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PI Ganesan

Professor and Head,
Department of Veterinary Public
Health and Epidemiology,
School of Veterinary and Animal
Sciences, Centurion University of
Technology and Management,
Paralakhemundi Campus,
Odisha, India

Dashrath Khemada

Assistant Professor, Department
of Veterinary Medicine, Apollo
College of Veterinary Medicine,
Jaipur, Rajasthan, India

Sravani G

P.G. Scholar, Department of
Veterinary Microbiology, NTR
College of Veterinary and Animal
Science, Gannavaram,
Andhra Pradesh, India

Corresponding Author:

PI Ganesan

Professor and Head,
Department of Veterinary Public
Health and Epidemiology,
School of Veterinary and Animal
Sciences, Centurion University of
Technology and Management,
Paralakhemundi Campus,
Odisha, India

Co-infections of a Labrador dog with *Babesia canis* and *Babesia gibsoni* and its status for clinical and hemato-biochemical changes

PI Ganesan, Dashrath Khemada and Sravani G

Abstract

Studies on the pathophysiology of a 4 years old, male Labrador dog with clinical signs i.e apathy, weakness, anorexia, pale mucus membrane and poor health condition was admitted in a pet clinic in Jaipur. Clinical examination of the dog confirmed the infestation of *Rhipicephalus sanguineus* ticks. Based on the clinical signs microscopic examination of the blood smears carried out. The infected dog's blood smear revealed small forms of *Babesia gibsoni* and the larger form of *Babesia canis* organisms inside the erythrocytes and the hemato-biochemical parameters pronounced variations from the normal values. The study on the hematological parameters observed declined levels of hemoglobin, Red blood cell count, MCV, MCH, Packed cell volume & Platelet counts, while the Erythrocytes sedimentation rate was elevated. The neutrophil value were got elevated while lymphocytes values were at low level. The biochemical parameter studies showed high levels of SGPT, SGOT and Alkaline phosphatase, which indicated the liver damage due to the infection of both *Babesia canis* and *Babesia gibsoni*. The pathophysiological factors for changes in their hemato-biochemical parameters in co-infection of *Babesia canis* & *Babesia gibsoni* infected dog are discussed in this study.

Keywords: Canine-Labrador, co-infection, Hemato, biochemical changes

Introduction

Canine babesiosis is a wide spread hemo- protozoan disease of domesticated dogs and wild canids (Irwin 2010)^[12]. Solano-Gallego *et al.* (2016)^[29] reported both large and small forms of *Babesia* species (*B. canis*, *B. vogeli*, *B. gibsoni* and *B. microti* like isolates also referred to as *B. vulpes* and *Theileria annae*) infects dogs in Europe. The reports on their prevalence, transmission, clinical manifestations, treatment and prognosis vary for each species of the infected animals. Jain *et al.* (2017)^[13] reported *B. gibsoni* infection in dogs in South Kerala. Clinical cases infected with infection by *B. gibsoni* had been reported in Germany (Hodiz, *et al.* (2015)^[10]. The course of infection related to disease manifestation either acute or chronic. Uilenberg (2006)^[33] reported the large *Babesia canis* and the small *Babesia gibsoni* organisms to infect the dogs. Intra erythrocytic piroplasm stage is often common in *Babesia gibsoni*. Co-infections with *Babesia* sp rarely documented in dogs. Ganesan and Sravani (2023)^[9] reported co-infection state of *Anaplasma marginale* and *Babesia gibsoni* in a Pomeranian dog. Showkat Ahmad Shah, *et al.* (2011)^[25] investigated the hemato-biochemical changes in dogs under natural infection with *B. canis* and *B. gibsoni*. Miro *et al.* (2015)^[18] reported the co-infection status of *B. canis* and *B. gibsoni* in Spanish and further emphasized that the co-infection status is a major clinically important subject, since this status complicates diagnosis by showing enhanced clinical signs. This condition reduces the effective treatment aspects and hence worsen the prognosis. In this study the co-infection status of *Babesia canis* and *Babesia gibsoni* in a Labrador dog discussed with their clinical signs and hemato-biological changes.

Materials and Methods

Case report

A 4 years old, male Labrador dog, admitted in a private pet clinic in Jaipur city for its chronic ailments. Accordingly disease investigation was carried out. Clinical examinations, blood smear examinations followed by hematological studies were carried out in this infected dog.

