ASSESSMENT OF DONKEYS (*Equus asinus*) EXPERIMENTALLY INFECTED WITH *Trypanosoma brucei* (Federe isolate) AND TREATED WITH HOMIDIUM CHLORIDE AND ISOMETAMIDIUM CHLORIDE

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OCTOBER, 2016

DECLARATION

I declare that the work in this thesis entitled, **'Assessment of Donkeys (*Equus asinus*) Experimentally Infected with *Trypanosoma brucei* (Federe isolate) and Treated with Homidium chloride and Isometamidium chloride'** has been performed by me in the Department of Veterinary Medicine, Faculty of Veterinary Medicine, A.B.U. Zaria. Information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma at any university.

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CERTIFICATION

This thesis, entitled "ASSESSMENT OF DONKEYS (*Equus asinus*) EXPERIMENTALLY INFECTED WITH *Trypanosoma brucei* (FEDERE ISOLATE) AND TREATED WITH HOMIDIUM CHLORIDE AND ISOMETAMIDIUM CHLORIDE" by Nneka Queen, OPARAH meets the regulation governing the award of Doctor of Philosophy of the Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my loving parents, siblings, fiancé, close friends, the scientific research community, people who believe in hard work and to all men of goodwill.

The day may come, when the rest of animal creation may acquire those rights which never could have been withholden from them by the hand of tyranny…… (For)…the question is not, "Can they reason? Nor "Can they talk?", but "Can they suffer?"

Jeremy Bentham
(1789)

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ABSTRACT

This study investigated the course of *T. brucei* (Federe isolate) experimental infection in donkeys and the therapeutic efficacy of two trypanocides against the infection. Twenty-eight apparently healthy donkeys (*Equus asinus*) of equal sexes, aged between 8 – 9 months, were purchased from a livestock market in Maigatari town, Jigawa State and used for the study. They were housed in a tick and fly-proof pen, and allowed to acclimatize for 4 weeks during which they were screened for presence of diseases, and were fed *Imperata cylindrica* grass, *Andropogon gyanus* hay, cereal bran and cotton seed cake with clean water and salt lick provided *ad libitum*. Thereafter, they were assigned at random to 4 groups; A (Infected Homidium chloride-treated, n=8), B (Infected Isometamidium chloride-treated, n=8), C (Infected untreated/positive control, n=6) and D (uninfected untreated/negative control, n=6). Groups A and B donkeys were further divided into subgroups A_1 A_2 and B_1 B_2, of 4 donkeys each, to represent treatments at acute (day 12) and chronic (day 24) phases of infection, respectively. Twenty-two donkeys (groups A, B and C) were infected through jugular venipuncture with 2 ml inoculum containing 2×10^6 *T. brucei* (Federe isolate) and evaluated for clinical signs, pathology and response to treatment. Parasitaemia was evaluated using the wet mount, haematocrit and mouse inoculation techniques, and blood was sampled every 3 days for parasitology and haematology while sera for biochemistry were harvested once weekly. Treatment with the trypanocides was at dose of 1 mg/kg 2.5% Homidium chloride (Novidium®) and 0.5 mg/kg 1% Isometamidium chloride (Sécuridium®) on days 12 and 24 post-infection. Donkeys were evaluated pre-infection, post-infection and post-treatment for changes in physiological parameters, body weight, haemato-biochemical parameters and pathology. Carcasses and internal organs were examined for gross lesions. Tissue sections of the organs were prepared and examined

for changes in architecture. Organ impression, cerebrospinal fluid and brain squash smears were prepared and examined for tissue invasion by the parasite. *Trypanosoma brucei* produced clinical trypanosomosis in the donkeys with pre-patent and incubation periods of 2 – 3 and 2 – 6 days respectively. Two of the infected donkeys did not develop trypanosomosis. From day 4 post-infection, the infected donkeys manifested weakness, reduced feed intake, intermittent fever, tachycardia, increased respiratory rates, intermittent penile erection, dehydration, rough hair coat, lacrimation, weight loss, pale mucous membranes with recumbency. Respiratory rales on auscultation was predominant at the chronic stage. Parasitaemia recorded was 4.27 ± 0.45 parasites per field at day 3 post-infection and increased significantly ($p<0.0001$) to 26.20 ± 1.35ppf by day 12 pi. Weight loss was significant at the chronic stage. Post-infection, haematology revealed anaemia, with a significant ($p<0.005$) reduction in mean red cell count (5.87±0.40 to 3.35±0.30), haemoglobin concentration (11.88±0.61 to 8.50±0.61) and packed cell volume (38.13±3.36 to 24.38±1.88). There was significant ($p<0.0001$) increase in mean corpuscular volume (53.07±1.65 to 69.92±1.73) and a significant ($p<0.002$) decrease in mean corpuscular haemoglobin concentration (36.05±0.35 to 32.47±0.66) indicating a macrocytic hypochromic anaemia. Mean white blood cell count reduced significantly post-infection (11.62±0.90 to 7.55±0.71) while differentials revealed significant ($p<0.05$) reduction in neutrophils (44.79±1.31 to 31.23±1.06) and eosinophils (4.63±0.70 to 0.39±0.10), and a significant ($p<0.05$) increase in lymphocytes (47.29±1.50 to 67.20±1.09). Serum chemistry post-infection revealed minor changes in albumin, aspartate aminotransferase, alkaline phosphatase and blood urea nitrogen, suggesting the infection caused mild pathology. Alanine aminotransferase (ALT) levels increased post-infection and post-treatment. Creatinine levels significantly ($p<0.01$) increased (69.00±2.86 to 83.67±3.27) post-infection in group C donkeys.

There was significant ($p<0.05$) decrease in the total protein (67.28±2.93 to 60.43±0.69) and glucose levels (100.70±3.17 to 46.00±7.84) post-infection. Serum electrolyte alterations observed were hypocalcaemia post infection and hypercalcaemia post treatment, while sodium, chloride, potassium, phosphorus and bicarbonate levels showed non-significant ($p>0.05$) variations. Twenty-four hours following treatment of donkeys in groups A and B with Novidium® and Sécuridium® respectively, parasites were not detected. Relapse of infection with low parasitaemia levels was detected from day 27 post treatment in subgroups A_1, A_2, and B_1. Repeated treatment using Novidium® at 1 mg/kg and double the initial 0.5 mg/kg dose of the Sécuridium® (1 mg/kg), eliminated the parasites. Gross lesions observed in the untreated donkeys were hydropericardium (600 ml), atrophy of fat, mucoid exudates in the bronchi, congested lungs and spleen, and splenic haemorrhages. Histopathological findings were mononuclear perivascular cuffing in the brain, congestion of the lungs, kidney congestion with glomerulonephritis and splenic congestion with haemosiderosis. Impression smears revealed no parasites on microscopy. Tissue sections of treated donkeys showed less pathology. This study confirms susceptibility of donkeys to *T. brucei* (Federe) infection. Treatment with the two trypanocides at recommended concentrations and doses was effective in eliminating the parasites, with blood parameters returning to pre-infection values. However, the drugs produced some biochemical alterations in the treated donkeys. Isometamidium chloride was observed to have a better therapeutic effect than Homidium chloride, and is suggested as first drug of choice in the treatment of *T. brucei* infection in donkeys.

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ABBREVIATIONS

AAT	African Animal Trypanosomiasis
ABU	Ahmadu Bello University
AFLP	Amplified Fragment Length Polymorphism
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
A-T	Adenine Thymine
B	Basophils
Band	Band Neutrophils
BUN	Blood Urea Nitrogen
b.w.	Body Weight
Ca^{2+}	Calcium ion
CATT	Card Agglutination Tests for Trypanosomes
°C	Degree Celsius
CDC	Center for Disease Control
CFSPH	Center for Food Security and Public Health
CFT	Complement Fixation Test
Cl^{-}	Chloride ion
CR	Creatinine
CSF	Cerebrospinal Fluid
DDT	Dichlorodiphenyltrichloroethane
DG	Darkground Buffy Coat Technique
DIT	Drug-induced Thrombocytopenia
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleoside Triphosphate
DPI	Days Post Infection

DPT	Days Post Treatment
E	Eosinophils
ECF	Extracellular Fluid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FVM	Faculty of Veterinary Medicine
GFR	Glomerular Filtration Rate
Hb	Haemoglobin
HCO_3^-	Bicarbonate
HCT	Microhaematocrit Centrifuge Technique
H & E	Haematoxylin-Eosin
HR	Heart Rate
IFAT	Indirect Fluorescent Antibody Test
IgG	Immunoglobin G
ILRAD	International Laboratory for Research on Animal Diseases
K^+	Potassium ion
kDNA	Kinetoplast DNA
kg	kilogram
L	Lymphocytes
LAMP	Loop-mediated Isothermal DNA Amplification
LCD	Liquid Crystal Display
M	Monocytes
m-AECT	Miniature-Anion Exchange Centrifugation Technique
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
mg	milligram
MIT	Mouse Inoculation Test

N	Neutrophils
Na^+	Sodium ion
NARP	National Agricultural Research Project
NITR	National Institute for Trypanosomiasis and Onchocerciasis Research
NO	Nitric Oxide
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PFGE	Pulse-field Gel Electrophoresis
pi	post infection
PO_4^{-3}	Phosphate ion
pp	post parasitaemia
ppf	parasites per field
pt	post treatment
RAPD	Amplified Polymorphic DNA
RBC	Red Blood Cells
RDU	Rational Drug Use
RFLP	Restriction Enzyme Fragment Length Polymorphism
RNA	Ribonucleic Acid
RR	Respiratory Rate
RT	Rectal Temperature
SAT	Sequential Aerosol Technique
TNF	Tumor Necrosis Factor
TP	Total Protein
USD	United States Dollar
VR	Variable Region
WBC	White Blood Cells
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background to the Research

Livestock farming/production forms a major component of the socio-economic system of most rural communities in African countries with the pastoralists and semi-pastoralists who inhabit the arid and semi-arid areas mostly dependent on livestock (Micheal *et al.,* 2005). These people are estimated to own approximately 50% of Africa's livestock which is equivalent to about 225 million animals (de Leeuw *et al.,* 1995). Pastoralists are said to derive 50% of their food and income from their livestock (Msigwa and Mvena, 2014). In addition to their use as food and income providers, livestock also play major social and cultural roles in pastoral communities, whereby ownership influences wealth status and decision-making power (Catley, 2002). Thus, health care of these livestock (cattle, sheep, goats, horses, camels, donkeys, dogs, cats, pigs, rabbits, guinea pigs, poultry birds) is of paramount importance for the sustenance of their economy and livelihood.

Donkeys constitute 70% of the African equine species and are predominantly found in the arid and semi-arid areas providing a reliable, environmentally friendly and renewable source of traction power to millions of resource-poor communities worldwide (Fielding and Pearson, 1991). They are docile and easy to manage, providing transport at low costs amongst communities in both rural and urban areas with poor terrains or without access to motor transport, and are also used in small scale farming for light tillage, threshing, seeding and weeding of crops (Howe and Garba, 1997; Hassan *et al*., 2013). They have the ability to withstand harsh conditions in many rural settings in which they are reared under extensive husbandry. However, donkeys have received least attention, especially where they are kept with other livestock. This may

be due to the assumption that they are hardy and rarely affected by any disease (Hassan *et al*., 2013).

Trypanosomosis is a complex disease condition of vertebrates, affecting all domestic animals and is caused by several species of parasitic protozoans of the genus, *Trypanosoma* (Barret *et al*., 2003). African Animal trypanosomosis (AAT) is a major constraint to livestock and mixed crop-livestock production in tropical Africa (Mattioli *et al*., 2004; Holt *et al*., 2016). Also known as "Tsetse disease", it is mainly transmitted cyclically by the genus, *Glossina* (Tsetse flies), and sometimes mechanically by biting flies (Tabanids and Stomoxys). In Nigeria, the major species incriminated are *Trypanosoma congolense, T. vivax, T. brucei brucei, T. simiae* and *T. evansi* (Enwezor and Lawal, 2003). Tsetse-transmitted trypanosomosis is a classically acute or chronic disease that causes intermittent fever and is accompanied by anaemia, oedema, lacrimation, enlarged lymphnodes, abortion, decreased fertility, loss of appetite and weight, leading to early death in acute forms or to digestive and/nervous signs with emaciation and eventually death in chronic forms (Taylor and Authié, 2004). In cattle, the disease is called "nagana" a Zulu term meaning "to be depressed".

While other animal diseases have been controlled with great success, trypanosomosis continues to represent a major constraint to livestock productivity in sub-Saharan Africa (Mihret and Mano, 2007; Eyob *et al.,* 2011). Although signs exhibited by trypanosomosis in donkeys can be confused with other common infections such as equine infectious anaemia, equine piroplasmosis and parasitic gastroenteritis, unlike trypanosomosis these diseases usually have a chronic course (Takele and Nibret, 2014).

It is reported that tsetse flies are endemic in tropical Africa between the latitudes 15ºN and 29ºS, from the Southern edge of the Sahara Desert to Zimbabwe, Angola and

Mozambique (CFSPH, 2009). This disease is said to spread to about 10 million square kilometer of fertile land across nearly 40 countries in Africa and as such has a huge impact on food security as well as livelihoods of farmers in the affected areas. An estimated 50 million (38%) African cattle are considered to be at risk of contracting the disease (Swallow, 1999). Also at risk are 70 million sheep and goats, with some 3 million cattle dying annually from the disease (Vreysen and Marc, 2006).

Epidemiology of equine trypanosomiasis (Surra) in Africa is mainly governed by camel infections (Desquesnes *et al.,* 2013) with prevalence recorded ranging from 1.3% to 31% depending on the test technique employed (Dia *et al.,* 2000; Eyob *et al.,* 2011; Bedada and Dagnachew, 2012). However, there are no documented records of estimated equine population at risk of the infection annually.

African animal trypanosomosis reduces meat and milk production, prevents use of draft animals for land cultivation in tsetse infested areas and also hinders the upgrading of low yielding local breeds of livestock (Swallow, 1999). Agyemang and Rege (2004) reported that annual losses in meat production were estimated at 5 billion USD while losses in milk production, manure and traction if they could be prevented would amount to 50 billion USD. This is corroborated by Budd (1999) who estimated that, if it were possible to eradicate trypanosomosis from Africa, the benefit to overall agricultural production would gradually rise to 4.5 billion USD per year. Furthermore, treatment costs to farmers and governments was estimated at 35 million USD annually (Geerts and Holmes, 1998), with an estimated 17.5 million herd of cattle treated per year (Kristjanson *et al*., 1999).

Trypanosomes are flagellated protozoan parasites that live in the blood, plasma, the lymph and other body fluids, as well as various tissue organs of their vertebrate hosts.

The genus *Trypanosoma* belongs to the protozoan branch, phylum Sarcomastigophora, order Kinetoplastida, family Trypanosomatidae. Tsetse-transmitted trypanosomes belong to the salivarian group; subgenus Nannomonas for *Trypanosoma congolense,* Dutonella for *T. vivax* and Trypanozoon for *T. brucei. Trypanosoma congolense, T. vivax, T. simiae* and *T. brucei* are among the major trypanosomes affecting livestock in Nigeria with economic losses resulting from stunted growth, debility, poor reproductive performance or death in affected animals, being unquantifiable (Onyiah, 1997; Enwezor and Lawal, 2003).

Prevention and control of tsetse-transmitted trypanosomes depends essentially on minimizing contact between domestic animals, game animals and tsetse flies (Bouyer *et al*., 2013; Namangala and Odongo, 2014). Although highly variable so far methods, employed for the control of trypanosomes in tsetse infested areas include control of tsetse fly numbers (Aksoy, 2003, Abd-Alla *et al*., 2013), use of curative or prophylactic trypanocidal drugs (Jamal *et al*., 2005; Melaku and Birasa, 2013) and use of livestock breeds that tolerate the disease (Agyemang and Rege, 2004).

Trypanocidal drugs remain the principal method of animal trypanosomosis control in most African countries including Nigeria (Adamu *et al*., 2011; Shiferaw *et al*., 2015). However, with increasing knowledge and liberalization of veterinary drugs, control of the disease through the use of trypanocides is diminishing; hence there is a growing concern that their future effectiveness may be severely compromised by widespread drug resistance (Geerts and Holmes, 1998; Kroubi *et al*., 2011). In general, in areas where trypanocides have been used intensively drug resistance is more common than in areas where they have been used less intensively (Grace, 2003). The problems of drug resistance in domestic animals have been reported in 21 countries in Africa (Chitanga *et al.,* 2011; Melaku and Birasa, 2013), even in donkeys (Assefa and Abebe, 2001). For

effective control of tsetse transmitted trypanosomosis, knowledge of the tsetse biology and ecology (Leak, 1999), the disease epidemiology (Eisler *et al*., 2004), and trypanocidal drug efficacy are of utmost importance (Grace, 2003).

Homidium chloride (Novidium®), is an anti-trypanosomal drug and a known mutagenic compound with an uncertain mechanism of action (Eghianruwa, 2014). It is administered at the dose rate of 1 mg per kg body weight (b.w.) 2.5% solution, by deep muscular injection. Homidium chloride has been shown to interfere with glycosomal functions, the function of an unusual AMP-binding protein, trypanothione metabolism and replication of kinetoplast minicircles giving rise to dyskinetoplastic trypanosomes (Holmes *et al*., 2004).

Isometamidium chloride (Samorin®, Sécuridium®) also an antitrypanosomal drug, produces lethal effects in the trypanosomes by displacing magnesium ions and polyamines from ribosomes and thus modifies cytoplasmic membranes and the ribosomes (Kinabo and Bogan 'Late', 1988), resulting in the inhibition of phospholipid synthesis, basic amino acid transport, and oxygen uptake in the Tri-carboxylic Acid Cycle of the trypanosome (Kinabo, 1993). It is usually administered to trypanosome-infected animals by deep intramuscular (IM) injection of 1% or 2% solution at the dose rate of 0.25-1 mg per kg body weight (Aliu, 1981; Mamman, 1993).

1.2 Statement of the Research Problem

Sadly, despite their invaluable contributions to agricultural economy, donkeys are accorded low status and consequently neglected. Knowledge of diseases in donkeys is scanty and often extrapolated from the knowledge of diseases in horses (Pearson *et al.,* 1999).

Trypanosomosis is a well-documented problem of livestock and productivity in Nigeria (Sackey, 1998; Agu and Amadi, 2001; Enwezor and Sackey, 2005; Adamu *et al.,* 2009) but information on the effect of the disease in donkeys is fragmentary and not readily available, despite the fact that these animals play a key role as a means of transportation in the agricultural communities of the country where poor infrastructure and road network have made transportation by vehicle inaccessible.

Also, though considered as work animals, in some parts of the world, donkey meat and milk continues to be traded and consumed (Blench *et al.,* 2004; Mwenya and Keib, 2004; Cosentino *et al*., 2015). Thus this never diminishing demand for donkey bye-products, coupled with a potential expansion of its markets in the near future necessitates a study into the possible dynamics of tsetse-transmitted trypanosome infections in the donkeys and probable chemotherapeutic solutions which have hitherto not been investigated.

This study hopes to evaluate the clinical manifestation and response to treatment of donkeys experimentally infected with *Trypanosoma brucei* (Federe) and treated with Homidium chloride and Isometamidium Chloride.

1.3 Justification for the Research

Despite its rank as being among the first animal species to be domesticated by humans, and its immense contribution to the development of civilization, the donkey in comparison to other domestic animal species has been relegated to the background in the subject of scientific enquiry (Starkey, 1997; Getachew, 1999). Donkeys have been widespread in Northern Nigeria for many years but have received little research or development attention, with cognizance to the very important role they play in the socio-economic life of the rural farming population. Invariably known and emerging

health challenges of these animal species are also being neglected. This brings to fore the importance of this present study.

Notwithstanding the increase in mechanization throughout the world, donkeys still do have a prominent position in the agricultural systems of many developing countries (Ayele *et al*., 2006). Contrary to the size of the world donkey population, 44 million estimated (Fernando and Starkey, 2004), and the service they provide to society and national economies, particularly in developing countries, the level of care given to these animals is low (Gebreab *et al*., 2004).

Working equines are prone to painful, debilitating and often fatal conditions (Stringer, 2014; Herago *et al*., 2015). In addition, these animals especially donkeys in our local environ and probably the world over, work under difficult environmental conditions with inadequate food and water, and suffer from resultant exhaustion, dehydration, malnourishment, harness lesions and hoof problems (Biffa and Woldemeskel, 2006: Svendsen *et al*., 2008).

Kanchula and Abebe (1997) and Githiori *et al*. (1998) reported that where donkeys are found in tsetse infected areas, trypanosomosis is an important constraint to animal health, working capacity and productivity. It could be that donkeys infected with tsetse-transmitted trypanosomes may or may not show clinical signs of the disease, and thus act as reservoir of infection or carriers for susceptible animals. A gap exists in the knowledge of the severity of highly pathogenic trypanosome infections in donkeys in Nigeria, which this study seeks to address.

Homidium chloride and Isometamidium chloride have been reported to confer both prophylactic and curative effects against most trypanosome infections, and have less development of drug resistance (Radostits *et al.,* 2006).

1.4 Aim of the Research

This study was aimed at investigating the course of experimental *Trypanosoma brucei* (Federe) infection in donkeys using standard parasitological, hematological, serobiochemical and pathological methods, and evaluating therapeutic efficacy of Homidium chloride (Novidium®) and Isometamidium chloride (Sécuridium®) in treatment of the infection.

1.5 Objectives of the Study

1. Determine percentage susceptibility as well as parasitaemia profile of infection in donkeys experimentally infected with *T. brucei* (Federe).
2. Evaluate clinical manifestations observed in donkeys experimentally infected with *T. brucei* (Federe).
3. Evaluate haematological and serum biochemical profile in *T. brucei* (Federe) infected donkeys.
4. Evaluate gross and histopathological changes due to *T. brucei* (Federe) experimental infection in donkeys.
5. Evaluate therapeutic effect of Novidium® and Sécuridium® trypanocides on parasitaemia and haematological changes in donkeys experimentally infected with *T. brucei* (Federe).
6. Evaluate effects of the trypanocides on infection-induced pathology in the tissues and serum chemistry of donkeys experimentally infected with *T. brucei* (Federe).
7. Observe and document relapses of infection due to *T. brucei* (Federe) in donkeys, if any.

1.6 Research Questions

1. Are donkeys susceptible to experimental infection with *Trypanosoma brucei* (Federe)?
2. What clinical signs are manifested by donkeys experimentally infected with *T. brucei* (Federe)?
3. What are the haematological, biochemical and pathological changes observed in donkeys experimentally infected with *T. brucei* (Federe)?
4. Is there any evidence of tissue invasion by *T. brucei* (Federe) in experimental infection in donkeys?
5. What are the therapeutic effects of Novidium® and Sécuridium® on *T. brucei* (Federe) trypanosomosis in donkeys?
6. Do relapses occur in *T. brucei* infected donkeys treated with Novidium®/Sécuridium®-treated?

CHAPTER 2

LITERATURE REVIEW

2.1 The Donkey

The donkey (*Equus asinus*) also known as "ass" or "burro" was first described by Linneaus in 1758 (Grinder *et al*., 2006). It is a domesticated breed of the wild African ass (*Equus africanus*) and a separate species from the horse (*Equus caballus*) (Vilá *et al*., 2006). The name donkey came from an old English word "dunkey" meaning an animal that is grayish-brown in colour (Hagstrom, 2004). Donkeys and horses are close enough to genetically produce viable, but sterile hybrid offspring; mules and hinnies. However, they differ in certain ways as the donkey has 62 chromosomes while the horse has 64 (Herren, 2011). The ear of the donkey is much longer in proportion to their size than a horse's. Furthermore, the mane and tail in the donkey are coarser (Flanders, 2011).

Two wild ass subspecies are thought to have had a role in the development of the modern donkey. These are the Nubian specie (*Equus africanus africanus*) and the Somali specie (*E. africanus somaliensis*) though recent DNA analysis suggests that only the Nubian ass contributed genetically to the domestic donkey (Vilá *et al*., 2006; Rossel *et al*., 2008; Kimura *et al*., 2013). The estimated 44 million donkeys (Fernando and Starkey, 2004) in the world are said to be found mostly in developing and underdeveloped countries, where they are used as draught or pack animals (Fielding, 1991).

Domesticated donkeys are generally smaller than the wild ones (Hutchins and Hutchins, 1981), and characteristically vary considerably in size depending on their breed and management (Maurice, 2010). The male is known as jack while the female is referred to

as jenny/jennet (Flanders, 2011). Their dentition comprises 40 – 42 teeth: 6 upper and lower incisors, 2 upper and lower canines, 6 upper and 6 – 8 lower premolars, 6 upper and lower molars (Reynolds, 2013). Colours in the donkeys range from gray-dun to brown, a rare bay, black, light-faced roan, cream/albino white and spotted (Sponenberg, 2009). There are typical markings such as the dorsal stripes and shoulder crosses, dark ear marks, white belly and inner legs (Hutchins and Hutchins, 1981). Height at the withers ranges from 79 – 160cm, with a body length of about 200cm, and tail length of about 42cm while weight ranges from 80 – 280kg (Geddes, 1988). On the nape of the neck, there is a stiff upright mane with tipped black hairs, tail terminates in a brush "broom tail", and their hooves are slender (Flanders, 2011). They are herbivores and naturally live in arid and semi-arid areas (Yilmaz *et al*., 2012) with a gestation period of 11 – 14 months, and usually giving birth a single foal (Maurice, 2010). Life span is 12 – 15 years for working donkeys, and 30 – 50 years for donkeys in captivity (Quaresma *et al*., 2014).

It is important to realise that over 97% of the world's donkeys used for work (Fielding and Krause, 1998). For thousands of years, donkeys have been the "helping hooves" of mankind - the original beast of burden (Pearson *et al*., 1999), providing valuable services in many parts of the world where they could be used for transporting farm produce to markets by the farmers (Gebreab *et al*., 2004), for agricultural activities (Gebregziabher *et al*., 2006), for domestic chores such as fetching wood and water in rural communities (Starkey, 1994), and as guard animals for ruminant herds because of their natural aversion to canines thus helping to keep them away (Houpt, 2011).

Characteristically, donkeys are highly intelligent and have a strong sense of self-preservation, a trait that has frequently given them the underserved reputation of being stubborn (Fernando and Starkey, 2004; Smith, 2011). They are also thought to have a

calming effect on distressed horses (McGreevy, 2004). Despite the increased use of fossil fuel, donkeys appear to be a continued source of assistance and one, which plays a significant role in the economy of most developing countries (Ebenezer, 1991).

2.1.1 Uses of donkeys in Nigeria

The primary function of donkeys in Nigeria has traditionally been as pack animals. Two groups of people are seen to own most of the donkeys; the village farmers and pastoralists. Blench *et al*., (2004) reported that prior to the oil boom era of the 1970s, the use of donkeys was a very popular and efficient means of transport for goods and farm produce in the North leading to the proverbial 'jakin Kano' of Kano State. Their use declined drastically with the oil boom due to the massive importation of small pickups and motor bikes which became the preferred means of transport. Market prices for both vehicles and fuel were so low that many farmers sold their donkeys and breeders turned to other businesses (Blench, 2000).

Donkeys were seen as a symbol of the old-fashioned 'backward' rural ways and were thought to be permanently disappearing. As a result, initiatives to study donkeys or assist smallholders with their purchase, or to develop equipment for them, were poorly received (Blench *et al*., 2004). However, economic circumstances changed and the recession of the late 1980s led to the donkeys becoming popular once again among the farmers (Fielding and Starkey, 2004).

Breeds of donkeys recognized in Nigeria include: *Auraki* (rust/red), *Idabari* (grey), *Duni* (dark brown/black), *Fari* (pale cream/white), *Bakin jaki* (black in colour and not very common), *Goho* and *Jaba* (Hassan and Ibitoye, 1993; Hassan *et al*., 2009). Average herd size is 6, sex ratio is 1:1 and management is extensive, usually an open yard housing or under tree shades (Hassan and Ibitoye, 1993). Donkeys are fed mainly

on crop residues (rice and wheat stover, cereal stalks, sugarcane top) supplemented with cereal grains and salt, although they are sometimes allowed to graze in the fields, and are usually watered in streams, rivers or ponds. Selection for work is based mainly on age, size and health status (Blench *et al*., 2004).

2.1.2 Diseases of donkeys in Nigeria

According to Pearson *et al*. (1999), donkeys harbour uncounted infections and parasitic agents which have not all been exhaustively investigated. Various reports made on varying diseases and infectious agents affecting donkeys include those of Pinchbeck *et al*. (2008) whom reported that *T. brucei* causes acute diseases in donkeys and Chahan *et al*. (2006) who reported that donkeys are susceptible to *Theileria equi* and *B. caballi*. Mediannikov *et al*. (2010) reports of *Rhipicephalus evertsi evertsi* found on donkeys while Hamblin *et al*. (1998) and Mellor and Hamblin (2004) report of susceptibility of donkeys to African Horse Sickness. Other notable infections recorded include rabies (Ali *et al*., 2006; Xie *et al*., 2012), equine herpes virus (Patel and Heldens, 2005), equine influenza (Van Maanen and Cullinane, 2002; Qi *et al*., 2010), horse pox (Fox *et al*., 2012), mange (de Pennington and Colles, 2011), glanders (Mota *et al*., 2010; Khan *et al*., 2013) and helminth infections (Matthee *et al*., 2000; Uslu and Guclu, 2007; Getachew *et al*., 2010).

A survey in Yobe State, Northeast of Nigeria, recorded that most frequent conditions observed in donkeys are *jini* (blood loss from wounds and sores), *rinkyau* (ear disease) and *annumur* (cuts on the tongue which bleed and do not heal quickly) (Hassan *et al*., 2009). Other problems of donkeys commonly encountered by farmers and donkey owners in Nigeria include: colic (anomari), ulcerative lymphangitis, inflammation of the tongue, hoof rot, pneumonia, fistulous withers, helminthosis, unknown causes of

lameness and nervous system disorder known as “chinkai” (Bale *et al*., 2003; Ahmed *et al.,* 2008). Generally, identification and treatment of donkey diseases is often taken from the knowledge of the disease in horses, whereas susceptibility or resistance to the effects of disease agents may not necessarily be same for these two species.

2.2 African Animal Trypanosomosis

2.2.1 Definition and aetiology

African Animal Trypanosomosis (AAT) is a disease complex caused by tsetse fly-transmitted *Trypanosoma brucei brucei, T. congolense* or *T. vivax*. The disease is also transmitted mechanically by haematophagous flies such as the *Tabanus, Haematopota*, *Stomoxys* and *Chrysops* (Kone *et al*., 2011). African animal trypanosomosis is most important in cattle but can cause serious losses in pigs, camels, goats, and sheep (Spickler, 2010). Infection of cattle by one or more of the three African animal trypanosomes results in subacute, acute, or chronic disease characterized by intermittent fever, anaemia, occasional diarrhea, rapid loss of condition and often terminates in death (Goodwin, 2009).

Trypanosoma brucei brucei resides in the subgenus *Trypanozoon.* Horses, dogs, cats, camels and pigs have been reported to be very susceptible to *T. b. brucei* infection (Maudlin *et al*., 2004). Spickler (2010) observed that *T. b. brucei* infection in cattle, sheep, goats and sometimes pigs results in a mild or chronic disease. Although this last observation is widely accepted, Hotez and Kamath (2009) showed evidences that *T. b. brucei* is widespread in East and West Africa and can cause serious disease and high mortality in cattle, sheep, and goats.

In East Africa, *T. congolense* is considered to be the single most important cause of AAT (Anene *et al*., 2001; Ng'ayo *et al*., 2005). This trypanosome is also a major cause of the disease in cattle in West Africa, and sheep, goats, horses and pigs may also be seriously affected (Losos and Ikede, 1972). In domestic dogs, chronic infection of AAT often results in a carrier state (Gow *et al*., 2007).

Although *T. vivax* is considered to be less pathogenic for cattle than *T. congolense*, it is nevertheless the most important cause of AAT in West African cattle (Geerts *et al*., 2001; Steverding, 2008). This trypanosome readily persists in areas free of tsetse flies for instance, in Central and South America, and the Caribbean (Garraud *et al*., 2007: van den Bossche *et al*., 2010), where it is transmitted mechanically by biting flies (Jones and Dàvila, 2001; Desquesnes and Dia, 2004) or contaminated clinical and surgical instruments (Spickler, 2010).

2.2.2 Morphology of Trypanosomes

2.2.2.1 General Morphology

A robust knowledge of the basic features of the various trypanosomes enables identification of each species and thus the precise cause of the disease (Uilenberg, 1998). Morphologically, trypanosomes are unicellular microscopic and elongated protozoa that move by the help of a single flagellum at the base of which is found a characteristic structure known as kinetoplast (Kohl and Bastin, 2005; Ugochukwu, 2008; Vargas-Parada, 2010). Taxonomically, they are classified in the phylum Sarcomastigophora, the order Kinetoplastida and the family Trypanosomatidae (WHO, 1998). They are divided into three subgenera that include *Nannomonas*, *Dutonella* and *Trypanozoon.*

The trypanosome consists of a single cell (Englund *et al*., 1982) varying in size from 8 to over 50 µm (Callejas *et al*., 2006; Sharma *et al*., 2008). African trypanosomes are

characterized based on their size, shape, position of the nucleus, size and location of the kinetoplast, host range and geographical distribution. There are distinct differences in appearance, shape and size between the various species, allowing specific identification (Uilenberg, 1998). The Salivaria group of trypanosomas may or may not have a free flagellum, the kinetoplast is terminal or sub-terminal, and the posterior end of the body is usually blunt (Lumsden, 1974). They develop as trypomastigotes within the mammalian host and are usually pathogenic (Vickerman, 1985; Barret *et al*., 2003).

2.2.2.2 The subgenus Nannomonas

Species of trypanosomes found in this group include *Trypanosoma congolense, T. simiae* and *T. godfreyi*. These are the smallest of the pathogenic trypanosomes with a length of 8-24 μm, and defined by their development in the tsetse fly, which involves the midgut and proboscis (Kreier and Baker, 1987). The blood forms are monomorphic, in that they lack a free flagellum (Uilenberg, 1998). Generally, two variants are seen, a shorter form (9-18 μ), the typical *congolense* type and a longer form (up to 25 μ), with individuals intermediate in length between the two (Hoare, 1972). There is evidence indicating that strains with the longest forms, the supposed 'dimorphic' strains, cause a more severe form of trypanosomosis and in stained specimens, *T. congolense* cytoplasm stains a diffuse, even, pinkish colour and seldom granular (Uilenberg, 1998).

Characteristically, the nucleus is centrally placed, kinetoplast is of medium size and usually situated at the margin of the body, just in front of the posterior extremity (marginal and subterminal), the undulating membrane is poorly developed and inconspicuous (Hoare, 1972). Recent studies have resulted in a subdivision of the *T. congolense* species into several 'types', which can be distinguished by iso-enzymatic differences using molecular techniques (Uilenberg, 1998). These are designated as *T.*

congolense savannah type, *T. congolense* Tsavo type, *T. congolense* riverine-forest type, *T. congolense* Kilifi type (McNamara *et al*., 1990; Majiwa *et al.,* 1993; Nthiwa, 2013).

Trypanosoma simiae species are polymorphic, with a length of 15-19 µm, the kinetoplast which is of medium size, is marginal and subterminal as in *T. congolense* and there may be a free flagellum present (Hoare, 1972; Stephen, 1986). *Trypanosoma godfreyi,* another specie of the Nannomonas, is described as a parasite of the Suidae and was identified to be separate from *T. congolense*, on the basis of iso-enzymatic and DNA differences (McNamara *et al*., 1994; Masiga *et al*., 1996). Morphologically, *T. godfreyi* is relatively small with a mean length of 13.7 µm (9.1 – 21.8 µm), with no free flagellum but a subterminal marginal kinetoplast and a conspicuous undulating membrane (McNamara *et al*, 1994).

2.2.2.3 The subgenus Duttonella

This subgenus has two species, *Trypanosoma vivax and Trypanosoma uniforme.* Their development in tsetse is confined to the proboscis (Moloo and Gray, 1989). They parasitize predominantly wild and domestic ungulates in Africa, Central America and South America, and are transmissible both cyclically by tsetse flies and mechanically by other bloodsucking insects (Jones and Dávila, 2001; Mbaya *et al*., 2009a).

Trypanosoma vivax is the principal species in this subgenus and was named because of the vigour of its activity under the microscope when examined in fresh preparations (Gardiner and Wilson, 1987). The parasite moves rapidly across the field of view (Picozzi *et al*., 2002). In the blood of mammals, it is also essentially monomorphic, with a free flagellum (Silva, 2012). Its length, inclusive of the free flagellum (which is 3-6 µm long), varies from 18 to 31 µm, kinetoplast is large and terminal, undulating membrane is inconspicuous while the nucleus is centrally placed and the posterior

extremity is swollen and blunt (club-shaped). It has a distinguishing feature of being larger than other pathogenic species (Hoare, 1972; Uilenberg, 1998).

Trypanosoma uniforme are the smaller trypanosomes of this subgenus, with length of about 12 to 20 µm, but otherwise similar to *T. vivax* (Gibson, 2007).

2.2.2.4 The subgenus Trypanozoon

The subgenus Trypanozoon is said to be the most homogeneous group of Salivarian trypanosomes, represented customarily by morphologically indistinguishable species which, however, differ in biological features (Gibson, 2003). This group comprises three members namely *T. brucei, T. evansi* and *T. equiperdum* (Hoare, 1972) with a host range of wild and domestic animals that include camels, horses, dogs and bovids (Gibson, 2003).

Studies show that *T. brucei* consists of 3 morphologically indistinguishable subspecies which can only be biologically differentiated by host range variation and geographical distribution (Hoare, 1972; Gibson and Wellde, 1985, Uilenberg, 1998). These are: *T b. brucei,* predominant in Tropical Africa and infects only livestock causing 'nagana', *T. b. gambiense* and *T. b. rhodesiense* which infect humans, and are predominant in West/Central Africa and East/Southern Africa respectively (Latif and Adam, 1973). The typical East African form of human trypanosomosis is characterized by a rapid and acute development of the disease, and untreated patients can die within weeks or months of the infection, whereas the West African form of this disease is more chronic and can last for several years (Baral, 2010). *Trypanosoma brucei* is polymorphic, with three main forms, all of which have a small kinetoplast and a conspicuous undulating membrane. The forms include;

(i) Long slender forms (23-30 µm in length) with a free flagellum, posterior pointed end and centralized nucleus. The kinetoplast is placed up to 4 µm in front of the posterior extremity.

(ii) Short stumpy forms (17-22 µm in length) normally without a free flagellum, but in which there may occasionally be individuals with a short free flagellum. The kinetoplast is usually subterminal. Here 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).

(iii) Intermediate forms, varying in length between the two previously mentioned types. A free flagellum, of varying length, is always present (Langousis and Hill, 2014). Nucleus is centrally placed and the posterior end is somewhat variable in shape, but usually bluntly pointed. The kinetoplast is close to the posterior extremity (Matthews, 2005).

During the course of the 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 long thin forms are the true mature blood forms which die in the gut of the insect (Uilenberg, 1998).

Trypanosoma evansi is typically represented almost exclusively by thin trypomastigotes comprising slender and intermediate forms corresponding to those in *T. brucei* (Brun *et al.,* 1998; Misra *et al.,* 2015). The slender forms have a long free flagellum and a narrow posterior extremity, which may be rounded or truncated, with the kinetoplast situated at some distance from the tip. The intermediate forms have a shorter free

flagellum and a short, frequently pointed, posterior extremity, with the kinetoplast lying near this end (Hoare, 1972). *Trypanosoma equiperdum* is morphologically indistinguishable from *T. evansi* (Brun *et al.,* 1998; Lai *et al.*, 2008). Like the latter species, it is typically monomorphic, being represented by thin (slender and intermediate) trypomastigotes possessing a free flagellum and its size likewise within the range of that of *T. evansi* (Brun *et al.,* 1998). As in the case of *T. evansi,* it is also liable to become pleomorphic, exhibiting typical and postero-nuclear stumpy forms (Hoare, 1972).

2.2.3 Host range of trypanosomes

Livestock exhibit a range of susceptibility to infection, from refractory to highly vulnerable (Bishop and Woolliams, 2014). Cattle, sheep, goats, pigs, horses, camels, dogs, and cats are susceptible to AAT (Taylor and Authié, 2004). Ruminants are extensively known to be active reservoirs of the trypanosomes (Abenga and Lawal, 2005; Desquesnes *et al.,* 2009; Funk *et al.,* 2013). Susceptibility of cattle to trypanosomosis is based on breed, age, habitat, previous exposure and health status (Murray *et al.,* 1984; Mattioli *et al.,* 2000; Orenge *et al.,* 2012). The indigenous zebu cattle are trypanosusceptible and West African *Bos taurus* breeds, commonly referred to as N'dama, Muturu and Keteku in Africa, are trypanotolerant (Murray *et al.,* 1982; Mattioli *et al.,* 2000; Freeman *et al*., 2004). Exotic imported ruminants (like improved dairy cattle) are more severely affected than local ones (Taylor and Authie, 2004).

Laboratory animals which include rats, mice, guinea pigs and rabbits can also be infected by trypanosomes. Wildlife (monkeys, wild equidae, lions, leopards, wild pigs, warthog, bush pig, duiker, bush buck, kudu, buffalo and monitor lizard) are the natural hosts of tsetse and may acquire prolonged, symptomless trypanosome infections (Taylor

and Authié, 2004; Maudlin, 2006; Munangàndu *et al.,* 2012). The wildlife in Africa generally tolerates infection and often serves as a reservoir for human and livestock-infective trypanosomes (Taylor and Authié, 2004; OIE, 2005).

2.2.4 Transmission and distribution of *Trypanosoma* species

In Africa, the prime vector for *T. congolense* and *T. brucei* is the tsetse fly (Glossina species) (Cattand *et al*., 2006). These trypanosomes replicate in the tsetse fly and are transmitted through tsetse saliva when the fly feeds on an animal (Majiwa *et al*., 1994; van den Abbeele *et al*., 2010). The Centre for Disease Control (CDC) of the United States of America reported that tsetse flies are found only in Africa however, Elsen *et al*. (1991) reported a discovery of two species of tsetse flies in Saudi Arabia. They are the biological and/or mechanical vector of trypanosomes and constitute a potent and constant threat to humans and livestock over much of sub-Saharan Africa (Gooding and Krafsur, 2005).

Tsetse flies are classified into a single genus *Glossina* Weidemann 1830, comprising of thirty-one species and subspecies. They are divided into three subgenera/groups (Krafsur, 2009) which include:

(i) The Fusca group flies (subgenus *Austenina* Townsend) which tend to occur in the lowland rainforests of West and Central Africa.

(ii) The Palpalis group (subgenus *Nemorhina* Robineau-Desvoidy) found in the riverine galleries of West and Central Africa but can extend into savannah regions between river systems; *G. palpalis* and *G. tachinoides* are important AAT vectors in this group.

(iii) The Morsitans group (subgenus *Glossina* Weidemann) occurring in a variety of savannah habitats lying between the forest edges and desert, and includes several important vectors of AAT such as *Glossina morsitans* spp., *G. pallidipes* and *G. austeni* (Rogers and Robinson, 2004).

In Nigeria, 11 species of the tsetse fly, which are capable of transmitting both animal and human trypanosomiasis, are found (Davies, 1977). Surveys have shown that these tsetse flies still infest about 74% - 80% of the nation's landmass, covering all the agro ecological zones of the country (Onyiah *et al*., 1983; Jawonisi, 1988). The highlands of Jos, Mambila and Obudu plateaux which were hitherto described as tsetse and trypanosomosis free have also been re-invaded (Anene *et al*., 1991; NITR/NARP, 1995). The four most important species of *Glossina* in Nigeria include *G. palpalis*, *G. tachinoides*, *G. morsitans,* a submortisans and *G. longipalis* (Davies, 1977). Tsetses feed exclusively on blood and rely on one such intracellular microbe for nutritional provision and fecundity (Akman *et al*., 2002). They are holometaboulos insects with females giving birth to full-grown larvae which rapidly pupate in the soil (Leak, 1999; Awoke and Kassa, 2006). As a result of their longevity, actions and habitual feeding, these flies' make highly efficient vectors. However, due to their low population growth rate, slight increases in mortality rate may result in dwindling populations and a likely extinction (Hargrove, 2003).

Trypanosomosis is also mechanically transmitted by tsetse and other biting flies through the transfer of blood from one animal to another (Cherenet *et al*., 2006). The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota, Liperosia, Stomoxys*, and *Chrysops* flies have also been implicated (Ahmed *et al*., 2005; Mammo *et al*., 2013). In Africa, both *T. vivax* and *T. b. brucei* have spread past the "tsetse fly belts" (D'Amico *et al.*, 1996), where transmission is notably by tabanid and

hippoboscid flies (Desquesnes and Dia, 2003). The activity of biting flies is responsible for the persistence of *T. vivax* in areas of Africa free from tsetse flies (Anene *et al*., 1991), as well as in several South American countries like Brazil, Colombia, Peru and French Guyana (Dávila and Silva, 2000; Cadioli *et al*., 2012). For instance, two fatal outbreaks of bovine trypanosomosis due to *T. vivax* were described in closed herds of Wadara cattle breed maintained in Maiduguri, a tsetse free sahelian region in Nigeria, (Nawathe *et al*., 1988).

2.2.5 Pathogenesis and clinical signs of animal trypanosomosis

The changes which occur in an animal during trypanosome infections are determined by several factors such as the specie of the infecting trypanosome, size of the infective dose, virulence of the parasite (ability/inability to invade extravascular spaces and organs), intraspecies variation, susceptibility of the host, age of the host, physiologic status of the host, nutrition and environmental factors (Anosa, 1983; Murray, 1989; Holmes *et al*., 2000; Taylor and Authié, 2004; Dalal, *et al*., 2008). African animal trypanosomosis typically manifests in three phases that include hyperacute, acute and chronic. However, under natural challenge, disease manifestation may be more complex (Taylor and Authié, 2004). There is considerable variation in the pathogenicity of different strains and the susceptibility of different host species (Barbour and Restrepo, 2000).

The trypanosomes undergo cyclical development in flies lasting 12 – 35 days before they become infective (Ugochukwu, 2008). Initial replication in the host happens at the site of inoculation in the skin. This causes an inflammatory reaction (a swelling and a sore) called ‘chancre’ with regional lymphadenitis (Zeledón, 1996; Kennedy, 2008). Trypanosomes then spread to the lymph nodes and blood, and continue to replicate

(Giddings *et al*., 2006) causing damage as they do. *Trypanosoma congolense* and *T. vivax* localize in small blood vessels and capillaries. *Trypanosoma brucei brucei* and *T. evansi* localize also in tissues aside blood vessels (Hill, 2003: Langousis and Hill, 2014).

Antibodies developed to the glycoprotein coat of the trypanosome lyse the trypanosome and consequently lead to the development of immune complexes (Taylor and Authié, 2004). These antibodies however, does not clear the infection, for the trypanosome has genes that can code for many different surface-coat glycoproteins and change its surface glycoprotein to evade the antibody, a phenomenon referred to as antigenic variation (Rudenko, 2011). Thus, there is a persistent infection that results in a continuing cycle of trypanosome replication, antibody production, immune complex development, and changing surface-coat glycoproteins (Hall *et al.,* 2013).

Immunological lesions are significant in trypanosomosis, and it has been suggested that many of the lesions (e.g. glomerulonephritis) in these diseases may be the result of the deposition of immune complexes that interfere with, or prevent normal organ function (Pentreath, 1995). The most significant and complicating factor in the pathogenesis of trypanosomosis is the profound immunosuppression that occurs following infection by these parasites. This marked immunosuppression lowers the host's resistance to other infections and thus results in secondary disease, which greatly complicates both the clinical and pathological features of trypanosomosis (Barrett *et al.,* 2003). Lymph node enlargement and splenomegaly develop, associated with plasma cell hyperplasia and hypergammaglobulinaemia which is primarily due to an increase in IgM (Aquino *et al*., 2002; Omotainse and Anosa, 2009). Concurrently there is a variable degree of suppression of immune responses to other antigens such as microbial pathogens or

vaccines. In infections of long duration, the lymphoid organs and spleen eventually become shrunken due to exhaustion of their cellular elements (Prowse, 2005).

Simultaneous infections with more than one *Trypanosoma* species are common (Pinchbeck *et al.,* 2008). Also simultaneous infections with trypanosomes and other haemoparasites (*Babesia* spp., *Theileria* spp., *Anaplasma* spp., and *Ehrlichia* spp.) frequently occur, and it is difficult to conclude which clinical signs are attributable to a given parasite (Cox, 2001). Few adequately controlled studies have been made, and thus a "typical" clinical response to each trypanosome is difficult to reconstruct (Uzcanga *et al*., 2004). Anaemia is for example seen in a whole series of diseases caused by blood parasites (*Babesia* and *Anaplasma* infections inclusive) as well as in certain gastro-intestinal helminth infections (*Haemonchus contortus*) and therefore not typical of trypanosomosis by itself (Nyeko *et al*., 1990).

Many of the clinical and pathological manifestations of trypanosomosis are common to domestic animals, irrespective of the host and the species of trypanosomes involved (Taylor *et al*., 2004). The crucial clinical symptom observed in AAT is anaemia which in its initial stage of the manifestation is proportional to the degree of parasitaemia (Igbokwe and Mohammed, 1992; Ohaeri and Eluwa, 2011). Studies on the mechanisms of anaemia in trypanosomosis indicate that though essentially haemolytic, the anaemia is complex and multifactorial in origin, and the relative contribution of each mechanism differs according to the host-parasite model, the phase of anaemia development and the severity of infection (Naessens *et al*., 2005; Mbaya *et* al., 2012; Shimelis, 2015). Most common among these factors are erythrocyte injury caused by lashing action of trypanosome flagella, undulating pyrexia, platelet aggregation, toxins and metabolites from trypanosomes, immunochemical reactions, oxidative stress, lipid peroxidation, disseminated intravascular coagulation, idiopathic and tumor necrosis factors (TNF),

bone marrow nitric oxide (NO) activity and malnutrition (Igbokwe, 1994; Nok and Balogun, 2003; Mbaya *et al*., 2012). Within a week of infection with the haematic trypanosomes (*T. congolense* and *T. vivax*) there is usually a pronounced decrease in packed cell volume (PCV), haemoglobin concentration and red blood cells count, and within 2 – 3 months the PCVs may drop to below 30 percent of their pre-infection values (Ohaeri and Eluwa, 2011). Also, invariably, present are intermittent fever, oedema and loss of condition (Desquesnes *et al*., 2013). Abortion may be seen, and infertility of males and females may be a sequel (Tibary *et al.,* 2006). Severity of clinical response is dependent on breed and species of affected animals, as well as the dose and virulence of the infecting trypanosome. Stress due to poor nutrition or a concurrent disease, also plays a prominent role in the disease process (Taylor and Authié, 2004).

