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Comparative Assessment of Dogs Experimentally Infected with Single *Trypanosoma brucei*, *Ancylostoma caninum* and Conjunct *Trypanosoma brucei* and *Ancylostoma caninum* Infections and Treated with Diminazene Aceturate and Mebendazole

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

A comparative serum biochemistry assessment of experimental infection with single and conjunct *Trypanosoma brucei (T. brucei),* and *Ancylostoma cannium (A. caninum)* was carried out in a total of sixteen mongrel breed of dog. The dogs were randomly assigned into 4 groups with 4 animals in each group as follows: GP I: Uninfected (control), GP II*: Ancylostoma caninum* infected, GP III: *Trypanosoma brucei* infected, GP IV: Conjunct *Trypanosoma brucei* and *A. caninum* infection. Post acclimatization *Ancylostoma caninum* infection was done on GPII and GPIV alone. Two weeks

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later, *Trypanosoma brucei* infection was done on GPIII and GPIV. Three weeks post trypanosome infection; GPIII and GPIV were treated with diminazene aceturate. Mebendazole was used only on GPII and GPIV and a repeat treatment given 2 weeks later. The serum biochemistry parameters showed a significant ($p< 0.05$) increase in Total protein in GPIII and GPIV. A significant ($p< 0.05$) decrease in Total protein was recorded in GPII. Conversely, there were significant ($p< 0.05$) decrease in albumin in all the infected groups (GPII, GPIII and GPIV). Significant ($p< 0.05$) increases in the levels of cholesterol and bilirubin were observed in GPII, GPIII and GPIV. However there was no significant ($p > 0.05$) alteration in calcium level in all the infected groups compared with control.

Keywords: Biochemical changes; Trypanosoma brucei; Ancylostoma caninum; diminazene aceturate; mebendazole.

1. INTRODUCTION

Trypanosomosis and ancylostomosis are parasitic diseases which may occur in mixed infection in dogs especially in the southeastern region of Nigeria [1,2]. The effect of these parasites are best known by proper assessment of biochemistry profiles to reveal the clinical status of animal, the organ function, nutritional state and determine therapeutic prescription [3,4]. Biochemical assessment of infected animals would also helps to determine the prognosis of the diseases and the specific therapeutic protocols and their accompanying effects on the body [5]. It may be used in determining the effect of prolonged use of therapy on vital organs of the body including liver or kidney [4]. In clinical biochemistry, the use of wide range of properly chosen tests are preferred to a specific test in order to reveal the effect of a disease condition in different systems of the body through the process of pattern recognition. Hence in trypanosomosis, serum biochemical 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. Regrettably, previous studies on the effects of trypanosomes on biochemical parameters had focused on single parasite species infections with very little information available on ancylostomosis or conjunct infection of ancylostomosis and trypanosomosis in dogs. It is therefore imperative to assess the serum biochemistry changes in dogs with single *Trypanosoma brucei* and in combination with *A. caninum.*

2. MATERIALS

2.1 Experimental Animals

Sixteen indigenous breed of dogs of both sexes weighing between 4.0 and 8.0 kg were used in this experiment. The dogs were acclimatized for 3 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 [6]. 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*.

2.2 Care of Experiment Animals

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.

2.3 Parasites and Infections

2.3.1 *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.

Estimated 2.5×10^6 of *T. brucei* suspended in 1ml of normal saline was used to infect each experimental dog in the group, and 1 ml of whole blood containing an estimated 2.5×10^6 parasites. The quantity of parasites inoculated was estimated using the rapid matching method of [7].

2.3.2 *Ancylostoma caninum* infection

The concentration of larval suspension was estimated using an automatic pipette (Biotht Peoline®), according to the method of [8]. 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 1 ml of distilled water were delivered *per os* to each of the experimental dogs, using a 2 ml syringe without needle.

2.3.3 Reconstitution of diminazene aceturate

A 2.36 g Veribin® a brand of trypanocide containing 1.05 g 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. Treatment was done in all the groups (GPII, GPIII and GPIV) on weeks 6, 8, and 9.

2.3.4 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 100 mg twice daily for 3 consecutive days. Treatment was repeated 2 weeks later.

