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Haemoassessment of Dogs Experimentally Infected with Single and Conjunct *Trypanosoma congolense* and *Ancylostoma caninum* and Treatment with Diminazene Aceturate and Mebendazole

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Authors' contributions

This work was carried out in collaboration between both authors. Author BMA designed the study and wrote the protocol. Author RION wrote the first draft of the manuscript, managed the literature searches, analyses of the study performed the spectroscopy analysis and managed the experimental process. Both authors read and approved the final manuscript.

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ABSTRACT

The haemoassessment of dogs with single *Trypanosoma congolense (T. congolense*) and conjunct *T. congolense /A. caninum* was determined in this study. Twelve mongrels of both sexes weighing between 4 to 8 kg were grouped into 3 of 4 members each. The group i (GPI) was the uninfected (control), group ii (GPII) was infected with *T. congolense* and group iii (GPIII) was conjunct infection of *T. congolense/A. caninum.* Post acclimatization GPIII was infected with 200 infective L_3 of *A. caninum*, 2 weeks later both GPII and GPIII were given $2.5x10^6$ trypanosomes intraperitoneally. Three weeks post trypanosome infection, treatment was done with 100 mg of mebendazole twice daily for 3 days and 7 mg/kg of diminazene aceturate. Result showed a significant decrease ($p<0.05$) in PCV, Hb and Rbc of both GPII and GPIII. The decrease in GPIII was more compared to that in GPII. There was significant decrease ($p<0.05$) in neutrophil, monocyte, lymphocyte and

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eosinophil counts of both GPII and GPIII except in basophil count which showed no significant difference ($p<0.05$) from GPI (control) throughout the experiment. Treatment with both diminazene aceturate and mebendazole cause significant haematological improvement.

Keywords: Trypanosoma congolense; Ancylostoma caninum; dogs.

1. INTRODUCTION

Haematological alterations are one of the consistent findings in trypanosomosis and ancylostomosis in animals [1,2]. These alterations could contribute to the eventual immunosuppression which renders infected animals susceptible to opportunistic infections [3]. Several researchers have consistently recorded anaemia as a cardinal sign in trypanosomosis which is believed to exert profound influence on the pathogenesis of the disease in animals [4,5,1,6]. Some of the hypothesis on the mechanism of anaemia in trypanosomosis includes: increased red cell destruction; extravascular and intravascular haemolysis by immune system; splenic pooling; direct traumatic effect of trypanosome and splenic phagocytosis [7,8]. Several reports in different species of animals show consistent decreases in pack cell volume (PCV), haemoglobin concentration (Hb) and red blood cell count (RBC) [5,9,10,6]. Similar observation was made in *T. rhodesiense* infection in Vervet monkey [1]. Available reports on dogs recorded sharp decline in PCV and Hb [11-14]. [12] observed decreases in PCV and Hb with abnormal erythrocytes in a dog with natural infection of trypanosome. Similar report was made by [11] who recorded decrease in PCV and thrombocytopaenia in *T. cruzi* infection in dogs, and [13] who observed decrease in PCV and RBC in dogs with *T. evansi* infection. The importance of haematology in health assessment prompted the study of haemoassessment of dogs experimentally infected with single and conjunct *T. congolense* and *A. caninum.*

2. METHODOLOGY

Twelve mongrels of both sexes weighing between 4.0 and 8.0kg were used in the experiment. The dogs were acclimatized for 4 weeks prior to commencement of experiment during which they were screened for blood parasites and confirmed negative by Giemsa-stain, thin blood smears and haematocrit buffy coat method [15]. 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 100 mg twice daily for 3 days and also treated with sulfadimidine at the dose of 48 mg/kg intramuscularly against systemic opportunistic bacterial infections. The experiment commenced a week post treatment. The animals were kept in clean cages in a fly proof house and fed twice daily. Water was given *ad libitum*.

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 passage in a donor dog from where parasites were collected for infection of the experimental dogs. Estimated 2.5×10^6 of *T. congolense* suspended in 1 mL 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 [16].

