SERUM CHANGES IN DOGS EXPERIMENTALLY INFECTED WITH SINGLE *TRYPANOSOMA CONGOLENSE, TRYPANOSOMA BRUCEI* AND CONJUNCT *TRYPANOSOMA CONGOLENSE* AND *ANCYLOSTOMA CANINUM* INFECTIONS AND TREATED WITH DIMINAZENE ACETURATE AND MEBENDAZOLE

[A]Nwoha R. I. O. and [B]Anene B. M.

[A]Department of Veterinary Medicine

[B] Department of Veterinary Medicine

[A]Michael Okpara University of Agriculture Umudike

[B]University of Nigeria Nsukka

[A]Micheal Okpara University of Agriculture Umudike. P.o.box 824, Nigeria. E.mail rosemarynwoha@yahoo.com phone 08030987115.

Abstract

Trypanosoma brucei (*T. brucei*) and *Trypanosoma congolense* (*T. congolense*) are two species of trypanosomes that pose a serious threat to the health of animals especially dogs in Nigeria. These species are often in mixed infection with *Ancylostoma caninum* (*A. caninum*). This prompted the study of serum changes in dogs experimentally infected with single *T. congolense, T. brucei* and conjunct *T. congolense* and *A. caninum* infections and treatment with diminazene aceturate and mebendazole. Twenty four mongrels of both sexes were randomly grouped into six of 4 members and used in the

study. Group I was the uninfected control, Group II was infected with *A. caninum,* Group III was infected with *T. congolense*, Group IV was conjunct infection of *T. congolense/A.caninum*, Group V was infected with *T. brucei*, and Group VI was conjunct infection of *T. brucei/A. caninum.* First *A. caninum* infection was done on GPII, GPIV and GPVI. Two weeks later *T. brucei* and *T. congolense* were done on GPIII/GPIV and GPV/GPVI respectively. Treatment with 7 mg/kg diminazene aceturate stat and 100mg mebendazole given twice daily for 3 days was done on all the infected groups (GPIII, GPIV, GPV and GPVI). Results show significant increases ($p<0.05$) in blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) of all the infected groups (GPII, GPIII, GPIV, GPV and GPVI). Treatment did not improve the altered serum analytes. A significant decrease ($p<0.05$) in blood sugar was observed only in the trypanosome infected groups (GPIII, GPIV, GPV and GPVI). It therefore show that both trypanosome species and *Ancylostoma caninum* cause significant damage to the kidney and liver tissues which was not resolved with treatment. The effect was more severe in combined infection of these parasites in dogs. Hence conceited effort should be enforced in prevention of occurrence of mixed infection of trypanosome/*A. caninum* in dogs.

Keywords: blood urea nitrogen, creatinine, ALP, ALT, AST, *Ancylostoma caninum,* trypanosome

Introduction

Trypanosomosis is a big scourge that is adversely affecting animals' well being and productivity in sub- Saharan Africa (Onditi *et al.,* 2007; Adewoga *et al.,* 2010). There is usually massive destruction of the erythrocytes by both *T. brucei* and *T. congolense* in dogs resulting in anaemia which is the cardinal sign of the disease in animals (Anosa and Kaneko, 1983; Eloy and Lucheis, 2009; Nwoha and Anene, 2011). Trypanosomes affect different body components such as the

