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## Pathological studies of canine ehrlichiosis in two coastal districts of Odisha

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### Abstract

Present study was aimed to evaluate the clinic-pathological alterations in Ehrlichiosis affected dogs in two coastal districts like Puri and Ganjam in Odisha over a period of four months i.e. November, 2021 to February, 2022. A total of 53 pet dogs (n=19, Puri; n=34, Ganjam) was included for screening which were either presented to local veterinary dispensaries as well as Veterinary Clinical Complex (VCC) of the college with non-specific clinical signs like dullness, in-appetence with pyrexia for more than 10 days. Screening was done on basis of blood smear examinations by observing morulae in leucocytes showing. Present study recorded an overall prevalence of 28.57% (n=16) of Ehrlichiosis among dogs. Prevalence of disease in Puri and Ganjam recorded 26.31% (n=5) and Ganjam (n=11, 32.35%) respectively. There was highest prevalence of ehrlichiosis among male dogs (n=11, 68.75%), dogs between 1-3 year ages (n=10, 62.5%), German Shepherd breeds (n=43.75%). Hematological evaluations revealed a decline in total leucocytes and total platelet count. Serum biochemical examinations revealed an altered liver and kidney function tests. One dog died during the study period subjected for detail post-mortem examination showing degenerative changes in liver and kidney. Microscopic examinations of representative tissues collected during necropsy revealed necrosis of hepatocytes, sinusoidal congestion with vacuolar to fatty degenerate changes and increased bowman's spaces with accumulations of pinkish exudates suspected of proteinaceous deposits in the tubular lumens.

**Keywords:** Dog, districts, ehrlichiosis, haemato-biochemical, necropsy, Odisha

### Introduction

Canine Ehrlichiosis is one of the emerging tick-borne tropical disease with characteristic multi-systemic effect occurring in every part of the globe. Hot and humid conditions in tropical climate favor the proliferation of ticks thus helps in increasing occurrence of disease. Etiology of disease linked to *Ehrlichia canis*, belonging to a rickettsia organism, transmitted mostly by *Rhipicephalus sanguineus*, popularly termed as brown dog tick<sup>[1]</sup>. Predilection site for the organism mostly owes to liver and lymph node altering the mononuclear phagocytic system of the host thus resulting most of the related pathology. In most of the cases, affected dogs may show non-specific clinical signs like dullness, fever, muscle pain with reduced body weight<sup>[2]</sup>. Typical symptoms like bleeding from nose, edema of hind limbs, ocular-nasal discharges, ulcerative stomatitis etc. may also be recorded<sup>[3]</sup>. Affected dogs show a great decline in white blood cells and platelets<sup>[4]</sup> attributing to myeloid depression<sup>[5]</sup>. Liver disorders in affected dogs reflected with increased liver specific enzymes and other renal related biochemical parameters<sup>[6]</sup>. Post mortem examination revealed hepatomegaly and lymphadenopathy along with hemorrhages in various visceral organs<sup>[7]</sup>. Diagnosis for Ehrlichiosis mostly done by examination of the giemsa stained blood smear and observing morulae in monocytes<sup>[8]</sup>. There is poor documentation of this disease in this part of globe which will be addressed by the present study to give awareness as well as in refreshing the knowledge among the field veterinarians to have a proper planning for mitigation of the disease with early diagnosis and advocating suitable therapy.

### Materials and Methods

Dogs of different ages, breeds and sexes, presented to different local veterinary dispensaries of two coastal districts i.e. Puri (n=29) and Ganjam (n=44) with a complain of persistent fever and dullness were subjected for Ehrlichiosis screening through giemsa stained blood smear examinations to observe the presence of morulae in leucocytes.

Further, these dogs were subjected for detail haemato-biochemical examination through automatic hematological analyzer (Medonic M51, Sweden) and automatic serum biochemical analyzer (CPC, I-track) at College of Veterinary Science and Animal Husbandry, Bhubaneswar with a request by the pet owners as well as concerned field veterinarians. A thorough blood smear examination was conducted by adopting giemsa protocol. During the study period, one affected dog died and presented for detail necropsy. Representative tissue samples collected during post-mortem examination were further processed for routine hematoxylin and eosin staining (H&E) in Department of Veterinary Pathology. Epidemiological risk factors were analyzed to find the level of significance responsible in occurrences of disease among dogs.

