

## CLINICO- HEMATOBIOCHEMICAL ALTERATIONS IN DOGS INFECTED WITH CANINE MONOCYTIC EHRLICHIOSIS

L. NARAYANA RAO, B. SHOBHAMANI<sup>1</sup>, V. VAIKUNTA RAO<sup>2</sup> and K.V. SUBRAMANYAM<sup>3</sup>

Department of Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram,  
Sri Venkateswara Veterinary University, Tirupati-517502, India

<sup>1</sup>Department of Veterinary Medicine, <sup>2</sup>Department of Veterinary Clinical Complex, CVSc, Tirupati-517 502

<sup>3</sup>Department of Veterinary Microbiology CVSc, Proddatur-516 360

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### ABSTRACT

The retrospective study was carried out during the period from January 2018 to October 2018. Fifty ehrlichiosis suspected dogs were subjected to various diagnostic methods and 17 dogs were confirmed positive for canine ehrlichiosis. All these canine ehrlichiosis positive dogs were subjected to hemato-biochemical analysis, radiography, abdominal ultrasonography and morphological tick identification. Most frequently observed clinical signs of *Ehrlichia* infected dogs were tick infestation, anorexia, lymphadenopathy, pale mucosa, pyrexia and hemorrhagic tendencies i.e. melena, petechial hemorrhages and epistaxis. Radiography and ultrasonography revealed that 35.29 percent dogs had internal organ changes like nodular interstitial lung pattern, splenomegaly and hepatomegaly with a distended gallbladder, ascites, pericardial effusion and ascites. The hemato-biochemical alterations included significant increase in mean total leukocyte, lymphocyte, granulocyte, alanine amino transferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine. A significant decrease was recorded in mean hemoglobin (Hb), hematocrit (HCT), total erythrocyte count (TEC) and platelet count. Correlating clinical signs and hemato-biochemical alterations with radiographic and ultrasonographic findings will be very much useful to spot the disease condition and prognosis.

**Keywords:** Canine ehrlichiosis, Hematology, Melena, *Rhipicephalus sanguineus*, Splenomegaly.

Ehrlichiosis is a multi-systemic, transmissible, infectious disease of dogs. It is caused by a small, Gram-negative, coccoid bacterium described by the cellular tropism of the infecting host i.e., *Ehrlichia canis*. *E. canis* parasitizes cytoplasm of the circulating monocytes in form of distinct clusters termed as “Morulae” (Bhadesiya and Raval, 2015). The disease is mainly transmitted by the brown tick of dogs i.e., *Rhipicephalus sanguineus* which passes the organism into the blood following the bite. The course of ehrlichiosis can be divided into three phases, viz., acute, subclinical and chronic. Acute ehrlichiosis is clinically characterized by sudden onset of fever, depression and abdominal pain. Physical examination of infected dogs shows pale mucous membranes, lymphadenomegaly, splenomegaly and hemorrhagic tendencies such as dermal petechiae, ecchymosis, and epistaxis. Thrombocytopenia is the most common hematological finding while non-regenerative anemia and a decline in leukocyte count may also occur. Subclinical infections are more common in naturally occurring ehrlichiosis, this phase is characterized by persistent rickettsemia and there are no visible alterations in clinical signs and hematology. The chronic stage includes distinct clinical findings with changes in hematological indices including non-regenerative anemia, thrombocytopenia and leucopenia while biochemical abnormalities may represent hypoalbuminemia, hyperglobulinemia and hypergammaglobulinemia. The present paper describes clinical signs, hemato-biochemical findings and internal organ changes in dogs infected with ehrlichiosis.

### MATERIALS AND METHODS

The retrospective study was carried out during the period from January 2018 to October 2018. Fifty ehrlichiosis

suspected dogs were subjected to different diagnostic methods like peripheral blood smear examination, buffy coat smear examination (Fig. 2), anigen rapid *E. canis* Ab test kit and nested PCR. Seventeen dogs were confirmed positive for canine ehrlichiosis. All positive dogs of canine ehrlichiosis were subjected to hemato-biochemical analysis, radiography, abdominal ultrasonography and morphological tick identification.

Blood samples were collected in EDTA vials from *Ehrlichia* positive dogs, complete hematology (total leucocyte count, differential leucocyte count, total erythrocyte count, hemoglobin, hematocrit and platelet count.) by Automatic haematology analyzer (MINDRAY, BC-2800Vet, China) and serum samples were used for biochemical analysis (total serum protein, serum albumin, serum globulin, Alanine aminotransferase (ALT) Alkaline phosphatase (ALP), BUN and creatinine) by semiautomatic serum analyzer (ERBA Mannheim, CHEM-5 PLUS V2, Germany). Six apparently healthy dogs brought for routine checkup and vaccination were selected as control group for obtaining normal data for comparison of parameters under study. The results were analyzed using paired ‘t’ test for comparing healthy and diseased groups. Statistical analysis was carried out according to the principles of Snedecor and Cochran (1994).