serum biochemical constituents (Igbokwe and Mohammed, 1992; Taiwo *et al*., 2003; Rashid *et al*., 2008; Eloy and Lucheis, 2009). Serum biochemistry analysis though variable and highly inconsistent reveals effects on vital organs of the body and results correlate to the degree of damage thus serves as substantial prognostic and diagnostic tool in clinical practice. For instance, trypanosomes have been associated with alteration of glucose metabolism in the islet of langerhans which results to hypoglycaemia in guinea pigs (Locatelli, 1930). Hypoglycaemia was also recorded in *T. brucei* infection in rabbits (Takeet and Fagbemi, 2009) and in *T. evansi* infection in donkey (Cadioli *et al.*, 2006). It was also recorded in *T. brucei* infection in dogs (Aquino *et al*., 2002). Trypanosomes have also been associated with enormous sugar consumption directly causing hypoglycaemia in infected fish (Joshi, 1982). On the other hand, Sandoval *et al.* (1994) observed no change in blood sugar in dogs with trypanosomes infection. Alterations in liver enzymes have also been reported. There are cases of abnormal elevations in serum liver enzymes in rabbits with *T. congolense* infection (Egbe-Nwiyi *et al*., 2005) and in *T. brucei* infection in rats (Orhue *et al*., 2005). Others include increases in blood urea nitrogen BUN in *T. brucei* infection in rats (Wellde *et al.*, 1974; Abenga and Anosa, 2007). There may also be fluctuating levels of BUN in *T. brucei brucei* infection in dogs (Nwoha *et al.* 2013). In sub-Saharan Africa where trypanosomes and *Ancylostoma caninum* are endemic, both diseases often occur naturally in mixed infections (Goossens *et al.*, 1997). This has the potential of enhancing susceptibility and severity of secondary infections. Hence the need to determine the serum changes in dogs experimentally infected with single *Trypanosoma congolense, Trypanosoma brucei* and conjunct *Trypanosoma congolense* and *Ancylostoma caninum* infections and treatment with diminazene aceturate and mebendazole

Materials/Methodology

Experimental Animals

Twenty four mongrel breed of dogs of both sexes weighing between 4.0 and 8.0kg were used in this experiment. The dogs were acclimatized for 4 weeks before commencement of the experiment during which they were screened for blood parasites and confirmed negative by Giemsa-stain, thin blood smears and haematocrit buffy coat method (Woo, 1970). They were dewormed with tablets of mebendazole (Vermin®, Janssen-Cilag Ltd 50 - 100 Holmers Farm Way, High Wycombe, Bucks, HP12 4EG UK) at the dose of 100mg twice daily for 3 days and also treated with sulfadimidine at the dose of 48mg/kg intramuscularly against systemic opportunistic bacterial infections. The experiment commenced a week later. The animals were kept in clean cages in a fly proof house and fed twice daily. Water was given *ad libitum*.

Ethical Approval

The care of the animals was in conformity with the guideline for animals' experimentation of Council for International Organization of Medical Sciences (CIOMS) for biomedical research involving animals. The dogs were humanely cared for and treated throughout the study. They were comfortably housed in properly ventilated pens in good hygienic condition and provided good and adequate feeding with clean portable drinking water. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Parasites and infections

***Trypanosoma brucei* isolate**

Trypanosoma brucei isolate used in the study was a local isolate obtained from a clinically infected dog from Nsukka area of Enugu State. The isolate was typed and confirmed in the department of Veterinary Parasitology and Entomology, University of Nigeria Nsukka. The parasites were maintained in rats and subsequently passage in a donor dog from where the experimental dogs were inoculated.

Trypanosoma congolense

Kilifi strain of *T. congolense* obtained from the National Institute of Trypanosomosis and Oncocerciasis Research (NITOR), Nigeria was used. The parasite was a primary isolate from a cow in Kaduna. It was maintained in rats, and subsequently passaged in a donor dog from where parasites were collected for infection of the experimental dogs.

Estimated 2.5×10^6 of *T. brucei / T. congolense* suspended in 1ml of normal saline was used to infect each experimental dog in the group. The quantity of parasites inoculated was estimated using the rapid matching method of Herbert and Lumsden (1976).

***Ancylostoma caninum* Infection**

The concentration of larval suspension was estimated using an automatic pipette (Biotht Peoline®), according to the method of MAFF (1977). Small doses of 20 µL larval suspensions were placed as drops on a microscope slide and counted under ×4 objective of a light microscope (Ozympu®). Dogs were starved prior to infection so as to establish infection. A dose of 200 infective L_3 suspended in 1mL of distilled water were delivered *per os* to each of the experimental dogs, using a 2 mL syringe without needle.

Reconstitution of Diminazene aceturate

A 2.36g Veribin® a brand of trypanocide containing 1.05g of diaminazene aceturate was reconstituted with 15 mL distilled water according to manufacturer's recommendation. The volume of diminazene acetutate administered to individual dog in GPIII and GPIV, were calculated from their weight at the dose of 7 mg/kg via the intramuscular route.

Administration of Mebendazole

Tablets of mebendazole (Vermin®, Janssen-Cilag Ltd 50 - 100 Holmers Farm Way, High Wycombe, Bucks, HP12 4EG UK) was given at the dose of 100mg twice daily for 3 consecutive days. Treatment was repeated 2 weeks later.

