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Electrocardiographic and haemato-biochemical findings in sheep with babesiosis

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Abstract
The present study was carried out to record the clinical, hematological, biochemical, and electrocardiographic changes during the naturally occurring babesiosis in sheep. Out of 131 sheep in a flock, 18 adult sheep were identified as suffering from clinical babesiosis. Confirmation of the *Babesia* organisms was done by microscopic examination of the stained peripheral blood smears. Affected sheep showed anorexia, suspended rumination, prolonged capillary refill time, blanched mucus membranes, fever, tachycardia, and hemoglobinuria. Hematology revealed reduced hemoglobin concentration, packed cell volume, red blood cell count, and increased total leukocyte count. Elevated levels of serum total proteins, blood urea nitrogen, creatinine, and lower levels of albumin were noticed. Electrocardiography abnormalities were reduced P wave amplitude and QRS voltage, prolonged PR, and QT interval. Recorded arrhythmias were sinus tachycardia, low voltage QRS complex, ST depression, atrial fibrillation, sinus arrest, and ventricular tachycardia. All the affected sheep were treated with diminazene aceturate, hematinic along with electrolyte therapy. The present study supports the renal damage during clinical babesiosis and also the development of cardiac changes in naturally occurring babesiosis in sheep.

Keywords: Sheep, babesiosis, haematology, biochemistry, electrocardiography

Introduction
In recent years, economic loss due to parasitic infections has been increasing in small ruminants (Sivajothi and Reddy, 2018) [4]. A greater number of small ruminants are suffering from haemoprotozoan diseases due to the presence of vectors such as ticks. *Babesia ovis* is one of the important parasitic diseases in small ruminants. It is a small form of *Babesia* parasite approximately 1 to 1.5 µm in diameter, intra-erythrocytic in nature, and transmitted by tick-borne disease (Moreau *et al.*, 2009) [7]. It causes acute, sub-acute, or chronic forms of infection and causes severe economic losses to the former. Small ruminants affected by clinical babesiosis show variable clinical signs including fever, anemia, and hemoglobinuria (Esmaeilnejad *et al.*, 2012) [4]. Duration varies with the individual animal, with a long convalescent period, acute shock, and progressive renal failure and death (Tufani *et al*., 2017) [15]. *Babesia ovis* plays an important role in causing anemia and kidney dysfunction in affected animals. Analysis of the hematological components provides information on the health status of individual animals and helps in diagnosis, differential diagnosis, and formulation of therapeutic regimen (Reddy *et al.*, 2016) [10]. The present study describes the clinical, hematological, biochemical, and electrocardiographic findings in sheep with babesiosis.

Material and Methods
The present study was carried out at the College of Veterinary Science, YSR District, Andhra Pradesh, India. Eighteen sheep out of 131 in a flock showed the passing of red-colored urine, anorexia, and the presence of *Babesia* organisms in peripheral blood smears. Clinical samples including whole blood and serum, were collected for laboratory analysis. Blood collected in ethylene diamine tetraacetic acid tubes was used to estimate hematological parameters including red blood cell count, hemoglobin, packed cell volume, white blood cell count, and differential count as per standard procedures. Serum obtained from clotted blood samples was used to determine biochemical parameters including total protein, albumin, alanine aminotransferase, creatinine, and blood urea nitrogen (Hadadazodeh *et al.*, 2002) [5]. Electrocardiography recording was done in a quiet standing position by the standard base apex lead system. Recordings were done on a three-channel electrocardiographic machine (Marks electronics, Chennai) with a paper speed of 25 mm/sec and calibration of 10 mm equal to 1

mV. The amplitude of P, QRS, and T waves was recorded for lead I and expressed in millivolt (mV). The duration of P waves, PR interval, QRS complex, ST interval, and T waves were calculated and expressed in seconds. Data were expressed as the mean±standard error (SE) and comparisons among clinically apparently healthy and diseased groups were evaluated using a student t-test (Reddy *et al.*, 2018) [9]. After confirmation of the disease, they were treated with injection diminazene aceturate (@ 3.5 mg/ kg, deep IM, OD for two consecutive days), injection oxytetracycline dehydrate (@ 10 mg/ kg, IM, OD for 3 days), hematinic (Injection Feritas, Intas Pharmaceuticals) @ 1 ml/50 kg, IM, twice weekly for 2 weeks, oral electrolyte solution for 2 weeks (Ijaz *et al.*, 2013; Sevinc *et al.*, 2007) [6, 12].

Results and Discussion

Clinical signs recorded were elevated rectal temperature, tachycardia, tachypnoea, blanched mucus membranes, increased capillary refill time, suspended rumination, and hemoglobinuria. *Babesia* organisms were detected in the stained peripheral blood smears. Out of 131 sheep, 18 showed the presence of the organisms in the stained blood smears. The degree of *Babesia* infection was assessed based on the percentage of RBCs infected with the organisms. It was reported that infection intensity was mild, moderate, and severe (mild 8, moderate 2, and severe 8). These observations were in accordance with the findings by (Aktas *et al.*, 2007, Tufani *et al.*, 2017) [1, 15].