## Results and Discussion

### Epidemiological Risk factors

Present study recorded an overall prevalence of 28.57% (n=16) of Ehrlichiosis among dogs in two coastal districts with 26.31% (n=5) in Puri and Ganjam (n=11, 32.35%) respectively. Relatively higher occurrences in these areas owes to tropical climatic conditions favouring the tick populations to flare up which is in accordance with Melo *et al.*, 2011 [9] and Guedes *et al.* 2015 [10]. Present study indicated about higher prevalence's of Ehrlichiosis among male dogs (n=11, 68.75%) which attributed to their higher exposure to ticks during outdoor activities for locating and protecting their habitats which corroborates with Saniz *et al.*, 2015 [11]. Age wise prevalence recorded the maximum affection among dogs belonging to 1-3-year age groups owing to large sample size as well as their excess behavioral characteristics and movement, supposed to expose them to maximum risks for tick infestation [12]. Relatively higher occurrences of canine Ehrlichiosis in this study were recorded among German Shepherd (n=7, 43.75%) followed by Saint Bernad (n=4, 25%), Labrador Retriever (n=3, 18.75%), Spitz (n=2, 12.5%) which is in agreement with the findings of Milanjeet *et al.*, 2014 [13]. Higher incidences among larger breeds attributed to their relatively higher risk of more tick infestations owing to more body surface area. Clinical signs recorded in the affected dogs were bleeding from nose, blood in stool, severe depression, ascites, rough body conditions and ticks in skin.

### Blood Smear Examination

Blood smears were subjected for Giemsa staining to observe morulae in the leucocytes which recorded over all prevalence of 28.57% (n=16). Observation of morulae in the blood smears were considered as an important diagnostic approach for screening of dogs against Canine Ehrlichiosis [14].

### Haemato-biochemical Evaluations

A through blood examinations (Control-37, Affected-16) through automatic hematological analyzer revealed a significant ( $p<0.05$ ) decrease in hemoglobin, total erythrocyte count, packed cell volume, total leucocyte counts, total platelet counts and lymphopenia in affected dogs as compared to Ehrlichiosis negative control dogs in present study (Table-1). Present findings related to the hematological findings are corroborative to Lara *et al.*, 2020 [15] which is mostly due to tropism of *E. canis* organism towards lympho-reticular organs in host.

**Table 1:** Alterations in hematological parameters in Ehrlichiosis affected dogs

Parameters	Control Dogs (N=37)	Affected Dogs (N=16)	P Value
Hb (g/dl)	12.06±0.20 <sup>a</sup>	7.48±0.25 <sup>b</sup>	$p<0.05$
TEC ( $\times 10^6$ $\mu$ l)	6.35±1.05 <sup>a</sup>	3.6±0.31 <sup>b</sup>	$p<0.05$
PCV (%)	32.10±0.58 <sup>a</sup>	18.36±0.65 <sup>b</sup>	$p<0.05$
TLC ( $\times 10^3$ $\mu$ l)	11.88±0.42 <sup>a</sup>	8.44±0.22 <sup>b</sup>	$p<0.05$
PLT	462.20±27.36 <sup>a</sup>	82.40±3.42 <sup>b</sup>	$p<0.05$
N (%)	58.20±0.48 <sup>a</sup>	73.37±0.63 <sup>b</sup>	$p<0.05$
L (%)	34.70±0.45 <sup>a</sup>	16.50±0.79 <sup>b</sup>	$p<0.05$
E (%)	6.17±0.26 <sup>a</sup>	2.53±0.93 <sup>b</sup>	$p<0.05$
M (%)	0.93±0.68 <sup>a</sup>	2.60±0.23 <sup>b</sup>	$p<0.05$

<sup>a</sup>Mean  $\pm$ SE with different superscripts differ significantly ( $p\leq 0.05$ )

Details of alterations related to serum biochemical parameters in dogs affected with Ehrlichiosis compared with Ehrlichiosis negative dogs considered as control group are depicted in Table-2. Present study showed a significant increase in ALT concentrations while other biochemical parameters like AST, creatinine and Blood Urea Nitrogen (BUN) showed a non-significant increasing trend among the Ehrlichiosis affected dogs as compared to dogs screened negative for Ehrlichiosis considered as control. Increase in liver specific enzymes and kidney related biochemical parameters mostly attributed to hepatic and kidney disorders in Ehrlichiosis affected dogs [16].

**Table 2:** Alterations in serum biochemical parameters in dogs affected with Ehrlichiosis

Parameters	Ehrlichiosis Negative (N=37)	Ehrlichiosis Positive (N=16)	P Value
AST	56.86±1.44 <sup>a</sup>	68.18±1.35 <sup>a</sup>	0.3
ALT	58.60±1.24 <sup>a</sup>	112.44±2.76 <sup>b</sup>	$p<0.05$
Creatinine	0.78±0.34 <sup>a</sup>	1.64±0.24 <sup>a</sup>	0.36
BUN	20.68±0.94 <sup>a</sup>	51.66±0.65 <sup>a</sup>	0.74

<sup>a</sup>Mean  $\pm$ SE with different superscripts differ significantly ( $p<0.05$ )

