HAEMATOLOGICAL CHANGES AND EVIDENCE OF MULTIPLE ORGAN INVOLVEMENT IN NATURAL BABESIOSIS IN NIGERIAN DOGS

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ABSTRACT

This study describes some haematological changes and the multiple organ damage observed in Nigerian dogs that died of canine babesiosis. 17 infected dogs with babesiosis, diagnosed by the detection of parasites in Giemsa stained thin blood smears and another 17 apparently healthy large breeds of dogs presented at the University of Ibadan Veterinary Teaching Hospital were enrolled in this study. Infected dogs were further sub-divided into the uncomplicated and complicated groups based on disease manifestations, while the full blood count and erythrocyte morphology were done using standard techniques. Tissue samples (brain, heart, lungs, kidneys, spleen and liver) were taken from five dogs that died of natural canine babesiosis and histopathological processed using standard techniques. Babesia negative dogs had lower neutrophil/lymphocyte ratio when compared with Babesia positive dogs. Complicated groups had higher neutrophil/lymphocyte ratio. Anisocytosis was the commonest encountered morphological abnormality. All of the five dogs used for the histopathological study had multiple organ lesions that involved the lungs 3(60 %), kidney 2(40 %), heart 2(40 %), brain 1(20 %), spleen 2(40 %) and liver 5(100 %). In conclusion, this study showed that neutrophil/lymphocyte ratio is a good diagnostic index to detect complications in babesiosis and also that multiple organ dysfunction is a major phenomenon in the pathophysiology of babesiosis in Nigerian dogs.

Keywords: Canine babesiosis, Neutrophil/lymphocyte ratio, Multiple organ damage, Nigerian dogs

INTRODUCTION

Babesiosis is a tick-borne disease of worldwide importance. In the dog, the disease is caused by intra-erythrocytic protozoan parasites of the Genus *Babesia*, Family Babesiidae, Order Piroplasmida and Phylum Apicomplexa (Vial and Gorenflot, 2006). Two species of *Babesia* are known to commonly infect the domestic dog. These are *Babesia canis* and *Babesia gibsoni* (Nel *et al.*, 2004). *B. canis* occurs in three antigenically distinct subtypes namely *Babesia canis canis, B. canis rossi and B. canis vogeli* (Uilenberg *et al.*, 1989). Infection of the dog with these organisms causes a condition known as canine babesiosis which may be manifested in two different forms; the uncomplicated and the complicated forms (Welzl *et al.*, 2001; Lobetti, 2005). Uncomplicated babesiosis refers to the clinical manifestation which is mainly attributable to haemolytic anaemia (Jacobson and Clark, 1994) while in the complicated form, the observable clinical signs are those which cannot be directly linked to haemolysis but appear to result from the host's inflammatory response (Jacobson and Clark, 1994). The term systemic inflammatory response syndrome (SIRS) was coined to describe massive inflammatory response to an insult which may be either infectious or non-infectious (Bone *et*

al., 1992). This systemic inflammatory response syndrome eventually leads to multiple organ dysfunction syndrome (MODS) (Weltzl, 2001). Multiple organ dysfunctions in babesiosis is believed to be the end result of tissue inflammation that is initiated by several factors which include hypotension, septic shock and infectious organisms (Lobetti, 2005). Babesiosis is capable of causing severe tissue hypoxia which in turn, leads to widespread tissue damage and release of inflammatory mediators (Lobetti, 2005) but in a lot of cases, the severity of hypoxia is not proportional to the degree of anemia observed (Taylor *et al*., 1993). This is due to the fact that in canine babesiosis caused by *B. canis*, a qualitative and quantitative deficit of haemoglobin can occur and the haemoglobin that is remaining in intact cells functions abnormally. This is seen especially in acidic and hypercapnic conditions (Taylor *et al*., 1993). In addition to these, *B. canis* has been shown to produce enzymes which are capable of cleaving haemoglobin (Taylor and Van Rensberg, 1995).

