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Co-factors associated with hemato-biochemical changes in canine *Babesia gibsoni* infection

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Abstract
Tick borne pathogens such as *Babesia canis, Babesia gibsoni* and *Ehrlichia canis* can cause diseases in canines. Different parasites are associated with different hematological parameters in the affected dogs. Hematological changes developed during infection may be diagnosed by laboratory examination of blood samples from dogs showing clinical signs. This study was carried out in dogs naturally infected with *Babesia gibsoni* infection for hemato-biochemical changes that are associated as co-factors. The hematological alterations in virtue of their associated factors experienced the changes in Hb level and in RBC count. These factors are associated with erythrocytes damage, removal of the parasitized RBCs and by the changes in the function of the immune system. Low levels of MCV & MCH values observed in this study indicated the microcytic hypochromic anemia, due to acute infections in the bone marrow. In the biochemical reactions, changes in SGPT, SGOT values were observed which could be due to liver damage. The elevated ALP values indicated the biliary system damage and the increased level of BUN is considered as a change in the RBCs catabolism.

Keywords: Co-factors-biochemical changes-*B gibsoni*

Introduction
Tick borne pathogens cause illness in dogs. The parasitic diseases, babasiosis & ehrlichiosis transmitted by *Rhipicephalus sanguineous* (Shaw S.E *et al.* 2001) [19]. Various authors reported the prevalence of babesia in general (Sorawat Thongsahuan *et al.* (2020) [24] and *babesia gibsoni* infection in dogs in particular. Dogs are infected by various ticks. These dogs develop varying clinical signs. The clinical signs such as high fever, drowsiness, loss of appetite, pale mucus membrane, vomiting and weight loss & inco-ordination are observed in these cases (Dantas-Torres C, *et al.* 2008, Sorawat Thongsahuan *et al.* (2020) [5, 24]. B*abesia canis* and *babesia gibsoni* is large pear shaped and small round to oval in shapes respectively (Salgeno-Gallego L. *et al.* 2016) [22]. Shaw S.E *et al.* 2001 [19]; Irwin PJ, (2004) [10] reported red blood cell destruction and anemia in this infection. Diagnoses are being carried out based on the clinical manifestations and laboratory findings. Microscopic examination of the blood smear allows the identification of blood parasites by their morphology (Franco-Zetina *et al.* 2019; Harrus S., *et al.* 2002; Mylonakis ME, 2003) [7, 9].
All tick borne pathogens develop hematological changes directly or indirectly. Screening for hematological alteration is important in routine laboratory diagnosis for blood parasites. (Ullah *et al.* 2018; Awoke N, *et al.* 2019; Latif I & Jamal A, 2015) [29, 3, 13]. Meinkoth *et al.* (2002) [14] reported low RBC count which could be associated with direct damage of erythrocytes by the parasites, removal of the parasitized RBCs and destruction of erythrocytes by immune process. Jain *et al.* (2017) [11] reported low level of Hb, total RBCs and platelet count in canine *B gibsoni* infection.
Vishnurahav *et al.* 2014; Anju S. *et al.* 2022) [30, 1] reported anemia, inconsistent leucocyte counts, differential leucocyte count & mild lymphocytosis in canine *B gibsoni infection.* Vishnurahav *et al.* (2014) [30] opined that these reactions are due to enhanced immune response of the body due to chronic *B. gibsoni* infection. Solano-Gallego *et al.* (2008) [23] reported anemia in all species because of vascular hemolysis, injury and rupture of RBCs, increased osmotic fragility of the cells, and due to the activity of secondary immune mediated processes. Reported oxidation in the erythrocyte, anti-erythrocyte antibodies development, osmotic fragility etc. to the damage of erythrocytes.
Thrombocytopenia in *Babesia gibsoni* infection occurs and persisted in the infected dogs in the system even after the anemia was reported. (Anju S, *et al.* 2022) [1].

Solano-Gallego L *et al.* (2011) [21] reported varying level of thrombocytopenia, and mild to severe anemia. Thomas *et al.* & Preena *et al.* (2019) [16] reported varying degree of thrombocytopenia, a consistent finding in *B. gibsoni* infection, but bleeding was not observed in all infected animals. It could be due to the altered functional activity of the thrombocytes (Kettener *et al.* 2003) [12]. Rafaj *et al.* (2013) [17] reported leucopenia in *B. gibsoni* infected dogs, due to the binding ability of the platelet to the endothelial cells. Further interaction with leucocytes takes place and the secondary capture induced in low level of leucocytes. Sohoeman *et al.* (2009) [20] reported decreased levels of MCV and MCH. These levels indicating normocytic normochromic anemia, since acute infections of the bone marrow takes 3-5 days to respond to the red blood cell damage and so the blood picture reflect non regenerative anemia.