Various pathological disorders at varying degrees have been reported consequent upon natural or experimental animal trypanosomosis. They include: emaciation, haemorrhage, pulmonary congestion, hepatomegaly, splenomegaly, atrophy of fat, atrophy and tissue necrosis of reproductive organs, mononuclear perivascular cuffing, enlarged Bowman's spaces, haemosiderosis, meningoencephalitis, nephritis, loss of tissue architecture, and presence of inflammatory changes (Ikede *et al*., 1977; Morrison *et al*., 1981; Omotainse and Anosa, 2009; Allam *et al*., 2011; Ukpai and Nwabuko, 2014).

2.2.6 Epidemiology of African Animal Trypanosomosis

The epidemiology of AAT is dependent on three factors which include vector distribution, virulence of the parasite and the affected host response (Taylor and Authié, 2004). When dealing with the tsetse-transmitted trypanosomosis, much depends on the

distribution and the vectorial capacity of the *Glossina* species responsible for transmission (Tadese, 2010; Apaatah, 2014; Grace *et al*., 2015). The savannah and riverine groups of *Glossina* are the most crucial since they inhabit areas appropriate for grazing and watering of livestock (Taylor *et al*., 2013). Although the infection rate of *Glossina* with trypanosomes is usually low, ranging from 1 – 20% of the flies, each is infected for life, and their presence in any number makes the rearing of cattle, pigs and horses extremely difficult (Rogers and Williams, 1993). Where savannah tsetses are the vectors, the danger of contracting the disease is great (Uilenberg, 1998). When the riverine species are the culprits (in many parts of West and Central Africa), transmission occurs particularly along rivers that have dense vegetation along their banks (FAO, 2006).

The proportion of a tsetse population found infected with pathogenic trypanosomes depends not only on its vector capability, but also on the host on which it mainly feeds (Geiger *et al*., 2015). For example, reptiles do not carry pathogenic trypanosomes, and there are also major differences between suids and bovids, as the former will infect the flies particularly with *T. simiae* and *T. godfreyi*, while bovids are mainly the source of *T. vivax* and *T. congolense* (Uilenberg, 1998). Biting flies may act as mechanical vectors, but their significance in Africa is still undefined. However, in Central and South America, *T. vivax* is thought to be transmitted readily by such flies (Gardiner, 1989).

The parasite virulence, immunogenicity and response to chemotherapeutics are also important factors in the epidemiology of trypanosomosis as the trypanosome species occur in a remarkable variety of genotypes (Eisler *et al*., 2004). Since parasitaemic animals commonly survive for prolonged periods, there are ample opportunities for fly transmission, especially of *T. brucei* and *T. congolense* (Taylor and Authié, 2004). In

contrast, some strains of *T. vivax* in cattle and *T. simiae* in domestic pigs kill their hosts within 1 – 2 weeks, so that the chances of fly infection are more limited (Gardiner, 1989). Species and breed susceptibility are also important in the epidemiology of trypanosomosis as animal hosts differ in their response to trypanosome infection, depending on the species and breed of the individual animals (Taylor *et al*., 2004). The level of animal husbandry practices, nutritional status, workload and stress exacerbate the severity of the disease (Uilenberg, 1998). The fact that the parasite affects not only cattle but also wild animals which constitute reservoirs of the disease, makes the epidemiology of animal trypanosomosis extremely complicated (Hoare, 1972; Uilenberg, 1998).

In 2005, a case report emerged in India of a human trypanosomosis caused by *Trypanosoma evansi*, first ever to be documented (Joshi *et al*., 2005). The patient who had fluctuating parasitaemia associated with febrile episodes for about 5 months was treated with suramin and a cure achieved. Patient follow-up 6 months after treatment showed a complete cure indicating that the drug was well tolerated (Joshi *et al*., 2006). A further investigation on human trypanosomosis using card agglutination tests on 1806 persons from the village of origin of the patient revealed 22.7% of the persons positive, indicating a probable frequent exposure of the human population in the study area to *T. evansi* (Shegokar *et al.* 2006).

2.3 Molecular Biology of Trypanosomes

Trypanosomes exhibit considerable intra-species genetic diversity and variation which can be defined at the level of both the genome and of individual genes (Myler, 1993). The nuclear genome shows considerable inter- and intra-species plasticity in terms of chromosome number and size whereas, the mitochondria (kinetoplast DNA) genome

varies considerably in terms of minicircle size and organization, and within the variable region (VR) of maxicircles (Myler, 1993).

2.3.1 Trypanosomal Nucleus

Trypanosomes have a genome size estimated to be 3-4 x 107 bp (Borst and Cross, 1982), and the DNA is organized into multiple, linear chromosomes, ranging in size from 25 kbp to several Mbp (Van der Ploeg *et al.,* 1992). The DNA content has been shown to vary among species, subspecies and strains (Melville *et al.,* 1999). The use of pulsed-field gel electrophoresis (PFGE) has enabled understanding of protozoan genome organization, which was hampered because of the absence of chromosome condensation during metaphase (Schwartz and Cantor, 1984).

The genomes of African trypanosomes contain variable numbers of minichromosomes (25- 50 kbp), which vary widely between species and even within species (McCulloch and Horn, 2009; Horn, 2014). *Trypanosoma congolense*, *T. brucei* and *T. b. rhodesiense* contain about 100-120 kbp, *T. b. gambiense* contains about 10 kbp, *T. equiperdum* contains one and *T. vivax* and *T. cruzi* contain none (Van der Ploeg *et al.,* 1984; Majiwa *et al.,* 1985; Gibson and Borst, 1986). The absence of minichromosomes suggests that they may represent an adaptation to the molecular mechanisms used for antigenic variations in African trypanosomes (Donelson, 2003; Morrison *et al.,* 2009). In addition to minichromosomes and the large chromosomes, which contain housekeeping genes, a majority of trypanosome species also contain intermediate-sized chromosomes that differ in number and size (150-700 kb) amid stocks of the same parasite species (Melville *et al.,* 2000; Donelson, 2003).

2.4 Diagnosis of Animal Trypanosomosis

The prime reason for diagnosis of animal trypanosomosis is for the appropriate application of therapeutic and prophylactic measures (Grace, 2003; Machila *et al*., 2007). Other reasons include the need to target and monitor tsetse control or eradication operations, investigations into the efficacy of chemotherapy and especially trypanocidal drug resistance, and pathophysiological, epidemiological and socio-economic studies (Uilenberg, 1998; Otte *et al*., 2004). The type of diagnostic test used in the detection of infections caused by the animal trypanosomosis will vary according to the epidemiological characteristics of the disease and the strategy for control (Cattand *et al*., 2006). Where tsetse-transmitted trypanosomosis occur and where disease prevalence is high, even tests of low diagnostic sensitivity will suffice if chemotherapy and chemoprophylaxis are administered on a herd basis (Luckins, 1992). However, in many situations where mechanically transmitted trypanosomosis is found, drugs are often administered therapeutically to individual infected animals and it is essential that more sensitive diagnostic tests be used in order to detect active infections (Luckins, 1993).

Similar considerations also apply after control campaigns. As the disease prevalence declines, the need for individual treatment as opposed to herd treatment becomes an important issue. When chemotherapy has been applied in areas where drug resistance is known to exist, it is also necessary to detect rapidly any failure in treatment (WHO, 1998).

It is often laborious to have a definitive diagnosis of trypanosomosis, especially in areas with limited veterinary services (Nantulya, 1990; Uilenberg, 1998). The methods frequently employed for diagnosis are clinical, parasitological and serological (Luckins, 1993). However due to poor sensitivity and specificity of these methods, molecular

techniques which are more precise and sensitive, are now being relied upon for more accurate diagnosis and identification of the parasites (Thekisoe *et al*., 2007).

2.4.1 Clinical diagnosis

Clinical diagnosis is somewhat problematic because the disease has no pathognomonic signs and a variety of other diseases have similar clinical manifestations with it (Barret *et al*., 2003). Often when an animal in an endemic area is febrile, anaemic and in poor condition, trypanosomosis is usually suspected (Herenda *et al.,* 1994). The clinical picture depends to some extent on the species of infecting trypanosomes and the susceptibility of the host (Luckins, 1993). Owing to the fact that the disease may present in acute, chronic or subclinical forms, diagnosis based on clinical manifestations may be presumptive. Confirmation, therefore, depends on the demonstration of the organism in blood or lymph node smears using the parasitological methods available (Menon and Mathew, 2008).

2.4.2 Parasitological diagnosis

Examination of the blood by light microscopy (direct microscopic examination) is the most readily applied technique for diagnosis of trypanosomosis and, more importantly, a technique which can be easily applied in the field. The basic technique, examination of fresh or stained blood films at x400 magnification, has been modified to improve diagnostic sensitivity, by concentrating the blood by centrifugation in a haematocrit tube, namely the microhaematocrit centrifuge technique (HCT) or the darkground buffy coat technique (DG) (Woo, 1970; Murray *et al.,* 1977; Paris *et al*., 1982). Other modifications suggested, but not widely applied include separation or removal of blood cells prior to centrifugation by anion exchange chromatography, miniature-anion

exchange centrifugation technique (m-AECT), or hypotonic lysis (Lumsden *et al*., 1979; Nantulya, 1990). Freshly collected blood can also be inoculated into laboratory rodents which can then be examined for periods of up to 30 to 60 days to determine if trypanosome infections develop in them (Luckins, 1993; Balows *et al*., 2012).

Evaluation of some of these techniques under experimental conditions has given an indication of their detection limits in relation to the numbers in blood of trypanosomes of different species (Luckins, 1993). In the field, practical considerations determine which technique can be used; remoteness of location could prevent maintenance of rodents and lack of generator sets, centrifuges and work bench may preclude use of HCT and DG techniques (Luckins. 1992).

2.4.2.1 Wet mount technique: The use of this technique in diagnosis of trypanosomosis was described by Herbert and Lumsden in 1976. It is the simplest and easiest technique involving the direct examination of blood samples collected from live animals or within minutes after death. In this technique, fresh blood samples are examined between a coverslip and a slide with the microscope, using medium magnification (usually a dry objective of 40x magnification, and eyepieces of 5–10x). Trypanosomes are seen either directly, moving between the blood cells, or indirectly, as they cause the blood cells to move. The lower the magnification, the larger the field (and the quantity of blood) observed, and the faster the examination.

This technique has the advantage of being inexpensive and rapid, with the parasites easily detected during the acute phase of the disease and diagnosis promptly made on the spot. However, it has limited sensitivity due to a detection limit of around 10^4 trypanosomes per ml of blood and the inability to identify the exact trypanosome

species using this technique (*T. brucei* and *T. vivax*, being larger and the latter also more mobile, are more easily noticed than *T. congolense*) (Uilenberg, 1998).

2.4.2.2 Microhaematocrit centrifugation technique: This technique was first described by Woo (1970) and usually employed when parasitaemia is low and cannot be detected by wet mount. Here, EDTA-coated microhaematocrit capillary tubes which are open at both ends are filled to three-quarters their length. One end of the tube is then sealed over a burner (taking care not to char the blood) or by special wax or plasticine. The tubes are 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 being thrown out during centrifugation), the cover is closed and screwed down, and the timing is set for five minutes. After centrifugation at 3000 x *g* for 5 minutes, the tubes are removed, care being taken that it remains known to which animal each of the tubes corresponds.

Upon centrifugation, the blood sample is separated into layers (plasma, buffy coat and red blood cell). The tubes are then examined for the presence of trypanosomes, either by direct examination of the buffy coat/plasma junction, or after making a smear of this area. For direct examination, the spun capillary tubes are placed on a microscope slide. Immersion oil is placed over the region of the tube (buffy coat, and buffy coat/plasma junction) where the parasites, if present are concentrated and will be visible, and examined using the oil immersion objective lens (Uilenberg, 1998).

This technique has the advantage of having a higher sensitivity than the direct wet mount method, can be used in the field with the aid of a portable generator to run the centrifuge, and packed cell volume can be obtained at the same time. Its disadvantage is that a special equipment is needed and specie identification may not be possible.

2.4.2.3 Thick blood smear: In this method, 2 – 3 drops of blood are placed together on a clean slide and spread out with the corner of another slide or wooden applicator stick, to produce a circular area to a thickness such that, when dry, the hands of a watch or small print can be seen through the film. One may also let the drops run over the inclined slide until the appropriate thickness is attained. Next, the film is thoroughly air-dried, and in the laboratory stained without fixation, usually with Giemsa stain. Giemsa stain is aqueous and removes the haemoglobin by lysing the red blood cells. Fixation with methanol is not carried out, as it would prevent lysis, which is necessary in order to see through the several layers of blood cells, which otherwise would obscure the parasites. If lysing and staining are done before the blood has thoroughly dried, the film may be washed off the slide. On the other hand, if many days elapse between the preparation of the film and lysis and staining, lysis may be incomplete, especially if the film has been exposed to heat or sun. It may help to lyse such films in distilled (or at least clean) water before staining, but it may difficult to obtain satisfactory results. Examination is carried out under the microscope, preferably using objective of 40–50x. Specific diagnosis of the trypanosomes is sometimes possible using the 100x oil immersion, but is usually difficult or impossible because the process of lysis distorts the parasites (Uilenberg, 1998).

This method is used to confirm results of direct wet mounts. It is simple, inexpensive and does not require a field microscope, as the blood films are taken back to the laboratory, for processing and examination at ease. It is sometimes (but mostly not) possible to identify the trypanosome species seen. The disadvantage of this method is that an immediate diagnosis of trypanosomosis on the spot is not possible, and the sensitivity of the method is limited.

2.4.2.4 Thin blood smear: Here, a pin-head drop of blood is made on one end of a clean glass slide. A spreader is held firmly in horizontal position at an angle of 30° to the glass slide, touching the drop of blood such that the blood runs along the line of contact between the spreader and the glass slide. The spreader is then pushed gently but firmly along the surface of the horizontal slide so that the blood is dragged behind the spreader to form the film with a feathered edge. Next, the smear is air-dried, fixed with methyl alcohol and stained with Giemsa. After staining, it is allowed to air dry and then examined using the 100x objective oil immersion lens for presence of parasites (Uilenberg, 1998).

This method is advantageous in specific diagnosis of trypanosomes, however, the sensitivity is very low. Thus it is mainly used for specie identification of trypanosomes found using other methods.

2.4.3 Immunodiagnostic techniques

Although direct demonstration of trypanosomes in the infected animal gives conclusive proof of infection, the limitations of parasitological diagnosis have been the driving force for a great deal of research into alternative techniques which provide indirect evidence of infection, namely immunodiagnostic techniques (Luckins, 1993). There are many reports of the use of immunodiagnostic techniques for diagnosis but invariably most of them have been retrospective surveys, intended to add further information rather than as an integral part of a control programme (Rebeski *et al*., 1999; Ndao, 2009). The one exception to this generalization is in the application of the complement fixation test (CFT) for the diagnosis of *T. equiperdum*, the cause of dourine in horses (Cauchard *et al*., 2014).

Serology has always played a major role in the diagnosis of this disease since the trypanosomes are rarely found in blood or other body fluids (Luciani *et al*., 2013). The CFT was used successfully in the control and eradication of dourine in North America (Derbyshire and Neilsen, 1997; Maudlin *et al*., 2004). A modified form of this assay is still in use today in testing sera for import and export of horses between different countries (Ogunremi *et al*., 2007). The test has not been used extensively for the other animal trypanosomiases due to problems in antigen preparation, standardization of the assay and interference by anti-complementary activity in sera from several animal species (Luckins, 1992). Problems in the control and standardization of another sensitive test, the indirect haemagglutination test (IHA) have hindered its general use despite its use in the diagnosis of *T. evansi* in camels in Central Saudi Arabia, and in a control programme in buffalo and cattle (Nantulya, 1990; Omer *et al*., 1998). In tests with *T. vivax* it was considered too unreliable (Clarkson *et al*., 1971).

Luckins (1993) had the opinion that the breakthrough in immunological diagnosis came with the introduction of primary binding assays; Card Agglutination Tests for Trypanosomes (CATT), Indirect Fluorescent Antibody Test (IFAT) and Enzyme-Linked Immunosorbent Assays (ELISA), for the detection of trypanosomal antibodies. He stated that these tests measure directly the interaction between antigen and antibody rather than relying on a secondary reaction consequent upon the initial binding, thus having a higher sensitivity and specificity. The IFAT has been used extensively in the detection of trypanosomal antibodies in animals and man (Stevens and Brisse, 2004; Kaufmann, 2013). Antigens are usually prepared from blood smears which are fixed in acetone and are then stored at a low (-20°C or -70°C) temperature (Luckins, 1993). The IFAT has been shown to be both specific and sensitive in detecting trypanosomal antibodies in infected cattle and camels (Magona *et al*., 2003; Ngaira *et al*., 2003).

However, cross-reactions between different trypanosome species do occur, sometimes producing false-negative results. Modifications in the preparation of antigens involving fixation of the parasites in acetone and formalin (Katende *et al*., 1987) have provided antigens which are stable even at 4°C, and can be kept in suspension until required and are capable of discriminating among different trypanosome species. The major drawback of IFAT, apart from its requirement for sophisticated microscopy, is its subjectivity which can make comparison of results quite difficult (Luckins, 1993).

Undoubtedly the introduction of enzyme-linked immunosorbent assays (ELISA) for use as diagnostic tests for animal trypanosomiases (Masake and Nantulya, 1991) has increased interest in the possibility of a universally applied immunodiagnostic assay and their modification and refinement brings field tests a little closer (Eisler *et al*., 1998; Nantulya *et al*., 1992). The tests are carried out in 96-well polystyrene micro-ELISA plates to which trypanosomal antigen are adsorbed. An indirect assay is routinely used in which serum from test cases is reacted with the antigen, followed by incubation of the resulting antigen/antibody complex with an enzyme-conjugated anti-globulin to the IgG fraction of the particular host species (Luciani *et al*., 2013). The test is visualized by the addition of enzyme substrate and chromogen, with the resulting colour change allowing interpretation photometrically (Nantulya and Lindqvist, 1989; Luckins, 1993).

2.4.4 Molecular diagnostic techniques

The introduction of molecular techniques/DNA-based diagnostic techniques such as restriction enzymes, sequencing and synthesis of DNA, DNA probing and polymerase chain reaction (PCR), have increased the specificity and sensitivity in trypanosome diagnosis, compared to the above mentioned diagnostic tools (Desquesnes and Dávila,

2002: Singh *et al*., 2004). The DNA diagnosis is either based on hybridization profiles of parasite DNA with DNA probes or polymerase chain reaction (PCR) technology (Weiss, 1995). A DNA-probe is a known DNA sequence which can be obtained by cloning or by PCR with labelled nucleotides (enzymes or isotopes) (Lockhart and Winzeler, 2000). A DNA probing entails exposing a denatured DNA sample fixed on nitro-cellulose to a labelled DNA-probe under specific salt and temperature conditions.

Where the complementary DNA sequence is present in the sample, the probes will bind to it and remain on the nitro-cellulose where they can be visualized (Desquesnes and Dàvila, 2002). Probes have been developed for the main pathogenic trypanosomes (Gibson, 2009), but the sensitivity of this technique is limited to 100 parasites (Masiga *et al.,* 1992), which is not sufficient for trypanosome detection in mouthparts of the vectors or in host blood when the parasitaemia is low.

Other approaches to investigate the molecular variation between various *Trypanosoma* species include methods such as randomly amplified polymorphic DNAs (RAPD), amplified fragment length polymorphisms (AFLP), and restriction enzyme fragment length polymorphism (RFLPs). According to Eisler *et al*. (2004), these methods may be effective for characterization of trypanosoma though they have not been applied largely in the detection of parasites because they generally require large amounts of purified parasite DNA. These DNA-based methods were later modified to species-specific DNA probes and then eventually improved to species-specific PCR tests (Adams *et al*., 2008). Species-specific PCR tests greatly improved the accuracy of identification and increased our understanding and knowledge of trypanosome diversity. In particular, the high prevalence of mixed infections with multiple trypanosome species was documented for the first time using PCR techniques (Masiga *et al*., 1996; Lehane *et al*., 2000).

The polymerase chain reaction (PCR) is an *in vitro* technique which allows the amplification of a specific deoxyribonucleic acid (DNA) region that lies between two regions of a known DNA sequence. The technique exploits a thermostable enzyme, *Taq* polymerase, which synthesizes a new strand of DNA by copying an original DNA template (Mullis *et al.,* 1986). The PCR was originally developed to amplify sequences of interest and to increase the sensitivity of detection using DNA-probes (Saiki *et al.,* 1986). However, since the DNA-probe technique is laborious and time consuming (Desquesnes and Dàvila, 2002), in recent years the development of PCR alone has mainly been devoted to diagnosis without the need of probes. Thus, PCR amplification of DNA is achieved by using oligonucleotide primers. These are short, single-stranded DNA molecules which are complementary to the ends of a defined sequence of DNA template. The primers are extended on a single-stranded denatured DNA template by a DNA polymerase, in the presence of deoxynucleoside triphosphates (dNTPs) under suitable reaction conditions. This results in the synthesis of a new strand complementary to the template strands. As described by Desquesnes and Dàvila (2002), following 30 – 40 cycles, the DNA template will have been amplified several million times, and the resulting PCR product can be visualized on an agarose or polyacrylamide gel, after staining with ethidium bromide and exposing it under ultraviolet light. The specific size of the PCR product is evaluated by simultaneous migration of molecular size markers and a positive control. A negative control is run together to expose any DNA contamination. The actual sequence amplified can be analyzed by sequencing.

Generally, once the specificity of the primers has been established, the size of the PCR product is sufficiently characteristic for diagnostic purposes. Species-specific primers have been developed for the main pathogenic trypanosomes: *Trypanozoon* (Moser *et al.,* 1989), West African *T. vivax* (Dickin and Gibson, 1989), *T. vivax* and *T. congolense*

savannah, forest and kilifi subgroups (Masiga *et al.* 1992), *T. simiae* (Majiwa and Webster, 1987), *T. simiae* Tsavo (Majiwa *et al*., 1993), *T. godfreyi* (Masiga *et al*., 1996) and *T. evansi* (Artama *et al*., 1992). Because of the diversity of *Trypanosoma* species potentially present in a single host, PCR diagnosis carried out on host material requires several PCR reactions (Desquesnes and Davila, 2002).

For diagnostic purposes, PCR must be performed with various biological materials in both vectors and host. In vectors, it is generally recommended to dissect out the organs of the insect where the parasite is thought to occur (in tsetse fly: mouthparts, salivary glands and midgut) and these organs have to be homogenized prior to DNA extraction (Desquesnes and Dávila, 2002). According to them, in mammalian hosts, the parasites are most often present in the blood, but other body secretions such as lymph node, cerebrospinal fluid (CSF), genital secretion (with regards to *T. equiperdum*), or any material derived from other organs can be investigated as well for the presence of trypanosome parasites. It is also recommended that samples be obtained from fresh material and if possible the fresh samples may be fixed on either filter paper or on slides (Lennette *et al*., 2012). The limitation of this technique is the cost implications for routine detection, and the inability to be conducted in the field due to cumbersomeness and likely DNA contamination (Yang and Rothman, 2004). There is still a need to design primers based on repetitive sequences for a sensitive detection and specific identification of *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense* for epidemiological studies in vectors, domestic and wild reservoirs of human sleeping sickness (Luckins, 1993).

The Loop-mediated isothermal DNA amplification (LAMP) method is another molecular technique that is simple, sensitive, rapid and highly specific (Parida *et al*., 2008). It is run under isothermal conditions (63-65 °C), requires simple equipment

(water bath or block heater) for amplification reaction and is cost effective (Notomi *et al*., 2000; Njiru, 2012). The technique (LAMP) requires two specially designed inner and outer primers, and depends on auto-cycling strand displacement DNA synthesis that is executed by a *Bacillus stearothermophilus* (Bst) DNA polymerase. Unlike *Taq* DNA polymerase, it is barely inhibited by impurities such as haemoglobin and/or myoglobin contaminants in blood and tissue derived DNA samples which are known to be inhibitors in PCR (Thekisoe and Inoue, 2011). An added benefit of LAMP over PCR-based methods is that DNA amplification can be monitored spectrophotometrically and/or with the naked eye without the use of dyes (Kuboki *et al*., 2003). All these advantages noted above indicate that LAMP has the potential to be used as an alternative molecular diagnostic method particularly in under-funded laboratories in *Trypanosoma* species endemic areas and in field conditions (Parida *et al*., 2008). The LAMP assays have been developed for the detection of *T. congolense*, *T. evansi*, *T. cruzi* as well as *T. b. gambiense* (Kuboki *et al*., 2003; Thekisoe *et al*., 2007).

2.5 Control of African Animal Trypanosomosis

Direct annual losses from AAT are currently estimated to be about US$1.5 billion and, overall, have the effect of limiting Africa's agricultural income to approximately US$4.5 billion a year, below its potential level (FAO, 1997). The financial benefit of controlling this disease has been calculated to range from below US$500 per km^2 to well over US$5,000 per km^2 (Shaw *et al*., 2006). Thus, there is a need for concerted efforts to control the disease and improve the lives of millions of families and communities who rely on their livestock for livelihood.

Prevention and control of AAT depends on methods directed at the parasites, the vectors and the host. Though useful, all of these approaches have important limitations

such as expense, environmental pollution and drug resistance (Uilenberg, 1998). They are further discussed as follows.

2.5.1 Parasite control

Conventional parasite control measures are based on the use of curative (chemotherapy) and preventive (chemoprophylaxis) drugs. In areas where large-scale, structured control operations are not undertaken, drugs are often the sole tool farmers can use to limit the impact of 'nagana' on their animals (Kristjanson *et al*., 1999). Isometamidium chloride, diminazene aceturate and homidium (bromide and chloride) are the only three drugs readily available, and these compounds have been on the market for over four decades (Hassen, 2008). Despite the high demand for trypanocides by African farmers, the cost of developing new compounds stops pharmaceutical companies from pursuing the quest for new drugs. The development and spread of drug resistance in parasite populations is perhaps the greatest risk to the sustained use of existing trypanocides (Holmes *et al.,* 2004). Until 1998, resistance to one or more of the trypanocidal drugs used in cattle had been reported in 13 countries of sub-Saharan Africa, including Burkina Faso, Central African Republic, Chad, Côte d'Ivoire, Ethiopia, Kenya, Somalia, Sudan, United Republic of Tanzania, Uganda, Zambia and Zimbabwe (Geerts and Holmes, 1998). Subsequently, resistance has also been reported from Nigeria (Anene *et al*., 2001), Guinea (Barry *et al*., 2007), Cameroon (Mamoudou *et al*., 2008), Ghana (Steverding, 2008), and Mali (Mungube *et al.,* 2012).

2.5.2 Vector control

Tsetse control has long been an important option for reducing the impact of African trypanosomosis and many methods have been used successfully, however, sustainability

of the results following interventions has often proved much more challenging (Allsopp, 2001).

Many methods widely used for tsetse control in the past have ceased to be used in the last 10-20 years, either because they were ineffective, or because they have become environmentally unacceptable (Uilenberg, 1998). Tsetse populations can be targeted either directly by affecting their mortality and/or fertility, and indirectly, by acting on their hosts and/or on their habitat (Rogers *et al*., 1994).

Insecticides are the basis of most tsetse suppression measures, acting by increasing natural tsetse mortality rates (Rogers *et al*., 1994). Organochlorines (banned in some countries) notably dichloro-diphenyltrichloroethane (DDT) and dieldrin, have been in use. However, the more environmentally friendly organophosphates such as 2, 2-dichlorovinyl dimethyl phosphate, Dichlovos (Biovan 1000EC®, Sargasso Worldwide Limited, United Kingdom) are more accepted. These two chemical groups have had a wide success in the control of tsetse flies (Uilenberg, 1998; Allsopp and Hursey, 2004). Methods of application include:

(i) depositing residual insecticide either discriminatively (on to the resting and breeding sites of tsetse by ground spraying) or less selectively (treating the entire habitat from the air) (Allsopp and Hursey, 2004)

(ii) application of insecticides on animals (‘dipping’, ‘pour-on’ or live-bait technique) (Hargrove *et al*., 2000)

(iii) repeated spraying of non-residual insecticide, either over large areas using aircraft (sequential aerosol technique - SAT) (Allsopp and Phillemon-Motsu, 2002) or in more localized areas using hand-held or vehicle mounted fogging machines

(iv) attracting tsetse to devices such as screens or traps treated with insecticide (Vale, 1993; Van den Bossche *et al*., 2004).

Traps, targets and screens are often destroyed by flood, strong wind, wild animals, fire as well as being damaged or stolen by humans, and where the terrain is bad and several species are involved, application of traps is limited (Adamu *et al*., 2011).

With regards to the use of insecticides technique which affects tsetse mortality, the Sterile Insect/Male Technique also used, affects birth rate of the tsetse flies (Klassen and Curtis, 2005). Reproductively sterile male insects are released among the indigenous target population of the flies so that upon mating, female insects become infertile (Feldmann, 2004). It is based on the fact that tsetse females copulate only once, and if the male of a copulating pair is sterile the female will not produce during her lifetime. This technique is cost intensive (Klassen and Curtis, 2005).

In the past, some tsetse control methods targeted the tsetse habitat and hosts, and included total deforestation/bush burning (Hocking *et al*., 1963) or a partial clearing of vegetation (Ford *et al*., 1970), as well as elimination of wild animals (Wooff, 1969). These methods are no longer deliberately used to control tsetse, although it is said that anthropogenic land cover change and reduction of wild animal populations may have similar effects (Bourn *et al*., 2001).

2.5.3 Host management

Host management includes the avoidance of tsetse-infested areas by animal hosts that are susceptible, a limited but continued contact to favour the emergence of some degree of immunity, and the use of trypanotolerant breeds (Luckins, 1993; ILRAD, 1994).

It has long been recognized that certain breeds of West African cattle poses the ability to survive and to be productive in tsetse infested areas where other breeds rapidly succumb to the disease. This trait is termed trypanotolerance (Murray *et al*., 2004). This is especially true of the West African short-horned cattle (Muturu, Baoulé, Lagune, Keteku, N'dama, Ghana shorthorn and Borgou) (Naessens, 2006). Mechanisms responsible for this ability include control of parasite proliferation and limitation of pathological effects of the parasite (Murray *et al*., 1982).

Susceptibility studies have shown N'dama to be the most resistant breed followed the Muturu (Mortelmans and Kageruka, 1983). The mechanisms of trypanotolerance have been extensively studied, and it is now well established that trypanotolerance has a genetic basis (Murray *et al.,* 1984; Naessens, 2006). Apart from cattle, breeds of sheep and goats (and even of horses) living in tsetse areas are also relatively trypanotolerant, particularly the Djallonke sheep and dwarf goats in West Africa (Geerts *et al*., 2009). The mechanisms of the tolerance phenomenon have not been fully defined (Baker, 1995). Knowledge of the resistance of small ruminants is still fragmentary compared to the knowledge on resistance in cattle (Uilenberg, 1998). Kalu (1995) reports that trypanotolerance as a trait is relative rather than absolute, because it is affected by heavy challenge, malnutrition, stress, breed, age, season and concurrent infections. The major constraint to a more widespread use of trypanotolerant breeds of cattle is the limited number of animals available. Information has it that about 200,000 out of the 10 – 14 million cattle in Nigeria are trypanotolerant (Adeniji, 1993).

One limitation to the use of West African trypanotolerant cattle breeds in husbandry practice is their small size, low productivity and traction power compared with the larger zebu breeds though, genetic studies might lead in the future to the transfer of the trypanotolerance trait to more productive breeds (Hanotte *et al*., 2003).

2.5.4 Chemoprophylaxis

A programme to eradicate tsetse flies from some 9 million km^2 of Africa asides being very high-reaching, will be complex, take many years and cost well over the US$20 billion reported by Budd in 1999. Thus, the application of antitrypanosomal agents (trypanocides) has been the most universally practiced means of controlling trypanosomosis in domestic livestock since the early 1950s, either as curative or prophylactic drugs (Delespaux and Koning, 2007). Over most of sub-Saharan Africa, animal trypanosomosis continues to be controlled primarily by trypanocides (Holmes *et al*., 2004).

Livestock farmers majorly depend on three trypanocide compounds that include isometamidium chloride, homidium (bromide and chloride) and diminazene aceturate, in treating cattle trypanosomosis (Geerts and Holmes, 1998; Geerts *et al*., 2001; Wilkinson and Kelly, 2009). All these drugs have been on the market for over 40 years and several generic forms of them from a wide range of companies have become available on the African market (Holmes *et al*., 2004; Wilkinson and Kelly, 2009).

Isometamidium is principally used as a prophylactic drug and can provide protection against trypanosomosis for up to 6 months whilst homidium has limited prophylactic properties, and is primarily used as a therapeutic agent. On the other hand, diminazene aceturate provides also a short term protection of 2 to 3 weeks, and is mainly used for therapeutic purposes (Holmes *et al*., 2004). Quinapyramine, suramin and melarsomine are primarily used as therapeutic drugs for infections caused by *T. evansi* in equidae, camels and buffaloes, although quinapyramine can also be used for prophylactic purposes (Gutiérrez *et al*., 2013). It has been estimated that US$30 – 90 million worth of trypanocides are sold annually in the African market as a result of the continuous

demand for trypanocides by livestock keepers (Borne, 1996; FAO, 2008). However, due to the high cost of development and licensing of new animal trypanocides (estimated to cost from US$200 to US$800 million), large pharmaceutical companies do not consider it to be a profitable venture hence investment in drugs against the disease is low (Veeken and Pecoul, 2000; Sones, 2001; Di Masi *et al*., 2003; Shiferaw *et al*., 2015).

Consequently, the control of trypanosomosis in livestock will continue to depend on the use of the currently available compounds, because it is unlikely that new trypanocides will be developed and be released in the near future (Melaku and Birasa, 2013). So the challenge remains to make optimal use of the three relatively old compounds until new methods of chemoprophylaxis/chemotherapy emerge (Holmes *et al*., 2004). As such, trypanocides will probably remain the mainstay in the control of African animal trypanosomosis for the foreseeable future, and the development of resistance to the small number of available compounds would generally be a cause of considerable concern (Geerts and Holmes, 1998).

2.6 Chemotherapy for African Animal Trypanosomosis

Chemotherapy against AAT involves using veterinary formulations that contain any one of the currently available trypanocides. Trypanocides are drug agents lethal to trypanosomes, and trypanocidal drugs are the most widely applied method farmers use to treat and prevent trypanosomosis in sub-Saharan Africa (Delespaux *et al*., 2008a). These drugs are popular because farmers can directly treat and, if successful, cure their own animals without relying on the efforts of others. They are often the first drugs tried by the farmers when their livestock develop (any) symptoms of the disease (Geerts *et al*., 2001). It has been estimated that about 35 – 50 million doses of trypanocides,

costing in the range of US$0.50 to US$3.00 per dose, are administered each year to domestic ruminants in sub-Saharan Africa (Kristjanson *et al*., 1999; Mattioli *et al*., 2004).

Trypanocides are frequently used in the absence of diagnosis or to treat conditions for which they are not effective against (Holmes *et al*., 2004). Unfortunately, the abundance of poor-quality products, inappropriate storage, their incorrect usage together with incorrect estimation of weight of animals to be treated and the use of contaminated syringes, can lead to under-dosage and treatment failure, and contribute to resistance in trypanosome populations. Consequently, treatment given by livestock owners is not without serious drawbacks as most farmers do not have adequate knowledge on diagnosis and appropriate use of the drugs.

In endemic areas/countries of the sub-Sahara, trypanocidal drugs represent 40% to 50% of the total animal health market, providing a large and attractive target for the sale of sub-standard or counterfeit products (Merial unpublished report). Internationally agreed standards for trypanocides, either as documented product specification or pharmacopoeia, are lacking in the public domain, thereby making quality control and compliance in the use of veterinary trypanocides in sub-Saharan Africa difficult to regulate (Sutcliffe *et al*., 2014).

The use of sub-standard or counterfeit trypanocides has severe implications for animal health, public health and the local economy, and increases the risk of emergence of drug resistance in trypanosome populations (Geerts and Holmes, 1998; Van Gool, 2008). On the other hand, proper use of trypanocides has been shown to provide effective treatment and reduce the risk of drug resistance. It is therefore necessary that quality trypanocides be

obtained and administered correctly (Uilenberg, 1998). Also, it is advised that regulations on the use of these products be strictly adhered to.

2.6.1 The Primary Trypanocides in Use

Only three compounds belonging to two chemical classes are widely available to treat trypanosomosis: diminazene aceturate (an aromatic diamidine), isometamidium chloride and homidium salts (phenanthridines).

2.6.1.1 Diminazene aceturate

Chemistry: Diminazene is an aromatic diamidine derived from Surfen (Jensch, 1958). The molecule is marketed as the diaceturate salt and consists of two amidinophenyl moieties linked by a triazene bridge: p,p-diamidinodiazoaminobenzene diaceturate tetrahydrate; N-1,3-diamidinophenyltriazene diaceturate tetrahydrate (Figure 2.1).

It is a yellow to orange powder that dissolves completely in water to give a yellow solution. In aqueous solution, the compound is stable for 2-3 days (Fairclough, 1962). Thus, because of this short duration of stability, diminazene is marketed in combination with the stabilizer phenyldimethyl pyrazolone (antipyrine). Solutions of the preparations can be used without loss of activity for up to 10-15 days when stored at room temperature (Fairclough, 1962). It is categorized as both a trypanocidal and babesicidal agent.

Antitrypanosomal activity: The compound was introduced into the market as a trypanocide and babesiacide for domestic livestock in 1955 (Jensch, 1955). Following its preliminary use in the field, it was concluded that intramuscular (i.m.) treatment with diminazene aceturate at a dose of 3.5 mg/kg body weight (b.w.) eliminated *T. congolense* and *T. vivax* infections in cattle. However, infections with *T. brucei* required a dose rate of 5 mg/kg b.w. and the drug is currently marketed under the trade names of Azidine®, Berenil®, Ganaseg®, Ganasegur® and Veriben® as both trypanocide and babesiacide for domestic livestock. It is

recommended only for use as a therapeutic agent since it is rapidly excreted and therefore thought to have little prophylactic activity (Fussgänger and Bauer, 1958). For all animals the general i.m. dosage is 3.5 mg/kg b.w. However, twice this amount is recommended for *T. brucei* infections (Hoechst Veterinär, Germany).

Standard therapeutic dosages of diminazene aceturate (3.5 – 7.0 mg/kg b.w.) rarely results in signs of toxicity in domestic animals (Peregrine and Mamman, 1993), and cattle are said to tolerate doses as high as 21 mg/kg b.w. (Fairclough, 1963). In camels, however, a single dosage of 7.0 mg/kg b.w. can be highly toxic (Leach, 1961). Diminazene is also relatively toxic to horses (Ezeokonkwo *et al.*, 2007; Taylor *et al.*, 2013) and contraindicated in dogs (Losos and Crockett, 1969; Irwin, 2010; Han *et al.*, 2014).

Mechanism of action: Diminazene binds to the trypanosomal kinetoplast DNA (MacAdam and Williamson, 1972; Newton, 1972). This binding does not occur by intercalation (Newton, 1972) but via specific interaction with sites rich in adenine-thymine (A-T) base pairs (Newton, 1972; Brack and Delain, 1975). Non-intercalative binding of diminazene to DNA, with strong affinity for A-T base-pair regions, has similarly been demonstrated *in vitro*, using DNA obtained from various sources (Lane *et al.,* 1991). Such studies have shown that the molecule binds with higher affinity to 5'-AATT-3' than to 5'-TTAA-3' regions of DNA (Hu *et al.,* 1992). Through this specific interaction with trypanosomes, diminazene inhibits synthesis of RNA primers, resulting in accumulation of replicating intermediates, thereby inhibiting kDNA replication (Newton, 1972; Brack and Delain, 1975). In another work, Shapiro and Englund (1990) have shown that diminazene specifically inhibits mitochondrial type II topoisomerase in viable trypanosomes. Thus, inhibition of DNA replication may also occur via this intercalation. Although diminazene probably exerts its action at the level of the DNA, this has not been proven *in vivo* and other mechanisms of action cannot be excluded (Peregrine and Mamman, 1993).

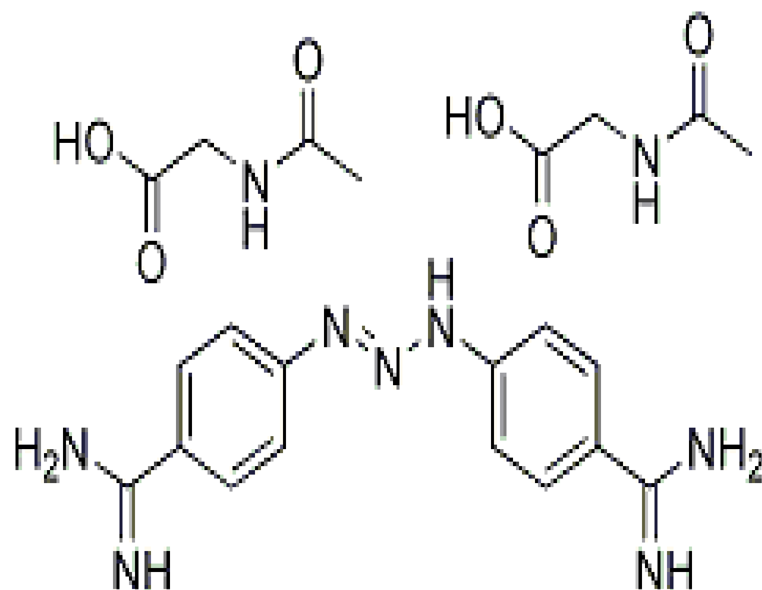

Figure 2.1 Chemical Diagram: Diminazene aceturate ($C_{22}H_{29}N_9O_6$).
Molecular weight: 515.52.
Source: http://www.chemicalbook.com/CAS%5CGIF%5C908-54-3.gif

2.6.1.2 Isometamidium chloride and Homidium salts

Chemistry: isometamidium chloride (Samorin®, Sécuridium®), marketed since 1961 as a prophylactic and therapeutic drug (Berg *et al*., 1961), and homidium (chloride salt: Novidium®; bromide salt: Ethidium®), marketed since 1952 (Watkins and Woolfe, 1952), are phenanthridinium compounds, whose antitrypanosomal activity was demonstrated more than 60 years ago (Browning *et al.,* 1938; Woolfe, 1956; Franklin and Snow, 2013).

Isometamidium differs from homidium by an additional moiety of *m*-amidinophenyl-*azo*amine, a part of the diminazene molecule, and thus can be seen as a 'hybrid molecule' which exhibits some of the properties of homidium and diminazene (Wragg *et al.,* 1958). More so, the drug product of isometamidium as marketed contains 70% isometamidium, and the remaining fraction (30%) is a mixture of its two isomers, a small proportion of a bis-compound and homidium (FAO, 1990). Both drugs are categorized as trypanocidal agents (Figs. 2.2 and 2.3).

Isometamidium is sold as a dark purple to brown powder that dissolves completely in water to give a brownish red solution while homidium chloride is sold as a red crystalline powder or tablets which also dissolves in water to give a red solution.

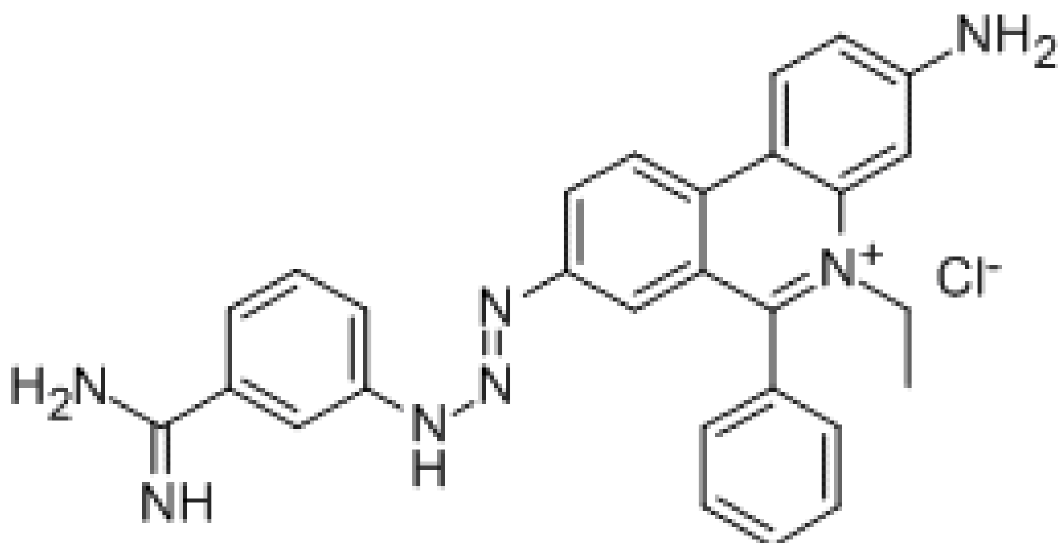

Figure 2.2 Chemical Diagram: Isometamidium chloride ($C_{28}H_{26}N_7Cl$).
Molecular Weight: 496.04
Source:http://www.chemicalbook.com/CAS/GIF/34301-55-8.gif

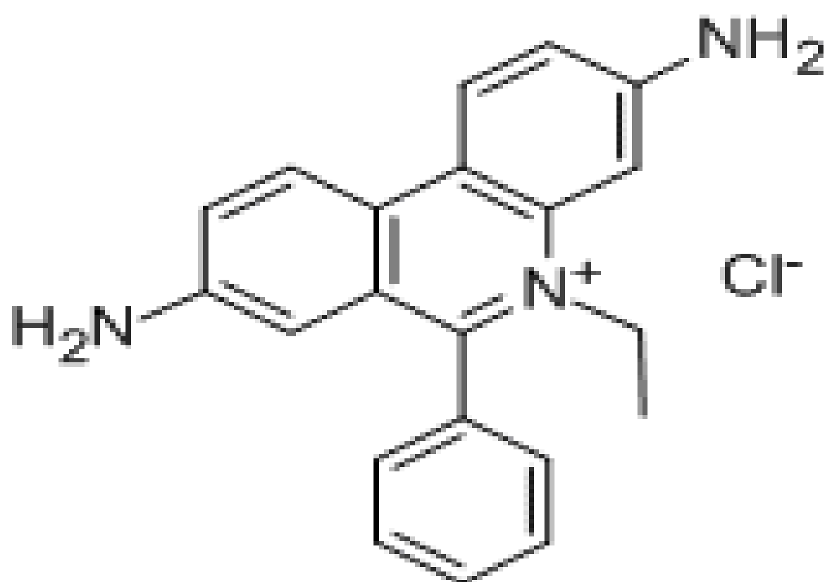

Figure 2. 3 Chemical Diagram: Homidium chloride ($C_{21}H_{20}N_3Cl$).
Molecular weight: 349.86
Source:http://www.chemicalbook.com/CAS/GIF/602-52-8.gif

Antitrypanosomal activity: Both isometamidium and homidium are active against *T. congolense* and *T. vivax*. Additionally, isometamidium is also of value against infections caused by *T. brucei* and *T. evansi* in donkeys, horses and camels (Desquesnes *et al*., 2013). Some studies on *T. evansi* and *T. equiperdum* have shown that the minimum effective concentration of isometamidium which killed trypanosome population by 100% within 24 hours of drug exposure (MEC100) was 1-4 µg/ml (Zhang *et al.,* 1991), and with 96 h drug exposure, 1-300 ng/ml (Brun and Lun, 1994). In the dose range recommended for prophylactic purposes (0.5-1.0 mg/kg b.w.), the compound has been used successfully to maintain the productivity of Zebu cattle exposed to tsetse challenge in both village and ranch management systems in East Africa (Trail *et al.,* 1985; Maloo *et al*., 1988). However, considerable variation in prophylactic activity has been observed in that a dosage of 1.0 mg/kg b.w. has been shown to confer prophylaxis to cattle for 2-22 weeks (Peregrine *et al.,* 1991). Variation in prophylactic activity appears to be independent of both the level of trypanosome challenge and the presence or absence of infection at the time of treatment, and the variation in drug susceptibility between different trypanosome populations appears to be a major factor determining the duration of prophylaxis (Peregrine *et al.,* 1988).

Homidium was extensively used in the 1960s and 1970s but its usefulness has been greatly reduced due to widespread resistance (Geerts *et al.,* 2001; McDermott *et al*., 2003; Jamal *et al*., 2005; Melaku and Birasa, 2013). Over the years, it has remained essentially a curative drug in the field, despite claims that the drug has some prophylactic activity, varying from 2 to 19 weeks against field challenge (Melaku and Birasa, 2013). de Deken *et al*. (1989) have reported success in protecting rabbits for more than 300 days against seven challenges of *T. congolense* using a slow release device implanted subcutaneously. This finding suggests that the commonly used drug

products, Novidium® or Ethidium®, lack prolonged prophylactic activity because the active principle, homidium is rapidly eliminated from the body (Murilla *et al.,* 1999). Therefore, should this slow release device technology prove cost-effective in the field, it will undoubtedly be widely accepted in many areas with a yearlong high incidence of trypanosomosis (ELsiddig, 2015).

Mechanism of action: The primary mode of action currently considered to account for the molecular mechanisms of antitrypanosomal activity of phenanthridine drugs is blockade of the nucleic acid synthesis through intercalation between DNA base pairs, inhibition of RNA polymerase, DNA polymerase and incorporation of nucleic acid precursors into DNA and RNA (Wagner, 1971; Lantz and Van Dyke, 1972; Richardson, 1973; Marcus *et al.,* 1982). Other biochemical reactions that may account partly due to their effects include modulation of glycoprotein biosynthesis, lipid metabolism, membrane transport and selective cleavage of kinetoplast DNA minicircles (Dixon *et al.,* 1971; Girgis-Takla and James, 1974; Casero *et al.,* 1982; Shapiro and Englung, 1990). The mechanism that is considered primary, that is, blockade of nucleic acid synthesis (Schnitzer and Hawking, 2013), does not explain the basis of their selective toxicity. However, there are a number of biochemical peculiarities that have been demonstrated in trypanosomes that appear to be candidate targets for drug modulation and that might explain the basis of selective toxicity (Opperdoes, 1985).

2.7 Drug Resistance in Animal Trypanosomosis

Uilenberg (1998) defined drug resistance (or drug fastness) as a loss of sensitivity by a strain of an organism to a compound to which it had previously been susceptible. He went further to explain that it implies failure of treatment and prevention by the drug in

question, and as there are no other active drugs, the animal has to rely on its immune defenses alone to combat the disease. Several studies have shown that the use of trypanocides is the cheapest method of trypanosomosis control, and trypanocidal drugs make cattle keeping profitable even in areas of high incidence and possible risk of drug resistance (Affognon, 2007).

Consequently, due to the privatization of veterinary services in most parts of Africa, the heavy reliance on trypanocides (Delespaux *et al*., 2008b), a rampant misuse and under-dosage by livestock farmers (Delespaux *et al*., 2002), trypanocidal drug resistance came to be. Used properly, trypanocides prevent losses and permit higher levels of production. However, when used improperly they promote drug resistance (Geerts *et al*., 2001). While the costs of inappropriate drug use and lost production are met largely by the farmer who misuses the drug, the costs of drug resistance are met by the society and future generations (Affognon, 2010).

