EFFICACIES OF BUPARVAQUONE AND ISOMETAMIDIUM CHLORIDE IN THE TREATMENT OF EXPERIMENTAL *TRYPANOSOMA EVANSI* INFECTION IN DONKEYS

BY

Umaru Musa GARBA

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

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BY

Umaru Musa GARBA
DVM, MSc.
Ph.D/VETMED/18715/07 – 08

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DEPARTMENT OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY
ZARIA

JUNE, 2015

DECLARATION

I declare that the work in this dissertation entitled 'Efficacies of Buparvaquone and Isometamidium chloride in the Treatment of Experimental *Trypanosoma evansi* Infection in Donkeys' has been performed by me in the Department of Veterinary Medicine under the supervision of Professor A.K.B. Sackey, Professor I. A. Lawal and Professor K.A.N. Esievo.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation had been previously presented for another degree or diploma at any university.

Umaru Musa GARBA _____ _____

Signature Date

CERTIFICATION

This dissertation titled "Efficacies of Buparvaquone and Isometamidium chloride in the Treatment of Experimental *Trypanosoma evansi* Infection in Donkeys" by Umaru Musa GARBA meets the regulation governing the award of the degree of Ph.D in Large Animal Medicine of Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

---------- Date----------
Prof. A.K.B. Sackey
Chairman, Supervisory Committee

---------- Date----------
Prof. Idris A. Lawal
Member, Supervisory Committee

---------- Date----------
Prof. K.A.N. Esievo
Member, Supervisory Committee

---------- Date----------
Prof. L.B. Tekdek
Head, Department of Veterinary Medicine,
Faculty of Veterinary Medicine,
Ahmadu Bello University, Zaria.

---------- Date----------
Prof. A. Z. Hassan
Dean, School of Postgraduate Studies,
Ahmadu Bello University, Zaria.

DEDICATION

This work is dedicated to my late father, Alhaji Garba Musa who died on 15 April, 2009 and my late sister Mrs Amina Garba Mohammed who died on 26 October, 2010. May Allah grant their souls Al-jannat Fiddaus, ameen.

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I seek Allah's blessings for those who contributed in various capacities towards the success of this study. Ameen!

ABSTRACT

The aim of this study was to evaluate the efficacies of Buparvaquone (an anti-theilerial drug) and Isometamidium chloride (a standard trypanocide) in the treatment of experimental *Trypanosoma evansi* infection in donkeys. Twenty four apparently healthy donkeys between 11 -15 months old were used for the experiment. Six animals were assigned to each of the experimental groups A_1, A_2, A_3 and B at random. Baseline values of vital parametres, body weight, haemogramme and serum biochemicals/electrolytes were evaluated 4 times at 7 days interval pre-infection. Donkeys in groups A_1, A_2 and A_3 were infected with 2mL of inoculum (about $2.0x10^6$ *Trypanosoma evansi*, Sokoto isolate) through the jugular vein, while group B was un-infected control. On day 28 post-infection, groups A_2 and A_3 were treated with Isometamidium chloride (1%), 0.5mg/Kg I.M. once and Buparvaquone (5%), 2.5mg/Kg I.M. twice, 3 days apart respectively. The animals were re-evaluated for parasitaemia post-infection and post-treatment using Haematocrit Centrifugation Technique and Mice Inoculation Test. Clinical signs, body weight changes, haemogramme picture and serum biochemical/electrolytes parametres were also re-evaluated. At post-mortem, internal organs were examined for gross lesions and tissue samples were taken, prepared and examined for histopathological changes. Organs impression smears were also prepared and examined for parasites. System Analytical Statistics (SAS) corporation-2010 was used to determine means of parametres which have been presented on tables and graphs. Analysis of Variance was used to compare group means of variables at 95% confidence level. Probability values less than 0.05 ($p<0.05$) were considered statistically significant. From the results, it was concluded that the pre-patent and incubation periods were 3-4 days and 3-7 days respectively. Parasitaemia was

undulating in the acute stage of infection but disappeared in the chronic stage from peripheral blood of infected-untreated animals from day 100 post-infection. Mice inoculation test revealed latent parasitaemia in the infected-untreated group with death of all mice used within 15 days post-inoculation from heavy parasitaemia. Isometamidium chloride, incompletely cleared parasitaemia in treated donkeys on day 11 post-treatment but showed 60% relapse on day 100 pos-treatment with low (only 1+) parasitaemia but no mice died during the 48 days of observation. Buparvaquone treated animals also showed disappearance of parasites from peripheral blood on day 49 post-treatment (depression) but recorded relapse in all treated donkeys and all mice used died from heavy parasitaemia within 23 days post-inoculation. The acute clinical signs were mild pyrexia, hyperpnoea, tarchycadia and lethargy, urticaria with alopecia and lymphadenopathy. Penile erection, rectal prolapse, rough hair coat, urticaria with alopecia, anaemia, isolation behavior, bilateral epiphora, pale mucous membranes were also recorded with acute mortality of 2/18(11%) and chronic mortality of 1/18(5.6%). The chronic signs were progressive emaciation, ventral abdominal oedema and recumbency. The haemopathological changes were anaemia, lymphopenia. Serum biochemical alterations were hypoglycemia, hypoproteinaemia, hypoalbuminaemia and elevated alkaline phosphatase. Electrolyte changes were hypocalcaemia and declined serum bicarbonate level. However, Isometamidium-treated animals showed hyperphosphatemia, hyperkalemia post-treatment. The gross pathological lesions were lungs congestion, frothy exudates in trachea and bronchi, hydrothorax, hydroperitoneum, adherence of renal capsule to the renal cortex and serous atrophies of mesenteric, peri-renal and coronary fats. The histopathological findings were congestion and mononuclear cellular infiltrations in lungs and liver.

Haemosiderosis and lymphocytes depletion were also observed in the spleen as prominent features of *Trypanosoma evansi* infection in donkeys. In conclusion, Isometamidium caused incomplete cure while buparvaquone treatment resulted in depression of parasitaemia.

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LIST OF ABBREVIATIONS

1. AAT	African Animal Trypanosomosis
2. A.B.U.	Ahmadu Bello University
3. ALB	Albumin
4. ALP	Alkaline Phosphatase
5. ALT	Alanine aminotransferase
6. AMP	Adenosine monophosphate
7. AST	Aspartate aminotransferase
8. Band	Band cell
9. bd wt	Body weight
10. BUN	Blood Urea Nitrogen
11. Buparv.	Buparvaquone
12. Ca^{2+}	Calcium ion
13. CATT	Card agglutination test
14. CFT	Complement fixation test
15. CG	Congestion
16. Cl^{+}	Chloride
17. CNS	Central nervous system
18. CR	Creatinine
19. ^{O}C	Degrees Celsius
20. DMEM	Dulbecco's Modified Eagle Medium
21. DNA	Deoxyribonucleic acid
22. EDTA-K^{+}	Potassium salt of Ethylene diamine tetra-acetic acid

23.	ELISA	Enzyme linked immunosorbent assay
24.	EC_{50}	Effective Concentration 50; is the concentration of a drug in blood that will cure 50% of treated animals
25.	Eos.	Eosinophil
26.	FAO	Food and Agricultural Organisation
27.	Fig.	Figure
28.	FCS	Foetal calf serum
29.	g	Gramme
30.	g/L	Grammes per liter
31.	HAT	Human African Trypanosomosis
32.	Hb	Haemoglobin
33.	HCO_3^{2-}	Bicarbonate ion
34.	HCT	Haematocrit Centrifugation Technique
35.	H-E	Haematoxylin and Eosin stain
36.	IFAT	Indirect fluorescent antibody test
37.	I.H.	Intermediate host
38.	ILRAD	International Laboratory for Research on Animal Diseases
39.	ILRI	International Livestock Research Institute
40.	I.M.	Intramuscular
41.	IP	Incubation period
42.	Isomet.	Isometamidium chloride
43.	IU/L	International Unit per Litre
44.	ITM	Institute of tropical medicine
45.	K^+	Potassium ion

46.	kDNA	Kinetoplast Deoxyribonucleic acid
47.	Kg	Kilogramme
48.	LD_{50}	Lethal Dose 50; is the quantity of a substance that will kill 50% of animals administered with the substance.
49.	Lymph.	Lymphocytes
50.	Mg/dL	Grams per deciliter
51.	Mg/kg	Milligramme per kilogramme
52.	Mg/L	Milligramme per liter
53.	MIT	Mice Inoculation Test
54.	µg/mL	Microgramme per milliliters
55.	ML	Milliliters
56.	MNC	Mononuclear cells
57.	MPE	Methanolic plant extract
58.	mmol/L	Millimole per liter
59.	Mono.	Monocytes
60.	µmol/L	Micromole per liter
61.	n	Group sample size
62.	N	Overall sample size
63.	Na^+	Sodium ion
64.	Neut.	Neutrophils
65.	NGL	No gross lesions
66.	OIE	International Organisation for Epizootics
67.	PCR	Polymerase Chain Reaction
68.	PCV	Packed cell volume
69.	PI	Post-infection

70.	PPP	Pre-patent period
71.	Pre-inf.	Pre-infection
72.	PM	Post-mortem
73.	PO_4^{2-}	Inorganic phosphate
74.	PT	Post-treatment
75.	QC	Quality control
76.	RBC or rbc	Red blood cells
77.	SC	Subcutaneous
78.	TP	Total proein
79.	USA	United States of America
80.	VAT	Variable antigen type
81.	pVAT	Predominant variable antigen type
82.	VSG	Variable Surface Glycoprotein
83.	WAD-goat	West African Dwarf- goat
84.	WBC	White blood cells
85.	WF	Wet Film
86.	WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Features of *Trypanosoma Species*

Trypanosomes are flagellated, protozoan parasites belonging to the genus *Trypanosoma* that live and multiply extra-cellularly in blood and tissue fluids of their mammalian hosts where they cause the disease termed trypanosomosis (Radostits *et al.*, 2000). Transmission of *Trypanosoma spp* is cyclical by haematophagous insects such as the tsetse fly, *Glosina spp* (Molyneux *et al.*, 1996) or by mechanical means via the serial bite of insect vectors, especially the horsefly (*Tabanus sp*) and stable fly (*Stomoxys sp*) as in the transmission of *Trypanosoma evansi* (Urquhart *et al*., 1996; Luckins, 1999). In a specie of *Trypanosoma* (*T. equiperdum*), transmission is venereal (Smyth, 1996).

Trypanosoma evansi is a slender trypanosome, monomorphic but may be pleomorphic in some strains with length of about 15 to 34 μm and is characterized by a long free flagellum which may be up to one half of the length of the organism with narrow and drawn out posterior end of the parasite (Queiroz *et al*., 2000). *Trypanosoma evansi* is morphologically identical with *T. equiperdum* and indistinguishable from slender forms of other members of the subgenus *Trypanozoon* (Van der Ploeg *et al*., 1985). *Trypanosoma evansi* has kinetoplast which is an aggregate of deoxyribonucleic acid (DNA) that is small, compact and disc-shaped situated within a large mitochondrion (Brun *et al.*, 1998). In most *Trypanosoma spp* structure, the aggregates of kinetoplast DNA (kDNA) are linked in a network to form larger aggregates refered to as maxicircles and smaller aggregates known as minicircles (Smyth, 1996). *Trypanosoma evansi* and *T. equiperdum* on the other hand, have only the minicircles and lack the maxicircles (Vickerman, 1985).

In a mammalian host, the cell surfaces of African trypanosomes are protected by a monolayer of a single variant surface glycoprotein (VSG) which is central to antigenic variation. One VSG gene is expressed at a time and it is switched to express a different VSG gene as a mechanism for resistance (Hutchinson *et al.,* 2007). The genome of *Trypanosoma brucei* contains a repertoire of more than 1000 VSG sequences (Hutchinson *et al.,* 2007).

It is reported that some of the trypanosomes such as *T. lewisi* are non-pathogenic to the hosts in wild rats of the genus *Rattus* (Smyth, 1996). Other well studied species such as *T. evansi* in wide range of animals is reported in several studies to be pathogenic to its hosts (Cadioli *et al.,* 2006; Habila *et al*., 2012).

1.2 Taxonomy

Trypanosoma species belong to the Phylum *Sarcomastigophora (Protozoa),* Subphylum *Mastigophora (Flagellata),* Class *Zoomastigophorea,* Order *Kinetoplastida,* Suborder *Trypanosomatina,* Family *Trypanosomatidae* and Genus *Trypanosoma* (Gluenz *et al.* 2011). The genus *Trypanosoma* consists of species of parasites that live in blood and tissue of vertebrates with invertebrates as intermediate hosts (I.H.) and are digenic (have two hosts in their life cycle) involving *trypomastigote, promastigote, amastigote* and *epimastigote* stages. Also, the infective stage for vertebrate hosts is the blood stream form, *trypomastigote* which is usually passed in blood meal taken by the insect vector as revised by Levine *et al.* (1980) and reported by Smyth (1996). The Family *Trypanosomatidae* is further classified into two namely:

(a) *Stercoraria* consisting of genera of *Trypanosoma* which complete their development in the hindgut (posterior station) of the intermediate host such that the infective stages are discharged in faeces and final hosts are infected through contaminative route (Smyth, 1996).

(b) *Salivaria* consists of genera of pathogenic species of *Trypanosoma.*They complete their development in the foregut (anterior station) of the intermediate hosts (I.H.) and the infective form (metacyclic stage) which is transmitted via inoculation of parasites-containing saliva of the I.H. into the final animal host during bite by the former (Smyth, 1996). The genus *Trypanosoma* is further classified into 3 subgenera namely *Nannomonas, Duttonella* and *Trypanozoon* (Hoare, 1972).

Nannomonas: This subgenus consists of *T. congolense* and *T. simae* (Smyth, 1996). Charracteristically, *T. congolense* exhibits relatively low parasitaemia in its main host (cattle), a behavior which does not favour mechanical transmission of the parasite (Desquesnes and Dia, 2003; Desquesnes *et al.*, 2009a).

Duttonella: The subgenus has *T. vivax,* found in Latin America (Jones and Dávila, 2001; Desquesnes, 2004) and Africa (Desquesnes and Dia, 2004). *Trypanosoma vivax* is cyclically transmitted mainly to bovine, ovine, caprine, camelids, wild animals and to a lesser extent the equidae (*D*ehoux *et al*., 1996; Da Silva *et al*., 2011). The blood stream form exhibits dimorphism with club-shaped and slender forms (Hoare, 1972).

Trypanozoon: This subgenus consists of three major trypanosome species that include *Trypanosoma brucei, T. evansi* and *T. equiperdum. Trypanosoma brucei* is further

divided into three subspecies namely *Trypanosoma brucei rhodesiense*, *T. brucei gambiense* and *T. brucei brucei* (Kirsten Gillingwater aus Hornussen, 2007; WHO, 2012).

1.3 Brief History of African Trypanosomosis

The disease which was formerly known as trypanosomiasis but now known as trypanosomosis, exists in Africa before 14^{th} century with episodes of epidemics as cited by Fèvre *et al.* (2008). It affected humans and animals with resultant annual deaths of 55,000 and 3,000,000 respectively (ILRAD, 1990; Abenga *et al.,* 2003; Mulumba, 2003). The distribution of trypanosomosis in Africa corresponds to the range of tsetse flies and comprises currently an area of 8 million km^2 between latitudes 14 degrees North and 20 degrees South (Molyneux *et al.,* 1996) covering areas in 36 countries with more than 60 million people infected in sub-Saharan Africa (Simarro *et al.,* 2012). In all susceptible mammals, the disease is characterized by parasitaemia, fever, anemia, poor body condition, reduced productivity, performance and mortality in affected population which together with factors negatively affect economic development of the rural areas in tropical Africa (Abenga *et al*., 2002; Fajinmi *et al*., 2007).

In Nigeria, 11species of the tsetsefly (out of 22 globally) infest over 80% of the 928,300 Km^2 of land mass involving the area across latitude $4^{O}N$ and $13^{O}N$ (NITR, 1987; Onyia, 1997). These include *Glosina spp* include *Glosina palpalis* (*G. palpalis*), *G. tachinoides*, *G. morsitans*, *G. submortisans* and *G. longipalis* (Davis, 1977). Surveys have shown that there is a wide geoecological distribution of animal trypanosomosis in Nigeria stretching from the mangrove forest to the Sudan savanna, owing to the presence of tsetse flies in these areas (Davis, 1977). Only about $1/5^{th}$ of the northern Sahel savanna and the

plateaus of Mambilla, Jos and Obudu are free from testes fly (ILRAD, 1990). However, Jos, Mambilla and Obudu highlands which were earlier declared as free of tsetse flies and trypanosomosis now have re-emergence of the vector and disease Dede *et al*., 2005) likewise in the Sahel around Maiduguri (Maxie *et al.,* 1979). These spread of trypanosomes and *Glosina spp* is enhanced by movement of cattle from tsetse fly infested to tsetse fly free areas (Anene *et al*., 1999). Recent report on study conducted in Sokoto Nigeria using polymerase chain reaction to evaluate trade cattle for trypanosomes, showed that amongst positive cases, (66.7%) had *Trypanosoma vivax and* 22 (4.4%) positive cases *Trypanosoma brucei* group infection (Fajinmi *et al.,* 2011) wich signals further danger especially with the *T. brucei* group which may include *T. evansi*.

Ilemobade (1971) in addition to earlier investigators reported the existence of *Trypanosoma evansi* incfection in horses, donkeys and cattle in Nigeria.

1.3.1 African Animal Trypanosomosis

African animal trypanosomosis (AAT) is a disease complex, commonly known as *Nagana* in East, Central, Southern Africa and *Samore* by Fulani ethnic group in Nigeria (Radostits *et al*., 2000). The four main species of trypanosomes responsible for the disease in livestock (cattle, pigs, camel, sheep and goats) are *T. brucei brucei* which is an animal pathogen only, amongst the subspecies of *T. brucei* (Kirsten Gillingwater aus Hornussen, 2007; WHO, 2012), *T. congolense, T. vivax (*Geerts *et al.,* 2001; Winkle, 2005) and *T simiae* (Radostits *et al.,* 2000). The disease is prevalent in countries where Tsetse fly, *Glosina spp* is endemic which transmits the trypanosomes cyclically (Da Silva *et al.,* 2011). The tsetse-fly-infested area of Africa extends from the southern edge of the Sahara

desert (latitude 15°N) to Angola, Zimbabwe, and Mozambique (latitude 29°S). However, *T. vivax* is also transmissible mechanically, thereby, extending its geographical spread beyond 'tse-tse fly belt' to Western Hemisphere in at least 10 countries in the Caribbean, South and Central America where there is no tse-tse fly (Anonymous, 2009)

In Kenya, it occurs with prevalence of 41% and 29% around Suba and Teso districts respectively (Thumbi *et al*., 2010), In domestic animals, AAT causes severe, often fatal disease whose major signs include fever, listlessness, emaciation, alopaecia, epiphora, oedema, anaemia, and paralysis (Winkle, 2005). As the illness progresses, the animals become weak and unfit for work, hence the name of the disease "N'gana" which is a Zulu word that means loss of spirit, "powerless/useless", thereby, making livestock farming very difficult in the tsetse belt (Winkle, 2005). In wild animals, the causative parasites of AAT cause relatively mild infections (Herrera *et al.*, 2002; Winkle, 2005).

1.3.2 Human African Trypanosomosis

Human African Trypanosomosis (HAT), also referred to as 'sleeping sickness' disease is charracterised by weakness and central nervous system symptoms in the infected human (WHO, 2012). It is caused by *Trypanosoma brucei rhodesiense* in Eastern and Southern Africa causing acute form of HAT which accounts for less than 5% of reported cases of the disease (Igbokwe, 1989; WHO, 2012). *Trypanosoma brucei gambiense*, on the other hand, is the causative agent of the chronic form of HAT in Western and Central Africa which accounts for greater than 95% of reported cases of the disease (Kirsten Gillingwater aus Hornussen, 2007). *Trypanosoma brucei rhodesiense* is a phenotypic variant of *T. brucei brucei* and genetically *T. brucei gambiense* has 2 groups:

The group I, is genetically more diverse while the group II is closely related to *T. b. brucei* (Balmer *et al*., 2011). According to the World Health Organization report (2012), countries where the disease is currently epidemic include Angola, Democratic Republic of the Congo, Uganda & Sudan. Countries with high levels endemicity of including Cameroon, Congo, Cote d'Ivoire, Central African Republic, Guinea, Mozambique, Tanzania, & Chad. African sleeping sickness can also be found in low endemic levels in Benin, Burkina-Faso, Gabon, Ghana, Equatorial Guinea, Kenya, Mali, Nigeria, Togo, & Zambia. Because of poor disease surveillance and reporting, epidemiological information in Burundi, Botswana, Ethiopia, Liberia, Namibia, Rwanda, Senegal, & Sierra-Leone is scanty and not well understood.

Another form of human trypanosomosis is caused by *T. cruzi* and is referd to as "chagas" disease which occurs mainly in Latin America (WHO, 2013).

1.3.3 *Trypanosoma evansi* infection

Trypanosoma evansi infection in animals causes a progressively wasting and ultimately fatal animal trypanosomosis (Mahmoud and Gray, 1980; OIE, 2004). The disease is known with over 30 different ethnic terms which describe the chronic devastating nature of the infection in domestic camels and equids in different geographical regions of the world as reviewed by Hoare (Stephen, 1986). Such names include '*surra'* in Asia and Middle East (OIE, 2004) which is an *Indi* word meaning 'rotten' (Vittoz, 1955), *El-debab* in Algeria (Atarhouch *et al*., 2003), *Mbori* in Sudan, *Guifar* in Chad and *Pmenchaca* amongst Tuaregs of Niger (Antoine-Moussiaux *et al*., 2007).

Trypanosoma evansi has the widest distribution of all species of trypanosomes and the greatest range of domestic and wild mammalian hosts throughout the tropical and subtropical areas of the world (Eyob and Matios, 20013). It occurs in places such as across Asia, China, Philipines, parts of Indonesia and Russia, Middle East (Pacholek *et al*., 2001), Africa, Central and South America (Dia *et al*., 1997). Such infections result in great economic losses due to death of thousands of affected animals annually (Herrera *et al*., 2004; OIE, 2004; Da Silva *et al.,* 2009; Fernandez *et al*., 2009) as well as reduced traction power of infected animals (ILRI, 1997). Zoonotic status of the parasite has been reported in India (Fernandez *et al*., 2009). Surra affects mainly camels but cattle and equidae (horses, donkeys, zebra and mules) are also affected in Africa while other hosts of the parasite are dogs and pigs (Mahmoud and Gray, 1980; OIE, 2004).

Surra is endemic in donkeys in Nigeria (Ilemobade, 1971). *Trypanosoma equiperdum* is a dyskinetoplastic variant of *T. evansi* and infects only equids (horses, donkeys and mules) causing venereal disease referred to as “Dourine" in horses (Hoare, 1972; Smyth, 1996).

Because *T. evansi* had lost the genetic ability to undergo cyclical transmission (Ventura *et al.,* 2000), transmission is mechanical in nature, by biting flies mainly *Tabanus sp, Stomoxys sp, Haematopota sp, Lyperosia sp* and *Chrysops sp* (Luckins, 1999). Transplacental transmission also occurs in donkeys without the transfer of maternally-derived, *T. evansi*-induced antibodies to neonatal foals (Kumar *et al*., 2015).

1.3.4 Review of treatment of *Trypanosoma evansi* infection

At the moment, chemotherapy and chemoprophylaxis are the main measures against *T. evansi* infection in camels, cattle and horses, relying on diminazene aceturate, quinapyramine, cymelarsan and suramin sodium (FAO, 2012). The limiting factors to the control of the disease include emerging resistance by *T. evansi* strains (El Rayah *et al.*, 1999; Zhou *et al.,* 2004) and other species of *Trypanosoma* (De Koning, 2001; Suswam *et al.,* 2001) to available drugs, toxicity of the drugs to treated animals as well as exhibition of antigenic variation which hampers vaccine production (Doua and Yapo, 1993).

1.4 Statement of Research Problem

Donkeys are widespread in Nigeria and owned by both pastoralists and farmers especially in the Northern part as source of traction power or means of transport (Blench *et al.,* 2013) because they are easy to manage as they tolerate some tropical diseases and parasites, survive on poor quality feeds and thrive under adverse climatic conditions (Aganga *et al.,* 2000). Despite the benefits and advantages of keeping donkeys, surra remains a protracted and fatal disease of camels and equids in Nigeria (Ilemobade, 1971), that is limiting the work efficiency and productivity of affected animals (ILRI, 1997).

There is also the problem of reported cases of resistance by *T. evansi* strains to the available range of trypanocides namely Diminazene aceturate, quinapyramine, cymelarsan and suramin in different countries (De Koning, 2001; Suswam *et al.,* 2001; Zhou *et al.*, 2004; FAO, 2012).

Data available on donkeys is also scanty due to little research attention accorded the animal specie (Blench *et al.,* 2013).

These drawbacks in the control of surra in Nigeria, stimulated the desire to conduct a study on the efficacies of other antiprotozoal drugs like Buparvaquone (an anti-theilerial drug) and Isometamidium chloride (a standard trypanocide) in the treatment of experimental *T. evansi* infection in donkeys with a view to making recommendations on the use of the drugs for treatment as well as provide additional information on surra in donkeys in Nigeria.

1.5 Justification of the Study

In Nigeria, donkeys are used as source of traction power in transport and ploughing (Blench *et al.,* 2013). Unfortunately, *T. evansi* infection causes protracted and fatal disease in donkeys and camels (horses are also affected) in the country thereby reducing their performance at work (ILRI, 1997).

The existence of reports on resistance of several strains of trypanosomes to standard trypanocides such as diminazene diaceturate and homidium compounds in the treatment of African Animal Trypanosomosis 'ngana' (Afewerk *et al.,* 2000; Anene *et al.,* 2001; Geerts *et al.,* 2001) reduces treatment successes. Also there are reports of increased risks of toxicities of Isometamidium chloride in camels with *T. evansi* infection (Aliu, 2007) and of diminazene aceturate in horses and mules (Tuntasuvan, *et al.,* 2003), thereby limiting the use of the drugs in treatments which further necessitates the quest for new, better and cheaper trypanocides (Dietmer, 2008) .

Despite the value and widespread nature of donkeys globally, The animal has received only little attention in terms of research and development (Starkey, 1994; Blench *et al.,* 2013) resulting in availability of only scanty scientific data on donkeys.

Buparvaquone is both a curative and prophylactic drug as a single dose, at the rate of 2.5 mg/kg bd wt intramuscular against *Theileria parva, T. sergenti, T. annulata* in cattle with 86% to 91% cure (Dhar *et al.,* 1987; Mutugi *et al.,* 1988). It is also parasite-specific and acts by disrupting energy generation by *Theileria spp* to cause progressive cytoplasmic vacuolation and death of schizonts (Fry *et al.,* 1984). It has high in vitro activity with EC_{50} of 0.0003 mg/L (10^{-9}M) against *T. parva* (Muguga; E174 strain), Long plasma half-life of at least 7 days. Its oral LD_{50} in rats is greater than 8,000 mg/kg (Kinabo and Bogan, 1988) and, therefore, can be described as a practically non-toxic substance (Satish, 2002). It is also safe in donkeys based on the activities of haematological and serum biochemical parametres following its administration (Kumar *et al.* 2003).

Isometamidium chloride, on the other hand, is a standard trypanocide with curative and prophylactic actions, is registered for use principally in cattle, sheep, goat, camel, horse and dog with efficacies against *T. congolense, T. vivax*, *T. brucei* and *T. evansi* (Touratier, 1981; Aliu, 2007) at the standard dose rates of 0.25–0.5mg/kg bd wt to achieve cure and at 1mg/kg bd wt for prophylaxis (FAO, 2012).

Therefore, based on the above information, efficacies study on buparvaquone and isometamidium in the treatment of experimental *T. evansi* infection in donkeys is justified.

