



Experimental Infestation of Dogs with *Rhipicephalus sanguineus* (Brown Dog Tick) and Its Effects on Haematological and Biochemical Parameters

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SUMMARY

Ticks as vectors are responsible for the maintenance and transmission of many pathogens affecting domestic animals and humans. This study was conducted to investigate the effects of experimental infestation of *R. sanguineus* (brown dog ticks). Four dogs of about eight months of age were used in this study. There was significant ($p < 0.05$) reduction in RBC, PCV and Hb concentration of dogs experimentally infested with *R. sanguineus* from week one through week eight of the experiment. Also, the activities of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) were significantly ($p < 0.05$) increased from week one through week eight of the experiment. Similarly, lipid profiles such as triglycerides (TAG), total cholesterol and metabolites such blood urea nitrogen (BUN) and creatinine were significantly ($P < 0.05$) higher from week one through week eight of the experiment. In addition, significant electrolyte loss was also observed in this study. In conclusion, experimental *R. sanguineus* tick infestation could result in anaemia and predispose to hepatic and renal damage with resultant cardiovascular dysfunction and ultimately death in both domestic and wild animals.

Keywords: *Rhipicephalus sanguineus*, anaemia, liver damage, renal injury, cardiovascular dysfunction

INTRODUCTION

Ticks (suborder Ixodida) are the most important group of vectors of pathogens. They are responsible for the maintenance and transmission of many pathogens affecting domestic animals and humans, including several species of bacteria, helminths, protozoa, and viruses (Jongejan and Uilenberg, 2004). The brown dog tick *Rhipicephalus sanguineus* is the most widespread tick in the world. *R. sanguineus* is a vector of many disease agents, some of them (e.g., *Coxiella burnetii*, *Ehrlichia canis*, *Rickettsia conorii*, and *Rickettsia rickettsii*) being of zoonotic concern (Dantas-Torres, 2008). The domestic dog is the main host of *R. sanguineus* in both urban and rural areas (Szabó *et al.*, 2001, Dantas-Torres *et al.*, 2004, Dantas-Torres *et al.*, 2009). Occasionally, *R. sanguineus* can also infest a wide range of domestic and wild hosts, including cats, rodents, birds, and humans (Dantas-Torres *et al.*, 2006, Dantas-Torres *et al.*, 2010). In tropical and subtropical areas, *R. sanguineus* ticks are prevalent throughout the year (Jacobs *et al.*, 2001, Louly *et al.*, 2006) whereas in temperate regions they are most active from the late spring to early autumn (Papadopoulos *et al.*, 1996).

Throughout the development of *R. sanguineus*, this tick prefers dogs as a host but it can also feed on other animals (Sadiq *et al.*, 2001). In the same vein, *R. sanguineus* was found to be commonly known tick infesting dogs in Makurdi, Nigeria as reported by Amuta *et al.*

(2010). Due to its veterinary and public health relevance, *R. sanguineus* is one of the most studied ticks. This study was conducted to investigate the haematological and biochemical parameters in dogs experimentally infested with of *R. sanguineus*.

MATERIALS and METHODS

Four naive dogs (which were not infested with ticks) of about eight months of age were used for this study. The dogs were treated for helminth and protozoan parasites and allowed to acclimatize for a week. *Mebendazole* (100 mg) was administered for 3 days consecutively. Protozoan and bacterial infections were treated with short acting (5%) *oxytetracycline* for 3-5 days consecutively. Blood sample was collected for haematology so as to obtain base line values. Blood smear and faecal samples were examined at intervals to make sure that the dogs were free of parasite. After obtaining the base line values, the dogs were allowed to be experimentally infested by keeping them in infested kennel for two weeks. Care was taken to remove the ticks with the aid of pairs of thumb forceps (Adejinmi, 2011) and the dogs were treated with ivermectin injection subcutaneously (1ml/50kg) at the end of the experiment for ethical purpose.

Animal ethics

All of the animals received humane care according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health (USA). The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments (PHS, 1986).

Blood collection for haematology and biochemistry

Haematology

For haematological studies, 5ml of whole blood were also collected from the same animals into

bijou bottles containing ethylenediamine tetraacetic acid (EDTA). The packed cell volume (PCV), haemoglobin (Hb) concentration, total erythrocyte (RBC) count and total leucocyte (WBC) count were determined by standard techniques as described by Jain (1986).