The concentration of larval suspension was estimated using an automatic pipette (Biotht Peoline®), according to the method of [17]. Small doses of 20 µL larval suspensions were placed as drops on a microscopic slide and counted under ×40 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 was delivered *per os* to each of the experimental dogs, using a 2 mL syringe without needle.

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

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 *per os* twice daily for 3 days. Treatment was repeated 2 weeks later.

Dogs were randomly divided into 3 groups of 4 members in each group. GROUP I was uninfected dogs (control), GROUP II was infected with *T. congolense*, and GROUP III was mixed infections of *T. congolense and A. caninum.* Post acclimatization, *Ancylostoma caninum* infection was done on GPIII alone. Two weeks later, *T. congolense* infection was done on GPII and GPIII. Three weeks post trypanosome infection; GPII and GPIII were treated with diminazene aceturate. Repeat treatments were done on week 8 and 9. Mebendazole was used only on GPIII and a repeat treatment given 2 weeks later.

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.

Five mililiter of blood was collected through the cephalic vein of each of the experimental dogs and 2 mL was dispensed into an ethylenediamine tetra-acetic acid (EDTA) bottle for haematology. The remaining 3 mL was delivered into dried, 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 pack cell volume was determined using capillary tubes as described by [18].

Haemoglobin was determined using the Drabkins cyanmethemoglobin method as described by [19].

Automatic pipette was used to dispense 4 mL of RBC's diluting fluid into clean and dry tubes. Approximately 0.2 µL of test blood sample was added and both were thoroughly mixed together. The mixture was allowed to stand for 5 minutes after which the Nauber chamber was charged. The charging entails first, proper cleaning of the surface of the chamber to clear dust particles which often form artefacts on the chamber. A clean cover slip was then properly fitted on the chamber and with a clean Pasteur pipette, the mixture was stirred and an aliquot collected and gently dispensed "charging" from one edge under the cover slip, carefully avoiding passage of bubbles. The charged chamber was then placed under the microscope and read at low magnification (x10). Only red blood cells found within the primary, secondary and tertiary squares at the centre of the Nauber chamber were counted and result obtained was recorded.

Using a WBC pipette of a haemocytometer, blood was drawn to fill the 0.5 mark. The tip of the tube was cleaned and thereafter the WBC diluting fluid was drawn up the 1.1 mark of the WBC pipette. The fluid and blood were mixed gently avoiding bubbles. A cover slip was appropriately placed on the counting chamber. The fluid-blood mixture was then transferred using a fine bore Pasteur pipette on the counting chamber. The charged chamber was allowed to stand for 2 minutes to ensure adequate settling of cells at the bottom of the chamber and then viewed under the microscope at low power objective (x10). The WBCs uniformly observed in the four larger corner squares were counted and cells within the margin of two squares were counted only on one side while avoiding those present on the opposite.

Thin blood smear was made by placing a drop of blood at one end of a clean slide and with a spreader placed just close to the tip of the blood to allow a uniform spread through out the width and with a swift move blood was uniformly spread down the slide leaving out thin edges. Adequate volume of methanol was used to fix the smear onto the slides for 5 minutes. A reconstituted Geimsa stain in the ratio of 1:100 mL of distilled water was used to cover the entire surface of the fixed slides. The stain was allowed for 10 minutes and then washed out with jets of water. The stained slides were left to dry and then packed in a slide pack. Using oil immersion, a minimum of 100 cells was counted by moving the slide in a systematic fashion to capture the central and peripheral areas of the smear. The differential cells: - neutrophil, lymphocyte, monocyte, eosinophil and basophil were counted at low power magnification x 40 with the aid of cell counter [20].

3. RESULTS

The results of PCV are shown in Table 1. There was a significant (p<0.05) increase in the PCV of both GPII and GPIII by week one of the experiment. By week 4, there was significant (p< 0.05) decrease in PCV in GPIII compared to the control. By week 5, significant (p<0.05) decrease was observed in GPII. The decrease in week 7 was more in GPIII compared to that in GPII. The decreases in both GPII and GPIII progressed up to week 8. By week 10, there was no significant difference observed in both GPII and GPIII when compared with the control (GPI).