Reports, though seemingly restricted to areas where the disease is endemic, state that about 18 countries have been reported to have trypanocidal drug resistance, and more recently in Benin Republic, Ghana and Togo summing it up to about 21 countries (Delespaux and de Koning, 2007; Delespaux *et al*., 2008b; Chitanga *et al*., 2011). Nevertheless, it is not evident whether these reports are due to an actual increase in trypanosome resistance or due to an increased interest and awareness by scientists. However, a report by Delespaux *et al.* (2008a) of a five-fold increase in the prevalence of diminazene aceturate resistance over a seven-year period in the Eastern Province of Zambia, suggests that there is indeed an aggravation of the phenomenon. Even more worrying are the recent reports of multiple drug resistance to isometamidium and diminazene aceturate, because this threatens the last stand to overcome drug resistance through the use of the sanative pair (Mamoudou *et al*., 2008).

The concept of the sanative pair recommends the use of two trypanocides (e.g. diminazene aceturate and homidium) unlikely to induce cross-resistance (Anene *et al.*, 2001). Hastings (2001) identified: (i) the mutation rate from wild type to resistant genotype, (ii) the level and pattern of drug use and (iii) the parasitaemia within the host i.e. the number of parasites exposed to the drug after the treatment of the host, as some of the important factors determining the rate of evolution of drug resistance in a parasite population.

The exact mechanisms of drug resistance are imperfectly known, but reduced drug uptake has emerged as a common characteristic of drug-resistant trypanosomes (Borst and Ouellette, 1995; Ouellette, 2001). The origin of multiple resistances to trypanocides by trypanosomes in the field is unclear, though cross-resistance between the different compounds probably due to their closely related molecular structures was implicated (Tsegaye *et al.*, 2015). Thus, drug resistance in trypanosomes poses a serious problem to livestock productivity in countries where it has been reported, unless checked and brought under control. The development and spread of drug resistance to the point where drugs become ineffective over large areas of Africa is probably the greatest risk to the future use of the existing three trypanocides (Melaku and Birasa, 2013). It is also possible that the market will shrink and manufacturers will have less profit because of the risk of drug resistance (Anene *et al.*, 2001). Additionally, the spread of generic products, some of which are of doubtful quality, may undermine farmers´ confidence in trypanocides (Holmes *et al.,* 2004).

Affognon (2010) recommended that management of trypanocidal drug resistance includes three components:

(i) the detection and the surveillance of resistance using recommended methods such as field tests, laboratory animal tests, *in vitro* and *in vivo* tests (WHO, 1998)

(ii) the prevention of resistance through Rational Drug Use (RDU), an indispensable and fundamental basis to combat resistance, given that trypanocidal drugs will be used long into the future (Grace, 2003; Affognon and Randolph, 2009)

(iii) the containment or eradication of resistance by vector control/eradication.

Methods for eradication of localized tsetse populations are highly effective and community-based bait methods, using insecticide-treated cattle and traps, are particularly attractive, being low-cost, environmentally-friendly and empowering to local communities. These could be used to eliminate 'pockets' of resistance, although the low sustainability of vector control throws doubt on its appropriateness as a long-term, large-scale trypanosomosis control operation (Brightwell *et al.*, 2001; Randolph *et al.*, 2003; Grace, 2005).

2.8 Vaccination in African Animal Trypanosomosis

In the past few years, tremendous advances have been made in the area of immunology. However, in the case of protozoa in general, some of which occupy the very arsenals of the immune response (trypanosomes in particular), many problems still remain unsolved (Shimelis, 2015). The almost unlimited antigenic variation during infection by one single strain of trypanosome and the antigenic strain diversity within each of the several trypanosome species and types, are the main obstacles preventing vaccine development against trypanosomes (Magez *et al.*, 2010). Some attempts are still being made at

vaccine development using internal non-variable antigens or at immunizing against proteins causing pathogenic effects, instead of against the parasite itself (Lubega *et al*., 2002; Silva *et al*., 2009). Targeting internal non-variable antigen has the problem that such antigens are out of reach of the host antibodies as long as the trypanosomes are alive and intact (Uilenberg, 1998). Therefore, only little progress has been made in the development of a vaccine against trypanosomes and no effective vaccine is likely to be marketed in the near future (Geerts and Holmes, 1998).

In West Africa, the recommended strategy for controlling the disease has been an integrated approach combining vector suppression in epidemiological hot spots and disease management at the herd level through the strategic use of trypanocides combined with the keeping of local trypanotolerant breeds (Hendrickx *et al*., 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Site/Location

The experiment was carried out at the experimental tick and fly-proof animal housing unit of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University (A.B.U.) Zaria, Kaduna State. Geographically, Zaria is located at latitude 11°11´04´´ N, longitude 7°73´21´´E and 644m above sea level (Zaria, 2014).

3.2 Experimental Animals

3.2.1 Acquisition and transportation

3.2.1.1 Donkeys (Equus asinus)

Due to the absence of breeding centres for experimental large animals in the country, it was impossible to obtain animals with known history, as seen in other laboratory animal species. Therefore, for this study, donkeys used were bought in the open market. Twenty-eight (28) apparently healthy donkeys, aged between 8 – 9 months (Muylle *et al*., 1999; Du Toit *et al*., 2008) and comprising of 14 males (Jacks) and 14 females (Jennies) were purchased from Maigatari International Livestock Market in Jigawa State. The donkeys were transported in an open truck to the Faculty of Veterinary Medicine, A. B. U Zaria, where they were accommodated throughout the period of the research.

3.2.1.2 Wistar Rats and Mice

A total of 12 rats and 80 mice were sourced from the breeding colony of the Laboratory Animal Unit, Department of Veterinary Pharmacology, A. B. U. Zaria. The Wistar rats (*Rattus norvegicus*) aged 3 weeks, were apparently healthy and with mean weight of

190 g served for propagation of *T. brucei* (Federe) for infection of the donkeys. The mice aged 4 weeks and with mean weight of 21 g were used for animal subinoculation tests at different stages of the experiment.

3.2.2 Experimental animal management

3.2.2.1 Housing

On arrival, the donkeys were temporarily kept in a paddock belonging to the Department of Veterinary Parasitology and Entomology, A.B.U. Zaria, for about 30 hours prior to movement into their pens where they were conditioned for four weeks before experimental infection. The pens were initially cleaned, washed and fumigated against pests. Wood shavings, as bedding, were used as padding on the floor to protect the animals from the cold bare floor, injuries from the hard surface, and to ease cleaning of waste so as to maintain a high standard of hygiene. The pens were cleaned daily and beddings replaced with new ones as soon as it was wet from urine and spilled drinking water. Adequate lighting and ventilation were also ensured. The Wistar rats and mice were housed in laboratory animal cages in a clean and well ventilated room. Wood shavings also were used as bedding and were changed as the need arose.

3.2.2.2 Feeding

The donkeys were kept under intensive husbandry system and fed twice daily with feed consisting of hay from Gamba grass (*Andropogon gayanus*) obtained from the College of Agriculture Samaru-Zaria, Livestock Section, cereal bran plus cotton seed cake as concentrates and freshly cut Spear grass (*Imperata cylindrica*) from the A. B. U. green areas when available. Mineral salt lick blocks, fresh drinking water in water troughs were provided *ad libitum.* Care and feeding management as recommended by Pearson (2005) were maintained throughout the duration of the experiment.

For the Wistar rats and mice, pelleted feed and water were provided in wire-bar lid feeders and bottles with sipper tubes respectively. They were fed on commercial diet in recommended rations (Garber *et al*., 2010) with water provided *ad libitum.*

3.2.3 Animal screening, acclimatization and grouping

3.2.3.1 Animal Screening

In the temporary paddock where the donkeys were held and rested on arrival, each animal was administered Vitamin C tablet (500 mg) orally to alleviate transportation stress (Minka and Ayo, 2010; Ake *et al.,* 2013; Ayo *et al*., 2016), and then tagged for ease of identification. They were physically and clinically examined for ectoparasites and any disease conditions which may be present. For clinical evaluation, blood and faecal samples were collected as described below to screen for haemo and endoparasites respectively.

Blood collection and examination: Three milliliters (3 ml) blood from each animal was collected via jugular venipuncture using a 5ml plastic syringe with an 18" G needle, and transferred into sterile EDTA coated sample bottles which were immediately taken to the laboratory for pre-infection blood analyses. Blood samples were examined immediately after collection using the wet mount technique as described by Herbert and Lumsden (1976), haematocrit centrifugation technique (HCT) as described by Woo (1970), Giemsa-stained thin blood film technique as described by Thrall *et al.* (2012) and mouse inoculation test (MIT) as described by Koprowski (1996), for the presence of haemoparasites (*Babesia* sp, *Theileri*a sp, *Trypanosoma* sp). Blood samples not processed immediately were stored at 4°C until analyzed later in the day or the next day. Mice (2 per donkey) were inoculated with the collected donkey blood samples to observe for manifestation of any subclinical haemoparasitic infections. They were

observed for a period of about 30 days with the blood screened for haemoparasites every 48 hours for the first 15 days and then every 72 hours afterwards.

Faecal sample collection and Examination*:* Using a clean gloved hand, with the index and middle fingers lubricated with water, approximately 5 g of faecal matter was collected from each of the donkeys by inserting the gloved fingers into the rectum of the animal and scooping its faecal content. After collection, the glove was inverted to enclose the faeces in order to keep the faecal sample encased within and airtight, to prevent dessication. This was then labelled accordingly with an indelible marker, placed in a cooler and immediately taken to the Department of Veterinary Parasitology and Entomology, Helminthology Laboratory for processing. Faecal samples were first examined physically for consistency, worm segments and presence of blood or any foreign material, and thereafter screened for worm eggs, larvae, protozoa trophozoites and cysts using the direct saline wet mount (Parija and Prabhakar, 1995) and standard centrifugation faecal examination technique (Dryden *et al*., 2005).

3.2.3.2 Acclimatization

Upon moving the donkeys into the prepared fly-proof experimental housing units, they were allowed 4 weeks to adapt to the environment during which they were fed twice daily, with salt lick and clean drinking water provided *ad libitum.*

Blood samples on examination were negative for haemoparasites and the subinoculated mice remained negative till the end of observation period (30 days), indicating that the animals were free of any haemoparasitic infection. However, faecal sample results showed the presence of strongyle eggs and oocysts in some of the animals. This was considered a herd problem and thus on day 4 of acclimatization, all the animals were dewormed with oral Albendazole 600 bolus at 10 mg/kg body weight (b.w.) (Aliu,

2007) and Vetcotrim bolus (containing Sulphadiazine 2 g and Trimethoprim 0.4 g). Topical Deltamethrin® pour-on was applied on the donkeys to clear them of possible ectoparasites which might have not been observed grossly. At the end of this period mouse inoculation test (MIT) was repeated to further confirm the absence of any trypanosomal infection.

3.2.3.3 Grouping and Identification

To group the donkeys, they were first separated into males and females and assigned serial numbers. Plastic sheets (1 x 2" size) of light weight (2 g) engraved with the serial numbers were prepared and used to tag each of the donkeys by hanging them loosely around their necks using a twine rope. Using a simple stratified random sampling design, by drawing the serial numbers "out of a hat", the donkeys were assigned at random in a logical fashion to the experimental groups A, B, C and D (Johnson and Besselsen, 2002). Group setup was as follows;

- Group A: 8 donkeys, 4 each of the male and female sexes served as the Infected-Homidium chloride treated group.
- Group B: 8 donkeys, 4 each of the male and female sexes served as the Infected- Isometamidium chloride treated group.
- Group C: 6 donkeys, 3 each of the male and female sexes served as the infected untreated group (Positive Control).
- Group D: 6 donkeys, 3 each of the male and female sexes served as the uninfected untreated group (Negative Control).

Mice for subinoculation were assigned to each of the groups and caged separately with indelible markings of different colours for each group for proper identification.

3.3 Ethical Clearance

Ethical clearance sought from the Ethics Committee on Animal Use and Care, Ahmadu Bello University, Zaria-Kaduna, was approved and the approval number ABUCAUC/2016/006 assigned.

3.4 Agents

3.4.1 Infecting agent: *Trypanosoma brucei* (Federe isolate)

Trypanosoma brucei used in this experiment were obtained as frozen stabilates from the National Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria. This isolate of *T. brucei* is unique in its characteristics of being highly virulent and has been used extensively in trypanosomosis research in Northern Nigeria. Originally, it was isolated from a bovine specie in a survey conducted by staff of the National Institute for Trypanosomiasis Research (NITR) in Federe, Plateau State in 1995. The isolates were cryopreserved in liquid nitrogen for characterization and further studies (Dede 2013, personal communication, March 29, 2013). Preliminary studies in Wistar rats showed the parasite to have the following characteristics: -

- Appearance of parasitaemia (patency) 24 hours post inoculation in rats.
- Rapid multiplication in rat blood exhibiting very high parasitaemia (≥ 50 parasites per microscope field) 24 hours after patency.
- Hundred percent (100%) mortality in rats 48 – 72 hours post patency.

Stabilates were prepared from heparinized blood of the parasitaemic cattle, using 8% glycerol (1:1) and cryopreserved in liquid nitrogen (-80°C) in 1ml aliquots. The stabilates on removal from nitrogen and thawing were re-suspended in physiological normal saline and examined under the light microscope at the institute's parasitology laboratory, for viability of the trypanosomes (presence of fully developed

trypomastigotes, flagellar motility and integrity of cell membrane) (Broadhead *et al.*, 2006). On confirmation of viability, 0.2 ml of the parasite suspension (1×10^2 trypanosomes) was inoculated intraperitoneally using 1ml tuberculin syringes, into two adult Wistar rats which were then housed in laboratory animal cages and transported under stringent conditions to the Department of Veterinary Parasitology and Entomology of Faculty of Veterinary Medicine, A. B. U. Zaria. On arrival, the Wistar rats were managed and monitored until they developed high parasitaemia (≥ 40 parasites per microscope field) levels. At high parasitaemia, the two rats were humanely sacrificed after being anaesthesized in a jar containing 1% chloroform and bled through cardiac puncture. Harvested blood was pooled and passaged into 9 other Wistar rats for propagation of the trypanosomes and used for the experimental infections (Maina, 2006).

3.4.2 Treatment agents: Trypanocides

Homidium chloride (Novidium®) and Isometamidium chloride (Sécuridium®) used were obtained from a Veterinary Pharmaceutical store in Zaria, Kaduna State.

Homidium chloride, a phenanthridine compound was obtained as a 250 mg dark-red coloured tablet which produced a clear ruby-red solution when dissolved in water. Prior to each administration, a freshly prepared 2.5% solution was made by crushing the tablet to a powder form and reconstituting it in sterile water to give a 10 ml injectable solution. This was then administered intramuscularly at the dose rate of 1 mg/kg body weight (b.w.).

Isometamidium chloride, a phenanthridine aromatic amidine compound was obtained as maroon-coloured granules dispensed in a 1 g sachet. The granules produced a brownish red solution when dissolved in water. It was prepared fresh before each use as a 1%

solution by reconstituting 1 g in 100 ml of sterile water. Administration was intramuscular at the dose rate of 0.5 mg/kg b.w. (Knottenbelt, 2006).

3.5 Experimental Design

The experiment was conducted in 4 phases and lasted for a period of 26 weeks (185 days);

I. Pre-infection evaluation of the animals.
II. Initiation and Establishment of the infection.
III. Post infection evaluation
IV. Treatment of the infection and assessment of treatment.

3.5.1 Pre-infection evaluation (Phase I)

At pre-infection, the experimental animals were observed for a period of 4 weeks for manifestation of any clinical disease. Baseline vital parameters (rectal temperature, respiratory rate and heart rates), body weight measurements as well as haematological parameters were obtained for all the donkeys during this period of conditioning.

Vital Parameters and Body Weight Measurements: Physiologic parameters such as the heart rate (HR), respiratory rate (RR) and rectal temperatures (RT) were recorded between 7 – 9am every 3 days, blood and faecal samples were collected and analyzed on a weekly basis while body weights were taken once a week. Measurements were taken according to standard laboratory procedures (Thrall *et al.,* 2012). Briefly, each donkey was restrained properly and all measurements completed within 5 – 6 minutes.

Using a suspension weighing scale, body weight readings were taken between 7 – 9am before feeding, according to the method of Pearson and Ouassat (2000).

Haematological and Biochemical Parameters*:* For the evaluation of haematological parameters; packed cell volume (PCV), Haemoglobin concentration (Hb), total red cell count (RBC0, total white cell (WBC) count and differentials, blood glucose, and biochemical parameters; serum enzymes like Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase, Total plasma proteins (Tp) and Electrolytes of the donkeys, a 10 ml syringe with an 18" G needle attached was used to obtain 6 ml of blood from each animal via the jugular vein. Each blood sample was divided into two aliquots of 3ml each. One aliquot was dispensed gently into sterile sample bottles containing EDTA at the rate of 1.5mg/ml of blood for haematology while the other was dispensed gently into anticoagulant-free vacutainer tubes for serum harvest which was used for biochemical assay. Blood collected in plain vacutainer tubes was centrifuged at 3000 x *g* for 5 minutes to obtain serum. The supernatant serum was harvested using pipette into sterile serum sample bottles and analyzed same day.

Briefly, for blood glucose, approximately 0.7 µl of obtained blood was used for instant blood glucose evaluation by dropping it onto the absorbent tip of the test strip which was then inserted into a handheld digital blood glucose monitor (OneTouch Ultra, Johnson and Johnson, Leeds England) and the values (mg/dL) displayed on the Liquid Crystal Display (LCD) screen recorded. One glucose test strip was used per test sample.

Packed cell volume was measured using standard microhaematocrit method (CLSI, 2000), erythrocyte and leucocyte counts were evaluated using an improved Neubauer counting chamber (haemocytometer) as described by Yakubu and Chafe (2008). Haemoglobin concentration was evaluated using the cyanmethaemoglobin method (Mori *et al*., 2004) while total protein values were obtained using the clinical protein

hand-held refractometer (RHC-200 ATC, Lumen Optical Instrument Co. Limited Fujian, China).

The serum samples were analyzed at the Chemical Pathology Laboratory, Ahmadu Bello University (A.B.U.) Teaching Hospital Shika Zaria, for Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) activities, and Creatinine (CR), Urea (BUN), Albumin (ALB), Total Protein (Tp) levels, using fully-automated clinical chemistry analyzer (Selectra XL®, Vital Scientific, Netherland), while the electrolytes; Calcium (Ca), Sodium (Na), Chloride (Cl), Potassium (K), Phosphorus (PO_4) and Bicarbonate (HCO_3) concentrations were measured with the aid of an electrolyte auto analyzer (Audicom AC9900® model, Audicom Medical Technology Limited Jena, Germany).

3.5.2 Initiation and establishment of infection (Phase II)

3.5.2.1 Propagation of Trypanosoma brucei (Federe isolate)

Blood samples (2 µl) from tail nip of the two Wistar rats infected with the *T. brucei* Federe isolate obtained from NITR, were examined every 48 hours using the wet mount technique to establish presence of the trypanosomes. Parasitaemia level was quantified using rapid matching method (Herbert and Lumsden, 1976). Using a sterile 1ml disposable syringe, blood obtained from the two Wistar rats was used to inoculate nine (9) other rats which served as donors for propagation of the parasite (Maina, 2006). Each of the donor rats was inoculated intraperitoneally with 0.5 ml (1×10^4 trypanosomes) of the infected blood. The rats were monitored for presence of the parasites using blood from tail vein until peak parasitaemia was recorded on day 9 post infection (pi).

3.5.2.2 Inoculation of Donkeys

The parasitaemic donor rats were anaesthesized and blood collected as previously described in (3.4.1). The harvested blood was pooled and expanded in a 50ml glass beaker by diluting with phosphate-buffered saline glucose (pH 7.8), to give a standardized inoculum with a concentration of 1 x 10^6 trypanosomes per ml of blood. Parasite concentration was quantified using the haemocytometer counting method as described by Sannusi (1977). Donkeys in the groups to be infected (A, B and C) were intravenously infected with 2ml of the inoculum, with each donkey receiving 2 x 10^6 trypanosomes (Table 3.1) while group D donkeys remained uninfected.

Table 3.1: Donkey groupings and infection with *Trypanosoma brucei* (Federe isolate) via jugular venipuncture.

Group	No. of Donkeys	Concentration of parasite and volume of blood administered per donkey
A	8	2×10^6 *T. brucei* in 2 ml blood
B	8	2×10^6 *T. brucei* in 2 ml blood
C	6	2×10^6 *T. brucei* in 2 ml blood
D	6	Uninfected

Key: A = Infected Novidium®-Treated; B = Infected Sécuridium®-Treated; C = Infected Untreated; D = uninfected. Members of each group were selected at random.

3.5.2.3 Establishment of Infection

Following infection of the donkeys, monitoring of parasitaemia using blood from the ear vein and employing the wet mount technique was done daily until parasites were detected in peripheral circulation in all the donkeys, thus establishing the infection.

3.5.3 Post infection evaluation (Phase III)

After parasitaemia was established, donkeys were bled twice weekly at three-day intervals, up to day 160 post-infection. At each occasion, six millilitre of blood was collected and divided into two aliquots for haematological and serum biochemical analyses. All the animals were weighed on day of infection and once weekly subsequently. Degree of parasitaemia on Giemsa stained thin blood smear slides were classified as described by Woo (1970) as follows: -

+ means < 10 trypanosomes seen per microscope field.

++ means ≥10 - 20 trypanosomes seen per microscope field

+++ means ≥20 - 30 trypanosomes seen per microscope field.

++++ means ≥30 - 40 trypanosomes seen per microscope field.

Infected donkeys were examined daily for clinical signs of change in appetite, changes in RT, RR and HR, body condition, color of mucous membranes, size of peripheral lymph nodes, coat condition, faecal consistency, lacrimation and subcutaneous oedema. For these physiological parameters, each donkey was restrained calmly and measurements completed within 5-6 minutes. The RT were taken with the aid of a rectal digital thermometer (Omron EcoTemp Basic Digital Thermometer, Omron Healthcare, Hoofddrop, The Netherlands). The RR was taken by counting the number of respiratory

flank movements (breath cycles) of each donkey in one minute while the HR was measured by auscultation of the heart, by placing a stethoscope (3M™ Littmann® Classic II S. E Stethoscope, U.S.A) between the fourth and fifth ribs on the left side of the animal and counting the number of heart beats per minute. In anaemic conditions, the heart rate increases to compensate for shortage due to blood loss. This informed the need to evaluate heart rates of the donkeys in this study. Body weight readings were taken weekly using a suspension weighing scale according to the method of Pearson and Ouassat (2000).

3.5.4 Treatment of infection and assessment of treatment (Phase IV)

3.5.4.1 Treatment of Infection

Using the test drugs Novidium® and Sécuridium® for groups A and B respectively, treatment was administered in two stages; acute and chronic, at days 12 and 24 post-infection respectively. Donkeys of groups A and B were further divided into 2 subgroups i. e. A_1, A_2 and B_1, B_2, each with 4 donkeys. At the acute phase of infection (day 12), subgroup A_1 donkeys were administered 1mg/kg b.w. freshly reconstituted 2.5% Novidium® while subgroup B_1 donkeys which were in same acute phase had 0.5mg/kg b.w. of freshly reconstituted 1% Sécuridium® administered to them, both by deep intramuscular injection. Group C donkeys were not treated. Twenty-four (24) hours post-treatment, donkeys were examined for elimination of the parasites using the wet mount method, and disappearance of clinical signs by physical observation. At day 24 post infection, subgroups A_2 and B_2 donkeys were treated for chronic infection using the respective drugs at same dosage as used previously for the acute phase, and evaluation also conducted as for the acute phase (Table 3.2).

Table 3.2: Donkey groups and treatment regimen following infection with *Trypanosoma brucei* (Federe isolate).

Groups	No. of Donkeys	Subgroups (No. of Donkeys)	Description of Procedure
A	8	A_1 (4)	Infected with *T. brucei* followed by treatment with Novidium® at 1 mg/kg b.w. on day 12 post infection
		A_2 (4)	Infected with *T. brucei* followed by treatment with Novidium® at 1 mg/kg b.w. on day 24 post infection
B	8	B_1 (4)	Infected with *T. brucei* followed by treatment with Sécuridium® at 0.5 mg/kg b.w. on day 12 post infection
		B_2 (4)	Infected with *T. brucei* followed by treatment with Sécuridium® at 0.5 mg/kg b.w. on day 24 post infection
C	6	-	Infected Untreated
D	6	-	Uninfected Control

Key: (4) = number of animals in subgroup. Period of observation was 22 weeks.

3.5.4.2 Assessment of Treatment

Monitoring of the animals commenced 24 hours post treatment. Blood from the treatment groups was subjected to haematological, parasitological and serum biochemical analyses twice weekly. The donkeys were also observed for clinical manifestations and their weights measured weekly.

To confirm parasite elimination post-treatment, 0.2ml blood from the ear vein of each of the donkeys of the treatment groups (A and B) which had maintained an aparasitaemic status for about 8 days, was subinoculated into mice (2 mice per donkey). The mice were tagged appropriately and monitored for a period of 50 days for parasitaemia, if any. A negative status of the mice confirmed elimination of the parasites and indicated effective treatment. Post-treatment observation for relapse was done when it was established that the drugs had eliminated the parasites from circulation in the treatment groups.

3.6 Post-Mortem Evaluation

Post mortem examinations were conducted on the following categories of donkeys: uninfected (1); infected untreated (1); infected Novidium®-treated (1) and infected Sécuridium®-treated (1) based on guidelines approved by the A.B.U Animal Use and Care Committee, 2015.

3.6.1 Gross pathological examination

Macroscopic changes involving the muscles and visceral organs were evaluated at necropsy. Gross pathological abnormalities such as changes in size, weight, colour, consistency and texture were noted on each of the examined organs. A representative animal from each of the experimental groups was necropsied for comparative evaluation. For euthanasia, animals were first sedated with 1% acepromazine at 0.1

mg/kg followed 10 minutes later with a barbiturate general anaesthesic, thiopental agent at 15mg/kg and euthanized as indicated by Thurmon (2009). Organ impression smears of the liver, kidney, lungs, spleen, lymph nodes, muscles, and brain squash smears were made on clean grease-free microscopic slides, fixed and stained with Giemsa. The slides were examined microscopically using the oil immersion (x100) objective lens for the presence of *T. brucei* in the tissues (Bancroft and Gamble, 2008).

3.6.2 Histopathological examination

Histopathology was done by examination of Haematoxylin-Eosin (H & E) stained slides from tissue samples taken from the spleen, liver, lungs, kidneys, brain, heart muscle, testes, ovary and lymph nodes. Tissues were placed in appropriately labelled bijou bottles until processed. In preparation for processing, samples from the spleen, liver, heart muscle, lungs and kidneys were fixed in freshly prepared 10% formalin while the brain, testes and ovary were fixed in freshly prepared Bouin's solution as described by Bancroft and Gamble (2008).

At processing, the tissues were trimmed, embedded in wax paraffin and cut into 5μm thickness. These were mounted on clean grease-free microscopic slides and stained with H & E as described by the same authors above. Prepared sections were examined under the digital light microscope (OMAX MD82ES10 40X – 2000X Digital LED Compound Microscope, MicroscopeNet Canada) at x10 x100 and x400 magnifications.

3.7. Data Analysis

Data generated were summarized as Means ± Standard Error of Means with variation among means subjected to analysis of variance (ANOVA) followed by Tukey's Multiple Range Test for variables. Means with a probability of less than or equal to 0.05 ($p \leq 0.05$) from same population were considered as significantly different. Graph pad Prism® software (Graph Pad Software, San Diego California, USA) version 6.0 of the year 2015 for windows® was used for analysis of data statistically, while the Microsoft Excel 2016 version was used for graphical presentation of results.

CHAPTER 4

RESULTS

4.1 Observations at Pre-Infection Evaluation

Physical examination revealed mild tick infestations in 9/28 (3%) of the donkeys. This was considered a herd problem and thus all the donkeys were treated with topical Deltamethrin® pour-on to get rid of the ticks and any other parasites which might have not been observed grossly. Blood samples on examination were negative for haemoparasites and mice subinoculated with blood samples from the donkeys also remained negative till the end of observation period (30 days), indicating that the donkeys were free of any haemoparasitic infection including *Trypanosoma* species. However, faecal sample results showed the presence of strongyle eggs and oocysts in 12/28 (43%) of the donkeys. This was considered a herd problem and treatment with oral Albendazole 600 bolus at 10mg/kg body weight (b.w.) eliminated the endoparasites. Assessment of physiological parameters revealed values within established normal range.

4.2 General Appearance/Clinical Signs

All the infected donkeys in group A (n=8), 6 out of 8 infected donkeys in group B (n=8) and all those in group C (n=6), developed clinical trypanosomosis with early signs of intermittent fever, pica appetite, tachycardia, lacrimation (with some being unilateral and others being bilateral), sternal recumbency, mucopurulent nasal discharge, dullness and transient intermittent penile erection in the males. In the later stages, diarrhoea, rough hair coat, respiratory rales on auscultation, depression, reduced feed intake, pale oral and occular mucous membranes, weakness, weight loss, lateral recumbency and emaciation were recorded while anorexia was predominant at the chronic stage. The

finding above indicates that of the 22 infected donkeys, parasitaemia was observed in 20 (90.9%) while 2 (9.1%) remained aparasitaemic (Table 4.1)

The two infected animals of group B which did not develop clinical trypanosomosis were aparasitaemic throughout the experimental period. Wistar rats sub-inoculated with blood samples from these donkeys remained negative for trypanosomes till the end of observation period (50 days).

There was pyrexia in all the infected donkeys, with a significant ($p<0.0001$) rise in rectal temperature from day 0 of infection (37.60±0.17 °C) to day 4 post infection (38.95±0.19 °C) and a peak at day 5 (39.91±0.23 °C) post infection (pi). Temperatures, however, dropped to 37.72±0.25 °C at day 9 pi but began to rise again at day 12 pi (38.05±0.18 °C), and remained fluctuating throughout the course of the experiment.

At day 6 pi, infected donkeys began to show signs of weight loss despite having a good appetite, with rough hair coat observed in all of them by day 7 pi. After day 15 pi, generalized sternal recumbency previously observed was no longer noted except in Group C (infected untreated) donkeys at chronic stage from day 150 and beyond. As the infection persisted, there was progressive weakness, dullness and debility by day 21 post infection in the infected animals (Groups A, B and C). At the chronic stage in Group C donkeys, muscles of the flank and hindquarters appeared to be wasting with the ribs and hip bones becoming prominent. Some of the infected males exhibited transient and intermittent protrusion of the penis early in the infection at periods of high parasitaemia. Terminal signs included drooping of the head, isolation, reduced appetite, sternal and lateral recumbency. Four (4) severely moribund cases made up of one from subgroup A_1 on day 113 post infection (day 101 post-treatment), one from subgroup B_1 on day 111 post infection (day 99 post-treatment), and two from group C (infected

untreated) on days 16 and 113 post infection (early and late chronic stage) were sacrificed. Group D (uninfected control) animals remained active till the end of the experiment.

4.3 Pattern of Parasitaemia

4.3.1 Post infection parasitaemia

Parasites were first detected day 2 pi following intravenous inoculation of the donkeys. Parasite count on examination of jugular vein blood was 0-3 parasites per microscopic field (ppf) on wet mount and approximately 11 parasites on haematocrit tube examination while ear vein blood was negative for parasites at this time. However, at day 3 pi, parasites were detectable in the ear vein blood and thereafter; infected donkeys showed fluctuating parasitaemia till the end of the experiment (Figure 4.1). Parasitaemia level which varied between the groups and individual animals, was characterized by transient peaks (≥ 40ppf) and sustained low levels (≤ 10ppf) with 9 – 14 days intervals between peaks. In the infected untreated group C donkeys, parasitaemia was undulating and persistent all through the study period (Figure 4.2).

Table 4.1: Percentage Positivity (%) of donkeys experimentally infected with *T. brucei* (Federe isolate).

Group	No. Infected	No. (%) Positive
A	8	8 (100)
B	8	6 (75)
C	6	6 (100)
Total	22	20 (90.9)

Key: A = Infected Novidium®-Treated; B = Infected Sécuridium®-Treated; C = Infected Untreated

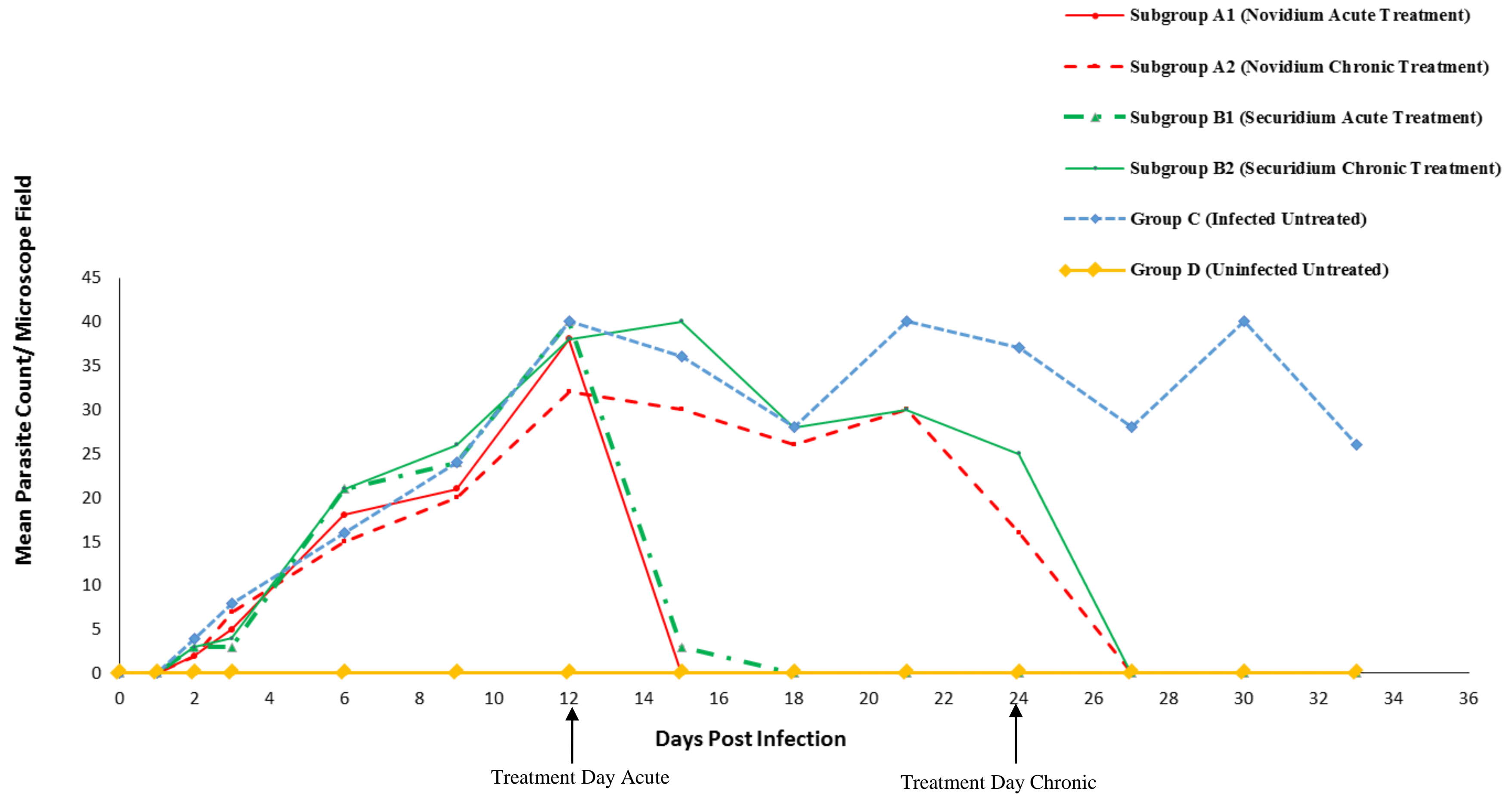

Figure 4.1: Pre and Post infection parasitaemia profile of donkeys experimentally infected with *Trypanosoma brucei* and following treatment on days 12 (acute phase) and 24 (chronic phase).

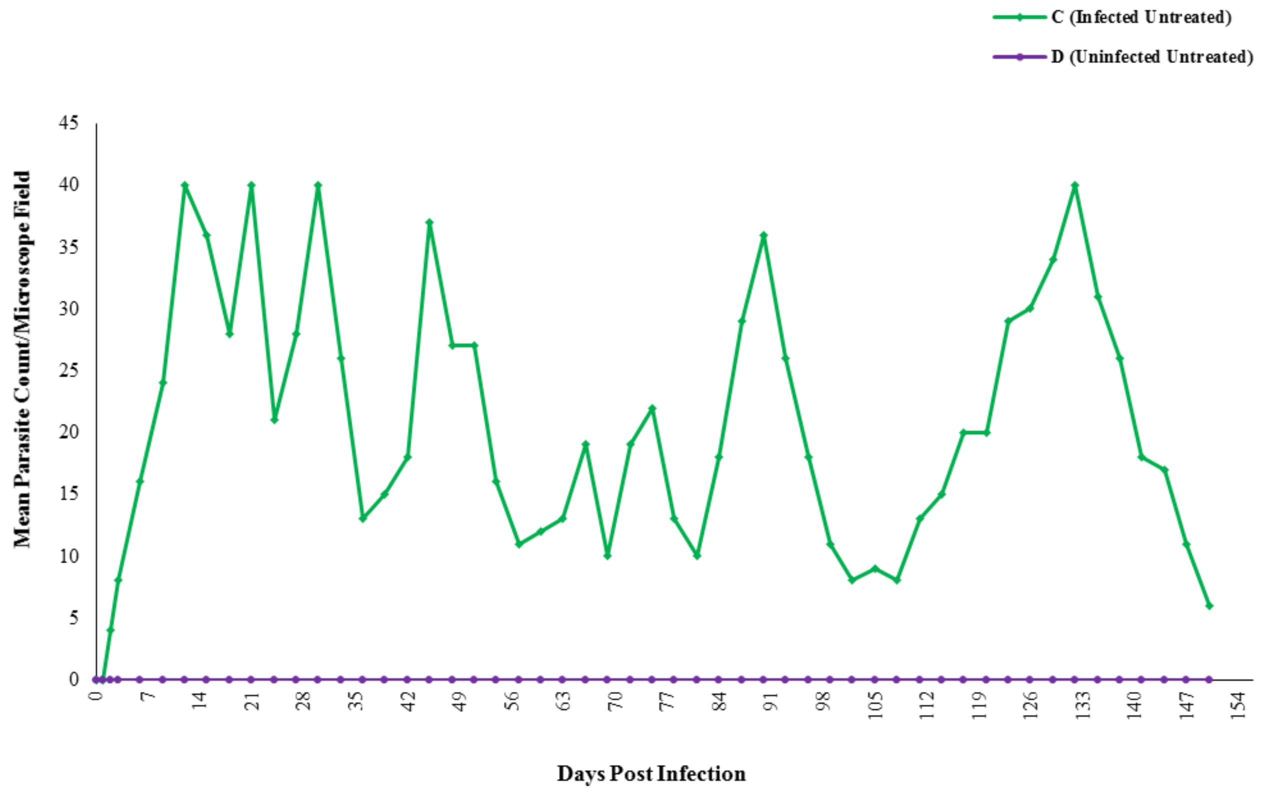

Figure 4.2: Mean parasitaemia pattern of Group C donkeys experimentally infected with *Trypanosoma brucei* and untreated, exhibiting undulating (waves of) parasitaemia.

4.3.2 Post-treatment parasitaemia

Parasites could not be detected from the venous blood 24 hours after treatment in the treatment groups. Also ear vein blood on wet mount and thin smear showed no parasites on examination.

4.3.2.1 Parasitaemia in donkeys treated with Novidium® at acute stage (Subgroup A_1).

Donkeys in this group (n=4) had a gradually rising mean parasitaemia of ≤2ppf at day 2 pi to ≥18ppf at day 6 pi and a peak of 38ppf by day 12 pi (Figure 4.3) before treatment was instituted. One of the four donkeys (25%) in this group showed no parasite on blood screening post infection and remained aparasitaemic till the termination of the experiment and mouse inoculation test (MIT) of its blood sample was negative for parasites throughout the 50-day observation period. Twenty-four (24) hours post treatment (pt) on day 12 pi, parasites were not detected in venous blood of the treated donkeys, however, a relapse parasitaemia was detected 27 days post treatment (27 dpt) in one of the donkeys, with a parasitaemic level of ≥ 20ppf on wet mount (Figure 4.4). The relapse infection was treated 21 days (58 days post initial treatment) after detection with same dose (1.0mg/kg b.w.) of Novidium® injectable solution and the parasites eliminated 24 hours later. At day 63 pt for first relapse, the treated donkey had another relapse (secondary relapse) with parasite count ≥ 15ppf. The donkey was treated same day with Novidium® at same dose as before and the parasites cleared 24hours after (Figure 4.4). No further relapses were recorded either on wet mount or MIT till termination of the experiment at day 160.

4.3.2.2 Parasitaemia in donkeys treated with Sécuridium® at acute stage (Subgroup B_1).

Prior to treatment, the animals in this group (n=4) had a rising mean parasitaemia of ≥ 3ppf at day 2 pi to ≥ 40ppf by day 12 pi (Figure 4.3). Twenty-four (24) hours post treatment at day12 pi, parasites were not detected in peripheral blood except in one of the donkeys in which parasitaemia persisted at low levels until 72 hours later (day 3 post treatment) (Figure 4.4). A relapse of infection was recorded at day 69 pt with parasite count of ≥ 20ppf which cleared 24 hours following treatment same day with Sécuridium® at an increased dose of 1 mg/kg b.w. No further relapses were recorded till the termination of the experiment.

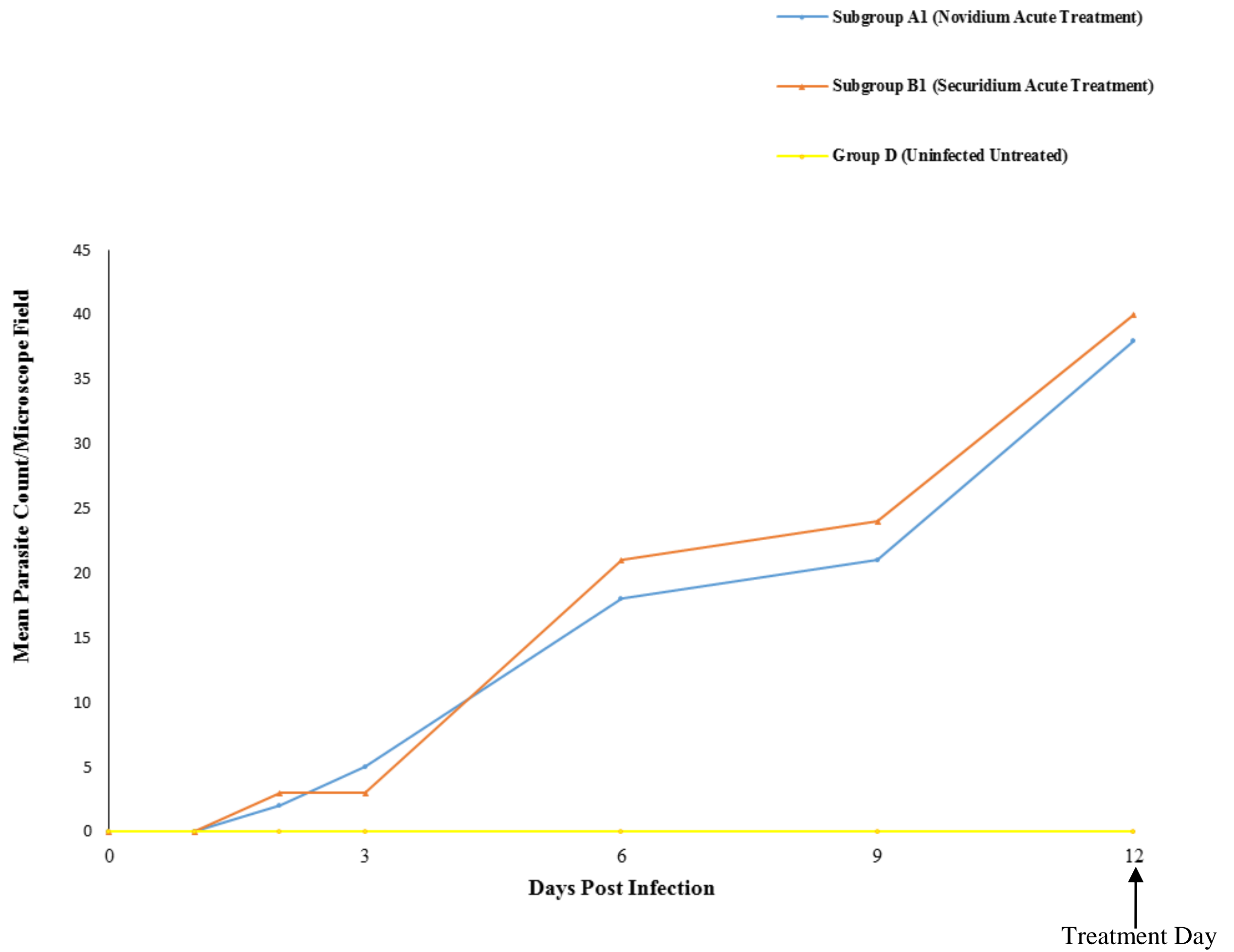

Figure 4.3: Parasitaemia profile of donkeys experimentally infected with *Trypanosoma brucei* at the acute infection stage before treatment on day 12.

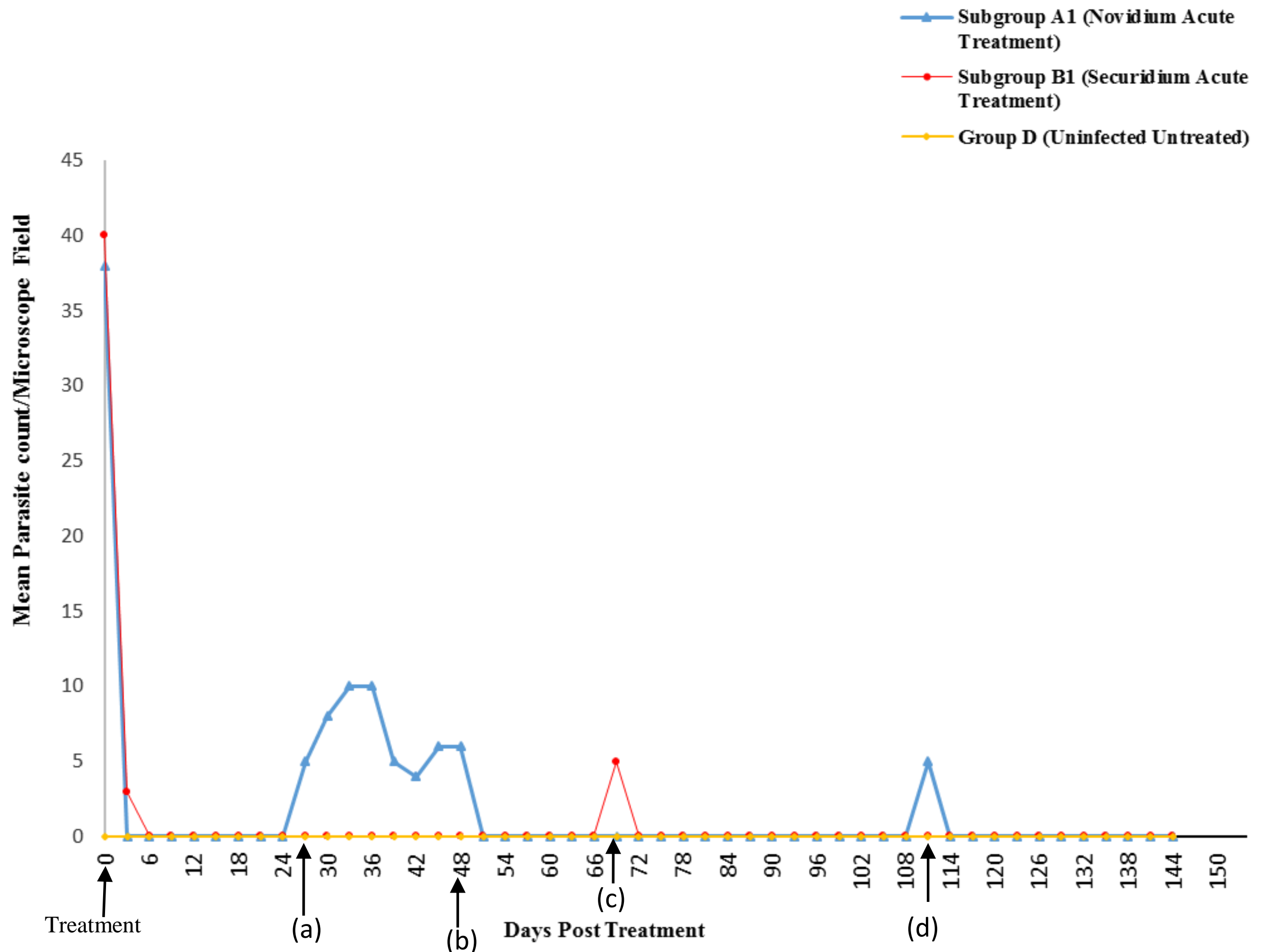

Figure 4.4: Parasitaemia profile of donkeys experimentally infected with *Trypanosoma brucei* at acute infection stage following treatment on day 12 and showing relapses.

Key:

0 = treatment at day 12 post infection

(a) = subgroup A1 first relapse at day 27 post treatment (dpt).

(b) = treatment of subgroup A1 first relapse.

(c) = subgroup B1 relapse at 69 dpt, treatment was administered same day.

(d) = subgroup A1 second relapse at 111 dpt (day 63 post 1st relapse treatment), treatment was administered same day.

4.3.2.3 Parasitaemia in donkeys treated with Novidium® at chronic stage (Subgroup A2).

Donkeys in this group (n=4) had fluctuating mean parasitaemia starting from day 3 pi with parasite count increasing from ≥ 3ppf to ≥ 32 at day 12 pi and ≥ 16ppf by day 24 pi. There were peaks at day 12 pi (≤ 32 ppf) and day 21 pi (≤ 30 ppf) (Figure 4.5). Treatment was instituted at day 24 pi and the parasites were not detected 24 hours after.

One of the four donkeys (25%) of the group, however, had very low (≤ 3ppf) parasitaemia which was only detected between days 9 to 12 pi and thereafter remained aparasitaemic till the termination of the experiment. Mouse inoculation test (MIT) of blood sample from the donkey collected by jugular venipuncture at day 15 pi was negative throughout the 50-day observation period. A primary relapse of infection was detected in one of the donkeys at day 30 post treatment, with parasite count of ≥ 5 ppf on wet mount (Fig 4.6). Treatment was repeated in the affected donkey 6 days after detected relapse and the parasites disappeared from circulation within 24 hours. A secondary relapse with parasite count of ≤ 3 ppf on wet mount was however detected in same donkey at day 27 post treatment of first relapse and treatment was instituted same day. No further relapses were recorded till the termination of the experiment. Mouse inoculation test afterwards was negative for all animals through the 50-day observation period.