Experimental Design

Dogs were randomly divided into 6 groups of 4 members in each group. GROUP I was uninfected dogs (control), GROUP II was *Ancylostoma caninum*, GROUP III was *Trypanosoma congolense* infection, GROUP IV was *Trypanosoma congolense /A. caninum* infection, GROUP V was *Trypanosoma brucei* infection and GROUP VI was mixed infections of *Trypanosoma brucei and A. caninum.*

Post acclimatization, *Ancylostoma caninum* infection was done on GPII, GPIV and GPVI alone. Two weeks later *Trypanosoma brucei* /*Trypanosoma congolense* infections was done on GPIII, GPIV, GPV and GPVI. Three weeks post trypanosome infection; the groups were treated with diminazene aceturate. Mebendazole was used only on GPII, GPIV and GPVI and a repeat treatment given 2 weeks later.

Serum Biochemistry

Blood Collection and Preparation of Serum Samples

Five millilitre of blood was collected through the cephalic vein of each of the experimental dogs and dispensed into dried appropriately labelled sterile test tubes with screw caps and kept slanted

and allowed to clot. The blood samples were immediately transported to the Department of Veterinary Medicine Laboratory. The samples were left at room temperature for about 2 hours to yield sera and then centrifuged at 11000 revolutions per minute for 5 minutes and sera obtained were separated into clean labelled tubes and stored at -20^{o}C until analyzed for biochemical constituents.

Biochemical Analysts

The serum aspertate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine and blood sugar were determined using Randox Text Kits according to the manufacturer's prescriptions.

Determination of Blood Sugar

The blood sugar of the infected dogs were determined with a glucometer (ACCU-CHEK active serial no. GN: 10023338). The instrument was first charged with a chip before use. A single glucose strip was later inserted into its position and the glucometer allowed to zero. Immediately a drop of blood sample was made on the appropriate position on the glucose stripe and instrument allowed few seconds to record. The blood sugar of the samples were read only when the result on the glucometer remains steady for 2 seconds but not more than that.

Results

Blood Urea Nitrogen

The result of blood urea nitrogen is shown in table 1. By week 3 there were significant ($p< 0.05$) increases in blood urea nitrogen in GPII, GPIV and GPVI. The increase in GPVI was higher compared to that in GPII, while the increase ($p<0.05$) in GPII correlates with GPVI.

Apart from week 7-9 when no significant difference was detected in GPII, BUN concentrations significantly differed in all the infected groups compared with the control. The significant ($p< 0.05$) increases in the infected groups progressed up to week 12. The increases ($p<0.05$) were higher in both GPIV and GPVI while that observed in GPV correlates with GPIII and GPII.

Creatinine

The result of creatinine level is shown in table 2. There was no significant change in the creatinine level of the infected groups up to week 3. By week 4, there was significant ($p< 0.05$) increase in GPV compared to others. From week 6 to 12, there were significant ($p< 0.05$) increases in GPII, GPIII, GPIV, GPV and GPVI which was greater in GPIV and GPVI on week 6 compared to others.

Aspartate transaminase

The table of serum aspartate transaminase is shown in table 3. Significant ($p< 0.05$) increases were observed in AST at week 3, in GPII, GPIV and GPVI. Starting from week 4 to 8, there were significant ($p< 0.05$) increases in all the infected groups compared to GPI. The increases were significantly higher ($p<0.05$) in GPIV and GPVI between weeks 4 to 6 compared to the other groups (Table 3). By week 9 to 12 there was no significant ($p< 0.05$) difference between the groups and control.

Alanine amino transferase

The result of serum alanine amino transferase is shown in table 4. Significant ($p<0.05$) increase in the ALT was observed in GPVI at week 5. By week 6, significant ($p<0.05$) increases were observed in GPIV and GPVI. By week 7, there were significant ($p<0.05$) increases in all the infected groups (GPII, GPIII, GPIV, GPV and GPVI) and subsequently in GPIV, GPV and GPVI

up to week 9. The increases in GPIV and GPVI were greater compared to GPII, GPIII and GPV on week 7. By week 10 to 11, there was no significant ($p<0.05$) difference in the infected groups

Experimental Period(Weeks)	GPI (control)	GPII (*Ac)*	GPIII (*Tc)*	GPIV *(Tc/Ac)*	GPV *(Tb)*	GPVI *(Tb/Ac)*

compared to GPI but by week 12, there was significant ($p<0.05$) increase in GPV.

