P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Review Article

Haematological, Serum Biochemical and Electrolyte Profile of Donkeys Experimentally Infected with *Trypanosoma evansi* and the Effects of Isometamidium Chloride and Buparvaquone Treatments

Garba UM[1]*, AKB Sackey[2], KAN Esievo[3] and AI Lawal[4]

[1]Veterinary Clinic, Equitation Department, Nigerian Defence Academy, Kaduna, Nigeria; [2]Department of Veterinary Medicine; [3]Department of Veterinary Pathology; [4]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria
***Corresponding author:** garbaum@yahoo.com

Article History: Received: June 01, 2016 Revised: June 16, 2016 Accepted: June 29, 2016

ABSTRACT

The objective of this study was to evaluate the haematological, selected serum biochemicals and electrolytes parameters of donkeys experimentally infected with *Trypanosoma evansi* as well as determine the effects of isometamidium chloride and buparvaquone treatments. Twenty four healthy donkeys, about 11 to 15 months old participated in the experiment. Six animals were assigned to each of groups A_1 (Infected-untreated), A_2 (Infected, isometamidium-treated), A_3 (Infected, buparvaquone-treated) and B (Un-infected-untreated; control) at random. Two (2) ml inoculum ($2.0x10^6$ *Trypanosoma evansi* parasites) was used to infect each animal in groups A_1, A_2 and A_3 through jugular vein. Groups A_2 and A_3 animals were treated on day 28 post-infection with isometamidium chloride (1%) using $0.5mgKg^{-1}$ I.M. once and buparvaquone (5%) using $2.5mgKg^{-1}$ I.M. twice at 72 hours apart. Mean parasitaemia counts were evaluated post-infection and post-treatment while haematological, serum biochemical and electrolytes parametres were evaluated pre-infection, post-infection and post-treatment. Data was analysed using Analysis of Variance at 95% confidence level ($P<0.05$ = statistically significant). Means of parasitaemia counts and blood/serum parameters are presented on graphs and tables respectively. From the result, groups A_1 donkeys had declined mean serum total Protein, Albumin, Calcium ion and bicarbonate ion levels but increased Alkaline phosphatase activity. Group A_2 showed elevations in Albumin and bicarbonate ion, and declines in Alkaline phosphatase, Creatinine and blood urea nitrogen to pre-infection levels. Also, increases in Phosphate ion, and Potassium ion were observed following treatment. The group A_3 did not show reversal of elevations in the levels of Alkaline phosphatase and blood urea nitrogen activities to pre-infection levels. In conclusion, the changes in donkeys experimentally infected with *Trypanosoma evansi* were haematological (anaemia and lymphocytopenia), serum biochemical (hypoproteinaemia, hypoalbuminaemia, Alkaline phosphatase and Blood urea nitrogen activities) and serum electrolyte (hypocalcaemia and decreased bicarbonate ion level). Isometamidium chloride treatment restored the levels of Albumin, bicarbonate ion, Alkaline phosphatase, Creatinine and Blood urea nitrogen to pre-infection (normal) levels with accompanying hyperphosphatemia and hyperkalaemia. Treatment with buparvaquone did not restore altered serum parameters to pre-infection levels.

Key words: Biochemical, Buparvaquone, Donkeys, Electrolyte, Isometamidium chloride, *Trypanosoma evansi*-infected

INTRODUCTION

Trypanosoma evansi is a pathogenic trypanosome belonging to *salivarian* group and subgenus *Trypanozoon.* It is reported in several studies to be pathogenic to wide range of animal hosts (Cadioli *et al.*, 2006; Habila *et al.*, 2012) mainly camels but cattle, equidae (horses, donkeys, zebra and mules), dogs and pigs are also affected in Africa (Mahmoud and Gray, 1980; OIE, 2004). The parasite also has the widest geographical distribution amongst *Trypanosoma species* affecting domestic and wild mammalian hosts in the tropical and subtropical areas of the world (Eyob and Matios, 2013). Affected areas include 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 are known by several names such as '*surra'* in Asia and Middle East which is an *Indi* word meaning

Cite This Article as: Garba UM, AKB Sackey, KAN Esievo and AI Lawal, 2016. Haematological, serum biochemical and electrolyte profile of donkeys experimentally infected with *Trypanosoma evansi* and the effects of isometamidium chloride and buparvaquone treatments. Inter J Vet Sci, 5(4): 189-199. www.ijvets.com

'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). The disease results in great economic losses due to annual deaths of thousands of mammalian livestock (Cadioli *et al.*, 2006; Fernandez *et al*., 2009) and reduced traction power of affected animals (ILRI, 1997). In Nigeria, natural infection occurs in horses, donkeys and cattle (Ilemobade, 1971). Reports from other parts of the world such as Brazil showed that diagnosis of *surra* in donkeys depends also on results of serum biochemical tests from laboratory (Mori *et al*., 2003). However, data on changes in haematological, serum biochemical and electrolyte parameters and the effect of treatment with Isometamidium chloride or trial of buparvaquone in the treatment of donkeys with experimental *T. evansi* infection, is scanty in Nigeria.

MATERIALS AND METHODS

Location of research

The experiment was conducted in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, A.B.U., (located between latitude 11 ° 15'N to 11° 3'N and longitude 7° 30'E to 7° 45'E), Zaria, Nigeria (Abbas and Arigbede, 2012).

Animals

Apparently healthy donkeys (n=24) comprising of 9 males and 9 females, sourced from Maigatari International Livestock Market, Jigawa State, Nigeria with an estimated age range (based on Wayne and Melvin, 2000 and Joe, 2012 dental features) of 11 to 15 months, participated in this experiment as models.