Serum biochemistry

For serum samples, about 5 ml of blood was withdrawn from the cephalic vein into sterile universal bottles with screw caps. They were transported slanted on ice packs to the Department of Veterinary Medicine Laboratory within 2 hours. They were left at room temperature 27-29°C for another 2 hours to clot. They were later centrifuged at 3000rpm and sera obtained stored at -20°C until analyzed.

The sodium potassium ions determination in the sera were done using the flame photometer (Corning model 400, Corning Scientific Ltd., England) and serum calcium level was measured by cresolphthalein complexone technique. Serum phosphate level was determined using the photoelectric colorimeter (Gallenkamp and Sons Ltd, England) while serum chloride level was estimated by mercury titration method (Schales and Schales, 1941). Serum bicarbonate level was determined by back titration with sodium hydroxide by flame photometer.

The serum total protein and albumin level were determined by the Biuret method as described by Lowry *et al.* (1951) while globulin was calculated according to Coles (1986). Serum urea, creatinine and total bilirubin level were determined using photoelectric colorimeter (Gallenkamp and Sons, Ltd, England) as described by Harrison (1947) and Keyser (1961).

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined using a photo electric colorimeter (Gallenkamp and Sons, Ltd, England) as described by (Reitmann and Frankel, 1957). Serum was also analyzed for total cholesterol (TC) and triglycerides

(TAG) by an enzymatic method coupled with spectrophotometry using assay kit (Randox Lab. Ltd., Co. Antrim, UK).

Statistical analysis

Statistical analysis was performed with the use of Graph-Pad Prism (version 3.0; GraphPad Software, San Diego). Dunnet post-hoc analysis was used where appropriate to test the differences between the groups. Difference of means were considered significant at $p < 0.05$ (GraphPad Software, 2003)

RESULTS

The results of this study has revealed a significant ($p < 0.05$) reduction in PCV values from one week (35.25 ± 2.50), two weeks (26.20 ± 3.55) and three weeks (27.58 ± 2.23)

post infection respectively when compared to the control (38.50 ± 0.58). Similarly, there was significant ($p < 0.05$) reduction in RBC values at two weeks (4.50 ± 0.08), and three weeks (3.23 ± 0.49) post infection respectively when compared to the control (5.07 ± 0.12). Also, values obtained for haemoglobin concentration were significantly reduced at two week (9.63 ± 1.49) and three weeks (9.65 ± 1.45) post infection respectively when compared to the control (12.35 ± 0.37). The values of WBC reduced significantly from week one (11.83 ± 0.94), week two (11.63 ± 0.95) and week three (8.23 ± 0.35) post infection respectively when compared to the control (13.43 ± 0.29). MCV and MCH values obtained decreased at two weeks post infection compared to the control values (Table I).

TABLE I shows haematological parameters of dogs naturally infected with *Rhipicephalus sanguineus*

Parameter	PCV (%)	RBC ($10^3/\mu\text{L}$)	Hb (g/dL)	WBC ($10^6/\mu\text{L}$)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Pre infection	38.50 ± 0.58	5.07 ± 0.12	12.35 ± 0.37	13.43 ± 0.29	75.64 ± 0.84	24.38 ± 1.20	32.07 ± 0.78
post infection:							
One week	35.25 $\pm 2.50^a$	4.50 $\pm 0.08^a$	11.75 ± 1.11	11.83 $\pm 0.94^a$	78.32 ± 5.04	28.50 ± 6.11	33.37 ± 1.32
Two weeks	26.20 $\pm 3.55^b$	4.93 ± 0.68	9.63 $\pm 1.49^b$	11.63 $\pm 0.95^b$	53.23 $\pm 1.22^b$	19.52 $\pm 0.73^b$	36.66 ± 0.82
Three weeks	27.58 $\pm 2.23^c$	3.23 $\pm 0.49^c$	9.65 $\pm 1.45^{c*}$	8.23 $\pm 0.35^c$	85.39 $\pm 4.55^c$	29.88 ± 2.96	34.98 ± 0.65

Values expressed as mean \pm S.D where (n=4). Different superscripts indicate significant ($P < 0.05$) within each column.