Furthermore, white blood cell populations play a significant role in the SIRS, and thus the differential white blood cell counts would represent a simple method of assessing SIRS severity (Zahorec, 2001). An earlier study had detailed the neutrophil/lymphocyte ratio in both complicated and uncomplicated babesiosis in dogs in Croatia (Kučer *et al.*, 2008). Information on this aspect and that of MODS is lacking in most tropical setting. Therefore, this study describes the haematological changes and the multiple organ damage observed in Nigerian dogs that died of canine babesiosis.

MATERIALS AND METHODS

Study Area: The study was carried out in Ibadan, Oyo State in the South Western part of Nigeria (7° 23' 16" North, 3° 53' 47" East). The area is located in the humid and tropical wet and dry climate with a lengthy wet season daily temperature ranges of 19 – 32 ^{o}C.

Animals and Selection Criteria: A total of 17 dogs presented at the University of Ibadan Veterinary Teaching Hospital with naturally occurring canine babesiosis, diagnosed by the detection of parasites in Giemsa stained thin blood films between February and July 2009 were enrolled in this study. Seventeen other apparently normal dogs with no parasites in the blood that were presented for routine clinical health assessment and/or vaccination served as control. In all the 17 infected dogs selected two (2) were Nigeria local indigenous dog breeds, while the exotic dog breeds selected were: Alsatian (4), Boerboel (2), Rottweiler (2), Pitbull (3), Neopolitan mastiff (2), Caucasian (1) and Doberman (1). All the dogs selected both infected and apparently healthy normal dogs were on a good plan of nutrition. Infected dogs were further sub-divided into the uncomplicated and complicated groups (Welzl *et al.,* 2001). Dogs in the uncomplicated group were those that had fever with or without anaemia, while those in the complicated group had manifestations of organ involvement resulting in haemoglobinuria, dehydration, tachycardia, dyspnea, convulsion and ataxia.

Haematology: Full blood count, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin concentration, total and differential white blood cell count and thrombocyte counts were done on each blood sample collected using standard haematological techniques (Sood, 2006).

Erythrocyte Morphology: The quantification of the morphological abnormalities was as described by Adekola *et al.* (2016). The assessment of the peripheral blood smear for the erythrocyte morphology was done by viewing the thin blood film under magnification of x1000 of an Olympus light microscope. The morphological abnormalities seen were counted using a tally counter. The values obtained were then converted to percentage abnormalities using the formula: Echinocytes (%) = number of echinocytes counted in 200 erythrocytes ÷ 200 x 100. This was repeated for all the observed morphological abnormalities to obtain the percentage count of each abnormality. The percentage morphological abnormalities obtained were scored using the reference guide by Adekola *et al.* (2016) to determine the severity of the infection.

Tissue Pathology: The carcasses of five dogs that died of natural canine babesiosis diagnosed by the identification of *Babesia* species in Giemsa-stained thin smears were submitted for necropsy. Tissue samples were taken from the brain, the heart, the lungs, the kidneys, the spleen and the liver for routine histopathological study (Bancroft *et al.,* 1994).

Data Analysis: Data obtained were analyzed using student t-test to compare differences between haematological parameters of *B. canis* positive and *B. canis* negative Nigerian dogs, and between *B. canis* positive complicated and uncomplicated cases. Probability value of $p<0.05$ was accepted as significant.

RESULTS

Haematological Parameters: *Babesia* negative dogs had higher values for the haemoglobin concentration, packed cell volume, red blood cell count, mean corpuscular haemoglobin concentration and segmented neutrophils, while the mean corpuscular volume, white blood cell count, band neutrophils, lymphocytes, monocytes, platelets and neutrophil/lymphocyte ratio values were lower when compared with *Babesia* positive dogs (Table 1). However, between the *Babesia* positive and *Babesia* negative dogs, statistically significant differences ($p<0.05$) were recorded only for the packed cell volume, red blood cell count, mean corpuscular volume, white blood cell counts, segmented neutrophils, lymphocytes and platelet.