Healthy and haemoparasites-free adult albino Wistar rats (n=4) and and adult Wistar mice were used for propagation of *Trypanosoma evansi* and animal inoculation test respectively.

Management of experimental animals

Housing: This study was approved by Institutional Committee on the use and Welfare of Experimental Animals of Ahmadu Bello University, Zaria. Inputs on care for the experimental animals were also obtained from Appendix-A of Council Directive 86(609) of European Economic Community (revised 2007). The donkeys were housed in compartmentalised, fly-proof experimental animal pen using their pre-determined mean wither height (height from the fore-hoof to point of wither) and body weight in determining stocking density. Wood shavings and corn stalk were used as bedding, faecal droppings were mucked thrice daily and the bedding was changed as soon as the old one was becoming damp. The albino rats and Wister mice were maintained in separate, labeled cages (Wolfensohn and Lloyd, 2013).

Feeding of animals: The experimental donkeys were provided with roughage feed (equivalent to 5% of their mean body weights) in the forms of sorghum and maize stovers, baled mixture of Rhodes grass and groundnuts hay (4:1 ratio). Concentrate feed consisting of 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) was also rationed. 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* (Aganga *et al*., 2000).

Identification and grouping of animals: Serially numbered neck tags were assigned to the donkeys at random for identification. A sample frame of each gender was prepared and the animals were assigned to experimental groups at random, given rise to a total of 6 animals (3 male and 3 female) in each of groups A_1, A_2, A_3 and B (Aviva and Poul, 2013).

The rats and mice were caged according to the groupings of donkeys they represented and within a group; they were marked with assorted colours of permanent marker pens.

Conditioning of experimental animals

The donkeys were acclimatized for 14 days based on recommendations of Wolfensohn and Lloyd (2013). While acclimatizing, about 5g of faecal sample was collected from each animal's rectum and examined for gastro-intestinal parasites using floatation and sedimentation methods (Charles, 2007). The animals were de-wormed using Fenbendazole bolus (Fenacure®, Ashish Life Sciences PVT ltd, India), 10mgkg-^{1} bd wt orally, once (Aliu, 2007).

Four millilitres (4ml) of whole blood was collected from each donkey through the jugular vein using 5ml syringe and 18G needle. Three millilitres of the collected blood in sample bottle was anticoagulated using 1mg of liquid Ethylene Diamine Tetra-acetate (EDTA+K_3) mL^{-1} of whole blood (Elaine and Margi, 2007) and examined immediately for haemoparasites especially *Trypanosoma species.* Two (2) preparations were examined each, of Wet Blood Film at 40x objectives, HCT and Giemsa Stained thin blood smear at 100x objectives (Weiser, 2012a). An average of 20 microscope fields of each preparation was examined to ensure that the animals were free from haemoparasites infection.

Mice inoculation test was conducted using the balance of 1mL of blood (out of the 4mL) anti-coagulated in sodium heparin-coated 1mL sterile disposable syringe with 29G x ½ inch needle. Half a millilitre (0.5mL) of the blood was inoculated per mice intra-peritoneally (Wolfensohn and Lloyd, 2013) using 2 representative mice per donkey (OIE, 2010). The inoculated mice were monitored for haemoparasites on HCT and Wet Film using tail tip blood at 48 hours intervals for 14 days (Monzon *et al*., 1990) to ensure that the experimental donkeys were not latent carriers of haemoparasites from field challenge. The mice were further observed for 60 days post-inoculation.

Pre-infection evaluation of animals for haemogramme, serum biochemical and electrolytes parametres

In order to determine the pre-infection values of haematological, specific serum biochemical and electrolyte parameters of the experimental animals, 6mL of blood was collected from each donkey through jugular vein (Weiser, 2012b; Wolfensohn and Lloyd, 2013) once

a week (4x), over a period of 28. Three mL of the collected blood was anticoagulated in EDTA for haemogramme analysis and the balance of 3mL saved in anticoagulant-free vacutainer tube for serum harvest.

***Haematological parameters*:** The parametres evaluated were packed cell volume (PCV) from haematocrit centrifugation (HCT) as described by Woo (1970), Haemoglobin concentration in g/dL from PCV divide by 3, total red blood cells (RBC) and total leucocytes (WBC) counts using haemocytometre techniques and differential leucocytes counts from the values of the total as reported by Elaine and Margi (2007) and Weiser (2012a).

***Serum biochemical and electrolyte parameters*:** Sera for evaluation of biochemical and electrolyte parameters were harvested from clotted and centrifuged blood samples with each sample yielding 0.8mL to 1.5mL of serum collected into serum vial and stored at -20ºC until analysed (Charles, 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 sera samples using fully-automated clinical chemistry analyzer (Selectra XL®, Vital Scientific, Netherland) (Margi, 2007). From each serum sample, 250µL was collected using Eppendoff pipette into sample cup. The filled cups were in turn loaded into the sample-rotor of the 80 samples-capacity (in single run) Selectra XL® machine as recommended by the manufacturer and analysed.

Calcium (Ca^{2+}), Phosphate (PO_4^-), Sodium (Na^+), Potassium (K^+), Chloride (Cl^-) and Bicarbonate (HCO_3^-) ions were assayed with the aid of automated electrolyte analyzer (Audicom AC9900®, Audicom Medical Technology ltd, Jena, Germany) (Margi, 2007). Using Eppendoff pipette, 200µL of each of the sera samples was collected and transferred into sample cup. The filled cups were in turn loaded into sample rotor of the 20 samples-capacity (in single run) Audicom AC9900® machine as recommended by the manufacturers and the electrolytes were assayed.