4.3.2.4 Parasitaemia in donkeys treated with Sécuridium® at chronic stage (Subgroup B2).

Prior to treatment, animals in this group (n=4) had a rising mean parasitaemia from day 2 post infection with parasite count ≥ 3ppf to a peak of ≥ 40ppf by day 15 pi (Figure 4.5). Parasitaemia remained fluctuating till day 24 pi when treatment was administered. There were no detectable parasites 24 hours post treatment and no relapses were observed till the termination of the experiment (Figure 4.6).

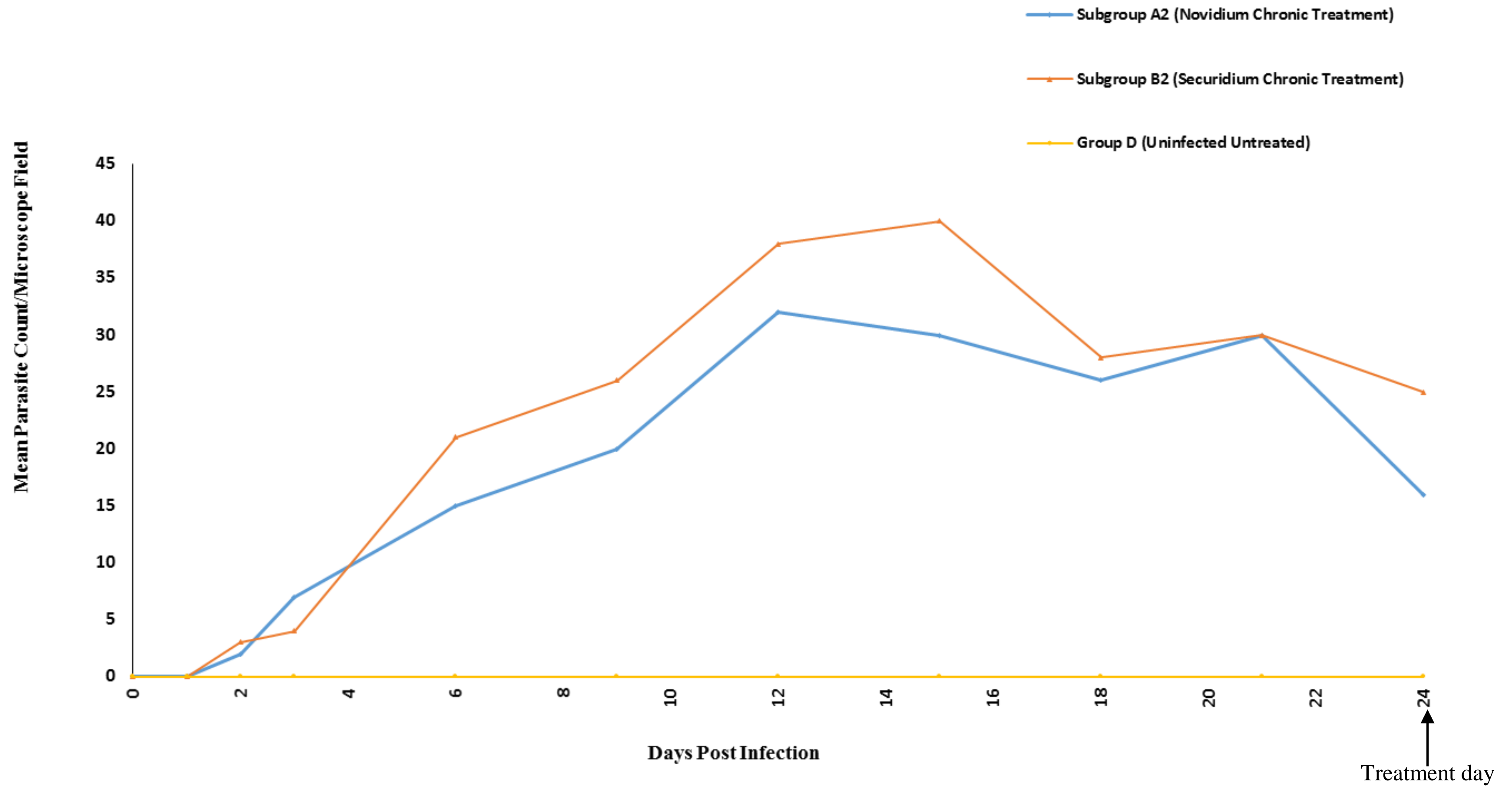

Figure 4.5: Parasitaemia profile of donkeys experimentally infected with *Trypanosoma brucei* at chronic infection stage before treatment on day 24.

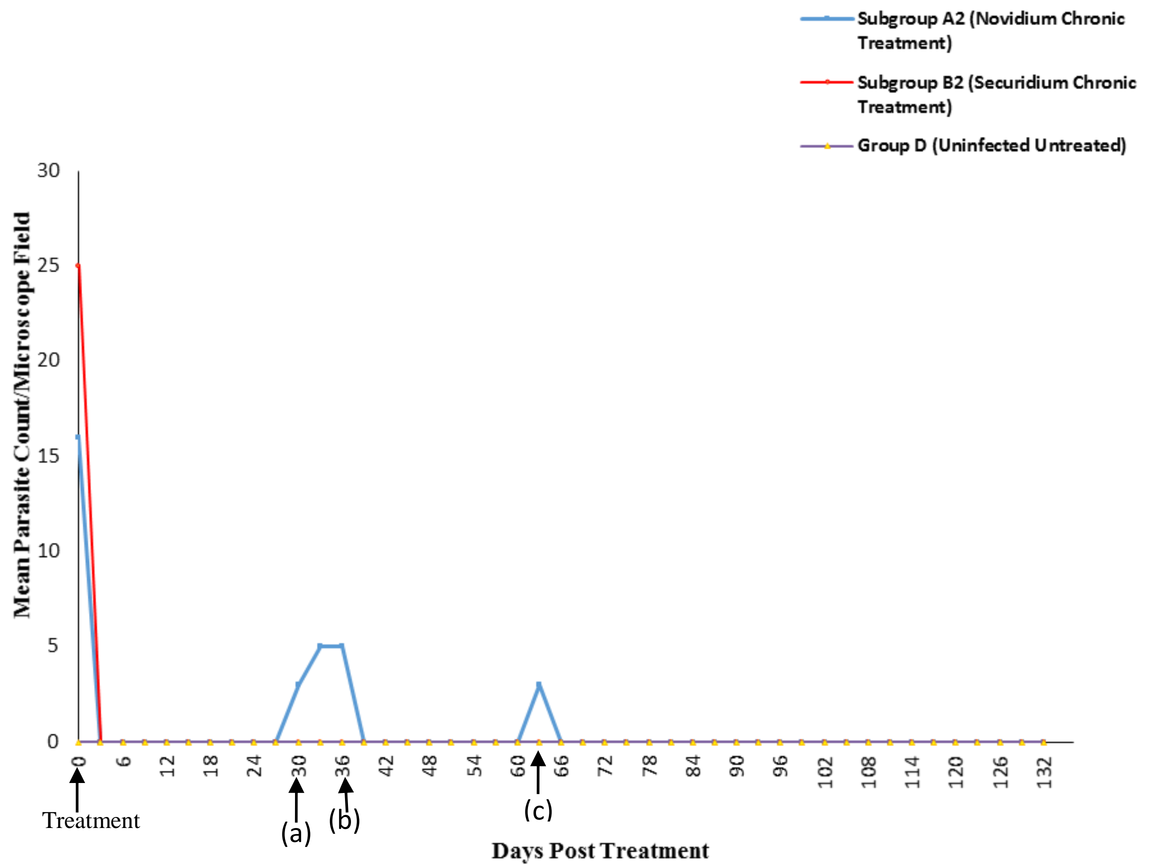

Figure 4.6: Parasitaemia profile for donkeys experimentally infected with *Trypanosoma brucei* at chronic infection stage following treatment on day 24 and showing relapses.

Key:

0 = treatment at day 24 post infection

(a) = subgroup A2 first relapse at 30 days post treatment (dpt)

(b) = treatment of subgroup A2 first relapse at 36 dpt (day 6 post relapse)

(c) = subgroup A2 second relapse at 63 dpt (day 27 post treatment of first relapse), treatment was administered same day.

4.4 Rectal Temperature Profile

Pre-infection, mean rectal temperatures of the donkeys in all the groups (A_1, A_2, B_1, B_2, C and D) varied from a mean of 35.73±1.29 °C to 37.58±0.47 °C (established normal range is 35.0 – 39.0 °C). Post infection, mean rectal temperatures began to rise at day 3 pi (mean 38.3 °C), peaked at day 5 pi (mean 40 °C), persisted for about 5days before it started to fluctuate, in relation to parasitaemia level. In the course of the infection, normal temperatures were sometimes observed despite detectable parasitaemia. Differences between means of the infected groups (A_1, A_2, B_1, B_2 and C) were not statistically significant ($p>0.05$) post infection but became significant ($p<0.0001$) post treatment.

4.4.1 Subgroup A_1 (Novidium® treatment at acute infection stage)

The mean rectal temperature of the donkeys for subgroup (A_1) increased significantly ($p=0.0062$), from pre-infection value of 36.94±0.24 °C to 37.93±0.24 °C by day 7 pi. Post infection, mean rectal temperature of this subgroup (37.73±0.18 °C) was significantly lower ($p<0.01$) than for the infected untreated group C donkeys (38.33±0.15 °C) but significantly higher ($p=0.0317$) than for the uninfected group D mean (37.30±0.08 °C). Post treatment, differences between A_1 mean rectal temperature (37.27±0.12 °C) and group C mean (38.33±0.15 °C) was highly significant ($p<0.0001$). Also A_1 mean was significantly higher ($p<0.03$) than group D mean (36.62±0.14 °C) (Figure 4.7).

4.4.2 Subgroup B_1 (Sécuridium® treatment at acute infection stage)

Mean rectal temperature values for this subgroup significantly ($p<0.002$) increased from pre-infection value of 37.17±0.20 °C to 38.33±0.15 °C by day 7 pi. Post infection, differences between means of B_1 (38.20±0.19 °C) and group C (38.33±0.15 °C) were

non-significant (p=0.59), but B_1 was significantly higher (p<0.0001) than group D mean (37.30±0.08 °C). Post treatment, B_1 mean (37.27±0.13 °C) was significantly lower (p<0.0001) than group C mean (38.16±0.17 °C) and significantly higher (p<0.001) than group D mean (36.62±0.14 °C) at termination of the experiment (Figure 4.7).

4.4.3 Subgroup A_2 (Novidium® treatment at chronic infection stage)

The mean rectal temperature values for this subgroup (A_2) significantly (p<0.001) increased to a mean temperature of 38.43±0.27 °C at day 7 pi from pre-infection value of 36.73±0.38 °C. Post infection, A_2 mean rectal temperature (38.09±0.13 °C) though increased, was non-significantly lower (p=0.4282) than the group C mean rectal temperature (38.23±0.11 °C) but significantly higher (p<0.0001) than that for group D donkeys (37.07±0.08 °C). Post treatment, A_2 mean (37.15±0.16 °C) dropped and was significantly lower (p<0.02) than that for group C donkeys (37.61±0.11 °C) but non-significantly higher (p=0.4993) than that group D donkeys (37.01±0.14 °C) (Figure 4.8).

4.4.4 Subgroup B_2 (Sécuridium® treatment at chronic infection stage)

The rectal temperature mean values increased significantly (p=0.0130) from 37.01±0.22 °C at pre-infection to 37.98±0.30 °C by day 7 pi. Subgroup B_2 mean value (38.28±0.13 °C) at post infection was non-significantly (p=0.7631) different from that for group C donkeys (38.23±0.11 °C) but significantly higher (p<0.0001) than that for group D donkeys (37.07±0.08 °C). Post treatment, there was a decrease in mean rectal temperature with B_2 mean (36.81±0.20 °C) significantly lower (p=0.0007) than group C mean (37.61±0.11 °C) and non-significantly lower (p=0.4066) than group D mean (37.01±0.14 °C) (Figure 4.8).

4.4.5 Group C (Infected Untreated)

In this group, there was a significant ($p<0.0001$) difference between mean rectal temperatures at pre-infection (37.35±0.13 °C) and post-infection (38.64±0.20 °C). Also, the difference between the mean of this group (37.87±0.11 °C) and group D (36.95±0.09 °C) was highly significant ($p<0.0001$) at termination of the experiment (Figure 4.8).

4.4.6 Group D (Uninfected Control)

Donkeys in this group maintained mean rectal temperature value between 35.87±0.4 °C to 37.52±0.26 °C throughout the study period (Figure 4.8).

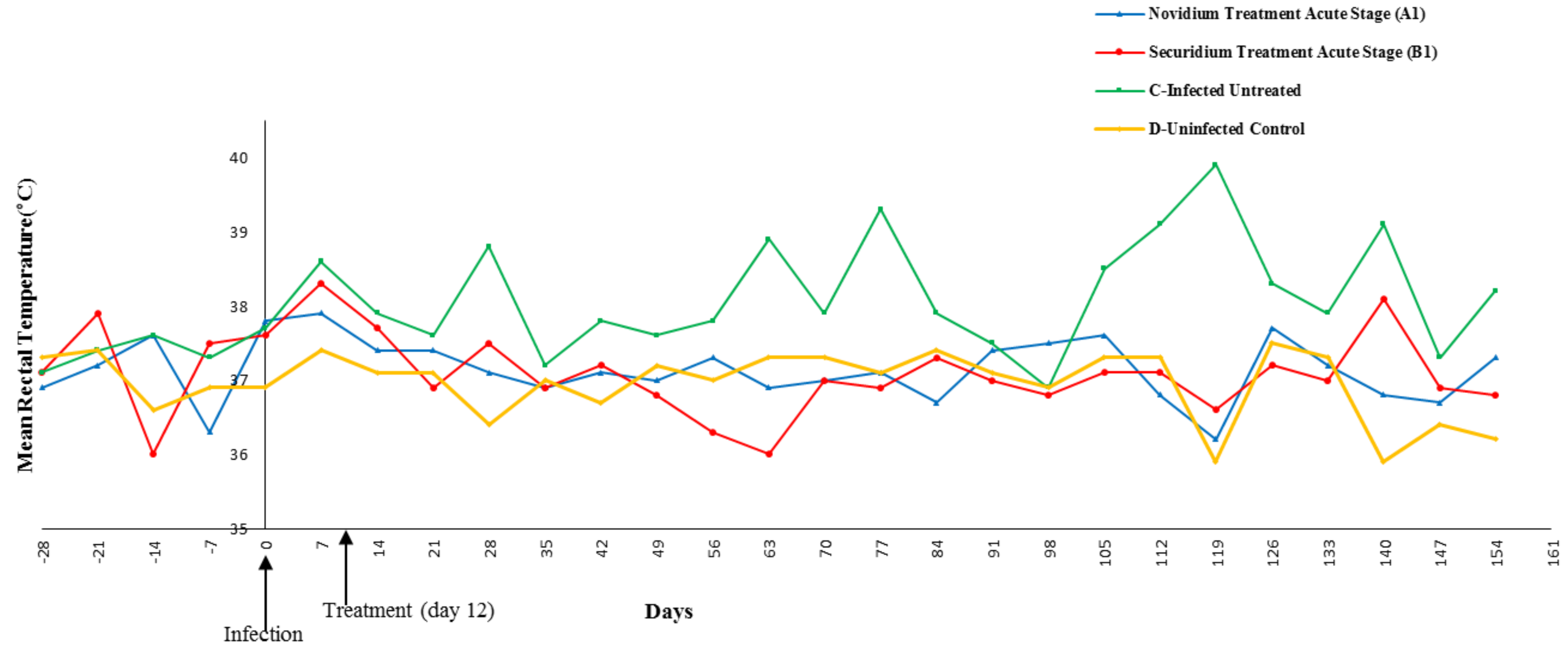

Figure 4.7: Mean rectal temperature pre-infection, post-infection and post-treatment at the acute stage (day 12 of infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 12 = post infection period
Day 12 = treatment day
Days 12 – 154 = post treatment period

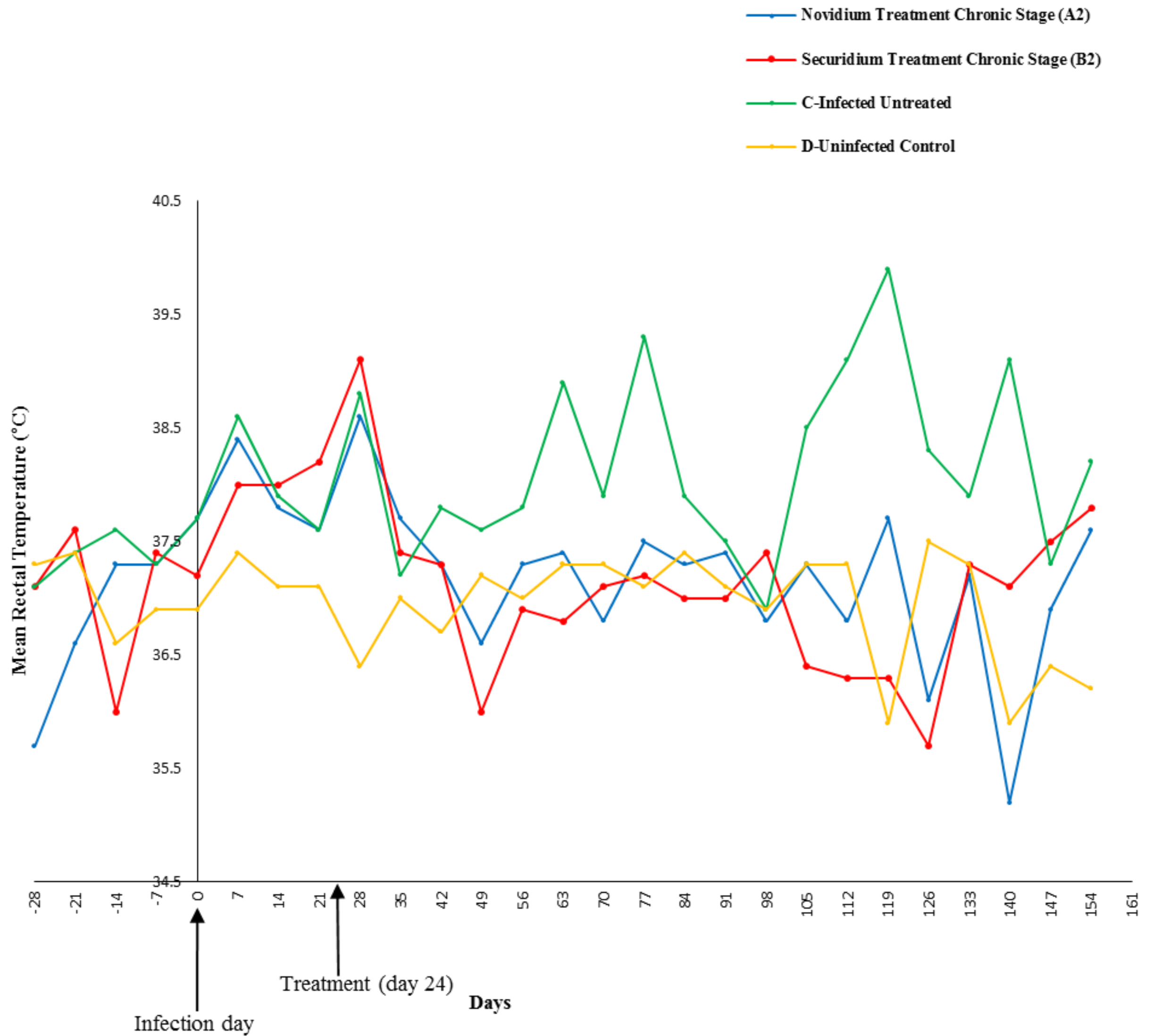

Figure 4.8: Mean rectal temperature pre-infection, post-infection and post-treatment at the chronic stage (day 24 of infection) for donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 24 = post infection period
Day 24 = treatment day
Days 24 – 154 = post treatment period

4.5 Heart Rate Changes

Prior to infection, mean heart rates varied between 36±5.35 beats per minute (bpm) to 44.75±0.85 bpm (established normal range is 38 – 45 beats/min). Post infection, mean values increased to a peak of 71.96±1.84 bpm by day 21 after which it varied between 34.75±1.38 bpm and 60.63±5.29 bpm post treatment. Mean values for the infected-treated groups (A and B) at post treatment were higher than for the uninfected control group D. At terminal stages in the infected untreated group C donkeys, values of up to 71.83±2.6 bpm were counted and beats were arrhythmic.

4.5.1 Subgroup A_1 (Novidium® treatment at acute infection stage)

The mean heart rates for this group increased non-significantly (p=0.1096) from pre-infection value of 44.75±0.85 to 51.25±2.93 bpm by day 7 pi. Also at day 7 pi, differences between the mean heart rate for this group (51.25±2.93 bpm) and the uninfected control group D (47.17±3.63 °C) was statistically non-significant (p=0.4066). Post infection, A_1 mean heart rate (52.75±2.45 bpm) though increased was non-significantly (p=0.2059) higher than that of group C (48±2.66 °C) and group D donkeys (46.50±1.85 °C). Post treatment, however, the mean heart rate (51.04±1.70 bpm) for this subgroup was significantly lower (p<0.0001) than that for the infected untreated group C donkeys (67.88±1.02 bpm) but significantly higher (p<0.0001) than for group D donkeys (42.06±0.52 bpm) (Figure 4.9).

4.5.2 Subgroup B_1 (Sécuridium® treatment at acute infection stage)

At day 7 pi, mean heart rate values for this group decreased non-significantly (p=0.7886), from pre-infection mean of 45.25±6.45 to 43±4.73 bpm. Post infection, differences between means of B_1 (46.50±3.02 bpm), group C (48±2.66 bpm) and group D (46.50±1.85 bpm) were statistically non-significant with p=0.8795. Similarly, post

treatment B_1 mean heart rate (64.57±1.47 bpm) was non-significantly ($p=0.0673$) lower than for group C donkeys (67.88±1.02 bpm) though it was significantly ($p<0.0001$) higher than for group D donkeys (42.06±0.52 bpm) (Figure 4.9).

4.5.3 Subgroup A_2 (Novidium® treatment at chronic infection stage)

Mean heart rate for subgroup A_2 recorded a non-significant ($p=0.0544$) increase from pre-infection value of 44.75±0.85 bpm to 55.50±3.59 bpm by day 7 pi. A non-significant ($p=0.1437$) decrease was also recorded when A_2 mean (55.50±3.59 bpm) was compared to group D mean (47.17±3.63 bpm) over the same period. Post infection, subgroup A_2 mean (56.67±2.79 bpm) was significantly higher ($p<0.04$) than group C mean (49.22±1.83 bpm) and also significantly higher ($p<0.002$) than group D mean (44.78±1.48 bpm). Post treatment, subgroup A_2 mean (50.13±1.25 bpm) was significantly ($p<0.0001$) lower than group C mean (68.98±0.99 bpm) but significantly ($p<0.0001$) higher than group D mean (42.05±0.49 bpm) (Figure 4.10).

4.5.4 Subgroup B_2 (Sécuridium® treatment at chronic infection stage)

In this group, mean heart rate values increased non-significantly ($p=0.1872$) from 41±1.00 bpm at pre-infection to 50±5.29 bpm by day 7 pi. Post infection, differences between the means of B_2 (49.58±2.67 bpm), group C (49.22±1.83 bpm) and group D (44.78±1.48 bpm) donkeys were statistically non-significant ($p=0.1437$) while post treatment, subgroup B_2 heart rate (67.75±1.55 bpm) was non-significantly ($p=0.5064$) lower than group C mean (68.98±0.99 bpm) but was significantly ($p<0.0001$) higher than the group D mean (42.05±0.49 bpm) (Figure 4.10).

4.5.5 Subgroup C (Infected Untreated)

For this group, the mean heart rate increased non-significantly ($p=0.5174$) from 43±2.66 bpm at pre-infection to 46±3.58 bpm by day 7 pi. Also, a significant difference ($p<0.0001$) was recorded between the means of this group C (64.12±1.13 bpm) and group D (42.67±0.50 bpm) at termination of the experiment (Figure 4.10).

4.5.6 Subgroup D (Uninfected Control)

Heart rate values for this group varied between 37.47±1.21 bpm and 48.17±1.57 bpm throughout the study period (Figure 4.10).

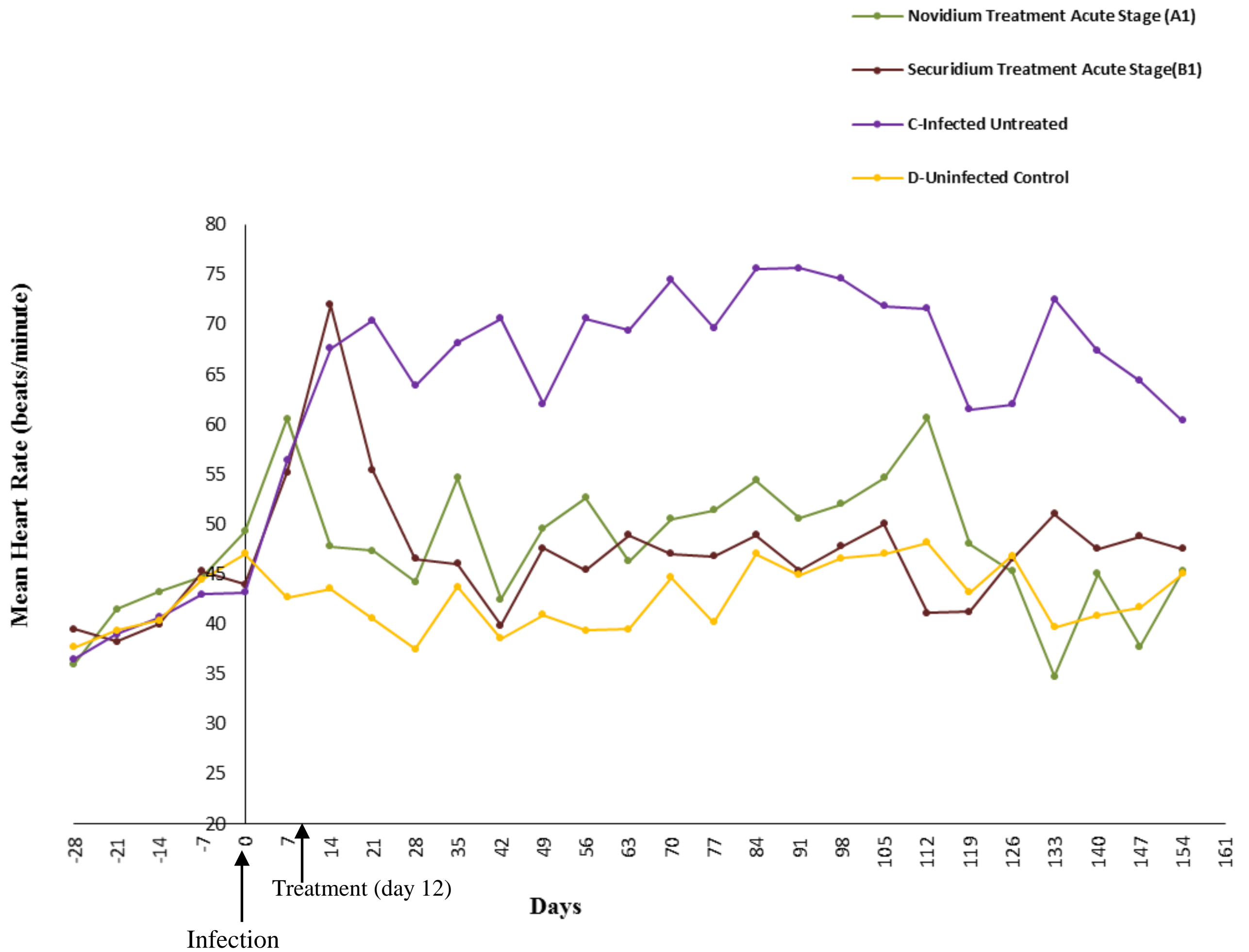

Figure 4.9: Mean heart rate values pre-infection, post infection and post treatment at the acute stage (day 12 post infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 12 = post infection period
Day 12 = treatment day
Days 12 – 154 = post treatment period

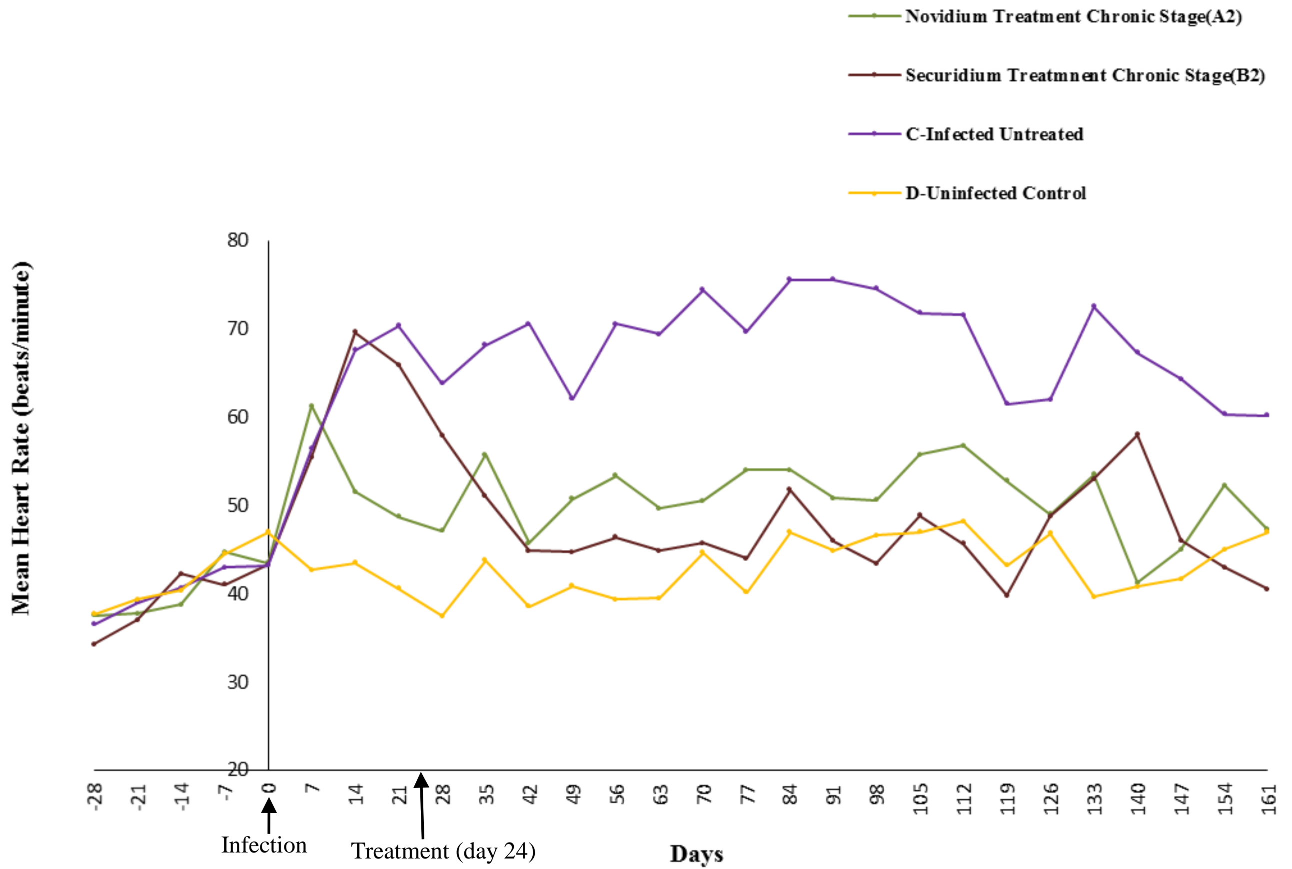

Figure 4.10: Mean heart rate values pre-infection, post-infection and post-treatment at the chronic stage (day 24 post infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 24 = post infection period
Day 24 = treatment day
Days 24 – 154 = post treatment period

4.6 Respiratory Rate Changes

Pre-infection, mean respiratory rate of all the donkeys in the four groups varied from 14±0.82 to 19.61±2.29 breaths/min (established normal range is 20 – 30 breaths/min). There was a marked increase in respiratory rate up to 22.83±1.19 breaths/min in all the infected groups post infection. At terminal stages for donkeys of the infected untreated group, breathing was shallow and partly abdominal. Moist rales were heard on auscultation.

4.6.1 Subgroup A1 (Novidium® treatment at acute infection stage)

Respiratory rate mean values increased significantly ($p<0.002$) from 14±0.82 breaths/min at pre-infection to 19.50±0.50 breaths/min by day 7 pi. Also at day 7 pi, the mean respiratory rate for subgroup A1 donkeys (19.50±0.50 breaths/min) significantly ($p=0.0108$) increased in comparison to group D donkeys (14.83±1.22 breaths/min) with the increase persisting till treatment on day 12. Post infection, differences between the mean respiratory rate of subgroup A1 donkeys (21.54±1.12 breaths/min) and that for the infected untreated group C donkeys (23.57±0.94 breaths/min) were statistically non-significant ($p=0.1690$), but significantly higher ($p<0.0001$) when compared with values for the infected untreated group D donkeys (16±0.30 breaths/min). Differences between means of respiratory rates of subgroup A1 donkeys (20.67±3.48 breaths/min), group C donkeys (37.00±7.80 breath/min) and group D donkeys (15.50±1.34 breaths/min) post-treatment were statistically non-significant ($p>0.05$). (Figure 4.11).

4.6.2 Subgroup B1 (Sécuridium® treatment at acute infection stage)

The mean values for this group increased non-significantly ($p=0.2473$) from 16±2.04 breaths/min at pre-infection to 19.75±2.10 breaths/min by day 7 pi. Also the values (19.75±2.10 breaths/min) non-significantly increased ($p=0.0982$) when compared to

those for group D donkeys (14.83±1.22 breaths/min) at day 7 pi. Similarly, their post infection mean respiratory rate (20.13±0.71 breaths/min) was significantly lower ($p<0.005$) than for the group C donkeys (23.57±0.94 breaths/min), and significantly higher ($p<0.0001$) than for the group D donkeys (16±0.30 breaths/min). Post treatment, however, the corresponding mean respiratory rate (18.05±0.66 breaths/min) was significantly lower ($p=0.0044$) than for the group C donkeys (20.84±0.69 breaths/min) but significantly higher ($p<0.02$) than for the group D donkeys (16.22±0.37 breaths/min) (Figure 4.11).

4.6.3 Subgroup A_2 (Novidium® treatment at chronic infection stage)

At day 7 pi, the pre-infection value of the mean respiratory rate (16.50±1.56 breath/min.) increased non-significantly ($p=0.4465$) to 18.50±1.89breaths/min. Also subgroup A_2 mean respiratory rate (18.50±1.89 breaths/min) was non-significantly higher ($p=0.1597$) than for the group D donkeys (14.83±1.22 breaths/min). Post infection, significant differences were recorded between mean respiratory rates of A_2, C and D. Subgroup A_2 mean respiratory rate (20.89±0.67 breaths/min) was significantly lower ($p<0.0001$) than for the group C donkeys (24.78±0.72 breaths/min), but significantly ($p<0.0001$) higher than for the group D donkeys (16±0.23 breaths/min). Post treatment, subgroup A_2 mean respiratory rate (19.13±0.72 breaths/min) was significantly lower ($p<0.0001$) than for group C donkeys (34.05±1.44 breaths/min) but however significantly higher ($p<0.02$) than for group D donkeys (17.01±0.38 breaths/min) (Figure 4.12).

4.6.4 Subgroup B_2 (Sécuridium® treatment at chronic infection stage)

The mean respiratory rate for subgroup B_2 increased significantly ($p<0.02$) by day 7 pi from 14.25±0.85 breaths/min to 18.25±0.85 breaths/min. However, difference between

B2 mean respiratory rate (18.25±0.85 breaths/min) and group D mean respiratory rate (14.83±1.22 breaths/min) was non-significant (p>0.05) at day 7 pi. Post infection, B2 mean (21.52±0.58 breaths/min) was significantly lower (p<0.001) than group C mean (24.78±0.72 breaths/min) and significantly higher (p<0.0001) than group D mean (16±0.22 breaths/min). Post treatment, B2 mean (17.75±0.61 breaths/min) was also significantly lower (p<0.0001) than group C mean (34.05±1.44 breaths/min) but non-significantly higher (p=0.3476) than group D mean (16±0.22 breaths/min) (Figure 4.12).

4.6.5 Group C (Infected Untreated)

Donkeys in this group had respiratory rate mean values increase non-significantly (p=0.0967) from 15.33±0.67 breaths/min to 20.33±2.43 breaths/min by day 7 pi. Same day (day 7 pi) also, group C mean (20.33±2.43 breaths/min) was non-significantly (p=0.0809) higher than group D mean (14.83±1.22 breaths/min). However, by day 10 pi, a significant (p<0.0001) difference was recorded between group C mean (36±1.77 breaths/min) and group D mean (16.17±0.70 breaths/min), persisting till termination of the experiment (Figure 4.12).

4.5.6 Group D (Uninfected Control)

Respiratory rate mean values for this control group varied from 14.5±0.56 breaths/min to 19.67±2.29 breaths/min all through the study period (Figure 4.12).

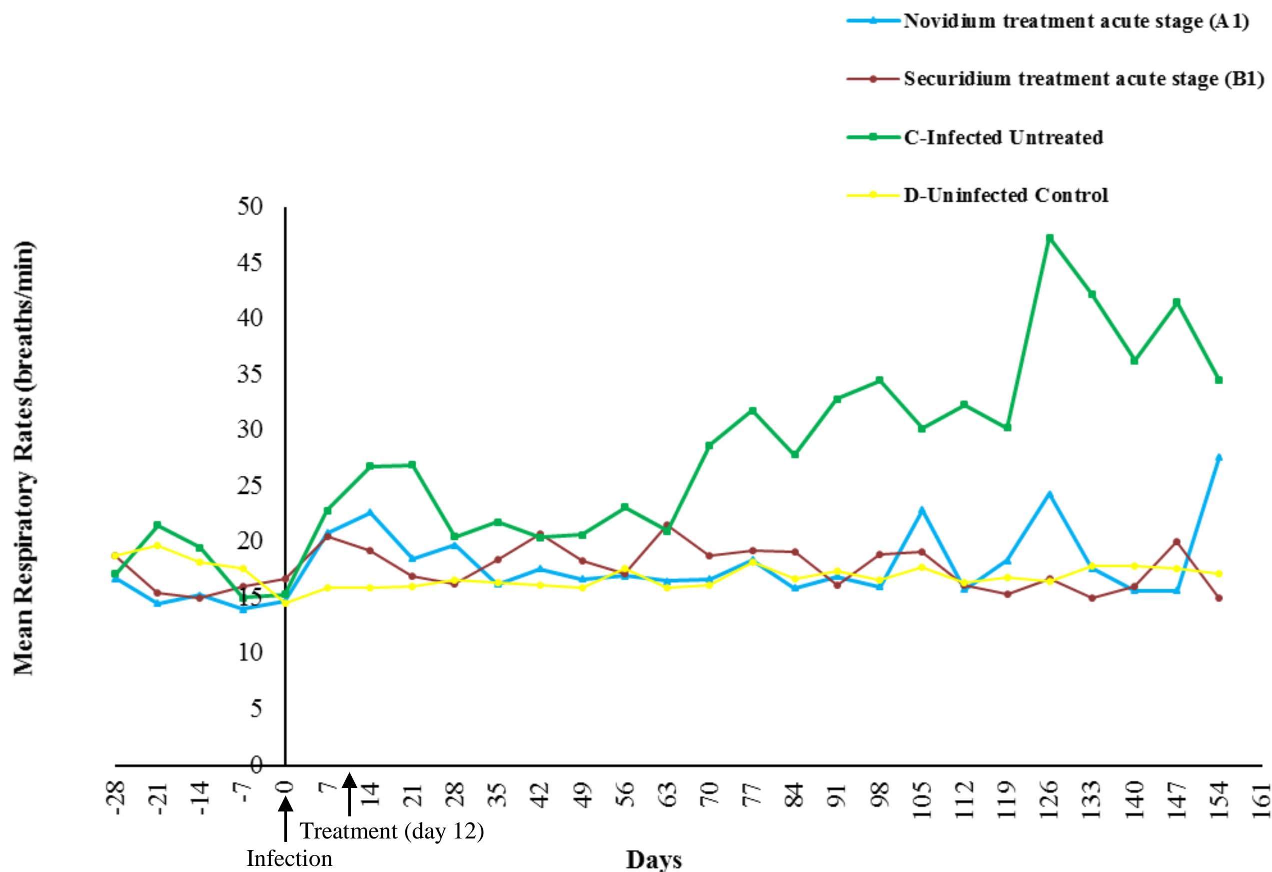

Figure 4.11: Mean respiratory rate values pre-infection, post-infection and post-treatment at the acute stage (day 12 post infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 12 = post infection period
Day 12 = treatment day
Days 12 – 154 = post treatment period

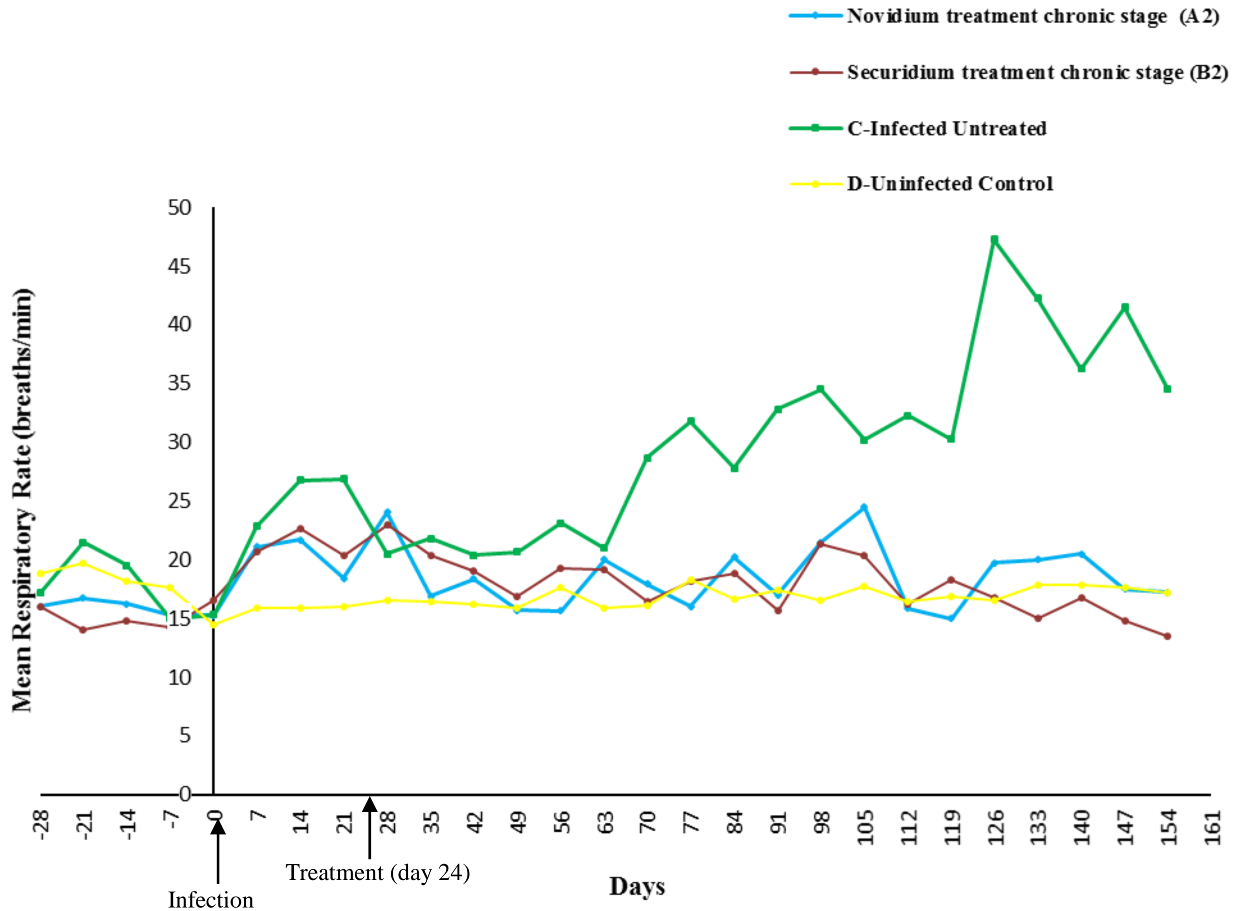

Figure 4.12: Mean respiratory rate values pre-infection, post-infection and post-treatment at the chronic stage (day 24 post infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 24 = post infection period
Day 24 = treatment day
Days 24 – 154 = post treatment period

4.7 Body Weight Changes

Following infection, parasitaemic donkeys were observed to lose weight when compared with the uninfected control group, and weight loss was marked in the infected untreated group than in the other groups. Post treatment, the donkeys were observed to regain weight. However, the mean weight values were less than for the uninfected control donkeys.

4.7.1 Subgroup A1 (Novidium® treatment at acute infection stage)

Post infection, mean weight values for subgroup A1 donkeys decreased non-significantly (p=0.1269) from the pre-infection value of 63±3.19 kg to 56±2.20 kg by day 7 pi. Also A1 mean weight (56±2.20 kg) at day 7 pi was non-significantly (p=0.1541) lower than the mean weight for group D (64.17±4.61 kg). These values, however, improved after treatment with a significant difference recorded at day 9 post treatment (day 21 pi), when the mean weight for the subgroup A1 donkeys (63.5±1.71 kg) significantly (p=0.0076) increased in comparison to the mean weight of the infected untreated group C (54.2±1.83 kg). At day 16 post treatment (day 28 pi), the mean weight of A1 (66±1.83 kg) was significantly higher (p<0.003) than for the corresponding sécuridium treatment acute infection subgroup B1 (54.75±1.25 kg). Following treatment, A1 mean weight value (64.29±1.60 kg) remained significantly higher (p=0.0001) than group C values, but significantly lower (p<0.003) than group D mean weight (73.17±2.29 kg) (Figure 4.13).

4.7.2 Subgroup B1 (Sécuridium® treatment at acute infection stage)

Post infection, subgroup B1 mean weight (56±2.20 kg) at day 7 was non-significantly (p=0.1269) lower than pre-infection mean weight (63±3.19 kg). Also, comparison between B1 mean weight (56±2.20 kg) and the group D mean weight (64.17±4.61 kg) at

day 7 pi, showed a statistically non-significant difference (p=0.1541). Post treatment, B_1 mean weight (62.31±1.49 kg) was significantly lower (p=0.0003) than the mean weight for group D donkeys (73.17±2.29 kg) but significantly higher (p=0.0002) than the mean weight for group C donkeys (53.87±1.44 kg) (Figure 4.13).

4.7.3 Subgroup A_2 (Novidium® treatment at chronic infection stage)

Weight loss recorded in this subgroup post infection only lasted a few days, day 7 – 14 pi. At day 21 pi prior to treatment on day 24, the mean weight values began to increase for this subgroup. Post treatment, A_2 mean weight (64.77±1.66 kg) was significantly higher (p=0.0001) than the mean weight for group C donkeys (53.87±1.44 kg). Significant differences were recorded between A_2 mean weight values and corresponding B_2 mean weight values. At day 4 post treatment (day 28 pi), subgroup A_2 mean weight (66±2.45 kg) was significantly higher (p=0.0012) than the B_2 mean weight (46±1.29 kg), also at day 39 post treatment (day 63 pi), A_2 mean weight (69.25±1.65 kg) was significantly higher (p=0.0182) than the B_2 mean weight (60.75±2.02 kg). (Figure 4.14).

4.7.4 Subgroup B_2 (Sécuridium® treatment at chronic infection stage)

Mean weight values for B_2 recorded significant difference at day 7 pi when mean weight recorded (50.75±2.59 kg) was significantly ($p<0.01$) lower than the pre-infection mean weight (62±1.47 kg), and also significantly (p=0.0370) lower than the uninfected group D mean weight (64.17±4.61 kg). Post treatment, the mean weight for B_2 increased (61.05±1.99 kg) but was significantly lower ($p<0.0003$) than the mean weight for group D donkeys (73.52±2.38 kg) and significantly higher (p=0.0068) than the mean weight for group C donkeys (53.85±1.52 kg) (Figure 4.14).

4.7.5 Group C (Infected Untreated)

Post infection, mean weight for donkeys in this group decreased significantly ($p=0.0002$) from 66.17±1.01 kg at pre-infection to a mean of 58.5±0.76 kg by day 7 pi. Also, mean weight for these donkeys (54.15±1.33 kg) was significantly lower ($p<0.0001$) than mean weight for the uninfected control group D donkeys (72.37±2.16 kg) at termination of the experiment (Figure4.14).

4.7.6 Group D (Uninfected Control)

Mean body weight values recorded for this group ranged from 57±2.03 kg to 84.33±2.35 kg all through the study period (Figure 4.14).

