COMPARATIVE STUDIES ON THE INFECTIVITY AND PATHOGENICITY OF EXPERIMENTAL *TRYPANOSOMA BRUCEI BRUCEI* INFECTION IN MICE, RATS, RABBITS AND GUINEA FOWLS

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JANUARY, 2018

DECLARATION

I declare that the work in this thesis entitled **"Comparative studies on the infectivity and pathogenicity of experimental *Trypanosoma brucei brucei* infection in mice, rats, rabbits and guinea fowls"** has been carried out by me in the Department of Veterinary Parasitology and Entomology, under the supervision of Professor Idris A. Lawal and Professor Sani Adamu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution

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Name of Student	Signature	Date

CERTIFICATION

This thesis entitled **"COMPARATIVE STUDIES ON THE INFECTIVITY AND PATHOGENICITY OF EXPERIMENTAL *TRYPANOSOMA BRUCEI BRUCE* INFECTION IN MICE, RATS, RABBITS AND GUINEA FOWLS"** by Karimat HUSSAIN meets the regulations governing the award of the degree of Master of Protozoology (M.Sc.) of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

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

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ABSTRACTS

The study compared the infectivity and pathogenecity of experimental *Trypanosoma brucei brucei* infection in mice, rats, rabbits and guinea fowls. A total of 10 each of the following animals' mice, rats, rabbits and guinea fowls of both sexes were used for the study. Each group of the animal species was divided into two groups (infected and control) of five animals each. The mice, rats, rabbits and guinea fowls in the infected groups were individually inoculated with blood containing 1 x10^6 *Trypanosoma brucei brucei* (Fadere stock). All animals in the control groups were not infected. Patent parasitaemia as determined by haematocrit centrifugation technique was between 3-4 days in the mice and rats, while it was 7-8 days in rabbits. No parasitaemia was seen in the infected Guinea fowl throughout the study. The mean body weights of mice, rats and rabbits decreased in the infected group as compared to the control group. All animals in the infected group with the exception of guinea fowls showed increase in body temperature. In the mice and rats there were significance diferrences (SD) ($p < 0.05$) in the overall mean PCV, HGB and RBC between the infected groups (IG) as compared to the control group (CG). In the rabbits there were SSD ($P < 0.05$) in the overall mean PCV, HGB and RBC between the IG (36.1 ± 0.6%, 12.11 ± 0.8 g/dl and 5.9 ± 0.4×10^6) and CG (43.7 ± 0.5%, 14.1 ± 0.9 g/dl and 7.23 ± 0.3×10^6 respectively). There was no SD ($P > 0.05$) between the overall mean PCV, HGB and RBC of the infected guinea fowls and those of the control group throughout the period of the experiment. Also, no mortality was recorded among the infected guinea fowls as

consequence of the infection. A decrease in mean total white blood cell (WBC) counts of the rats and the rabbits were observed while mice and guinea fowls groups showed no significance difference between the WBC of the infected and control groups. Microscopic lesions observed in the mice, rats and rabbits included congested central vein and perivascular cuffing, congestion and mononuclear cellular infilteration around blood vessels of the lungs, depletion of lymphoid cells, congested inter tubular spaces and focal necrosis of renal tubular epithelium. In the guinea fowls, the spleen revealed heamosiderosis. The study thus demonstrated that mice, rats and rabbits are, in order of susceptibility better laboratory models than the guinea fowls which tend to show some measure of resistance to *Trypanosoma brucei brucei* used in the experiment. Therefore rabbits could be use in our laboratory to preserve *Trypanosoma brucei brucei.*

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Trypanosomosis is a disease of man, domestic (Fajinmi *et al.*, 2011; WHO 2015) and wild animals (Abenga *et al.,* 2006; Mbaya *et al.,* 2009; 2011). It is transmitted by tsetse flies (*Glossina* spp.) and characterized by anaemia, oedema, cachexia, intermittent fever and death (Yakubu *et al.,* 2014). In Africa including Asia and South America it is transmitted by several biting flies like *Tabanus, Hippobosca, Stomoxys* (WHO 2015; Yakubu *et al.,* 2014).

The *Trypanosoma species* affecting man and his domestic animals have been subdivided into two groups, the haematic group (*Trypanosoma congolense, T. vivax*) which remain in the blood plasma and the tissue invading group (*T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense and T. equiperdum*) that are found in extra and intra vascular spaces (Abubakar *et al.,* 2005; Chretien and Smoak, 2005; Ngure *et al.,* 2008). Because of their presence in the blood, these invading parasites produce numerous changes in the cellular and biochemical constituents of blood (Taiwo *et al.,* 2003). Infection with *Trypanosoma brucei* infection like other *Trypanosoma* infection precipitate red blood cell destruction which results in anaemia as well as tissue damage (Ekanem and Yusuf, 2008; Akanji *et al.,* 2009).

Clinically, the effects of trypanosomosis in animals range from anaemia, immunosuppression, depression with inability to rise, pyrexia directly associated with parasitaemia, paleness of mucous membrane, rapid pulse beat, retarded growth, roughness

of haircoats. Reduced capacity to work leading to morbidity and mortality in the absence of treatment (Batista *et al*., 2012; Silva *et al*., 2013).

In addition are enlargement of peripheral lymph nodes, reduced milk production, low meat quality, and weight loss as well as reproductive disorders such as infertility, abortion, stillbirth.

In a review by Ibrahim *et al.,* (2015), some of the pathological changes in animals (mice, rats and rabbits) include splenomegaly, hepatomegaly and nephritis, in addition, cardiomegaly has been reported in rabbits. The histopathology changes include degenerative changes in both liver and kidneys, The spleen of the mice showed giant macrophages, that of the rats showed epithelioid giant cells, while those of the rabbits showed haemosiderosis and eosinophilic infiltrations. However, exclusive to the rabbits was hydropic degeneration, necrosis and mononuclear cellular infiltrations in the myocardium. Also observed was that the liver of the mice showed central vascular congestion with sheathed artery, that of the rats showed sinusoidal haemorrhages and nuclear vacuolation while that of the rabbits showed very mild, but widespread vacuolar degeneration of hepatocytes with periportal mononuclear cellular aggregations and presence of scattered megalocytes. The report also indicated that the kidneys of the mice infected with *T. brucei* showed interstitial haemorrhages and necrosis of the tubular epithelial cells, that of rats showed multi-focal glomerular degenerations and tubular ballooning in the cortex while that of rabbits showed mild but diffuse interstitial mononuclear cell infiltration especially at the corticomedullary junction.

1.2 Statement of Research Problem

Although *T. brucei brucei* is primarily a parasite of domestic animals as well as man, laboratory animals are often used to establish the course of infection and the degree of pathogenecity of the parasite, and these animals therefore serve as models in different experimental designs to study the disease it causes. However, it has been reported from experimental studies that the course of infection and severity depends on some factors such as the host species and the strains of the parasite. These studies have not given much Information on the degree of infectivity and pathogenecity of *T. brucei brucei* infection in laboratory animals such as mice, rats, rabbits and guinea fowls.

Laboratory mice and rats especially have been used in laboratories to preserve *Trypanosoma brucei brucei* for experimental studies and some degree of pathogenecity and sometimes death have been reported (Lawal *et al.,* 1999). It is therefore important to explore the possibility of using other animal species as experimental models for the *Trypanosoma brucei brucei* infection for appreciable periods with minimum loss of such animals and hence the parasites.

1.3 Justifications

- Extensive work has been done on animal trypanosomosis due to *Trypanosoma* vivax, *Trypanosoma congolense* and *Trypanosoma brucei* (Adeiza *et al.,* 2008; Adamu *et al.,* 2008) but little has been done to determine and compare the degree of pathogenecity of these parasites in different laboratory animals. Laboratory animals (mice and rats) are used as models in many researches. Therefore, there is need to

establish the infectivity rate and pathogenecity of *Trypanosoma brucei brucei* infection in these animals with the view of establishing the best laboratory animal model for use in trypanosomosis research.

The ability of laboratory animals to serve as *in vivo* models depends partly on the capacity to withstand experimental infection. This needs to be established in different laboratory animal models.

Laboratory rats and mice have over the years been used extensively in preservation of *Trypanosoma brucei* parasites but the pathogenecity of the parasites sometimes result in sudden loss of both the animals and the parasite hence the need to explore the possibility of using other animal models in comparism with mice and rats for this purpose.

1.4 Aim and Objectives of the study

1.4.1 Aim of the study

The aim of this research was to compare the infectivity and pathogenecity of *Trypanosoma brucei* in mice, rats, rabbits and guinea fowls

1.4.2 Specific objectives of the study

The objectives of the study were to determine and compare the:

a. Parasitaemia and its onset in mice, rats, rabbits and guinea fowls following experimental infection with *Trypanosoma brucei*.
b. Changes in rectal temperature induced by experimental *Trypanosoma brucei* infection in mice, rats, rabbits and guinea fowls.
c. Hematological changes induced by experimental *Trypanosoma brucei* infection in mice, rats, rabbits and guinea fowls.

a. Microscopic lesions and their distribution in the laboratory animals experimentally infected with *Trypanosoma brucei*

1.5 Research Hypothesis

Ho; There is no difference on the course of experimental *Trypanosoma brucei* infection in mice, rats, rabbits and guinea fowls.

Ha; There is difference on the course of experimental *Trypanosoma brucei* infection in mice, rats, rabbits and guinea fowls.

CHAPTER TWO

LITERATURE REVIEW

2.1 African Trypanosomosis

African trypanosomosis is a disease of humans (HAT) and animals (AAT) caused by several species of trypanosomes and spread by *Glossina* (tsetse flies) in 37 countries within the sub-saharan region (Welburn *et al.,* 2001). The disease is mainly transmitted cyclically by the genus *Glossina spp*., but can also be transmitted by several biting flies like tabanids, hippoboscids, stomoxys (Votypka *et al.* 2012). The culex mosquito has also been indicated in the transmission of an avian *Trypanosoma species, Trypanosoma culicavium*. Two tsetse- transmitted parasites, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodensiense*, cause Human African Trypanosomosis (HAT), which is commonly known as sleeping sickness, while Animal African Trypanosomosis (AAT), is mainly caused by *Trypanosoma congolense, T. vivax* and *T. brucei brucei* and *T. simiae* (Abenga *et al.,* 2002) and in known as Nagana.

2.2 Morphology and Taxonomy of Trypanosomes

A sound knowledge of the basic features of the various trypanosomes enables the identification of each species and so the exact cause of the disease. Trypanosomes are classified in the Phylum Sarcomastigophora; the Order Kinetoplastida, the Family Trypanosomatidae and the Genus: *Trypanosoma* (Mulligan *et al*., 1970). The trypanosome consists of a single cell varying in size from 8 to over 50 μm. There are distinct differences in appearance, shape and size among the various species of *Trypanosoma*, allowing specific identification.

The salivarian group of trypanosomes develop in the anterior gut of their vector, development occurs in the proboscis and midgut, forming eprmastigotes which then invade the hypopharynx and develop into trypomastigotes and then infective metetrypanosomes form. They may or may not have a free flagellum, the kinetoplast is terminal or sub-terminal or marginal and the posterior end of the body is usually blunt, they develop as trypomastigotes within the mammalian host and are usually pathogenic (Mulligan *et al*., 1970).

2.2.0 Subgenera of *Trypanosoma species*

2.2.1 The Subgenus *Nannomonas*

This consists of the smallest of the pathogenic *Trypanosoma species*, with a length of 8-24 μm. The blood forms are monomorphic, in that they lack a free flagellum. Generally, two variants are to be seen, a shorter form (9-18μm), which is the typical *congolense* type and a longer form (up to 25μm), and intermediate in length individuals between the two (Hoare, 1972). There is evidence that strains with the most forms, the so-called dimorphic'strains, cause a more severe form of trypanosomosis. In stained specimens of *T. congolense*, the cytoplasm stains a diffuse, even, pinkish colour and is seldom granular. The nucleus is centrally placed. The kinetoplast is of medium size and is usually situated at the margin of the body, just in front of the posterior extremity (marginal and subterminal), with poorly developed and inconspicuous undulating membrane (Hoare, 1972). *Trypanosoma simiae a* member of this subgenus is polymorphic, with a length of 15-19 μm. Though in typical cases some are without a free flagellum and the kinetoplast is of medium size, marginal and subterminal, as in *T. congolense* (Hoare, 1972). Another member, *Trypanosoma godfreyi* has been separated recently from *T. congolense* in the Gambia, not only on the basis of isoenzymatic and DNA differences, as well as the facts that the disease it causes is different

(Hoare, 1972). It is pathogenic in pigs, but the resultant disease is more chronic than the one caused by *T. simiae*. Morphologically it is similar to *T. congolense*, with a length of 9-22 μm, but the undulating membrane is described as being usually conspicuous (Hoare, 1972).

2.2.2 The Subgenus *Duttonella*

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

2.2.3 The Subgenus *Trypanozoon*

The subgenus *Trypanozoon* is the most homogeneous group of salivarian trypanosomes, represented conventionally by species, which are morphologically indistinguishable but differ in biological features (Hoare, 1972). This group comprises of five members: *T brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. evansi* and *T. equiperdum*. *Trypanosoma brucei* members are polymorphic, with three main forms, all of which have a small kinetoplast and a conspicuous undulating membrane. These three forms are

(a) Long slender forms (23-30 μm in length) with a free flagellum, which may be up to one half of the length of the organism. The posterior end is pointed and the nucleus is central. The kinetoplast is placed up to 4 μm in front of the posterior extremity (Hoare, 1972).

(b) Short stumpy forms (17-22 μm in length) normally without a free flagellum, but in which there may occasionally be individuals with short free flagella. The kinetoplast is usually subterminal. The position of the nucleus varies greatly and it is in some cases in the posterior part of the cell, sometimes so far posterior that the kinetoplast is anterior to it (so-called postero nuclear forms).

(c) Intermediate forms, varying in length between the two other forms with the following features: a free flagellum, of varying length, is always present, centrally placed nucleus, some what variable posterior end but usually blunt, the kinetoplast is close to the posterior extremity (Hoare, 1972). During the course of infection, there is a change in the trypanosome population from the long thin forms, through the intermediate, to the short stumpy forms, and this altered appearance is accompanied by a change in the type of respiration, as the trypanosome prepares for its period within the tsetse fly. The short stumpy forms are adapted to living and developing in the tsetse, while the long thin forms are the true mature blood forms which die in the gut of the insect (Hoare, 1972).

Trypanosoma evansi is a salivarian trypanosome belonging to the Family: *Trypanosomatidae*; Order: kinetoplastida. This tissue parasite is thought to have evolved from *T. brucei* and is closely related to the subspecies: *T. brucei brucei*, the aetiologic agents of animal and *T. brucei rhodesiense* and *T. brucei gambiense*, the aetiologic agents of human trypanosomosis in Africa, respectively (Brun *et al.*, 1998). The current hypothesis is that when *T.brucei brucei*-infected camels were moved outside the tsetse fly zone in Africa via travelling caravans, the trypanosomes were spread by other haematophagous flies to other animal species. Recent molecular evidence supports the close genetic relationship between *T. brucei* and *T. evansi*, leading others to suggest that *T. evansi* is a subspecies of *T. brucei* (Jensen *et al.*, 2008; Lai *et al.*, 2008). *Trypanosoma*

evansi is morphologically indistinguishable from the slender form of *T. brucei.* It is generally monomorphic in its slender form, although stumpy and intermediate forms may be observed in some isolates (Losos, 1980). *Trypanosoma evansi* is 14-33 μm long and 1.5-2.2 μm wide, with a free flagellum and a subterminal kinetoplast (Brun *et al.*, 1998). Isolates without kinetoplast have been identified in livestock from Brazil (Ventura *et al.*, 2002).

2.3 Host Range

Cattle, sheep, goats, pigs, horses, camels, dogs, cats and monkeys are susceptible to Africa Animal Trypanosomosis (AAT) and may suffer syndromes ranging from subclinical, mild or chronic infection to acute fatal disease (Molyneux and Ashford, 1983). Rats, mice, guinea pigs and rabbits are susceptible and useful laboratory species (Molyneux and Ashford, 1983). More than 30 species of wild animals can be infected with pathogenic trypanosomes, and many of these remain carriers of the organisms (Molyneux and Ashford, 1983). Ruminants are widely known to be active reservoirs of the trypanosomes (Molyneux and Ashford, 1983). Wild equidae, lions, leopards and wild pigs are all susceptible and can also serve as carriers of trypanosomes (Molyneux and Ashford, 1983).

2.4 Transmission and Distribution

In Africa, the primary vector of *T. congolense*, *T. vivax* and *T. brucei* is the tsetse fly (Hoare, 1972). These trypanosomes replicate in the tsetse fly and are transmitted through tsetse fly saliva when the fly feeds on an animal. The three main species of tsetse flies for transmission of trypanosomes are *Glossina morsitans*, which favours the open woodland savanna; *G. palpalis*, which prefers the shaded habitat immediately adjacent to rivers and

lakes; and *G. fusca*, which favours the high, dense forest areas (Hoare, 1972). The distribution of the tsetse-transmitted African trypanosomes is governed by that of their tsetse vectors, which infest an area of sub-Saharan Africa that extends from the southern edge of the Sahara desert (latitude 15° N) to Angola, Zimbabwe and Mozambique (latitude 20° S). Trypanosomosis is also mechanically transmitted by tsetse and other biting flies through the transfer of blood from one animal to another. The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota*, *Liperosia*, *Stomoxys* and *Chrysops* flies have also been implicated (Hoare, 1972). In Africa, both *T. vivax* and *T. b. brucei* have spread beyond the "tsetse fly belts" (WHO, 2003), where transmission is principally by tabanid and hippoboscid flies. Of the three African animal trypanosomes, only *T. vivax* occurs in the Western Hemisphere in at least 10 countries in the Caribbean, South and Central America (Hoare, 1972).

In Nigeria, animal trypanosomosis is distributed from the mangrove forest to the Sudan savanna due to the presence of tsetse flies in these areas. The Sahel savanna, the plateaux, including Mambila, Jos and Obudu areas are classified as tsetse-free, however cases of trypanosomosis have been reported in Jos Plateau and the Sahel north of Maiduguri (Anosa, 1991a). Anosa, (1991a) reported that between 75- 80 percent of the landmass area of Nigeria is infested with eleven species of tsetse fly, which are capable of being vectors of both animal and human trypanosomosis (Anosa, 1991a). Several surveys have been carried out in Nigeria to evaluate the situation with regards to the incidence of bovine trypanosomosis (Folkers and Jones-Davies, 1966). According to Anosa (1991a), prevalence of trypanosomosis dropped from 89.6 percent in 1915 to between 30-45 percent in the 1950s and 1960s as a result of introduction of trypanocidal drugs. The incidence of bovine

trypanosomosis in northern Nigeria was reported as 33 percent based on sampling of 925 cattle using the blood smear techniques (Glover, 1961) with the relative frequency of *T. vivax* as 92*%, T. brucei,* 5% and *T. congolense* 3%. Tsetse risk and trypanosomosis acquired along a major trade cattle route from Jibiya in the north to the south-west region of Nigeria have been described (Yesufu and Mshelbwala, 1973) and the investigators found infection rates of 100%, 42.8% and 28.6% for *T. vivax, T. brucei* and *T. congolense,* respectively. Godfrey *et al*. (1965) also found an infection rate of 42% in another herd of trade cattle at Ilorin. In another study of cattle (4,043) that trekked from northern Nigerian to south-west of Nigeria, rates of trypanosomosis acquired along the trade route were 54.7%, 6.7%, 2.7% and 5.3% for *T. vivax, T. brucei,T.congolense* and unidentified species of trypanosomes respectively (Folkers and Jones- Davies, 1966). The Northern Nigerian Veterinary Tsetse and Trypanosomiasis Unit Report of 1954 –55 showed a marked rise in the relative incidence of *T.congolense,* which were in accordance with results of Folkers and Jones-Davies (1966). Yesufu and Mshelbwala (1973) also confirmed the findings of Godfrey *et al*. (1965) that *T.vivax* was the most common *Trypanosoma species* infecting trade cattle, while *T. congolense* was more common in the migratory cattle in northern Nigeria (Killick–Kendrick and Godfrey, 1963).*Trypanosoma congolense*, which is the major cause of cattle trypanosomosis in East Africa, is the second most important cause of bovine trypanosomosis after *T. vivax* in Nigeria (Anosa, 1991b).According to Griffin (1978), many of the accounts of naturally occurring trypanosomosis in sheep and goats came from Eastern Africa, and are due to *T. congolense* which is clearly the most widespread and economically important species in the region (Robson and Ashkar, 1972). Kalu (1991) screened three hundred and fifty-five (355) blood samples for trypanosomes using six diagnostic techniques and recorded prevalence of 51.6% in sheep, 33.3% in goats

and 24.6% in cattle. *Trypanosoma vivax* and *T. congolense* were the most prevalent species encountered, accounting for 51.7% and 11.9% of all positive cases, respectively. In another study on trypanosomiasis, using sheep and goats slaughtered at Bodija abbatoir, Ibadan, Kalejaiye *et al.,* (1995) reported prevalence of2.28% and 4.20%in goats and sheep respectively, with *Trypanosoma vivax* accounting for 1.65%, *T. brucei* 0.4% and *T. congolense* 0.2% of the infections in goats.