Propagation of *Trypanosoma evansi* and infection of experimental donkeys

Trypanosoma evansi originally isolated from camel in Sokoto city and maintained in Wister rat was used. One mL of blood was collected from a highly parasitaemic (>20 parasites per microscope field using HCT) rat via ocular vein. The blood was heparinised in sample bottle and diluted with 1mL phosphate buffered glucose saline solution given a total of 4 mL diluted inoculum. Four donor Wister rats were each inoculated with 0.5 mL of the diluted inoculum, intra-peritoneally, using sterile 1 mL disposable syringe 25G x $1^1/_2$ inch needle (Monzon *et al*., 1990; Wolfensohn and Lloyd, 2013). The rats were monitored for rising parasitaemia using HCT at 48 hours intervals. By day 12 post-inoculation, the 4 donor rats had attained parasitaemia levels of +2 (6-10/field) to +4 (>20 parasites/field) parasitaemia levels. All rats were bled and about 15 mL of parasitaemic blood was pooled into a sterile conical flask containing heparin (20 U/mL of blood) as anticoagulant. The harvested blood was mixed by gently rotating the flask, parasitaemia was re-evaluated using haemocytometre (Elaine and Margi, 2007) and parasite concentration was adjusted to 1.0 x10^6 *T. evansi* /mL inoculum by the addition of phosphate buffered glucose saline solution (Monzon *et al*., 1990). Immediately, all the donkeys in groups A_1, A_2 and A_3 were, each infected with 2 mL inoculum (2.0x10^6 *T. evansi*) through the jugular vein.

Treatment of specific infected animals

On day 28 post-infection, groups A_2 and A_3 were chosen at random and treated using isometamidium chloride 1%w/v (Securidium®, Laprovet, France), 0.5mgkg^{-1} bd wt by deep intramuscular injection once and buparvaquone 5% w/v (Butalex®, Coopers K-brand Ltd, Kenya), 2.5 mgkg^{-1} bd wt by deep intramuscular injection twice at 72 hours apart as recommended by the manufacturers respectively. Group A_1 animals were infected but untreated group while group B remained as un-infected, untreated control. Treatment day was considered as day 0 of treatment.

Monitoring of parasitaemia levels in donkeys

Ear vein blood was collected from experimental animals using sterile needle-prick on the ear vein. Two prepared microhaematocrit tubes and another two Giemsa -stained thin blood smear slides per blood sample were examined for parasitaemia as recommended by OIE, (2010) and FAO, (2014). The parasitaemia levels were evaluated post-infection, post-treatment and scored as +1, +2, +3 and +4 representing 1-5, 6-10, 11-20 and >20 parasites per microscopic field respectively (Woo, 1970).

Post-infection monitoring: Parasitaemia was evaluated daily from day 1 to day 7 Post-infection (PI) and twice per week from day 8 to day 28 PI.

Post-treatment monitoring: Parasitaemia was evaluated post-treatment (PT) over 148 days as follows; on daily basis from day 1 to day 7 PT, twice per week from day 8 to day 28 PT and once a weekly from day 29 to day 148 PT. The Infected, untreated (group A_1) and control (group B) donkeys was evaluated side by side those of the 2 treated groups up to day 176 PI (equivalent to day 148 PT).

Mice inoculation test: Mice inoculation test (MIT) was performed for infected-untreated and infected but variously treated groups of donkeys as a support for HCT and Thin blood smear. The MIT was performed for groups of donkeys that showed sustained aparasitaemia for up to 10 days or more to detect any case of subclinical, latent or relapsed infection. One millilitre of blood was collected from each donkey through jugular vein using heparin-coated 2mL syringe with 19Gx $1^1/_2$ inch needle. Two (2) representative mice were sub-inoculated with the collected blood. Each mouse was inoculated with 0.5 mL of the collected donkey-blood, intra-peritoneally using sterile 27G needles. The mice were identified as described earlier monitored for parasitaemia using tail tip blood on HCT and Giemsa-stained thin blood smears at 48 hours intervals (OIE, 2010) as follows:

Group A₁ (Infected-Untreated) Donkeys: The Infected, untreated donkeys were subjected to MIT on day 110 PI.

Group A₂ (Infected, Isometamidium chloride-treated) Donkeys: The Isometamidium chloride-treated donkeys were subjected to MIT on days 21, 50 and 100 PT.

Group A₃ (Infected, Buparvaquone-treated) Donkeys: The donkeys in this group were tested using MIT on day 59 PT.

Post-infection and Post-treatment evaluation of haematological, serum biochemical and electrolytes parametres

A total of Six millilitres of whole blood sample was collected once per week post-infection (4x) over 28 days PI and post-treatment (PT) once per week post-treatment (21x) from day 29 PI (equivalent to day 1 PT) today 176 PI (equivalent to day 148 PT) from every animal in all experimental groups. Three mL of the blood was anticoagulated with EDTA and used for evaluation of haematological parameters as described in pre-infection stage while the remaining 3 mL was allowed to cloth in plain vacutainer bottle, centrifuged and serum was harvested (Elaine and Margi, 2007). The levels of the serum biochemical and electrolyte parameters were also assayed PI and PT using automated XL®, Vital Scientific, Netherland) (Margi, 2007) and (Audicom AC9900®, Audicom Medical Technology ltd, Jena, Germany) (Margi, 2007) respectively.

