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Haematological response of dogs with natural haemo-parasitic infections in Abeokuta, Ogun State

KA Adetula, OA Akinkuotu, OS Omotainse

ABSTRACT

My Undergraduate Project was carried out in Abeokuta, Ogun State where a hundred blood samples were collected from dogs from the Veterinary Teaching Hospital Abeokuta and also from other locations across Abeokuta. Haematological examination was carried out on all blood samples with the use of an Auto Haemo-analyzer. Reticulocyte counts were also done manually with the aid of a special stain (New Methylene Blue).

Parasitological examination was carried out on each of the blood samples with the use of Giemsa stain to detect the presence of haemoparasites. Out of the 100 blood samples that were collected, 21 samples were with infection while 79 samples were without infection. Out of the 21 samples, 4 of them were infected with trypanosomes (19.05%) while 17 of them were infected with Babesia (80.95%).

There was marked anaemia and increase in Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) in the samples with both trypanosome and babesia infection. The only notable change in the mean values of samples with trypanosome infection was an increase in Mean Corpuscular Haemoglobin. There was marked anaemia in the mean value of the samples with babesia infection as well as a decrease in the Red Blood Cell count. The level of reticulocytosis in the samples with trypanosomes were higher than the samples with babesia.

Results from this study showed that infection with babesiosis inserted more harm in terms of anaemia than in trypanosomiasis infection.

KEYWORDS: Haematological, Reticulocytes, Haemo-parasites, Babesia, Trypanosomiasis, Infection, Dogs

INTRODUCTION

Haemoparasites are parasites living in the blood of animals; examples are *Babesia spp, Trypanosoma spp, Ehrlichia spp, Eperythrozoon spp, Anaplasma spp, Leptospira spp, Hepatozoon spp (*James, 2001). The method of transmission varies, depending on the parasite, but often they are transmitted through the bites of ticks or flies.

African animal trypanosomiasis (AAT) is caused by protozoa in the family Trypanosomatidae genus *Trypanosoma*. *Trypanosoma congolense* resides in the subgenus *Nannomonas*, a group of small trypanosomes with medium-sized marginal kinetoplasts, no free flagella, and poorly developed undulating membranes. This trypanosome is also a major cause of the disease in cattle in West Africa. Sheep, goats, horses, and pigs may also be seriously affected. In domestic dogs, chronic infection often results in a carrier state (Suliman *et al.,* 1989). Initial replication of trypanosomes is at the site of inoculation in the skin; this causes a swelling and a sore (chancre). Trypanosomes then spread to the lymph nodes and blood and continue to replicate. *Trypanosoma congolense* localizes in the endothelial cells of small blood vessels and capillaries.

Immunologic lesions are significant in trypanosomiasis, and it has been suggested that many of the lesions (e.g., anaemia and glomerulonephritis) in these diseases may be the result of the deposition of immune complexes that interfere with, or prevent, normal organ function (Roder *et al.,* 1984). The most significant and complicating factor in the pathogenesis of trypanosomiasis is the profound immunosuppression that occurs following infection by these parasites.

Anaemia is a cardinal feature of the disease in which red blood cells are removed from the circulation by the expelled mononuclear phagocytic system. Later, in infection of several months of duration, when the parasitaemia become low and intermittent, anaemia may resolve to a variable degree (Urquhart *et al.* 2002).

No pathognomonic change is seen in African Animal Trypanosomosis. Anaemia, oedema, and serous atrophy of fat are commonly observed. Subcutaneous oedema is particularly prominent and is usually accompanied by ascites, hydropericardium, and hydrothorax.