In the complicated and uncomplicated groups, statistically significant differences ($p<0.05$) were found in the haemoglobin concentration, packed cell volume, red blood cell counts, mean corpuscular haemoglobin concentration, total white blood cell count, band neutrophils, platelet counts and the neutrophil/lymphocyte ratio.

Haematocrit below reference values was observed in 14(82.4 %) dogs. Out of these 14 dogs, 5(35.7 %) had the complicated form of canine babesiosis, while 9(64.3 %) had the uncomplicated form of canine babesiosis. Two of the dogs that had the complicated form of canine babesiosis had severe anaemia with PCV of 9.0 % each. Leucocytosis was observed in 5(35.7 %) out of the 14 *Babesia* positive dogs. Complicated babesiosis accounted for 3(60.0 %) of the cases, while the remaining 2(40.0 %) had uncomplicated babesiosis. Thrombocytopaenia was found in only 1(5.9 %) dog which had uncomplicated babesiosis.

Erythrocyte Morphology: Anisocytosis was the commonest encountered morphological abnormality observed in 14(82.4 %) of the dogs. Macrocytosis and microcytosis were each found in 9(52.9 %) dogs, while Howell jolly body and spherocytosis were each found in 4(23.5 %) dogs. Polychromasia, leptocytosis and poikilocytosis were found in 6(35.3 %), 3(17.6 %) and 2(11.8 %) dogs respectively. Roleaux formation and hypochromatia were found in 1(5.9 %) dog (Table 3).

Tissue Pathology: All the dogs used in this study had multiple organ damage. The distribution of lesions encountered was presented in Figure 1. Pulmonary lesions were found in 3(60.0 %) of the dogs, renal lesions were found in 2(40.0 %), cardiac lesion in 2(40.0 %), brain damage in 1(20.0 %) splenic lesions in 2(40.0 %) and hepatic lesions in 5(100.0 %) dogs. Edema and haemorrhage were seen in 2 of the dogs with pulmonary lesions (Figure 2A), while an acute purulent pneumonia was found in the third dog (Figure 2B). Renal lesions encountered were tubulo-interstitial nephritis (Figure 2C) with diffuse tubular degeneration, multifocal lymphoplasmacytic, interstitial infiltrates and multifocal haemorrhages. Cardiac lesions observed were myocardial necrosis (Figure 2D) and focal areas of calcification. In the brain, diffuse neuronal necrosis was observed. In the examined spleens, there was severe lymphoid depletion, while the hepatic lesions had severe centrilobular necrosis (Figure 2E), diffuse perivascular lymphoplasmacytic infiltration and haemosiderosis (Figure 2F).

Table 1: Haematological parameters of *Babesia canis* positive and negative Nigerian dogs presented at the University of Ibadan Veterinary Teaching Hospital for routine check-up and therapy

Haematological parameters	*Babesia* status	
	Negative	Positive
Haemoglobin concentration (g/dl)	14.1± 0.5	8.2±0.9
Packed cell volume (%)	42.9+1.3	25.4±2.6*
Red blood cell count ($x10^6$)	7.1±0.3	4.1±0.5*
Mean corpuscular volume (fl)	60.5±1.3	64.4±2.9*
Mean corpuscular haemoglobin concentration (g/dl)	32.9±0.5	31.7±0.5
White blood cell count ($x10^3$/ul)	10.1±0.6	15.7±2.0*
Segmented neutrophils (%)	72.7±2.2	65.0±6.6*
Band neutrophils (%)	2.4±0.6	7.7±3.1
Lymphocytes (%)	19.5±2.0	21.5±4.7*
Monocytes (%)	2.4±0.3	3.6±0.5
Eosinophils (%)	3.1±0.6	2.2±0.7
Platelets ($x10^3$/µl)	233.4±11.8	319.8±40.1*
Neutrophil/lymphocyte ratio	5.3±1.0	8.3±2.2

**Significantly different means at p<0.05*

Table 2: Haematological parameters analyzed in the complicated and uncomplicated groups of *Babesia canis* positive Nigerian dogs presented at the University of Ibadan Veterinary Teaching Hospital for routine check-up and therapy

