PARASITOLOGICAL AND MOLECULAR CHARACTERIZATION OF *TRYPANOSOMA VIVAX* AND *TRYPANOSOMA CONGOLENSE* ISOLATES AND THE EFFICACY OF TRYPANOCIDES IN EXPERIMENTALLY INFECTED YANKASA SHEEP IN NIGERIA.

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF DOCTOR OF PHILOSOPHY IN VETERINARY PARASITOLOGY

DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY,
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MARCH, 2014

DECLARATION

I declare that the work in the dissertation entitled "Parasitological and Molecular Characterization of *Trypanosoma vivax* and *Trypanosoma congolense* Isolates and the Efficacy of Trypanocides in Experimentally Infected Yankasa Sheep in Nigeria" has been carried out by me in the Department of Veterinary Parasitology and Entomology under the supervision of Professors I. A. Lawal, O.J.Ajanusi and J.K. P. Kwaga. The information provided from the literature has been duely acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or Diploma at this or any University.

OGBAJE, Igoche Christopher …….. ……

Signature Date

CERTIFICATION

This dissertation entitled "**Parasitological and Molecular Characterization of *Trypanosoma vivax* and *Trypanosoma congolense* Isolates and the Efficacy of Trypanocides in Experimentally Infected Yankasa Sheep in Nigeria"** meets the regulations governing the award of Doctor of philosophy Degree of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to Almighty God, my wife, children and my late mother who passed away a week after my enrolment into this programme. Mum, I appreciate you for your love, care, support and prayer for me in your life time, may Almighty God grant you eternal rest.

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ABSTRACT

Based on reports from farmers of occurrence of relapse infections after treatment of infected livestock with trypanosomosis, a study was conducted to characterize field isolates of *Trypanosoma congolense* and *Trypanosoma vivax* of Nigeria origin *in vitro* (PCR) and *in vivo* (inYankasa Sheep) as well as to assess the efficacy of the commonly used trypanocides (isometamidium chloride, homidium chloride and diaminazene aceturate) at their recommended dosages. A total of 36 Yankasa Sheep of both sexes were acquired from tsetse fly free areas (Karfur) of Kastina state, screened for haemo-, ecto- and endo-parasites, treated for Anaplasma species, coccidia and dewormed and then conditioned in fly-proof pens for two weeks. The animals were randomly divided into six groups of six animals each. Two groups were infected intravenously with infected blood of approximately 2.0×10^6 of *Trypanosoma vivax* and two other groups were also infected intravenously with blood containing the same number of *Trypanosoma congolense*. The remaining two groups served as uninfected controls. Of the two each of *Trypanosoma vivax* and *Trypanosoma congolense* infected groups, one group from each was further divided into three sub-groups of two animals each and each of the sub-groups was treated with different trypanocides 12 days post infection at high parasitaemia. The other infected groups were left untreated. All the animals were monitored for 8 weeks post infection. Pre-patent period of 6 days was recorded for both parasite species. The common clinical signs observed were pyrexia, rough hair coat, emaciation, anorexia, weakness, depression, oedema of the eyelids, ocular discharge, diarrhoea, pale mucous membrane, teeth grinding, central nervous system disorder (only in the *Trypanosoma vivax* infected animals) and death. The signs were more severe in *Trypanosoma vivax* infected group. There were decreases in all the haematological indices (PCV= 14.17±0.94 %, Plasma Protein= 4.62 g/dl, WBC= $3.62\pm0.51\times 10^9$/L and weight=

11.00±0.34 kg) and body weights of all the infected groups. Gross pathology of *Trypanosoma vivax* infected, untreated and infected, treated animals revealed serous atrophy of pericardial fat, focal haemorrhagic area of the right lung, hydropericardium, diffuse pinpoint haemorrhagic areas on the liver and kidney surfaces, empty gall bladder, congestion and hyperemia of the lungs. The *Trypanosoma congolense* infected untreated and infected treated groups revealed pale carcasses and slight congestion of the liver. Histopathology of the *Trypanosoma vivax* infected-untreated revealed areas of neuronal degeneration in the brain, haemosiderosis of the spleen, necrotic renal tubule and degeneration of glomerular in the kidney, haemorrhagic congestion of the lungs and thickened alveolar membranes, atrophy and degeneration of the skeletal and cardiac muscles, absence of sperm cells in the epididymis but the *Trypanosoma vivax* infected and treated animals revealed thickened alveolar membranes, uterine necrosis, glomerular necrosis of the kidney and haemosiderosis of the spleen. All the organs of the uninfected control animals revealed normal structures. The *Trypanosoma congolense* infected untreated animals revealed necrosis of adrenal glands and pancreas, haemosiderosis of the spleen, pulmonary congestion in the lungs, infiltrative myositis of the skeletal muscle and congestion of the liver. In the infected and treated animals, changes such as necrosis in most of the organs like uterus, kidney and skeletal muscle, haemosiderosis of the spleen and thickened alveolar membranes were noticed. In *Trypanosoma vivax* infected and teated groups, it was only the group treated with the recommended dosage of homidium chloride that had relapse. Conversely, there were relapse infections in all the *Trypanosoma congolense* infected treated with all the three trypanocides at their recommended dosages. RAPD-PCR revealed band size of chronic form of the infection that differed from those of the acute and relapse forms of *Trypanosoma congolense* infections, whereas the band size of the relapse form of the *Trypanosoma vivax* infection differed from those of acute and chronic

forms. Multiplex PCR and *Trypanosoma vivax* - specific PCR showed that the isolates were *Trypanosoma congolense* and *Trypanosoma vivax* at molecular band sizes of 750 bp and 175 bp, respectively.

This study revealed torticolis like nervous sign and pathological lesion in the brain of Yankasa Sheep infected with *Trypanosoma vivax*, occurrence of strains of *Trypanosoma vivax* and *Trypanosoma congolense* species that form relapse after treatment with the commonly available trypanocides. It also showed that the *Trypanosoma vivax* and *Trypanosma congolense* used amplified at the same band weights with *T. vivax* and *T. congolense* in other geographical areas using the same primers sequence and infection with *Trypanosoma vivax* is more severe than *Trypanosoma congolense* infection.

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6. Mean ± SE weekly total plasma protein of *Trypanosoma vivax* infected, treated (A), infected-untreated (B) and uninfected control (C) Yankasa Sheep ….. ….. 136

7. Mean ± SE weekly temperature of *Trypanosoma congolense* infected, treated (E), infected-untreated (F) and uninfected control (2C) Yankasa Sheep ….. ….. 137

8. Mean ± SE weekly body weight of *Trypanosoma congolense* infected, treated (E), infected-untreated (F) and uninfected control (2C) Yankasa Sheep ….. ….. 138

9. Mean ± SE weekly Packed Cell Volume (PCV) of *Trypanosoma congolense* infected, treated (E), infected-untreated (F) and uninfected control (2C) Yankasa Sheep ….. 140

ABBREVIATIONS

AAT	African Animal Trypanosomosis
ANOVA	Analysis of Variance
Bp	Base pair
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
DEAE	Di- Ethyl- Amino- Elthyl
DPX	Diphynyphthalate Polysterene Xylene
ELISA	Enzyme Linked Immunosorbent Assay
EDTA	Ethylene Diamine Tetraacetic acid
HCT	Haematocrit Centrifuge Technique
H & E	Haematoxylin and Eosin
ITSI	Information Technology In Science Inquiry
ITS	Internal Transcribed Spacer 1
IgG	Immunoglobulin G
KCL	Potasium Chloride
$MgCL_2$	Magnesium Chlorde
M & B	May and Baker
PCR-RFLP	Polymerase Chain Reaction Fragment Length Polymorphism (RFLP) assays

PPR	Peste des Petits Ruminants
PCV	Packed Cell Volume
Pi	Post infection
PBS	Physiological Buffer Saline
PP	Percentage Positivity
RAPD- PCR	Random Amplify Polymorphic DNA Analysis
RBC	Red Blood Cell
SEM	Standard Error Mean
SDS	Sodium dodecylsulphate
UV light	Ultra Violet Light
VSG	Variant Surface Glycoprotein
WBC	White Blood Cell

CHAPTER ONE

INTRODUCTION

1.1 Background Information

African trypanosomosis is a devastating disease of humans and animals (Hu and Aksoy, 2005; Ezeokonkwo *et al*., 2012). The disease constitutes a major constraint to livestock development. *Trypanosoma vivax* and *Trypanosoma congolense* are the main species responsible for African Animal Trypanosomosis (AAT) or Nagana in West Africa especially Nigeria (Losos and Ikede, 1972; Losos, 1986; D'Archivio *et al*., 2011). The disease causes about 3 million deaths annually and has a marked impact on agriculture in Sub- Sahara and South America endemic countries, leading to annual livestock production losses of about 1.2 billion US dollars (Osorio *et al*., 2008; WHO, 2010).

Trypanosoma vivax accounts for up to half of total prevalence of African Animal trypanosomosis prevalence in West Africa especially in Nigeria where it is considered to be the predominant pathogen for domestic animals ((Osorio *et al*., 2008). This could be ascribed to the mechanical transmission or shorter development cycle in the anterior part of the tsetse fly (Daniel, 1994). The trypanosome readily persists in areas free of tsetse flies (for example, in Central and South America and in the Caribbean), where it is transmitted mechanically by biting flies or contaminated needles, syringes, and surgical instruments, (Logan-henfrey *et al*., 1992). *Trypanosoma vivax* and *Trypanosoma congolense* infect large variety of domestic and wild animals (D'Avila *et al.,* 1997).

The cardinal clinical sign observed in AAT is anemia (Anosa, 1983a; 1983b; 1988). Within one week of infection with the haematic trypanosomes (*Trypanosoma congolense* and *Trypanosoma vivax*) there is usually a pronounced decrease in packed cell volume, hemoglobin, red blood cell, and white blood cell levels, and within 2 months they may drop to below 50 percent of their pre-infection values (Anosa and Isoun, 1980). Also invariably

present are intermittent fever, oedema and loss of condition. Abortion may be seen, and infertility of males and females may be a sequelae. The severity of the clinical response is dependent on the species, the breed of affected animal as well as the dose and virulence of the infecting trypanosome (Anosa *et al.*, 1992). Stress, such as poor nutrition or concurrent disease, play prominent role in the disease process, and under experimental conditions, where stress may be markedly reduced; it is difficult to elicit clinical disease (Losos and Chouinard, 1979; Anosa *et al.*, 1992). *Trypanosoma vivax* has a variable incubation period, and, although it is considered to be less virulent for cattle than *Trypanosoma congolense*, mortality rates of over 50 percent can occur (Losos, 1986; Anosa *et al,.* 1992). There seems to be a marked variation in the virulence of different strains of *Trypanosoma vivax*, but it remains the most important cause of trypanosomosis of cattle, sheep, and goats in West Africa (Hoare, 1972; Losos, 1986; Anosa *et al,.* 1992; Abenga *et al.*, 2004). It causes mild disease in horses and chronic disease in dogs (Da Silva *et al*., 2010).

Control of African animal trypanosomosis is dependent largely on vector control and use of drugs (chemotherapy and chemoprophylaxis). The drug control of trypanosomosis is dependent on three compounds, isometamedium chloride, homidium bromide or chloride and diminazene aceturate (ILRAD, 1990; Geerts and Holmes, 1998).

The use of drugs for the prevention and treatment of trypanosomosis has been of importance for many decades, but the rapidity with which the trypanosomes developed resistance to each drug introduced has tremendously complicated this approach to controlling the disease (Willianson, 1970; Dwinger, 2000). In spite of this, some of the older chemoprophylactic drugs such as the quinapyramine derivatives, antrycide and antrycide prosalt are still used and give effective protection against *Trypanosoma brucei brucei* infection in horses, camels, and cattle for up to 3 months. The drug pyrithidium bromide is useful in the prophylaxis of *Trypanosoma vivax* and *Trypanosoma congolense* infections in cattle, sheep, and goats and

can give protection for up to 6 months (Losos and Ikede, 1972). The most widely used of the newer chemoprophylactic drugs (and also the least expensive) is isometamidium chloride (Ogunyemi and Ilemobade, 1989). This drug has been in use for over three decades and sold under the trade names Samorin, Trypamidium, and M&B 4180A; it is excellent for the prophylaxis of all the three species of African animal trypanosomes, and gives protection for 3-6 months. The development of resistance to this drug has been reported in both East and West Africa (Codjia *et al*., 1993; Leak *et al.,* 1993; Peregrine *et al*., 1994). Homidium bromide has also been found to be an effective chemoprophylactic drug in Kenya, and the newly introduced arsenical compound, cymelarsan, is effective in the treatment of *Trypanosoma brucei brucei* infection. A very widely used chemotherapeutic drug is diminazine aceturate (Berenil^{R}), which is effective against all the three African animal trypanosomes such as *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei brucei* (Whitelaw *et al*., 1989; Katunguk-Rwakishaya *et al*., 1997).

1.2 Statement of Research Problems

Despite years of efforts by researchers to eliminate trypanosomosis, it still constitutes a major constraint for livestock development in Sub-Saharan Africa (Gyening, 2005). Over a decade ago, it was estimated that over 3 billion British pounds was lost annually from agricultural production in Africa as a result of the effects of trypanosomosis (Hursey, 2000). The huge economic losses due to trypanosomosis result from livestock deaths, reproductive disorders, reduced efficiency of work animals and large amount of money required for chemotherapeutic and prophylactic control of the disease (Swallow, 2000).

It has been reported that the most important pathogenic trypanosomes of ruminants in Africa and Nigeria are *Trypanosoma congolense* and *Trypanosoma vivax* (Anosa *et al*., 1992; Lejon *et al.,* 2003).

Controlling the disease relies on few old drugs such as Novidium chloride, Isometamedium chloride and Diminazene aceturate, which have been heavily used over the past 50 years, with one exception (Cymelarsan), (Geerts and Holmes, 1998). Consequently, this has led to increased drug resistance resulting in high prevalence of the disease (Geerts and Holmes, 1998).

Immunological control of the disease which involves the use of antigens looks promising and would have economic and environmental benefits as it would help in reducing the use of expensive trypanocidal drugs (Connor and Van den Bossche, 2005). Knowledge on the genetic polymorphisms of the trypanosomes will greatly assist in the possible development of vaccine against the disease. Anosa *et al*. (1992) have reported that about eleven different pathogenic trypanosomes including *Trypanosoma vivax* and *Trypanosoma congolense*, exist in endemic regions of Africa. There are marked variation in the virulence of different strains of *Trypanosoma vivax* and *Trypanosoma congolense*, but *Trypanosoma vivax* remains the most important cause of trypanosomosis of cattle, sheep, and goats in West Africa (Anosa *et*

al., 1992; Lejon *et al.*, 2003). Consequently, there is a need to carry out studies on *Trypanosoma vivax* and *Trypanosoma congolense* originating from cattle in the field to establish the parasitological and molecular characteristics in experimental infections at different stages of the disease. The resurgence and difficulty in the control of the disease due to antigenic variation and development of drug- resistant strains have necessitated the need for more research in the study of the gene and nature of DNA elements within the genome of the parasites for possible vaccine and new drug development (Brun and Balmer, 2006).

1.3 Justification of the Study

It has been reported that animal protein supply intake in Nigeria is low compared to what obtains in the developed world, resulting in increasing import of live animals and animal products yearly (Adesehinwa *et al*., 2007). According to the report of FAO (2008), among all the diseases affecting humans and livestock, trypanosomosis straddles the ground between human and livestock health, agricultural production and rural development. Consequently, tackling trypanosomosis has the potential to impact on all the eight millennium development goals of the Country which include eradication of extreme poverty and hunger (FAO, 2008).

Despite years of efforts to prevent trypanosomosis in sub-saharan countries (Losos and Ikede, 1972; Saror, 1975, Anosa *et al.*, 1992; Sackey, 1998; Gyening, 2005; Olaoluwa *et al.*, 2006), little has been done in the areas of characterization of *Trypanosoma congolense* and *Trypanosoma vivax* at different stages of the disease in Yankasa sheep in Nigeria to check if there are alteration of the parasite gene as the disease progresses from stage to stage and after the used of trypanocides. The increasing prevalence rates of ruminant trypanosomosis in Nigeria (Anosa *et al*., 1992; Omotanise *et al.*, 2001) require more research works in area of infectivity and molecular characterisation of the two species of trypanosomes in different domestic animals in Nigeria. There is also the need to ascertain the efficacy of the most

commonly used trypanocidal drugs (isometamedium chloride, diminazene aceturae and homidium Chloride) *in vitro* and to check whether there is alteration of the DNA sequences of the trypanosomes at different stages (acute, relapse and chronic) of infection using PCR. Information generated from these studies will greatly contribute to the epidemiological understanding of the disease and possible vaccine development.

1.4 Aim of the Study

The aim of this research was to carry out parasitological, molecular characterization and to determine the efficacy of trypanocides to *Trypanosoma congolense* and *Trypanosoma vivax* of Nigerian origin.

1.5 Objectives of the Study

The objectives of the studies are to;

i. Characterize isolates of *Trypanosoma vivax* and *Trypanosoma congolense* using Yankasa sheep in terms of prepatent period, parasitaemia, clinical signs, total plasma protein, body weight changes, haematological values, gross and histopathological changes.
ii. Characterise field isolates of *Trypanosoma congolense* and *Trypanosoma vivax* using Random Amplified Polymorphic DNA polymerase chain reaction, Multiplex primers and *Trypanosoma* species- specific primer PCR.
iii. Assess the efficacy of the three different trypanocidal drugs (Diminazene aceturate, Isometamedium Chloride and Homidium Chloride) commonly used in the field to treat *Trypanosoma congolense* and *Trypanosoma vivax* due to the reports of the high incidence of the disease.

1.6 Research Questions

1. Do isolates of *Trypanosoma vivax* and *Trypanosoma congolense* of Nigeria origin have the same behavior in experimental infections of Yankasa sheep with isolates from other countries?

2. Are Nigeria isolates of *Trypanosoma vivax* and *Trypanosoma congolense* genetically the same?

3. Does relapse infection of infected animals treated with any of the commonly used trypanocides occur in the field?

CHAPTER TWO

LITERATURE REVIEW

2.1 Trypanosomosis

African trypanosomosis is a parasitic infection caused by flagellated extracellular parasites that survive in the tissue fluids and the bloodstream that encompasses a number of diseases affecting both humans and animals (Hu and Aksoy, 2005). Trypanosomosis is a disease complex caused by several species of protozoan parasites of the genus *Trypanosoma*, order kinetoplastida and transmitted cyclically by the genus *Glossina* and mechanically by several biting flies such as *Tabanus* and *Stomoxys* (Jones and Davila, 2001; Osorio *et al*., 2008; OIE, 2008). *Trypanosoma* species infecting mammals are divided into two distinct groups, the salivaria and stercoraria, which differ in their mode of transmission (Hoare, 1972). The salivaria have *Duttonella*, *Nannomonas* and *Trypanozoon* as subgenera. *Trypanosoma* species in this group either undergo cyclic development in the insect vector before being transmitted through the saliva, or are transmitted mechanically (Cherenet *et al*., 2004). Stercorarian trypanosomes also undergo development in the insects, but the infective forms are deposited in the faeces of the vector and have *Schizotrypanum*, *Megatrypanum* and *Herpetosoma* as subgenera (Connor, 1994; Kettle, 1995; Seifert, 1996). Different species of trypanosomes occur throughout the world and in all classes of vertebrates which include fish, frogs, reptiles, birds and mammals (Losos, 1986; Connor, 1994). Most are well adapted to their host and cause no appreciable harm. Some trypanosomes that parasitize mammals are less well adapted to their hosts and they commonly cause diseases (Losos, 1986; Connor, 1989; 1994).

Most *Trypanosoma* species that parasitize animals are unable to infect human and are a burden for the economic development in endemic areas (FAO, 1994). This feature of the

disease is due to the presence of two trypanolytic factors (TLF) in human serum (TLF1 and TLF2) that provide a level of innate resistance, preventing human infections (FAO, 1994). Although the animal diseases cause heavy losses of domestic livestock, there is no evidence that any single disease has had the same profound influence on the distribution of livestock in Africa as has trypanosomosis (FAO, 1994).

Transmission of animal trypanosomiosis occurs mainly through its natural insect vector, the tsetste fly (*Glossina* species), but can also be accomplished through mechanical transmission by biting insects. The latter has allowed certain animal trypanosomes to propagate outside the African continent, as is the case for *Trypanosoma evansi* and *Trypanosoma vivax* that have become endemic to Asia and South America (Cherenet *et al*., 2004). Sexual transmission of trypanosomiosis so far has only been confirmed in the case of *Trypanosoma equiperdum* of horses.

Control of African trypanosomosis is dependent largely on vector control and the use of drugs (chemotherapy and chemoprophylaxis). In animals, the drug control of trypanosomosis is dependent on three compounds, isometamedium chloride, homiduim bromide or chloride and diminazene aceturate (ILRAD, 1990; Geerts and Holmes, 1998).

2.1.1 Morphology of *Trypanosoma vivax*

Trypanosoma vivax is a monomorphic trypanosome. The typical blood stream form has a somewhat expanded or club- shaped posterior end, which tapers towards the anterior (Hoare, 1972). The large, generally terminally placed kinetoplast is a distinctive feature that separates it from other trypanosomes (Hoare, 1972). The movement of the trypanosome species in wet blood films is rapid and distinctive (Ana *et al*., 2008). *Trypanosoma vivax* also differs from other species of the group by its length (means from 21- 25.4 µm). However, more recent research on strains that naturally infect rodents or that have been adapted to the laboratory rodents has revealed the existence of longer, more granular blood stream forms of

Trypanosoma vivax with a clearly subterminal kinetoplast which appear at the peak of parasitaemia, known as 'late' forms, (Gardiner, 1989). Plate I, shows the typical structure of *Trypanosoma vivax* in thin blood smear.

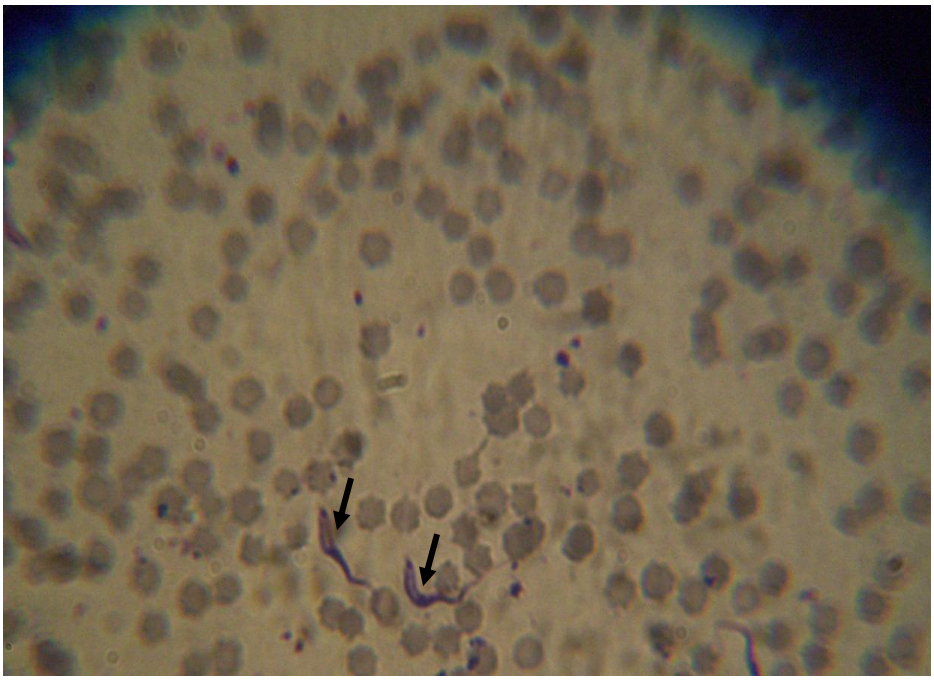


Plate I: *Trypanosoma vivax* in thin blood smears from Yankasa sheep (× 1000)

2.1.2 Morphology of *Trypanosoma congolense*

Trypanosoma congolense has other names (synonym). These are *Trypanosoma dimorphon* (Laveran and Mesnil, 1904), *T. nanum* (Laveran, 1905), *T. confusum* (Montgomery and Kinghorn, 1909*), T. montgomerryi* (Laveran, 1909), *T. pecorum* (Bruce *et al*., 1910).

Trypanosoma congolense is a small trypanosome measuring 8- 24µm in length. It lacks a free flagellum and in most cases, the undulating membrane is inconspicuous. It possesses a medium- sized kinetoplast which is generally marginally positioned. The posterior end of the body is either rounded (in the smaller forms) or pointed (in the longer forms), (Hoare, 1972). The species, *Trypanosoma congolense* has been divided on morphological basis by some workers into three types, the relatively short *congolense* form, the *intermediate*, and the longer *dimorphon* form (Godfrey, 1960).

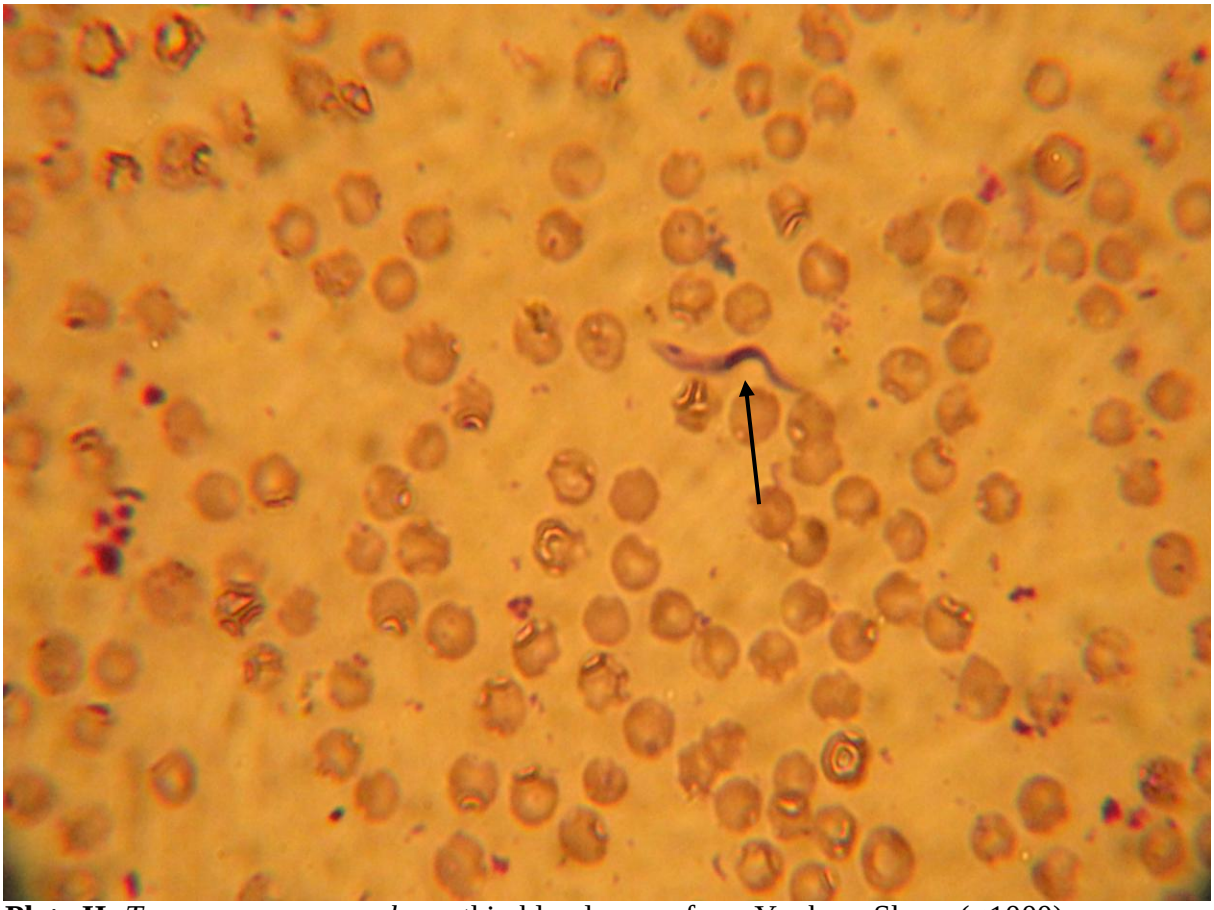


Plate II: *Trypanosoma congolense* thin blood smear from Yankasa Sheep (×1000)

2.1.3 General life cycle of trypanosomes

The African trypanosomes reside almost exclusively in the bloodstream and are transmitted by the bite of the tse-tse fly (*Gossina species*) which acquires *the* infection while taking a bloodmeal, and returns the trypanosome to a vertebrate host in its saliva when it takes another bloodmeal. Because this mode of transmission is by inoculation during biting, these groups of trypanosomes are also referred to as "Salivarian" (Hoare, 1970).

The life cycle is mostly the same for all the *Trypanosoma* species (*Trypanosoma brucei*, *Trypanosoma vivax* and *Trypanosoma congolense)*. The life cycle of *Trypanosoma brucei* and *Trypanosoma congolense* is similar except that after *Trypanosoma brucei* has developed in the midgut it goes forward to the proboscis instead of salivary glands like *Trypanosoma congolense* and *Trypanosoma vivax* which does not have a midgut stage but develops in the proboscis only (Mathew, 1999). The ingested form that is infectious for the fly is termed the bloodstream trypomastigote, which is a non-dividing form. Following ingestion, the bloodmeal is retained within the midgut, and the parasite differentiates into a procyclic form and divides by binary fission. After 2 weeks, some procyclics migrate from the midgut through the hemocoel eventually reaching the salivary glands in *Trypanosoma brucei*. At this point, they differentiate through an epimastigote stage into a metacyclic trypomastigote stage, which is a non-dividing form and infectious for the mammalian host (Mathew, 1999). Metacyclic trypomastigotes are found in the salivary glands 20 days after the bloodmeal, and there are 40,000 trypomastigotes/bite, but it takes only 400 to initiate an infection (Mathew, 1999).

In the mammalian host, the metacyclic trypomastigotes replicate at the site of infection. There may be an immune response causing inflammation (trypanosomal chancre) at the site of the bite (Dwinger, 1985). From there the trypomastigotes move via the lymphatics to the

lymph nodes and then to the bloodstream (Greenwood, 1980). Long- slender bloodstream trypomastigotes divide by binary fission in the bloodstream, generating, on occasion, short-stumpy forms to continue the cycle in the tse-tse fly. The long-slender trypomastigotes are not infectious for the fly (Mathew, 1999).

2.2 Epidemiology of African Animal Trypanosomosis

The distribution of trypanosomosis in Africa corresponds to the range of tsetse flies and comprises currently an area of 8 million km^2 between 14^0N and 20^0S Latitude (Molyneux, *et al*., 1996). African animal trypanosomosis (Nagana) is caused by *Trypanosoma congolense, Trypanosoma vivax* and to a lesser extent *Trypanosoma brucei* species (Morrison *et al*., 1981). The parasites cause relatively mild infections in wild animals, while in domestic animals they cause a severe, often fatal disease (Losos, 1986; Connor, 1994). All domestic animals can be affected by the disease (D' Avila *et al*., 1997; Damillo *et al*., 2012). With the appearance of tsetse flies some 35 million years ago, trypanosomes have been transmitted to mammals by these blood sucking insects (Vickerman, 1985). The long co-existence of both tsetse flies and game animals may explain why African wild life species are resrvior hosts of trypanosomes infections; they become infected by the parasite but show no ill effects (Vickerman, 1985).

In contrast, most domestic animals have yet been unable to develop tolerance or resistance to trypanosome infections within the 13,000 years of their breeding.

Trypanosoma vivax was introduced to Central and South America, the West Indies and Mauritius from Africa (Levine, 1973). The parasite was reported in the New World for the first time in French Guyana (Lerger and Vienne, 1919) and later in other parts of South America, Central America and some Caribbean islands (Melendez *et al*., 1995). Studied showed that South American *Trypanosoma vivax* originated from West Africa (Dirle *et al*., 1993).

In Nigeria, it has been reported that tsetse flies still infest 80% of the nation's aerable land mass including the high land areas previously known to be tsetse-free (Onyiah, 1997). *Trypanosoma vivax* is widely found in tsetse-free areas due to adaption of the parasite to mechanical transmission carried out by hematophagous dipterians, such as *Tabanus spp* and *Stomoxys spp.* (Jones and Davila, 2001; Osorio *et al*., 2008).