The acute phase of the disease in dogs can be diagnosed by direct demonstration of the presence of trypomastigote forms in the blood, by a blood smear stained by the Giemsa method, or by a thick smear. Currently, the direct detection of *Trypanosoma evansi* is usually performed by microscopic examination of fresh blood between a slide and coverslip, by blood smear, or even by using centrifuge methods that concentrate parasites for later viewing. Such techniques include searching for the parasite in leukocyte aggregations, the microhematocrit method (MHCT), the Strout concentration method, and others (Jones *et al.*, 2000). ELISA and hemagglutination tests are also used (Jones *et al.*, 2000). The polymerase chain reaction (PCR) is used to amplify fractions of the protozoan's DNA, which are then used to identify it. The reported high sensitivity and specificity of PCR have increased its diagnostic potential. Using two of the cited techniques allows reliable diagnosis in most cases. With the introduction of molecular diagnosis techniques, various assays have been developed based on DNA detection by PCR, and this technique has proved more sensitive than conventional parasitological methods in diagnosing various trypanosome species in different hosts. However, its high cost and complexity are still drawbacks to its routine use in diagnosis.

The severity of babesiosis ranges from subclinical infection to widespread organ failure and death. Most dogs with babesiosis develop haemolytic anaemia and/or thrombocytopenia, together with varying degrees of anorexia, fever, splenomegaly, icterus, and pigmenturia. The main determinant of this variable pathogenesis is the species of piroplasm responsible for the infection, but other factors such as the age and immune status of the host and the presence of concurrent infections also influence clinical outcome. The presence of multiple co-infections (as can readily occur when pathogens share the same vector) confounds the attribution of clinical signs to the babesiosis alone (Kordick *et al.,* 1999). Puppies tend to develop more severe clinical disease than adult dogs, and the unnamed Babesia spp in the United States has been reported only in immuno-compromised (by cancer or splenectomy) dogs (Holm *et al.,* 2006).

In anaemic conditions, the bone marrow of the animals should respond positively in an attempt to alleviate it. Despite the fact that anaemia is consistent in experimental trypanosomal and babesia infections, the level of erythropoiesis is not compensatory enough as reported by Igbokwe and Anosa (1989), Omotainse and Anosa, (1992). In acute babesiosis, microscopy is reasonably sensitive for detecting intra-erythrocytic piroplasms, provided that the blood films are well prepared and suitably stained. Visual detection of piroplasms confirms the diagnosis and is sufficient to warrant specific treatment in most cases (see later), but the species (or genotype) of the organism cannot be determined by morphology alone; this requires PCR and genomic sequence analysis. Despite improvements in laboratory diagnostic methodologies in recent years, there is no testing procedure that offers a 100% certainty of detecting a piroplasmic infection. The combination of serologic testing and PCR is considered to offer the greatest sensitivity; the current recommendation is to screen suspected cases or blood donors initially by serology and subsequently test seronegative dogs with an appropriate piroplasm PCR (Zahler *et al*., 1998).

To date, two studies have recorded poor regenerative responses in dogs naturally infected with *Babesia rossi*, showing moderate to severe anaemia (Reyers *et al*., 1998, Scheepers *et al*., 2011). The first study noted that the mean reticulocyte percentage (RET%) of the admission blood samples was non-regenerative (RET% = 0.74%) in moderately anaemic dogs (haematocrit; HCT = 0.15–0.30 L/L) and mildly regenerative (RET% = 3.43%) in severely anaemic dogs (HCT < 0.15 L/L) (Reyers *et al*., 1998). However, the absolute reticulocyte count (ARC) was not determined in these cases. The second study described the regenerative response as mild (ARC = 60–150 × 10^9/L) to moderate (ARC = 150–300 × 10^9/L) in dogs requiring a blood transfusion (Scheepers et al., 2011). The transfused group had a median HCT of 0.09 L/L at presentation. Similarly, a mild to moderate regenerative response was seen in dogs that did not receive a blood transfusion. Nonetheless, the overall regenerative response, recorded over a period of 6 days, was never marked, despite the severity of anaemia observed.

Another study recorded those dogs experimentally infected with *Trypanosoma evansi* showed marked decrease in erythrocyte count, but reticulocytes proportion didn't until the 12th day (Mario *et al.*, 2000).

Therefore, there is the need to investigate the haematological changes and responses to natural infections of babesiosis and trypanosomiasis in Nigerian Dogs.

