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Clinico-diagnostic and Therapeutic Management of Ehrlichiosis in Dog

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ABSTRACT

Background: The research work was undertaken to investigate the efficacy of various diagnostic tests and therapeutic efficacy of different treatment protocols on Canine Monocytic Ehrlichiosis (CME) infected dogs from in and around Navsari, Gujarat, India.

Methods: A total of 69 dogs were suspected for ehrlichiosis based on clinical signs and hematological values and further subjected to rapid Ab detection test and PCR assay targeting 16S rRNA gene. The pre and post-treatment haematology and biochemical analysis of treatment groups along with all the healthy dogs were compared. Further, Modified Clinical Sign Grading (MCSG) score for CME-infected dogs was carried out to evaluate therapeutic responses based on minimizing the clinical score.

Result: The hospital based CME incidence was 49.28% among suspected dogs.The anemia along with leucopenia were the most significant hematological alteration recorded while ALP, creatinine and BUN were elevated and albumin was significantly decreased in CME-infected dogs.The significant improvements were observed in haemato-biochemical parameters towards normalcy in both treatment groups. However, a higher percentage of reduction was observed in the clinical score of imidocarb treated CME affected dogs than the oxy-doxycycline therapy after 14 days.

Key words: Dogs, Ehrlichiosis, Immidocarb, Thrombocytopenia, Treatment.

INTRODUCTION

Canine Monocytic Ehrlichiosis (CME) is an important vector-borne zoonotic disease of canines with a worldwide geographical distribution, observed particularly in tropical and subtropical areas. The main cause of canine monocytic ehrlichiosis (CME) is recognized as *Ehrlichia canis* (Sainz *et al*., 2015). Leukocytes are the main target of infection by *Ehrlichia* spp., which produce intracytoplasmic, membrane-bound bacterial aggregates known as morulae (Mylonakis and Theodorou, 2017).

The prevalence of *E. canis* is determined by the distribution of *Rhipicephalus sanguineous* tick, the vector that is prevalent in tropical and subtropical areas (Megat Abd Rani *et al*., 2010). Hematologic abnormalities observed in CME are moderate to severe thrombocytopenia, mild anemia and mild leukopenia during the acute stage, mild thrombocytopenia in the subclinical stage and pancytopenia in the severe chronic stage. Hypoalbuminemia, hyperglobulinemia, elevated level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) is the promising biochemical alteration observed in CME (Mylonakis and Theodorou, 2017). Diagnosis of CME is based on anamnesis, clinical presentation and detection of morula in peripheral blood or buffy coat smear examination. The tetracyclines are the drug of choice for the treatment of canine ehrlichiosis and among all the tetracyclines, doxycycline is probably the most effective drug for use in dogs. Besides this, imidocarb is also another choice of treatment for rickettsial organisms. In view of this, the current study was undertaken to investigate the haemato-biochemical alterations, efficacy of various diagnostic techniques used for the identification of *Ehrlichia* infection in dogs along with the therapeutic efficacy of different protocols used for management of canine monocytic ehrlichiosis in dogs.

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MATERIALS AND METHODS

The study was carried out at Veterinary Clinical Complex, College of Veterinary Science and A.H., Navsari of south Gujarat region during October 2022 to June 2023. A total of 1040 were registered as fresh cases. Among them 69 dogs were suspected for ehrlichiosis based on clinical signs and hematological values and further subjected to Anigen Rapid CaniV-4 (Leish) test (Fig 1 and 2) (manufactured by BIONOTE Inc., Republic of Korea) and PCR assay. Out of these, 12 dogs found positive in rapid diagnostic test were randomly divided in two groups comprising 6 animals each

for therapeutic trials. Group II was treated with Inj. Oxytetracycline @ 22 mg/kg body weight intravenously diluted with normal saline once a day for 3 days followed by Tab. Doxycycline @ 10 mg/kg body weight orally once a day for a period of 14 days along with supportive treatment. Group III was treated with Inj. Imidocarb dipropionate @ 6.6 mg/kg body weight subcutaneously (if required repeat after 14 days) along with supportive treatment.