2.2.1 Transmission of *Trypanosoma vivax* and *Trypanosoma congolense*

In Africa, the primary vector of *Trypanosoma vivax* and *Trypanosoma congolense* is Glossina species (Losos and Ikede, 1972). The three main species of tsetse flies for transmission of trypanosomes (*Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei, brucei*) are *Glossina morsitans,* which favours the open woodland of the savanna; *Glossina palpalis*, which prefers the shaded habitat immediately adjacent to rivers and Lakes; and *Glossina fusca*, which favours the high, dense forest areas (Rogers *et al.*, 1996). Trypanosomosis is also mechanically transmitted by other biting flies through the transfer of blood from infected animal to another susceptible animal (Cherenet *et al*., 2004). This mode of transmission requires close proximity of two animals involved. The most important mechanical vectors are flies of the genus *Tabanus* (Jones and Davila, 2001; Osorio *et al*., 2008), but *Haematopota, Lyperosia, Stomoxys*, and *Chrysops* flies have also been implicated, (Roder *et al*., 1984; Luckins, 1999a).

In Africa, *Trypanosoma vivax* have spread beyond the "tsetse fly belt'' (Roder *et al*., 1984), where transmission is principally by *tabanid* and *hippoboscid* flies.

In the western Hemisphere, the biological vectors for *Trypanosoma vivax* remain unknown, (Paiva *et al*., 2000; Osorio *et al*., 2008), but several species of hematophagous (especially *tabanid* and *hippoboscid*) flies are believed to serve as mechanical vectors (Luckins, 1999a).

The simple life cycle of *Trypanosoma vivax* enable its easy adaptation to different vectors and hosts (D'Archivio *et al*., 2011).

2.2.2 Hosts of *Trypanosoma vivax* and *Trypanosoma congolense*

Trypanosoma vivax and *Trypanosoma congolense* infect a large variety of wild and domestic mammals among which ruminants are the most susceptible in Africa and Latin America (D'Avila *et al*., 1997; Legon *et al*., 2003; Abenga *et al*., 2004). Horses are susceptible (Da Silva *et al*., 2010); but *Trypanosoma vivax* does not readily infect laboratory rodents. However, more recent research on strains of *Trypanosoma vivax* that naturally infect rodents or that have been adapted to laboratory rodents has revealed the existence of larger, more granular blood stream forms of *Trypanosoma vivax* with a clearly sub-terminal kinetoplast (Gardinar, 1989). In endemic areas, asymptomatic buffaloes, bovines and sheep could serve as reservoir hosts of *Trypanosoma vivax* (Ventura *et al*., 2001; Davila *et al*., 2003; Garcia *et al*., 2006; Osorio *et al*., 2008).

Trypanosoma congolense infects ruminants, horses, camels, pigs and dogs (D' Archivio *et al*., 2011). Wild animals like buffaloes, antelopes etc, act as reservoir hosts for both *Trypanosoma congolense* and *Trypanosoma vivax* (OIE, 2009). The most preferential hosts of each trypanosomes species may differ, but *Trypanosoma congolense* and *Trypanosoma vivax* have a wide host range among domesticated animals.

2.2.3 Pathogenesis of animal african trypanosomosis

There are many factors involved in the pathogenesis of animal African trypanosomosis and these have been described by many researchers (Taylor and Authie, 2004; Olaoluwa, 2006; Batista *et al*., 2009). Natural infection with trypanosomes occur following subcutaneous inoculation of the host with metacyclic forms of the trypanosomes by an infected tsetse fly

where they multiple in the skin leading to the development of an inflammatory swelling known as ‘chancre’. The trypanosomes enter the blood from this site either directly or through the lymphatics and are disseminated through out the body by circulating blood thereby causing parasitaemia which is normally low in most natural hosts with some *Trypanosoma* species, especially *Trypanosoma congolense* (Buscher, 2001). Trypanosomosis, like other infectious diseases, starts with an increase of the body temperature, a hyperthermia. This is as a result of the contact between the trypanosomes multiplying in the host and the defense system of the host. The surface proteins of the trypanosomes provoke the host to produce specific antibodies against these proteins, and after a few days almost all of the trypanosomes in the blood are destroyed by these antibodies and the body temperature drops. However, a few parasites survive as they have been able to replace their surface proteins with different ones, against which the antibodies cannot act (Zambrano *et al*., 2002; Deitsch *et al*., 2009). These surviving trypanosomes are able to multiply, and cause a new peak of parasitaemia and hyperthermia, until the mechanism of the host makes specific antibodies against the new surface proteins. This seesawing process continues for a long time, as the trypanosome is able to make an almost unlimited number of antigenic variants, and the host responds to each of them, until either the antigenic repertoire of the trypanosome is finally exhausted, in which case self-cure of the host follows, or the ability of the host to react to all of the antigenic variants is overwhelmed, and the host dies (Strickeler *et al*., 1987; Morrison *et al*., 2009).

Trypanosoma congolense localizes in the endothelial cells of small blood vessels and capillaries whereas *Trypanosoma vivax* is primarily a vascular parasite, but has also been found in extravascular, locations (Taylor and Authie, 2004).

There is remarkable intraspecies variation in the pathogenicity of different parasite stocks, especially stocks isolated from distinct geographical regions.

Among other host factors that determine the severity of the disease are genetic, physiological status of the host as well as nutritional and environmental factors (Taylor and Authie, 2004). The most significant and complicating factor in the pathogenesis of trypanosomosis is the profound immunosuppression that occurs following infection by these parasites. This marked immunosuppression lowers the host's resistance to other infections and thus results in secondary disease, which greatly complicates both the clinical and pathological features of trypanosomosis (Taylor and Authie, 2004).

2.2.4 Clinical features of trypanosomosis

Infections by pathogenic trypanosomes (*Trypanosoma vivax, Trypanosoma congolense* and to a lesser extent *Trypanosoma brucei*) cause diseases, which vary in severity depending on the species of infecting organism and susceptibility of the host (Losos, 1986). It has been stated that the course and severity of the clinical signs are modified by the type of husbandry, breed, and individual susceptibility of the animal infected (Wells *et al*., 1982; Desquesnes, 1997b). The infection may produce peracute, acute, and chronic syndromes. The clinical manifestations are insidious in onset without the development of either remarkable or pathognomonic signs. The common clinical manifestation of animal African trypanosomosis have been summarized by several authors (Bawa *et al*., 2000; Taylor and Authie, 2004; Olaoluwa, *et al*., 2006; Osorio *et al*., 2008; Batista *et al*., 2009). These signs include intermittent pyrexia, anorexia, increased respiratory and heart rates, marked depression, lacrimation, photophobia, nasal discharge, rough hair coat, pale mucous membrane, subcutaneous edema, prominent jugular pulse, weigh loss, enlargement of superficial lymph nodes, corneal opacity and blindness, central nervous system disorders and disturbances in reproduction resulting to reduced libido, infertility, abortion, stillbirth and death. Majority of the authors believed that the disease was mainly as a result of anemia. In the acute form of

the disease, drop in the red blood cell level was rapid and quickly became incompatible with life. The severity of anemia at any time throughout the course of infection determined the severity of clinical signs (Anosa *et al*., 1992).

Trypanosoma brucei infections have two stages of the clinical diseases (Potera, 1985). These are haematolymphatic (the early) stage, which is characterized by symptoms that occur before Central Nervous System (CNS) invasion by trypanosomes and the late (meningoencephalic) Stage. The early stage signs include the development of a chancre, which is followed later by periodic pyrexia, joint pains, muscle aches, palpable enlargement of lymph nodes, generalized oedema which also extends to appendages, anemia, reduced libido, impotence, abortion and infertility. The late stage signs are those associated with CNS involvement and include ataxia, convulsions and other nervous disorders**.**

2.2.5 Gross pathology in african animal trypanosomosis

There is no pathognomonic gross lesion seen in animals that died of trypanosomosis (Dwinger, 1985; Taylor and Authie, 2004). In the peracute disease of 2 to 4 weeks duration, there is no observable loss of condition, but as the infection becomes chronic the carcass becomes anaemic, emaciated and usually cachetic. Hemorrhages, petechial and ecchymotic, were reported in the lymph nodes and on the mucosal and serosal surfaces (Taiwo *et al*., 2003; Takeet and Fagbemi, 2009). The lymph nodes and spleen may be enlarged, normal, atrophied, or fibrosed (Masake and Morrison, 1981; Morrison *et al*., 1981; Mwangi *et al*., 1996). The changes in the spleen have been considered to be dependent on the duration and severity of the disease (Morrison *et al*., 1983). Most often the spleen has been noted as being obviously enlarged. Subcutaneous edema, particularly in horses and sheep; excessive fluid in the body cavities (more fluid than normal in the chest, lungs, abdomen and pericardium) and pulmonary edema may also be seen. The liver is enlarged and congested (Taiwo *et al*., 2003;

Fatihu *et al.*, 2008). The heart may be somewhat enlarged and may show a few hemorrhages on the muscle surface (flabby heart) and its weaker pumping action may have contributed to circulatory disturbances and increased fluid in the tissues (oedema).The kidneys are pale and swollen. Wasting or emaciation and dehydration are common (Takeet and Fagbemi, 2009). Some trypanosomes can directly damage tissues, resulting in lesions such as keratitis or cardiac damage. Immune complexes also cause inflammation and damage in a variety of tissues, including the kidneys and blood vessels (Murrary and Dexter, 1988; Taylor, 1998).

The skin may show pressure sores and ulcers, when the animal has been unable to stand up for some time. The fat reserves under the skin would have been used up and the skin is closely adherent to the underlying muscles and bone.

2.2.6 Histopathology in african animal trypanosomosis

It is believed that trypanosomosis is a disease that results from anaemia and that the main changes occurred in blood and hematopoietic tissues (Saror, 1980; Mwangi *et al*., 1991; Andrianarwo *et al*., 1995). In some bones especially in the femur, usually the active marrow is only present in the distal epiphysis in some animals and serous atrophy of fat is mostly found in the rest of the marrow as a result of trypanosomosis (Fatihu *et al*., 2008; Takeet and Fagbemi, 2009).

Anosa *et al*.(1992) found damage to the endothelium causing oedema, petechial, and ecchymotic haemorrhages. An accompanying hyperplasia of the myeloid tissue and multiple perivascular accumulations of lymphocytes were evident in the liver, kidney, skin, and testicle. Tissue necrosis was also reported. Round cell infiltration accompanied by degeneration and necrosis of parenchymatous cells occurred, and these changes were correlated to liver function (Egbe-Nwiyi *et al*., 2005; Takeet and Fagbemi, 2009). Erythrophagocytosis and haemosiderosis have also been reported in all the components of the

reticuloendothelial system (Taylor and Authie, 2004; Fatihu *et al*., 2008). It was reported that in Nagana, cerebral capillary haemorrhages is considered to be caused by the aggregations of trypanosomes (*Trypanosoma congolense* infection), in the blood vessels (Masake *et al*., 1984). They made the generalization that there was abundant evidence that trypanosomes in the blood do not injure vessels except by capillary embolism that occurred when the blood swarmed with the parasites.

A second interpretation given to the microscopic observations was presented by Anosa *et al*. (1997a; 1997b); they postulated that the disease process could not be the result of the anaemia alone. They concluded that the damage to the blood vessels, characterized by denudation of the endothelium, resulted in a widespread thrombosis of the smaller arteries and veins, and this in turn caused infarction as shown by the perivascular inflammatory cell infiltrations and parenchymal necrosis particularly in the kidney and adrenal glands (Fatihu *et al*., 2008).

In the cryptic form of the disease, they observed that trypanosomes accumulated in the capillaries of the myocardium, and postulated that the parasites were responsible for the necrosis of myocardial fibres. Mononuclear cell infiltrations of the epicardium, myocardium, and endocardium have been described by others in association with infections by *Trypanosoma congolense* and *Trypanosoma vivax* (Morrison *et al*., 1983; Anosa *et al*., 1997a). Losos and Ikede (1972), reported accumulation of *Trypanosoma brucei* in the brain. Brain lesions have also been reported by Losos and Ikede (1972) in an infected animal. There was atrophy and terminal central lobular necrosis of the liver, and an obvious hypertrophy of haemolymph nodes and of the red pulp of the spleen (Taiwo *et al*., 2003; Takeet and Fagbemi, 2009). Mononuclear-cell infiltrations, both diffuse and nodular, were present in the interstitial tissues of the kidney, and were most often found around large blood vessels and glomeruli.

2.2.7 Diagnosis of african animal trypanosomosis

A variety of diagnostic tests are available for trypanosomosis (Toure, 1976) and researchers are still trying to improve existing tests and to develop new ones. The current diagnostic tests vary in their sensitivity and specificity, the ease with which they can be applied and their cost (Paris *et al*., 1982). The choice of a particular test will be guided by the economic principles and the availability of expertise, but especially by the diagnostic requirement. Reliable diagnosis may be achieved by combining appropriate diagnostic tests. Reliable interpretation of results from diagnostic tests will depend on test validity as well as on proper sample selection/collection, the sample size and the way the diagnostic tests are conducted (Paris *et al*., 1982).

2.2.7.1 Parasite detection techniques

These techniques are highly specific, but their sensitivity is relatively low (i. e. the proportion of false negative results recorded is high). Sensitivity is especially low when results are considered at the individual animal level rather than the herd level. Sensitivity is highly variable during the cause of the infection, (Bailey and Smith, 1992) in the early phase, the sensitivity is high as parasites are actively multiplying in the blood in the absence of immunological control; (Bengaly *et al*., 2002). During the chronic phase the sensitivity is low as a result of immune response of the host; the parasites are scanty and are rarely seen in the blood (Bengaly *et al*., 2002). Sensitivity is almost nil in healthy carriers, where parasites are never seen. At the population level these variations mean that parasite detection techniques are highly sensitive during epizootic outbreaks (when most of the animals are in early stages of infection), and are of low or very low sensitivity in enzootic areas where most of the

animals are in the chronic stages of infection, especially during subclinical phases of the infection (healthy carriers), (Bengaly *et al*., 2002).

Due to this low sensitivity the apparent parasitological prevalence of trypanosomosis is a little or much lower than the true parasitological prevalence. The low diagnostic sensitivity also makes it difficult to detect trypanosomosis when present at low parasitological prevalence and it is impossible to establish the absence of the disease with a high degree of confidence (Bengaly *et al*., 2002). More over, in areas where trypanocidal drugs are used extensively, parasites may not be detected (OIE, 2008).

Several parasite detection techniques such as wet mount, thin, thick smears, buffy coat and haematocrit centrifuge techniques are available, each with varying sensitivity. The choice of the techniques will depend on the laboratory facilities available and the aim of the diagnosis (Bengaly *et al*., 2002).

2.2.7.1.1 Direct parasite detection techniques**.**

The simplest techniques are examination of wet, thick or thin films of fresh blood, usually obtained from the ear vein, jugular vein or the tail. Amongst the direct examination techniques, stained thin blood films are usually regarded as more specific but less sensitive than the other two (Paris *et al*., 1982). The actual specificity and sensitivity of these techniques is directly dependent on the volume of blood actually examined and the skill and the experience of the microscopist (Paris *et al*., 1982).

2.2.7.1.2 Wet blood films.

The diagnostic sensitivity of the method is generally low but depends on the examiner's experience and the level of parasitaemia. Sensitivity can be improved significantly by lysing the RBCs before examination using a haemolytic agent such as sodium dodecylsulphate (SDS) (Paris *et al*., 1982; OIE, 2008).

2.2.7.1.3 Thick blood films

Trypanosomes are easily recognized by their general morphology, but may be damaged during the staining process. This may make it difficult to identify the species (Paris *et al*., 1982).

2.2.7.1.4 Thin blood smears films

Thick smears contain more blood than thin smears and, hence, have a higher diagnostic sensitivity. Thin smears on the other hand allow *Trypanosoma* species identification (Paris *et al*., 1982).

The following morphological characteristics can be used to identified *Trypanosoma* species;

i. *Trypanosoma vivax* is about 20- 27 µm long (large species), the undulating membrane is not obvious, free flagellum is present at the anterior end, posterior end is rounded, the kinetoplast is large and terminal (Hoare, 1972).

ii. *Trypanosoma congolense* is about 8- 25µm (small species), undulating membrane is not obvious (no undulating membrane), free flagellum is absent, posterior end is rounded; kinetoplast is medium sized and terminal or sub-terminal. Although *T. congolense* is considered to be monomorphus, a degree of morphological variation is sometimes observed. Within *T. congolense*, different types or subgroups exist (savannah, forest, Kilifi, tsavo) that have a different pathogenicity, (Bengaly *et al*., 2002). However, these types can only be distinguished by using PCR (Geysen *et al*., 2003).

2.2.7.1.5 Parasite concentration techniques

The probability of detecting trypanosome in a sample from an infected animal depends largely on the amount of blood examined and the level of parasitaemia. The amount of blood

examined with direct examination techniques is little and parasites are often very scanty in the blood of an infected animal. These factors contribute to the low sensitivity of direct examination techniques. Sensitivity can be improved by increasing the volume of blood to be examined and by concentrating the trypanosomes (Woo, 1970).

2.2.7.1.6 Microhaematocrit centrifugation technique

This technique is widely used for the diagnosis of animal trypanosomosis. It is based on the separation of the different components of the blood sample depending on their specific gravity (Paris *et al.*, 1982).

The microhaematocrit centrifugation technique is more sensitive than the direct examination techniques because it concentrates the parasites (Kratzer and Ondiex, 1989).

In the case of *T. vivax* infections, the sensitivity of the HCT approaches 100% when the parasitaemia is >700 trypanosomes/ml of blood. Sensitivity decreases to 50% when parasitaemia varies between 60 and 300 trypanosomes/ml of blood. Trypanosomes become very difficult to detect when the parasitaemia is lower than 60 trypanosomes/ml blood (Desquesnes and Tresse, 1996). Identification of trypanosome species is difficult.

As for *Trypanosoma congolense*, the specific gravity is similar to that of RBCs and parasites are often found below the buffy coat in the RBC layer. To improve the separation of RBCs and the parasites, and increase the sensitivity for *Trypanosoma congolense*, the specific gravity of RBCs can be increased by the addition of glycerol (Desquesnes and Tresse, 1996).

2.2.7.1.7 Buffy coat technique.

This technique represents an improved method for the detection of trypanosomes and is widely used (Murrary *et al.*, 1977).

As with the microhaematocrit centrifuge technique, the buffy coat technique is more sensitive than direct examination techniques (Murrary *et al*., 1977). Compared with the microhaematocrit centrifugation technique, the buffy coat technique has an added advantage in that preparation can be fixed and stained for more accurate identification of species and for retention as a permanent record.

Both methods give direct results and can be used for screening large numbers of animals. They require specialized equipment and an electricity supply making the test more expensive compared with the examination of the wet blood film. However, this is compensated for by increased sensitivity. However, both techniques rely on the detection of motile, live, trypanosomes. Because trypanosomes can lose their vigour and die rather quickly once the blood sample is drawn, samples collected in capillary tubes should be cooled immediately and should not be allowed to over heat in the microhaematocrit centrifuge or on the microscopic stage. Samples should be examined as soon as possible after collection, preferably within an hour or two hours (Murrary *et al*., 1977).

2.2.7.1.8 Animal Inoculation

The sub-inoculation of blood into rodents, usually mice or rats, is particularly useful in revealing subpatent infections (Toure, 1976). It is more sensitive than direct examination of the wet blood film. Nevertheless, the method is not practical; it is expensive and diagnosis is not immediate. The method is highly sensitive in detecting *Trypanosoma brucei brucei* infections. However, some *Trypanosoma congolense* strains are not easily transmitted and *Trypanosoma vivax* rarely infects laboratory animals (Toure, 1976).

2.2.7.2 Indirect methods of diagnosis/Trypanosomal antigen detection test (Serology).

During the past 20 years, immunoassay methods have been widely used for diagnosis of bovine trypanosomosis in the field (Nantulya, 1990; Luckins, 1992). These indirect techniques, detecting specific antibodies or antigens, offered a sufficiently simple, robust, and inexpensive tool which appeared accurate and reliable for diagnosing trypanosomosis.

Several antibody detection techniques have been developed to detect trypanosomal antibodies for the diagnosis of animal trypanosomosis, with variable sensitivity and specificity (IAEA, 1993).

2.2.7.2.1 Indirect Fluorescent antibody test.

The original method for this test (Luckins, 1977) has been replaced by a new technique for the preparation of trypanosomal antigens (Katende *et al.*, 1987), which involves fixation of live trypanosomes using a mixture of 80% cold acetone, the enzyme-link and 0.25% formalin in normal saline.

2.2.7.2.2 Antibody- detection enzyme- linked immunosorbent assay

The original antibody ELISA (Luckins, 1977) has recently been further developed for use in large- scale surveys of bovine trypanosomosis (Desquesnes, 1997b; Hopkins *et al.*, 1998). Recommendations have been made that allow antigen production and standardization of the test on a local basis (Greiner *et al.*, 1997; Desquesnes, 2004).

The standard antigen for trypanosomosis antibody tests is derived from blood stream- form trypanosomes. Trypanosomes are purified by DEAE anion- exchange chromatography of parasites from whole blood of infected rats. Antigens are prepared as a soluble fraction with lysis using seven freeze- thaw cycles and centrifugation at 10,000g for 30 minutes. Antigens obtained from *in-vitro* propagated procyclic trypanosome forms can also be used (Greiner *et al.*, 1997). Soluble antigens can be stored at -80^0C or -20^oC for long and short periods, respectively, but they may also be lyophilized for conservation at room temperature

(Desquesnes, 1997a, b). ELISA using *Trypanosoma congolense* or *Trypanosoma vivax* pre-coated microtitre plates have been developed that have the advantage that a standardized denatured antigen is used that can be stored for long periods at room temperature (Rebeski *et al*., 2000).

Each ELISA micro-plate is run with strong positive, weak positive and negative reference sera, which are required to comply with pre- set values for quality assurance. The absorbance of each ELISA- sample tested is expressed as a percentage (percentage positivity; PP) of the strong positive reference standard (Wright *et al*., 1993), or the positive and negative reference standards (Desquesnes, 1997); results are therefore, quantifiable. The cut-off value is determined using known positive and negative field or experimental samples (Desquesnes, 2004).

Both antibody- detection tests have high sensitivity and genus specificity. Their species specificity is generally low, but may be improved by using a standardized set of the three species-specific tests (Desquesnes, 2004). They detect immune responses to current and past infection and can, therefore, only provide a presumptive diagnosis of active infection. However, persistence of antibodies after a curative treatment or a self-cure is estimated to be on the average of 3-4 months in young and adult cattle infections (Desquesnes, 2004); although it might take up to 13 months before all antibodies would have disappeared in some animals (Van den Bossche *et al*., 2000).

Immunodiagnosis needs expensive, sophisticated equipment and expertise, which is not always available. It has to be performed in specialized laboratories and there is a substantial delay between the actual sampling and availability of the results. Antibody ELISA is a very useful test for large- scale surveys in determining the distribution of tsetse-transmitted trypanosomosis because of the high degree of automation and standardization, in addition to the use of filter papers for sample collection and storage.

2.2.7.2.3 DNA amplification tests.

A polymerase chain reaction (PCR) method has been developed as a tool for the diagnosis of infection with African trypanosomes in humans and animals; as well as tsetse flies. Specific repetitive nuclear DNA sequences can be amplified for *Trypanosoma vivax* and three subspecies of *Trypanosoma congolense* (Masiga *et al*., 1992; Desquesnes, 1997; Desquesnes and Davila, 2002); however, current primers for *Trypanosoma vivax* seem not to be able to amplify some genotypes within this species. A common primer set is available for detection of three *Trypanosoma brucei* subspecies. The primer sets available for different trypanosome subgenus, species and types are referred to as follows: Trypanozoon subgenus- TBR1 and TBR2; *Trypanosoma congolense* (Savannah type) - TCN1 and TCN2; *Trypanosoma congolense* (forest type) - TCF1 and TCF2; *Trypanosoma congolense* (Kenya coast type) - TCK1and TCK2 and *Trypanosoma vivax* – TVW1 and TVW2. Due to the multiplicity of these taxon- specific primers in tsetse flies or cattle, a full trypanosome species identification requires that five PCR test be carried out per sample, which considerably increases the cost of diagnosis. Recently PCR restriction fragment length polymorphism (RFLP) assays and Information Technology In Science Inquiry (ITSI) of ribosomal DNA amplification have been developed that allow the identification of all *Trypanosoma* species as single or mixed infection using one single test (Desquesnes and Davila, 2002; Delespaux *et al*., 2003; Geysen *et al.*, 2003); however these tests are not yet suitable for routine diagnosis.

2.2.8 Treatment and control

Control of African trypanosomosis is generally dependent largely on vector control and use of drugs (chemotherapy and chemoprophylaxis). In animals, the drug control of trypanosomosis is dependent essentially on three drugs, namely; homidium (Homidium Chloride–Novidium® and Homidium Bromide–Ethidium®), diminazene aceturate (Berenil®) and isometamidium chloride (Samorin®,Trypamidium®) (Leach and Roberts,

1981; ILRAD, 1990; Molyneux, 1997; Geerts and Holmes, 1998). However, almost all of these trypanocides are gradually losing their efficacy due to drug resistance (Williamson, 1970).

The use of drugs for the prevention and treatment of trypanosomosis has been important for many decades, but the rapidity with which the trypanosomes have developed resistance to each drug introduced has tremendously complicated this approach to controlling the disease. In spite of this, some of the older chemoprophylactic drugs such as the quinapyramine derivatives Antrycide and Antrycide Prosalt are still in used and give effective protection against *T. b. brucei* infection in horses, camels, and cattle for up to 3 months (Robson, 1962). The drug pyrithidium bromide (Prothidium and AD2801) is useful in the prophylaxis of *Trypanosoma vivax* and *Trypanosoma congolense* infections in cattle, sheep, and goats and can give protection for up to 6 months. The most widely used of the newer chemoprophylactic drugs (and also the least expensive) is isometamidium chloride (Ogunyemi and Ilemobade, 1989). This drug has being in use for over 20 years and sold under the trade names Samorin, Trypamidium, and M&B 4180A, is excellent for the prophylaxis of all three African animal trypanosomes, and gives protection for 3-6 months (Mamman *et al*., 1995). The development of resistance to this drug has been reported in both East and West Africa (Sones *et al*., 1988; Clausen *et al*., 1992; Codjia *et al*., 1993; Leak *et al*., 1993; Peregrine *et al*., 1994). Although extensively used in trypanosomosis control, chemoprophylaxis is an expensive, time-consuming, and thus unsatisfactory long-term solution to the problem of African animal trypanosomosis.

Homidium bromide has also been found to be an effective chemoprophylactic drug in East Africa, and the newly introduced arsenical Cymelarsan is effective in treatment of *Trypanosoma brucei brucei* infection (Desqusnes *et al*., 1995; Geert *et al*., 1997, 2001).

A very widely used chemotherapeutic drug in West Africa is diminazine aceturate (Berenil), which is effective against all the three African animal trypanosomes (Williamson, 1970; Roberts, 1981). Diminazene aceturate has been used extensively across the African continent with very satisfactory results (Roberts, 1981). Its popularity in cattle has been largely due to its therapeutic efficacy with an index that was found to be higher than with other anti-trypanosomal drugs used in animals (Williamson, 1970). In addition, diminazene is often effective against infection that has proved refractory to treatment with other trypanocidal drugs (Williamson, 1970), and for about 2 decades after extensive field usage, the reported incidence of resistance to diminazene has remained low (Williamson, 1970).

The solutions of resultant formulation can be used for up to 10-15 days when stored at room temperature without loss of activity (Fairclough, 1962).

Controlling arthropod vectors is important in preventing new infections since trypanosomes cannot survive for long periods outside the host, and disappear (died) quickly from the carcass after death (FAO, 1994). In endemic areas of Africa, African animal trypanosomiosis can be controlled by reducing or eliminating tsetse fly populations with traps, insecticides and sterile male insect release technique (Allsopp, 1992). Although tsetse control has great potential and has aroused strong feelings, both for and against, realistically it must be appreciated that, except in a few countries, it has had minimal impact on animal trypanosomiosis compared to trypanocidal drugs (Geerts and Holmes, 1998). Under such circumstances, the application of control of trypanosomiosis should, as in the past, be considered as distinct from the control of other animal diseases. However, present day thinking is moving away from the concept of large area eradication of the vector and towards a reduction of disease risk by control of the vector, often over relatively circumscribed areas (FAO, 1994). With or without owner participation, tsetse control is still a specialised activity

and requires a separate organisation for the application of control measures against any other animal diseases (FAO, 1994).

In Nigeria, the control strategies of animal trypanosomosis over the years have been directed against both the parasites and the vector. Strategies developed for the control of the vectors have been those of bush clearing (Steiner, 1964 ;Oluwafemi *et al.*, 2007), the use of traps and screens (Challier and Laveissiere, 1973) and the use of sterile insect technique (SIT), (Oladunmade, 1990). The main approach of controlling the parasite in the host has been by chemotherapy and chemoprophylaxix (Oluwafemi *et al.*, 2007). Experence has however shown that no one single technology or approach will result in the eradication of tsetse flies from an area.

The natural resources are never affected by tsetse control alone but by a wide range of other factors occurring parallel with it. These include socio-economic, sociological and demographic processes which are inter-dependent and act in combination on the landscape. Opinions as to the actual influence of tsetse control measures on the natural resources range from the view that the "wilderness" can only be protected by refraining from all control measures to the claim that the consumption and degradation of the natural resources within the tsetse belt continues without any tsetse control measures (Jordan, 1986; Nagel, 1991). In the case of residual applications, considerable negative effects on non-target organisms must partly be expected, the effects of aerosol applications are usually easily identifiable but tolerable. The implementation of stationary targets currently comprises of the most environmentally sound control method (FAO, 1977a, 1977b).

Advances in recombinant DNA technologies have recently fueled the development of molecular genetic approaches for the control of vector-borne diseases (Aksoy *et al*., 2001). The most challenging application of this technology, transgenesis, aims to modulate vector competence of insects, that is, to eliminate the ability of insects to transmit pathogens by

introducing and expressing foreign genes with antipathogenic properties that interfere with pathogen viability, development or transmission (Aksoy *et al*., 2001). It is proposed that these genetically engineered refractory insects can then be driven into natural populations to replace their susceptible counterparts. The consequences and risks associated with field release of genetically modified organisms need to be carefully assessed before this approach can be used. In the meantime, the approaches proposed here stand to benefit the ongoing vector-control techniques by improving their efficacy and reducing their cost.

However, *Trypanosoma vivax* in South America does not require tsetse flies; this technique would not be effective there. Trypanotolerant cattle remain an important option but they have not been widely used beyond the geographical boundaries within which breeds like the N'dama are endemic (d'Iteren and Trail, 1988; Murray and Trail, 1983*)*. Animals given good nutrition and rested are more likely to recover rapidly than undernourished and stressed animals (Murray and Trail, 1983).

2.2.8.1 Vaccination

Despite all the anti-trypanosome trails reported (Greca and Magez, 2011) not a single 'promising' experimental result obtained in these studies has sparked off a positive field vaccination trial. Indeed, in reality it appears that trypanosomes have evolved two defensive mechanisms that protect them from antibody-mediated elimination by the immune system. The first mechanism involves the capacity to modulate its own antigen 'appearance', while the second mechanism relies on undermining the host's capacity to mount an efficient immune response and to maintain its immunological memory (Greca and Magez, 2011).

One of the most remarkable features of trypanosomes is their ability to regularly switch their surface coat and hence evade immune destruction. This mechanism is known as antigenic variation (Pays *et al*., 2004; Vanhamm *et al*., 2001).

Subsequent to the sequencing of the trypanosome genome, an enormous repertoire of Variant Surface Glycoprotein (VSG) was identified, made up out of full-length VSG genes as well as a huge array of pseudogenes (Berriman *et al*., 2005). The parasite greatly increases the variations of the expressed protein by creating a puzzle made of fragments of several genes. Trypanosomes exclusively express only one VSG out of that vast repertoire at a time. This, together with the fact that all trypanosomes survive extracellularly, would theoretically turn the parasite into an 'easy target' for antibody-mediated killing by the host immune system (Berriman *et al*., 2005). Even though the latter occurs upon infection, by the time the host mounts an efficient response against the most frequently encountered VSG, VSG switching has already taken place and a different VSG-expressing population has arisen. Hence, the mechanism of antigenic variation allows the parasites to continually escape the immune system of the host and in conclusion, it appears that even if many VSGs share combined epitopes, this molecule is unable to be targeted by an efficient destructive antibody response (Greca and Magez, 2011). Taken the fact that many VSGs are expressed as mosaic proteins of previously 'used' VSGs, it remains remarkable that this system of antigenic variation seems to be so effective in escaping immune recognition. The reason why this is the case, is most likely linked to the second defensive mechanism that trypanosomes have developed, i.e., the abrogation of B-cell homeostasis and the destruction of the hosts immunological memory (Greca and Magez, 2011). Together, these immune dysfunctions result in the lack of build up of anti-VSG memory, and hence allow the parasite to use over time very similar VSG molecules, or even re-use a surface coat protein that already has been encountered by the host (Greca and Magez, 2011).

