British Journal of Medicine & Medical Research
14(3): 1-13, 2016, Article no.BJMMR.24062
ISSN: 2231-0614, NLM ID: 101570965

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Blood Profil of Dogs Experimentally Infected with Single *Trypanosoma brucei, Ancylostoma caninum* and Conjunct *Trypanosoma brucei* 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.

Article Information

DOI: 10.9734/BJMMR/2016/24062
Editor(s):
(1) Sinan INCE, Department of Pharmacology and Toxicology, University of Afyon Kocatepe, Turkey.
Reviewers:
(1) M. Zamri Saad, Universiti Putra Malaysia, Malaysia.
(2) Nelida Virginia Gomez, Buenos Aires University, Argentina.
Complete Peer review History: http://sciencedomain.org/review-history/13504

Original Research Article

Received 1st January 2016
Accepted 9th February 2016
Published 2nd March 2016

ABSTRACT

The present study was designed to ascertain the level of haematological alterations in single *Trypanosoma brucei (T. brucei), Ancylostoma caninum (A. caninum)* and conjunct infections of both parasites in dogs and effect of treatment with diminazene aceturate and mebendazole on haematology. Sixteen dogs grouped into 4 of 4 members each were used in the study. Group 1 (GPI) was uninfected (control), GPII was infected with *A. caninum,* GPIII was infected with *T. brucei* and GPIV was infected with conjunct infections of *T. brucei / A. caninum.* Post acclimatization, GPII and GPIV were infected with *A. caninum,* 2 weeks after GPIII and GPIV were infected with *T. brucei.* By week 6 post infection, GPII and GPIV were treated with 100 mg of

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mebendazole given twice daily for 3 days and a repeat given 2 weeks later. GPIII and GPIV were also treated with diminazene aceturate at 7 mg/kg once. Treatment was repeated at week 8 and 9 of the experiment. There was a significant ($p < 0.05$) decreases in pack cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC) in all the experimental groups (GPII, GPIII and GPIV). The decreases were more in the conjunct group (GPIV) compared to the others. A significant ($p < 0.05$) decrease in white blood cell (WBC) count was recorded in all the experimental groups (GPII, GPIII and GPIV). It was reflected in significant ($p <0.05$) decreases in lymphocytes, neutrophil, monocyte, basophil counts in *T. brucei* infected group. Conversely there were significant ($p <0.05$) increases in neutrophil, eosinophil, monocyte and basophil count but a decrease in lymphocyte count in *A. caninum* group. The haematological alterations were more in *T. brucie* group compared to the *A. caninum* group. Similarly the effect was more in the conjunct *T. brucei /A. caninum* group compared to the single *T. brucei.* Treatment with 7 mg/kg diminazene aceturate and 100 mg mebendazole given once daily for 3 days caused some improvement in haematology. These findings would enhance clinicians' knowledge of the effect of single and mixed infections of *T. brucei* and *A. caninum* in dogs.

Keywords: Blood profile; Trypanosoma brucei; Ancylostoma caninum; conjunct; treatment; diminazene aceturate; mebendazole.

1. INTRODUCTION

Trypanosomes are one of the most important haemoparasites that affects dogs and livestock in sub- Sahara Africa [1]. The reports on effect of trypanosomosis on WBC counts in infected animals were highly variable and are either leucopaenia or leucocytosis. The variability in white blood cell parameters is dependent on the stage of the disease process [2]. Some of the reports in trypanosome infection in large animals and in *T. rhodesiense* infection in vervet monkey include distinct leucopaenia typified by neutropaenia and lymphocytopaenia [3,4]. Conversely, [5] recorded leucocytosis in *T. brucei* infection in deer mice and rabbits. Their findings somewhat corroborates the findings of [6] who observed leucocytosis with lymphocytosis and relative neutrophilia in experimental trypanosome infection in horse. Also [7] recorded leucocytosis with lymphocytosis in *T. congolense* infection in sheep. In dogs, [8] recorded leucopaenia in *T. brucei* infection. This was inline with the findings of [9] who recorded leucopaenia characterized by neutropaenia, eosinopaenia and lymphopaenia in cats experimentally infected with *T. brucei*. Leucopaenia with neutropaenia was also reported in *T. brucei* infection in rabbits [10]. Similarly neutropaenia and lymphocytosis was recorded in *T. brucei* infection in rabbits [11]. So far the existing information on haematological alterations in dogs was on either single or mixed heterologous species of trypanosome with limited information on mixed infection of trypanosomes and *A. caninum* often found in the field. Based on this premise, it became a matter of necessity to examine the blood profile of dogs experimentally infected with single *T. brucei, A. caninum* and conjunct infection of both parasites and treatment with diminazene aceturate and mebendazole.

2. METHODOLOGY

2.1 Experimental Animals

Sixteen mongrel breed of dogs of both sexes weighing between 4.0 and 8.0kg were used in the 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 [12]. 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 later. The animals were kept in clean cages in a fly proof house and fed twice daily. Water was given *ad libitum*.

2.2 Parasites and Infections

2.2.1 *Trypanosoma brucei* isolate

Trypanosoma brucei 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. The quantity of parasites inoculated was estimated using the rapid matching method of [13].

2.2.2 *Ancylostoma caninum* infection

The concentration of larval suspension was estimated using an automatic pipette (Biotht Peoline®), according to the method of [14]. 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 1mL of distilled water were delivered *per os* to each of the experimental dogs, using a 2 mL syringe without needle.

