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Sandeep
Post Graduation Student, Department of Medicine, Veterinary College Hebbal, Bengaluru, Karnataka, India

Basavaraj Vadari
Post Graduation Student, Department of Parasitology, Veterinary College Hebbal, Bengaluru, Karnataka, India

Sumathi BR
Assistant Professor, Department of Microbiology, IAH & VB Hebbal, Karnataka, India

Corresponding Author:
Sandeep
Post Graduation Student, Department of Medicine, Veterinary College Hebbal, Bengaluru, Karnataka, India

Diagnosis of canine babesiosis

Sandeep, Basavaraj Vadari and Sumathi BR

Abstract
The present study was conducted at Department of Veterinary Medicine, Veterinary College Hospital, Hebbal, Bengaluru with an objective of Diagnosis of canine babesiosis by blood smear and PCR, studying the haematological and biochemical parameters in canine babesiosis. The study was conducted for a period of six months from July 2022 to Dec 2022. A total of 50 blood samples indicative of canine babesiosis were collected. Characteristic clinical features observed among the positive cases were high fever, anorexia, inappetance, lethargy, vomiting, pale mucous membrane, jaundice and ascites. Fifty blood samples were collected and subjected to microscopic examination of stained blood smears and PCR, out of which 21 (42%) samples found positive by microscopy and 35 (70%) samples were found positive by PCR, indicating that PCR is more sensitive diagnostic tool for diagnosis of canine babesiosis. The haematological alterations observed were anaemia, thrombocytopenia and leucocytosis. Serum biochemistry changes included increased ALT, BUN and total bilirubin and decreased total protein. Serum creatinine is in the normal range.

Keywords: Babesia canis vogeli, *B. gibsoni*, PCR, blood smear examination, genus specific primers

Introduction
Babesiosis is a common and clinically significant tick-borne haemoprotozoan parasitic disease of domesticated dogs and wild canids with a worldwide distribution and typically characterized by haemolytic anaemia, thrombocytopenia, fever and splenomegaly. It is a very common life threatening and clinically significant disease caused by intraerythrocytic apicomplexan parasites of the genus *Babesia*. It is one of the most important vector-borne diseases of dog caused by *Babesia canis* and *Babesia gibsoni* throughout the globe. *B. canis* is the large form (3.0-5.0 µm), while *B. gibsoni* is a small pleomorphic organism (1.5-2.5 µm) and appears most commonly as ring form. It is found in almost all parts of Asia, Europe, Africa, America and Australia and transmitted by *Dermacentor reticulatus* in Europe, *Rhipicephalus sanguineus* in tropical and subtropical regions and *Haemaphysalis leachi* in South Africa (Uilenberg *et al.*, 1989) [30].

Acute *Babesia* infections are typically associated with fever, progressive anaemia, lethargy, thrombocytopenia, haemoglobinuria, marked splenomegaly and hepatomegaly (Wozniak *et al.*, 1997 and Goo *et al.* 2008) [34, 13]. Chronic infections are more common and infected dogs remain as carriers without any overt clinical signs (Conrad *et al.*, 1991) [7]. Cases of canine babesiosis may be presented with a wide variation of clinical signs. Lethargy is the most common symptom, followed by anorexia, pale mucous membrane, vomiting, amber to brown urine, splenomegaly, jaundice, weight loss, tachycardia and tachypnoea.

Babesia canis is presumed to be the only large *Babesia* species to infect dogs throughout the world. Historically, the microscopic identification of large (3 - 5 mm) piroplasms in canine erythrocytes was sufficient for the diagnosis of a *B. canis* infection. But recent molecular studies have shown the existence of three subspecies of *B. canis*: *Babesia canis canis, Babesia canis rossi, and Babesia canis vogeli*. And these subspecies differ in their geographic location, pathogenicity, antigenicity and also in vector specificity. Pathogenicity and treatment are known to vary for each subspecies, hence there is a need for precise identification of subspecies. As far as the diagnosis of canine babesiosis is concerned, direct microscopic examination of the stained blood smear is the simplest, commonly used, conclusive, feasible and cost-effective diagnostic method (Caccio *et al.*, 2012) [6]. But molecular diagnosis is more sensitive, specific and rapid method for diagnosis of babesiosis and has allowed further characterization by genotype into several subspecies.