Alkaline phosphatase is higher in *B. gibsoni* infection in dogs by serum biochemistry. Total protein, albumin level, and albumin: Globulin ratio, creatinine and BUN values not unaltered. The alteration observed in the complicated babesiosis cases when more than two organs infected. Anju S, *et al.*, (2022) [1] reported that majority of the animals presented with *B. gibsoni* infection were without any organ impairment.

Salgeno-Gallego L. *et al.* (2016) [22] reported elevated values of SGPT& SGOT in moderate to severely affected *babesia gibsoni* infected dogs both in acute and chronic form. Anemia in dogs with babesiosis is considered as one of the factors causing hypoxia and hypoxic liver injury. It resulted in increased level of ALT, AST & ALP. (Zyger W, *et al.* 2011 [32]; Taboada JJ & Lobetti R 2006) [25]. Both ALT and AST are present in high concentrations in hepatocytes. Any damage to hepatocytes can result in the leakage of ALT and AST in to the blood circulation. (Giannini EG *et al.* 2005 [8]; Wadhwa DR, *et al.* 2011) [31]. Increased level of ALP may also be due to damage or abnormal function of the biliary system. Thankachan *et al.* (2020) [26] reported significant variations in A:G ratio, ALT/SGPT and GGT values in severe organ dysfunctions in complicated babesia cases and elevated serum biochemical values with organ failure in *B. gibsoini* cases. De Scally *et al.* (2004) [6] reported a case with elevated BUN and creatinine levels and an unaltered creatinine values in some other animals, and suggested that uneven rise in BUN level might be associated with increased catabolism in the infected RBCs.

Materials and methods

Case reports

Two numbers of dogs' i.e. German Sphered and Pomeranian each one, brought to a private veterinary clinic in Jaipur were taken for this study. The dogs infested with ticks along with the clinical signs of pyrexia, pale mucus membrane, drowsiness, in-coordination, congested mucus membrane & poor appetite.

Laboratory investigation

The blood smears stained with Giemsa to see the blood parasites. Hematological values i.e. erythrocyte count, Hb, MCV, MCH, WBC count, platelet count and WBC differential count were analyzed. The biochemical parameters i.e. SGPT, SGOT, alkaline phosphatase, blood urea nitrogen (BUN) and creatinine levels analyzed with their normal range values.

Results and Discussion

Table 1: Hemato-biochemical values in *B. gibsoni* infection (GSD & Pomeranian breeds)

Parameters	units	Observation ranges		Reference ranges
		GSD	Pomaranian	
Hb	g/dl	11.8	8.40	12.0-18.0
TLC	thou/mm^3	8.4	9.40	6.0-17.0
DLC				
Neutrophils	%	80.0	66.00	60.0- 70.0
Lymphocytes	%	14.00	27.00	12.0-30.0
Eosinophil	%	2.00	3.00	2.0-10.0
Monocytes	%	4.00	4.00	3.0-10.0
Basophils	%	0.00	0.00	0.00-1.00
ALC				
Neutrophils	thou/mm^3	6.72	6.20	3.0-11.0
Lymphocytes	thou/mm^3	1.18	2.54	1.0-4.80
Eosinophil	thou/mm^3	0.17	0.28	0.1-1.3
Monocytes	thou/mm^3	0.34	0.38	0.1-1.40
Basophils	thou/mm^3	0.00	0.00	0.0-0.1
RBC	mill/mm^3	5.00	4.48	5.50-8.50
PCV	%	43.80	24.50	37.0-55.00
MCV	FL	53.00	54.70	60.00-77.00
MCH	PG	18.000	18.80	19.00-24.50
MCHC	g/dl	33.80	34.30	32.0-36.00
Platelet count	thou/mm^3	215.0	215.0	200.0- 900.00
Bio-chemical Reactions				
SGOT	u/l	49.70	160.3	9.00-49.0
SGPT	u/l	269.70	92.10	8.00-57.00
Alkaline phosphatase	u/l	431.70	374.80	10.00-10.00
BUN	mg/dl	26.42	60.47	8.8-25.9
Creatinine	mg/dl	0.83	0.78	0.5-1.6

Blood samples collected from the above dogs' i. e GSD and Pomeranians infested with *Rhipicephalus sanguineus* showed the clinical signs of pyrexia, pale mucus membrane, drowsiness, in-coordination, congested mucus membrane & poor appetite. The blood smears from these dogs revealed the infection of *Babesia gibsoni.* No other blood parasites were detected in these blood smears. Various authors reported the prevalence of babesia in general (Sorawat Thongsahuan *et al.* (2020) [24] and *babesia gibsoni* infection in dogs in particular. The hemato-biochemical values obtained from the naturally infected dogs with certain hematological changes presented in the table.