Clinical examination of the affected dog: Clinical examination of the dog showed i.e. apathy, weakness, anorexia and pale mucus membrane. The dog infested with *Rhipicephalus sanguineus* ticks.

Blood smear studies by staining technique: Infected dog's blood smear was examined after staining the smear by Giemsa as per the procedure of Wang *et al.* 2019 and [35]

Solano-Gallego *et al.* (2016) [29].

Hemato-biochemical studies: Studies on the hemato biochemical values were carried out as per standard protocol.

Results and Discussion: Examination of the blood smear under the microscopic view showed the co-infection of *B. canis* and *B. gibsoni*. (Figure 1 & 2, Table 4).

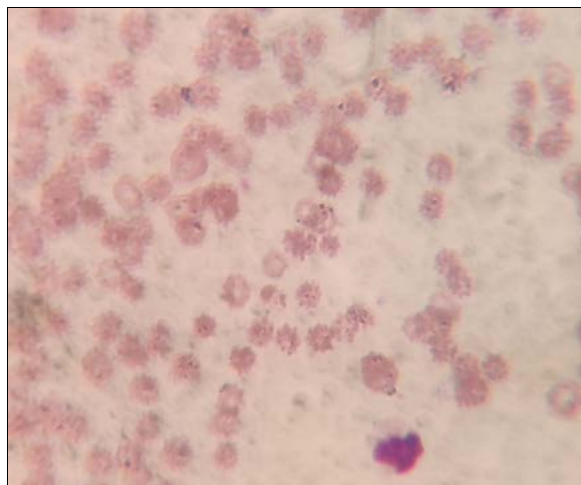


Fig 1: *B. canis*

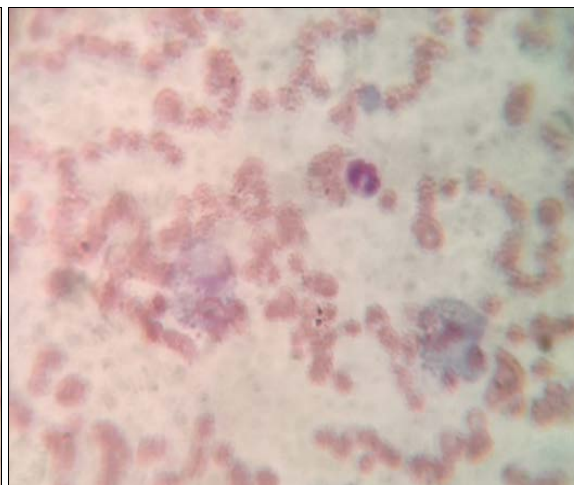


Fig 2: *B. canis* & *B. gibsoni*

Table 1: Hematology

Parameters	Result	Units	Normal range
HGB	5.00	g/dl	12.5-18
E.S.R	48	MM 1 st hour	0-5
PCV	15	%	35-45
RBC	2.01	Million/Cu mm	3.5-6.0
MCV	75	fL	80-95.0
MCH	23	pg	26.5-33.5
PLT	0.50	Lakhs/C mm	1.5-6.5
WBC	16.43	10 ⁹ /L	6.00- 17.00
Neutrophils	70.00	%	50-65
Lymphocytes	22	%	25-45
Basophils	00	%	0-1
Monocytes	01	%	0-5

Table 2: Biochemical-Liver function tests

Parameters			
Total protein	5.7	mg/dl	5.5-8
Albumin	3.30	mg/dl	2.5-5.5
Globulin	2.40	mg/dl	1.5-4.5
A/G ratio	1.37:-:1	mg/dl	1.3.1:2.5:1
Alk.phos	240	mg/dl	70-290
Bilirubin total	0.32	mg/dl	0-0.5
SGOT	98.0	U/L	10-70
SGPT	108	U/L	10-75

Table 3: Biochemical-Kidney function tests

Parameters	Result	Units	Normal Range
Creatinine	0.70	mg/dl	0.1-1.5
Blood urea	42.0	mg/dl	5-50
BUN	20.1	mg/dl	2-25
Calcium	8.70	mg/dl	8.5-11.5
Uric acid	0.32	mg/dl	0.10-0.40
Phosphorus	14.3	mg/dl	1.5-6.0