MATERIALS AND METHODS

Sample Collection

A total of one hundred blood samples were collected from one hundred different dogs. The dogs whose bloods were collected were from the Veterinary Teaching Hospital and also from other locations across Abeokuta. 5ml of blood was collected from the cephalic vein of each dog and put in Ethylene Diamine Tetra-acetic Acid (EDTA) sample to maintain the morphological integrity of the blood cells as well as the plasma. The samples upon collection were transported to the laboratory in ice packs so as to prevent the blood cells from lysing which could hamper accurate results from being gotten.

Haematology

Haematological examination was carried out on the one hundred blood samples with the use of an Auto Haemo-analyzer, haematological values determined were; Red Blood Cell count, Haemoglobin concentration, Packed Cell Volume, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Mean corpuscular Haemoglobin Concentration, White Blood Cell count and Differential White Blood Cell Count (which includes lymphocyte, monocyte and granulocyte).

Reticulocyte counts were also done manually with the aid of a special stain (New Methylene Blue). A drop of freshly collected blood was put on a clean glass slide and an equal quantity of new methylene blue was also put on the blood and mixed before leaving for 12 to 15 minutes to allow the reticulocytes pick up the stain. After the specified period, the blood was smeared and allowed to dry before it was viewed with the binocular microscope at a magnification of ×1000 using immersion oil. The total

number of reticulocytes visible were counted in a field of 200 red blood cells and the percentage calculated (Caroll *et al*., 2017).

Parasitology

Parasitological examination was carried out on each of the blood samples with the use of Giemsa stain to detect the presence of haemoparasites according to Esonu *et al* (2019) and Murray (1977). Thin blood smears were made on clean glass slides and the blood was fixed with methanol and air-dried before the smeared slides were stained with giemsa for about 20 minutes to allow the parasites (if present) pick up the stain. After the specified period the slides were rinsed with clean water and air-dried before they were viewed at magnification of ×1000 with immersion oil. The absence of molecular characterisation is the limitation of the current study in regards to the identification of the parasites.

Analysis

The results obtained were presented as Mean ± Standard Deviation with the use of Microsoft Excel (2007 version). Separate tables were also made for the samples with parasitic infection and those without infection. A multiple bar chart was made showing the differences in haematological values of the samples with infection, without infection, with trypanosomes and also with babesia. Specific values compared were: Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Reticulocyte count (RET), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cell count (WBC)

RESULTS

PARASITOLOGY

Values were grouped into five groups namely;

A.) Whole Blood samples- WS

B.) Samples with infection- WI

C.) Samples without infection- WOI

D.) Samples with Trypanosomes- WT

E.) Samples with Babesia- WB

Out of the 100 blood samples that were collected, 21 samples were with infection while 79 samples were without infection. Out of the 21 samples, 4 of them were infected with trypanosomes while 17 of them were infected with Babesia.

HAEMATOLOGY

Haematology showed that in group A, the mean values of packed cell volume, red blood cell count, haemoglobin count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were within normal range while the reticulocyte count was above the normal value.

In group B, the mean values of mean corpuscular volume and haemoglobin count were within the normal range while the packed cell volume and red blood cell count were reduced with the mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration above the normal range.

In group C, the mean values of packed cell volume, red blood cell count, haemoglobin count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were within normal range.

Comparison of values between groups D and E showed that there was increase in the value of mean corpuscular haemoglobin in group D. In group E, the packed cell volume and red blood cell count were reduced but the mean corpuscular haemoglobin concentration was above the normal range.

TABLE 1 Showing the haematological indices (Mean ± SD) of dogs in different groups.