Data analysis

Data was analysed using System Analytical Statistics (SAS) corporation-2010. Means of parasitaemia levels were determined and presented graphically, means of haematological serum biochemical and electrolyte parametres are presented in tables. Analysis of Variance (ANOVA) was used to compare means of variables between experimental animal groups and within groups at 95% confidence level; Probability less than 0.05=statistically significant (Mead *et al*., 2002).

RESULTS

Findings from pre-infection evaluation of experimental animals

Examination of faecal samples from the animals showed that all animals finally used for this experiment were negative for helminthes after deworming with fenbendazole and negative for any existing subclinical haemoparasitic including *Trypanosoma spp* infection before experimental challenge.

Post-infection parasitaemia

Groups A₁, A₂ and A₃ (Trypanosoma evansi-infected): Parasitaemia was first detected in some of the animals infected with *T. evansi* on day 3 and by day 4, all infected animals were positive. Mean parsitaemia counts per microscope field increased gradually with peaks on days 7 PI (group A_1= 13/field, A_2 = 10/field, A_3 = 9/field) and day 18 PI (A_1= 20/field, A_2 = 19/field, A_3 = 23/field). On day 28 PI (day 0 of treatment), parasitaemia levels in the groups had risen further with counts in groups A_1= 10/field, A_2 = 16/field and A_3 = 8/field (Fig.1a).

Group B (un-infected, untreated): In this group (control), all the animals remained negative for *T. evansi* throughout the experiment (Fig.1a).

Post-treatment parasitaemia

Group A₁ (Infected-Untreated): The infected-untreated animals maintained an undulating wave of parasitaemia and became aparasitemic on day 100 PI (that is day 72 PT) using HCT (Fig.1b). However, mice inoculation test (MIT) conducted for the donkeys in this group, on day 110 PI resulted in the death of mice due to heavy parasitemia (between +3 and +4/field) on HCT and by day 15 post-inoculation, all mice had died. This indicated that there was subclinical infection in this group as at 100 days PI.

Group A₂ (Infected, Isometamidium chloride-treated): The *T. evansi*-infected, Isometamidium chloride-treated animals showed sharp drop in mean parasitaemia on HCT from about 12/field on day 1 PT to about 2/field on day 2 PT and they became aparasitaemic on day 11 PT (Fig.1b). Mice inoculation tests on days 21 and 50 were negative but was positive on day 100 PT in 6/10(60%) of mice used representing 3/5 (60%) donkeys in the group. The 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 on HCT during 48 days of post-inoculation observation. The remaining 4 mice were negative on HCT during the same period of observation.

Group A₃ (Infected, Buparvaquone treated): The Infected, Buparvaquone treated group became aparasitemic on HCT on day 49 PT (Fig.1b). Following MIT on day 59 PT, all inoculated mice tested positive for *T. evansi* and died by day 23 post-inoculation observation.

Group B (un-infected, untreated): The group of animals was the control group and remained negative for *T. evansi* throughout the experiment (Fig.1b).

Means of Packed cell volume, haemoglobin and total red blood cells values

Group A₁ (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. 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 1).

Table 1: 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	27.3±0.5cd	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.

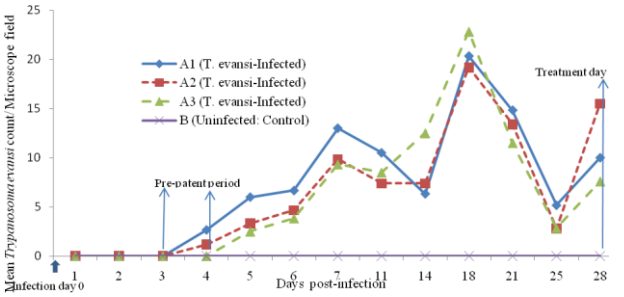

Fig. 1a: Mean levels of parasitaemia in donkey experimentally infected with *Trypanosoma evansi*

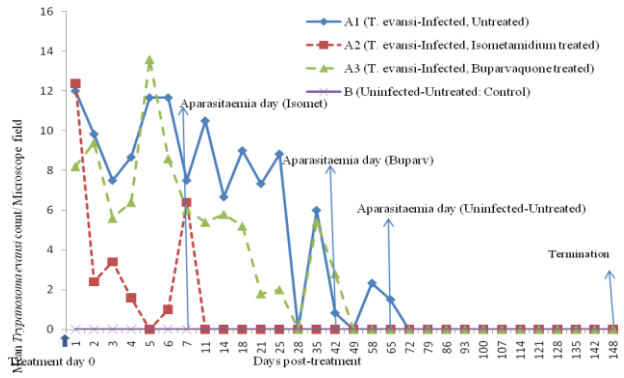

Fig. 1b: Mean levels of Trypanosoma evansi observed in experimentally infected donkeys post-treatment

***Group A₂ (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%. The PCV rose significantly (P<0.05) to 37.0±0.0% PT. 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±1.2x10^{12}/L) significantly (P<0.05) declined PI to 5.3±1.0x10^{12}/L. However, the PT value (5.7±0.6x10^{12}/L) was not significantly different (P>0.05) from the pre-inf. value (Table 1).

***Group A₃ (T. evansi-Infected, Buparvaquone-treated*):** The buparvaquone-treated animals showed insignificant (P>0.05) decline in PCV from pre-infection value of 39.3±0.4% to PI value of 36.4±3.8%. Despite treatment on day 28 PI, there was further significant (P<0.05) decline in PCV to 27.3±0.5% PT. 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±1.1x10^{12}/L), PI (5.6±1.1x10^{12}/L) and PT (6.1±0.8x10^{12}/L) values (Table 1).