A number of studies reported four decades ago have already described the following key facts of *Trypanosoma* infections: (1) the immune system is unable to react against unrelated new antigens upon infection (Goodwin, 1970); (2) there is a non-specific activation of

immunoglobulin production (Urquhart *et al*., 1973); (3) the cellular components of the humoral response are no longer coordinated upon infection (Terry *et al*., 1973); (4) despite a sustained plasma cell hyperplasia, failure of antibody production (both IgM and IgG) at the cellular level occurs at 2–4 weeks post infection (Jennings *et al*., 1973). Those findings were directly associated to the presence of living trypanosomes, since the immune competence was restored after treatment (Murray *et al*., 1974), and define the basis of immunosuppression which is a hallmark of trypanosome infections.

Further insight into B cell dysfunction was gained through the study of morphological and qualitative changes in secondary lymphoid organs (Terry *et al*., 1973). The first peak of parasitemia and a marked splenomegaly were observed simultaneously, the latter was due to plasma cell proliferation in the white pulp and erythrocyte expansion in the red pulp of the spleen. These events were followed by a gradual disorganization of the white pulp caused by the increasing number of red blood cells, whereas the amount of plasma cells grew smaller with time (Greca and Magez, 2011). Under no circumstances were germinal centers observed (Morrison *et al*., 1981). As confirmed by Radwanska *et al*. (2008), not only is the architecture of the spleen severely affected by *Trypanosomal* infection, but the study of the cellular composition revealed a drastic disappearance of marginal zone B cells as well. This cell subset is the main mediator of T-cell independent immune responses. Finally, the same authors showed that B-cell memory was gravely impaired, due to either depletion or hindered reactivation. Important to stress is that the destruction of the B-cell memory compartment appears to affect not only anti-trypanosome responses, but immunological memory in general. Using this knowledge, it was later shown that during experimental trypanosome infections, exposure to a particular VSG does not provide the host with the capacity to mount a protective memory response against this given VSG. Indeed, re-challenge with a previously encountered trypanosome stock is possible within weeks after encountering the same VSG

antigenic variant (Magez *et al*., 2008). The ability of the parasites to change their surface coat antigens continuously, blocking the efficiency of the specific host antibodies has hindered the prospects of developing a protective vaccine against the disease (Seifert, 1996).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The studies were conducted in Makurdi and Idon in Benue State and Kaduna respectively. *:* Makurdi the administrative headquarter of Benue state, lies approximately between latitude 7°44^{1}N and longitude 8°54^{1}E. The town is located along the coast of the River Benue. The climatic condition in Makurdi is influenced by two air masses: the warm, moist south westerly air mass, and the warm, dry northeasterly air mass. The southwesterly airmass is a rain – bearing wind that brings about rainfall from the months of May to October. The dry northeasterly airmass blows over the region from November to April, thereby bringing about seasonal drought. The mean annual rainfall in Makurdi is about 1,290 mm (Akintola, 1986). Temperature in Makurdi is however, generally high throughout the year, with February and March as the hottest months. Temperature in Makurdi varies from a daily of 40℃ and a maximum of 22.5℃.

Idon is located in Kachia Local Government in Southern part of Kaduna State in Northern Gunea Savanna zone. It is located between latitude 9^{0}08’ and 11^{0}07’N and longitude 6^{0}10’ and 8^{0}48’E with annual rain fall of about 1,016 mm. It has five months of wet period (May - October) and temperature range of 41^{0}C to 27^{0}C (Ammani *et al*., 2012). Based on previous report and consultation with the farmers, the two places were selected for isolation because they are tsetseflies infested areas. Two herds of cattle farms were visited and the animals were examined and blood samples were collected from them.

3.2 Source of Parasite

3.2.1 Field Isolation of the *Trypanosoma vivax* and *Trypanosoma congolense*

Trypanosoma vivax and *Trypanosoma congolense* were isolated from clinical cases in a sedentary farm comprising of white Fulani in Makurdi, Benue State and Idon, Kaduna State. Twenty four (24) cattle with pyrexia were identified and sampled from a farm in Makurdi. About 3 mls of blood sample was collected from the jugular vein of each animal into sample bottle containing EDTA and placed on ice. The samples were analyzed immediately after collection on the field using wet mount and microhaematocrit centrifugation/ buffy coat techniques. Three of the samples were positive using wet mount technique. Two of the positive wet mounts were mixed infections of *Trypanosoma vivax* and *Trypanosoma congolense*, while the third was a mono infection of *Trypanosoma vivax*. The samples were then transported on ice to the Parasitology laboratory of the Department of Veterinary Parasitology and Entomology, College of Veterinary Medicine, Federal University of Agriculture, Makurdi where thin blood smears of all the blood samples collected were prepared. The single positive *Trypanosoma vivax* sample was then inoculated immediately into one of the pre-screened goats through the jugular vein for parasite multiplication. The goat became positive four (4) days post inoculation (pi). A week later, the parasitaemic goat was transported by road to Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria where they were kept in an fly-proof pen and monitored. The infected goat was left as donor for the experiment.

The *Trypanosoma congolense* was isolated from cattle in Kaduna State. A trip was made to cattle herds in Idon village situated 80km on Kaduna-Kachia road. Fourteen cattle with a history of pica appetite and emaciation were identified and separated. Rectal temperatures of these animals were taken and little quantity of blood sample was taken from the jugular vein of each animal with pyrexia. Each sample was collected into sample bottle containing 1mg/

ml of EDTA kept on ice. The samples were analyzed immediately after collection as described by Woo, (1970). One of the samples was positive and was suspected to be *Trypanosoma congolense* based on the movement while the rest were negative for trypanosome parasite. The positive blood sample was immediately kept on ice and transported by road to Zaria where it was immediately inoculated into a sheep previously prepared for isolation of the parasite. The sheep was kept in fly-proof pen in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and monitored. The sheep became parasitaemic twelve (12) days pi. The characteristic morphological features of the trypanosome in Giemsa stained thin blood smears confirmed it to be *Trypanosoma congolense*.

3.3 Experimental Animals

Thirty six (36) Yankasa sheep of similar age and of both sexes were purchased from an open market at Karfur in Kastina State. On arrival, the sheep were then introduced into fly-proof pens of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and screened for ecto-, endo- and haemo-parasites. Physical examination was used to check for presence of ecto-parasites. Two (2ml) of blood was obtained from the jugular vein of each of the sheep, and examined for haemoparasites using thin blood smear, wet mount and microhaematocrit centrifugation methods as described by Woo (1971). Facal samples were collected from the rectum of each sheep using a clean polythene bag and taken to helminthology laboratory for detection of helminth eggs using floatation and sedimentation methods as described by Cole (1986).

All the experimental sheep were dewormed using Albendazole® at the dose rate of 7.5 mg/kg. Ecto-parasites infestations were treated and controlled with deltamethrin® pour-on preparation and asuntol® spray. Those found with *Anaplasma* infections were treated with

oxytetracycline long acting at the dose rate of 20mg/kg body weight. *Amprolium* at dose rate of 10mg/kg body weight for 5 days was given to those that were infected with Coccidia. The sheep were also vaccinated each with 1 ml subcutaneous injection of monoclonal *pestes de petit ruminantes* (PPR) vaccine against PPR and pre-conditioned for two weeks before the commencement of the experiment.

3.3.1 Management

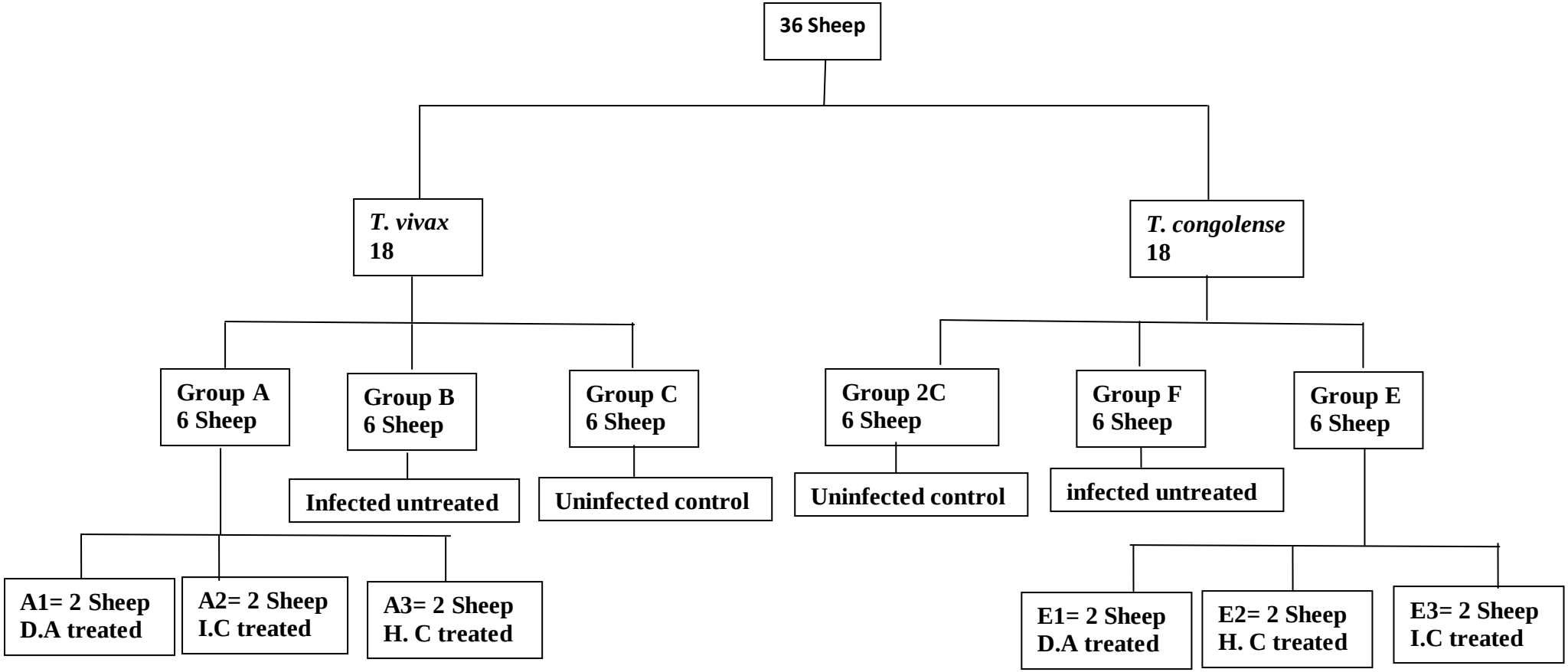
The animals were fed on cotton seed cake and maize offal, ground nut husk, *Digiteria* hay, salt lick and water was provided *ad libitum*. The feed were sourced from National Animal Production Research Institute (NAPRI), Shika, Zaria.

3.4 Experimental Design

3.4.1 *Trypanosoma vivax* and *Trypanosoma congolense*

The experimental animals were tagged and randomly divided into three groups (A, B and C) of six animals, each. Base line data were obtained from each of the animal in all the groups for a period of one week prior to inoculation. Two (2 ml) of blood containing approximately 2.0×10^6 *Trypanosoma vivax* as quantified using the improved Naubauer haemocytomter (Petana, 1963) with gram's of iodine as diluent after first multiplying the parasite in a donor sheep was inoculated through the jugular vein into each of the sheep in groups A and B. Group C served as uninfected control. Group A animals were further divided into 3 sub-groups (A_1, A_2, and A_3) of two animals each. Animals in each of the sub-groups were treated with one of 3 different trypanocides (diminazene aceturate at dose rate of 3.5 mg/kg body weight, isometamidium chloride at dose rate of 0.5 mg/kg body weight and homidium chloride at dose rate of 1mg/kg for 2.5% solution) when the parasitaemia was very high.

The *Trypanosoma congolense* experimental sheep were also divided into three groups (E, F and 2C) of six animals each. Base line data were obtained from each of the animal in all the groups for a week prior to infections. Each sheep in groups E and F was infected through intravenous inoculation of 2 ml of blood containing approximately 2.0×10^6 *Trypanosoma congolense* as quantified described for *Trypanosoma vivax*. Group 2C served as uninfected control. All the groups were provided with feed, while salt licks and water were provided *ad libitum*. Group E animals were further divided into 3 sub-groups (E_1, E_2 and E_3) of two animals each and each of the sub-group was treated with either one of the 3 different trypanocides (diminazene aceturate at dose rate of 3.5 mg/kg body weight, isometamedium chloride at dose rate of 0.5 mg/kg body weight and homidium chloride at dose rate of 1mg/kg for 2.5% solution) when the parasitaemia was very high.



4. 1 Flow Chart of experimental design

3.4.2 Post- infection monitoring

3.4.2.1 Clinical observations

All the experimental sheep (infected and non- infected) were monitored daily through physical examination (for change in gait, posture, possible discharges from the eyes and nose, cutaneous edema, enlargement of superficial lymph node and appetite) and laboratory tests (parasitaemia, haematology and serum proteins estimation).

3.4.2.2 Rectal temperature (0^{C})

Daily rectal temperature was taken early morning consistently with the aid of clinical digital thermometer by restraining and inserting the thermometer into the rectum of each of the sheep.

3.4.2.3 Weight changes (Kg).

Live weights of all the experimental sheep were determined once a week with the aid of bathroom weighing scale.

3.4.2.4 Parasitaemia

Parasitaemia was determined every 3 days using wet mount and buffy coat as described by Murray *et al*. (1983).

3.4.2.5 Packed Cell Volume (PCV) (%)

Packed Cell Volume was determined twice weekly by haematocrit centrifuge technique as described by cole (1986)

3.4.2.6 Total Plasma Protein (g/dl)

Serum protein concentration was estimated using Goldberg Refractometer (Kerr, 1989) as follows: After reading the PCV, the microhaematocrit tube was broken using a diamond tipped pencil at the buffy coat-plasma interface. The piece containing plasma was drained through establishing capillary flow onto the refractometer prism. The protein value was read directly from a scale inside the refractometer and expressed in gram per dl of plasma (g/dl).

3.4.2.7 White Blood Cell Counts (WBC)

Total and differential white blood cell counts were determined as described by Schalm *et al*. (1975).

3.4.2.8 Gross and Histopathological Examination

At day 56 post- infection (pi), one each of the treated sheep in groups A, B, E and F as well as one animal each from the control groups C and 2C were picked at random, sacrificed and necropsied. Samples of the spleen, lungs, brain, liver, heart, kidney, testicle, ovary, uterus, adrenal gland and pancreas were taken, fixed in 10% formal saline, embedded in paraffin wax, cut at 5µ thickness and stained with Heamatoxylin and Eosin (H and E) as described by Drury and Wallington (1976). Tissue sections were examined under light microscope (Olympus Optical Co. ltd, Olymp Tokyo, Japan) at ×40 objective lens.

3.4.2.9 Molecular Characterization of Trypanosoma vivax and Trypanosoma congolense using Polymerase Chain reaction Technique (PCR).

Isolates from different stages of the infections were collected and preserved at -20^{o}C and later subjected to RAPD-PCR, Multiplex *Trypanosoma* PCR and *Trypanosoma* species-specific PCR.

1 Acute stage form of *Trypanosoma vivax* and *Trypanosoma congolense*

2 Relapse stage form of *Trypanosoma vivax* and *Trypanosoma congolense*

3 Chronic stage form of *Trypanosoma vivax* and *Trypanosoma congolense*

The multiplex PCR primers for *Trypanosoma* and *Trypanosoma vivax* species-specific primers were supplied from South Africa by Inqaba Biotechnical Industries LTD, Hatfield-Pretoria, S/Africa, and RAPD-PCR primers were obtained from United State of America (Bioneer-Biotech Company, Wanstington DC, USA).

The experiment was divided into three different phases.

i. RAPD- PCR was conducted on the three different forms (the field isolate, the relapse and the chronic forms) of the two isolates.
ii. Each of the two isolates was subjected to multiplex PCR using primers for identification of mixed infections.
iii. *Trypanosoma vivax* species-specific PCR was conducted on the two field isolates for species identification of the isolates (i.e. confirmatory test).

3.4.2.10 DNA extraction from whole blood:

Buffy coat was first obtained from the whole blood and then two hundred and fifty microlitres (250µl) of the buffy coat was mixed with 250µl of lysis buffer (0.31 M sucrose, 0.01 M Tris–Cl, pH 7.5, 5 mM MgCl2, 1% Triton X-100). The mixture was centrifuged at 13,000×g (^{0}c) for 20 secs, the supernatant was removed and 500µl of lysis buffer was added and mixed by vortexing. Centrifugation and addition of lysis buffer was repeated twice. After the last centrifugation and removal of the supernatant, the pellet was resuspended in 250µl of 1× PCR buffer (10 mMTris–HCl, pH 8.4, 50 mMKCl, 1% Triton X-100) and 1.5µl of proteinase-K (10 mg/ml) was added, mixed and incubated at 56 ^{0}C for 1 hr. Finally the mixture was incubated at 95 ^{0}C for 10 minutes to inactivate the proteinase-K. The resulting eluate was stored frozen at -20^{0}C until used (Clausen *et al*., 1998).

3.4.2.11: Experiment 1: RAPD-PCR of Trypanosoma vivax and Trypanosoma congolense

Procedure: Stabilates of the two samples (*Trypanosoma vivax* and *Trypanosoma congolense*) were retrieved from the freezer (-20).

3.4.2.12 Characterization of the Extracted DNA Using Randomly Amplified Polymorphic DNA PCR (RAPD-PCR).

Procedure: The DNAs extracted from the two isolates were subjected to RAPD-PCR using short random primers (10 bases) as described by Welsh and McClelland (1990) and modified by Tibayrenc *et al*. (1993). Genomic DNA samples (20ng) were amplified in 60µl of specific buffer (10mM Tris- HCL, 50Mm KCL, 1.5mM $Mgcl_2$, PH 8.3), in presence of 0.2µM of primers, 4x 100mM, dNTP and 0.9µl of Taq DNA polymerase. The RAPD-PCR was based on the use of ten base pairs primers (Pbs-5'GGAAACAGCTATGACCATGA-3' and Tpnr-5' CCAAGTCGACATGGCACAAC-3').The total number of amplification was 45 cycles at

94°C for 1 min and 72°C for 2 min and a final elongation at 72°C for 7min. The amplification was repeated three times for the pair of the primers. The amplified product was separated by electrophoresis on 1.6% agarose gels with TAE buffer (40mM Tris- acetate PH 7.5, 1mM EDTA) at 5V/cm and DNA fragments were visualized after staining with ethidium bromide. The patterns of amplified fragments of each isolate were used to compare the isolates and stages of the disease.

3.4.2.13 Experiment 2: Identification of different Trypanosoma species using multiplex Trypanosoma primers (PCR).

This method focused on the internal transcribed spacer 1 (ITS) of ribosomal DNA as described by Desquesenes *et al*. (2001).

Briefly, a pair of primers designed for ribosomal DNA was used. The amplification was performed in a final volume of 50 µl containing 5 µl of DNA extract (template), (20 µM= 1µL) of each primer (F: 5'GCGTTCAAAGATTGGGCAATG-3' R: 5'-CGCCCGAAAGTTCACC-3'), 2 mM of each dNTP, 32.5µL of Nuclease-free water and 0.5 unit of Taq DNA polymerase. Initial denaturing step at 94 °C for 5 minutes was followed by 30 amplification cycles. Each cycle consisted of a denaturation step at 94 °C for 30 seconds, an annealing step at 58 °C for 30 seconds, 54°C for 30 secs and an extension step at 72 °C for 1 minute and a final extension at 72 °C for 10 minutes. The total number of cycles was 42 (4 cycles for 58°C, 8 cycles for 56°C and 30 cycles for 54°C).

Then 10 micro-liters of the amplified products of the PCR were resolved on 2% agarose gel at 100 Volts for 60 minutes containing 1.0 mg/µl of ethidium bromide. The gels were observed on ultraviolet light and photographed.

In order to confirm the presence or absence of *Trypanosoma vivax* in any of the samples, they were subjected to *Trypanosoma vivax*- specific primers PCR.

3.4.2.14 Experiment 3: Trypanosoma vivax species-specific PCR

Extraction of DNA was performed as described above for experiment one.

Primers. A Pair of specific primers for *Trypanosoma vivax* was used in this study

TVW A- d: 5'-GTG CTC CAT GTG CCA CGT TG-3'

TVW B- d: 5'-CAT ATG GTC TGG GAG CGG GT-3' (Desquesenes *et al*., 2001)

DNA amplification:

The amplification was performed in a final volume of 25µl containing 2 µl of DNA extract (template), (1 µM= 1µL) of the primer (TVW A- d: 5'-GTG CTC CAT GTG CCA CGT TG-3' TVW B- d: 5'-CAT ATG GTC TGG GAG CGG GT-3'), 10 mM Tris–Cl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 200 µM of dNTPs, 10µL of Nuclease free water, chelex 10µl. Initial denaturing step was carried out at 94 ℃ for 5 minutes and then annealing step at 60^0C for 60 secs and 72^oC for another 30 secs. The total number of amplification cycles was 30 cycles. The final extension was at 72 ℃ for 5 minutes.

3.4.3 Statistical analysis

Group means ± S.E.M was calculated for each of the group's temperature, PCV, total serum protein, RBC and live body weight. Significance difference between the means was calculated by one-way analysis of variance (ANOVA). Post test analysis was done where P-values were <0.05, using Minitab.

CHAPTER FOUR

RESULTS

4.1 Pre-infection Observation

4.1.1 Temperature

The pre- infection mean rectal temperature of the sheep was 38.4^{o}C, which is within the normal range (normal= 38^{o}C-38.8^{o}c).

4.2. Post-infection Observations

4.2.1 Prepatent period of *Trypanosoma vivax* and *Trypanosoma congolense* infections

All the infected sheep became positive for presence of the parasite six (6) days post infection. But the levels of parasitaemia differed in the sheep.

4.2.2 Rectal temperature of *Trypanosoma vivax* and *Trypanosoma congolense* infected Yankasa sheep

The pre-infection values of mean rectal temperature of the *Trypanosoma vivax* infected-untreated (group B), infected-treated (group A) and uninfected control (C) sheep were 38.33±0.26^{0}c, 38.63±0.17^{0}c and 38.18±0.15^{0}c respectively. There were statistically significant differences ($P<0.05$) in the values of mean temperatures of the groups (A, B and C), (39.25±0.11^{0}c, 39.58±0.01^{0}c and 38.80±0.14^{0}c) at week 2 post infection shortly before treatment of group A animals. However, the value of the infected, treated group (Group A) dropped (38.32±0.03^{0}c), while that of infected untreated animals (39.29±0.14^{0}c) remained high and the uninfected control (38.58±0.21) was within normal range at the end of the experiment at week 8 post infections (Figure 4.1).

Pre-infections values of the mean temperature of the *Trypanosoma congolense* infected, untreated (group F), infected treated (group E) and uninfected control (2C) were

38.35±0.22^{0}c, 38.17±0.16^{0}c and 38.28±0.17^{0}c respectively. However, there were statistically significant differences (P<0.05) between group F and groups E and 2C at weeks 4 and 5 (39.44±0.09^{0}c, 39.08±0.22^{0}c, 38.75±0.09^{0}c and 39.12±0.19^{0}c, 39.05±0.22^{0}c, 38.84±0.21^{0}c) respectively post infections. At week 8 post infections the values of groups E, and F (38.48±0.12^{0}c and 38.81±0.13^{0}c) dropped. The values of the uninfected control remained relatively unchanged throughout the period of the experiment (38.58±0.12^{0}c at week 8), (Figure 4.2).

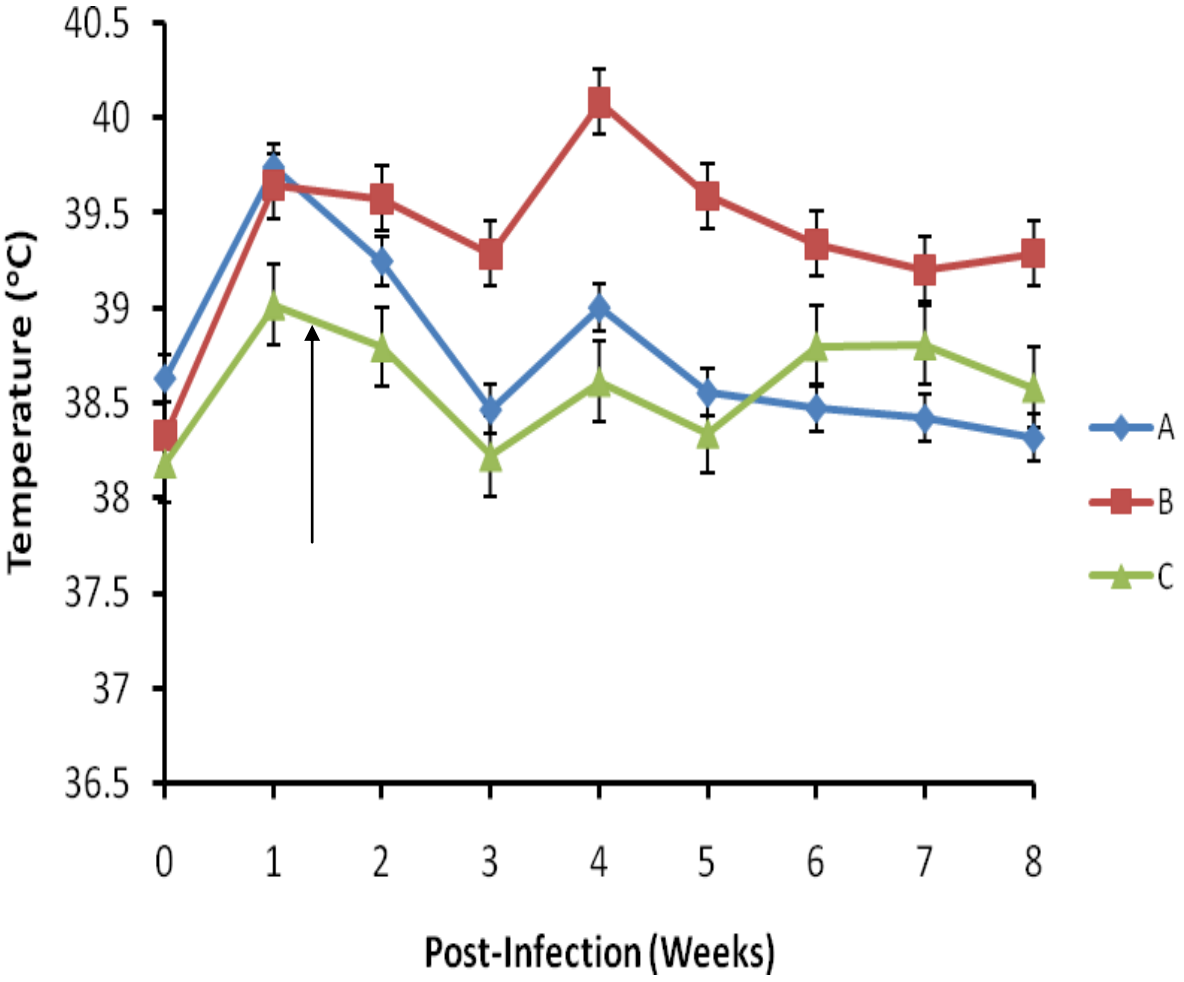


Figure 4.1: Mean ± SEweekly temperature of *Trypanosoma vivax*-infected and treated (A), infected, untreated (B) and uninfected control (C) Yankasa sheep.

↑ The arrow indicates point of treatment of group A animals.

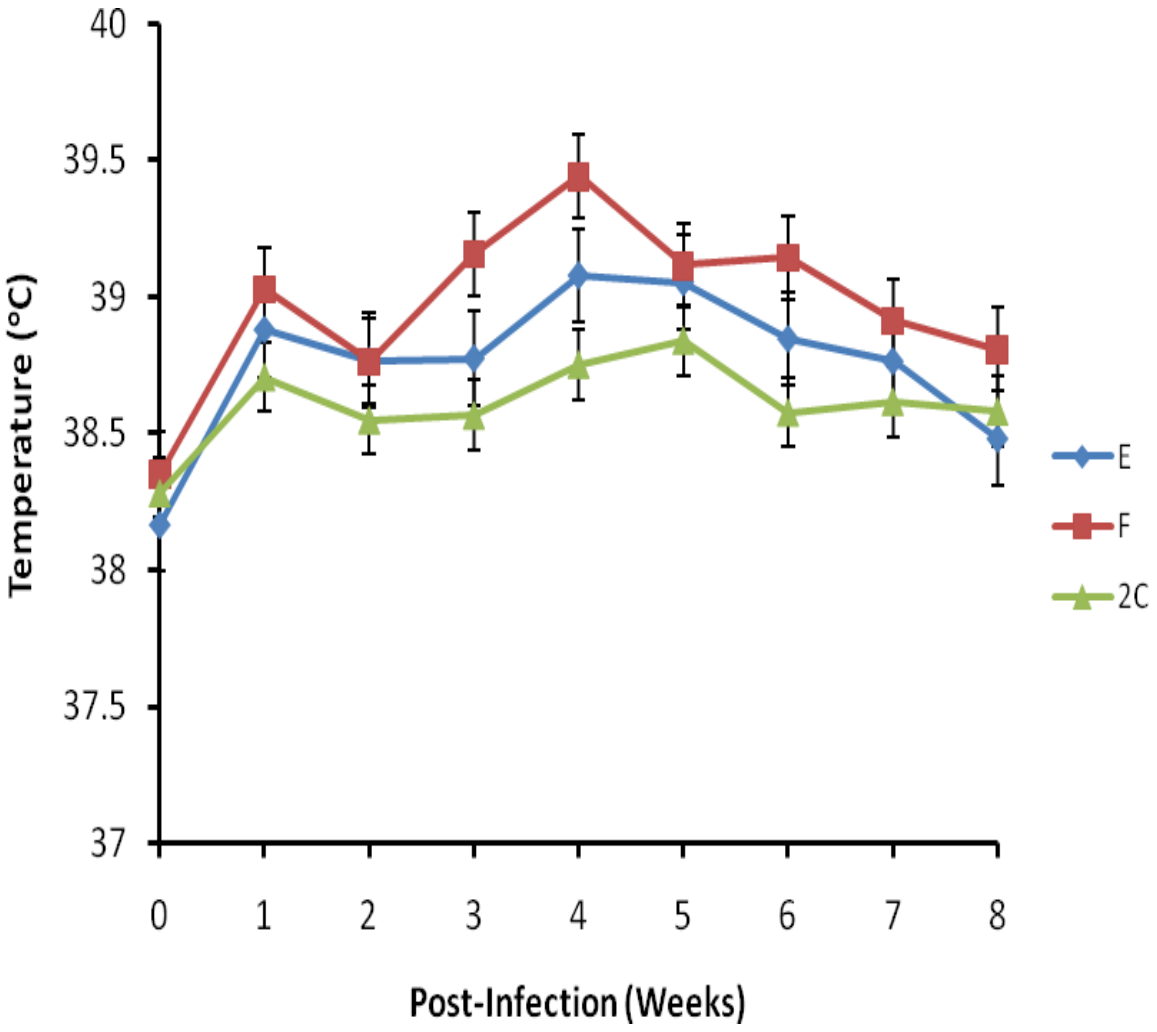


Figure 4.2: Mean ± SE weekly temperature of *Trypanosoma congolense* infected- treated (E), infected, untreated (F) and uninfected control (2C) Yankasa sheep.

↑ The arrow indicates point of treatment of group E animals.

4.3 Mean ± SE weekly body weight of *Trypanosoma vivax* and *Trypanosoma congolense* infected Yankasa Sheep.

The values of mean body weights of the *Trypanosoma vivax* infected and treated (A), infected untreated (B) and uninfected control (C) Yankasa sheep decreased by 1.5kg, 4.34kg and 1.75kg from week 1 post infections to week 8 post infections respectively. The infected untreated animals (B) had the highest decrease in value (4.34 kg) in weight lost. There were statistically significant differences ($P<0.05$) in the values of mean weight lost of group F animals and those of groups E and 2C at weeks 3, 5-8 post infections (Figure 4.3).