2.4 Experimental Design

Dogs were randomly divided into 3 groups of 4 members in each group. GROUP (GP) I was uninfected dogs (control), GROUP (GP) II was infected with *Ancylostoma caninum*, GROUP (GP) III was *Trypanosoma brucei* infection, and GROUP (GP) IV was mixed infections of *Trypanosoma brucei and A. caninum.*

One week post acclimatization, *Ancylostoma caninum* infection was done on GPII and GPIV alone. Two weeks later *Trypanosoma brucei* infections was done on GPIII and superimposed on GPIV. Three weeks post trypanosome infection; GPIII and GPIV were treated with diminazene aceturate. Mebendazole was used only on GPII and GPIV and a repeat treatment given 2 weeks later.

2.5 Serum Biochemistry

2.5.1 Blood collection and preparation of serum samples

Five milliliters of blood was collected through the cephalic vein of each of the experimental dogs and dispensed into dried appropriately labeled 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 labeled tubes and stored at -20℃ until analyzed for biochemical constituents.

2.6 Biochemical Analysts

The serum Total protein, Bilirubin, albumin, cholesterol, and calcium were determined using Randox Text Kits (55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom) according to the manufacturer's prescriptions.

2.7 Statistical Analysis of Data

Data obtained from this study were presented as mean± standard error of mean (SEM). Statistical significance were analyzed using one way analysis of variance (ANOVA) and Duncan's multiple range test with SPSS version 16 soft ware package. The acceptance of level of Significance was at $P < 0.05$ [9].

3. RESULTS

3.1 Total Protein

The result of total protein is shown in Table 1. There was significant ($p< 0.05$) decrease in total protein by week 3 in GPII compared to other groups (Table 1). The decreases in GPII progressed up to week 5. Conversely a significant ($p< 0.05$) increase was observed by

week 4 in GPIII and by week 5 in GPIV. Increases in both groups progressed up to week 7 in GPIII and to week 9 in GPIV. Treatment was instituted on week 6 in all the infected groups (GPII, GPIII and GPIV). Subsequently from week 6 there was no difference between GPII compared to GPI. From week 10 to 12, there was no significant difference between both GPIII and GPIV compared to GPI.

3.2 Total Albumin

The result of the serum albumin level is shown in Table 2. From the result a significantly ($p< 0.05$) decrease in Albumin concentration was only observed in GPII on week 3 compared to GPI through out the experimental period. However from week 3 there was a significant decrease in GPIII and GPIV compared to GPI. The decreases in both groups progressed up to week 7 in GPIII and week 8 in GPIV. From week 9 onwards no significant difference was observed between GPIII and GPIV compared to GPI. However by week 12 there was a sudden decrease in both GPIII and GPIV compared to GPI.

3.3 Total Bilirubin

The result of total bilirubin is shown in Table 3. A Significant ($p < 0.05$) increase in bilirubin concentration was observed on week 6 In GPII compare to GPI through out the experiment. Nevertheless by week 3 there were significant ($p< 0.05$) increases in both GPIII and GPIV. The increases progressed up to week 6 in the groups. Subsequently there was no significant difference between GPIII and GPIV compared to GPI.

3.4 Cholesterol

The result of serum cholesterol level is shown in Table 3. Significant ($p< 0.05$) increase in cholesterol level occurred in GPII on week 3 to 6. There was a sudden decrease were observed in both GPIII and GPIV on weeks 4, 5 and 6. Subsequently there was no significant difference between the groups compared to GPI.

3.5 Calcium Ion

The result of serum calcium ion level is shown in Table 2. There was no significant alteration in calcium ion concentration in all the experimental groups (GPII, GPIII and GPIV) except on week 3 which shows a significant ($p<0.05$) increase. Afterwards there was no observable difference between the groups (GPII, GPIII and GPIV) compared to GPI.