2.3 Reconstitution of Diminazene Aceturate

A 2.36 g Veribin®, CEVA Sante Animale- La Ballasteiére 33501 Libourne Cedex, France) a 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, was calculated from their weight at the dose of 7 mg/kg via the intramuscular route.

2.4 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 100mg twice daily for 3 days. Treatment was repeated 2 weeks later.

2.5 Experimental Design

Dogs were randomly divided into 4 groups of 4 members in each group. GP I was uninfected dogs (control), GP II was infected with *A. caninum,* GP III was infected with *T. brucei* and GP IV was conjunct infections of *T. brucei and A. caninum.* Post acclimatization, *A. caninum* infection was done on GPII and GPIV alone. Two weeks later *T. brucei* infections was done on GPIII and superimposed on GPIV. Three weeks post trypanosome infection; GPIII and GPIV were treated with diminazene aceturate on weeks 6, 8 and 9. Mebendazole was used only on GPII and GPIV and a repeat treatment given 2 weeks later.

2.6 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.

2.7 Blood Collection

Three milliliter of blood was collected through the cephalic vein of each of the experimental dogs and dispensed into an ethylenediamine tetra-acetic acid (EDTA) containing bottle for haematology. Blood sample for haematology was collected at weekly interval and analyzed.

2.8 Haematology

2.8.1 Packed cell volume

Blood filled capillary tubes were centrifuged in a microhaematocrit centrifuge at 11000 revolutions per minute for 5 minutes, the PCV values were read using the PCV reader [15].

2.8.2 Haemoglobin concentration

Haemoglobin concentration was determined using the cyanmethaemoglobin method. Sixteen test tube bottles containing 5 mL of cyanide including the blank were assembled on the rack. Approximately 0.02 mL of individual blood samples were added in each of the test tubes except in the blank. The tubes were thoroughly mixed and allowed to stand for not less than 3 minutes after which the results were read using the electronic colorimeter. The colorimeter was

first zeroed using the blank by adjusting both the fine and coarse adjusters. Subsequently the absorbances of the samples were recorded. The final results were obtained using the Hb reference table [16].

2.8.3 Red blood cell count

Automatic pipette was used to dispense 4 mL of RBCs diluting fluid into sixteen clean and dry tubes. Approximately 0.2 µL of individual blood samples was added in each of the test tubes and 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 artifacts 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 was gently dispensed "charging" from one edge of 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 center of the Nauber chamber were counted and result obtained was recorded.

2.8.4 White blood cell count

Using a WBC pipette of a haemocytometer, blood was drawn up to the 0.5 mark into a clean and dry container. Thereafter the WBC diluting fluid was drawn up to the 1.1 mark of the WBC pipette into the same container. The mixture was thoroughly mixed together and allowed to stand. A cover slip was placed appropriately on the counting chamber and then charged with an aliquot of the mixture with a fine bore Pasteur pipette. The procedure was carefully done to prevent overflow and bubbles. 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 found within the lines are counted only on one side of the square.

2.8.5 Thin blood smears techniques for differential count

Thin blood smear was made by placing a drop of blood at one end of a clean slide and with a spreader the blood was swiftly 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 after which it was washed out with jets of water. The stained slides were left to dry and safely packed in a slide pack. Using oil immersion, at least 100 cells were counted by viewing the slide in a systematic fashion as to include the central and peripheral areas of the smear. The cell counter was used to count the differential counts: neutrophil, lymphocyte, monocyte, eosinophil and basophil [17].

3. RESULTS

3.1 Pack Cell Volume (PCV)

The results of the PCV (%) are shown in Table 1. There was a significant ($p< 0.05$) decrease in PCV of GPII and GPIV by week 4 of the experiment (Table 1). By week 5, there was a significant ($p< 0.05$) decreases in the PCV of all the experimental groups (GPII,GPIII and GPIV) which progressed up to week 8 of the experiment. The decreases recorded in GPIII and GPIV were more compared to that in GPII. By week 9, there was no significant ($p< 0.05$) difference between GPII, GPIII and GPIV when compared to GPI. It remains so up to week 12 of the experiment.

3.2 Haemoglobin Concentration

The results of the haemoglobin concentration are shown in Table 2. Similarly there were significant ($p< 0.05$) decreases in Hb concentrations in GPII and GPIV observed at week 4 of the experiment. The decreases progressed up to week 7 in GPII and up to week 8 in GPIII and GPIV. Subsequently from week 9 to 12, there was no significant ($p< 0.05$) difference between GPII, GPIII and GPIV compared to GPI.

3.3 Red Blood Cells Count

The results of the Red Blood Cells count are shown in Table 3. By week 3, there was a significant ($p< 0.05$) increase in Rbc of GPIII compared to GPI. By week 4, there was a significant ($p< 0.05$) decrease in all the experimental groups (GPII, GPIII and GPIV) compared to GPI. The decreases progressed up

to week 7 in GPII and week 8 in GPIII and GPIV. The decreases were more in GPIII and GPIV compared to GPII. By week 9, there was no significant difference between all the groups (GPII, GPIII and GPIV) compared to GPI.

