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Research Article

EFFICACY OF POLYHERBAL FORMULATION AS AN ADJUNCT THERAPY IN RESOLUTION OF THROMBOCYTOPENIA IN EHRLICHIOSIS AFFECTED DOGS

SURAJ GULMAIRE, MUKESH KUMAR SHRIVASTAV, ASHISH SRIVASTAV, SHANKER KUMAR SINGH, BARKHA SHARMA, JITENDRA TIWARI, ARPANA RAIKWAR*, ALOK KUMAR CHAUDHARY, P.N. PANIGRAHI, JITENDRA SINGH GANDHAR
TVCC, Kothari Hospital & Department of Veterinary Medicine, DUVASU, Mathura (U.P.)

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ABSTRACT

Canine monocytic ehrlichiosis (CME) is a potentially fatal tick-borne disease of dogs caused by an obligate, pleomorphic rickettsial organism of genus *Ehrlichia* and family Anaplasmataceae. CME is primarily caused by *Ehrlichia canis*, having worldwide distribution in domesticated as well as wild canids. In the present study important clinical signs exhibited by the *Ehrlichia* positive dogs were fever and mucosal pallor followed by lymph adenomegaly, melena, depression, tick infestation, weight loss and ecchymotic and petechial hemorrhages. Out of sixty-two (62) screened samples, 42 samples were found positive for ehrlichiosis on the basis of nested PCR. The study shows that nested PCR is a reliable diagnostic method for *Ehrlichia canis* infection. Animal treated with standard and self-prepared polyherbal platelets booster containing extracts of *Carica papaya*, *Tinospora cordiofolia*, *Withinia somnifera* and *Asparagus racemosus* shows significant improvement in thrombocytopenia, total protein, albumin, ALT, AST and BUN as compared to other treatment group.

Keywords: Canine ehrlichiosis, Thrombocytopenia, Platelet booster, Polyherbal formulations

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Canine monocytic ehrlichiosis (CME) is a potentially fatal tick-borne disease of dogs caused by an obligate, pleomorphic rickettsial organism of genus *Ehrlichia* and family *Anaplasmataceae* (Harrus & Waner, 2010). CME is primarily caused by *Ehrlichia canis*, having worldwide distribution in domesticated as well as wild canids (Ebani *et al.*, 2011). The disease is also known as tracker dog disease, canine typhus, canine rickettsiosis and canine hemorrhagic fever. *E. canis* is transmitted trans-stadially by tick vector *Rhipicephalus sanguineus* (a brown dog tick) (Bremer *et al.*, 2005). On the basis of different diagnostic methods, the prevalence in the south and east Asia ranges from 0.0% (South Korea) to 86.9% (India) (Truong *et al.*, 2021). The disease in dogs occurs in three forms acute (2-4 weeks), subclinical (several months to years) and chronic but the distinction among these phases is not straight forward in the naturally occurring cases (Mylonakis and Theodorou, 2017). The acute phase is characterized by pyrexia, depression, lethargy, anorexia, lymph adenomegaly and splenomegaly, hemorrhagic tendencies (epistaxis, hematoma, dermal petechiae and ecchymoses) (Leiva *et al.*, 2005). The chronic form, being more dangerous is characterized by pale mucous membranes, weakness, bleeding and death (Harrus & Waner, 2011). Dogs in chronic phase of disease with severe symptoms may be less responsive to therapy.

Thrombocytopenia is one of the most commonly encountered (appearing in 80% of cases) and consistent hematological abnormalities in naturally or experimentally *E. canis* infected dogs regardless of phase of disease (Mylonakis & Theodorou, 2017). Researchers claimed that during acute phase of disease various mechanisms like enhanced platelets consumption due to inflammatory changes in vascular endothelium along with sequestration of platelets in spleen as a result of platelet migration inhibition factor are primarily associated with thrombocytopenia in CME affected dogs.