The results of haemoglobin concentration are presented on (Table 2). By week 5 and 6, significant (p<0.05) decreases was recorded in both GPII and GPIII. By week 6, the decrease in GPIII was more compared to that in GPII. The decreases (p<0.05) recorded by week 6 was more in GPIII when compared to GPII. The decreases persisted up to week 7 and 8. By week 10, no significant (p< 0.05) difference was observed in both GPII and GPIII compared to the control.

The results of RBC count are shown in Table 3. Significant (p<0.05) increase in RBC of GPIII was observed on first week of experiment. By week 3 to 6, there were significant (p< 0.05) decreases in the RBC in GPII and GPIII when compared to GPI. By week 7, the decrease in GPIII was more compared to GPII. By week 10, no significant (P<0.05) difference was observed in both GPII and GPIII compared with the control (GPI).

The results of WBC count are presented in Table 4. At week 3, significant (p< 0.05) decreases were observed in the WBC count of GPII and GPIII compared to GPI. The decreases persisted in both groups up to week 6. By week 10, no significant (p<0.05) difference was observed between GPII and GPIII compared with the control (GPI).

The result of lymphocyte count is shown in Table 5. By week 3, there was a significant (p< 0.05) decrease in lymphocyte count in GPIII compared to the other groups (GPII and GPI). The decreases continued and progressed up to week 9. Similarly by week 5, significant (p< 0.05) decrease was observed in GPII which progressed up to week 10. By week 11, no significant (p< 0.05) difference was observed between the infected groups compared with the control.

The result of the neutrophil count is shown in Table 6. By week 4, there were significant (p< 0.05) decreases in neutrophil count in both GPII and GPIII compared to GPI. The decrease progressed in both groups up to week 9 in GPIII and week 10 in GPII. By week 6 and 7, the decrease recorded in GPIII was more compared to GPII. By week 10, no significant (p<0.05) difference was observed between GPII and GPIII compared with the control.

Table 1. Mean ± SE PCV (%) of dogs with experimental single *T. congolense* and conjunct *T. congolense/ A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (Week)	GPI (control)	GPII (*Tc*)	GPIII (*Tc*/*Ac*)
0	39.80±2.10[a]	36.00±2.10[a]	25.00±2.00[a]
1 ⇧	33.80±1.20[a]	34.10±0.10[a]	34.10±0.10[a]
2	34.70±1.00[a]	35.00±0.00[a]	32.00±0.10[a]
3 ⇳	35.00±1.20[a]	35.80±3.60[a]	30.30±3.00[a]
4	35.50±2.00[a]	34.30±2.20[a]	24.00±0.40[b]
5	34.70±2.20[a]	23.50±2.90[b]	22.30±0.30[b]
6 * +	36.70±0.90[a]	17.60±1.40[b]	19.20±2.10[b]
7	35.30±2.00[a]	19.00±0.70[d]	15.00±0.60[c]
8 * +	36.00±1.50[a]	23.80±1.70[b]	23.30±0.70[b]
9 *	37.40±1.20[a]	35.30±1.10[a]	37.00±0.60[a]
10	34.30±0.30[a]	34.00±1.80[a]	----
11	36.00±1.50[a]	33.00±4.80[a]	-----
12	39.00±1.50[a]	34.90±1.70[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; Tc Trypanosoma congolense*