The rapid test detects antibodies of *Ehrlichia canis*, *Leishmania infantum*, *Anaplasma platys and Dirofilaria immitis* antigen. The genomic DNA of the ehrlichiosis organism was isolated from whole blood by using the standard phenol/chloroform extraction method as per John *et al*. (1991) with necessary modifications. Purified DNA from the blood samples was subjected to molecular analysis with PCR using genus-specific primers EHR16SF (5′-GGTACCYACAGAAGAAGTCC-3′) and EHR16SR (5′-TAGCACTCATCGTTTACAGC-3′) which was amplified at 345 bp (Alhassan *et al*., 2021). The pre and post-treatment haematology and biochemical analysis of treatment were compared between and within groups along with healthy dogs.Besides this, the six healthy dogs presented for vaccination or regular health check-ups were also included in study as control after obtaining the owner's consent. Modified Clinical Sign Grading (MCSG) score for CME-infected dogs was carried out based on the modification of 17- points scale clinical score of dogs (Table 1) for tick-borne diseases given by (Himalini *et al*., 2018). The same was used to evaluate therapeutic responses based on minimizing the clinical score from day 0 to day 14 where day 0 was pre-treatment and day 14 was post-treatment score. The finalresponse of treatment was determined based on the percentage of reduction which was calculated using the following formula.

Percentage of reduction =

$$\frac{\text{Pre-treatment - Post-treatment}}{\text{Pre-treatment}} \times 100$$

The hematological and biochemical parameters were subjected to descriptive statistical analysis to obtain mean±SE. One-way analysis of variance (ANOVA) followed by DMRT was performed to determine differences in means. Student t-test was also used to compare differences between means wherever applicable. The criterion for statistical significance was considered both for $p \leq 0.05$ as well as $p \leq 0.01$.

RESULTS AND DISCUSSION

The overall incidence of canine monocytic ehrlichiosis was 3.26% in and around the Navsari district whereas hospital based incidence among the suspected cases was found 49.28% (34/69). The result was in accordance with Senthil *et al*. (2020) who also reported an overall incidence of ehrlichiosis in 36% and 56.43% cases of dogs, respectively. On the contrary, a higher incidence of CME was detected by Bhadesiya and Raval (2015) and Kottadamane *et al*. (2017) in 62.07% and 86.90% of the dogs, respectively might be due to the large size of the population studied. While very low prevalence (1.33% and 0.63%) of *E. canis* in cases in dogs was reported by Roopali *et al*. (2019) and Prajapati *et al*. (2023), respectively. The variation in the incidence rate of CME might be attributed to distribution of the vector, geographical area, climatic condition, sample size and time of the sample collection (Selim *et al*., 2020). Among 69 suspected cases, 34 (Thirty-four) dogs were found positive Anigen Rapid CaniV-4(Leish) test and 15.94% (11) dogs were found positive for *Ehrlichia* genus using primers of 16S rRNA gene which produced an amplicon at 345 bp (Fig 3). Similarly, Sarawade *et al*. (2023) also found 16.66% dogs positive for *E.canis* among 60 suspected dogs. The 72.5 per cent of dogs were found positive for antibodies to *E. canis*. The serological prevalence for CME found in our study was similar to the findings of Sosa-Gutierrez *et al*. (2013) who reported 74.5% cases positive for *E. canis* antibodies using the IDEXX snap 4Dx test.