Alkaline phosphatase

The result of serum alkaline phosphate is shown in table 5. By week 4, there was significant ($p<0.05$) increase in ALP in GPV. By week 5 to 9, there were significant ($p<0.05$) increases in the ALP in all the experimental groups. By week 10 to 12, there was no significant ($p<0.05$) difference in ALT in the infected groups GPII, GPIII, GPVI and GPV compared with control (GPI).

Blood sugar

The result of serum blood sugar level is shown in table 6. Significant ($p<0.05$) decrease in blood sugar level was recorded only in the trypanosome infected groups (GPIII, GPIV, GPV and GPVI) by weeks 8 and 9 of experiment.

Table 1. Mean ± SE Blood urea nitrogen (mg/dl) of dogs with experimental single *T. brucei*

Experimental Period(Weeks)	GPI (control)	GPII (*Ac*)	GPIII (*Tc*)	GPIV (*Tc/Ac*)	GPV (*Tb*)	GPVI (*Tb/Ac*)
0	11.5±1.20[a]	18.5±4.80[a]	17.9±1.70[a]	20.0±10.30[a]	18.6±11.80[a]	19.8±3.10[a]
1 ⇧	13.3 ±3.40[a]	13.0 ± 3.00[a]	14.1±0.10[a]	13.0±0.00[a]	14.8 ±0.20[a]	13.9±1.10[a]
2	12.4 ±2.00[a]	11.34 ±4.00[a]	12.3±0.00 [a]	14.5 ±30.00[a]	15.2±9.00 [a]	14.3 ±6.00[a]
3 ⇳	11.4±4.90[a]	20.4±0.70[b]	14.9±2.70[a]	21.9±3.30[bc]	15.6±5.00[a]	28.5±2.70[c]
4	12.0±2.00[a]	11.0±1.50[a]	16.0±5.00[a]	28.0±2.00[b]	28.2±6.00[b]	23.1±2.00[b]
5	15.6±10.40[a]	15.0±0.20[a]	25.8±4.60[b]	29.0±2.30[b]	29.1±4.50[b]	29.1±1.00[b]
6 * +	13.0±2.00[a]	19.0±49.00[ab]	28.9±3.00[b]	100.0±9.00[c]	30.0±4.00[b]	99.0±14.00[c]
7	12.0±13.00[a]	75.8±6.90[b]	83.8±25.30[b]	108.3±23.10[c]	82.7±13.80[b]	109.0±7.20[c]
8 * +	13.0±5.70[a]	60.3±7.30[b]	75.3±8.00[b]	67.0±1.60[b]	58.0±8.00[b]	63.0±7.00[b]
9 *	11.8±2.80[a]	56.5±6.00[b]	67.9±0.40[b]	62.6±9.00[b]	50.2±9.00[b]	60.0±4.80[b]
10	14.0±3.00[a]	55.0± 6.80[b]	60.9±0.60[b]	----	49.0±8.00[b]	59.0±4.90[b]
11	12.0±7.90[a]	54.0±7.00[b]	52.0±0.70[b]	----	30.0±45.00[b]	50.0±9.00[b]
12	13.0±0.70[a]	52.0±15.90[b]	77.0±3.30[b]	----	65.3±9.00[b]	81.0±17.30[b]

and *T.congolense* and conjunct with *A. caninum* infections and treatment with diminazene aceturate and mebendazole.

Superscripts a b c represents the homogeneity between the experimental groups at probability P≤ 0.05

⇧ Infection with *A. caninum*
⇳ Infection with trypanosomes
+ Treatment with mebendazole
* Treatment with diminazene aceturate
Ac *Ancylostoma caninum*
Tb *Trypanosoma brucei*
Tc *Trypanosoma congolense*

Table 2. Mean ± SE Creatinine concentration (mg/dl) of dogs with experimental single *T. brucei* and *T.congolense* and conjunct with *A. caninum* infections and treated with diminazene aceturate and mebendazole.

