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Hematobiochemical changes in babesiosis and ehrlichiosis suspected Dogs

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Abstract
The present study was aimed to know the hematobiochemical changes in the dogs affected with Babesiosis and Ehrlichiosis in Dogs. Dogs presented to small animal OPD, Department of Veterinary Medicine were selected for the study and grouped as control and diseased group with 10 Dogs in each group. All the dogs considered under the diseased group were exhibiting classical clinical signs of blood parasitic diseases such as Anorexia/inappetence, Pale mucus membranes, Lymphadenopathy, Tick infestation, Pain in the limbs and blood mixed urine. Highest number of dogs which were affected by Blood parasitic diseases were of the age between 1 to 3 years, Labrador retriever was the predominant breed affected, Male dogs were slightly more in the diseased group. There was no significant difference in Rectal temperature and Heart rate between the control group and diseased group. Hematology revealed increase in total leukocyte count (TLC) and decreased total erythrocyte count (TEC), Hemoglobin (Hgb), platelets and packed cell volume (PCV) in the diseased group. Serum biochemistry revealed nonsignificant difference in alanine amino transaminase (ALT), total protein, Albumin, globulin, creatinine and blood urea nitrogen (BUN) levels.

Keywords: Babesiosis, ehrlichiosis, hematology, serum biochemistry

Introduction
Canine vector borne diseases especially blood parasitic diseases such as Babesiosis and Ehrlichiosis has gained much importance in the Pet industry due to the higher morbidity and Mortality rates. As the pets are exposed to Vectors especially ticks when they are taken outside for walks or Vacations or when they are boarded in the kennels there are high chances that pets are affected with blood parasitic diseases.
Canine vector borne diseases (CVBDs) are a group of diseases transmitted by several arthropod vectors, including fleas, biting and secretophagous flies, mosquitoes, sand flies and, especially, ticks (Otranto *et al*., 2009) [28]. In the past two decades the distribution of CVBDs has been investigated mainly in industrialized countries of the northern hemisphere, whereas data about the occurrence and the impact of these infections in developing countries is minimal (Maggi and Kramer, 2019) [23].

Review of Literature
Babesia spp. are classified as members of the order Piroplasmida, which is part of the phylum Apicomplexa. Early studies identified two morphologically distinct forms of the erythrocytic stage in the canine host, leading to the designation of the larger form (3-5 m) as *B. canis* and the smaller (1-3 m) as *B. gibsoni* (Adaszek *et al*. 2010) [2]. *B. canis* was reclassified into three subspecies based on cross-immunity, serological testing, vector specificity, and molecular phylogeny (*B. canis*, *B. rossi*, and *B. vogeli*). They are currently all recognized as distinct species (Zahler *et al*. 1998, Carret *et al*. 1999, Costa-Junior *et al*. 2009) [36, 9, 10].
It is typical for dogs to have both Ehrlichiosis and Babesiosis at the same time. The occurrence of concurrent infection varies and is most likely related to the geographic distribution of the agents. Multiple organ dysfunction syndrome (MODS) develops from systemic inflammatory response syndrome (SIRS), which is a hallmark of Babesiosis (Matijatko *et al*., 2010) [25].
A tick stage and a mammalian host stage are both part of the Babesia life cycle. The vector tick picks up *Babesia* spp. parasitized erythrocytes while feeding on an affected animal. The parasites undergo gamogony within the tick gut. The resultant zygotes mature into kinetes, which move to various tissues and multiply. Kinetes in the salivary glands go through sporogony, which requires a molt to the next tick stage to evolve into infective sporozoites.

The tick transfers these infective sporozoites to its next host animal while eating after molting. Transstadial transmission is the name given to this type of transmission (Holman and Snowden, 2009) [18].

