadima *et al.,* 2000) and in trypanosomes infected camels (Padmaja, 2012). The hypoglycemia that was recorded in this study could be explained by the parasite's need for glucose as an energy source (Opperdoes *et al.,* 1986; Kadima *et al.,* 2000). It has been shown that parasite count is inversely proportional to glucose concentration (Jatkar and Singh, 1974). Increased metabolic rate caused by fever and hepatocyte degeneration could be another reason for the hypoglycemia in trypanosome infection (Cadioli *et al.,* 2006).

Low levels of serum total protein, globulins and albumin as observed in this study is consistent with the findings of Sadique *et al.,* (2001) who reported lower values for total protein in cattle infected with *T. congolense* and those of Kamal, (2008) and Gaber *et al.,* (2012) in *T. evansi* infected camels. The results however contradicts the observations made in sheep infected with *T. brucei* by Taiwo *et al.,* (2003), who observed no change in the levels of total plasma proteins from pre-infected values at the initial stage of the infection, but in the later stage the levels increased significantly above pre-infection levels. Hypoproteinaemia, hypo-albuminaemia and hypo-globulinaemia is said to arise due trypanosomes uptake of albumin bound fatty acids and lipo proteins or due to increased catabolism of body proteins (Gutierrez *et al.,* 2006) and immunologic response due to trypanosome infection (Jatkar *et al.,* 1973; Igbokwe, 1995).

The hypocholesterolaemea observed in this study agrees with previous reports of experimental *T. vivax* infection in cattle (Espinoza *et al.,* 1980) and sheep (Schenk *et al.,* 2001) respectively. Similar findings were also reported by Katunguka Rwakishaya *et al.,* (1997) working with sheep experimentally infected with *T. congolense* and Eze *et al.,* (2015) who reported decreased serum cholesterol levels in pigs experimentally infected with a single and mixed *T. b. brucei* and *T, congolense*. Low cholesterol levels are often found in many types of anaemia due to greater use of plasmatic cholesterol to replace erythrocyte lipids in cases of profound haemolysis (Naoum, 1999). Glucose, triglycerides and cholesterol from the host are the primary energy source for trypanosomes (Coppens *et al.,* 1995; Katunguka Rwakishaya *et al.,* 1997).

High and Low density lipoproteins (HDL and LDL) showed a significant reduction in the serum of infected cattle. This finding was in agreement with previous studies in *T. congolense* and *T. brucei* experimentally infected sheep (Biryomumaisho *et al.,* 2003); *T. brucei* infected pigs (Adamu *et al.,* 2008); and single and mixed infection of *T. b. brucei* and *T, congolense* in pigs (Eze *et al.,* 2015). Many mechanisms have been advanced to be responsible for the low level of serum HDL and LDL in the trypanosome infected animals. High and Low density lipoproteins (HDL and LDL) along with phospholipids and total lipids are required by the blood-stream trypanosomes for synthesis of their membranes and for their growth (Katunguka-Rwakishaya *et al.,* 1991; Green *et al.,* 2003; Nok *et al.,* 2003).

Hyper-triglyceridemia as reported in this study concurs with several experimental animal models. Hypertriglyceridemia have been reported in rabbits experimentally infected with *T. b. gambiense* (Diehl and Risby, 1974)*;* monkeys experimentally infected with *T. b. rhodesiense* (Ngure *et al.,* 2008); rabbits experimentally infected with *T. brucei* (Nakamura, 1998) and goats experimentally infected with *T. vivax* (Hamminga *et al.,* 1996). The increase in triglycerides could be due to marked inhibition of the enzyme lipoprotein lipase (LPL) responsible for clearing lipids from plasma, a process that is markedly inhibited by tumor necrosis factor (TNF) (Beutler and Cerami, 1988). Marked increase in pro-inflammatory cytokines including TNF has been observed in trypanosome infection (Netea *et al.,* 2000; Maina *et al.,* 2004). According to Nakamura (1998), the continuous release of this TNF-α, suppresses the normal lipoprotein lipase synthesis which leads to hyper-triglyceridemia.

The lowered Na, Cl and HCo^3 seen in the infected cattle are in agreement with previous studies. Such as in cattle (Da Silva *et al*. 2011), sheep and goats (Neils *et al.,* 2006; Ogunsanmi and Taiwo, 2004), equines (Yadav *et al.,* 2012), camels (Chaudhry and Iqbal, 2000; Kamal, 2008; Sazmand *et al.,* 2011), Swine (Allam *et al.,* 2011), rabbits (Amole *et al.,* 1990; Sivajothi *et al.,* 2013) and Dogs (Velayudhan *et al.,* 2015). These observations were all made during experimental infection.