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

Experimental period (week)	GPI (control)	GPII (*Ac*)	GPIII (*Tb*)	GPIV (*Tb*/ *Ac*)
0	39.8±2.10[a]	39.5±2.40[a]	27.5±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]	34.5±2.30[a]	30.3±2.30[a]	33.0±0.90[a]
4	35.5±2.00[a]	27.0±1.10[b]	37.0±0.30[a]	23.8±0.60[b]
5	34.7±2.20[a]	26.3±0.10[b]	23.7±1.90[b]	22.8±0.80[b]
6 * +	36.7±0.90[a]	24.5±1.30[b]	16.0±1.50[c]	15.8±2.00[c]
7	35.3±2.00[a]	25.8±1.50[b]	22.0±1.00[bc]	17.8±2.00[c]
8 * +	36.0±1.50[a]	27.3±1.40[b]	26.7±2.00[b]	22.5±1.90[b]
9 *	37.4±1.20[a]	35.8±1.30[a]	35.3±0.90[a]	35.3±4.80[a]
10	34.3±0.30[a]	36.8±1.60[a]	36.3±2.80[a]	33.3±0.90[a]
11	36.0±1.50[a]	36.0±1.50[a]	37.0±2.80[a]	32.0±2.00[a]
12	39.0±1.50[a]	38.7±0.80[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 2. Mean ± SE haemoglobin concentration (mg/dl) of dogs with experimental single *T. brucei*, *A. caninum* and conjunct *T. brucei*/ *A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (weeks)	GPI (control)	GPII (*Ac*)	GPIII (*Tb*)	GPIV(*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[cd]	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 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*

3.4 White Blood Cell Count

The results of the White Blood Cell count are shown in Table 4. By week 4, there were significant ($p< 0.05$) decreases in all the experimental groups (GPII, GPIII and GPIV) compared to the control (GPI). The decreases progressed up to week 6 in GPII and to week 8 in GPIII and GPIV. The decrease observed in GPIV was more compare to that in GPII and GPIII. By week 9 to 12, there was no significant difference observed in all the groups (GPII, GPIII and GPIV) compared to control (GPI).

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

Experimental period (weeks)	GPI (control)	GPII (*Ac*)	GPIII (*Tb*)	GPIV *(Tb/Ac)*
0	4.28±15.30^a	5.03±60.40^a	4.74±59.10^a	4.88±46.40^a
1 ⇑	4.89±67.90^a	4.58±34.60^a	4.78±25.80^a	4.85±67.00^a
2	5.77±45.30^a	5.45± 34.30^a	5.56± 21.00^a	5.23±12.10^a
3 ⇕	4.69±82.90^{ab}	4.09±33.90^{ab}	6.47±31.90^b	4.40±99.70^a
4	5.72±33.70^a	4.11±32.50^b	3.50±32.80^b	3.20±34.40^b
5	5.90±58.90^a	4.69±25.10^b	3.20±13.50^c	3.34±23.60^c
6 * +	8.20±35.90^a	3.56±28.60^b	2.00±8.90^c	2.08±14.90^c
7	5.86±85.60^a	4.53±12.70^{ab}	4.38±33.90^{ab}	3.38±45.00^b
8 * +	5.87±8.40^{ab}	6.50±58.90^a	4.34±25.40^c	4.13±36.50^c
9 *	6.48±25.50^a	6.59±60.60^a	6.27±17.60^a	6.00±56.80^a
10	6.13±25.90^a	6.02±52.80^a	6.33±114.50^a	6.38±75.40^a
11	6.65±53.80^a	6.46±52.30^a	6.82±122.90^a	6.76±67.60^a
12	6.78±58.20^a	6.48±50.90^a	6.50±76.00^a	6.17±60.10^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; Ac- Ancylostoma caninum; Tb- Trypanosoma brucei*

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

Experimental period (weeks)	GPI (control)	GPII (*Ac*)	GPIII (*Tb*)	GPIV (*Tb/Ac*)
0	3.93±58.70^a	4.37±84.00^a	3.18±55.70^a	3.57±27.90^a
1 ⇑	3.01±21.10^a	3.23±35.00^a	2.88±38.90^a	3.01±21.00^a
2	3.89±56.80^a	3.21±43.00^a	2.90±34.90^a	3.21±45.00^a
3 ⇕	4.47±72.50^a	3.62±46.20^a	2.89±39.70^a	3.65±30.50^a
4	4.07±48.40^a	2.96±36.10^b	1.23±62.70^c	1.67±25.10^{bc}
5	4.28±31.30^a	2.69±25.10^b	1.20±73.50^b	1.34±23.60^b
6 * +	4.97±49.80^a	3.40±19.50^b	1.72±11.10^c	1.83±50.50^c
7	4.03±39.70^a	3.67±40.30^a	3.14±43.70^a	2.30±79.20^b
8 * +	4.28±35.40^a	3.47±23.20^a	3.30±57.90^{ab}	2.27±18.90^b
9 *	4.32±37.40^a	4.08±54.70^a	3.82±66.90^a	3.79±12.50^a
10	4.21±18.10^a	3.97±27.80^a	4.28±33.50^a	3.17±64.90^a
11	4.93±2.30^a	4.10±41.80^a	4.42±103.50^a	3.90±107.10^a
12	5.43±38.40^a	4.96±47.40^a	5.14±29.10^a	4.47±24.00^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*

3.5 Lymphocyte Count

The results of the lymphocyte count are shown in Table 5. By week 3, there were significant ($p< 0.05$) decreases in all the experimental groups (GPII, GPIII and GPIV) compared to control (GPI). The decreases in the groups progressed up to week 11 in all the groups except on week 12 of the experiment.