Material and Method

A total of 50 dogs with clinical signs suggestive of babesiosis presented to the Department of Veterinary Medicine, Veterinary College Hospital, Hebbal, Bengaluru with a history of anorexia, tick infestation, lethargy, weakness and red coloured urine (haemoglobinuria) were included for the study.

Clinical examination of the affected dogs revealed pyrexia (105.2 ºF), enlarged lymph nodes and pale mucous membrane. Blood samples were collected from infected animals in a separate EDTA vial and serum vials for preparation of blood smear, estimation of hematological and biochemical parameters for haemato-biochemical study. About 1 ml blood of sample from each case was utilised for haematological estimation and blood smear preparation and PCR and remaining was processed for serum extraction. Haematological parameters (haemoglobin, packed cell volume, total erythrocyte count, total leucocyte count and platelet count) were estimated with the help of BC-2800 Vet, Auto Haematology Analyzer. Serum samples were used for the estimation of biochemical parameters like Alanine transaminase. blood urea nitrogen, creatinine, total protein, bilirubin. semi-automatic serum biochemistry Analyzer (RX-50 of Micro Lab).

The diagnosis of babesiosis was confirmed by the demonstration of parasites within the infected erythrocytes in Giemsa- stained thin blood smears. DNA was extracted from the whole blood sample using QIA amp DNA blood mini kit (M/s QIAGEN Germany) and subjected to genus specific PCR for babesia organisms.

PCR protocol I.

The PCR for the amplification of 18S rRNA gene of *Babesia* organism in the blood samples, was carried out as per the method of Duarte *et al.* 2011 [9].

Genus specific primer for *Babesia* (Duarte *et al.*, 2011) [9]

Forward primer Bab7: F51 GGCTACCACATCTAAGCAAG 31

Reverse primer Bab9: R51 CTAAGAATTTCACCTCTGACAG 31

Extracted DNA (2.5 µl) was used as a template to amplify a fragment of the 18S rRNA gene using the following reaction mixture

Reagents	Volume (µL)
Deoxy-nucleoside triphosphates	2.5
Buffer	2.5
Enzyme (Taq DNA polymerase)	0.5
Forward primer Bab7 (10 pmol/µL)	1.5
Reverse primer Bab9 (10 pmol/µL)	1.5
Template DNA (sample)	2.5
Sterile Nuclease free distilled water	14
Total volume (µL)	25

The PCR was carried out for *Babesia* in a thermal cycler (Eppendorf, Germany) using the following cycle condition profile as per the procedure described by Duarte *et al.*, 2011 [9]

Step 1	Initial denaturation	94 °C for 4 minutes	
Step 2	Denaturation Annealing	94 °C for 30 seconds 50 °C for 30 seconds	35 cycles
	Extension	68 °C for 30 seconds	
Step 3	Final extension	68 °C for 7 minutes	
	4°C thereafter		

Analysis of the PCR product

Analysis of the PCR product was done by submarine gel electrophoresis as follows:

The gel tray was prepared by placing the comb at one edge of the tray. Fifty ml of 1.0

% agarose gel (w/v) containing 3.5 µl of Ethidium bromide was poured in to the tray and allowed to solidify. After the gel was completely set, the comb and the tape were carefully removed. The tray with the gel was mounted in the electrophoresis tank and 1X TAE buffer was added to the tank sufficient to cover the gel.

The PCR product (10 µl) was loaded in to the wells alongside 100 bp DNA marker (10 µl). Submarine gel electrophoresis was carried out at 150 volts for 45 min. The PCR products were visualized using an UV transilluminator and photographed using gel documentation system for the expected sized PCR product of 490 bp for *Babesia* organisms.

Mindray Auto Haematology Analyzer BC 2800 Vet

Analyzer BC 2800 Vet

Results

Clinical signs

Most common clinical signs reported in *Babesia* infected cases were fever (77%), anorexia (74%), pale mucous membrane (66%), lethargy (54%) followed by vomiting (43%), inappetence (29%), jaundice (17%) and ascites (6%). Ticks were found on the body of 69 per cent (24/50) dogs (Table 1).

