

HAEMATOBIOCHEMICAL AND ACID BASE STATUS OF HAEMOPROTOZOAN AND RICKETTSIAL INDUCED RENAL DISORDERS IN DOGS

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ABSTRACT

The present study was aimed to find out haemato-biochemical and acid base status of haemoprotozoan and rickettsial induced renal disorders in dogs. A total of 25 dogs (25/2945; 0.85%) tested positive for babesiosis, ehrlichiosis and anaplasmosis using the *Ehrlichia* - *Anaplasma* - *Babesia gibsoni* - *Babesia canis* Combo Test kit. The most common clinical signs were dullness and depression (88%), inappetence/anorexia (84%), followed by weight loss (60%), lymph node enlargement (56%), pale conjunctival mucous membranes (40%). Haemato-biochemical changes included leucocytosis, thrombocytopenia, increased values of BUN (blood urea nitrogen), Creatinine along with increased activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP). Blood gas and acid-base status showed significantly lower levels of pCO_2 and HCO_3 in diseased dogs indicative of metabolic acidosis.

Keywords: *Anaplasma*, *Babesia*, Combo test kit, *Ehrlichia*

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Tick-borne diseases, namely Babesiosis, Ehrlichiosis and Anaplasmosis, pose a significant threat to canine health globally, with many cases leading to renal complications (Solano-Gallego *et al.*, 2016; Adaszek *et al.*, 2016; Aziz *et al.*, 2022). Transmitted primarily through tick bites, these diseases present with a range of symptoms from mild lethargy and anorexia to severe renal failure, evidenced by elevated levels of BUN, Creatinine and proteinuria (Baneth 2018; Martinescu *et al.*, 2021). Key haematobiochemical markers such as anemia and thrombocytopenia are frequently observed, indicating the involvement of renal dysfunction in the disease process (Hasan *et al.*, 2019; Gojska-Zygnier *et al.*, 2022). The use of serological kits like SNAP 4DX Plus and specific antibody tests is critical for diagnosing these infections and successfully identifying pathogens like *Babesia gibsoni*, *Ehrlichia canis* and *Anaplasma* spp. in diseased dogs (Chawla *et al.*, 2020; Martinescu *et al.*, 2021; Turna *et al.*, 2022). This evidence highlights the crucial role of serological diagnostics in managing tick-borne diseases and their renal manifestations in dogs.

MATERIALS AND METHODS

Between September 15, 2022 and November 15, 2023, a total of 2945 dogs were presented in the Department of Veterinary Medicine, VCC, DGCN, COVAS, CSKHPKV, Palampur, (H.P). Based on the history, clinical signs such as fever, inappetence or anorexia, vomiting, epistaxis, presence of ticks, enlarged lymph nodes and physical examination, cases suspected for either Haemoprotozoan or Rickettsial diseases were screened for the presence of

Haemoprotozoan and Rickettsial induced renal disorders. Further parasitological studies by rapid test combo kits, were employed for the confirming diagnosis. Final confirmation was made after carrying out a haemato-biochemical profile, electrolyte estimation, blood gas and Acid-base analysis and imaging studies.

Approximately 2 ml of blood was drawn from either the cephalic or saphenous vein into K2 EDTA-lined sterile vials (NexTube®, Nexamo Technoplast Pvt. Ltd.) for a complete blood count, analyzed by the BC-5000 Vet Auto-Hematology Analyser from Mindray Animal Care Medical Technology Co. Ltd. For biochemical analyses, 4 ml of blood was collected in clot activator vials (Hemo Tube™, MB Plastic Industries, Noida) and tested using the Agappe Mispa CXL Pro Plus (Agappe Diagnostics Ltd.). Blood gas and acid-base levels were measured from 2 ml samples using a heparinized syringe and analyzed within 10 minutes by the IDEXX electrolyte and blood gas analyzer (Idexx Co., USA).

Clinical signs were recorded upon presentation and data from ten healthy control dogs were statistically analyzed using Instat (Graphpad software, 2008), comparing mean values via “t” test and “ANOVA” at significant levels.

RESULTS AND DISCUSSION

a) Prevalence

A total of 25 dogs were positive by rapid combo test kits (Table 1 and Fig. 1) with a prevalence rate of 0.85% (25/2945). The majority of cases occurred during the monsoon season (July to October) with 76% followed by summer season (March to June) with prevalence of 24%.