***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. The initial values of Hb (14.3±0.0g/dL) and total RBC (6.4±1.1x10^{12}/L) counts did not differ significantly (P>0.05) from their final values of 14.7±0.0g/dL and 6.4±0.3x10^{12}/L respectively (Table 1).

Leucogramme picture of groups of donkeys

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.2).

Group A₂ (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±2.3x10^9/L to PI value of 4.5±0.8x10^9/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±1.3x10^9/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±0.0x10^9/L. However, PT value of 2. 0.0±0.0x10^9/L was observed. Eosinophils values declined insignificantly (P>0.05) between pre-infection value of 0.0±0.1x10^9/L to PI value of 0.0±0.0x10^9/L while the PT value was of 0.0±0.0x10^9/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±0.1x10^9/L and the PT value was 0.0±0.0x10^9/L (Table.2).

Group A₃ (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±0.7x10^9/L. However, a significant (P<0.05) elevation in the WBC was observed PT to 10.6±4.1x10^9/L. Neutrophils count did not change significantly (P>0.05) between pre-infectin value of 4.8±1.8x10^9/L and PI value of 4.5±0.6x10^9/L while the PT value was 4.3±2.1x10^9/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±0.4x10^9/L but had a significant elevation (P<0.05) PT to 2.8±1.4x10^9/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±0.0x10^9/L and an insignificant (P>0.05) elevation PT to 0.1±0.1x10^9/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±0.0x10^9/L and an insignificant (P>0.05) elevation PT to 0.1±0.1x10^9/L. Band cells count significantly increased (P<0.05) from pre-infectin value of 0.0±0.0x10^9/L to PI value of 0.1±0.1x10^9/L and declined insignificantly (P>0.05) PT to 0.0±0.1x10^9/L (Table.2).

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.2).

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

***Group A₁ (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 3a).

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 3b).

***Group A₂ (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 3a). 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 3b).

Group A₃ (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. (Table 3a). 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 3b).

***Group B (Uninfected-Untreated; control*):** This was the control group and the activities of the assayed serum biochemical parameters did not show significant changes throughout the experiment (Tables 3a and 3b).

Mean serum levels of some selected electrolytes

***Group A₁ (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).

Group A₂ ***(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).

***Group A₃ (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).

***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).

Table 2: 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.

Table 3a: 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	Pre-infection	68.9±3.6abc	37.6±3.9cde	36.4±14.8bc	51.3±13.7bc
(Infected- isometamidium	Post-infection	67.9±5.3abc	36.7±6.1e	41.8±12.6bc	44.4±3.3cd
treated)	Post-treatment	66.3±6.8bc	40.1±3.4ab	43.5±2.8bc	50.4±5.0bcd
A_3 (Infected-	Pre-infection	69.5±3.4ab	41.8±3.8a	41.5±16.9bc	51.3±16.6bc
buparvaquone treated)	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;	Initial value	68.7±3.6abc	39.3±3.4abcde	41.8±16.8bc	51.3±16.2bc
control)	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.

DISCUSSION

The declines in the mean packed cell volume (PCV), haemoglobin concentration (Hb) and total red blood cell (RBC) values in all *T. evansi*-infected groups of donkeys post-infection (PI) are attributed to experimental *T. evansi* infection. This finding agrees with earlier report that declines in the three parametres occur side by side high parsasitaemia in *T. evansi*-infected animals (Aquino *et al.*, 2001) resulting in anaemia in donkeys and other animal species in other parts of the world (Herrera *et al*., 2002; Cadioli, *et al.*, 2006; Berlin *et al*., 2010). The anaemia is due to synergism amongst pro-inflammatory cytokines whose levels are increased in *T. evansi* infection (Paim *et al*., 2011), free fatty acids, haemodilution, coagulation disorders, depression of erythrogenesis, release of trypanosomal sialidase enzyme in infected animal (Esievo and Saror, 1991; Omer *et al*., 2007; Adamu *et al*., 2008).

Post-treatment, values of PCV and other erythrogramme parametres, slightly improved but not reverted to pre-infection range of values in isometamidium-treated animals. The improvement in erythrogramme values is in line with observation by Berlin *et al.* (2010) who reported that improvement is can be due to lowered parasitaemia as a result of cure, haemoconcentration precipitated by loss of intravascular fluid to the interstitials as in dehydration and subcutaneous oedema associated with chronic infection (Omer *et al*., 2007; Wolkmer *et al*., 2009). Buparvaquone group showed steady reduction in PCV and Hb values PI and PT suggesting absence of reduction in parasitaemia in the group while the later-stage increase in total RBC suggests haemoconcentration.

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 suppression of leucocytes even amongst the buparvaquone treated animals, despite the report that buparvaquone potentiates immunity in its prophylactic effect against bovine theileriosis (Mutugi *et al*, 1988).