The life cycle of *Ehrlichia* species in the vector is still unknown. However, there are three intracellular forms in the host. Initial bodies are small spherical structures (1-2 microns) that are thought to grow into bigger numerous morulae. Morula is hypothesized to decompose into microscopic grains known as elementary bodies. The infective stage is the elementary body, which enters the monocyte or other leukocyte types by phagocytosis. Individual *Ehrlichia* around one meter in diameter and frequently coccoid or ellipsoid in shape are classified as elementary bodies. Once inside the phagosomes, the pathogens reproduce through binary fission, generating clusters of closely packed elementary entities known as initial bodies. Additional growth and replication results in the establishment of the morula, the genus's defining structure. The host cell rupture allows the elemental bodies to infect additional cells (Nicholson *et al*., 2010) [27].

Parasitic infection triggers a systemic inflammatory response, which is thought to be a key component of canine Babesiosis pathogenesis and leads to a variety of clinical symptoms (Schetters *et al*., 2009) [31]. Cytokines, which mediate and regulate all elements of the immune response to infection, play a significant role in causing systemic inflammation (Borghetti *et al*., 2009) [7]. Canine Babesiosis immunopathogenesis is not fully understood. Tumor necrosis factor alpha (TNF), the sole cytokine identified but related with *B. canis* infection, was found in higher amounts in dogs with higher peripheral parasitemia and more severe illness (Brown *et al*., 2009; Vaughan-Scott, 2001) [8, 35].

The pathophysiology of *E. canis* infection has received the greatest attention. Infection occurs by the tick's salivary secretions at the attachment site during a blood meal or through blood transfusions. Adult *Rhipicephalus sanguineus* can transmit the disease to other dogs for at least 155 days after separation if it engorges on the dog during the acute stage. *Rhipicephalus sanguineus* transmits the disease trans-stadially, which means that the tick acquires the bacterium by feeding on an infected dog as a larvae or nymph, and then transfers the disease to another dog as a nymph or adult. *Ehrlichia*'s life cycle is not fully understood, however it is thought to exist in three intracellular forms. Small spherical structures (1-2 micrometers in diameter) are thought to grow into bigger numerous membrane-bound units known as morulae. Morulae are inclusions seen in the cytoplasm of leukocytes. This morula then dissociates into microscopic granules known as elementary bodies (Azmi *et al*., 2013) [4].

Clinical symptoms of *B. gibsoni* infection vary; they are affected not only by parasitemia but also by the immunological response of the diseased dog (Kraje, 2001) [22]. The most serious sickness affects puppies and canines under the age of two. Mild fever, anorexia, depression, pale mucous membranes, lethargy, vomiting, loss of stamina, enlarged lymph nodes, and splenomegaly may occur in certain dogs.

In a study, Parmar *et al*. (2013) [29] found that depression and anorexia were the top concerns of most pet owners when they presented their cases to veterinarians. Bleeding tendencies such as epistaxis, hematuria, and melena were observed in 50% of the dogs. Paleness of visible mucus membranes indicating anemia in 52.5% of cases of proven canine Ehrlichiosis, and petechial/ecchymotic hemorrhages on oral mucosa, penis, and conjunctiva in 50% of cases.

The most notable finding in the study was thrombocytopenia, with all of the dogs (100%) exhibiting levels below the normal range. Four (50%) of the eight dogs had total erythrocyte count, hemoglobin percentage, and packed cell volume values that were on the lower end of the reference range, indicating an anemic trend. Three dogs (cases 2-4) had total erythrocyte counts below the normal range; four dogs (cases 1-4) had subnormal hemoglobin percentages; and five (62.5%) of the eight dogs exhibited leukocytosis. Differential leukocytic counts revealed relative lymphocytosis in two cases and relative neutrophilia in one (Parmar *et al*., 2013) [29].

The mean values of hematological parameters of dogs suffering from concomitant TBICDs are There was significant decrease in the Hb, TEC, TLC and platelet count level in concomitant infection in comparison with healthy group (Sarma, *et al*., 2015) [30].