The mean values with standard error of the hematological and serum biochemical findings were presented in Table-1. Recorded anemia indicative of microcytic hypochromic type is due to destruction of red blood cell hemolysis resulting from the replication of *Babesia* within the RBCs. Hemolysis results in profound anemia, jaundice, and hemoglobinuria. The presence of Howell-Jolly bodies, anisocytosis, and pallor of RBCs observed in most of these cases represent severe anemia. A very significant elevation of the blood urea nitrogen, creatinine, and total protein with a reduction in the albumin levels. Reduction in the levels of RBCs, PCV, and Hb was recorded and these findings were in associated with the previous reports (Hadadazadeh *et al.*, 2002) [5]. Recorded red blood cell abnormalities were anisocytosis (spherocytosis) and polychromatophilic erythrocytes have a role in the development of the abnormal indices (Shiono *et al.*, 2003) [13]. The leukogram revealed a significant increase in WBC and it might be due to extended tissue damage by the parasites, and maturation of the neutrophil and lymphocytes. Eosinophilia was due to the sensitivity to the foreign protein of a parasite which may be a part of an immune phenomenon (Okon *et al.*, 2011) [8].

In the current study, as parasitemia increased, a significant elevation was evident in BUN and creatinine levels it might be due to kidney dysfunction, increased muscle catabolism, and localization of the *B. ovis* in the renal blood circulation. Death in Babesiosis due to hypoxia caused by hemoglobinuria, which further leads to systemic hypotension and disseminated intravascular coagulation (DIC). The observed hypoalbuminemia in the current study is in agreement with those reported earlier (Ijaz *et al.*, 2013) [6]. Reduction of albumin level probably corresponds to a disturbance in liver function, urinary loss of albumin associated with renal failure (proteinuria), and anorexia. The observed hyperproteinemia can be attributed to an increase in the globulin concentration in response to parasitic antigen and released hemoglobin from destructed erythrocytes. The reported serum biochemical alterations were in accordance with the previous reports (Esmaeilnejad *et al.*, 2012; Hadadazadeh *et al.*, 2002; Ijaz *et al.*, 2013 and Tufani *et al.*, 2017) [4, 5, 6, 15].

Electrocardiography abnormalities were reduced P wave amplitude and QRS voltage, prolonged PR interval, QT interval, and heart rate (Table-2). Arrhythmias recorded in the present study were sinus tachycardia (100%, 18/18), low voltage QRS complex (66.67%, 12/18), ST depression (50%, 9/18), atrial fibrillation (44.45%, 8/18), sinus arrest (22.23%, 4/18), ventricular tachycardia (22.23%, 4/18) and ventricular premature complexes (16.67%, 3/18) and second degree AV block (11.11%, 2/18). Present findings are indicative of changes in the electrolyte imbalance and myocardial changes during the *Babesia* infection. Literature available on electrocardiography is very limited and the present study's electrocardiographic findings were nearer to the findings in dogs with *Babesia* (Chaudhuri *et al.*, 2017) [2].

Further studies are required to assess cardiac function, myocardial issues in sheep infected with babesiosis, and serum protein response in *B. ovis* infection.

Table 1: Haemato-biochemical findings in sheep (Mean±SE)

S. No	Parameters	Apparently healthy group (n=6)	Babesia infected sheep (n=18)	P value
1	Hb (g/dl)	11.5±1.10	6.87±0.93	0.006**
2	PCV (%)	35.12±2.16	21.56±1.98	0.008**
3	TEC x10^6/cumm	6.82±0.81	4.21±0.98	0.006**
4	TLC /cumm	8,894±314.4	12,310±288.8	0.042**
5	Neutrophils (%)	30.12±1.02	24.09±1.05	0.082 NS
6	Lymphocytes (%)	62.81±2.10	65.3±2.83	0.066 NS
7	Eosinophils (%)	5.3±0.04	8.1±0.10	0.038*
8	Monocytes (%)	2.2±0.02	2.1±0.10	0.091 NS
9	Total protein (g/dL)	7.38±0.25	7.12±1.15	0.088 NS
10	Albumin (g/dL)	3.10±0.07	1.21±0.88	0.042*
11	Globulin (g/dL)	3.94±0.11	5.91±0.09	0.006**
11	SGOT (AST) (µ/L)	100±0.87	111.88±18.66	0.072NS
12	BUN (mg/dL)	22.5±0.87	98.95±4.11	0.044*
13	Creatinine (mg/dL)	2.38±0.12	3.08±0.82	0.038*

NS – Non Significant (p>0.05); * Significant (p<0.05); * * Highly Significant (p<0.01)

Table 2: Electrocardiographic parameters in sheep (Mean±SE)

S. No	Parameters	Apparently healthy group (n=6)	Babesia infected sheep (n=18)	P value
1	P wave amplitude (mV)	0.179±0.004	0.112±0.004	0.041*
2	QRS complex amplitude (mV)	0.972±0.052	0.782±0.042	0.018*
3	T wave amplitude (mV)	0.342±0.011	0.312±0.007	0.078 NS
4	P wave duration (Sec)	0.058±0.007	0.062±0.004	0.059 NS
5	QRS complex duration (Sec)	0.051±0.002	0.054±0.005	0.091 NS
6	T wave duration (Sec)	0.095±0.004	0.099±0.005	0.072 NS
7	P-R interval (Sec)	0.196±0.018	0.241±0.006	0.031*
8	Q-T interval (Sec)	0.294±0.021	0.311±0.019	0.039*
9	Heart rate (bpm)	91.42±2.23	112.8±9.12	0.004**

NS – Non Significant (p>0.05); * Significant (p<0.05); * * Highly Significant (p<0.01)

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