The Infected-untreated group showed significant decline in serum TP and ALB post-infection. This suggests decrease in the synthesis of the proteins due to enzyme disorders, poor utilisation of dietary protein and insignificant immunoglobulin response to infection (Allison, 2012). Earlier report showed that chronic surra in donkeys is associated with hypoproteinaemia including hypoalbuminaemia (Cadioli *et al*., 2006). However, the observed low levels of ALT, AST and TP in the present study contradict an earlier report by Sow *et al.* (2014) that serum levels of ALT, AST and TP are elevated in donkeys with trypanosomosis. The opposing finding may probably be due to variations in host susceptibility, parasite strain, environmental factors, existence of concurrent infection and stress which are known to determine the severity 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, brief significant rise in ALP activity and its later reversal to pre-infection levels sugest that liver function was not significantly altered in the infected but untreated group of animals. Similarly, insignificant rise in CR and unaltered BUN activity recorded in the infect-untreated group, point that there was no chronic kidney dysfunction in the infected-untreated group of animals (Allison, 2012). This is unlike the observation in horses were surra results in liver disorders (Stephen, 1986) perhapse because horses show less resistance to *T. evansi* infection than donkeys (Desquesnes *et al*., 2013).

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

Alkaline 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). In camels, surra is characterised by deposits of immune complexes in internal organs of infected animal Enwezor and Sackey, 2005). Deposits of immune complexes might be amongst the pathologies in experimental donkeys causing cholestasis and consequently, the transient elevation (reverted to pre-infection levels in the later stage of the disease in treated and untreated groups) of ALP activity PI. The normal levels of CR and BUN observed in the groups of animals suggest that there was no kidney dysfunction (Allison, 2012).

Table 3b: 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	Pre- infection	76.0±11.2ab	71.7±4.5abc	4.5±0.9a
treated)	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	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
treated)	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.

Table 4: 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
Treated	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
	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.

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) resulting in reduced muscle contraction due to lack of calcium activity (Bohn, 2012). This might have contributed to the lethergy and intermittent recumbency reported in donkeys (Garba *et al*., 2015). 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. 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).

Conclusion

It was concluded that the haemopathological pictures associated with *T. evansi* infection in donkeys were anaemia, lymphopenia in acute and early chronic phases of the infection. Serum biochemical and electrolyte alterations were hypoproteinaemia, hypoalbuminaemia, hypocalcaemia and decreased bicarbonate ion level. Elevated Alkaline phosphatase and Blood urea nitrogen activities were also observed.

Treatment with isometamidium chloride restored Albumin, bicarbonate ion, Alkaline phosphatase, Creatinine and Blood urea nitrogen values to pre-infection (normal) levels. Hyperphosphatemia and hyperkalaemia were also observed in animals after treatment with Isometamidium chloride. Treatment with buparvaquone did not ameliorate the alterations in the levels of serum biochemical and electrolytes parametres of *T. evansi*-infected donkeys to pre-infection (normal) levels.

Acknowledgement

We appreciate the roles of the following staff of Ahmadu Bello University, Zaria, Nigeria; Mallam Yunusa of Veterinary Clinical Pathology Laboratory for haemogramme analysis, Mr Ayegbusi Olu of Chemical Pathology laboratory in Teaching Hospital, Shika, for sourcing the reagents and assisting in serum biochemical/electrolytes assays. Also, Mr John Obiamata of Data Processing Unit, Institute of Agricultural Research, for the analysis of all bio-data.

REFERENCES

Abbas II and YA Arigbede, 2012. Green area mapping of Ahmadu Bello University Main Campus, Zaria, Nigeria using remote sensing (Rs) and geographic information system (Gis) techniques. J Geog Regional Plann, 5: 287-292. DOI: 10.5897/JGRP12. 024.

Adamu S, NDG Ibrahim, AJ Nok and KAN Esievo, 2008. Sialyltransferase activity probably counteracts that of sialidase as one of the possible mechanisms of natural recovery of stabilization of erythrocyte mass in Trypanosome-infected animals- A perspective, Afri J Biotech, 7: 4992–5001.

Aganga AA, M Letso and AO Aganga, 2000. Feeding donkeys. Livestock Res Rural Dev, 12(2). http://www.cipav.org.co/lrrd/lrrd12/2/agan122.htm. Accessed 12 July 2012.

Aliu YO, 2007. Nigerian Veterinary Formulary; Handbook of Essential Veterinary Drugs, Biologics and Pesticide Chemicals. 1st edition. Veterinary Council of Nigeria, Nigeria, p: 67.

Allison RW, 2012. Laboratory evaluation of the liver/Laboratory evaluation of plasma and serum proteins, in: Thrall MA, G Weiser, RW Allison and TW Campbell (eds), Veterinary Hematology and Clinical Chemistry. 2nd edition. Wiley-Blackwell, United Kingdom, pp: 401-424 and 460-479.

Antoine-Moussiaux N, B Faye and GF Vias, 2007. Tuaregs ethno-veterinary treatments of camel diseases in Agadez area (Niger). Trop Anim Hlth Prod, 39: 83-89.

Antoine-Moussiaux N, A Cornet, F Cornet, S Glineur, M Dermine and D Desmecht mail, 2009. A Non-Cytosolic Protein of *Trypanosoma evansi* Induces CD45-Dependent Lymphocyte Death, *PLoS ONE* 4: e5728. Doi: 10.1371/journal.pone.0005728.

Aquino LPCT, RZ Machado, AC Alessi, AE Santana, MB Castro, LC Marques and EB Malheiros, 2001. Hematological, biochemical and anatomopathological aspects of experimental infection with *Trypanosoma evansi* in dogs, *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia,* 54: Belo Horizonte fev. 2002. Doi: 10.1590/S0102-09352002000100002

Atarhouch T, M Rami, MN Bendahman and A Dakkak, 2003. Camel trypanosomosis in Morocco 1; result of a first epidemiological survey. Vet Parasitol, 111: 277-286.

