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Clinical findings and hemato biochemical alterations in dogs affected with *Ehrlichia canis*

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Abstract

776 dogs showing pyrexia, anorexia, hemorrhagic tendencies and pale conjunctiva which were suggestive of clinical signs of *E. canis* were identified at Veterinary Clinical Complex, College of Veterinary Science, Rajendranagar, Hyderabad during the period January 2020 to February 2021 and subjected for detailed clinical examination. 124 dogs were screened for ehrlichiosis by different diagnostic methods. Out of 124 samples 5 (4.03%), 109 (87.5%), 120 (96.77%) samples were detected as positive for ehrlichiosis by peripheral blood smear examination, rapid antigen test kit and nested PCR respectively. The clinical signs of *Ehrlichia canis* infected dogs were tick infestation, anorexia, pale mucosa, pyrexia and hemorrhagic tendencies (melena, petechial hemorrhages and epistaxis). Other signs observed were respiratory distress, scleral hemorrhage, recumbency and emesis. The haematological and bio-chemical alterations in *Ehrlichia canis* infected dogs include significant increase in mean total leukocyte count, ALT, ALP, BUN and creatinine and significant decrease was recorded in mean Hb, HCT, TEC and platelet count and A/G ratio.

Keywords: Canine ehrlichiosis, clinical findings, haematological alterations, and biochemical profile

1. Introduction

Among the haemo-protozoal diseases, canine ehrlichiosis was found to be the most rapidly emerging disease, endangering the lives 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) [4].

The disease in dogs is mainly transmitted by brown tick *Rhipicephalus sanguineus* (Venkatesa *et al.*, 2018) [24]. The disease could also be transmitted through blood transfusion from an infected dog (Ettinger and Feldman, 2005) [8]. The incubation period of *Ehrlichia canis* varied from 8 to 20 days in dogs (Salib and Farghali, 2015) [18]. The clinical signs are mainly characterized by high body temperature, ophthalmic lesions, epistaxis and lymphadenopathy (Barman *et al.*, 2014) [3]. Other clinical signs exhibited in the affected dogs are weakness, depression, anorexia, bleeding, neurological signs, inflammation of the eye, chronic weight loss, edema in hind legs, pale gums and emaciation (Buhles *et al.*, 1974) [5]. Significant haemo-pathological findings determined in dogs affected with *E. canis* were anemia, thrombocytopenia and leukopenia (Alexander *et al.*, 2016) [2]. Among the alterations in the hematological parameters, anemia was the most prevalent followed by monocytosis and thrombocytopenia (Senthil *et al.*, 2020) [20]. Biochemical abnormalities like hypoalbuminemia (Anuchai *et al.*, 2006) [25], hyperglobulinemia and hypergamma globulinemia (Akhtardanesh *et al.*, 2010) [1]. The present paper describes clinical signs and hemato-biochemical findings in dogs infected with ehrlichiosis.

2. Materials and Methods

The present research was undertaken to study the clinical signs and hemato-biochemical findings, in ehrlichiosis in dogs. The present study was carried out at Veterinary Clinical Complex, College of Veterinary Science, Rajendranagar, during the period January 2020 to February 2021. Pet dogs referred from Veterinary Hospital, Bhoiguda were also included in the study. 124 dogs which exhibited the clinical signs suggestive of Ehrlichiosis were further subjected to detailed study.

Five ml blood was collected from forty animals (randomly divided in Group-I and Group-II with 20 dogs in each group for further comparative study) for hematology, serum

biochemistry. Five ml blood was also collected from apparently healthy dogs (20) for comparative study. Whole blood from suspected dogs was collected from cephalic or lateral/ medial saphenous veins. The blood was collected in vacutainer with EDTA and analyzed using ABX Micros ESV 60 automatic hematological analyzer supplied by HORIBA, India. Whole blood was transferred into in clot activator coated sterile serum vials. Serum vacutainers were kept undisturbed till serum separation and analyzed using EM DESTINY 180 automatic biochemical analyzer supplied by TRANSASIA Bio-Medicals, Mumbai, India.