There was a decreased of 2.59 kg mean weekly weight of the *Trypanosoma congolense*-infected untreated (F) sheep between weeks 1 and 8 post infection. However, the values of the infected-treated (E) and uninfected control (2C), increased slightly by 0.34 kg and 0.29 kg respectively from weeks 1 and 8 post infections. There were statistically significance differences ($P<0.05$) in the mean weight of group F animals and those of groups E and 2C from weeks 2- 8 post infection (Figure 4.4).

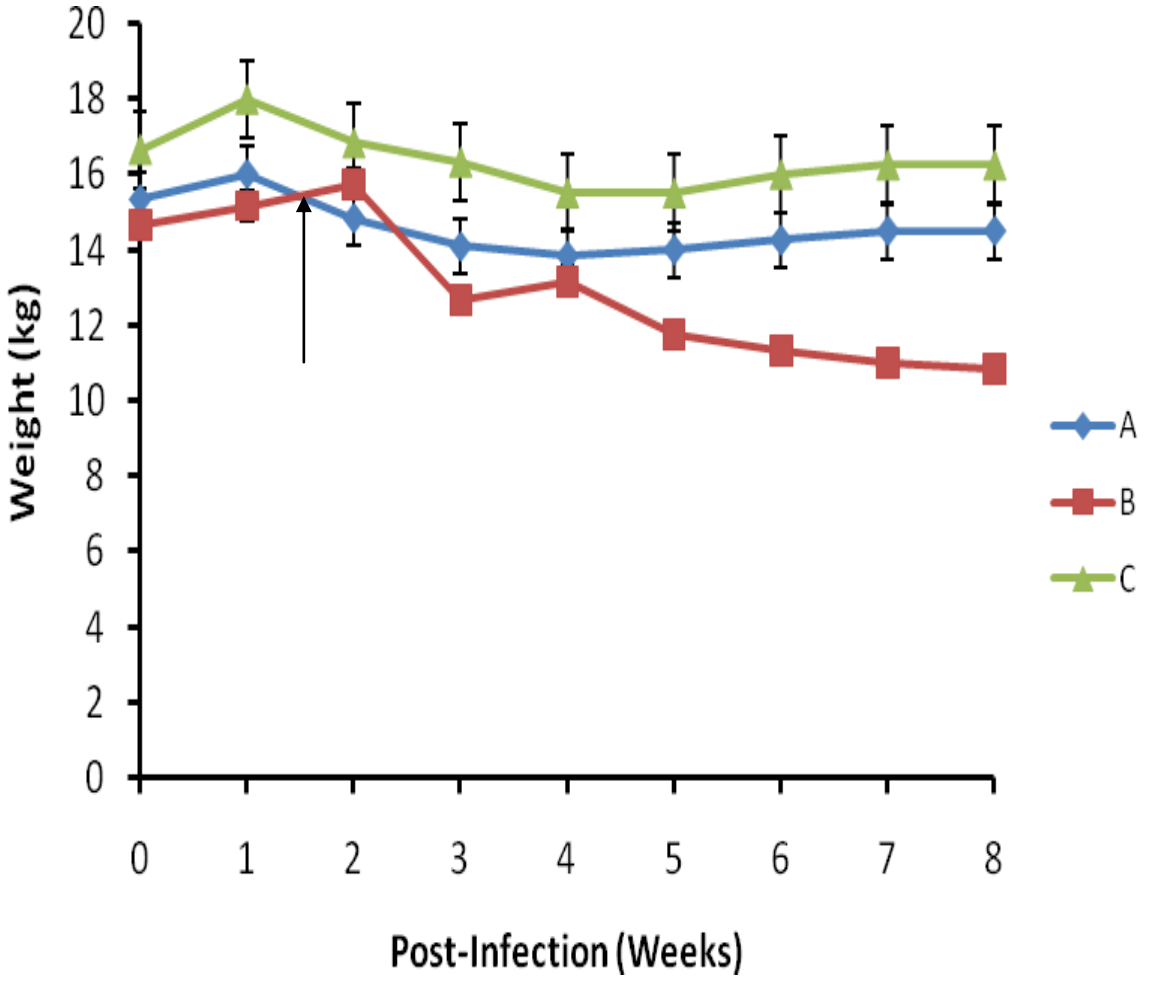


Figure 4.3: Mean ± SE weekly body weight of *Trypanosoma vivax*-infected and treated (A), infected, untreated (B) and uninfected control (C) Yankassa sheep.

↑ The arrow indicates point of treatment.

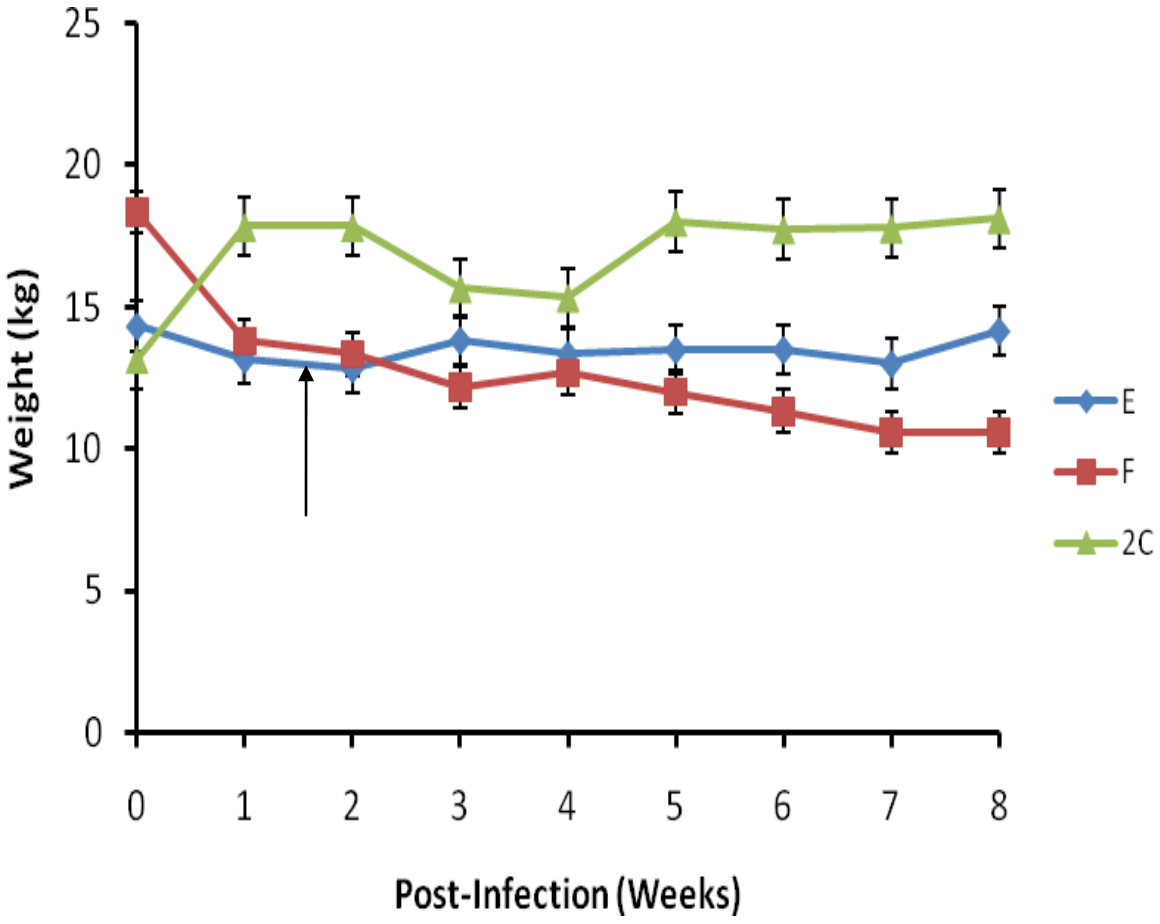


Figure 4.4: Mean ± SE weekly body weights of *Trypanosoma congolense* infected and treated (E), infected, untreated (F) and uninfected control (2C) Yankassa sheep.

↑ The arrow indicates point of treatment.

4.2.4 Daily parasitaemia of *Trypanosoma vivax* and *Trypanosoma congolense* infected Yankasa sheep.

There were relapse infections in the homidium chloride-treated animals on days 27 post infections (ie day 15 post treatment) and 32 post infections (ie day 20 post treatment) respectively. The two relapsed infections were treated with diminazene aceturate at dose rate of 3.5mg/kg body weight, which cleared the parasite from the peripheral blood within 24 hours post secondary treatment and the animals remained negative through out the period of observation. The experiment was terminated after monitoring for eight (8) weeks post infection (Figure 4. 5).

In *Trypanosoma congolense* infected and treated group, all the treated animals had relapse infections on day 37 post infections (ie day 25 post treatment). They were then treated for the second time with diminazene aceturate 3 days post relapse. A second relapse occurred day 6 post secondary treatment (pst) and all the relapsed animals were later treated for the third time (tertiary treatment) with homidium chloride at the dose rate of 1mg/kg (2.5% solution). The parasite disappeared from the peripherial circulation after 24 hours post tertiary treatment and the experiment was terminated after monitoring for 8 weeks post infections (Figure 4.6).

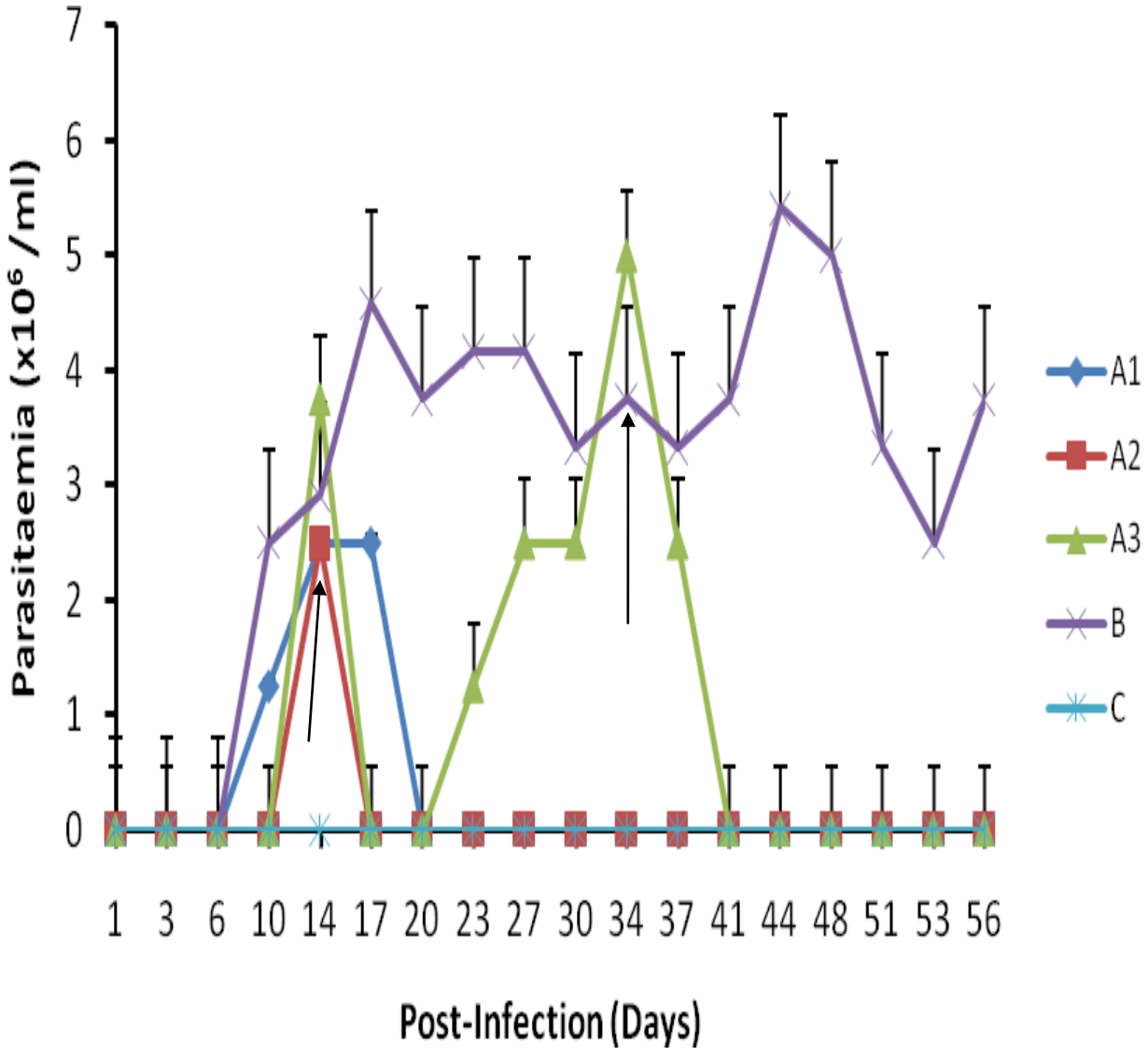


Figure 4.5: Mean ± SE daily parasitaemia of *Trypanosoma vivax* **A1**= Infected-diaminazene aceturate treated; **A2**= Infected-isometamedium chloride treated; **A3**= Homidium chloride treated; **B**= Infected, untreated; **C**= Uninfected control.

↑ The arrow indicates point of treatment.

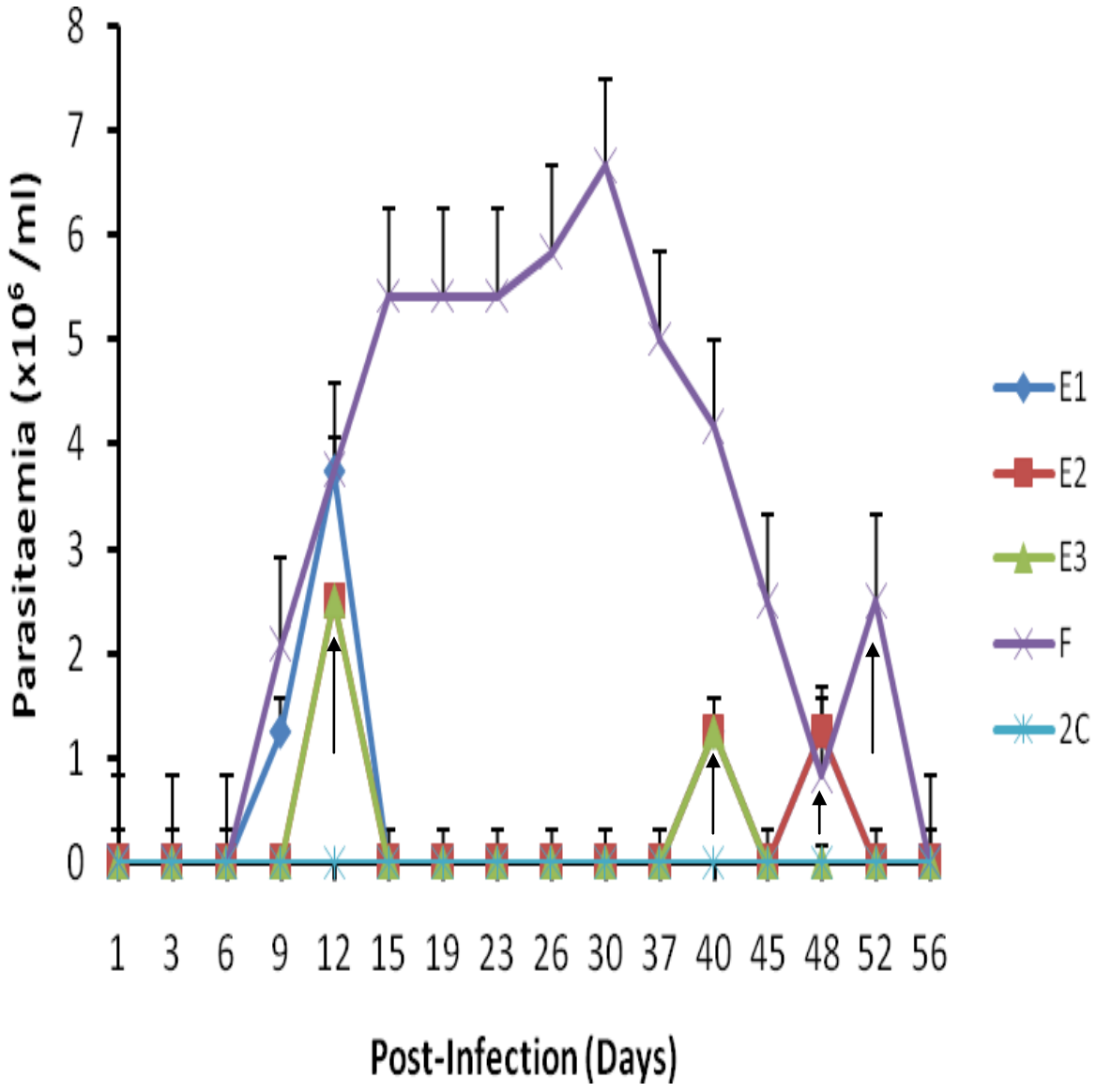


Figure 4.6: Mean ± SE daily parasitaemia of *Trypanosoma congolense* **E1**= Infected-diaminazene aceturate treated; **E2**= infected-homidium chloride treated; **E3**= infected-isometamedium chloride treated; **F**= infected untreated; **2C**= uninfected control.

↑ The arrow indicates point of treatment.

4.2.5 Clinical manifestaions of *Trypanosoma vivax* and *Trypanosoma congolense* infected animals

The following clinical signs were observed in *Trypanosoma vivax* experimentally infected sheep. These were; anorexia, rough hair coat, ocular discharge, pale mucous membrane, diarrhea, severe weakness, depression, edema of the eye lids, teeth grinding, emaciation and central nervous system (CNS) disorder. Three of the animals in group B died before treatment at week 8 post infections. In *Trypanosoma congolense* infected animals, apart from central nervous disorder and teeth grinding, all other signs seen in *Trypanosoma vivax* infected animals were also observed but in mild forms.

4.2.6 Packed cell volume of *Trypanosoma vivax* and *Trypanosoma congolense* infected animals

There was a decrease in values of 9.58±0.96% in mean packed cell volume (PCV) of the *Trypanosoma vivax*-infected untreated sheep (Group B) between weeks 1 and 7 post infection. At 8 weeks post infection, slight increase of 2.25% was observed after treatment. However, the mean PCV of the *Trypanosoma vivax* infected-treated group (A) dropped slightly by 1.67% between weeks 1 and 8 post infection. The mean PCV of the uninfected-control group (C) increased by 2.16% between weeks 1 and 8 post infections. There were statistically significant differences ($P<0.05$) in the mean values of the PCV of the three groups at week 2 post infection and weeks 5-8 post infections (Figure 4. 7).

There was a decrease of 8.75% in the value of the mean PCV of the *Trypanosoma congolense*-infected untreated sheep (Group F), between weeks 1 and 6 post infection. The highest decrease occurred at week 2 post infection. At week 8, a week after treatment, the mean PCV value increased by 2.18%. The infected and treated animals (E) also had their lowest value at week 2 post infections, (24.00±1.29%), following 4.58% loss in values. However, there was an increase of 1.47% between weeks 1 and 8 post infection in the mean PCV of the uninfected control animals (2C). There were statistically significant differences ($P<0.05$) in the mean PCV of group F and those of groups E and 2C between weeks 3-8 pi (Figure 4.8).

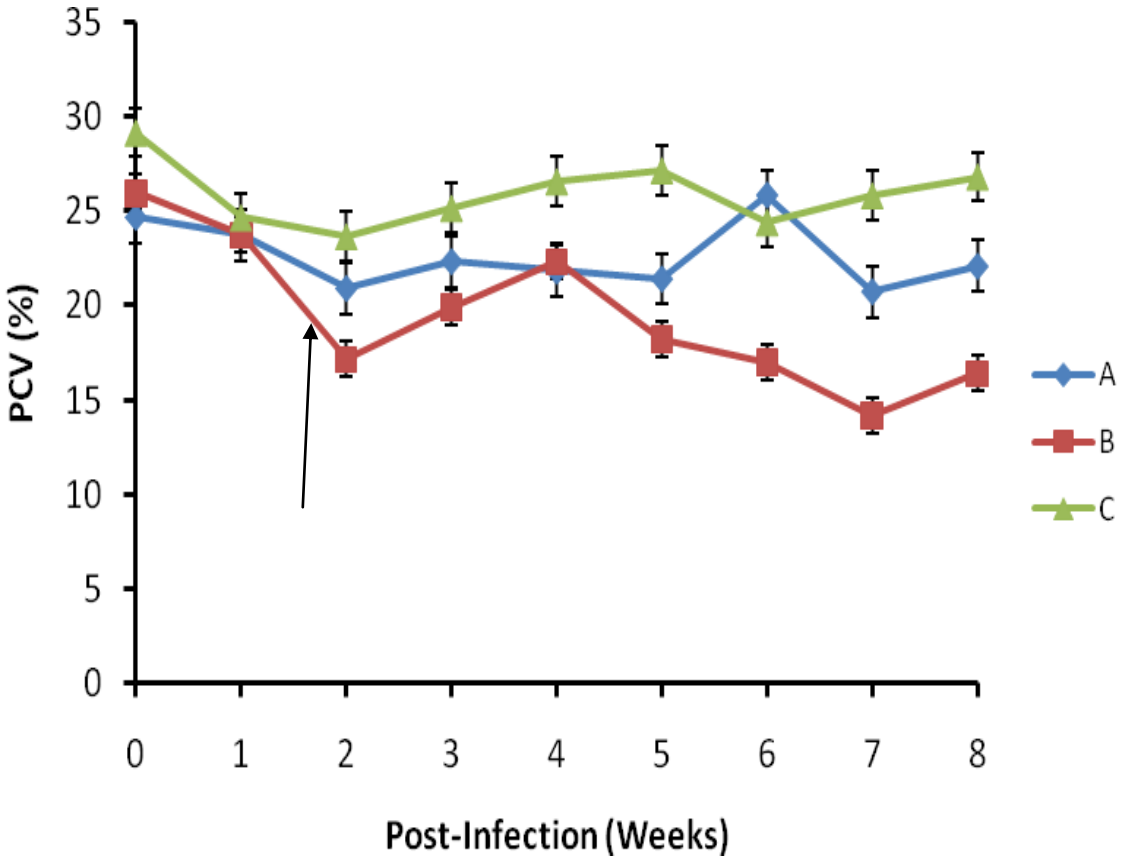


Figure 4.7: Mean ± SE weekly Packed Cell Volume of *Trypanosoma vivax*-infected and treated (A), infected, untreated (B) and uninfected control (C) Yankasa sheep.

↑ The arrow indicates point of treatment.

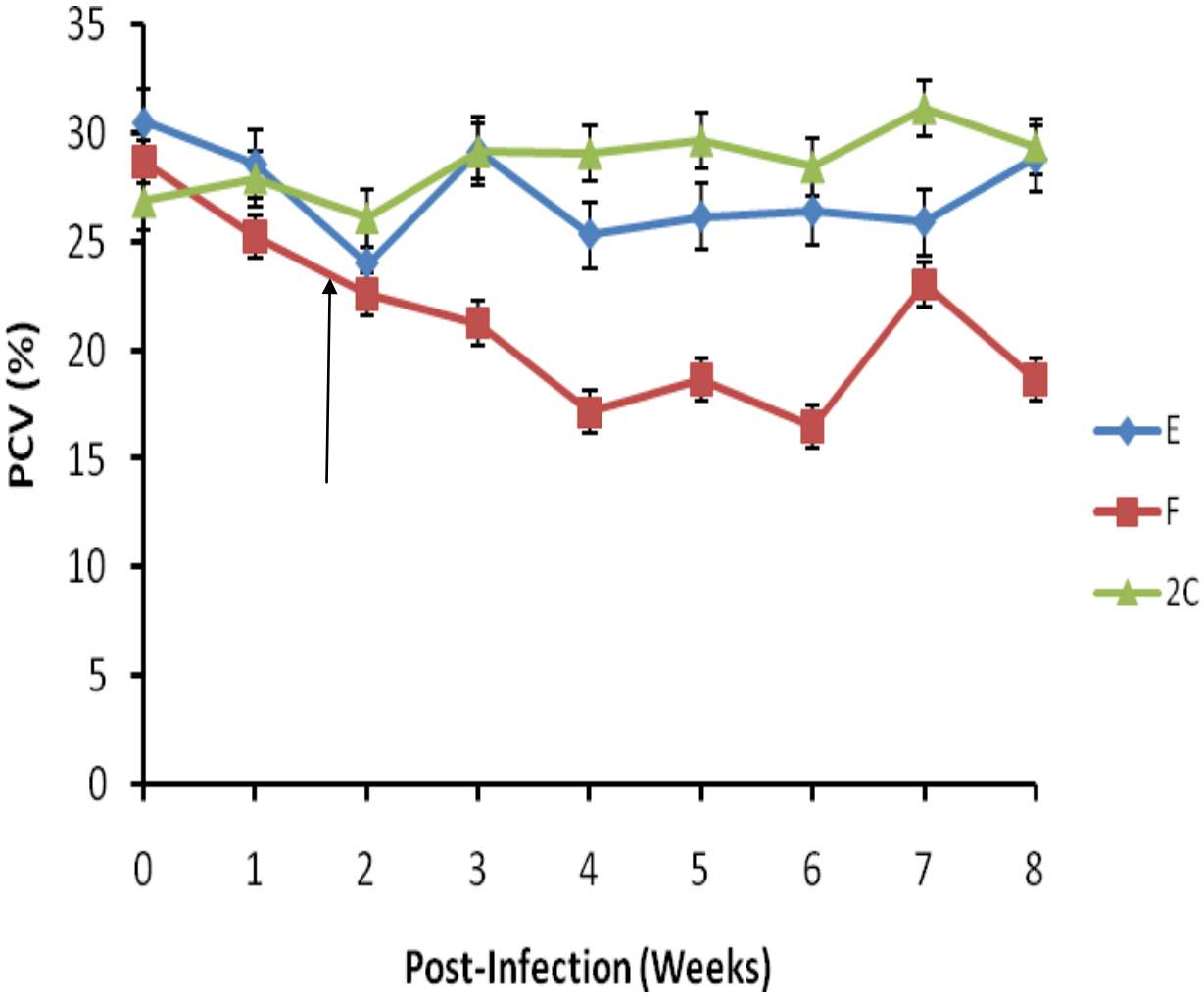


Figure 4.8: Mean ± SE weekly Packed Cell Volume of *Trypanosoma congolense*-infected and treated (E), infected, untreated (F) and uninfected control (2C) Yankasa sheep.

↑ The arrow indicates point of treatment.

4.2.7 Total plasma protein of *Trypanosoma vivax* and *Trypanosoma congolense* infected animals

There was a decrease of 0.95g/dl in the value of mean total plasma protein of the *Trypanosoma vivax*-infected untreated sheep (group B) between weeks 1 to 8 post infection. The mean values of this parameter in the infected treated sheep (group A), and uninfected control group (C) slightly decreased by 0.92 g/dl and 1.03 g/dl respectively throughout the period of the experiment. There were statistically significant differences ($P<0.05$) at weeks 1 and 3 to7 post infection between groups B, A and C (Figure 4.9).

In the *Trypanosoma congolense* infected animals, the variation in the decrease in values of plasma proteins were higher than in *Trypanosoma vivax.* There was a decrease of 0.87 g/dl in the value of mean total plasma protein from weeks 1 to 6 post infections. Conversely, increases were observed in the values of the infected-treated (E) (0.85 g/dl) and uninfected control animals (2C) (1.64 g/dl) at the end of the experiment. Statistically significant differences ($P<0.05$) exist in the values of mean total plasma protein between the groups at weeks 2-4 and 6-8 post infections (Figure 4.10).

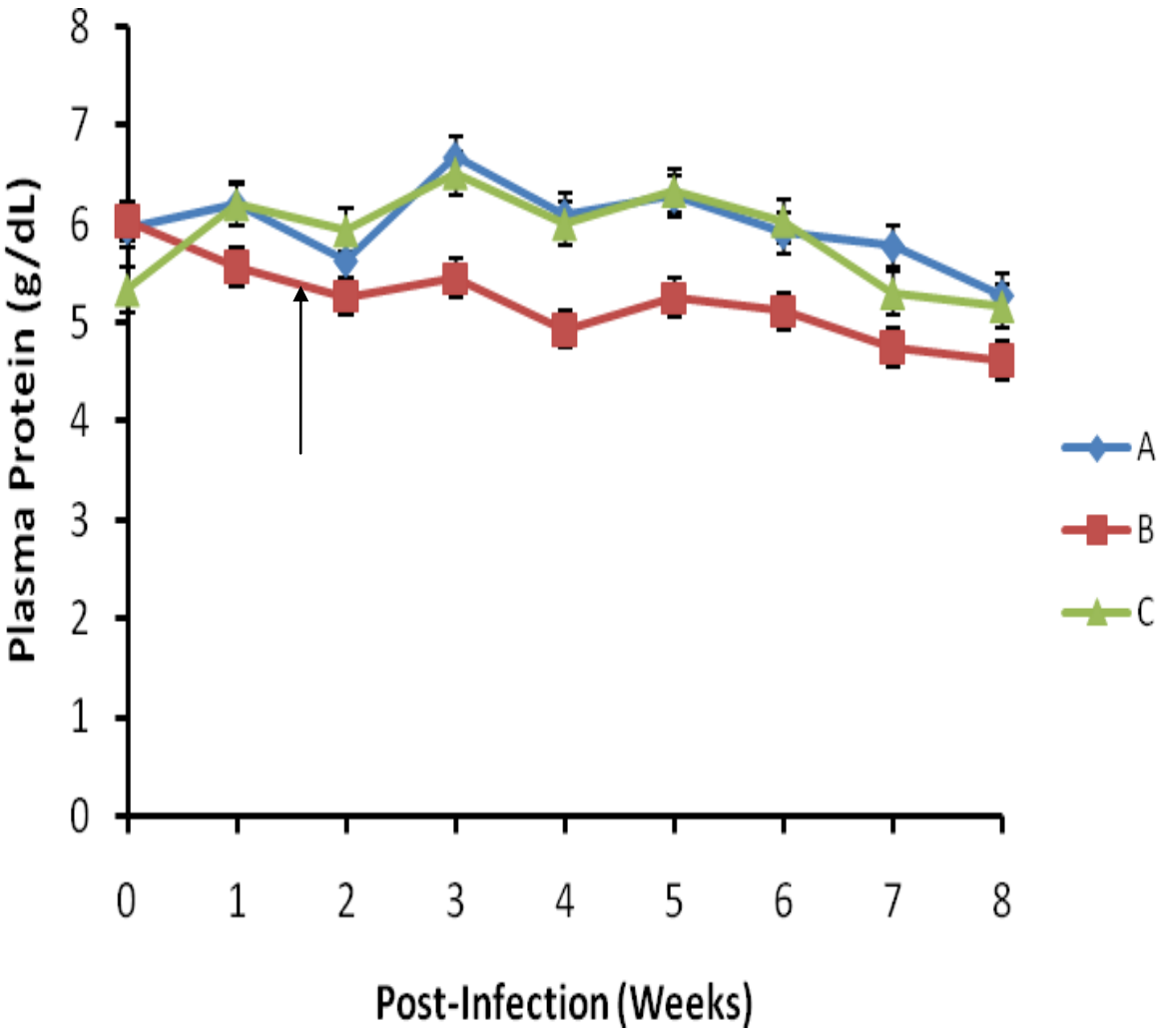


Figure 4.9: Mean ± SE weekly total plasma protein of *Trypanosoma vivax*-infected and treated (A), infected, untreated (B) and uninfected control (C) Yankassa sheep.

↑ The arrow indicates point of treatment.