Ticks collected from *Ehrlichia* infected dogs were processed and their permanent mount was prepared as per the standard method of fixation, dehydration, staining and mounting. Internal organ changes were studied by radiography (Care stream, direct view classic CR radiographic Machine, U.S.A.) and abdominal ultrasonography (ALOKA PRO SOUND α6LT ultrasound machine). Linear array 3.5 MHz, 5.0 MHz, and 7.5 MHz probes were used for large,

\*Corresponding author: lnrao402@gmail.com

medium and small dogs (Nyland and Mattoon, 1995).

## RESULTS AND DISCUSSION

**Clinical findings:** The naturally occurring cases of ehrlichiosis were manifested by a wide variety of clinical signs. In the present study, detailed clinical signs exhibited by 17 *Ehrlichia* infected dogs are depicted in Table 1, Fig. 1. The most frequently observed clinical signs in canine ehrlichiosis were anorexia (100%) and history of tick infestation (100%), lymphadenopathy (88.23%). Observations of pale conjunctival mucous membrane (88.23%) and pyrexia (82.35%) corroborated with Kumar and Varshney (2006). Hemorrhagic tendencies like melena was noticed in 64.70 per cent, petechial hemorrhages in 52.94 percent and epistaxis in 35.29 percent cases which was in similar to the findings of Harrus and Waner (2011). This might have occurred due to reduction in the number of platelets or platelet dysfunction and altered vascular permeability induced by the organism. Edema of limbs or scrotum was exhibited in 17.64 percent of dogs, this could be because of hypoalbuminemia or altered vascular permeability induced by the organism. Digestive disturbances like emesis (11.76 %) and ophthalmic lesions (11.76%) were less frequently recorded.

**Morphological identification of ticks:** After processing, the ticks were observed under an oil immersion microscope and were noticed for short mouth parts with hexagonal basis capitulum, bifid coxae and festones and was confirmed as *Rhipicephalus sanguineus*. These ticks may act as a vector for ehrlichiosis. These findings corroborated with Bhadesiya *et al.* (2014).

**Hematological findings:** In the present study leucocytosis was significant ( $P<0.01$ ) when compared to healthy control dogs (Table 2). However, Bhadesiya *et al.* (2015) reported normal leucocyte count while Waner (2008) documented significant leucopenia. Significant variations in the leucocyte indices might be due to the collection of blood samples at different stages of infection. Amongst differential leucocyte count, the level of lymphocytes decreased significantly ( $P<0.01$ ) and granulocytes and monocytes increased significantly ( $P<0.05$ ). These findings were parallel with Bhadesiya *et al.* (2015). Lymphocytopenia is suggestive of myelosuppression while granulocytosis might be due to neutrophilia. In the current study, anemic changes (decreased levels of TEC, Hb, HCT,) and thrombocytopenia were significant ( $P<0.01$ ) when compared with healthy control, as endorsed by earlier workers (Devi *et al.*, 2015; Kasondra *et al.*, 2017). Decreased Hb and TEC could be due to epistaxis, petechial hemorrhages and bone marrow hypoplasia by the parasites leading to impaired production of cellular components of blood. Thrombocytopenia occurs due to increased platelet consumption and decreased platelet half-life due to immune-mediated splenic sequestration and destruction.

**Serum biochemistry:** As compared to healthy control, the

**Table 1**

**Frequency of clinical signs in *Ehrlichia* infected dogs (n=17)**

Clinical signs	Number of cases	Percent
Tick infestation	17	100
Anorexia	17	100
Lymphadenopathy	15	88.23
Pale conjunctival mucous membrane	15	88.23
Pyrexia	14	82.35
<b>Hemorrhagic tendencies</b>		
Melina	11	64.70
Petechial hemorrhages	9	52.94
Epistaxis	6	35.29
Respiratory distress	5	29.41
Limb/scrotal edema	3	17.64
Ataxia	3	17.64
Recumbence	3	17.64
Emesis	2	11.76
Corneal opacity	2	11.76