Table 4: Canine: Microscopic examination

Infectious agents	
<i>B.canis</i>	Positive
<i>B.gibsoni</i>	Positive
<i>H.canis</i>	Negative
<i>E.canis</i>	Negative
<i>Ana plasma Sp</i>	Negative
Mycoplasma hemo canis	Negative
Others (Spiroketes, Bacteria, Microfilaria)	Negative

Studies on clinical signs in Babesiosis

Rojas *et al.* (2014) [23] reported that all *Babesia* species can cause fever, anemia lymphadenopathy, splenomegaly, thrombocytopenia, jaundice and pigmenturia. Solano-Gallego *et al.* 2011 [28]; Irwin *et al.* (2009) [11] reported the clinical signs and varying clinico-pathological parameters which are common to all *Babesia* species infection in dogs and the clinical signs, hematological and biochemical parameter changes could be due to the difficulties in establishing the immune system to eliminate the parasites. Solano-Gallego *et al.* (2016) [29] reported that the anemia is caused by a combination of intravascular and extravascular hemolysis resulting from injury because of the parasites and rupture of red blood cells. It is observed by the author that the osmotic fragility increases with the activity of secondary immune-mediated processes in all species of animals. Jacobson *et al.* (2006) [14] reported the clinico-pathological abnormalities such as weight loss, chronic nephropathy, glomerulonephritis, coagulation disorders, and jaundice from liver disease, thrombocytopenia, hemo-concentration, shock, respiratory acidosis/alkalosis, pancreatitis, vomiting, diarrhea, ocular lesions & myalgia in *B.rossi* infected dogs. Further it is mentioned by the author that some clinical signs and clinico-pathological abnormalities varies in the *Babesia* species infecting dogs. In this case the dog showed anorexia, apathy, weakness and pale mucus membrane as major clinical signs, which was discussed by Jacobson *et al.* (2006) [14].

Studies on the co-infection status of Babesia sp

The study conveyed the blood picture in a co-infection state of *Babesia canis* and *Babesia gibsoni* in the affected Labrador dog. Ganesan *et al.* (2023) [8] reported the co-infection state of *Ehrlichia canis* and *Babesia gibsoni* in a German Sphered dog. Jayabal *et al.* (2019) [15] reported the co-infection state of *B. canis* with *Ehrlichia canis* due to a single species tick infestation i.e. *Rhipicephalus sanguineous*, which is in concurrence with the observation of this study. Santos *et al.* (2018) [24] reported co-infection status of *Ehrlichia/Anaplasma* and *Babesia Sp* or more than two pathogens in the dog population and the blood values indicated thrombocytopenia and this observation is in concurrence with the present study.

Hematology of B. gibsoni

Hematological study showed the declined levels in hemoglobin, RBC and platelet count in *B. gibsoni* infected dogs. (Jain *et al.* 2017) [13]. Vishnurahav *et al.* (2014) [34] conveyed anemia as one of the clinical sign in *Canine babesiosis*. A moderate to severe thrombocytopenia was in picture in *B. gibsoni* infection (Anju, *et al.* 2022) [1]. The reasons for declined levels of the above parameter changes were attributed to immune mediated destruction of erythrocytes (Meinkoth *et al.* 2002) [17]. Schoeman *et al.* (2009) [26] reported declined levels of MCV and MCH in *B. gibsoni* cases indicating normocytic normochromic anemia which was explained for infection of the bone marrow in

acute cases. Dawn Ruben (2018) [5] suggested iron deficiency also for the development of anemia. Furlananello *et al.* (2005) [7] opined that the thrombocytopenia, and the normocytic normochromic anemia, both for re-regenerative and non-regenerative anemia in the *Babesia gibsoni* infected dogs. These observations observed in this study also.