Haematological parameters	*Babesia canis* Positive Dogs	
	Uncomplicated	Complicated
Haemoglobin concentration (g/dl)	8.1 ± 0.5	8.3 ± 1.9*
Packed cell volume (%)	25.2 ±1.4	25.5 ± 5.5*
Red blood cell count ($x10^6$)	4.0 ± 0.4	4.2 ± 0.9*
Mean corpuscular volume (fl)	66.3 ± 4.3	62.2 ± 4.1
Mean corpuscular haemoglobin concentration (g/dl)	32.0 ± 0.5	31.4 ± 0.9*
White blood cell count ($x10^3$/ul)	13.2 ± 1.4	18.4 ± 3.8*
Segmented neutrophils (%)	56.2 ± 8.1	74.9 ± 10.0
Band neutrophils (%)	4.6 ± 0.4	11.2 ± 6.5*
Lymphocytes (%)	31.1 ± 7.2	10.7 ± 3.1
Monocytes (%)	4.3 ± 0.8	2.7 ± 0.7
Eosinophils (%)	3.7 ± 1.1	0.5 ± 0.5
Platelets ($x10^3$/µl)	280.1 ± 28.7	364.0 ± 78.9*
Neutrophil/lymphocyte ratio	3.5 ± 1.0	13.6 ± 3.7*

**Significantly different means at p<0.05*

Table 3: Erythrocyte morphology observed in *Babesia canis* positive Nigerian dogs presented at the University of Ibadan Veterinary Teaching Hospital for routine check-up and therapy

Morphology	Number Affected
Anisocytosis	14(82.4)
Macrocytosis	9(52.9)
Microcytosis	9(52.9)
Howell jolly body	4(23.5)
Spherocytosis	4(23.5)
Polychromasia	6(35.3)
Leptocytosis	3(17.6)
Poikilocytosis	2(11.8)
Roleaux formation	1(5.9)
Hypochromatia	1(5.9)

Number in parenthesis = percentage infection

DISCUSSION

This study describes the haematological changes and the multiple organ dysfunctions observed in Nigerian dogs that died of naturally occurring canine babesiosis. Anaemia which is defined by a low red blood cell count, haematocrit and haemoglobin concentration was found in 76.5, 82.4 and 82.4 % respectively of *Babesia* infected dogs examined. In a similar study conducted in Poland, Zygner *et al.* (2007) recorded much lower percentages of anaemic dogs in a population of *Babesia* positive dogs.

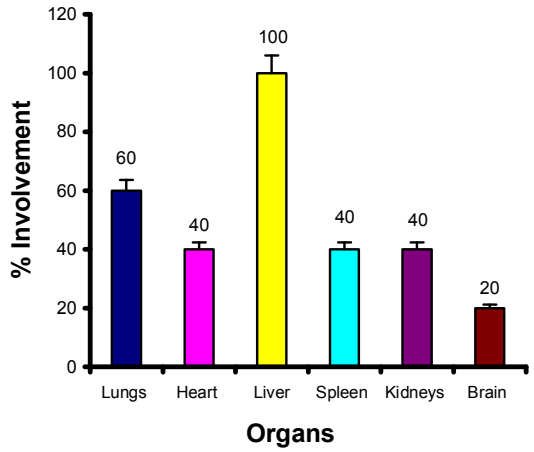

Figure 1: Organ involvement in naturally occurring canine babesisosis

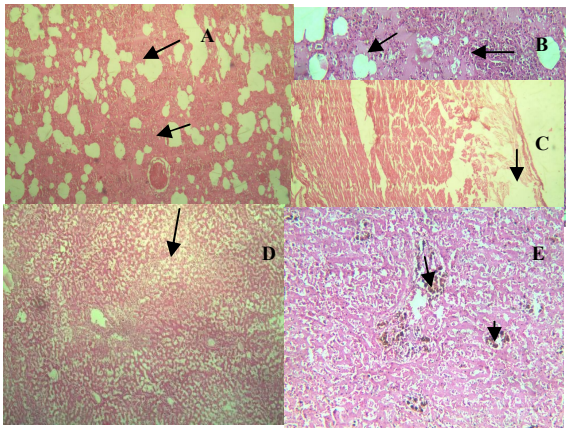

Figure 2: A = Pulmonary edema and haemorrhage, B = Acute pneumonia, C = Myocardial necrosis, D = Centrilobular necrosis and E = Haemosiderosis