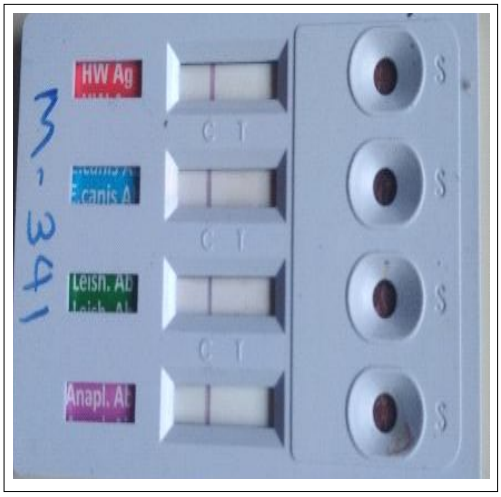
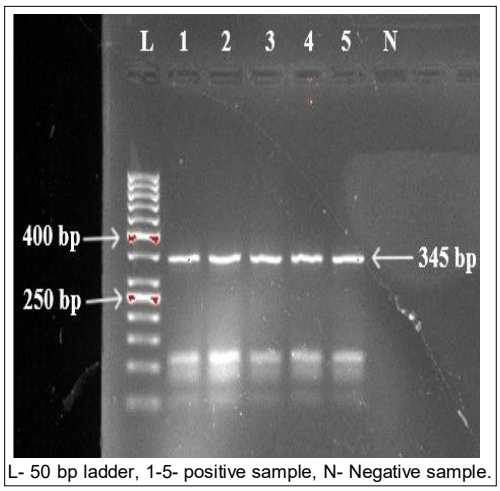


Fig 1: Negative anigen rapid CaniV-4 (Leish) test.

Fig 2: Positive anigen rapid CaniV-4 (Leish) test.

However, Roopali *et al*. (2020) and Van Hai and Khuong, (2021) detected *E. canis* antibodies in 100% and 95.7% cases, respectively through a dot-ELISA kit. The difference in sero-prevalence might be due to regional variations, different climates, sample sizes, sampling periods, methodology and the differences in the dog populations (outdoor, indoor, stray, or owned dog) (Cetinkaya *et al*., 2016). This rapid test detects IgM produced by the body against the ehrlichial organism, hence, the presence of adaptive immunity suggests the presence of acute infection. Therefore, the dogs were found positive in rapid antibody tests and if showing clinical signs and other defined laboratory findings, then such dogs might be infected with *E. canis* infection. Further, a low number of *E. canis* infections detected using PCR technique might be due to *E. canis* being captured in tissues such as bone marrow and spleen resulting in low concentrations of DNA of organism in systemic circulation which was inadequate for PCR amplification (Cetinkaya *et al*., 2016). Looking at the literature, it was found that Nested PCR is a more efficient technique in the detection of CME rather than conventional PCR because Nested PCR is a modification of conventional PCR which increases the specificity of any reaction. Besides this, Kalaivanan *et al*. (2020) stated that any previous treatment of the clinically suspected cases and prevalence of transmitting ticks in local regions could also be a cause for the variation in the results of *Ehrlichia canis* detection using various techniques.



L- 50 bp ladder, 1-5- positive sample, N- Negative sample.

Fig 3: Positive PCR.

In the current study, tick infestation was noticed in 23 (67.65%) dogs which were found positive for CME and the significant ($p \leq 0.05$) difference was observed between the tick-infested and non-infected dogs (Table 2). Moreover, the relative risk of owning ticks on the body was found >1 (Carneiro, 2011) in the present study which suggests that harbouring the ticks is relatively more prone to be affected by CME. Game *et al*. (2019), Rao *et al*. (2020) and Singh *et al*. (2021) also reported similar results in their studies.

Hematological alterations in ehrlichia affected dogs

There was a significant ($p \leq 0.05$) decrease in Hb level, TEC count and PCV in the infected dogs on the day of presentation compared to healthy dogs. These results suggested anemia in dogs infected with CME. The results of the present studies were in accordance with Dhavalagi *et al*. (2021b) and Singh *et al*. (2021). Anaemia found in dogs with CME could be due to loss of blood on account of

Table 1: Modified 17-points scale clinical sign grading score of dogs with CME.