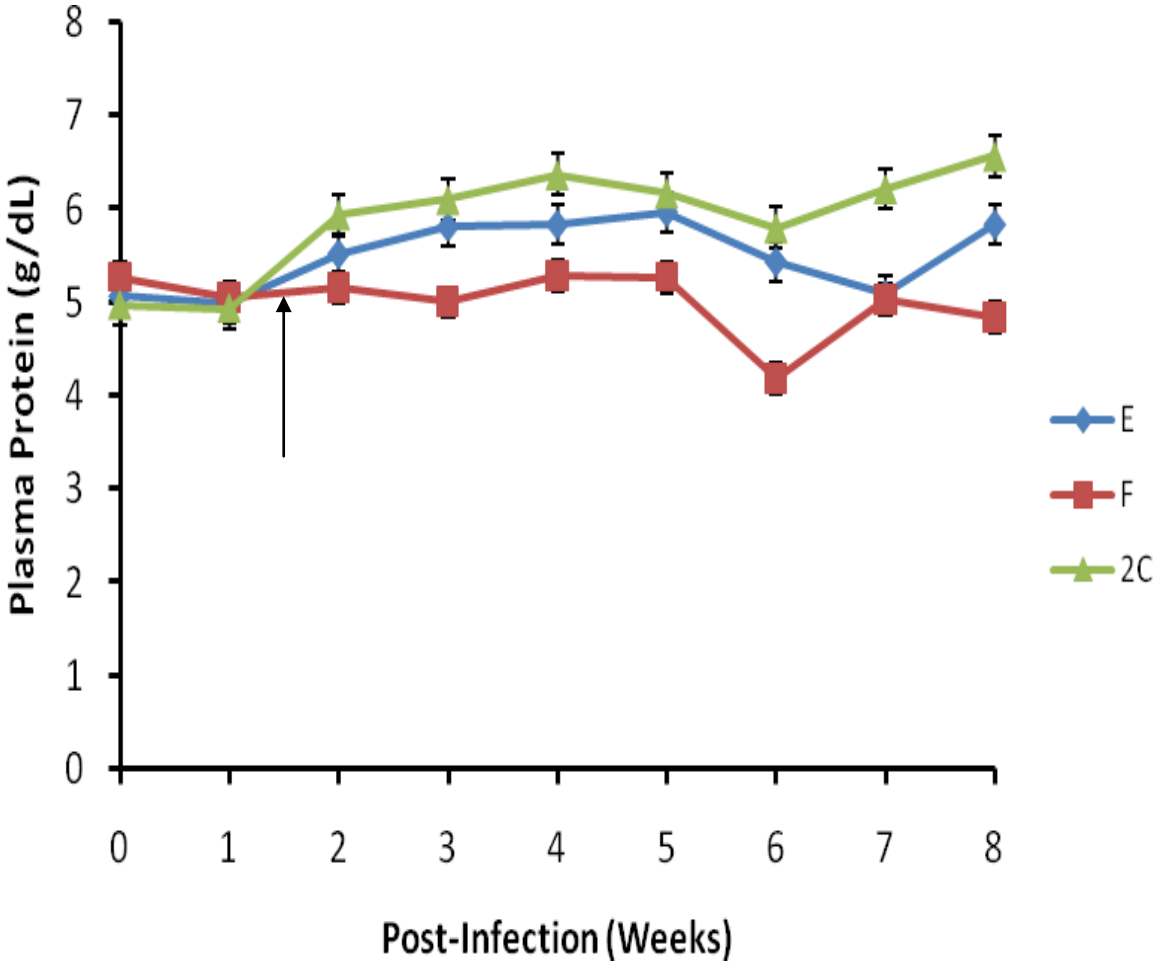


Figure 4.10: Mean ± SE weekly total plasma protein of the *Trypanosoma congolense*-infected and treated (E), infected, untreated (F) and uninfected control (2C) Yankassa sheep.

↑ The arrow indicates point of treatment.

4.2.8 Total white blood cell Count (WBC) of Yankasa sheep experimentally infected with *Trypanosoma vivax* and *Trypanosoma congolense*.

The mean WBC count of the *Trypanosoma vivax*-infected untreated (B) sheep dropped from $6.80\pm0.54\times10^9$/L at week 1 post infection to $3.62\pm0.51\times10^9$/L at week 2 pi. The value later rose to $7.23\pm0.40\times10^9$/L at the end of the experiment. However, the infected and treated animals (A), had value of $6.85\pm0.50\times10^9$/L at week 1 post infection, which later rose to $7.05\pm0.41\times10^9$/L at the end of the experiment. The mean WBC of the uninfected control group dropped slightly from week 1 post infections ($7.77\pm0.34\times10^9$/L) to $5.45\pm1.08\times10^9$/L at week 8 post infections. There were statistically significance difference ($P<0.05$) in the mean values of group B, A and C at week 2 post infection (Table 1).

In the *Trypanosoma congolense* infected animals, the WBC counts were characterized by flutuctions in values in all the groups during the experiment. There was a loss of $7.79\pm0.85\times10^9$/L in the mean WBC of the *Trypanosoma congolense*-infected untreated sheep (F) between pre-infection week and week 1 post infections. There was slight increase of $2.22\pm0.41\times10^9$/L at the end of the experiment. The infected treated animals (E) had a decreased values of $7.71\pm1.16\times10^9$/L between pre-infection week and week 1 post infections. There were statistically significance differences ($P<0.05$) in the mean values of WBC of group F animals and those of groups E and 2C at weeks 5 and 8 post infection (Table 2).

Table 1: Mean ± SE weekly WBC of *Trypanosoma vivax* infected-treated (A), infected-untreated (B) and uninfected-control (C) Yankasa sheep

Class	Pre-Infection	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
A	7.33±1.23	6.85±0.50	**5.90±0.64**[ab]	5.25±0.80	6.72±0.62	5.48±0.81	4.93±0.57	3.72±0.50	7.05±0.41
B	5.15±0.63	6.80±0.54	**3.62±0.51**[b]	4.77±0.86	6.37±0.71	5.92±0.61	5.47±0.91	5.95±1.39	7.23±0.40
C	5.68±0.16	7.77±0.34	**7.80±1.34**[a]	6.15±0.97	8.52±1.02	7.08±0.56	7.02±0.67	5.45±1.08	8.22±0.23

$\times 10^9$/L

* Mean +-SEM based on three observations.

Table 2: Mean ± SE weekly WBC of *Trypanosoma congolense* infected-treated (E), infected-untreated (F) and uninfected-control (2C) Yankasa sheep

Class	Pre-Infection	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
E	**14.33±1.31^{b}**	**6.20±0.39^{b}**	**6.62±0.15^{b}**	**5.87±0.57^{c}**	**6.17±0.67^{a}**	**7.73±0.36^{a}**	7.20±0.48	8.28±1.54	**6.72±0.32^{ab}**
F	**13.17±1.01^{b}**	**5.38±0.16^{c}**	**5.93±0.27^{c}**	**7.44±0.43^{b}**	**4.28±0.46^{b}**	**5.30±0.35^{b}**	7.65±2.21	6.88±0.76	**6.50±0.29^{b}**
2C	**18.33±0.76^{a}**	**8.02±0.37^{a}**	**8.42±0.20^{a}**	**9.31±0.36^{a}**	**7.07±0.51^{a}**	**8.67±0.16^{a}**	9.44±1.15	5.38±0.75	**8.38±^{a}**

$\times 10^{9}$/L

* Mean +-SEM based on three observations.

4.3 Observations on the Gross Pathology of Yankasa Sheep Experimentally Infected With *Trypanosoma vivax* and *Trypanosoma congolense*

The gross pathological lesions observed in *Trypanosoma vivax* infected, untreated animal were serous atrophy of pericardial fat, focal haemorrhagic area on diaphragmatic lobe of the right lung, hydropericardium (17mls), diffuse pin point haemorrhagic areas on the liver and kidney surfaces and empty gall bladder. The infected and treated revealed serous atrophy of pericardial fat, congestion of the entire right apical lobe and parts of the diaphragmatic lobe and focal areas of hyperemia.

The *Trypanosoma congolense*-infected untreated and Isometamedium treated sheep showed pale carcasses and slight congestion of the liver. There were no pathological changes seen in the uninfected controls *Trypanosoma vivax* and *Trypanosoma congolense* groups.

4.3.1 Histopathology of Yankasa sheep experimentally infected with *Trypanosoma vivax* and *Trypanosoma congolense*

Histological section of the brain of *Trypanosoma vivax* infected untreated Yankasa sheep (group B) revealed areas of neuronal degeneration (plate xix), the spleen showed areas of haemosiderosis in the red and white pulps (plate xx), the Kidney revealed necrotic renal tubules and glomerular degeneration (plate xxi). The Lung was congested with areas of haemorrhages and thickened alveoli membranes (pneumonia) (plate xxii). There was atrophy and degeneration of the skeletal muscles with absence of sperm cells and normal epithelium of the epididymis.

The Trypanosoma vivax infected-treated animal (group A) lungs revealed thickened alveolar membranes (Pneumonia) and uterine necrosis in the uterus.There were focal areas of tubular and glomerular necrosis in the kidneys. The spleen revealed haemosiderosis of the red and white pulps.

Histological sections of the *Trypanosoma congolense* infected untreated sheep (group F)

adrenal glands and pancreas revealed areas of necrosis in the glands and pancreatic cells. The spleen showed areas of haemosiderosis and pulmonary congestion of the lungs. There was infiltrative myositis in the skeletal muscles and congestion of the liver. Most of the organs (Uterus, Kidney, and Skeletal muscle) of the *Trypanosoma congolense* infected-treated with Isometamedium chloride sheep (E) revealed areas of necrosis and there were thickened alveolar membranes of the lungs. The spleen revealed haemosiderosis of the red and white pulps. Histopathology of all the organs from uninfected control sheep (groups C and 2C) revealed normal cells and tissues. The lesions are shown on plates III to XXVIII.

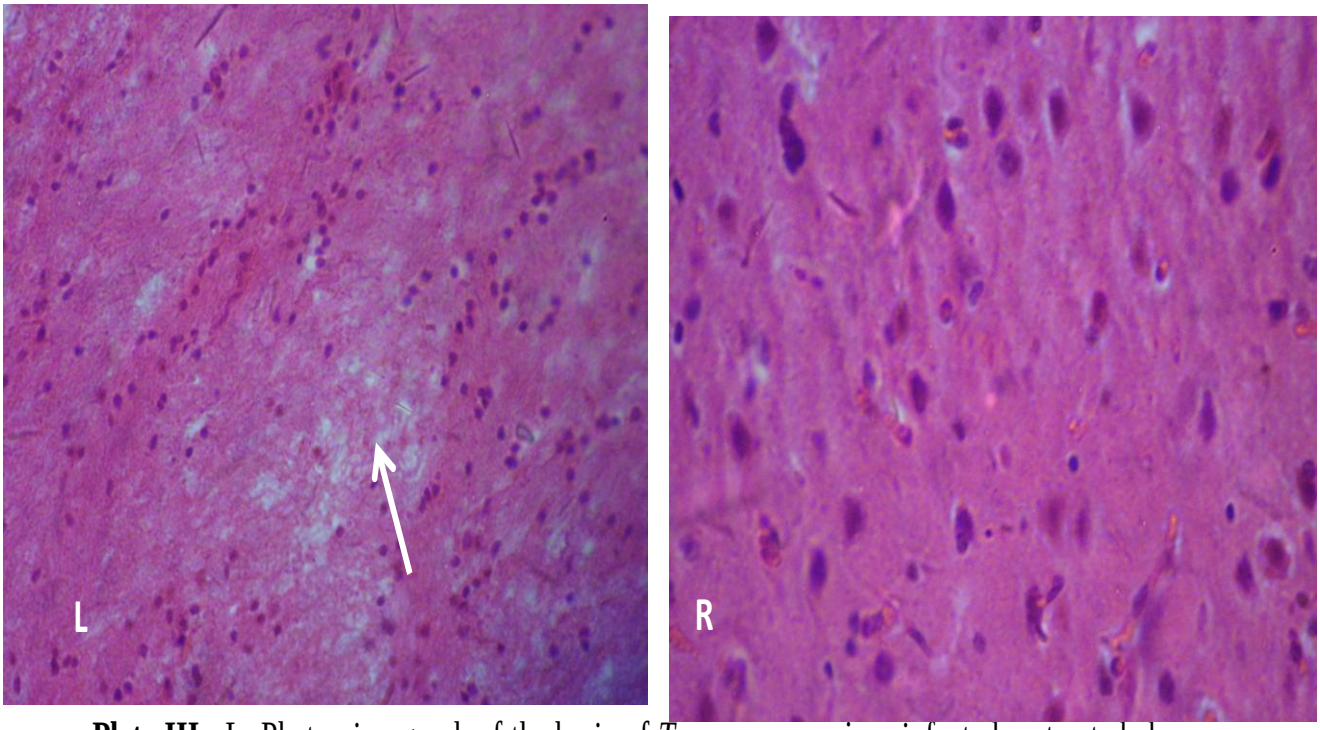


Plate III : L- Photomicrograph of the brain of *Trypanosoma vivax*-infected, untreated sheep showing areas of neuronal degeneration (arrow); R- showing brain of uninfected control sheep with normal brain (H & E,× 400).

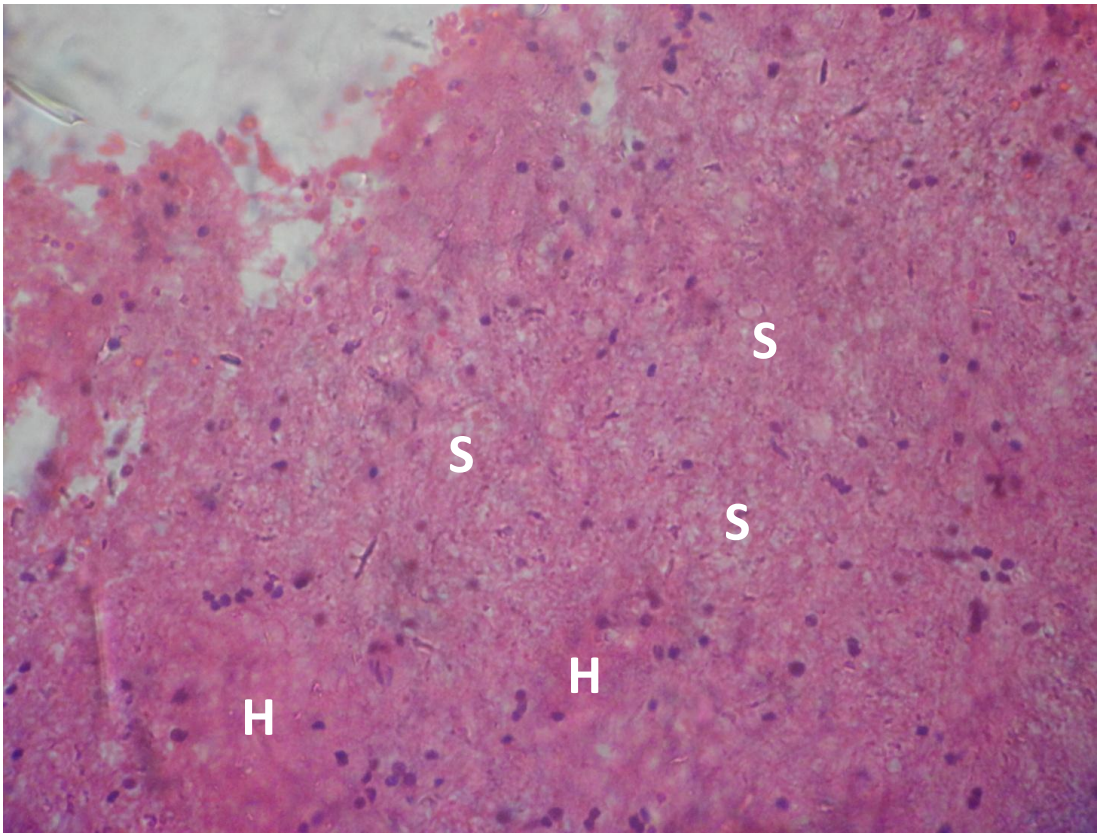


Plate IV: Photomicrograph of the brain of *T. congolense*-infected, Isometamedium chloride- treated sheep, showing areas of haemorrhages (H) and neuronal degeneration (S) (H & E ×400).

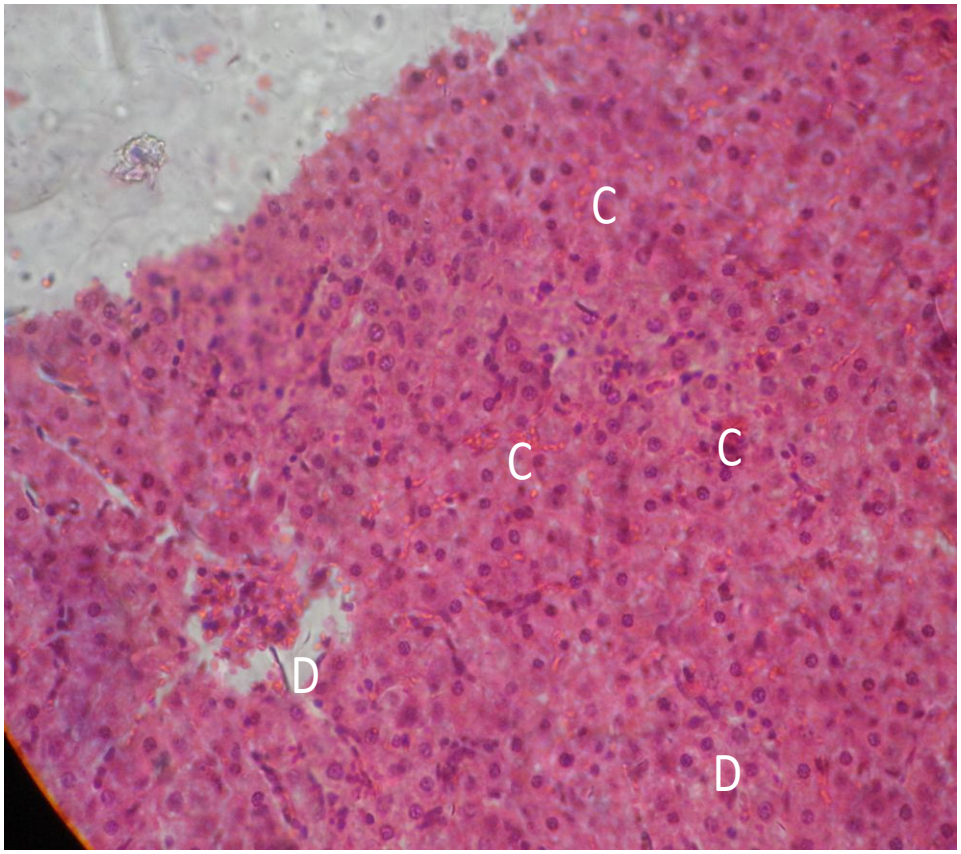


Plate V: Photomicrograph of the liver of *T. congolense*-infected, untreated sheep showing areas of congestion of sinuses (C) and hepatic degeneration (D), (H & E, × 400).

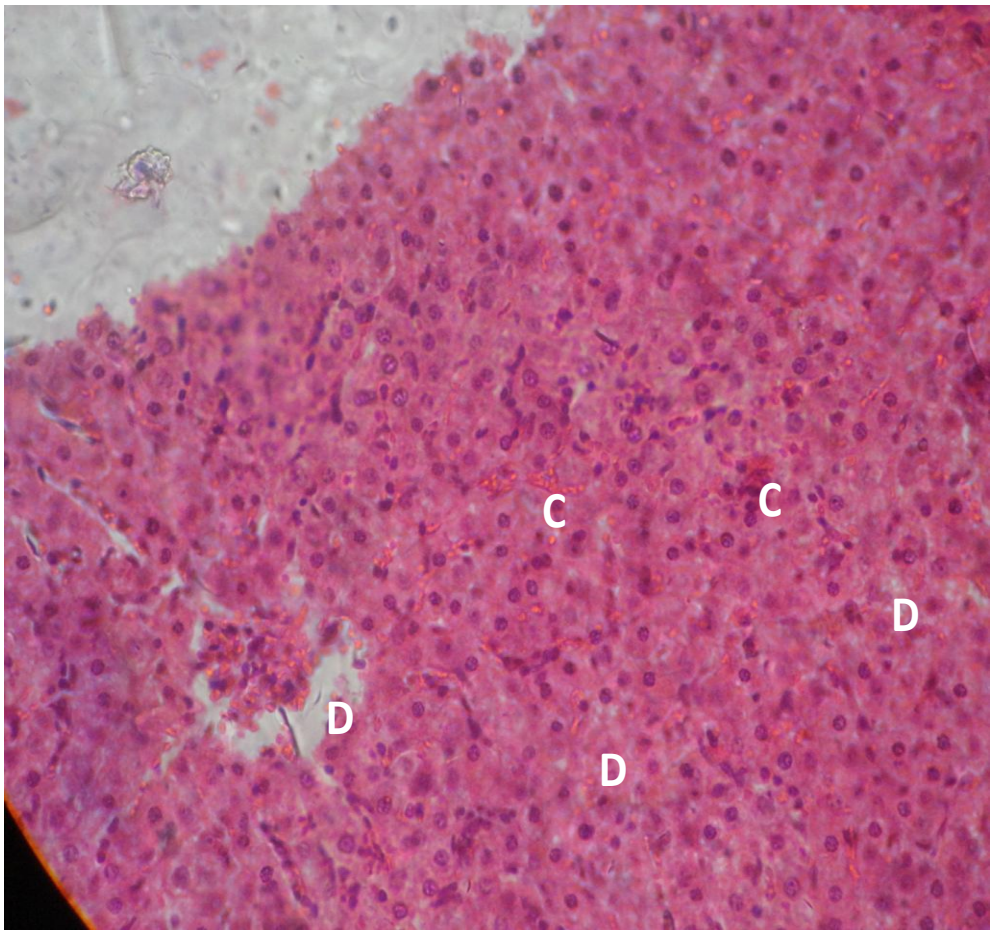


Plate VI: Photomicrograph of the liver of *T. congolense*-infected, isometamidium chloride-treated sheep showing congested sinuses (C) and hepatic degeneration (D), (H & E, × 400).

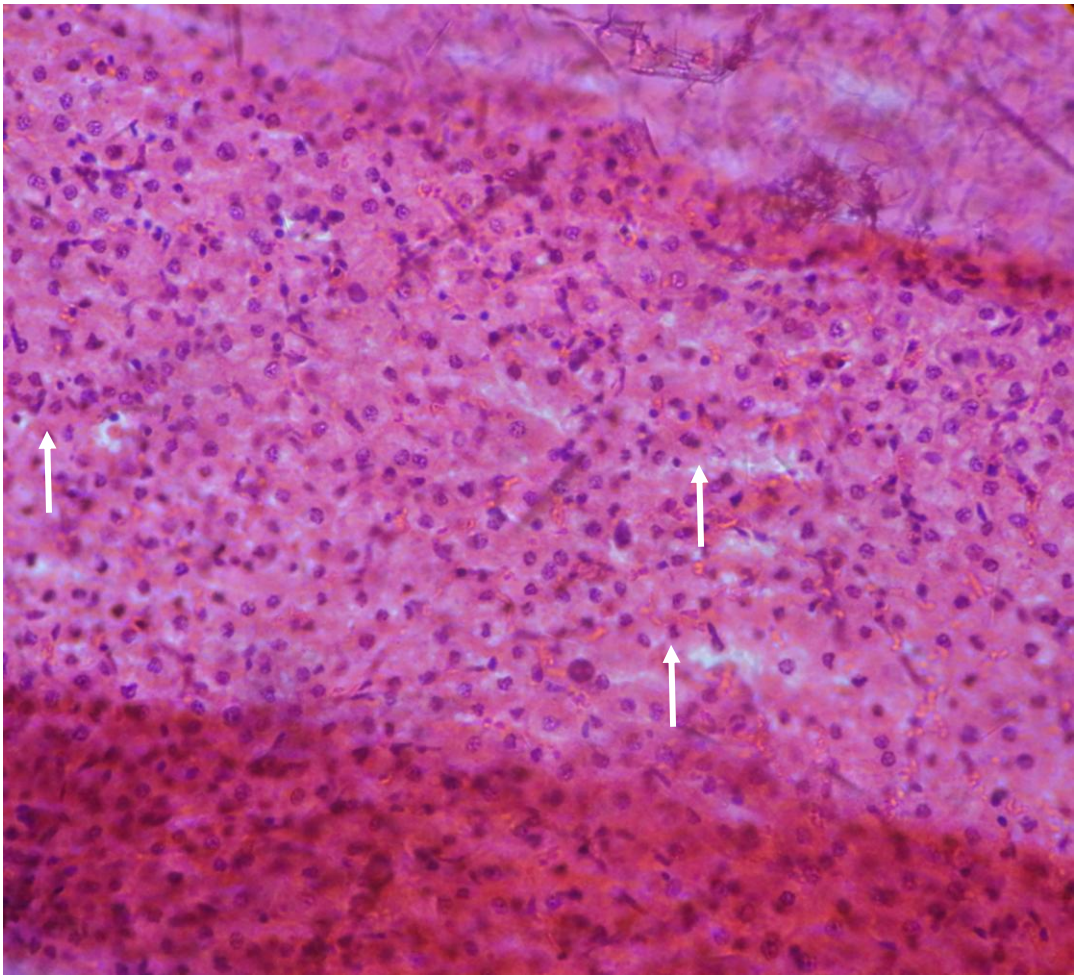


Plate VII: Photomicrograph of the liver of *T. congolense* infected, Isometamedium chloride-treated sheep, showing mild hepatic degeneration (arrow), (H & E, ×200)

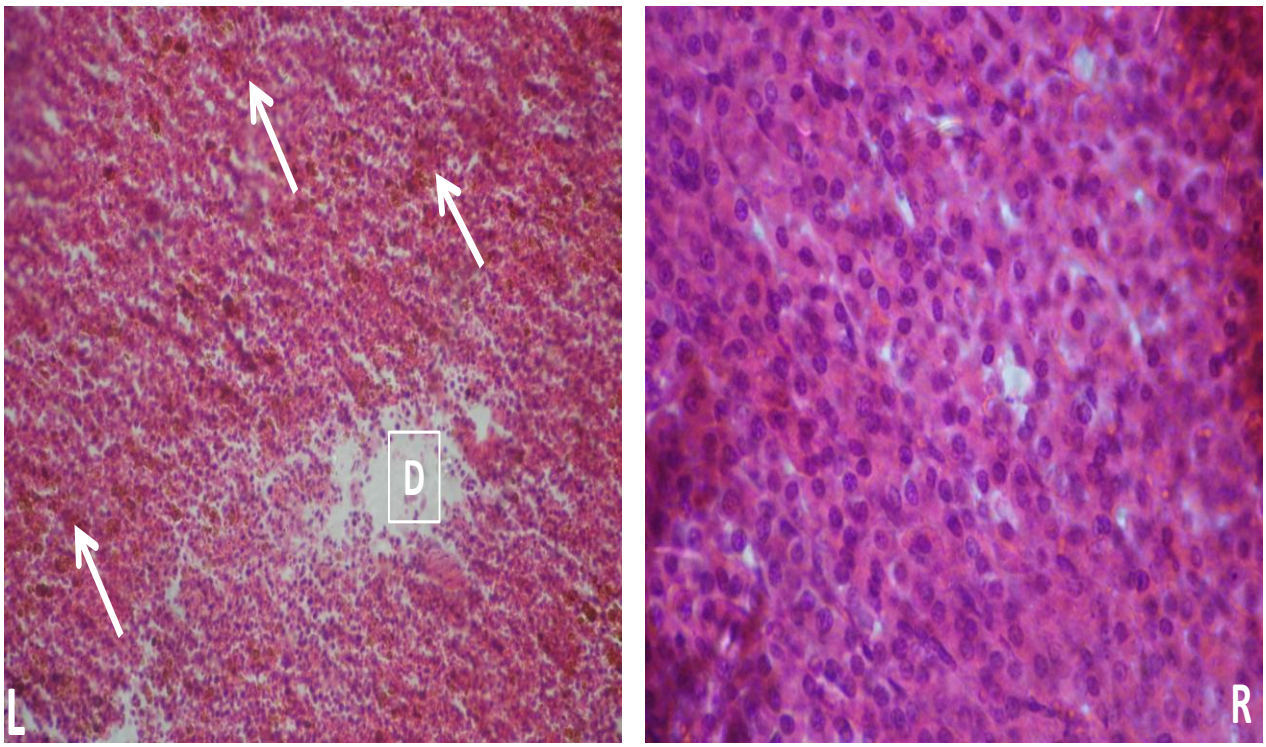


Plate VIII: L- Photomicrograph of the spleen of *T. vivax*-infected, untreated sheep showing areas of haemosiderin (arrows) and depleted follicles (D); R- showing a normal spleen from control sheep (H & E, × 400).

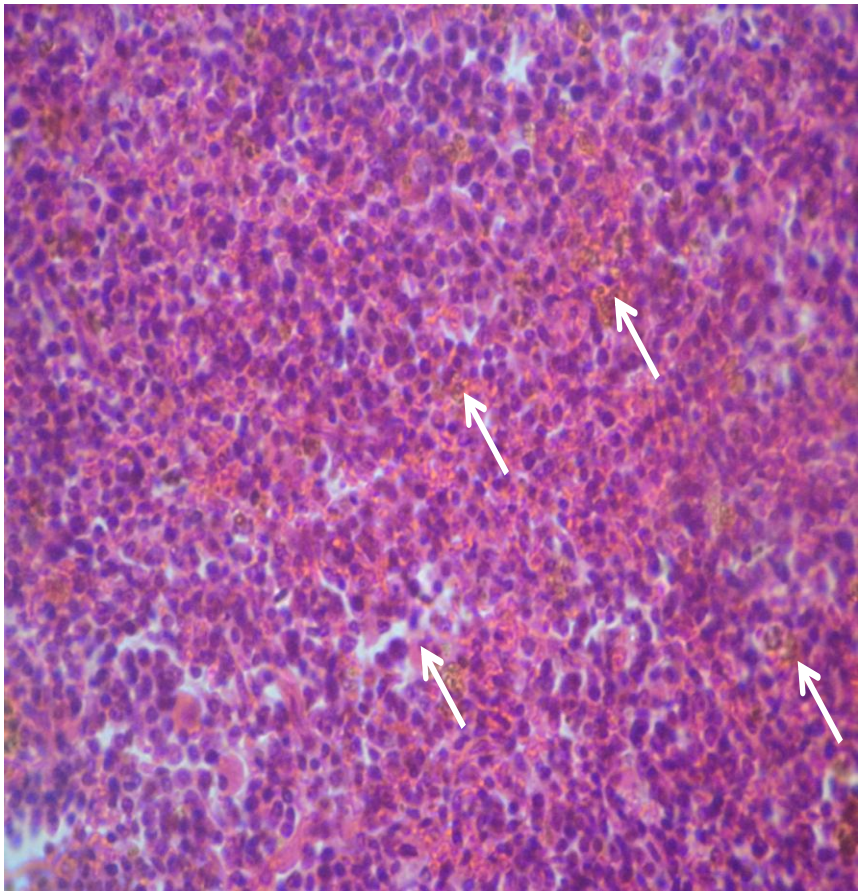


Plate IX: Photomicrograph of the spleen of *T. congolense*-infected, untreated sheep showing areas of haemosiderin (arrow), (H & E, × 400).