3.6 Pack Cell Volume (PCV)

There was a significant ($p<0.05$) decrease in PCV of GPII at week 3 post infection which progressed up to week 7. Subsequently there was no significant ($p <0.05$) difference between GPII and control (GPI). Similarly significant

Table 1. Mean ± SE total protein (mg/dl) of dogs with experimental single *T. brucei, T. brucei* and conjunct *T. brucei* / *A. caninum* infections and treated with diminazene aceturate and mebendazole

Experimental period (weeks)	GPI (control)	GPII (*Ac*)	GPIII (*Tb*)	GPIV (*Tb/Ac*)
0	6.4±0.90^{a}	5.0±1.00^{a}	5.2±1.00^{a}	6.0±0.50^{a}
1 ⇧	6.7±0.30^{a}	6.2±0.30^{a}	6.0±0.40^{a}	5.8±0.20^{a}
2	6.8±0.20^{a}	6.0±0.70^{a}	6.1±0.30^{a}	5.9±0.10^{a}
3 ⇳	6.9±0.50^{a}	5.0±0.30^{b}	6.1±0.60^{a}	6.0±0.30^{a}
4	6.1±0.20^{a}	4.9±0.20^{b}	7.0±0.50^{c}	6.2± 0.40^{a}
5	6.0±0.30^{a}	4.0±0.30^{b}	7.7±0.70^{b}	6.6±0.20^{b}
6 * +	6.7±0.80^{a}	5.9±0.60^{a}	7.6±0.60^{b}	7.8± 0.50^{b}
7	6.9±0.10^{ab}	6.0±0.20^{a}	7.0±0.40^{ab}	8.0±0.40^{b}
8 * +	6.5±0.20^{a}	5.2±0.40^{a}	6.1±0.80^{a}	8.0±0.60^{b}
9 *	6.8±0.60^{a}	5.9±0.70^{a}	6.3±0.20^{a}	8.4±0.40^{b}
10	6.5±0.50^{a}	6.0±0.30^{a}	6.5±0.50^{a}	6.5±0.20^{a}
11	6.6±0.20^{a}	6.3±0.20^{a}	6.6±0.30^{a}	6.5±0.30^{a}
12	6.2±0.20^{a}	6.0±0.20^{a}	7.0±0.30^{a}	6.0±0.30^{a}

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*

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

Experimental period (Weeks)	GPI (control)	GPII *(Ac)*	GPIII (*Tb*)	GPIV (*Tb/Ac*)
0	1.9±0.50[a]	1.5±0.30[a]	1.9±0.20[a]	2.2±0.10[a]
1 ⇧	2.6±0.20[a]	2.4±0.10[a]	2.1±0.40[a]	2.1±0.20[a]
2	2.7±0.20[a]	2.3±0.30[a]	2.3 ±0.20[a]	2.1±0.40[a]
3 ⇳	2.8±0.50[a]	2.2±0.20[b]	2.4±0.10[a]	2.2±0.30[b]
4	2.3±0.20[a]	2.5±0.20[a]	2.0±0.10[ab]	1.6±0.20[b]
5	2.6±0.20[a]	2.4±0.30[a]	1.5±0.10[c]	1.3±0.00[c]
6 * +	2.0±0.50[a]	2.0±0.20[a]	1.4±0.20[b]	1.3±0.30[b]
7	2.4±0.20[a]	2.3±0.20[a]	1.7±0.10[cb]	1.4±0.20[cd]
8 * +	2.2±0.20[a]	2.6±0.30[a]	2.0±0.10[a]	2.0±0.10[b]
9 *	2.5±0.10[a]	2.5±0.40[a]	2.2±0.10[a]	2.3±0.20[a]
10	2.5±0.20[a]	2.3±0.10[a]	2.3±0.20[a]	2.3±0.10[a]
11	2.4±0.10[a]	2.4±0.10[a]	2.3±0.10[a]	2.2±0.20[a]
12	2.6±0.10[a]	1.9±0.40[ab]	1.6±0.10[b]	1.1±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*

Table 3. Mean±SE Total Bilirubin (mg/dl) of dogs with experimental single *T. brucei, T. brucei* and conjunct *T. brucei* / *A. caninum* infections and treated with diminazene aceturate and mebendazole