3.6 Neutrophil Count

The results of the neutrophil count are shown in Table 6. There was a significant ($p< 0.05$) increase in the neutrophil count in GPII by week 2 of the experiment. This progressed up to week 5. Conversely by week 4, there was a significant ($p< 0.05$) decrease in both GPIII and GPIV which progressed up to week 10. The decrease was

Table 5. Mean ± SE Lymphocytes count (µl) of dogs with experimental single *T. brucei*, *A. caninum* and conjunct *T. brucei* / *A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (weeks)	GPI (control)	GPII (*Ac*)	GPIII (*Tb*)	GPIV (*Tb/Ac*)
0	63.3±5.30^{a}	60.8±2.60^{a}	58.5±4.10^{a}	63.8±10.70^{a}
1 ⇧	38.3±1.90^{a}	36.2±1.00^{a}	38.3±3.90^{a}	36.0±1.50^{a}
2	30.1±1.00^{a}	29.7±0.10^{a}	30.1±2.00^{a}	27.2±2.30^{a}
3 ⇳	26.8±5.50^{a}	14.0±1.50^{b}	29.7±2.30^{b}	19.3±3.30^{b}
4	29.0±2.50^{a}	13.0±4.40^{b}	26.7±6.70^{a}	18.5±4.30^{b}
5	30.0± 3.50^{a}	12.0±4.30^{b}	20.0± 4.80^{bc}	17.0±5.70^{bc}
6 * +	40.0±2.00^{a}	10.0±3.60^{c}	19.5± 6.80^{b}	16.0±3.70^{b}
7	50.0±1.20^{a}	25.0±2.80^{b}	29.0±7.00^{b}	26.0±5.80^{b}
8 * +	63.0±3.60^{a}	25.5±4.60^{b}	30.0±5.80^{b}	33.0±4.80^{b}
9 *	62.0±3.20^{a}	30.0±2.70^{b}	35.0± 4.70^{b}	39.0±3.80^{b}
10	60.0±5.70^{a}	40.0±4.70^{ab}	40.0± 8.00^{ab}	40.0±5.80^{ab}
11	63.0±2.60^{a}	44.0±4.60^{ab}	45.0±3.60^{ab}	43.0±0.00^{b}
12	63.5±0.00^{a}	50.0±0.00^{a}	59.5±0.10^{a}	45.0±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*

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

Experimental period (weeks)	GPI (control)	GPII (*Ac*)	GPIII (*Tb*)	GPIV (*Tb/Ac*)
0	30±3.30^{a}	32±2.70^{a}	28.8±3.40^{a}	38.5±8.80^{a}
1 ⇧	56.0±0.10^{a}	59.4±0.20^{a}	54.1±0.10^{a}	49.2±2.50^{a}
2	48.2±3.90^{a}	60.1±9.00^{b}	50.1±2.10^{a}	47.1±1.00^{a}
3	45.0±4.30^{a}	62.8±1.50^{b}	46.0±2.00^{a}	46.0±3.10^{a}
4 ⇳	43.0±4.60^{a}	63.3±6.40^{b}	38.0±5.00^{cb}	30.0±10.00^{c}
5	42.0±4.60^{a}	67.0±6.60^{b}	39.0±7.00^{ac}	26.0±8.00^{c}
6 * +	46.0±3.20^{a}	41.0±7.60^{a}	40.0±6.00^{a}	30.0±8.00^{c}
7	33.0±3.60^{a}	43.0±5.60^{a}	45.0±8.00^{a}	20.0±9.80^{c}
8 * +	45.0±3.30^{a}	42.0±5.70^{a}	65.0±5.50^{bc}	29.0±6.90^{c}
9 *	35.0±3.50^{ab}	45.0±5.50^{a}	34.0±6.90^{ab}	20.0±7.90^{b}
10	39.0±4.80^{ab}	38.0±6.70^{a}	33.0±5.40^{b}	35.0±7.80^{b}
11	40.0±4.50^{a}	49.0±6.70^{a}	46.0±4.50^{a}	43.0±6.90^{a}
12	41.0±5.60^{a}	49.0±7.00^{a}	47.0±5.70^{a}	45.0±7.70^{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; Ac- Ancylostoma caninum; Tb- Trypanosoma brucei*

more in GPIV compared to GPIII. Subsequently there was no significant change between GPII, GPIII and GPIV compared to GPI.

3.7 Monocyte Count

The results of the monocyte count are shown in Table 7. By week 5 and 6 significant decreases in monocyte count were recorded in GPII. Similarly by week 4, significant decreases were recorded both in GPIII and GPIV. This progressed up to week 6. Subsequently there were no changes observed between GPII, GPIII and GPIV compared to GPI.

3.8 Eosinophil Count

The results of the eosinophil count are shown in Table 8. By week 2, there was a significant

Table 7. Mean ± SE Monocytes count (µl) of dogs with experimental single *T. brucei*, *A. caninum* and conjunct *T. brucei* / *A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental period (weeks)	GPI (control)	GPII (*Ac*)	GPIII(*Tb*)	GPIV (*Tb/Ac*)
0	5.3±1.90^{a}	6.2±3.80^{a}	5.8±1.70^{a}	5.3±5.60^{a}
1 ⇧	4.7±0.10^{a}	3.8±3.80^{a}	3.9±2.00^{a}	3.2±3.10^{a}
2	3.0±0.20^{a}	3.3±2.00^{a}	3.8±1.10^{a}	3.9±0.20^{a}
3 ⇳	2.8±0.60^{a}	2.8±0.30^{a}	3.7±0.90^{a}	3.8±1.30^{a}
4	6.3±3.40^{a}	4.8±2.00^{a}	1.3±2.00^{b}	2.0±5.20^{b}
5	5.6±1.30^{a}	1.0±3.80^{b}	1.0±5.40^{b}	1.9±3.60^{b}
6 * +	5.9±3.50^{a}	2.0±4.50^{b}	1.5±4.60^{b}	2.0±4.60^{b}
7	3.4±4.60^{a}	4.5±5.40^{a}	5.0±4.40^{a}	5.4±4.40^{a}
8 * +	5.7±3.60^{a}	5.0±3.60^{a}	6.0±3.60^{a}	5.9±3.50^{a}
9 *	3.5±5.40^{a}	4.5±4.50^{a}	5.5±4.40^{a}	6.0±5.60^{a}
10	4.0±5.80^{a}	5.4±3.50^{a}	4.5 ±3.30^{a}	3.5±2.50^{a}
11	4.9±4.50^{a}	6.7±3.60^{a}	5.6.3±4.60^{a}	4.8±4.50^{a}
12	5.9±3.50^{a}	5.5±0.00^{a}	5.4±5.30^{a}	6.5±5.60^{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; Ac- Ancylostoma caninu; Tb- Trypanosoma brucei*