1.6 The Aim and Objectives of the Study

1.6.1 Aim

The aim of this study was to assess the efficacies of buparvaquone and isometamidium chloride in the treatment of experimental *T. evansi* infection in donkeys (*Equus africanus asinus*).

1.6.2 Objectives

The objectives of the study were to:

1. Determine the parasitaemia pattern, and disease course including vital parametres and body weight changes in donkeys experimentally infected with *T. evansi*.

2. Determine the effect of experimental surra on haematological picture, some serum biochemical and electrolyte levels, gross and histopathological features as well as level of invasion of major organs by *T. evansi* in donkeys.

3. Determine the effect of treatment with Buparvaquone and Isometamidium in the amelioration of experimentally-induced surra in donkeys.

4. Observe the infected animals for resurgence of parasitaemia (relapse) after treatment.

1.7 Research Questions

1. Has experimental *T. evansi* (Sokoto isolate) infection in donkeys any effect on pattern of parasitaemia?

2. Are Buparvaquone (Butalex®) and Isometamidium chloride (Securidium®) effective against experimental *T. evansi* infection in donkeys?

3. Are there pathological changes associated with experimental *T. evansi* infection in donkeys at post-infection and post-treatment phases as well as any evidence of invasion of major internal organs?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Trypanosoma Evansi* Infection

Trypanosoma evansi infection (Surra) is a progressively wasting and ultimately fatal animal trypanosomosis that affects mainly camels, cattle and equidae (horses, donkeys, zebra and mules) in Africa (Mahmoud and Gray, 1980; OIE, 2004), Asia, South America and Middle East (El Rayah *et al.,* 1999; Giardina *et al.,* 2003).

2.1.1 Aetiology

The causative agent of surra, *Trypanosoma evansi* (*T. evansi*), is a member of the subgenus, *Trypanozoon* which also includes other subspecies of the Genus, *Trypanosoma* like *T. equiperdum, T. b. brucei, T. b. rhodesiense* and *T. b. gambiense* (Hoare, 1972). Molecular characterization studies indicate that various strains of *T. evansi* isolated from Asia, Africa and South America are very homogeneous and may have a single origin (Ventura *et al.,* 2002). Some findings suggest that *T. evansi* could have emerged from *T. brucei* in several instances and microscopically resembles the slender forms of the tsetse-transmitted *T. brucei brucei, T. b. rhodesiense* and *T. b. gambiense* (Jensen *et al.*, 2008; Lai *et al.,* 2008).

Biologically *T. evansi* is very similar to *T. equiperdum* and the latter is the causative agent of the sexually transmitted trypanosomosis referred to as “dourine” in horses (Brun *et al.,* 1998; Claes *et al*., 2003a). Both parasites are thought to be derived from *T. brucei*

which has abundant maxicircles and minicircles present in its kinetoplastic DNA (Li *et al*., 2006) while the maxicircle is lost in *T. evansi* (Smyth, 1996). Also the minicircle complexity, which is very high in *T. brucei* (hundreds of minicircle sequence classes) is on the other hand, scarce, if any, in *T. evansi* and *T. equiperdum* (Gibson and Stevens, 1999).

Molecular characterization, using random amplified polymorphic DNA techniques and endonuclease fingerprinting showed that isolates of *T. evansi* and *T. equiperdum* formed a closely homogeneous group (Zablotskij *et al.,* 2003). The difficulties in differentiating *T. equiperdum* from the other *Trypanozoon* species have been stressed and the existence of *T. equiperdum* was even questioned (Claes *et al*., 2005). This is because, like all pathogenic trypanosomes, *T. evansi* is covered by a dense protein layer consisting of a single protein called the variable surface glycoprotein (VSG). This acts as a major immunogen and elicits the formation of specific antibodies. Studies on *T. brucei* showed that the parasites are able to evade the deleterious effect of these host-immune reactions by switching from one VSG to another in a repertoire of >1,000 VSG sequences coded for in its genome; a phenomenon known as antigenic variation (Hutchinson *et al.,* 2007). *Trypanosoma evansi* also undergoes antigenic variation (Van der Ploeg *et al*., 1985; Borst *et al*., 1987).

Following antigenic variation, there is the formation of variable antigen types (VAT) of *T. evansi*. A particular type will predominate in abundance and so tagged "predominant variable antigen type" (pVAT) coded RoTat 1.2. This RoTat 1.2 is in abundance in early phase of *T. evansi* infection. Most *T. equiperdum* strains are reported to express isoVATs

of RoTat 1.2. This explains, in part, why antibody tests based on *T. evansi* RoTat 1.2 cannot reliably distinguish between infections caused by *T. evansi* and those caused by *T. equiperdum* unless it can be proven that most described *T. equiperdum* are actually misclassified *T. evansi* (Claes *et al.*, 2003b).

2.1.2 Morphological features of *Trypanosoma evansi*

The features of classical *T. evansi* in camel blood studied using microscopy are those of *Trypanozoon* parasites and include large size (compared to *T. congolense*) of about 25-35µm, slender body with mean length of 24±4µm (minimum15 µm, maximum 33µm), small size when compared with *Trypanosoma theileri* (Tejero *et al.*, 2008). *Trypanosoma evansi* has small and sub-terminal kinetoplast (smaller than in *T. vivax*), thin posterior extremity, conspicous undulating membrane, a central nucleus, long free flagellum and moves actively in blood producing limited displacements of blood cells when examined in wet film under the microscope (Tejero *et al.*, 2008). On a Giemsa stained thin smear, *T. evansi* has generally been described as a monomorphic, slender trypomastigote parasite (Losos, 1980). Some intermediate forms may appear stumpy with short free flagellum and posterior extremity that has almost terminal kinetoplast suggesting polymorphism in some isolates which is an inconsistent finding and appears only sporadically in *T. evansi* (Hoare, 1972; Losos, 1980). The size and shape of the blood forms of *T. evansi* are not in relation with genetic characteristics, but more or less with the growing conditions of the parasite and the immune response of the host (Tejero *et al.*,

2008). The sporadically appearing forms of the parasite creat confusion during species identification on blood smears, since the truncated parasite may look like *T. vivax* as observed in a recent case in Spain. However, the kinetoplast is larger in *T. vivax* than in *T. evansi* (Tamarit *et al.,* 2010).

2.1.3 Transmission

Trypanosoma evansi is primarily transmitted mechanically by haematophagous flies mainly *Tabanus spp* (Horse flies, March flies) and *Stomoxys spp* (Stable flies) which are endemic in Africa, Asia and South America; although in America the vampire also acts as a vector as well as reservoir hosts (Urquhart *et al*., 1996). Horseflies (*Tabanus spp*), is the most important, large, aggressive feeders whose sucking act causes avoidance behaviour in its host and the half-fed fly moves to another host animal for more blood meal by bite (Gruvel, 1980). These acts of the fly result in simple and direct pathogens transfer from an infected host to another susceptible animal by inoculation along with the saliva of the biting insect (Losos, 1980; Foil, 1989). Other flies like *Stomoxys spp, Haematopota spp, Lyperosia spp, Chrysops spp, Musca spp* and *Atylotus spp* are also mechanical transmitters of *T. evansi* (Gruvel, 1980). Transplacental transmission occurs in donkeys (Kumar *et al.,* 2015). Consumption of milk and meat of infected animals by carnivores, coital, iatrogenic means, Vampire bats and ticks are being proposed as means of transmission of the parasite (Herrera *et al.,* 2004).

It is reported that *T. evansi* losses viability after 7 minutes in the mouthpart of *Stomoxys niger niger* (Sumba *et al.,* 1998) and 15 minutes in *Tabanus striatus* (Mitzmain, 1914). *Trypanosoma evansi* parasite also losses viability and infectivity when exposed to ultraviolet light for one minute as well as after 8 hours of the death of an infected animal and in such situations, it cannot be transmitted by haematophagous flies (OIE, 2004). For transmission to be successful, parasitemia in the infected host must be at least 1 parasite per 6nL of blood and the interval between successive blood meals by the vector must be short (Dargantes, 2010).

2.1.4 Life cycle of *Trypanosoma evansi*

Trypanosoma evansi differs fundamentally from *T. brucei* in that it cannot complete normal cyclic development in tsetse fly, because strains of *T. evansi* lack maxicircles in their kDNA networks which they had lost genetically (Lun and Desser, 1995; Ventura *et al.,* 2000). It is established that transmission through the tsetse fly requires a functioning mitochondrion, which explains the failure of the cyclical process and, instead, *T. evansi* is mechanically transmitted by other blood sucking flies in enzootic areas of the world (Brun *et al.,* 1998; Rodgers, 2009). The insect vector takes up trypomastigotes (blood stream form of the trypanosome unique to species in the genus *Trypanosoma*) along with blood meal from an infected vertebrate host but no significant transformation of the parasite occurs in the mouthpart of the vector. Within minutes, the parasitemic blood is inoculated into another susceptible host by the vector in an effort to take another blood meal (Foil, 1989). At the site of inoculation in natural *T. evansi* infection, blood stream form

transforms and divides by binary fission, followed by distribution to other organs and tissues like blood, lymphatic and cerebrospinal fluid. Another set of infective trypomastigotes would have emerged and ready for another cycle of infection (Smyth, 1996).

Moreover, in marked contrast to the situation in *T. brucei*, the minicircles of *T. evansi* show great deal of homology in strains from different parts of the World, suggesting restrictions on the ability of *T. evansi* to mate (Al-Taqi, 1989), whereas in *T. brucei*, the highly heterogeneous minicircles are postulated to have arisen from much more frequent exchanges of kinetoplast Deoxyribonucleic Acid (kDNA) at the insect stage (Van der Ploeg *et al.,* 1985; Borst *et al.,* 1987).

2.1.5 Epidemiology

2.1.5.1 *Hosts and distribution of Trypanosoma evansi infection*

Trypanosoma evansi is the most widespread pathogenic trypanosome (Luckins, 1988; Cadioli *et al.,* 2006) and has the widest mammalian host range globally (Herrera *et al.,* 2002; Lun *et al.,* 2004; Zhou *et al.,* 2004). It is also reported to establish in pigeon nestling Manda *et al*., 2008). The ability to be transmitted by other blood-sucking insects apart from *Glossina spp*, enhanced *T. evansi* spread into several African nations in northern part of the Sahara desert (Elamin *et al*., 1999) such as Nigeria (Losos, 1980), Mauritania (Dia *et al*., 1997), Niger (Pacholek *et al*., 2001), Kenya (Njiru *et al*., 2001) and Ethiopia (Zeleke and Bekele, 2001). *Trypanosoma evansi* infection is also present in Sudan (Ali *et al*., 2011), Algeria, Tunisia, Libya, Egypt, northern parts of Mali, Burkina

Faso, Chad and Somalia (Hoare, 1972). Other endemic areas include Asia Minor, Pakistan, India, the USSR, China, Sumatra, Java, the Philippines, Mauritius, Madagascar, South and Central America (Elamin *et al*., 1999), Indonesia and Middle East countries such as in Iran (Zarif-Fard *et al*., 2001) and Israel amongst camels, donkeys and horses (Berlin *et al.*, 2010). The disease causes great economic losses due to thousands of deaths of affected animals annually (OIE, 2004; Mihret and Mano, 2007). Camels' movement as means of transport introduced *T. evansi* into Australia, North America and South-West Africa, thereby, extending the geographical distribution of surra (Elamin *et al*., 1999).

In Africa, Camels, cattle and equidae are the most susceptible and important hosts for *T. evansi* (Mahmoud and Gray, 1980; OIE, 2004). It causes progressively wasting and ultimately fatal animal trypanosomosis called *Surra* in India. Other terms by which the disease is referred to include *Mal de Caderas* in South America, *El debab* in the Middle East and Africa and *Gufar* in Sudan (El Rayah *et al.*, 1999; Giardina *et al.*, 2003). An outbreak of surra in horses, camels and donkeys in a farm in Israel with the highest prevalence in camels (80%), followed by donkeys (46%) and horses (43%), the affected animals showed anaemia and parasitaemia (detected on blood smears), some were asymptomatic latent carriers and others were only seropositive and thus considered to be suspected carriers (Berlin *et al.*, 2010).

2.1.5.2 *Trypanosoma evansi infection in Nigeria*

Studies on *surra* in Nigeria showed that natural clinical disease occurs with high incidence in camels (Losos, 1980), horses, donkeys (Ilemobade, 1971). *Trypanosoma*

evansi infection has its lowest infection rate in cattle compared to other animal trypanosome species and extends to the southern part of Nigeria were it occurs in trade cattle (Takeet *et al.,* 2013). Savannah Brown goats have been reported to be susceptible to *T. evansi* infection under experimental condition (Shehu *et al*., 2006) but infection was subclinical in West African Dwarf (WAD) goats who gain weight despite infection with the Sokoto isolates of the parasite (Ogbaje *et al.,* 2011).

Zoonosis of *T. evansi* was first reported following a human case in India in 2005 and was treated successfully using suramin which was well tolerated by the patient (Joshi, *et al.,* 2006). Therefore, suramin should be considered in the treatment of cases of human *T. evansi* infection (WHO, 2005; Joshi, *et al.,* 2006). There is no report yet of human case of *T. evansi* infection in Nigeria.

2.1.5.3 *Course of Trypanosoma evansi infection and risk factors*

The clinical signs in horses naturally infected with *T. evansi* include a clinical course of 2 to 20 days, marked ataxia, blindness, head tilt and circling, hyperexcitability, obtundity, proprioceptive deficits, head pressing, and paddling movements (Rodrigues *et al.,* 2009). The disease may be acute or chronic, and in the latter case may persist for several months, possibly years (OIE, 2010). In horses, donkeys, mules, dogs and cats, surra is mostly acute and rapidly fatal (Hoare 1972). However, these host species may develop mild or subclinical infections as in wild animals such as deer, capybara and coati which may also constitute a reservoir (Anonymous, 2009). Severerity of surra depends on parasite strain, host specie, immune staus of the host animal and stress factors such as

malnutrition and pregnancy (OIE, 2010). *Trypanosoma evansi* is reported to be viable in dead animal hosts for up to 13 hours in rabbits and 14 hours in rats and mice (Mandal *et al*., 2014).

2.1.6 Pathogenesis

Trypanosoma evansi is inoculated into susceptible host from the contaminated mouthpart of biting flies or saliva of vampire bats during bite of the host resulting in the formation of localized skin swelling known as *chancre* within which the first trypanosomes multiply by binary fission (Luckins *et al*., 992). The parasites then multiply further in the blood and body fluids causing pyrexia, inflammation and anaemia as in *T. brucei* infection (Radostits *et al.,* 2000). During high parasitaemia period the host animal develop hypoglycaemia due to utilization of host glucose reserves by the parasites and increased demand by host animals cells for metabolism (Habila *et al*., 2012). Similarly, the anaemia causes pale mucous membranes as a result of reduced peripheral circulation (Rickman and Cox, 1983), tissue anoxia which results in complications such as weakness and gradual weight loss in *T. evansi*-infected animals (Gardiner and Mahmoud, 1990). Hyperpnoea and tachycardia are also observed when anaemia had set in and are usually compensatory responses to tissue anoxia and hypovolemia (Silva *et al.,* 1995).

The animal also may develope conditions like lymphadenopathies due to immunologic response by lymphoid tissue to the infecting *T. evansi* (Klaus, 1996), Rough hair coat, urticaria, and bilateral seromucous epiphora also sets in due to the effects of immune complexes deposition, tissue inflammation and anoxic tissue damage characteristic of *T. evansi* infection in animals (Damayanti, 1993; Enwezor and Sackey, 2005). Epiphora may be observed in some infected animals due to irritation and

inflammatory tissue reaction that accompany the entry of *T. evansi* into the anterior chamber of the eye as reported for horses (Stephen, 1986). Intravascular coagulopathy occurs in horses with *T. evansi* infection and is reported to be responsible for persistent penile erection that occurs in horses with the infection as reported by Lingard (1896) and cited by Stephen (1986). Some animals develope diarrhea which may partly be due to dietary nutrients maldigestion and malasorption (Allison, 2012) as well as metabolic enzymes disorder reported in surra (Bal *et al*., 2012). The infection causes immunosuppression because of the parasite ability switch its antigenic coat in antigenic variation which allows for secondary infection with agents such as *Salmonella* serovars and *Clostridium perfringens* (Stewart, 2013).

Ventral abdominal oedema may be observed in the affected animal such as in donkeys with chronic infection (Desquesnes *et al*., 2013) which is precipitated by hypoalbuminaemia with loss of vascular fluid to the interstitial tissue, especially in dependent areas (Allison, 2012).

Mortality in horses is over 50% in acute stage of natural surra (Silva *et al.,* 1995). There is considerable variation in the pathogenicity of different strains of the parasite and the susceptibility of different host species (Kaminsky *et al.,* 1997). The disease may manifest as an acute or chronic form, and in the latter case may persist for several months, possibly years (OIE, 2010). In the absence of adequate treatment, surra is often acute and rapidly fatal in horses, donkeys, mules, dogs and cats (Hoare 1972). However, these host species may develop mild or subclinical infections as in wild animals such as deer, capybara and coati which may also constitute a reservoir (Anonymous, 2009).

2.1.7 Clinical signs

In horse, incubation period of *Trypanosoma evansi* infection varies from 7 to 28 days and sometimes up to 56 days (Stephen, 1986), however, 5 to 60 days have been reported for equidae (Anonymous, 2009). Clinical signs in most animal species (camels, horses, donkeys, buffalo, cattle and pigs) include recurrent fever that can be as high as 44°C directly associated with intermittent episodes of parasitaemia, anaemia, weight loss or reduced weight gain, lethargy, lymphadenopathy, dependent oedema, urticaria, jaundice, petechial haemorrhages of the mucous membranes of the eyes and anus (Cadioli *et al.,* 2006). There is usually a loss of appetite, reluctance to move, rough hair coat and cases of abortion have been reported in buffalos and camels (Gutierrez *et al.,* 2005).

In advanced cases, the parasites invade the central nervous system (CNS), which can lead to nervous signs such as progressive paralysis of the hind quarters and exceptionally, paraplegia, especially in horses as well as in other host species before complete recumbency and death ensue. (Gutierrez *et al*., 2005). There are also indications that the disease causes immunodeficiency (Onah *et al.,* 1998; Dargantes *et al.,* 2005a) and the severity of the disease generally depends on the strain of *T. evansi*, health status of the host and stress (Kaminsky *et al.,* 1997).

The clinical signs in horses naturally infected with *T. evansi* include a clinical course of 2 to 20 days, marked ataxia, blindness, head tilt and circling, hyperexcitability, obtundity, proprioceptive deficits, head pressing, and paddling movements (Rodrigues *et al.,* 2009).

2.1.8 Pathology

At postmortem, lesions are non-specific and may include emaciated carcass, anaemia, petechial haemorrhages on some internal organs, hydrothorax, ascites, lymphadenopathy and splenomegaly (OIE, 2010). Donkeys experimentally infected with the Brazilian strain of *T. evansi* developed a chronic disease and the pathological changes included enlargement of the spleen and its white pulp, mediastinal lymph nodes and lungs congestion. Haematological picture included marked decline in haemoglobin, packed-cell volume, and erythrocyte count (Cadioli *et al.*, 2006). Biochemical alterations include increased levels of icterus index, serum globulins and decreased serum albumin and glucose values as in camels (Mbaya *et al*., 2014) and Swiss Albino mice (Bal *et al*., 2012). The main histopathological features consisted of meningoencephalitis, demyelination in some areas of the cerebellum pediculus and neuropil vacuolization in donkeys (Soodan *et al*., 1996) and horses (Marques *et al.*, 2000).

Gross pathology due to *T. evansi* infection in horses includes symmetric leukoencephalomalacia with yellowish discoloration of white matter and flattening of the gyri in the brain tissue (Rodrigues *et al.*, 2009). Histopathologically, necrotizing encephalitis especially in the white matter with oedema, demyelination, and lymphoplasmacytic perivascular cuffs occur as well as mild to moderate meningitis or meningomyelitis in the spinal cord (Rodrigues *et al.*, 2009).

2.1.9 Diagnosis

On the field, presence of fever and anaemia could lead to clinical suspicion of *surra*. However, anaemia is not pathognomonic and animals with subclinical infection can have

low parasitemia without anaemia (Dargantes *et al.,* 2005b) and thus, diagnosis must be confirmed by laboratory methods (Dia *et al.,* 1997).

2.1.9.1 *Identification of the parasite by direct microscopic examinations*

The classical direct parasitological methods for the diagnosis of trypanosomosis, namely microscopic examination of blood or lymph node material, are not highly sensitive, but a number of techniques, including enrichment of the sample, rodent inoculation and DNA methods may increase the sensitivity (Ramírez-Iglesia *et al*., 2011). In regions where *Trypanosoma equiperdum* occurs in addition to *T. evansi*, specific identification by microscopy is not possible. Thus molecular tool such as polymerase chai reation (PCR) are then very useful for species specific diagnosis of *T. equiperdum* in horses (Clausen *et al*., 2003). It is recommended that blood for diagnosis of trypanosomes is obtained from peripheral vein by puncturing the ear or tail vein with a sterile needle. However, when larger volume of blood is required or for the reason that sometimes the parasites leave small peripheral vessels for deeper blood vessels, the jugular vein is recommended to be used (Elaine and Margi, 2007). Direct microscopy is reported to be poorly sensitive but highly specific (as much as greater than 94%) for the diagnosis of *T. evansi* and other trypanosomes infection in animals (Herbert and Lumsden, 1976; Takeet *et al*., 2013). It can be supported with mice inoculation test which is 50% to 74% more sensitive (Luckins, 2014).

Wet blood films procedure: A small drop of blood (2–3 µl) is placed on to a clean grease-free glass slide and a cover-slip is placed over it and the blood is spread as a

monolayer of cells. The slide is examined using light microscopy (200×) to detect motile trypanosomes and study their fashion of movement. Improved visualisation can be obtained with dark-ground or phase-contrast microscopy at 200–400× (Elaine and Margi, 2007). The sensitivity of this method is low, approximately 10 trypanosomes per µl, which is frequent in early or acute infections only (OIE, 2010).

Stained thick smears: A large drop of blood (10 µl) is placed on the centre of a clean grease-free microscope slide and spread with a tooth-pick or the corner of another slide so that an area of approximately 1.0–1.25 cm in diameter is covered (Lumsden, *et al*., 1973). The preparation is air-dried for 1 hour or longer, while protecting the slide from insects. The slide is placed in a horizontal position and the unfixed smear is stained with Giemsa's Stain (one drop of commercial Giemsa + 1 ml of phosphate buffered saline, pH 7.2), for 25 minutes. After washing and drying the stained slide, it is examined by light microscopy at a magnification of 500× with oil immersion. The advantage of the technique is that it concentrates the drop of blood into a small area, and thus less time is required to detect the parasites, which are more visible owing to the haemolysis of the unfixed red cells but the disadvantage is that the trypanosomes may be clumped together or overlap one another, and the method is, therefore, not suited for species identification in case of mixed infections (OIE, 2010).

Stained thin smears procedure: Place a small drop of blood (3–5 µl) at one end of a clean microscope slide and draw out a thin film using a spreader or a another slide with the edge placed ahead and in contact with the drop of blood at an acute angle formed between the slide and spreader. The spreader is drawn forward to spread out a thin film of the blood. The slide is air-dried briefly and fixed in methyl alcohol for 1 minute and

allowed to dry. The slide is then stained in Giemsa (one drop Giemsa + 1 ml PBS, pH 7.2) for 25 minutes after which the stain is poured off, washed in tap water, air dried again and examined at a magnification of 400–1000× with oil immersion. This technique permits detailed morphological studies and identification of the *Trypanosoma* species, but the disadvantage is that it is of a very low sensitivity as it detects parasitaemia greater than 500,000 trypanosomes/ml of blood (Lumsden, *et al*., 1973).

2.1.9.2 *Concentration methods*

In most hosts, *T. evansi* can induce mild clinical or subclinical carrier state infections with low parasitaemia in which it is difficult to demonstrate the parasites (Desquesnes *et al*., 2013). In these circumstances, concentration methods are necessary, as they increase the sensitivity of microscopic examination (Elaine and Margi, 2007).

Haematocrit centrifugation technique (*also known as Woo's technique*): The blood (70 µl) is collected into two heparinized capillary tubes (75 × 1.5 mm). The wet end is closed with plasticine and the tubes are centrifuged at 3000*g* for 5 minutes (generally 12,000 rpm in a haematocrit centrifuge machine). The capillary tubes are then examined and the value of the haematocrit is expressed as a percentage of packed red blood cells (RBCs) to total blood volume. The capillary tube is then placed in a groove made with pieces of slide glued to a slide. Trypanosomes are large cells that concentrate at the junction between the buffy coat and the plasma, which is observed under the microscope (100–200×). Light conditions must be set to induce refringence of the cells to increase the visibility of the moving trypanosomes. This can be obtained by lowering the position of the light condenser or with intermediary positions of the turret light condenser. The

fresher the sample, the better is the sensitivity as strong parasitic movements make trypanosomes more visible. This technique can detect around 50– 200 trypanosomes/ml of blood (Woo, 1970). The buffy coat can also be collected in a microtube and frozen. Also the sample can be prepared for polymerase chain reaction, PCR (OIE, 2010).

Other concentration methods include Dark-ground/phase-contrast buffy coat method (also known as Murray's technique) and Mini-anion exchange centrifugation technique.

2.1.9.3 *Animal inoculation*

Trypanosoma evansi has a broad spectrum of infectivity for small rodents, and so rats and mice are often used to reveal subclinical (non-patent) infections in domesticated animals (Monzon *et al.,* 1990). In horses and camels with *T. evansi* infection, mice inoculation test is 88% (Monzon *et al.,* 1990) and 100% (Godfrey and kellick-Kendrick, 1962) sensitive respectively, but further improvement in its efficacy can be obtained by the use of buffy coat material (Reid *et al.,* 2001). Such a procedure was able to detect as few as 1.25 *T. evansi* per ml blood (Reid *et al.,* 2001). Heparinised blood (1–2 ml) is inoculated intraperitoneally into rats or mice (0.25–0.5 ml). A minimum of two rodents are inoculated per animal being investigated. The animals are bled from the tail after every 48 hours to detect parasitaemia. The incubation period before appearance of the parasites and their virulence depends on the strain of trypanosomes, their concentration in the inoculum, and the strain of laboratory animal used. However, in most cases it is very short (5 ± 2 days), but can extend to 2 weeks in rare cases (Monzon *et al.,* 1990). Sensitivity of this *in vivo* culture system may be increased by use of immunosuppressed laboratory animals. Drugs such as cyclophosphamide or hydrocortisone acetate, X-ray irradiation, or

splenectomy have been used for this purpose. Such a procedure is only justified when the detection of a potentially infected host is of high importance (for example, importation into a disease-free area) (OIE, 2010).

2.1.9.4 *Detection of trypanosomal DNA*

Detection of minute amounts of trypanosomal *Deoxyribonucleic acid* (DNA) is a possible means of identifying animals with active infections as the parasitic DNA does not remain for more than 24–48 hours in the blood of the host after the trypanosomes are killed (Desquesnes, 1997). The DNA-based techniques are used in the detection of parasites in both clinical and subclinical carrier animals (OIE, 2010) and include;

Deoxyribonucleic acid probes: Specific DNA probes have been used to detect trypanosome DNA in infected blood or tissue but are not routinely applied as further evaluation needs to be made (Basagoudanavar *et al.*, 2001; Reid *et al.*, 2001).