2.5 Life Cycles and Modes of Transmission of *Trypanosoma species*

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

inflammatory swelling called a chancre (FAO, 2000). Thereafter, they enter the bloodstream, multiply and a parasitaemia, detectable in the peripheral blood, usually becomes apparent 1-3 weeks later. Subsequently, the parasitaemia may persist for many months although its levels may change due to the immune response of the host (Urquhart *et al.*, 1996). Trypanosomes use cyclic transmission where the arthropod vector is a necessary vector, in which the trypanosomes multiply, undergoing a series of morphological transformations before metacyclic forms (trypomastigotes) infectious to the next mammalian host are produced. When multiplication occurs in the digestive tract and proboscis, such that the new infection is transmitted when feeding, the process is known as anterior station development and the various species of trypanosomes which use this process are often considered as a group, the salivaria. All salivaria are trypanosomes transmitted by tsetse flies, the main species being *T. congolense, T. vivax* and *T.brucei* (Urquhart *et al.*, 1996; FAO, 2000). In other trypanosomes, multiplication and transformation occurs in the gut and the infective forms migrate to the rectum and are passed with the faeces.This is posterior station development and the trypanosome species using this process are the stercoraria. The human infective *T. cruzi* of South and Central America belongs to this group. In domestic animals these are all relatively non-pathogenic trypanosomes such as *T. theileria* and *T. melophagium,* transmitted by tabanid flies and keds respectively.

Non-cyclical transmission is essentially mechanical transmission in which the trypanosomes are transferred from one mammalian host to another by the interrupted feeding of biting insects, notably tabanids and *Stomoxys*. The trypanosomes in or on the contaminated proboscis do not multiply and die quickly so that cross contamination is only possible for a few hours (Urquhart *et al.*, 1996).The salivarian trypanosomes, normally

transmitted cyclically in tsetse flies, may on some occasions be transmitted mechanically (FAO, 1998; 2000). The importance of this latter mode of transmission is variable from place to place, depending on the numbers of hosts and biting insects present, and also on the species of trypanosome. Large biting insects such as tabanids carry more blood and are more likely to act as mechanical vectors than for example mosquitoes. Tsetse themselves can also act as mechanical vectors (FAO, 1998).The life cycle of *T. evansi* is simple and direct. It does not undergo any development or multiplication in its insect vectors. The parasite multiplies by binary fission only in the animal host (Brun *et al.*, 1998). *Trypanosoma evansi* does not develop in its insect vectors because it lacks maxicircle DNA that is necessary for development in vectors (WHO, 2009).Apart from the classical cyclical and non-cyclical transmissions, dogs, cats and wild carnivores may become infected by eating fresh carcasses or organs of animals which have died of trypanosomosis; the parasite penetrating oral abrasions (Urquhart *et al.*, 1996).

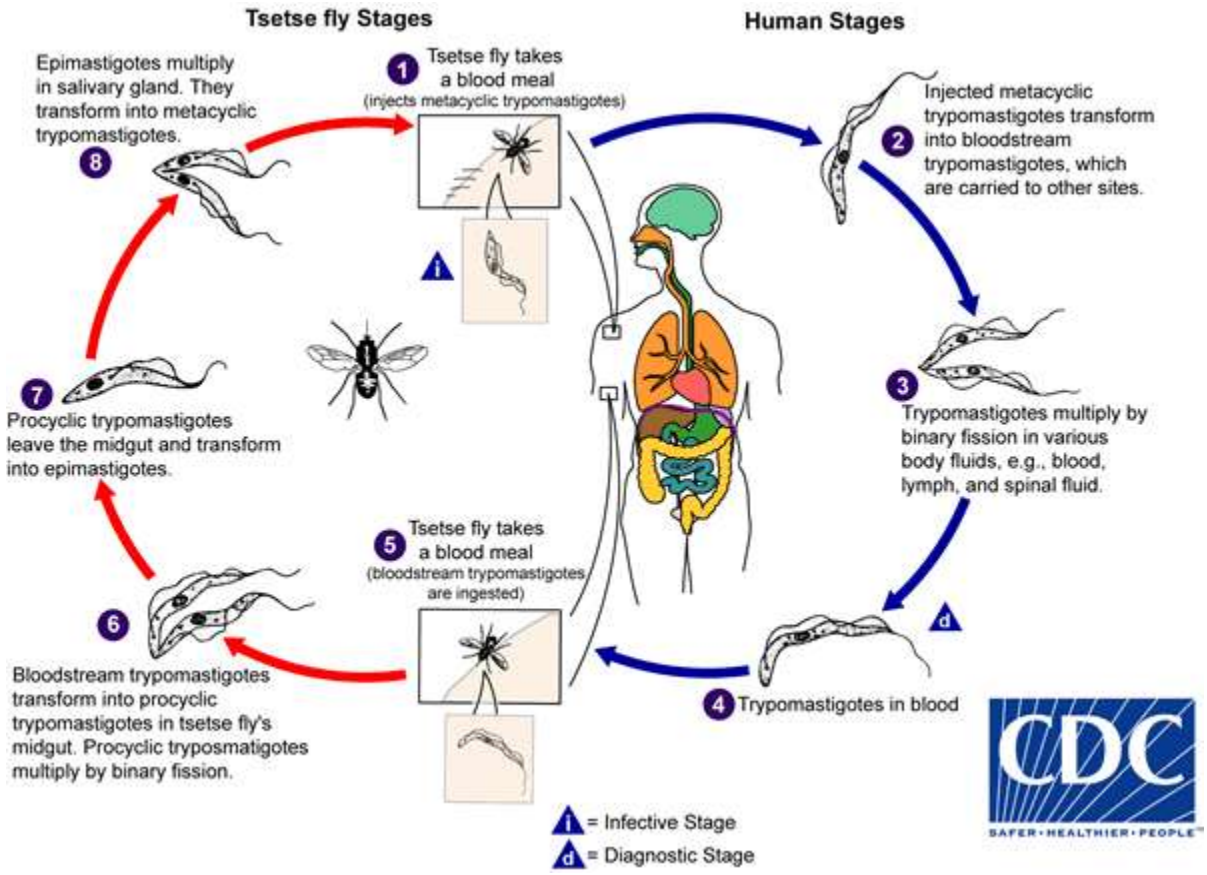


Figure 2.1: Life Cycles and Modes of Transmission of *Trypanosoma species*

Adapted from WHO, 2009

2.6 Antigenic Variation

Trypanosomes undergo antigenic variation that enables them to evade the hosts' immune system (Morrison *et al*., 2009). Antigenic variation occurs when a new generation of trypanosome arises that expresses a different variable surface glycoprotein (VSG). The VSG is a glycoprotein with a molecular weight of about 65,000 KDa that forms a 12-15 nm thick external monolayer over the plasma membrane and is attached to it by a glycosyl-phosphatidyl inositol (GPI) anchor (Blum *et al.*, 2008). It covers the entire parasite including the flagellum presumably as a protective shield against host antibodies (Morrison *et al*., 2009). This protective coat determines the antigenic features of the parasite; is easily recognised by the host and is highly immunogenic. Perhaps the most important aspect of trypanosomosis which accounts for the persistent parasitaemia is the way in which the parasite evades the immune response of the host through antigenic variation (Urquhart *et al.,* 1996). The repeated switching of the glycoprotein coat is now known to depend on a loosely ordered sequential expression of an undefined number of genes, each coding for a different glycoprotein coat. This, together with the finding that metacyclic trypanosomes may be a variation of antigenic types each expressing a different genetic repertoire, explains why domestic animals even if treated successfully, are often immediately susceptible to reinfection (Urquhart *et al.,* 1996). Once a host is infected with a trypanosome manifesting a specific antigen, known as variable antigen type (VAT), the host responds by producing specific antibodies to eliminate the proliferating trypanosomes. Successive waves of parasitaemia occur when a new VAT becomes predominant. These cyclical waves of parasitaemia are characteristic of trypanosome infections and are associated with clinical signs of pyrexia (Losos, 1980; Dargantes *et al.*, 2005a). Two or several VATs may be present during the second and subsequent parasitaemic waves

(Dargantes *et al.*, 2005a). Whilst some authors believe that the host's immune system triggers antigenic variation, others believe that antibodies selectively regulate rather than initiate VSG changes. This is because VSG switches have been observed *in vitro* in the absence of antibodies, in immuno-compromised animals and in early infections before antibodies are produced. There are over 1,000 genes encoding different VSGs but only 10, one gene coding for a particular VSG, is expressed at any point in time (WHO, 2009; Morrison *et al*., 2009). The expression of a new VSG may also involve genomic rearrangement (Morrison *et al*., 2009).

2.7 Pathogenesis of Trypanosomosis

Initial replication of trypanosomes is at the site of inoculation in the skin which results in a swelling and a sore (chancre) and then spread to the lymphnodes and blood and continues to replicate (FAO, 2000). *Trypanosoma vivax, T.congolense* and *T.brucei* are characteristically present in the bloodstream. *Trypanosoma brucei* is also found extravascularly in tissues, for example, the myocardium, the central nervous system and the reproductive tract (FAO, 1998). Lymphoid enlargement and splenomegaly develop, associated with plasma cell hyperplasia and hypergammaglobulinaemia, which is primarily due to an increase in immunoglobulin M. Concurrently there is a variable degree of suppression of immune responses to other antigens such as microbial pathogens or vaccines (FAO, 1998). Ultimately, in infections of long duration, the lymphoid organs and spleen become shrunken due to exhaustion of their cellular elements. Anaemia is a cardinal feature of the disease, particularly in cattle, and initially it is proportional to the degree of parasitaemia (FAO, 2000). It is haemolytic in that the red blood cells are removed from

circulation by the expanding mononuclear phagocytic system. Cell degeneration and inflammatory infiltrates occur in many organs such as the skeletal muscles and the CNS, but perhaps most significantly in the myocardium, where there is separation and degeneration of the muscle fibres (Urquhart *et al.,* 1996). The responses of antibodies developed against the glycoprotein coat of the trypanosomes kill the parasites and result in the development of immunocomplexes. Antibodies however do not clear the infection, since trypanosomes have genes that can code for a number of different surface-coat glycoproteins and therefore change their surface antigenic makeup to evade the antibodies. Thus there is a persistent infection that results in a continuing cycle of trypanosome replication, antibody production, immunocomplex development and changing surface-coat glycoproteins (Urquhart *et al.,* 1996).

2.8 Prepatent Period and Parasitaemic Waves

The prepatent period varies from species to species and depends on the susceptibility of the host, pathogenicity of the isolate, route and dose of infection and the diagnostic method used to detect parasitaemia. The prepatent period in mice infected with approximately 10^4 trypanosomes was 4 to 12 hours, 1 day and 2 to 5 days post-infection using polymerase chain reaction (PCR), micro-haematocrit centrifugation technique (MHCT) and direct blood examination, respectively (Ijaz *et al.*, 1998; Fernández *et al.*, 2009). Likewise, data from experimental studies indicated that trypanosomes could be detected in blood as early as 24 hours in horses (Wernery *et al.*, 2001), 48 hours in goats and cattle (Payne *et al.*, 1993; Dargantes *et al.*, 2005b), 3 days in Rusa deer, pigs and sheep (Audu *et al.*, 1999; Reid *et al.*, 1999), 6 days in Australian wallabies (Reid *et al.*, 2001), and 14 days in buffalo (Holland *et al.*, 2001a) using MHCT. The prepatent period was 3 days in buffalo if mouse

inoculation test (MIT) was used (Holland *et al.*, 2001a). The prepatent period of as early as 2 days, have been reported in South American coati (Herrera *et al.*, 2002), 5 days in rats (Al-Mohammed, 2006), and 9 days in dogs (Aquino *et al.*, 2002) after intravenous infection with *T.evansi*. Also, Raina *et al*. (1985) observed a prepatent period of 8 to 12 days and 2 to 10 days, respectively, following oral challenge of dogs and mice with *T. evansi*. The characteristic fluctuating parasitaemia in animals infected with *T. evansi* is due to the interplay of the immune response of the host and the ability of the parasite to evade it by antigenic variation. These waves of parasitaemia are directly related to the rise and fall of the body temperature of the host and occur at intervals of 6 to 7 days in cattle (Payne *et al.*, 1993), 8.3 days in sheep (Audu *et al.*, 1999) and 13 to 19 days in goats (Dargantes *et al.*, 2005b).

2.9 Clinical Signs

Because of their presence in the blood, the invading parasites produce numerous changes in the cellular and biochemical constituents of blood (Igbokwe and Mohammed, 1992; Taiwo *et al*., 2003). *Trypanosoma brucei* infection like other trypanosome infections precipitate increased red blood cell destruction which results in anaemia as well as tissue damage (Ekanem and Yusuf, 2008;Akanji *et al.*, 2009).

There is also a very high significant decrease in live body weight observed in rat infected with *Trypanosoma brucei* which is an indication of the severity of the disease infection (Ajakaye *et al.*, 2013). This phenomenon may possibly be as a result of parasite induced anorexia. Because simultaneous infection with more than one trypanosome species are very common and simultaneous infection with trypanosomes and other hemoparasites (*Babesia* ,*Theileria*, *Anaplasma* and *Ehrlichia*) frequently occurs it is difficult to conclude which

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

2.9.1 Pathogenesis of anaemia in trypanosomosis

At an early stage there is phagocytosis of red cells by the white cells of the host. The red cells apparently become coated with toxins and material from trypanosomes which tricks the phagocytes into mistaking them for foreign invaders and remove them (auto-immunity). And it is also possible that the anaemia caused by phagocytosis is increased by toxic substances emanating from the trypanosomes, which destroys cells directly by lysis (FAO, 1998).The haematopoietic system tries to compensate for loss of erythrocytes by increasing its activity but later in the chronic stages of trypanosomosis, other toxins from the parasite exert a depressing effect on the haematopoietic system and the host is unable to produce as many red cells as are removed (FAO, 1998) and the resultant anaemia means a reduction in

haemoglobin and therefore in the oxygen carrying capacity of the blood. Insufficient oxygen is available to the cells and the efficiency of their normal activities is reduced leading to deterioration of health condition (FAO, 1998).

The mechanism or pathophysiology of anaemia in trypanosomosis is complex and multifactorial in origin (Naessens *et al*., 2005). It initiates a cascade of events leading to haemolytic anaemia and cardiovascular collapse (Anosa, 1988). In human trypanosomosis, disseminated intravascular coagulation has been reported (Barret-Connor *et al*., 1973).Among the complex and multifactorial etiologies associated with the anaemia is haemolysin, a sensory/excretory product of living trypanosomes. This product is known to lyse red blood cells in the absence of antibodies (*in-vitro*) and causes haemodilution (*in-vivo*). This mechanism has been adequately described in gold fish (*Carassius auratus*) infected with *Trypanosoma dahilewskyi* (Nazrul-Islam and Woo, 1991) and in murine models infected with *T. b. rhodesiense* (Naessens *et al*., 2005). Haemolytic anaemia caused by animal and human trypanosomes has been reported in *T. brucei* infection of red fronted gazelles (*Gazella rufifrons*) (Mbaya *et al*., 2009a), vervet monkeys (*Cercopethicus aethiopes*) (Abenga and Anosa, 2006), and baboons (*Papio anubis*) (Mbaya *et al*., 2009b), sheep and goats (Ikede and Losos, 1972; Anosa, 1977).

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

2.9.1.1 Phase I: Acute crises

This phase begins with the initial appearance of trypanosomes in peripheral circulation. The parasitaemia in this case is usually high, fluctuating and evident in most days (Abenga and Anosa, 2006; Mbaya *et al*., 2009a; Mbaya *et al*., 2010;Mbaya *et al*., 2011). During this

phase the anaemia is morphologically classified as macrocytic and normochromic (Maxie and Losos, 1979; Anosa and Isoun, 1980). At this stage death commonly occurs due to severe pancytopoenia and other pathologies (Anosa, 1988). Sub-acute cases have been produced experimentally in rodents infected with *T. congolense* (Isoun and Esuroso, 1972) and with *T. brucei* (Mbaya *et al*., 2011).

2.9.1.2 Phase II: chronic

This phase follows the acute crises phase and is characterized by low levels of parasitaemia with low to moderate erythrocyte values at this point and this persists with minor fluctuations. This period ranges from several weeks to months. With the *T. brucei* groups which mostly invade tissues, this is the aparasitaemic phase when the parasites establish extravascularly and are less numerous in peripheral circulations (Rabo, 1995) or absent (Mbaya *et al*., 2007, 2009a). In this chronic phase, the morphological classification of the anaemia is normocytic normochromic (Maxie and Losos, 1979).

2.9.1.3 Phase III: recovery

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

2.9.2 The mechanism of anaemia in trypanosomosis

The interplay of several factors acting either individually or synergistically contributes to the development of haemolytic anaemia in human and animal trypanosomosis. Most common among these factors are erythrocyte injury caused by lashing action of

trypanosome flagella, undulating pyrexia, platelets aggregation, toxins and metabolites from trypanosomes, lipid peroxidation and malnutrition (Saror, 1982; Igbokwe, 1994).

2.9.2.1 Anaemia through mechanical injury to erythrocytes

Anaemia caused by mechanical injury to erythrocyte occurs by the lashing action of the powerful locomotory flagella and microtubule reinforced bodies of the millions of the organisms during parasitaemia (Vickerman and Tetley, 1978). Erythrocyte membrane damage has also been associated with adhesion of erythrocytes, platelets and reticulocytes to trypanosome surfaces via sialic acid receptors leading to damages to erythrocyte cell membranes (Bungener and Muller, 1976; Banks, 1980; Anosa and Kaneko, 1983; Shehu *et al*., 2006). As such, several areas of discontinuity occur along the surface of erythrocyte membranes where they adhere to the trypanosomes. Mechanical damage to vascular endothelium has been reported when tissue-invading trypanosomes such as the *T. brucei* group penetrate tissues via the interstices (Anosa and Kaneko, 1983).

2.9.2.2 Anaemia through undulating pyrexia

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

2.9.2.3 Anaemia through platelets aggregation (Microangiopathy)

Intact trypanosomes or fragments of trypanosomes may cause platelet aggregation commonly called microangiopathy (Davies *et al*., 1974). This can lead to the release of platelets autoantibodies that in turn releases procoagulants and thereby causing fibrin deposits. Subsequently, microthrombi formation or disseminated intravascular coagulation occurs (Igbokwe, 1994). During trypanosomosis, erythrocytes with weak cell membranes become fragmented and lysed as they squeeze through the fibrin deposits of the micro thrombi (Anosa and Kaneko, 1983; Murray and Dexter, 1988). Disseminated intravascular coagulation has been reported in *T. b. gambiense* infection of the baboon (*Papio Anubis*) (Mbaya *et al*., 2009b), *T. vivax* infection of cattle (Isoun and Esuroroso, 1972) and in goats (Vanden Ingh *et al*., 1976; Anosa and Isoun, 1983).