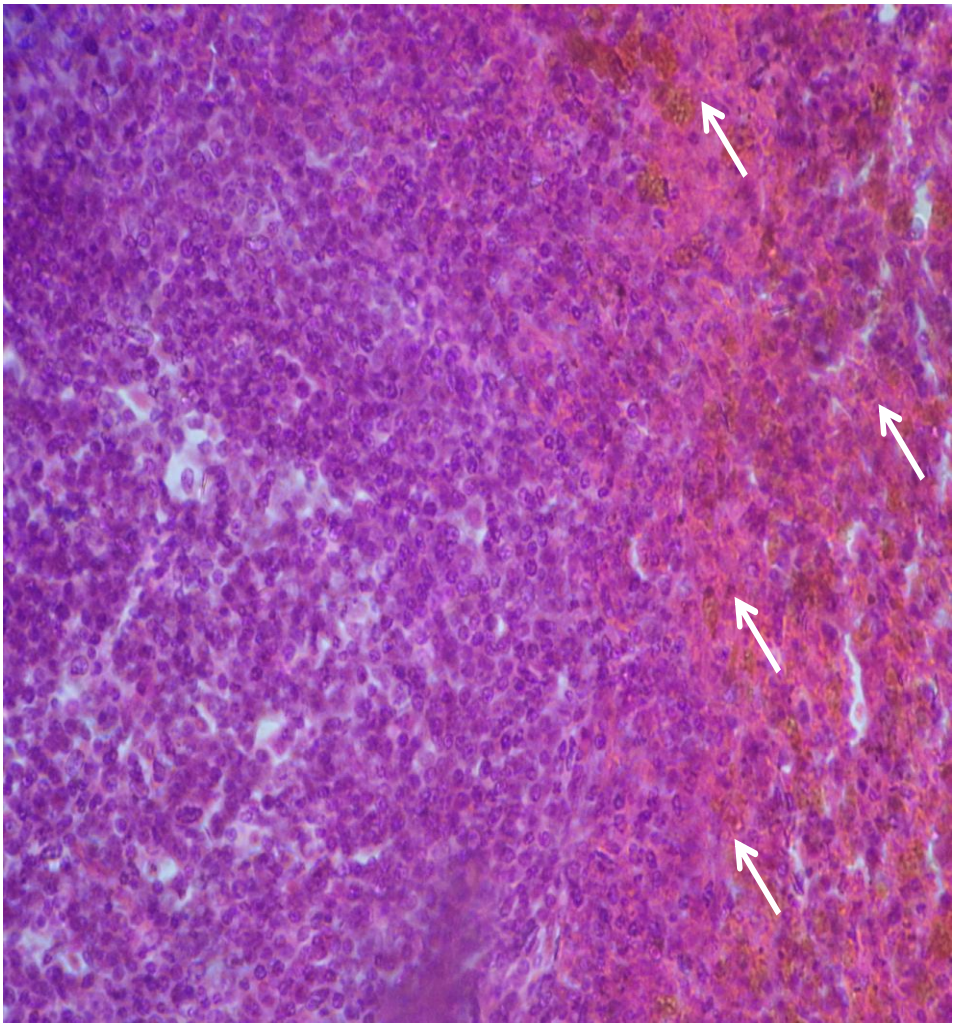


Plate X: Photomicrograph of the spleen of *T. vivax*-infected, diaminazene aceturate-treated sheep showing areas of haemosiderin (arrow) in the white and red pulps. (H & E, × 400).

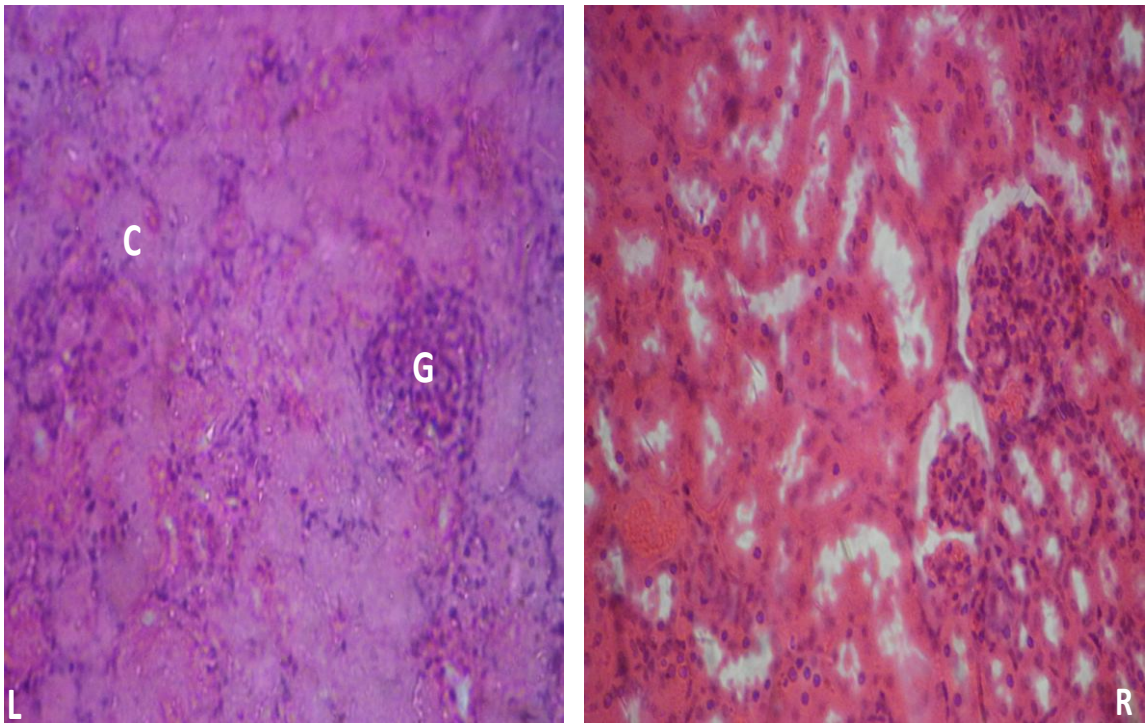


Plate XI : L- Photomicrograph of the Kidney of *T. vivax*-infected and untreated sheep, showing foci areas of necrosis of tubules (C) and glumeruli (G); R- showing apparently normal kidney from the kidney of control sheep (H & E, × 400).

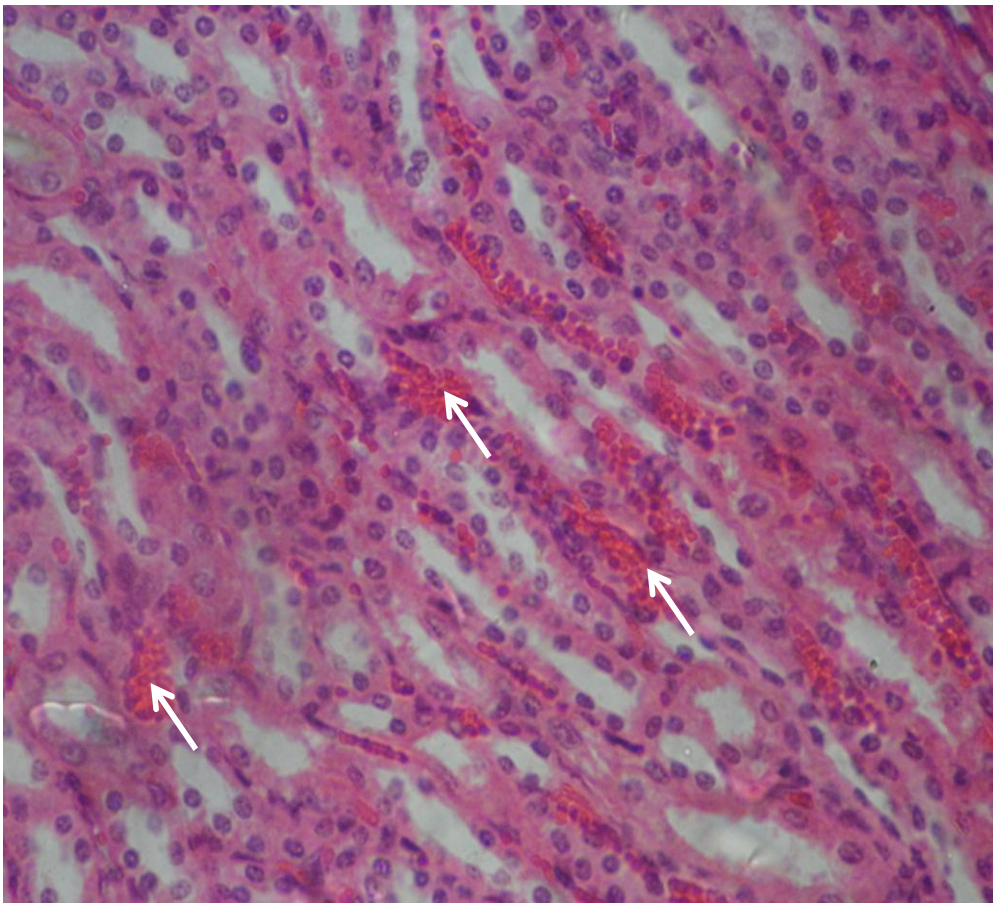


Plate XII: Photomicrograph of the kidney of *T. congolense*-infected and Isometamedium chloride treated sheep, showing congested vessels (arrow) (H & E, × 400)

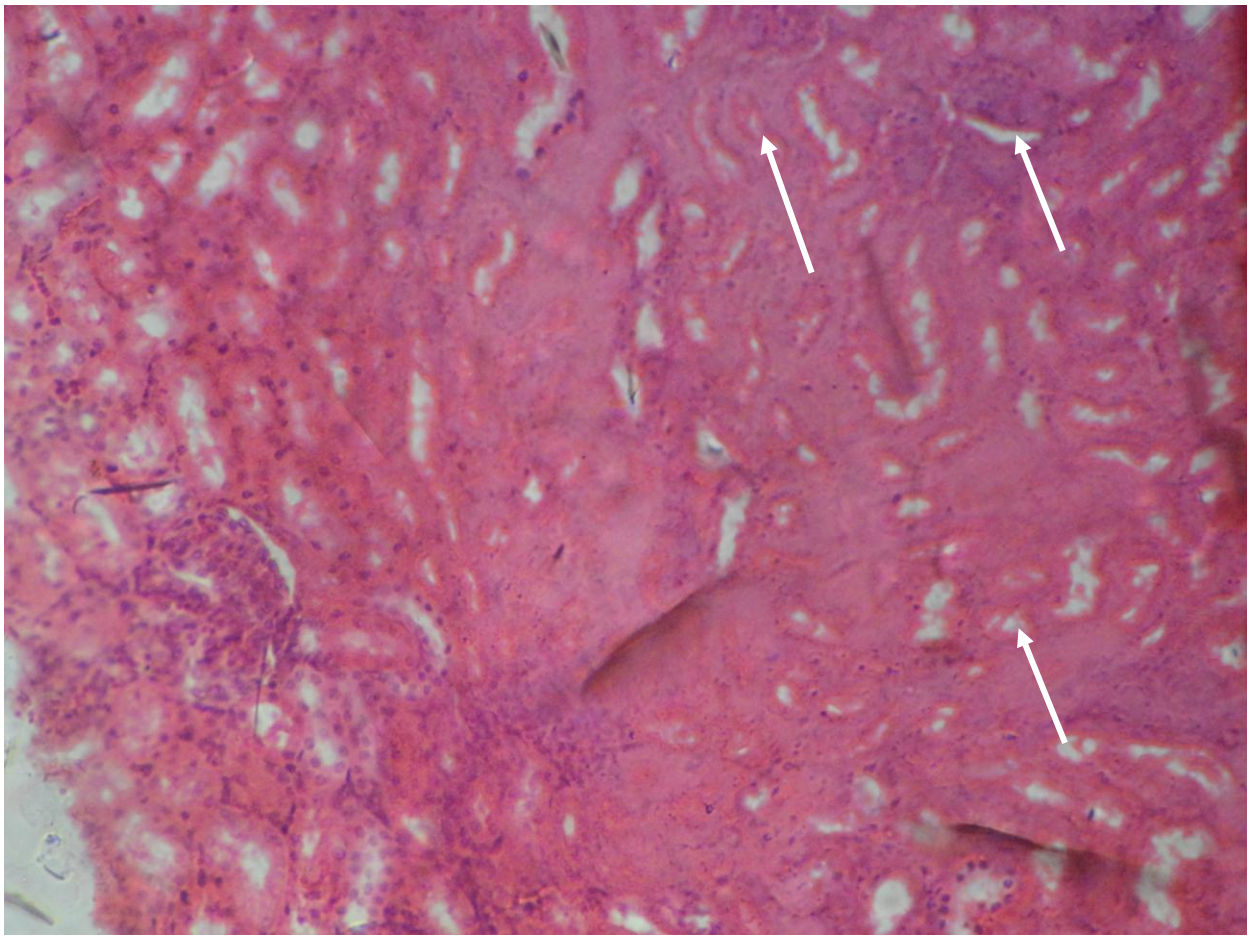


Plate XIII: Photomicrograph of the kidney of *T. vivax*- infected, diaminazene aceturate- treated sheep, showing focal areas of necrosis of the tubules and glomeruli (arrows), (H & E, × 400)

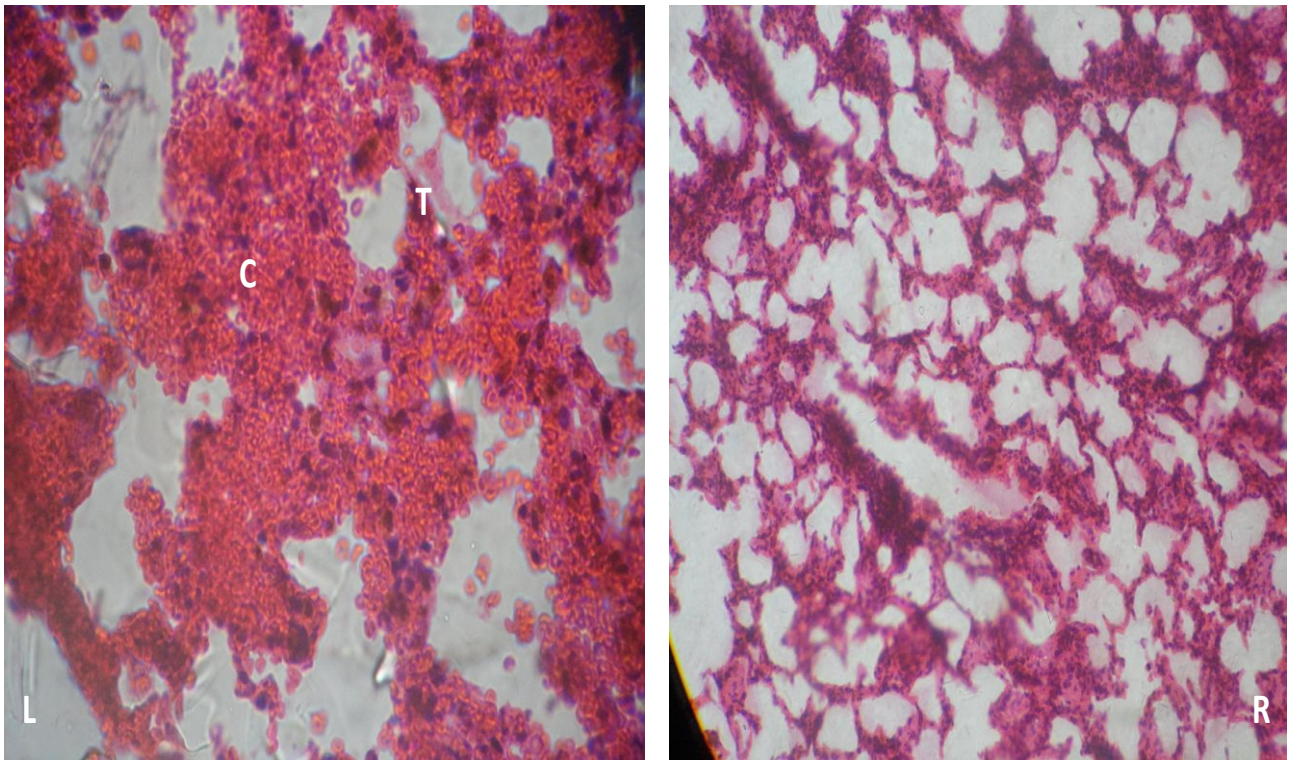


Plate XIV : L- Photomicrograph of the Lung of sheep infected with *T. vivax* for 8 weeks, showing congestion with haemorrhages (C) and thickened alveoli membranes (T); R-showing Photomicrograph of the lung of sheep from the uninfected control sheep. (H & E, ×400)

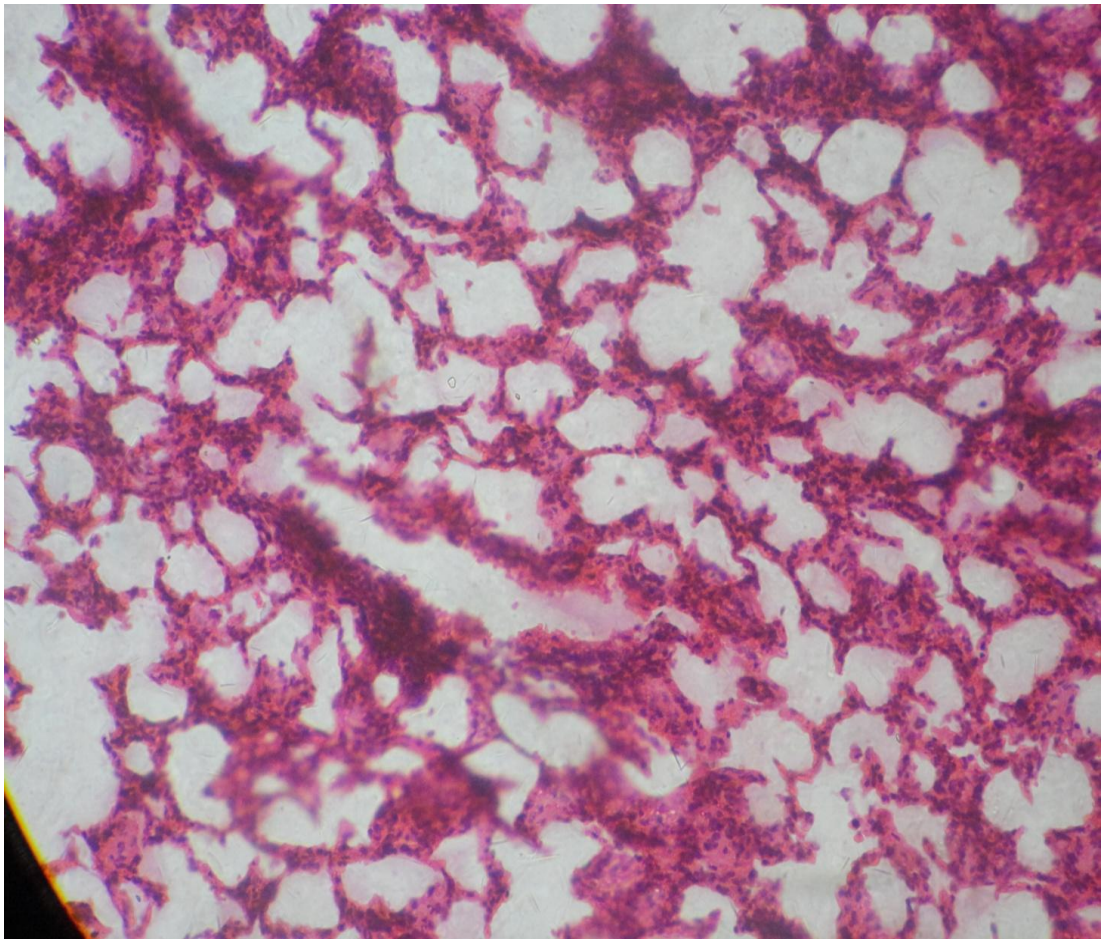


Plate XV : Photomicrograph of the lung of *T. congolense*-infected , Isometamidium chloride treated-sheep, showing mild pneumonia (H & E, × 200)

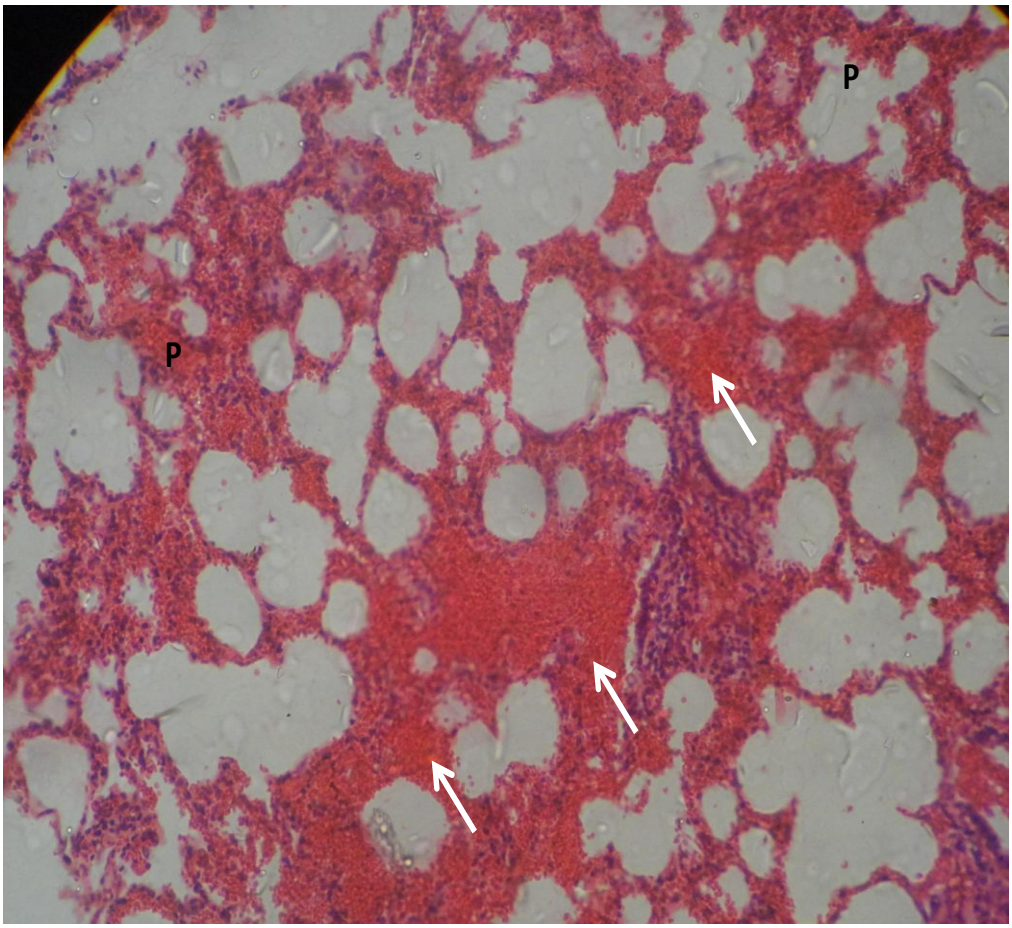


Plate XVI: Photomicrograph of the lung of *T. congolense* infected and untreated sheep showing pulmonary congestion (arrows), (H & E, × 400).

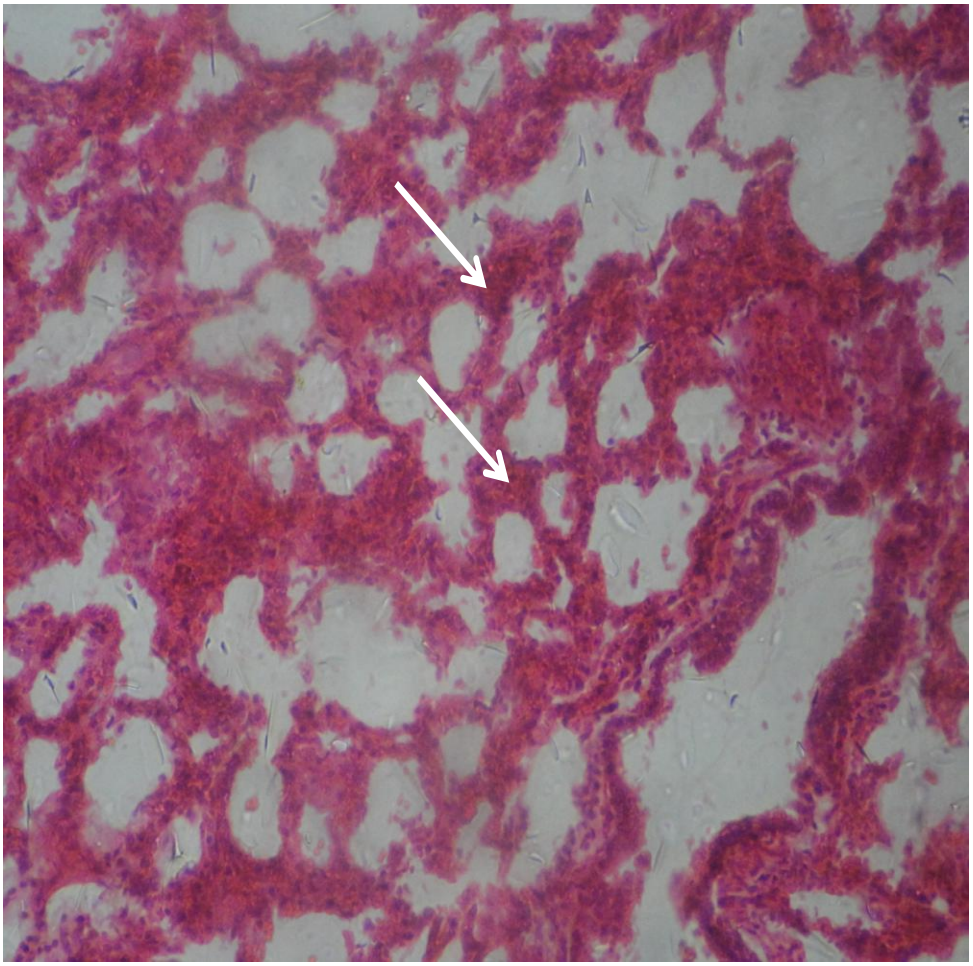


Plate XVII: Photomicrograph of the lung of *T. vivax* –infected, diaminazene aceturate treated sheep, showing thickened alveolar membranes (arrow), (H & E, 400).

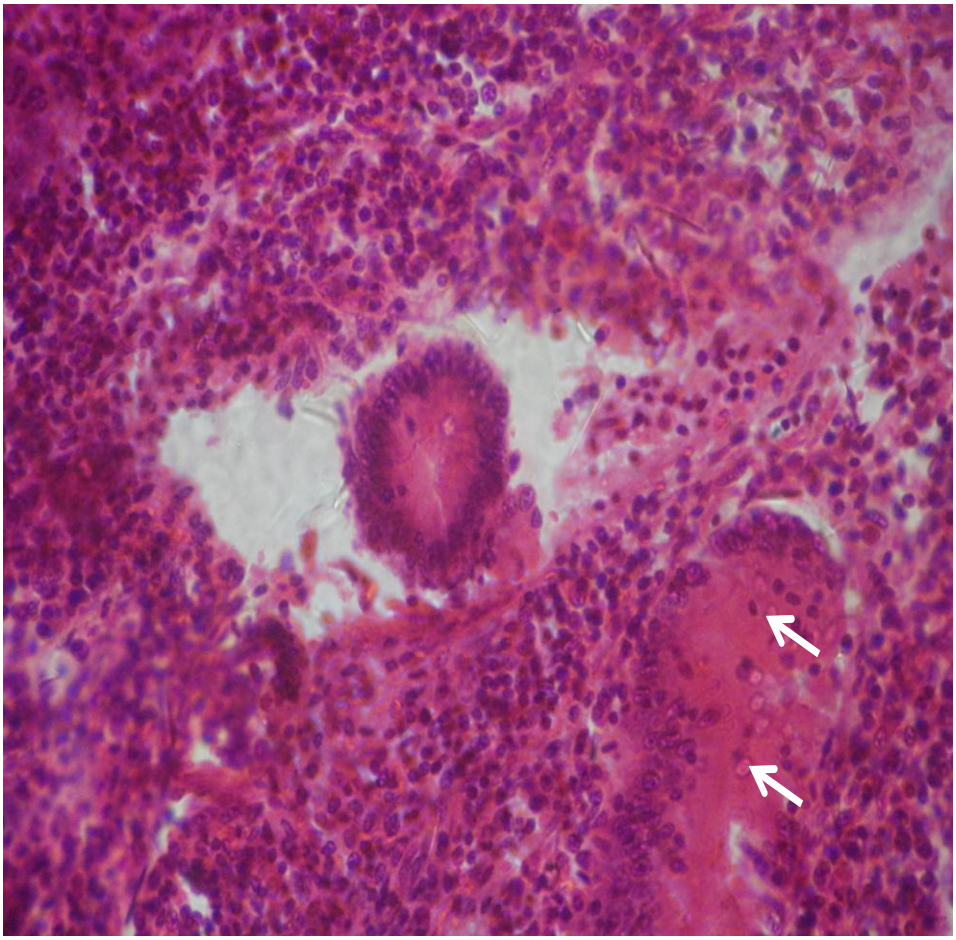


Plate XVIII : Photomicrograph of the pancreas of *T. congolense*-infected and untreated sheep, showing necrosis of the pancreatic cells (arrow) (H & E, × 400)

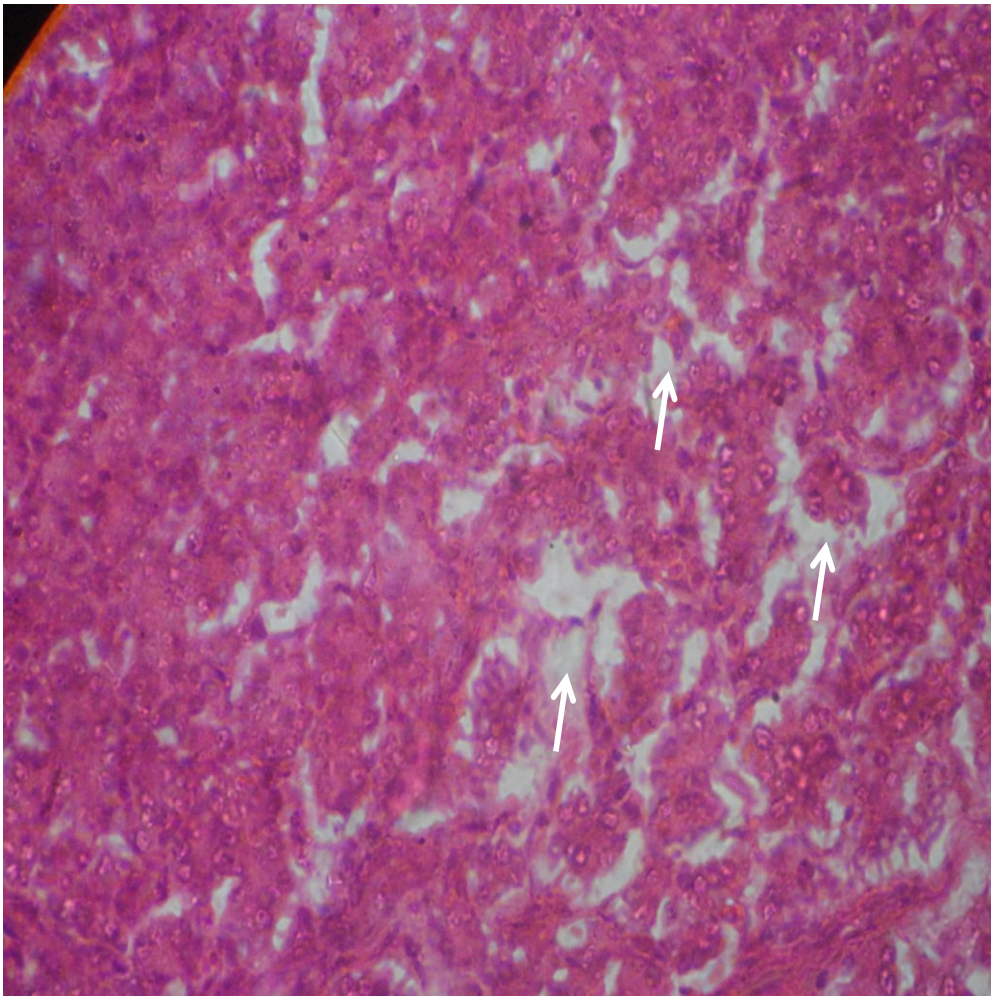


Plate XIX: Photomicrograph of the pancreas of *T. congolense* -infected, isometamedium chloride-treated sheep, showing mild pancreatitis (arrow) (H & E, × 400).