Polymerase Chain Reaction (PCR) techniques are generally the preferred DNA-based methods and are routinely used in some laboratories test (Desquesnes & Davila, 2002). The first step is DNA preparation from collected blood sample which is a determinant of the success and the sensitivity of the PCR. It can be done on anticoagulated blood, buffy coat to increase the sensitivity of the test (Desquesnes and Davila, 2002), blood preserved 1:1 in 70% alcohol, or on drop of dry blood on filter paper (Holland *et al*., 2002; Desquesnes, 2004). It is suitable for detection of pre-patent parasitaemia and carrier status in horses with *T. evansi* infection (Fernandez *et al*., 2009)

2.1.9.5 *Serological tests*

Historically, different methods have been used to detect non-specific humoral antibodies present in cases of *surra* infection (Uilenberg, 1998). These methods are biochemical tests and amongst them in current use to detect specific humoral antibodies to trypanosomal antigens are direct or indirect agglutination tests, complement fixation test (CFT), indirect fluorescent antibody test (IFAT) and the trypanolysis test (Uilenberg, 1998). The IFAT is still useful for small-scale surveys.

The trypanolysis test is used for individual confirmation of positivity because of its high specificity. The recent and more easily standardized techniques of enzyme-linked immunosorbent assay (ELISA) have replaced most of the older methods (Reid and Copeman, 2002; Reid and Copeman, 2003) and card agglutination test 'CATT' (Njiru *et al*., 2004). Attempts to develop new techniques such as latex agglutination test have not been successful so far (Gutierrez *et al*., 2004; Holland *et al*., 2005).

Evaluations of ELISA and CATT have been carried out in camels, horses, cattle, buffaloes and pigs (Reid and Copeman, 2003; Holland *et al*., 2005; Desquesnes *et al*., 2009b). Tests should preferably be carried out on plasma or serum, but the collection of samples should be simplified by using filter paper blood spots for later use in the ELISA, while for the CATT whole blood can be substituted for serum (Holland *et al*., 2002). It is vitally important that serological tests be validated and standardised if they are to be suitable for correctly identifying infected animals; cross evaluation in different laboratories are thus required. Standard criteria for interpreting the tests might have to be developed for each animal species and standardized at least at a regional level (Desquesnes, 1997). It is also necessary to take into consideration the various

Trypanosoma species and strains (RoTat *versus* non-RoTat for example) present in a given area (OIE, 2010).

2.1.10 Differential diagnosis

Other diseases that cause oedema, anaemia, and wasting /or neurological signs should be considered when suspecting trypanosomosis. In horses (related to donkeys), the differential diagnoses include African horse sickness, equine viral arteritis, equine infectious anemia, equine babesiosis and chronic parasitism (Radostits *et al*., 2000). Other diseases to be considered include encephalitis, equine herpesvirus type 1 myelo-encephalopathy, Eastern, Western or Venezuelan equine encephalitis, equine protozoal myeloencephalitis, West Nile virus infection and rabies should also be considered as reported by Rodrigues *et al.* (2009).

2.1.11 Treatment and control of *Trypanosoma evansi* infection

Treatment of trypanosomosis depends on the use of chemotherapeutic agents (FAO, 2012). At the moment, standard trypanocides for the treatment of trypanomosis including surra, in various species of animals include Diminazene aceturate, Homidium, Quinapyramine, Cymelarsan, Isometamidium chloride and Suramin (El Rayah *et al.*, 1999; Zhou *et al.,* 2004). However, the effectiveness of the available standard trypanocidal drugs is limited by resistance of *T. evansi* strains to the available range of trypanocides namely; Diminazene aceturate, quinapyramine, cymelarsan (De Koning, 2001; Suswam *et al.,* 2001) and suramin (FAO, 2012). In a mouse model experiment,

suramin, diminazene, cymelarsan, and quinapyramine performed poorly compared to three synthesized diamidine compounds tagged DB 75, DB 867 and DB 1192 when evaluated for efficacies against *T. evansi* and the synthetic compounds were considered qualified as preclinical candidates to be further evaluated in large animals (Gillingwater *et al*., 2009).

The best strategy for the control of trypanosomosis in areas with multiple drug resistance should include area-wide community vector control, control of co-infections to exploit self-cure against resistant trypanosome populations and the rational use of trypanocidal drugs which should be urgently promoted at all levels as a way of containing or reversing resistance (Mungube *et al*., 2012).

2.1.11.1 *Diminazene aceturate* treatment

Diminazene aceturate (Berenil®, Farbwerke-Hoechst A.G., Germany) is an aromatic diamidine active against protozoa (*Trypanosoma, Leishmania and Babesia*), *Pneumocystis carinii* and some bacteria (Aliu, 2007). The mechanism of action involves coupling to nucleic acid and then inducing partial uncoiling denaturation of the DNA double helix. It selectively blocks kinetoplast DNA and produces dyskinetoplastic trypanosomes. It is highly effective against *T. congolense* and *T. vivax* but less effective against *T. brucei brucei* (Odika *et al*., 1995) and *T. evansi* (Gillingwater *et al*., 2009). The drug is available as water- soluble granules in sachet for dissolution to form 7% solution or as liquid of 7% aqueous solution. The dose rate is 3.5mg/kg body weight intramuscularly (I.M.) or subcutaneously (S.C.) once, while in *T. brucei and T. evansi* infections, the dose is doubled and given once. Isometamidium chloride is recommended

for treatment of relapse from diminazene therapy (FAO, 2012). It is formulated with phenazone, an antipyretic-analgesic to reduce pain at the I.M. injection site. There is good tolerance to diminazene by cattle, sheep and goat. General reactions to the drug occur in horses and camels (Aliu, 2007; FAO, 2012).

2.1.11.2 *Homidium*

Homidium is an antitrypanosomal phenanthridinium derivative active against *T. vivax and T. congolense* but less active against *T. brucei brucei*. Homidium compounds act by interfering with glycosomal function, the function of adenosine monophosphate (AMP)-binding protein, trypanathione metabolism and replication of kinetoplast minicirclesgiving rise to dyskinetoplastic trypanosomes. Homidium is mutagenic and trypanosomes exposed to it for 1 hour may retain motility for 24 hours but are no longer infective. Homidium is available in two water-soluble tablet forms viz: Homidium chloride (Novidium®, May and Baker ltd, Nigeria) and Homidium bromide (Ethidium®, Boots Pure Drugs ltd, UK). Each 250mg tablet of the two forms is dissolved in 10ml of injection water (2.5% solution) and then administered at the dose rate of 1mg/kg bd wt, I.M., once. For treatment of relapse, diminazene aceturate is recommended for alternate use with homidium. There is good tolerance by cattle, sheep and goats. Toxicity effects of Homidium include transient liver damage and local tissue reaction at injection site in horses. Ethidium® is potentially carcinogenic as reported by Aliu (2007) and FAO (2012).

2.1.11.3 *Isometamidium chloride*

Isometamidium chloride (Samorin®, May and Baker, Nigeria; Securidium®, Laprovet, France) belongs to the same chemical group as homidium (FAO, 2012), the group of the phenanthridine aromatic amidine compounds (Fig.1). It is a dark red powder, producing a brownish red solution in water for injection as 1% or 2% concentration. It is an antitrypanosomal drug with curative and prophylactic actions, registered for use principally in cattle, sheep, goat, camel, horse and dog with efficacies against *T.congolense, T. vivax, T. brucei* and *T. evansi* (Touratier, 1981) at the standard dose rates of 0.25–0.5mg/kg bd wt I.M. to achieve cure and a sanative dose of 1mg/kg bd wt I.M. for prophylaxis. Local tissue reaction occurs in cattle and diminazene is recommended as the sanitive pair for treatment of relapse from Isometamidium chloride therapy (Aliu, 2007).

The mode of action of Isometamidium is not fully understood, however, it is evident that the enzyme, kinetoplastic topoisomerase type II of *Trypanosoma spp* is selectively inhibited by the drug (Kaminsky *et al*., 1997; Boibessot *et al*., 2002). The reported protection duration of Isometamidium is as long as 5 months (Mehlhorn, 2008) and has been used in many parts of the world in the treatment of trypanosomosis in animals as cited by Delespaux and de Koning (2007) and Karaye (2012).

Some recent studies showed that:

a. Isometamidium hydrochloride treatment of rats experimentally infected with *Trypanosoma evansi* resulted in 90 percent anti-trypanosomal efficacy. Haematological studies of all the experimental groups, 3 days post infection and on day 7 post-treatment showed that *T. evansi* infection significantly decreased haematological values, however Isometamidium treatment significantly ($P < 0.05$) improved Hb and PCV without any

significant changes in total erythrocytes and total leucocytes values. Serum aspertate aminotransferase and alkaline aminotransferase activities were significantly ($P < 0.01$) higher in the treated rats compared to healthy rats. Treated rats also showed significantly improved blood glucose level, decreased creatinine level but though blood urea nitrogen was elevated after treatment, the rise was not significant (Kumar *et al*., 2009).

b. Pharmacokinetic study of Isometamidium chloride/hydrochloride injected intravenously to Indian Buffalo calves using high performance liquid chromatography showed that the pharmacokinetic of the drug is independent of dose because at doses of 0.25mg/Kg, 0.5mg/Kg and 1.0mg/Kg body weight, the time for maximum plasma conconcentration to be attained was 0.08 hours (4.8 minutes=Tmax) irrespective of dose. The maximum plasma concentration (Cmax) also declined rapidly such that after 8 hours (for 0.25mg/kg) and 24 hours (for 0.5mg/Kg and 1.0mg/Kg) the drug could not be detected in the calves (Anand *et al*., 2013).

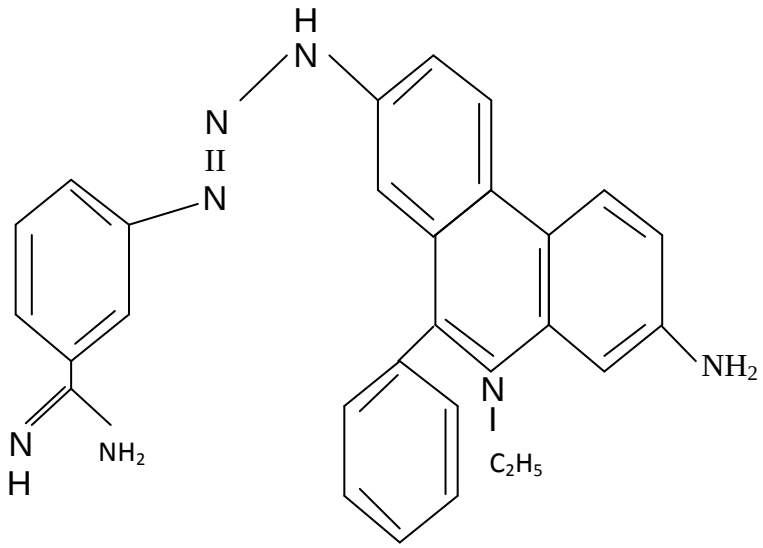


Fig.1: The Chemical Structure of Isometamidium (Source: Anene *et al.*, 2001)

2.1.11.4 *Quinapyramine*

Quinapyramine (Antricide prosalt®, Dynamic Medicine Services, India) is a white, cream or pale yellow coloured, sterile crystalline powder containing quinapyramine sulphate, 1.5g and quinapyramine chloride, 1.0g (total 2.5g) as described by the manufacturer (Aliu, 2007). It is dissolved in cold injection water to produce a 10% solution and dosed at the rate of 5mg/kg bd wt., S.C with effecacies against *T. vivax, T. brucei brucei, T. congolense and T. evansi.* It is tolerated well by cattle, sheep, goat and camel but it causes local tissue reaction in horses. Isometamidium chloride is recommended for use in treatment of relapse that may result from quinapyramine therapy but it (FAO, 2012).

2.1.11.5 *Melarsamine hydrochloride, MelCy*

Melarsamine hydrochloride (Cymelarsan®, BOC Sciences, USA) is a melamino-phenylarsine made by conjugation of one equivalent of melarsen oxide and two equivalents of cysteamine (Aliu, 2007). Immediately after it is dissolved in water, the compound exists as an equilibrium mixture containing MelCy (43%), MelCy which had lost one cysteamine moiety (MelCy -1; 24%), melarsen oxide (33%), and free cysteamine. Small amounts (< 1%) of the oxidation products derived from the last two components were also formed (cystamine and sodium melarsen) (Berger and Fairlamb, 1994). Melarsamine hydrochloride (Cymelarsan®) is used as a 0.5% solution at the dose rate of 0.25- 0.5mg/kg bd wt I.M. or S.C. and it has proved to be efficatious against *T. evansi*

infection of dairy cattle in Thailand (Desquesnes *et al*., 2011) and *T. b. brucei* in camel and cattle (Zweygarth and Kaminsky, 1990) but registered only for use against *T. evansi* in camels (FAO, 2012).

2.1.11.6 *Suramin sodium*

Suramin sodium (Germanin®, Bayer, Germany) is a polysulphonated naphthylurea compound with antitrypanosomal, antineoplastic and antinematodal effects. The molecular formular is $C_{15}H_{40}N_6Na_6O_{23}S_6$ (FAO, 2012). It acts by inhibiting the activity of topoisomerase II by blocking its binding to DNA and consequently inhibiting the proliferation of the target cell. A 10% solution in water is administered at the dose rate of 10mg/kg bd wt.,I.M. It is effective against *T. brucei brucei* and *T. evansi* with good tolerance by camels and horses but is registered for use only in camels. Quinapyramine sulphate is recommended for treatment of relapse (FAO, 2012).

2.1.12 Recent research for trypanocides search

Piper nigrum (Black pepper) fruits have been used in the treatment and prevention of diarrhoea, large intestine toxins, asthma, respiratory disorder, different gastric problems, cancer, fever, common cold and stomach disorder in traditional medical practice (Chatterjee *et al.,* 2007; Scott *et al.,* 2008). As part of search for antitrypanosomal compounds from medicinal plants, methanolic extract in methanol concentration of 250-1000 µg/mL from dried fruits of *Piper nigrum* was prepared. The extract was tested against *Trypanosoma evansi* for trypanocidal activity on Vero cells grown in Dulbecco's Modified Eagle Medium (DMEM)

and supplemented with foetal calf serum (FCS) 20-40% at appropriate conditions. *In vitro* cytotoxicity test of *P, nigrum* fruits extract at concentrations (1.56-100 µg/ml) was done on Vero cells but without FCS. *In vitro* trypanocidal activity varied from immobilization, reduction in the number of trypanosomes and to the killing of trypanosomes in corresponding ELISA plate wells. At 750 µg/ml of MPE, there was reduction of mean trypanosomes count in extract as observed. At 1000 µg/ml of the test extract of *P. nigrum*, there was complete killing of trypanosomes at 7 h of incubation. Trypanosomes counts decreased in concentration and time –dependent manner with significant difference ($P<0.05$). MPE of *P, nigrum and* diminazine aceturate, standard drug, were cytotoxic to Vero cells except at concentrations of 1.56-3.13 and 1.56-6. 25 µg/ml. Moderate anti-trypanosmal activity of MPE of *P. nigrum* was observed (Shaba *et al.,* 2012).

2.2 Buparvaquone

Buparvaquone (Butalex®, Coopers K-Brand Limited, Kenya) is the trial drug in this experiment. It is a second-generation injectable hydroxynaphthoquinone (Fig.2) in aqueous solution with both curative and prophylactic effects against the causative agents of Bovine theileriosis; *Theileria parva* (*T. parva*), *T. sergenti* and *T. annulata* (Dhar *et al.,* 1987). Physically, buparvaquone is a clear ruby-red aqueous solution. It is administered at the dose rate of 2.5 mg/kg bd wt, I.M. once or in severe infections a repeat dose is administered within 48 to 72 hours as recommended by the manufacturer. A single dose against theileriosis of cattle has cure rate of 86% to 91% (Dhar *et al.,* 1987; Mutugi *et al*, 1988). The drug is parasite-specific and acts by disrupting energy generation by *Theileria spp* to cause progressive cytoplasmic vacuolation and death of schizonts (Fry *et al.,* 1984).

Buparvaquone is known to have high *in vitro* activity: EC_{50} of 0.0003 mg/L (10^{-9}M) against *T. parva* (Muguga; E174 strain), the cause of East Coast Fever (Bovine theileriosis), long plasma half-life (at least 7 days) with oral LD_{50} in rats greater than 8,000 mg/kg (Kinabo and Bogan, 1988). Thus, practically a non-toxic substance (Satish, 2002). It is a water-soluble lipophilic drug (Antti *et al.*, 2004).

Haematological (white blood cells, red blood cells, packed cells volume, granulocytes and haemoglobin) as well as Serum biochemical (alanine aminotransferases, aspartate aminotransferases, alkaline phosphatase, albumin/globulin ratio) parametres have shown that buparvaquone was safe in donkeys experimentally infected with *Theileria equi* and medicated with buparvaquone in an efficacy trial (Kumar *et al.,* 2003). In horses when used against *Theileria equi* infection at the dose rate of 5mg/kg bd wt x4 at 48 hrs interval, it induced transient therapeutic efficacy (Zaugg and Lane, 1992). Based on the properties listed, buparvaquone may be effective against *T. evansi* infection in donkeys.

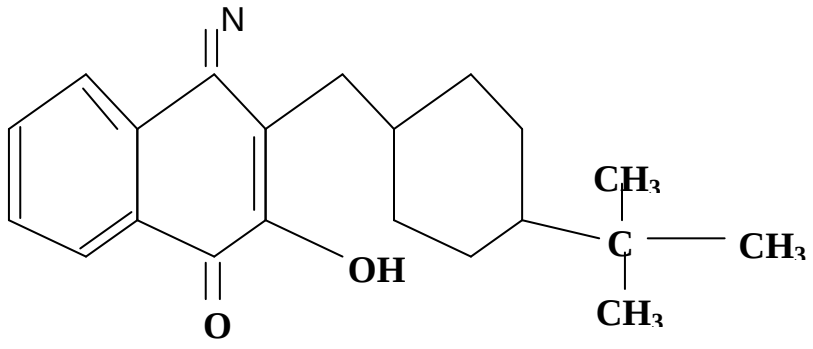


Fig. 2: The Chemical Structure of Buparvaquone (Source: Antti *et al.*, 2004)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of Research

The Experiment was conducted in the Department of Veterinary Parasitology and Entomology Research Pen, Faculty of Veterinary Medicine, Ahmadu Bello University, Main campus, Samaru, Zaria, North-Western Nigeria. The main campus in Samaru is located on latitude 11 ° 15'N to 11° 3'N of the equator and longitude 7° 30'E to 7° 45'E of the Greenwich Meridian as cited by Abbas and Arigbede (2012).

3.2 Experimental Animals

Twenty four apparently healthy donkeys were purchased from International Livestock Market in Maigatari, Jigawa State, North-Western Nigeria and used for this experiment. The donkeys comprised of 12 males and 12 females, between the ages of 11 to 15 months as estimated at point of purchase using dental eruption pattern of the incisor teeth as described by Wayne and Melvin (2000) and Joe (2012).

Rats and mice were sourced in batches from Animal Laboratory, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria and were used for parasites propagation and mice inoculation test respectively as follows:

Four (4) apparently healthy and haemoparasites-free, adult albino Wistar rats, with body weight range of 200g to 220g were used as donors for the propagation of *Trypanosoma evansi* (*T. evansi*) to obtain sufficient inoculum for the experimental infection.

Mice inoculation tests (MIT) were conducted using apparently healthy and haemoparasites-free, adult Wistar mice (100 only) with body weight range of 14g-16g. Two mice were used for each donkey screened, each time, as support test for HCT at pre-infection phase to ensure that they were haemoparasites-free, post-infection and post-treatment to determine relapse, ascertain effectiveness or otherwise of the treatment drugs against *T. evansi* infection.

3.3 Pre-infection Management and Evaluation of Experimental Animals

3.3.1 Housing and acclimatisation

On arrival in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, the donkeys were further examined for signs of disease and external parasites infestation. Wither height (height measured from floor to point of wither while the donkey is standing) range of the donkeys was determined using wooden ruler. Topical sweat-resistant fly-repellant spray (Endure®, Farnam co. inc., USA) was applied on the animals once which kept flies away from accompanying the donkeys while they were lead into the fly-proof and prepared experimental animal pen. The main entrance into the pen had first (outer; completely wooden) door which opened outwards and a second (inner; fly-proof) door which opened into a corridor that provided access to all compartments within the 3.0m high pen. The compartments were in a row with partitioning walls that were 1.5m high for groups of animals. The floor of pen was made of hard core concret, flat, slightly rough to prevent slippery floor and each compartment had floor dimension of $10m^2$. Each of the compartments had a wooden door (1.0m high) that allowed animals to see through and opened into the common corridor. The roof

structure comprised of wooden framework and asbestoes sheets. The pen had no ceiling and was constructed with sufficient vents which allowed optimal air circulation/ventilation in the pen. The pen had shallow floor drain along the perimetre wall of each compartment. The drain allowed liquid wastes to be swept and drained into an external septic tank. Wood shaving was used as bedding material to prevent damp floor, protect the animals from excessive cold and prevent bruising when they lay down. Faecal droppings were mucked daily and the bedding was changed as soon as the old one was becoming damp. The experimental donkeys were acclimatized for 14 days (Wolfensohn and Lloyd (2013).

The albino rats and Wister mice were maintained in separate, labeled cages (A_1, A_2, A_3 and B) according to the group of donkeys whose blood was used to sub-inoculate them (Wolfensohn and Lloyd (2013).

The housing was in accordance with the revised June 2007 Guidelines for the Accomodation and care of Animals used for Experiment and other Scientific Purposes of the European Communities, contained in Appendix-A of Council Directive 86(609)EEC.

3.3.2 Feeding of experimental animals

The experimental donkeys were provided with feed daily equivalent to 5% of their mean body weight, in the forms of Sorghum/Maize stovers and Rhodes grass/groundnuts hay (4:1 ratio). They were also fed with concentrate feed consisting of a mixture of sorghum grain (500g), dried groundnut cake (130g) and Maize/Wheat bran (2.5kg) per 200-300kg bd wt of animal (Aganga *et al.,* 2000). Salt lick blocks, rich in dicalcium phosphate and clean drinking water were provided *ad-libitum.*

The rats and mice were provided with commercially obtained pelletised feed in feeders and clean drinking water in sipper tubes *ad libitum* (Wolfensohn and Lloyd, 2013).

3.3.3 Identification and grouping of animals

The donkeys were identified using Light-weight (about 5g) aluminum plates with engraved serial numbers were prepared and used as neck tags for the donkeys.

The donkeys were first separated into male and female groups. Animals in these 2 groups were assigned serial numbers at random. A sample frame of each gender was prepared and treated one after the other by random picking of animals' numbers and placing in experimental groups A_1, A_2, A_3 and B to obtain a total of 6 animals (3 male and 3 female) in each experimental group (Putt *et al.*, 1988). Choice of groups of animals inoculated with *T. evansi* as well as assigning of either Isometamidium chloride or Buparvaquone for group treatment was made at random (Aviva and Poul, 2013).

The rats and mice were caged according to the group of donkeys they represented. Marks were applied on the various body parts of the rats and mice for identification using different colours of permanent marker pens. The marks were coded to represent individual donkey's number such rats or mice represented.

3.3.4 Faecal sample collection and examination

About 5g of faecal sample was collected from the rectum of each animal and examined for gastro-intestinal parasites using floatation and sedimentation methods as reported by Charles (2007). The animals were dewormed using Fenbendazole bolus

(Fenacure®, Ashish Life Sciences PVT ltd, India) at the dose rate of 10mg/kg bd wt orally, once (Aliu, 2007).

3.3.5 Blood sample collection and examination

Four millilitres (4ml) of blood was collected from each animal through the jugular vein using 5ml syringe and 18G needle. Three millilitres of the blood was placed in blood sample bottle containing liquid tripotassium salt of Ethylene Diamine Tetra-acetate (EDTA+K_3). The EDTA+K_3 was used at the rate of 1mg/mL of whole blood as anticoagulant (Elaine and Margi, 2007) and the blood was examined immediately for haemoparasite infections using 2 preparations each of Wet Blood Film at 40x magnification, HCT and Giemsa Stained thin blood smear as reported by Weiser (2012a). An average of 20 microscope fields of the stained thin smear slide was examined to enable a conclusive decision on the sample.

3.3.6 Animal inoculation

One mL of blood from the 4mL collected from each donkey for examination was transferred into sodium heparin-coated 1mL sterile disposable syringe with 29G $_X$ ½ inch needle. Half a millilitre (0.5mL) of the blood was injected into a mouse intra-peritoneally (Wolfensohn and Lloyd, 2013) using 2 representative mice per donkey (OIE, 2010). The inoculated mice were monitored for haemoparasites, particularly *Trypanosoma species* on HCT and Wet Film using tail tip blood at 48 hours intervals for 14 days (Monzon *et al.*, 1990), and remained under observation up to 60 days post-inoculation.

3.3.7 Baseline data collection

Baseline information was collected weekly over a period of 28 days. These data included:

3.3.7.1 *Vital parametres*

The vital parameters (respiratory rate, pulse rate and rectal temperature) were evaluated for all animals between 06.00 a.m. and 08.00 a.m. once per week. The *respiratory rate* (breaths/minute) was evaluated by counting complete circles of costo-abdominal movement of each animal at a distance while the animals were at rest for 30 seconds and the obtained value was multiplied by 2 to determine value for 1minute. P*ulse rate* (beats/minute) was assessed using two finger tips of the examiner, placed on the medial aspect of the donkey's mandibular angle to feel the pulsation of the External Maxillary artery and was counted for 30 seconds and converted per minute as for respiration. The *rectal temperature* (degrees Celsius) was evaluated for each donkey using digital clinical thermometer with the bulb inserted into and tilted sideways to establish contact with the rectal mucosa until beep sound was heard which signaled attainment of maximum reading (Wosu, 2002).

3.3.7.2 *Body weight*

Body weights of the donkeys were determined at weekly intervals using sling scale as reported by FAO (2014) between the hours of 06.00 and 08.00 to minimize the influence of gut-fill on weight values (De Aluja *et al*., 2005).

3.3.7.3 *Blood Sampling and Serum Harvest*

In order to evaluate haematological, specific serum biochemical and electrolyte constituents, 6mL of blood was collected through the jugular vein, using 10mL syringe with 19G $_X$ $1^1/_2$ inch needle as reported by Weiser (2012b) and Wolfensohn and Lloyd (2013), once a week. About 3mL of the blood was dispensed into sample bottles containing 1mg of liquid EDTA+tripotassium (K_3) per 1mL of blood as anticoagulant for haematological evaluation (Elaine and Margi, 2007; Weiser, 2012b). The remaining was dispensed into anticoagulant-free vacutainer tube for serum harvest. The blood was allowed to clot for 30 minutes and then centrifuged at 5,000*g* for 5 minutes to improve serum yield. Sera were decanted into labelled vials and between 0.8mL to 1.5mL of serum were harvested from the samples. The EDTA-blood and sera were stored at 4^{o}C and -20^{o}C respectively until analysed (Charles, 2007; Weiser, 2012a).

3.3.7.4 *Laboratory protocols*

Haematological evaluations: Thin blood smears as reported by Weiser (2012a) and haematocrit centrifugation (HCT) tubes as described by Woo (1970) were prepared. Packed cell volume (PCV) was determined from HCT as a percentage of the whole blood. Haemoglobin concentration in g/dL was estimated by dividing PCV value by 3 (Weiser, 2012a). Total red blood cells (RBC) and total leucocytes (WBC) counts were determined using haemocytometre techniques while differential leucocyte counts were deduced from the values of the total as reported by Elaine and Margi. (2007).

Serum biochemical assay: Blood glucose was evaluated with the aid of handheld digital blood glucose monitor (Fora®G20, Fora Care Inc., U.S.A.) in mg/dL using a tiny

drop of whole blood (about 0.7µL) placed on the tip of the test strip as recommended by the manufacturer- Fora Care Inc., U.S.A. (Margi, 2007).

Total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine (CR) levels were determined from the stored sera with the aid of a fully-automated clinical chemistry analyzer (Selectra XL®, Vital Scientific, Netherland) as reported by Margi (2007). The analyzer holds 80 samples, 30 standard reagents cups, 24 cuvets or cups (contained in 2 reaction chambers) in a single reaction run and 2 wash stations for self cleaning after each sampling. During each analysis, 250µL of each serum sample was collected using Eppendoff pipette and dispensed into a sample cup. The cups with samples were loaded into the machine sample rotor as recommended by the manufacturer. The machine used between 6µL to 12µL of each sample to determine the level of a substance. Results were generated, recorded and printed automatically. After dispensing of each sample, the auto-pipettes were cleaned at the 'Wash Station' within 3 seconds before collection of another set of sample and reagents as recommended by the manufacturer.