Sodium and Potassium are largely intracellular cation with over 98% of the exchangeable ions located intracellularly. Their distribution across the cell membrane plays a significant role in the maintenance of cardiac and neuromuscular excitability (Carlson, 1989; Ogunsanmi *et al.,* 1994b). Some of the clinical sign presented by the trypanosome infected animals like disorientation and instability might be related to the changes in concentrations of sodium and potassium (Marques *et al.,* 2000; Herrera *et al.,* 2004; da Silva *et al.,* 2011) since these minerals are involved in the regulation of the acid-base balance and in the transmission of nerve impulses and in muscle contraction (McDowell, 1992).

Decrease level of calcium as reported in this study can be attributed to decrease in both intake and absorption of these mineral due to anorexia and gastrointestinal atony which were usually associated with trypanosomosis or due to hypoalbuminaemia as calcium is found in the serum in protein bound that could not be replenished by re-absorption from the kidneys (Besarab *et al*. 1981; Bienzle *et al*. 1993). Diminished plasma concentration of parathyroid hormone also may cause hypocalcaemia due to profound degeneration in the parathyroid glands in trypanosome infected animals**.**

Calcium is directly involved in muscle contraction and depletion of calcium store could have been the cause of in-coordination and instability of hind limbs, atrophy of the large muscles of the limbs, difficulty to stand up and muscle weakness observed in cattle with trypanosomosis as reported by Marques *et al.,* (2000) and Herrera *et al.,* (2004). Similarly, coagulopathies (De La Rue *et al.,* 1997) and emaciation as a consequence of trypanosome infection may have direct relationship with the low serum calcium concentration.

Hypoferremia as reported in this study agree with the findings of decreased serum iron levels in mice experimentally infected with *T. cruzi* (Lalonde and Holbein 1984), animals experimentally infected with *T. evansi* (Gutierrez *et al.,* 2006; Wolkmer *et al.,* 2013).

Decrease in iron concentrations causes a reduction in mitotic activity of T-lymphocytes, reduction in the production of lymphokynes, decreased production of antibodies and reduced phagocytic activity (Saker, 2006). The Hypoferremia may be due to increasesd iron utilization by the microphages, (Stijlemans *et al.,* 2008; 2015) and also due to the trypanosome itself, since this mineral is used for trypanosoma growth and multiplication (Weinberg, 1978; Lalonde and Holbein 1984).

The significant low Zinc level observed in infected cattle is in agreement with thework of Fraker *et al.,* (1982) who reported hypozincaemia in experimentally infected rats with *T. cruzi* and that of Wolkmer *et al.,* (2011) in rats experimentally infected with *T. evansi.* However, in contrast to that of Isaac *et al.,* (2011) reported hyperzincaemia in humans infected with *T. b. gambiense*. Alteration in Zn level could be associated with disfunctioning of the immune system (Keen and Gershwin, 1990; Schlesinger *et al.,* 1993), thymus atrophy, reduced number of circulating leukocytes (Cousins, 1985), iron deficiency anemia and hepato-splenomegaly (Prasad, 1983). Zn

deficient animals show an increased susceptibility and prolongation of trypanosome infection (Fraker *et al.,* 1982; Lee *et al.,* 1983; Da Silva *et al.,* 2009a). Certain clinical signs that have long been associated with African trypanosomosis such as immune-suppression (Amole *et al.,* 1982), testicular degeneration (Ikede, 1979), hepato-splenomegaly (Amole *et al.*, 1982), alopecia and dermatitis (Clegg *et al.*, 2006) could be due to hypozincaemia.

Copper is known as an essential nutrient for iron metabolism and biologic activation of some enzymes (Rogers 2005), such as superoxide dismutase and cytochrome C oxidase (Nelson and Cox 2008; Berger, 2002) and is known to participate in the antibody generation process that is involved in cellular immunity (Saker 2006). Significant low serum copper level as repoeted in this study is in agreement with Wolkmer *et al.,* (2013) and Da Silva *et al.,* (2009a) where they reported decrease serum copper level in rats and cats experimentally infected with *T. evansi* respectively. However, Saror (1976), Joshua *et al.,* (1994) and Neils *et al.,* (2006) earlier on observed no change in serum copper level in cattle and sheep experimentally infected with *T. vivax* or *T. congolense*.