	WS	WI	WOI	WT	WB	REFERENCE RANGE
PCV (%)	39.99±17.54	34.41±16.24	41.48±17.66	38.65±14.88	33.42±16.81	37-55
RBC ($\times10^{12}$/L)	5.75±2.49	4.77±2.19	6.01±2.52	5.52±2.80	4.59±2.09	5.3-8.8
MCV (fl)	71.04±11.84	73.24±7.97	70.46±12.65	74.25±11.26	73.01±7.43	60-77
HB (g/L)	127.98±11.84	126.57±34.94	128.35±41.99	122.75±8.58	127.47±38.83	120-180
MCH (pg)	250.58±104.02	307.33±143.03	235.49±85.97	311.50±185.09	306.35±138.36	195-245
MCHC (g/L)	346.00±119.63	411.90±169.79	328.48±96.40	350.75±117.08	426.29±179.75	320-360
RET (%)	3.80±2.69	3.24±2.27	3.95±2.78	3.75±1.50	3.12±2.44	0.0-1.5
WBC ($\times10^{9}$/L)	16.96±10.22	15.23±10.84	17.42±10.07	17.30±11.33	14.75±11.02	6-17
LYMPH ($\times10^{9}$/L)	5.65±4.26	5.75±5.00	5.62±4.07	7.73±6.56	5.29±4.69	1.0-4.8
MONO ($\times10^{9}$/L)	1.08±0.92	0.78±0.66	1.14±0.96	0.98±0.76	0.73±0.65	0.15-1.35
GRAN ($\times10^{9}$/L)	10.28±7.46	8.71±6.85	10.69±7.60	8.60±5.80	8.74±7.24	

Figure 1 showing mean Hb values in different groups of blood samples.

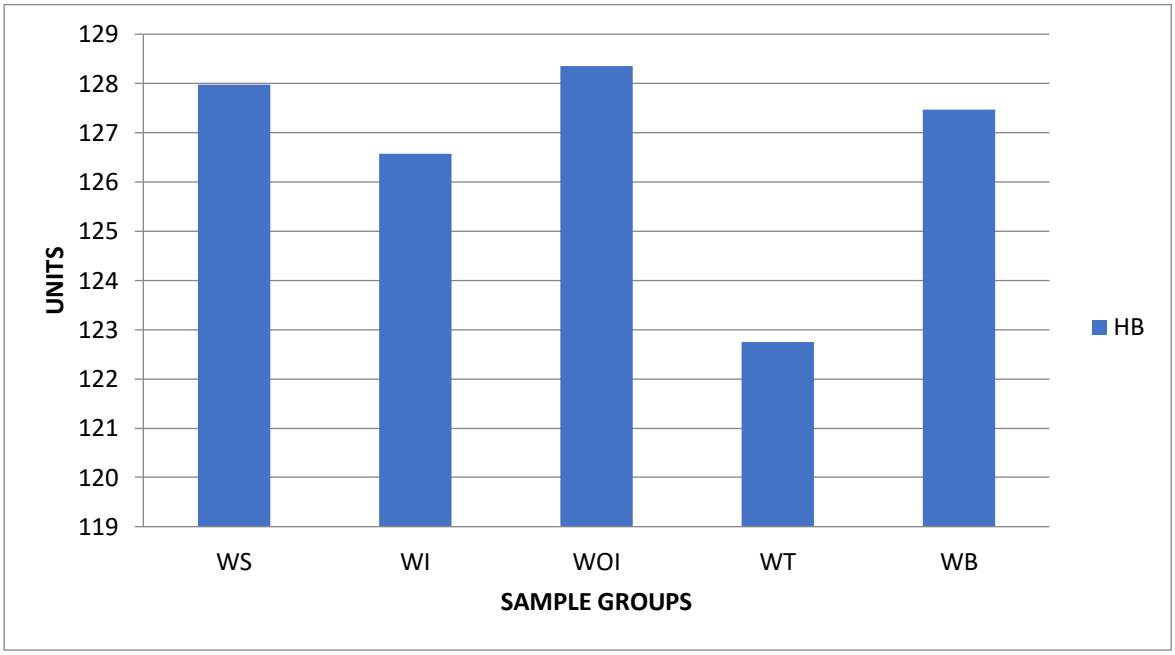


Figure 2 showing mean values of MCV in different groups of blood samples.