Experimental period (weeks)	GPI (control)	GPII *(Ac)*	GPIII *(Tb)*	GPIV *(Tb/Ac)*
0	0.3±0.10[a]	0.4±0.00[a]	0.5±0.10[a]	0.4±0.40[a]
1	0.3±0.10[a]	0.3±0.10[a]	0.2±0.10[a]	0.2±0.10[a]
2	0.2±0.10[a]	0.3±0.10[a]	0.3±0.10[a]	0.3±0.00[a]
3 ⇳	0.3±0.10[a]	0.3±0.00[a]	0.7±0.10[b]	0.7±0.10[b]
4	0.2±0.10[a]	0.2±0.00[a]	0.7±0.20[b]	0.4±0.10[a]
5	0.2±0.10[a]	0.3±0.10[a]	0.3±0.10[a]	0.2±0.00[a]
6 * +	0.2±0.10[a]	0.4±0.00[b]	0.5±0.10[b]	0.5±0.10[ab]
7	0.4±0.10[a]	0.5±0.10[a]	0.3±0.10[a]	0.4±0.10[a]
8 * +	0.3±0.10[a]	0.3±0.10[a]	0.3±0.10[a]	0.3±0.10[a]
9 *	0.2±0.10[a]	0.4±0.20[a]	0.3±0.20[a]	0.3±0.10[a]
10	0.3±0.30[a]	0.2±0.20[a]	0.3±0.10[a]	0.2±0.20[a]
11	0.2±0.10[a]	0.3±0.20[a]	0.2±0.10[a]	0.3±0.10[a]
12	0.3±0.10[a]	0.3±0.10[a]	0.3±0.10[a]	0.2±0.00[a]

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*

(p<0.05) decrease in PCV was first observed in GPIV at week 4 post infection. The decrease continued in both GPIII and GPIV from week 5 up to week 8. Subsequently there was no significant difference between GPIII and GPIV when compared to GPI.

3.7 Haemoglobin Concentration (Hb)

As in PCV, there was a significant decrease (p<0.05) in Hb of GPII starting from week 4 up to week 7 post infection. Also significant decreases were equally observed in trypanosomes infected groups starting from week 4 in GPIV and week 5 in GPIII up to week 8. Subsequently no difference was seen between the groups (GPIII and GPIV) compared to the control.

4. DISCUSSION

The decrease (p<0.05) in total protein (TP) observed in *A. caninum* infected group (GPII) agrees with [10] who recorded hypoproteinaemia in ancylostomosis. This may be due to protein

Table 4. Mean ± SE Cholesterol level (mg/dl) of dogs with experimental single *T. brucei*, *T. brucei* and conjunct *T. brucei* / *A. caninum* infections and treated with diminazene aceturate and mebendazole

Experimental period (weeks)	GPI (control)	GPII (*Ac*)	GPIII (*Tb*)	GPIV (*Tb/Ac*)
0	78.3±15.90[a]	198.0±55.00[a]	182.0±19.40[a]	154.0±39.30[a]
1 ⇧	138.1 ±2.00[a]	140.0±25.00[a]	131.1 ±0.80[a]	110.0 ± 0.10[a]
2	128.0± 3.70[a]	130.2±8.90 [a]	123.2±0.10[a]	119.3±3.00[a]
3 ⇳	121.0±10.80[a]	138.0±13.00[ab]	124.0±9.00[a]	120.0±5.70[a]
4	121.0±3.00[a]	132.0±3.00[a]	184.0±20.00[b]	146.0±18.70[ab]
5	141.0±26.80[a]	222.0±23.10[b]	140.0±3.70[a]	190.0±28.80[ab]
6 * +	111.0±6.30[a]	177.0±15.00[b]	200.0±38.40[b]	161.0±18.10[ab]
7	130.0±10.80[a]	116.0±19.00[a]	160.3±20.00[a]	120.0±10.00[a]
8 * +	127.0±6.90[a]	88.0±19.30[b]	170.0±16.50[a]	130.0±15.30[a]
9 *	125.0±3.00[a]	121.0±12.00[a]	134.0±3.00[a]	125.0±3.20[a]
10	130.0±2.90[a]	125.0±3.60[a]	136.0±2.00[a]	126.0±2.50[a]
11	124.0±2.00[a]	136.0±3.40[a]	132.0±2.00[a]	130.0±1.00[a]
12	114.0±23.30[a]	134.0±3.00[ab]	119.0±30.00[a]	108.7±29.00[a]