Serum electrolyte assay: Calcium (Ca^{2+}), Phosphate (Po^{4-}), Sodium (Na^{+}), Potassium (K^{+}), Chloride (Cl^{-}) and Bicarbonate (HCO_3^{-}) ions were assayed with the aid of an automatic electrolyte analyzer (Audicom AC9900®, Audicom Medical Technology ltd, Jena, Germany) as reported by Margi (2007). The system holds 20 sample cups, has 2 quality control (QC) reagent loading points (QC_1 for electrode activation reagent and QC_2 for calibration reagent), one wash station containing electrode deproteinizing standard reagent and 1 emergency call. From each sample, 200µL of the thawed sample was

collected using Eppendoff pipette, dispensed into a sample cup and the filled cups were loaded into serially numbered cups holders and the machine was set to run the samples as recommended by the manufacturers. Different electrodes determine the concentration levels of each element in a sample. Analysis of a sample for 5 elements and generation of printed result was completed within 120 seconds before collection of another set of samples as recommended by the manufacturer.

3.3.8 Propagation of *Trypanosoma evansi*

Trypanosoma evansi (Sokoto Isolate) originally isolated from camel in Sokoto city was propagated and used for this experiment. The parasite was isolated by Dr Mohammed Aminu Alhaji of The Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Sciences, Usumanu Danfodio University, Sokoto, Nigeria and maintained using passage in Wister mice in the Veterinary Protozoology laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

The level of parasitemia of an infected Wistar rat was first determined to be +4 (representing greater than 20 parasites per field of microscope after examining 20 fields), using the haematocrit centrifugation technique (HCT) and Giemsa stained thin blood smear procedures (Plate. 1). One millilitre of parasitaemic blood was collected via ocular vein of the Wister rat into heparinised sample bottle and diluted with 1mL phosphate buffered glucose saline solution to obtain sufficient inoculum. The 4 donor rats were inoculated with 0.5 mL of the inoculum per rat, intra-peritoneally using sterile 1mL disposable syringe, $25G_X$ $1^1/_2$ inch needle (Monzon *et al.,* 1990; Wolfensohn and Lloyd, 2013).

The rats were monitored for parasitaemia at 48 hours intervals using haematocrit centrifugation technique (HCT). Twelve days post-inoculation, three (3) of the 4 rats had attained +4 (i.e >20 parasites per microscope field) while the fourth rat had +2 (6-10/field) parasitemia. The four donor rats were sacrificed and their blood was pooled into a sterile conical flask containing heparin sodium at 20 U/mL of blood. A total of 15mL of the pooled blood was obtained and the parasitemia was re-evaluated using haemocytometre (Elaine and Margi, 2007). The blood was diluted with phosphate buffered glucose saline solution to obtain an inoculum containing 1.0 $x10^6$ *T. evansi* /mL (Monzon *et al.*, 1990).

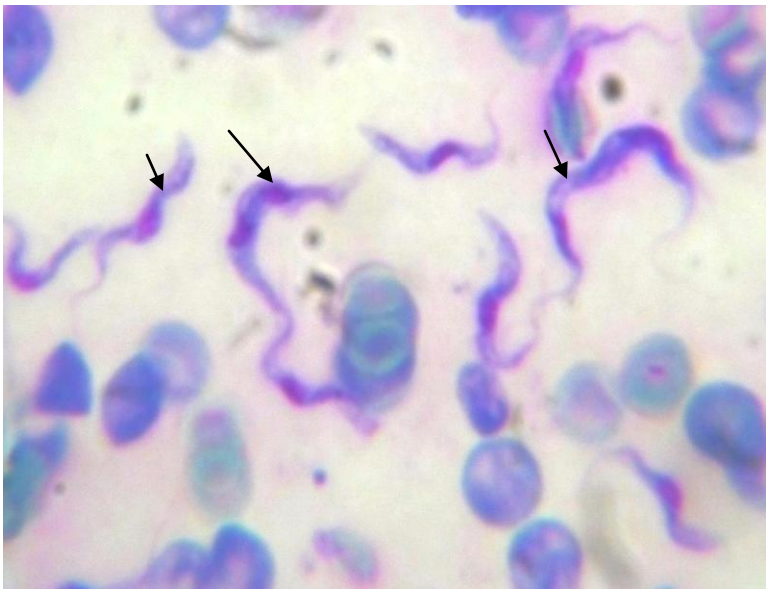


Plate. I: *Trypanosoma evansi;* Sokoto isolate (arrowed) used for this experiment from donor Wister rat blood and stained with Giemsa stain on thin blood smear (100x objective)

3.3.9 Experimental infection of animals

The prepared inoculum was immediately used to infect the experimental animals in groups A_1, A_2 and A_3 through the jugular vein using 2mL ($2.0x10^6$ *T. evansi*) of inoculum per animal and the day was considered as day 0 of infection. Group B animals remained as un-infected (control) group.

3.3.9.1 *Post-infection monitoring*

During the period of post-infection monitoring, 6 mL of blood from each donkey was collected weekly for evaluations with 3mL in EDTA bottle and 3mL in plain vacutainer bottle (Elaine and Margi, 2007) for haematological analysis and serum harvest (for serum chemistry analysis) respectively (Margi, 2007).

Day1 to day7: The infected animals were monitored daily for parasitaemia and PCV using ear vein blood examined on HCT and Giemsa-stained thin blood smears. Their vital parametres were similarly monitored (OIE, 2010; FAO, 2014). Levels of parasitemia were scored using +1, +2, +3 and +4 representing 1-5, 6-10, 11-20 and >20 parasites per microscopic field respectively (Woo,1970). Packed cell volume values were also determined as reported by Elaine and Margi (2007).

Day8 to day28: Parasitaemia, PCV and vital parameters were monitored twice weekly. Changes in behaviour and physical appearance of the animals were also recorded as they were observed (Wosu, 2002). Live body weight (Kg) of the animals using sling weighing scale (FAO, 2014) and also blood glucose (g/dL) using digital blood glucose monitor were evaluated at weekly intervals.

3.3.10 Treatment of specific infected animals

On day 28 post-infection (PI) of groups A_1, A_2 and A_3 animals, the animals in group A_2 were treated with Isometamidium chloride (Securidium®, Laprovet, France) 1% solution at the dose rate of 0.5mg/kg bd wt by deep intramuscular injection once. Similarly animals in group A_3 were treated with Buparvaquone (Butalex®, Coopers K-brand Ltd, Kenya) 5% w/v at dose rate of 2.5 mg/kg bd wt by deep intramuscular injection twice at 72 hours apart as recommended by the manufacturers while group A_1 animals left as infected-untreated. Group B animals were maintained as Uninfected-Untreated group of animals (Control). Treatment day was considered as day 0 of treatment.

3.3.10.1 *Post-treatment monitoring*

Post-treatment (PT) monitoring of experimental donkeys was conducted for 148 days during which levels of parasitaemia on HCT and thin blood smear, PCV, respiratory rate, pulse rate, rectal temperature and clinical manifestations were evaluated to determine the effectiveness or otherwise of the drugs in eliminating the infection.

From day 1 to day 7 PT; the parametres were evaluated daily, while from day 8 to day 28 PT, the parametres were evaluated twice per week and finally from day 29 to day 148 PT; the parametres were evaluated at weekly intervals.

Body weight, haemogramme picture and serum chemistry were evaluated at weekly intervals throughout the whole period of the experiment.

3.3.10.2 *Laboratory animal inoculation*

Mice inoculation test (MIT) was performed for infected-untreated and infected but variously treated groups of donkeys as a support for direct microscopic methods in-order to detect any case of subclinical infection. In this regard, blood was collected from infected groups of donkeys that exhibited sustained aparasitemic status for the first time for 10 days or more. One millilitre (1mL) of blood was collected through jugular vein from each donkey in the groups concerned using heparin-coated 2mL syringe 19Gx $1^1/_2$ inch needle. Half millilitre (0.5mL) of the collected blood was sub-inoculated into mouse intra-peritoneally using sterile 27G needles for 2 representative mice per donkey. The mice were identified and monitored for parasitaemia using tail blood on HCT and Wet Film at 48 hours intervals (OIE, 2010) as follows:

Group A_1 (Infected-Untreated) Donkeys; the Infected, untreated donkeys were subjected to mice inoculation test (MIT) on day 110 PI.

Group A_2 (Infected, Isometamidium chloride-treated) Donkeys; the Isometamidium chloride-treated donkeys were subjected to mice inoculation tests on days 21, 50 and 100 PT.

Group A_3 (Infected, Buparvaquone-treated) Donkeys; the donkeys in this group were tested using MIT on day 59 PT.

3.3.11 Postmortem examination

Postmortem (PM) examination was conducted on both dead and exsanguinated animals in order to determine the gross pathological changes due to *T. evansi* infection in

the donkeys with lungs, liver, heart, spleen, kidney, brain, testes, ovaries and muscles being given top priority.

3.3.11.1 *Gross pathological* e*valuation*

The carcasses of animals were evaluated for gross pathological changes involving musculoskeletal system and internal organs.

Post-infection: During the post-infection period, animals that died were examined at post-mortem and represented the sacrifice of animals at post-infection phase of the experiment.

At Termination of Experiment: At termination of the experiment on day 148 PT (equivalent to day176 PI), one animal was picked from each of the 4 experimental groups at random, exsanguinated and examined at postmortem. Findings regarding lesions in organs of animals from infected groups were compared with the organs of the uninfected-untreated group to determine those lesions that might be due to *T. evansi* infection, effects of subsequent various treatments and non-treatments.

3.3.11.2 *Histopathological evaluation*

For histopathological evaluation of organs, tissue samples from the liver, kidney, spleen, heart and muscle were collected and fixed in 10% formaldehyde. Brain, testes and ovary samples were fixed in Bouin's solution. All samples were stored in labeled plastic sample bottles until processed. At processing, the tissues were embedded in paraffin wax, cut at 5µ thickness, mounted on microscope slides and stained with Haematoxylin-Eosin (H-E) as described by Drury and Wallington (1976). The prepared sections were examined under light microscope (Zeiss Microscope®, Olympus Optical Co. ltd, Japan).

3.3.12 Determination of organ invasion by *T. evansi*

During autopsy, organ impression smears were prepared on microscope slides from the liver, kidney, lungs, muscle, spleen, ovary, testes, gluteal and heart muscles. Brain squash smear was also made on microscope slides, fixed and stained with Giemsa stain. The prepared slides were examined microscopically using 100x (Oil Immersion) objective for presence of *T. evansi* in the tissues (Elaine and Margi, 2007).

3.3.13 Ethical considerations

The care for the experimental donkeys and procedures performed on them were according to the guidelines on accommodation and care for animals used for experiment and other scientific purposes of the European Communities, contained in Appendix-A of Council Directive 86(609)EEC, 'Directive 2010/63' and approved by the Ahmadu Bello University Research and Ethics Committee.

3.4 Data Analysis

Data was analysed using:

$$\text{Prevalence of clinical sign} = \frac{\text{Number of animals with the clinical sign in a group}}{\text{Total number of animals in that group}} \times 100$$

Statistical Package for Social Scientists (SPSS) Version 17 and SAS corporation-2010 were used for analyses of all numerical data generated from this study. Means of parametres were determined and presented in tables and graphs using Microsoft Excel 2010.

Analysis of Variance (ANOVA) was used to compare means of variables between experimental animal groups and within groups at 95% confidence level, where $P < 0.05$ was considered statistically significant (Mead *et al.*, 2002).

CHAPTER FOUR

4.0 RESULTS

4.1 Findings from Pre-infection Evaluation of Experimental Animals

Faecal sample examination showed Strongyle eggs load of +1 in 6/24 (25%) of the experimental animals and +2 in 4/24 (16.7%) of the animals which necessitated deworming the animals with fenbendazole before aimals were experimentally infected with *Trypanosoma evansi.*

Blood samples collected and examined using haematocrit centrifugation technique, Giemsa-stained thin blood smear and mice inoculation test to determine any existing subclinical infection before experimental challenge showed that the animals were all negative for haemoparasites including *Trypanosoma spp.*

Height range of all the donkeys was 0.6 to 0.9metres which was used to decide the number of animals assigned per compartment of the pen.

4.2 Clinical Signs of *Trypanosoma Evansi* Infection in Groups of Donkeys

The clinical signs of experimental *Trypanosoma evansi* infection in the donkeys, started with mild pyrexia (38.5^0C; baseline=36.7^0C) and lethargy between days3-7 post-infection. These were followed by rough hair coat in 3-5/6(50-83%), urticaria with alopaecia in 2-4/6(33-67%), and prominent ribs/hip-bones in 6/6(100%) of animals in all infected groups within 7 days PI (Table4-1, plates IIa and IIb). Other observed signs were pica appetite, pale ocular and oral mucosal membranes (mild) and lymphadenopathies.

Occasionally observed clinical signs included penile erection, rectal prolapse, intermittent diarrhoea, bilateral sero-mucous epiphora and self isolation behaviour in early stage while fever was ongoing. Ventral abdominal oedema was a finding in the later stage of infection in these animals (Tables 4.1and plate IIb). Two animals died PI in the order of one each from groups A_3 and A_2 on days 11 and 28 respectively accounting for mortality of 2/18(11%) during the acute stage of the disease. Another 1/18(6%) animal from group A_1 (Infected-Untreated) also died on day138 PI (Table 4.1). Overall mortality amongst infected animals was 3/18(16.7%) (Table4.1). There was no mortality post-treatment from the variously treated groups. Isometamidium chloride-treated group of animals only exhibited ventral abdominal oedema in 1/5(20%) and relapse in 3/5(60%) animals PT. In the the buparvaquone-treated animals, the PI signs persisted to PT phase and and all animals had relapsed infection (Table 4.1).

Table 4.1: Clinical signs observed in donkeys experimentally infected with *Trypanosoma evansi* based on their infection and treatment status

Clinical signs	Day3 – 28 Post-infection			Day29-176 Post-infection (Day1-148 PT)		
	A_1 (n=6)	A_2 (n=6)	A_3 (n=6)	A_1 (I-U,n=6)	A_2 (I-Isomet,n=5)	A_3 (I-Buparv,n=5)
Mild pyrexia, 38.5^{0}C (control=36.7^{0}C)	6(100)	6(100)	6(100)	NA	NA	NA
Lethargy/recumbency	6(100)	6(100)	6(100)	6(100)	NA	5(100)
Rough hair coat	3(50)	5(83.3)	5(83.3)	6(100)	NA	5(100)
Urticaria/alopecia on trunk	3(50)	4(66.7)	2(33.3)	2(33.3)	NA	2(40)
Prominence of ribs and hip bones	6(100)	6(100)	6(100)	6(100)	NA	2(40)
Pica appetite	6(100)	6(100)	6(100)	NA	NA	NA
Penile erection (males only)	3(100)	3(100)	3(100)	NA	NA	NA
Rectal prolapse	1(16.7)	1(16.7)	1(16.7)	NA	NA	NA
Pale ocular and oral mucosae (mild)	6(100)	6(100)	6(100)	NA	NA	NA
Intermittent diarrhoea	1(16.7)	NA	2(33.3)	NA	NA	NA
Bilateral sero-mucoid epiphora	NA	NA	2(33.3)	NA	NA	2(40)
Isolation (seclusion)	NA	NA	2(33.3)	NA	NA	2(40)
Ventral abdominal oedema	NA	NA	NA	6(100)	1(20)	1(20)
Lymphadenopathy	6 (100)	6(100)	6(100)	6(100)	NA	2(40)
Morbidity	6 (100)	6(100)	6(100)	--------	------	-------
Mortality	NA	1(16.7)	1(16.7)	1(16.7)	NA	NA

Keys: Letter A_1, A_2 and A_3 represented the experimental groups of animals
A_1 (I-U) = Infected-Untreated group
A_2 (I-Isomet)= Infected, Isometamidium chloride- treated group
A_3 (I-Buparv)=Infected, Buparvaquone-treated group
n= sample size in group
NA = Not applicable (i.e the clinical sign was not observed in the experimental group).
Values in parentheses are percentages.
------Clinical sign or parameter was not evaluated for the group
*Prevalence of 'penile erection' is specific for male animals only and calculated based on the total number of males in a group



Plate.IIa: Normal skin without gross lesions of a Group B (Uninfected-untreated, control) donkey

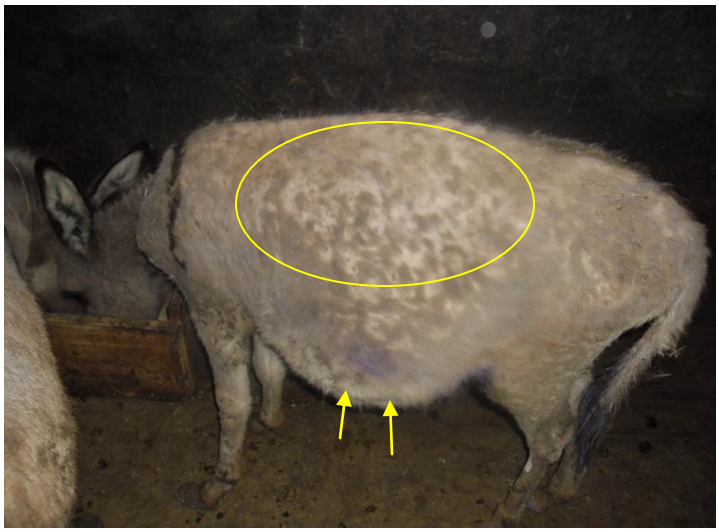


Plate.IIb: Skin with urticaria-like lesions, alopecia (circled) and ventral abdominal oedema (arrowed) exhibited by a Group A (*T. evansi*-infected, untreated) donkey.

4.3 Parasitaemia Levels in Groups of Donkeys Infected with *Trypanosoma Evansi*

4.3.1 Post-infection parasitaemia

Some of the animals in the *T. evansi*-infected groups (A_1, A_2 and A_3) were positive for the parasite on day 3 and by day 4 all infected animals were positive. Parsitaemia levels increased gradually from day 3 with undulating waves that peaked on days 7 and 18 with intervals of 10 to 11 days between peaks of parasitaemia PI. On day 28 PI, a third rising wave of parasitaemia was observed in all the three groups and animals in groups A_2 and A_3 were treated (arrowed on X-axis of Fig.3) and the day was considered as day 0 of treatment (arrowed on X-axis of Fig.4).

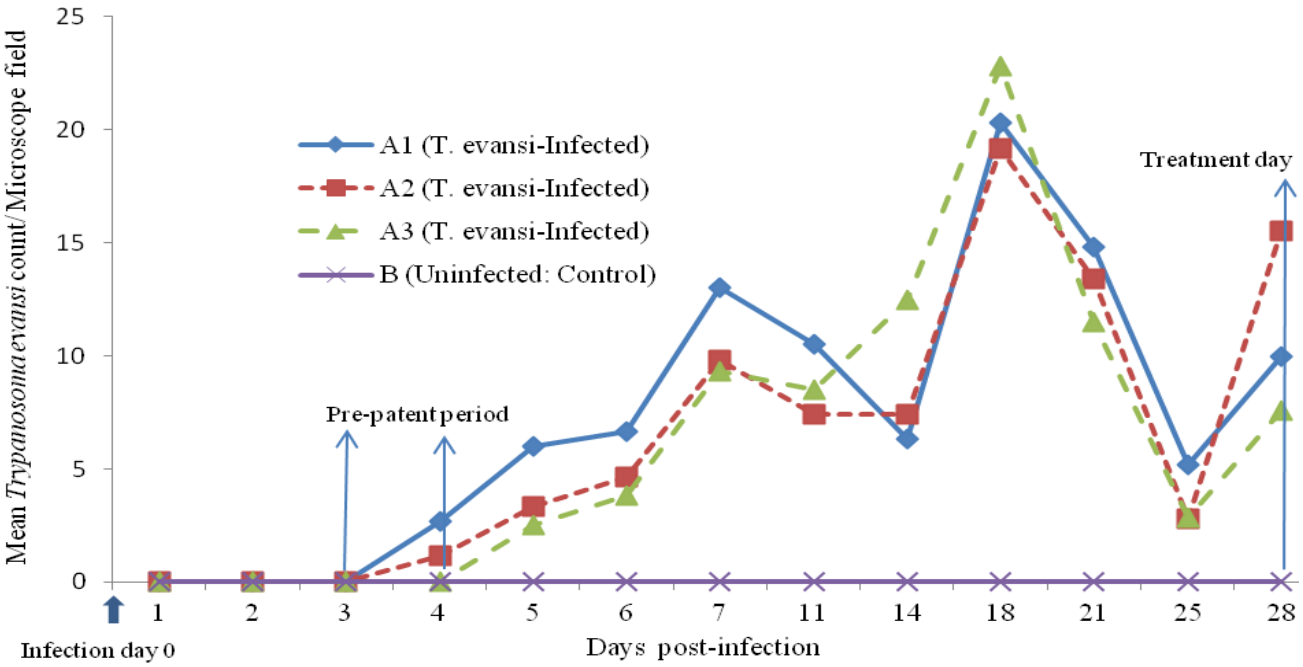


Fig. 3: Mean levels of parasitaemia in donkey experimentally infected with *Trypanosoma evansi*

4.3.2 Post-treatment parasitaemia

4.3.2.1 *Group A_1* (*Infected-Untreated*)

The infected-untreated group of animals maintained an undulating wave of parasitaemia until it became aparasitemic on HCT examination on day 100 PI, equivalent to day 72 PT, (Fig.4). However, mice inoculation test (MIT) on day 110 PI resulted in death of all mice, by day 15 post-inoculation from heavy parasitemia (between +3 and +4/field) on HCT. This indicated that there was subclinical infection in this group by 100 days PI.

4.3.2.2 *Group A_2* (*Infected, Isometamidium chloride-treated*)

The *T. evansi*-infected, Isometamidium chloride-treated group of animals had sharp drop in mean parasitaemia on HCT from approximately 12/field on day 1 to about 2/field on day 2 PT and they became aparasitaemic on day 11 PT (Fig.4). Mice inoculation tests on days 21 and 50 were negative but became positive on day 100 PT in 6/10(60%) of mice used representing 3/5 (60%) donkeys in the group. The test (MIT) showed that there was no detectable parasitaemia within 50 days PT and that by day 100 PT, subclinical status was detectable in 3/5(60%) of the donkeys treated with isometamidium. The inoculated mice showed only 1 parasite (1+) per microscope field both on Wet Film and HCT during 48 days of post-inoculation observation. The remaining 4 mice were negative both on WF and HCT for the same period of observation.

4.3.2.3 *Group A_3* (*Infected, Buparvaquone treated*)

The Infected, Buparvaquone treated group became aparasitemic on HCT on day 49 PT (Fig. 4). Following MIT on day 59 PT, all inoculated mice tested positive for *T. evansi* and died by day 23 post-inoculation observation.

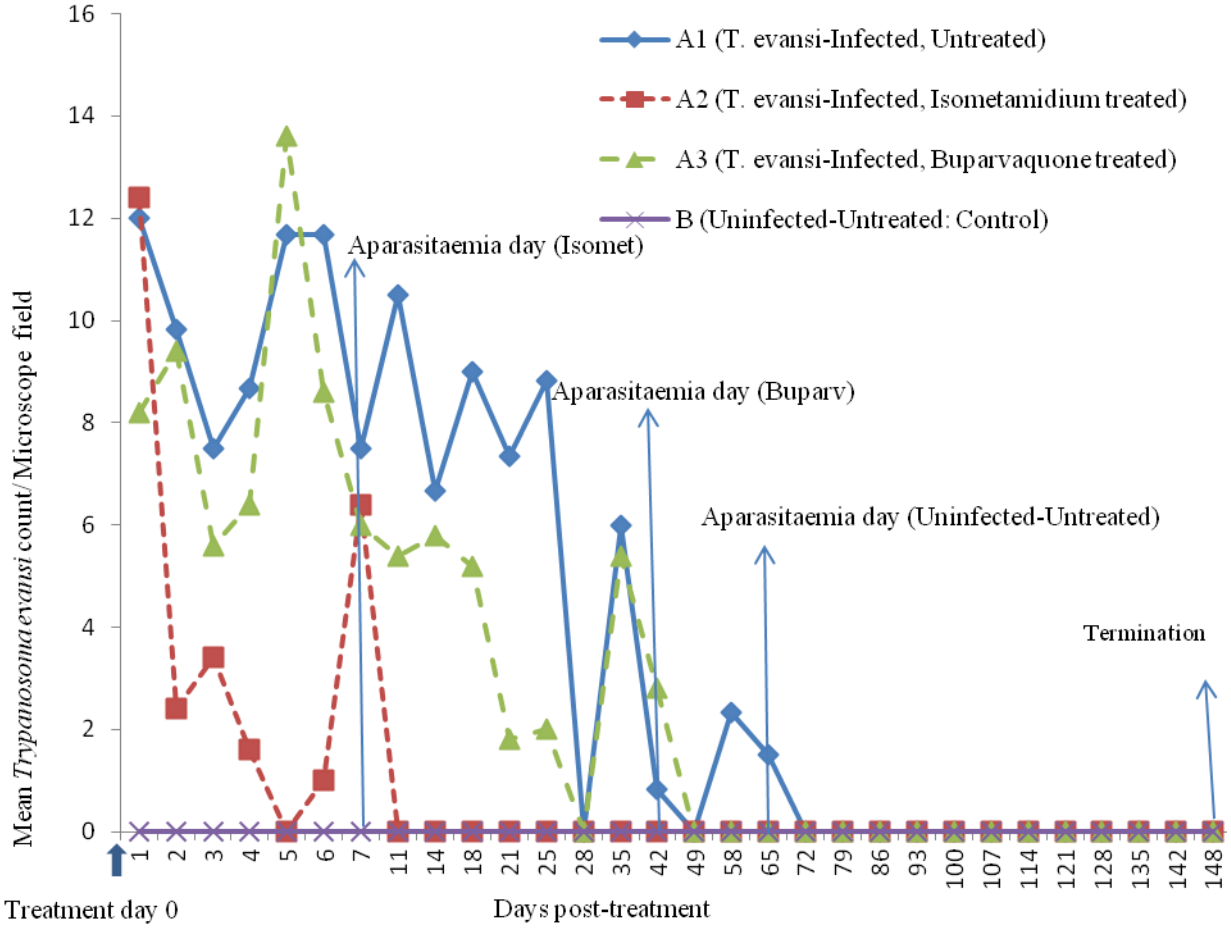


Fig. 4: Mean levels of *Trypanosoma evansi* observed in experimentally infected donkeys post-treatment

4.4 Vital Parametres of Donkeys Infected with *Trypanosoma Evansi*

4.4.1 Mean respiratory rate changes

4.4.1.1 *Group A1* (*T. evansi-Infected-Untreated*)

Significant($P<0.05$) increase in respiratory rate occurred from a pre-infection mean of 20.5±2.5 breaths/min to PI mean of 26.5 ±0.5 breaths/min from day 28 -35 PI. On day 42 PI, the value declined to 21±1.0 breaths/minute to the termination of experiment on day 176 PI, equivalent to day 148 PT (Fig.5).

4.4.1.2 *Group A_2* (*T. evansi-Infected, Isometamidium-treated*)

The respiratory rate value increased significantly ($P<0.05$) from pre-infection value of 19±6.0 breaths/min to PI value of 29±0.0 breaths/min on day 28 PI. Following treatment on day 28 PI, the value declined to 18±0.0 breaths/min on day 35 PI (1 week PT) and staggered at pre-infection level to the end of the experiment (Fig.5).