**Table 2**

**Hemato-biochemical alterations in *Ehrlichia* infected dogs (Mean±S.E)**

Parameter	Control group (n=6)	Diseased group (n=17)
WBC ( $10^3/\mu\text{l}$ )	9.98±0.73	21.50±1.56**
Lymphocyte (%)	17.27±0.85	13.56±1.09**
Monocyte (%)	4.22±0.32	6.12±0.55*
Granulocyte (%)	78.08±0.59	80.33±0.91*
Erythrocyte ( $10^6/\mu\text{l}$ )	6.83±0.24	3.66±0.33**
Haemoglobin (g/dl)	15.08±0.67	7.65±0.70**
HCT (%)	40.72±5.93	25.05±1.94**
Platelet ( $10^3/\mu\text{l}$ )	326.33±24.94	74.08±14.24**
Total serum protein (g/dl)	6.55±0.22	5.00±0.27**
Serum Albumin (g/dl)	2.93±0.10	1.02±0.09**
Serum globulin (g/dl)	3.62±0.19	4.0±0.16ns
ALT (IU/L)	30.13±3.86	94.08±6.54**
ALP (IU/L)	32.41±3.5	117.02±5.84**
BUN (mg/dl)	9.50±1.06	31.1±3.05*
Creatinine (mg/dl)	0.88±0.07	1.80±0.13**

\*\*Statistically highly significant ( $P\leq0.01$ ); \*Statistically significant ( $P\leq0.05$ )

serum biochemistry revealed significantly ( $P<0.01$ ) decreased levels of total serum protein and serum albumin (Table 2) and it was in accordance with previous reporters (Bhadesiya *et al.*, 2015) where there was significant increase in the BUN, creatinine, ALT and ALP levels and these findings were comparable with the result of Kasondra *et al.* (2016). However, there is no statistical difference in globulin level. On the contrary, Heebheather *et al.* (2003) reported hyperglobulinemia. Hypoproteinemia and hypoalbuminemia may be due to anorexia, peripheral loss to the edematous inflammatory fluid as a consequence of vasculitis, decreased protein production because of concurrent liver diseases or proteinuria. *E. canis* affects other internal organs like liver and kidney, hence there was an elevation in ALT, ALP, blood urea nitrogen and creatinine level. The elevation of ALT and creatinine levels might be due to immune complex-





Fig. 1. Clinical signs exhibited by *Ehrlichia* infected dogs

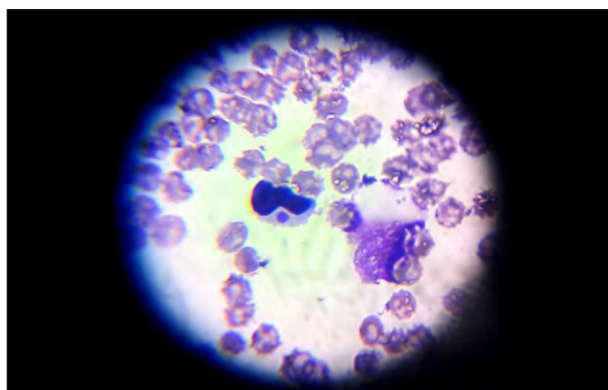


Fig. 2. *E. canis* morula in buffy coat smears (Purple colored inclusion bodies within the cytoplasm of monocyte suggestive of *E. canis* morula, Giemsa stain X1000) Hematology

**Table 3**

**Radiographic and abdominal Ultrasonographic findings**

Effected organs	No. of cases (n=17)	Percentage
Hepatomegaly	3	17.64%
Hepatomegaly with distended gallbladder	2	11.76%
Ascites	1	5.88%
Pericardial effusion +Ascites	1	5.88%
Nodular interstitial lung pattern	5	29.41%
Splenomegaly	5	29.41%