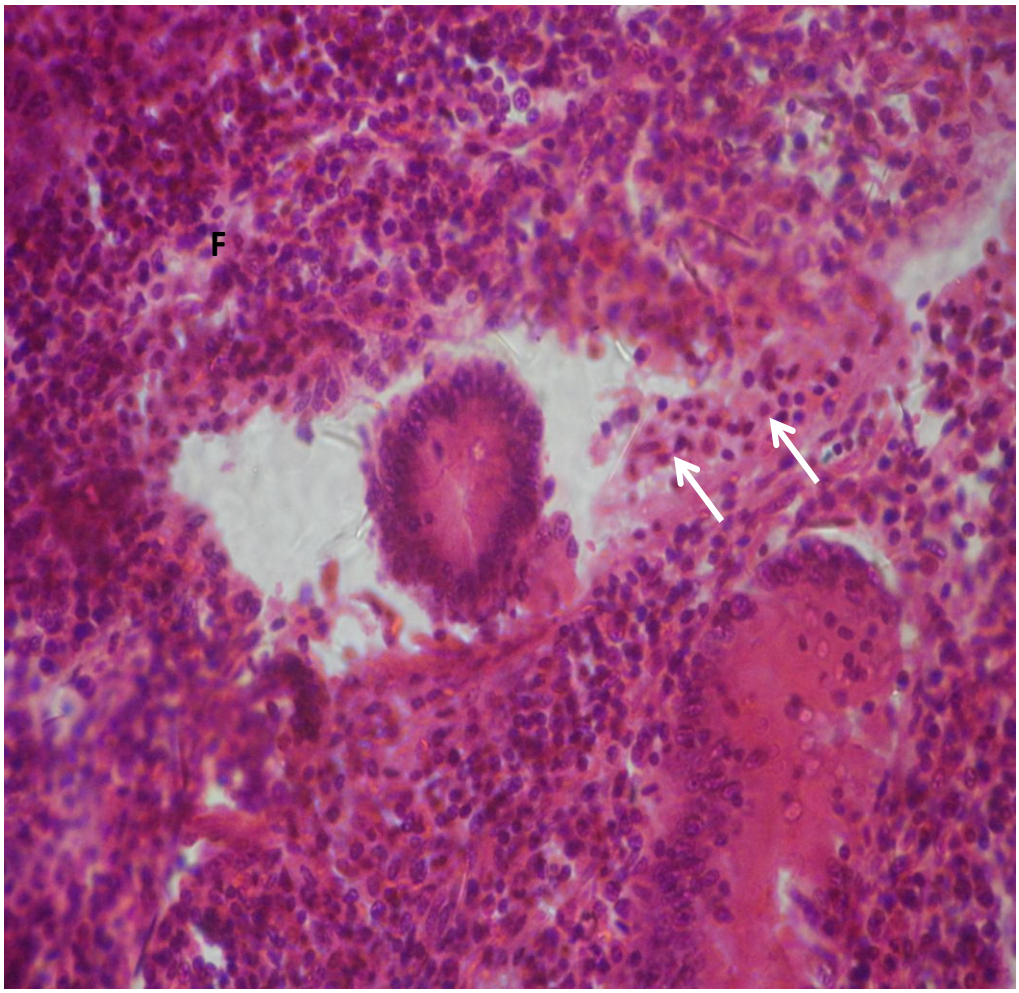


Plate XX: Photomicrograph of the pancreas of *T. congolense*-infected and untreated sheep, showing infiltration of the inflammatory cells (arrow), (H & E, × 400)

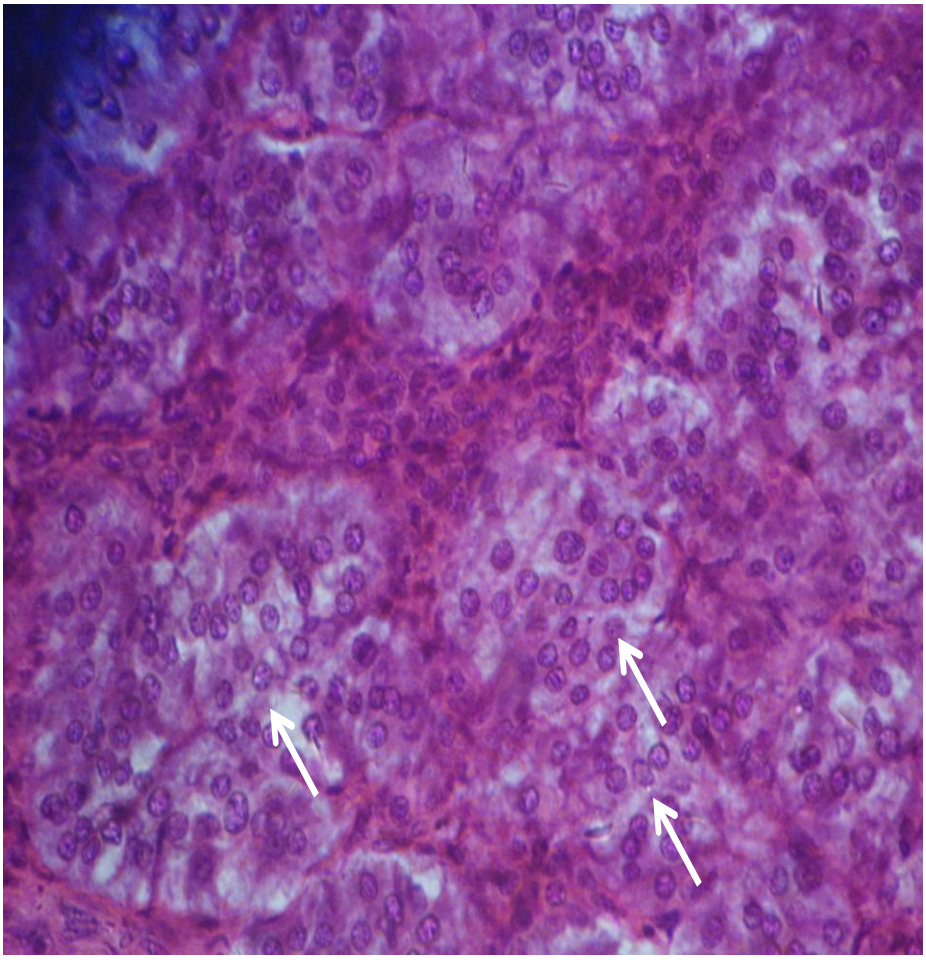


Plate XXI : Photomicrograph of the adrenal gland of *T congolense*-infected, isometamidium chloride-treated sheep, showing mild areas of necrosis (N), (H & E, × 400).

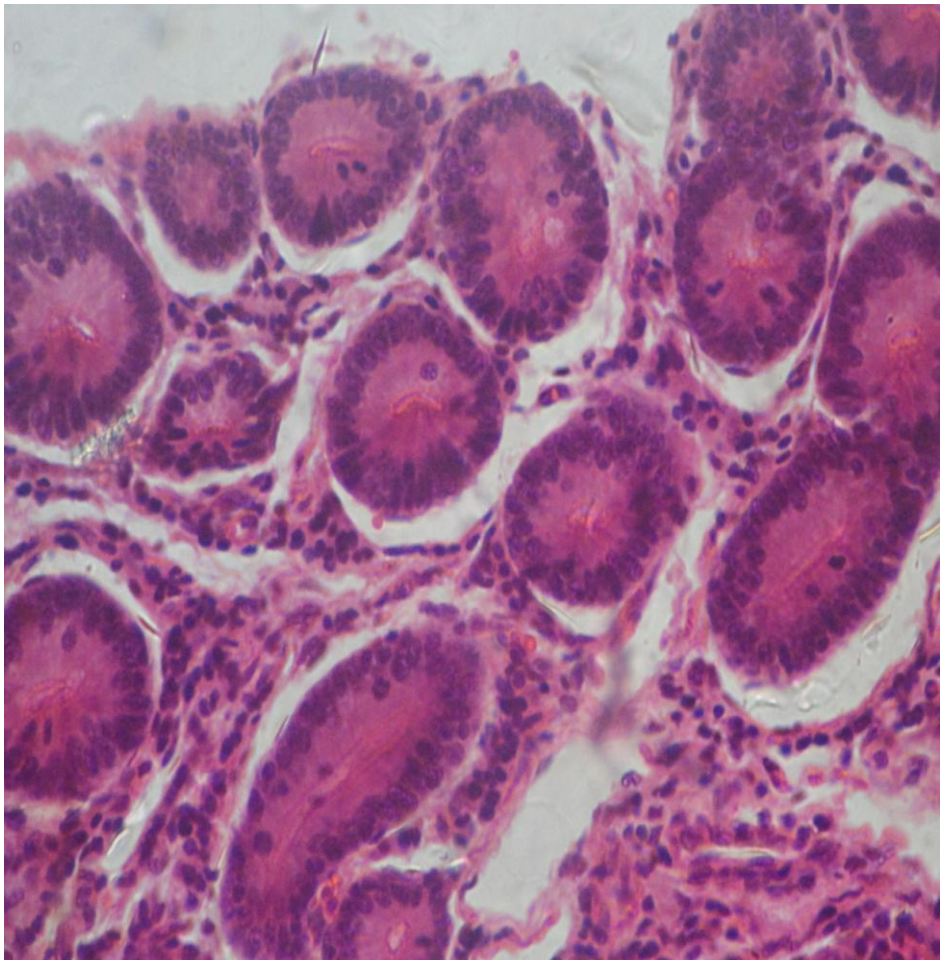


Plate XXII : Photomicrograph of the adrenal gland of *T congolense*-infected, untreated sheep, showing normal cells (H & E,× 400).

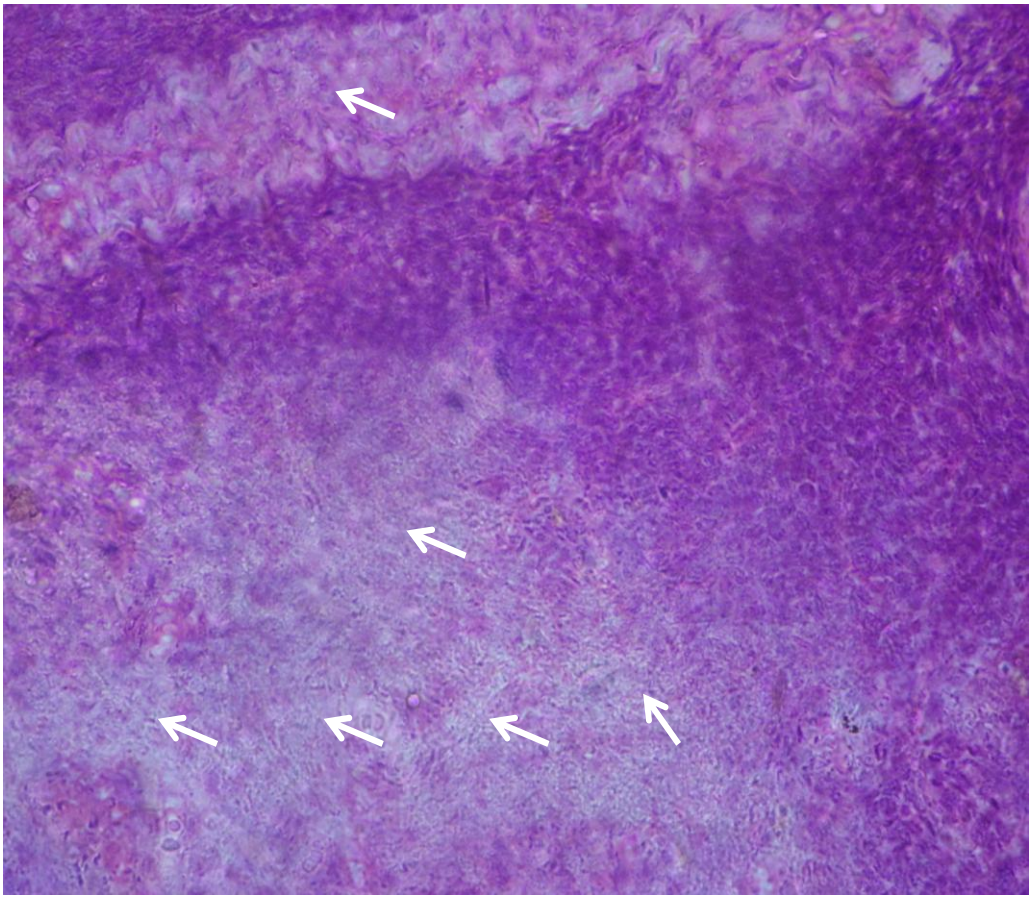


Plate XXIII: Photomicrograph of the uterus of *T. vivax*-infected, diminazene aceturate-treated sheep, showing necrosis of the uterus (arrow). (H & E, ×400)

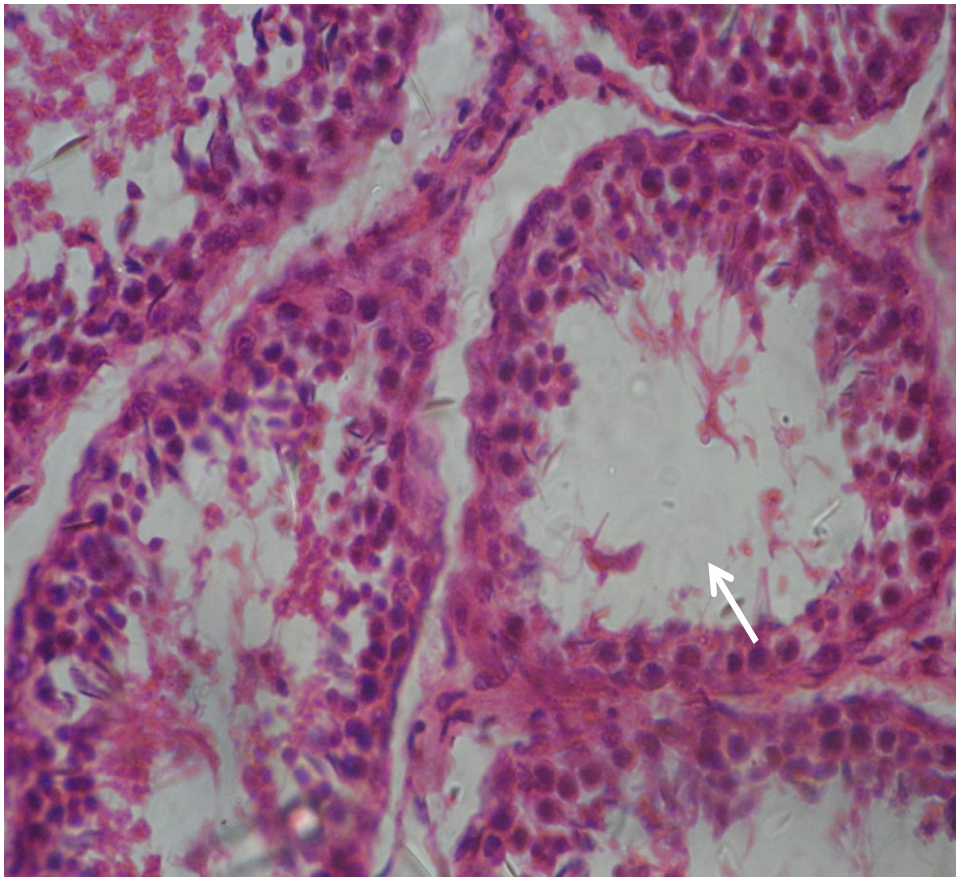


Plate XXIV: Photomicrograph of the testis of *T. congolense*-infected , Isometamedium chloride treated-sheep, showing seminuferous tubules (arrow), with low spermatogenic activity (H & E,× 400).

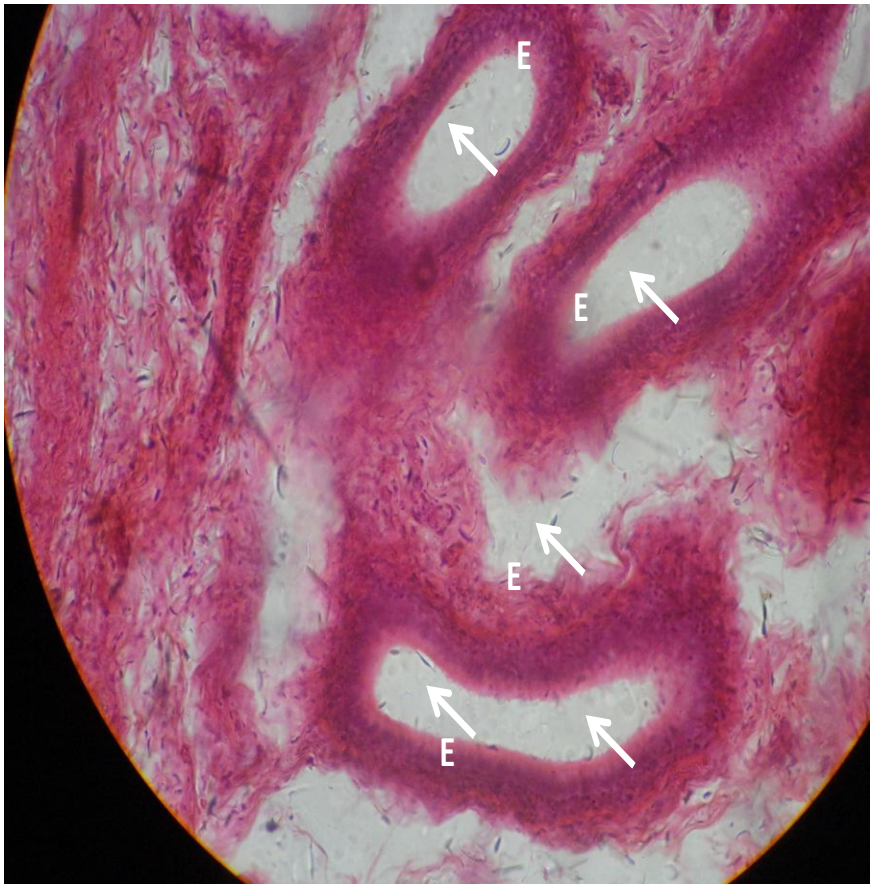


Plate XXV: Photomicrograph of the epididymis of *T. vivax*-infected , untreated sheep devoid of sperm cells (arrow), (×200)

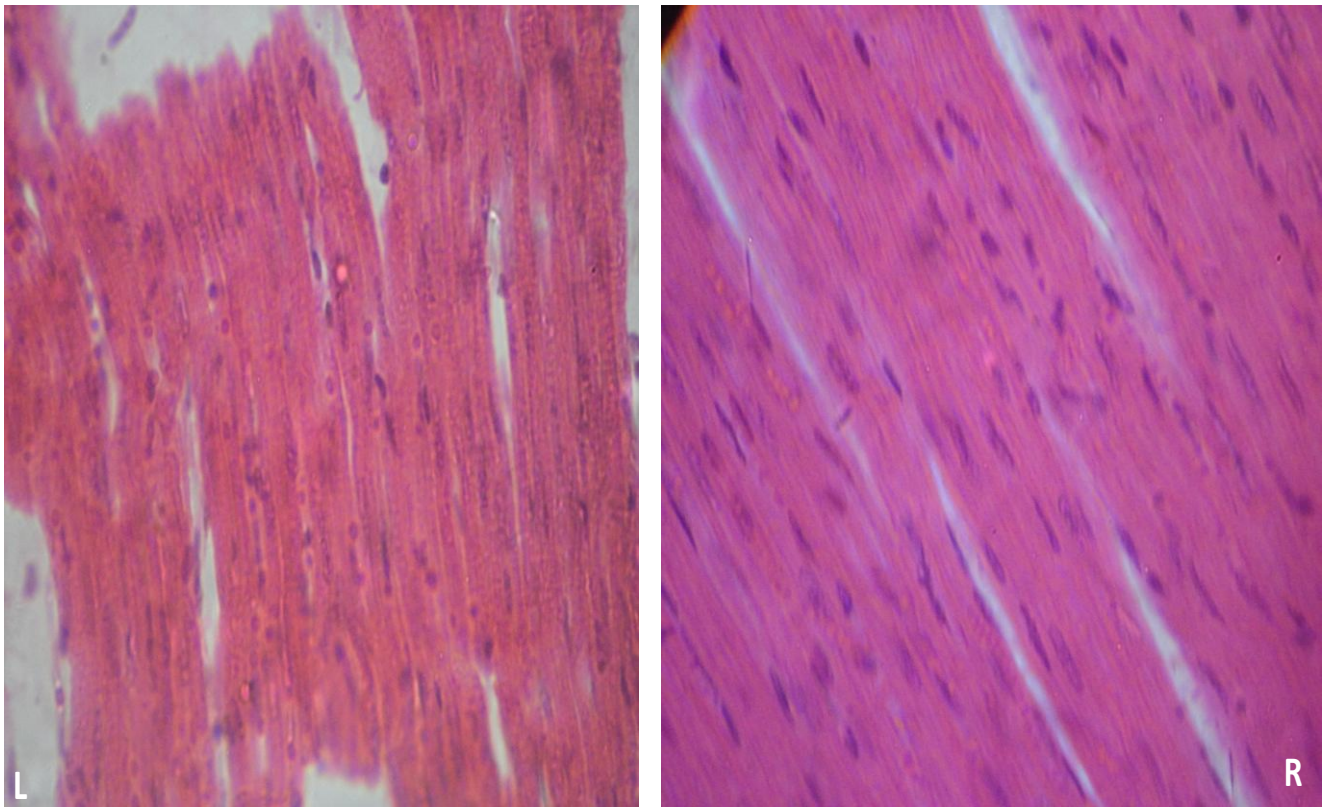


Plate XXVI : L- Photomicrograph of the skeletal muscle of *T. vivax*-infected, untreated sheep showing muscle atrophy and degeneration; R- Photomicrograph of the skeletal muscle from uninfected control sheep (H & E, ×400)

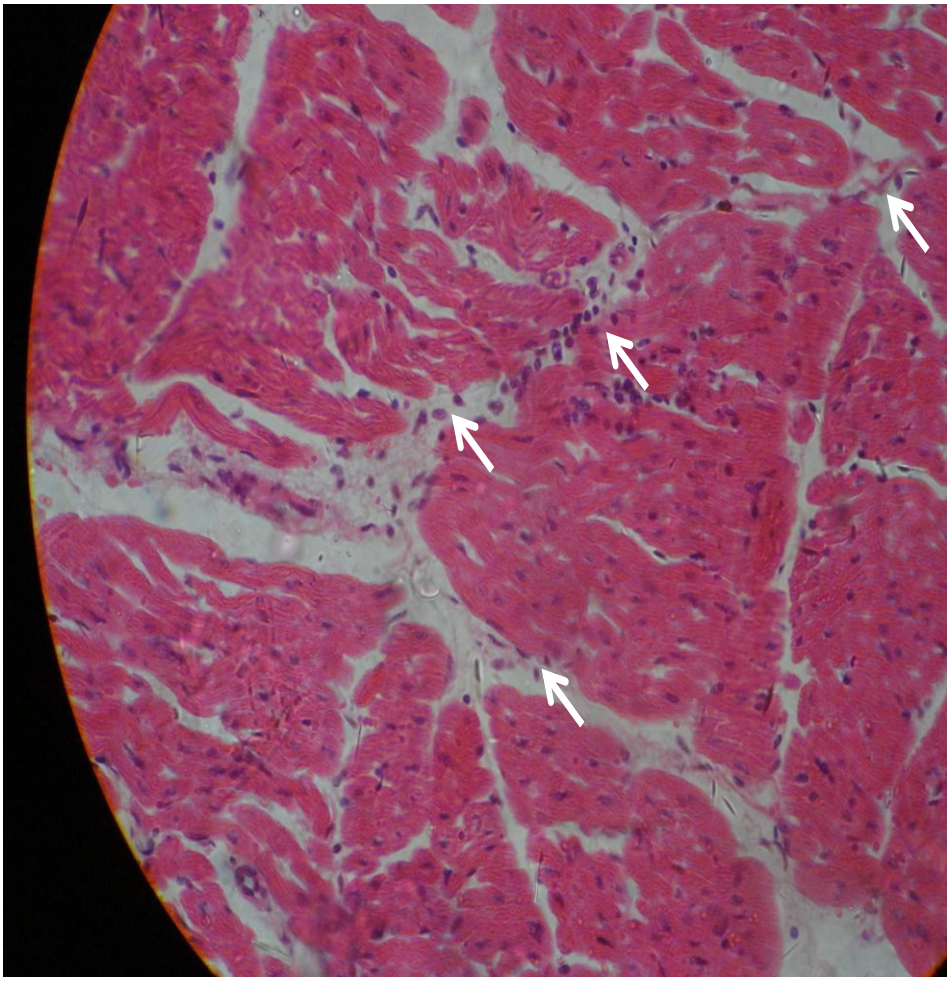


Plate XXVII: Photomicrograph of the skeletal muscle of *T. congolense*-infected, untreated sheep, showing areas of cellular infiltration (arrow), (H & E, × 400).

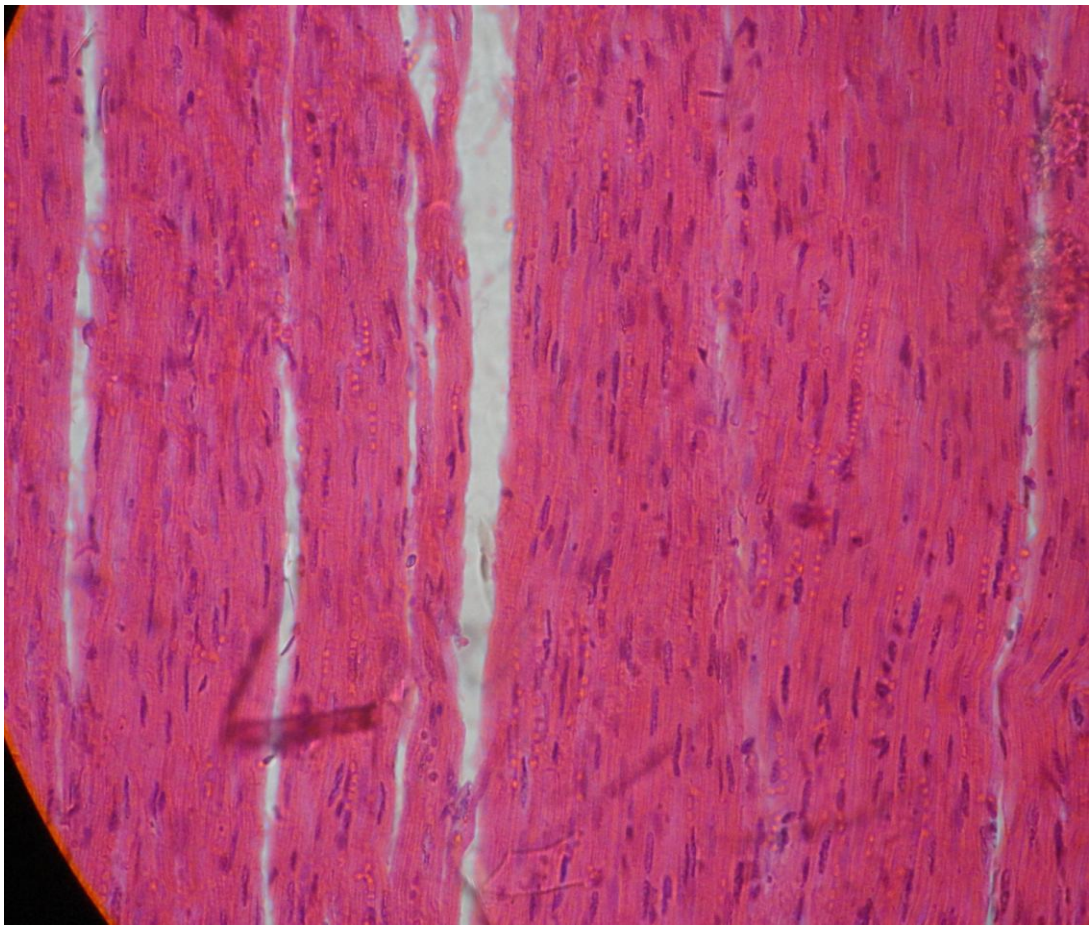


Plate XXVIII : Photomicrograph of the heart muscle of *T. congolense*-infected, Isometamedium chloride treated-sheep, showing mild atrophy of the muscle (H & E, × 400)

4.4 Molecular Characterization of *Trypanosoma vivax* and *Trypanosoma congolense* using Polymerase Chain reaction Technique (PCR).

4.4.1 Observation on the RAPD-PCR of *Trypanosoma vivax* and *Trypanosoma congolense*

The different forms (acute, relapse and chronic forms) of both *Trypanosoma vivax* and the *Trypanosoma congolense* were subjected to RAPD-PCR before Multiplex and *Trypanosoma* species specific PCR were carried out on them. The results of the experiment revealed that the amplicons of the acute (N) and chronic forms (C) of the *Trypanosoma vivax* were the same, whereas that of the relapse form (K) was different.

However, in *Trypanosoma congolense*, the acute (A) and the relapse forms (D) yielded bands of the same molecular sizes but the chronic form (R) had a different pattern from the other two forms (Plate XLVIII)

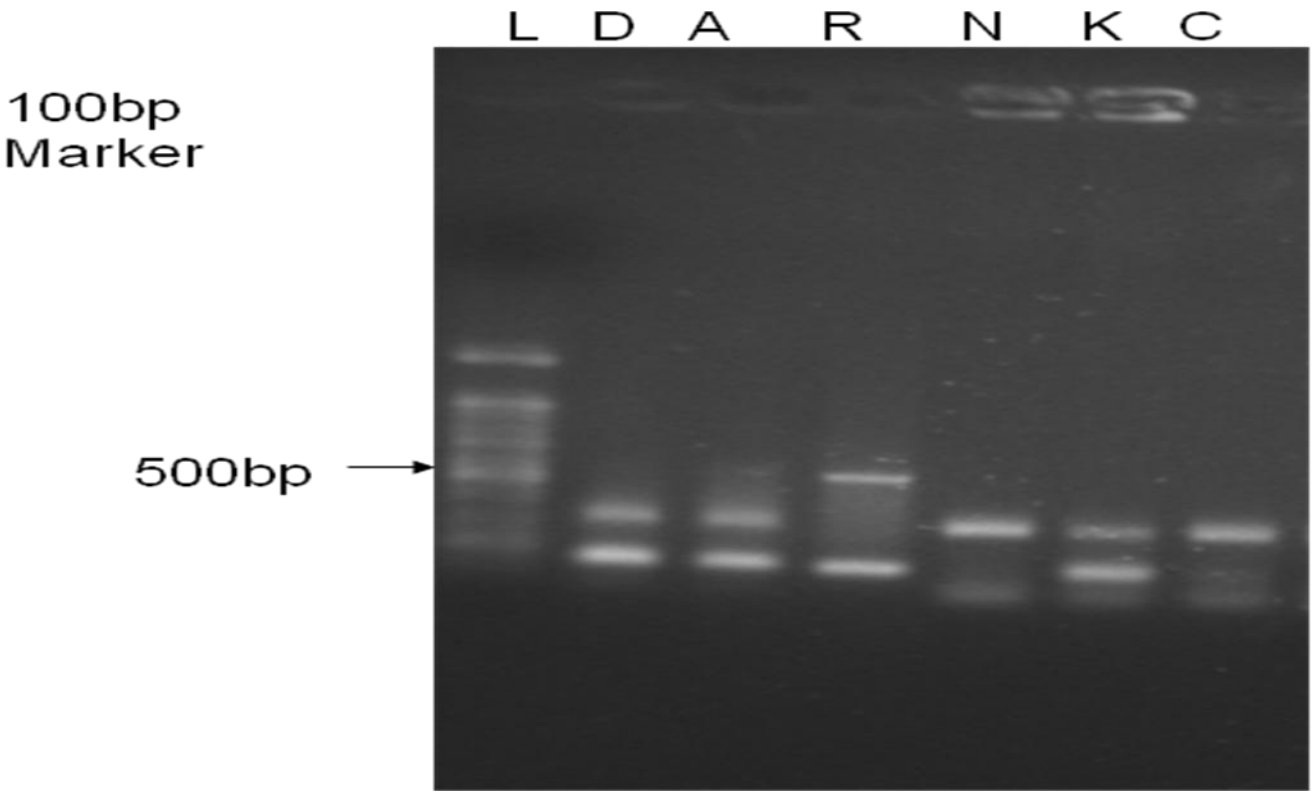


Plate XXIX: RAPD-PCR of *Trypanosoma vivax* and *Trypanosoma congolense* different forms.

L= Marker (100bp)

D= relapse form of *Trypanosoma congolense* (Approximately 420bp & 290bp)

A= Acute Isolate of *Trypanosoma congolense* (Approximately 420bp & 290bp)

R= Chronic form of *Trypanosoma congolense* (Approximately 510bp & 290bp)

N= Acute Isolate of *Trypanosoma vivax* (Approximately 390bp & 220bp)

K= Relapse form of *Trypanosoma vivax* (Approximately 390bp & 290bp)

C= Chronic form of *Trypanosoma vivax* (Approximately 390bp & 220bp)

4.4.2 Identification of the species of trypanosomes using multiplex *Trypanosoma* primers PCR.

The result shows that the isolate from the Northern Guinea savannah (Idon in Kaduna state) is *Trypanosoma congolense* with a band weight of 750bp and the one from Makurdi is *Trypanosoma vivax*.

