

*Original Research***Clinical and Haematobiochemical Response in Canine Ehrlichiosis in Puducherry Region**

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Abstract

The present research work was undertaken to study the importance of hematobiochemical changes in canine ehrlichiosis. Blood samples were collected from 35 dogs with clinical signs suggestive of ehrlichiosis. Out of 35 dogs 16 were positive for canine ehrlichiosis in species specific nested PCR. The common clinical findings observed in the positive animals were fever, swollen superficial lymphnodes and tick infestation. Haematology evinced the Hb, PCV, RBC and thrombocyte levels were significantly reduced ($P \leq 0.01$) in comparison with apparently healthy animals. In differential leukocyte count monocytosis was noticed ($P \leq 0.05$). Serum biochemistry reveals significantly increased levels of globulin and AST ($P < 0.01$) than apparently healthy animals. In conclusion, the prevalence of canine ehrlichiosis was 45.71% in puducherry region and this following article discussed about the clinical and hemotobiochemical changes.

Key words: Dogs, *Ehrlichia canis*, Monocytosis, Nested PCR, *Rhipicephalus Sanguineus*, Thrombocytopenia

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Introduction

Canine ehrlichiosis is a tick borne disease caused by *Ehrlichia canis*, which has been termed “silent killer” as it is inapparent during the early and subclinical stages of infection. It is transmitted by brown dog tick *Rhipicephalus sanguineus*. The disease is clinically characterized by anorexia, dullness, swollen superficial

lymph nodes, fever, dark coloured urine, epistaxis, ecchymosis, organomegaly, hind leg weakness along with history of tick infestation (Moreira *et al.*, 2003).

The haematological changes in all stages of canine ehrlichiosis included decreased packed cell volume, hemoglobin, red blood cell count, leukocyte and platelet count. Biochemical abnormalities may represent hyperglobulinemia and hypoalbuminemia, mild elevation in alkaline phosphatase and alanine amino transferase activity (Ettinger and Feldman, 2005). Demonstration of *Ehrlichia spp. morulae* in monocytes of Romanowsky stained blood smear / buffy coat smear and less frequently, smear made from lymph node aspirate is helpful in establishing a definite diagnosis of acute CME. PCR is found to be a highly sensitive in early diagnosis of the disease, when compared to serological test (Iqbal and Rikihisa, 1994).

Effective therapeutic management of canine ehrlichiosis depends on the stage of the disease, its underlying pathophysiology and alterations in organ functions. The present study was carried out in order to evaluate the diagnostic significance of clinical and hematobiochemical parameters of ehrlichiosis in dogs of Puducherry region.

Materials and Methods

This study was conducted over a period of six months (Dec- May) at the Small Animal Medicine Unit, Teaching Veterinary Clinical Campus, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry India. Based on the clinical signs suggestive of canine Ehrlichiosis suspect 35 dogs were selected and screened for ehrlichiosis by both blood smear examination and/ or polymerized chain reaction and the samples were also subjected for hematobiochemical studies. The dogs that turned positive for ehrlichiosis were grouped under diseased and apparently healthy dogs were made as control group. Blood samples collected from both control and diseased group were subjected for complete hematology (hemoglobin [Hb], Packed cell volume, red blood cell count, total leukocyte count [TLC], differential leukocyte count, and platelet count) by blood cell counter and serum samples for biochemical analysis which includes aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], gamma glutamyl transferase (GGT), total protein, albumin, globulin, blood urea nitrogen [BUN] and creatinine by standard methods. The data were statistically analysed by one – way analysis of variance (ANOVA) using SPSS software version 17 for windows.

Results and Discussion

Among 35 dogs screened for Canine Ehrlichiosis 16 were found to be positive. The overall incidence was 45.71%. Which are in concurrence with the findings of Lakshmanan *et al.* (2007) from Chennai (50%) and

63.01% by Kartika (2013) from Puducherry region using a species-specific nested PCR. Dogs which were positive showed prominent clinical signs such as inappetance, fever, pale / blanched (Fig.1) to congested mucus membrane, tick infestation (Fig.2), swollen lymphnodes and high coloured urine (Fig.3). Waner and Harrus (2013) opined that the stage of the disease varies with clinical signs and described the disease in three stages as acute, subacute and chronic. Other signs noticed were epistaxis, petechiae/ecchymosis (Fig.4), ocular and nasal discharge.