Following treatment with diminazene aceturate or isomethamidium chloride, a remarkable increase in the levels of PCV and other erythrocyte parameters was observed. On day 14 post treatment, the PCV, total erythrocyte count, haemoglobin concentration and the thrombocytes counts were almost the same with the values of the control. This improvement in the erythrocyte parameters was primarily due to the trypanolytic property of diminazene aceturate and isomethamidium chloride. However, trypanosome clearance was not solely due to destruction of the parasite by the trypanoside but also could have been due to immune modulation effect of

drug. Studies have shown that diminazene aceturate modulates the host immune response by reducing the serum level of pathology-promoting inflammatory cytokines thereby promoting faster and effective parasite clearance (Kuriakose *et al.*, 2012).

Similarly, following treatment with diminazene aceturate, the leukocytes recovered. Rapid destruction of trypanosomes by the diminazene aceturate releases antigens, which are likely to provide a stimulus for further recruitment and mobilisation of more white blood cells from the primary haematopoietic centre, the bone marrow as well as the secondary centres such as spleen and lymph nodes into the general circulation (Morrison *et al.*, 1985). This may explain the lymphocytosis and monocytosis that is seen soon after treatment with diminazene aceturate, whereas granulocyte counts took much longer time for it to recover (Ngotho *et al.*, 2011).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 C onclusions

The following conclusions could be made from this study:

1. The study showed that *Trypanosoma congolense, T. vivax and T. brucei* were species of trypanosomes responsible for bovine trypanosomosis in selected LGAs in Niger State accounting for 5.5%, each for *T. congolense* and *T. vivax* while *T. brucei* accounted for 2.0%.

2. This study to the best of our knowledge has documented for the first time the following clinical signs; corneal opacity, cutaneous haemorrhages, dark hair coat, coughing, agalactia, pica, muscle tremors and ataxia to be associated with natural cases of trypanosomosis in cattle in selected LGAs in Niger State.

3. Haematological parameters: total red blood cells, heamoglobin concentration, platelets counts, total leucocyte counts, neutriphils, eosinophils, and basophils of the infected cattle were significantly lower than those of the control except for lymphocytes and monocytes which were significantly higher.

4. Serum biochemical parameters of infected cattle were significantly ($P<0.05$) lower in infected cattle when compared to the control. Serum enzymes like ALT, ALP, and CK were significantly ($P<0.05$) higher in the infected cattle while AST was higher in the infected cattle but was not statistically significant ($P\geq0.05$).

5. Serum biomolecules like glucose, total protein, globulins, albumin, total cholesterol and low density lipoproteins were significantly ($P<0.05$) lower in

infected cattle while high density lipoproteins were in-significantly (P≥0.05) low in the infected cattle. However, triglyceride was significantly higher in infected cattle when compared to the control. All the serum minerals analysed differed significantly (P ≤0.05) between the infected and their control except potassium which was insignificantly (P ≥0.05) higher in the infected cattle.

6. Serum minerals like sodium, chloride, calcium, iron, copper, and Zinc were significantly (P ≤0.05) lower in the infected cattle while potassium was in-significantly(P ≥0.05) higher in the infected cattle. Bicarbonate was significantly higher in infected cattle when compared to their control.

7. Treatment of trypanosoma infected cattle with Diminazene aceturate and Isomethamidium chloride showed that these drugs were effective in the treatment of naturally occurring trypanosomosis in selected LGAs in Niger State even though some cases of relapse infection occurred.

8. A total of 19 clinical signs were recorded in cattle with trypanosomosis in the course of this study. Following treatment with diminazene aceturate by day 14, anorexia, dark/rough coat, pyrexia, corneal opacity and cutaneous haemorrhages were not observed in the infected cattle while on day 28 clinical signs like emaciation, pica, teeth grinding and diarrhea were still persistent. Similarly, for the group treated with isomethamidium chloride anorexia, dark/rough coat, pyrexia and cutaneous haemorrhages were not observed in the infected animals while on day 28, emaciation, pica, teeth grinding and dysentery still persisted.

9. Following treatment with either diminazene aceturate or isomethamidium chloride an increase in the haematological parameters in the infected cattle was observed in both groups. On day 14 and day 28 post treatments, the PCV, total erythrocyte count, haemoglobin concentration, platelets counts, leucocytes count (total and differential) had risen to level close to the controls. The increase in the haematological values were however not statistically significant but suggested that infected animals recovered from the infection.