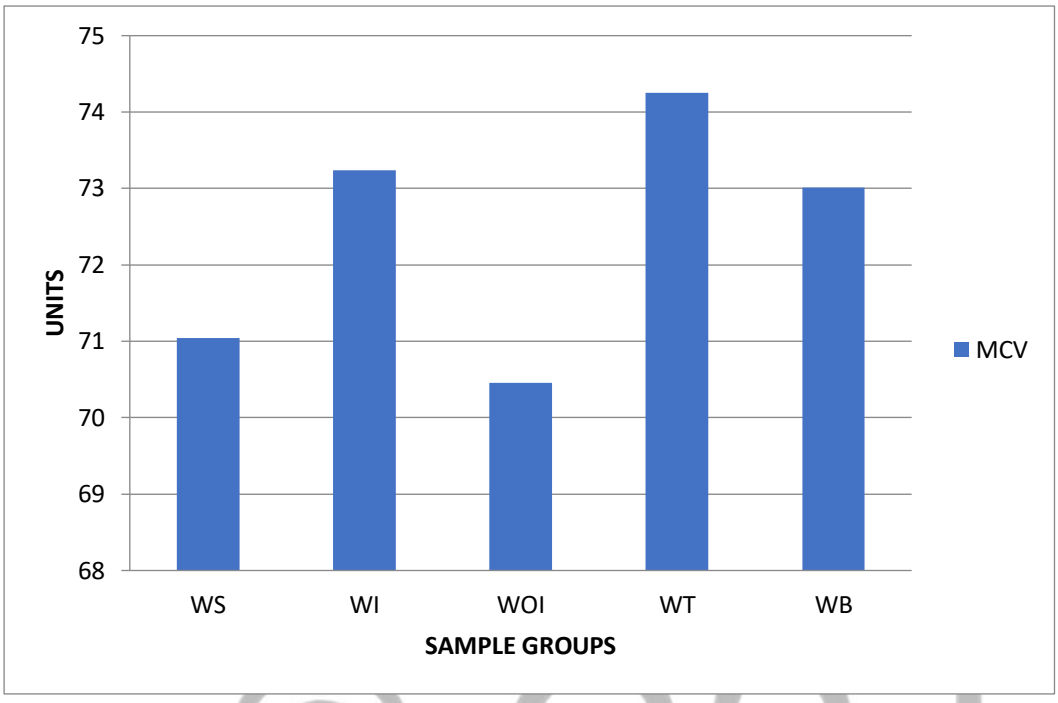


Figure 3 showing the mean values of RBC and RET in different groups of blood samples .

