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

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**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 A1 (Infected-untreated), A2 (Infected, isometamidium-treated), A3 (Infected, buparvaquone-treated) and B (Un-infected-untreated; control) at random. Mean parasitaemia counts were evaluated post-infection and post-treatment while haematological, serum biochemical and electrolytes parameters 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 A1 donkeys had declined mean serum total Protein, Albumin, Calcium ion and bicarbonate ion levels but increased Alkaline phosphatase activity. Group A2 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 A3 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 (hydroproteinæmia, hypoalbuminæmia, 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, Philippines, 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...
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 pelleted 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 A1, A2, A3 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<sup>®</sup>, Ashish Life Sciences PVT ltd, India), 10mgkg<sup>-1</sup> 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 1mL of liquid Ethylene Diamine Tetra-acetate (EDTA+K<sub>3</sub>·H<sub>2</sub>O) m<sup>-1</sup> 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 parameters**

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 haemogram analysis and the balance of 3mL saved in anticoagulant-free vacutainer tube for serum harvest.

**Haematomal 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 -20°C 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 Eppendorf 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 (Ca²⁺), Phosphate (PO₄³⁻), Sodium (Na⁺), Potassium (K⁺), Chloride (Cl⁻) and Bicarbonate (HCO₃⁻) ions were assayed with the aid of automated electrolyte analyzer (Audicom AC9900®, Audicom Medical Technology ltd, Jena, Germany) (Margi, 2007). Using Eppendorff 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 1½ 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 x10⁶ T. evansi/mL inoculum by the addition of phosphate buffered glucose saline solution (Monzon et al., 1990). Immediately, all the donkeys in groups A₁, A₂ and A₃ were, each infected with 2 mL inoculum (2.0x10⁶ T. evansi) through the jugular vein.

**Treatment of specific infected animals**

On day 28 post-infection, groups A₂ and A₃ were chosen at random and treated using isometamidium chloride 1% w/v (Securidium®, Laprovit, 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 A₁ 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 Giemsastained 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 A₁) 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 1½ 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-peritonealy 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 parameters**

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 clot 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 XL9®, 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 parameters 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 parasitaemia counts per microscope field increased gradually with peaks on days 7 PI (group A₁= 13/field, A₂ = 10/field, A₃ = 9/field) and day 18 PI (A₁= 20/field, A₂ = 19/field, A₃ = 23/field). On day 28 PI (day 0 of treatment), parasitaemia levels in the groups had risen further with counts in groups A₁= 10/field, A₂ = 16/field and A₃ = 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 aparasitaemic 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 parasitaemia (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 (+) 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 aparasitaemic 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±5.5% to PI values of 34.0±1.0% up to day 28 and a further significant (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 x10¹²/L to PI value of 5.4±0.5 x10¹²/L and later final value of 5.6±0.5 x10¹²/L (Table 1).
Standard deviation of levels of Trypanosoma evansi observed in 3-0.1±0.1 x10^9-0.9±1.1x10^9-9...

...PCV rose significantly (P<0.05) to 193 on day 28 PI, there was further significant (P<0.05) decline in PCV from pre-infection mean value of 45.0±1.0% to PI value of 10.8±0.2g/dL and final value of 14.7±0.0g/dL. The PT value declined to 10.6±1.7g/dL. The 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, Isometamidium-treated): The pre-infection values of WBC (7.1±3.9x10^9/L), neutrophils (4.9±2.3x10^9/L) and monocytes (0.1±0.1 x10^9/L) counts declined insignificantly (P>0.05) PI to 6.4±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 insignifcant 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, Buparvaquone-treated): The buparvaquone-treated animals showed insignificant (P>0.05) decline in PCV from pre-infection value of 39.3±0.4cd to PI value of 36.4±3.8c. 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±1.0g/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) WBC (7.2±2.5 x10^9/L) and neutrophils (5.2±2.2 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 insignifcant 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 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).

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Group A2 (T. evansi-Infected, Buparvaquone-treated): The buparvaquone-treated animals showed insignificant (P>0.05) decline in PCV from pre-infection value of 39.3±0.4cd to PI value of 36.4±3.8c. 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±1.0g/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) WBC (7.2±2.5 x10^9/L) and neutrophils (5.2±2.2 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 insignifcant 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 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).