Experimental	GPI	GPII	GPIII	GPIV	GPV	GPVI
0	0.5±0.10[a]	0.6±0.10[a]	0.5±0.10[a]	0.5±0.10[a]	0.6±0.10[a]	0.5±0.10[a]
1 ⇧	0.2±0.00[a]	0.3±0.10[a]	0.2±0.00[a]	0.3±0.10[a]	0.3±0.10[a]	0.2±0.00[a]
2	0.3±0.10[a]	0.2±0.00[a]	0.3±0.10[a]	0.2±0.00[a]	0.2±0.00[a]	0.3±0.10[a]
3 ⇳	0.2±0.00[a]	0.3±0.00[a]	0.2±0.00[a]	0.3±0.10[a]	0.3±0.10[a]	0.3±0.10[a]
4	0.2±0.10[a]	0.2±0.00[a]	0.2±0.00[a]	0.2±0.00[a]	0.4±0.10[b]	0.2±0.00[a]
5	0.2±0.20[a]	0.3±0.10[a]	0.3±0.10[a]	0.2±0.10[a]	0.3±0.10[a]	0.3±0.10[a]
6 * +	0.3±0.20[a]	1.0±0.20[b]	1.0±0.40[b]	1.4±0.10[c]	1.0±0.10[b]	1.8±0.10[c]
7	0.4±0.40[a]	1.1±0.10[b]	1.0±0.20[b]	1.2±0.10[b]	1.1±0.20[b]	1.0±0.10[b]
8 * +	0.2±0.10[a]	1.3±0.10[b]	1.0±0.10[b]	1.1±0.10[b]	1.0±0.10[b]	1.3±0.10[b]
9 *	0.3±0.20[a]	1.2±0.10[b]	0.8±0.10[b]	1.0±0.10[b]	0.9±0.10[b]	1.2±0.20[b]
10	0.2±0.10[a]	1.1±0.10[b]	0.8±0.20[b]	-----	0.9±0.10[b]	1.1±0.10[b]
11	0.2±0.10[a]	1.0±0.10[b]	0.9±0.20[b]	-----	1.0±0.10[b]	1.1±0.20[b]
12	0.4±0.20[a]	1.0±0.30[b]	1.1±0.10[b]	----	1.0±0.10[b]	1.2±0.20[b]

Superscripts a b c represents the homogeneity between the experimental groups at probability P≤ 0.05.

⇧ Infection with *A. caninum*
⇳ Infection with trypanosomes
\+ Treatment with mebendazole
* Treatment with diminazene aceturate
Ac *Ancylostoma caninum*
Tb *Trypanosoma brucei*
Tc *Trypanosoma congolense*

Table 3. Mean ± SE Aspartate transaminase (AST) (IU/L) level of dogs with experimental single *T. brucei* and *T.congolense* and conjunct with *A. caninum* infections and treated with diminazene aceturate and mebendazole.

Experimental Period(Weeks)	GPI (Control)	GPII (*Ac*)	GPIII (*Tc*)	GPIV (*Tc/Ac*)	GPV (*Tb*)	GPVI (*Tb/Ac*)
0	35.8±6.70^{a}	29.5±4.00^{a}	29.0±4.10^{a}	30.3±6.90^{a}	30.0±10.00^{a}	33.5±8.30^{a}
1 ⇧	20.1±2.00^{a}	23.2 ±2.00^{a}	23.0 ±3.40^{a}	20.3±0.20^{a}	23.3 ±2.00^{a}	28.4±13.80^{a}
2	23.2±1.60^{a}	26.7±4.60 ^{a}	18.2±1.20^{a}	30.4±13.80^{a}	21.1±2.00^{a}	30.4±11.80^{a}
3 ⇳	24.0±6.70^{a}	30.0±1.00^{b}	17.8±1.80^{a}	43.6±14.20^{b}	24.0±9.00^{a}	40.0±3.70^{b}
4	11.0±2.60^{a}	36.0±5.20^{b}	24.0±1.20^{b}	47.0±14.10^{c}	30.0±4.70^{b}	46.8±18.30^{c}
5	12.0±3.60^{a}	39.8±1.20^{b}	26.5±7.30^{b}	55.8±8.90^{c}	36.6±11.90^{b}	56.9±10.70^{c}
6 * +	18.0±1.00^{a}	56.3±6.30^{c}	30.0±6.30^{b}	6 0.0±5.20^{c}	46.0±7.80^{b}	60.0±14.30^{c}
7	24.0±9.30^{a}	79.0±18.20^{b}	64.0±10.00^{b}	61.3±7.50^{b}	62.7±21.40^{b}	62.3±2.30^{b}
8 * +	25.0±12.00^{a}	26.0±2.00^{a}	29.0±4.60^{a}	40.9±3.80^{b}	46.7±5.30^{b}	45.3±7.00^{b}
9 *	24.0±3.00^{a}	26.1±4.00^{a}	27.9±3.70^{a}	30.0±4.90^{a}	31.0±3.00^{a}	34.0±3.90^{a}
10	23.0±7.90^{a}	29.0±1.90^{a}	23.0±6.90^{a}	----------	30.0±7.00^{a}	25.8±7.90^{a}
11	23.1±7.00^{a}	24.9±5.90^{a}	20.0±6.90^{a}	----------	23.0±7.90^{a}	22.0±5.90^{a}
12	26.0±5.30^{ab}	25.0±7.60^{a}	22.0±3.80^{a}	----------	36.3±4.70^{b}	33.3±4.30^{ab}