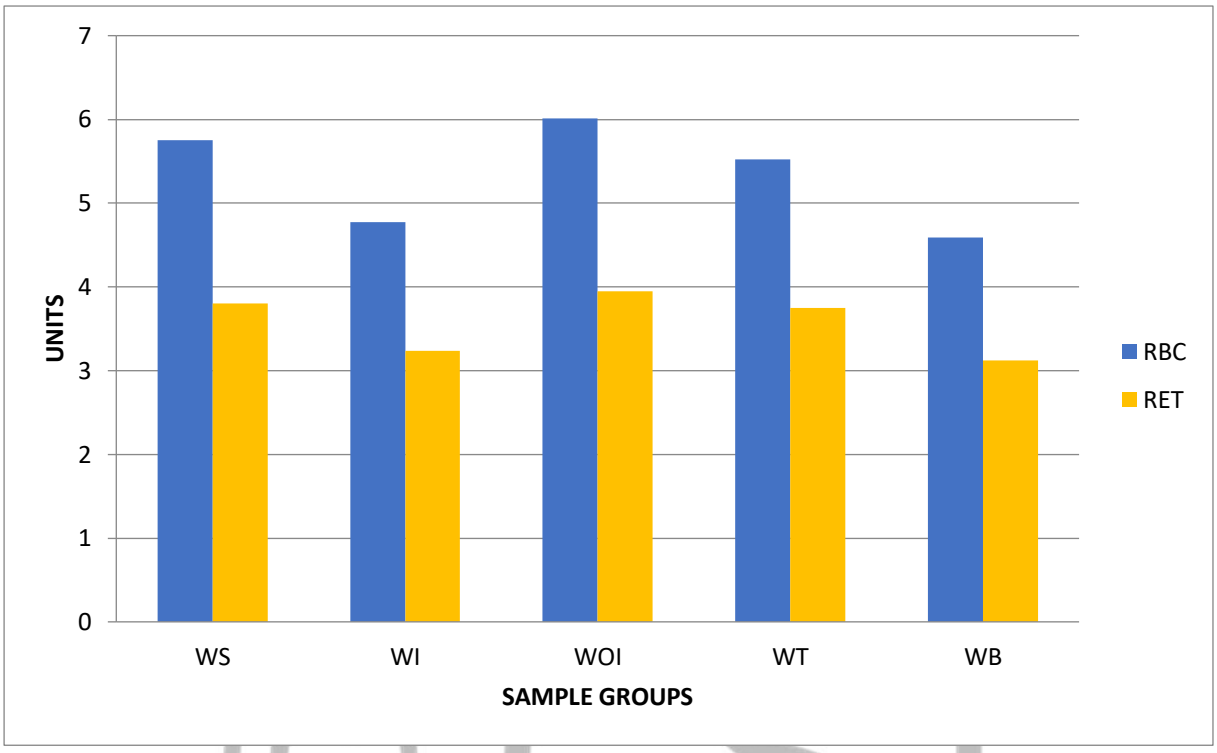


Figure 4 showing the mean leucocytic values in different groups of blood samples.