6.2 Recommendations

1. There is urgent need for effective control of trypanosomosis and its vectors in selected LGAs in Niger state,
2. Clinicians should acquaint themselves with current trend in diagnosis, treatment and management of trypanosomosis to avoid speculative diagnosis which potentiates drug resistance.
3. Further work should be done to establish the parasitological and pathological bases for each of the clinical signs that were observed in this study.
4. Public awareness through extension services should further be supported by Ministry of Agriculture and rural development and other relevant agencies towards control of tsetse flies and trypanosomosis in the state.
5. Due to the dynamic nature of tsetse distribution, it has become pertinent to conduct periodic country-wide surveys of tsetse and trypanosomosis using more sensitive diagnostic techniques to determine the extent of tsetse and trypanosomosis distribution and their impact on the economy.

6. Research on trypanosomosis should continue to receive priority in financial allocation, to focus attention on sites where studies can be carried out and provide financial support to such centers, the mobilization of large-scale financial and technical support for future long term activities in the field.
7. The State Veterinary office should be equipped with the necessary logistics to monitor the Tsetse fly population, Tsetse fly species, species of trypanosomoses and proper use of drugs and chemicals.
8. There is need for gathering adequate information on the current status of tsetse, distribution of resistant trypanosomes and economic impact of the disease through geographical information system and national surveillance.

6.3 Limitation of the study

The limitations encountered during this study were sensitivity of the parasitological test used, disproportionate size of the infected and uninfected group of animals, possibility of co-infection with helminthoses and other haemoparasites.

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APPENDIX 1

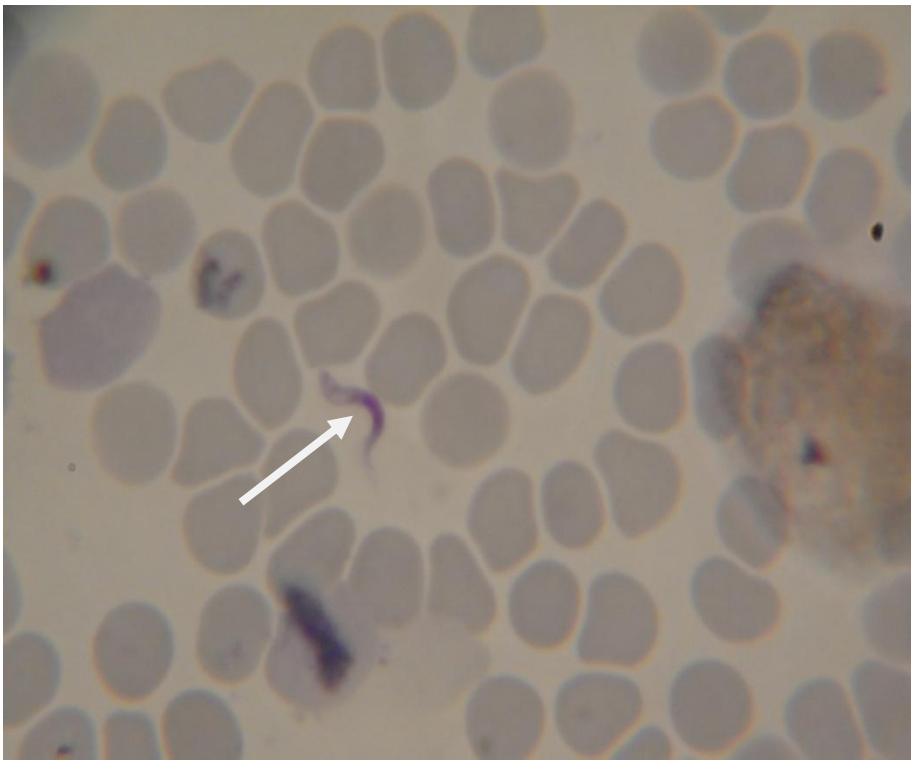

Plate 1: ***Trypanosoma congolense*** **in stained blood smear of an infected cow (Slides were stained with Giemsa and examined under oil immersion at 100x)**