3. Results and Discussion

3.1 Clinical findings

The clinical findings in *Ehrlichia canis* affected Group I dogs were current tick infestation or history of tick infestation (100%), anorexia (100%), followed by lethargy (90.00%), pyrexia (90.00%), pale mucous membranes (90.00%), general debility (85.00%), melena (55.00%) and petechial hemorrhages (45.00%). Other less frequently recorded clinical findings were dehydration (30.00%), respiratory distress (30.00%), emesis (15.00%), epistaxis (15.00%), scleral hemorrhage (10.00%), recumbency (10.00%) and ataxia (5%) presented in Table 1, Fig. 1, 2, 3, 4, 5.

Table 1: Clinical Findings in Group I *Ehrlichia canis* affected dogs (n=20)

Clinical Signs	Number of Cases	Percentage
Tick Infestation	20	100.00
Anorexia	20	100.00
Lethargy	18	90.00
Pyrexia	18	90.00
Pale Mucous Membranes	18	90.00
General Debility	17	85.00
Melena	11	55.00
Petechial Hemorrhages	9	45.00
Dehydration	8	40.00
Respiratory Distress	6	30.00
Emesis	3	15.00
Scleral Hemorrhage	3	15.00
Epistaxis	2	10.00
Recumbency	2	10.00
Ataxia	1	5.00

The clinical findings in *Ehrlichia canis* affected Group II dogs were current tick infestation or history of tick infestation (100%), anorexia (100%), followed by lethargy (95.00%), pyrexia (90.00%), pale mucous membranes (85.00%), general debility (75.00%), melena (65.00%) and petechial hemorrhages (50.00%). Other less frequently recorded clinical findings were dehydration (45.00%), respiratory distress (35.00%), emesis (25.00%), epistaxis (20.00%), scleral hemorrhage (20.00%), recumbency (20.00%) and ataxia (10.00%) presented in Table 2, Fig. 6, 7, 8, 9.

observations corroborated with Kumar and Varshney *et al.* (2006) [10]. These symptoms were followed by bleeding tendencies, i.e. melena and petechial hemorrhages that were in line with the findings of Devi *et al.* (2015) [7] and Palacios *et al.* (2017) [14]. Other less frequently recorded clinical findings were dehydration, respiratory distress, emesis, scleral hemorrhage, ataxia, recumbency, epistaxis, which were in accordance with the findings of Senthil *et al.* (2020).

Table 2: Clinical Findings in Group II *Ehrlichia canis* affected dogs (n=20)

Clinical Signs	Number of Cases	Percentage
Tick Infestation	20	100.00
Anorexia	20	100.00
Lethargy	19	95.00
Pyrexia	18	90.00
Pale Mucous Membranes	17	85.00
General Debility	15	75.00
Melena	13	65.00
Petechial Hemorrhages	10	50.00
Dehydration	9	45.00
Respiratory Distress	7	35.00
Emesis	5	25.00
Scleral Hemorrhage	4	20.00
Epistaxis	4	20.00
Recumbency	4	20.00
Ataxia	2	10.00

In the present study, the most frequent clinical findings in dogs affected with *Ehrlichia canis* was current tick infestation or history of tick infestation, anorexia followed by lethargy, pyrexia, pale mucous membranes, general debility and these