Clinical signs	MCSG score	
	Present	Absent
Fever	1	0
Anorexia/Inappetence	1	0
Mucous membrane (Pale/Congested)	1	0
Depression/ Lethargy	1	0
Lymphadenopathy	1	0
Vomiting	1	0
Diarrhoea	1	0
Melena	1	0
Haemorrhage (Petechiae/Ecchymosis/Epistaxis)	1	0
Ascites	1	0
Limb oedema		
Respiratory affection	1	0
Ocular affection	1	0
Nervous sign	1	0
Tick infestation	1	0
Palpable splenomegaly	1	0
Palpable hepatomegaly	1	0
Total	17	0

thrombocytopaenia and immune-mediated destruction of red blood cells as well as suppression in production of colony forming erythroid cells resulting in decreased RBC production under the effect of bone marrow suppression by *Ehrlichia* organism (Game *et al*., 2019).

The platelet count of CME-infected dogs of the present study was significantly ($p \leq 0.01$) decreased in comparison with healthy dogs. The thrombocytopaenia in *Ehrlichia* infected dogs corroborates with the earlier reports of Dhavalagi *et al*. (2021b) and Singh *et al*. (2021). Thrombocytopaenia in CME might be observed due to decreased platelet production by bone marrow, increased platelet destruction and the presence of antiplatelet antibodies reduces adhesiveness of platelets because of immune-mediated response stimulated by *Ehrlichia* organism. Besides, platelet consumption was increased and inversely platelet half-life was decreased due to immune-mediated splenic sequestration (Dhavalagi *et al*., 2021b).

The mean TLC count of CME-infected dogs significantly ($p \leq 0.01$) decreased when compared with healthy control dogs. Game *et al*. (2019) also similarly reported leucopenia in their studies. While Rao *et al*. (2020) and Singh *et al*. (2021) observed leucocytosis in ehrlichiosis infected dogs as result of a hypersensitized immune system in the acute phase of infection. The variation in the same parameters among the authors could be associated with the presentation of dogs in various stages of CME (acute, sub-clinical and chronic) during blood sample collection. It has been reported that in the acute or subclinical phase there will be leucocytosis as a result of body defence whereas leukopenia is observed in chronic stage due to myelosuppression (Rao *et al*., 2020).

Table 2: Relative risk of tick infestation in CME infected dogs.

Particulars	Number of dogs	Percentage	*p* value	Relative risk
Tick infested	23	67.65	0.003**	1.94
Non infested	11	32.35		

($p \leq 0.01$**: Highly significant).

Table 3: Haematological assessment in therapeutic groups of CME affected dogs.

Parameter	Treatment	Healthy group-I (n=6)	Treatment groups		*p* value
			Infected group-II (n=6)	Infectedgroup-III (n=6)	
Haemo-globin (g/dl)	Before	12.35^{a}±0.46	08.90^{b}±0.87	10.12^{ab}±0.84	0.02
	After	12.35^{a}±0.46	09.91^{b}±0.24	09.66^{b}±0.91	0.01
	p value	-	0.17	0.54	-
TEC (10^6/µl)	Before	05.89^{a}±0.30	05.01^{ab}±0.36	04.60^{b}±0.29	0.03
	After	05.89±0.30	06.13±0.66	05.26±0.50	0.47
	p value	-	0.11	0.26	-
PCV (%)	Before	39.72±1.14	34.23±4.19	37.24±3.26	0.48
	After	39.72±1.14	35.57±1.38	38.45±3.22	0.39
	p value	-	0.69	0.77	-
Platelets (10^3/µl)	Before	326.35^{a}±25.74	97.67^{by}±5.02	64.50^{by}±10.63	0.00
	After	326.35^{a}±25.74	177.17^{bx}±21.19	171.67^{bx}±15.34	0.00
	p value	-	0.01	0.00	-
TLC (10^3/µl)	Before	10.30^{a}±0.70	06.75^{by}±0.71	05.82^{by}±0.90	0.00
	After	10.30±0.70	11.53^{x}±1.46	08.97^{x}±0.56	0.22
	p value	-	0.05	0.02	-
Neutro-phils (%)	Before	70.83±1.99	65.00±3.67	71.17±4.80	0.43
	After	70.83±1.99	68.00±2.71	66.83±3.86	0.63
	p value	-	0.45	0.44	-
Lympho-cytes (%)	Before	25.50±2.13	29.50±3.97	24.17±4.27	0.56
	After	25.50±2.13	28.17±2.75	30.00±4.01	0.59
	p value	-	0.76	0.28	-
Monocytes (%)	Before	02.00±0.37	03.83±0.75	03.17±0.79	0.18
	After	02.00±0.37	02.33±0.21	01.83±0.40	0.58
	p value	-	0.14	0.10	-
Eosino-phils (%)	Before	01.67±0.21	01.67±0.21	01.50±0.22	0.82
	After	01.67±0.21	01.50±0.34	01.17±0.17	0.38
	p value	-	0.61	0.18	-