Aviva P and W Poul, 2013. Statistics for Veterinary and Animal Science. 3rd edition, Wiley-Blackwell ltd, United Kingdom, 391pp.

Nasereddin DA, K Azmi, S Eregat, Z Abdeen and G Baneth, 2010. Longitudinal study of an outbreak of *T. evansi* infection in equids and dromedari camels in Israel. Vet Parasitol, 174: 317-322. Doi: 10.1016/j.vetpar.2010.09.002.

Bohn AA, 2012. Laboratory evaluation of electrolytes, in: Thrall MA, G Weiser, RW Allison and TW Campbell (eds), Veterinary Hematology and Clinical Chemistry. 2nd edition. Wibey-Blackwell, United Kingdom, pp: 378-392.

Brun R, H Hecker and ZR Lu, 1998. *Trypanosoma evansi* and *Trypanosoma equiperdum*: Distribution, biology, treatment and phylogenetic relationship (A review), Vet Parasitol, 79: 95-107.

Cadioli FA, LC Marques, RZ Machado, AC Alessi, LPCT Aquino and PA Baznabe, 2006. Experimental *T. evansi* infection in donkeys: Haematological, biochemical and histopathological changes. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, doi.org/10.1590/S0102-09352006000500008.

Charles MH, 2007. Internal parasites, External parasites, in: Charles MH and S Margi (eds), Laboratory procedures for veterinary technicians, 5th edition. Mosby Elsevier Inc, St Louis Missouri, pp: 187-252.

Da Silva AS, MM Costa, C Marques, CM Moreira, RA Zanette, GR Thomé, MA Otto, EMM Flores, ST dos Anjos Lopes and SG Monteiro, 2011. Experimental Infection by *Trypanosoma evansi* in Rabbits: Levels of Sodium, Potassium, Calcium and Phosphorus in Serum, Acta Vet Sci, 39: 959.

Da Silva AS, RA Zanette, P Wolkmer, MM Costa, HA Garcia and SG Lopes, 2009. Diminazene aceturate in the control of *Trypanosoma evansi* infection in cats. Vet Parasitol, 65: 47–50.

Desquesnes M, P Holzmuller, D Lai, A Dargantes, Z Lun and S Jittaplapong, 2013 *Trypanosoma evansi* and surra: A review and perspective on origin, history, distribution, taxonomy, morphology, host and pathogenic effects, ID-194176, 22pp. htm:/dx.doi.org/10.1155/2013/194176. Accessed 20 July, 2014.

Dia ML, N Van Meirvenne, E Magnus, AG Luckins, C Diop, A Thiam, P Jacquiet and R Hamers, 1997. Evaluation de 4 tests de diagnostic: Frottis sanguins, CATT, IFI et ELISA-Ag dans l'étude de l'épidémiologie de la trypanosomose cameline à *Trypanosoma evansi* en Mauritanie. Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux, 50: 29–36.

Dobre M, W Yang, J Chen, P Drawz, LL Hamm, E Horwitz , T Hostetter, B Jaar, CM Lora, L Nessel, A Ojo, J Scialla, S Steigerwalt, V Teal, M Wolf and M Rahman, 2013. Association of serum bicarbonate with risk of renal and cardiovascular outcomes in CKD: A report from the Chronic Renal Insufficiency Cohort (CRIC) study, Am J Kidney Dis, 62: 670-678. DOI: 10.1053/j.ajkd.2013.01.017.

Elaine A and S Margi, 2007. Hematology and hemostasis, in: Charles MH and S Margi (eds), Laboratory Procedures for Veterinary Technicians. 5th edition. Mosby Elsevier Inc, St Louis Missouri, pp: 27-73.

Enwezor FNC and AKB Sackey, 2005. Camel Trypanosomosis – A Review, Vet Arhiv, 75: 439-452.

Esievo KAN and D Saror, 1991. Immunochemistry and immunopathology of animals' trypanosomiasis. Vet Bull, 61: 765–777.

Eyob E and L Matios, 20013. Review on camel trypanosomosis (surra) due to *Trypanosoma evansi*: Epidemiology and host response. J Vet Med Anim Hlth, 5: 334-343. DOI: 10.5897/JVMAH2013.0236.

FAO (Food and Agricultural organization), 2014. Animal production: Section 1, Modul 5, Livestocks Systems Research Manual-Volume 1. Produced by International Livestock Research Institute (ILRI). www.fao.org/wairdocs/ilri/x5469e/5469e08.htm. Accessed 16 December 2014.

Fernández D, B González-Baradat, M Eleizalde, E González-Marcano, T Perrone and M Mendoza, 2009. *Trypanosoma evansi*: A comparison of PCR and parasitological diagnostic tests in experimentally infected mice. Exp Parasitol, 121: 1-7. DOI:10.1016/ j.exppara.2008.09.013.

Garba UM, AKB Sackey, IA Lawal, KAN Esievo, 2015. Clinical signs of experimental *Trypanosoma evansi* infection in donkeys: Ameliorative effects of isometamidium chloride and buparvaquone treatments, J Vet Adv, 5: 691-701. DOI:10.5455/ jva.20150413121927.

Habila N, MH Inuwa, IA Aimola, MU Udeh and E Haruna, 2012. Pathogenic mechanisms of *Trypanosoma evansi* infection. Res Vet Sci, 93: 13–17.

Herrera HM, AC Alessi, LC Marques, AE Santana, LP Aquino, RF Menezes, MA Moraes and RZ Machado, 2002. Experimental *Trypanosoma evansi* infection in South American coati (*Nasua nasua*): hematological, biochemical and histopathological changes. Acta Trop, 81: 203–210.