Fig 1: Ticks in the ear

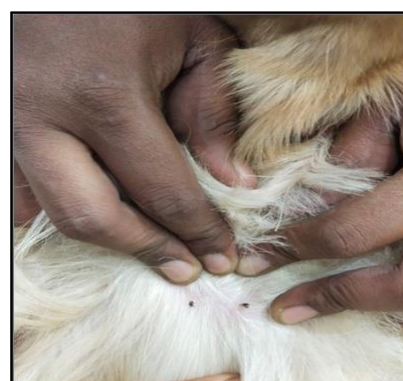


Fig 2: Ticks on body

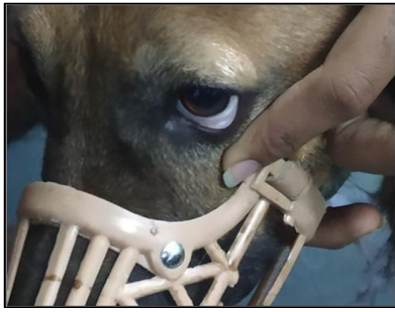


Fig 3: Pallor of CMM



Fig 4: Pallor of BMM



Fig 5: Scleral hemorrhages in *Ehrlichia canis* affected dogs



Fig 6: Petechial hemorrhages on abdomen in *Ehrlichia canis* affected dogs



Fig 7: Petechial hemorrhages on the ear pinna



Fig 8: Melena in *E. canis* affected dogs



Fig 9: Epistaxis in *E. canis* affected dogs

3.2 Hematological findings

The mean values of hematological parameters of 20 healthy dogs and 40 *Ehrlichia canis* affected dogs (Group I- 20) and dogs (Group II- 20) are presented in Table 3 and Table 4.

Table 3: Hematological alterations in Group I *Ehrlichia canis* affected dogs

Parameter	Healthy Control (n =20)	Group I (n =20)
Hemoglobin g/dL	14.05±0.54	6.97±0.41**
Packed cell Volume %	45.4±2.63	18.82±0.65**
Total Erythrocyte Count× 10 ⁶ /µl	7.08±0.31	3.21±0.19**
Total Leucocyte Count× 10 ³ /µl	9.17±0.82	18.28±0.64**
Lymphocytes %	25.52±1.95	25.82±1.28ns
Monocytes %	4.17±0.27	6.20±0.15**
Neutrophils %	66.08±3.18	75.33±3.84 s
Eosinophils %	3.45±0.52	3.12±0.22 ns
Basophils %	0.14±0.06	0.15±0.06 ns
Platelets × 10 ³ /µl	320.67±6.65	81.16±6.63**

** - Statistically highly significant ($p \leq 0.01$)

* - Statistically significant ($p \leq 0.05$)

ns - Non significant

Table 4: Hematological alterations in Group II *Ehrlichia canis* affected dogs

Parameter	Healthy Control (n =20)	Group II (n =20)
Hemoglobin g/dL	14.05±0.54	6.53±0.13 **
Packed cell Volume %	45.4±2.63	18.05±0.58**
Total Erythrocyte Count× 10 ⁶ /µl	7.08±0.31	3.72±0.06**
Total Leucocyte Count× 10 ³ /µl	9.17±0.82	18.23±0.67**
Lymphocytes %	25.52± 1.95	25.00±0.69 ns
Monocytes %	4.17±0.27	6.65±0.13**
Neutrophils %	66.08±3.18	75.67±1.32 ns
Eosinophils %	3.45±0.52	3.25±0.19 ns
Basophils %	0.14±0.06	0.15±0.06 ns
Platelets × 10 ³ /µl	320.67±6.65	78.16±3.55**

** - Statistically highly significant ($p \leq 0.01$)

* - Statistically significant ($p \leq 0.05$)

ns - Non significant

In the present study, anemic changes (low TEC, Hb, HCT,) and thrombocytopenia were significant ($P<0.01$) when compared with healthy control as endorsed by earlier workers (Bhadesiya and Raval 2015, Laxmi Bai *et al.*, 2016, Sangeetha *et al.*, 2017 and Senthil *et al.*, 2020) [4, 11, 19, 20]. 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 (Neer *et al.*, 2002) [13]. Thrombocytopenia occurs due to increased platelet consumption and decreased platelet half-life due to immune mediated splenic sequestration and destruction (Sangeetha *et al.*, 2017) [19]. Variations in haematological profiles in *Ehrlichia canis* infected dogs may be related to differences in the virulence of *Ehrlichia canis* strains, antigen heterogeneity of this bacterial agent and the clinical form of the disease (Tsachev *et al.*, 2013) [23]. In the present study, leukocytosis was significant ($p<0.01$). However, Laxmi Bai *et al.* (2016) [11] reported normal leukocyte counts. In contrary Singla *et al.* (2011) [21] and Sainz *et al.* (2015) [17] documented significant leukopenia.

