Clinico-haematobiochemical alterations and Electrocardiography findings in Babesia infected dogs

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Abstract
Canine babesiosis is a worldwide tick borne disease caused by Babesia species. Dogs with fever, inappetance, lymphnode enlargement, tick infestation were screened for the haemoproteozoan diseases during the period under study at College of Veterinary Science, Tirupati. The dogs were subjected for routine clinical laboratory evaluation including peripheral blood smear examination and PCR. Out of 235 samples examined 23 samples were found positive for Babesia species. Clinical examination revealed anorexia, pyrexia, lymphadenopathy, weakness, lethargy, pale conjunctival/buccal mucous membranes, change in urine colour, petechial haemorrhages, congested mucus membranes, vomiting and icterus. Laboratory examination of the clinical samples revealed reduction in Haemoglobin, PCV, TEC and platelets when compared to the healthy control. There was significant elevation of ALT, ALP, BUN and creatinine along with significant decrease in the albumin and glucose levels in the affected dogs. The colour of the urine was red brown, yellowish orange and yellow in affected dogs while the urinalysis revealed increase in pH and specific gravity of urine, proteinuria and bilirubinuria. Electrocardiographic findings revealed significant increase in T wave amplitude. However, reduced “R” wave amplitude, arrhythmia, ventricular premature complex (VPC), tachycardia, deep Q and T waves and ST coving were recorded in individual dogs affected with babesiosis. Haemato-biochemical observations were indicative of severity of babesiosis in dogs.

Keywords: Babesia, PCR, haemato-biochemical findings, electrocardiography

1. Introduction
Canine babesiosis is a clinically significant and geographically widespread haemoproteozoan disease of domesticated dogs and wild canids [1]. The commonly occurring Babesia species in dogs are Babesia canis and Babesia gibsoni. Large pear shaped organisms usually present in pairs are indicative of B. canis infection, whereas smaller, singular, round to oval organisms are B. gibsoni. The parasite was mainly transmitted through tick vector and also transmitted by blood exchange, dog bite and transplacental route [2]. The life cycle of Babesia includes two stages inside the host RBCs, in which the sporozoites convert into piroplasms, and the other inside the tick vector [3]. Once inside the host Babesia organisms are attached to the erythrocyte by endocytosis. Due to the parasites invading and replicating in the erythrocyte, babesiosis results in destruction of the erythrocyte. The destruction of the erythrocyte is multifactorial, including direct parasite damage to the infected erythrocyte and antibody-mediated cytotoxic destruction of erythrocytes. The immunological response plays the most important role in pathogenesis of canine babesiosis. These parasites initiate a mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes. Autoantibodies are directed against components of the membranes of infected and uninfected erythrocytes which causes intravascular and extravascular hemolysis. Clinical signs can vary from peracute and fatal to chronic and subclinical, depending on microbial virulence and host resistence. The clinical-pathological changes, including hematology and blood chemistry, are nonspecific. The various clinical symptoms regularly depend on the severity of the disease in infected animals. The typical clinical findings include anaemia, thrombocytopenia, leukocyte abnormalities, increased liver enzymes, and Hyperbilirubinemia. Hypokalemia, hyperglobulinemia, azotemia, metabolic acidosis, and abnormalities of urinalysis may be observed in some severely affected dogs [4]. The disease can be clinically classified into uncomplicated and complicated forms. For some time,
uncomplicated babesiosis has been suggested to be a consequence of haemolysis [5] while complicated canine babesiosis has been suggested to be a consequence of the development of systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS), both of which are cytokine-mediated phenomena [5, 6]. Both uncomplicated and complicated babesiosis appear to be the result of host inflammatory responses [7, 8].

Electrocardiographic findings like low amplitude R waves, prolonged QRS interval, ST segment deviation, large T waves and notching of R waves first degree AV block, sinoatrial-block, escape rhythms, ventricular premature complexes and ventricular tachycardia [9, 10] can support diagnosis. ECG changes are common in canine babesiosis but most changes are nonspecific and appear to have little clinical significance. Bradycardia and irregular rhythm might be poor prognostic indicators [11]. Supportive treatment is usually given and includes fluid therapy, anti-inflammatory and antipyretics, gastroproteants, oxygen supplementation, and blood transfusion should be employed when necessary. The objectives of this study were to describe the clinical signs and hematobiochemical alterations, urinalysis and electrocardiography findings in dogs infected with babesiosis.