Hematology of B. canis

Solano-Gallego *et al.* (2008) [27] reported anemia in the position of regenerative or non-regenerative and it depends on the infective species and the course of infection. In *B. canis* infection and it is non-regenerative form of anemia, due to dysfunctional bone marrow. Omobowale *et al.* (2017) [21] reported higher neutrophil/lymphocyte ratio in complicated babesiosis cases due to *B. canis* infection and this observation is concurrence with the findings of this study. Booser and McIntire (2003) [3] conveyed changes in neutrophils and lymphocytes ratio in canine *B. canis* infection and suggested altered neutrophil/ lymphocyte ratio can be taken as a as a diagnostic feature in complicated *Babesia* cases. Sorawat Thongsahuan *et al.* (2020) [30] reported anemia, thrombocytopenia, eosinopenia and lymphopenia in *B. canis* infected dogs.

Hematology of co-infection status of B. gibsoni and B. canis in a Labrador dog

In this Labrador dog the blood parameters i.e. hemoglobin level, packed cell volume value, RBC count, platelet count & MCV, MCH levels were declined from the normal values. Increased level of ESR denoted the poor erythrocyte production and it could be due to bone marrow depression and poor functional status of the infected liver. Elevated neutrophil and low level lymphocytes i.e. altered neutrophil/lymphocyte ratio were observed in this Labrador dog and the MCV and MCH values were observed in a declined phase in this case. Schoeman *et al.* (2009) [26] indicated normocytic normochromic anemia because of acute infection of the bone marrow due to the infection of *B. gibsoni*. Niwetpathomwat *et al.* (2006) [20] studied the hemato-biochemical parameters occurred in dogs naturally infected with *Babesia* and mentioned it as microcytic hypochromic anemia. It was opined by the author that the anemia in babesiosis could be due to enhanced osmotic fragility of erythrocytes and thrombocytopenia due to immune mediated platelets destruction. Makinde and Bobade 1994 [16]: Onishi and Suzuki (1994) [22] reported these information in their studies on babesiosis. Murase *et al.* 1996 [19]; Tvedten (2004) [32] suggested oxidative stress as a cause to damage the erythrocytes, that can result in increased susceptibility of phagocytosis. Brockus and Andraeasen (2003) [3] suggested declined phase of hemoglobin value due to extra vascular hemolysis. Booser and Macintire (2003) [3] mentioned that the platelet sequestration in the spleen or immune mediated platelet destruction and intravascular coagulation could be the reasons for thrombocytopenia. Eichenberger *et al.* (2016) [6] reported thrombocytopenia was a characteristic feature in *Babesia* positive cases. Rojas *et al.* (2014) [23] mentioned thrombocytopenia in all *Babesia* species affected dogs and it varies from mild to moderate as doe's anemia. Meinkoth *et al.* (2002) [17] reported declined phase of the hematological changes and it is opined for poor performance of the immune system because of destruction of erythrocytes. Showkat Ahmad Shah, *et al.* (2011) [25] investigated the blood value changes in dogs naturally infected with *B. canis* and *B. gibsoni* in decreased levels. The authors reported declined

phase level of hemoglobin, red blood cells, packed cell volume, MCV, and platelets in their studies. The hematological studies in this case are in concurrence with the findings of the above mentioned authors.

Biochemical studies in co-infection status of *B. gibsoni* and *B. canis*

In this case the SGOT and SGPT levels (Table3) were increased, which indicated the direct damage to the liver by the blood parasites (Torres-Velez *et al.* (2003) [31]. Bilawal *et al.* (2018) [2] attributed the reason for the high level of alkaline phosphatase due to hepatic dysfunction in *Babesia canis* infection. Solano-Gallego *et al.* 2011[28]; Irvin (2009) [11] narrated varying hematological and biochemical parameter changes in *Babesia* parasite infected populations and opined it could be due to the decreased functioning level of the immune system in the elimination process of the blood parasites or when the immune system is in abatement and these statements are in concurrence with the findings of the present case studies.

Conclusions

The co-infection of *Babesia canis* and *B. gibsoni* in a Labrador dog with the clinical manifestations of anorexia, lethargy, pale mucus membrane and dullness was studied. With these clinical signs the blood parameter were studied and the major changes occurred in the hematology and biochemistry values in the co-infection status of *B. gibsoni* and *B. canis* infection regarded in a Labrador dog. The risk factors associated for the development of variable clinical manifestations and hemato-biochemical parameters were attributed to poor management of the dog and repeated exposure of the dogs for tick infestation and thereby rendering the dog's system for abatement of the immune process.

Conflict of Interest

Not available

Financial Support

Not available

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