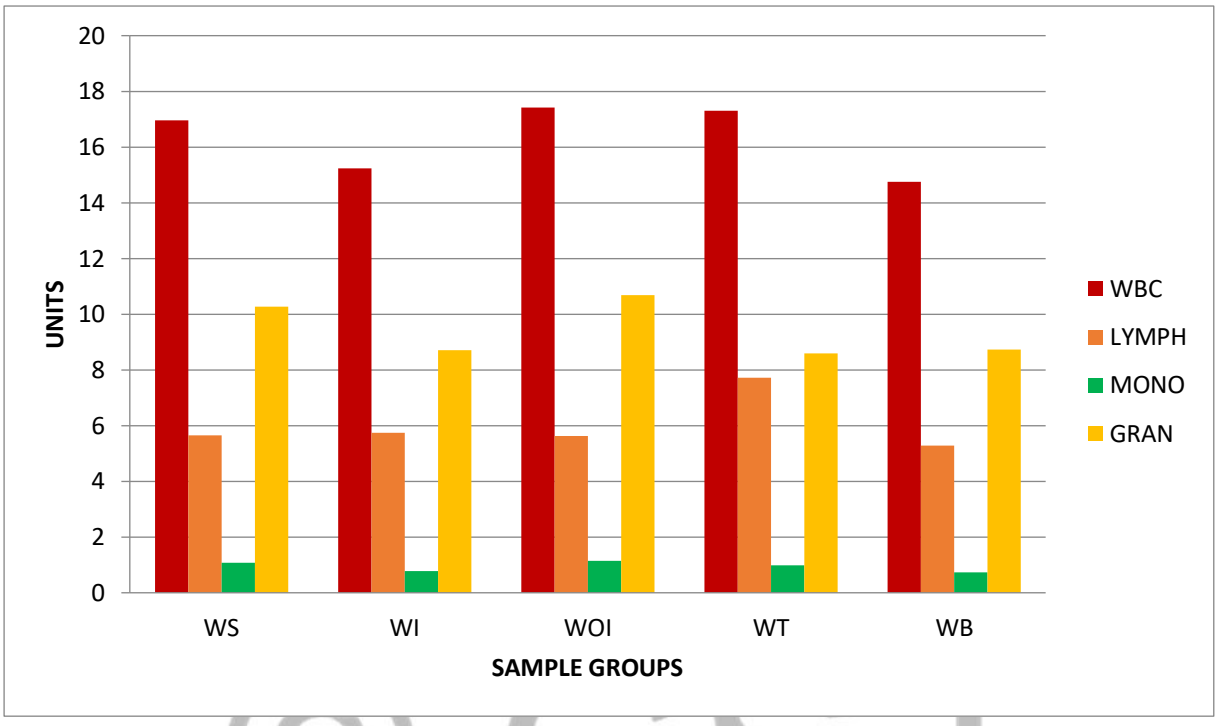
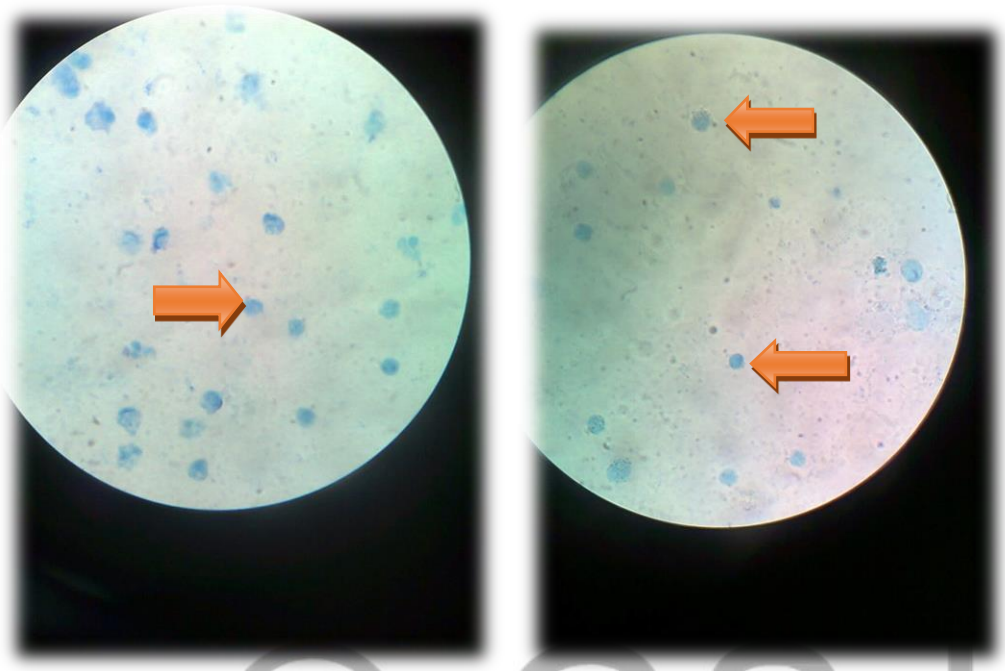
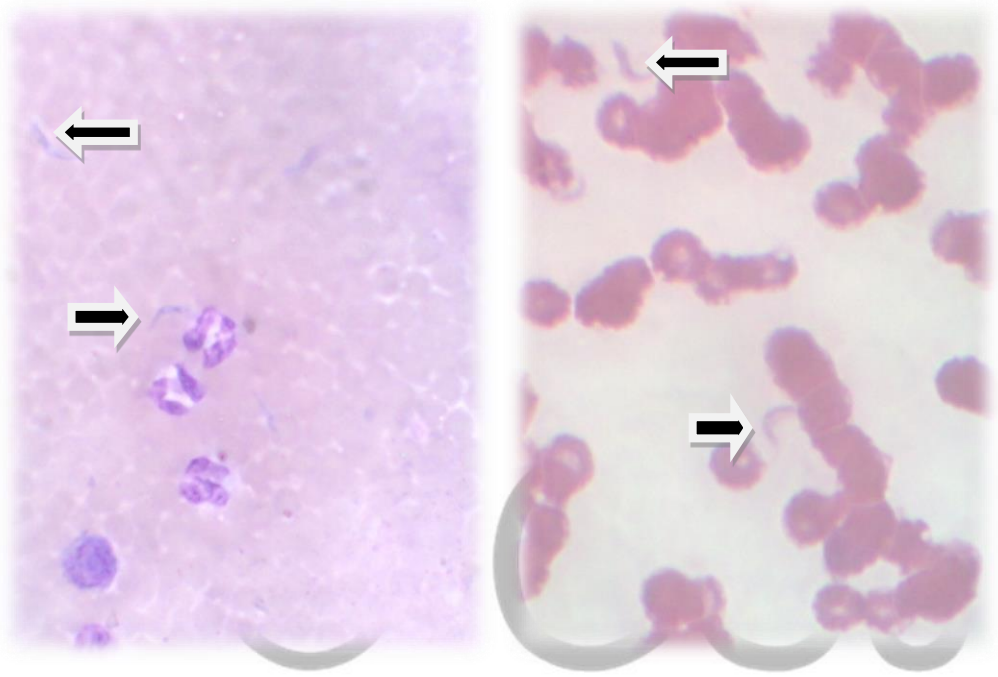