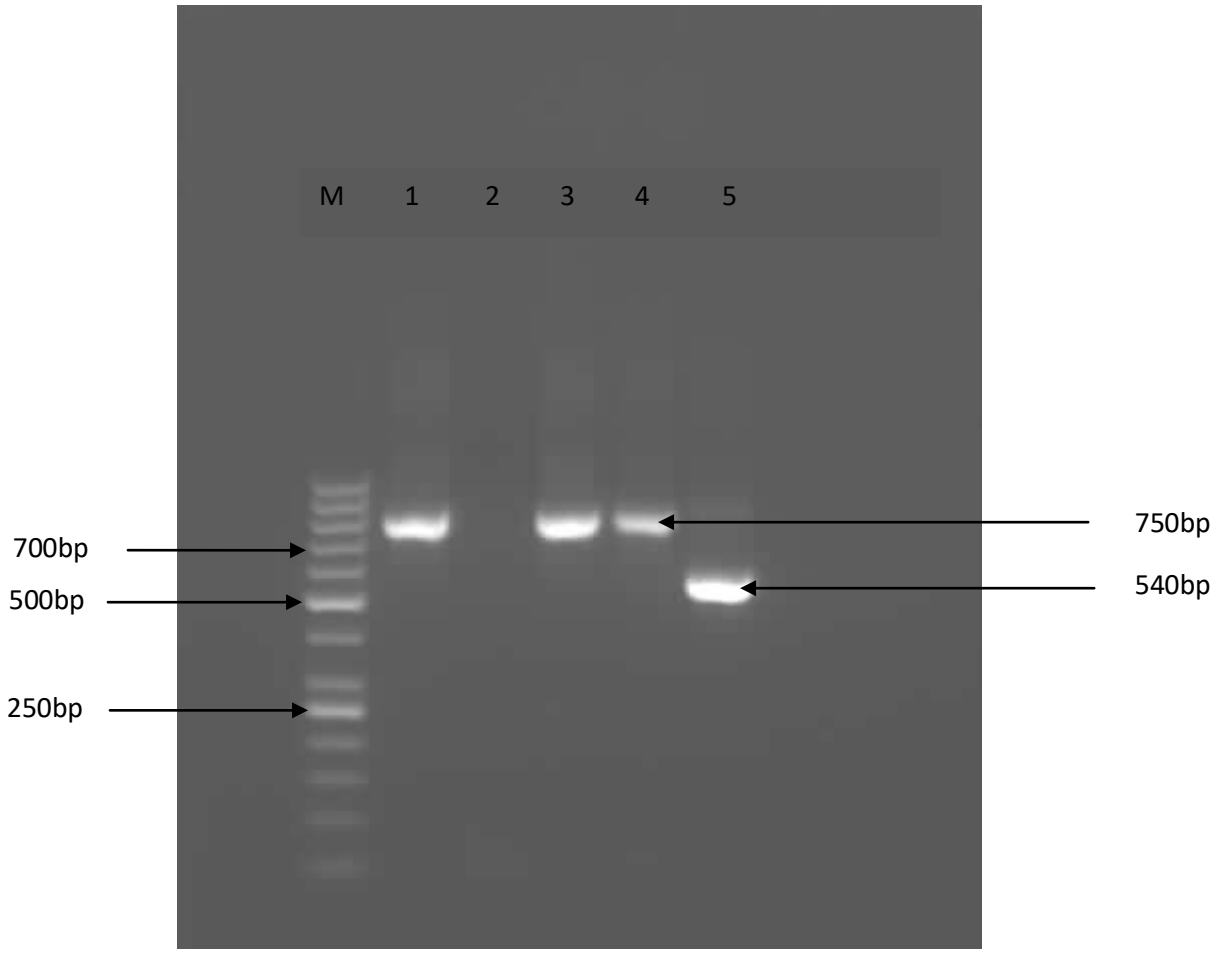


Plate XXX: Identification of different species of trypanosomes using multiplex *Trypanosoma* primers PCR.

Lane M - 50bp DNA Molecular Wight Marker (Thermo-Scientific)

This result confirms the species of the parasite. It also confirms that they are right species and no mix infection.

4.4.3 *Trypanosoma vivax* species-specific PCR

In order to confirm the presence or absence of *Trypanosoma vivax* in any of the samples (Kaduna and Makurdi), the samples were subjected to *Trypanosoma vivax* specific primers PCR. The *Trypanosoma vivax* yielded the species specific amplicon of 175 bp (plate XLVII).

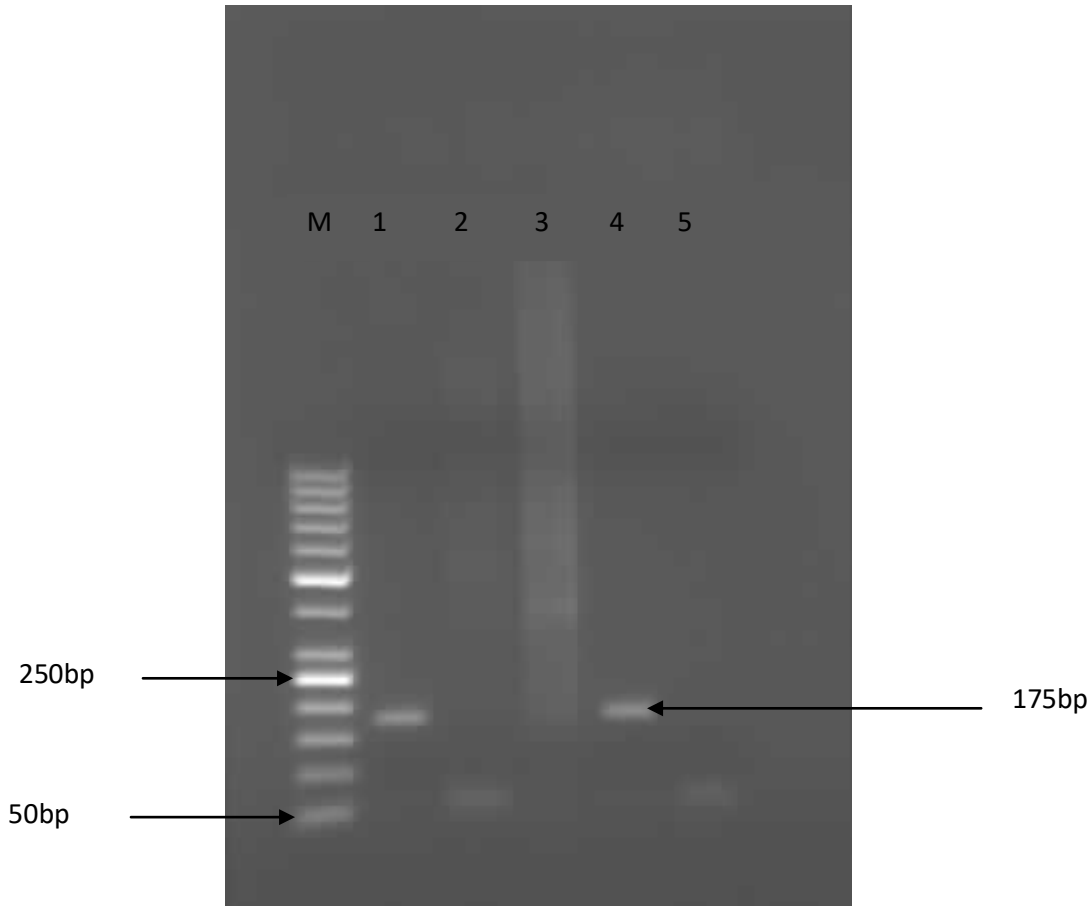


Plate XXXI: *Trypanosoma vivax* PCR with TVWA/TVWB Primer

Lane M - 50bp DNA Marker (Thermo Scientific)

The results revealed that sample from Southern Guinea Savannah (Makurdi) was pure *Trypanosoma vivax* isolate since it was negative to multiplex primers PCR for other trypanosomes, but positive for *Trypanosoma Vivax* by yielding the expected amplicon of 175 bp.

CHAPTER FIVE

DISCUSSION

The pre-patent period of *Trypanosoma vivax* observed in this study was 6 days post infection. This is in agreement with the 4-12 days reported by Hoof *et al* (1948) in sheep and goats in Congo, although, it contrasts the 3-4 days that was reported by other authors in Nigeria (Ogwu *et al*., 1986; Bawa *et al*., 2000; Akinwale *et al*., 2006). However, it has been reported (Hoare, 1972) that the incubation period of *Trypanosoma vivax* in sheep and goats vary depending on the level of host resistant and the virulence of the parasite strains/isolates involved. They further explained that, the more virulent the parasite, the shorter the incubation period and the more resistant the host, the longer the incubation period (Batista *et al*., 2007).

The incubation period of 6 days for *Trypanosoma congolense* that was observed in this study disagrees with 7-11 days reported by other researchers (Anene *et al*., 1997; Sackey, 1998; Takeet and Fagbemi, 2009). The fairly short incubation period recorded in this experiment revealed that the two isolates are probably highly pathogenic.

Pyrexia, observed in this study which was suggested to be due to circulating trypanosomes and their by-products corresponded with increase in rectal temperature in the first two weeks post infection for the animals treated with the three different trypanocidal drugs. However, the same animals treated with Novidium chloride experienced secondary pyrexia during relapse. This finding is in line with similar observations made by Anosa and Isoun (1974; 1980) and Batista *et al*. (2009). Pyrexia in trypanosomosis usually resulted from increased basal metabolic rate of the host's body system. The pyrexia seen in the relapse may be due to increase pathogenicity of the resurgent parasitaemia and the response of defence mechanism to curtail it.

Weight loss was observed in all the infected untreated animals, an observation that has been reported by many authors (Silva *et al*., 1999; Akinwale *et al.*, 2006; Batista *et al*., 2007; Damillo *et al*., 2012). The decrease in weight may be due to poor absorption and utilization of nutrient and also from the utilization of body fat to meet up the energy demand of the animal's body. In addition, the decreased feed intake and diarrhoea will definitely lead to loss of weight.

It has been reported that parasitaemia increased concomitantly with temperature (Batista *et al*., 2009). This is in agreement with the finding in this work because high parasitaemia were observed in most of the infected animals at week 2 post infection which corresponded with the period of high temperature.

The relapse infection observed in the two sheep infected with *Trypanosoma vivax*- treated with Novidium chloride was an indication of the resistance of the isolate to the recommended therapeutic doses (1 mg/kg body weight of 2.5^ solution) of the drug which is in support of the earlier reports by other authors (Peregrine, 1994; Geerts and Holmes, 1998), of the resistance of trypanosomes to most of the commonly used trypanocidal drugs in most African countries especially in East Africa. The resistant problem was seen to be more serious with *Trypanosoma congolense* since more than three relapses were experienced in all the *Trypanosoma congolense* infected animals treated with the three different trypanocides. High parasitaemia were recorded in the *Trypanosoma congolense* infected animals 12-24 hours before death.

Over the years, diminazene aceturate and isometamidium chloride have been used as the best therapeutic and prophylactic trypanocides respectively. Diminazene aceturate 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 more persistent prophylactic drug,

isometamidium chloride (Aliu *et al.,* 1981). However, the findings from the present study are suggestive of necessary update of the isolates used in this experiment developed resistance to all the trypanocidal drugs. Treatment failures with shortened prophylaxis have been observed and attributed to infections with drug-resistance trypanosome species (Chitambo and Arakawa, 1992; Peregrine and Mamman, 1993). The outcome of this work confirmed the reports of other researchers that high drugs resistance are encountered in the field with *Trypanosoma congolense* than *Trypanosoma vivax* (Geerts and Holmes, 1998; McDermott *et al.,* 2003). Most of these cases of resistance may be that these parasites probably could be in the past been exposed to subtherapeutic dosages therapy by farmers or non veterinarian in the field.

Although clinical signs were more severe in the *Trypanosoma vivax*-infected animals but more mortality was recorded in the *Trypanosoma congolense*-infected animals. At week 7 post infection, only two infected, untreated sheep survived in the *Trypanosoma congolense* group while four animals survived in the *Trypanosoma vivax* group. This observation agreed with the earlier report that infection with *Trypanosoma congolense* is more fatal than infection with *Trypanosoma vivax* (Lejon *et al.*, 2003).

The decrease in Packed Cell Volume (PCV) in all the infected untreated animals observed in this study is an indication of anaemia which is in agreement with the earlier findings, that animal suffering for trypanosomosis usually develop anaemia (Batista *et al*., 2007; Damillo, *et al.*, 2012). It was reported that most of the clinical signs observed in trypanosomosis are associated with anaemia and that when the rate of blood destruction by the parasites and its products are high, the infection becomes incompatible with life and the infected animal dies within a week or two post infections (Anosa and Isoun, 1974). The *Trypanosoma congolense*-infected untreated group had their lowest PCV at week 6 post infection when the animal's immune system had been overwhelmed with the infections (i.e. immunosuppression of the

host by the parasite). It has been reported that hemolysis is usually from the irritation by the parasites or by the enzymes produced by the parasites which are recognized as foreign bodies by the body defensive system (Losos and Ikede, 1972).

The observation of the gradual drop of plasma protein of all the *Trypanosoma vivax* and *Trypanosoma congolense*-infected untreated animals from week 1 post infection to week 8 post infection is in tandem with the previous report that animals infected with trypanosomes develop at a stage, an irreversible loss of appetite, total plasma protein and other haematological indices even after treatment (Egbe-Nwiyi and Anita, 2003). Another reason why plasma protein may decline in the infection is as a result of diarrhoea. This affects normal absorption of nutrient from the small intestine.

The study shows that the mean White Blood Cell (WBC) counts of the *Trypanosoma vivax*-infected untreated animals increased at week 2 post infections and later dropped at week 8 post infection. This type of initial increase and decrease at the late stage of infections has been reported in other parasitic infections by Coles (1986). It was observed that the initial leukocytosis is as a result of massive mobilization of the body defensive cells in response to the infection but as the disease lingers, leucopenia sets in because most of the cells were been destroyed thereby decreasing the cells population. Consequently, leukopenia is a consistent finding in the chronic form of trypanosomosis because of the immunosuppressive action of the parasites (Batista *et al*., 2006; Bezerra *et al*., 2008).

The gross pathological changes were more severe in *Trypanosoma vivax* infected group than *Trypanosoma congolense* in line with observations on the clinical manifestations which were more severe in the *Trypanosoma vivax* infected animals. This observation is a confirmation of previous report by Losos and Ikede, (1972) that pathology in tissues is associated with the relative ability of *Trypanosoma vivax* to invade both vascular and extravascular systems and

Trypanosoma congolense remain confined to the vascular system. This explains why lesions were more severe in *Trypanosoma vivax* infected groups. Visceral organs were variously affected because of the strategic physiological roles. Consequently, the liver was more affected grossly because of its important function as the major site of erythrocytes and some times parasites clearance from the body system while splenomegaly was also seen at the acute stage of the disease as a result of red cell and lymphocyte sequestration (Losos and Ikede, 1972).

The nervous signs observed in the two *Trypanosoma vivax* infected and untreated sheep manifested as areas of neuronal degeneration in the histopathological section of the brain whereas the *Trypanosoma congolense*-infected untreated had normal brain cells. The *Trypanosoma congolense*-infected, diminazene aceturate-treated animal's brain revealed areas of haemorrhages, depletion of purkinje cells, as have been observed by other authors (Losos and Ikede, 1972; Batista *et al*., 2007). These lesions may be due to hypoxia from anaemia or partial occlusion of the brain vessels by the parasites or their products.

Histologically, the liver in both *Trypanosoma vivax* and *Trypanosoma congolense* infected animals revealed hepatic necrosis, degeneration and congested sinuses. The hepatic necrosis may have resulted from the insufficient blood supply to the liver due to anaemia and partial blockage of liver vessels by the parasites and their products at a certain time (Fatihu *et al*., 2008). The haemosiderin and follicular depletion seen in the spleen of both *Trypanosoma vivax* and *Trypanosoma congolense*-infected animals might have been caused by hemolysis and anaemia which are associated with the disease. Similar observations have been reported by Losos *et al*. (1973), Valli and Forsberg, (1979).

In the kidney of the *Trypanosoma vivax* infected- untreated animals, there were foci areas of necrosis in the renal tubules and glomeruli while that of *Trypanosoma congolense* infected

diminazene treated animal, there was congestion of the renal vessels. All these findings agree with the early report by Kaliner, (1974).

The respiratory involvements were exhibited in both *Trypanosoma vivax* and *Trypanosoma congolense*-infected animals by the thickened alveolar membranes, congested and haemorrhagic lungs. The associated pneumonia may be due to secondary bacterial infections as a result of immunosuppression commonly seen in trypanosomosis (Tizard *et al*., 1978).

In the pancreas of the *Trypanosoma vivax*-infected animals, there were areas of necrosis in the glands, unlike those of the *Trypanosoma congolense* infected group that revealed mild pancreatitis. Similar inflammatory changes in the glands of the body have been reported by Losos and Ikede, (1972). There was also necrosis of the adrenal glands of the *Trypanosoma vivax*-infected, untreated animals. Enlarged adrenal glands, characterized by inflammatory changes have been reported by Ogwu *et a*l. (1992). Such inflammatory reaction if prolonged, can lead to cells necrosis, so this finding is in agreement with the former report.

The histological sections of *Trypanosoma vivax* and *Trypanosoma congolense* infected females uterus revealed uterine necrosis. This may be one of the reasons for abortion that have been widely reported in the disease by other researchers (Maikaje *et al*., 1991; Bawa *et al*., 2000; Akinwale *et al*., 2006; Batista, *et al*,. 2007). Section of the epididymis from both *Trypanosoma vivax* and *Trypanosoma congolense*-infected males revealed normal epithelium that was devoid of spermatozoa. This finding is in disagreement with the earlier report that trypanosomosis causes epididymitis (Batista *et al*., 2007; Bezerra *et al*., 2008).

There was mild atrophy of the skeletal and cardiac muscles associated with both infection and could be as a result of anorexia and poor utilization of the feed taken by the animals. This is one of the reasons behind the disease being referred to as wasting disease (Losos and Ikede, 1972).

The results obtained from the Randomly amplified Polymorphic DNA analysis-PCR (RAPD-PCR) shows that acute and chronic forms (about 390bp and 220bp) were the same but differed from that of the relapse form (about 390bp and 290bp) for *Trypanosoma vivax*. In *Trypanosoma congolense*, the bands observed with the acute and relapse forms (420bp and 290) were the same and differed from the bands of the chronic form (510bp and 290bp). The difference in band weights of the three forms of the two isolates may be responsible for the differences in the pathogenicity of the parasites.

The samples were subjected to multiplex and *Trypanosoma vivax* species-specific PCR which confirm the species of the isolates. It also confirms that the parasites were the right species and proved that the samples were not contaminated by other species of Trypanosomes (ie no mixed infections). It is known that multiplex PCR can simultaneously detect multiple infections with *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei brucei* in ruminants (Nyeko *et al*., 1990). The isolates were confirmed through the detection of bands width of 750bp and 175bp for the primers used in amplifying the fragments from *Trypanosoma congolense* and *Trypanosoma vivax* respectively.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

The study shows pre-patent periods of 6 days for both *Trypanosoma vivax* and *Trypanosoma congolense* infections. This study confirms the existence of resistance strains of *Trypanosoma vivax* and *Trypanosoma congolense* in the study areas to the commonly used trypanocides (diminazene aceturate, Isometamidium chloride and homidium chloride) at the recommended therapeutic dosages and the resistance is found to be more serious in *Trypanosoma congolense* than *Trypanosoma vivax* infections.

The study also revealed that clinical manifestations and pathological lesions were more severe in *Trypanosoma vivax* infections but *Trypanosoma congolense* infections caused more fatality than *Trypanosoma vivax*. Infection of the *Trypanosoma vivax* has both vascular and extravascular effects, thereby causing more organ necrosis than infection with *Trypanosoma congolense* which causes problems mainly in the system. The study also shows that chronicity in trypanosomosis is associated with weight loss, gradual drop in all the haematological indices and leucopenia. The pathology in the reproductive system observed in both *Trypanosoma vivax* and *Trypanosoma congolense* infections is indicative of possible economic losses.

The study revealed that the two isolates of the parasites used were the right species and that they have specific gene of interest. It also shows that the samples were pure and free of contamination by other species of trypanosomes (ie no mixed infections).

6.2 RECOMMENDATIONS.

► More work should be done on the area of chemotherapy to review the current efficacy of the commonly used trypanocidal drugs to stem the rising problem of drug-resistance.

► Isolates from different geographical locations should be tested *in vivo* to characterise the existing strains in the country.

►More isolates from different geographical areas should be assess for existence of drugs resistance strains.

► Studies should be conducted on the pathogenesis of organ necrosis in trypanosomosis.

► Isolates of *Trypansoma vivax* and *Trypanosoma congolense* from different geographical areas should be characterize molecularly to check whether all the existing species in Nigeria will be amplify at the same band weight using the same primers sequence.

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APPENDIX 1: Summary of comparative *in vivo* characteristics of *Trypanosoma vivax* and *Trypanosoma congolense* isolates of Nigeria origin.

S/N	Variables	*Trypanosoma vivax*	*Trypanosoma congolense*
1.	Rectal Temperature (0^{C})	Infected untreated group had higher temperature than normal ($38^{O}C$) through out the period of the experiment ($39.5^{0}C$-$40.1^{0}C$)	Higher than normal ($38^{O}C$) but lower ($39.0^{0}C$) than *T. vivax* infected group ($40.1^{O}C$)
2.	Incubation period	6 days	6 days
3.	Parasitaemia	Massive (++++). At day4 post parasitaemia	Moderate (++ to +++). At day 4 post parasitaemia.
4.	Packed Cell Volume	Dropped with about 6.5% from wk 1-6 pi. Progressive decrease from 23% to 17% at week 6 pi.	Lowest value at week 6 pi (16.50±0.61%) with about 8.75% drop from week 1 pi. More profound decline from 25% at week 1 pi to 16% at week 6 pi.
5.	Clinical signs	Anorexia, rough hair coat,ocular discharge,pale mucous membrane,diarrhoea,severe weakness, depression, oedema of the eyelids,teeth grinding, emaciation and CNS disorder.	As for *T. vivax* but no nervous system involvement.
6.	Relapses	Resistant to Novidium chloride only. Relapsed seen only in the Novidium treated group.	Relapses seen in all the drugs (Novidium chloride, Isometamidium chloride and diaminazene aceturate) treated groups.
7.	Total plasma protein	Lowest values at weeks 4 & 7 pi (4.9 and 4.7 respectively).	Lowest values at week 6 pi (4.18±0.30).
8.	Total WBC	Lowest value at week 2 pi (3.62±0.51)	Lowest value at week 4 pi (4.28±0.46)
9.	Neutrophil	Lowest values at weeks 3 & 7 pi	Lowest value (21.83±0.95) at week 5 pi
10.	Lymphocyte	The value dropped at week 2 pi (0%).	Was high (73- 76%) through out the period of observation
11.	Monocyte	Recorded low values through out the	Low through out.

		experiment.	
12.	Eosinophil	Recorded zero from weeks 1-6 pi then 0.17 and 1.17 at weeks 7 and 8 respectively.	Low to zero through out.
13.	Basophil	Recorded zero through out the observation period	Low to zero through out
14.	Band Neutrophil	No band at weeks 4 & 6 pi then value of 2.33 was observed at week 7 pi.	Low through out.
15.	Weight Variations	About 3.5kg decrease from weeks 1-8 pi.	About 2 kg drop at week 4 pi.
16.	Gross Pathology	Serous atrophy of pericardial fat, focal haemorrhagic area on diaphragmatic lobe of the right lung, hydropericardium, diffuse pin point haemorrhagic ares on the liver and kidney surfaces and empty gall bladder.	Pale carcasses and slight congestion of the liver were the only gross pathology observed.
17.	Histopathology	Neuronal degeneration in the brain, pneumonia, haemosiderosis of the red and white pulps of the spleen, degeneration of the glomeruli and necrosis of the kidney tubules and uterus. There was haemosiderosis of the spleen, atrophy and degeneration of the skeletal muscles with absence of sperm cells in the epididymis.	Necrosis of the adrenal glands, pancreas and infiltrative myositis of the skeletal muscles.There was congestion of the lungs, liver and haemosiderosis of the spleen.
18.	Multiple primers PCR	Negative for *Trypanosoma vivax*.	Positive for *Trypanosoma vivax* at amplicon of 750bp.
19.	*T. vivax* species-specific PCR	Positive for expected 175bp amplicon.	Negative at 175bp amplicon.
20.	RAPD-PCR	Acute and chronic forms have the same amplicons but differ from the amplicon of relapse form.	Relapse and acute forms have the same amplicons.

APPENDIX 2 : Mean ± SE weekly Temperature of *Trypanosoma vivax* infected-treated (A), infected-untreated (B) and uninfected-control (C) Yankasa sheep

Pre-Infection	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	W
38.63±0.17	39.74±0.06^{a}	39.25±0.11^{a}	38.47±0.19^{b}	39.00±0.29^{b}	38.56±0.13^{b}	38.48±0.11^{b}	38.42±0.08	38
38.33±0.26	39.65±0.07^{a}	39.58±0.10^{a}	39.29±0.25^{a}	40.08±0.21^{a}	39.59±0.18^{a}	39.34±0.14^{a}	39.21±0.19	39
38.18±0.15	39.02±0.17^{b}	38.80±0.14^{b}	38.22±0.20^{b}	38.62±0.25^{c}	38.34±0.11^{b}	38.80±0.30^{ab}	38.81±0.35	38

* Mean +-SEM based on three observations.

APPENDIX 3: Mean ± SE weekly body weight of *Trypanosoma vivax* infected-treated (A), infected-untreated (B) and uninfected-control (C) Yankasa sheep

Pre-Infection	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	W
15.33±0.92	16.00±0.89	14.83±0.87	14.08±0.67^{b}	13.83±0.70	14.00±0.58^{a}	14.25±0.60^{b}	14.50±0.67^{b}	1
14.67±0.33	15.17±0.54	15.75±0.36	12.67±0.56^{b}	13.17±0.40	11.75±0.36^{b}	11.33±0.31^{c}	11.00±0.34^{c}	1
16.67±0.96	18.00±0.86	16.83±1.08	16.33±0.99^{a}	15.50±1.15	15.50±1.15^{a}	16.00±1.00^{a}	16.25±1.05^{a}	1

* Mean +-SEM based on

hree observations.

APPENDIX 4: Mean ± SE weekly Parasitaemia of *Trypanosoma vivax* infected-treated (A), infected-untreated (B) and uninfected-control (C) Yankasa sheep

	D1	D3	D7	D10	D14	D17	D20	D23	D27
A	0.00±0.00	0.42±0.42^{b}	2.92±0.42^{a}	0.83±0.53^{b}	0.00±0.00^{b}	0.42±0.42^{b}	0.83±0.83^{b}	0.83±0.83^{b}	1.67±
B	0.00±0.00	2.50±0.00^{a}	2.92±0.42^{a}	4.58±1.00^{a}	3.75±1.07^{a}	4.17±0.83^{a}	4.17±1.05^{a}	3.33±0.53^{a}	3.75±
C	0.00±0.00	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±

* Mean +-SEM based on three observations.

APPENDIX 5: Mean ± SE weekly Packed Cell Volume of Trypanosoma vivax infected-treated (A), infected-untreated (B) and uninfected control (C) Yankasa sheep.

e-Infection	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	We
.67±1.89	23.75±1.80	20.92±1.54[b]	22.33±2.11	21.83±1.42	21.42±0.81[b]	25.83±1.13[a]	20.75±1.06[b]	22.
.00±0.97	23.75±0.88	17.17±0.47[c]	19.92±0.95	22.33±1.76	18.25±0.77[c]	17.00±0.70[c]	14.17±094[c]	16.
.17±1.35	24.67±0.85	23.67±0.67[a]	25.17±1.35	26.58±1.09	27.17±0.79[a]	24.42±1.57[b]	25.83±2.02[a]	26.

* Mean +-SEM based on three observations.

APPENDIX 6: Mean ± SE weekly Total Plasma Protein of *Trypanosoma vivax* infected-treated (A), infected-untreated (B) and uninfected-control (C) Yankasa sheep

Pre-Infection	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	W
5.97±0.22	6.20±0.21[a]	5.63±0.20	6.68±0.32[a]	6.10±0.31[a]	6.28±0.16[a]	5.92±0.15[a]	5.78±0.16[a]	5
6.03±0.06	5.57±0.10[b]	5.27±0.30	5.45±0.13[b]	4.93±0.17[b]	5.25±0.25[b]	5.12±0.25[b]	4.75±0.28[b]	4
5.33±0.47	6.20±0.22[a]	5.93±0.12	6.52±0.21[a]	6.00±0,20[a]	6.33±0.24[a]	6.03±0.17[a]	5.30±0.21[a]	5

* Mean +-SEM based on three observations.

APPENDIX 7: Mean ± SE weekly Temperature of *Trypanosoma congolense* infected-treated (E), infected-untreated (F) and uninfected-control (2C) Yankasa sheep

Pre-Infection	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	W
38.17±0.16	38.88±0.11	38.77±0.09	38.78±0.29	39.08±0.22^{ab}	39.05±0.22	38.85±0.19^{ab}	38.77±0.15	38
38.35±0.22	39.03±0.12	38.77±0.14	39.16±0.23	39.44±0.09^{a}	39.12±0.19	39.15±0.15^{a}	38.91±0.13	38
38.28±0.17	38.71±0.08	38.55±0.19	38.57±0.13	38.75±0.09^{b}	38.84±0.21	38.58±0.07^{b}	38.62±0.08	38

* Mean +-SEM based on three observations.

APPENDIX 8: Mean ± SE weekly body weight of *Trypanosoma congolense* infected-treated (E), infected-untreated (F) and uninfected-control (2C) Yankasa sheep

Pre-Infection	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	W
13.93±0.17^{b}	13.17±0.87^{b}	12.83±0.79^{b}	13.83±0.83	13.33±1.05	13.50±1.06^{b}	13.50±0.81^{b}	13.00±0.68^{b}	14
13.97±0.17^{a}	13.80±0.76^{b}	13.33±0.80^{b}	12.17±0.98	12.67±0.80	12.00±0.52^{b}	11.33±0.56^{b}	10.58±0.63^{c}	10
16.20±0.25^{b}	17.83±0.79^{a}	17.83±0.79^{a}	15.67±1.26	15.33±1.31	18.01±0.92^{a}	17.74±0.89^{a}	17.78±1.21^{a}	18

* Mean +-SEM based on three observations.

APPENDIX 9: Mean ± SE weekly Parasitaemia of *Trypanosoma congolense* infected-treated (E), infected-untreated (F) and uninfected-control (2C) Yankasa sheep

	D1	D3	D6	D9	D12	D15	D19	D23
E	0.00±0.00	0.00±0.00	0.83±0.53[b]	3.33±0.53[a]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
F	0.00±0.00	0.00±0.00	2.08±0.42[a]	3.75±0.56[a]	5.42±0.77[a]	5.42±1.19[a]	5.83±1.54[a]	6.67±1.39[a]
2C	0.00±0.00	0.00±0.00	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]

* Mean +-SEM based on three observations.

APPENDIX 10: Mean ± SE weekly Packed Cell Volume (PCV) of *Trypanosoma congolense* infected-treated (E), infected-untreated (F) and uninfected-control (2C) Yankasa sheep

Pre-Infection	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	W
30.50±1.12	28.58±1.65	24.00±1.29	29.17±1.70[a]	25.33±1.57[a]	26.17±1.83[a]	26.42±1.46[a]	25.92±1.93[b]	28
28.67±1.41	25.25±1.36	22.58±0.52	21.25±0.67[b]	17.17±1.37[b]	18.67±1.31[b]	16.50±0.61[b]	23.03±0.81[b]	18
26.83±2.06	27.92±2.03	26.08±1.64	29.17±1.12[a]	29.08±1.21[a]	29.67±1.17[a]	28.44±0.68[a]	31.16±0.64[a]	29

* Mean +-SEM based on three observations.

APPENDIX 11: Mean ± SE weekly Total Plasma Protein of *Trypanosoma congolense* infected-treated (E), infected-untreated (F) and uninfected-control (2C) Yankasa sheep

Pre-Infection	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	W
5.07±0.15	4.98±0.19	5.52±0.15[b]	5.81±0.24[a]	5.83±0.33[ab]	5.97±0.30	5.43±0.12[b]	5.08±0.14[b]	5.8
5.27±0.08	5.05±0.11	5.15±0.06[c]	5.00±0.10[b]	5.28±0.18[b]	5.27±0.27	4.18±0.30[c]	5.02±0.14[b]	4.8

4.97±0.31	4.93±0.23	5.93±0.15[a]	6.10±0.27[a]	6.37±0.21[a]	6.17±0.26	5.79±0.24[a]	6.22±0.18[a]	6.5

* Mean +-SEM based on three observations.