2. Materials and Methods
The entire work involved in the present study was carried out in Department of Veterinary Medicine, College of Veterinary Science, Tirupati. Blood smears from a total of 234 suspected dogs were screened for intraerythrocytic piroplasms of Babesia species. Diagnosis was made based on clinical signs, demonstration of Babesia species on giemsa stained blood smears and PCR. The clinical signs were recorded and blood samples were subjected for haematobiochemical analysis. Two millilitres of blood was collected from the saphenous or cephalic vein of the dog using sterile disposable syringes into vacutainers containing EDTA as anticoagulant and haematological parameters such as packed cell volume, haemoglobin, total erythrocyte count, total leukocyte count, platelet count, and differential leukocyte count were estimated as per the standard procedures described by [12]. For separation of serum, 3 ml of blood was collected in clot activator vacutainer. Serum was separated immediately after clotting by centrifugation at 2000 rpm for 5 minutes. Serum biochemical parameters viz., alanine amino transferase (ALT), alkaline phosphatase (ALP), total serum protein, albumin, glucose, blood urea nitrogen and creatinine were estimated with semi auto analyser (Star 20, Rapid Diagnostics, New Delhi) by employing the biochemical kits supplied by M/S ERBA diagnostics, Mumbai at the wave lengths specified in the procedures. Urine samples were collected from the dogs in sterile vial either from mid stream free collection or by catheterization [13]. Urinalysis was done by using Urine test strips (Aspen Diagnostics Pvt. Ltd., Delhi) and urine analyser. The electrocardiography (ECG) was recorded using the standard bipolar and augmented unipolar limb leads at 25 mm/sec and interpreted as described by [14]. The ECG was recorded in dogs in right lateral recumbency on a non conductive top table. The leads were connected proximal to the olecranon process on the caudal aspect of the appropriate forelimb and over the patellar ligament on the cranial aspect of the appropriate hind limb. Lead II recordings were taken for the purpose of comparison with apparently healthy dogs. Abnormal ECG rhythms were recorded and interpreted accordingly.

3. Results and Discussion
Among 235 suspected samples 23 samples were confirmed positive based on presence of intra-erythrocytic Babesia organisms in giemsa stained blood smears and confirmed by PCR. The most frequently observed clinical signs in canine babesiosis were anorexia (100%), pyrexia (95.65%), lymphadenopathy (91.30%), weakness (82.60%), lethargy (78.26%), pale conjunctival/buccal mucous membranes (78.26%), change in urine colour (52.17%), petechial haemorrhages (17.39%), congested mucus membranes (13.04%), vomiting (13.04%) and icterus (8.69%). Out of 23 Babesia infected dogs two dogs died (8.69%). These two dogs were presented to clinics in recumbent stage with icteric mucus membranes, subnormal temperature and lymph node enlargement which died on the day of presentation. In the present study anorexia was observed in 100 per cent dogs affected with babesiosis. This observation was in concurrence with that of with [15, 16, 17]. Pyrexia in the present study might be due to release of endogenous pyrogens from erythrolysis, parasitic destruction and activation of inflammatory mediators [18]. The present finding was in agreement with that of [15, 19]. In the present study lymphadenopathy was seen in 91.30 per cent cases. This finding was in agreement with [16, 18] and this might be due to more amount of inflammatory products or antigens draining through the lymph node [20]. Weakness was seen in 82.60 per cent dogs. The present finding was in agreement with [12, 17]. In the present study weakness might be due to anaemia causing decrease in oxygen delivery to the tissues and also due to electrolyte disorders [22]. Lethargy was seen in 78.26 per cent dogs. The present finding was in agreement with [21]. Lethargy noticed in the present study might be because of the delayed presentation of cases to clinic, more severity of infection and subsequent development of hypoglycemia due to anorexia [16]. In the present study paleness of the conjunctival/buccal mucosa was observed in 78.26 per cent of dogs which might be due to intravascular and extravascular hemolysis [18] (Fig 1a). Congested mucus membranes was recorded in 13.04 per cent cases which was in agreement with the findings of [23]. The occurrence of haemoconcentration and congestion in canine babesiosis is considered as ‘red biliary’ which is a syndrome of paradoxical phenomenon of severe intravascular haemolysis combined with haemoconcentration and the cause was thought to be due to vasculitis and fluid shifts leading to relative haemoconcentration despite severe haemolysis [3, 10]. Change in urine colour was seen in 52.17 per cent cases, this finding were in agreement with [4, 15] and [18]. A colour of urine that is red, brown or black suggesting that the presence of blood or haemoglobin (Fig 1b). Haemoglobinemia is suggestive of intravascular hemolysis resulting from immune mediated as well as parasitic-mediated destruction of red blood cells which causes renal tubular damage, renal hypoxia and capillary sludging [19]. Petechial haemorrhages were observed in 17.39 per cent cases, (Fig. 1d) similar findings were reported by [15, 19] which might be due to thrombocytopenia predisposing to disseminated intravascular coagulopathy [24]. Observations of less frequent clinical signs such as icterus (8.69%) (Fig. 1c) and vomiting (13.04%) were in agreement with that of [23, 19] and this might be due to hepatopathy which causes increase in bilirubin level above normal range and accumulation in the blood and extravascular space as a result of increased production, reduced clearance [10]. Vomiting is a non specific sign observed in babesiosis which might be due to direct stimulation of the vomiting
center via the chemoreceptor trigger zone by endotoxins from GI system that bypasses the liver [22]. Thus the clinical signs observed in the present study seems to be the result of concomitant systemic inflammatory response syndrome caused by marked cytokine release [30].