Table 1: Clinical signs of canine babesiosis

Clinical signs/findings	*Babesia* infected (n=35)	Total (Percentage)
Fever	27	77
Anorexia	26	74
Pale mucous membrane	23	66
Lethargy	19	54
Vomiting	15	43
Inappetence	10	29
Jaundice	6	17
Ascites	2	6

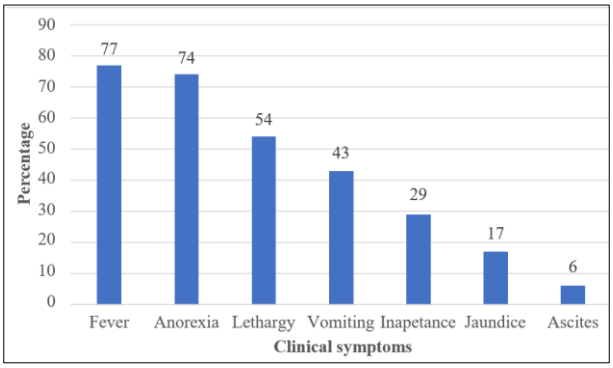


Fig 1: Clinical symptoms of canine babesiosis

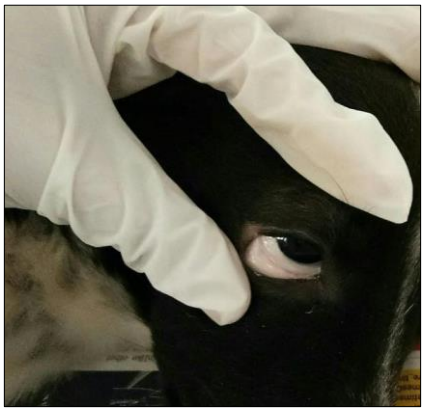


Plate 1: Dog with Babesiosis showing pale conjunctival mucous membrane

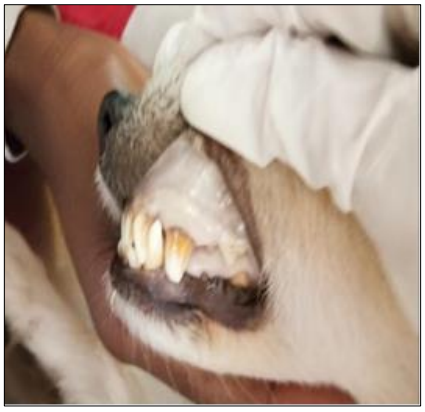


Plate 2: Dog with Babesiosis showing pale oral mucous membrane

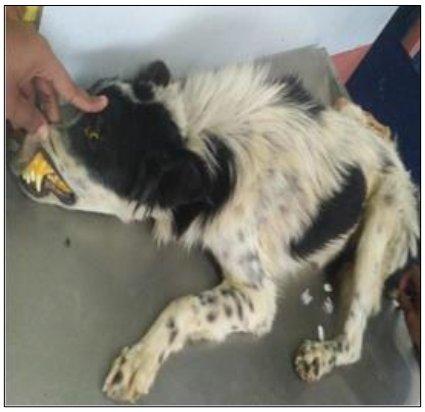


Plate 3: Dog with Babesiosis showing icteric oral mucous membrane

Plate 4: Dog with Babesiosis showing icteric conjunctival mucous membrane

Plate 5: Dog with Babesiosis showing icteric ear

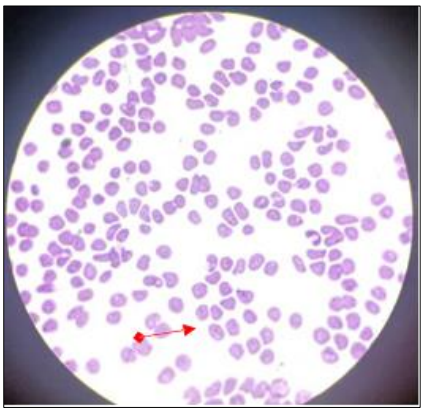


Plate 6: Giemsa-stained blood smear showing *Babesia* organism

Plate 7: Dog with Babesiosis showing ascites

Plate 8: Dog with Babesiosis showing tick infestation

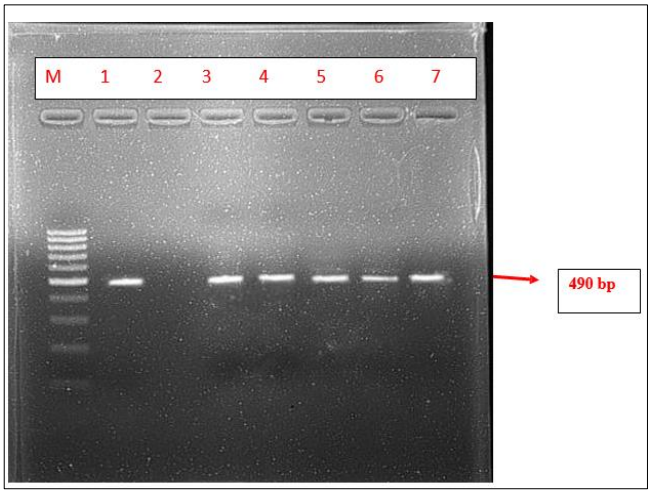
By blood smear examination

A total of 50 blood samples (randomly) from dogs showing clinical symptoms suggestive of babesiosis were collected. All the blood sample were screened by thin blood smear examination by using Giemsa-stain. Microscopic examination of the stained blood smear revealed several pleomorphic forms of *Babesia* organisms in 21 out of 50 *i.e.*, 42 per cent dogs were positive for canine babesiosis (Plate 6).

Polymerase Chain Reaction (PCR)

In the present study, DNA was extracted from the same fifty blood samples and were subjected to polymerase chain targeting 18S rRNA gene fragment using genus specific primers. Thirty-five samples out of fifty were positive for canine babesiosis.

Identification of *Babesia* organism was based on the band produced in accordance with band of positive control of 490 bp for *Babesia* organisms. In all the positive samples of Babesiosis 490 bp bands was produced which were visualised by using UV transilluminator



Genus- specific PCR product showing positive for *Babesia* (490 bp)