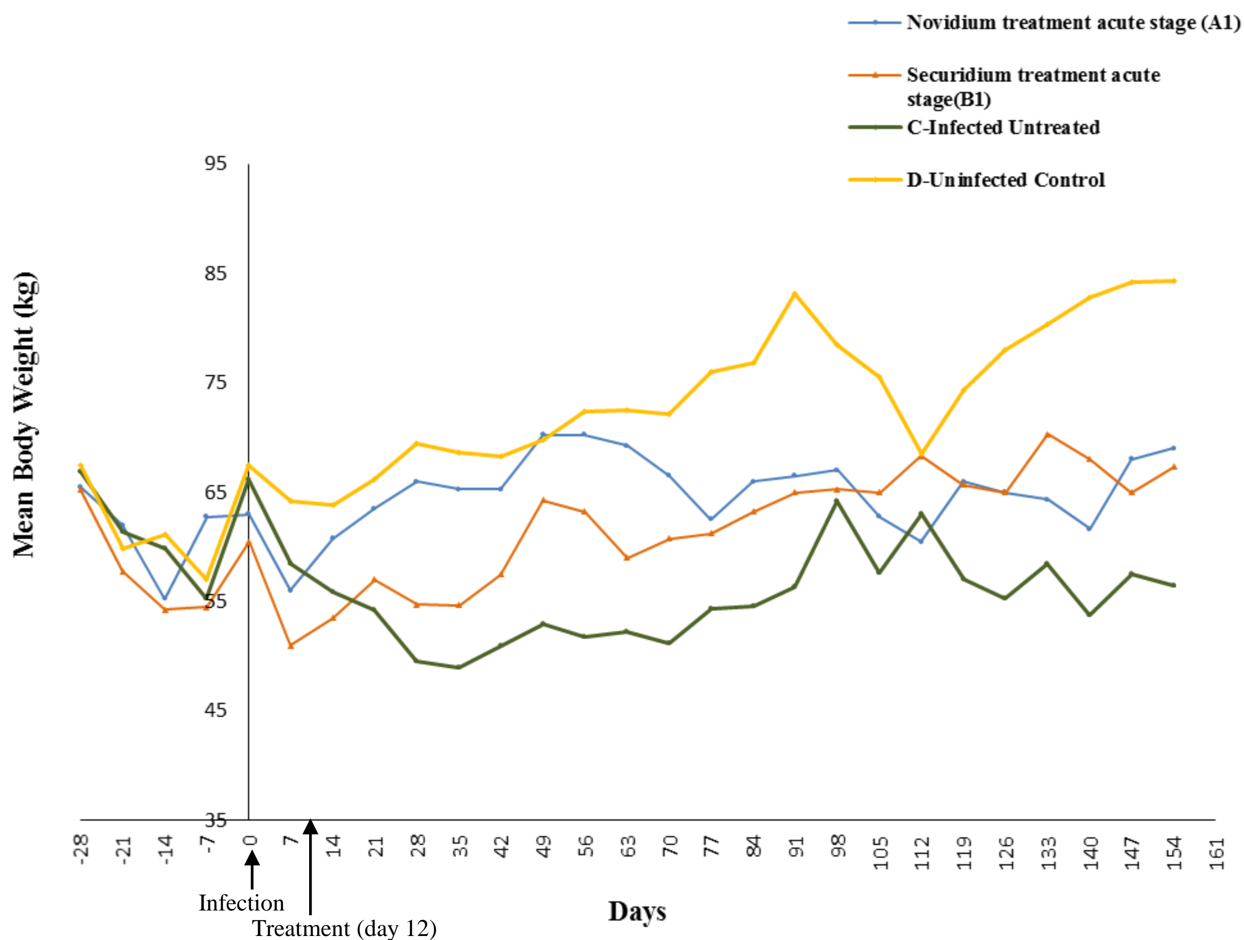

Figure 4.13: Mean body weight values pre-infection, post-infection and post-treatment at the acute stage (day 12 post infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 12 = post infection period
Day 12 = treatment day
Days 12 – 154 = post treatment period

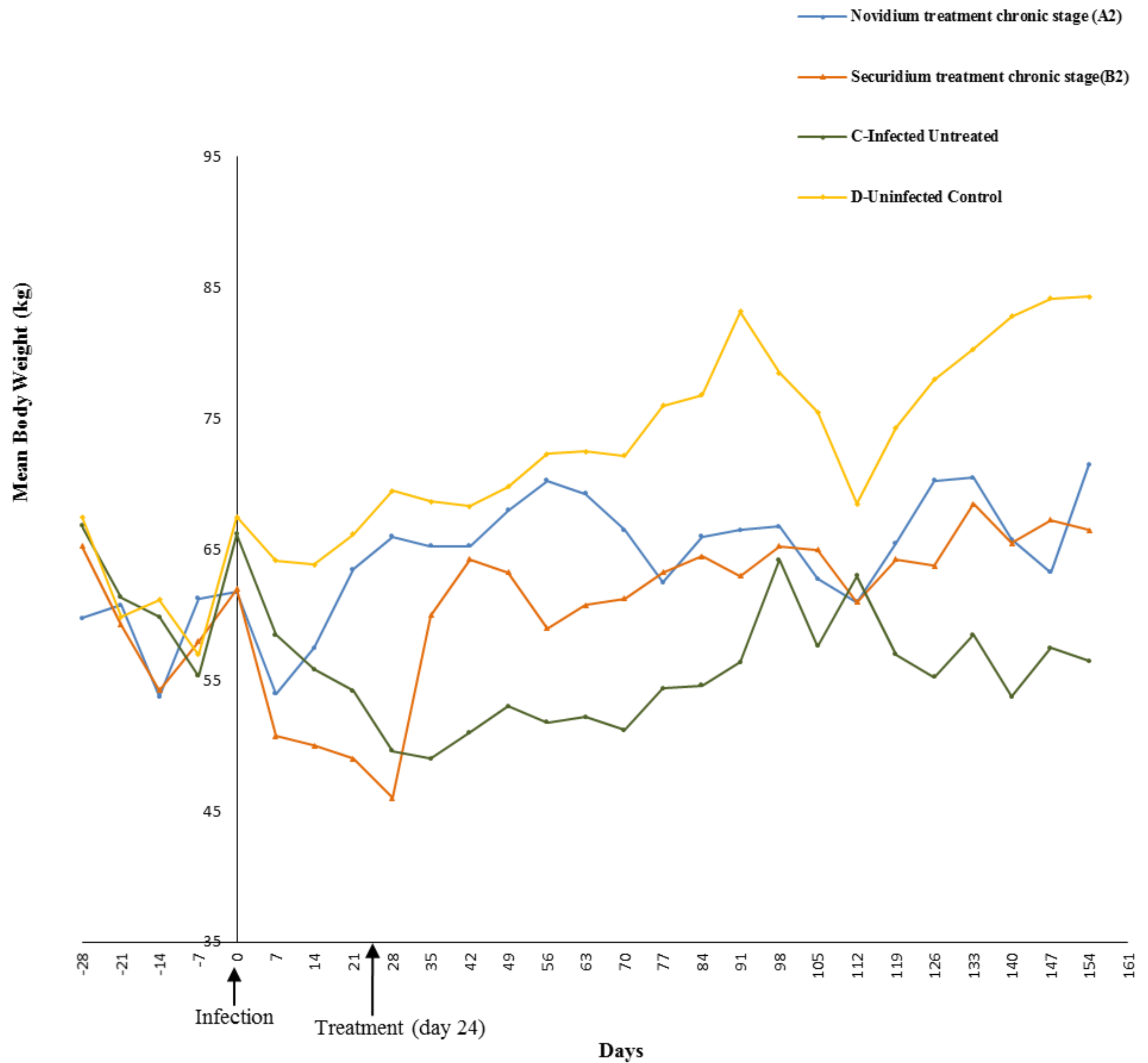

Figure 4.14: Mean body weight values pre-infection, post-infection and post-treatment at the chronic (stage day 24 post infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 24 = post infection period
Day 24 = treatment day
Days 24 – 154 = post treatment period

4.8 Haematological Changes

Results of haematological analyses as presented in Tables 4.2 – 4.4, show that *T. brucei* (Federe) infection in the study donkeys produced a reduction in values of haematological parameters from pre-infection values. Post treatment with Novidium® and Sécuridium®, increase in blood components were recorded with some returning to pre-infection levels while others, though increased, did not fully return to pre-infection levels. Anaemia, evidenced by significant reduction in RBC, Hb concentration and PCV values post infection, was present. The PCV values were significantly ($p<0.05$) reduced post infection in all the infected animal groups and returned to pre-infection range post treatment (Table 4.2). There was an increase in MCV post infection and a decrease to pre-infection range post treatment, while MCH and MCHC values reduced significantly ($p<0.05$) post infection in all the infected donkeys (Table 4.3) indicating the anaemia to be macrocytic and hypochromic in nature. Platelet counts reduced significantly ($p<0.05$) post infection and post treatment (Table 4.3).

4.8.1 Red blood cells (RBC) count and Haemoglobin (Hb) concentration

At day 7 post-infection, the mean RBC count reduced significantly ($p<0.0002$) from pre-infection value of $5.99\pm0.25\times10^6/\mu L$ to $4.60\pm0.23\times10^6/\mu L$ while the mean Hb concentration reduced significantly ($p<0.005$) from pre-infection value of 10.52 ± 0.32 g/dL to 9.33 ± 0.24 g/dL in the infected donkeys. Post treatment in the treated groups, the mean RBC counts were non-significantly different from pre-infection range while the mean Hb concentration increased but were lower than pre-infection range (Table 4.2).

4.8.1.1 Subgroup A_1 (Novidium® treatment at acute infection stage)

Post infection in this subgroup, there was a significant ($p=0.0320$) reduction in the mean RBC count from $5.79\pm0.29\times10^6/\mu L$ at pre-infection to $4.61\pm0.27\times10^6/\mu L$ at day 7 post

infection. Also, the mean Hb concentration reduced significantly ($p<0.04$) from 11.55±0.41 g/dL to 9.25±0.37 g/dL. Post treatment by day 154, the mean RBC count increased non-significantly ($p>0.05$) to 5.00±0.15 $\times10^6$/µL while the mean Hb increased significantly ($p>0.05$) to 9.62±0.17 g/dL (Table 4.2).

4.8.1.2 Subgroup B_1 (Sécuridium® treatment at acute infection stage)

In this subgroup, the mean red cell count significantly ($p<0.0001$) reduced from pre-infection value of 6.32±0.20 $\times10^6$/µL to 4.10±0.33 $\times10^6$/µL post infection and also significantly ($p<0.03$) increased post treatment to 5.02±0.09 $\times10^6$/µL. The mean Hb concentration recorded a significant ($p<0.05$) reduction from 10.64±0.18 g/dL at pre-infection to 9.33±0.30 g/dL at day 7pi and increased significantly ($p<0.05$) to 9.62±0.17 g/dL by day 150 (Table 4.2)

4.8.1.3 Subgroup A_2 (Novidium® treatment at chronic infection stage)

Post infection in this subgroup, there was a non-significant ($p=0.1343$) reduction in the mean RBC counts from 5.48±0.24 $\times10^6$/µL at pre-infection to 4.91±0.53 $\times10^6$/µL at day 21 pi. This The mean Hb concentration significantly ($p<0.05$) reduced from pre-infection value of 12.28±0.71 g/dL to 8.15±0.83 g/dL at day 24 pi and increased non-significantly ($p>0.05$) to 9.55±0.57 g/dL by day 154 post treatment (Table 4.2).

4.8.1.4 Subgroup B_2 (Sécuridium® treatment at chronic infection stage)

In this subgroup, mean RBC count at day 10 pi reduced significantly ($p<0.05$) to 5.22±0.29 $\times10^6$/µL from pre-infection value of 5.92±0.23 $\times10^6$/µL, and increased significantly ($p<0.05$) to 5.17±0.09 $\times10^6$/µL by day 101 post treatment. The mean Hb concentration reduced significantly ($p<0.003$) from pre-infection value of 11.01±0.18 g/dL to 9.33±0.30 g/dL at day 24 pi. However, a significant ($p<0.05$) increase to 9.97±0.18 g/dL was recorded at day 154 post treatment (Table 4.2).

4.8.1.5 Group C (Infected Untreated)

In this group, the mean RBC count post infection ranged between 3.35±0.30 $\times 10^6/\mu L$ and 6.52±0.46 $\times 10^6/\mu L$. A significant ($p<0.001$) reduction in the mean RBC count was recorded on day 49 pi when the mean RBC count reduced from pre-infection value of 5.87±0.40 $\times 10^6/\mu L$ to 3.35±0.30 $\times 10^6/\mu L$. The mean Hb range at post infection was 8.35±0.52 g/dL to 10.37±0.26 g/dL. There was a significant ($p<0.004$) reduction in the mean Hb concentration from 11.32±0.61 g/dL at pre-infection to 8.35±0.52 g/dL by day 154 post infection (Table 4.2).

4.8.1.6 Group D (Uninfected Control)

The initial (beginning of the experiment) and final (end of the experiment) mean RBC count for this group were 6.08±0.21 $\times 10^6/\mu L$ and 5.90±0.08 $\times 10^6/\mu L$ while the mean Hb concentration recorded 9.67±0.50 g/dL and 9.13±0.32 g/dL (Table 4.2).

Table 4.2: Changes in mean RBC, Hb and PCV parameters of donkeys experimentally infected with *Trypanosoma brucei* (Federe isolate) and treated with Novidium® and Sécuridium®.

Groups/Subgroups	N	Phase of Experiment	RBC±SEM(x10^6/µL)	Hb±SEM(g/dL)	PCV±SEM (%)
	4	Pre-infection	5.79 ± 0.29[a]	11.55 ± 0.41[a]	37.50 ± 0.65[a]
		Post-infection	4.61 ± 0.27[ab]	9.25 ± 0.37[b]	25.00 ± 1.23[b]
A1 (Infected Novidium®-treated Acute Stage)		Post treatment	5.00 ± 0.15[a]	9.62 ± 0.17[c]	46.00 ± 4.42[ac]
	4	Pre-infection	5.48 ± 0.24[a]	12.28 ± 0.71[a]	39.50 ± 1.56[a]
		Post-infection	4.91 ± 0.53[a]	8.15 ± 0.83[b]	26.00 ± 2.04[b]
A2 (Infected Novidium®-treated Chronic Stage)		Post treatment	5.21 ± 0.10[a]	9.55 ± 0.57[ab]	44.75 ± 3.28[ac]
	4	Pre-infection	6.32 ± 0.20[a]	10.64 ± 0.18[a]	37.50 ± 0.65[a]
		Post-infection	4.10 ± 0.33[b]	9.33 ± 0.30[b]	24.00 ± 1.58[b]
B1 (Infected Sécuridium®-treated Acute Stage)		Post treatment	5.02 ± 0.09[c]	9.62 ± 0.17[a]	39.50 ± 2.75[ac]
	4	Pre-infection	5.92 ± 0.23[a]	11.01 ± 0.18[a]	39.50 ± 1.56[a]
		Post-infection	5.22 ± 0.29[b]	9.33 ± 0.30[b]	26.25 ± 1.03[b]
B2 (Infected Sécuridium®-treated Chronic Stage)		Post treatment	5.17 ± 0.09[a]	9.97 ± 0.18[c]	43.75 ± 5.07[ac]
	6	Pre-infection	5.87 ± 0.40[a]	11.32 ± 0.61[a]	36.83 ± 0.91[a]
C (Infected Untreated)		Post-infection	3.35 ± 0.30[b]	8.35 ± 0.52[b]	27.00 ± 2.07[b]
	6	Initial Value	6.08 ± 0.213.[a]	9.67 ± 0.50[a]	39.00 ± 0.86[a]
D (Uninfected Control)		Final Value	5.90 ± 0.08[a]	9.13 ± 0.32[a]	41.67 ± 2.79[a]

KEY: White Blood Cells (WBC), Red Blood Cells (RBC), Haemoglobin concentration (Hb), Packed Cell Volume (PCV), N = Number of animals in each group, Standard Error of Mean (SEM). Means with different letters in same column for each group are significantly different at $p < 0.05$.

4.8.2 Packed cell volume (PCV)

The mean PCV at the 4-week pre-infection period was between 33±1.18% and 46.25±3.55% for all the donkeys. Post infection, the mean PCV of the infected donkeys significantly ($p<0.0001$) dropped below pre-infection range, from a mean of a 40.17±1.31% at day of infection, day 0 to a mean of 31.04±1.91% at day 7 pi. Post treatment, the mean PCV for all the treated donkeys increased to a mean of 38.95±1.02% (Figure 4.15 and 4.16).

4.8.2.1 Subgroup A_1 (Novidium® treatment at acute infection stage)

The mean PCV of donkeys in this group dropped significantly ($p<0.0001$) from pre-infection value of 37.50±3.23% to 25.50±2.04% by day 7 post infection. Post infection, the difference between PCV means of subgroup A_1 donkeys (25.50±2.04%) and group C donkeys (36.33±2.51%) was statistically non-significant ($p>0.05$) but was siginificantly lower ($p<0.005$) than that of group D donkeys (39.00±0.86%). Furthermore, post treatment, an increase in mean PCV values for subgroup A_1 donkeys (46.00±4.42%) was recorded but was non-significantly ($p=0.4421$) higher than the mean for group D (41.67±2.79%) and significantly ($p<0.02$) higher than for group C (27.00±2.07%) (Figure 4.15).

4.8.2.2 Subgroup B_1 (Sécuridium® treatment at acute infection stage)

The mean PCV for this subgroup significantly ($p<0.001$) reduced from pre-infection value of 37.50±0.65% to 24.00±1.58% by day 7 pi. Minimum mean PCV recorded post infection for this subgroup was 23±1.58%. Also at day 7 pi, the mean PCV for subgroup B_1 donkeys (24.00±1.58%) was non-significantly lower ($p>0.05$) than the mean PCV for group C (30.00±2.84%) and significantly lower ($p<0.006$) than for group D donkeys (39.00±0.86%). Post treatment, a significant ($p<0.002$) increase in mean PCV was

recorded for subgroup B_1 (39.50±2.75%) when compared with the mean PCV for group C donkeys (27.00±2.07%), and in comparison with the group D mean PCV (41.67±2.79%) recorded a non-significant ($p=0.6017$) increase (Figure 4.15).

4.8.2.3 Subgroup A_2 (Novidium® treatment at chronic infection stage)

The mean PCV for this subgroup declined significantly ($p<0.01$) from pre-infection value of 39.50±1.56% to 26.00±2.04% by day 7 pi. The minimum value recorded for A_2 post infection was 23±0.71%. At day 7 pi, the mean PCV for A_2 (26.00±2.04%) was non-significantly different ($p>0.05$) from that for group C donkeys (30.00±2.84%) but significantly ($p=0.0070$) lower than for group D donkeys (39.50±2.75%). Post treatment, A_2 mean value (44.75±3.28%) increased and was significantly higher ($p=0.0053$) than mean PCV for group C (27.00±2.07%) and non-significantly higher ($p>0.05$) than mean PCV for group D (41.67±2.79%) (Figure 4.16).

4.8.2.4 Subgroup B_2 (Sécuridium® treatment at chronic infection stage)

The mean PCV for this subgroup significantly ($p<0.0001$) decreased from pre-infection value of 39.50±1.56% at day 0, to 26.25±1.03% at day 7 post infection. The minimum value recorded post infection was 26.25±1.03%. In comparison with the positive and negative (groups C and D) controls, subgroup B_2 mean PCV (26.25±1.03%) at day 7 pi was non-significantly ($p=0.1594$) lower than group C mean (30.00±2.84%) and significantly ($p=0.0005$) lower than group D mean (39.00±0.86%). Post treatment, B_2 mean PCV (43.75±5.07%) was significantly higher ($p=0.0377$) than group C mean (27.00±2.07%) but non-significantly higher ($p=0.7341$) than group D mean (41.67±2.79%) (Figure 4.16).

4.8.2.5 Group C (Infected Untreated)

Post infection, there was a significant ($p<0.0001$) reduction in the mean PCV for this group from pre-infection value of 36.83±0.91% at day 0, to 21.67±1.33% by day 28 post infection. The minimum and maximum recorded mean PCV for this group post infection was 21.67±1.33% at day 18 pi and 31.83±1.35% at day 147 pi. At termination of the experiment, the difference between group C mean PCV (27.00±2.07%) and the group D mean PCV (41.67±2.79%) was highly significant ($p<0.002$) (Figure 4.16).

4.8.2.6 Group D (Uninfected Control)

For this group, PCV recorded ranged from 33±1.18% to 44.67±2.89% throughout the course of the experiment (Figure 4.16).

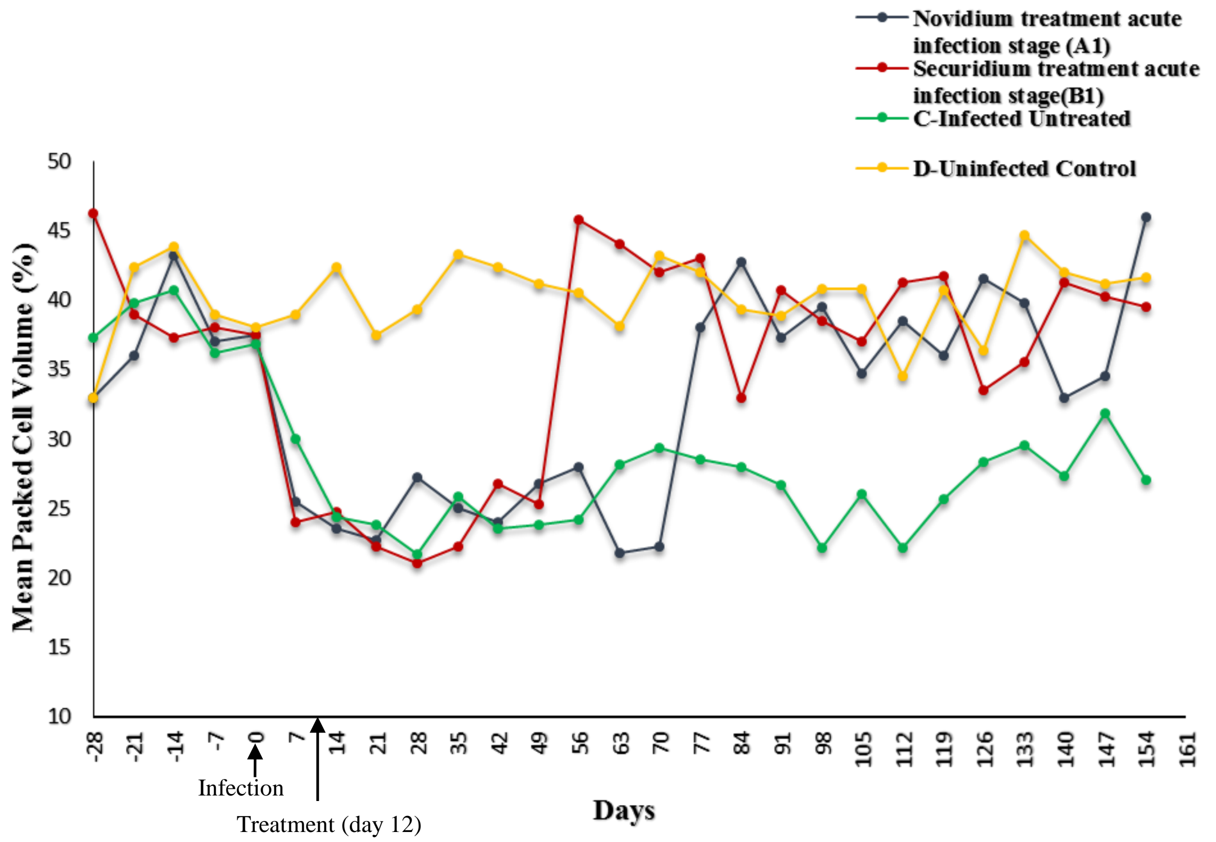

Figure 4.15: Mean packed cell volume (%) pre-infection, post-infection and post-treatment at acute stage (day 12 post infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 12 = post infection period
Day 12 = treatment day
Days 12 – 154 = post treatment period

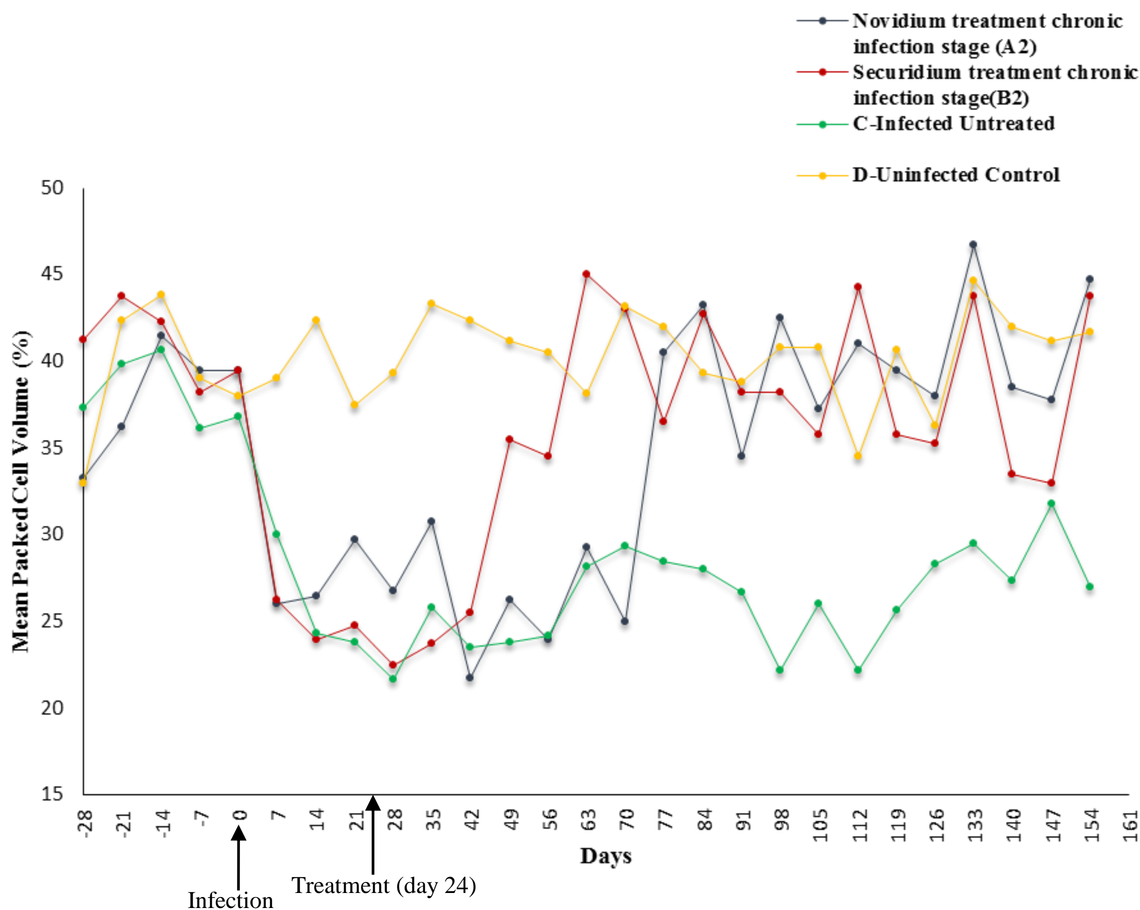

Figure 4.16: Mean packed cell volume (%) pre-infection, post-infection and post-treatment at the chronic stage (day 24 post infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 24 = post infection period
Day 24 = treatment day
Days 24 – 154 = post treatment period

4.8.3 Erythrocytic indices (Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) and Platelet counts.

The mean corpuscular volume (MCV) for the infected donkeys in this study significantly ($p<0.0001$) increased from 53.40±0.85 fl at day 0 pre-infection to 64.95±1.19 fl by day 12 post infection. The mean corpuscular haemoglobin (MCH) on the other hand recorded a non-significant ($p>0.05$) decrease from 19.94±0.35 pg pre-infection to 18.21±0.80 pg at day 7 pi, while the mean corpuscular haemoglobin concentration (MCHC) significantly ($p<0.04$) decreased from 34.60±0.52 g/dL to 33.09±0.30 g/dL at day 12 pi.

The mean platelet counts were observed to reduce significantly ($p>0.05$) in all the infected donkeys post infection with a further reduction recorded post treatment in the treated donkeys (Table 4.3).

4.8.3.1 Subgroup A1 (Novidium® treatment at acute infection stage)

In this subgroup, MCV values increased significantly ($p<0.03$) from 54.30±1.72 fl at pre-infection to 62.05±2.12 fl by day 11 pi, and reduced non-significantly ($p>0.05$) to 55.95±2.48 fl post treatment. The MCH significantly ($p<0.04$) reduced from 20.85±1.21 pg at pre-infection to 16.85±0.76 pg post infection, and increased non-significantly ($p>0.05$) to 20.25±0.80 pg post treatment while the MCHC significantly ($p<0.05$) reduced from pre-infection value of 35.23±0.25 g/dL to 33.07±0.47 g/dL at day 8 pi, and increased non-significantly ($p>0.05$) to 33.45±0.18 g/dL post treatment (Table 4.3). The mean platelet counts for this subgroup significantly ($p<0.05$) reduced post infection from 318.13±13.96 $\times10^6$/µL at day 0, to 215.13±13.59 $\times10^6$/µL at day 7 pi, and increased significantly ($p<0.05$) to 215.30±13.10 $\times10^6$/µL post treatment (Table 4.3).

4.8.3.2 Subgroup B1 (Sécuridium® treatment at acute infection stage)

Donkeys in this subgroup had MCV increased significantly ($p<0.04$) from 53.99±1.43 fl at pre-infection to 64.61±3.03 fl at day 9 pi, and reduced non-significantly ($p>0.05$) to 59.89±3.73 fl at day 154 post treatment. The MCH significantly ($p<0.05$) reduced from 20.39±0.37 pg at pre-infection to 19.09±0.18 pg post infection, and then increased significantly ($p<0.05$) to 19.67±0.12 pg post treatment. The MCHC significantly ($p<0.05$) reduced from pre-infection value of 35.14±0.36 g/dL to 33.47±5.41 g/dL at day 8 pi, and decreased non-significantly ($p>0.05$) to 33.45±0.18 g/dL (Table 4.3). The mean platelet counts for this subgroup significantly ($p<0.05$) reduced post infection and treatment. At pre-infection, the mean platelet count was 314.81±13.42 $\times 10^6/\mu L$, this reduced to 247.38±14.73 $\times 10^6/\mu L$ post infection, and further reduced to 215.30±14.10 $\times 10^6/\mu L$ post treatment (Table 4.3).

4.8.3.3 Subgroup A2 (Novidium® treatment at chronic infection stage)

The MCV for this subgroup significantly ($p<0.04$) increased from 50.70±0.36 fl at day 0 to 66.08±4.24 fl at day 22 pi, and thereafter, reduced significantly ($p<0.05$) to 50.95±0.86 fl post treatment. The MCH non-significantly ($p>0.05$) reduced from 20.88±0.43 pg at pre-infection to 17.69±0.53 pg post infection, and then increased significantly ($p<0.05$) to 18.76±0.17 pg post treatment. The MCHC significantly ($p<0.05$) reduced from pre-infection value of 34.73±0.32 g/dL to 33.07±0.47 g/dL at day 24 pi, and then increased significantly ($p<0.05$) to 33.33±0.20 g/dL (Table 4.3). In this subgroup, significant differences were only recorded between mean platelet counts at pre-infection (281.56±12.66 $\times 10^6/\mu L$) and post-infection (215.13±18.59 $\times 10^6/\mu L$) whereby there was a significant ($p<0.05$) reduction in the platelet numbers. Post treatment, a further reduction in mean platelet counts (210.50±9.02 $\times 10^6/\mu L$) was

recorded. However, this was significantly different from the pre and post infection values (Table 4.3).

4.8.3.4 Subgroup B_2 (Sécuridium® treatment at chronic infection stage)

In this subgroup, the MCV increased significantly ($p<0.02$) from 51.73±1.18 fl at pre-infection to 65.30±3.02 fl at day 24 pi, and then reduced non-significantly ($p>0.05$) to 56.15±4.90 fl post treatment. The MCH significantly ($p<0.05$) reduced from 20.41±0.44 pg at pre-infection to 19.09±0.18 pg day 20 pi, and increased non-significantly ($p>0.05$) to 19.58±0.11 pg post treatment. The MCHC significantly ($p<0.05$) reduced from pre-infection value of 35.00±0.36 g/dL to 33.47±0.41 g/dL at day 19 pi, and decreased significantly ($p<0.05$) to 33.38±0.19 g/dL (Table 4.3). In this subgroup also, significant differences were only recorded between mean platelet counts at pre-infection (304.56±11.47 $\times 10^6$/µL) and post-infection (247.38±18.73 $\times 10^6$/µL) whereby there was a significant ($p<0.05$) reduction in the platelet numbers. Post treatment, a further reduction in mean platelet counts (219.01±7.99 $\times 10^6$/µL) was recorded. However, this was significantly different from the pre and post infection values (Table 4.3).

4.8.3.5 Group C (Infected Untreated)

In this group, the MCV recorded post infection ranged from 60.85±2.14 fl to 69.98±1.63 fl. A significant ($p<0.05$) increase from pre-infection value of 53.07±1.65 fl to 69.92±1.73 fl was recorded by day 21 pi. The MCH recorded post infection ranged from 16.42±0.76 to 18.55±0.69 pg, and a significant ($p<0.05$) reduction was recorded between pre-infection value of 20.37±0.76 pg and 17.23±0.61 pg at day 21 pi. The MCHC ranged between 31.85±0.62 g/dL and 35.38±0.46 g/dL post infection. There was a significant ($p<0.05$) reduction between pre-infection (36.13±0.22 g/dL) and post infection (33.64±0.15 g/dL) values for MCHC (Table 4.3). The mean platelet counts in

this group significantly reduced from 204.60±15.82 $\times10^6/\mu L$ at pre-infection to 180.70±7.00 $\times10^6/\mu L$ post infection (Table 4.3).

4.8.3.6 Group D (Uninfected Control)

The initial and final values of erthrocytic indices recorded for this group were: MCV (57.72±3.97 and 59.43±3.70 fl), MCH (18.97±0.27 and 19.07±0.16 pg) and MCHC (35.25±0.79 and 34.58±0.56 g/dL), Platelet counts (302.70±12.29 and 294.80±4.84 $\times10^6/\mu L$) (Table 4.3).

4.8.4 White blood cell and differential counts

White blood cell (WBC) counts reduced early in the infection but increased post treatment. Differential counts revealed increased lymphocytes (lymphocytosis), and reduced neutrophils (neutropenia), monocytes and eosinophil post infection in the donkeys. Post treatment, neutrophil count significantly ($p<0.05$) reduced but in the acute stage only (subgroups A1 and B1), there was further increase in lymphocyte count and significant ($p<0.05$) reduction in eosinophil count. Band cells recorded non-significant variations. Basophils were not found in blood in this study (Table 4.4).

4.8.4.1 Subgroup A_1 (Novidium® treatment at acute infection stage)

In this subgroup, the WBC count reduced non-significantly ($p>0.05$) from 8.38±1.19 $\times10^9$/L at pre-infection to 6.80±0.26 $\times10^9$/L at day 7 pi, and increased non-significantly ($p>0.05$) to 8.35±1.31 $\times10^9$/L post treatment. Neutrophil count significantly ($p<0.05$) reduced from 45.56±1.20% at day 0 to 41.06±1.14% by day 7 pi, and further reduced significantly ($p<0.001$) to 30.56±1.90 post treatment. There was significant ($p<0.001$) increase in lymphocyte count from 43.44±1.27% pre-infection (day 0) to 55.94±1.33%

at day 7 pi, and a further increase to 67.83±1.95% post treatment. The eosinophil count reduced significantly ($p<0.001$) from 7.75±0.81% pre-infection to 0.81±0.45% at day 7 pi, and also at post treatment to 0.32±0.10. The monocytes and band cell counts non-significantly ($p>0.05$) decreased post infection and post treatment (Table 4.4).

4.8.4.2 Subgroup B_1 (Sécuridium® treatment at acute infection stage)

Donkeys in this subgroup had WBC counts non-significantly ($p>0.05$) reduced from pre-infection value of 9.78±2.08 $\times 10^9$/L to 6.08±0.98 $\times 10^9$/L at day 7 pi, and increased significantly ($p<0.03$) to 12.38±1.03 $\times 10^9$/L post treatment. There was a significant ($p<0.05$) reduction in neutrophil count from 45.69±1.55% at pre-infection to 38.25±5.97% at day 7 pi, and a further reduction to 30.56±5.90% post treatment. The lymphocyte counts significantly ($p<0.002$) increased from 45.31±1.54% pre-infection to 60.75±5.97% at day 7 pi, and 67.83±5.95% post treatment. The eosinophils significantly ($p<0.05$) reduced from 5.88±0.98% pre-infection to 0.56±0.33% at day 7 pi, and 0.32±0.10% post treatment. The monocytes count non-significantly ($p>0.05$) decreased post infection and post treatment, while Band cells also recorded a non-significant ($p>0.05$) reduction post infection and treatment (Table 4.4).

4.8.4.3 Subgroup A_2 (Novidium® treatment at chronic infection stage)

In this subgroup, the WBCs significantly ($p<0.02$) reduced from 11.70±1.58 $\times 10^9$/L at pre-infection to 5.35±0.40 $\times 10^9$/L by day 7 pi, and then increased to 11.80±1.67 $\times 10^9$/L post treatment. The neutrophils non-significantly ($p<0.05$) reduced from 47.25±4.55% at pre-infection to 40.75±7.09% by day 21 pi, and then increased to 51.00±3.94% post treatment. The lymphocyte counts significantly ($p<0.03$) increased from 46.81±1.72% at pre-infection to 55.94±2.33% by day 21 pi, and 66.50±1.35% post treatment. The eosinophils count significantly ($p<0.05$) reduced from 3.81±0.81% at pre-infection to

0.81±0.45% by day 21 pi, and then 0.14±0.07% post treatment. Monocytes count non-significantly ($p>0.05$) decreased post infection and significantly ($p<0.05$) decreased post treatment, while the Band cells also recorded a non-significant ($p>0.05$) increase post infection and non-significant ($p<0.05$) reduction post treatment (Table 4.4).

4.8.4.4 Subgroup B_2 (Sécuridium® treatment at chronic infection stage)

For this subgroup, WBCs non-significantly ($p>0.05$), reduced from 8.75±1.99 $\times10^9$/L at pre-infection to 6.60±0.73 $\times10^9$/L by day 21 pi and increased to 9.63±1.19 $\times10^9$/L post treatment. Neutrophils non-significantly ($p<0.05$), reduced from 48.50±4.52% at pre-infection to 33.75±3.73% by day 21 pi, and increased to 35.00±6.46% post treatment. Lymphocyte counts significantly ($p<0.04$) increased from 44.69±1.50% at pre-infection to 60.75±1.97% by day 21 pi, and then 65.13±1.04% post treatment. Eosinophils significantly ($p<0.05$) reduced from 5.75±0.89% at pre-infection to 0.56±0.33% at day 21 pi, and 0.34±0.11% post treatment. Monocytes count non-significantly ($p>0.05$) decreased post infection and post treatment, while Band cells also recorded a non-significant ($p>0.05$) reduction post infection and treatment (Table 4.4).

4.8.4.5 Group C (Infected Untreated)

Donkeys in this group had WBCs significantly ($p<0.006$) reduce from 11.62±0.90 $\times10^9$/L at pre-infection to 7.55±0.71 $\times10^9$/L post infection. Neutrophils significantly ($p<0.02$) reduced from 44.79±1.31% at pre-infection to 31.23±1.06% post infection. Lymphocytes significantly ($p<0.003$) increased from 47.29±1.50% at pre-infection to 67.20±1.09% post infection. Eosinophils significantly ($p<0.02$) reduced from 4.63±0.70% at pre-infection to 0.39±0.10% post infection. Monocytes and Band cells were non-significantly ($p>0.05$) different pre and post infection (Table 4.4).

4.8.4.6 Group D (Uninfected Control)

Initial and final values of WBC and differential counts recorded for this group were: WBC (10.45±1.67 and 11.03±1.18 $\times 10^9$/L), Neutrophils (48.17±1.65 and 48.38±0.75%), Lymphocytes (44.58±1.64 and 45.33±0.76%), Monocytes (1.96±0.18 and 1.83±0.07%), Eosinophils (5.21±0.71 and 5.95±0.32%) and Band Cells (0.83±0.31 and 0.83±0.40%) (Table 4.4).

Table 4.3: Changes in mean Platelets count, MCV, MCH and MCHC indices of donkeys experimentally infected with *Trypanosoma brucei* (Federe isolate) and treated with Novidium® and Sécuridium®.

Groups/Subgroups	N	Phase of Experiment	Platelets±SEM($x10^3/\mu L$)	MCV±SEM(fl)	MCH±SEM(pg)	MCHC±SEM(g/dL)
A1 (Infected Novidium®-treated Acute Stage)	4	Pre-infection	318.13 ± 13.96^a	54.30 ± 1.72^a	20.85 ± 1.21^a	35.23 ± 0.25^a
		Post-infection	215.13 ± 13.59^b	62.05 ± 2.12^b	16.85 ± 0.76^b	33.07 ± 0.47^b
		Post treatment	215.30 ± 13.10^c	55.95 ± 2.48^{ab}	20.25 ± 0.80^{ab}	33.45 ± 0.18^b
A2 (Infected Novidium®-treated Chronic Stage)	4	Pre-infection	281.56 ± 12.66^a	50.70 ± 0.36^a	20.88 ± 0.43^a	34.73 ± 0.32^a
		Post-infection	215.13 ± 18.59^b	66.08 ± 4.24^b	17.69 ± 0.53^{ab}	33.07 ± 0.47^b
		Post treatment	210.50 ± 9.02^{ab}	50.95 ± 0.86^{ac}	18.76 ± 0.17^{ac}	33.33 ± 0.20^c
B1 (Infected Sécuridium®-treated Acute Stage)	4	Pre-infection	314.81 ± 13.42^a	53.99 ± 1.43^a	20.39 ± 0.37^a	35.14 ± 0.36^a
		Post-Infection	247.38 ± 14.73^b	64.61 ± 3.03^b	19.09 ± 0.18^b	33.47 ± 5.41^b
		Post treatment	215.30 ± 14.10^c	59.89 ± 3.73^b	19.67 ± 0.12^c	33.45 ± 5.18^b
B2 (Infected Sécuridium®-treated Chronic Stage)	4	Pre-infection	304.56 ± 11.47^a	51.73 ± 1.18^a	20.41 ± 0.44^a	35.00 ± 0.36^a
		Post-infection	247.38 ± 18.73^b	65.30 ± 3.02^b	19.09 ± 0.18^b	33.47 ± 0.41^b
		Post treatment	219.01 ± 7.99^{ab}	56.15 ± 4.90^{ab}	19.58 ± 0.11^{ab}	33.38 ± 0.19^c
C (Infected Untreated)	6	Pre-infection	204.60 ± 15.82^a	53.07 ± 1.65^a	20.37 ± 0.76^a	36.13 ± 0.22^a
		Post-infection	180.70 ± 7.00^b	69.92 ± 1.73^b	17.23 ± 0.61^b	33.64 ± 0.15^b
D (Uninfected Control)	6	Initial Value	302.70 ± 12.29^a	57.72 ± 3.97^a	18.97 ± 0.27^a	35.25 ± 0.79^a
		Final Value	294.80 ± 4.84^a	59.43 ± 3.70^a	19.07 ± 0.16^a	34.58 ± 0.56^a

KEY: Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), N = Number of animals in each group, Standard Error of Mean (SEM). Means with different letters in same column for each group are significantly different at $p < 0.05$.

Table 4.4: Changes in mean WBC and leukocytes indices of donkeys experimentally infected with *Trypanosoma brucei* (Federe isolate) and treated with Novidium® and Sécuridium®.

Groups/Subgroups	N	Phase of Experiment	WBC±SEM(x 10^9/L)	N±SEM%	L±SEM%	M±SEM%	E±SEM%	Band±SEM %
A1 (Infected Novidium®-treated Acute Stage)	4	Pre-infection	8.38 ± 1.19^{a}	45.56 ± 1.20^{a}	43.44 ± 1.27^{a}	2.00 ± 0.41^{a}	7.75 ± 0.81^{a}	1.25 ± 0.48^{a}
		Post-infection	6.80 ± 0.26^{a}	41.06 ± 1.14^{b}	55.94 ± 1.33^{b}	1.50 ± 0.65^{a}	0.81 ± 0.45^{b}	0.75 ± 0.48^{a}
		Post treatment	8.35 ± 1.31^{a}	30.56 ± 1.90^{c}	67.83 ± 1.95^{c}	1.25 ± 0.75^{a}	0.32 ± 0.10^{c}	0.75 ± 0.75^{a}
A2 (Infected Novidium®-treated Chronic Stage)	4	Pre-infection	11.70 ± 1.58^{a}	47.25 ± 4.55^{a}	46.81 ± 1.72^{a}	1.75 ± 0.48^{a}	3.81 ± 0.81^{a}	0.50 ± 0.50^{a}
		Post-infection	5.35 ± 0.40^{b}	40.75 ± 7.09^{a}	55.94 ± 2.33^{b}	0.50 ± 0.50^{ab}	0.81 ± 0.45^{b}	1.25 ± 0.75^{a}
		Post treatment	11.80 ± 1.67^{a}	51.00 ± 3.94^{a}	66.50 ± 1.35^{c}	0.00 ± 0.00^{b}	0.14 ± 0.07^{c}	0.00 ± 0.00^{a}
B1 (Infected Sécuridium®-treated Acute Stage)	4	Pre-infection	9.78 ± 2.08^{a}	45.69 ± 1.55^{a}	45.31 ± 1.54^{a}	2.75 ± 0.75^{a}	5.88 ± 0.98^{a}	2.00 ± 0.71^{a}
		Post-infection	6.08 ± 0.98^{ab}	38.25 ± 5.97^{b}	60.75 ± 5.97^{b}	0.50 ± 0.50^{a}	0.56 ± .33^{b}	0.50 ± 0.50^{a}
		Post treatment	12.38 ±1.03^{ac}	30.56 ± 5.90^{a}	67.83 ± 5.95^{c}	1.00 ± 0.71^{a}	0.32 ± .10^{c}	1.75 ± 1.03^{a}
B2 (Infected Sécuridium®-treated Chronic Stage)	4	Pre-infection	8.75 ± 1.99^{a}	48.50 ± 4.52^{a}	44.69 ± 1.50^{a}	1.25 ± 0.75^{a}	5.75 ± 0.89^{a}	1.00 ± 0.71^{a}
		Post-infection	6.60 ± 0.73^{a}	33.75 ± 3.73^{a}	60.75 ± 1.97^{b}	0.00 ± 0.00^{a}	0.56 ± 0.33^{b}	0.25 ± 0.25^{a}
		Post treatment	9.63 ± 1.19^{a}	35.00 ± 6.46^{a}	65.13 ± 1.04^{c}	0.50 ± 0.50^{a}	0.34 ± 0.11^{c}	0.50 ± 0.50^{a}
C (Infected Untreated)	6	Pre-infection	11.62 ± 0.90^{a}	44.79 ± 1.31^{a}	47.29 ± 1.50^{a}	2.00 ± 0.52^{a}	4.63 ± 0.70^{a}	1.83 ± 0.48^{a}
		Post-infection	7.55 ± 0.71^{b}	31.23 ± 1.06^{b}	67.20 ± 1.09^{b}	1.00 ± 0.68^{a}	0.39 ± 0.10^{b}	0.50 ± 0.50^{a}
D (Uninfected Control)	6	Initial Value	10.45 ± 1.67^{a}	48.17 ± 1.65^{a}	44.58 ± 1.64^{a}	1.96 ± 0.18^{a}	5.21 ± 0.71^{a}	0.83 ± 0.31^{a}
		Final Value	11.03 ± 1.18^{a}	48.38 ± 0.75^{a}	45.33 ± 0.76^{a}	1.83 ± 0.07^{a}	5.95 ± 0.32^{a}	0.83 ± 0.40^{a}

KEY: Neutrophils (N), Lymphocytes (L), Monocytes (M), Eosinophils (E), Basophils (B), Band Neutrophils (Band), N = Number of animals in each group, Standard Error of Mean (SEM). Means with different letters in same column for each group are significantly different at $P < 0.05$.

4.9 Blood Glucose Levels

Pre-infection, the mean blood glucose levels for all the donkeys varied between 55.0±1.63 mg/dL and 100.67±0.73 mg/dL. Post infection, mean blood glucose levels reduced significantly ($p<0.0001$) from 100.67±0.73 mg/dL at day 0 to 49.27±3.24 mg/dL at day 7 post infection. Post treatment for the donkeys treated, increases in the glucose levels were observed, with values sometimes higher than those for the uninfected control donkeys. In the infected untreated group C donkeys, blood glucose levels recorded throughout the experiment were relatively low (Figure 4.17 and 4.18).

4.9.1 Subgroup A_1 (Novidium® treatment at acute infection stage)

The mean blood glucose level of donkeys in subgroup A_1 dropped significantly ($p<0.05$) from 86.25±13.09 mg/dL on day of infection to 47.5±6.02 mg/dL by day 7 pi. Post infection, differences between the mean blood glucose for A_1 (47.5±6.02 mg/dL) and group C donkeys (57.33±5.29 mg/dL) were non-significant ($p>0.05$), but highly significant ($p<0.003$) when compared to donkeys of group D (86.67±3.37 mg/dL). The mean glucose levels increased post treatment with the mean blood glucose for A_1 (87.00±1.43 mg/dL) significantly higher ($p<0.004$) than the mean for group C (46.00±7.84 mg/dL) and non-significantly lower ($p=0.1070$) than the mean for group D (91.33±2.24 mg/dL) (Figure 4.17).

4.9.2 Subgroup B_1 (Sécuridium treatment at acute infection stage)

The mean blood glucose level for this subgroup dropped significantly ($p<0.0001$) from 91.5±4.09 mg/dL at pre-infection to 41.25±10.36 mg/dL by day 7 pi. Post infection, differences between the mean blood glucose for B_1 (41.25±10.36 mg/dL) and group C

(52.33±5.29 mg/dL) were non-significant ($p=0.3232$), but significant ($p<0.001$) when compared to group D (86.67±3.37 mg/dL). Post treatment, blood glucose values increased, with the mean value for B_1 (86.50±1.15 mg/dL) being significantly higher ($p<0.01$) than the mean for group C (46.00±7.84 mg/dL) and non-significantly lower ($p>0.05$) than mean for group D (91.33±2.24 mg/dL) (Figure 4.17).

4.9.3 Subgroup A_2 (Novidium® treatment at chronic infection stage)

The mean blood glucose level of subgroup A_2 dropped significantly ($p<0.03$) from 90.25±13.09 mg/dL on day of infection to 45.00±6.18 mg/dL at day 7 pi. Post infection, differences between the mean blood glucose for A_2 (45.00±6.18 mg/dL) and group C (52.33±5.29 mg/dL) were non-significant ($p=0.3987$), but significant ($p<0.0002$) when compared to group D (86.67±3.37 mg/dL). Post treatment, the mean value for A_2 (78.25±7.41 mg/dL) was significantly higher ($p<0.02$) than for group C donkeys (46.00±7.84 mg/dL) and non-significantly lower ($p=0.4410$) than for group D donkeys (91.33±2.24 mg/dL) (Figure 4.18).

4.9.4 Subgroup B_2 (Sécuridium treatment at chronic infection stage)

In this subgroup, the mean blood glucose level at pre-infection, 84.00±4.24 mg/dL dropped non-significantly ($p>0.05$) to 58.75±9.47 mg/dL by day 7 pi. Post infection, differences between the mean blood glucose for B_2 (58.75±9.47 mg/dL) and that for group C (52.33±5.29 mg/dL) were non-significant ($p=0.5388$), but significant ($p<0.01$) when compared to group D mean (86.67±3.37 mg/dL). Post treatment, blood glucose values increased, with the mean value for B_2 (95.75±3.82 mg/dL) being significantly higher

($p<0.002$) than the mean for group C (46.00±7.84 mg/dL) and non-significantly higher ($p>0.05$) than the mean for group D (91.33±2.24 mg/dL) (Figure 4.18).

4.9.5 Group C (Infected Untreated)

There was a significant ($p<0.0001$) reduction in the mean blood glucose for this group from the pre-infection value of 100.70±3.17 mg/dL at day 0 to 52.33±5.29 mg/dL at day 7 post infection. Post infection, mean blood glucose ranged from 46±1.99 mg/dL to 71.83±4.37 mg/dL in these donkeys. In comparison to the uninfected controls post infection, differences between group C mean blood glucose (46.00±7.84 mg/dL) and the mean blood glucose for group D donkeys (91.33±2.24 mg/dL) were highly significant ($p<0.01$) (Figure 4.18).