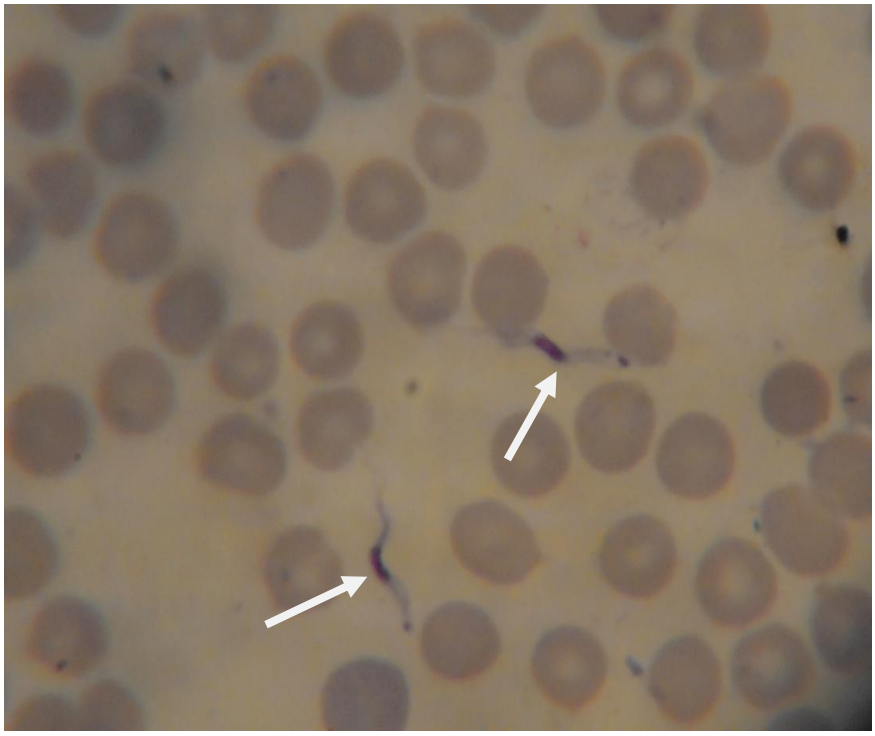

Plate 2: *Trypanosoma vivax* in stained blood smear of an infected cow (Slides were stained with Giemsa and examined under oil immersion at 100x)

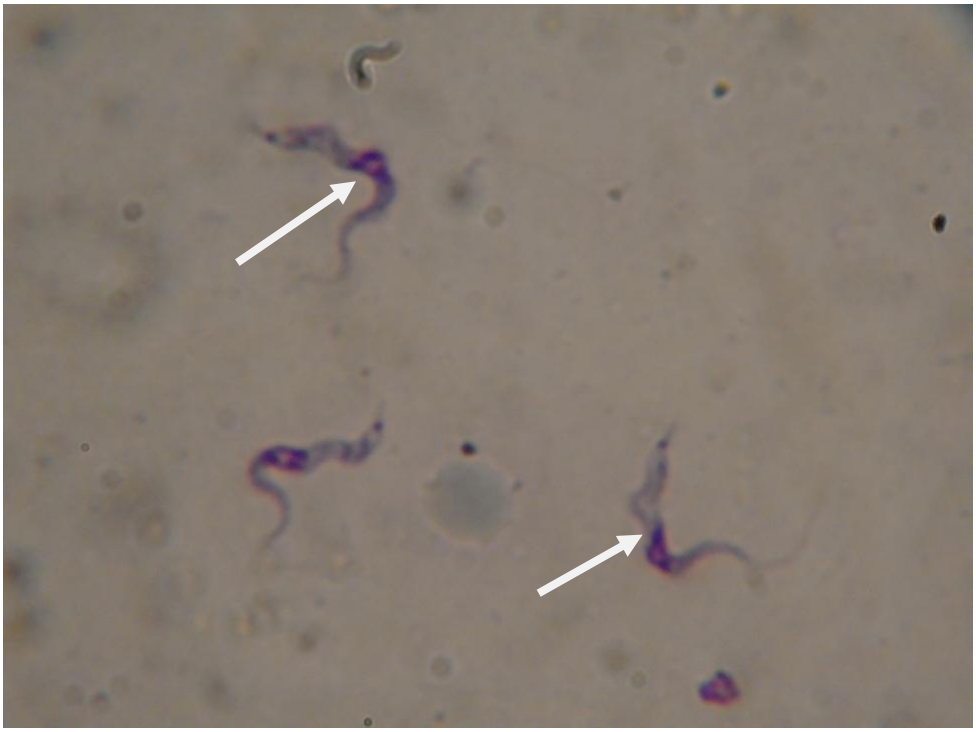

Plate 3: *Trypanosoma brucei* in stained blood smear of an infected cow (Slides were stained with Giemsa and examined under oil immersion at 100x)