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Group A2 (T. evansi-Infected, Buparvaquone-treated): The buparvaquone-treated animals showed insignificant (P>0.05) decline in PCV from pre-infection value of 39.3±0.4cd to PI value of 36.4±3.8c. Despite treatment on day 28 PI, there was further significant (P<0.05)
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\(_3^-\) (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\(_3^-\) (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; 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 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 67.8±4.6mmol/L and towards the end of the study had significant (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 Pt 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 insignificantly (P>0.05) (Table 3b).

**Group B (Uninfected; 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).

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1.6±0.4 x 10\(^{9}\)/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 x 10\(^{9}\)/L to PI value of 0.0±0.0 x 10\(^{9}\)/L. However, PT value of 2. 0.0±0.0 x 10\(^{9}\)/L was observed. Eosinophils values declined significantly (P>0.05) between pre-infection value of 0.0±0.1x10\(^{9}\)/L to PI value of 0.0±0.0 x 10\(^{9}\)/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 0.9±0.0 x 10\(^{9}\)/L to PI value of 0.9±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 x 10\(^{9}\)/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-infection 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 x 10\(^{9}\)/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-infection value of 0.1±0.3±0.1 x 10\(^{9}\)/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-infection value of 0.0±0.1 x 10\(^{9}\)/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-inf. value of 0.0±0.0 x 10\(^{9}\)/L to PI value of 0.0±0.1x10\(^{9}\)/L and declined insignificantly (P>0.05) PT to 0.0±0.1x10\(^{9}\)/L (Table 2).

**Group B (Uninfected; control):** The initial and final values of all leucocytes parameters were not significantly different (P>0.05) in the control group (Table 2).

**Group A2 (T. evansi-Infected, Isometamidium-treated):** There was insignificant (P>0.05) decrease in creatinine (CR) activity from pre-infection value of 69.9±45mmol/L to PI value of 67.8±4.6mmol/L and towards the end of the experiment 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 A3 (T. evansi-Infected, Buparvaquone-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 Pt 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 B (Uninfected; 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).
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 parameters occur side by side high parasitaemia 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 erythropoiesis, release of trypanosomal salidase enzyme in infected animal (Esievo and Saror, 1991; Omer et al., 2007; Adamu et al., 2008).

Post-treatment, values of PCV and other erythrogramme parameters, 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-uninfected 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 phase of experiment increase in total RBC followed by lymphopaenia in the chronic) stage of the experiment increase in total RBC (chronic) stage of the experiment. Berlin, 2007; Adamu 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 monocytes levels.
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 insufficiency suggests decrease in the synthesis of the proteins due to enzyme disorders, poor utilisation of dietary protein and 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 infection.

<table>
<thead>
<tr>
<th>Experimental animals groups</th>
<th>Phase of experiment</th>
<th>ALP±SD (IU/L)</th>
<th>CR±SD (mmol/L)</th>
<th>BUN±SD (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (Infected-untreated)</td>
<td>Pre- infection</td>
<td>70.2±4.1cde</td>
<td>69.9±5.1abcd</td>
<td>4.3±0.8ab</td>
</tr>
<tr>
<td></td>
<td>Post-infection</td>
<td>78.5±5.5a</td>
<td>67.8±4.6bC</td>
<td>4.4±0.3ab</td>
</tr>
<tr>
<td></td>
<td>Final value</td>
<td>62.8±13.9de</td>
<td>72.1±5.5ab</td>
<td>4.3±0.3ab</td>
</tr>
<tr>
<td>A2 (Infected- isometamidium treated)</td>
<td>Pre- infection</td>
<td>76.0±11.2ab</td>
<td>71.7±4.5abc</td>
<td>4.5±0.9a</td>
</tr>
<tr>
<td></td>
<td>Post-infection</td>
<td>76.8±7.3ab</td>
<td>69.8±4.9bC</td>
<td>4.5±0.7a</td>
</tr>
<tr>
<td></td>
<td>Post-treatment</td>
<td>65.2±2.7c</td>
<td>64.9±2.3d</td>
<td>4.0±0.5c</td>
</tr>
<tr>
<td>A3 (Infected- buparvaquone treated)</td>
<td>Pre- infection</td>
<td>73.3±8.9bcd</td>
<td>75.3±8.5a</td>
<td>4.0±0.6c</td>
</tr>
<tr>
<td></td>
<td>Post-infection</td>
<td>79.6±7.7a</td>
<td>69.8±4.3abc</td>
<td>4.5±0.8a</td>
</tr>
<tr>
<td></td>
<td>Post-treatment</td>
<td>72.2±4.9bcd</td>
<td>68.2±3.8bcd</td>
<td>4.4±0.1ab</td>
</tr>
<tr>
<td>B (Uninfected-untreated)</td>
<td>Pre- infection</td>
<td>69.8±6.5cde</td>
<td>69.4±11.6bC</td>
<td>4.2±0.8abc</td>
</tr>
<tr>
<td></td>
<td>Final value</td>
<td>72.9±8.0bcd</td>
<td>66.8±11.9bC</td>
<td>4.1±0.9abc</td>
</tr>
</tbody>
</table>