Lane M	100 bp DNA ladder
Lane 1	Positive control of *Babesia*
Lane 2	Negative control of *Babesia*
Lane 3, 4, 5, 6, 7	Positive sample of *Babesia*

Haematological analysis

Results of the haematological analysis of all the fifty blood samples which were positive both by microscopy and PCR, are presented in Table 3.

Table 3: Mean ± SE of haematological values of apparently healthy dogs (control group) and dogs infected with babesiosis

Parameters	Control group of dogs (n=10)	*Babesia* infected dogs (n=50)
Total erythrocyte count (×106/ µL)	6.50±0.17	1.99*±0.12
Total leucocyte count (×103/ µL)	13.90±0.40	21.44*±1.79
Haemoglobin (g/dL)	14.20±0.45	4.92*±0.24
Packed cell volume (%)	42.88±2.61	14.93*±0.67
Platelet count (×103/ µL)	275±3.47	48.22*±3.26
Mean corpuscular volume (fl)	69.58±2.61	72.80±2.23
Mean corpuscular haemoglobin (pg)	25.30±0.61	23.35±0.44
Mean corpuscular haemoglobin concentration (mg/dL)	34.12±0.33	32.45±0.54

***** Indicates statistically significant

Mean ± SE values of TEC among dogs which were diagnosed as babesiosis was 1.99±0.12×106/ µL. On the contrary Mean ± SE value of TEC in apparently healthy dogs (control) was 6.50±0.17×106/ µL (Table 3). The TEC value was significantly lower among *Babesia* infected dogs as compared to the control group.

Mean ± SE values of TLC in dogs infected with *Babesia* organism was 21.44±1.79×103/ µL. Whereas Mean ± SE value of TLC in apparently healthy dogs was 13.90±0.40×103/ µL (Table 3). The TLC values were significantly higher in *Babesia* infected dogs as compared to the control group.

Dogs which were positive for babesiosis had a Mean ± SE haemoglobin value of 4.92±0.24 g/dL. Whereas the same among control group dogs was 14.20±0.45 g/dl (Table 3).

Significantly lower mean haemoglobin value was noticed in *Babesia* infected dogs as compared to the control group.

14.93±0.67% was the Mean ± SE of PCV in dogs which were positive for *Babesia* whereas Mean ± SE of PCV value in apparently healthy dogs was 42.88±2.61% (Table 3). The mean PCV value was significantly lower in *Babesia* infected dogs.