According to Parmar *et al*. (2013) [29], serum samples from confirmed *Ehrlichia* cases were subjected to assessment of enzymes and other components that indicate liver and kidney functioning. All of the dogs' bilirubin, SGPT, and SGOT levels were within the normal range, with one or two exceptions showing a minor increase.

Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and blood urea nitrogen (BUN) levels were considerably higher in dogs with Babesiosis than in healthy dogs (Bilwal *et al*., 2017) [6].

Bilirubin, ALT, AKP, BUN, and creatinine levels were significantly elevated in dogs infected with *B. gibsoni* and *B. canis*. In five dogs infected with *B. gibsoni*, hypoglycemia was found (Gonde *et al*., 2017) [17].

Materials and Methods

Dogs with clinical signs suggestive of Haemoprotozoan diseases, such as anorexia/inappetence, high temperature, lymphadenopathy, dullness, pale mucous membranes, icterus, emaciation, diarrhoea, haemoglobinuria, and a history of tick infestation, were selected for the study.

To collect the control values for the parameters under research, healthy dogs (N=10) which were presented to the Small Animal Medicine Unit, Department of Veterinary Medicine, Veterinary College Hospital, Veterinary College, Hebbal, Bengaluru for vaccination, deworming, and routine health check-ups. Epidemiological risk factors such as Age, breed, gender were assessed in the dogs which were suspected for Babesiosis and Ehrlichiosis.

Blood collection for hematological and microscopic evaluation To prevent clotting, two milliliters of whole blood were drawn from the cephalic/saphenous vein with an EDTA-coated vacutainer for hematology and four ml of blood was collected into serum vacutainer tube with clot activator for serum biochemistry. The BC-2800 Vet, Auto Hematology Analyzer is then used to assess various hematological parameters and semi-automatic serum biochemical analyzer RX-50 of Micro Lab., India for serum biochemical parameters on this sample.

Results and discussion

It was observed that highest percent of the dogs considered for this study were of the age group between 1 – 3 years, followed by ≤ 1 year age group, 3 – 5 years and ≥ 5 years. These results were in agreement with Abd Rani *et al.*, (2011),

Singh *et al*., (2014) [32], Kottadamane *et al*., (2017) [21] and Manoj *et al*., (2019) [24]. Incidence rate is lower in the age group less than 1 year could be due to the fact that the dogs are not much exposed to external environment so the chances of tick infestation is lower and in older dogs tick control measures will be regular so the incidence rate may be lower.

In the present study the highest number of dogs in the study group were Labrador retriever, followed by German shepherd, Rottweiler, Shih Tzu and Siberian husky Indie and Golden retriever. The results are concordant with Singh *et al*., (2014) [32], Bhadesiya & Modi (2015) [5] and Kottadamane *et al*., (2017) [21]. There is no any breed predilection for hemoprotozoan diseases but German shepherd dog is more predisposed to Ehrlichiosis due to inherent breed inability of blast formation and leucocyte migration inhibition factor. This may be due to the defective cell-mediated immune response seen in this breed. (Huxsoll *et al*., 1972) [19].

Male dogs were affected than female dogs in the present study which was in similarity with Singh *et al.*, (2014) [32], Singla *et al*., (2016) [33] and Manoj *et al*., (2019) [24] and Kopparthi *et al*., (2023) [20] which can be attributed to Male dogs may fight more often and can acquire this parasite when bitten by a carrier dog (Singla *et al*., 2016) [33].

In the present study **t**he mean rectal temperature of the dogs in the control group was 101.8±0.212 ^{0}F, whereas the mean rectal temperature recorded in the study group was 102.5±0.357 ^{0}F. Similar findings were reported by Sarma *et al*., (2015) [30], Bilwal *et al*., (2017) [6] and Kottadamane *et al*., (2017) [21]. Pyrexia is not a cardinal sign in canine Babesiosis due to *B. gibsoni*. (Bilwal *et al*., 2017) [6].