| 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 infection.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Phase of Experiment</th>
<th>Ca±SD (mmol/L)</th>
<th>PO4±SD (mmol/L)</th>
<th>Na±SD (mmol/L)</th>
<th>K±SD (mmol/L)</th>
<th>Cl±SD (mmol/L)</th>
<th>HCO3±SD (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected-untreated</td>
<td>Pre- infection</td>
<td>2.4±0.1bcd</td>
<td>1.1±0.1b</td>
<td>138.9±2.5a</td>
<td>4.0±0.4bc</td>
<td>99.2±2.9abc</td>
<td>24.0±2.0ab</td>
</tr>
<tr>
<td></td>
<td>Post- infection</td>
<td>2.4±0.1abcd</td>
<td>1.1±0.0b</td>
<td>138.1±2.5a</td>
<td>3.9±0.4bc</td>
<td>101.5±4.5a</td>
<td>23.7±2.3a</td>
</tr>
<tr>
<td></td>
<td>Final value</td>
<td>2.3±0.1e</td>
<td>1.2±0.2b</td>
<td>138.0±2.3a</td>
<td>3.7±0.5c</td>
<td>100.4±3.0a</td>
<td>21.9±7.6c</td>
</tr>
<tr>
<td>Infected- isometamidium</td>
<td>Pre- infection</td>
<td>2.3±0.1d</td>
<td>1.1±0.2b</td>
<td>140.4±3.6a</td>
<td>3.9±0.5bc</td>
<td>99.5±3.5ab</td>
<td>23.9±2.2ab</td>
</tr>
<tr>
<td>Treated</td>
<td>Post- infection</td>
<td>2.4±0.1bcd</td>
<td>1.1±0.1b</td>
<td>138.8±2.3a</td>
<td>4.0±0.3bc</td>
<td>97.3±3.4bc</td>
<td>24.1±3.5ab</td>
</tr>
<tr>
<td>Infected- buparvaquone</td>
<td>Pre- infection</td>
<td>2.4±0.1abcd</td>
<td>1.1±0.1b</td>
<td>139.4±2.7a</td>
<td>4.1±0.5bc</td>
<td>97.2±4.0bcd</td>
<td>24.0±2.3ab</td>
</tr>
<tr>
<td>Treated</td>
<td>Post- infection</td>
<td>2.5±0.1a</td>
<td>1.1±0.1b</td>
<td>140.0±4.3a</td>
<td>4.3±0.3ab</td>
<td>99.5±1.9b</td>
<td>23.3±1.3abc</td>
</tr>
<tr>
<td>Uninfected-untreated</td>
<td>Initial value</td>
<td>2.4±0.1bcd</td>
<td>1.2±0.1b</td>
<td>138.9±2.7a</td>
<td>4.1±0.6bc</td>
<td>99.3±3.0abc</td>
<td>24.0±2.6ab</td>
</tr>
<tr>
<td>(Control)</td>
<td>Final value</td>
<td>2.4±0.1bcd</td>
<td>1.1±0.1b</td>
<td>139.3±3.5a</td>
<td>4.0±0.8bc</td>
<td>99.2±2.8abc</td>
<td>24.6±1.5a</td>
</tr>
</tbody>
</table>

P-Value                    | 0.000               | 0.054          | 0.098           | 0.014          | 0.000         | 0.000          |

Keys: Total calcium (Ca²⁺), phosphate ion (PO₄³⁻), sodium (Na⁺), potassium (K⁺), chloride (Cl⁻), bicarbonate (HCO₃⁻) 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 lethargy and intermittent recumbency reported in donkeys (Garba et al., 2015). The serum levels of PO₄, 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 (HCO₃⁻) 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 HCO₃⁻ 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, hypoalbuminemia, 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 electrolyte parametres of *T. evansi*-infected donkeys to pre-infection (normal) levels.

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