4.9.6 Group D (Uninfected Control)

Mean blood glucose values recorded for this group ranged between 58.17±6.25 mg/dL and 103.33±10.93 mg/dL through the experimental period (Figure 4.18).

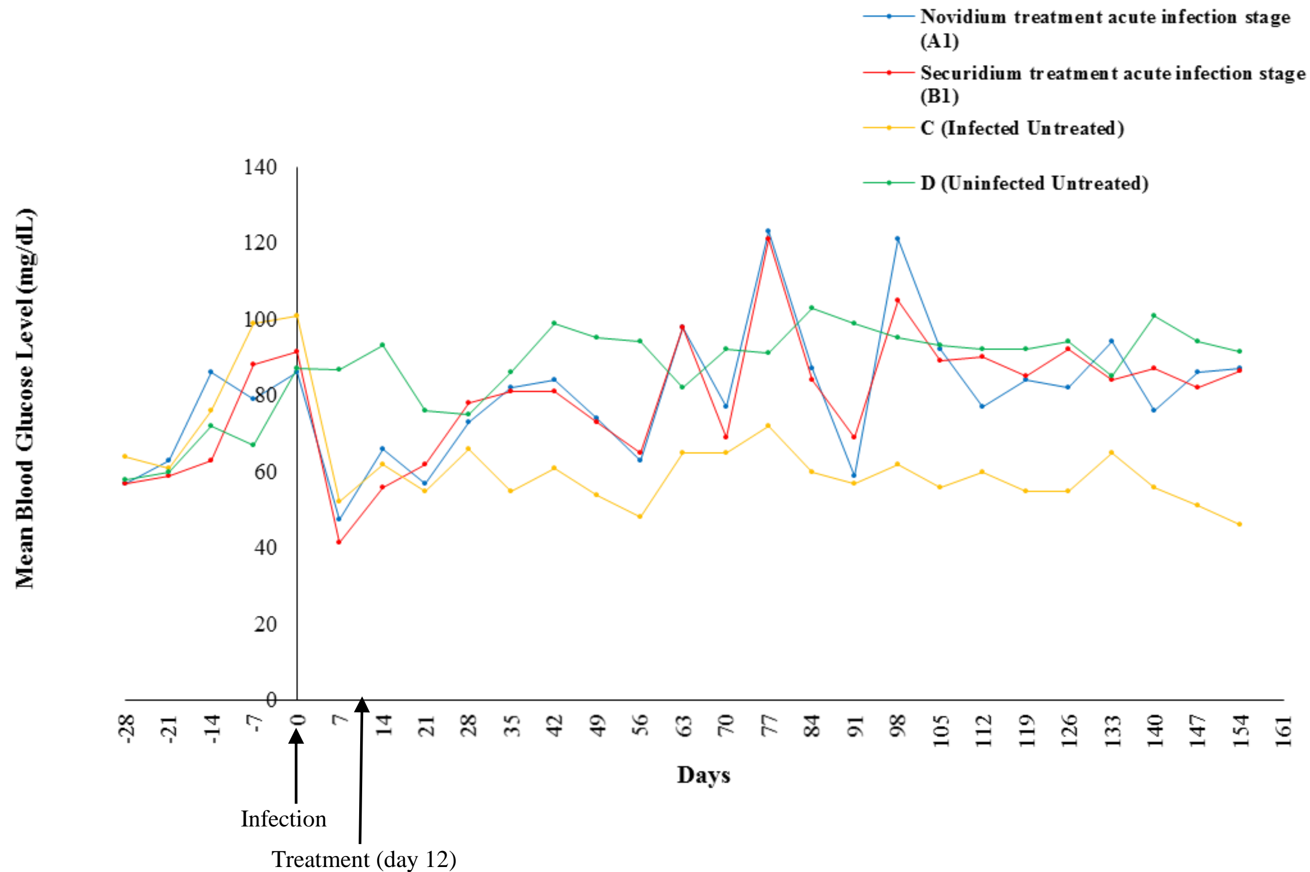

Figure 4.17: Mean blood glucose levels pre-infection, post-infection and post-treatment at the acute stage (day 12 post infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 12 = post infection period

Day 12 = treatment day
Days 12 – 154 = post treatment period

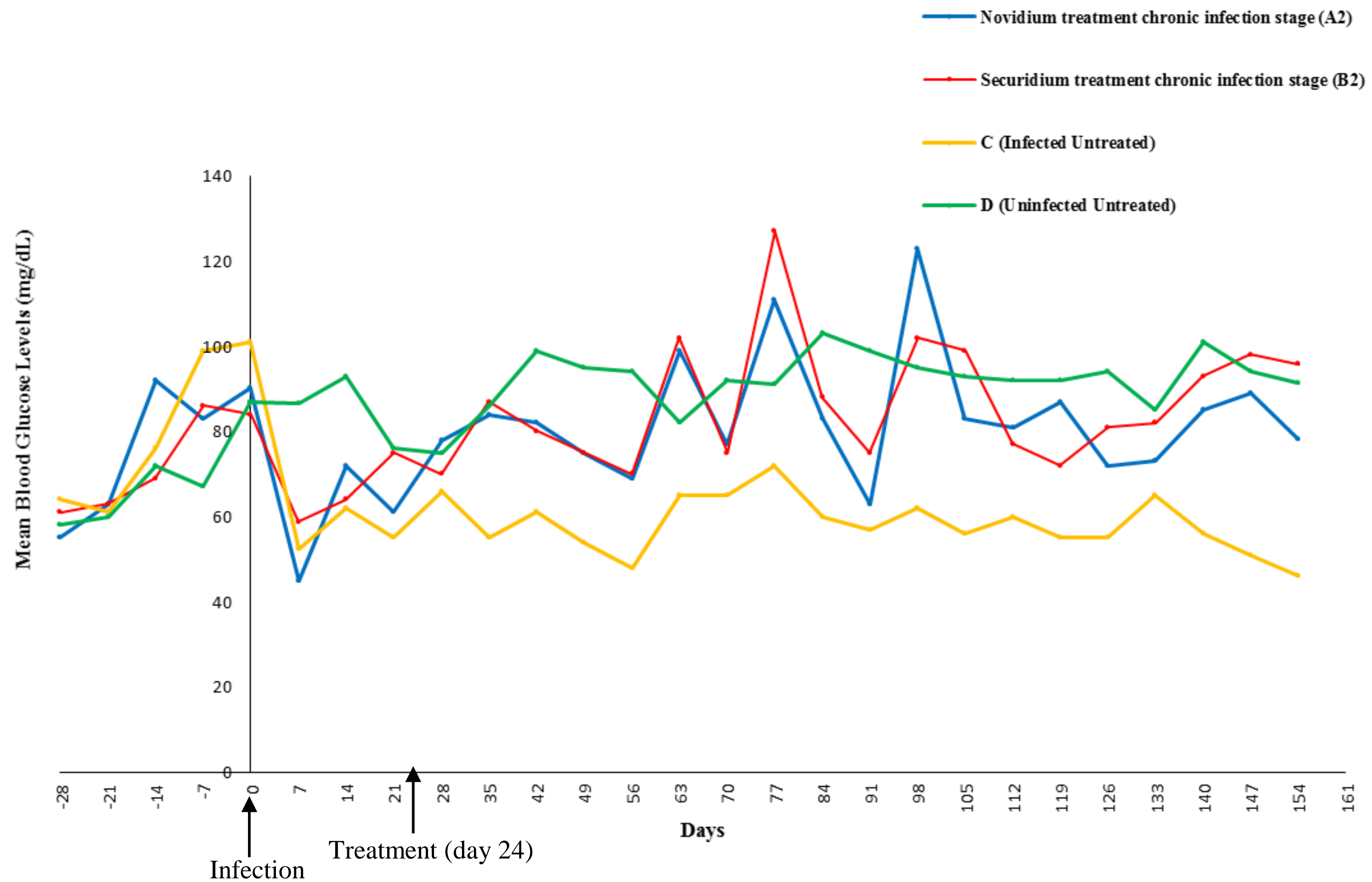

Figure 4.18: Mean blood glucose levels pre-infection, post-infection and post-treatment at the chronic stage (day 24 post infection) of donkeys experimentally infected with *Trypanosoma brucei* and treated with Novidium® and Sécuridium®.

Key:

Days -28 – 0 = pre-infection period
Day 0 = infection day
Days 0 – 24 = post infection period
Day 24 = treatment day
Days 24 – 154 = post treatment period

4.10 Serum Biochemical parameters, Enzymes and Electrolytes Changes

Results of serum biochemical analyses are presented in Tables 4.5 – 4.7. *Trypanosoma brucei* (Federe isolate) infection produced changes in levels of liver enzymes, plasma proteins, serum biochemical components and serum electrolytes. Values for Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Albumin (ALB), Blood Urea Nitrogen (BUN), Sodium (Na^+), Potassium (K^+), Chloride (Cl^-), Phosphate (PO_4^{3-}) and Bicarbonate (HCO_3^-) though varying, showed non-significant ($p>0.05$) differences amongst the different experiment phases across the groups (Tables 4.5 – 4.7). Alanine aminotransferase (ALT) activity recorded significant ($p<0.003$) increase from 36.64±0.87 IU/L at pre-infection to 45.86±2.71 IU/L at day 7 post infection in all the infected donkeys, and non-significant ($p>0.05$) increases from 42.50±2.49 IU/L post infection to 47.13±3.06 IU/L post treatment in the treated groups (A and B). Creatinine (CR) levels though increased post infection was only significant ($p<0.05$) in the infected untreated group with pre-infection value of 69.00±2.86 µmol/L increasing to 83.67±3.27 µmol/L post infection. There was further increase in CR post treatment though non-significant in the treated groups (Table 4.5). Total protein (Tp) concentration significantly ($p<0.0001$) reduced from 67.32±0.83 g/L at pre-infection to 57.45±1.55 g/L at day 7 post infection in all the infected donkeys, and returned to pre-infection range following treatment in the treated groups (Table 4.6). Calcium ion (Ca^{2+}) ion significantly ($p<0.05$) reduced post infection and increased post treatment, with values recorded post treatment significantly higher ($p<0.05$) than the pre-infection values in all the treated groups (Table 4.6).

Table 4.5: Mean changes in some serum biochemical parameters and enzymes of donkeys experimentally infected with *Trypanosoma brucei* (Federe isolate) and treated with Novidium® and Sécuridium®.

Groups/Subgroups	N	Phase of Experiment	CR±SEM(µmol/L)	BUN±SEM(mmol/L)	AST±SEM(IU/L)	ALT±SEM(IU/L)	ALP±SEM(IU/L)
A1 (Infected Novidium®-treated Acute Stage)	4	Pre-infection	69.50 ± 4.92[a]	3.65 ± 0.43[a]	21.00 ± 2.12[a]	38.13 ± 1.40[a]	73.25 ± 3.38[a]
		Post-infection	75.75 ± 4.89[a]	4.08 ± 0.50[a]	25.50 ± 2.60[a]	37.50 ± 1.87[b]	76.50 ± 3.10[a]
		Post treatment	78.25 ± 6.22[a]	4.08 ± 0.45[a]	28.25 ± 4.70[a]	49.08 ± 1.37[bc]	73.64 ± 0.97[a]
A2 (Infected Novidium®-treated Chronic Stage)	4	Pre-infection	75.00 ± 1.47[a]	4.53 ± 0.19[a]	31.75 ± 0.85[a]	37.56 ± 0.92[a]	67.25 ± 2.43[a]
		Post-infection	77.50 ± 5.95[a]	3.30 ± 0.17[a]	27.50 ± 4.66[a]	46.00 ± 2.77[ab]	77.25 ± 5.96[a]
		Post treatment	79.00 ± 5.87[a]	4.65 ± 0.57[a]	24.00 ± 2.94[a]	50.36 ± 1.37[b]	79.50 ± 2.66[a]
B1 Infected Sécuridium®-treated Acute Stage	4	Pre-infection	68.50 ± 4.92[a]	3.55 ± 0.43[a]	20.00 ± 2.12[a]	37.88 ± 0.66[a]	72.25 ± 3.38[a]
		Post-infection	71.25 ± 5.44[ab]	4.80 ± 0.45[a]	23.25 ± 0.95[a]	41.50 ± 3.08[bc]	72.75 ± 6.36[a]
		Post treatment	86.25 ± 1.44[b]	3.98 ± 0.46[a]	23.25 ± 0.63[a]	50.02 ± 1.52[c]	72.00 ± 5.73[a]
B2 Infected Sécuridium®-treated Chronic Stage	4	Pre-infection	74.00 ± 1.47[a]	4.26 ± 0.20a	26.50 ± 1.62a	39.50 ± 1.50[a]	66.25 ± 2.43[a]
		Post-infection	65.25 ± 2.46[a]	4.55 ± 0.17a	25.60 ± 0.81a	51.50 ± 4.91[ab]	75.75 ± 6.76[a]
		Post treatment	75.50 ± 4.56[a]	4.14 ± 0.09a	26.20 ± 0.31a	37.00 ± 3.34[ac]	74.75 ± 2.66[a]
C Infected Untreated	6	Pre-infection	69.00 ± 2.86[a]	4.50 ± 0.34[a]	25.17 ± 0.98[a]	33.83 ± 1.70[a]	72.67 ± 2.46[a]
		Post-infection	83.67 ± 3.27[b]	4.52 ± 0.25[a]	26.83 ± 1.01[a]	51.00± 3.17[b]	71.17 ± 1.99[a]
D Uninfected Control	6	Initial Value	77.50 ± 3.63[a]	4.37 ± 0.11[a]	28.33 ± 1.98[a]	35.83 ± 1.54[a]	69.00 ± 1.88[a]
		Final Value	75.00 ± 2.91[a]	4.00 ± 0.29[a]	26.33 ± 2.28[a]	42.83 ± 3.63[a]	75.17 ± 3.09[a]

KEY: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Creatinine (CR), Blood Urea Nitrogen (BUN), N = Number of animals in each group, Standard Error of Mean (SEM). Means with different letters in same column for each group are significantly different at $P < 0.05$.

Table 4.6: Mean changes in some serum proteins and electrolytes of donkeys experimentally infected with *Trypanosoma brucei* (Federe isolate) and treated with Novidium® and Sécuridium®.

Groups/Subgroups	N	Phase of Experiment	ALB±SEM(g/L)	TP±SEM(g/L)	Ca^{2+}±SEM(mmol/L)	PO_4^{3-} ±SEM(mmol/L)	Na^{+}±SEM(mmol/L)
A1 Infected Novidium®-treated Acute Stage	4	Pre-infection	42.75 ± 1.93[a]	71.25 ± 2.78[a]	2.41 ± 0.66[a]	1.01 ± 0.05[a]	140.80 ± 1.70[a]
		Post-Infection	39.75 ± 2.69[a]	57.00 ± 3.76[b]	1.66 ± 0.07[b]	1.04 ± 0.04[a]	142.80 ± 1.38[a]
		Post treatment	41.00 ± 3.16[a]	64.00 ± 0.71[ab]	2.67 ± 0.10[a]	1.06 ± 0.05[a]	139.30 ± 1.44[a]
A2 Infected Novidium®-treated Chronic Stage	4	Pre-infection	39.75 ± 1.11[a]	69.00 ± 1.73[a]	2.39 ± 0.04[a]	1.23 ± 0.10[a]	141.30 ± 2.02[a]
		Post-Infection	44.25 ± 2.69[a]	57.25 ± 4.31[b]	1.70 ± 0.07[b]	1.09 ± 0.05[a]	138.50 ± 2.40[a]
		Post treatment	43.00 ± 3.76[a]	68.50 ± 2.02[ab]	2.86 ± 0.17[c]	0.99 ± 0.05[a]	138.50 ± 2.02[a]
B1 Infected Sécuridium®-treated Acute Stage	4	Pre-infection	41.75 ± 1.93[a]	68.13 ± 1.02a	2.38 ± 0.04[a]	1.00 ± 0.05[a]	139.80 ± 1.70[a]
		Post-Infection	36.75 ± 1.11[a]	61.13 ± 3.23b	1.61 ± 0.05[b]	1.09 ± 0.04[a]	139.30 ± 3.20[a]
		Post treatment	38.75 ± 2.66[a]	67.50 ± 0.58ac	2.88 ± 0.06[c]	1.03 ± 0.04[a]	139.00 ± 1.96[a]
B2 Infected Sécuridium®-treated Chronic Stage	4	Pre-infection	38.75 ± 1.11[a]	68.00 ± 1.73[a]	2.36 ± 0.04[a]	1.20 ± 0.82[a]	140.30± 2.02[a]
		Post-Infection	40.00 ± 4.02[a]	54.50 ± 3.84[b]	1.74 ± 0.06[b]	1.13 ± 0.02[a]	138.00 ± 2.80[a]
		Post treatment	40.00 ± 2.61[a]	69.75 ± 2.63[a]	3.04 ± 0.13[c]	1.04 ± 0.04[a]	136.00 ± 2.04[a]
C Infected Untreated	6	Pre-infection	38.68 ± 1.75[a]	67.28 ± 2.93[a]	2.41 ± 0.05[a]	1.06 ± 0.05[a]	139.70 ± 1.71[a]
		Post-infection	40.99 ± 0.48[a]	60.43 ± 0.69[b]	1.69 ± 0.05[b]	0.99 ± 0.03[a]	137.80 ± 1.08[a]
D Uninfected Control	6	Initial Value	37.17 ± 1.47[a]	67.00 ± 1.61[a]	2.48 ± 0.05[a]	1.17 ± 0.04[a]	137.20 ± 1.58[a]
		Final Value	39.17 ± 2.23[a]	69.00 ± 1.69[a]	2.80 ± 0.11[a]	1.00 ± 0.09[a]	137.30 ± 1.54[a]

KEY: Albumin (ALB), Total plasma protein (TP), Calcium ion (Ca^{2+}), Phosphate ion (PO_{43-}), Sodium ion (Na^{+}), N = Number of animals in each group, Standard Error of Mean (SEM). Means with different letters in same column for each group are significantly different at $P < 0.05$.

Table 4.7: Mean changes in some serum electrolytes of donkeys experimentally infected with *Trypanosoma brucei* (Federe isolate) and treated with Novidium® and Sécuridium®.

Groups/Subgroups	N	Phase of Experiment	K^+±SEM(mmol/L)	Cl^-±SEM(mmol/L)	HCO_3^- ±SEM(mmol/L)	Glucose±SEM(mg/dL)
A_1 Infected Novidium®-treated Acute Stage	4	Pre-infection	4.03 ± 0.25^{a}	99.00 ± 2.20^{a}	24.25 ± 2.02^{a}	86.25 ± 13.09^{a}
		Post-Infection	3.88 ± 0.11^{a}	101.3 ± 1.11^{a}	24.00 ± 1.63^{a}	47.50 ± 6.02^{b}
		Post treatment	3.98 ± 0.46^{a}	98.00 ± 1.23^{a}	25.75 ± 1.65^{a}	87.00 ± 1.43^{a}
A_2 Infected Novidium®-treated Chronic Stage	4	Pre-infection	4.20 ± 0.25^{a}	100.8 ± 2.06^{a}	23.25 ± 1.49^{a}	90.25 ± 13.09^{a}
		Post-Infection	4.13 ± 0.33^{a}	101.8 ± 0.75^{ab}	27.25 ± 0.75^{b}	45.00 ± 6.18^{b}
		Post treatment	3.36 ± 0.36^{a}	95.25 ± 0.63^{c}	25.25 ± 0.48^{ab}	78.25 ± 7.41^{a}
B_1 Infected Sécuridium®-treated Acute Stage	4	Pre-infection	3.93 ± 0.25^{a}	98.00 ± 2.20^{a}	24.25 ± 2.02^{a}	91.50 ± 4.09^{a}
		Post-Infection	4.55 ± 0.13^{a}	99.75 ± 2.18^{a}	25.75 ± 1.25^{a}	41.25 ± 10.36^{b}
		Post treatment	3.95 ± 0.29^{a}	99.50 ± 1.66^{a}	25.50 ± 1.85^{a}	86.50 ± 1.15^{a}
B_2 Infected Sécuridium®-treated Chronic Stage	4	Pre-infection	4.10 ± 0.25^{a}	99.75 ± 2.06^{a}	23.25 ± 1.49^{a}	84.00 ± 4.24^{a}
		Post-Infection	3.48 ± 0.17^{a}	100.8 ± 2.29^{a}	23.75 ± 1.38^{a}	58.75 ± 9.47^{ab}
		Post treatment	3.68 ± 0.28^{a}	99.25 ± 1.65^{a}	24.25 ± 1.44^{a}	95.75 ± 3.82^{ac}
C Infected Untreated	6	Pre-infection	4.08 ± 0.20^{a}	100.0 ± 1.29^{a}	22.83 ± 1.05^{a}	100.70 ± 3.17^{a}
		Post-infection	3.95 ± 0.18^{a}	100.5 ± 0.85^{a}	25.33 ± 1.05^{a}	46.00 ± 7.84^{b}
D Uninfected Control	6	Initial Value	4.13 ± 0.21^{a}	100.2 ± 1.14^{a}	24.17 ± 1.18^{a}	87.17 ± 3.41^{a}
		Final Value	3.72 ± 0.23^{a}	100.5 ± 0.56^{a}	25.67 ± 1.28^{a}	91.33 ± 2.24^{a}

KEY: Potassium ion (K^+), Chloride ion (Cl^-), Bicarbonate ion (HCO_3^-), N = Number of animals in each group, Standard Error of Mean (SEM). Means with different letters in same column for each group are significantly different at $P < 0.05$.

4.11 Gross Lesions Observed at Necropsy

4.11.1 Lungs

Comparative pathology of the lungs of donkeys experimentally infected with *T. brucei* and treated with Novidium® and Sécuridium® presented as follows: a representative donkey from the uninfected control group D had normal lungs at necropsy (A) while the one from infected untreated group C had congested lungs (B). Group A_1 (Novidium® treatment acute infection stage) donkey revealed no pathology while group A_2 (Novidium® treatment chronic infection stage) donkey had slightly congested lung (C) with mucoid exudate in the bronchi. In group B_1 (Sécuridium® treatment acute infection stage) donkey, no gross lesions were observed in the lungs while B_2 (Sécuridium® treatment chronic infection stage) at necropsy had slightly congested lung (D) (Plate I).

4.11.2 Spleen

Comparative pathology of the spleens of donkeys experimentally infected with *T. brucei* and treated with Novidium® and Sécuridium® presented as follows: a representative of group D donkeys necropsied had a normal spleen (A) while the group C donkey had a congested spleen (B). No gross splenic lesions were observed for group A_1. However, in group A_2, there were mild splenic haemorrhages (C) while groups B_1 and B_2 revealed no gross lesions (D) (Plate II).

4.11.3 Heart

Comparative pathology of the hearts of donkeys experimentally infected with *T. brucei* and treated with Novidium® and Sécuridium® presented as follows: group D donkey at necropsy revealed normal heart (A). Group C donkey revealed no gross lesions (B).

Hydropericardium and pericardial haemorrhages were observed in group A_2 donkey while endocardial haemorrhage was seen in group B_2 donkey (Plate III).

4.11.4 Liver

Representative donkeys of groups D and B had normal liver architecture. In group A donkey, liver was slightly congested while in group C donkey, the liver was moderately congested.

4.11.5 Kidneys

No gross lesions were observed in the representative donkeys for all the groups.

4.11.6 Brain

No gross lesions were observed in all the groups.

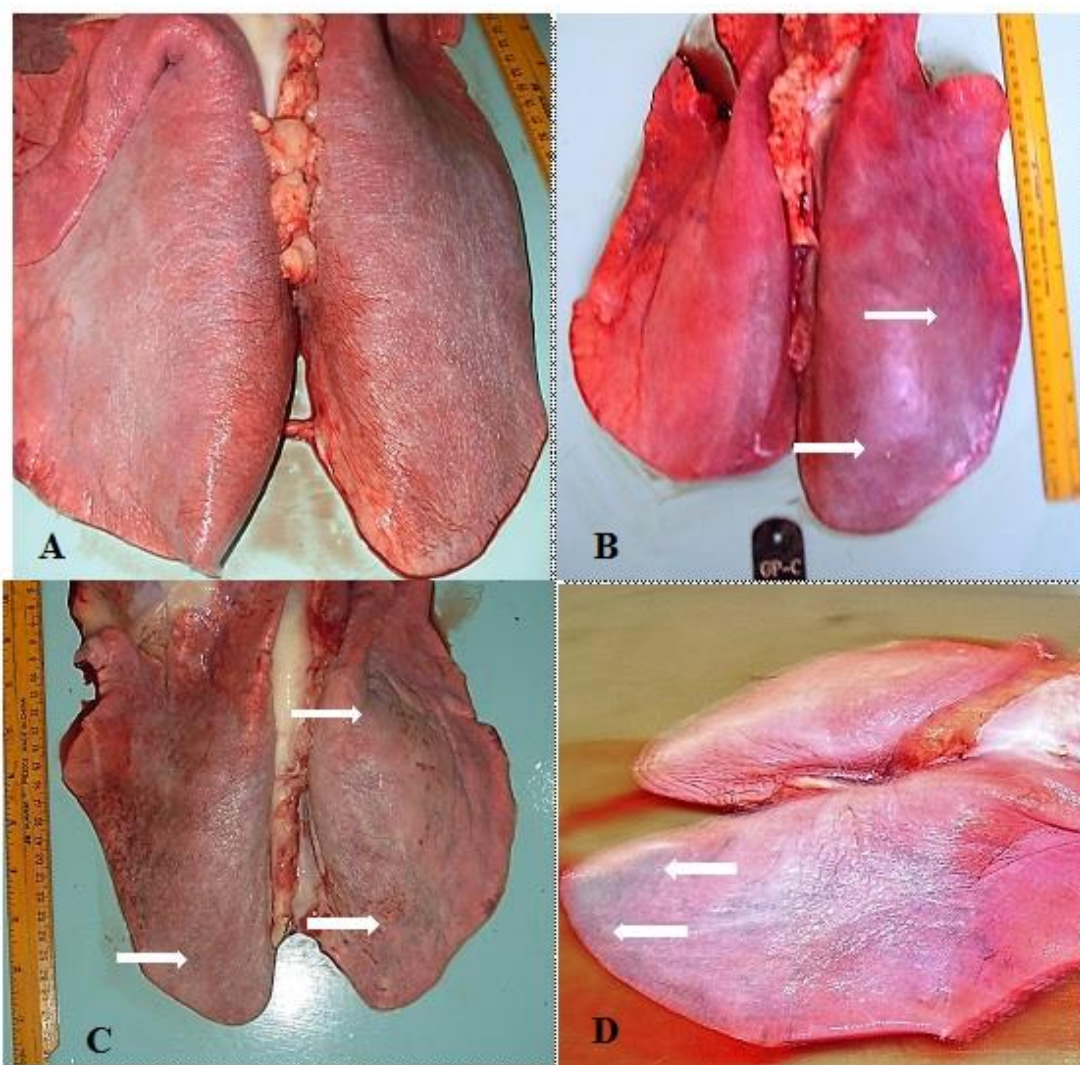

Plate I: Pathology of lungs of donkeys experimentally infected with *T. brucei* and treated with Novidium® and Sécuridium®.

A = Normal lungs from an uninfected (control) donkey at termination of the experiment

B = Lungs of one of the *T. brucei* infected untreated donkeys at termination of the experiment showing areas of congestion and bluish discolouration (arrows).

C = Lungs showing areas of congestion (arrows) from *T. brucei*-infected, Novidium®-treated donkey post treatment.

D = Lungs showing some areas of congestion (arrows) from *T. brucei*-infected, Sécuridium®-treated donkey post treatment.

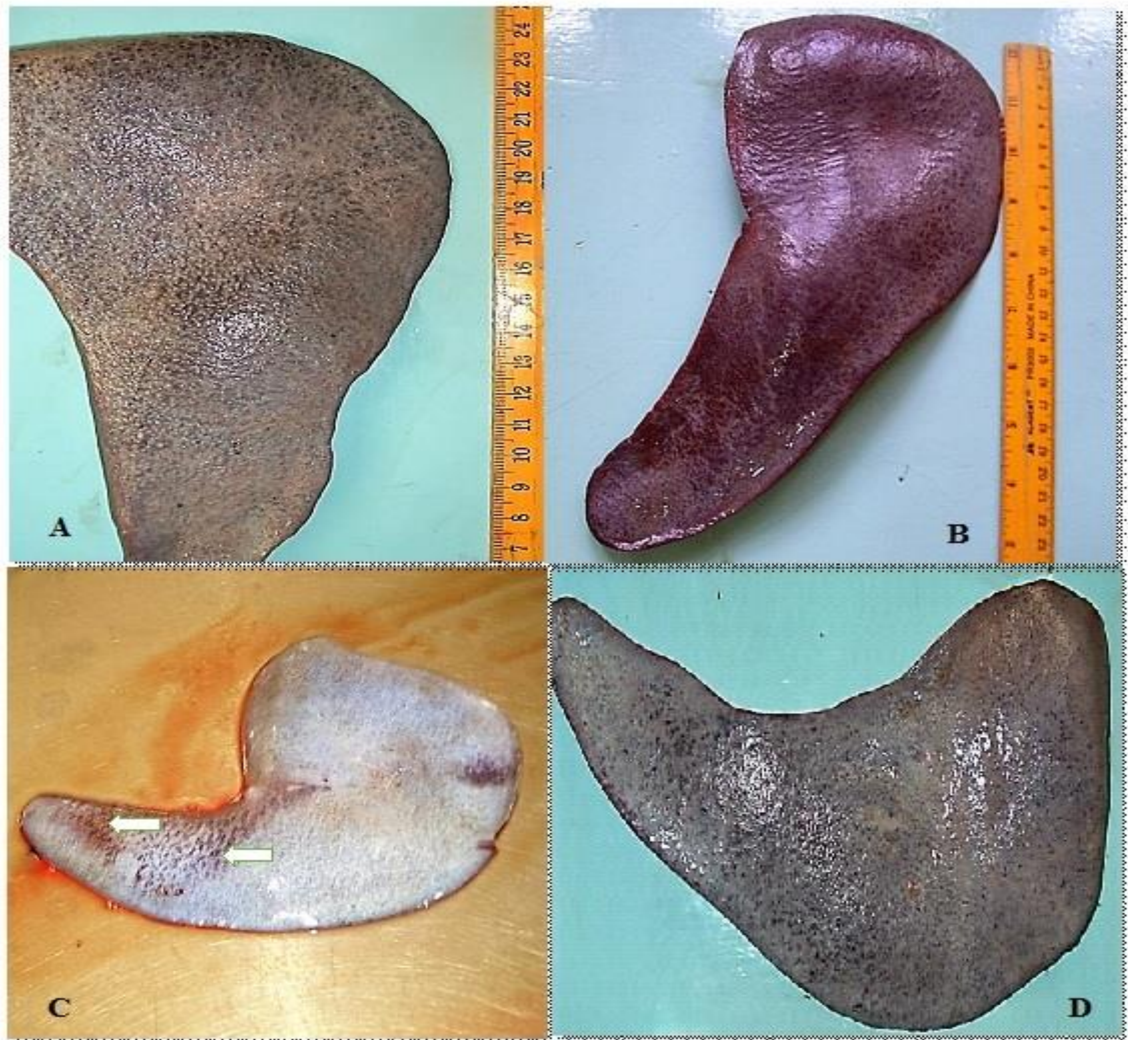

Plate II: Pathology of spleens of donkeys experimentally infected with *T. brucei* and treated with Novidium® and Sécuridium®.

A = Normal spleen of an uninfected (control) donkey at termination of the experiment.
B = Spleen from a *T. brucei* infected untreated donkey at termination of the experiment showing congestion.
C = Spleen from a *T. brucei*-infected, Novidium®-treated donkey post treatment. Note the areas with haemorrhages (arrows).
D = Spleen from *T. brucei*-infected, Sécuridium®-treated donkey post treatment showing no gross lesions.

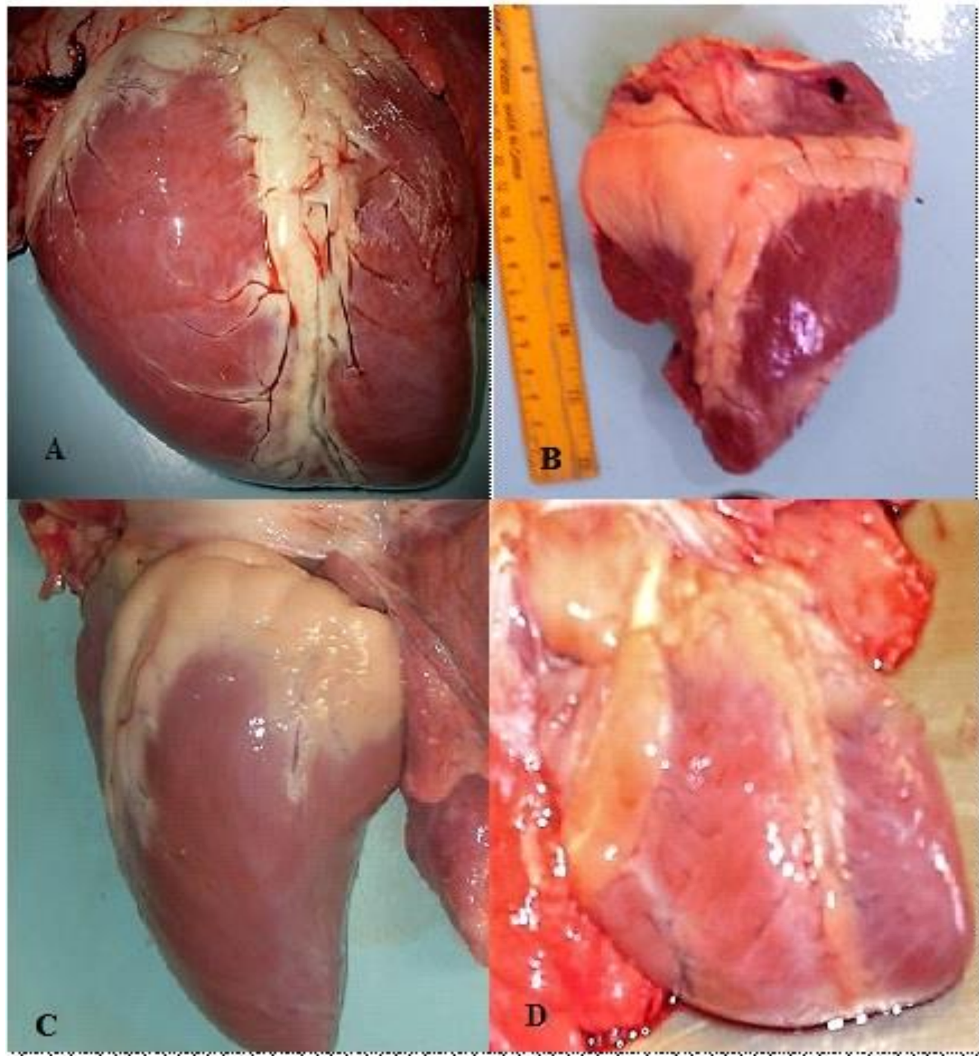

Plate III: Pathology of hearts of donkeys experimentally infected with *T. brucei* and treated with Novidium® and Sécuridium®.

A = Normal heart from an uninfected (control) donkey (group D) at the termination of the experiment.

B = Heart from an infected untreated donkey (group C) at termination of the experiment showing no gross lesions.

C= Apparently normal heart from a *T. brucei*-infected Novidium®-treated donkey (A_1) post treatment.

D=Apparently normal heart from a *T. brucei*-infected Sécuridium® treated donkey (B_1) post treatment.

4:12 Organ Impression Smears and Histopathology of Donkeys Experimentally Infected with *T. brucei* (Federe isolate)

4.12.1 Organ Impression smears

Giemsa-stained organ impression, cerebrospinal fluid and brain squash smears of the infected donkeys, revealed no parasites on examination.

4.12.2 Histopathology

Histopathologically, lesions were observed in the brain, skeletal muscles, lungs, spleen, liver, kidney, pancreas, and testes, at the end of the experiment:

4.12.2.1 Brain

Brain tissue section from the uninfected control group D donkey revealed normal histology (A) while the infected untreated group C donkey revealed eosinophilic perivascular cuffing without neuronal degeneration (B). Group A (Novidium® treatment) donkey had an apparently normal brain section (C) while group B (Sécuridium® treatment) donkey had brain tissue showing eosinophilic perivascular cuffing and neuronal degeneration (D) (Plate IV).

4.12.2.2 Lungs

Normal lung tissue was observed in group D (A). The group C donkey had lung tissue section showing an obliteration of alveoli with inflammatory cells, haemorrhages and congestion (B). There were thickened interalveolar walls occupied by massive red blood cells and some infiltrated inflammatory cells in the group A donkey while in the group B donkey, lung tissue was characterized by pulmonary congestion (Plate V).

4.12.2.3 Spleen

Splenic tissue sections for the group D donkey had normal histology (A). A tissue section of group C donkey showed massive haemosiderin deposits (B) while splenic congestion and haemosiderin deposits were observed in groups A (C) and B (D) donkeys (Plate VI).

4.12.2.4 Liver

A normal hepatic architecture was observed in the group D donkey (A). Liver tissue section of group C donkey revealed hepatic degeneration with mild cholestasis (B). In group A donkey, no abnormal histology was observed while in the group B donkey, mitotic dividing cells were seen in the tissue section (Plate VII).

4.12.2.5 Kidney

Kidney sections of donkey from group D were normal (A) while the kidney of group C donkey revealed tubular necrosis with areas of congestion (B). Congestion and glomerulonephritis with dilated Bowman's space were observed in the tissue section of group A donkey (C). In the group B donkey, glomerulonephritis was also observed (D) (Plate VIII).

Other histological lesions observed were mild Zenker's necrosis in muscle tissue sections of group A donkey. An infiltrative pancreatitis in group B donkey, while sections of the testis and ovary revealed no pathology except in group B donkey where dead spermatogenic cells and necrosis of testicular tissues were observed.

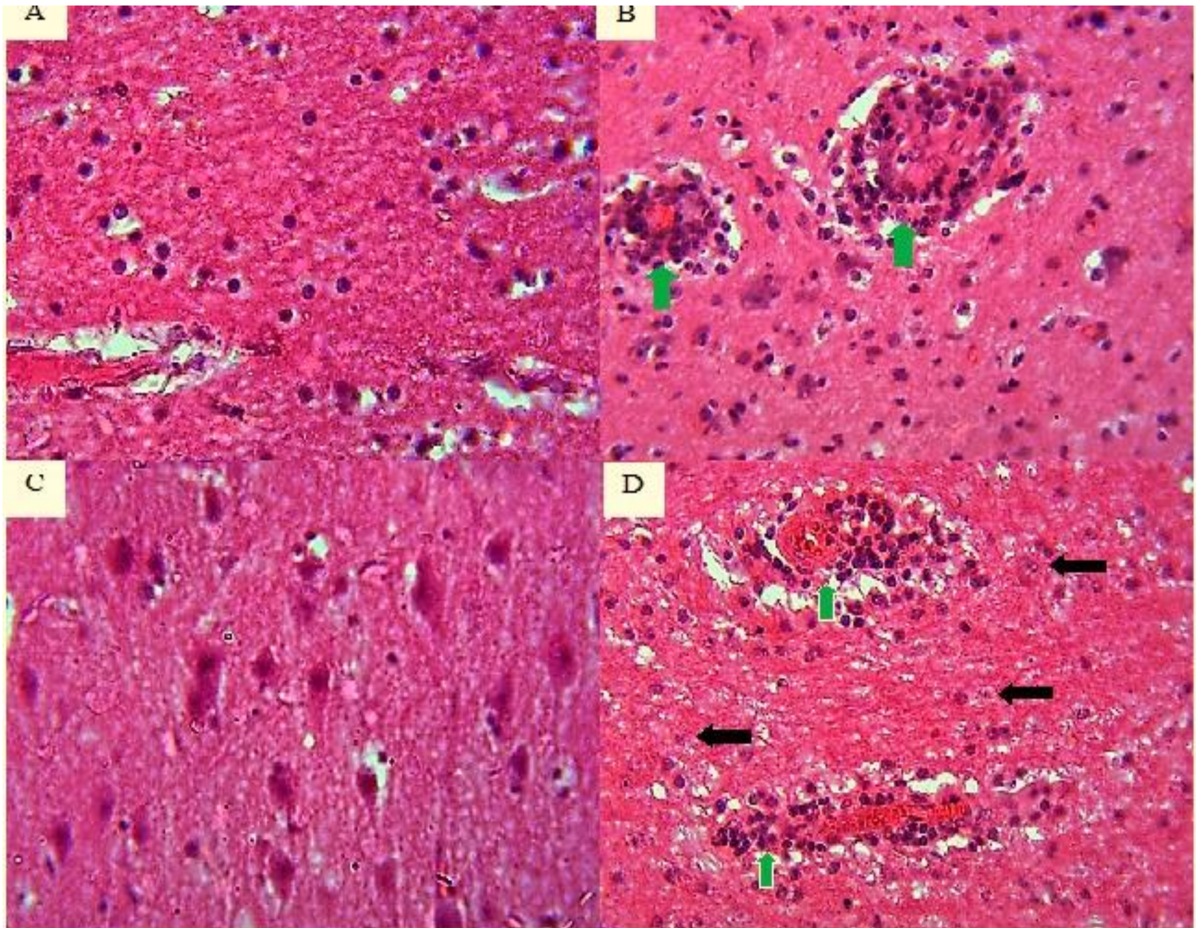

Plate IV: Photomicrograph of brain sections of donkeys experimentally infected with *T. brucei* and treated with Novidium® and Sécuridium®

A = Section of brain from uninfected (control) donkey at termination of the experiment showing normal histology. (H&E x400).

B = Section of brain showing eosinophilic perivascular cuffing (green arrows) in *T. brucei*-infected untreated donkey at termination of the experiment. (H&E x400).

C = Section of apparently normal brain in T. brucei-infected Novidium®-treated donkey post treatment. (H&E x400).

D = Section of brain showing eosinophilic perivascular cuffing (green arrows) and neuronal degeneration (black arrows) in *T. brucei*-infected Sécuridium®-treated donkey post treatment (H&E x400).

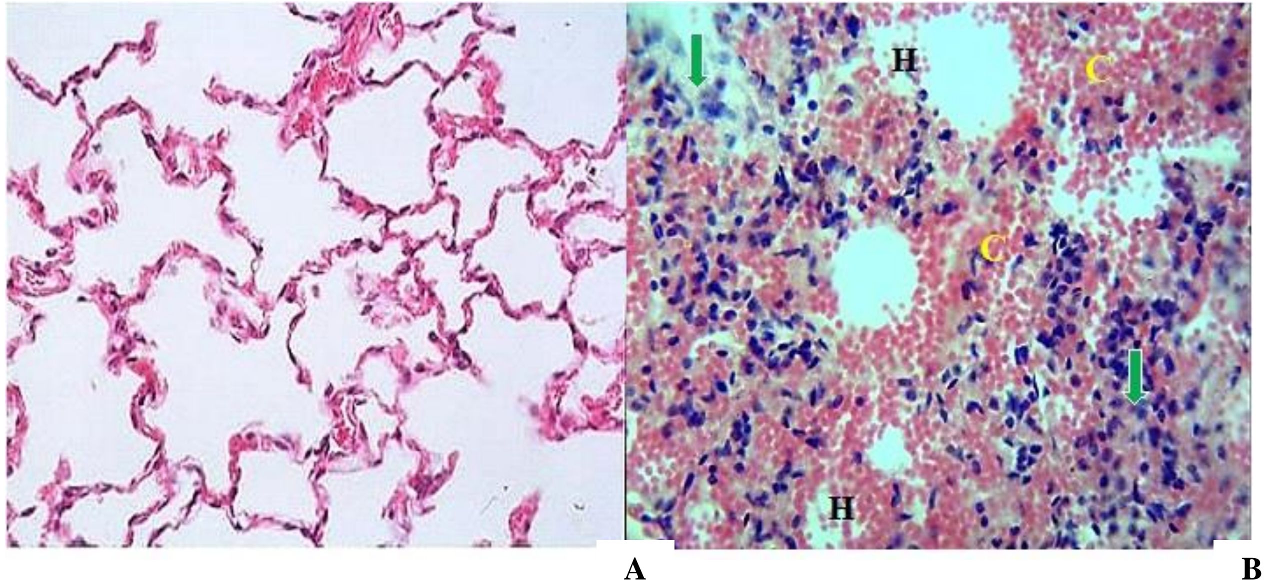

Plate V: Photomicrograph of lung sections of donkeys experimentally infected with *T. brucei*

A = Normal lung tissue section from uninfected (control) donkey at the of termination of the experiment. (H&E x400).

B = Lung tissue section showing obliteration of alveoli with inflammatory cells (green arrows), haemorrhages (H) and congestion (C) in *T. brucei*-infected untreated donkey at termination of the experiment. (H&E x400).

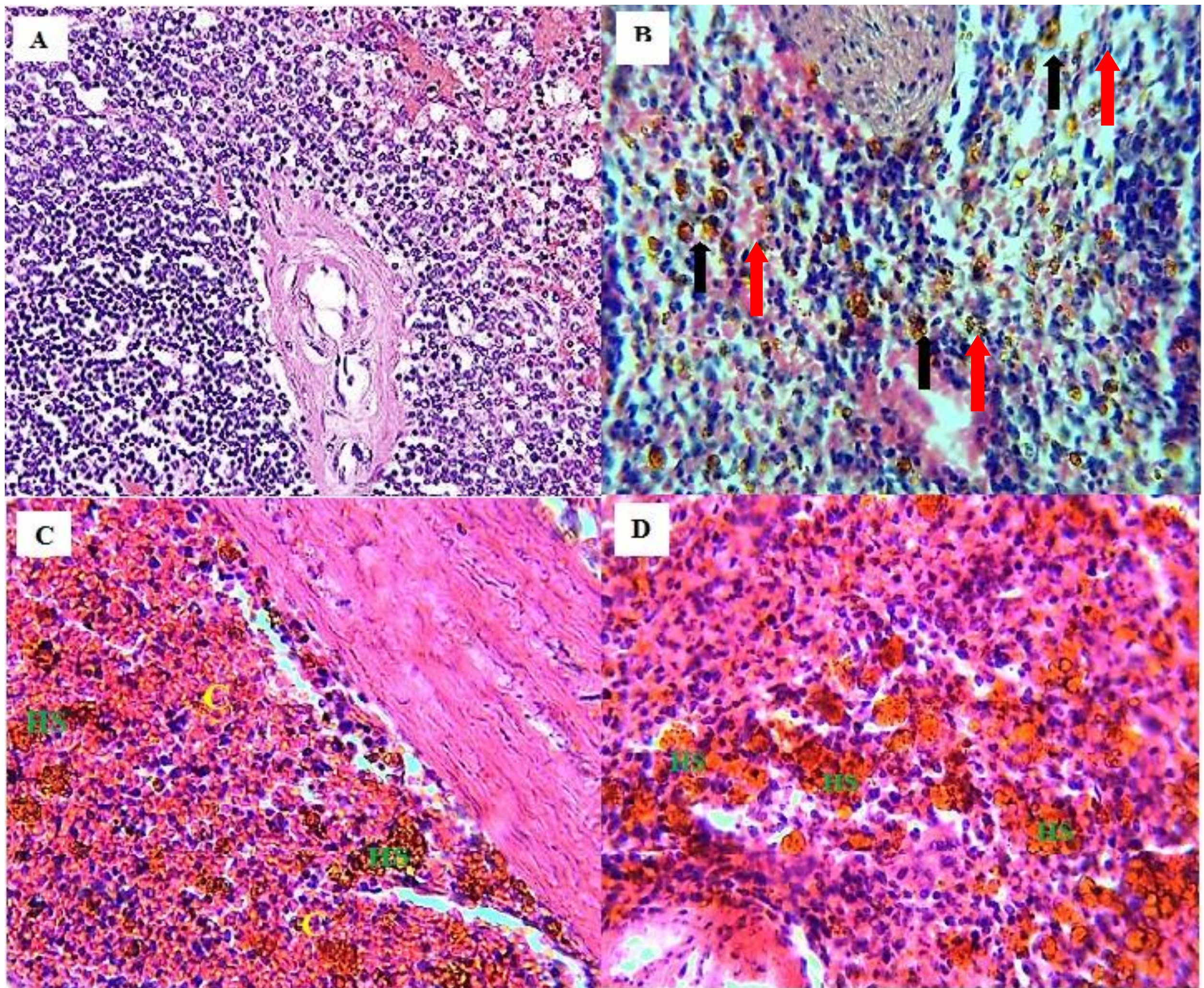

Plate VI: Photomicrograph of spleen sections of donkeys experimentally infected with *T. brucei* and treated with Novidium® and Sécuridium®

A = Normal spleen tissue section. (H&E x400).

B = Tissue section of the spleen from *T. brucei*-infected untreated donkey at termination of the experiment showing massive haemosiderin deposits (red arrows). (H&E x400).

C = Spleen section from *T. brucei*-infected Novidium®-treated donkey post treatment showing splenic congestion (yellow C) with haemosiderin deposits (green HS) (H&E x400).

D = Spleen section from *T. brucei*-infected Sécuridium®-treated donkey post treatment showing hemosiderin deposits (green HS) (H&E x400).

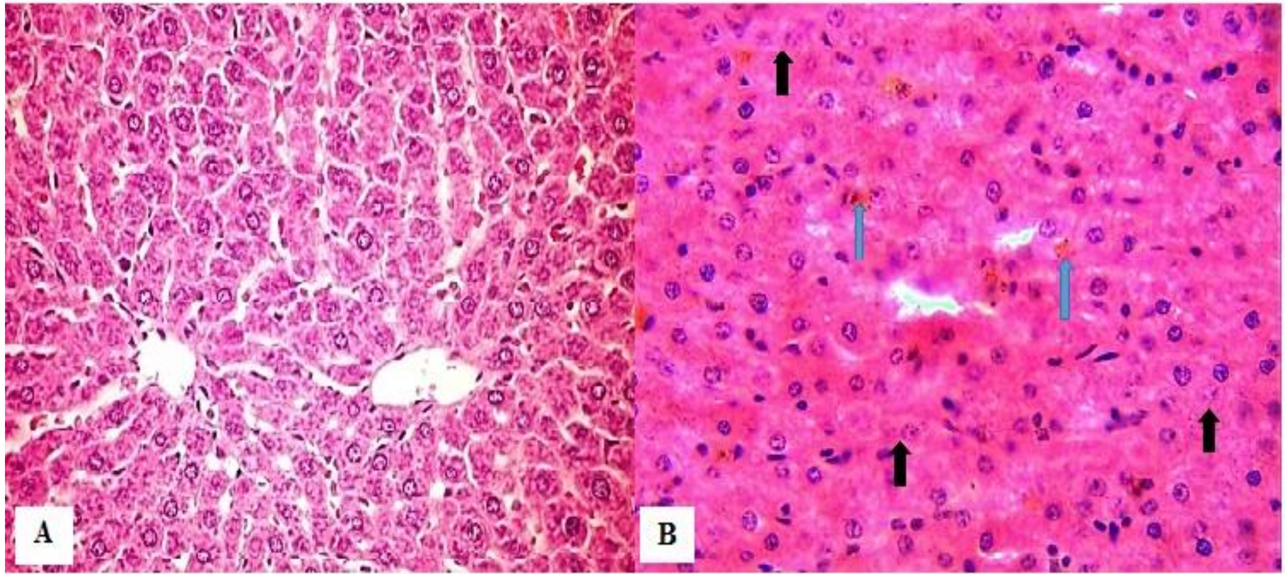

Plate VII: Photomicrograph of liver sections of donkeys experimentally infected with *T. brucei*

A = Liver tissue section showing normal hepatic architecture. (H&E x400).