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

Experimental period (weeks)	GPI (control)	GPII (*Ac)*	GPIII (*Tb)*	GPIV (*Tb/Ac)*
0	1.8±0.80^{a}	2.3±1.60^{a}	2.0±1.80^{a}	2.5±0.60^{a}
1 ⇧	3.0±0.40^{a}	4.8±1.00^{a}	3.2±0.20^{a}	3.5±0.30^{a}
2	2.9±0.20^{a}	5.6±2.00^{b}	3.0±0.60^{a}	3.3±0.40^{a}
3 ⇳	2.8±2.10^{a}	5.7±0.60^{b}	2.7±0.70^{a}	3.2±0.50^{a}
4	2.3±2.80^{a}	5.8±1.10^{b}	1.3±0.30^{ac}	2.0±0.90^{a}
5	3.0±2.00^{a}	5.2±1.80^{b}	0.5±0.80^{c}	0.5±0.50^{c}
6 * +	2.6±3.00^{a}	5.71±2.00^{b}	1.0±0.50^{ac}	1.0±0.30^{ac}
7	3.6±1.00^{a}	2.0±0.50^{a}	2.0±0.40^{a}	0.2±0.20^{b}
8 * +	2.7±0.50^{a}	2.5±0.80^{a}	0.1±0.30^{b}	0.1±0.10^{b}
9 *	2.6±0.60^{a}	2.0±0.80^{a}	1.0±0.70^{ab}	0.5±0.20^{b}
10	2.8±0.90^{a}	2.5±3.00^{a}	1.2±0.80^{ab}	1.0±0.20^{b}
11	2.7±1.60^{a}	2.4±1.60^{a}	1.9±0.90^{a}	2.0±0.10^{a}
12	2.6±2.60^{a}	2.3±0.20^{a}	1.9±0.10^{a}	2.0±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 9. Mean ± SE Basophyl count (µl) of dogs with experimental single *T. brucei*, *A. caninum* and conjunct *T. brucei* / *A. caninum* infections and treatment with diminazene aceturate and mebendazole

Experimental Period (Weeks)	GPI (control)	GPII *(Ac)*	GPIII *(Tb)*	GPIV *(Tb/Ac)*
0	0.4±0.00[a]	0.5±0.50[a]	0.3±0.50[a]	0.4±0.30[a]
1 ⇧	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]
2	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]
3 ⇳	0.0±0.00[a]	1.3±0.50[b]	0.0±0.00[a]	0.0±0.00[a]
4	0.3±0.30[a]	0.2±0.20[a]	0.0±1.00[a]	2.0±0.00[a]
5	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]	0.0±0.00[a]
6 * +	0.2±0.10[a]	0.0±0.00[a]	0.3±0.30[a]	0.0±0.00[a]
7	0.0±0.00[a]	0.2±0.10[a]	0.2±0.20[a]	0.0±0.00[a]
8 * +	0.0±0.00[a]	0.0±0.00[a]	0.1±0.10[a]	0.0±0.00[a]
9 *	0.0±0.00[a]	0.0±0.00[a]	0.3±0.30[a]	0.3±0.30[a]
10	0.4±0.20[a]	0.2±0.20[a]	0.2±0.20[a]	0.2±0.20[a]
11	0.2±0.10[a]	0.0±0.00[a]	0.1±0.10[a]	0.1±0.10[a]
12	0.0±0.00[a]	0.1±0.10[a]	0.3±0.30[a]	0.3±0.30[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; Ac- Ancylostoma caninum; Tb- Trypanosoma brucei*

(p< 0.05) increase in eosinophil count in GPII. This progressed up to week 6. Subsequently no significant difference was observed up to week 12. Conversely significant (p< 0.05) decrease was observed in GPIII and GPIV by week 5 up to week 10. By week 11 and 12, there was no significant change observed in GPII and GPIV compared to GPI.

3.9 Basophil Count

The results of the basophil count are shown in Table 9 above. By week 3 a significant (p< 0.05) increase in basophil count was recorded only in GPII compared to all the groups (GPI, GPIII and GPIV). Subsequently no significant difference was recorded up to week 12 of the experiment.