Means bearing different superscript in row wise (a, b) and column wise (x, y) differ significantly at $p \leq 0.05$.

Biochemical alterations in ehrlichia affected dogs

The elevation of total protein value could be due to either dehydration or increased gamma globulin response after *Ehrlichia* organism infection (Smitha and Vijayakumar, 2014). Similar findings were alsonoted by Mondal *et al*. (2019) Further, the albumin value was significantly ($p \leq 0.01$) lower in CME-infected dogs than in healthy control dogs. This finding was in accordance with the study of Smitha and Vijayakumar, (2014); Dhavalagi *et al*. (2021a) and Singh *et al*. (2021). The hypoalbuminemia in CME-infected dogs might be due to anorexia and reduced protein intake, loss of protein into the oedematous inflammatory fluid as a result of vasculitis which increases vascular permeability as well as protein excretion in urine because of glomerulopathy, or decreased production of protein due to liver damage in some cases (Smitha and Vijayakumar, 2014; Dhavalagi *et al*. 2021a).

In the present study, SGPT was non-significantly higher in CME-infected dogs as compared to healthy control dogs. This was in agreement with the earlier reports of Singh *et al*. (2021). Besides, Dhavalagi *et al*. (2021a) reported significantly higher levels of SGPT in *Ehrlichia-infected* dogs. Additionally, the SGOT values were found non-significantly higher in CME-infected dogs as compared to healthy control dogs. This finding was in accordance with Parashar *et al*. (2015) who also reported same results.

The ALP value was significantly ($p \leq 0.01$) higher in CME-infected dogs of the present study in comparison with healthy control. Smitha and Vijayakumar, (2014), Dhavalagi *et al*. (2021a) and Singh *et al*. (2021) also reported the same results in their study. Hepatocytes were compressed and injured by development of many expanding foci of reticuloendothelial cells in the hepatic sinusoids which compressed the function of hepatocytes leading to their necrosis and rise in the levels of serum SGPT, SGOT and

Table 4: Biochemical assessment in therapeutic groups of CME affected dogs.

Parameter	Treatment	Healthy group (n=6)	Treatment groups		*p* value
			Affected group-II (n=6)	Affected group-III (n=6)	
Total protein (g/dl)	Before	06.00±0.11	07.15±0.47	07.25±1.23	0.45
	After	06.00±0.11	06.88±0.22	07.44±0.70	0.08
	p value	-	0.55	0.81	-
Albumin (g/dl)	Before	04.18^{a}±0.16	01.87^{by}±0.25	01.68^{by}±0.12	0.00
	After	04.18^{a}±0.16	03.35^{bx}±0.23	03.02^{bx}±0.21	0.00
	p value	-	0.00	0.01	-
SGPT (IU/L)	Before	39.80±2.76	35.86^{y}±4.30	99.93±38.13	0.11
	After	39.80±2.76	61.66^{x}±8.92	82.90±24.97	0.18
	p value	-	0.01	0.26	-
SGOT (IU/L)	Before	33.10±2.37	38.17±13.38	61.48±27.86	0.51
	After	33.10±2.37	34.68±8.42	49.46±19.53	0.60
	p value	-	0.56	0.22	-
ALP (IU/L)	Before	68.06^{b}±1.34	120.75^{ax}±14.93	167.92^{ax}±25.22	0.00
	After	68.06^{b}±1.34	85.18^{ay}±4.72	99.62^{ay}±7.38	0.00
	p value	-	0.03	0.03	-
BUN (mg/dl)	Before	21.80^{b}±0.82	80.72^{ax}±7.63	81.06^{ax}±3.81	0.00
	After	21.80^{c}±0.82	57.51^{ay}±5.84	38.75^{by}±4.29	0.00
	p value	-	0.01	0.00	-
Creatinine (mg/dl)	Before	00.89^{b}±0.04	01.53^{ax}±0.20	01.53^{ax}±0.24	0.04
	After	00.89±0.04	00.97^{y}±0.05	00.83^{y}±0.05	0.14
	p value	-	0.05	0.04	-