4.4.1.3 *Group A_3* (*T. evansi-Infected, Buparvaquone-treated*)

Mean respiratory rate value also increased significantly ($P<0.05$) from pre-infection value 16.5±5.5 breaths/min to PI value of 30±0.0 breaths/min on day 28 PI. Following treatment on day 28 PI, the value declined to 22±0.0 breaths/min on day 49 PI (3 weeks PT). Beyond this day, the value remained at the level of pre-infection to end of PT observation (Fig.5).

4.4.1.4 *Group B* (*Uninfected-Untreated*)

The control animals maintained mean respiratory rate of 18±7.0 (that is 11-25) breaths/min throughout the experimental period (Fig.5).

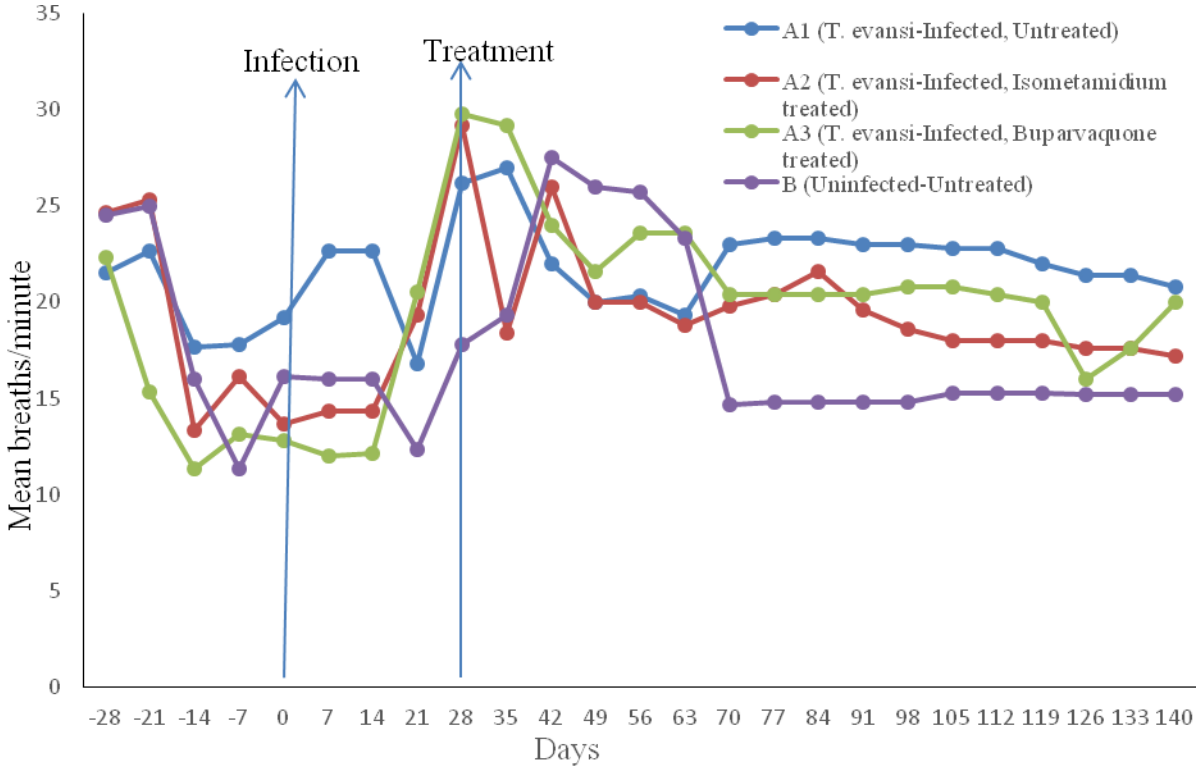


Fig.5: Mean respiratory rates; pre-infection, post-infection and post-treatment of donkeys experimentally infected with *Trypanosoma evansi*

4.4.2 Mean pulse rate changes

4.4.2.1 *Group A_1 (T. evansi-Infected-Untreated*)

There was no significant (P>0.05) rise in pulse rate from pre-infection value of 41±6.0 to the PI value of 46±0.0 beats/min on day 35 PI (Fig.6).

4.4.2.2 *Group A_2 (T. evansi-Infected, Isometamidium-treated*)

In this group, there was insignicant (P>0.05) increase from pre-infection value of 37±1.0 to PI value of 40±0.0 beats/min on day 35 PI (Fig.6).

4.4.2.3 *Group A_3 (T. evansi-Infected, Buparvaquone-treated*)

In this group, there was signicant (P<0.05) increase from pre-infection value of 39.5±6.5 to PI value of 51±0.0 beats/min on day 35 and51.5±1.0 beaths/min on days 49 to 63 PI (Fig.6).

4.4.2.4 *Group B (Uninfected-Untreated*)

This control group, maintained mean pulse rate of 41.5±4.5beats/min throughout the experimental period (Fig.6).

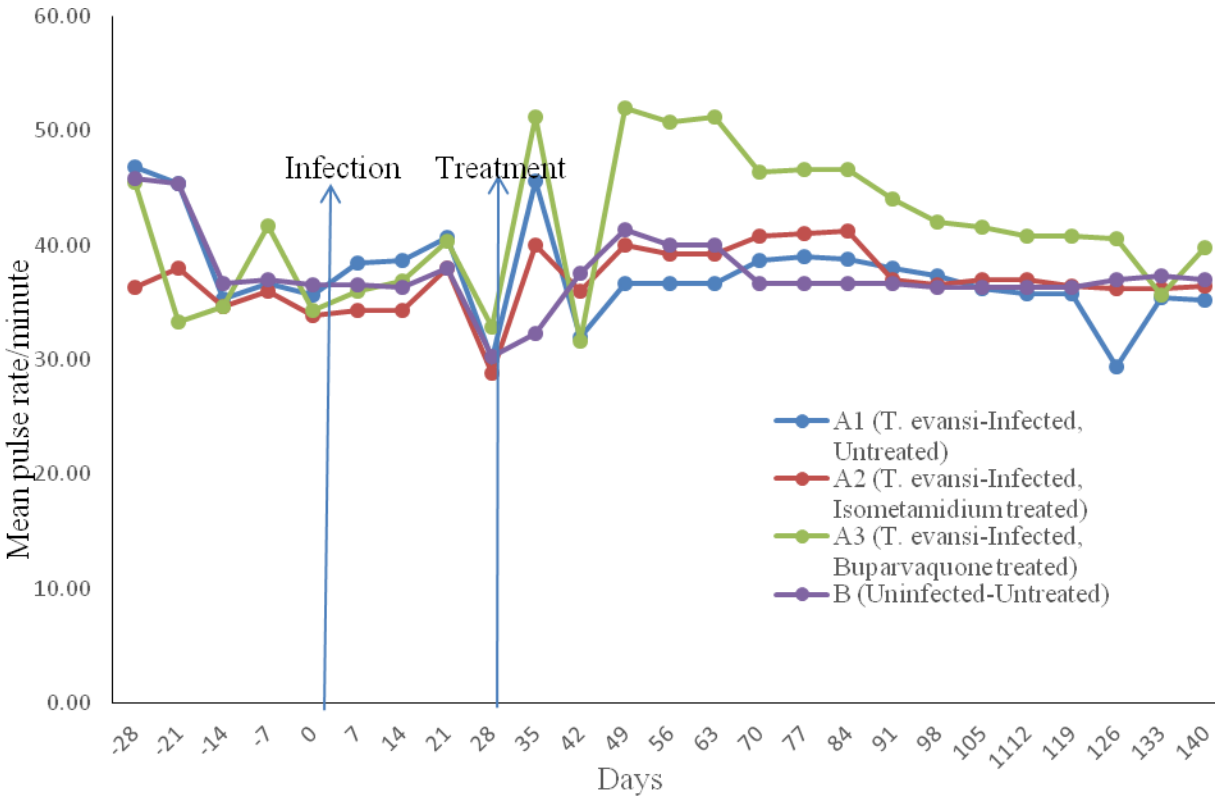


Fig.6: Mean pulse rate; pre-infection, post-infection and post-treatment of donkeys experimentally infected with *Trypanosoma evansi*

4.4.3 Mean rectal temperature changes

4.4.3.1 *Group A_1 (T. evansi-Infected-Untreated*)

There was significant ($P<0.05$) difference between pre-infection rectal temperature value of 36.5 ± 0.5^0C and PI peak value of 38 ± 0.0^0C on day 14 and later insignificantly ($P>0.05$) fell to 37 ± 0.0^0C as observed on day 49 PI (Fig.7).

4.4.3.2 *Group A_2 (T. evansi-Infected, Isometamidium-treated*)

In this group, there was insignicant ($P>0.05$) increase from pre-infection value of 36.8 ± 0.2^0C to PI value of 37 ± 0.0^0C on day 14 PI (Fig.7).

4.4.3.3 *Group A_3 (T. evansi-Infected, Buparvaquone-treated*)

In this group, there was signicant ($P<0.05$) increase from pre-infection value of 36.6 ± 0.4^0C to PI peak value of 38 ± 0.0^0C on day 14. The temperature later fell to 37 ± 0.0^0C on day 42 and rose slightly to 37.5 ± 0.0^0C on days 56 to 63 and reverted to pre-infection levels till end of PT obsrvation period (Fig.7).

4.4.3.4 *Group B (Uninfected-Untreated*)

This control group, maintained mean rectal temperature value of 36.5 ± 0.5^0C throughout the experiment period (Fig.7).

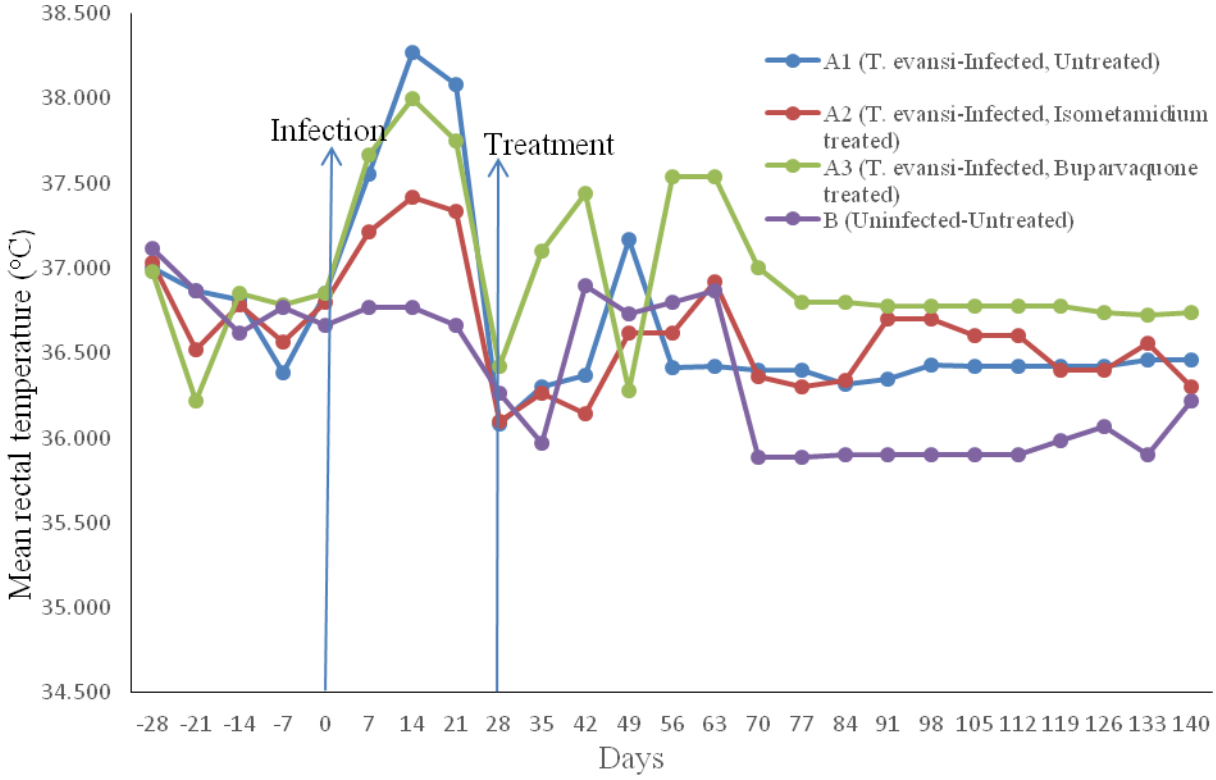


Fig.7: Mean rectal temperature; pre-infection, post-infection and post-treatment of donkeys experimentally infected with *Trypanosoma evansi*

4.4.4 Mean body weight changes

4.4.4.1 *Group A_1 (T. evansi-Infected-Untreated*)

There was significant (P<0.05) decline in mean body weight from pre-infection value of 69.5±0.5Kg to 66.5±0.5Kg from day 7 to 21 (2 weeks) PI. The mean weight increased to 70.5±2.5Kg between days 28 to 84 PT and declined again to 63.0±0.0Kg on day 91 till the end of the experiment (Fig.8).

4.4.4.2 *Group A_2 (T. evansi-Infected, Isometamidium-treated*)

This group showed, signicant (P<0.05) decline in body weight from pre-infection value of 62.5±2.5Kg to PI value of 54±0.0Kg from days 14 to 21 PI (a peak) and by day 28, animals improved in weight to 66.0±00kg. This was followed by a gradual increase in weight to 70.0±0.0Kg by day133 PI (Fig.8).

4.4.4.3 *Group A_3 (T. evansi-Infected, Buparvaquone-treated*)

The mean body weight decreased significantly (P<0.05) from pre-infection value of 66.5±2.5Kg to PI value of 55.0±3.0^{0}C, especially from day 7 to 21. Post-treatment on day 28 to end of PT observation, the animals significantly (P<0.05) gained mean weight of 67.0±5.0Kg (about 12Kg in 21 weeks of PT observation) when compared with PI value. However, the graph showed a gentle downward trend after day 49 PT side-by-side the trend in Group A animals signifying stunted growth (Fig.8).

4.4.4.4 *Group B (Uninfected-Untreated*)

This group showed a significant (P<0.05) weight gain from 65.0±1.0Kg at the commencement of the experiment to 79.0±0.0Kg at the end of experiment. Animals gained14.0 ± 00Kg throughout the duration of the experiment (Fig.8).

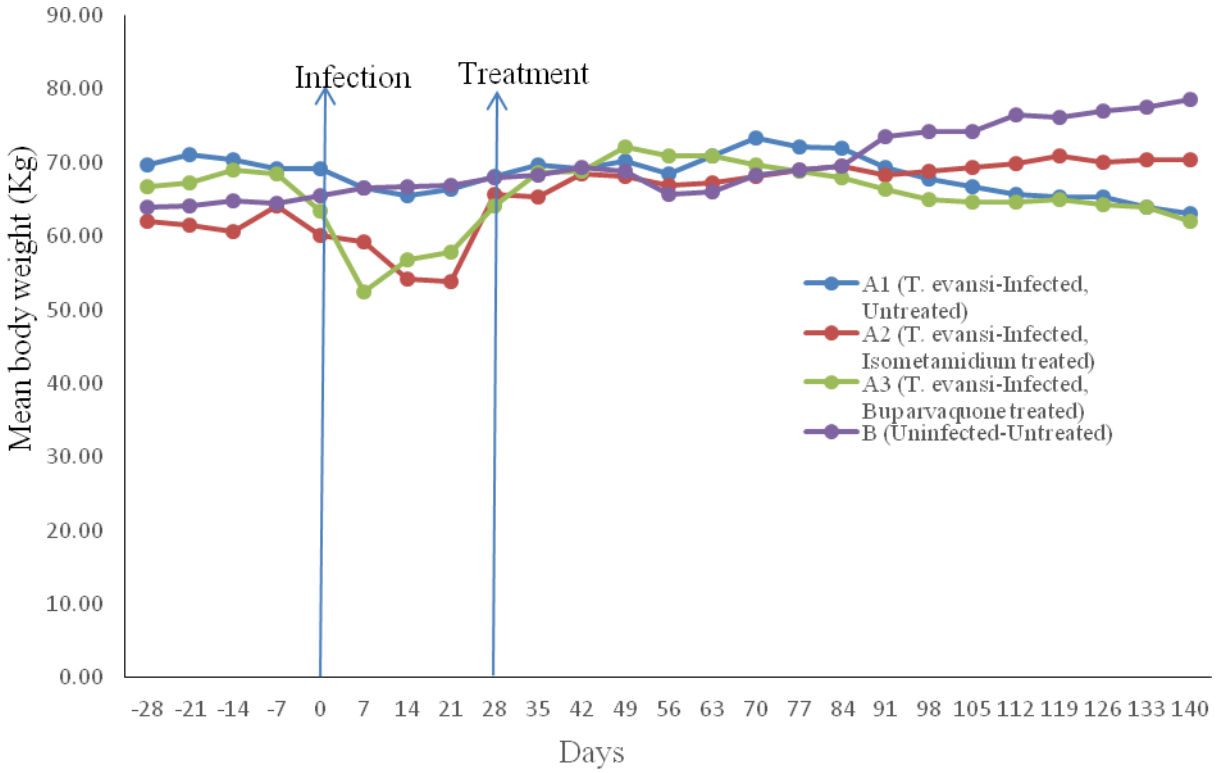


Fig.8: Mean body weight; pre-infection, post-infection and post-treatment of donkeys experimentally infected with *Trypanosoma evansi*

4.5 Observed Haemogramme Picture of Donkeys Infected with *Trypanosoma Evansi*

4.5.1 Mean packed cell volume changes

4.5.1.1 *Group A_1 (T. evansi-Infected-Untreated*)

There was insignificant (P>0.05) decline in mean PCV from pre-infection value of 35.5±0.5% to PI values of 34.0±1.0% upto day 28 and a further signicant (P<0.05) decline to 29.5±3.5% PI up to the end of experiment (Fig.9).

4.5.1.2 *Group A_2 (T. evansi-Infected, Isometamidium-treated*)

The group exhibited signicant (P<0.05) decline in PCV from pre-infection mean value of 45.0±1.0% to PI value of 32.5±0.5% between days 49 and 63 PI (i.e days 21 and 35 PT). Between days 70 and 84 PI (i.e days 42 and 56 PT), the PCV rose significantly (P<0.05) to 37.0±0.0% and the value ended at 30.0±0.0% by day 140 (Fig.9).

4.5.1.3 *Group A_3 (T. evansi-Infected, Buparvaquone-treated*)

The buparvaquone-treated animals showed insignificant (P>0.05) decline in PCV from pre-infection value of 39.2±0.4% to PI value of 32.6±0.0% between day 21 and 28 PI. Despite treatment on day 28 PI, there was further significant (P<0.05) decline in PCV to 27.3±0.5% between days 35 and 63 was observed but insignificantly (P>0.05) improved to 30.4±0.0% on day 140 (Fig.9).

4.5.1.4 *Group B (Uninfected-Untreated*)

This group maintained mean PCV value of 43.0±0.0% within the first 28 days of experiment and finally showed PCV value of 44.0±0.0% at the end of experiment (Fig.9).

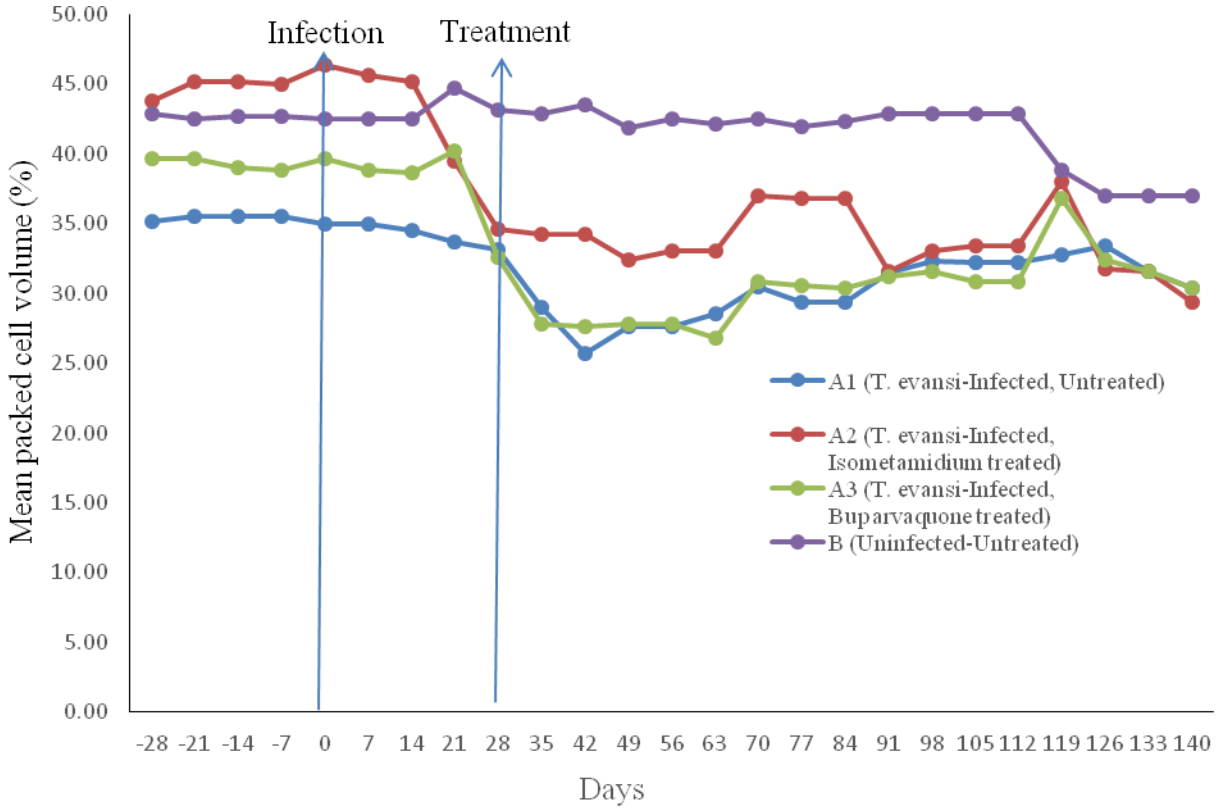


Fig. 9: Mean packed cell volume; pre-infection, post-infection and post-treatment of donkeys experimentally infected with *Trypanosoma evansi*

4.5.2 Means of haemoglobin and total red blood cells values

4.5.2.1 *Group A_1* (*T. evansi-Infected-Untreated*)

The pre-infection values of Hb (11.3±1.1 g/dL) was not different significantly ($p>0.05$) from those of day 28 PI (11.3±0.3g/dL). The final mean values after day 28 PT fell to 9.8±1.2g/dL but was not significantly different ($p>0.05$). The total red blood cell (RBC) value did not change significantly ($p>0.05$) from pre-infection value of 5.9±0.8 $x10^{12}$/L to PI value of 5.4±0.5 $x10^{12}$/L and later final value of 5.6±0.5 $x10^{12}$/L (Table 4.2).

4.5.2.2 *Group A_2* (*T. evansi-Infected, Isometamidium-treated*)

The pre-inf values of Hb (15.0±0.3g/dL) significantly ($p<0.05$) declined PI to 10.8±0.2g/dL and improved PT (beyond day28 PT) to 12.3±0.0g/dL. Total rbc count pre-inf. (6.4±1.2$x10^{12}$/L) significantly ($p<0.05$) declined PI to 5.3±1.0$x10^{12}$/L. However, the PT value (5.7±0.6$x10^{12}$/L) was not significantly different ($p>0.05$) from the pre-inf. value (Table 4.2).

4.5.2.3 *Group A_3* (*T. evansi-Infected, Buparvaquone-treated*)

The pre-inf values of Hb(13.1 ±0.1g/dL) insignificantly ($p<0.05$) declined PI to 12.1±1.3g/dL. The PT values (beyond day28 PT) further declined to 10.6±1.7g/dL. Total rbc count did not differ significantly ($p>0.05$) between pre-inf. (5.9±1.1$x10^{12}$/L), PI (5.6±1.1$x10^{12}$/L) and PT (6.1±0.8$x10^{12}$/L) values (Table 4.2).

4.5.2.4 *Group B* (*Uninfected-Untreated*)

The initial values of Hb (14.3±0.0g/dL) and total RBC (6.4±1.1$x10^{12}$/L) counts did not differ significantly ($p>0.05$) from their final values of 14.7±0.0g/dL and 6.4±0.3$x10^{12}$/L respectively (Table 4.2).

Table 4.2: Means of some selected erythrogramme parametres of donkeys experimentally infected with *Trypanosoma evansi* based on treatment status and phase of experiment

Experimental Groups	Phase of Experiment	PCV±SD (%)	Hb±SD (g/dL)	RBC±SD $x10^{12}$/L
A1 (Infected-Untreated)	Pre-infection	35.5±0.5c	11.3±1.1c	5.9±0.8ab
	Post-infection	34.0±1.0c	11.3±0.3c	5.4±0.5b
	Final Value	29.5±3.5bc	9.8±1.2bc	5.6±0.5ab
A_2 (Infected,Isometamidium-treated)	Pre-infection	45.0±1.0a	15.0±0.3a	6.4±1.2a
	Post-infection	32.5±0.5cd	10.8±0.2c	5.3±1.0b
	Post-treatment	37.0±0.0c	12.3±0.0b	5.7±0.6ab
A_3 (Infected,Buparvaquone-treated)	Pre-infection	39.3±0.4ab	13.1 ±0.1b	5.9±1.1ab
	Post-infection	36.4±3.8c	12.1±1.3b	5.6±1.1ab
	Post-treatment	31.8±5.0cd	10.6±1.7ab	6.1±0.8ab
B (Uninfected-Untreated; control)	Initial Value	43.0±0.0a	14.3±0.0a	6.4±1.1a
	FinalValue	44.0±0.0a	14.7±0.0a	6.4±0.3a
P-Value	----------	0.054	0.051	0.053

Keys: Packed cell volume (PCV), Haemoglobin concentration (Hb), Total red blood cells (RBC) count and Standard deviation of mean (SD), Means in the same column with the same letter are not significantly different.' P-value <0.05= significant.

4.5.3 Leucogramme picture of groups of donkeys

4.5.3.1 *Group A1 (T. evansi-Infected-Untreated)*

The pre-infection values of WBC (7.1±3.9x109/L), neutrophils (4.9±2.3x109/L) and monocytes (0.1±0.1 $x10^9$/L) counts declined insignificantly (P>0.05) PI to 6.1±0.9 $x10^9$/L, 4.5±0.8 $x10^9$/L and 0.0±0.0 $x10^9$/L respectively. The final values of WBC (7.2±2.5 $x10^9$/L) and neutrophils (5.2±2.2 $x10^9$/L) were insignificant improved while there was no change in monocytes counts (0.0±0.0 $x10^9$/L). Lymphocytes count significantly declined from pre-infection value of 2.0±1.7 $x10^9$/L to PI value of 1.6±0.4 $x10^9$/L and final value of 1.9±0.8 $x10^9$/L. Monocytes values declined insignificantly (p>0.05). There was no significant change (P>0.05) in eosinophil values despite the decline from 0.0±0.1 $x10^9$/L pre-inf to 0.0±0.0 $x10^9$/L PI and the improvement observed (0.0±0.1 $x10^9$/L) in the final count. Band cells count did not change significantly (P>0.05) (Table.4.3).

4.5.3.2 *Group A_2 (T. evansi-Infected, Isometamidium-treated)*

There was insignificant (P>0.05) WBC decline from pre-infection value of 8.7±4.9 $x10^9$/L to PI value of 5.9±0.6 $x10^9$/L. There was an insignificant (P>0.05) improvement PT in the WBC value of 6.6±1.7 $x10^9$/L.

Neutrophils count showed insignificant (P>0.05) decline from pre-infection value of 4.9±2.3$x10^9$/L to PI value of 4.5±0.8$x10^9$/L and there was an insignificant (P>0.05) improvement in the PT value of 4.4±1.0 $x10^9$/L.

Lymphocytes showed insignificant (P>0.05) decline from pre-infection value of 2.8±2.1 $x10^9$/L to PI value of 1.6±0.4 $x10^9$/L and there was an insignificant (P>0.05) improvement in lymphocytes 2. 1±1.3$x10^9$/L PT.