Hematology

The Hb values were 11.8 & 8.40 g/dl in GSD& Pomeranian dogs respectively (As against the normal values of 12.0-18.0). Jain *et al.* (2017) [11] reported Hb, total RBCs and platelet count at low level in canine *B. gibsoni* infection which coincides with the observation of this study. The RBC count was 5.00 in GSD & 4.48 in Pomeranian respectively (As against the normal values of 5.5-8.5 mill/mm^3). Meinkoth *et al.* (2002) [14] reported low RBC count and asserted that could be associated with direct damage of erythrocytes by the parasites, removal of the parasitized RBCs and destruction of erythrocytes by immune process. Vishnurahav *et al.* (2014) [30] reported anemia in dogs infected with canine babesiosis infection due to red blood cells destruction. Solano-Gallego *et al.* (2008) [23] reported anemia in all species caused by vascular hemolysis because of parasite injury and rupture of RBCs.

In this study no thrombocytopenia was observed. Solano-Gallego L *et al.* (2011) [21] reported thrombocytopenia in mild to severe form, as doe's anemia. Thomas *et al.* & Preena *et al.* (2019) [16] also reported varying degree of thrombocytopenia and it was a consistent finding in *B. gibsoni* infection, but spontaneous bleeding not observed in any all infected animals. Kettener *et al.* (2003) [12] suggested that thrombocytopenia could be due to the functional activity of the thrombocytes. No leucopenia was observed in this study, even though Rafaj *et al.* (2013) [17] reported it in *B. gibsoni* infected dogs.

The MCV values were 53.00 in GSD & 54.70 in Pomeranian respectively (As against the normal values of 60.0- 77.00 FL) and the MCH values were 18.00 in GSD & 18.80 in Pomeranian respectively (As against the normal values of 19.5-24.5 pg).The MCV & the MCH values were lower than the normal range of values in all these infected cases. Sohoeman *et al.* (2009) [20] also reported low levels of MCV and MCH levels which indicating normocytic normochromic anemia, since acute infections of the bone marrow takes 3-5 days to respond to the red blood cell damage and thus the blood picture reflect non regenerative anemia.

Hemato-biochemical studies

The biochemical studies revealed high level SGOT i.e. 49.70 in GSD & 160.30 in Pomeranian respectively. (As against the normal values of 9.0-49.0 u/l).The SGPT levels were 269.70 in GSD & 92.10 in Pomeranian respectively (As against the normal values of 8.0-57.0 u/l). An elevated value of SGPT& SGOT in babesia gibsoni infected dogs was reported by Salgeno Gallego L. *et al.* (2016) [22]. Thankachan *et al.* (2020) [26] also reported significant variations in A: G ratio, ALT/SGPT and GGT with severe organ dysfunctions in complicated babesiosis and expressed the elevated serum biochemical values mostly associated with the organ failure in *B. gibsoni* cases. Zyger W, *et al.* 2011 [32]; Taboada JJ & Lobetti R (2006) [25] reported anemia in babesia infected dogs due to liver injury that resulted in increased levels of ALT, AST & ALP. In addition Giannini EG, *et al.*, 2005 [8] and Wadhwa DR *et al.*, (2011) [31] also reported high levels ALT and AST are present in the blood due to hepatocytes damage that can result in the leakage of ALT and AST in to the blood circulation. The elevated alkaline phosphatase level was 431.70 & 374.8 in GSD & Pomeranian respectively (As against the normal values of 10.0-100 u/l) which is in agreement with the findings of who reported increased level of ALP due to the abnormal function of the biliary system.

The BUN level was 26.42 in GSD & 60.47 in Pomeranian respectively (As against the normal values of 8.8-25.9 mg/dl). De Scally *et al.* (2004) [6] studied a case with elevated BUN and creatinine levels and an unaltered creatinine values in some other animals and suggested that the uneven rise in BUN level might be associated with increased catabolism of RBCs.

Conclusions

The dogs German sphered & Pomeranian breeds brought to a private veterinary clinic, in Jaipur were taken for this study. The above dogs were infested with *Rhipicephalus sanguineus* with the clinical signs of; pyrexia, pale mucus membrane, drowsiness, in-coordination, congested mucus membrane & poor appetite. Blood smear examination revealed *Babesia gibsoni* infection in the above dogs, followed on that, a detailed hemato-biochemical studies carried out to assess the changes occurred in these *B. gibsoni* infected dogs. The study revealed altered hemato-biochemical changes in the *B. gisoni* infected dogs. The co-factors associated for the altered hemato-biochemical values in these *Babesia gibsoni* infected dogs discussed for better diagnostic approach.

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