The standard treatment protocols currently used for CME advocate the use of some antimicrobials (tetracyclines and amphenicols) that may cause adverse effects as well as antibiotic resistance. Various combination of herbal plant extract including *Carica papaya*, *Cissampelos pareira*, *Tinospora cordifolia* and *Azadirachta indica* have been used in human medicine and been reported as potent platelets booster (Sailor *et al.*, 2021).

The aim of the study was to evaluate the diagnostic relevancy of canine specific platelet migration inhibition factor (PMIF) in ehrlichiosis and to assess the role of self-prepared polyherbal formulation in ameliorating the thrombocytopenia associated with ehrlichiosis.

MATERIAL AND METHODS

Selection of study animals

The study included the animals that were free from any concomitant diseases, positive for ehrlichiosis on blood smear and buffy coat smear examination, had received the recommended vaccinations and deworming and exhibiting

*Corresponding author: arpanabind@gmail.com

two or three clinical signs of ehrlichiosis along with thrombocytopenia and anemia. A total sixty-two (62) dogs were screened in the present investigation, which were presented to TVCC, DUVASU, Mathura between August, 2020 to June, 2021. Out of sixty-two (62) screened samples, 42 samples were found positive for ehrlichiosis on the basis of nested PCR. The 42 CME infected dogs divided in three groups namely; Group-1 (conventional treatment group) receiving Doxycycline @ 5mg/kg BW twice a day, pantoprazole @ 1mg/kg BW once daily, carprofen @ 2.2 mg/kg BW twice daily in case of fever and sucralfate @ 1gm thrice a day; Group-2 (Conventional treatment + Prednisolone @ 2 mg/kg BW/day tapering doses); Group-3 (Conventional treatment+Self prepared polyherbal platelets booster). Six healthy dogs of any breed and sex have been taken as healthy control or Group-0.

The primary confirmation of canine monocytic ehrlichiosis was done by detection of morulae in monocytes in the blood smear which was taken from ear tip and stained by Leishman stain for detection of morulae in monocytes. Dogs having symptoms of CME or having hematological changes signifying for CME but negative for blood and buffy coat smear examination were further confirmed by polymerase chain reaction test.

Amplification of *Ehrlichia* specific genes

Specific primer pairs were used for *E. canis* gene (16 S rRNA) for their identification (Nakaghi *et al.*, 2008). The sequences of the primers which were used for the amplification of *E. canis* is are adopted from Waner *et al.*, 1997 and mentioned as primary PCR assay (ECC: 5' AGAACGAACGCTGGCGGCAAGC 3'; ECB: 5' CGTATTACCGCGGCTGCTGGCA 3') and Nested PCR assay (ECAN5: 5'- CAATTATTTATAGCCTCTGGCTCT GGCTATAGGA-3' HE3: 5'- TATAGGTACCGTCATTAT CTTCCCTAT- 3').

Blood Sampling and Processing

Approximately 5.0 mL blood was collected from cephalic or saphenous vein in three clean, sterile and dry blood collection vials one containing EDTA (dipotassium ethylene diaminetetraacetic acid) as anticoagulant (for Hematology) and one containing trisodium citrate as anticoagulant (for coagulation profile) and another plain vial without any anticoagulant (for biochemical) on day 0 (before initiation of treatment), day 7th and 14th after initiation of treatment.

Hematology and Serum Biochemistry

Hematological parameters like total erythrocyte count, hemoglobin, packed cell volume, total leucocyte count, differential leucocyte count, platelet count, platelet indices (MPV, PDW, PCT) and erythrocyte indices (MCV, MCH, MCHC) were done by fully automated hematology analyzer (Celltac Alpha VET MEK-6550) and biochemical parameters (serum creatinine, blood urea nitrogen, alanine aminotransferase, aspartate amino transferase, alkaline phosphatase, total protein, albumin) were analyzed by biochemical analyzer (AKRAY Healthcare Pvt. Ltd.). Globulin was calculated by subtracting albumin from total protein. Assay of serum Canine Platelet migration inhibition factor (C-PMIF) were done using compatible ELISA kits.