Monocytes values did not change between pre-inf. value of 0.0±0.1 $x10^9$/L to PI value of 0.0±0.0$x10^9$/L. However, PT value of 2. 0.0±0.0$x10^9$/L was observed.

Eosinophils values declined insignificantly (P>0.05) between pre-infection value of 0.0±0.1$x10^9$/L to PI value of 0.0±0.0$x10^9$/L while the PT value was of 0.0±0.0$x10^9$/L.
Band cells count values did not change significantly (P>0.05) between pre-infection value of 0.0±0.0 $x10^9$/L to PI value of 0.0±0.1$x10^9$/L and the PT value was 0.0±0.0$x10^9$/L (Table.4.3).

4.5.3.3 *Group A_3 (T. evansi-Infected, Buparvaquone-treated)*

Total WBC values declined insignificantly (P>0.05) between pre-infection value of 6.8±2.6±0.1 $x10^9$/L and PI value of 6.2±0.7$x10^9$/L. However, a significant (P<0.05) elevation in the WBC was observed PT to 10.6±4.1$x10^9$/L.
Neutrophils count did not change significantly (P>0.05) between pre-infectin value of 4.8±1.8$x10^9$/L and PI value of 4.5±0.6$x10^9$/L while the PT value was 4.3±2.1$x10^9$/L. Lymphocytes values declined though insignificantly (P>0.05) between the pre-infection value of 1.8±0.9±0.1 $x10^9$/L and PI value of 1.6±0.4$x10^9$/L but had a significant elevation (P<0.05) PT to 2.8±1.4$x10^9$/L.

Monocytes count declined significantly ($P<0.05$) from pre-infectin value of 0.1±0.3±0.1 $x10^9$/L to PI value of 0.0±0.0$x10^9$/L and an insignificant ($P>0.05$) elevation PT to 0.1±0.1$x10^9$/L.

Eosinophils count declined insignificantly ($P>0.05$) from pre-infectin value of 0.0±0.1 $x10^9$/L to PI value of 0.0±0.0$x10^9$/L and an insignificant ($P>0.05$) elevation PT to 0.1±0.1$x10^9$/L.

Band cells count significantly increased ($P<0.05$) from pre-infectin value of 0.0±0.0$x10^9$/L to PI value of 0.1±0.1$x10^9$/L and declined insignificantly ($P>0.05$) PT to 0.0±0.1$x10^9$/L (Table.4.3).

4.5.3.4 *Group B (Uninfected-Untreated; Control)*

The initial and final values of all leucocytes parameters were not significantly different ($P>0.05$) in the control group (Table.4.3).

Table 4.3: Mean leucogramme picture of donkeys experimentally infected with *Trypanosoma evansi* based on treatment status and phase of experiment

Experimental groups	Phase of experiment	WBC±SD ($x10^9$/L)	Neut±SD ($x10^9$/L)	Lymph±SD ($x10^9$/L)	Mono±SD ($x10^9$/L)	Eos±SD ($x10^9$/L)	Band±SD ($x10^9$/L)
A_1 (Infected-Untreated)	Pre-infection	7.1±3.9ab	4.9±2.3b	2.0±1.7a	0.1±0.1ab	0.0±0.1ab	0.0±0.0b
	Post-infection	6.1±0.9b	4.5±0.8bc	1.6±0.4b	0.0±0.0b	0.0±0.0b	0.0±0.0b
	Final Value	7.2±2.5ab	5.2±2.2b	1.9±0.8b	0.0±0.0b	0.0±0.1ab	0.1±0.1a
A_2 (Infected, Isometamidium-treated)	Pre-infection	8.7±4.9ab	5.8±3.0b	2.8±2.1a	0.0±0.1b	0.0±0.1ab	0.0±0.0b
	Post-infection	5.9±0.6b	4.2±0.5bc	1.6±0.4b	0.0±0.0b	0.0±0.0b	0.0±0.1ab
	Post-treatment	6.6±1.7ab	4.4±1.0bc	2. 1±1.3a	0.0±0.0b	0.0±0.0b	0.0±0.0b
A_3 (Infected, Buparvaquone-treated)	Pre-infection	6.8±2.6ab	4.8±1.8bc	1.8±0.9ab	0.1±0.3a	0.0±0.1ab	0.0±0.0b
	Post-infection	6.2±0.7b	4.5±0.6bc	1.6±0.4b	0.0±0.0b	0.0±0.0b	0.1±0.1a
	Post-treatment	10.6±4.1a	4.3±2.1bc	2.8±1.4a	0.1±0.1ab	0.1±0.1a	0.0±0.1ab
B (Uninfected-Untreated; control)	Initial Value	6.1±2.6b	4.3±2.1bc	1.7±0.8b	0.0±0.0b	0.0±0.0b	0.0±0.0b
	FinalValue	6.5±2.1ab	4.5±1.4bc	1.9±0.9ab	0.1±0.1ab	0.0±0.0b	0.1±0.1a
P-Value	----------	0.046	0.000	0.000	0.051	0.078	0.043

Keys: Total white blood cells (WBC), Neutrophils (Neut), Lymphocytes (Lymph), Monocytes (Mono), Eosinophils (Eos), Band cells (Band), Standard deviation of mean (SD), Means in the same column with the same letter are not significantly different. P-value <0.05=significant.

4.6 Blood Biochemical Parametres in Donkeys Infected with *Trypanosoma Evansi* with or without Treatment

4.6.1 Mean blood glucose levels changes

4.6.1.1 *Group A_1 (T. evansi-Infected-Untreated)*

There was significant (P<0.05) decline in mean blood glucose level from pre-infection value of 70.0±1.0mg/dL to PI value of 35.5±0.5mg/dL between days 35 and 37 PI. The glucose level, however, rose on day 70 PI to 65.0±0.0mg/dL but did not return to pre-infection values (Fig.10).

4.6.1.2 *Group A_2 (T. evansi-Infected, Isometamidium-treated*)

There was signicant (P<0.05) decline in mean blood glucose level from pre-infection value of 62.0±2.0mg/dL to PI value of 47.5±0.5mg/dL between days 14 and 21 PI. Treatment on day 28 was followed by rise in blood glucose level to 77±0.2mg/dL on days 35 to 42 PI but declined later by day 49 to 64mg±0.0/dL and up to the end of experiment (Fig.10).

4.6.1.3 *Group A_3 (T. evansi-Infected, Buparvaquone-treated*)

The buparvaquone-treated animals showed significant (P<0.05) decline in mean blood glucose level from pre-infection value of 67.0±3.0mg/dL to PI value of 41.0±1.0mg/dL between days 35 and 37 PI. The glucose level then rose on day 70 PI to 66.0±00mg/dL. The pre-infection value was not attained despite the treatment up to the end of the experiment (Fig.10).

4.6.1.4 *Group B (Uninfected-Untreated*)

This group maintained mean blood glucose level of about 69.3±10.0mg/dL throughout the experiment period (Fig.10).

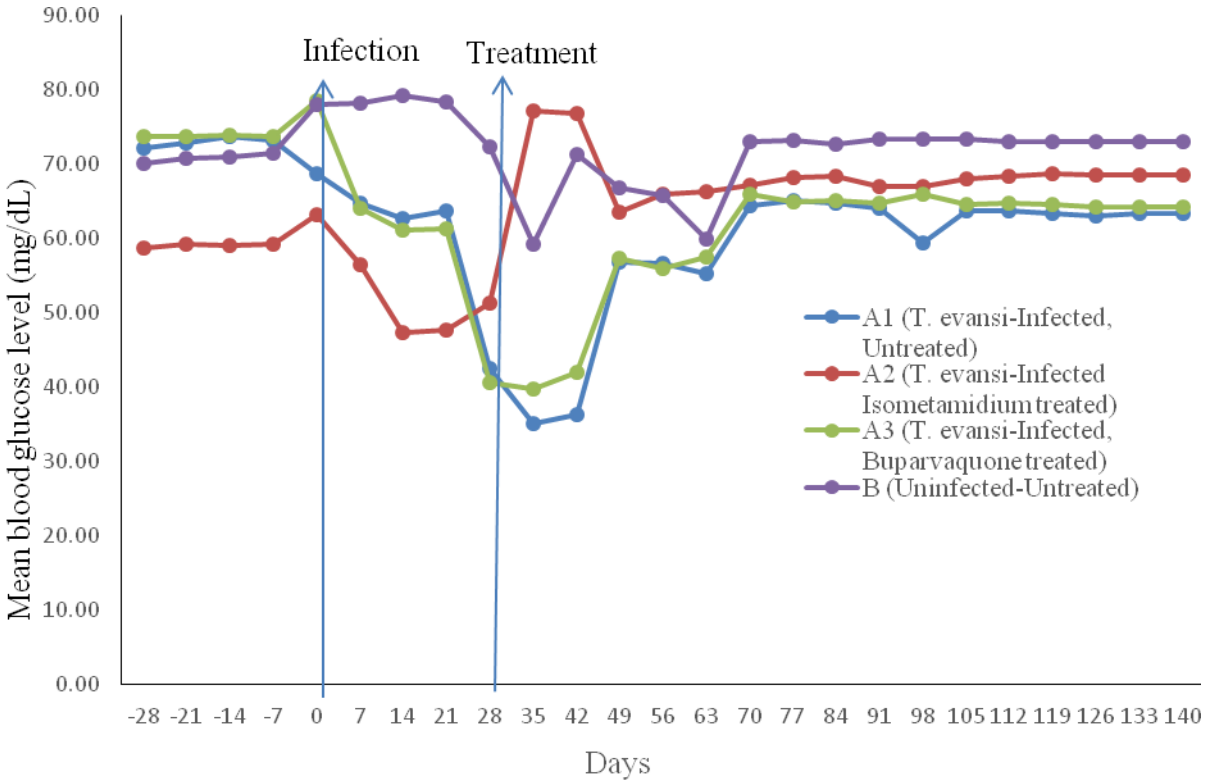


Fig. 10: Mean blood glucose levels; pre-infection, post-infection and post-treatment lof donkeys experimentally infected with *Trypanosoma evansi*

4.6.2 Mean serum levels of total protein, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine and blood urea nitrogen

4.6.2.1 *Group A_1 (T. evansi-Infected-Untreated*)

This group showed significant ($p<0.05$) steady decline in the pre-infection values of total protein (TP) (70.0±3.8 g/dL) and albumin (ALB) (40.4±3.7 g/dL) to final values of (65.8±4.9 g/dL) and (37.0±4.2 g/dL) respectively. The pre-infection values of aspartate aminotransferase (AST), (34.4±9.9 IU/L) and alanine aminotransferase (ALT), (46.2±11.4 IU/L) insignificantly ($p>0.05$) increased PI to 42.4±10.6 IU/L and 51.8±3.2 IU/L respectively and fell insignificantly ($p>0.05$) in the final stage of the experiment to 43.8±5.1 IU/L and 42.6±3.0 IU/L respectively to level up with pre-inf value (Table 4.4a).

There was significant ($p<0.05$) increase in alkaline phosphatase (ALP) from the pre-infection value of 70.0±4.1IU/L to PI value of 78.5±5.5 IU/L which later declined insignificantly ($p>0.05$) to 68.2±13.9 IU/L in the final stage of experiment to return to pre-infection value. There was insignificant ($p>0.05$) decrease in creatinine (CR) activity from pre-infection value of 69.9±5mmol/L to PI value of 67.8±4.6mmol/L and towards the end of the study had insignificant ($p>0.05$) rise to 72.1±5.5mmol/L. There was no significant change in blood urea nitrogen (BUN) activity in all stages of the experiment (Table 4.4b).

4.6.2.2 *Group A_2 (T. evansi-Infected, Isometamidium-treated*)

There was no significant change ($p>0.05$) in the serum levels of TP, AST and ALT. The ALB activity pre-infection (37.6±3.9g/dL) declined PI to 36.7±6.1g/dL and then significantly ($p<0.05$) increased PT to 40.1±3.4g/dL (Table 4.4a). Pre-infection values of

ALP (76.0±11.2 IU/L), CR (71.7±4.5mmol/L) and BUN (4.5±0.9mmol/L) increased though insignificantly ($p>0.05$) PI to 76.8±7.3 IU/L, 69.8±4.9mmol/L and 4.5±0.7mmol/L respectively but declined significantly ($p<0.05$) PT to 65.2±2.7 IU/L, 64.9±2.3mmol/L and 4.0±0.5mmol/L respectively (Table 4.4b).

4.6.2.3 *Group A_3 (T. evansi-Infected, Buparvaquone-treated)*

Animals in this group showed steady but insignificant ($p>0.05$) decline in their mean serum TP, ALB, ALT and AST activity during pre-infection, PI and PT phases of the experiment. (Table4.4a). The pre-infection values of serum ALP (73.3±8.9 IU/L) and BUN (4.0±0.6mmol/L) significantly ($p<0.05$) increased PI to 78.5±5.5 IU/L and 4.5±0.8mmol/L respectively. The PT values of ALP (72.2±4.9 IU/L) and BUN (4.4±0.1 mmol/L) declined insignificant ($p>0.05$) (Table 4.4b).

4.6.2.4 *Group B (Uninfected-Untreated*)

The activities of the above described serum biochemical parameters did not show significant changes throughout the experiment (Tables 4.3a and 4.4b).

Table 4.4a: Mean serum levels of some selected biochemical parametres of donkeys experimentally infected with *Trypanosoma evansi* based on treatment status and `phase of experiment

Experimental Groups	Phases of Experiment	TP±SD (g/dL)	ALB±SD (g/dL)	AST±SD (IU/L)	ALT±SD (IU/L)
A_1(Infected-Untreated)	Pre-infection	70.0±3.8a	40.4±3.7abc	34.4±9.9c	46.2±11.4cd
	Post-infection	67.8±2.9abc	36.9±2.1de	42.4±10.6bc	51.8±3.2bc
	Final value	65.8±4.9c	37.0±4.2e	43.8±5.1bc	42.6±3.0d
A_2 (Infected-Isometamidium Treated)	Pre-infection	68.9±3.6abc	37.6±3.9cde	36.4±14.8bc	51.3±13.7bc
	Post-infection	67.9±5.3abc	36.7±6.1e	41.8±12.6bc	44.4±3.3cd
	Post-treatment	66.3±6.8bc	40.1±3.4ab	43.5±2.8bc	50.4±5.0bcd
A_3 (Infected- Buparvaquone treated)	Pre-infection	69.5±3.4ab	41.8±3.8a	41.5±16.9bc	51.3±16.6bc
	Post-infection	68.0±3.6abc	39.3±4.7abc	41.8±7.9bc	48.3±6.1bcd
	Post-treatment	67.6±7.1abc	39.5±3.9abcde	44.7±8.7b	47.2±2.6cd
B (Uninfected-Untreated; Control)	Initial value	68.7±3.6abc	39.3±3.4abcde	41.8±16.8bc	51.3±16.2bc
	Final value	69.3 ±3.0abc	39.9±3.0bcd	51.5 ±10.1b	62.1±8.7b
P- Value	----------	0.000	0.000	0.000	0.000

Keys: Total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and standard deviation of mean (SD). Means in the same column with the same letter are not significantly different.

Table 4.4b: Mean serum levels of some selected biochemical parametres of donkeys experimentally infected with *Trypanosoma evansi* based on treatment status and phase of experiment

Experimental Animals Groups	Phase of Experiment	ALP±SD (IU/L)	CR±SD (mmol/L)	BUN±SD (mmol/L)
A_1 (Infected-Untreated)	Pre- infection	70.0±4.1cde	69.9±5.1abcd	4.3±0.8ab
	Post-infection	78.5±5.5a	67.8±4.6bcd	4.4±0.3ab
	Final value	68.2±13.9de	72.1±5.5ab	4.3±0.3ab
A_2 (Infected-Isometamidium Treated)	Pre- infection	76.0±11.2ab	71.7±4.5abc	4.5±0.9a
	Post-infection	76.8±7.3ab	69.8±4.9bcd	4.5±0.7a
	Post-treatment	65.2±2.7e	64.9±2.3d	4.0±0.5bc
A_3 (Infected- Buparvaquone Treated)	Pre- infection	73.3±8.9bcd	75.3±5.2a	4.0±0.6bc
	Post-infection	79.6±7.7a	69.8±4.3abcd	4.5±0.8a
	Post-treatment	72.2±4.9bcd	68.2±3.8bcd	4.4±0.1ab
B (Uninfected-Untreated)	Pre- infection	69.8±6.5cde	69.4±11.6bcd	4.2±0.8abc
	Final value	72.9 ±8.0bcd	66.8±11.9bcd	4.1±0.9abc
P- Value	----------	0.000	0.000	0.000

Keys: Alkaline phosphatase (ALP), creatinine (CR), urea nitrogen (BUN) and standard deviation of mean (SD). Means in the same column with the same letter are not significantly different. P-value <0.05=significant.

4.6.3 Mean serum levels of some selected electrolytes

4.6.3.1 *Group A_1 (T. evansi-Infected-Untreated*)

This group showed significant ($p<0.05$) decline in pre-infection values of Ca^{2+} (2.4±0.1mmol/L) and HCO_3^- (24.0±2.0mmol/L) ions to final values (towards end of experiment) of 2.3±0.1mmol/L and 21.9±7.6mmol/L respectively. However, the levels of PO_4^-, Na^+, K^+ and Cl^- ions did not change significantly ($p>0.05$) in all phases of the experiment (Table 4.5).

4.6.3.2 Group A_2 *(T. evansi-Infected, Isometamidium-treated)*

Pre-infection values of PO_4^- (1.1±0.2mmol/L), K^+ (3.9±0.5mmol/L) and HCO_3^{2-} (23.9±2.2mmol/L) did not differ significantly ($p>0.05$) from PI values of 1.1±0.1mmol/L, 4.0±0.3mmol/L, and 24.1±1.3mmol/L respectively. There was, however, a significant ($p<0.05$) increase in values of PO_4^- (1.3±0.0mmol/L), K^+ (4.5±0.6mmol/L) and HCO_3^{2-} (19.8±2.7mmol/L) respectively PT. There was no significant change in the levels of Ca^2, Na^+ and Cl^- ions (Table 4.5).

4.6.3.3 *Group A_3 (T. evansi-Infected, Buparvaquone-treated*)

There was no significant change ($P>0.05$) in the levels of all of the electrolytes under consideration in this group in all phases of the experiment (Table 4.5).

4.6.3.4 *Group B (Uninfected-Untreated; control*)

Animals in this control group maintained insignificantly different ($P>0.05$) blood levels of all the electolytes in the initial and final phases of the experiment (Table 4.5).

Table 4.5: Mean serum levels of some selected electrolytes in donkeys experimentally infected with *Trypanosoma evansi* based on treatment status and phase of experiment

Experimental Animals Groups	Phase of Experiment	Ca^{2+}±SD (mmol/L)	PO_4^{-}±SD (mmol/L)	Na^{+}±SD (mmol/L)	K^{+}±SD (mmol/L)	Cl^{-}±SD (mmol/L)	HCO_3^{-}±SD (mmol/L)
Infected-Untreated	Pre- infection	2.4±0.1bcd	1.1±0.1b	138.9±2.5a	4.0±0.4bc	99.2±2.9abc	24.0±2.0ab
	Post-infection	2.4±0.1abcd	1.1±0.0b	138.1±3.5a	3.9±0.4bc	101.5±4.5a	23.7±2.3a
	Final vlue	2.3±0.1e	1.2±0.2b	138.0±3.3a	3.7±0.5c	100.4±3.0a	21.9±7.6c
Infected- Isometamidium	Pre-infection	2.3±0.1d	1.1±0.2b	140.4±3.6a	3.9±0.5bc	99.5±3.5ab	23.9±2.2ab
	Post-infection	2.4±0.1bcd	1.1±0.1b	138.8±2.3a	4.0±0.3bc	97.3±3.4abc	24.1±1.3ab
Treated	Post- treatment	2.4±0.1ab	1.3±0.0a	112.8±5.4a	4.5±0.6a	96.0±1.1cd	19.8±2.7d
Infected- Buparvaquone	Pre- infection	2.4±0.1abcd	1.1±0.1b	139.4±2.7a	4.1±0.5bc	97.2±4.0bcd	24.0±2.3ab
Treated	Post-infection	2.4±0.1bcd	1.1±0.1b	140.0±3.8a	4.3±0.3ab	99.5±1.9ab	23.3±1.3abc
	Post treatment	2.5±0.1a	1.1±0.1b	136.7±2.9a	4.0±0.3bc	97.0±2.1bcd	22.6±1.8bc
Uninfected-Untreated	Initial value	2.4±0.1bcd	1.2±0.1b	139.8±2.7a	4.1±0.6bc	99.3±3.0abc	24.0±2.6ab
(Control)	Final value	2.4 ±0.1bcd	1.1±0.1b	139.3±3.5a	4.0±0.8bc	99.2 ±2.8abc	24.6±1.5a
P –Value	----------	0.000	0.054	0.098	0.014	0.000	0.000

Keys: Total calcium (Ca^{2+}), phosphate ion (PO_4), sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), bicarbonate (HCO_3) and standard deviation of mean (SD). Means in the same column with the same letter are not significantly different. P-value <0.05=significant.

4.7: Gross Pathological Lesions in Donkeys Infected with *Trypanosoma Evansi* Based on Treatment Status

4.7.1: Group A_1 (Infected-untreated)

Post-mortem conducted on dead animals on days 11 and 28 PI, showed frothy exudates in the trachea and bronchi as well as lung congestion. While exsanguinated animal on day 176 PI equivalent to day 148 PT (termination of experiment) showed more lesions notably gray hepatisation of the lungs, hydrothorax (30ml), hydroperitoneum (straw coloured) and serous atrophies of mesenteric, peri-renal and coronary fats. Others were chicken fat clot in auricles and adherence of renal capsule to the renal cortex (Table 4.6, Plates IV, VII, VIII and X).

4.7.2: Group A2 (Infected, Isometamidium-treated)

Lung congestion and frothy exudate in trachea and bronchi were observed on day 148 PT (Table 4.6, Plate V).

4.7.3: Group A3 (Infected, Buparvaquone-treated)

Lung congestion, frothy exudate in trachea and bronchi, haemorrhagic enteritis and impaction of stomach were observed PT (Table 4.6, Plate VI).

4.7.4: Group B (Uninfected-untreated; control)

The control group did not show gross lesions at termination of experiment (Table 4.6, Plate III.

Table 4.6: Gross postmortem lesions observed in donkeys experimentally infected with *Trypanosoma evansi*

Experimental group	Days11 and 28 Post- infection (PI) lesions	Day176 PI (i.e Day148 Post-treatment Period) lesions
A_1 (Infected- Untreated)	-Frothy exudates in trachea and bronchi -Lung congestion	-Lung congestion - Frothy exudates in trachea and bronchi -Gray hepatisation of lungs -Hydrothorax (300ml) -Hydroperitoneum (straw coloured) -Serous atrophies of mesenteric, peri-renal and coronary fats. -Chicken fat clot in auricles -Adherence of renal capsule to the renal cortex
A_2 (Isometamidium treated)		-Lung congestion - Frothy exudates in trachea and bronchi
A_3 (Buparvaquone treated)		-Lung congestion - Frothy exudates in trachea and bronchi -Haemorrhagic enteritis -Impaction of stomach
B (Uninfected-Untreated; control)		-No gross lesions (NGL)

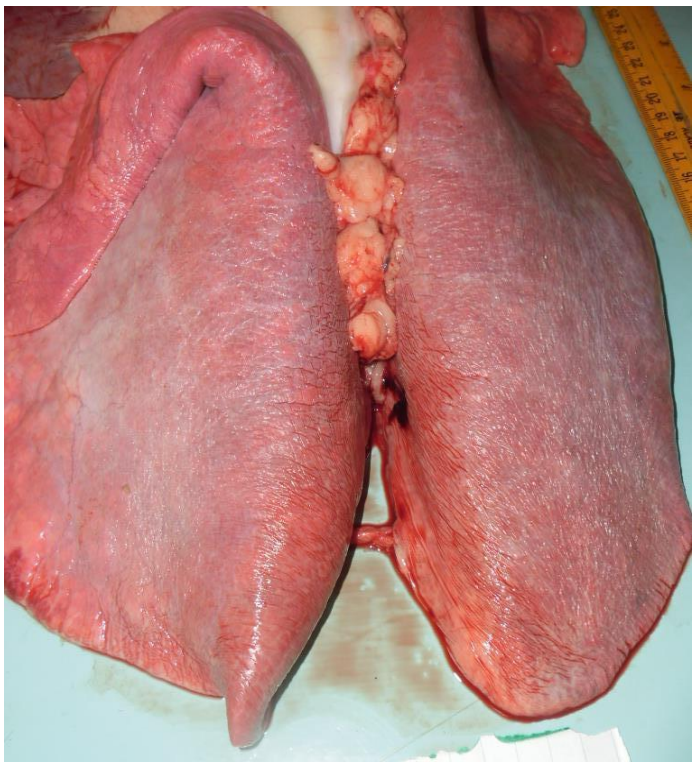


Plate.III: Normal lungs of an Uninfected-untreated (control) donkey; at termination of experiment.

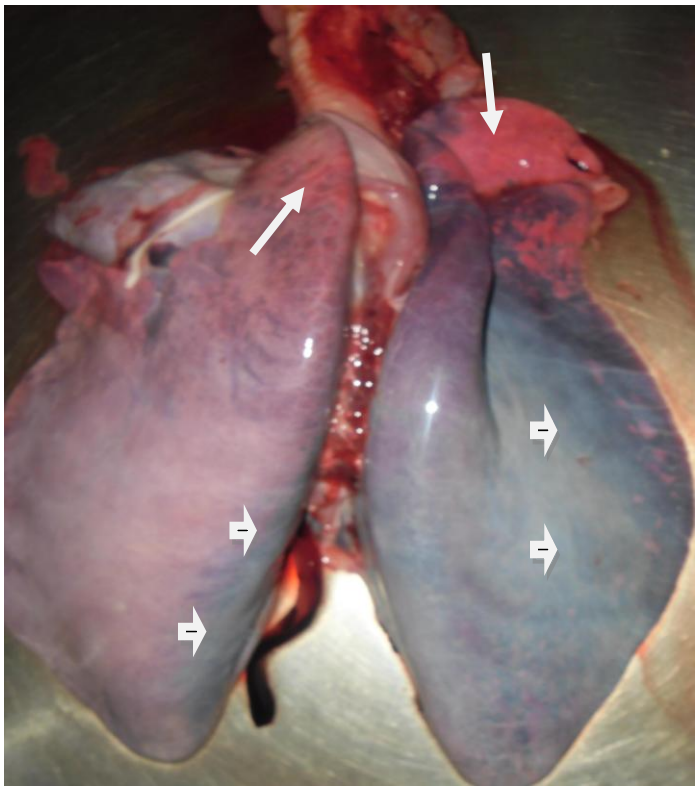


Plate. IV: Lungs with congestion (arrowed) and gray hepatization (arrow heads) from a *T. evansi*-infected, untreated donkey; day28 post-infection.