3.1 Haematological Study
There was a significant (P<0.01) decrease in the values of Hb, PCV and TEC were recorded in dogs with babesiosis when compared to apparently healthy dogs suggestive of anaemia (Table. 1). These findings are in agreement with the reports of [25, 26, 17]. Decreased Hb and TEC might be due to direct damage to the erythrocyte cell membrane by Babesia, consequently resulting in increased osmotic fragility and subsequent intravascular hemolysis [27], immune mediated destruction of RBC due to production anti-erythrocyte membrane antibodies, inhibition of erythrocyte 5'-nucleotidase, development of methemoglobinemia secondary to oxidative stress, induction of serum hemolytic proteins, and increased macrophage erythropagocytic activity [24, 29].

The mean values of total leucocyte count in dogs infected with babesiosis showed no significant difference when compared with healthy control group. These findings are in accordance with [29]. On contrast [28] and [18] observed leukopenia in Babesia infected dogs. This might be due to depressed lymphocyte blastogenesis [30]. Whereas [31] and [26] reported significant increase in total leucocyte count in Babesia gibsoni infected dogs. There was a significant decrease in platelet count in dogs affected with babesiosis suggestive of thrombocytopenia. These findings are endorsed by [28, 26, 17]. Thrombocytopenia in babesiosis might be due to platelet sequestration in the spleen or immune mediated platelet destruction and development of disseminated intravascular coagulopathy [32].

In the present study among differential leukocyte count, mean values of neutrophils decreased significantly whereas lymphocytes and monocytes increased significantly. These findings are in accordance with [33, 34, 18, 15]. Neutropenia in the present study might be due to immune mediated activity as mentioned by [35] or as a result of acute infection [36]. Similar findings were reported by [16, 18]. On contrast [25, 31, 28] documented neutrophilia in dogs with babesiosis. The reason might be due to secondary bacterial infection. In the present study lymphocytosis was noticed which is concurrence with that of [36].

3.2 Biochemical study
In the present study, there was significant increase in ALT, ALP, BUN and creatinine, whereas significant decrease in albumin and glucose levels was noticed in the infected dogs (Table 2). These findings concur with [37, 16, 38]. In contrast, [28] reported normal biochemical values in dogs affected with babesiosis. However there is no statistical difference in total serum protein and these findings agreed with [33].