Superscripts a b c represents the homogeneity between the experimental groups at probability P≤ 0.05
⇧ *Infection with A. caninum* ⇳ *Infection with trypanosome; + Treatment with mebendazole;*
* *Treatment with diminazene aceturate; Ac Ancylostoma caninum; Tb Trypanosoma brucei*

loss enteropathy often encountered in cases of ancylostomosis. The marked increase (P<0.05) recorded by the 4th week in GPIII agrees with [11,12] who reported hyperproteinaemia in cases of *T. brucei brucei* infection in various species of animals. This increase may be due to increase inflammatory reactions associated with trypanosome infection [12,13]. In humans, inflammatory reactions cause release of variety of proteins with associated influx of proteins into the blood stream leading to hyperproteinaemia [14]. [15,16] recorded no significant difference (p>0.05) in the total protein of pigs and West Africa Dwarf Goat (WADG) infected with *T. brucei brucei,* respectively. The persistent increase in total protein in the conjunct group (GPIV) was due to relapse of infection with persistent inflammatory reactions. Hyperproteinaemia has been associated with series of untowards effects among which includes oxygen deficit. Negatively charged proteins attract positively charged rbcs forming a protein cover chain. This process though does not completely eliminate oxygen uptake but severely inhibits oxygen carrying capacity by rbcs [14]. This often led to cell aging and degeneration seen in trypanosomosis [17,13] and may account for the severe disease in the conjunct group. The significant increase in albumin in the experimental groups at week one was most probably due to improved diet and efficient food absorption. Dogs purchased from the local market are more or less ill fed and bound with both external and internal parasites which compromise their health status. These parasites were adequately treated during the period of acclimatization and certified healthy for experimental purpose. Treatment enabled efficient and proper food assimilation and absorption thus influenced the rapid increase in albumin concentration."

The significant (p<0.05) decreases observed in total albumin in the trypanosome infected groups (GPIII and GPIV) may be attributed to the loss of albumin in urine which was documented in cases of trypanososmosis [18]. The decrease recorded in the *Ancylostoma* group (GPII) agrees with the previous record of hypoalbuminaemia which was attributed to enteropathy and loss of essential nutrients for synthesis of albumin [19].

Transitory hyperbilirubinaemia in the trypanosome infected groups could be attributed to trypanosome-induced liver damage. Sequestration of *Trypanosoma brucei* in the liver tissues could induce liver dysfunction affecting its excretory function. This agrees with [20] in *T. brucei* infection in dogs. It may also be due destruction of red blood cells through diverse means including hemorrhages as observed in significant (p<0.05) decrease in both the PCV and Hb of the experimental groups (Tables 5 and 6). Increase in cholesterol level in both trypanosomes (GPIII and GPIV) and *Ancylostoma* (GPII) groups were probably due to both liver and kidney impairment in the dogs.

Liver tissues produce clearance enzymes that aids in excretion and elimination of triglycerides and these lipids tends to accumulate in cases of liver impairment [21]. Trypanosomes induced liver dysfunction previously seen in hyperbilirubinaemia down played the excretory function of the liver resulting to increase in cholesterol. Similarly, hepatotracheal migration of *Ancylostoma caninum* in young dogs in GPII induced hepatic damage and resultant increase in serum cholesterol [22]. In addition for some unexplained reasons, cholesterol levels increases in cases of kidney damage. The triglycerides clearance enzymes produced in the liver could become less active and perhaps resistant in kidney damage and therefore could be the cause of increases in cholesterol levels in GPII, GPIII, and GPIV [21]. Increase observed in trypanosome groups agree with the findings of [23] in *T. brucei* infection in rabbits and also with [24,25] in *T. b. gambiense* infection in Monkey and *T. congolense* infection in rabbits,

Table 5. Mean ± SE Calcium ion conc.(mg/dl) of dogs with experimental single *T. brucei*, *T. brucei* and conjunct *T. brucei* / *A. caninum* infections and treated with diminazene aceturate and mebendazole