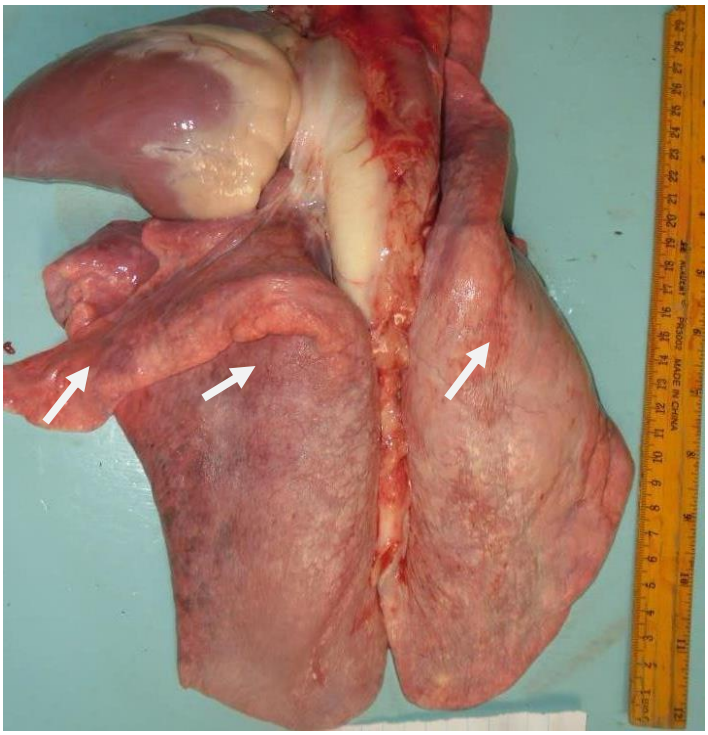


Plate. V: Lungs with congestion (arrowed) from a *T. evansi*-infected, isometamidium-treated donkey on day 148 post-treatment

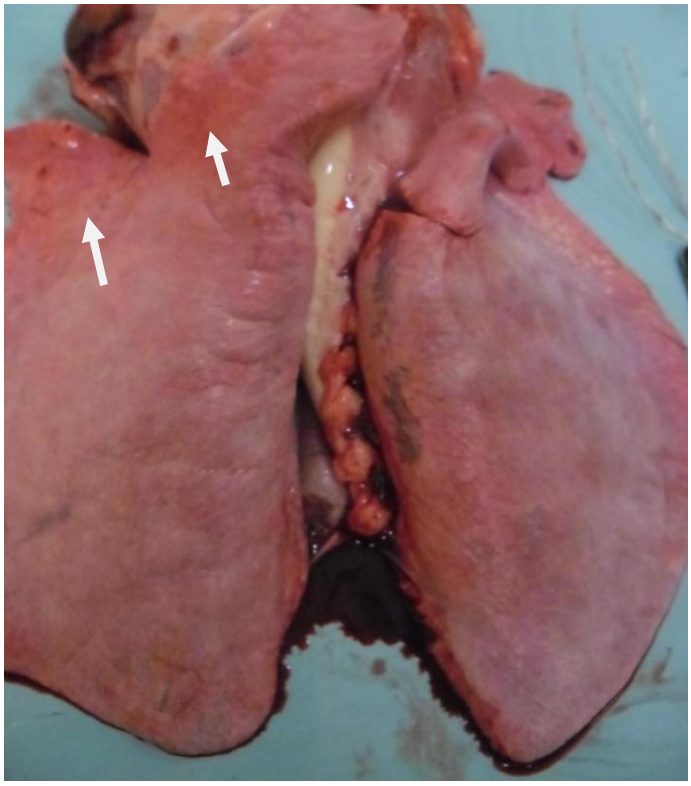


Plate. VI: Lungs with congestion (arrowed) from a *T. evansi*-infected, buparvaquone-treated donkey on day 148 post-treatment

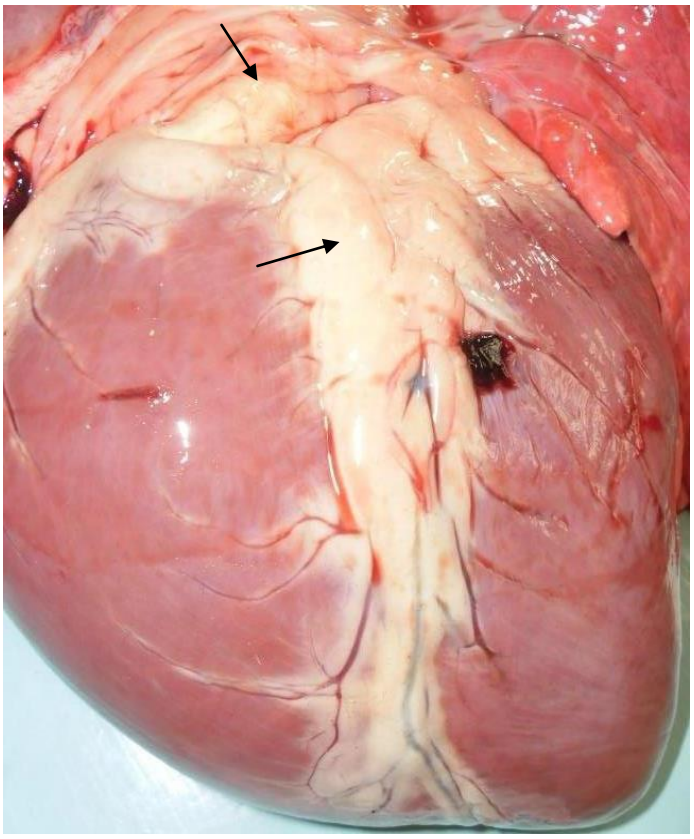


Plate.VII: Heart with intact coronary fat (arrowed) around from an Uninfected-untreated (control) donkey at termination of experiment.

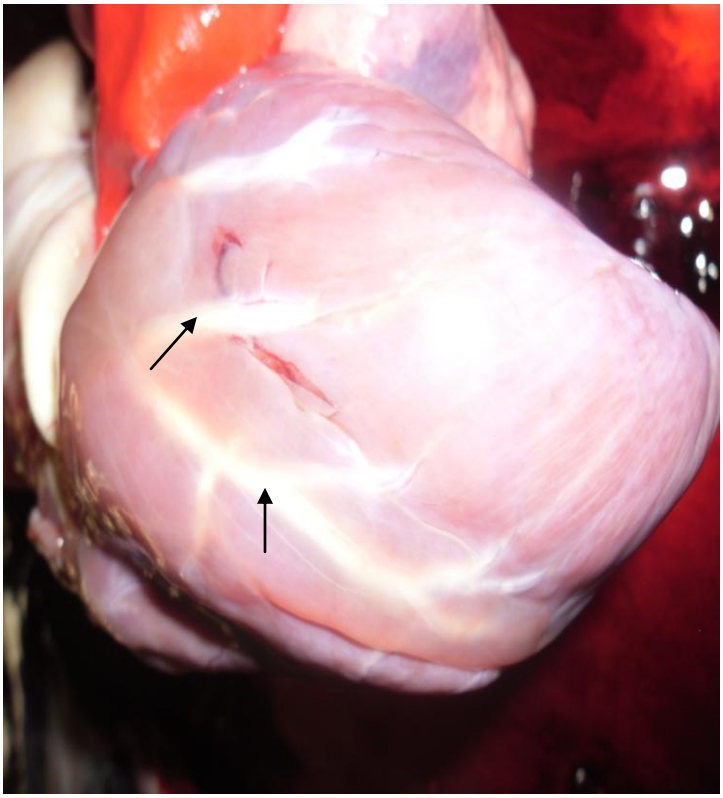


Plate. VIII: Heart with serous atrophy of coronary fat (arrowed) from a *T. evansi*-infected, untreated donkey on day 176 post-infection.

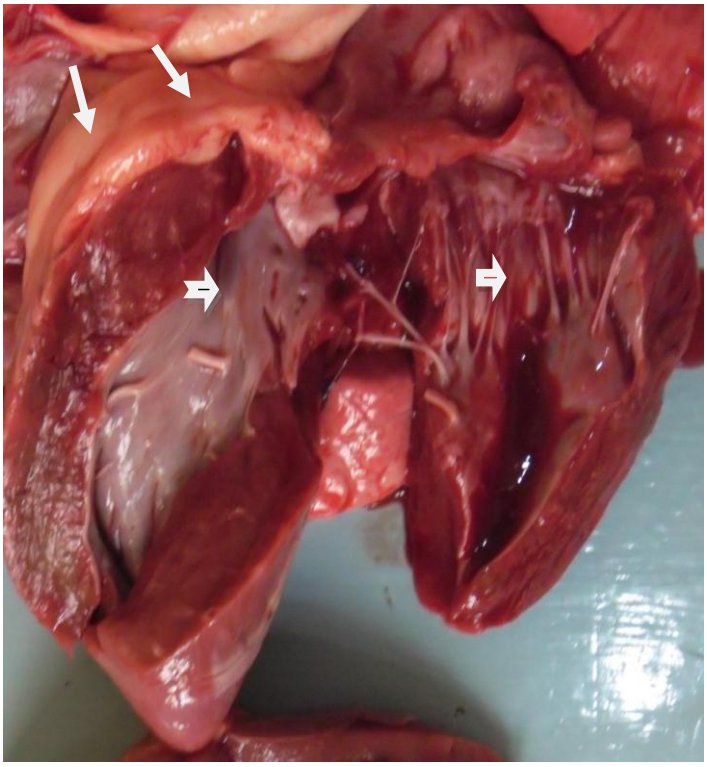


Plate. IX: Heart with coronary fat (arrowed), empty ventricle/ auricle (arrow head) of a *T. evansi*-infected, buparvaquone-treated donkey on day 148 post-treatment, similar to observation in Isometamidium- treated donkey.

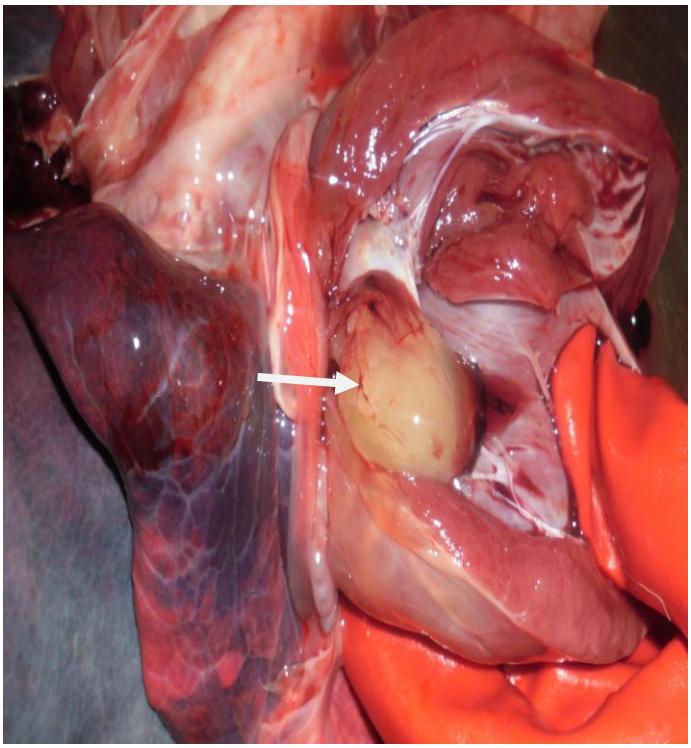


Plate. X: Heart with chicken fat clot (arrowed) within auricle of a *T. evansi*-infected, untreated donkey on day 176 post-infection.

4. 8 Histopathological Lesions of Donkeys Infected with *Trypanosoma Evansi* Based on Treatment Status

4.8.1 Group A_1 (Infected-untreated)

Lung tissue sections of Infected-untreated animals showed congestion and mononuclear cellular infiltration (Plate. XII).

In the spleen, haemosiderosis and depletion of lymphocytes at germinal centres were prominently observed and most severe among all infected groups (Plate. XVI).

Liver lesions observed in this group were congestion, focal areas of necrosis of hepatocytes and mononuclear cellular infiltrations (Plate. XX).

4.8.2 Group A2 (Infected, Isometamidium-treated)

Lungs of the infected, isometamidium treated (Plate. XIII) showed only mononuclear cellular infiltration as a prominent finding.

In the spleen, haemosiderosis and depletion of lymphocytes at germinal centres were observed also but of least severity amongst all infected groups (Plate. XVIII).

There were no significant histopathological lesions in the liver sections of animals from this group.

4.8.3 Group A3 (Infected, Buparvaquone-treated)

Lungs of the infected, buparvaquone treated (Plate. XIV) groups showed only mononuclear cellular infiltration as a prominent finding.

In the spleen, haemosiderosis and depletion of lymphocytes at germinal centres were prominent but less severe than observed in the infected-untreated group (Plate. XVII).

There was no significant histopathological lesions in the liver sections of animals frm this group.

4.8.4 Group B (Uninfected-untreated; control)

The lungs, spleen and liver of uninfected-untreated (control) animals were normal.

4.9 Studies on Organs Invasion by *Trypanosoma Evansi*

Light microscopic investigation of Giemsa-stained impression smears prepared from gluteal muscles, lungs, liver, spleen, heart, kidneys, ovary, testes and brain squash smears of infected animals did not reveal any parasite in any of them post-infection, post-treatment and at termination of the experiment.

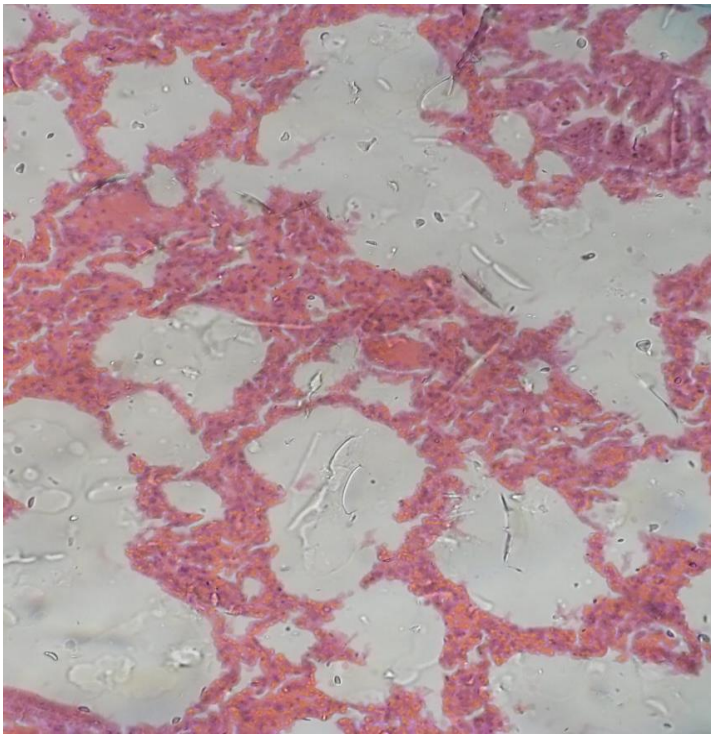


Plate. XI: Normal architecture of lung section of an uninfected-untreated (control) donkey (Haematoxylin-Eosin; H-E stain) (x200).

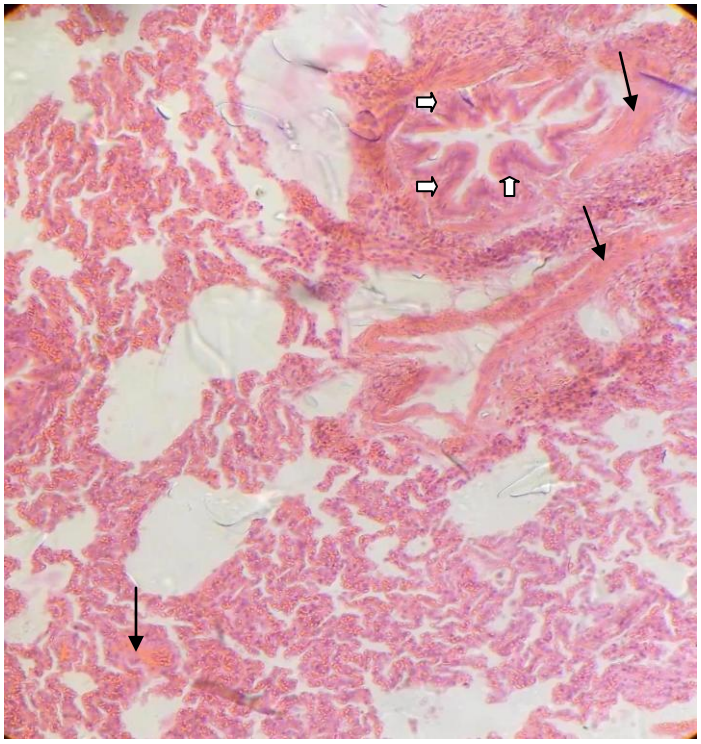


Plate. XII: Congestion (arrowed), mononuclear cellular infiltration (arrow heads) in histopathological section of lungs of a *T. evansi*-infected, untreated donkey; day 176 post-infection (H-E, x200).

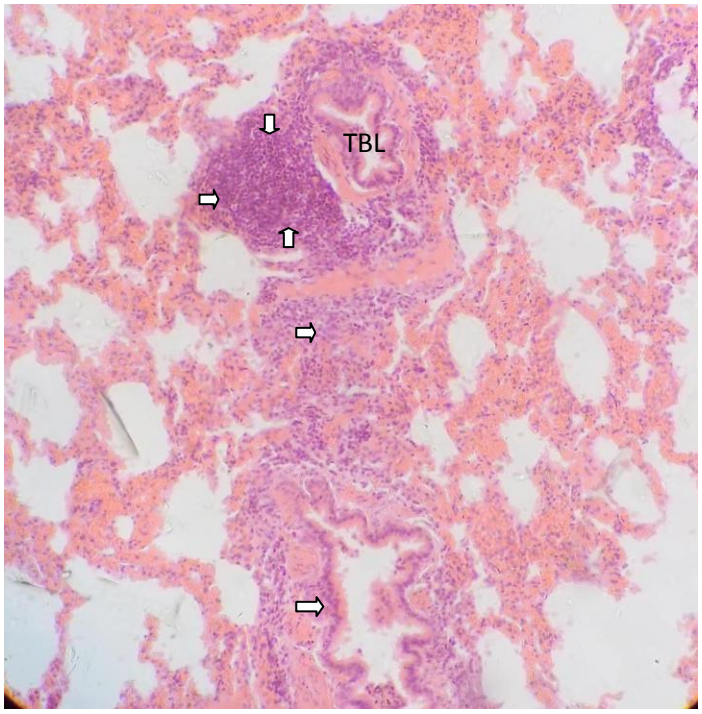


Plate. XIII: Mononuclear cellular infiltrations (arrow heads) around terminal bronchiole (TBL) in histopathological section of lung of a *T. evansi*-infected, isometamidium-treated donkey; day148 post-treatment (H-E, x200**).**

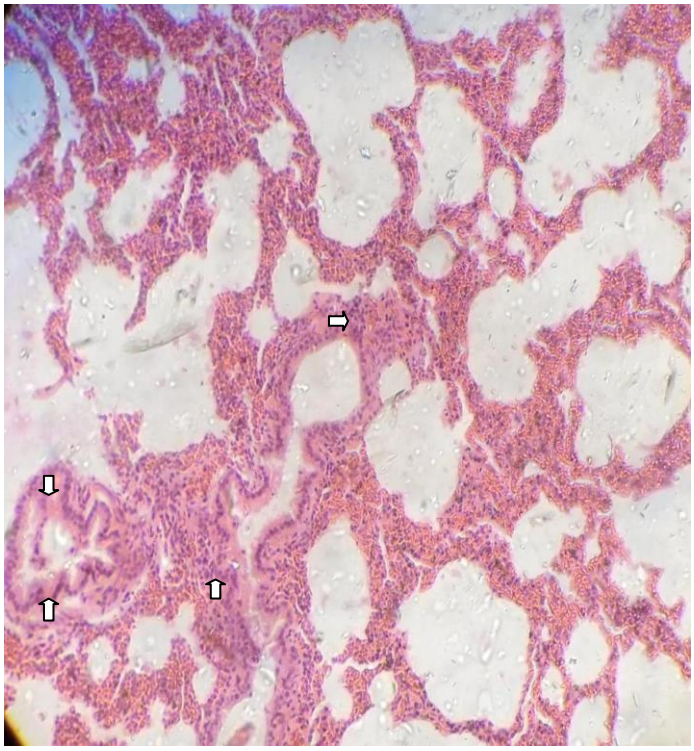


Plate. XIV: Mononuclear cellular infiltration (arrow heads) in histopathological section of lung of a *T. evansi*-infected, buparvaquone-treated donkey; day 148 post-treatment (H-E, x200).

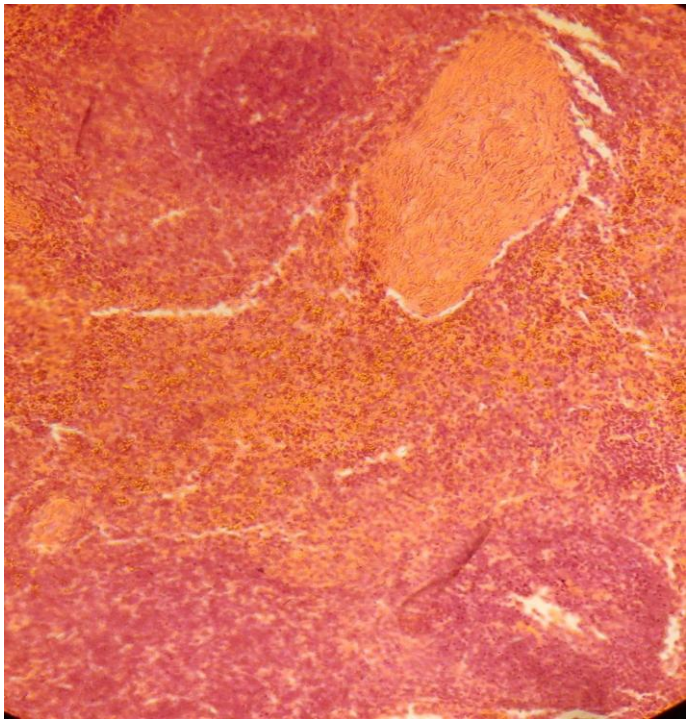


Plate. XV: Normal histopathological section of spleen of an Uninfected-untreated (control) donkey (H-E, x400).

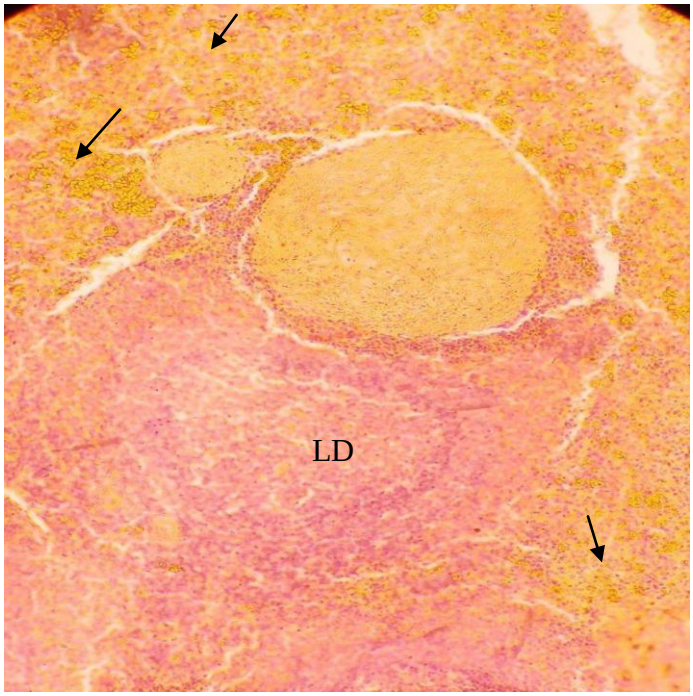


Plate. XVI: Haemosiderosis (arrowed) and lymphocytes depletion at germinal centres (LD) in a histopathological section of spleen of a *T. evansi*-infected-untreated donkey; day 176 post-infection (H-E, x200).

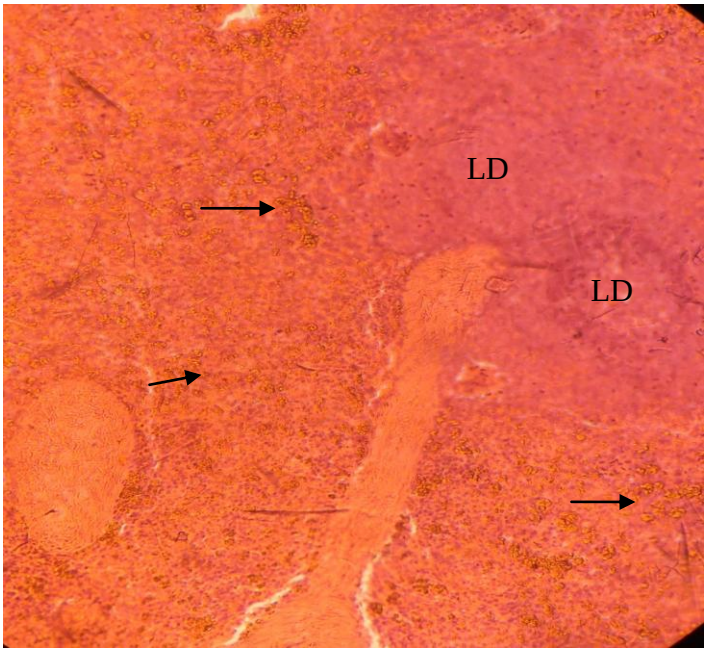


Plate. XVII: Haemosiderosis (arrowed) and lymphocytes depletion at germinal centres (LD) in a histopathological section of spleen of a *T. evansi*-infected, buparvaquone-treated donkey; day 148 post-treatment (H-E, x200).

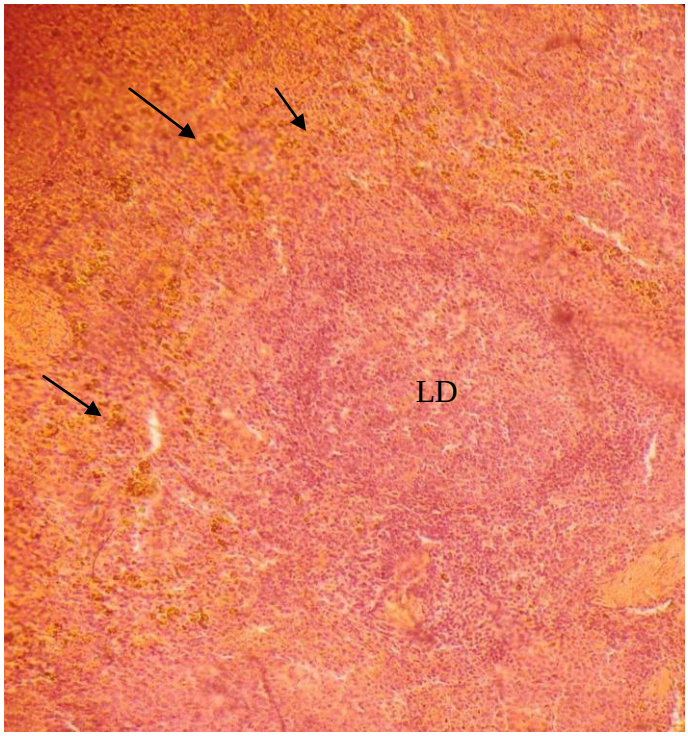


Plate. XVIII: Haemosiderosis (arrowed), and lymphocytes depletion at germinal centres (LD) in histopathological section of spleen of a *T. evansi*-infected, Isometamidium-treated donkey; day 148 post-treatment (H-E, x400).

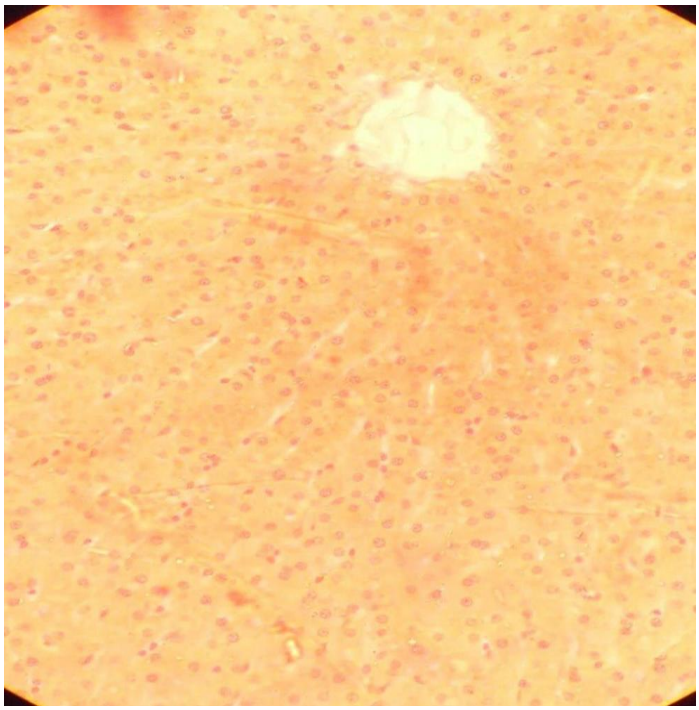


Plate. XIX: Normal histopathological section of the liver of an Uninfected-untreated (control) donkey (H-E, x400).