In the present study increased serum activity of transaminases suggested hepatopathy. Centrilobular hepatitis with hypoxic liver damage could be the possible mechanism that resulted in significant changes in hepatic enzymes [39]. Hypoaalbuminaemia could be due to peripheral loss of albumin to edematous inflammatory fluids as a result of increased vascular permeability, blood loss or decreased plasma production due to concurrent hepatopathy that occurred with the disease [40, 41]. In the present study acute renal failure might have resulted in significant increase in BUN and creatinine levels. Renal impairment was noticed due to damage to the renal cells caused by inflammatory mediators, or possibly due to development of refractory hypotension resulting in reduced renal tissue perfusion and glomerular filtration [42]. In the present study anorexia, impaired hepatic function and increase in breakdown system resulted in hypoglycemia in Babesia infected dogs [43]. According to [44] increased non-insulin mediated glucose consumption believed to be induced by inflammatory mediators, especially in macrophage-rich tissues like spleen, liver and lungs was the cause of hypoglycaemia and regarded as a poor prognostic indicator in the affected dogs.

3.3 Urinalysis
Analysis of the urine samples from the dogs affected with babesiosis revealed red brown colour of urine in 5 dogs, yellow orange colour in 3 dogs and yellow in 6 dogs. Urobilinogen was 34 umol/L in three dogs, trace amounts of protein was present in 6 dogs, pH of the urine samples was 5.5 in 6 dogs, 6 in 9 dogs, 6.5 in 4 dogs and 7 in 4 dogs. Specific gravity of urine was 1.015 in 7 dogs, 1.025 in 6 dogs and 1.030 in 10 dogs. Other parameters like nitrates, ketone, leukocytes, bilirubin, and glucose were negative in the urine of all the dogs examined.

In the present study with regard to urinalysis, alteration in urine colour, proteinuria, increased urobilinogen, specific gravity and pH in babesiosis dogs are in agreement with [15]. Intravascular hemolysis of erythrocytes, damage of the renal epithelium might have shown traces of protein in urinalysis and the increased renal tubular cells and casts can be speculated as a reason for the increase in specific gravity in few dogs [15, 24, 19].

3.4 Electrocardiography
There was no significant (P>0.05) difference in electrocardiographic values between apparently healthy dogs and dogs affected with babesiosis except T wave amplitude which was significantly (P<0.05) increased in dogs affected with babesiosis when compared to apparently healthy dogs (Table.3). In the present study the mean value of T wave amplitude was significantly increased in diseased group. These findings are in agreement with [11]. T wave amplitude increases with hyperkalemia which was resulted from potassium ion efflux from RBC due to intravascular and extravascular hemolysis [17, 45]. Whereas the mean values of P wave and R wave amplitude, QRS interval, PR interval and QT interval did not show any significant variation in ECG values when compared to the apparently healthy dogs. Various findings like reduced R wave amplitude, arrhythmia, ventricular premature complex (VPC), tachycardia, deep Q and T waves, ST coving and tall R waves were recorded in individual dogs affected with babesiosis (Fig 2) [24]. Opined cardiac dysfunction as a rare occurrence in canine babesiosis and significant changes in ECG were not observed in their study.
Table 1: Haematological alterations in dogs affected with babesiosis (Mean ± S.E)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Apparently healthy dogs (n=8)</th>
<th>Dogs affected with babesiosis (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.79 ± 0.38</td>
<td>7.75 ± 0.21 **</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>41.13 ± 1.09</td>
<td>24.26 ± 0.68 **</td>
</tr>
<tr>
<td>Total erythrocyte count (×106/μl)</td>
<td>6.06 ± 0.30</td>
<td>3.59 ± 0.14 **</td>
</tr>
<tr>
<td>Total leucocyte count (×103/μl)</td>
<td>11.27 ± 0.78</td>
<td>10.25 ± 0.42 NS</td>
</tr>
<tr>
<td>Platelet count (×103/μl)</td>
<td>4.55 ± 0.27</td>
<td>1.72 ± 0.14 **</td>
</tr>
<tr>
<td>Differential leucocyte count (DLC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>74.63 ± 1.10</td>
<td>57.61 ± 1.49 **</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>24.38 ± 1.31</td>
<td>40.04 ± 1.45 **</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.38 ± 0.18</td>
<td>1.87 ± 0.29 **</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.50 ± 0.19</td>
<td>0.48 ± 0.21 NS</td>
</tr>
</tbody>
</table>