Among differential leukocyte count, levels of lymphocytes were non-significant ($p\geq 0.05$), which was in line with the findings of Rahul *et al.* (2016) [15]. The monocytes counts were significantly higher ($p<0.01$) than the normal values and this was in line with the findings of Laxmi Bai *et al.* (2016) [11] and Senthil *et al.* (2020) [20]. The counts of neutrophils were non-significant ($P\geq 0.05$) as compared to the normal

values, which were in agreement with the findings of Rahul *et al.* (2016) [15], while they were contrary to the findings of Laxmi Bai *et al.* (2016) [11], who reported neutropenia. The counts of basophils were non-significant ($P\geq 0.05$) as compared to the normal values which were in line with the findings of Das and Konar (2013) [6].

3.3 Serum biochemistry

The mean values of serum bio chemical parameters of 20 healthy dogs and 40 *Ehrlichia canis* affected dogs (Group I-20) and dogs (Group II -20) are presented in Table 5 and Table 6.

Table 5: Serum Biochemical Alterations in Group I *Ehrlichia canis* affected dogs

Parameter	Healthy Control (n =20)	Group I (n =20)
BUN (mg/dl)	19.75±0.59	37.81±1.21**
Serum Creatinine (mg/dL)	0.55±0.03	1.93±0.10**
SGPT/ ALT (IU/dL)	48.33±7.46	111.51±4.28**
SGOT/ AST (IU/dL)	31.83±1.53	75.83±2.30**
Total Protien (g/dL)	6.85±0.14	7.05±0.14 ns
Albumin (g/dL)	3.77±0.05	3.02±0.13**
A/G ratio	1.23±0.04	0.77±0.07**

*- Statistically highly significant ($p\leq 0.01$)

*- Statistically significant ($p\leq 0.05$)

ns- Non significant

Table 6: Serum Biochemical Alterations in Group II *Ehrlichia canis* affected dogs

Parameter	Healthy Control (n=20)	Group II (n = 20)
BUN (mg/dl)	19.75±0.59	38.83±1.72**
Serum Creatinine (mg/dL)	0.55±0.03	1.95±0.05**
SGPT/ALT (IU/dL)	48.33±7.46	119.33±1.93**
SGOT/AST (IU/dL)	31.83±1.53	73.33±3.72**
Total Protien (g/dL)	6.85±0.14	6.78±0.15 ns
Albumin (g/dL)	3.77±0.05	3.03±0.10**
A/G ratio	1.23±0.04	0.81±0.04**

** - Statistically highly significant ($p\leq 0.01$)

* - Statistically significant ($p\leq 0.05$)

ns - Non significant

In the present study, compared to healthy control, the serum biochemistry revealed significantly ($p<0.01$) increased levels of SGOT, SGPT and serum creatinine, and it was in line with previous reporters, (Bhadesiya and Raval 2015, Laxmi Bai *et al.*, Rao *et al.*, 2020) [4, 11, 16] and it could be due to immune complex- mediated glomerulo-nephritis indicating renal involvement in dogs with ehrlichiosis (Bhadesiya and Raval 2015) [4]. Hypoalbuminemia was observed in the affected dogs in the present study and was in line with previous workers (Mylonakis *et al.*, 2010 and Solomon *et al.*, 2019) [12, 22]. Values of BUN were significantly high ($p<0.01$), in agreement to the findings of the previous workers (Rao *et al.*, 2020) [16]. The total protein levels in the affected dogs were non-significant in comparison to the values of healthy dogs and the globulin levels were elevated and this finding agreed with Harrus *et al.* (1997) [9], who reported hyperglobulinemia. The values of the Albumin: Globulin (A/G) ratio decreased significantly ($p<0.01$), which may be attributed to hypo albuminemia and hyperglobulinemia as reported by Harrus *et al.* (1997) [9]. This was in contrary to the findings of Laxmi Bai *et al.* (2016) [11], who reported significant rise in the A/G values in the affected dogs.

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