4. DISCUSSION

Haemoconcentration is one of the causes of abnormal increase in the RBC count of animals and individuals [18] and could account for the abnormal increase in RBCs of GPIII by week 3 of the experiment. This could have resulted from assertion of territorialism common in canine population resulting to water deprivation. Prompt intervention by ensuring adequate watering of individual dogs in the group normalized the RBC count. In a healthy dog the total circulating RBCs remains constant [19], and significant decreases as observed in the study would signify anaemia which is the leading cause of death in trypanosomosis [20,21,22] and in ancylostomosis in humans and animals [23,24] respectively. Anaemia in this study was upheld at PCV below 30% as recorded by [25]. Anaemia manifested by significant (p <0.05) decrease in the PCV and haemoglobin concentration observed in the *A. caninum* infected dogs may have resulted from intestinal blood loss from blood sucking activities of the parasite [23,24]. A matured female *Ancylostoma* parasite takes up about 0.01 mL of blood meal [26]. The amount of blood meal consumed increases during oviposition and in case of heavy infestation adversely depreciates the PCV and haemogloblin concentration. Anaemia in the trypanosome infected groups (GPIII, and GPIV) arises from high parasitaemia recorded in the dogs. The red blood cells are rapidly destroyed during high parasitaemia occasioned by mechanical damage from the increased activities of trypanosomes [27]. Similar observations have been made by previous workers in trypanosomosis in animals [20,28,29]. Anaemia in trypanosomosis is described as the cardinal sign of the disease [20,9,30,28,11]. The continued decreases in PCV post treatment with trypanocides in (GPIII, and GPIV) was due to relapses and persistent parasitaemia in the trypanosome groups. There had been several reports on relapses due to resistant strains of trypanosomes in treated animals [31]. The continued decrease in *Ancylostoma* infected dogs (GPII) post treatment may be due to resumption of the blood sucking

activity of newly matured migratory juvenile L_3 larvae (Urquhart et al. 1998). Repeat anthelminthic treatment often given within 2 to 3 weeks post initial dose was essentially to mop up newly matured migratory L_3 from the intestines. Repeated treatment with diminazene and mebendazole resulted in total clearance of both parasitaemia and faecal *Ancylostoma* eggs from the dogs. Subsequent improvement in both the PCV and Hb could be attributed to efficacy of the drugs in eliminating both parasites. It therefore supports the report that treatment of dogs with diminazene aceturate against trypanosomosis revert pathological conditions [32,33]. The decreases in RBC counts, PCV and Hb in the conjunct *T. brucei* / *A. caninum* group seems more compared to that observed in the single *T. brucei* and *A. caninum* groups. This could be related to the combined effects of blood sucking activities of hookworm and the rate of RBC destruction by trypanosome in the dogs [34]. This supports the findings of [35] in interaction of *T. congolense* and *Haemonchus contortus* infection in N'Dama.

The significant decreases ($p< 0.05$) in WBC count in all the groups (GPII, GPIII, and GPIV) were probably due to a shift from circulatory to marginal cells especially the neutrophils and monocytes. Leucopaenia recorded in the study also arise from decreases in lymphocyte, neutrophils and monocyte counts which were more in the conjunct than in the single infected groups as a result of the combined effect of both parasites. Decrease in lymphocyte count in the *A. caninum* group was induced by iron deficiency anaemia [24]. Iron is an essential mineral for differentiation and proliferation of cells especially lymphocytes. Its deficiency in ancylostomosis inhibits proliferation and depreciates lymphocytes count [36]. In addition *A. caninum* causes the release of lymphocytes-specific suppressor factors which affects the lymphocyte count [37]. Lymphocytopaenia in trypanosomosis was induced by increased level of circulating glucocorticoids released in response to severe stress associated with the disease [9,30]. Leucopaenia have previously been recorded by other workers in trypanosomosis in dogs [8,38,39]. Leucopaenia has also been reported by [3,9] in *T. brcuei* infection in cats. On the contrary, [38] observed lymphocytosis in rabbits infected with *T. brucei* and in *T. congolense* infection in sheep [7].

The decrease in the relative number of neutrophil and monocyte counts was due to increased demand for phagocytosis of dead cells in the tissues. In *T. congolense* infections, neutrophils predominated in tissue where they are later destroyed [40]. Neutropaenia recorded in trypanosomes infected groups agrees with the findings of [9]. The neutrophilia recorded in ancylostomosis may have resulted from severe blood loss in the intestine and stomach manifested as melena from the activities of adult hookworms in the infected dogs [19]. In acute haemorrhage, neutrophilia initially occur from a shift from marginal to circulatory pool and later from massive marrow response [41]. Neutrophilia recorded in ancylostomosis contradicts earlier report of [42] who observed neutropeania and attributed it to secretion of neutrophils inhibitory factors by *Ancylostoma*.

Eosinophilia and basophilia recorded in GPII (*A. caninum*) may be associated with the presence of hookworm parasite in the dogs. Eosinophilia and basophilia often appear simultaneously in most allergic conditions, hypersensitivity and parasitism such as gastrointestinal parasitism [43]. Elevated blood eosinophils are clear marker of hookworm infection in humans [44,45]. On the contrary record of eosinopaenia and basopaenia in trypanosomosis could be related to elevated levels of glucocorticoids in the blood which suppress their number [46]. This agrees with the findings of [5,11] in cases of trypanosomosis in animals.

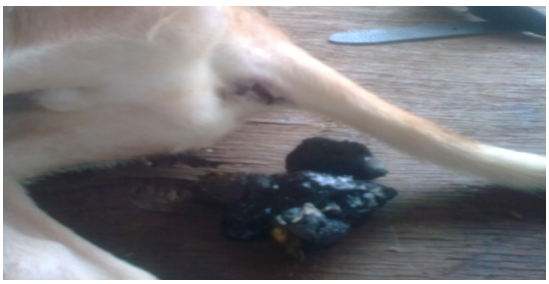

Fig. 1. Bloody faeces (melena) from a dog with conjunct *T. brucei* / *A. caninum* infection post infection

5. CONCLUSION

Significant haematological alterations were observed in dogs with single *T. brucei, A. caninum* and conjunct infection of both parasites. The changes were more severe in the conjunct infected group compared to the single infections. Treatment with diminazene aceturate and mebendazole gave some degree of

improvement in haematology. These findings though may not be pathogmonic to the conditions however would enhance the knowledge of effect of both parasites in dogs.