2.9.2.4 Anaemia caused by trypanosome toxins and metabolites

Living and dead trypanosomes can produce various forms of active chemical substances, which can elicit erythrocyte injury (Zwart and Veenendal, 1978; Naessens *et al*., 2005). Common among these chemical substances are proteases, neuraminidase, phospholipase, free fatty acids, pyruvates and aromatic byproducts. Neuraminidase has been generated *in-vitro* by *T. vivax* during periods of parasitaemia, making erythrocytes prone to phagocytosis (Esievo, 1979; 1983). One of the factors that make erythrocytes prone to phagocytosis by the expanded mononuclear phagocytic system (MPS) during trypanosomosis is associated with the activity of neuraminidase. This enzyme cleaves off sialic acids on the surface of

erythrocytes and thereby disabling them (Verma and Gautam, 1978; Igbokwe, 1994; Adamu *et al*., 2009) and by damaging erythropoietin (Igbokwe *et al.*, 1989).Trypanosomes are capable of releasing proteolytic lysosomal enzymes (proteases) from pockets on their flagella and from damaged or dead trypanosomes (Vickermon and Tetley, 1978; Rautenberg *et al*., 1982; Londsdale-Eccles and Grab, 1986; Igbokwe, 1994) and these enzymes when released into the general circulation are capable of damaging erythrocytes and vascular endothelium by cleaving sialic acid fractions from the cell membrane in the form of glycopeptides (Cook *et al*., 1966). It was also reported that aromatic amino acids could be metabolized by trypanosome to produce toxic by-products, which acts directly on the erythrocyte cell membrane to cause osmotic fragility and lyses (Igbokwe, 1994). Similarly, phenylalanine could be catabolized to phenylpyruvate, which is proteolytic in nature and inhibitory to mitochondrial gluconeogenesis (Igbokwe, 1994). Tryptophan can also be broken down during trypanosomosis to indole-ethanol, which damages erythrocyte cell membranes (Igbokwe, 1994).

2.10 Biochemical Changes

The implications of biochemical changes to the disease pathogenesis have been reported by several investigators. Taiwo *et al.* (2003) reported elevated levels of total protein, globulin and decrease in cholesterol and glucose levels in sheep experimentally infected with *T. b.brucei* and *Trypanosoma congolense.* Abenga and Anosa (2005) reported increased protein, creatinine and globulin levels in monkeys experimentally infected with *Trypanosoma brucei gambiense*. Vickerman and Tetley (1979) had also reported hypo-albuminaemia which may occur because of the uptake of albumin-bound fatty acid and lipoproteins and haemodilution (Katunguka- Rwakishaya *et al.*, 1992b) in Trypanosoma

infected animals. Awobode (2006) reported hypoalbuminaemia, normal total protein, decreased urea level and increased creatinine level in natural human trypanosomal infections.

Furthermore, increasing evidences indicate that free radical-induced oxidative stress plays an important role in the pathogenesis of African trypanosomiasis (Igbokwe, 1994; Ogunsanmi and Taiwo, 2007; Umar *et al.,* 2007; Akanji *et al.,* 2009). and host animal activated macrophages and monocytes (Schwacha and Loegering, 1992).

2.11 Trypanosome-Induced Pathology in Livestock

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

be seen (FAO, 1998).

2.12 Vector

Tsetse, sometimes spelled tzetze and also known as tik-tik flies, are large biting flies that inhabit much of mid-continental Africa between the Sahara and the Kalahari deserts (Rogers *et al.*, 1996). They live by feeding on the blood of vertebrate animals and are the primary biological vectors of *Wuchereria bancrofti*, which cause Elephantiasis, and trypanosomes, which cause human and animal trypanosomiasis, also known as sleeping sickness and nagana respectively. Tsetse, have been extensively studied because of their disease transmission. These flies are multivoltine, typically producing about four generations yearly, and up to 31 generations total over their entire lifespan (Cockerell, 1997). There are 23 species of tsetse flies(Cockerell, 1997).

Fossilized tsetse have been recovered from the Florissant Fossil Beds in Colorado, laid down some 34 million years ago (Cockerell, 1997). There are 23 species of tsetse flies. Diseases transmitted by tsetse flies kill 250,000–300,000 people per year (WHO, 2006).

2.13 Morphology of the Tsetse Fly

According to Jordan, (2006), Tsetse fly can be seen as independent individuals in two forms: as third instar larvae, and as adults. They first become separate from their mothers during the third larval instar, during which they have the typical appearance of maggots. However, this life stage is short, lasting at most a few hours, and is almost never observed outside of the laboratory.The tsetse then become puparia: small, hard shelled, oblongs with two distinctive, small, dark lobes at one end. Tsetse puparia are under 1.0 cm long within the puparial shell, tsetse complete the last two larval instars and the pupal stage. It then emerge as adult flies. Tsetse adults are relatively large flies, with lengths of ½–1½ cm

(Jordan, 2006), and have a recognizable shape or bauplan which makes them easy to distinguish from other flies. They have large heads, distinctly separated eyes, and unusual antennae. The tsetse thorax is quite large, while the abdomen is wide rather than elongated and shorter than the wings. (Fig 2.2)

Tsetse flies fold their wings completely when they are resting so that one wing rests directly on top of the other over their abdomen, and also have a long proboscis, which extends directly forward and is attached by a distinct bulb to the bottom of their head. (Jordan, 2006)



Figure 2. 2: **A typical Tsetse fly (*Glossina mortistan*).**

Like all other insects, Tsetse flies have an adult body comprising three visibly distinct parts: the head, the thorax and the abdomen:

(Jordan, 2006)

The head has large eyes, distinctly separated on each side, and a distinct, forward-pointing proboscis attached underneath by a large bulb. The thorax is large, made of three fused segments. Three pairs of legs are attached to the thorax, as are two wings and two halteres. The abdomen is short but wide and changes dramatically in volume during feeding (Gouteux, 1997).

Most tsetse flies are physically very tough. Houseflies are easily killed with a fly-swatter but it takes a great deal of effort to crush a tsetse fly. The internal anatomy of tsetse is fairly typical of the insects. The crop is large enough to accommodate a huge increase in size during the bloodmeal since tsetse can take a bloodmeal weighing as much as themselves. The reproductive tract of adult females includes a uterus which can become large enough to hold the third instar larva at the end of each pregnancy (Gouteux, 1997).

2.14 Life Cycle of Tsetse Fly

Tsetse has an unusual life cycle which may be due to the richness of their food source. Female tsetse only fertilizes one egg at a time and retains each egg within their uterus to have the offspring develop internally during the first larval stages, a strategy called adenotrophic viviparity. During this time, the female feeds the developing offspring with a milky substance secreted by a modified gland in the uterus. In the third larval stage, the tsetse larva finally leave the uterus and begin their independent life. However, the newly independent tsetse larva simply crawls into the ground, and forms a hard outer shell called the puparial case, in which it completes its morphological transformation into an adult fly.

This lifestage has a variable duration, generally twenty to thirty days, and the larva must rely on stored resources during this time. The importance of the richness of blood to this development can be seen since all tsetse development before it emerges from the puparial case as a full adult occurs without feeding, based only on nutritional resources provided by the female parent. The female must get enough energy for her needs, for the needs of her developing offspring, and to store the resources which her offspring will require until it emerges as an adult (Gouteux, 1997).

Technically the insects undergo the standard development process of insects which comprises oocyte formation, ovulation and fertilization, development of the egg, five larval stages, a pupal stage, and the emergence and maturation of the adult.

2.15 Diagnosis of Trypanosomosis

Routine diagnosis of trypanosomosis in the field is undertaken via clinical signs and knowledge of the endemicity of the disease in the area (WHO, 1998; FAO, 2000). However, other more accurate methods of diagnosis include: parasitology, serology and molecular biology. Some of the parasitological methods currently in use are: stained thin blood films, the buffy coat dark ground-phase contrast technique (BCT), the haematocrit centrifugation technique (HCT) and the miniature-anion exchange centrifugation technique (mAECT) (FAO, 2000). These methods are confirmatory since they depend on demonstration of trypanosomes. However, the parasitological detection methods have a limited analytical sensitivity (i.e. lower detection limit and may lead to under-reporting of the prevalence of disease (FAO, 2000).More sensitive diagnostic methods, including Polymerase chain reaction (PCR), Indirect fluorescent antibody test and the detection of trypanosome-specific antibodies and antigens have been developed (FAO, 2000).

2.16 Trypanosomosis Control

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

2.16.1 Trypanocidal drugs

The control of trypanosomosis in domestic livestock depends mainly upon the use of drugs, either curatively or as a prophylactic (WHO, 2005). Drugs currently recommended for chemotherapy of animal trypanosomosis come from only three closely related groups. These are the phenanthridines, isometamidium and homidium, and the aromatic diamidine, diaminazone. Only isometamidium and homidium are recommended for prophylaxis. The incidence of resistance to these drugs is apparently increasing and the main means of controlling the disease is therefore under threat (Peregrin, 1994). Resistance to the available drugs is on the increase and their continued use is expensive for livestock owners, it has been estimated that at least US$ 20 million (approximately 50 million doses) is spent annually to treat or protect animals exposed to trypanosomes in Africa. The actual amount of trypanocides used is difficult to estimate, particularly in recent years, since the distribution of trypanocides has become more decentralized, with a number of generic brands being sold, increasingly through traders and shopkeepers and less through official veterinary channels (WHO, 2005). Drug resistant trypanosomes develop through; under dosing which may occur for a variety of reasons such asunderestimation of animal body weight, over diluted solutions or incorrectly calculated dosevolume, incorrect (and therefore ineffective) injection or an incorrect strategy of drug use. Measures that may delay the development of drug resistance are to reduce the selection pressure on

trypanosome populations by avoiding exclusive reliance on drugs for trypanosomosis control and avoiding mass treatment of livestock at short intervals (Leak, 1998).

2.16.2 Tsetse vector control

Tsetse vector control methods relying on large scale bush clearing and aerialspraying methods are no longer used due to environmental concerns (Weidhaas and Haile, 1978). Tsetse control currently relies on two bait systems: insecticide-treated traps and targets and insecticide treated livestock. Sterile Insect Technique (SIT) has also been used in efforts to eradicate tsetse flies in some areas. Because of the stability of tsetse populations and their low reproductive rate, little sustained mortality pressure (additional to natural mortality) needs to be exerted on a population to cause its extinction (Weidhaas and Haile, 1978).That makes them good candidates for traps and target control methods. Not to be forgotten though are the risk of reinvasion or immigration into an area already cleared of tsetse flies. The theory behind this control method is simple: the flies are visibly attracted to a trap or target. This attraction may be further helped by the use of olfactory attractants. When the tsetse lands on a trap or target they either receive a lethal dose of insecticide, or are caught in the trap and subsequently die (Leak, 1998). The effectiveness of traps and targets will depend on when the flies are active, how they move in their active state, whether they will move into the vicinity of a trap or target and finally, whether they are trapped or killed (FAO, 2001).Insecticide-treated livestock was developed as a method of tsetse control from the concept of baited traps and targets (Baylis and Stevenson, 1997). It is widely accepted by a majority of stockowners in Africa. The method has been used to control tsetse and trypanosomosis with varying results in Kenya (Stevenson *et al*., 1991). Most commonly used are the synthetic pyrethroids, and of these deltamethrin, appears to be the most potent

and it is also low in mammalian toxicity and has minimal environmental impact (Thompson *et al*., 1991). Extensive use of insecticides on cattle for tsetse control appears to have the potential to interfere with endemic stability immunity of cattle to several tick borne diseases. Thus the long term use of these products may jeopardize control of tick borne diseases (FAO 2001).

2.16.3 Vaccination

Attempts to vaccinate cattle against trypanosomosis started at an early stage in trypanosomosis research (FAO, 2001). For example, efforts have been made to protect the animals by deliberately infecting them with known strains of trypanosomes and then treating them at various intervals following infection. These animals were said to be tolerant or to some extent ―premunized‖ but the resistence broke down relatively easy (FAO, 2001). Attempts were also made to vaccinate cattle with killed trypanosomes by giving part-curative doses of trypanocidal drugs after infection, or by injection of small numbers of living trypanosomes (FAO, 2001). These attempts failed and it is now recognized that the major obstacle to immunization lies in the phenomenon of antigenic variation in trypanosomes. Trypanosomes are covered by a dense coat of variant surface glycoproteins that stimulate antibody production in the host. The surface coat changes successively during the course of an infection, thus avoiding the immune response of the host. At present there are no drugs available that have the ability to interfere with antigen switching (FAO, 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out at the Protozoology laboratory of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Zaria is located between Latitude 11º11" N and Longitude 07º38" E, at an altitude of 686 metres above sea level. It lies within the Guinea Savanna zone, and has 3 distinct seasons namely cold and dry harmattan (Nov- Feb), hot dry/ dusty (March -May) and rainy (Jun- Oct) (Ayo *et al*., 1998). Its annual rainfall, average temperature and relative humidity are 1055 millimetres, 24.55º C and 43.6%, respectively (Meteorological Unit IAR, 2009).

3.2 Experimental Animals

The laboratory animals used were mice, rats, rabbits and guinea fowls. The mice, rats and rabbits were acquired from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, and the guinea fowls were acquired from the research farm of the Division of Agricultural College, Ahmadu Bello University, Zaria. The mice and rats were housed in plastic cages covered with wire mesh, while the rabbits and guinea fowls were kept on the floors with wood shavings litter at the Department of Veterinary Parasitology and Entomology Animal Pen. The animals were maintained on commercial growers poultry feed, maize bran (dusa) and groundnut cake (kuli kuli), compounded in a ratio appropriate for these types of laboratory animals. Water was provided *ad libitum*.

3.3 Source of *Trypanosoma brucei brucei* used

The *Trypanosoma brucei brucei* used for the study was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria. It was originally isolated from a natural infection in cattle, in Kaduna State. The *Trypanosoma brucei brucei* was inoculated into Wistar rats and transported to the Protozoology Research Laboratory, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for proper identification and propagation in rats. Using the Giemsa-stained thin blood smear diagnostic technique (Hoare, 1972), the parasites morphology was used for specific identifications. Consequently the *Trypanosoma brucei brucei* was identified by the presence of small subterminal kinetoplast, pleomorphic nature with some having long free flagellum, some with short free flagellum while some had none but all had conspicuous undulating membrane. The parasite was sub-inoculated intraperitoneally into 3 Wistar rats and 3 mice and kept in separate cages in the Department of Veterinary Parasitology and Entomology, Zaria. Blood samples were collected daily from each of the wister rats and mice through the eyes to determine the level of parasitaemia using the haematocrit centrifugation technique (HCT) as described by Woo (1969) and Biryomumaisho *et al*. (2013).

3.4 Animals Grouping and Inoculation with *Trypanosoma brucei brucei*

Each group consisted of 10 animals assigned into infected and control groups of five animals each, the 10 experimental animals per group were sub divided into two groups (infected and control) of 5 animals each.

Parasitaemia was detected in the donor rats 2-3 days post infection. They were subsequently monitored till the parasitaemia peaked at 30 -40 parasites per field with the heamatocrit centrifugation technique (HCT). Inoculum (infective material) for the infected group was prepared by bleeding rats using capillary tube through the peri orbital / stenous plexus to collect sufficient blood into ethylene diethyl tetra acetic acid (EDTA) Bijou bottles containing normal saline. The mice, rats and rabbits were experimentally infected intraperitonially while the guinea fowls were infected through the wing veins. Each of the mice, rats, rabbits and guinea fowls in the infected group were inoculated using 0.1ml, 0.3ml. 0.5ml and 0.5ml of *Trypanosoma brucei brucei* -infected blood estimated to be 1×10^6 parasites/ ml using haemocytometer.

3.5 Post Infection Monitoring of the Animals

The following indices were determined after the experimental infection of the animals.

3.5.1 Body temperature

The rectal temperature for each experimental animal was assessed on daily basis for mice and rats and three times weekly for rabbits and guinea fowls at 7.00 am in the morning using digital thermometer. The thermometer was inserted into the rectum and tilted to touch the rectal mucosa. After a beep, the thermometer was removed and the body temperature read and recorded in degrees centigrade (^oC).

3.5.2 Body weight

A portable weighing scale was used to determine the weights of the animals three times a week for mice and rats and on weekly basis for rabbits and guinea fowls and was recorded in gramme (g).

3.5.3 Blood collection for parasitological and heamatological analyses

Blood (0.5 ml) was collected from the median cantus of the eye of each infected mice and rats, 1ml from the ear vein of each rabbit and wing web vein of each of the guinea fowl. The blood samples were used for the estimation of parasitaemia and heamatological indices.

3.5.4 Determination of parasitaemia profile

The level of parasitaemia was estimated using haematocrit centrifugation technique (HCT) as described by Woo (1969). The procedure involved filling heparinised micro-capillary tubes (75x1.5mm) to approximately two-third of their volumes with each of the infected blood. The tubes were sealed using a Bunsen burner and thereafter, placed in a micro-haematocrit centrifuge in opposite direction to be balanced, while the sealed ends were allowed to face outwards and spuned for 3 minutes at 1500 revolutions per minute (rpm). The spuned capillary tubes were thereafter placed on a glass slide, oil immersion was applied on the Buffy coat area and viewed under the objective lens (X 40) to see the parasites and score the parasitaemia as described by Woo, (1969) as shown below:

1. + = less than 10 trypanosomes in buffy coat or plasma layer seen per field.

2. ++ = 10 - 20 trypanosomes in buffy coat or plasma layer seen per field.

3. +++ = Numerous (20 – 30) trypanosomes in buffy coat or plasma layer seen per field.

4. ++++ = massive (30 – 40) trypanosomes in buffy coat or plasma layer seen per field.

The parasite count was determined by examination of wet mount microscopically at x 40 magnification according to the method of Herbert and Lumsden (1976). This will involve counting of parasites per field in pure blood or in blood appropriately diluted with buffered phosphate saline.

3.5.5 Thin blood smear for identification of *Trypanosoma specie*

A drop of blood was placed on a clean and grease-free microscope slide. A thin smear of blood was made by sliding another slide on the first slide. The smear was air-dried and fixed in methanol for 3 minutes. The slide was stained in 10 % Giemsa stain for 25 to 30 minutes, rinsed in distilled water, air dried and examined microscopically under oil immersion at X 100 objective (Coles, 1986).The identification of the *Trypanosoma species* was done based on their morphological features as described by Hoare (1972).

3.5.6 Determination of packed cell volume (PCV)

The PCV was determined using standard microhaematocrit centrifugation technique and the values were read using Hawksley microhaematocrit reader (Gellman Hawksley Ltd, 92 England) as described by Coles (1986). The packed cell volume was determined daily for mice and rats, 3 times a week for a period of 14 and 8 weeks for rabbits and guinea fowls respectively. Blood from each of the animal was drawn up at one end of the heparinized capillary tube, until about three-quarters of the length of the tube was filled. The other end

of the tube was sealed over a Bunsen burner .The tubes were then placed in the grooves (individually identified by a number corresponding to the number of the blood sample) of the rotor plate, with the sealed end outwards (to prevent the blood from spilling during centrifugation); the cover was closed and screwed down, and the timing was set for three minutes. After centrifugation, the tubes were removed, care being taken that it remains known to which animal each of the tubes corresponded. The packed cell volume (PCV) value was then read directly with hematocrit reader, which was individually adjusted for the length of the blood column in each tube.

3.5.7 Determination of haemoglobin concentraion

The haemoglobin concentration was determined by cyanmethaemoglobin measurement 0.02 ml of blood was obtained into a clean cuvette and then diluted with 5ml of cyanmethaemoglobin reagent (modified drabkin fluid). After the diluents was added, it was stoppered and inverted 2-3 times and allowed to stand for ten minutes for maximum conversion of haemoglobin to cyanmethaemoglobin. The absorbance of the resulting mixture was read using spectrophotometer (Beckham coulter, model B U520, Austria) at a wavelength of 540 nm against reagent blank. The percentage transmission or optical density at 540 nm was recorded and compared with the reading obtained using a standard solution of cyanmethaemoglobin.