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

Experimental period (Week)	GPI (control)	GPII (*Tc*)	GPIII (*Tc/Ac*)
0	12.80±2.10[a]	11.00±2.10[a]	11.00±2.00[a]
1 ⇧	12.30±0.40[a]	12.30±0.30[a]	12.00±0.00[a]
2	12.40±0.20[a]	12.20±0.00[a]	12.10±0.10[a]
3 ⇳	12.50±1.20[a]	12.10±3.60[a]	12.30±3.00[a]
4	12.60±2.00[a]	9.20±0.40[b]	8.20±0.60[b]
5	12.40±2.20[a]	9.80±0.60[b]	7.50±0.50[b]
6 * +	12.60±0.90[a]	7.30±1.40[b]	6.60±0.70[c]
7	12.70±2.00[a]	9.30±0.70[b]	8.00±0.30[b]
8 * +	12.80±1.50[a]	8.60±0.30[b]	8.00±0.30[b]
9 *	12.50±4.00[a]	10.40±0.10[a]	9.50±0.10[a]
10	12.40±0.30[a]	11.40±1.80[a]	-----
11	12.60±1.50[a]	12.60±1.60[a]	-----
12	12.00±0.10[a]	12.70±0.20[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; Tc Trypanosoma congolense*

Table 3. Mean ± SE RBC counts (x10^6) of dogs with experimental single *T. congolense* and conjunct *T. congolense/ A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (Week)	GPI (control)	GPII (*Tc*)	GPIII (*Tc/Ac*)
0	4.28±15.30[a]	4.91±83.20[a]	5.03±70.10[a]
1 ⇧	4.89±67.90[a]	5.08±40.00[a]	4.56±37.90[a]
2	5.77±45.30[a]	4.89±45.10[a]	5.00±34.20[a]
3 ⇳	4.69±82.90[ab]	3.85±24.40[ab]	5.16±46.60[ab]
4	5.72±33.70[a]	4.47±47.40[b]	3.28±55.20[b]
5	5.90±58.90[a]	4.33±22.50[b]	3.80±33.80[b]
6 * +	8.20±35.90[a]	2.38±27.60[b]	1.87±18.60[b]
7	5.86±85.60[a]	4.26±61.80[ab]	3.90±39.50[b]
8 * +	5.87±8.40[ab]	4.68±35.30[b]	4.50±60.70[b]
9 *	6.48±25.50[a]	6.80±26.00[a]	6.34±31.40[a]
10	6.13±25.90[a]	6.58±49.50[a]	----
11	6.65±53.80[a]	6.93±68.10[a]	-----
12	6.78±58.20[a]	5.58±28.40[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; Tc Trypanosoma congolense*

The result of monocyte count is shown in Table 7. By week 4, there were significant ($p< 0.05$) decreases in monocyte count of both GPII and GPIII. The decreases continued up to week 6 in both groups. By week 10, no significant ($p<0.05$) difference was observed between GPII and GPIII compared with the control (GPI).

The result of the eosinophil count is presented in Table 8. By week 4, there were significant ($p< 0.05$) decreases in the eosinophil count in both GPII and GPIII compared to GPI. The decreases extended up to week 6 in GPIII and week 10 in GPII. By week 10 no significant ($p<0.05$) difference was observed between GPII and GPIII compared to the control (GPI).

The results of the basophil count are shown in Table 9. There was no significant ($p< 0.05$) difference in the basophil count between the infected groups (GPII and GPIII) and control (GPI) throughout the experimental period.

Table 4. Mean ± SE WBC count (x10^3) of dogs with experimental single *T. congolense* and conjunct *T. congolense/ A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (Week)	GPI (control)	GPII (*Tc*)	GPIII (*Tc/Ac*)
0	3.93±58.70^a	2.92±28.10^a	2.87±102.70^a
1 ⇧	3.01±21.10^a	3.22±46.90^a	3.00±21.00^a
2	3.89±56.80^a	3.02±32.90^a	3.14±34.00^a
3 ⇳	4.47±72.50^a	3.09±61.30^a	3.01±18.60^a
4	4.07±48.40^a	2.20±66.80^b	1.61±13.50^b
5	4.28±31.30^a	2.13±22.50^b	1.15±33.80^b
6 * +	4.97±49.80^a	1.28±10.00^b	2.05±71.20^b
7	4.03±39.70^a	3.70±54.60^a	2.03±44.70^b
8 * +	4.28±35.40^a	3.53±42.80^a	4.15±15.00^a
9 *	4.32±37.40^a	3.82±27.50^a	4.71±61.70^a
10	4.21±18.10^a	3.18±20.70^a	-----
11	4.93±2.30^a	4.92±117.90^a	-----
12	5.43±38.40^a	4.21±19.70^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; Tc Trypanosoma congolense***