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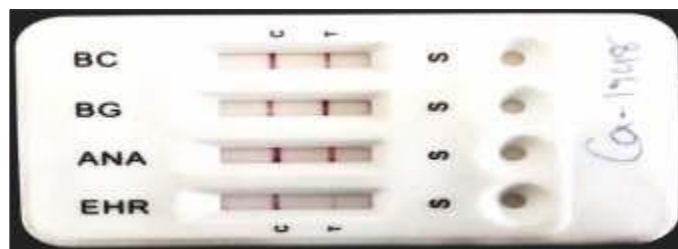


Fig. 1. Ehrlichia–Anaplasma–Babesia gibsoni–Babesia canis positive dogs by Combo Test kit

The findings were similar to Kumar *et al.* (2009) who reported maximum haemoprotozoan cases in monsoon season. Male dogs were predominantly affected (92%), especially those older than 5 years (60%), with the next most affected age group being 2-5 years (36%). Labradors were the most susceptible breed (24%), followed by non-descriptors (16%) and Rottweilers (12%). Findings were similar to Gonde *et al.* (2017) who also reported highest disease prevalence in Labrador breed. This might be due to greater percentage of Labradors being kept as pets in the specific area.

b) Clinical manifestations

The mean values of rectal temperature and heart rate were significantly higher in diseased dogs when compared to healthy dogs (Table 2) which were similar to those observed by Gonde *et al.* (2017), highlights increased rectal temperature and heart rate in dogs due to infection-induced cytokine release.

The clinical signs observed ranged widely, with dullness and depression (88%) and inappetence/anorexia (84%) being most common, followed by weight loss (60%), lymph node enlargement (56%), pale conjunctival mucous membranes (40%), congested conjunctival mucous membranes (36%), dehydration (36%), dental tartar and halitosis (32%), fever (40%), presence of ticks, vomiting and melena (28%) and epistaxis (16%). These findings were in accordance Chawla *et al.* (2020), Chirek *et al.* (2018) and contrast with Martinescu *et al.* (2021) in relation to fever where they recorded high grade fever in 88.1% of the affected cases.

c) Haemato-biochemical parameters

The mean values of total erythrocyte count, haemoglobin, and packed cell volume were significantly decreased in diseased dogs when compared with the healthy dogs. These findings were in accordance with Gonde *et al.* (2017). Low haemogram could be because of loss of blood due to bone marrow suppression, pathogen-mediated erythrocyte lysis, immune-mediated hemolysis and impaired erythropoiesis due to renal dysfunction.

The study observed a significant decrease in total

Table 1. Babesiosis, Ehrlichiosis & Anaplasmosis induced renal disorders by rapid test kits

S. No.	Babesiosis, Ehrlichiosis & Anaplasmosis induced renal disorders	Number (percentage)
1.	4 in 1 combo	
	<i>Anaplasma</i> spp.	25 (100%)
	<i>Babesia canis</i>	22 (88%)
	<i>Babesia gibsoni</i>	24 (96%)
	<i>Ehrlichia canis</i>	20 (80%)
	Total	25 (100%)

Table 2. Clinical parameters in diseased dogs

S. No.	Parameter	Healthy Dogs (n=10) (Mean \pm S.E.)	Babesiosis, Ehrlichiosis & Anaplasmosis (n=25) (Mean \pm S.E.)
1.	Rectal Temperature ($^{\circ}$ F)	101.18 \pm 0.25	102.58 \pm 0.32**
2.	Respiration Rate (per min)	27.1 \pm 0.59	27.8 \pm 0.03
3.	Heart Rate (beats per min)	102.6 \pm 3.01	116.02 \pm 1.58**

platelet count in diseased dogs compared to healthy ones, indicating severe thrombocytopenia. These results were similar to Roopali *et al.* (2018). Such thrombocytopenia might result from platelet dysfunction, the production of antiplatelet antibodies or a reduced half-life of platelets in circulation. The mean corpuscular hemoglobin concentration (MCH) value significantly increases in diseased dogs compared to healthy dogs, suggesting a macrocytic anemia, due to compensatory production of immature red blood cells in response to pathogen-induced anemia and renal dysfunction. Monocytes showed significant rise in diseased dogs when compared to healthy ones due to an elevated immune response to the infections. The total leucocyte count (TLC) showed non-significant increase from healthy dogs, contrasting with the findings of Bhardwaj (2013), who reported a decrease in TLC in affected dogs.

Diseased dogs exhibited a significant rise in mean blood urea nitrogen (BUN) and creatinine values compared to healthy dogs, indicating uremia (Adrian *et al.*, 2016). Immune complex-mediated glomerulonephritis in diseased dogs could be a factor in increased creatinine levels (Agnihotri *et al.*, 2012). The mean alanine aminotransferase (ALT), aminotransferase (AST) value was significantly higher 15 in diseased dogs compared to healthy dogs, suggesting hepatic dysfunction (Agnihotri *et al.*, 2012). Alkaline phosphatase (ALP) levels were also non-significantly higher in diseased dogs and were in accordance with Kottadamane *et al.*, 2017. The mean values of total bilirubin levels also increased, likely due to a rise in indirect bilirubin levels leading to immune-mediated RBC lysis. The findings were in accordance with Sharma *et al.* (2015).