Superscripts a b c represents the homogeneity between the experimental groups at probability P≤ 0.05.

⇧ Infection with *A. caninum*
⇳ Infection with trypanosomes
\+ Treatment with mebendazole
* Treatment with diminazene aceturate
Ac *Ancylostoma caninum*
Tb *Trypanosoma brucei*
Tc *Trypanosoma congolense*

Table 4. Mean ± SE Alanine Amino Transferase (ALT) (IU/L) level of dogs with experimental single *T. brucei* and *T.congolense* and conjunct with *A. caninum* infections and treated with diminazene aceturate and mebendazole.

Experimental Period (Weeks)		GPI (control)	GPII (*Ac*)	GPIII (*Tc*)	GPIV (*Tc*+*Ac*)	GPV (*Tb*)	GPVI (*Tb*+*Ac*)
0		18.3±3.00[a]	16.8±2.00[a]	13.8±2.00[a]	18.5±2.70[a]	18.3±4.50[a]	16.5±2.20[a]
1	⇧	11.3±3.00[a]	12.9±5.00[a]	15.3±11.00[a]	14.3±12.00[a]	12.6±3.00[a]	11.3±3.00[a]
2		12.3±3.00[a]	10.3±3.00[a]	12.3±2.00[a]	12.0±3.00[a]	12.3±3.00[a]	12.7±3.00[a]
3	⇧	13.7±9.70[a]	11.8±0.50[a]	16.3±0.80[a]	16.8±1.20[a]	19.3±10.40[a]	14.0±5.00[a]
0		40.3±5.70[a]	45.0±8.50[a]	39.3±5.90[a]	42.8±4.20[a]	39.5±7.80[a]	47.0±3.50[a]
4		12.3±3.00[a]	9.0±2.00[a]	5.0±0.30a	11.3±1.20[a]	10.3±0.30[a]	14.3±7.00[a]
5		15.7±1.80[a]	10.3±1.10[a]	16.0±3.00[a]	15.3±3.30[a]	18.7±3.30[a]	33.8±2.50[b]
6	* +	13.7±1.80[a]	22.0±7.00[a]	20.8±5.30[a]	30.0±2.10[b]	19.7±5.20[a]	38.3±6.00[b]
7		14.2±5.00[a]	25.3±5.00[b]	27.8±1.90[b]	49.7±5.00[c]	20.0±7.00[b]	48.0±2.00[c]
8	* +	16.3±6.00[a]	19.0±4.20[a]	10.0±1.50a	51.0±5.90[b]	47.3±2.30[b]	50.0±1.20[b]
9	*	13.5±4.00[a]	18.9±5.00[a]	19.0±0.10[a]	31.0±3.00[b]	32.1±0.10[b]	36.0±0.20[b]
10		14.7±0.40[a]	17.8±7.50[a]	21.0±0.30[a]	-----	28.2±10.40[a]	23.9±15.00[a]
11		12.0±5.80[a]	16.3±7.30[a]	19.6±0.90[a]	-----	19.0±1.00[a]	20.0±3.10[a]
12		13.9±0.60[a]	15.0±4.00[a]	23.0±2.00[ab]	-----	35.3±4.00[b]	16.0±3.10[a]

Superscripts a b c represents the homogeneity between the experimental groups at probability P≤ 0.05.