Mean ± SE values of total platelet count was 48.22±3.26 x103/µL, among *Babesia* infected dogs and the Mean ± SE value of total platelet count in apparently healthy dogs was 275±3.47x103/µL (Table 3). The mean total platelet value was significantly lower in *Babesia* infected dogs when compared to the control group.

Serum Biochemistry analysis

Results of the serum biochemistry analysis of all the thirty-five blood samples which were positive both by microscopy and PCR, are presented in Table 4.

Table 4: Mean ± SE of Serum biochemistry values of apparently healthy (control group) dogs and dogs infected with babesiosis.

Parameters	Control group (n=10)	*Babesia* infected dogs (n=50)
ALT (IU / L)	29.84±1.54	134.9*±9.47
Creatinine (mg/dL)	0.90±0.03	1.14±0.10
Blood urea nitrogen (mg/dL)	19.84±0.54	29.25*±0.61
Total protein (g/dL)	6.80±0.15	5.38±0.03
Bilirubin (mg/dL)	0.20±0.04	0.54*±0.05

***** Indicates statistically significant

Mean ± SE values of ALT in dogs infected with *Babesia* was 134.9±9.47 IU and apparently healthy dogs was 29.84±1.54 IU (Table 4). The mean ALT value was significantly higher in *Babesia* infected dogs as compared to the control group.

1.14±0.10 mg/dL was the Mean ± SE of serum Creatinine in dogs infected with *Babesia* whereas Mean ± SE value of Creatinine in apparently healthy dogs was 0.90±0.03 mg/dL (Table 4). On comparison the mean creatinine value of *Babesia* infected dogs was higher than the apparently healthy dogs.

Mean ± SE values of BUN in dogs infected with *Babesia* and apparently healthy dogs were 29.25±0.61mg/dL and 19.84±0.54 mg/dL respectively (Table 4). The mean BUN values were significantly higher in *Babesia* infected dogs.

Mean ± SE values of TP in dogs infected with *Babesia* was 5.38±0.03 g/dL and that of apparently healthy dogs was 6.80±0.15g/dL (Table 4). The mean TP value was significantly lower in *Babesia* infected dogs as compared to the control group.

Mean ± SE values of total bilirubin in dogs infected with *Babesia* was 0.54±0.05 mg/dL. Whereas mean value of Bilirubin in apparently healthy dogs was 0.2±0.04 mg/dL (Table 4). The Bilirubin values were significantly higher in *Babesia* infected dogs as compared to the control group.

Discussion

Clinical symptoms

Most common clinical signs reported in *Babesia* infected cases were fever (78%), anorexia (74%), pale mucous membrane (68%), lethargy (38%) followed by vomiting (30%), inappetence (20%), jaundice (12%) and ascites (4%), Ticks were found on the body of 70 per cent (35/50) dogs. These findings were in accordance with Varshney and Kumar, (2008) [31], Wadhwa *et al.* (2011) [33] and Preena *et al.* (2021) [23] who reported similar clinical signs.

These findings suggested that there is a large variation in clinical signs of canine babesiosis and the observed wide range of inconsistent clinical manifestations might be due to multi systemic effects of disease. (Wadhwa *et al.*, 2011) [33].

PCR

In the present study, a total of 50 cases with clinical signs suggestive of babesiosis in dogs, were screened during study period by blood smear examination and PCR. By microscopic examination the positivity was found to be 42 per cent in dogs, whereas 35 blood samples in this study were found positive based on PCR. The percent positivity by PCR is 70%. All the twenty-one samples that were positive by microscopic examination were also positive by PCR. Further another fourteen samples which were negative by microscopic examination were positive by PCR. This indicates that PCR is highly sensitive and can be used as a diagnostic tool for diagnosis of canine babesiosis. (Yamane *et al.* 1994) [35]. Percent positivity of 70% by PCR is in agreement with the findings of Samaradhani *et al.* (2005) [27], Mohan Kumar, (2016) [20], Mahalingaiah, *et al.* (2017) [17], Roopesh (2017) [26] who reported 64%, 69%, 64.7%, 69.09% positivity for *Babesia* infection using PCR respectively.