Experimental period (Weeks)	GPI (Control)	GPII *(Ac)*	GPIII *(Tb)*	GPIV *(Tb/Ac)*
0	10.2±3.70[a]	9.6±0.40[a]	9.0±1.20[a]	10.4±0.20[a]
1 ⇧	10.1±2.00[a]	9.5±1.00[a]	11.1±1.00[a]	9.3±1.00[a]
2	9.2±1.00[a]	10.3±5.00[a]	9.1±1.00[a]	9.0±1.00[a]
3 ⇳	10.4±0.10[a]	10.1±0.20[a]	9.0±0.40[a]	9.3±0.50[a]
4	9.2±0.20[ab]	10.2±0.30[b]	10.0±0.60[b]	9.0±0.40[ab]
5	8.0±1.10[a]	8.5±1.00[a]	7.6±2.20[a]	8.0±1.00[a]
6 * +	7.8±1.00[a]	4.6±1.20[a]	8.3±1.80[a]	3.6±2.00[a]
7	9.1±1.30[a]	9.0±0.70[a]	10.3±2.30[a]	9.9±0.80[a]
8 * +	7.8±2.40[a]	7.5±10.00[a]	7.7±1.00[a]	4.0±2.40[a]
9 *	8.3±5.70[a]	7.3±0.50[a]	9.0±1.00[a]	8.5±3.00[a]
10	9.0±2.90[a]	8.9±2.00[a]	9.8±1.30[a]	9.4±2.90[a]
11	10.2±1.00[a]	9.0±2.00[a]	9.8±0.20[a]	9.8±2.00[a]
12	10.3±0.20[a]	7.3±0.40[a]	7.7±1.00[a]	7.1±2.40[a]

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*

Table 6. Mean ± SE PCV (%) of dogs with experimental single *T. brucei* and conjunct with *A. caninum* infections and treated with diminazene aceturate and mebendazole

Experimental period (Week) ls)	GPI (control)	GPII (*Ac)*	GPIII (*Tb*)	GPVI (*Tb/ Ac)*
0	39.8±2.10[a]	39.5±2.40[a]	37.5±3.20[a]	39.0±2.00[a]
1 ⇧	33.8±1.20[a]	33.4± 2.09[a]	35.7±2.00[a]	35.0±1.10[a]
2	34.7±1.00[a]	34.9±0.10[a]	35.9±1.09[a]	33.9±0.10[a]
3 ⇳	35.0±1.20[a]	27.0±1.10[b]	30.3±2.30[a]	33.0±0.90[a]
4	35.5±2.00[a]	30.3±0.10[a]	37.0±0.30[a]	23.8±0.60[b]
5 * +	34.7±2.20[a]	24.5±1.30[b]	23.7±1.90[b]	22.8±0.80[b]
6	36.7±0.90[a]	25.8±1.50[b]	16.0±1.50[c]	15.8±2.00[c]
7 * +	35.3±2.00[a]	27.3±1.40[b]	22.0±1.00[bc]	17.8±2.00[c]
8 *	36.0±1.50[a]	35.8±1.30[a]	26.7±2.00[b]	22.5±1.90[b]
9	37.4±1.20[a]	36.8±1.60[a]	35.3±0.90[a]	35.3±4.80[a]
10	34.3±0.30[a]	36.0±1.50[a]	36.3±2.80[a]	33.3±0.90[a]
11	36.0±1.50[a]	38.7±0.80[a]	37.0±2.80[a]	32.0±2.00[a]
12	39.0±1.50[a]		38.0±2.00[a]	33.7±2.60[a]

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*

Table 7. Mean ± SE Haemoglobin concentration (mg/dl) of dogs with experimental single *T. brucei* and conjunct *A. caninum* infections and treated with diminazene aceturate and mebendazole