3.5.8 Determination of red blood cell counts

A well mixed heparinized blood was drawn to the 0.5 mark of a red blood cell diluting pipette, 0.9% normal saline was drawn to the “101” mark of same pipette. The pipette was

rotated between the thumb and the fore finger for 3 minutes to allow even distribution of the cells. The pipette was held upright to prevent air bubbles .The unmixed and relatively cell free fluid from the capillary portion of the pipette was expelled. The fore finger was placed over the top (short end) of the pipette and with the pipette held at 45^0 angle, the tip of the pipette was made to touch the junction of the cover glass and the counting chamber. The mixture was allowed to flow under the cover glass until the chamber was completely charged, similarly the opposite side of the hemocytometer was filled. The cells were allowed to settle for about three minute. The total number of cells in the center square and four corners square of the super square was determined, and multiplied by 10,000. This value represented the total number of erythrocytes per microlitre. (Decie and lewis 1991).

3.5.9 Determination of total white blood cell (Leucocyte) counts

Haemocytometer method as described by Dacie and Lewis (1991) was employed for the total leukocyte count. A well mixed heparinized blood was drawn to the 0.5 mark of a white blood cell diluting pipette, 2% glacial acetic acid was drawn to the "11" mark of same pipette. The pipette was rotated between the thumb and the fore finger for 3 minute to allow even distribution of the cells. The pipette was held upright to prevent air bubbles .The unmix and relatively cell free fluid from the capillary portion of the pipette was expelled. The fore finger was placed over the top (short end) of the pipette and with the pipette held at 450 angles, the tip of the pipette was touched to the junction of the cover glass and the counting chamber. The mixture was allowed to flow under the cover glass until the chamber was completely charged; similarly the opposite side of the hemocytometer was filled. The cells were allowed to settle for about three minutes and the ruled area was focused under low power magnification with light reduced and observed for even

distribution of cells. The white blood cells were counted in the four 1sq mm corner areas. All the white blood cells lying within the square and those touching the upper and right hand center lines were counted, the WBC within the four areas were counted in a snake - like manner and the value obtained was multiplied by the factor of 50 to obtain the total leukocyte count.

3.6.10 Histopathological examination

Tissue samples collected from the spleen, kidney, liver and lungs of dead and sacrificed animals were preserved in 10% buffered neutral formalin (BNF). After 48 hours of fixation, the tissue samples were processed (washed in 50% and then 70% alcohol), embedded in paraffin wax and sectioned at 5 microns using a microtome. The sections were mounted on clean grease-free glass slides and stained with Haematoxylin and Eosin (H and E) stains as described by Luna (1968). The stained slides were examined microscopically at X40 objective. Histopathological lesions were observed, recorded and photomicrographed with the aid of a digital camera.

3.7 Data Analyses

Data obtained were presented using chart and tables.

Quantitative variables were summarized as Mean ± Standard error of mean.

T-test was used to determine the statistical significance in the difference between the values of the infected and control groups.

All analyses were carried out using Graph Pad Prism, version 5.0 with P values < 0.05 considered significant.

CHAPTER FOUR

RESULTS

The clinical response of the experimental animals to inoculation of the parasites varied. Consequently, variables of susceptibility such as parasitaemia, body temperature, body weight gain, mortality, and pathology monitored differed among the animals.

4.1 Parasitaemia

The results of parasitaemia determination were as shown in Table 4.1. The onset, course, duration of parasitaemia varied among the infected groups. While the infected guinea fowls showed no detectable parasitaemia, the infected mice, rats and the rabbits were parasitaemic with the following pre-patent periods of 3.5 ± 0.5, 3.5 ± 0.5 and 7.5 ± 0.5 days respectively. The intensity of the parasitaemia was higher in mice and rats, which showed massive parasitaemia within 3 and 5 days, respectively, of patency. Parasitaemia in the infected mice and rats was progressive, while in rabbit undulating parasitaemic wave of 3 – 4 days interval was recorded. Parasitaemia was maintained in all the infected mice and rats up to day's 6 and 8 post-inoculation respectively, when they all died. The rabbits, however, maintained the parasitaemia throughout the experimental period of 14 weeks.

Table 4.1: Parasitaemia profile of mice, rats, rabbits and guinea fowls experimentally infected with *Trypanosoma brucei brucei*

Animals	prepatency or onset of Parasitaemia (days)	Course of parasitaemia	mortality
Mice	3.5 ± 0.5	2-3 parasites per field at the onset. Became massive (uncountable) 3 days after onset.	100%
Rats	3.5 ± 0.5	1-2 parasites per field at onset. massive 5 days pp	100%
Rabbits	7.5 ± 0.5	1 parasite per field at the onset of parasitaemia and increased to 3 per field after 4 days. Regressed and reappeared at longer intervals (4 days).	0
Guinea fowls	___	no detectable parasitaemia	0

4.2 Clinical Observations

4.2.1 Mean rectal temperature

The mean rectal temperature in the infected and control experimental animals were as presented in Figures 4.1 - 4.4. The rectal temperature fluctuated in all the infected animals when compared to those of the respective control groups, which remained relatively unchanged throughout the period of the experiment. The mean rectal temperature fluctuated between 37.3 and 38.9 ºC, 37.5 and 39.0 ºC and between 38.5 and 39.2ºC in the *T. brucei brucei* experimentally infected mice, rats and rabbits, respectively. The mean rectal temperature in the infected guinea fowls remained relatively unchanged throughout the period of the experiment.

There was a significant ($p < 0.05$) difference between the mean rectal temperatures (Figure 4.1) of the infected and control groups of mice at day 4 and 5 post inoculation.

The mean rectal temperature (Figure 4.2) progressively increased in the *T. brucei brucei*-infected rats and was significantly ($p < 0.05$) higher than those of the control beginning from day 3 post-infection and remained so until the death of all the rats during the experiment.

Significantly ($p < 0.05$) higher mean rectal temperature (Figure 4.3), than those of the control rabbits, was observed in the *T. brucei brucei*-infected rabbits at week 5 and 6 post-infection.

The mean rectal temperature (Figure 4.4) in the *T. brucei brucei*-infected and control guinea fowls were more or less the same up to week 4 post-infection. Thereafter, the mean rectal temperature of the *T. brucei brucei*-infected guinea fowls became significantly ($p < 0.05$) higher than those of the control at week 5 and 6 post-infection.

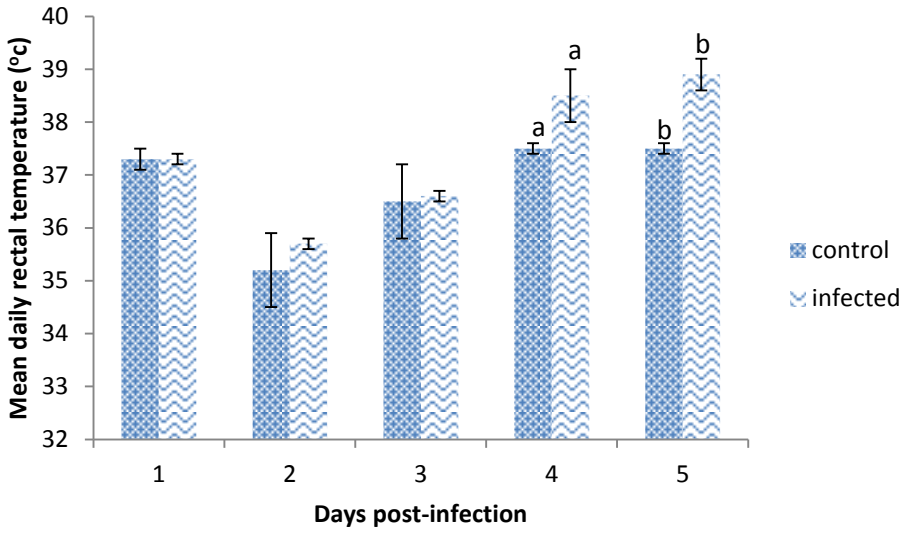


Figure 4.1: Mean daily rectal temperature (°c) profiles of *Trypanosoma brucei brucei* experimentally-infected and control mice.

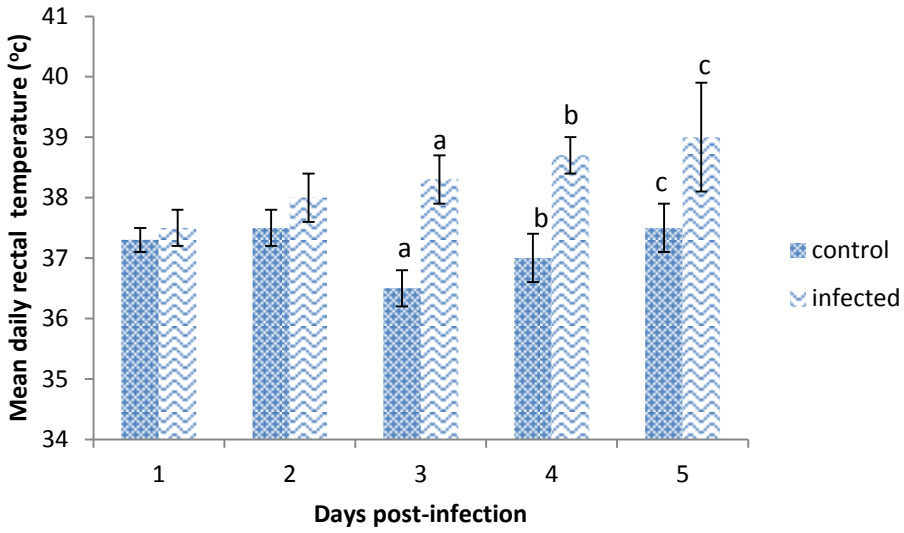


Figure 4.2: Mean daily rectal temperature (^{o}c) of *Trypanosoma brucei brucei* experimentally-infected and control rats.

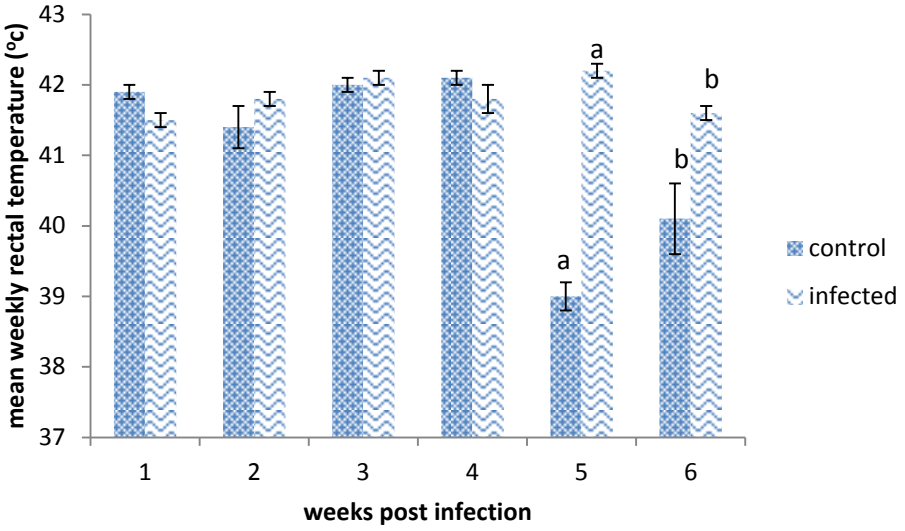


Figure 4.3: Mean weekly rectal temperature (^{o}c) profiles of *Trypanosoma brucei brucei* experimentally-infected and control rabbits.

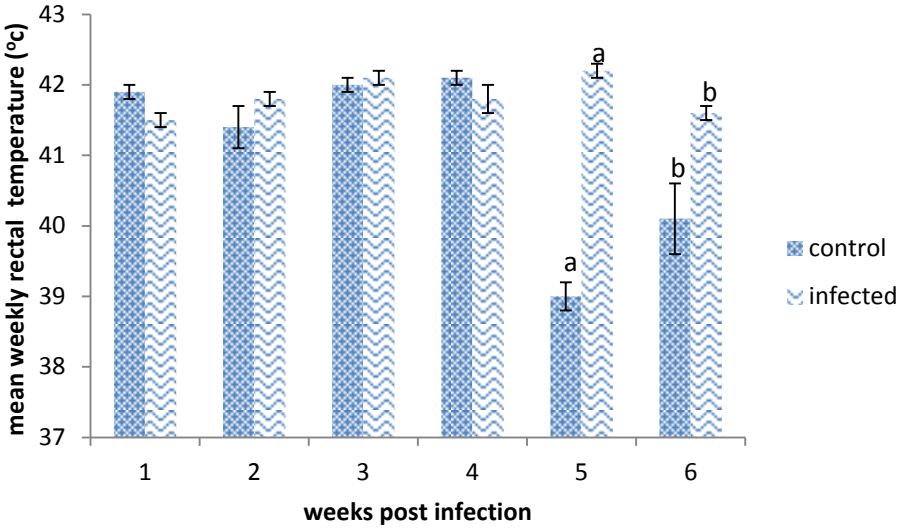


Figure 4.4: Mean weekly rectal temperature (^{o}c) in *Trypanosoma brucei brucei* experimentally-infected and control guinea fowls.

4.2.2 Body weight changes

The mean body weights of the *T. brucei brucei*-infected groups of the experimental animals, pre- and post-infection were as presented in Tables 4.2 and 4.3. Pre-infection, the mean body weights of all the experimental animals showed remarkable increase during the period of acclimatization that lasted 15 days. Post-infection, decreases in the mean body weights of the *T. brucei brucei*-infected mice, rats and rabbits were observed. The mean body weight of infected guinea fowls remained fairly unchanged until week 12 pi, when a drop in the mean body weight from1678g to 1500 g was observed. The mean bodyweights of the infected mice, rats, rabbits dropped from their day 0 values of 19.1g, 140.5g and 990.8 g to 14.2 g, 130.5 g and 725.7 g, respectively.

Table 4.2: Post-infection mean body weight profiles of mice and rats experimentally infected with *Trypanosoma brucei brucei*.

Days Post-infection	Mean Body Weights (g)	
	Mice	Rat
1	19.1±0.8	140.5±0.9
3	18.5±1.5	135.5±0.7
6	14.2±1.4	130.5±1.5
9	*mortality	*mortality

* = All experimental animals in these groups died

Table 4.3: Post-infection mean body weight profiles of rabbits and guinea fowls experimentally infected with *Trypanosoma brucei brucei*.

weeks Post-infection	Mean Body Weights (g)	
	Rabbits	Guinea fowls
1	990.8±1.1	1678±0.9
3	985±0.6	1675±1.7
6	850.7±0.8	1673±1.3
9	830±2.1	1675±0.8
12	750.5±1.4	1500±0.6
15	725.7±0.7	1450±0.9

4.3 Hematological Change in *T. brucei brucei*-infected and Control Mice

4.3.1 Mean packed cell volume, haemoglobin concentration and red blood cell count

The mean packed cell volume (PCV), haemoglobin concentration (HGB) and red blood cell (RBC) count of the infected and control groups of mice were as presented in Figures 4.5 – 4.7. The infection of the mice with *T. brucei brucei* resulted in significantly ($p < 0.05$) lower mean PCV in the infected, compared to the control group on days 5, 6 and 7, post-infection. The overall mean PCV (45.4 ± 1.1 %) of infected mice was significantly lower ($P < 0.05$) than that of the control group (48.9 ± 1.5 %), during the course of the experiment. The mean haemoglobin concentration (HGB) and red blood cell (RBC) count in both the *T. brucei brucei* infected mice followed a pattern similar to that of PCV. The mean PCV, HB and red blood cell count in the control mice remained relatively unchanged up to termination of theexperiment. The overall mean RBC count in the infected mice (7.5 ± 0.5 x 10^{12}/L) was significantly ($p < 0.05$) lower than that of the control (8.2 ± 0.7 x 10^{12}/L).

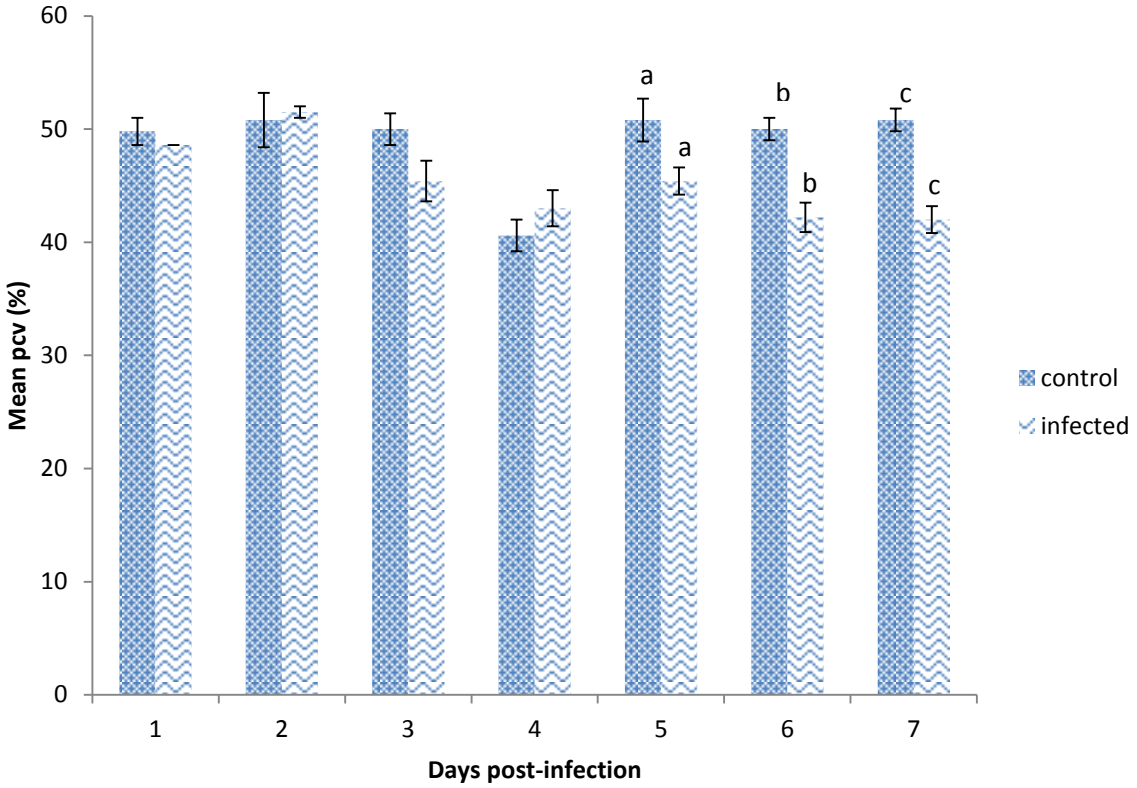


Figure 4.5: Mean daily (±SEM) packed cell volume profiles of *Trypanosoma brucei brucei* experimentally-infected and the control Mice.