Means bearing different superscript in row wise (a, b) and column wise (x, y) differ significantly at $p \leq 0.05$.

Table 5: Percentage of reduction observed in clinical score based on modified clinical sign grading score (MCSG).

Group		MCSG score		Percentage of reduction (%)
		Before treatment	After treatment	
Group-II	Total	68	14	79.41
	Mean±SE	11.33±0.56	02.33±0.21	
Group-III	Total	65	10	84.62**
	Mean±SE	10.83±0.60	01.67±0.21	

($p \leq 0.01$**: Highly significant).

ALP but this might be found for transient period, hence, the liver associated enzymes could not significantly altered in all the presented cases (Smitha and Vijayakumar, 2014). Further, infiltration of perivascular mononuclear cells because of hepato-biliary dysfunction might also contribute to the elevation of these enzymes (Nair *et al*., 2016).

The elevated values of BUN and creatinine found in the present study were similar to Rao *et al*. (2020) and Singh *et al*. (2021) but in contrast with the study of Kottadamane *et al*. (2017) and Mondal *et al*. (2019) who noted non-significantly increased value of BUN in CME infected dogs. The increased creatinine and BUN values in ehrlichiosis infected dogs might be due to immune complex-mediated glomerulonephritis which affect the GFR of nephron and resulted into renal pathology (Roopali *et al*., 2018).

A significant improvement towards normalcy was observed in platelet count, TLC and blood glucose before and after treatment values in both the treatment groups (Table 3). Likewise, a significant regression was also observed in albumin, ALP, BUN and Creatinine values while comparing before and after treatment data of both the treatment groups (Table 4). However, looking at Table 5, a significant percentage of reduction was observed in the clinical score of group III CME-affected dogs than group II after 14 days of treatment. The better efficacy of imidocarb was reported by Xaxa and Kumar, (2018), Roopali *et al*. (2018) and Game *et al*. (2020) which was similar to the present study. In contrary to present findings, Rao *et al*. (2022) stated that doxycycline was far greater in the normalcy of clinical signs in comparison with imidocarb, whereas Sainz *et al*. (2000) reported equal efficacy of both the drugs in returned to normal clinical signs. The better efficacy of imidocarb recorded in the present study might be due to the persistence of the drug for a longer period in plasma and tissue and therefore produces highly active concentration to destroy the intracellular *Ehrlichia* organism (Game *et al*., 2020).

CONCLUSION

It may be concluded that owing ticks on the body of dogs might have more probability of being found positive for CME. The Rapid Antibody-based serological assay can be used as primary screening for ehrlichiosis. Further, the thrombocytopenia was a good prognostic marker for suspecting disease. The anemia along with leucopenia were the most significant hematological alteration recorded while ALP, creatinine and BUN were elevated and albumin was significantly decreased in CME-infected dogs. Based on therapeutic trials, imidocarb was found more effective in minimizing the clinical signs of CME rather than oxy-doxycycline therapy.

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Conflict of interest

The authors have no conflict of interest for said article.

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