d) Blood gas and acid base status

Acid-base parameters showed significantly lower

Table 2. Haemato-biochemical parameters in diseased dogs

Parameters	Healthy Animals (n=10) (Mean ± S.E.)	Babesiosis, Ehrlichiosis & Anaplasmosis (n=25) (Mean ± S.E.)
Total Leucocytic Count ($\times 10^9/L$)	11.13 ± 0.6	16.95 ± 3.370
Lymphocytes (%)	15.3 ± 0.62	14.39 ± 3.36
Monocytes (%)	4.04 ± 0.27	10.57 ± 1.85**
Neutrophils (%)	79.68 ± 0.85	73.35 ± 3.96
Eosinophils (%)	1.35 ± 0.18	1.24 ± 0.75
Total Erythrocytic Count ($\times 10^{12}/L$)	6.49 ± 0.21	4.75 ± 0.39***
Haemoglobin (g/dL)	13.47 ± 0.34	10.26 ± 0.86**
Packed Cell Volume (%)	38.87 ± 1.04	29.52 ± 2.25***
Mean Corpuscular Volume (fL)	61.57 ± 0.47	63.43 ± 1.06
Mean Corpuscular Haemoglobin (pg)	21.88 ± 0.4	23.8 ± 0.38**
Mean Corpuscular Haemoglobin Concentration (g/dL)	35.18 ± 0.58	38.06 ± 2.7
Platelets ($\times 10^9/L$)	299.2 ± 23.72	118.16 ± 14.34***
Alanine aminotransferase (U/L)	33.92 ± 2.4	78.29 ± 18.04*
Aspartate aminotransferase (U/L)	38.05 ± 3.98	102.48 ± 27.81*
Alkaline Phosphatase (U/L)	79.25 ± 8.72	255.41 ± 89.17
Total Bilirubin (mg/dl)	0.22 ± 0.04	0.87 ± 0.61
Total Protein (g/dl)	6.6 ± 0.35	7.04 ± 0.22
Blood Urea Nitrogen (mg/dl)	25.14 ± 4.54	298.93 ± 38.39***
Creatinine (mg/dl)	1.01 ± 0.08	10.78 ± 1.605***
Glucose (mg/dl)	102.67 ± 2.66	106.73 ± 7.98

***Significant at 0.1% level ($P < 0.001$), ** Significant at 1% level ($P < 0.01$), * Significant at 5% level ($P < 0.05$)

Table 3. Acid-base parameters in diseased dogs

Parameters	Healthy dogs (n=6) (Mean ± S.E.)	Babesiosis, Ehrlichiosis & Anaplasmosis (n=10) (Mean ± S.E.)
pH	7.35 ± 0.02	7.243 ± 0.048
pCO ₂ (mm Hg)	38.15 ± 2.02	29.7 ± 1.136**
HCO ₃ (mmol/L)	20.16 ± 0.52	12.99 ± 1.698**
AnGap (mmol/L)	30.26 ± 1.25	25.12 ± 1.498*
tCO ₂ (mmol/L)	20.8 ± 0.65	13.88 ± 1.723**
BE (mmol/L)	-6.08 ± 0.84	-11.55 ± 2.335*
BEact (mmol/L)	-6.26 ± 1.12	-13.69 ± 2.582*
BEecf (mmol/L)	-5.98 ± 1.34	-12.65 ± 2.163*
BB (mmol/L)	42.92 ± 0.46	36.44 ± 2.334*
stHCO ₃ (mmol/L)	20.16 ± 0.42	15.3 ± 1.672*
st pH	7.34 ± 0.01	7.181 ± 0.05*
cH ⁺ (nmol/L)	49.63 ± 0.81	59.24 ± 8.137

** Significant at 1% level ($P < 0.01$), * Significant at 5% level ($P < 0.05$)

pCO₂, HCO₃ and Base Excess (BE) levels in diseased dogs compared to healthy ones, while pH levels were non-significantly lower than healthy dogs, indicative of metabolic acidosis. These findings were in agreement with Chawla *et al.*, 2020 and Chandrasekar *et al.* (2022). Typically, metabolic acidosis develops as a result of diminished renal excretion of acidic substances like phosphate and sulfate, in addition to reduced renal reabsorption of bicarbonate, and a decrease in renal ammonia production, as described

by Kogika *et al.* (2006).

CONCLUSION

Babesiosis, Ehrlichiosis and Anaplasmosis can be effectively identified early using a specialized combination of antibody detection kit, allowing for timely initiation of treatment. Delayed treatment can result in diminished kidney function and permanent damage in chronic cases. Therefore, recognizing clinical and hematobiochemical alterations during examination is crucial for a preliminary diagnosis of these diseases, enabling more effective clinical management.

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