The authors in the said work recorded 26.2, 31.4 and 29.0 % for the RBC count, haematocrit and haemoglobin concentration respectively, which were below the minimal reference values for normal dogs. In another study involving 350 dogs, Fabisiak *et al.* (2010) recorded lower than normal values of 45.0, 34.6 and 50.0 % for RBC count, haematocrit and haemoglobin concentration respectively. The much higher percentage of anaemic dogs recorded in this study is probably due to the pathogenicity of the prevalent sub-species of *Babesia canis* found in the study area, the highly virulent *Babesia canis rossi* (Sasaki *et al*., 2007), as against the mildly virulent *Babesia canis canis* reported by Fabisiak *et al.* (2010) in Poland.

Babesiosis used to be regarded as a disease of only the red blood cells but findings have shown that the disease can result into multiple organ dysfunctions and the disease is therefore classified into the uncomplicated and the complicated forms (Matijatko *et al*., 2007). Just like malaria parasite infection in man, babesiosis can be classified as a protozoal sepsis (Bone *et al*., 1992; Jacobson *et al*., 2002). The inflammatory mechanisms in this disease are thus similar to those found in other septic conditions that lead to SIRS and MODS.

The leukocytosis observed in this study is consistent with other findings that suggest a systemic inflammatory reaction which has been reported to be a major pathophysiologic mechanism in canine babesiosis (Welzl *et al*., 2001). Since neutrophillia and lymphocytopaenia are typical phenomena observed in canine babesiosis (Boozer and Mcintire, 2003), there is a need for a simple and fast method of distinguishing between complicated and uncomplicated babesiosis.

In this study, neutrophil/lymphocyte ratio was found to be very high in dogs with complicated babesiosis and statistically significant ($p<0.05$) when compared with the uncomplicated group. This is similar to an earlier work by Kučer *et al.* (2008), who found higher pre-treatment values for neutrophil/lymphocyte ratio in complicated canine babesiosis when compared with uncomplicated cases. Since differential leucocyte count is a simple diagnostic procedure, the neutrophil/lymphocyte ratio could serve as a ready diagnostic index for the clinician in differentiating between complicated and uncomplicated babesiosis hence the neutrophil/lymphocyte ratio could be a simple, rapid and reliable way of evaluating the extent of systemic inflammation (Zahorec, 2001) in canine babesiosis.

Although multiple organ dysfunction has been associated with a number of haematozan diseases including malaria (Gomes *et al*., 2011), this study is probably the first to document multiple organ involvement in complicated babesiosis in Nigerian dogs. Pulmonary edema observed may be as a result of increased endothelial permeability which results from the

release of cytokines, nitric oxide, free oxygen radicals, eicosanoids and platelet activating factor (Jacobson and Clark, 1994). In this study, there was hepatic involvement in all the animals examined. This is similar to a study carried out in South Africa on multiple organ involvement in canine babesiosis (Welzl *et al.*, 2001). This probably shows that the liver is highly sensitive to damage caused by systemic inflammatory response in canine babesiosis. An experimental study of *B. gibsoni* infection in dogs revealed similar histopathological lesions (Wozniak *et al.*, 1997) even with demonstration of *Babesia* antigens in the liver of infected animals. This probably indicates a direct damage by the parasite to the liver (Torres-Vélez *et al.*, 2003).

Conclusion: This study, though with smaller sample size showed that neutrophil/lymphocyte ratio is a good diagnostic index to detect complications in babesiosis and also that multiple organ dysfunction is a major phenomenon in the pathophysiology of babesiosis in Nigerian dogs.

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