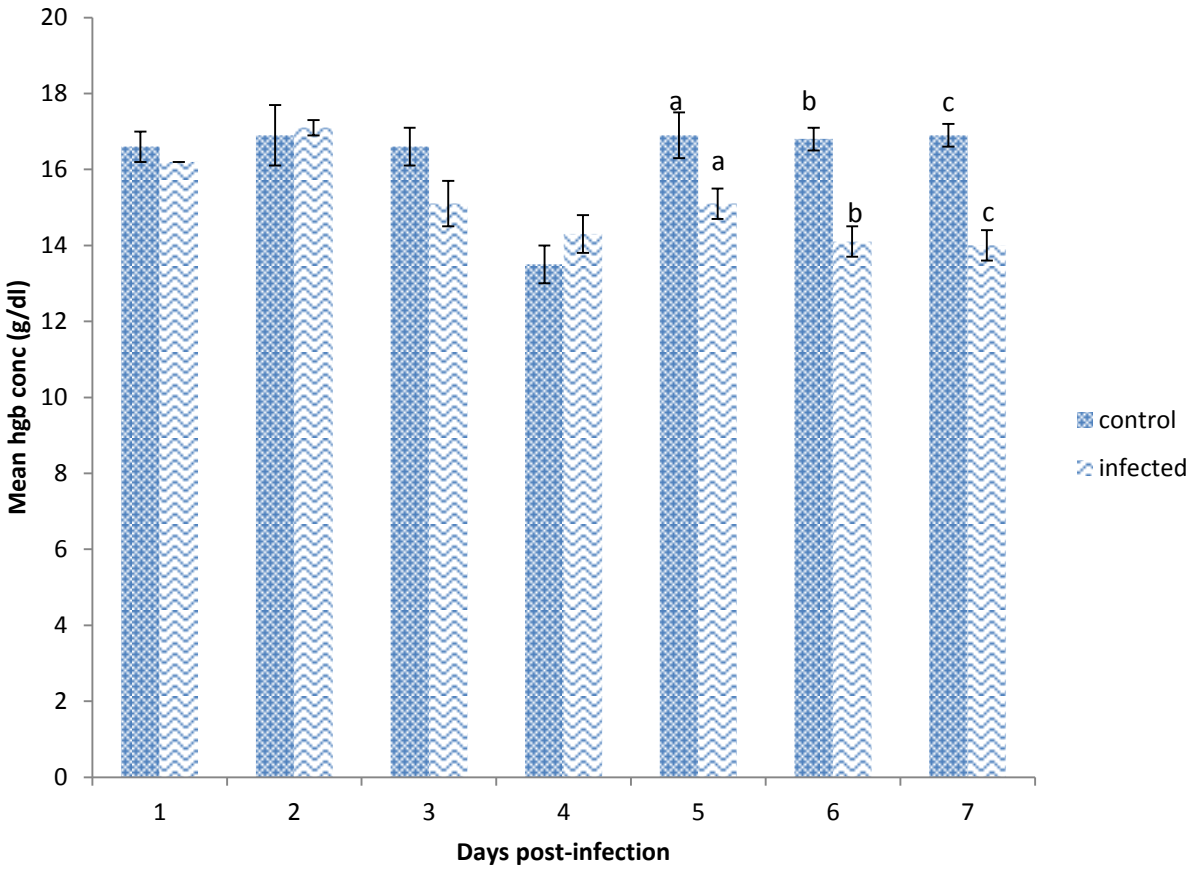


Figure 4.6: Mean daily (±SEM) haemoglobin concentration profiles of *Trypanosoma brucei brucei* experimentally-infected and the control mice.

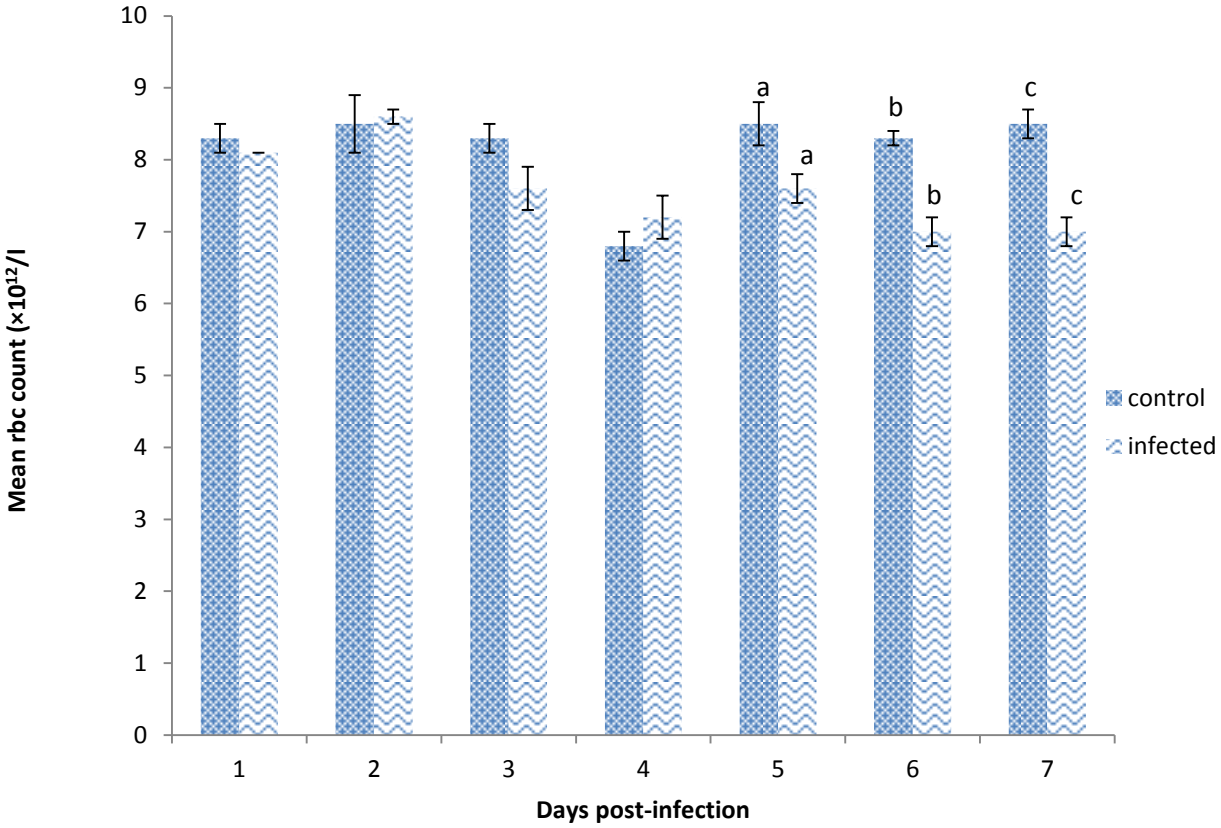


Figure 4.7: Mean daily (±SEM) red blood cell count profiles of *Trypanosoma brucei brucei* experimentally-infected and the control mice.

4.3.2 Mean total white blood cell (WBC) count of the *T. brucei brucei*-infected and control mice

Although the mean WBC was higher in the *T. brucei brucei*-infected mice on days 4, 5 and 6, post-infection, there was no significance ($P > 0.05$) difference between the pooled mean values in the two groups of animals (Fig 4.8). The overall mean WBC counts of infected and control were 9.6 ± 1.2 and $9.8 \pm 1.3 \times 10^9$/L, respectively.

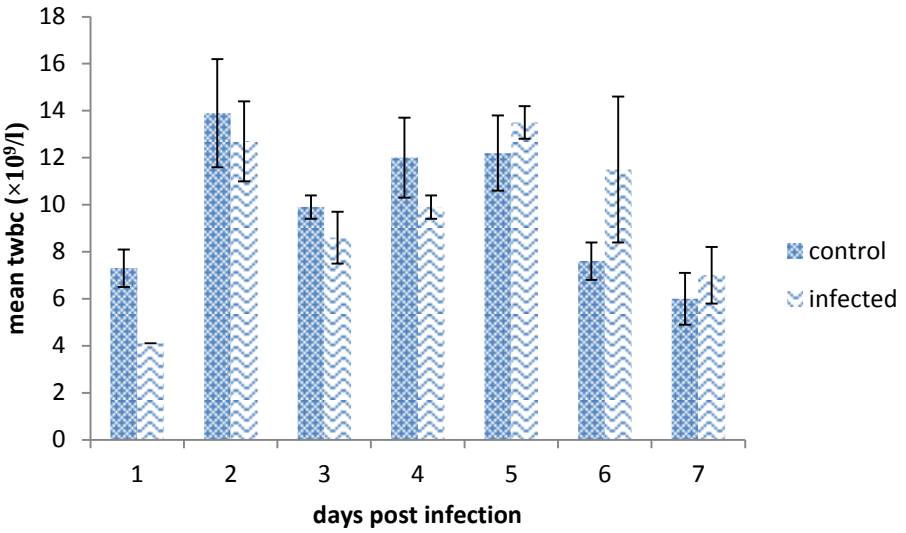


Figure 4.8: Mean daily (±SEM) total white blood cell count profiles of *Trypanosoma brucei brucei* experimentally-infected and control mice.

4.4 Haematological Changes in *T. brucei brucei*-infected and Control Rats

4.4.1 Mean packed cell volume, haemoglobin concentration and red blood cell count

The mean PCV, HB concentration and RBC count of the *T. brucei brucei*-infected and control rats were as presented in Figures 4.9 – 4.11. The infection of the rats with *Trypanosoma brucei brucei* resulted in significantly ($P< 0.05$) lower mean PCV in the infected group, when compared to that of the control group on days 5, 6 and 7 post-infection. The pooled mean PCV (41.3 ± 0.5 %) of infected rats was significantly ($P < 0.05$) lower than that (46.5 ± 0.2 %) of the control. The mean HGB and RBC count in both the *Trypanosoma brucei brucei* infected followed a pattern similar to that of the PCV. The mean haematological values in the control groups remained fairly unchanged during the experiment. The pooled mean HB and RBC count of the infected (13.8 ± 0.2 g/dl and 7.10 ± 0.2 $\times 10^{12}$/l) were significantly ($P < 0.05$) lower than the corresponding values of the control rats (15.5 ± 0.1g/dl and 7.95 ± 0.0 $\times 10^{12}$/l).

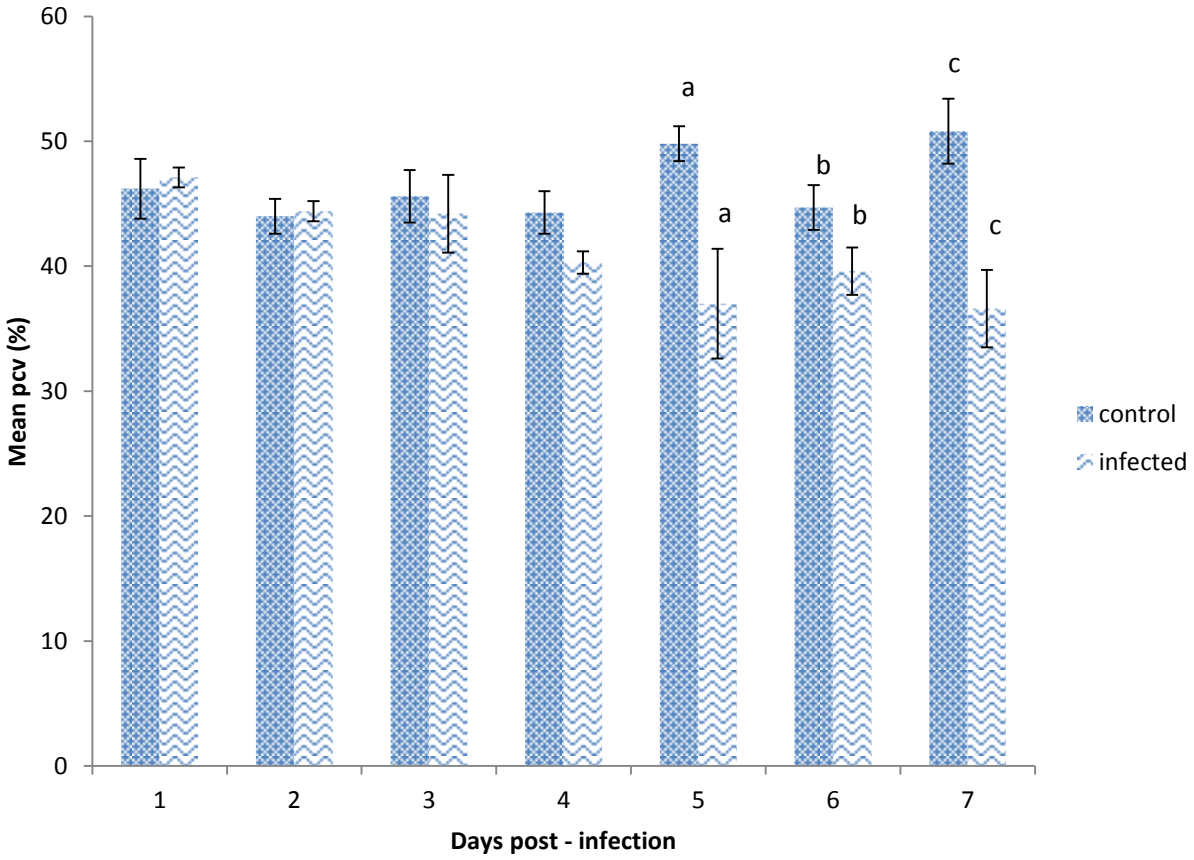


Figure 4.9: Mean daily (±SEM) packed cell volume of rat experimentally infected with *Trypanosoma brucei brucei* and the control

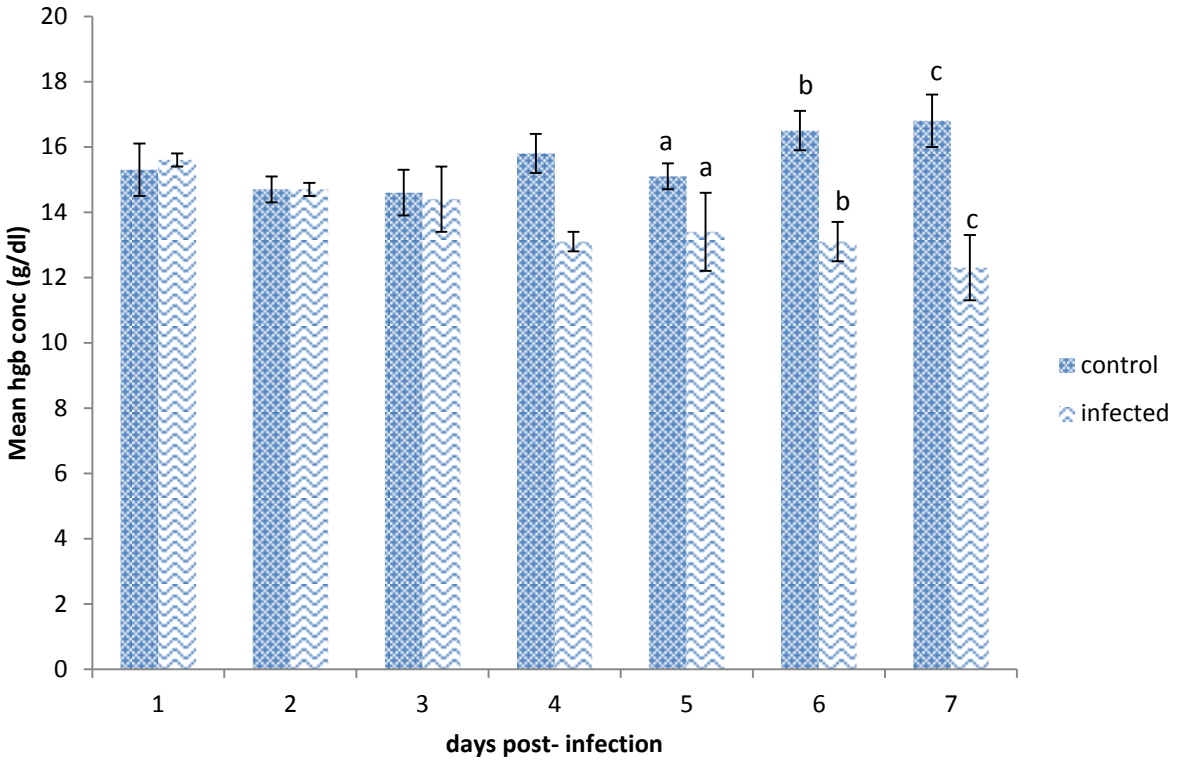


Figure 4.10: Mean daily (±SEM)haemoglobin concentration of rats experimentally infected with *Trypanosoma brucei brucei* and the control

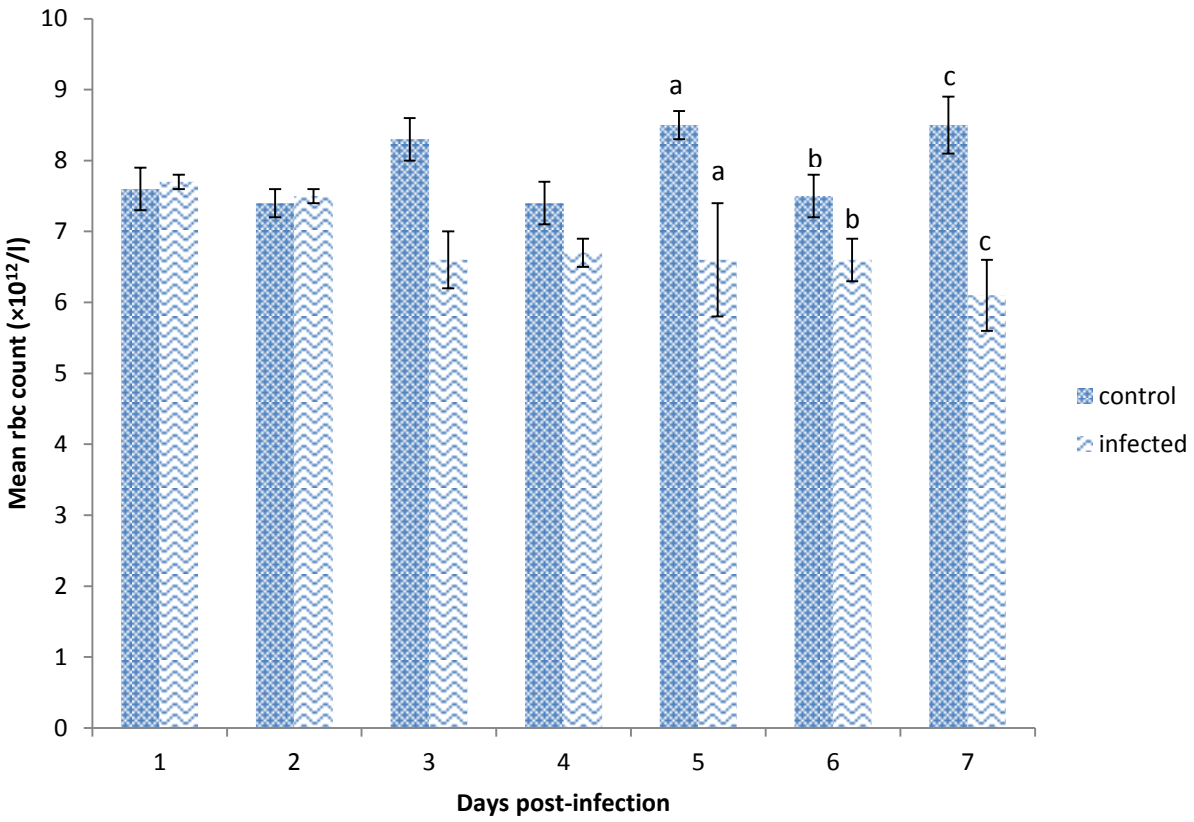


Figure 4.11: Mean daily (±SEM) red blood cell counts of rats experimentally with *Trypanosoma brucei brucei* and the control

4.4.2 Mean total white blood cell (WBC) count

The mean WBC count of the *T. brucei brucei*-infected and control rats was as presented in figures 4.12. The infection with *T. brucei brucei* resulted in significantly lower ($P < 0.05$) mean WBC in the infected group of rats, when compared to that of the control group on days 4 and 5, post-infection. However, the mean WBC was significantly higher in the infected than in the control rats on day 6 post-infection. The pooled mean WBC count (8.3 ± 0.2 x 10^9/L) in the infected was significantly lower than that of the control (10.8 ± 0.2 x 10^9/L).