Figure 5 A and B showing the stained reticulocytes on stained blood smears (arrowed).



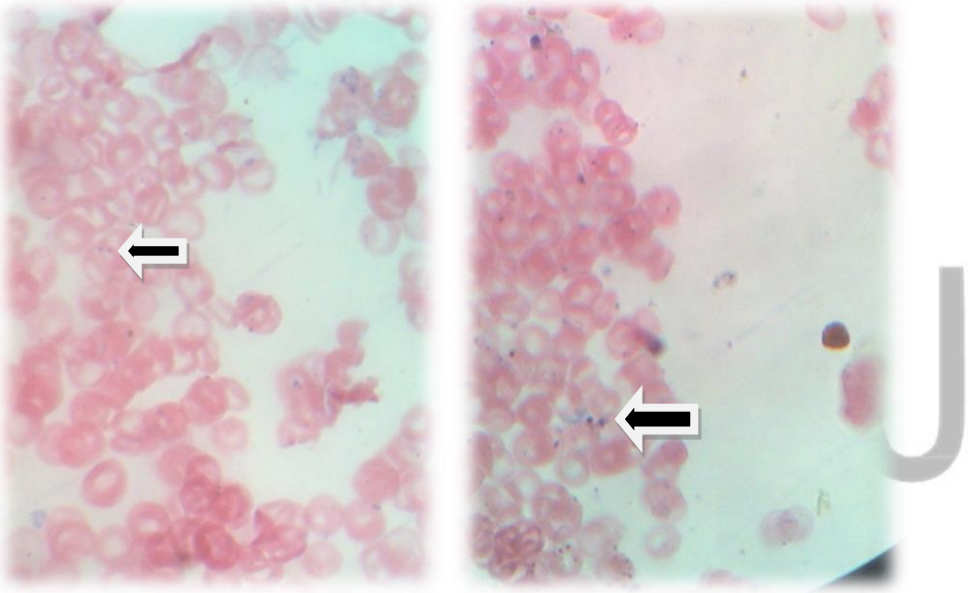
A **B**

Figure 6 C and D showing the trypanosomes on Geimsa stained blood smears (arrowed).



C **D**

Figure 7 E and F showing babesia parasites in Geimsa stained blood smears (arrowed).



E **F**

DISCUSSION

There was marked anaemia and increase in MCH and MCHC in the samples with both trypanosome and babesia infection. The only notable change in the mean values of samples with trypanosome infection was an increase in Mean Corpuscular Haemoglobin. There was marked anaemia in the mean value of the samples with babesia infection as well as a decrease in the Red Blood Cell count. There was also an increase in the Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration. The level of reticulocytosis in the samples with trypanosomes were higher than the samples with babesia.

The normocytic hyperchromic anaemia observed in this study was in contrast with Furlanello (2005) who observed normocytic normochromic anaemia in dogs with natural babesia infection.

CONCLUSION

The most common abnormality in the investigated parameters of babesiosis and trypanosomiasis was anaemia. The haematological findings of canine babesiosis in this study was in agreement with the findings of Furlanello *et al*., (2005) in experimental infected dogs with Babesia and trypanosomiasis (Omotainse and Anosa, 1992).

Results from this study showed that infection with babesiosis inserted more harm in terms of anaemia than in trypanosomiasis infection.

Factors involved in the pathogenesis of anaemia in African trypanosomosis include haemolysis, haemodilution, haemorrhages and dyshaematopoiesis (ANOSA, 1988).

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