Fig.1: Blanched mucosa



Fig.2: Severe tick infestation



Fig.3: High coloured urine



Fig.4: Petechiation in ventral abdomen

Pyrexia an important clinical finding observed in our study which was 43.75%, whereas Bhadesiya and Raval (2015) observed 100% and 71.8 % by Thirunavukkarasu *et al.* (1994), which is due to the release of endogenous pyrogens related to activation of inflammatory mediators as reported by Heerden (1982). Das and Konar (2013) reported that tick infestation or previous history of tick infestation was found in 100% cases of ehrlichia infection in dogs, whereas the present study recorded 75% .The predominant gastrointestinal signs were melena in 11.11% cases whereas Mylanokis *et al.* (2014) found gastrointestinal signs in approximately 10–15% of dogs, 14.06% by Thirunavukkarasu *et al.* (1994). Lymphadenomegaly (75%) is a common finding in this study, which is due to hyperplastic activity of both B and T lymphocytes in lymphnodes by stimulation of ehrlichia antigens as described by Waner and Harrus (2013). Bhadesiya and Raval (2015) reported lymphadenopathy in 94.4% cases. Bleeding disorders were observed as epistaxis and ecchymosis/petechiation which is the outcome of immune-mediated destruction, increased consumption of platelets by sequestration, decreased production, vasculitis, platelet function abnormalities (Lappin *et al.*, 2010) and antiplatelet antibodies in dogs with ehrlichiosis (Cortese *et al.*,2011.) The common clinical findings observed in the study were described in Fig. 1