B = Tissue section of the liver from *T. brucei*-infected untreated donkey at termination of the experiment showing hepatic cell degeneration (black arrows) and mild cholestasis (blue arrows). (H&E x400).

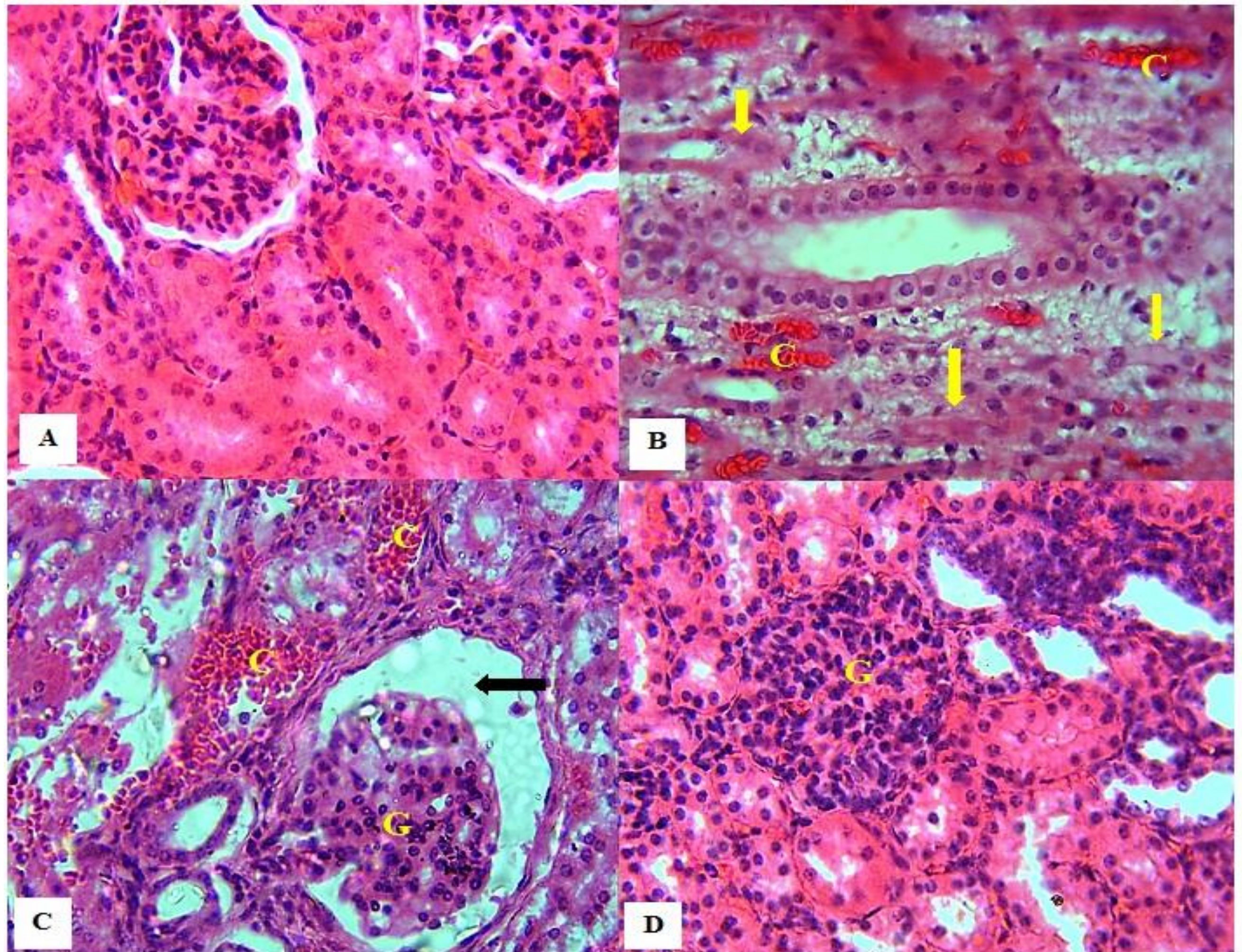

Plate VIII: Photomicrograph images of kidney sections of donkeys experimentally infected with *T. brucei* and treated with Novidium® and Sécuridium®

A = Normal Kidney tissue section from uninfected (control) donkey at the termination of the experiment (H&E x400).

B = Kidney tissue section from infected untreated showing tubular necrosis (yellow arrows) and areas of congestion (C). (H&E x400).

C = Kidney section from *T. brucei*-infected Novidium®-treated donkey showing congestion (yellow C) and glomerulonephritis (yellow G) with dilated Bowman's space (arrow). (H&E x400).

D = Kidney section from *T. brucei*-infected Sécuridium®-treated donkey at termination of the experiment showing glomerulonephritis (yellow G). (H&E x400).

CHAPTER 5

DISCUSSION

The clinical and pathological manifestations of *T. brucei* (Federe isolate) infection in donkeys in this experiment, are similar to those previously described for *T. brucei* infection in pigs (Allam *et al*., 2011), horses (Kihurani *et al*., 1994)), carnivores (Ezeokonkwo *et al*., 2012) and goats (Igbokwe and Mohammed, 1992; Sackey, 1998; Adeiza *et al*., 2008).

In this study, twenty (20) out of the twenty-two (22) *T. brucei* (Federe) experimentally infected donkeys developed clinical trypanosomosis as evidenced by parasitaemia, fever, anaemia, lymphocytosis and thrombocytopenia. Despite receiving same quantity of inoculum as all other infected animals, as well as being managed similarly, the two donkeys that remained infection-free, neither developed parasitaemia nor manifested clinical trypanosomosis. This could be attributed to their individual genetic composition which perhaps made them more tolerant to the infection (Kumar and Weatherall, 2008), as well as possibly being exposed previously to trypanocides which might have impacted some prophylaxis on them (Mehlhorn, 2008).

The observed early appearance of parasitaemia (2-3 days) in this study, compared to earlier report of 5-6 days (Ikede *et al.,* 1977), could be related to the higher virulence of the *T. brucei* (Federe isolate) used in this experiment and the intravenous route of inoculation in contrast to the subcutaneous route used by Ikede *et al*. (1977). Other workers on *T. brucei* studies however, reported variable pre-patent periods of 4 – 6 days, depending on the host, the parasite isolate and presence of a concurrent infection (Morrison *et al*., 1981; Sackey, 1998; Adamu *et al*., 2009; Opaluwa-Kuzayed *et al*., 2015). Of interest in this experiment, is the high parasitaemia levels recorded which

were generally higher than those earlier reported in savannah brown goats (Adeiza *et al*., 2008) and rabbit bucks (Opaluwa-Kuzayed *et al*., 2015) experimentally infected with *T. brucei*. This could possibly be attributed to virulence of the *T. brucei* isolate used in this study and host differences.

The fluctuating rectal temperatures and parasitaemia as noted in this experiment have also been observed in *T. brucei* infections in savannah brown goats and red fronted gazelles (Sackey, 1998; Mbaya *et al*., 2009b). These fluctuations are attributed to extravascular tissue localization of the parasites and alternate expression of variant surface glycoproteins with the emergence of new variant antigenic types (Antia *et al.,* 1996; Barry and McCulloch, 2001).

The observed anaemia in this experiment was a progressive and regenerative type. It was revealed by consistent decreases in mean PCV, Hb concentration and total RBC counts in all the infected donkeys when parasitaemia and pyrexia were high, indicating or suggesting the possible role of the large number of the parasites and high body temperature in reducing the levels of these blood parameters. This is consistent with the findings of Kihurani *et al*. (1994) in horses naturally infected with a mixed infection of *T. brucei*, *T. congolense* and *T. vivax*, Sackey (1998) in *T. brucei* experimentally infected savannah brown goats and Allam *et al*. (2011) in *T. brucei* experimentally infected gilts. Also, the increased MCV, and reduced MCHC recorded in this experiment indicated that the anaemia was generally macrocytic and hypochromic in nature, and similar to the findings in vervet monkeys experimentally infected with *T. brucei rhodesiense* (Ngotho *et al*., 2011). However, this finding is in contrast with reports of normocytic normochromic anaemia in dogs infected with *T. brucei* (Omotainse and Anosa, 1992) and macrocytic normochromic anaemia in *T. brucei* experimentally infected rabbits (Erah *et al*., 2003). The observed erythrophagocytosis

and massive haemosiderosis in the mononuclear phagocyctic system (e.g the spleen) suggest that the anaemia is haemolytic and could partly be due to extravascular haemolysis in these donkeys. This agrees with the findings of Ikede *et al*. (1977) also in donkeys experimentally infected with *T. brucei*.

The observed clinical signs shown by the infected animals in this experiment were consequent upon the presence and virulence of the trypanosome parasites in circulation, with the resultant loss of condition, dehydration and emaciation in the infected animals. This is similar to the reports by other researchers on animal trypanosomosis. For example, Igbokwe (1994) and Magez and Radwanska (2013) reported that trypanosomosis causes a drop in feed intake resulting in energy deficit and loss of tissue associated with catabolism of body fat, deficiencies of vitamin C, B and essential amino acids.

The observed lacrimation in the experimentally infected donkeys (groups A, B and C) strongly suggests the ability of *T. brucei* (Federe) to localize in extravascular tissues similar to reports by Sackey (1998) in savannah brown bucks infected with *T. brucei*. The lacrimation could be due to irritations caused by activities of the parasites as they localize in the tear duct during extravasation in the course of the infection. The irritation induces a metabolic reaction resulting in the flow of tears as the tear gland tries to eliminate these parasites.

The intermittent penile erection recorded in these young reproductively immature donkeys is similar to reports by *et al*. (2013) and Garba *et al*. (2015) in *T. evansi* infections in horses and donkeys respectively. Disseminated intravascular coagulation associated with *T. brucei* infections (Tizard, 1985 cited by Abenga, 2014) could possibly be responsible for intermittent penile erection, which may lead to gangrene at later stages as found by Ajape and Bello (2011).

The observed increase in heart rates of the parasitaemic animals could have been due to enhanced sympathetic activity of the cardiovascular system caused by changes induced by systemic and metabolic disturbances as reported by Ajibola and Oyewale (2014). Ollarves *et al*. (2000) also reported a transient increase in heart rate in rats inoculated with *T. cruzi*. Also it is probable that the anaemia observed in this experiment contributed to the increase in heart rate as the organ tries to compensate for the shortage due to blood loss.

The increased respiratory rate seen in this study are similar to observations of Ikede *et al*. (1977) in donkeys experimentally infected with *T. brucei*, and Lazim *et al*. (2015) in goats experimentally infected with *T. vivax*. It is suggestive of metabolic stress induced by the infection as supported by the observed lymphocytosis and eosinopenia. Also, due to the anaemia and the resultant shortage of oxygen-carrying blood, the lungs in the bid to ensure adequate oxygen distribution to the body organs and tissues, compensate by increasing the respiratory rate.

The reduction in the total WBC counts observed in this study agrees with the findings of Allam *et al*. (2011) in pigs experimentally infected with *T. brucei* and that of Shimelis (2015) in cattle experimentally infected with *T. vivax.* However, it is different from the findings by Yusuf *et al*. (2013) who recorded an increase in WBC count in wistar rats experimentally infected with *T. brucei* in both acute and chronic stages of the infection. The reduced WBC counts may be ascribed to the immunosuppressive actions of trypanosome infection (Ekanem and Yusuf, 2008, Allam *et al*., 2011). *Trypanosoma brucei* (Federe) infection in the donkeys caused lymphocytosis, thrombocytopenia, neutropenia and eosinopenia similar to the findings by Ezebuiro *et al*. (2012) in *T. brucei* infection in rabbits. These changes indicate immunosuppression caused by the infecting parasites. Davis *et al.* (1974), also reported severe thrombocytopenia without

changes in hematocrit concentration in rats intraperitoneally injected with *Trypanosoma rhodesiense*. The observed thrombocytopenia could be as a result of hypertrophy of the mononuclear phagocytic system and/or increased splenic sequestration of platelets (Yusuf *et al*., 2013), leading to a decrease in the number of platelets in peripheral circulation. Other mechanisms also implicated is the depletion of the bone marrow by *T. brucei* infection in mice and rats (Bockstal *et al*., 2011; David, 2013) and desialylation of the platelet surface sialic acids by *T. brucei* trans-sialidase, resulting in alteration of circulating platelets thereby reducing the life span of platelets and thus leading to the development of the thrombocytopenia (Tribullati *et al*., 2005; Ammar *et al*., 2013).

The observed lymphocytosis post infection in this experiment agrees with the report of Allam *et al*. (2011) in experimental *T. brucei* infected in pigs, and indicates an increased immunological response to the invading organisms by the donkeys possibly influenced by their genetic makeup or hardy nature.

The neutropenia observed in this experiment points to neutrophil apoptosis in trypanosomosis as reported by Happi *et al*. (2012) in *T. brucei* infected rats. Aoshiba *et al*. (1999) in a study on oxidative stress and influence of red blood cells (RBCs), suggested that RBCs protect against neutrophil apoptosis in circulation by reducing intracellular oxidative stress through catalase and glutathione metabolism. With the knowledge that anaemia is a consistent finding in trypanosomosis, the decreased RBC count will then lead to a decrease in the amount of antioxidants and consequently the decrease in protective effect against apoptosis of the circulating neutrophils.

The fluctuating changes in blood glucose manifested in this study was similarly reported in experimental *T. brucei* infection in pigs (Allam *et al*., 2011) and grasscutters (Omonona *et al*., 2013). The hypoglycaemia could be explained by any of the three factors: parasites' need for glucose as an energy source (Kadima *et al*., 2000);

hepatocyte fatty degeneration (Nayak *et al*., 2010) and the metabolic effect of fever (Sazmand *et al*., 2011). Subsequent increases in glucose levels following treatment, when parasites were no longer in circulation supports the suspected parasite-glucose energy relationship.

Tissue damage/reaction is one of the pathological indicators of animal trypanosomosis manifested by alterations in the serum enzyme levels (Herrera *et al*., 2002; Eloy and Lucheis, 2009; Eyob and Matios, 2013; Abenga, 2014). In this experiment, serum AST, ALP, CR and BUN levels in all the groups remained within pre-infection range throughout the course of the experiment, indicating that the infection caused a mild pathology. Previously, marked AST, ALT and ALP have been observed in dogs, pigs, rabbits and rats experimentally infected with *T. brucei* (Orhue *et al*., 2005; Ezeokonkwo *et al*., 2012) and *T. congolense* (Egbe-Nwiyi *et al*., 2005; Omeje and Anene, 2012).

Sackey (1998) in an experimental *Trypanosoma brucei, T. congolense, T.* vivax infection in savannah brown goats reported that damage to body cells lead to alteration of membrane permeability with consequent release of enzymes into the extracellular fluid (ECF). Similar reports on non-significant changes in serum enzymes were made by Taiwo *et al*. (2003) and Dagnachew *et al*. (2015) in experimental *T. brucei* infection in sheep and cattle respectively. This is contrary to the report by Egbe-Nwiyi *et al*. (2005) in experimental infections of rats with *T. congolense*, and the one in dogs with *T. brucei* (Ezeokonkwo *et al*., 2012). It suggests that though anaemia was present, it might not have been such to induce severe anoxic conditions with the resultant fall in tissue pH and vascular damage manifested by signs of organ dysfunction (Enwezor and Sackey, 2005). The ALT levels on the other hand significantly increased post treatment in all the infected groups indicating a possible effect of trypanosome lyses resulting from the host’s defense mechanisms (Enwezor and Sackey, 2005; Bezie *et al*., 2014)

rather than severe liver damage since no significant alterations were seen in the other liver enzymes such as AST and ALP.

The observed marginal increase in serum albumin (ALB) concentrations post infection especially at chronic infection stage in this experiment, agrees with a similar report by Adenike and Stephen (2010). However, post treatment ALB level was not significantly different from pre-infection and post-treatment levels. This could possibly be due to dehydration, hence haemoconcentration accompanying trypanosomosis, or a poor utilization of dietary proteins (Adenike and Stephen, 2010; Thrall *et al*., 2012).

The decrease in total proteins observed in this study is similar to those obtained in experimental *T. brucei* infected goats (Sackey, 1998; Adejinmi and Akinboade, 2000), but contrasts the elevated total protein levels as reported in experimental *T. brucei* infected rats (Adenike and Stephen, 2010). It is proposed that malabsorption of dietary proteins (Igbokwe, 1994) could be responsible for the low levels recorded in this experiment. Other reports indicate varying levels of total protein in animal trypanosomosis, and this could be normal, increase or decrease (Anosa, 1988; Taiwo *et al*., 2003; Adeiza *et al*., 2008; Sazmand *et al*., 2011, Allam *et al*., 2011; Garba *et al*., 2015).

A significant reduction in calcium levels observed post infection in the infected donkeys, agrees with the report of hypocalcaemia in donkeys experimentally infected with *T. evansi* (Garba *et al*., 2015) but is at variance with the report of hypercalcaemia in dogs experimentally infected with *T. brucei* (Ajibola and Oyewale, 2014). The hypocalcaemia is reported to be associated with a protein abnormality and hypoalbuminaemia recorded secondary to trypanosomosis infection, and resulting in the malabsorption of the calcium electrolyte (Thrall *et al*., 2012). However, in this

experiment, though total protein significantly reduced post infection, albumin levels were non-significantly altered. As such it cannot be stated that protein abnormality led to the hypocalcaemia observed. Da Silva *et al*. (2011) suggested that since calcium is directly involved in muscle contraction, the low levels observed may be responsible for the clinical signs of muscle wasting, weakness and lethargy often reported in animal trypanosomosis, and also reported in this experiment.

The reduction in serum phosphate levels (though mild) could be explained by the reduced feed intake exhibited by the animals following infection, with a consequent reduced intake of phosphorus (Da Silva *et al*., 2011). It could also be explained by a phosphate shift into the cells induced by respiratory alkalosis due to the increased respiratory rate and pulmonary insufficiency observed in this experiment, and similar to a report by Schrier (2007).

Serum Na^+, K^+, Cl^- and HCO_3^- ions levels did not change significantly across the infected groups at the different experimental phases, and these observations are supported by the findings of Otesile *et al*. (1991) in boars experimentally infected with *T. brucei*. This may point to the fact that the infection caused only mild tissue damage and had a mild effect on renal tubular function.

The observed dilated Bowman's space, glomerulonephritis, kidney tubular necrosis, testicular tissue necrosis, perivascular cuffing, haemorrhages, congestion and haemosiderosis in this experiment are a resultant effect of the invading parasites, as similarly reported in experimental *T. brucei* infection of donkeys (Ikede *et al*., 1977), sheep (Akpavie *et al*., 1987), gilts (Allam *et al*., 2011) and dogs (Obi *et al*. 2013). This underlines the similarity in pathomorphological changes in different species of animals infected with *T. brucei*. These lesions have been linked to the activities of trypanosomes

in tissues, thus attracting severe inflammatory reactions characterized by mononuclear cell infiltration in the various organs affected (Abenga, 2014). Also extravascular tissue invasion by the trypanosomes (Masocha *et al.,* 2008), infection-induced tissue changes (Ukpai and Nwabuko, 2014) and host response to the invading parasites have been implicated in the manifestation of these pathological lesions (Odeyemi *et al*., 2015).

Observations of tissue sections in treated animals showed less distortions of tissue architecture suggesting attempts by the host at restoration of cellular morphology. This might have been aided by the administered drugs as reported by Adeyemi and Sulaiman (2012) in rats experimentally infected with *T. brucei* and treated with Homidium and Berenil®.

The experiment also showed that treatment with 2.5% Homidium chloride (Novidium®) and 1% isometamidium chloride (Sécuridium®) at doses of 1mg/kg and 0.5mg/kg b. w. respectively was effective against *T. brucei* infection in donkeys, with parasites being eliminated from circulation 24 hours post administration of these trypanocides. Also, previously observed clinical signs were no longer exhibited by the donkeys, thereby demonstrating therapeutic efficacy of the drugs against *T. brucei* (Federe) infection in the donkeys. However, infection relapses were recorded. The pathogenesis of relapse infection in trypanosomosis though not fully understood has been linked to re-emergence of trypanosomes from drug inaccessible sites in the body (Dukes, 1984), a limitation of the trypanocides against the parasite when used at recommended doses, and possible trypanocidal drug resistance as previously reported by Shiferaw *et al*. (2015).

The treatment was observed to restore blood glucose and total protein levels in the infected and treated donkeys. The reported rapid PCV increase after treatment with trypanocidal agents in *T. congolense* infected cattle (Rowlands *et al*., 1990) and mice

(Tadesse *et al*., 2015), was not observed in this study. In all the treatment groups, RBC counts remained insignificantly lower than post-infection values. Also, there was non-return of Hb concentration and PCV to pre-infection range. Some authors postulate that this may be due malnutrition and/or reduced response of the bone marrow due to exhaustion (Dukes, 1984; Igbokwe, 1994; Aliu, 2007; Bockstal *et al*., 2011).

The reduced platelet counts following treatment, which was more pronounced in the Sécuridium® treatment group could be related to a drug-induced thrombocytopenia (DIT) (Visentin and Liu, 2007), or to a generalized myelosuppression as reported by Carey (2003) to be a common and anticipated adverse effect of cytotoxic chemotherapy.

The lymphocytosis observed post treatment across the treatment groups could possibly be due to development of immune responses following drug administration as reported by Mehlhorn (2008) or a nonspecific finding that is common during the convalescent period of many acute infectious diseases (Cunha, 2004). Adenike and Stephen (2010) also observed post treatment lymphocytosis in rats experimentally infected with *T. brucei* and treated with Novidium® and Berenil®.

The increases in serum calcium ion and creatinine concentration post treatment point to a mild azotemia though blood urea nitrogen were minimally altered. Azotemia is said to be due to a decrease in renal perfusion leading to volume depletion (reduction in extracellular fluid volume) or impaired renal function (Orsini and Divers, 2014). Hypercalcaemia has also been implicated by Thrall *et al*. (2012) in development of azotemia due to renal vasoconstriction with resultant decreased glomerular filtration rate (GFR). The study revealed that BUN levels were minimally altered and this was contrary to the findings of Oyewusi and Saba (2013) who reported azotemia in *T. brucei brucei* infected rabbits, with increased blood urea nitrogen (BUN) levels which was suspected to be due to dehydration following the infection in rabbits.

The increased ALT activities post-treatment in this study was similar to that described by Adeyemi and Sulaiman (2012) in *T. brucei* experimentally infected rats, in which it was suggested that elevated ALT activities could be due to ability of trypanocidal drugs or their metabolites to interact with and enhance secretion of the enzyme, or a stabilizing effect of the drugs on body tissues thus stimulating increased production of the enzyme. The liver pathosis could not have caused the increase since, Kreutzer *et al*. (2008) reported that ALT enzyme was not very useful in evaluating liver diseases in the equine and other large animals. Acute hepatic diseases, aflatoxicosis, muscle necrosis and glucocorticoids are likely factors said to influence elevated serum ALT levels in equine species (Valentine *et al*., 1990; Giannini *et al*., 2005; Lavoie and Hinchcliff, 2011).

Glucocorticoids are said to increase production and release of ALT enzyme in the absence of liver disease (Reed *et al*., 2009) and this could be responsible for post treatment elevated levels of ALT and reduced eosinophil counts seen in this study. The glucocorticoids may have been released in response to stress or the need for increased glucose metabolism as reported by Macfarlane *et al*. (2008) as well as by Ranabir and Reetu (2011). It is also suspected that the released glucocorticoids were responsible for the mild increase in ALP enzyme post treatment in this study (Shoemaker, 2012).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The experiment revealed that:

1. The donkeys used in the experiment were susceptible (90.9% positive) to experimental *T. brucei* (Federe isolate) infection and developed classical symptoms (anaemia, pyrexia) of trypanosomosis following the infection.

2. Transient intermittent penile erection in the male (reproductively immature) donkeys were observed and this was an unusual finding.

3. The pre-patent period (time lapse before appearance of the parasites in circulation) and incubation period (period between infection and manifestation of clinical signs) of experimental *T. brucei* (Federe isolate) infection in donkeys were 2 – 3 and 2 – 6 days respectively.

4. Level of parasitaemia was very high (≥ 40 parasites per field) early in the infection and remained fluctuating throughout the duration of the experiment while the commencement of pyrexia coincided with the beginning of high parasitaemia (temperature was 40°C at day 5 post infection when parasitaemia was ≥ 40 parasites per field).

5. Anaemia recorded was macrocytic hypochromic in nature.

6. There was pronounced weight loss in the infected donkeys as the infection ran a chronic course.

7. Experimental infection of *T. brucei* (Federe isolate) in donkeys caused reduction in haematological parameters and significant serum biochemical changes that led to pathological responses in organs and tissues.

8. Gross lesions observed during the experiment included hydropericardium, emaciation, atrophy of fat, mucoid exudates in the bronchi, congested lungs and spleen, with splenic haemorrhages while histopathological findings included mononuclear cell perivascular cuffing in brain tissues, congestion of the lungs, kidney congestion with glomerulonephritis and splenic congestion with haemosiderosis and that organ impression smears did not demonstrate the trypanosome parasites. Minor changes in ALB, AST, ALP, CR and BUN post infection suggests the infection caused a mild pathology in the donkeys.

9. The trypanocides when administered at the recommended concentrations and doses effected cure, but there were relapses of the infection post treatment in both Novidium® and Sécuridium® treated animals even though there were fewer relapses observed in the latter drug.

10. Novidium® administered at the acute stage of the infection provided a better therapeutic efficacy than when administered at the chronic stage. This is because the secondary relapse in the acute stage treatment developed 60 days after treatment of the primary relapse while in the chronic stage the secondary relapse developed 27 days after treatment of the primary relapse.

11. Return of normal PCV levels was not influenced by the stage of the infection (acute or chronic) at which drug therapy was initiated.

12. Post treatment there was significant thrombocytopenia, marked lymphocytosis and neutropenia, elevation of serum ALT activities, hypercalcaemia and a mild azotemia.

6.2 Recommendations

1. *Trypanosoma brucei* infection should be included as a differential in diagnosis of suspected haemoparasitic infections in donkeys living in tsetse endemic areas.

2. Isometamidium chloride should be the first drug of choice in the treatment of *T. brucei* infections in donkeys.

3. Repeat treatment (2 - 3 times) should be employed at 25 – 30 days interval for Novidium®, and 60 – 65 days interval for Sécuridium®, using same dose (Novidium®) or slightly increased doses (Sécuridium®) to prevent relapse of infection.

4. The pathogenesis of *T. brucei* infection relapse in the donkey post-treatment should be investigated.

5. There is the need for studies to determine the most effective doses of Novidium® and Sécuridium®, treatment regimens for *T. brucei* infections, as well as Novidium® and Sécuridium® toxicity studies in donkeys.

6. There's the need for further studies using *Trypanosoma congolense* to evaluate overall performance of donkeys in the tsetse infected areas into which they are now being moved to as livestock.

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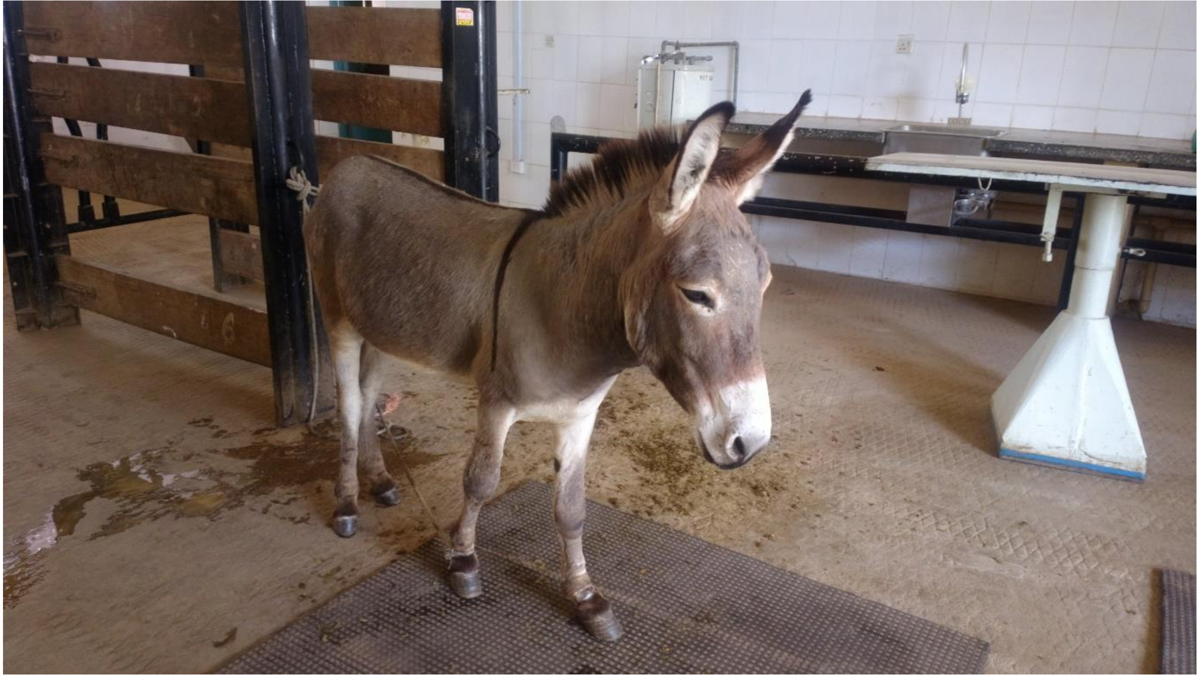
APPENDICES

Appendix I: A Somali Wild Ass (used with permission from www.ardea.com) showing black markings (red arrows) on the legs.

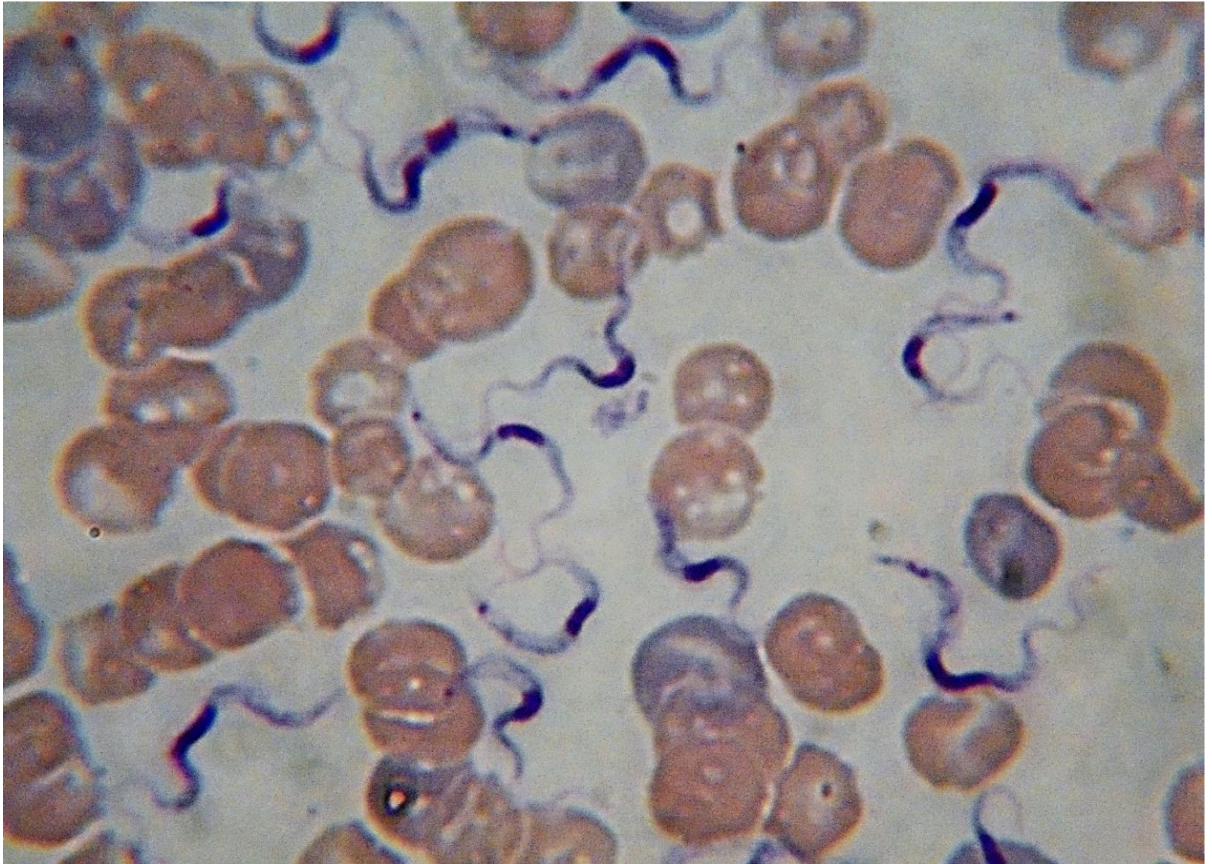


Appendix II: A Nigerian Domestic Donkey at the Large Animal Clinic floor of the Faculty of Veterinary Medicine (FVM) Ahmadu Bello University, Zaria. Note absence of black markings on the legs and a dorsal black strip across the shoulders

Appendix III: A long line of donkeys at Ezzangbo, Ebonyi State awaiting sale and slaughter. Culled from Daily Trust Newspaper, January 23rd 2016.

Appendix IV: CONSUMABLES USED IN THE EXPERIMENT

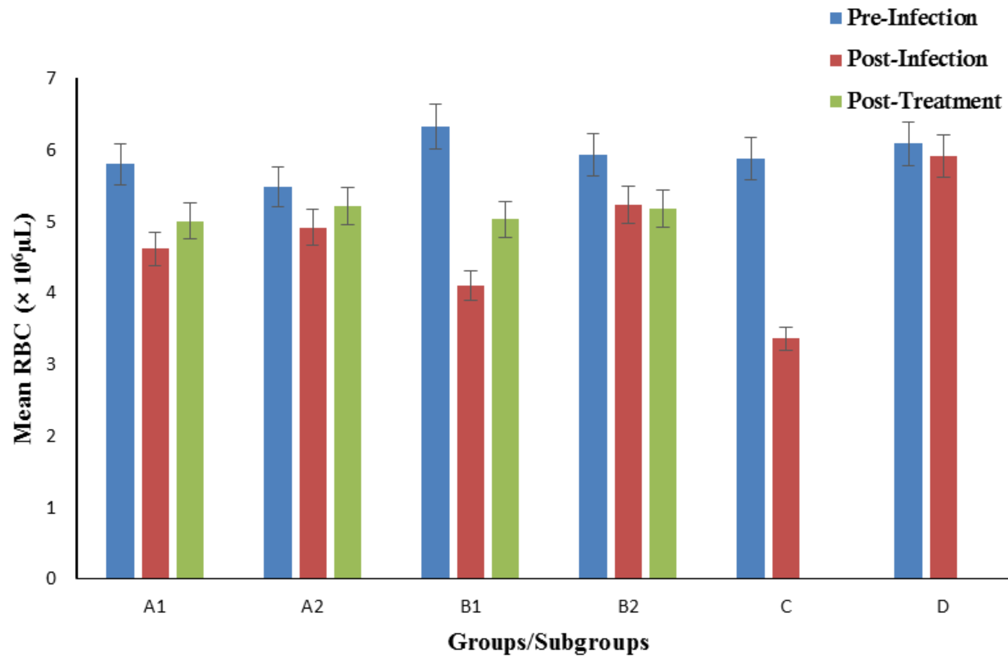
Homidium chloride (Novidium® Merial, France), Isometamidium choride (Securidium® LAPROVET, France), Giemsa powder (G4507, Sigma USA), Immersion Oil (No. 56822 Sigma-Aldrich, USA), Distilled water, Xylene (No. 296325 Sigma-Aldrich Co. USA), Ethanol 90% (SPECTRANAL™, Sigma-Aldrich, USA), Mineral Salt Lick (Royal®, Turkey), Hay from Rhodes Grass, Feed Concentrates, Albendazole 600 bolus (DAVATI® Georgia, Holland), Vetcotrim® bolus (Sam Pharmaceuticals Limited, Nigeria), Lysol® Disinfectant (Reckitt Benckiser, North America), Deltamethrin® 1% Pour-on (Targros Chemicals, India), DDVP® 20% EC(Loveland Products, Canada INC.), Fly-repellant (Endure® Farnam Co. Inc. USA), Hematocrit tubes, Glass slides, Cover Slips, Paraffin wax, Liquified Gas, Blood Sample bottles, Serum vials, Injectable 20% Oxytetracycline Antibiotics, Ethylenediaminetetraacetic acid (EDTA, Sigma-Aldrich Co LLC, USA), 1% Chloroform (Fluka-611778, Sigma-Aldrich Co LLC, USA), 1% Acepromazine injection (Acepran®, Univet S. A – São Paulo Brazil), Thiopental Injection (Thionembutal® Abbott Laboratories, North Chicago – Chicago, USA), 5ml and 10ml disposable plastic syringes, Cotton Wool.



Appendix V: ***Trypanosoma brucei*** **(Federe isolate) in thin blood smear of experimentally infected donkeys.**

Appendix VI: An infected untreated group C donkey at day 89 post infection showing muscle wasting with prominent hip bones

Appendix VII



Bar chart showing mean red blood cell count in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

Key:

A_1 = infected Novidium® acute stage treatment

A_2 = infected Novidium® chronic stage treatment

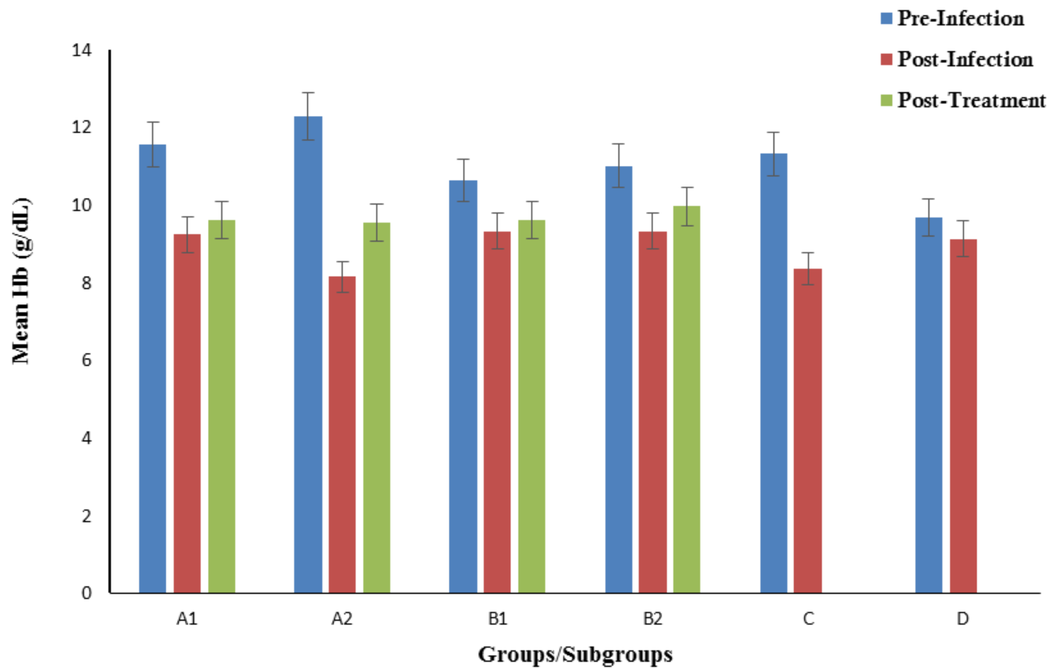
B_1 = infected Sécuridium® acute stage treatment

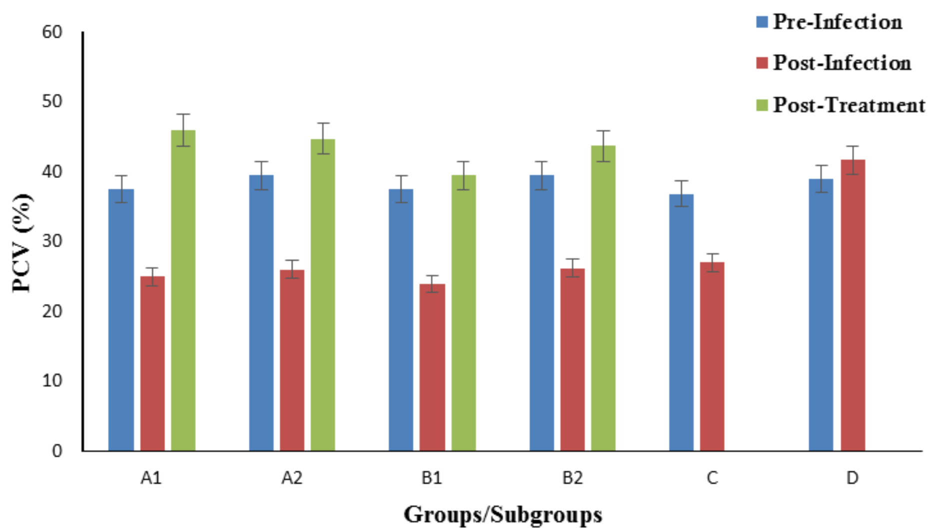
B_2 = infected Sécuridium® chronic stage treatment

C = infected untreated

D = uninfected control

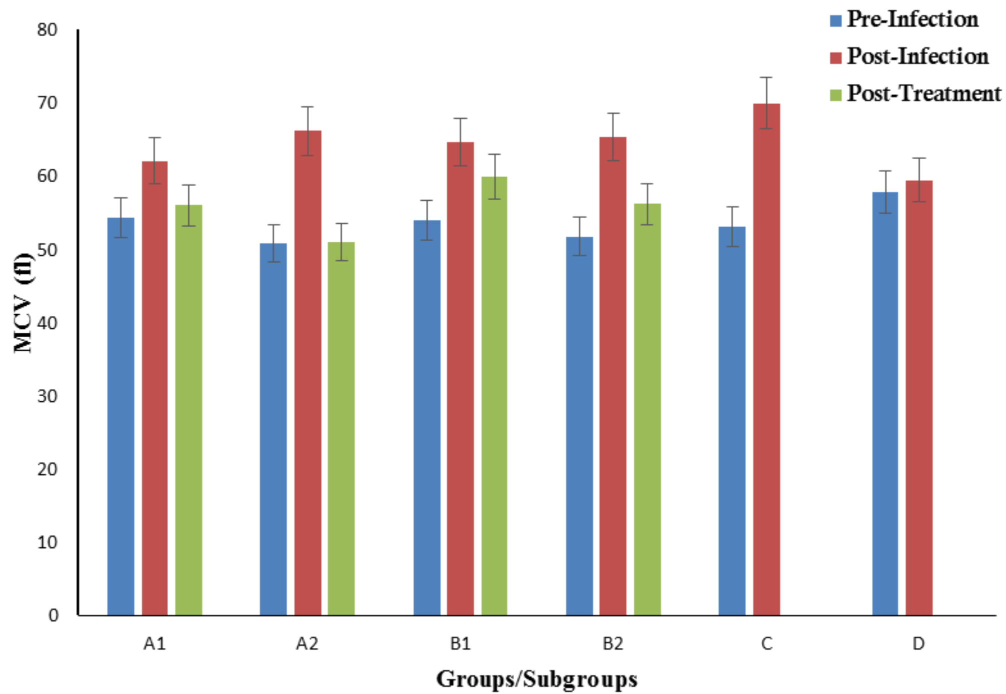
Appendix VIII

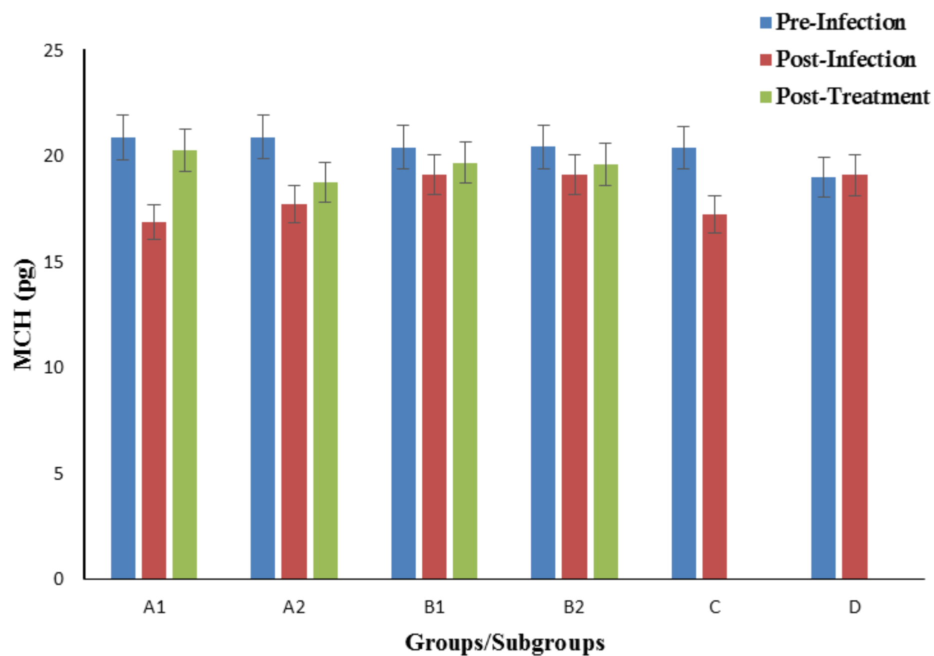




Bar charts showing mean haemoglobin concentration and mean packed cell volume in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

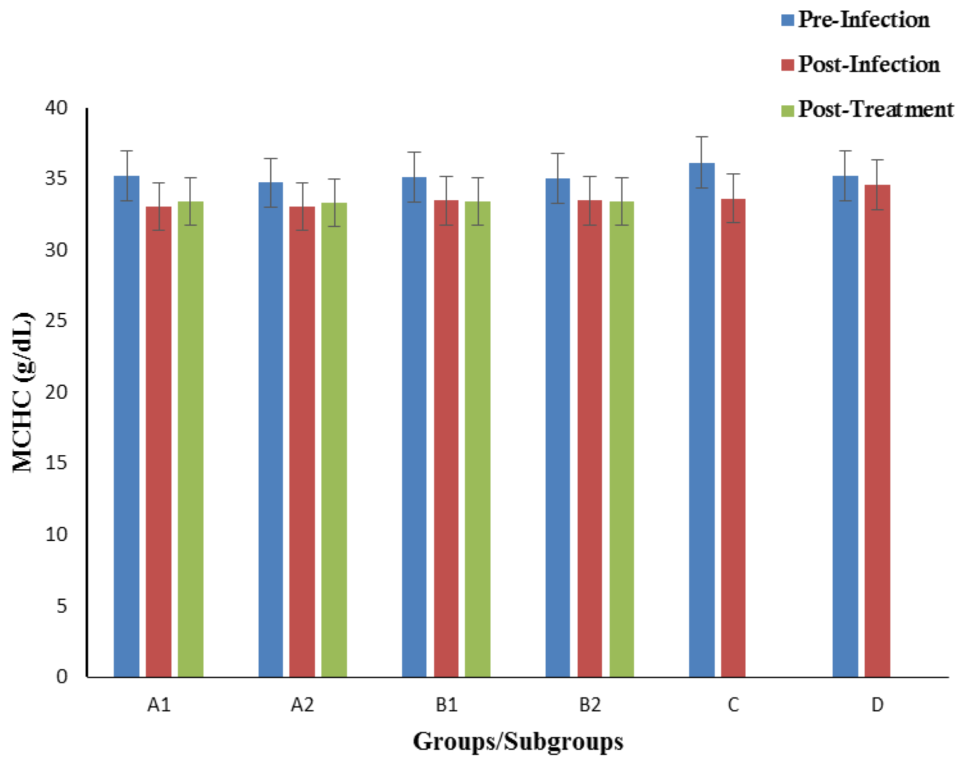
Appendix IX

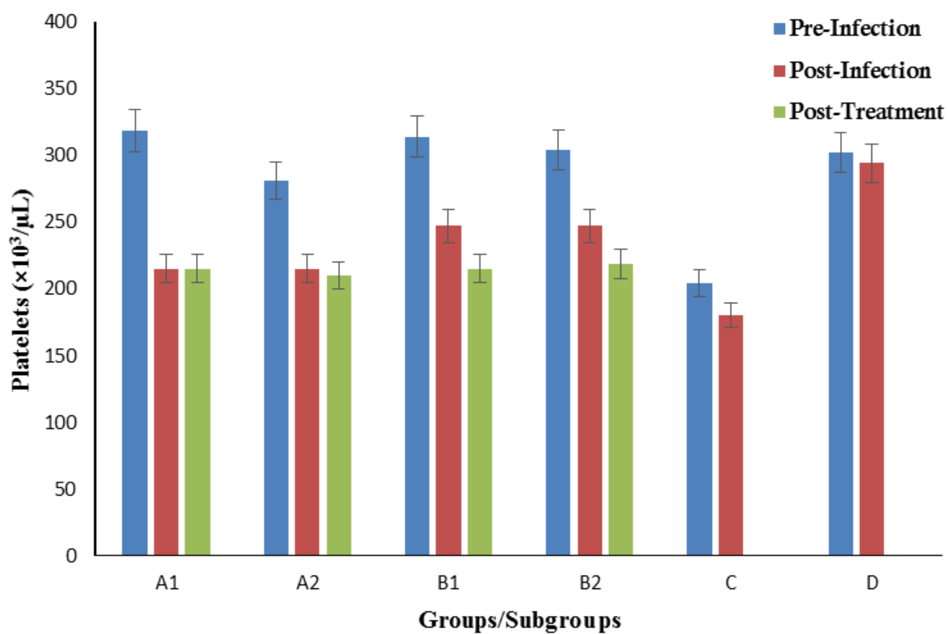




Bar charts showing mean corpuscular volume and mean corpuscular haemoglobin in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