Table 5. Mean ± SE Lymphocyte count (µL) of dogs with experimental single *T. congolense* and conjunct *T. congolense*/*A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (Week)	GPI (control)	GPII (*Tc*)	GPIII (*Tc/Ac*)
0	63.30±5.30^a	58.50±5.60^a	59.00±10.40^a
1 ⇧	38.30±1.90^a	40.00±2.00^a	42.40±2.00^a
2	30.10±1.00^a	30.10±1.00^a	30.10±1.00^a
3 ⇳	26.80±5.50^a	29.00±4.70^a	17.80±4.10^b
4	29.00±2.50^a	27.00±3.50^a	16.30±1.40^b
5	30.00± 3.50^a	20.00±9.00^b	15.00±5.80^b
6 * +	40.00±2.00^a	19.00±3.50^b	14.50±5.70^c
7	50.00±1.20^a	29.00±5.70^b	23.00±3.60^b
8 * +	63.00±3.60^a	37.00±3.60^b	25.00±3.30^b
9 *	62.00±3.20^a	39.00±4.60^b	25.00±0.10^c
10	60.00±5.70^a	39.00±3.70^b	----
11	63.00±2.60^a	43.50±3.30^{ab}	----
12	63.50±0.00^a	45.60±0.10^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; Tc Trypanosoma congolense*

4. DISCUSSION

Anaemia in this study was considered at PCV below 30% as recorded by [21]. Anaemia in *T. congolense* infected groups (GPII and GPIII) was due to high parasitaemia which enhanced rate of RBC destruction in the dogs. The Rbcs are rapidly destroyed through mechanical damage during high parasitaemia due to increased number and activities of trypanosomes in the blood [22]. Similar observations have been made by previous workers in trypanosomosis in animals [5,23,14]. Anaemia observed in the groups was in accordance with previous works and is described as thecardinal sign of trypanosomosis [5,24,6, 23,25].

Table 6. Mean ± SE Neutrophil count (µL) of dogs with experimental single *T. congolense* and conjunct *T. congolense A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (Week)	GPI (control)	GPII (*Tc*)	GPIII (*Tc/Ac*)
0	30.00±3.30[a]	27.30±7.20[a]	30.00±8.80[a]
1 ⇧	56.00±0.10[a]	50.00±0.20[a]	50.00±2.60[a]
2	48.20±3.90[a]	48.00±1.00[a]	47.20±2.90[a]
3 ⇳	45.00±4.30[a]	49.00 ±6.10[a]	44.80±4.40[a]
4	43.00±4.60[a]	30.80±6.30[b]	28.00±3.00[b]
5	42.00±4.60[a]	37.00±7.00[ab]	27.00±6.80[c]
6 * +	46.00±3.20[a]	49.00±6.80[a]	29.00±5.40[b]
7	33.00±3.60[a]	45.00±8.00[a]	26.00±6.90[b]
8 * +	45.00±3.30[a]	40.00±7.00[ab]	26.00±5.90[ab]
9 *	35.00±3.50[ab]	36.00±7.60[b]	25.00±5.50[b]
10	39.00±4.80[ab]	35.00±7.50[b]	-----
11	40.00±4.50[a]	47.00±7.50[a]	-----
12	41.00±5.60[a]	46.00±7.90[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; Tc Trypanosoma congolense*