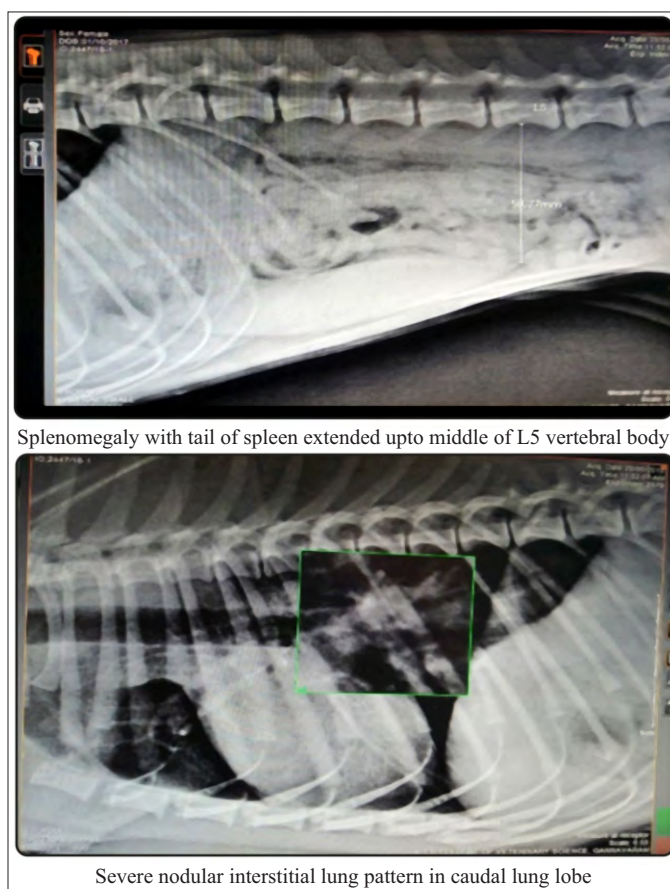


Fig. 3. Radiographic findings

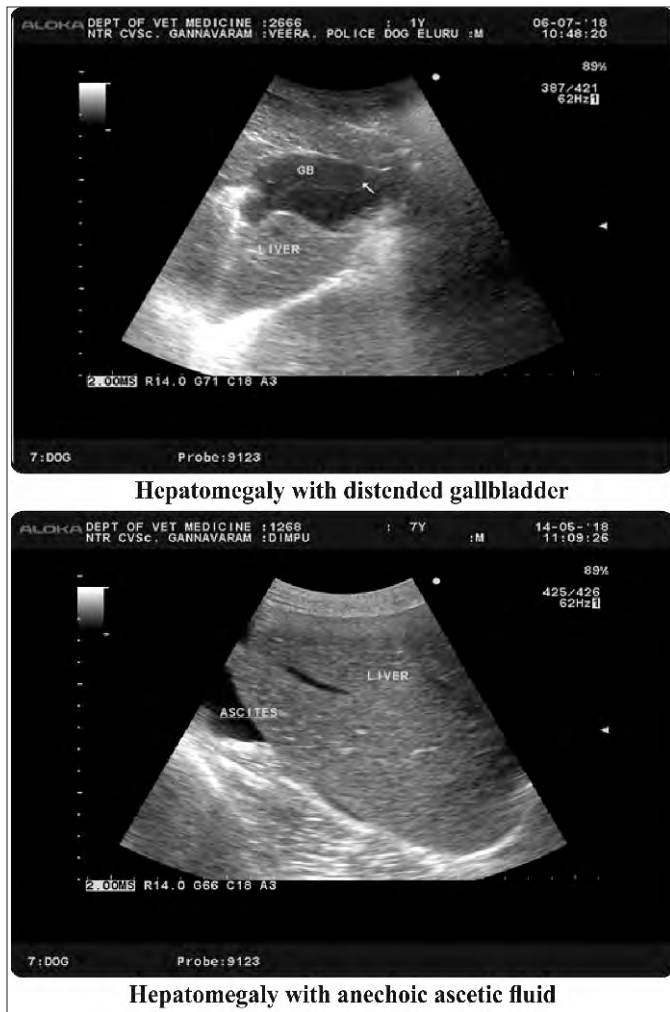


Fig. 4. Abdominal Ultrasonographic findings

mediated glomerulonephritis indicating renal involvement in dogs with ehrlichiosis.

**Radiographic findings :** The radiographic findings revealed that 6 dogs had internal organ changes (35.29%), amongst them 5 dogs (29.41%) had mild to severe interstitial lung pattern, two dogs had splenomegaly (extended up to middle of 5<sup>th</sup> lumbar vertebral body with increased opacity), three dogs had hepatomegaly (rounded caudo ventral margins with increased opacity of liver), one dog (5.88%) had ascites (generalized increased fluid density of abdomen and thorax) and one dog had ascites with pericardial effusion (5.88%), (Table 3, Fig. 3). These multi-systemic changes were suggestive of ehrlichiosis and comparable with the studies by Mylonakis *et al.* (2010) and Harrus and Waner (2011).

**Abdominal ultrasonographic findings :** Abdominal ultrasonography revealed splenomegaly in five dogs (29.41%) and hepatomegaly (hypoechoic texture with rounded edges) in three dogs (17.64%) and hepatomegaly with distended gall bladder in two dogs (11.76%). The details are presented in Table 3, Fig. 4. These observations are following the studies of Sarma *et al.* (2014) and Basavareddy *et al.* (2015). Splenomegaly might be attributed to the diffused proliferation

of lymphoreticular cells in the white pulp of the organ as well as reticuloendothelial cells in red pulp. Egenvall *et al.* (2000) opined that splenomegaly is because of reactive lymphoid hyperplasia and concurrent extramedullary hematopoiesis. As per Harrus *et al.* (1997), the spleen is the organ most likely to harbor *E. canis* parasites during the subclinical phase and the last organ to accommodate the parasite before elimination. Hence, splenomegaly is one of the ultrasonography alterations of dogs with ehrlichiosis. Hepatomegaly might be inflammatory infiltration in the hepatic tissue, the sonographic change in gall bladder included distention with the presence of sludge/clear bile which may be due to anorexia.

Correlating radiographic and USG findings with clinical signs and hemato-biochemical alterations will be very much useful to spot the disease condition and organ involvement in *Ehrlichia* infection in dogs.

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