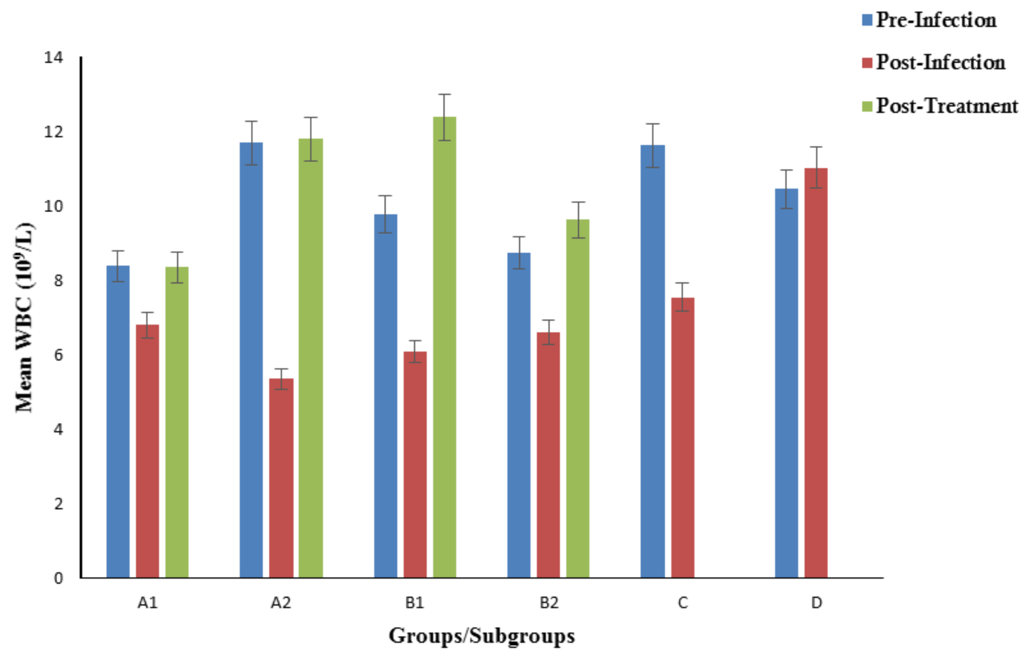
Appendix X

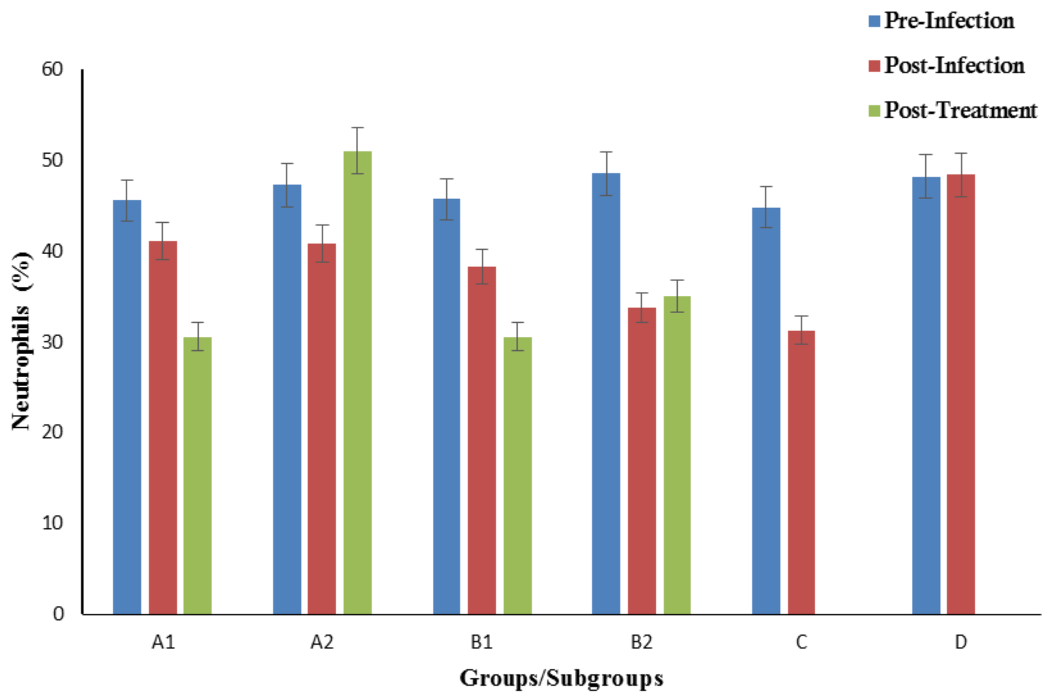




Bar charts showing mean corpuscular haemoglobin concentration and mean platelet counts in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

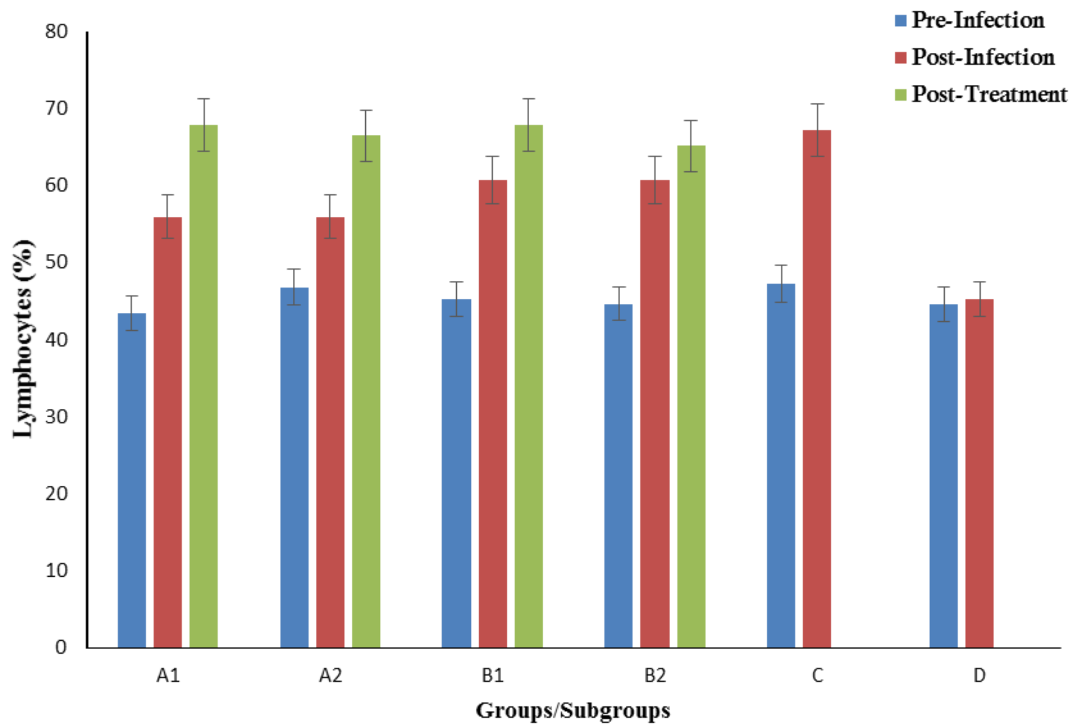
Appendix XI

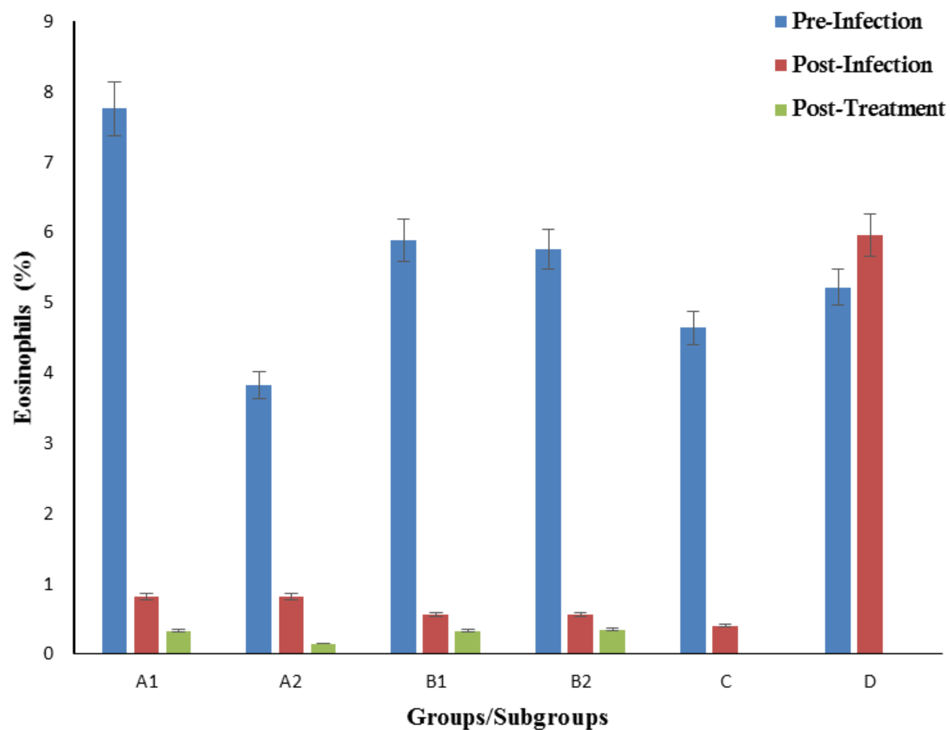




Bar charts showing mean total white blood cell and neutrophil counts in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

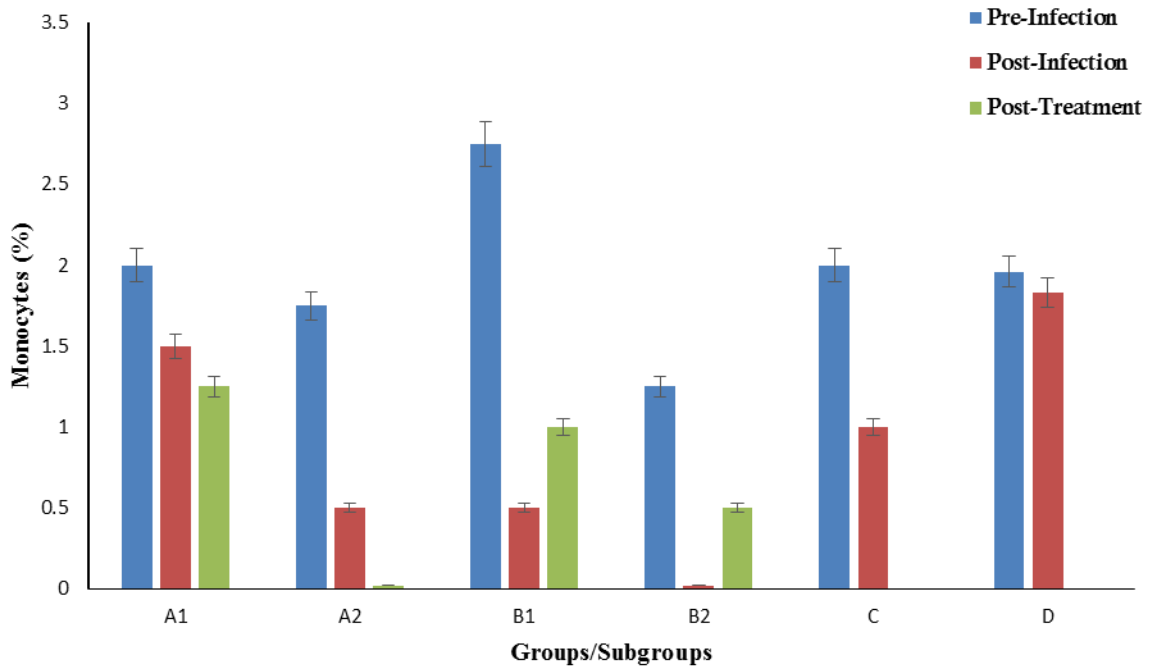
Appendix XII

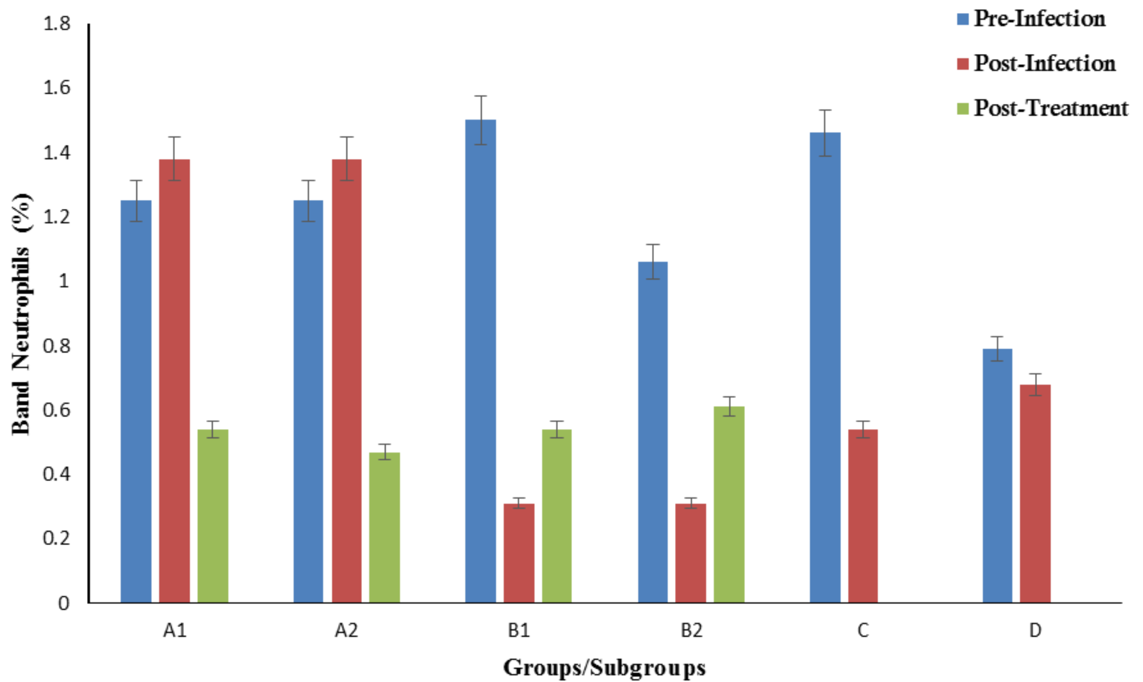




Bar charts showing mean lymphocyte and eosinophil counts in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

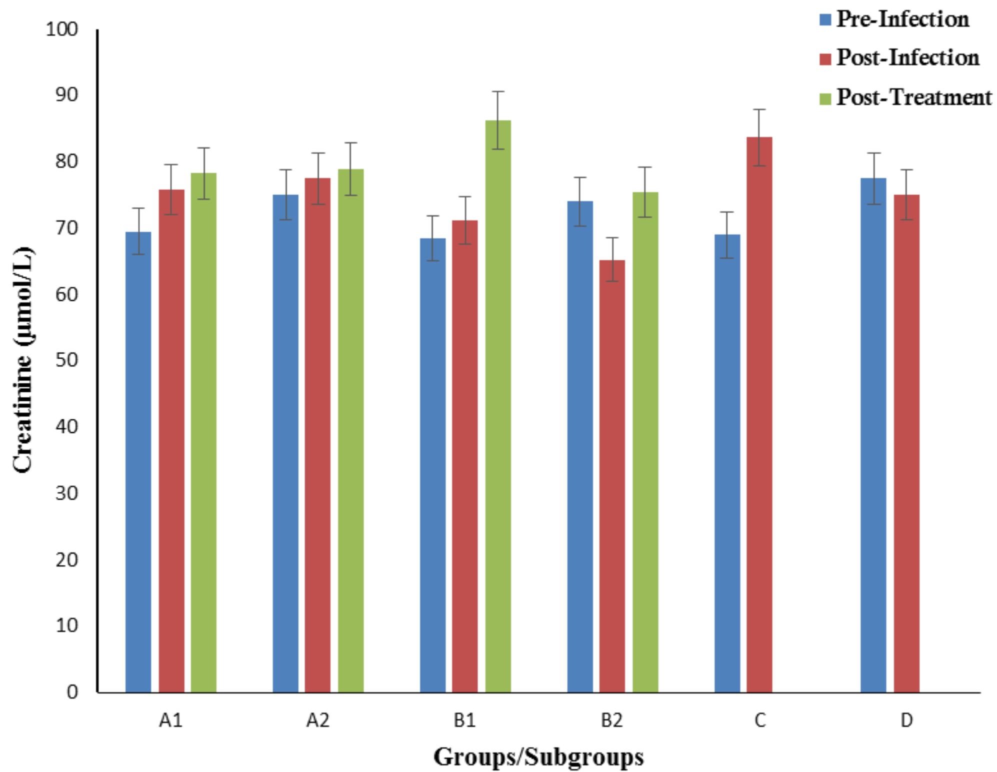
Appendix XIII

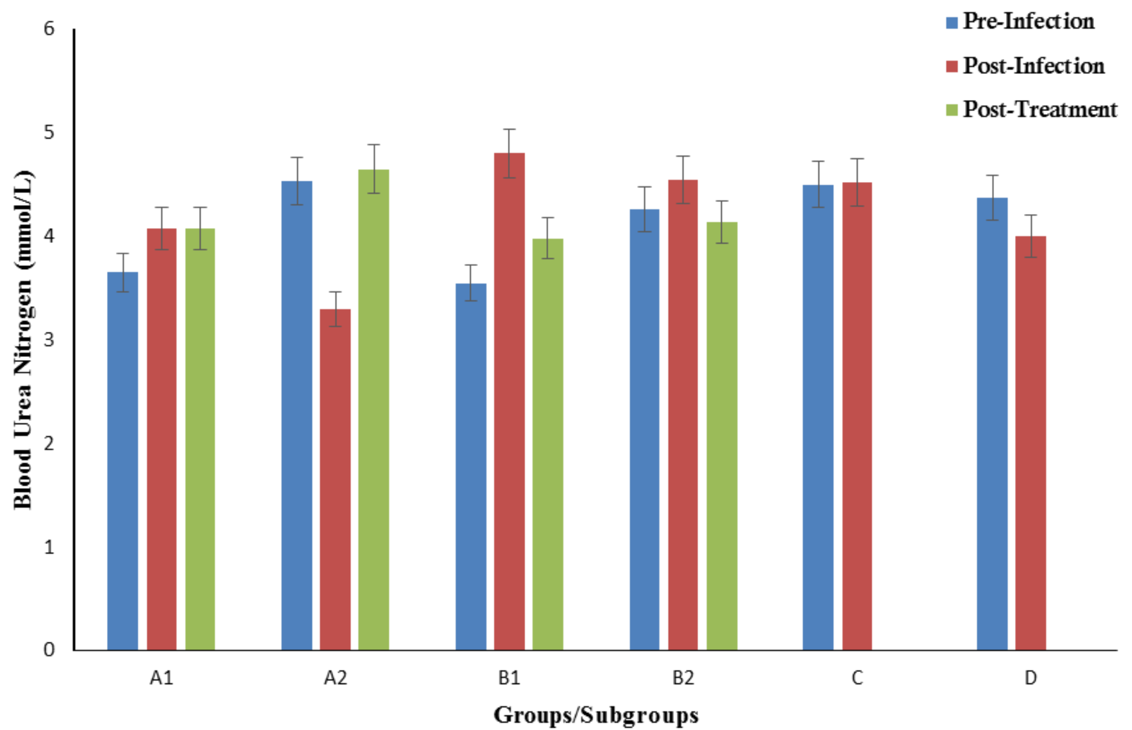




Bar charts showing mean monocyte and band neutrophil cell counts in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

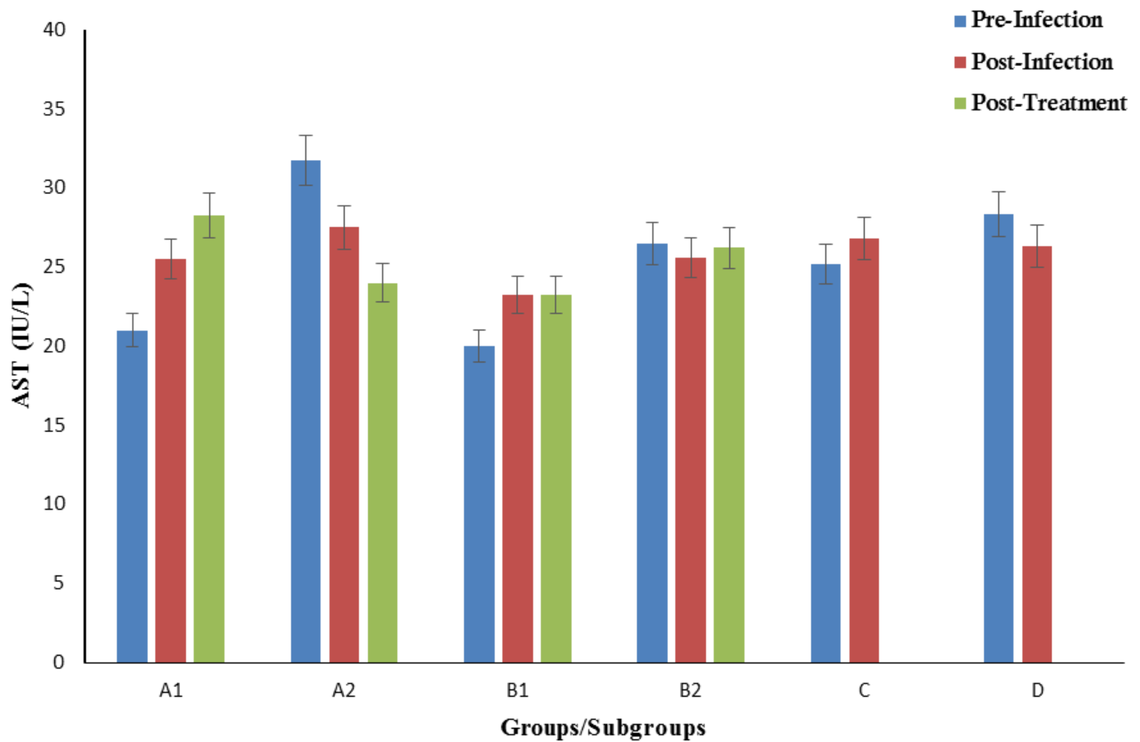
Appendix XIV

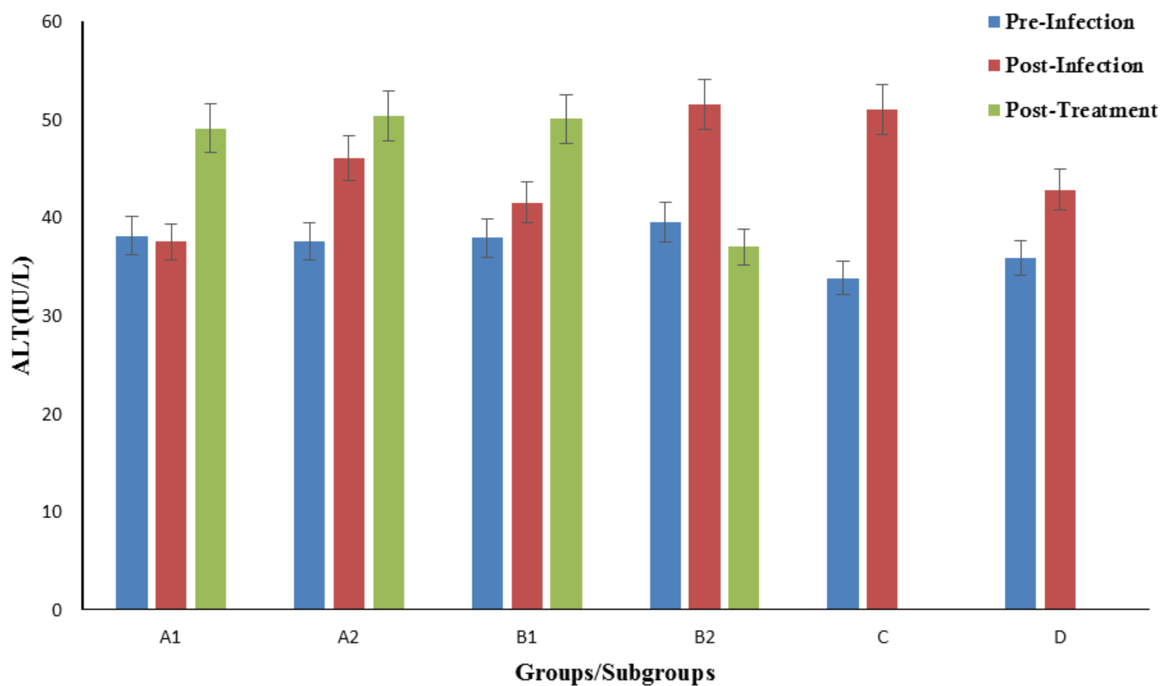




Bar charts showing mean creatinine and blood urea nitrogen concentrations in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

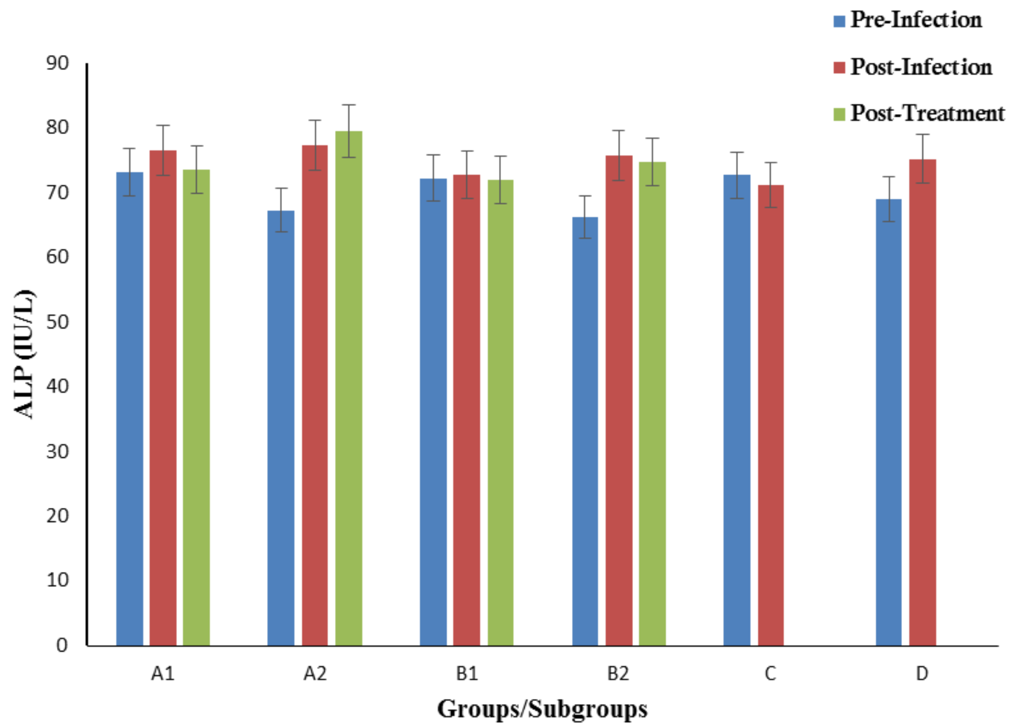
Appendix XV

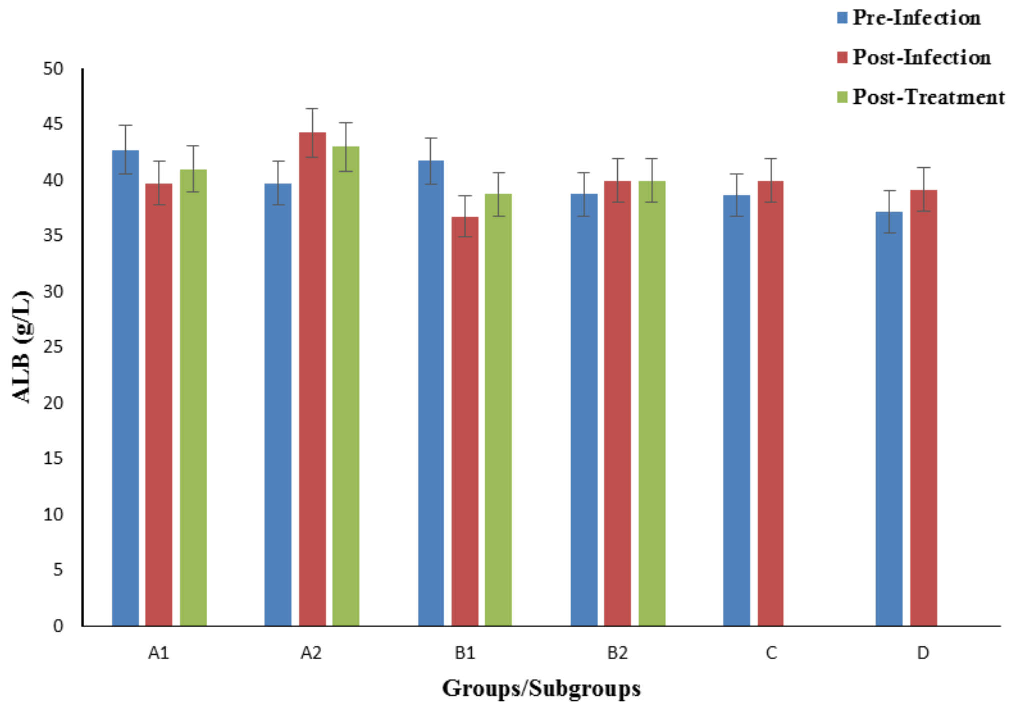




Bar charts showing aspartate aminotransferase and alanine aminotransferase activities in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

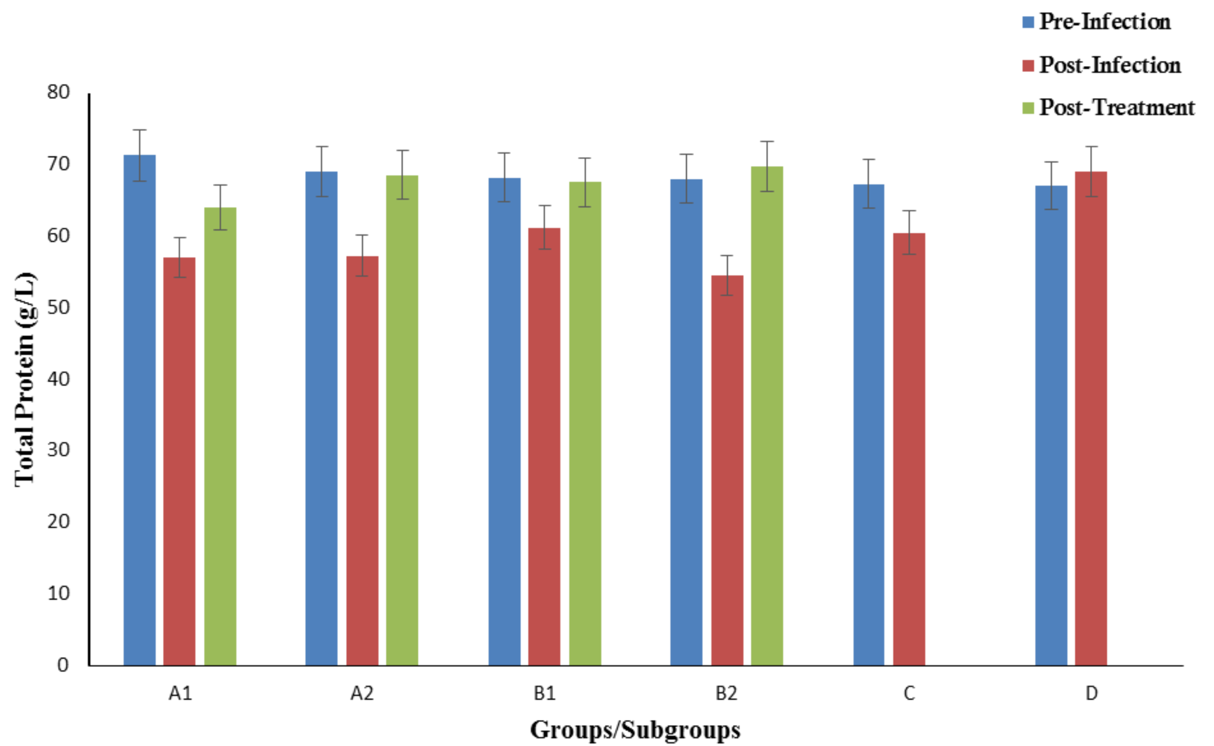
Appendix XVI

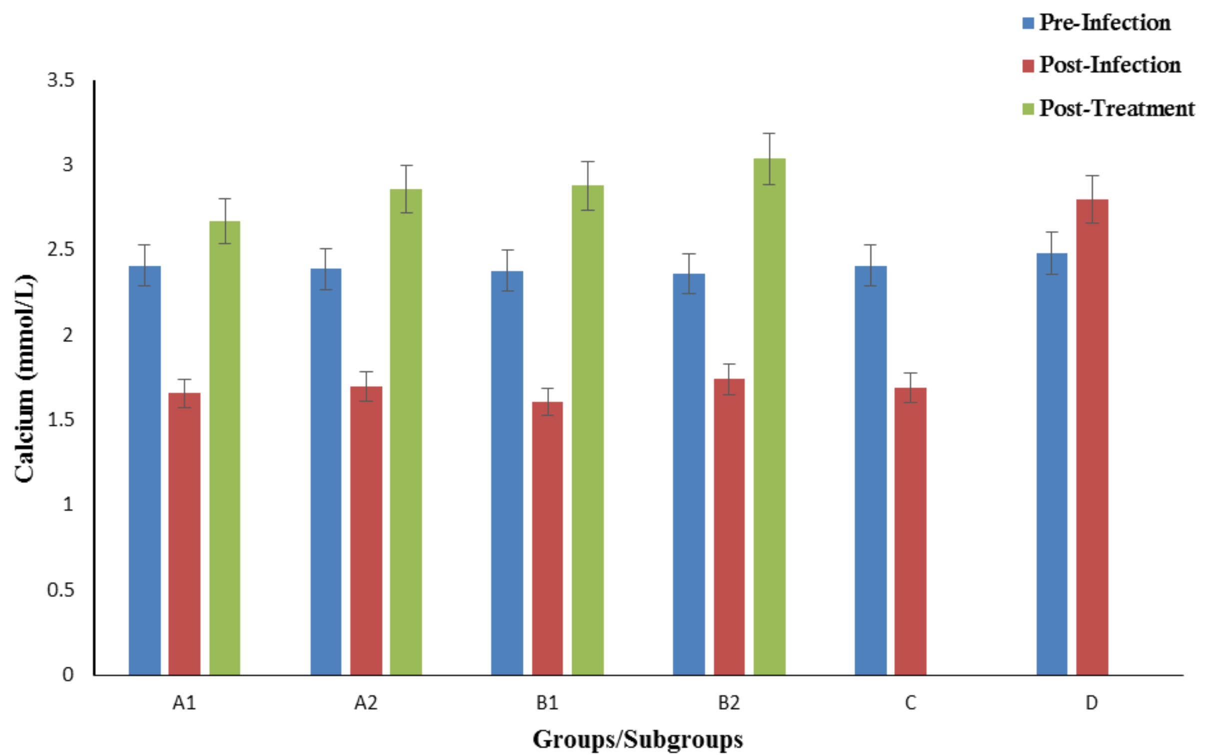




Bar charts showing alkaline phosphatase activity and albumin levels in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

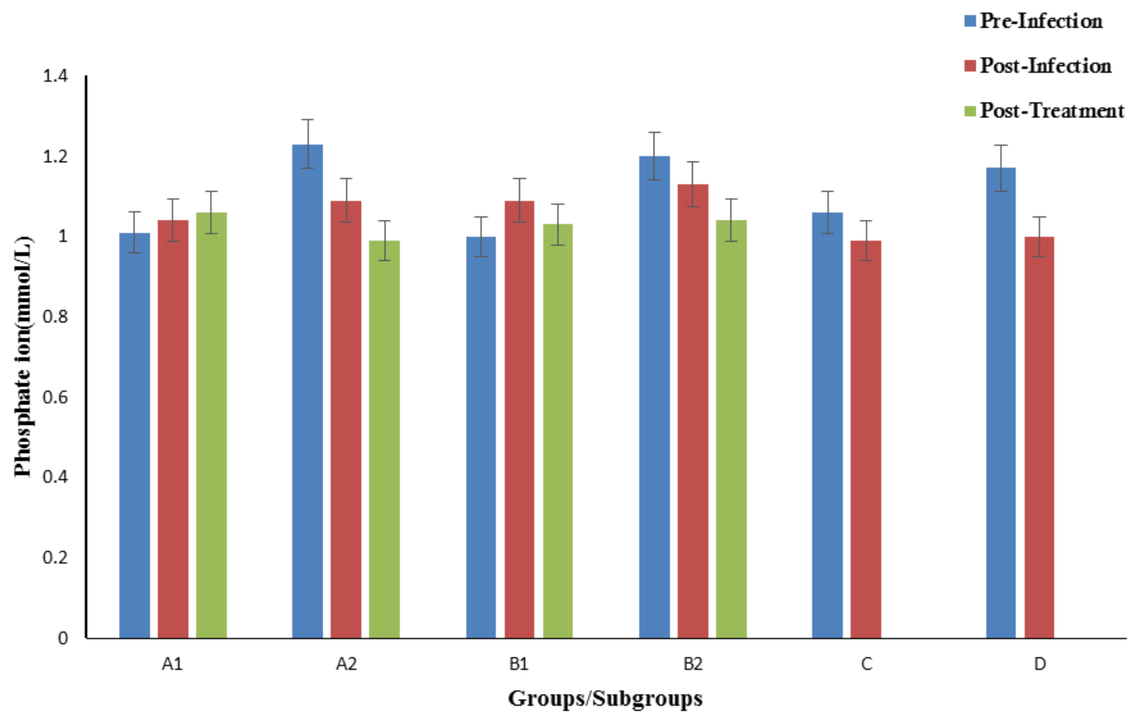
Appendix XVII

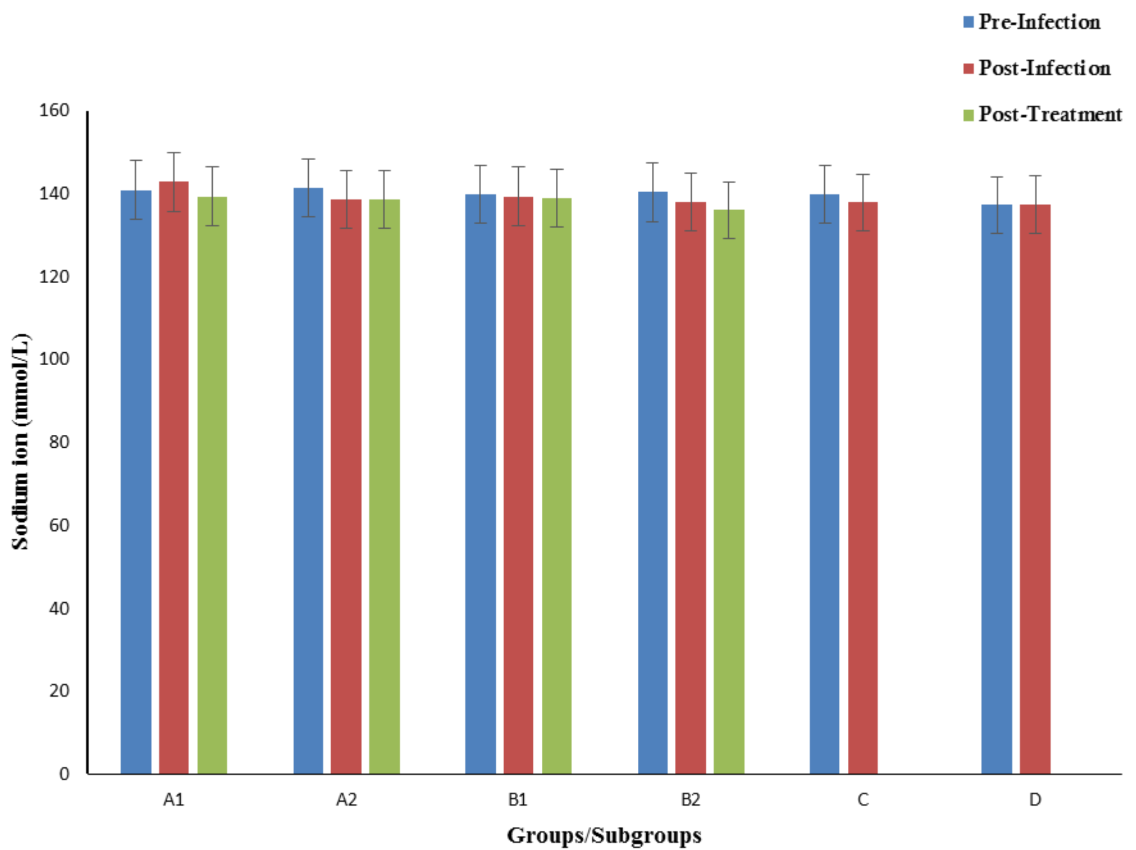




Bar charts showing mean total protein and calcium ion levels in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

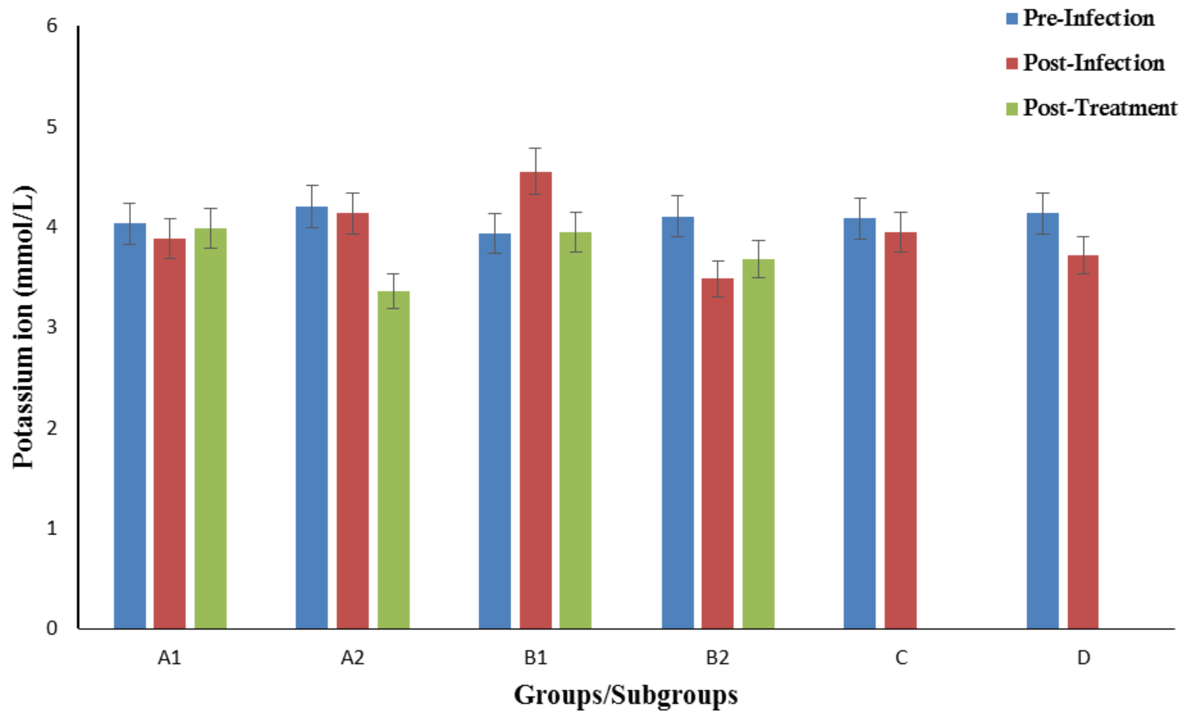
Appendix XVIII

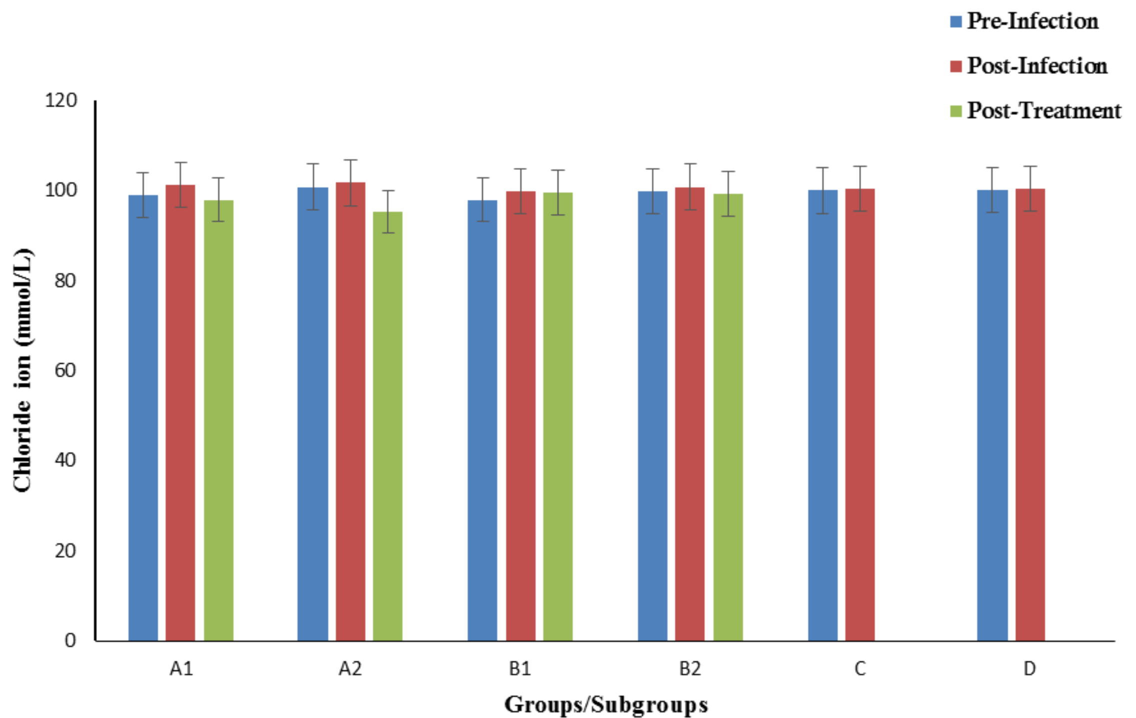




Bar charts showing mean phosphate and sodium ion levels in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

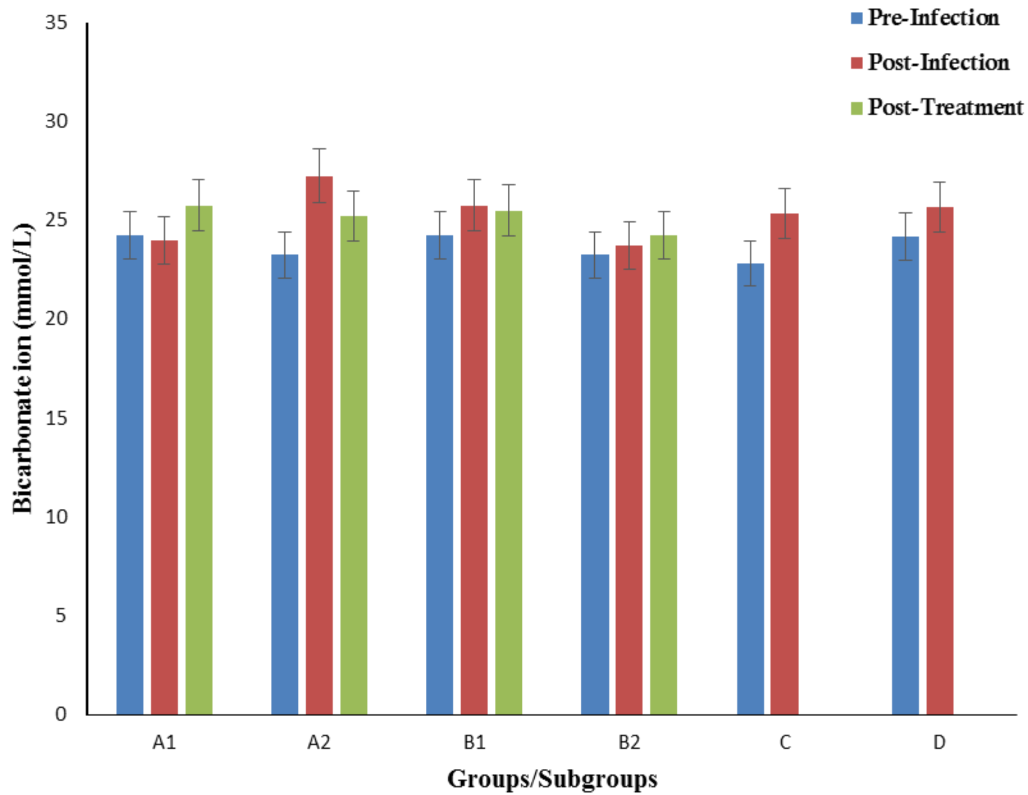
Appendix XIX

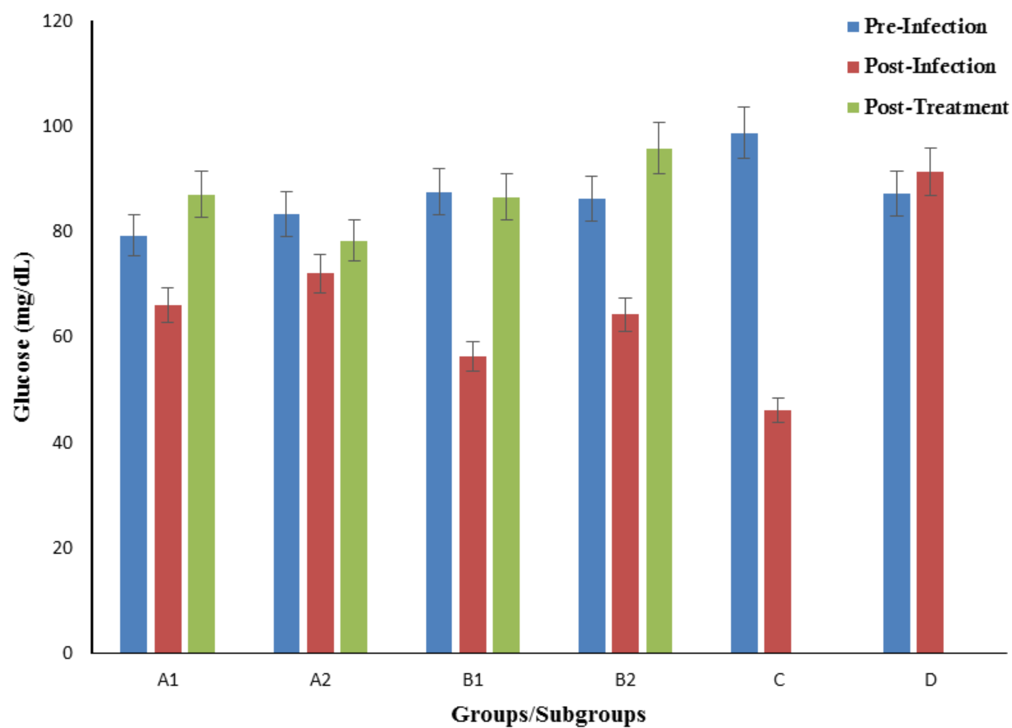




Bar charts showing mean potassium and chloride ion levels in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.

Appendix XX





Bar charts showing mean bicarbonate ion and glucose levels in donkeys infected with *T. brucei* (Federe) and treated with Novidium® and Sécuridium® at acute and chronic stages.