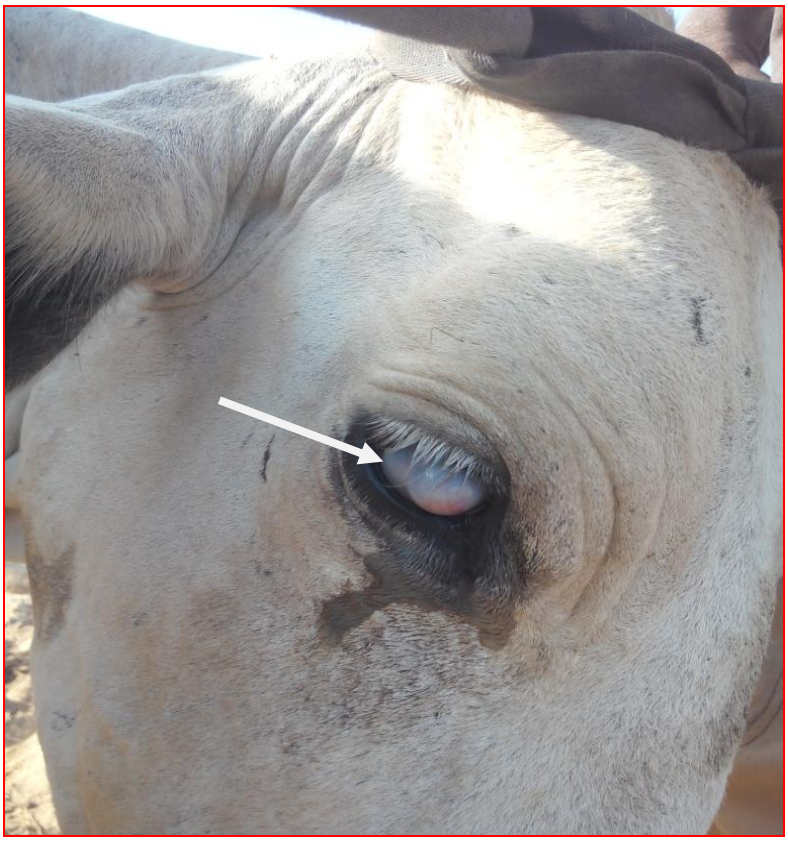

Plate 4: Picture of Corneal opacity observed in cattle with trypanosomosis

Plate 5: Picture of Epiphora observed in cattle with trypanosomosis

Plate 6: Picture of Tail Alopecia in cattle infected with trypanosomosis

Plate 7: Picture of alopecia and emaciation in cattle with trypanosomosis

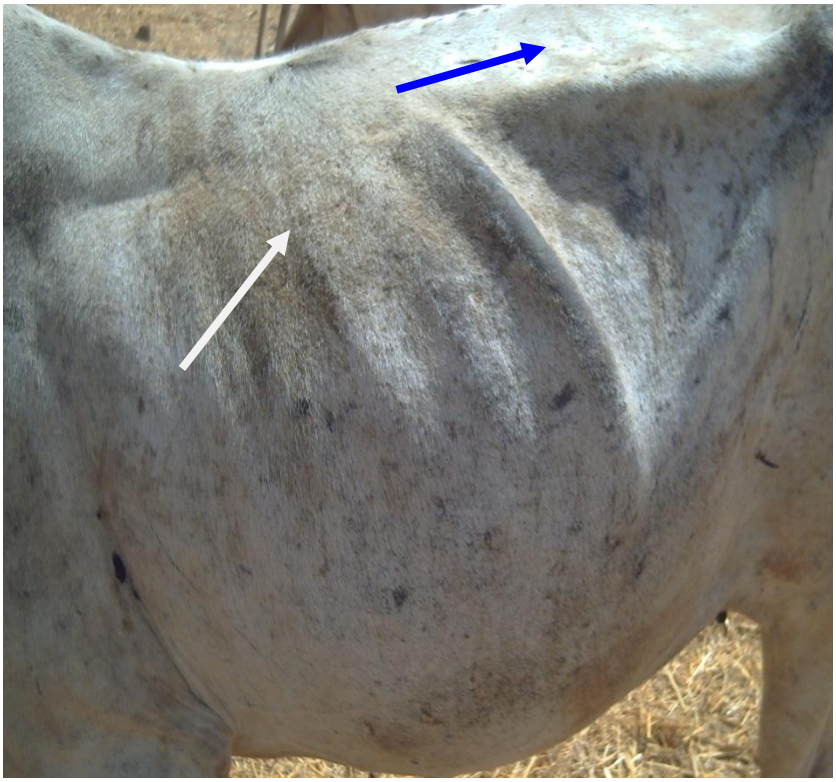

Plate 8: Picture of emaciation and dark hair coat on lateral side of a cow with trypanosomosis