CONSENT

It is not applicable.

ACKNOWLEDGEMENTS

We appreciate the effort of the Tertiary Education Trust Fund for sponsorship of this research through the Directorate of University Research Administration, Michael Okpara University of Agriculture, Umudike, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ukpai OM, Nwabuko OP. Effects on haematological parameters and pathology of internal organs of *Trypanosoma brucei* brucei infected albino rats. Nig J. Biotech. 2014;27:8-13.
2. Marques LL, Machado RZ, Alessi AC, Aquino LPCT, Pereira GT. Experimental infection with trypanosome evansi in horses: Clinical and haematological observations. Revise Brazilian Veterinary Parasitiology. 2000;9(1):11-15.
3. Valli VE, Forsberg CM, Lumsden JH. The pathogenesis of *Trypanosoma congolense* infection in calves. lII. Neutropenia and mycloid response. Veterinary Pathology. 1979;16:96-107.
4. Kagira JM, Thuita JK, Ngotho M, Madachi R, Mwangangi DM, Ndung'u JM. Haematology of experimental *Trypanosoma brucei rhodesiense* infection in vervet monkeys. African Journal of Health sciences. 2006;13(3-4):59-65.
5. Emeribe AO, Anosa VO. Haematology of experimental *Trypanosoma brucei gambiense* infection. II. Erythrocyte and leucocyte changes. Rev Elev Med Vet Pays Tropica. 1991;44(1):53-7.
6. Monzon CM, Manceso OA, Roux JP. Comparison Between six parasitological methods for diagnosis of trypanosomosis due to trypanosome evansi in the subtropical area of Argentina. Veterinary Parasitiology. 1991;36:141-146.
7. Katunguka-Rwakishaya E, Murray M, Holmes PH. The pathophysiology of ovine trypanosomosis: Haematological and blood biochemical changes. Veterinary Parasitology. 1992;45:17-32.
8. Egbe-Nwiyi TN, Anita RE. The effect of trypanocidal drug treatment on the haematological changes in trypanosome brucei brucei infected splenectomised dogs. Veterinary Parasitology. 1993; 50(1-2):23-33.
9. Nfon CK, Oyewunmi OB, Nottidge HO, Taiwo VO. Experimental *T. brucei* and *T. congolense* infection in cats: Clinicopathological study. Tropical Veterinarian. 2000;18:220-227.
10. Adenike SF, Adeyemi OL. Changes in Haematological indices and protein concentrations in *Trypanosome brucei* infected rats treated with homidium chloride and diminazene aceturate; 2009.
11. Takeet MI, Fagbemi BO. Haematological, pathological and plasma biochemical changes in rabbits experimentally infected with *Trypanosoma congolense.* Science World Journal. 2009;4:2.
12. Woo PTK. The Haematocrit centrifugation technique for the diagnosis of African trypanosomosis. Acta Tropica. 1970;27: 384-386.
13. Herbert WJ, Lumsden WHR. *Trypanosoma brucei,* a rapid matching method for estimating the hosts parasitaemia. Experimental Parasitology. 1976;40:427-428.
14. MAFF. Report on Straw Utilization Conference held at Oxford 24 and 25 February, London H.M.S.O; 1977.
15. Coles EH. Veterinary clinical pathology. Saunders W. B. Co. Philadelphia 4th Edition. 1986;145-151.
16. Van Kampen EJ, Zijlstra WG. Test-combination haemoglobin. Clinical Chemistry Acta. 1961;6:538.
17. Sood N, Coleman CI, Klucer J, White M, Padala A, Baker N. The association among blood transfusion, white blood cell count and the frequency of post-cardiothoracic surgery Atrial fibrillation. A vnested cohort study from the Atrial fibrillation suppression trials I, II and III. Journal of Cardio-Thoracic and Vascular Anesthesia. 2009;23(1):22-27.
18. Rasouli M, Kiasari AM, Arab S. Indicators of dehydration and haemoconcentration

are associated with the prevalence and severity of coronary artery disease. Clinical Experimental Pharmacology and Physiology. 2008;35(8):889-94.