### Pathological changes

Present study recorded one death of affected dogs during treatment which subjected for a detail post-mortem examination showing case fatality rate of 6.25%. Low death rate in the affected dogs related to good response to treatment and early diagnosis of the disease through observing morulae in blood smear examination [17]. Gross patho-morphological changes in affected dogs mostly recorded hepatomegaly (Fig.1) and nephritis (Fig.2) besides lymphadenomegaly and splenomegaly. Affected dogs mostly having rough body conditions with tick infestations on skin and enlarged abdomen. Mucus membrane of affected dogs show little icteric with petechial hemorrhages. There was history of melena and nasal bleeding. Ascitic fluid recovered approximately about two liters during necropsy. Lungs were congested showing some patchy consolidations. Present findings were corroborative with earlier researchers [18, 19].

Histopathological findings mostly concentrated with infiltrations of inflammatory cells and degenerative changes like cloudy swelling, fatty changes as well as disordered hepatic arrangement in liver (Fig.3) as well as kidney parenchyma with proteinaceous materials in renal tubules (Fig.4). Lympho-reticular tropism of this rickettsia organism results in splenic haemosiderosis and lymphoid follicular hyperplasia [20, 21]. Poor management and lack of awareness among the animal owners in Odisha are important cause for many such emerging and re-emerging disease conditions like PPR in goats [22, 23], heartworm diseases [24] and canine pyometra [25].

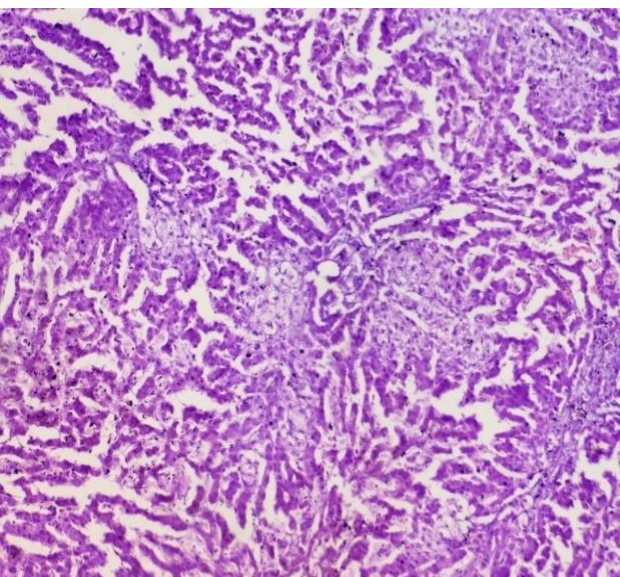
## Figures



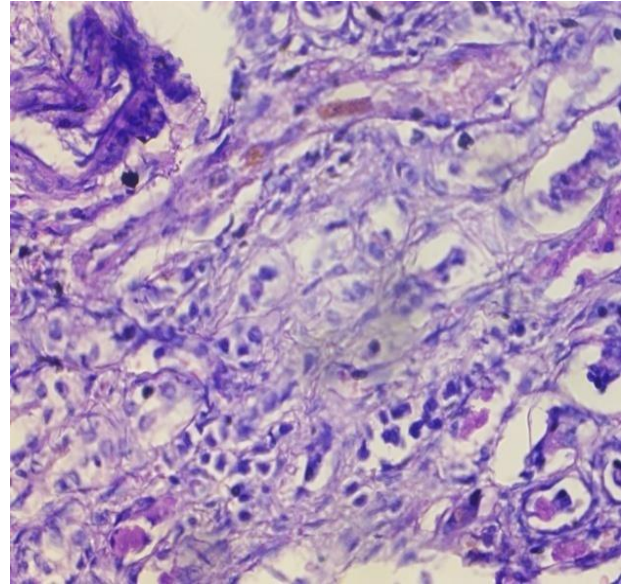
**Fig 1:** Hepatomegaly with congestion and distended Gall Bladder



**Fig 2:** Nodular and contracted kidney with few necrotic patches suggestive of severe nephritis



**Fig 3:** Photomicrograph showing centrilobular degeneration and necrosis with sinusoidal congestion (H&E-100X)



**Fig 4:** Photomicrograph showing infiltrations of mononuclear cells in the inter tubular spaces with fibrotic proliferations (H&E-40X)

### Conclusion

Present study recorded a significant high prevalence (28.75%) of canine Ehrlichiosis in two coastal districts of Odisha which was the first ever detail investigative approach on canine Ehrlichiosis in these part of the state. Detail epidemiological risk factors as well as diagnostic approach on the basis of haemato-biochemical parameters in affected dogs might be considered as an educative approach for the field veterinarians and pet owners for early diagnosis and advocating suitable treatment to mitigate this newly emerging disease in future. Documentation of this disease through present study will enrich the scientific literature about canine ehrlichiosis in these parts of the state.

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