TABLE II shows serum electrolytes of dogs naturally infected with *Rhipicephalus sanguineus*

Parameters	Pre-infection	Post infection period (weeks)		
		1	2	3
Na ⁺ (mmol/L)	145.00± 0.60	142.00± 1.75 ^a	134± 2.30 ^b	129.00±4.25 ^c
K ⁺ (mmol/L)	5.65±0.14	4.40± 0.28 ^a	4.50± 0.23 ^b	4.50± 0.25 ^c
CL ⁻ (mmol/L)	107.40± 2.95	105.00±0.50	101.00± 1.00 ^b	94± 2.00 ^c
Ca ²⁺ (mg/100ml)	8.800± 0.40	8.80± 0.08	8.70±0.10	7.80± 0.95
PO ₄ ²⁺ (mg/100ml)	3.96±1.11	5.10± 0.10 ^a	4.60±0.15	3.40± 0.03

Values expressed as mean±S.D where (n=4). Different superscripts indicate significant indicate significant (P<0.05) within each row.

Serum sodium and potassium ions obtained decreased (p<0.05) significantly from week one to week three in experimental animals compared to the control values. Chloride ions decreased at week two (101.00±1.00) and week three (94.00±2.00) respectively compared to the control (107.40± 2.95). In the same vein, values obtained for phosphate ions were significantly increased at one week (5.10± 0.10) compared to the control (3.96±1.11) (Table II).

The values of ALP increased significantly from week one (115.00±8.50), week two (132.00±2.40) and week three (225.00±10.00) post infection respectively when compared to the pre-infection group (94.32±7.16). The values of ALT increased significant from week one (16±0.33), week two (21±0.33) and week three (20.00± 0.30) post infection respectively when compared to the pre-infection group (15.00±0.56). (Table I) The values of AST increased significant at week three (34.00± 3.25) post infection respectively when compared to the pre-infection group (22.8± 5.20) (Table I). Also, the values of serum total protein increased significantly from week one (6.10±0.10), week two (6.9± 0.10) and week three (9.00± 0.30) post infection when compared to the pre-infection group (2.91± 0.43) (Table III). The values of albumin decreased significant at week three (1.5±0.30) post infection respectively when compared to the control (2.91±0.43) as shown in table III.

Serum cholesterol, triglycerides and creatinine increased significantly (p<0.05) from week one week three post infection compared to the pre-infection group (Table IV). Serum urea also increased significantly at two weeks (34.00±6.75) and three weeks (48.00±4.25) compared to the pre-infection group (15.05±3.52)(Table IV).

TABLE III shows aminotransferases, phosphatase and serum proteins

Parameters	Pre-infection	Post infection period (weeks)		
		1	2	3
ALP (IU/L)	94.32±7.16	115± 8.50 ^a	132±2.40 ^b	225± 10.00 ^c
AST (IU/L)	22.8± 5.20	30±8.75	35± 6.50	34.00± 3.25 ^c
ALT (IU/L)	15.00±0.56	16± 0.33 ^a	21± 0.33 ^b	20.0± 0.30 ^c
Total Protein (g/100ml)	2.91± 0.43	6.1±0.10 ^a	6.9± 0.10 ^b	9.0± 0.30 ^c
Albumin (g/100ml)	2.91± 0.43	3.0± 0.05	2.7±0.18	1.5±0.30 ^c

Values expressed as mean±S.D where (n=4). Different superscripts indicate significant indicate significant (P<0.05) within each row.

TABLE IV shows serum cholesterol and metabolites in dogs naturally infected with *Rhipicephalus sanguineus*

Parameters	Pre-infection	Post infection period (weeks)		
		1	2	3
CHOL (mg/100ml)	107.00±2.00	136.23±4.60 ^a	159.00± 13.00 ^b	203.00± 7.50 ^c
TRIG (mg/100ml)	65.00±5.00	115.36±6.37 ^a	111.00± 7.50 ^b	128.00±2.80 ^c
Urea (mg/100ml)	15.05± 3.52	15.50± 2.50 ^a	34.00± 6.75 ^b	48.00± 4.25 ^c
Creatinine (mg/100ml)	0.71±0.081	0.90±0.01 ^a	1.50± 0.13 ^b	2.80± 0.18 ^c

Values expressed as mean±S.D where (n=4). Different superscripts indicate significant (p<0.05) within each row.