Table 7. Mean ± SE Monocyte count (µL) of dogs with experimental single *T. congolense* and conjunct with *T. congolense/ A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (Week)	GPI (control)	GPII (*Tc*)	GPIII (*Tc/Ac*)
0	5.30±1.90[a]	5.20±3.80[a]	5.30±1.30[a]
1 ⇧	4.70±0.10[a]	3.60±0.20[a]	3.80±0.20[a]
2	3.00±0.20[a]	3.10±3.10[a]	3.20±0.20[a]
3 ⇳	2.80±0.60[a]	3.30±1.10[a]	2.50±1.00[a]
4	6.30±3.40[a]	2.00±3.00[b]	1.50±3.60[b]
5	5.60±1.30[a]	2.00±4.50[b]	2.00±4.50[b]
6 * +	5.90±3.50[a]	3.00±3.50[b]	2.00±3.60[b]
7	3.40±4.60[a]	4.30±5.60[a]	5.00±4.40[a]
8 * +	5.70±3.60[a]	5.00± 4.60[a]	6.40±4.40[a]
9 *	3.50±5.40[a]	6.00±4.60[a]	5.00±5.60[a]
10	4.00±5.80[a]	5.00± 3.50[a]	-----
11	4.90±4.50[a]	5.90± 4.70[a]	-----
12	5.90±3.50[a]	5.80±0.00[a]	-----

*Superscripts a b c represents the homogeneity between the experimental groups at probability P≤ 0.05. ⇧ Infection with trypanosomes; ⇳ Infection with A. caninum; + Treatment with mebendazole; * Treatment with diminazene aceturate; Tc Trypanosoma congolense*

Anaemia in trypanosomosis manifests as pallor of the mucous membrane [26,5,1,6]. The high degree of anaemia in conjunct *T. congolense/ A. caninum* group was probably due to complications of intestinal and stomach blood loss from the blood sucking activities of *A. caninum*. *Ancylostoma caninum* causes anaemia through its blood sucking activities in the infected host [27,2]. This supports the findings of [28] in interaction of *T. congolense* and *Haemonchus contortus* infection in N'Dama. It was also as observed in comparative haematological study of mixed *T. congolense* and *T. brucei* infection in dogs [29]. Continued decrease in PCV post treatment with diminazene aceturate was due to relapses and persistent parasitaemia. There had been several reports on relapses due to resistance strains of trypanosomes in treated animals [30]. Larval migration in ancylostomosis is the basis for repeat anthelminth treatment 2 to 3 weeks post-treatment to eliminate newly matured L_3 from the intestines. Repeated treatment with diminazene and mebendazole brought total clearance of

both parasitaemia and faecal egg production from the dogs and thus resulted in haematological improvement in the infected groups. It therefore supports the report of clinical improvement on use of diminazene aceturate against trypanosomosis and anthelmintic against ancylostomosis [31,12,2,23].

The decreases ($p<0.05$) in haemoglobin concentration (Hb) of the infected groups correspond to that in PCV. The decreases resulted from anaemia observed in the dogs. This was as previously observed by [9,1,13,32]. The significant ($p< 0.05$) decrease in RBC counts recorded in both GPII and GPIII correspond to the decreases recorded in both their PCV and Hb concentrations. It corroborates with [30]. In a healthy dog, the total circulating RBCs remains constant [33] and any associated decrease signifies anaemia which is a leading cause of death in animals with trypanosomosis [34-36,5,37,11].

Table 8. Mean ± SE Eosinophil count (µL) of dogs with experimental single *T. congolense* and conjunct *T. congolense/ A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (Week)	GPI (control)	GPII (*Tc*)	GPIII (*Tc/Ac*)
0	1.80±0.80^{a}	2.10±1.30^{a}	2.50±1.20^{a}
1 ⇧	3.00±0.40^{a}	4.00±3.10^{a}	3.00±0.10^{a}
2	2.90±0.20^{a}	3.50±0.10^{a}	2.60±0.80^{a}
3 ⇳	2.80±2.10^{a}	3.30±1.10^{a}	2.00±1.40^{a}
4	2.30±2.80^{a}	2.80±0.90^{ab}	0.50±0.30^{b}
5	3.00±2.00^{a}	2.00±0.40^{a}	0.50±0.70^{b}
6 * +	2.60±3.00^{a}	0.50±0.70^{b}	1.00±0.30^{ab}
7	3.60±1.00^{a}	1.00±2.00^{ab}	2.20±0.20^{a}
8 * +	2.70±0.50^{a}	0.50±1.00^{b}	2.00±0.50^{a}
9 *	2.60±0.60^{a}	1.00±0.50^{ab}	2.30±0.20^{a}
10	2.80±0.90^{a}	1.50±0.60^{ab}	-----
11	2.70±1.60^{a}	2.00±0.80^{a}	-----
12	1.60±2.60^{a}	1.50±0.70^{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; Tc Trypanosoma congolense*