Babesia species may be found in thin Giemsa-stained blood films during the early stages of infection. However, due to their low numbers in chronic carrier stages, parasites are difficult to visualize in blood smear. Yao *et al.* (2014) [36].

Haematological analysis

Results of haematological analysis of all the thirty-five blood samples were positive both by microscopic examination and PCR are discussed here.

Total erythrocyte count (TEC)

A significant decrease of TEC in dogs with babesiosis (1.99±0.12×106/µL) was observed in the present study as compared to the control group (6.50±0.17×106/µL). Mean TEC value during this study is in corroboration with Abdullah *et al.* (1990) [1], Yao *et al.* (2014) [36], Nalubamba *et al.* (2015) [21], Hasan *et al.* (2019) [14], Mittal *et al.* (2019) [19] and Lavanya *et al.* (2022) [16] who recorded Mean TEC value of 2.80±0.89×106/µL, 2.2±0.78×106/µL, 2.98±1.50×106/µL, 2.1±0.98×106/µL, 2.81±0.95×106/µL and 2.90±0.59×106/µL in babesiosis respectively.

On the contrary high TEC value was reported by Fabisiak *et al.* (2010) [10], Bilwal *et al.* (2017) [3], Vishnurahav *et al.* (2017) [32], Chandra *et al.* (2018a) [5] and Roopali *et al.* (2018) [25] recorded 5.53±0.91×106/µL, 4.82±1.08×106/µL, 5.32±1.34×106/µL, 4.59±1.21×106/µL, and 4.11± 1.18×106/µL in babesiosis respectively.

The decreased TEC value in dogs affected with babesiosis could be attributed to antibody mediated cytotoxic destruction of erythrocytes and/or by auto-antibody directed against components of the membranes of infected and uninfected erythrocytes (Aysul *et al.* 2013) [2] and could also be due to increased osmotic fragility of erythrocytes and intravascular and extravascular haemolysis.

Total leucocyte count (TLC)

Our study revealed a significant leucocytosis in dogs with babesiosis (21.44±1.79×103/µL) when compared to the control group (13.90±0.40×103/µL). The mean TLC value during this study is in agreement with Abdullahi *et al.* (1990) [1], Furlanello *et al.* (2005) [11], Fabisiak *et al.* (2010) [10], Gonde *et al.* (2017) [12], Vishnurahav *et al.* (2017) [32], Das *et al.* (2019) [8] and Lavanya *et al.* (2022) [16] who have recorded mean TLC value in the range of 18.65±2.79×103/µL to 22.49±2.6×103/µL in babesiosis.

Other authors *viz*., Niwetpathomwat *et al.* (2006) [22], Yao *et al.* (2014) [36], Zamokas *et al.* (2014) [38], Nalubamba *et al.* (2015) [21], Roopali *et al.* (2018) [25] recorded mean TLC value in the lower range of 9.67±2.19×103/µL to 14.2±1.29×103/µL.

Leucocytosis observed during present study could be due to *Babesia* infection and could also be due to stress resulting in elevated TLC levels (Tvedten, 2004) [28].

Haemoglobin

Present study revealed a significant decrease of haemoglobin in dogs with babesiosis (4.92±0.24 g/dl) compared to control group (14.20±0.45 g/dl). This low Mean haemoglobin i.e., anaemia during the study is in accordance with Abdullah *et al.* (1990) [1], shah *et al.* (2011), Yao *et al.* (2014) [36], Nalubamba *et al.* (2015) [21], Hasan *et al.* (2019) [14], Mittal *et al.* (2019) [19] and Camelia *et al.* (2020) [4] who recorded haemoglobin concentration of 5.3±0.51 g/dl, 4.9±0.33g/dl, 6.40±3.05 g/dl, 4.6±0.25 g/dl, 6.12±0.79 g/dl, 5.8±0.29 g/dl and 6.65±00.86 g/dl in babesiosis respectively.

On the contrary few earlier researchers namely Fabisiak *et al.* (2010) [10], Zamokas *et al.* (2014) [38], Vishnurahav *et al.* (2017) [32], Chandra *et al.* (2018a) [5], Roopali *et al.* (2018) [25] and Preena *et al.* (2021) [23] recorded 12.85±2.81 g/dl, 12.6±2.70 g/dl, 12.23±1.58 g/dl, 10.61±1.31 g/dl, 9.09±1.11 g/dl and 11.69±3.27 g/dl respectively.