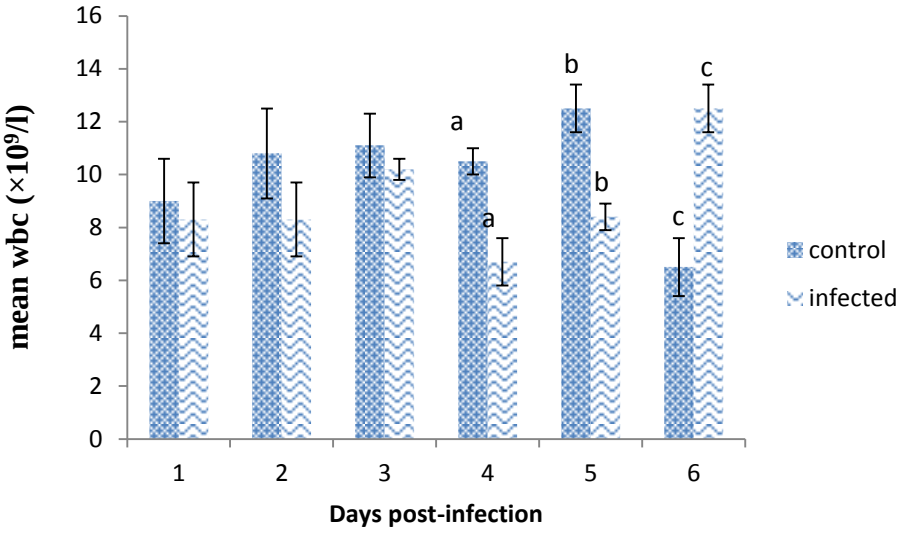


Figure 4.12: Mean daily (±SEM) WBC count in the *Trypanosoma brucei brucei*-infected and control rats

4.5 Haematological Changes in *T. brucei brucei*-Infected and Control Rabbits

4.5.1 Mean packed cell volume (PCV), haemoglobin concentration (HB) and red blood cell count (RBC)

The mean PCV, HB and RBC count of the infected and control groups of rabbits were as presented in figures 4.13- 4.15 respectively. Following infection of the rabbits with *Trypanosoma brucei brucei*, a progressive decline in the mean PCV was observed in the infected group of rabbits. The mean PCV in the infected group was significantly ($p < 0.05$) lower than that of the control group beginning from week 8 post-infection and remained so until termination of the experiment. Changes in HB and RBC count of this group of rabbits followed pattern similar to that of PCV. The mean values of all these erythrocyte parameters remained fairly unchanged in the control group throughout the experimental period. The pooled mean PCV, HB and RBC count of rabbits in the infected group (36.1 ± 0.6 %, 12.11 ± 0.8 g/dl and 5.92 ± 0.4 $\times 10^{12}$/L, respectively) were significantly ($P < 0.05$) lower than those of the control group (43.7 ± 0.5 %, 14.09 ± 0.9 g/dl and 7.23 ± 0.3 $\times 10^{12}$/L, respectively).

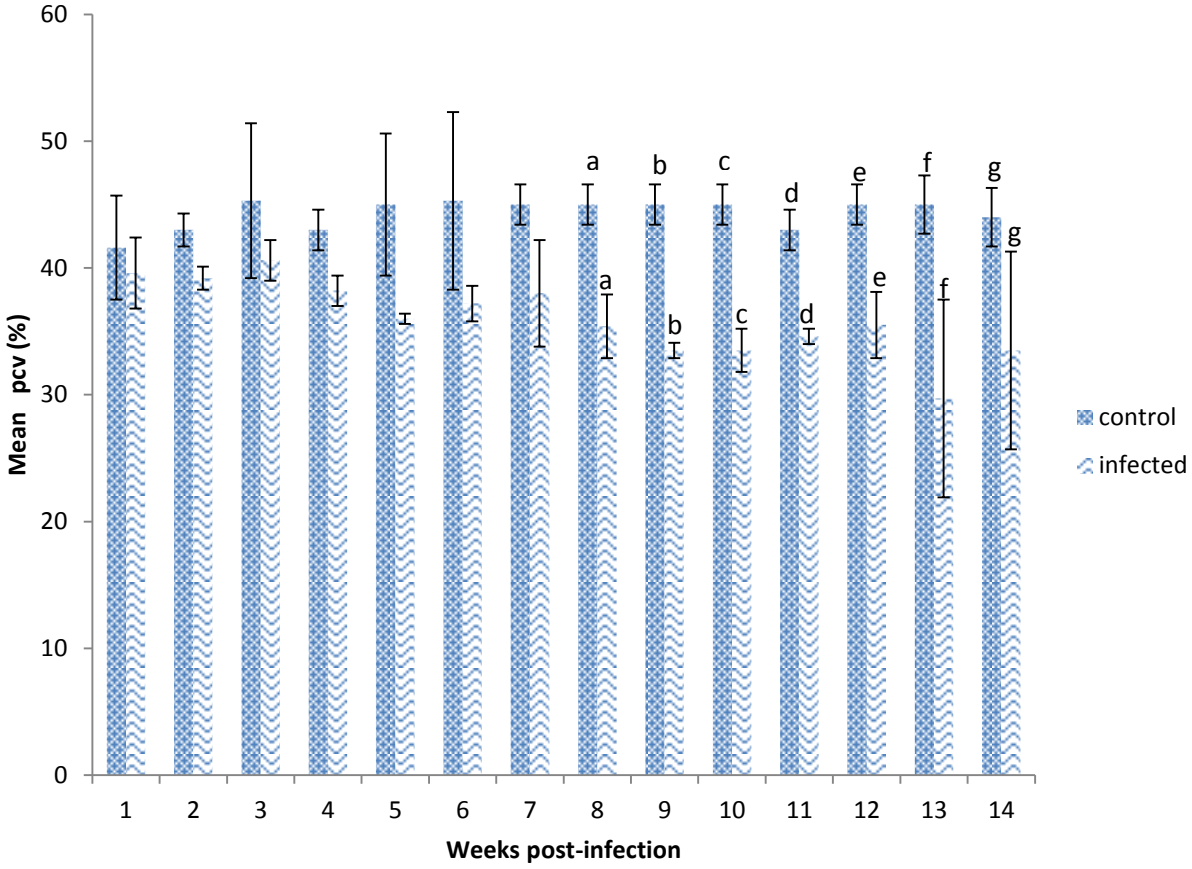


Figure 4.13: Mean weekly (±SEM) packed cell volume in *Trypanosoma brucei brucei*-infected and control rabbits

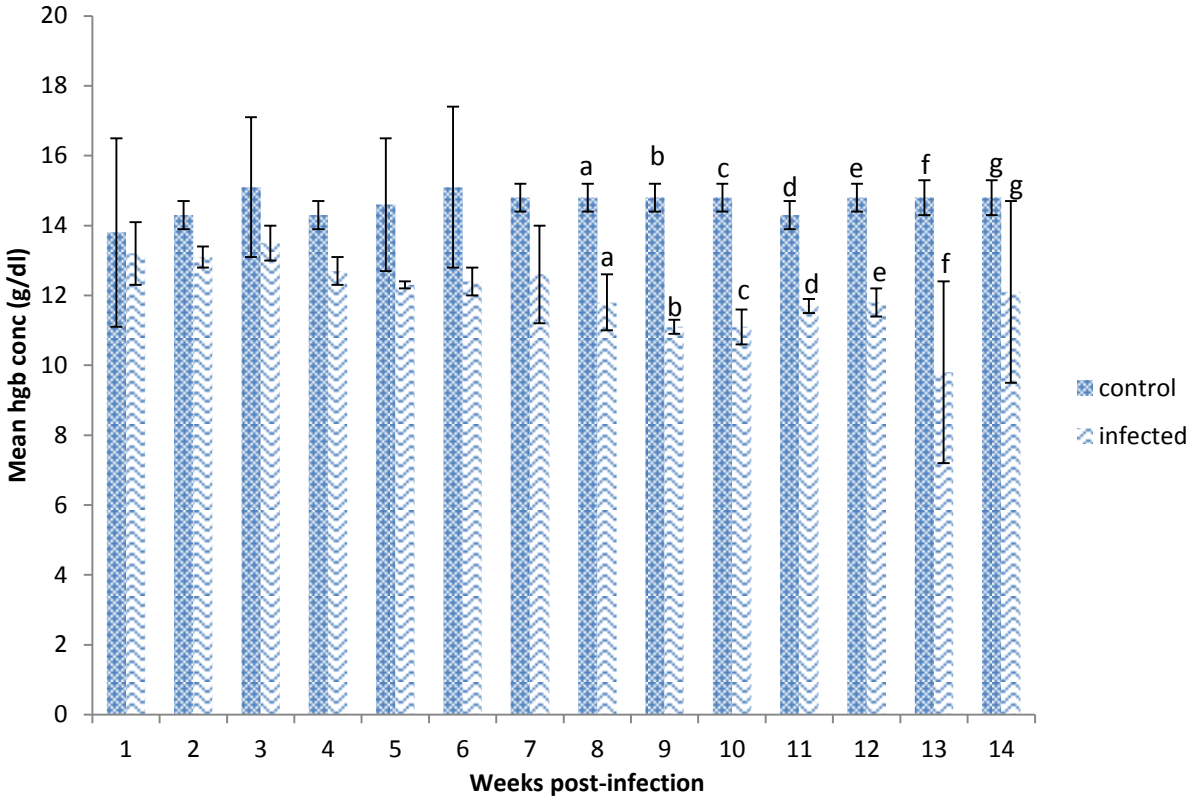


Figure 4.14: Mean weekly (±SEM) haemoglobin concentration of rabbits experimentally infected with *Trypanosoma brucei brucei* and control

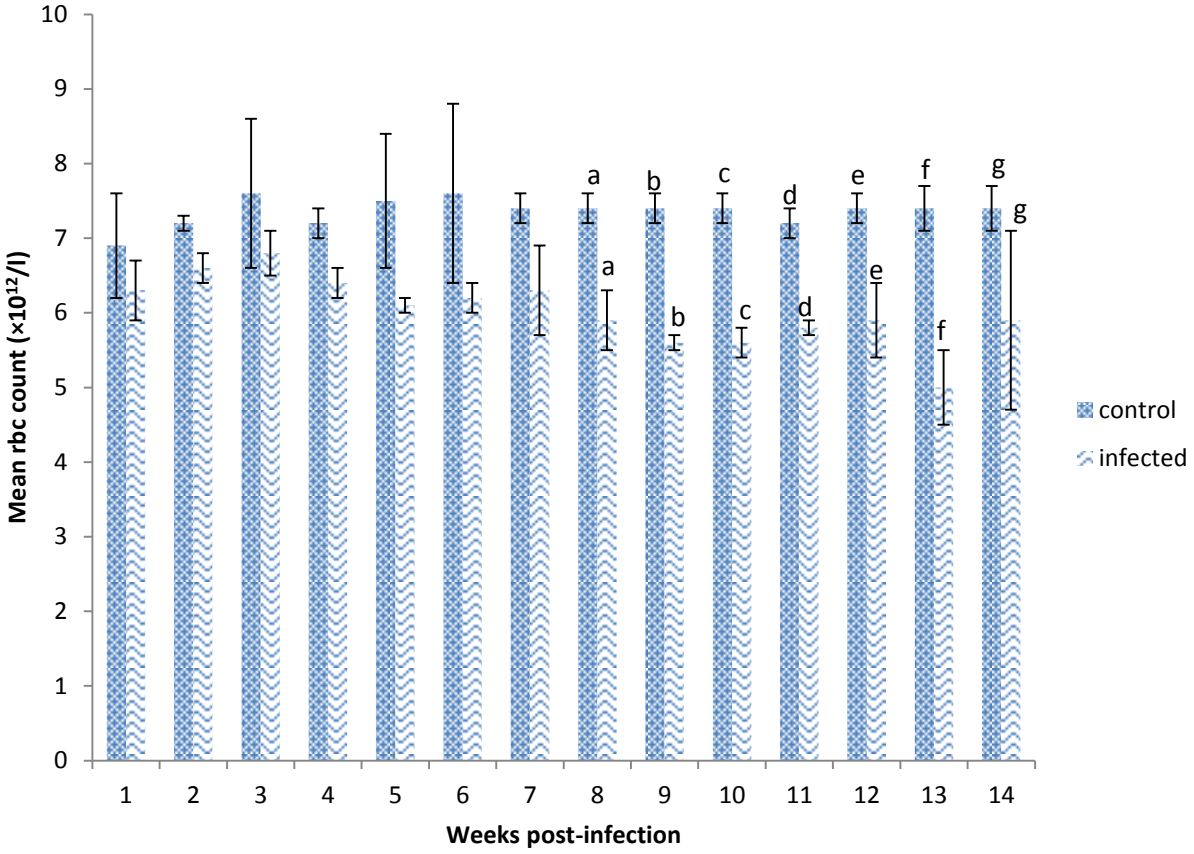


Figure 4.15: Mean weekly (±SEM) red blood cell counts of rabbits experimentally infected with *Trypanosoma brucei brucei* and control

4.5.2 Mean total white blood cell count (WBC)

There was no significant ($p > 0.05$) difference between the mean WBC of the *T. brucei brucei*-infected rabbits and control up to week 9 post-infection. However, the value in the infected rabbits was significantly ($p < 0.05$) lower than that of the control on week 10 to 14- post-infection. The pooled mean white blood cell count in infected $7.9 \pm 1.0 \times 10^{12}$/l was significantly lower than control $9.7 \pm 1.9 \times 10^{12}$/l. (Fig. 4.16)

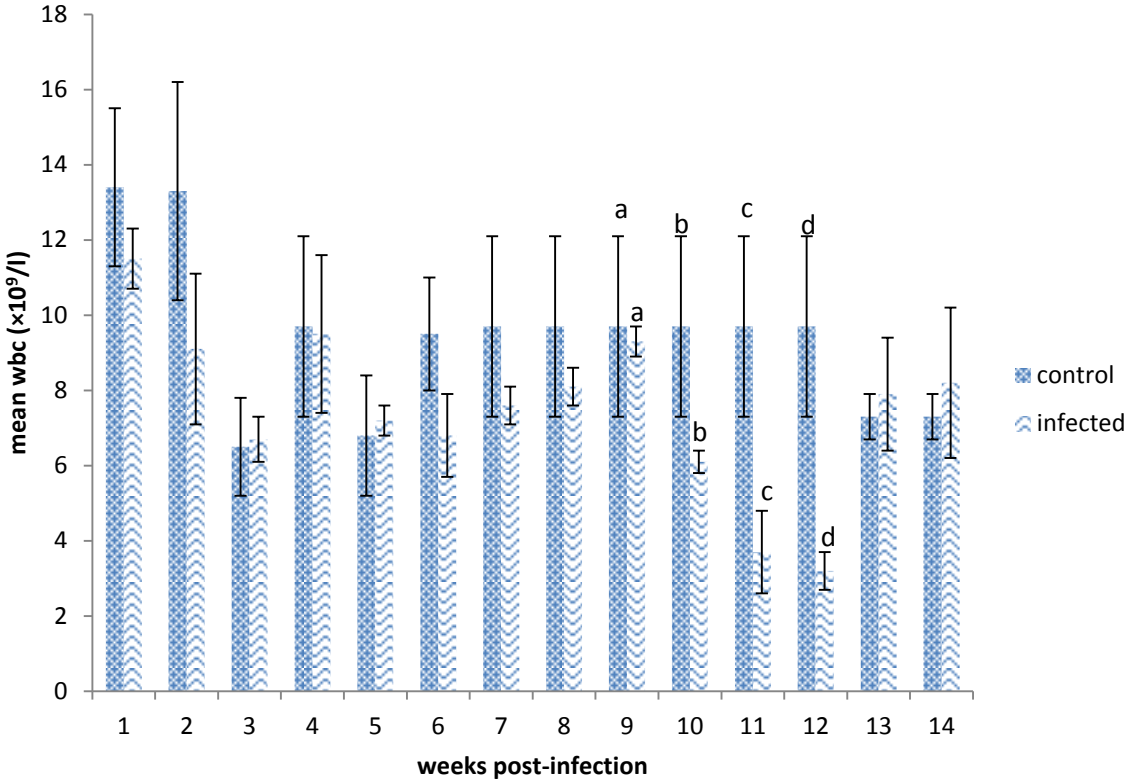


Figure 4.16: Mean weekly (±SEM) white blood cell counts of rabbits experimentally infected with *Trypanosoma brucei brucei* and control

4.6 Haematological Changes in *T. brucei brucei*-infected and Control Guinea fowls

4.6.1 Mean packed cell volume (PCV), haemoglobin concentration (HGB) and red blood cell (RBC) count

The infection of the guinea fowl with *Trypanosoma brucei brucei* resulted in no significant ($p > 0.05$) difference in the mean PCV, when compared with that of the control. The mean HGB and RBC count of the *T. brucei brucei*-infected guinea fowls follow similar pattern to that of the PCV ($p > 0.05$). There was no significant difference ($P > 0.05$) between the overall mean PCV, HGB concentration and RBC count of the infected guinea fowls and those of the control group throughout the period of the experiment. Also, no mortality was recorded in the infected as consequence of the infection. (Fig. 4.17- 4.19)

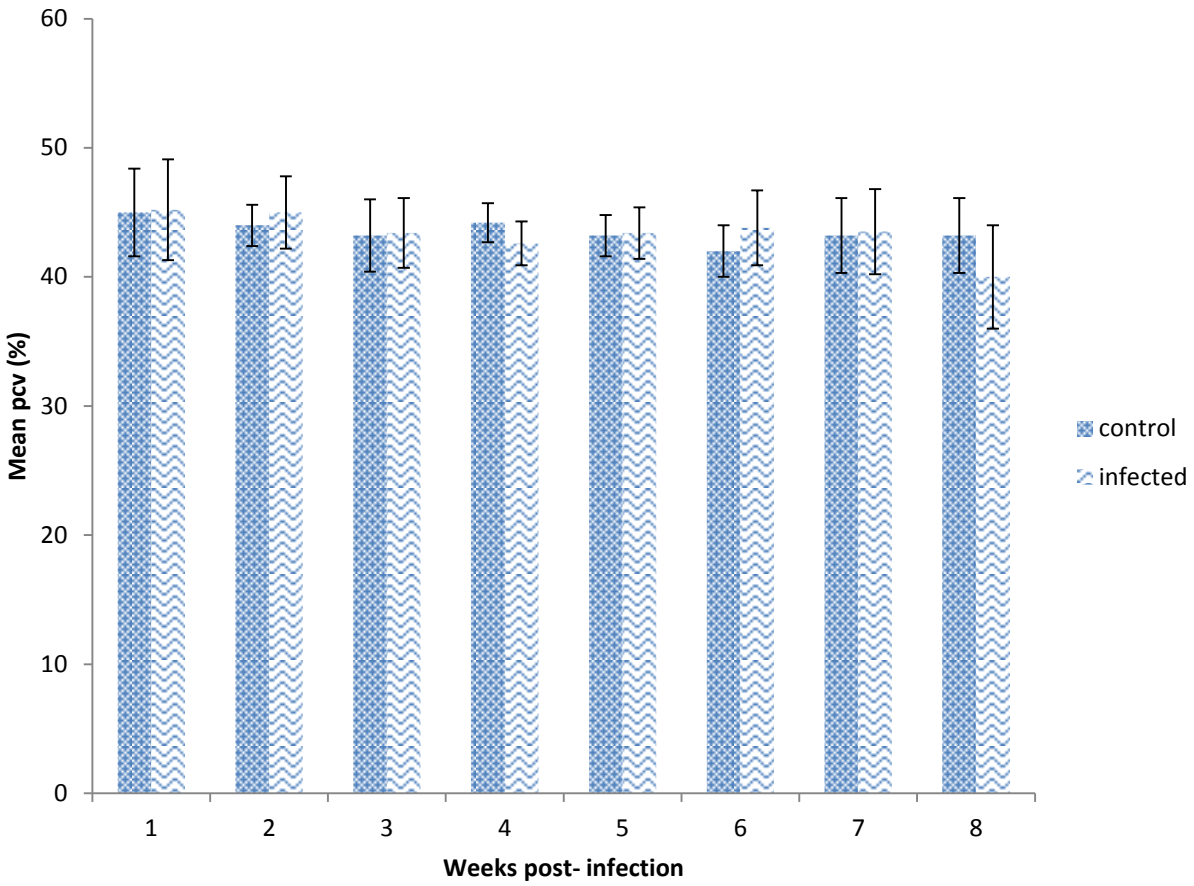


Figure 4.17: Mean weekly (±SEM) packed cell volume of guinea fowls experimentally infected with *Trypanosoma brucei brucei* and control