Table 9. Mean ± SE Basophyl count (µL) of dogs with experimental single *T. congolense* and conjunct *T. congolense/ A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (Week)	GPI (control)	GPII (*Tc*)	GPIII (*Tc/Ac*)
0	0.00±0.00^{a}	0.80±0.50^{a}	0.30±0.30^{a}
1 ⇧	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
2	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
3 ⇳	0.00±0.00^{a}	0.30±0.00^{a}	0.00±0.00^{a}
4	0.30±0.30^{a}	0.00±0.00^{a}	0.00±0.00^{a}
5	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
6 * +	0.20±0.10^{a}	0.20±0.20^{a}	0.10±0.10^{a}
7	0.00±0.00^{a}	0.10±0.00^{a}	0.20±0.20^{a}
8 * +	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
9 *	0.00±0.00^{a}	0.00±0.00^{a}	0.30±0.30^{a}
10	0.40±0.20^{a}	0.20±0.20^{a}	-----
11	0.20±0.10^{a}	0.30±0.30^{a}	-----
12	0.00±0.00^{a}	0.10±0.10^{a}	-----

Superscripts a b c represents the homogeneity between the experimental groups at probability P≤ 0.05.
⇧ *Infection trypanosomes,* ⇳ *Infection A. caninum; + Treatment with mebendazole;* ***** *Treatment with diminazene aceturate; Tc Trypanosoma congolense*

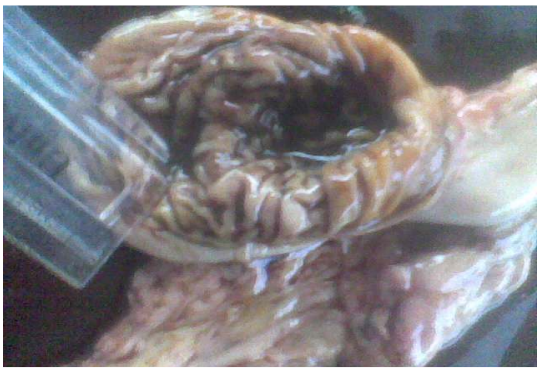

Fig. 1. Haemorrhages in the stomach of a dog with *T. congolense/A. caninum* infections

The significant decreases ($p < 0.05$) in WBC counts in (GPII and GPIII) was probably due to a shift from circulatory to marginal cells especially in neutrophils and monocytes counts. Leucopaenia in both groups was as a result of decreases in lymphocyte, neutrophils and monocyte counts which were more in conjunct *T. congolense/ A. caninum* group than in single *T. congolense* infected group probably due to the combined effect of *Ancylostoma* and trypanosomes. *Ancylostoma caninum* infection induces iron deficiency which inhibits cellular proliferation especially lymphocytes thus depreciates the count [38]. In addition they cause the release of lymphocytes-specific suppressor factors [39].

Leucopaenia have been recorded by other workers in trypanosomosis in dogs [40,13,30]. On the contrary, there was record of leucocytosis in *T. congolense* infection in sheep [41].

5. CONCLUSION

In conclusion, infections of single *T. congolense* and conjunct *T. congolense/ A. caninum* produced significant depreciations in the PCV, Hb concentrations, RBC counts, lymphocyte, neutrophil and monocyte counts of the infected dogs. The degree of depreciation was more in the conjunct *T. congolense/ A. caninum* compared to single *T. congolense* group. Treatment of the dogs with 7mg/kg of diminazene aceturate and 100 mg of mebendazole improved haematological alterations in the infected dogs.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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