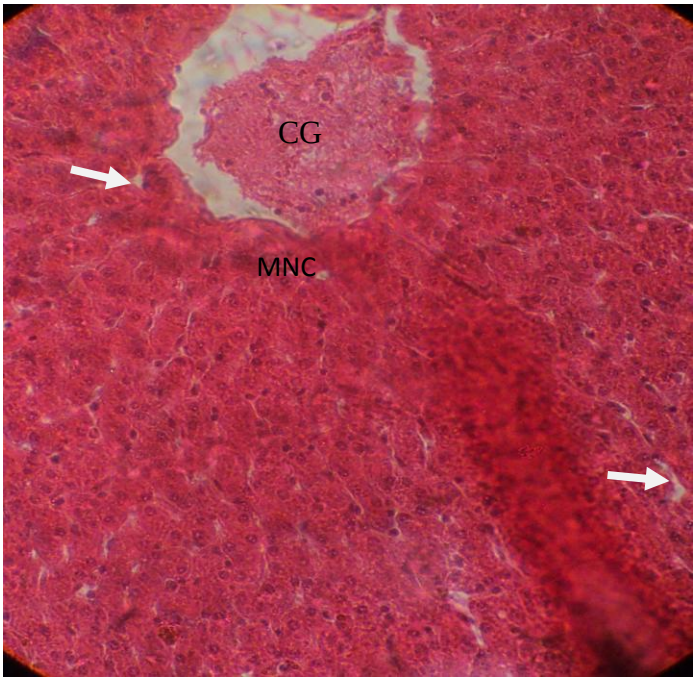


Plate. XX: Congestion (CG), focal necrosis (arrowed) and mononuclear cellular infiltration (MNC) in histopathological section of liver of a *T. evansi*-infected-untreated donkey; day 148 post-treatment (H-E, x400).

CHAPTER FIVE

5.1 DISCUSSION

From this study, *Trypanosoma evansi* parasites were observed in peripheral blood samples collected from the experimentally infected groups of donkeys and examined beginning from day 3 and by day 4 all infected animals had shown parasitaemia. This showed that the pre-patent period (PPP) in this study is 3 to 4 days which is earlier than the 4 to 13 days reported for domestic animals by Ramirez *et al.* (1979). Similarly, the clinical signs started manifesting on day 3 PI with mild pyrexia and by day 7 all infected animals had shown pyrexia and lethargy. This implies that the incubation period (IP) of *T. evansi* infection in this study is 3-7 days. These days are slightly earlier than those reported for infection under natural condition possibly due to difference in the routes of infection in which the jugular vein route used in this experiment allowed the parasites to reach blood stream earlier than if infection was aided by insect vector bite during blood meal. A mild form of pyrexia was observed and this could be due to the level of pathogenicity of the Sokoto isolate of *T. evansi* or susceptibility of the donkeys as a result of immunotolerance of donkeys to *T. evansi* infection, thereby, limiting the severity of acute signs (Brun *et al*., 1998; Herrera, *et al*., 2004) with tendency for the disease to progress to the chronic form of the disease (Desquesnes *et al*., 2013).

The signs observed in this study were similar to those described for *T. evansi* infection in donkeys by Desquesnes *et al.* (2013) and include lymphadenopathies which is due to immunologic response by lymphoid tissue to the infecting *T. evansi* (Klaus, 1996). The pale mucous membranes suggest reduced peripheral circulation of blood due to anaemia (Rickman and Cox, 1983) while the rough hair coat, urticaria, and bilateral seromucous

epiphora may partly be due to the effects of immune complexes deposition, tissue inflammation and anoxic damage characteristic of *T. evansi* infection in animals (Damayanti, 1993; Enwezor and Sackey, 2005). Furthermore, the epiphora might be triggered by irritation and inflammatory tissue reaction that accompany the entry of *T. evansi* into the anterior chamber of the eye as reported for horses (Stephen, 1986).

The lethargy, prominent hipbone/ribs and the isolation behaviour might be contributed by the hypoglycaemia (Habila *et al*., 2012) and the anaemia observed in this study resulted in tissue anoxia (Gardiner and Mahmoud, 1990) in all the *T. evansi*-infected donkeys.

Other signs observed in the acute phase were persistent penile erection which has not been reported previously, anywhere, in cases of surra in donkeys. The penile erection could not have been due to libido because at the age of 11 to 15 months of donkeys' life, they were not mature as to exhbit such behaviour, since donkeys attain maturity at the age of 3-4 years (Starkey, 1995). In horses, intravascular coagulopathy was suggested as responsible for similar persistent penile erection in Lingard report of 1896 and cited by Stephen (1986) and might be responsible for the same condition in these donkeys.

The diarrhoea observed in infected animals in this study could be due to the hypoalbuminaemia observed in this study and enhanced by dietary nutrients maldigestion and malasorption (Allison, 2012) since metabolic enzymes disorder is a characteristic finding in surra (Bal *et al*., 2012). The immunosuppression of the host animal by *T. evansi* could have allowed the flare up of intestinal microflora (*Salmonella* serovars and *Clostridium perfringens*), especially in the colon and caecum leading to hypermotility of the intestines and finally resulting in decreased transit time of intestinal content and

diarrhoea would ensue (Stewart, 2013). The rectal prolapse observed in this study might be due to the loss of rectal muscle tone during straining associated with intermittent diarrhoea (Stewart, 2013).

The ventral abdominal oedema observed in the chronic phase of the infection could have been precipitated by hypoalbuminaemia with loss of vascular fluid to the interstitial tissue, especially in dependent areas (Allison, 2012). The oedema occurred in all groups of infected animals but was most severe amongst Infected–Untreated group of donkey. The Infected but treated ones showed less severe oedema, possibly due to treatment in the latter 2 groups while the infected-untreated group suffered from full blown disease (OIE, 2010) due to parasites-induced metabolic enzymes failure (Bal *et al*., 2012).

The acute signs of infection observed in this study were mild pyrexia, hyperpnoea, tachycardia, lymphadenopathy, sudden weight loss, urticaria, diarrhea, rectal prolapse, lethargy, pale mucous membranes (anaemia), persistent penile erection and upto 11% acute mortality which was lower than over 50% acute mortality reported for natural surra in horses (Silva *et al.*, 1995).

The chronic signs recorded in this study were rough hair coat, severe urticaria, emaciation, lethargy/recumbency, bilateral seromucoid epiphora, isolation behavior and ventral abdominal oedema.

The observed 100% morbidity showed that *T. evansi* (Sokoto isolate) obtained from an infected camel, is pathogenic to donkeys. The low mortality rate in the acute phase as well as the relapse observed in the chronic phase of the study with majority of the infected-untreated animals surviving beyond the termination of experiment suggest that the course of surra in donkeys is predominantly chronic, if not treated or ineffectively treated in

Nigeria. Similar reports from other parts of the world have been made (Faye *et al*., 2001; Desquesnes *et al.,* 2013). The infected, Isometamidium chloride treated donkeys showed decrease in the over-all signs of the disease suggesting amelioration of signs following incomplete clearance of parasitaemia level by the drug (Gutiérrez *et al.,* 2013). This would lead to suggest the need for a second dose of the drug on day 50 post first treatment. As for the buparvaquone-treated group of animals there was persistence of the signs up to the chronic phase but with reduced severity suggesting that there was no amelioration of clinical signs by buparvaquone. This would tend to suggest that buparvaquone does not have therapeutic effect enough to substantially eliminate *T. evansi* from blood stream of donkeys. This seems the first report of the use of this drug in trying to determine the chemotherapeutic effect of buparvaquone on *T. evansi* infection in donkeys.

The trend of parasitaemia in all groups of animals infected with *T. evansi* was observed to be undulating and its disappearance from peripheral blood in chronic stage is consistent with parasitaemia pattern in donkeys, suggesting trypanosomes ability to reside intermittently in intra- or extra-vascular fluids (Sudarto *et al*., 1990). Switching of surface glycoprotein by trypanosomes also causes relapses of parasitaemia and remittent clinical signs (Ramirez *et al*., 1979; Stephen, 1986; Hutchinson *et al.,* 2007). The detection of positive parasitaemia status chronically in the Infected-Untreated group using mice inoculation test (MIT) and the death of all mice used for the test as a result of high parasitaemia proved that infected but untreated donkeys become chronic to subclinical carriers of low level parasitaemia and that the parasites from such carriers are virulent and pathogenic (OIE, 2010). From this study, the carrier status was assumed to be from day 100 PI but detectable on following the use of MIT which is 50% to 74% more sensitive

than direct microscopic methods such as HCT (Luckins, 2014). The negative parasitaemia status (on MIT) exhibited by Isometamidium-treated group of animals lasted for 50 days PT. There was, however, relapse on day100 in 60% of treated animals as revealled using MIT and recording no death in any of the inoculated mice suggesting that Isometamidium chloride, incompletely cured the *T. evansi* infection and that treatment might have rendered the *T. evansi* non-pathogenic and avirulent. This can also be explained by the report of Shapiro and Englund (1990) that Isometamidium acts by cleavaging kDNA-topoisomerase complexes, resulting in the disintegration of minicircle network within the *T. evansi* kinetoplast. Isometamidium chloride also utilizes mechanisms apart from those dependent on kDNA (Kaminsky *et al*., 1997). Buparvaquone-treated group became aparasitaemic earlier than the Infected-Untreated group on HCT. However, the detection of parasitaemia using MIT and the death of all mice used for MIT from heavy parasitaemia suggest depression of parasitaemia without cure or clearance of the parasitaemia. The donkeys become chronic subclinical carriers of virulent and pathogenic *T. evansi* PI, indicating non-effectiveness of the drug against the parasite.

The hyperpnoea observed in all infected groups of animals is apparently compensatory as accompanied by *T. evansi*-induced anaemia which reduced available haemoglobin to transport oxygen to tissues resulting in tissue anoxia (Gardiner and Mahmoud, 1990). The return of respiratory rate to pre-infection level amongst the isometamidium chloride treated animals in a week PT indicates amelioration by isometamidium as against persistence of hyperpnoea for longer period in other groups.

Although, only the buparvaquone-treatment group showed significant elevation of pulses rate (tachycardia) PI, there was also tachycardia in other infected groups PI. This suggests

a compensatory increase in heart rate to improve blood supply to tissues as a physiological effort to overcome hypovolaemia precipitated by *T. evansi*-induced anaemia (Gardiner and Mahmoud, 1990). This finding is similar to the tarchycadia in donkeys reported by (Desquesnes et al., 2013).

The pyrexia observed in all *T. evansi*-infected donkeys was mild possibly due to the relatively low susceptibility of the donkeys to *T. evansi* infection (Silva *et al*., 1995; Desquesnes *et al*., 2013) and their immunotolerance to *T. evansi* (Herrera, *et al*., 2004). The absence of pyrexia in the chronic phase of infection may be as a result of low parasitaemia in the phase since pyrexia in acute phase of infection is reported to accompany successive waves of high parasitaemia in camel surra (Rami *et al.*, 2003; Wolkmer *et al*., 2009). This might be the reason for absence of pyrexia in chronic phase of infection in the donkeys.

The decline in mean body weight values of all *T. evansi*-infected animals occurred side-by-side with high parasitaemia, resulting in the generation of peroxides and free radicals that damage all components of the cell, including proteins and lipids (Murray *et al*., 2003; Saleh *et al*., 2009). Similarly, complete dependence of *T. evansi* on the host animals for the glucose it requires for its matabolic activity (Maudlin *et al*., 2004; Hunt, 2010), mortility (Newton, 1978) and the parasites-induced metabolic enzymes insufficiencies in the host (Enwezor and Sackey, 2005) might be responsible for the weight loss (emaciation) observed in infected animals which agrees with the progressive weight loss that occurs in chronic form of surra as reported by Omer *et al.* (2007).

Isometamidium incompletely cured parasitaemia (Shapiro and Englund, 1990) thus allowed weight gain in treated group, though, resurgence of parasitaemia caused the rate

of weight gain to be very slow. The infected buparvaquone-treated and untreated groups experienced gentle but steady weight loss in the chronic stage, perhaps due to persistence of pathogenic parasite in the animals resulting in un-abated metabolic failures and tissue damage (Enwezor and Sackey, 2005).

The decline in the mean PCV, Hb and total RBC values in all *T. evansi*-infected groups could be attributed to the infection at least during the PI period, especially before treaments with the two study drugs. Similar observations of allignment of decline in the levels of the three parametres with high parsasitaemia in surra were made by Marques *et al.* (2000) and Aquino *et al.* (2001) and reported to be responsible for the anaemia associated with *T. evansi* infection in donkeys and other animal species (Herrera *et al*., 2002; Enwezor and Sackey, 2005; Cadioli, *et al.,* 2006; Berlin *et al*., 2010). Anaemia in Haemol surra is due to synergism amongst pro-inflammatory cytokines whose levels are increased in *T. evansi* infection (Paim *et al*., 2011), free fatty acids, hemodilution, coagulation disorders, depression of erythrogenesis, release of trypanosomal sialidase enzyme (Esievo and Saror, 1991; Omer *et al*., 2007; Adamu *et al*., 2008). The anaemia in animals' surra is progressive (Omer *et al*., 2007).

Post-treatment, values of PCV and other erythrogram parametres, reverted to pre-infection range of values in all infected and variously treated animals and this is in agreement with observation by Berlin *et al.* (2010) who regarded the changes to be due to low parasitaemia, haemoconcentration precipitated by loss of intravascular fluid to the interstitials as in dehydration and subcutaneous oedema which are pronounced in chronic stage of infection (Omer *et al*., 2007; Wolkmer *et al*., 2009). However, in the Isometamidium-treated group the parameters improved earlier and that the mean values

were relatively higher, suggesting cure and ameliorative effect of Isometamidium chloride which was closely followed by values from buparvaquone group where there was no cure.

Leucogramme change in this study was lymphopaenia in the Infected-Untreated group of animals that was observed PI and it progressed to the final (chronic) stage of the experiment. The PI decline in lymphocytes count could be due to the lymphotoxic substances released from *T. evansi* membranes into the host animals' body resulting in leucocytes death as reported on surra in donkeys (Antoine-Moussiaux *et al*., 2009). The gradual but steady progression of the decline in levels of the lymphocyte towards the later stage of the infection in infected-untreated animals also suggests immunosuppressive effect and the tendency of surra to progress to chronic stage in donkeys (Berlin, *et al*., 2010; Desquesnes *et al*., 2013).

The Isometamidium-treated group showed insignificant panleucopaenia PI which improved PT suggesting a decline in parasitaemia due to treatment with corresponding decline in lymphotoxin activity (Antoine-Moussiaux *et al*., 2009) consequently amelioration of the leucogramme picture.

The buparvaquone-treated group showed insignificant leucopaenia PI. There was also significant PT decline in neutrophils and monocytes levels, insignificant decline in lymphocytes and eosinophils. Band cells count increased significantly. This picture suggests supression of leucocytes even amongst the buparvaquone treated animals, despite the report that buparvaquone potentiates immunity in its prophylactic effect against bovine theileriosis (Dhar *et al.,* 1987; Mutugi *et al,* 1988).

The significant decline in blood glucose levels of all *T. evansi*-infected groups of animals started on day7 PI which coincided with the period of high parasitemia (day7-35)

in the groups of *T. evansi*-infected animals. This may be due to glucose uptake by the *T. evansi* parasites as *T. evansi* depends on host glucose for motility and multiplication as observed by Habila *et al.* (2012) and also reported in *T. evansi* –infected donkeys (Marques *et al*., 2002; Cadioli *et al*., 2006) and camels (Sazmand *et al*., 2011). Also, hypoglycemia in animals with surra is enhanced by increased metabolism in host animal due to fever (Stephen, 1986) and hepatocytes degeneration (Von Brand, 1973). Animals treated with isometamidium chloride showed improvement in mean blood glucose level within 1 week which agreed with similar report on rat by Kumar *et al*. (2009). However, the persistence of hypoglycemia in infected but buparvaquone-treated and the Infected-untreated groups up to day 63 PI (8 weeks period) reflects lack of parasite control or cure in the groups. Beyond day 70 PI to the end of the experimen, charracterised by 10 weeks period of low parasitaemia, the animals showed improved but slightly low blood glucose values that were below pre-infection values due to the low parasitaemia in the chronic stage of surra (OIE, 2010).

The Infected-Untreated group showed significant decline in serum TP and ALB post-infection. This suggests possible decrease in the synthesis of the proteins due to enzyme disorders, poor utilisation of dietary protein and insignificant immunoglobulin response to infection (Allison, 2012). Chronic surra in donkeys is associated with hypoproteinaemia including hypoalbuminaemia as reported earlier by Cadioli *et al.* (2006). However, the observed low levels of ALT, AST and TP contradicts an earlier report by Sow *et al*. (2014) that serum levels of the parameters are high in donkeys with trypanosomosis. The opposing finding may probably be due to variations in host susceptibility, parasite strain, environmental factors, concurrent infection and stress which

are important for in the determination of the severeity of trypanosomosis (Brun *et al*., 1998). The low TP also conforms to the pattern of decline by negative-acute-phase proteins in acute inflammation (Allison, 2012). The insignificant decrease in AST and ALT activity and the brief significant rise in ALP activity which, later (chronically) fell to pre-infection levels indicated that the overall liver function of the infected animals was not significantly altered in the Infected-Untreated animals. Similarly, the, insignificant rise in CR and unaltered BUN activity, suggest that there was no chronic kidney dysfunction in the infected-untreated group of animals (Allison, 2012).

In the Isometamidium treated group of animals TP, AST and ALT activity did not change. This could indicate that the liver function was normal in the group even though insignificant hypoalbuminaemia occurred PI which was significantly reversed following treatment (Margi, 2007).

Akaline phosphatase activity rose PI in the infected-untreated as well as infected-buparvquone-treated groups of animals which suggests obstructive lesion in the billiary duct (cholestasis). Report by Enwezor and Sackey (2005) on camel surra showed that immune complex deposits are found in host organs with *T. evansi* infection. Therefore, deposits of immune complexes might be responsible for cholestasis in this experimental donkeys causing transient elevation of ALP activity PI which reverted to pre-infection levels in the later stage of the disease (whether treated or untreated). The normal levels of CR and BUN observed in the groups of animals suggest that the Kidneys were devoid of chronic dysfunction (Allison, 2012).

The observed hypocalcemia in the chronic phase of infection amongst the infected-untreated group of donkeys might be due to uptake of calcium by *T. evansi* as reported in

rabbits (Da Silva *et al.*, 2011), thereby, giving rise to lethergy and intermittent recumbency because of lack of active role of calcium in muscle contraction as also observed by Bohn (2012). The serum levels of PO_4^-, Na^+, K^+ and Cl^- ions did not change significantly in most groups of animals except in the infected, isometamidium-treated donkeys where hyperphosphatemia and hyperkalemia were observed during the chronic stage of the disease suggesting decline in renal clearance of the electrolytes. It has been reported that increased dietary K^+ alone is unlikely to cause hyperkalemia, thereby, incriminating renal or post-renal (obstructive lesions in the ureter or ruptured bladder) disorder (Bohn, 2012). This may mean that the Isometamidium treated animals developed renal or post-renal (obstructive lesions) disorder. The significant decline in serum bicarbonate (HCO_3^-) level in infected-untreated and infected, Isometamidium-treated animals suggests that the animals in the groups were acidotic (Margi, 2007). Possibly, the animals also have no threatening heart failure since high HCO_3^- level in human is associated with cardiac failure (Dobre *et al.*, 2013).

The gross pathological lesions observed in the Infected-Untreated group of animals were limited during the acute phase and became pronounced with more lesions in the chronic phase of infection. Lesions in lungs included congestion, frothy exudates in the trachea and bronchi, hydrothorax, hydroperitoneum and adherence of renal capsule to the renal cortex. The pronounced gross lesions seen in the chronic disease suggest a more severe surra in the donkeys and this agrees with the documentation by OIE (2010). The chicken fat clot observed in the auricles and serous atrophies of the mesenteric, peri-renal and coronary fats are commomnly observed in debilitating equine diseases and this is an

indication of wasting syndrome that could be seen in chronic surra in donkeys. Wasting is a feature of chronic surra as reported by Cadioli *et al* (2006) and OIE (2010).

The infected-Isometamidium-treated animals did not show persistence of lungs congestion and frothy exudates in the trachea and bronchi after treatment, suggesting clearance of parasitaemia and amelioration of the signs. This might have been made possible because Isometamidium chloride is a standard trypanocides and cures infection (Touratier, 1981; Aliu, 2007).

The histopathological observations of congestion and mononuclear cellular infiltrations in the lungs and liver of all the *T. evansi*-infected groups of animals should be due to parasites aggregation, immune complexes deposition and inflammatory tissue formation in capillary beds resulting in vascular stasis in lungs and liver with resultant congestion as reported by Bal *et al* (2012) and Damayanti (1993). The haemosiderosis and lymphocytes depletion in spleen of all the *T. evansi*-infected animals were due to deposits of free iron released from severe hemolysis and RBC sequestration especially in the red pulp area as ealier reported by Bal *et al* (2012) as well as cells lysis due to lymphotoxins released by circulating *T. evansi* (Antoine-Moussiaux *et al.,* 2009).

CHAPTER SIX

6.0 CONCLUSION

In a study to determine the efficacies of Isometamidium chloride and Buparvaquone in the treatments of experimental *Trypanosoma evansi* infection in donkeys, it was observed that:

a) The pre-patent period (PPP) of experimental infection of *T. evansi* in donkeys was 3-4 days.

b) The incubation period (IP) of the infection was 3-7 days.

c) The trend of parasitemia in donkeys was undulating and infected-untreated animals became chronic carriers with parasitaemia that could not be detected on HCT from day100 PI. However, MIT detected parasitaemia and the relapsed parasites killed all mice used for the test confirming that the parasites remained virulent and pathogenic despite disappearance from peripheral circulation in the donkeys.

d) Isometamidium chloride treatment using 1% preparation at the dose rate of 0.5mg/kg body weight once, intramuscularly, resulted in cure and incomplete clearance of parasitaemia in donkeys. The residual parasitaemia was not detectable in the first 50 days PT using MIT but relapse of upto 60% was detected on day100 PT using the same MIT and the relapsed very low parasitaemia did not kill any of the mice used within 48 days of their observation. Therfore, the relapsed parasites were avirulent and non-pathogenic after exposure to Isometamidium treament.

e) Buparvaquone (5%) used at the dose rate of 2.5mg/kg body weight, intramuscularly, twice at 7 days interval caused disappearance (depression) of *T. evansi* from peripheral circulation at about day 49 PT which was 23 days earlier than

infected-untreated group showed aparasitaemic status on HCT. However, this group immediately showed relapse on first trial of MIT with death of all mice used for the test. Therefore, the relapsed parasites were virulent and pathogenic despite exposure to buparvaquone treatment.

f) The clinical course of *T. evansi* infection in donkeys was partly acute but predominantly chronic. Some of the acute signs included transient but mild pyrexia, hyperpnoea, tarchycadia, anaemia, lethargy, lymphadenopathy, intermittent diarrhea and low acute mortality of 11%. Other signs which have not been reported previously in donkeys in Nigeria to the best of our knowledge, but observed in this experiment were penile erection, urticaria with alopecia and rectal prolapse. Isolaton behaviour and bilateral epiphora were also observed in both the acute and chronic stages of the infection. The chronic signs observed were, progressive emaciation/stunted growth, anaemia, ventral abdominal oedema, recumbency and death.

g) Isometamidium chloride treatment significantly ameliorated all clinical signs, gross and histopathological lesions, serum biochemical and electrolytes alterations in the infected donkeys but buparvaquone did not achieve the same result.

h) The haemopathological pictures associated with *T. evansi* infection were anaemia, lymphopenia in acute and early chronic phases of the infection in the Infected-Untreated animals.

i) Serum biochemical alterations in the infected-untreated animals were hypoglycaemia, hypoproteinaemia, hypoalbuminaemia as well as significantly elevated ALP activity. The ALT AST, CR and BUN activities were not significantly elevated in surra of donkeys.

j) Serum electrolytes changes include hypocalcaemia amongst infected, untreated animals. However, Isometamidium-treated animals, showed hyperphosphatemia, hyperkalaemia and declined serum bicarbonate (HCO_3^-) levels in the chronic stage of infection. Treatment with both drugs did not restore the pre-infection levels of the electrolytes.

k) The gross pathological lesions in Infected-Untreated animals were more pronounced in the chronic than in acute disease and included lungs congestion, frothy exudates in the trachea and bronchi, hydrothorax, hydroperitoneum, adherence of renal capsule to the renal cortex, chicken fat clot and serous atrophies of mesenteric, peri-renal and coronary fats.

l) Histopathologically, *T. evansi*-infected but untreated as well as buparvaquone-treated donkeys manifested congestion and mononuclear cellular infiltrations in lungs and liver sections. Haemosiderosis and lymphocytes depletion in spleen were prominent features of *T. evansi* infection in Nigeria.

6.1 Recommendations

* Isometamidium chloride should be used for treatment of surra in donkeys with early treatment being more effective.

* A second curative dose of Isometamidium should be considered in the treatment of surra in donkeys but prophylactic repeat dosing in endemic areas should be administered at about 50 days apart.

* For now, Buparvaquone should not be used in the treatment of surra in donkeys since it only depressed parasitaemia in the present study.

*With anaemia, hypoglycaemia and wasting/stunted growth being major pathologies in *T. evansi* infection in donkeys, haematinics, high energy and proteinous feed rations should be provided during recovery of donkeys under treatment.

*****Antibacterial and non-steroidal anti-inflammatory agents should be considered in the treatment regimen in order to control secondary bacterial infection and the widespread inflammatory processes, especially associated with gastrointestinal and respiratory systems in the course of surra in donkeys.

6.2 Areas of Further Research

1. Further study on safe and effective application of 2 doses of Isometamidium chloride using 1% solution at the rate of 0.5mg/kg body weight is suggested.

2. Research into the effect of Buparvaquone (5%) at the dose rate of 5mg/Kg (doubled dose) and as a combination with other standard trypanocides against T. *evansi* infection in donkeys for possible synergism.

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APPENDICES

Appendix.I: Recommended minimum accommodation standards for equines used for experiment and other scientific purposes

Wither height (WH) (m)	Floor area/animal (m^2/animal)		Enclosure height (m)
	1-3 animals	4 animals	
1.0-1.4	9.0	6.0	3.0
1.4-1.6	12.0	9.0	3.0
>1.6	16.0	$(2x\ WH)^2$	3.0

Source: Guidelines for the Accommodation of Animals used for experiment and other Scientific Purposes, Council Directive 86(609)EEC of 4 November, 1986. Retrived from: www.EUR.lex-32007H0526-EN-EUR-lex

Appendix.II: Recommended minimum accommodation standards for rats and mice used for experiment and other scientific purposes

Animals	Body weight (g)	Enclosure size (cm^2)	Floor area/animal (cm^2)	Enclosure height (cm)
Rat	200-300	800	250	18
Mice	20-25	330	70	12

Source: Guidelines for the Accommodation of Animals used for Experiment and other Scientific Purposes, Council Directive 86(609)EEC of 4 November, 1986. Retrived from: www.EUR.lex-32007H0526-EN-EUR-lex