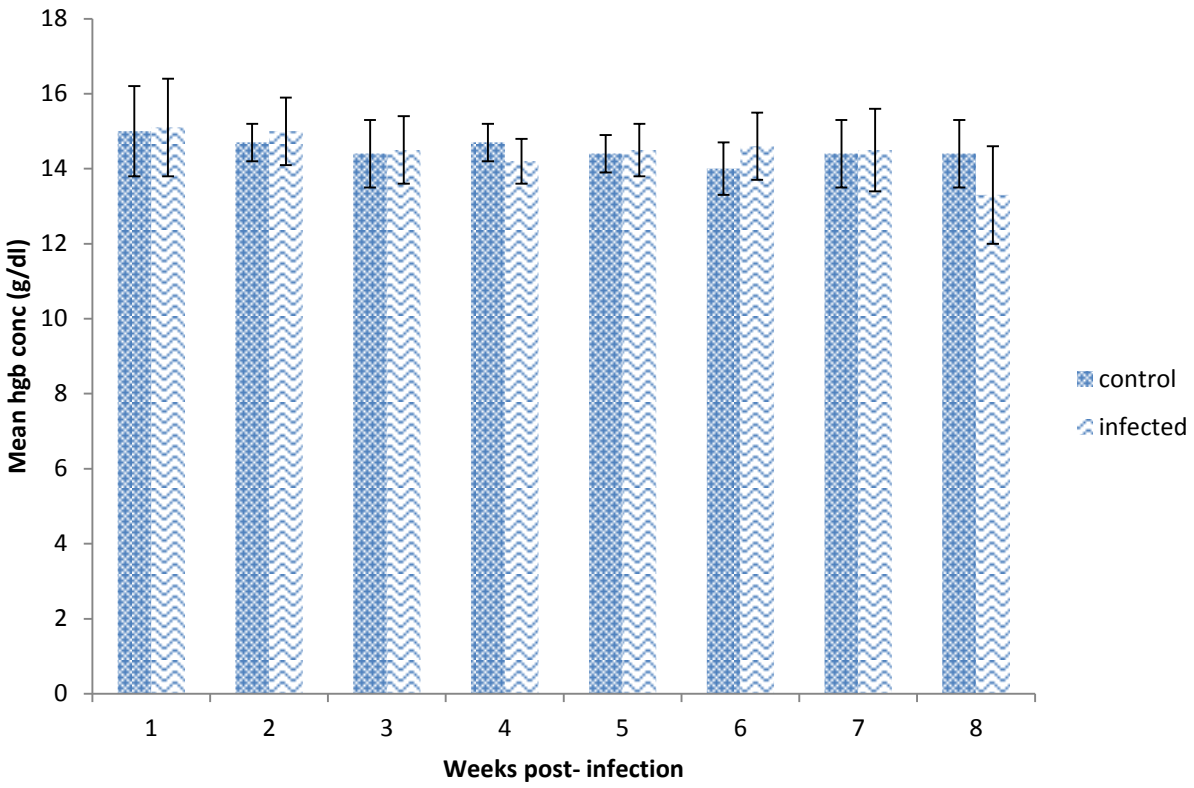


Figure 4.18: Mean weekly (±SEM) haemoglobin concentration of guinea fowls experimentally infected with *Trypanosoma brucei brucei* and control

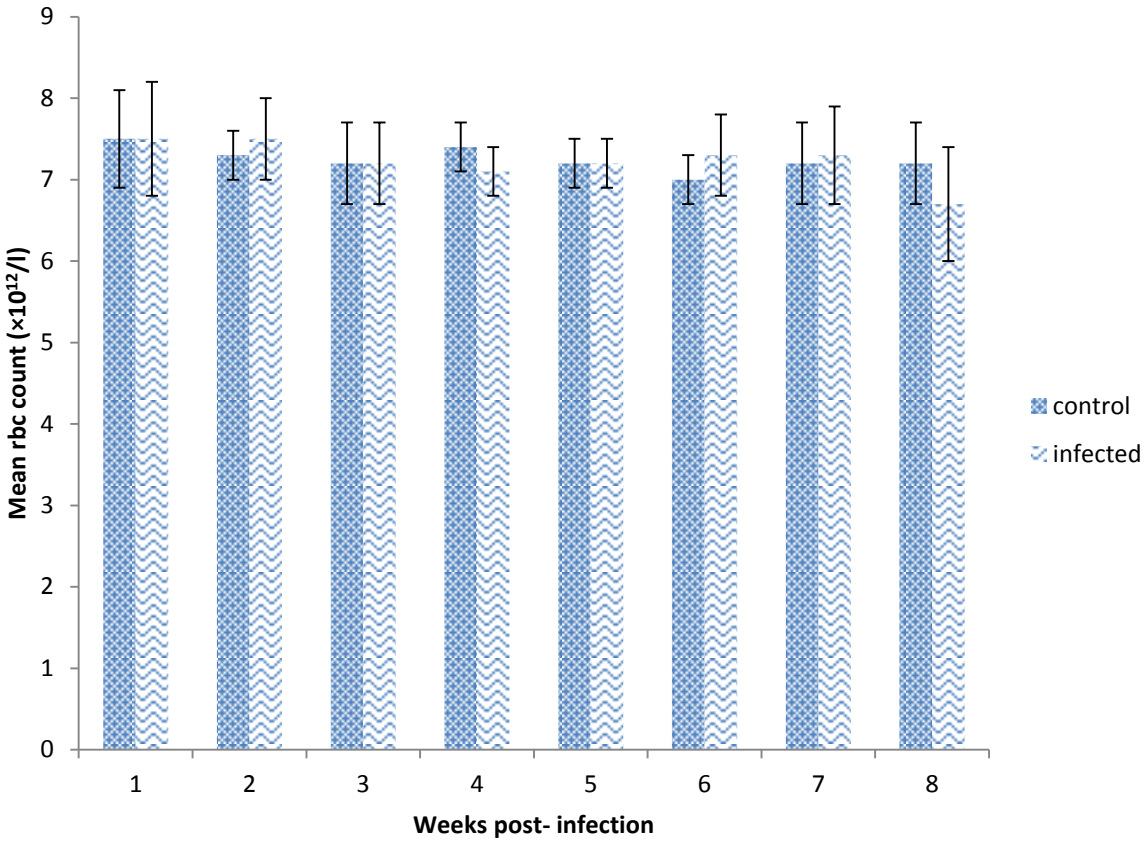


Figure 4.19: Mean weekly (±SEM) red blood cell counts of guinea fowls experimentally infected with *Trypanosoma brucei brucei* and control

4.6.2 Mean total white blood cell count (WBC)

The mean total white blood cell count in the *T. brucei brucei*-infected and control guinea fowls was as presented in figure 4.20. No significant ($p > 0.05$) changes were observed in the mean white blood cell count of the infected and control groups as fluctuations were similar in the two groups of the experimental birds.

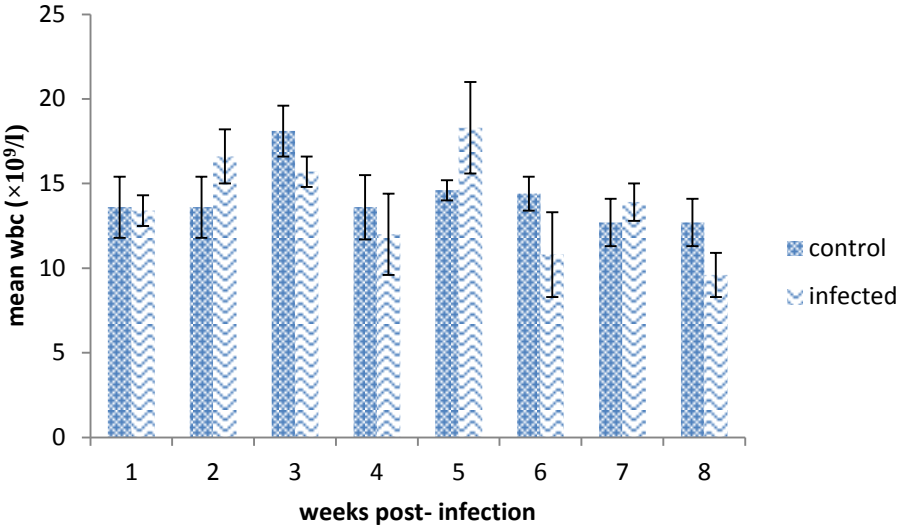


Figure 4.20: Mean weekly (±SEM) total white blood cell counts of guinea fowls experimentally infected with *Trypanosoma brucei brucei* and control

4.7 Histopathological Findings

4.7.1 Histopathological findings in tissues and organs of *Trypanosoma brucei brucei* – infected mice, rats, rabbits and guinea fowls

4.7.1.1 Lungs

The histological sections of the lungs of the infected mice and rats showed congestion and mononuclear cellular infilteration around the blood vessels respectively (plate I) No significant findings occured in the lungs of rabbits and guinea fowls experimentally infected with *Trypanosoma brucei brucei*.

4.7.1.2 Liver

The histological sections of the livers of the infected groups of mice, rats and rabbits revealed liver with congested central vein and perivascular cuffing, congested sinusoid dilated with erythrocyts (plate II). No significant hiostopathological findings occurred in the liver sections of guinea fowls infected with *Trypanosoma brucei brucei*

4.7.1.3 Kidney

Significant histological changes were observed in the kidney sections of *Trypanosoma brucei brucei*- infected rats and rabbits characterized by congested interlobular spaces and focal necrosis of epithelium of renal tubules (plate IV). No significant Histopathological findings were observed in the kidney sections of *Trypanosoma brucei brucei*- infected mice and guinea fowls

4.7.1.4 Spleen

Histological sections of the spleen of *Trypanosoma brucei brucei* infected rats and guinea fowls showed depletion of lymphoid cells and heamosiderosis (plate

VIII). while those of the *Trypanosoma brucei brucei*- infected mice and rabbits showed no significant changes**.**

Table 4.3: Observations on the histopathology of some visceral organs of mice, rats, rabbits and guineafowls experimentally infected with *Trypanosoma brucei brucei*

Organ	mice	rats	rabbits	guineafowls
Liver	Congestion and perivascular cuffing	Congested central vein and Perivascular cuffing	Congested sinusoid dilated with erythrocyte and peri-Vascular cuffing	___
Lungs	Congestion	Mononuclear cellular infilteration-around blood vessels.	____	___
kidney	_____	Congested interlobular spaces	Focal necrosis of epithe-lium of renal tubules	___
spleen	_____	depletion of lymphoid cells	_____	haemosiderosis

_____ No abnormal findings

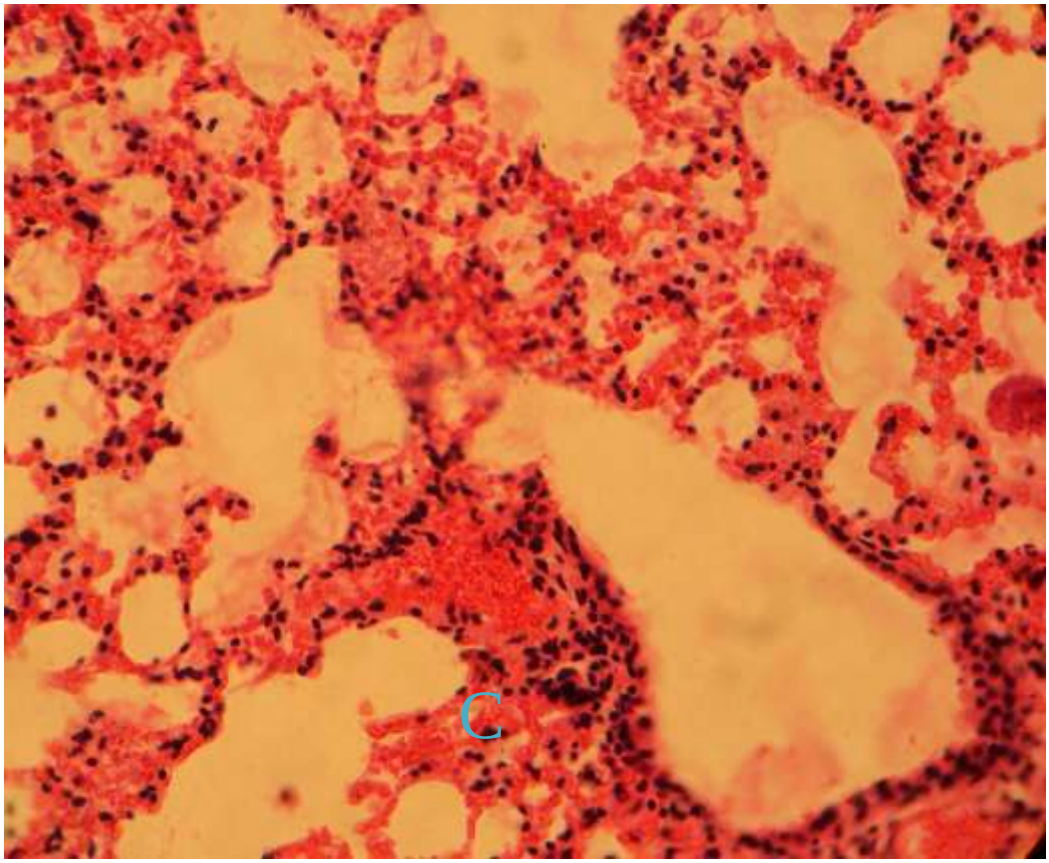


PLATE I: Section of the lung of mice infected with *T. brucei* showing congestion. (c). (H& E × 400).

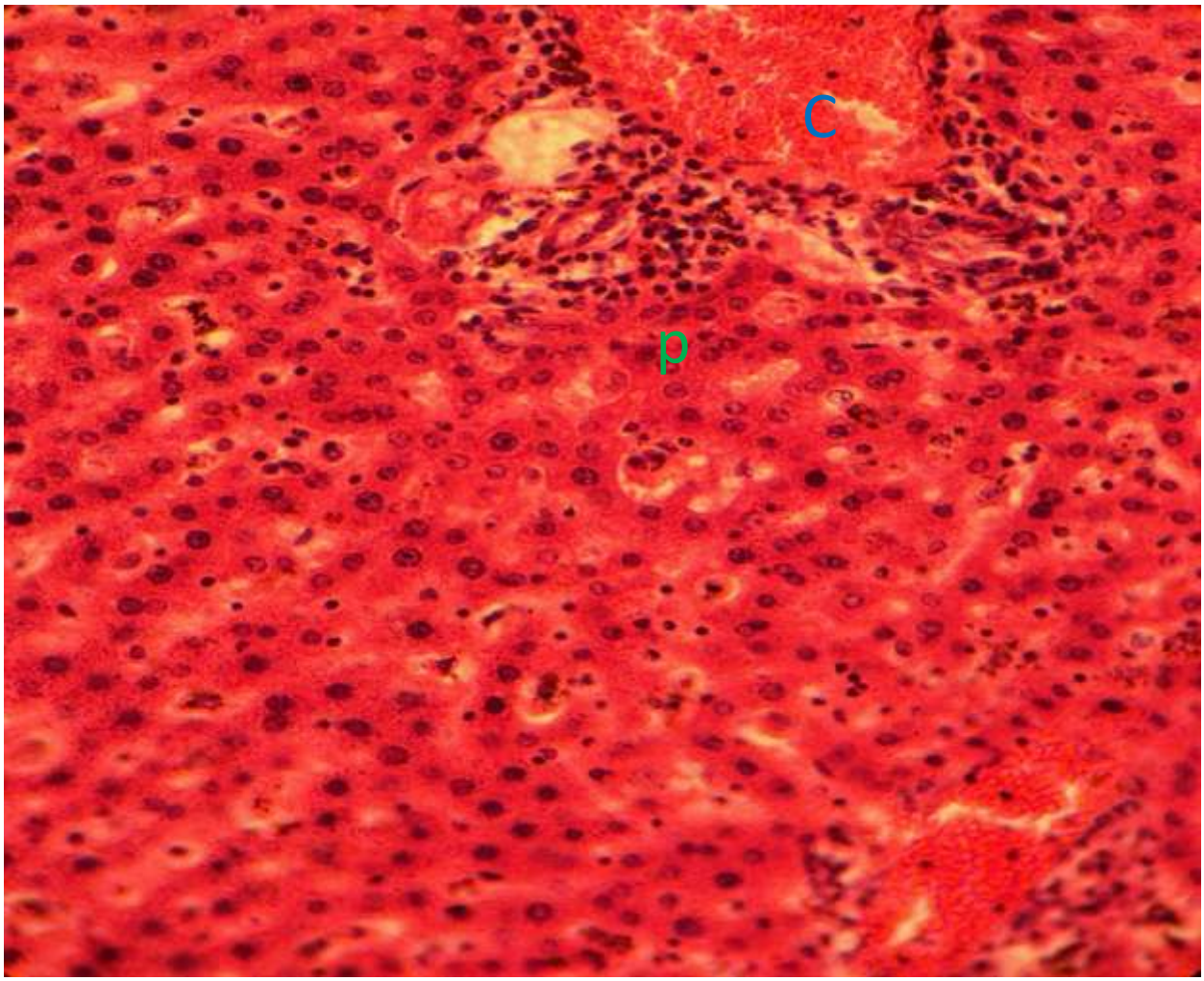


PLATE II: Section of the liver of a rat infected with *T. brucei* showing congested central vein (C) and perivascular cuffing (P) (H and E ×400)

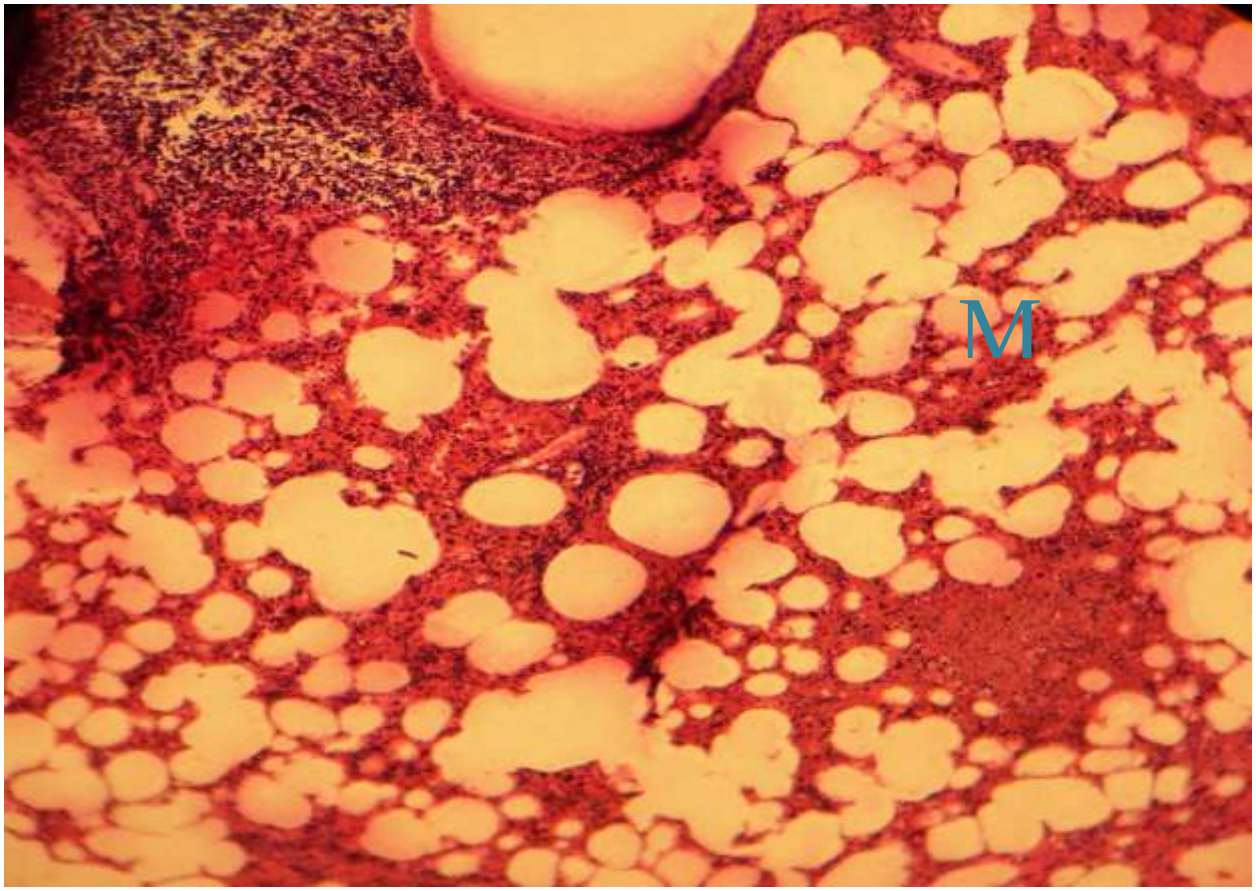


PLATE III: Section of the lung of rats infected with *T.brucei* showing mononuclear cellular infilteration around blood vessels (m). (H & E × 400).