Ilemobade AA, 1971. Studies on the incidence and pathogenicity of *Trypanosoma evansi* in Nigeria, 11: The pathogenicity of *Trypanosoma evansi* for equine and bovine species. ISCTRC, OAU/STRC, 105: 107-114.

ILRI (International Livestock Research Institute), 1997. Monograph: Disease resistance and protecting the environment, in: Livestock, people and the environment, pp: 10-11.

Joe, A, 2012. The Donkey Sanctuary. British Veterinary Association. www.bva.co.uk Accessed 11 February 2012.

Mahmoud MM and AR Gray, 1980. Trypanosomosis due to *Trypanosoma evansi* (steel, 1885), Balbani 1888: A review of recent research. Trop Anim Hlth Prod, 12: 35-47.

Margi S, 2007. Clinical chemistry, in: Charles MH, S Margi (eds), Laboratory procedures for veterinary technicians. 5th edition. Mosby Elsevier Inc, St Louis Missouri, pp: 74-113.

Mead R, RN Curnow and AM Hasted, 2002. Statistical Methods in Agriculture and Experimental Biology (Texts in Statistical Science, 3rd edition. Chapman and Hall/CRC, United Kingdom, 488pp.

Monzon CM, OA Mancebo and JP Roux, 1990. Comparison between 6 parasitological methods for diagnosis of *Trypanosoma evansi* in the subtropical area of Argentina. Vet Parasitol, 36: 141–146.

Mori E, WR Fernandes and RMS Mirandola, 2003. Reference values on serum biochemical parameters of Brazilian donkey (*Equus asinus*) breed. J Equine Vet Sci, 23: 356-364.

Mutugi JJ, AS Young, AC Maritim, SG Ndungu, DA Stagg, JG Grootenhuis and BL Leitch, 1988. Immunization of cattle against theileriosis using varying doses of *Theileria parva parva* and *Theileria parva lawrencei* sporozoites and oxytetracycline treatments, Inter J Parasitol, 18: 453-461.

OIE (Office International des Epizootes), 2004. Manual of diagnostic tests and vaccines for terrestrial animals [online]. Paris: OIE; *Trypanosoma evansi* infections (including surra). http://www.oie.int/eng/normes/ mmanual/2008/pdf/2.01.17_TRYPANO.pdf, Accessed 27 August 2009.

OIE (Office International des Epizootes), 2010. *Trypanosoma evansi* infection (*surra*),Version adopted by The World Assembly of Deligates of the OIE in May 2010. Chapter 2.1.17.

http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.17_TRYPANO.pdf. Accessed 24 November 2012.

Omer HO, HM Mousa and N Al-Wabel, 2007. Study on the antioxidant status of rats experimentally infected with *Trypanosoma evansi*, Vet Parasitol, 145: 142–145.

Pacholek XD, SG Gamatic and TR Franek, 2001. Prevalence of *Trypanosoma evansi* trypanosomosis in young camels in West Niger. Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux, 44 : 177-182.

Paim FC, MM Duarte, MM Costa, AS Da Silva, P Wolkmer, CB Silva, CB Paim, RT França, CM Mazzanti, SG Monteiro, A Krause and ST Lopes, 2011. Cytokines in rats experimentally infected with *Trypanosoma evansi*, Exp Parasitol, 128(4): 365-370. Doi: 10.1016/j.exppara.2011.04.007.

Stephen L, 1986. Trypanosomiasis: A Veterinary Perspective, Pergamon Press, New York, USA.

Sow A, I Sidibe, M Kalandi, A Bathily, NP Ndiaye, M Ouédraogo, MMM Mouiche and GJ Sawadogo, 2014. Biochemical changes induced by natural infection of trypanosomosis in Burkinabese local donkey breeds, Comp Clin Pathol, 23: 103-109. DOI: 1007/S00580-0121579-2.

Vittoz R, 1955. Prophylaxix of surra in Asia. Bul OIE, 49: 83-106.

Wayne L and B Melvin, 2000. Determining age of horses by their teeth, Extension and Agricultural Information, 1-98 Agriculture Building, Columbia, MO 65211; XPLOR. http://extension.missouri.edu/publications/index.aspx. Accessed 20 July 2011.

Weiser G, 2012a. Laboratory technology for veterinary medicine, in: Mary AT, W Glade, WA Robin and WC Terry (ed), Veterinary Hematology and Clinical Chemistry. 2nd edition. Wiley-BlackWell, United Kingdom, pp: 3-33.

Weiser G, 2012b. Sample collection, processing and analysis of laboratory service options, in: Mary AT, W Glade, WA Robin and WC Terry (eds), Veterinary Hematology and Clinical Chemistry. 2nd edition. Wiley-BlackWell, United Kingdom, pp: 34-50.

Wolfensohn S and M Lloyd, 2013. Handbook of Laboratory Animal Management and Welfare, 4th edition. Wiley-Blackwell Publishing ltd, United Kingdom: 371pp.

Wolkmer P, AS Da Silva, CK Traesel, FC Paim, JF Cargnelutti, M Pagnoncelli, ME Picada, SG Monteiro and ST Anjoslopes, 2009. Lipids peroxidation associated with anemia in rats experimentally infected with *Trypanosoma evansi*, Vet Parasitol, 165: 41–46.

Woo PTK, 1970. The haematocrit centrifugation technique for the diagnosis of African trypanosomiasis. Acta Trop, 27: 384-386.