Experimental period (Weeks)	GPI (control)	GPII (*Ac*)	GPIII (*Tb*)	GPVI (*Tb/Ac*)
0	12.8±2.10[a]	12.5±2.40[a]	12.5±3.20[a]	12.0±2.00[a]
1 ⇧	12.3±0.40[a]	12.0±0.30[a]	12.0±0.20[a]	12.2±0.10[a]
2	12.4±0.20[a]	12.1±0.20[a]	12.3±0.70[a]	12.3±0.20[a]
3 ⇳	12.5±1.20[a]	12.0±2.30[a]	13.5±2.30[a]	12.5±0.90[a]
4	12.6±2.00[a]	8.10±0.70[b]	11.2±0.50[a]	8.0±0.60[b]
5	12.4±2.20[a]	9.3±0.10[b]	8.7±0.90[b]	7.5±0.80[b]
6 * +	12.6±0.90[a]	9.0±1.30[b]	8.2±0.30[b]	6.0±2.00[c]
7	12.7±2.00[a]	11.9±0.90[ab]	8.60±0.90[c]	6.2±0.60[d]
8 * +	12.8±1.50[a]	12.0±0.70[a]	8.60±0.90[bc]	6.0±0.60[b]
9 *	12.5±4.00[a]	12.2±0.00[a]	10.2±0.10[a]	9.0±1.80[a]
10	12.4±0.30[a]	12.4±1.10[a]	11.3±1.20[a]	10.6±0.90[a]
11	12.6±1.50[a]	12.5±0.70[a]	12.4±0.70[a]	12.6±1.10[a]
12	12.0±0.10[a]	12.7±0.30[a]	12.0±0.30[a]	12.9±0.60[a]

Superscripts a b c d 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 rypanosoma brucei*

respectively. It however contradicts the findings of decrease level of cholesterol in experimental *T. brucei /T. congolense* infections in goats [16] and in *T. brucei brucei* infection in pigs [26]. The significant ($p<0.05$) increases observed in GPVI and GPV post treatment was due to inapparent relapses. The transient increase in calcium ion concentration in all the infected groups (GPII, GPIII and GPIV) could be disregarded as an important factor in trypanosomosis and ancylostomosis infection in dogs. Previous work of [27] observed a significant ($p<0.05$) increase in calcium ion of *T. cruzi* parasitized cells and attributed it to intracellular release of calcium deposit from the host mitochondria. Generally serum biochemistry parameters analyzed showed most severe alterations in the conjunct group compared to the single infection. This was extrapolated from the extended period of alteration in serum biochemistry of the group compared to others. Treatment with 7 mg/kg diminazene aceturate and 100 mg mebendazole twice daily for 3 days improved most of the altered serum biochemistry as previously observed by [22]. However the efficacy of diminazene aceturate was impaired by the resistant strains of *T. brucie*. Nevertheless repeated doses effectively improved the altered conditions.

5. CONCLUSION

Biochemical changes observed in single *Trypanosome brucei*, *Ancylostoma caninum* and conjunct infection of *Trypanosoma brucei/ A. caninum* were severe in the conjunct group compared to the single infections. Treatment of the infected group with diminazene aceturate and mebendazole improved the altered biochemical components.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abenga JN, Anosa VC. Clinical studies on experimental gambiense trypanosomosis in vervet monkeys. Veterinarski Arhiv. 2006;76(1):11-18.
2. Adamu D, Barde N, Abenga JN, Useh NM, Ibrahim NDG, Esievo KAN. Experimental *Trypanosome brucei* infection induced changes on the serum profiles of lipids and cholesterol and the clinical implications in pigs. Journal of Cell and Animal Biology. 2009;3(2):15-20.
3. Agu WE, Egbuji AN. Urine albumin level in mice infected with *Trypanosoma brucei.* Veterinary Arhives. 2002;72:101-108.