19. Rebar AH, MacWilliams PS, Feldman BF, Metzger FL, Pollock RVH, Roche J. Basophils: Overview, Quantity, Morphology. In: A Guide to Hematology in Dogs and Cats, Teton NewMedia, Jackson, WY, USA; 2005.
20. Silva RAMS, Herrera HMH, Domingos LB, Ximenes FA, Dávila AMR. Pathogenesis of *Trypanosoma evansi* infection in dogs and horses: hematological and clinical aspects; 1995. http://dx.doi.org/10.1590/S0103-84781995000200009
21. Roberto AMSS, Heitor MH, Luzimar, Borges da Silveira D, Flora AX, Alberto MRD. Pathogenesis of *Trypanosoma evansi* infection in dogs and horses hematological and clinical aspects. Cienc. Rural. 1995;25(2):5.
22. Kjos SA, Snowden KF, Craig TW, Lewis B, Ronald N, Olson JK. Distribution and characterization of canine chagas disease in Texas. Veterinary Parasitology. 2008; 152:249-256.
23. Simon B, Jeffrey B, Peter JH. Human hookworm infection in the 21st century. Advanced Parasitology. 2004;58:197-288.
24. Okewole EA, Omobowale OT, Ayoola OM. Iron supplementation therapy in the reversion of the haematological and immunological defects accompanying canine ancylostomosis. Veterinarski Arhiv. 2008;73(5):247-260.
25. Ward E. Anaemia in dogs. VCA Animal Hospital; 2009.
26. Urquhart GM, Armour J, Dunn JL, Jennings FW. Veterinary parasitology. Blackwell Science Limited. 1998;53-55.
27. Holmes PH, Katunguka-Rwakishaya E, Bennison JJ, Wassink GJ, Parkins JJ. Impact of nutrition on the pathophysiology of bovine trypanosomosis. Parasitology. 2002;120:73-85.
28. Noyes HA, Alimohammadian MH, Agaba M, Brass A, Fuchs H, Durner VG, Hulme H, Iraqi F, Kemp S, Rathkolb B, Wolf E, Angelis MH, Roshandel D, Naessens J. Mechanisms controlling anaemia in *Trypanosoma congolense* infected mice. PLoS ONE. 2009;4(4):e5170. DOI: 10.1371
29. Nwoha RIO, Anene BM. Clinical signs and pathological changes in dogs with single and conjunct experimental infections of *Trypanosoma brucei brucei* and *Ancylostoma caninum.* Journal of Veterinary Parasitology. 2011a;25(2):97-102.
30. Biryomumaisho S, Katunguka-Rwakishaya E. The pathogenesis of anaemia in goats experimentally infected with *Trypanosoma congolense* or *Trypanosoma brucei*: Use of myeloid: erythroid ratio. Veterinary Parasitology. 2007;143:354-357. Coles EH. Veterinary clinical pathology. Saunders W. B. Co. Philadelphia 4th Edition. 1986;145-151.
31. Samdi SM, Abenga JN, Attahir A, Wayo BM, Sumayin HM, Haruna MK, Jijitar AM, Ogunwale RT, Ramatu RA, Bizi RL. Constraints in the control of African trypanosomiasis the prevailing factors in Kurmin Kaduna, Northern, Nigeria (Review article). International Journal of Animal and Veterinary Advance. 2010;2(1):31-36.
32. Dempsay WL, Mansfield JM. Lymphocyte function in experimental African Trypanosomosis. Vi. Parasite-specific immunosuppression. The Journal of Immunology. 1983;130(6):2896-2898.
33. Rashid A, Rasheed K, Hussain A. Trypanosomiasis in Dog: A case report. Journal of Arthropod-Borne Diseases. 2008;2(2):48-51.
34. Nwoha RIO, Anene BM. Changes in packed cell volume and hemoglobin concentration in dogs with single and conjunct experimental infections of *Trypanosoma brucei* and *Ancylostoma caninum.* Philippine Journal of Veterinary Animal Science. 2011b;37(2):151-158.
35. Kaufmann J, Dwinger RH, Hallebeek A, Van Dijk B, Pfister K. The interaction of *Trypanosoma congolense* and *Haemonchus contortus* infections in trypanotolerant N'Dama cattle. Veterinary Parasitology. 1992;43(3-4):157-70.
36. Özcan A, Cakmak M, Toraman AR, Yazgan H, Demirdoven M, Yokus O, Gurel A. Evaluation of leucocyte and its subgroups in iron deficiency anaemia. International Journal of Medicine and Medical Sciences. 2011;3(5):135-138.
37. Cross J. Enteric Nematodes of Humans. Medical microbiology. 4th edition. University of Texas Medical branch at Galveston, Galveston, Texas. 1996;90: 701.
38. Gunaseelan L, Senthil Kumar P, Selvaraj K, Kathiresan D. Haemato biochemical

changes in a case of canine trypanosomiasis. Tamilnadu J. Veterinary & Animal Sciences. 2009;5(3):122-123.

39. 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.
40. Mwangi DM, Hopkins J, Luckins AG. Cellular phenotypes in *Trypanosoma congolense* infected sleep: The local skin reaction. Parasite Immunology. 1990;12: 647-658. Behnke JM. Immunology. In Gilles HM, Ball PAJ, (ed.), Human parasitic diseases: Hookworm infections. Elsevier, Amsterdam, the Netherlands. 1991;4: 93-155.
41. Nader DN. Neutrophilia. Drugs and Diseases. Medscape webMD LLC; 2013.
42. Moyle M, Foster DL, McGrath DE, Brown SM, Laroche Y, De Meutter J, Stanssens P, Bogowitz CA, Fried VA, Ely JA. A hookworm glycoprotein that inhibits neutrophil function is a ligand of the integrin CD11b/CD18. Journal Biology Chemistry. 1994;269:10008-10015.
43. Rebar HA, Macwilliams SP, Feldman FB, Metzger LF, Pollock HVR, Roche JA. Guide to hematology in dogs and cats. 1st edition. Teton NewMedia. 2002;39.
44. Pritchard DI, Quinnell RJ, Slater AF, McKean PG, Dale DD, Raiko A, Keymer AE. Epidemiology and immunology of *Necator americanus* infection in a community in Papua New Guinea: Humoral responses to excretory-secretory and cuticular collagen antigens. Parasitology. 1990;100:317-326.
45. Behnke JM. Immunology. In Gilles HM, Ball PAJ, (ed.), Human parasitic diseases: hookworm infections. Elsevier, Amsterdam, the Netherlands. 1991;4: 93-155.
46. Hines R. Why is my dog's eosinophil count high? 2014. Available:http://www.2ndchance.info/.www.vcahospitals.com/main/pet-health-information/...in-dogs/431

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13504