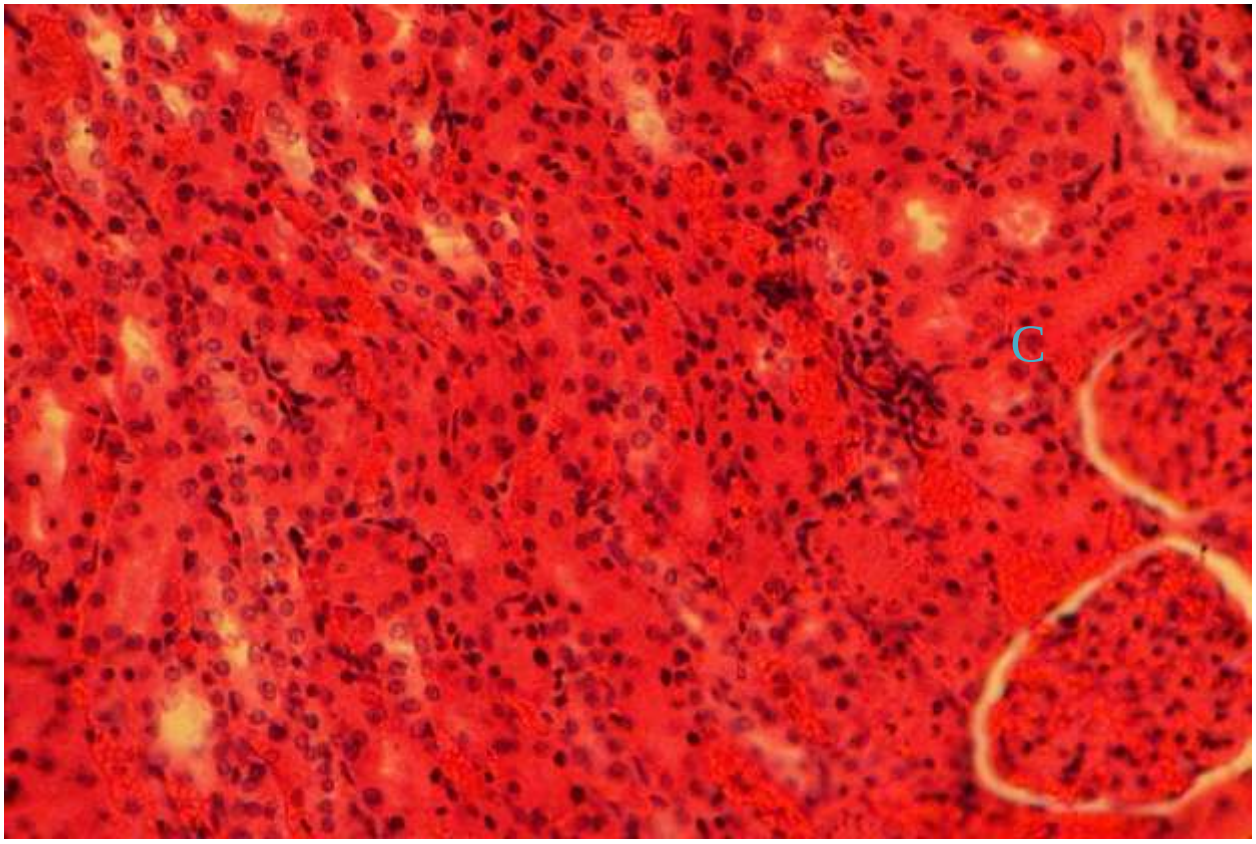


PLATE IV: Section of the kidney of rat infected with *T. brucei* showing congested intertubular spaces. (H & E × 400)

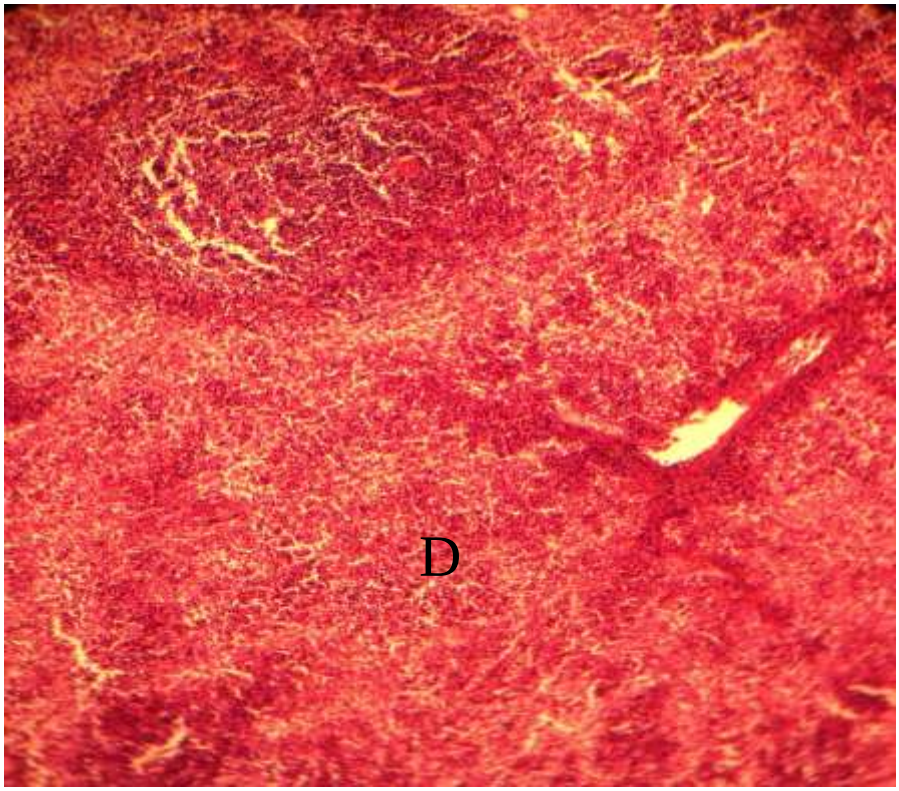


PLATE V: Section of the spleen of *T. brucei* infected rat showing depletion of lymphoid cells (D) (H and E × 200)

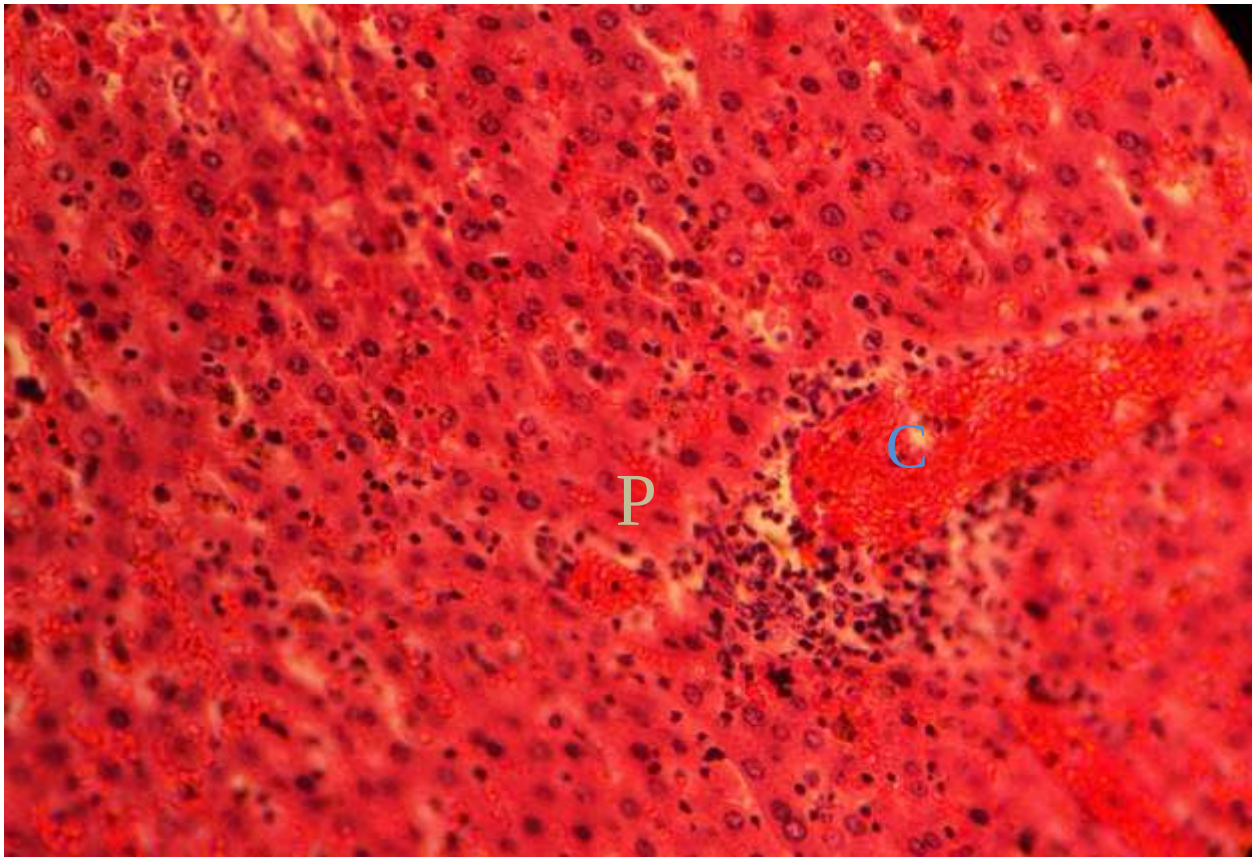


PLATE VI: section of liver of rabbit showing infected with *T. brucei* showing congested sinusoid dilated with erythrocytes and perivascular cuffing.

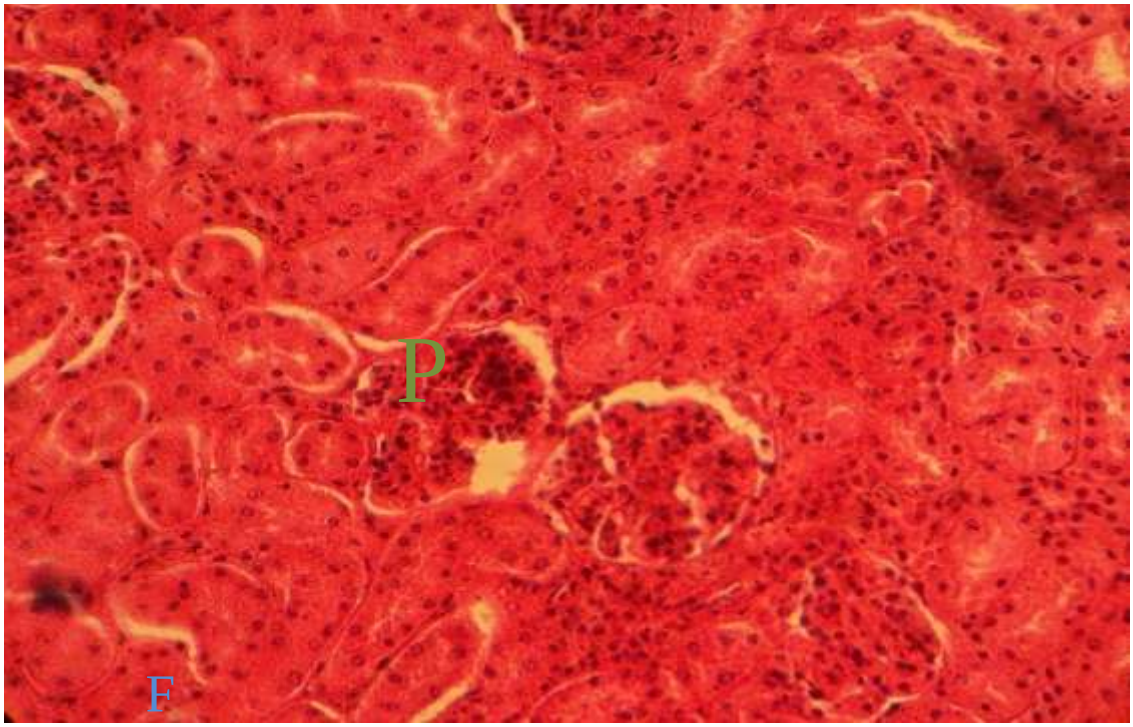


PLATE VII: section of kidney of rabbit infected with *T. brucei* showing pinkish material in the lumen of renal tubules and focal necrosis of renal tubular epithelium.

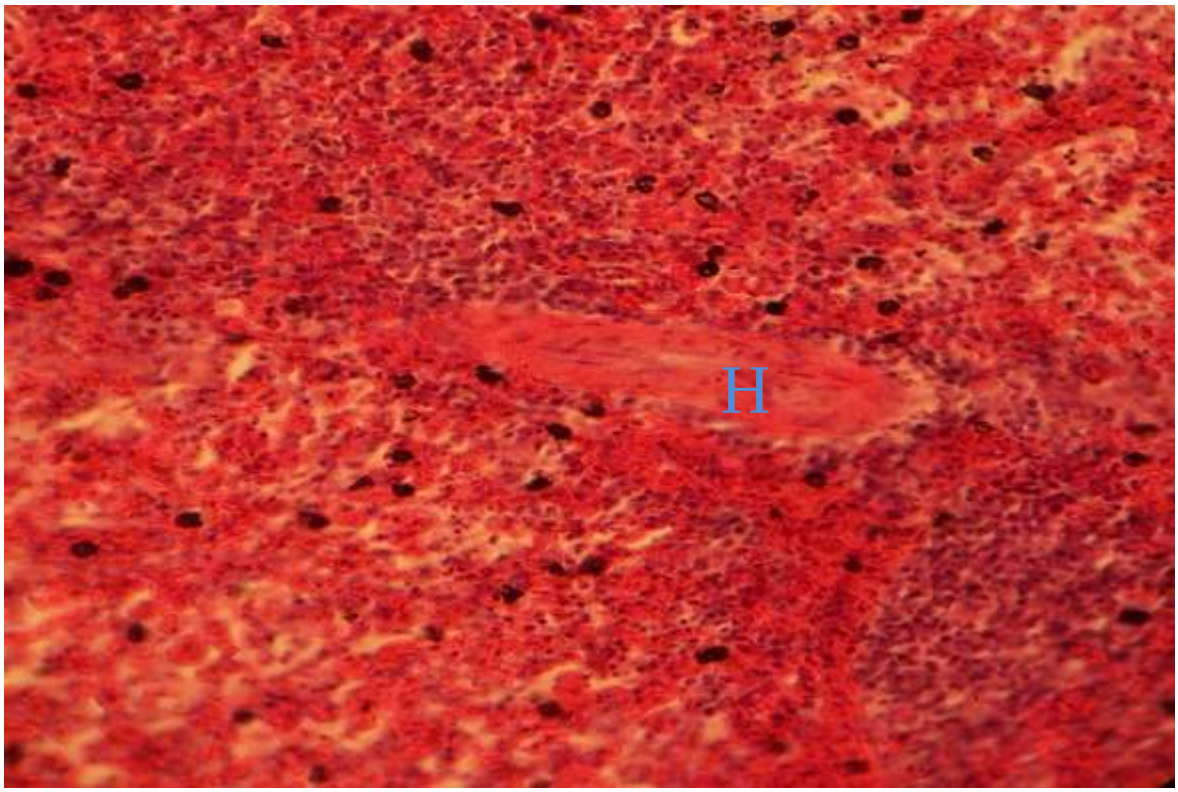


PLATE VIII: Section of the spleen of guinea fowl infected with *T. brucei* showing haemosiderosis. (H & E × 400)

CHAPTER FIVE

DISCUSSION

The prepatent periods in the present study were found to be shorter in the *Trypanosoma brucei brucei* infected mice and rats but longer in *Trypanosoma brucei brucei* infected rabbits and no parasitaemia in *Trypanosoma brucei brucei* infected guinea fowls. The shorter prepetent period observed for *Trypanosoma brucei brucei* infected mice and rats in the present study agrees with similar findings by Allam *et al*.(2006), Adeiza *et al*.(2008) and Mbaya *et al*.(2009b) that *T. brucei brucei* has a short prepatent period. The variation in the prepatent periods may be attributed to a variety of factors such as strains and virulence of the isolates, the immune status, nutritional requirement and degree of susceptibility of the hosts to the isolates.

There was also variation in the level of parasitaemia in the infected rat and mice compare to the infected rabbit and no parasitaemia was detected in guinea fowl. These discrepancies in variation in the level of parasitaemia is in agreement with Mohammed (2000) who reported that *T. evansi* (Sokoto isolate) was very pathogenic to Savanna brown goats, mice and rats. The peak parasitaemia was lower among the infected rabbit compared to the infected rat and mice. The ability of the host to limit the peak and the number of parasites is however dependent on whether the infection is acute, subacute or chronic. The characteristic nature of the parasitaemia may be attributed to the ability of the parasites to evade the immune response of the host through the phenomenon of antigenic variation. Trypanosomes are covered by a dense coat of variant surface glycoproteins that stimulate antibody production in the host as been reported by Morrison *et al*. (2009).

Increased body temperature observed in the infected group of animals is one of the known signs of Animal trypanosomosis. Adeiza *et al* (2008) also reported an increase and fluctuation in the

rectal temperature of goats experimentally infected with *Trypanosoma brucei* and *Trypanosoma congolense*.

Furthermore, it has been documented that changes in the body temperature regulating centre (hypothalamus) due to pyrogenic stimuli released during infection leads to pyrexia and oxidative stress observed in trypanosomiasis, because pyrexia is a direct response to successive waves of parasitaemia (Stephen, 1986; Baracos et al., 1987).

The significant decrease in live body weight observed in the *Trypanosoma brucei brucei*-infected mice, rats and rabbits is in accordance with the typical signs of weight loss and emaciation associated with African animal trypanosomosis (Mann *et al.*, 2009). This phenomenon may possibly be as a result of parasite induced anorexia. This observation agrees with the findings of Itard, (1989) which reported that the apparent weight loss was due to increased parasitaemia.

It has been established that the measurement of anaemia gives a reliable indication of the disease status and productive performance of trypanosome infected animals (Ekanem et al., 2005, 2006). Trypanosome infection may cause anaemia as a result of massive erythrophagocytosis by an expanded and active mononuclear phagocytic system (MPS) of the host (Igbokwe and Nwosu, 1997). Haematological results obtained in this study agree with earlier studies (Anosa, 1988; Igbokwe et al., 1994; Ekanem et al., 2008). The low PCV observed in the infected group may be as a result of acute haemolysis due to growing infection. Previous studies have shown that infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage arising from depletion of reduced glutathione on the surface of the red blood cell (Igbokwe *et al*., 1994, 1996; Taiwo *et a*l., 2003; Akanji *et al*., 2009). Severity of anaemia usually reflects the intensity and duration of parasitaemia. Several reports (Ogunsanmi and

Taiwo, 2001; Umar *et al*., 2007; Ekanem *et al*., 2008; Saleh *et al*., 2009) have also ascribed acute anaemia in trypanosomosis to proliferating parasites.

The lower counts of WBC and lymphocytes observed in the infected rats and rabbits agree with the work of Abubakar et al., 2005; Ekanem and Yusuf, 2008, who reported that this may be attributed to the immunosuppressive actions of trypanosome infection.

The histological lesions (cell proliferations and infiltration by lymphocytes, mononuclear infiltration of interstitial tissues) seen in other tissues and organs could be immunological in nature. Greenwood and Whittle (1980), considered trypanosomosis to cause immunoproliferative disorder of B-lymphocytes and plasma cells, with resultant direct or indirect impaired functions of various organs.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

- The indices of susceptibility and consequently infectivity and pathogenecity were pronouncedly altered in the infected rats, mice and rabbits while they remained within normal in guinea fowls.
- The study thus showed that mice, rats and rabbits are in order of susceptibility better laboratory models than the guinea fowls which showed absolute resistance to *Trypanosoma brucei brucei* used in the experiment.

6.2 Recommendation

- Due to the high mortality recorded in the infected laboratory mice and rats, rabbits should be used in our laboratory to preserve *Trypanosoma brucei brucei*
- Guinea fowls should not be used as experimental model for *Trypanosoma brucei brucei* because of their resistivity to the parasite

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