The decrease in the mean haemoglobin value could be due to intravascular and extravascular haemolysis, progressive anemia and haemoglobinemia recorded in canine babesiosis.

Packed cell volume (PCV)

A significant decrease of PCV value in dogs with babesiosis (14.93±0.67%) was observed as compared to control group (42.88±2.61%). Mean PCV value during this study is in the same range of observations of Abdullah *et al.* (1990) [1], Yao *et al.* (2014) [36], Nalubamba *et al.* (2015) [21], Das *et al.* (2019) [8] and Camelia *et al.* (2020) [4] who recorded Mean PCV value of 18±6.22%, 15.37±6.72%, 18.37±5.22%, 16±7.72% and 15.8±4.12%, in babesiosis respectively.

Contrary to this, high mean PCV value was reported by Fabisiak *et al.* (2010) [10], Bilwal *et al.* (2017) [3], Vishnurahav *et al.* (2017) [32], Preena *et al.* (2021) [23] recorded Mean PCV

value of 36±8.14%, 30.26±2.72%, 38.33±5.09%, 34.09±9.66% and 27±6.12%, in babesiosis respectively.
The decreased mean PCV value might due to hemolytic anemia and intravascular and extravascular haemolysis, progressive anemia and haemoglobinemia.

Platelet count
In the present study a significant decrease in platelet count i.e thrombocytopaenia in dogs with babesiosis (48.22±3.26×103/µL) was observed when compared to the control group (275±3.47×103/µL). Mean Platelet count during this study is in comparision with the earlier researchers Abdullahi *et al.* (1990) [1], Fabisiak *et al.* (2010) [10], Yao *et al.* (2014) [36], Yogeshpriya *et al.* (2018) [37], Ubah *et al.* (2019) [29] and Lavanya *et al.* (2022) [16] who have reported a mean platelet value of 49±7.98×103/µL, 42.14±13.89×103/µL, 63.5±20.2×103/µL, 43.125±12.18×103/µL, 45±8.1×103/µL and 78±5.82×103/µL in babesiosis respectively.
On the contrary high platelet count was reported by Bilwal *et al.* (2017) [3], Gonde *et al.* (2017) [12], Vishnurahav *et al.* (2017) [32], Chandra *et al.* (2018a) [5] recorded 246±35.68×103/µL, 194±24×103/µL, 258±27.81×103/µL, 338±35×103/µL and 234±10.8×103/µL respectively.
Thrombocytopenia could be attributed to the immune mediated destruction of thrombocytes, spleenic sequestration, coagulatory consumption of platelets from haemolytic or vascular injury and elevated body temperature (Reddy *et al.*, 2014) [24].

Serum biochemistry
Results of serum biochemistry analysis of all the thirty-five blood samples which were positive both by microscopic examination and PCR are discussed here.

Alanine aminotransferase (ALT)
The present study revealed a significant increase of ALT value in dogs with babesiosis (134.9±9.47 IU/L) when compared to control group (apparently healthy dogs). Mean ALT value during this study was in accordance with Abdullahi *et al.* (1990) [1], Furlanello *et al.* (2005) [11], Niwetpathomwat *et al.* (2006) [22], Chandra *et al.* (2018a) [5] and Mittal *et al.* (2019) [19] who reported mean ALT value of 159.82±14.17 IU/L, 168±22.1 IU/L, 132.98±12.8 IU/L, 153.78±15.82 IU/L, and 135.9±8.47 IU/L in babesiosis respectively.
Earlier workers Ubah *et al.* (2019) [29] recorded a very high mean ALT value of 31±4.17 IU/L and 45±12.1 IU/L, in babesiosis respectively.
Increased mean ALT value could be due to hepatic insufficiency, increased erythrolysis induced by parasites associated with the infection and hypoxia. (Nalubamba *et al.*, 2015) [21].