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Tb *Trypanosoma brucei*
Tc *Trypanosoma congolense*

Table 5. Mean ± SE Alkaline Phoshatase (ALP) (IU/L) level of dogs with experimental single *T. brucei* and *T.congolense* and conjunct with *A. caninum* infections and treated with diminazene aceturate and mebendazole.

Experimental Period(Weeks)	GPI (Control)	GPI (Ac)	GPI (Tc)	GPI (Tc/Ac)	GPV (Tb)	GPV (Tb/Ac)
1 ⇧	27.0±0.40^a	24.0±6.40^a	24.0±2.40^a	25.0±7.40^a	24.0±7.40^a	25.0±9.40^a
2	25.0±8.40^a	22.0±2.40^a	21.0±4.40^a	23.0±5.40^a	23.0±9.40^a	24.0±2.40^a
3 ⇳	28.7±6.40^a	24.3±3.20^a	22.0±1.00^a	28.9±0.80^a	29.7±8.50^a	29.0±6.60^a
4	27.3±3.50^a	32.0±8.20^a	34.3±4.10^a	33.1±1.10^a	68.3±3.00^b	38.0±4.00^a
5	30.0±15.90^a	82.8±25.10^b	55.0±11.00^b	82.3±15.90^b	55.0±15.60^b	75.8±23.50^b
6 * +	38.7±11.90^a	89.0±14.00^b	65.3±20.60^b	100.9±2.10^b	150.3±72.00^b	144.0±48.00^b
7	30.0±25.30^a	138.0±36.00^b	131.0±23.50^b	139.0±17.00^b	111.0±27.70^b	110.0±11.20^b
8 * +	36.4±50.10^a	102.3±25.00^b	103.3±33.10^b	190.0±34.00^b	147.3±33.10^b	189.0±12.00^b
9 *	27.0±3.50^a	89.3±34.00^b	87.4±35.30^b	99.0±24.10^b	86.9±24.90^b	90.9±35.90^b
10	34.0±3.60^a	56.9±45.00^a	63.9±23.00^a	-----	58.4±24.30^a	60.3±24.90^a
11	26.9±0.10^a	48.8±37.90^a	51.0±25.80^a	-----	46.0±34.00^a	49.9±45.90^a
12	35.9±7.00^a	46.0±10.20^a	53.0±15.20^a	------	44.0±1.50^a	46.0±5.00^a
1	95. 8 ± 6.10^a	94. 8 ± 3.00^a	92. 2 ± 6.20^a	95. 2 ± 2.00^a	92. 1 ± 6.20^a	92. 3 ± 4.00^a
0	97.0 ± 4.70^a	92. 8 ± 4.00^a	94.3± 4.00^a	86.0±5.80^a	79.3 ±17.40^a	87.8 ±4.20^a

Superscripts a b represent the homogeneity between the experimental groups at probability P≤ 0.05.

⇧ Infection with *A. caninum*
⇳ Infection with trypanosomes
\+ Treatment with mebendazole
* Treatment with diminazene aceturate
Ac *Ancylostoma caninum*
Tb *Trypanosoma brucei*
Tc *Trypanosoma congolense*

Table 6. Mean ± SE blood sugar (mg/dl) of dogs with experimental single *T. brucei* and *T.congolense* and conjunct with *A. caninum* infections and treated with diminazene aceturate and mebendazole.