The mean heart rate recorded in the dogs in the control group was 123.8±3.119 bpm, whereas the mean heart rate recorded in the study group was 135.5±7.542 bpm. The results are concordant with Dvir *et al*., (2004) [14], Bilwal *et al*., (2017) [6], Daste *et al*., (2013) [12] and Filippi *et al*., (2019) [15]. The increased heart rate is a consequence of an exacerbated physiological stimulation of the autonomic nervous system and may occur due to cardiac or extracardiac changes, such as excitement, fever, hypovolemia, pain or as an early sign of heart failure.

In the hematological parameters, total leukocyte counts were significantly lower in the study group, whereas all other parameters such as total erythrocyte count, hemoglobin levels, platelet count and PCV were significantly lower in the study group. Similar results were reported by Das and Konar (2013) [11], Sarma *et al*., (2015) [30] and Filippi *et al*., (2019) [15]. These elevated cell numbers were higher than those observed in other canine blood parasitic infections, which may be due to the inflammatory response induced by tissue invasion and multiplication in CVBD's. The results from RBC parameters suggested normocytic normochromic anemia, which is non-regenerative due to bone marrow dysfunction (Fleischman, 2012) [16], Severe microcytic-hypochromic anemia may have been initiated by antibody mediated cytotoxic destruction of erythrocytes and/or by auto-antibody directed against components of the membranes of infected and uninfected erythrocytes which has also been reported previously in *B. gibsoni* infection (Aysul *et al*., 2013) [3].

In serum biochemical parameters estimated ALT and globulin levels were non significantly higher, whereas total bilirubin, creatinine and BUN were significantly higher. Total protein, albumin and A/G ratio were non significantly lower. The results are in agreement with Sarma *et al*., (2015) [30], Bilwal *et al*., (2017) [6] and Dhavalgi *et al*., (2021) [13]. Significant elevation of bilirubin, ALT and AKP are indicative of hepatic hypoxia (Aysul *et al*., 2013) [3]. Whether the insult is due to inflammatory cytokines, hypoxic damage, or a combination of these is not known. Hypoproteinemia along with hypoalbuminemia, hyperglobulinemia and hyperbilirubinemia in concomitant TBDs in dogs might be due to a chronic inflammatory disease, anorexia or decreased protein intake (Mylonakis *et al*., 2010) [26]. Increase in the levels of Creatinine and BUN are indicative of degenerative changes due to inflammatory response during the disease process (Gonde *et al*., 2017) [17].

Table 1: Hematological parameters recorded in the present study in Group 1 and Group 2

Parameter	Group 1 (N=10)	Group 2 (N=15)	P value
TLC (X 10^3)	9.366±0.7837	17.69±2.854	*
TEC (X10^6)	6.186±0.06954	2.919±0.3857	***
Hgb (gm/dl)	13.35±0.2207	6.373±0.5855	***
PLT (X 10^3)	308.9±19.82	59.80±12.53	***
PCV (%)	39.89±0.6803	19.42±1.762	***

NS: Non-Significant at p>0.05 level;* Significant at P≤0.05 level;** Significant at p≤0.01 level

Table 2: Serum biochemical parameters recorded in the present study in Group 1 and Group 2

Biochemical parameter	Group 1 (N=10)	Group 2 (N=15)	P value
Alanine transaminase (IU/L)	38.20±4.343	69.93±24.49	NS
Total Bilirubin (mg/dL)	0.2800±0.03887	0.9067±0.08075	***
Total protein (mg/dL)	6.330±0.1989	6.200±0.1543	NS
Albumin (mg/dL)	3.340±0.1318	3.263±0.1676	NS
Globulin (mg/dL)	3.030±0.1136	3.133±0.1369	NS
Albumin/globulin ratio	1.102±0.02225	1.041±0.03702	NS
Creatinine (mg/dL)	1.050±0.06540	1.567±0.1157	**
Blood urea nitrogen (mg/dL)	19.85±1.532	28.49±1.398	*

NS: Non-Significant at p>0.05 level;* Significant at p≤0.05 level;** Significant at p≤0.01 level

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