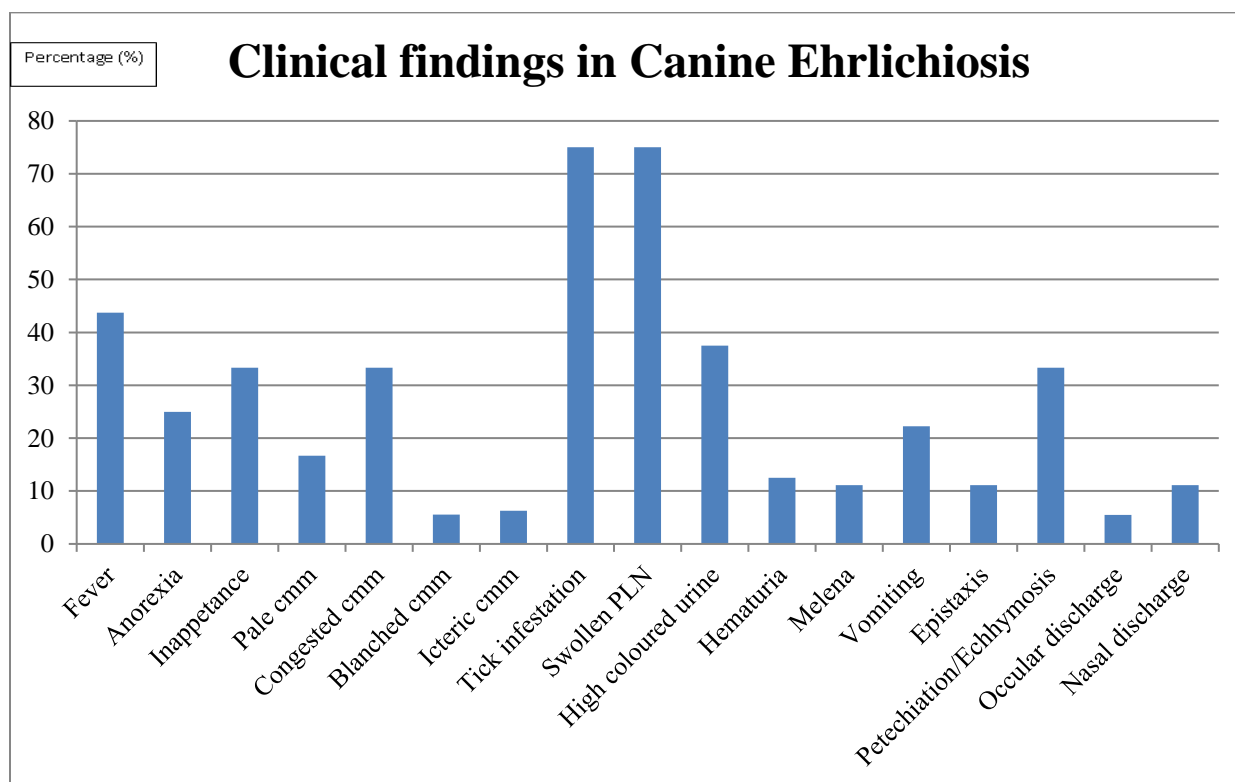


Fig. 1: Clinical findings in dogs with ehrlichiosis

Hematobiochemical Findings

The most common hematobiochemical findings in this study were anemia, thrombocytopenia and monocytosis (Table.1). Hematological abnormalities like anemia and thrombocytopenia were frequently encountered in CME (Neer and Harrus, 2006). Thrombocytopenia ($P \leq 0.01$) is the most common and consistent haematological finding in ehrlichiosis affected dogs. Similar findings have been documented by Macieira *et al.* (2005); Niwetpathomwat *et al.* (2006) and Silva *et al.* (2012). Nakaghi *et al.* (2008) recorded anemia, leucopenia and thrombocytopenia as marked hematological changes in dogs of ehrlichiosis. Levels of monocytes increased significantly ($p \leq 0.01$) in dogs with ehrlichiosis than healthy dogs. These findings were in agreement with Castro *et al.* (2004).

Table.1: Haematobiochemical parameters of control and diseased animals

Parameter	Apparently healthy dogs (n=7)	Dogs with ehrlichiosis (n=16)	P value
Hb (g/dl)	15.94±2.5	9.41±1	0.001**
PCV (%)	46.91±2.29	28.73±2.86	0.001**
RBC ($\times 10^6/\text{mm}^3$)	6.46±0.18	4.19±10	0.001**
WBC ($\times 10^3/\text{mm}^3$)	7242.86±516.79	10425±2102.12	0.337 ^{NS}
Platelet ($\times 10^5/\text{mm}^3$)	2.87±0.17	0.9±0.08	0.000**
Neutrophil (%)	77±2.06	69.75±3.36	0.187 ^{NS}
Lymphocyte (%)	21.14±1.28	27.94±3.44	0.216 ^{NS}
Monocyte (%)	0.29±0.18	1.38±0.26	0.015*
Eosinophils (%)	1.57±0.95	0.88±0.45	0.455 ^{NS}
BUN (mg/dl)	15.65±2.25	26.86±8.05	0.377 ^{NS}
Creatinine (mg/dl)	0.86±0.04	1.73±0.73	0.442 ^{NS}
Total protein (g/dl)	6.4±0.1	6.51±0.18	0.700 ^{NS}
Albumin (g/dl)	3.26±0.06	2.36±0.1	0.000**
Globulin (g/dl)	3.19±0.12	4.14±0.21	0.008**
ALT (IU/L)	50.71±5.64	57±5.82	0.520 ^{NS}
AST (IU/L)	40.86±3.75	75.56±5.28	0.000**
ALP (IU/L)	61.57±8.07	81.44±12.78	0.338 ^{NS}
GGT (IU/L)	4.93±0.18	6.24±0.81	0.303 ^{NS}

** : Significant ($P \leq 0.01$); * : Significant ($P \leq 0.05$); NS: Not significant (≥ 0.05)

Hypoalbuminemia, hyperbilirubinemia and increased alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were reported by Mylonakis *et al.*, (2010). The present study showed higher AST value significantly ($p < 0.01$) in dogs with ehrlichiosis than healthy dogs but ALT and ALP levels were within the normal range (Table: 2). These findings were in agreement with Agnihotri *et al.* (2012); Bhardwaj (2013) and Bhadesiya and Raval (2015) which is an indication of hepatic dysfunction. Biochemical abnormalities such as hypoalbuminemia ($P \leq 0.01$) and hyperglobulinemia ($P \leq 0.01$) is a significant finding which is in accordance with Irwin (2007). Hypoalbuminemia may be the consequence of peripheral loss of albumin to edematous inflammatory fluids as a result of increased vascular

permeability, blood loss or decreased protein production due to concurrent mild liver disease, or it may be due to minimal change glomerulopathy, the decreased albumin concentration act as compensatory mechanism for the hyperglobulinemic state to maintain the osmotic pressure and preventing increase in blood viscosity (Woody and Hoskins, 1991). BUN and serum creatinine values showed no alteration which is in accordance with Sharma *et al.* (2015).

Conclusion

The overall incidence of canine ehrlichiosis in Puducherry based on PCR was 45.71%. Classical clinical findings in the diseased animals were fever, swollen superficial lymph nodes and history of tick infestation. A decrease in Hb, PCV, RBC, platelet, albumin and increase in monocytes, globulin and AST levels were recorded in diseased.

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