2	93. 8 ± 2.00[a]	96. 8 ± 9.00[a]	93. 4± 2.00[a]	92. 3 ± 3.00[a]	94. 3 ± 6.10[a]	94. 8 ± 6.20[a]
3	92. 3 ± 6.00[a]	93. 8 ± 6.00[a]	92. 4 ± 7.00[a]	92. 8 ± 4.00[a]	93. 1 ± 3.00[a]	92. 6 ± 6.10[a]
4 ⇧	81. 8 ± 6.20[a]	81. 3 ± 2.00[a]	82. 2 ± 2.00[a]	80. 8 ± 2.00[a]	84. 2 ± 6.10[a]	82. 8 ± 6.50[a]
5	82. 4 ± 6.00[a]	85. 8 ± 3.00[a]	84. 2 ± 6.00[a]	82. 8 ± 6.30[a]	83. 8 ± 6.00[a]	82. 0 ± 2.00[a]
6 ⇳	84.0± 4.60[a]	85.5 ± 4.70[a]	88.8±3.80[a]	86.5 ±2.30[a]	90.7±4.80[a]	87.8±3.90[a]
7	86.8± 5. 40[a]	88.3 ± 2.20[a]	86.5± 2.30[a]	90.3 ± 4.00[a]	86.3± 0.70[a]	81.3± 1.30[a]
8	104.0± 3.70[a]	99.5± 2.20a	93.5± 3.90[b]	91.3± 2.00[b]	87.0± 5.60[b]	82.8± 1.40[b]
9 * +	102.0± 5.80[a]	101.0± 5.80[a]	98.1± 4.20[b]	93.0 ± 2.50[b]	91.0± 3.50[b]	91.3± 7.10[b]
10	103.0± 5.80[a]	100.0± 8.80[a]	104.0± 3.20[a]	101.0± 5.50[a]	100.0± 1.60[a]	101.0± 2.80[a]

Superscripts a b represent the homogeneity between the experimental groups at probability P≤ 0.05.

⇧ Infection with *A. caninum*
⇳ Infection with trypanosomes
\+ Treatment with mebendazole
* Treatment with diminazene aceturate
Ac *Ancylostoma caninum*
Tb *Trypanosoma brucei*
Tc *Trypanosoma congolense*

Discussion

Elevation in BUN observed in both species of trypanosomes and *A. caninum* result from blood loss and anaemia caused by the activities of trypanosomes and *A. caninum* in the body. The elevation was higher in the conjunct *T. brucei / A. caninum* compared to other groups due to the severe anaemia in

the group. Anaemia decreases renal perfusion resulting to renal impairment and consequent rise in BUN level (Deepak *et al*., 2007). *Ancylostoma caninum* induces massive gastrointestinal haemorrhages which increase the release of protein and its associated nitrogen by product, elevating BUN (Deepak *et al*., 2007). Treatment in dogs gave no appreciable improvement in the altered level of BUN in the blood due to initial ineffectiveness of diminazene aceturate to eliminate resistant strains of both *T. congolense* and *T. brucei*. Sudden increase in the treated groups could be due to relapses of infection.

The significant ($p<0.05$) increases in creatinine level of both A. *caninum* and trypanosomes infected groups' show significant impairment in the Kidney especially in the conjunct groups (GPIV; GPVI) as observed in BUN. Although high BUN serves as a pointer to renal disease, there however could be extra-renal causes. Conversely, creatinine has a high clearance rate in the body, and appreciable increase as observed in the infected groups clearly indicates significant renal damage due to anaemia. Higher creatinine level in the conjunct groups (GPIV and GPVI) corresponds to increased BUN. Azotemia recorded in the groups agrees with the records of Abenga and Anosa (2006).

The significant ($p<0.05$) increase in serum liver enzymes of AST, ALT and ALP in GPII, GPIII GPIV, GPV, and GPVI reveal significant damage to hepatic cells by both *Ancylostoma* and trypanosomes. Trypanosomes induce hepatic damage through sequestration of parasites within the tissue. This was as recorded in *T. brucei* infection in dogs (Akpa *et al.,* 2008; Nwoha *et al.,* 2013). In *T. congolense* infection in rabbits Takeet and Fagbemi (2009); and in *T. congolense* infection in rats (Egbe- Nwiyi *et al.,* 2005). *Ancylostoma caninum* induced hepatic damage through the release of inflammatory cells from larval migration in liver tissue. This corroborates previous observation in both single *A. caninum* and conjunct *T. brucei/A.caninum* infection in dogs (Nwoha *et al*., 2013), and in *A. caninum* infection in mice (Soh *et al*., 1969; Gollapudi and Viveka, 2013). The increases were

more in the conjunct groups (GPIV and GPVI) due to the combined effects of trypanosome and *A. caninum* in the liver.

Hypoglycaemia in the trypanosome infected groups could be attributed to some degree of glucose consumption by active trypanosomes. This corroborates the findings of Locatelli, (1930); Cadioli *et al*. (2006); Eloy and Lucheis (2009): Takeet and Fagbemi (2009) in different species of animals. On the other hand, it contradicts the findings of Sandoval *et al*. (1994) in trypanosomosis infection in dogs.

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