4. Akanji MA, Adeyemi OS, Oguntoye SO, Sulyman F. Psidium guava extract reduces trypanososis associated lipid peroxidation and raises glutathione concentration in infected animals. Excli, Journal. 2009;8: 148-154.
5. Akpa PO, Ezeokonkwo RC, Eze CA, Anene BM. Comparative efficacy assessment of pentamidine isethionate and diminazene aceturate in the chemotherapy of *Trypanosoma brucei brucei* infection in dogs. Veterinary Parasitology. 2008;151:139-149.
6. Anene BM, Ifebigh AO, Igwilo IA, Umeakuana PU. Prevalence and haemato-biochemical parameters of trypanosome-infected pigs at Nsukka, Nigeria. Comparative Clinical Pathology; 2010.
   DOI: 10.1007/s00580-009-0944-2
7. Biryomumaisho S, Katunguka-Rwakishaya, E, Rubaire-Akiiki CM. Serum biochemical changes in experimental *Trypanosoma congolense* and *Trypanosoma brucei* infections in East African sheep. Veterinarski Arhiv. 2003;73(3):167-180.
8. Bukowski JA. Serum biochemistry profile. Webvet Social Media Icons; 2012.
9. Cardon L. Incidences and causes of hyperproteinaemia. A study of 4,390 cases. Arch intern medicine (Chicago). 1943; 71(3):377-390.
10. Ekanem JT, Yusuf OK. Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *T. brucei* infected rats. African Journal of Biomedical Research. 2008;11:79-85.
11. Ezeokonkwo RC, Ezeh IO, Onunkwo JI, Obi PO, Onyenwe IW, Agu WE. Comparative haematological study of single and mixed infections of mongrel dogs with *Trypanosoma congolense* and *Trypanosoma brucei brucei*. Veterinary Parasitology. 2010;173(1-2):48-54.
12. Herbert WJ, Lumsden WHR. *Trypanosoma brucei,* a rapid matching method for estimating the hosts parasitaemia. Experimental Parasitology. 1976;40:427-428.
13. Kary. Cholesterol and your kidneys. A fatal connection. Report on Straw Utilization Conference held at Oxford 24 and 25 February, London H.M.S.O. 1977;2008.
    Available:http://www.healthcentral.com/cholestMAFF
14. MAFF. Report on Straw Utilization Conference held at Oxford 24 and 25 February, London H.M.S.O; 1977.
15. Mundim AV, Coelho OA, Hortêncio SM, Guimarães EC, Espindola FS. Influence of age and sex on the serum biochemical profile of Doberman dogs in the growth phase. Comparative Clinical Pathology. 2007;16:41–46.
16. Nakamura Y. Alteration of serum lipid, lipoprotein and inflammatory cytokines profile of rabbits infected with *Trypanosoma brucei*. Veterinary Parasitology. 1998; 80:117-125.
17. Nwoha RIO. Prevalence of gastrointestinal parasites in dogs from Umuahia city of Abia state. International Journal of Biological Sciences. 2011;3:3.
18. Nwoha RIO, Eze IO, Anene BM. Serum biochemical and liver enzymes changes in dogs with single and conjunct experimental infections of *Trypanosoma brucei brucei* and *Ancylostoma caninum.* African Journal of Biotechnology. 2013;12(6):618-624.
19. Ohaeri CC, Eluwa MC. Abnormal biochemical and haematological indices in trypanosomiasis as a threat to herd production. Veterinary Parasitology. 2011; 177:199–202.
20. Osuna A, Castanys S, Rodriguez-Cabezas MN, Gamarro F. *Trypanosoma cruzi*: Calcium ion movement during internalization in host HeLa cells. International Journal for Parasitology. 1990;20(5):673–676.
21. Payne JM, Payne S. The metabolic profile test. Oxford University Press, Oxford, UK. 1987;179.
22. Rashid A, Rasheed K, Hussain A. Trypanosomiasis in Dog: A case report. Journal of Arthropod-Borne Diseases. 2008;2(2):48-51.
23. Snedecor GW, Cochran WG. Statistical method (6th Edition) Iowa state University press Amess, Iowa, USA; 1973.
24. Taiwo VO, Olaniyi MO, Ogunsami AO. Comparative plasma biochemical changes and susceptibility of erythrocytes to invitro peroxidation during experimental *Trypanosome congolense* and *T. brucei* infections in sheep. Isreal Journal of Veterinary Medicine. 2003;58(4):10.
25. Takeet MI, Fagbemi BO. Haematological, pathological and plasma Biochemical changes in rabbits experimentally infected

with *Trypanosoma congolense*. Science World Journal. 2009;4:2.

26. Urquhart GM, Armour J, Dunn JL, Jennings FW. Veterinary parasitology. Blackwell Science Limited. 1998;53-55.

27. Woo PTK. The Haematocrit centrifugation technique for the diagnosis of African trypanosomosis. Acta Tropica. 1970;27: 384-386.

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