DISCUSSION and CONCLUSION

R. sanguineus ticks are known vectors of pathogens like *Babesia canis* and *Ehrlichia canis*, the etiological agents of canine babesiosis and canine monocytic ehrlichiosis, respectively. Interestingly, it has been suspected that *R. sanguineus* is involved in the transmission of other major pathogens such as *Leishmania infantum* the etiological agent of visceral leishmaniasis (Coutinho *et al.*, 2005). The role of *R. sanguineus* ticks in the transmission of pathogens to humans has been documented (Palmas *et al.*, 2001). Additionally, *R. sanguineus* ticks are vectors of *Rickettsia rickettsii*, the etiological agent of Rocky Mountain spotted fever (Demma *et al.*, 2005, Wikswow *et al.*, 2007). In the Mediterranean

region, *R. sanguineus* ticks are vectors and reservoirs of *Rickettsia conorii*, the etiological agent of Mediterranean spotted fever (Matsumoto *et al.*, 2005).

The blood is an important diagnostic tool for clinicians' in human and veterinary medicine and that almost every change that takes place in the mammalian body is reflected in the blood (Oyagbemi *et al.*, 2011). The main function of the RBC is the transportation of oxygen to the tissues of the body. Any pathological or physiological condition that affects the RBC will ultimately alters its function and is potentially detrimental to the body. Hence, anaemia is a disease which is characterized by a reduction in the concentration of haemoglobin, circulating red blood cell and packed cell

volume per unit of the peripheral blood below the normal (Oyagbemi *et al.*, 2011).

In this study, we obtained a significant increase in serum aminotransferases (AST and ALT) and phosphatase (ALP) from one to three weeks post infection. Increases in serum AST and ALT levels have been reported as a pointer to hepatic structural damage because these enzymes (aminotransferases) are normally localized within the cytoplasm and released into circulation after cellular damage has occurred (Kaplowitz, 2001). In the same vein, elevated activities of AST and ALT in plasma are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver and that their estimations are useful quantitative markers of the extent of hepatic damage (Rajesh and Latha, 2004). Hence, *R. sanguineus* infestation might be associated with liver damage in domestic animals. Decrease serum albumin and total protein are common phenomenon associated with liver disease (Kaplowitz, 2001). But according to our study, it was contrary. We only observed a decreased serum albumin and an increased serum total protein.

The reduced serum albumin is a good indicator of liver damage while increased serum total protein was found to be associated with chronic renal failure (CRF) accompanied by abnormal muscle protein degradation and synthesis, metabolic acidosis and ureamia (Johansen, 2009, Muscaritoli, 2009). The electrolyte loss/imbalance has been found to be associated abnormal renal function (Huang, 2007, Olgar, 2005) and as indicated from our finding it might also be associated with kidney failure. Increase in serum creatinine and blood urea nitrogen has also been associated with renal injury (Waikar and Bonventre, 2006). This was supported with significant increase in blood urea nitrogen (BUN) and creatinine as observed in this study. Hence, this therefore confirms the association between renal failure and elevated serum creatinine and blood urea nitrogen. In the same

vein, aberration in haematological parameters with resultant anaemia might not be unconnected with renal damage as previously reported (Anand, 2008, Kaldara-Papatheodorou *et al.*, 2010)

Previous studies have reported the association between total cholesterol and triglycerides to be inversely related to the risk of cardiovascular disease (Muscaritoli, *et al*, 2009). In our study, serum triglycerides and total cholesterol showed a significant elevated concentration. Therefore, hypercholesterolemia observed in this study might be associated with associated with obstructive jaundice and also predispose to cardiovascular dysfunction (Vaziri, 2006).

In conclusion, *R. sanguineus* tick infestation could result in anaemia and predispose to hepato-renal damage with resultant cardiovascular dysfunction and ultimately death. Therefore, *R. sanguineus* ticks are a major public health concern to domesticated animals in sub-Saharan Africa and the world in general. Hence, proper ectoparasite control methods should be put in place to circumvent diseases they transmit.

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