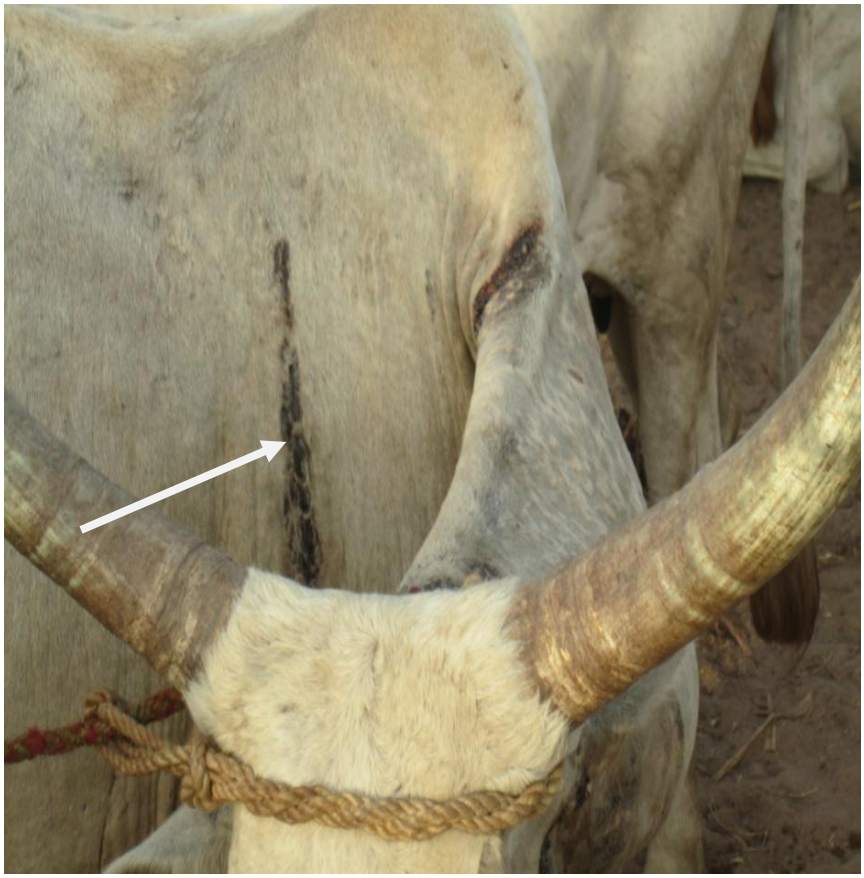

Plate 9: Picture of Cutaneous haemmorrhage on a cattle with trypanosomosis

APPENDIX 2

SUMMARY OF METHODS USED IN THE ESTIMATION OF HAEMATOLOGICAL AND SERUM BICHEMUCAL CONSTITUENTS;

Procedure for Determination Hemoglobin Concentration
Method: Acid hematin method
Requirements: Sahlis instrument, blood sample

Procedure

- Take 0.1N HCl (1%) into central graduated tube up to mark 2.
- Suck the blood exactly up to mark 20 (20 µl) with the help of sahlis pipette.
- Transfer the blood from pipette to central graduated tube of the hemometer.
- Mix it well with the help of stirrer or rod and allow it to react for two minute.
- Make up with distilled water by adding drop by drop until the color matches with the Standard comparator tube and mix well.
- When the color matches take out and record the values on the side as gm/100ml and or in percentage.

Procedure for Determination Some Serum Emzymes

Determination of AST emzyme activity level using Reitman-Frankel method as described by Cheesbrough (1991)
*Principle:*AST is incubated at 37^0C for 1 hour (60 minutes) in a pH 7.5 buffered substrate containing aspatate and ketoglutarate. AST catalyses the transfer of the animo group from aspatate to ketoglutarate to form oxaloacetate and glutamate. The former then reacts with 2,4-dinitrophenylhydrozone which in an alkaline medium gives a red-brown clour. The absorbance of this colour is measured using either colorimeter with a green filter or a spectrophotometer at 505nm wavelength.