** Significant (P<0.01);* Significant (P<0.05); NS Not Significant (P>0.05)

Table 2: Biochemical alterations in dogs affected with babesiosis (Mean ± S.E)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Apparently healthy dogs (n=8)</th>
<th>Dogs affected with babesiosis (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>29.28 ± 2.15</td>
<td>82.86 ±7.47 **</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>31.69 ±1.09</td>
<td>96.63 ± 8.86 **</td>
</tr>
<tr>
<td>Total serum protein (g/dl)</td>
<td>6.74 ± 0.16</td>
<td>6.27± 0.22 NS</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.59 ± 0.15</td>
<td>1.88 ± 0.08 **</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>18.21 ± 2.30</td>
<td>32.76 ± 3.69 **</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.85 ± 0.13</td>
<td>1.98 ± 0.14 **</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>98.02 ± 4.14</td>
<td>79.84 ± 2.32 **</td>
</tr>
</tbody>
</table>

** Significant (P<0.01); *Significant (P<0.05); NS Not Significant (P>0.05)

Table 3: ECG readings in dogs affected with babesiosis (Mean ± S.E)

<table>
<thead>
<tr>
<th>Reading</th>
<th>Apparently healthy dogs (n=8)</th>
<th>Dogs affected with babesiosis (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P wave amplitude (mV)</td>
<td>0.21 ± 0.01</td>
<td>0.20 ± 0.01 NS</td>
</tr>
<tr>
<td>P wave duration (seconds)</td>
<td>0.04 ± 0.002</td>
<td>0.05 ± 0.002 NS</td>
</tr>
<tr>
<td>R wave amplitude (mV)</td>
<td>1.19 ± 0.06</td>
<td>1.21 ± 0.08 NS</td>
</tr>
<tr>
<td>QRS duration (seconds)</td>
<td>0.04 ± 0.003</td>
<td>0.04 ± 0.002 NS</td>
</tr>
<tr>
<td>PR interval (seconds)</td>
<td>0.09 ± 0.007</td>
<td>0.09 ± 0.005 NS</td>
</tr>
<tr>
<td>T wave amplitude (mV)</td>
<td>0.23±0.02</td>
<td>0.38 ± 0.03 *</td>
</tr>
<tr>
<td>T interval (seconds)</td>
<td>0.17 ± 0.009</td>
<td>0.18 ± 0.007 NS</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>100.23 ± 1.58</td>
<td>110.22 ± 3.74 NS</td>
</tr>
</tbody>
</table>

** Significant (P<0.01); * Significant (P<0.05); NS Not Significant (P>0.05)

Fig 1: Clinical findings in Babesia infected dogs

1a. Pale buccal mucus membrane  
1b. Red brown colour urine  
1c. Icteric conjunctival mucus membrane  
1d. Petechial haemorrhages on inguinal
4. Conclusion
The presumptive diagnosis of canine babesiosis can be made based on a clinical signs such as fever, anaemia, anorexia, vomiting and pale mucous membrane. Microscopic examination may not be very revealing in the detection of very low parasitemia, but it can be considered as the most rapid confirmatory method. The main clinico-pathological findings in canine babesiosis indicate that it typically causes haemolytic anaemia, neutropenia, lymphocytosis, moderate to severe thrombocytopenia and multiple organ dysfunctions. Elevation of serum biochemical parameters such as ALT and ALP are indicative of hepatic hypoxia and increase BUN and creatinine are indicative of degenerative changes in kidneys. Significant decrease in albumin and glucose indicated impaired hepatic function. Results of this study suggest that haemato-biochemical changes could be beneficial in determination of the severity of babesiosis in dogs. Urinalysis revealed change in urine colour, proteinuria, bilirubinuria, rise in pH and specific gravity of urine indicating renal damage and intravascular hemolysis. Electrocardiographic findings revealed significant increase in T wave amplitude. ECG are common in canine babesiosis but most changes are non specific and appear to have less clinical significance.

5. Acknowledgements
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6. References


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