Serum Creatinine
An increase of mean Creatinine value in dogs with babesiosis (1.34±0.10mg/dL) was observed compared to control group (0.90±0.03 mg/dL). Mean Creatinine value during this study is in accordance with Furlanello *et al.* (2005) [11], Niwetpathomwat *et al.* (2006) [22], Zamokas *et al.* (2014) [38], Vishnurahav *et al.* (2017) [32], Mittal *et al.* (2019) [19].
Our observation is in contrary to the Creatinine value reported by Abdullah *et al.* (1990) [1], Yogeshpriya *et al.* (2018) [37], Das *et al.* (2019) [8] and Ubah *et al.* (2019) [29].
The reason for this increase in mean creatinine value though not significant is due to decreased renal elimination or urinary tract obstruction associated with advanced renal disease and reduction in glomerular filtration rate (GFR). Also, catabolic states such as starvation and severe infection with the intra-erythrocytic *Babesia* could be predisposing factors to tissue damage and could account for the observed increased serum creatinine level. (Niwetpathomwat *et al.*, 2006) [22].

Blood urea nitrogen (BUN)
The present study revealed a significant high BUN value in canine babesiosis (29.25±0.61 mg/dL) compared to control group (19.84±0.54 mg/dL). Mean BUN value during this study is in agreement with Furlanello *et al.* (2005) [11], Niwetpathomwat *et al.* (2006) [22], Zamokas *et al.* (2014) [38], Bilwal *et al.* (2017) [3], Gonde *et al.* (2017), Vishnurahav *et al.* (2017) [32], who recorded Mean BUN value of 30.15±2.31mg/dL, 33.80±3.13 mg/dL, 29.2±2.29 mg/dL, 29.96±4.41 mg/dL, 31.63±5.25 mg/dL and 29.5±4.34 mg/dL and 34.2±6.34 mg/dL in babesiosis respectively.
Other earlier researchers Matijatko *et al.* (2009) [18], Yogeshpriya *et al.* (2018) [37], Das *et al.* (2019) [8] and Camelia *et al.* (2020) [4] have recorded mean BUN value of 13.47±6.58 mg/dL, 19.58±5.59 mg/dL, 18±3.78 mg/dL, 26±10.79 mg/dL and 14.9±0.98 mg/dL in babesiosis respectively.
Hemolysis of erythrocytes is a common cause for renal insufficiency, which leads to azotemia and increased urea values in blood plasma (Niwetpathomwat *et al.*, 2006) [22].

Total protein (TP)
A significant decrease in TP value in dogs with babesiosis (5.38±0.03 g/dL) was observed when compared to control group (6.80±0.15 g/dL). Mean TP value during this study is in agreement with earlier workers Abdullahi *et al.* (1990) [1], Furlanello *et al.* (2005) [11], Shah *et al.* (2011), Bilwal *et al.* (2017) [3], Chandra *et al.* (2018a) [5] and Mittal *et al.* (2019) [19] who recorded Mean TP value of 5.73±0.39 g/dL, 5.60±0.41 g/dL, 5.46±0.59 g/dL, 5.6±0.39 g/dL, 5.81±0.39 g/dL and 5.65±0.34 g/dL respectively. Whereas high TP value was reported by Niwetpathomwat *et al.* (2006) [22] Matijatko *et al.* (2009) [18], Gonde *et al.* (2017) [12], Vishnurahav *et al.* (2017) [32] and Yogeshpriya *et al.* (2018) [37], who recorded Mean TP value of 6.38±0.48 g/dL, 6.32±0.32 g/dL, 6.43±0.69 g/dL, 6.73±0.89 g/dL and 7.88±0.94 g/dL in babesiosis respectively.
Decreased mean TP value could be due to hepatic insufficiency, increased erythrolysis induced by parasites associated with the infection (Nalubamba *et al.* 2015) [21].

Total bilirubin
In the present study a significant increase of total bilirubin value in babesiosis affected dog (0.54±0.05 mg/dL) was noticed when compared to control group (0.20±0.04 mg/dL). Our observations during this study is in corraboration with Zamokas *et al.* (2014) [38], Bilwal *et al.* (2017) [3] and Chandra *et al.* (2018a) [5] who recorded an increase in the mean total bilirubin value almost in the range of our observations.
On the contrary total bilirubin value is reported by Furlanello *et al.* (2005) [11], Gonde *et al.* (2017) [12] and Camelia *et al.* (2020) [4], who have recorded Mean total bilirubin value of 0.2±
0.08 mg/dL, 0.32±0.02 mg/dL and 0.28±0.09 mg/dL in babesiosis respectively.

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