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

PROCEDURE FOR DETERMINATION SOME SERUM BIOMOLECULES

Determination of serum Protein Level by Biuret method (Cheesbrough, 1991)

Principle: Protein molecules are made up of amino acids in long chain (peptide chains). The links between the amino acids are the peptide bonds. In the Biuret reaction, the cupric ions in the reagent join with the peotide bonds to form a blue violet cooured complex. The absorbane to the colour produced a measured in a clorimeter using yellow green filter or in a spectrophotometer at 500nm wavelength. Blank solution used to avoid errors due turbidity.

Determination of Serum Glucose level by the Glucose oxidase-peroxidase method as described by Cheesbrough, (1991).

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

produced is measured via a colorimeter using a green filter or a spectrophotometer at 515nm.

These are then well mixed and left at 37^0C for 10minutes or at room temperature for 30 minutes. Absorbance is then read using either colorimeter (Yellow-Green fiter) or spectrophotometer at 540nm. These instruments are zeroed with blank solutions. The control and test blanks are read. The blank reading is then substracted.

Detmination of serum Albumin by the BCG method as described by Cheesbrough (1991).

Principle: Bromocresol green (BCG) albumin method is used. BCG is an indiator. It is yellow between pH3.5-4.2. The colour changes from yellow to blue-green BCG binds to albumin.

The test tubes are shaken to mix well (in such a way ton avoi frothing0. Absorbance is read immediately using colorimeter (orange filter) or spectrophotometer at 632nm. The instrument is zeroed with blank solution in tube B.

Determination of Serum Globulin level

Globulin concentration in serum samples was calculated by total protein minus albumin difference.

Determination of serum Cholesterol level

All cholesterol esters present in serum or plasma are hydrolyzed quantitatively into free cholesterol and fatty acids by microbial cholesterol esterase. In the presence of oxygen, free cholesterol is oxidized by cholesterol oxidase to cholest-4-en-3-one. The H_2O_2 reacts in the presence of peroxidase (POD) with phenol and 4-aminophenazone to form an o-quinone-imine dye. The intensity of the color is proportional to the cholesterol concentration and is measured photometrically.

Determination of serum triglycerides level

This method uses microbial lipase to promote rapid and complete hydrolysis of triglycerides to glycerol with subsequent oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The peroxide reacts with 4-aminophenazone and 4-chlorophenol in a Trinder reaction to a colorimetric endpoint.

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Determination of serum HDL level.

HDL fraction was extracted from the serum after precipitating very low density (VLDL) and low density lipoprotein (LDL) with heparin-manganese chloride solution. LDL-cholesterol was calculated from the values of total cholesterol, triglycerides and HDL-cholesterol using the formula described by Friedewald et al. (1972).

PROCEDURE FOR DETERMINATION SOME SERUM ELECTROLYTES

Determination of serum Na^+ and K^+ by flame Emission Spectrometry (Cheesbrough, 1991).

Using compresed air, diluted serum is sprayed as a fine mist of droplets into a non luminous pas flame. These then give the colour of emission characteristics of Na^+ or K^+ metallic ions. A light filter selects the ligh of a wavelength corresponding to the metal being measured. This is then allowed to fall on a photosensitive detector system which then emits light; the amount of light depends on the concentration of metallic ion. The valu is then evaluated in mmol/l.

Determination of Serum Bicarbonate level by Titrimetric method (Cheesbrough, 1991)

Principle: A known amount of strong HCl is added to a sample of serum. The reaction forms CO_2 which is expelled by shaking. The H^+ remaining is titrated against NaOH, with phenol red as the indicator.

Determination of Serum Iron level

Iron (Fe^{3+}) is separated from transferrin by means of guanidinium chloride in the weakly acidic pH range and reduced to Fe^{2+} with ascorbic acid. Fe^{2+} then forms a colored complex with ferrozine.

Detemination of serum Calcium level

Calcium reacts with o-cresolphthalein complexone in the presence of 8-hydroxyquinoline-5-sulfonic acid to form a purple complex. The intensity of the final reaction color is proportional to the amount of calcium in the specimen.

Detemination of Serum Copper and Zink levels

Copper and Zink were determined using atomic absortion spectrophotometry as described by Neils *et al.,* (2007) and Wolkrman *et al.,* (2013). Sera wer digested with Hypochloric ($HClO_4$) and diluted with deionized water to a factor of 5 and 1 respectively.