Statistical Analysis

The values of various parameters were expressed as mean ±S.E. and datas were analyzed by one-way (ANOVA) Analysis of Variance followed by the Post-HocTukey HSD test using Statistical Package for the Social Sciences (SPSS 20.0). The level of statistical significance for all comparisons was established at ($P<0.05$).

RESULTS AND DISCUSSION

Diagnosis of *Ehrlichia canis* infection

In present investigation total 62 dogs were screened for ehrlichiosis which was based on the clinical diagnosis criteria as stated earlier in materials and methods section. All the screened dogs underwent for blood smear and buffy coat smear examination and hematology to confirm the state of clinical ehrlichiosis. Blood smear examination results showed 3 dogs positive for ehrlichiosis, while buffy coat smear examination results showed 6 dogs positive for ehrlichiosis. In the same cases, primary PCR revealed 18 positive dogs, however nested PCR confirmed 42 dogs positive for ehrlichiosis of all screened dogs. All the 42 nested PCR positive dogs were included for the therapeutic studies (Figs. 1, 2).

Changes in Hematology and Serum Biochemistry

Before commencement of treatment (day 0), total leukocytes count (TLC), total erythrocytes count (TEC), hemoglobin (Hb), packed cell volume (PCV) and lymphocyte concentration were significantly reduced ($p<0.05$) in all treatment groups as compare to healthy control group (Table 1 & 2). Whereas neutrophils concentration was significantly increased in all treatment groups on day of presentation as compare to healthy control group. In the current study, marked decrease in total erythrocyte count, hemoglobin concentration, packed cell volume and total leucocyte count is due to anemia as a result of bone marrow aplasia (Weiss, 2005), extensive hemorrhages throughout the body which is due to thrombocytopenia and platelets dysfunction, blood loss by ticks, immune mediated destruction of erythrocytes

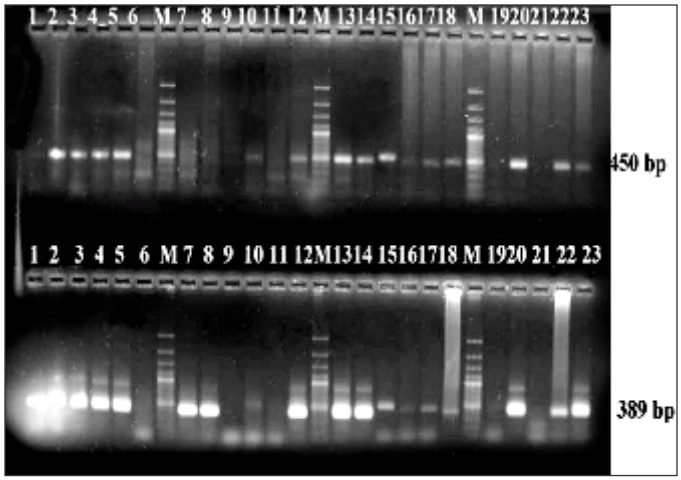

Fig. 1. PCR analysis of *Ehrlichia canis* positive cases on iBright 750 Imaging system

through production of antibodies and their succeeding binding on the membranes of erythrocytes and platelets which may result in obliterations of these cells (Taylor *et al.*, 2007). Decreased hemoglobin and TEC levels could be due to epistaxis, petechial hemorrhages, myelosuppression or due to severe anemia (Bhardwaj, 2013; Dixit *et al.*, 2012). Phagocytosis of antibody-opsonized erythrocytes has been reported by Woody and Hoskins, (1991) in *E. canis* infected animals which might be a factor conducive to anemia. Bone marrow dysfunctioning in ehrlichial dogs is due to tropism for hematopoietic cells and bone marrow hypoplasia such as suppression in myeloid, erythroid and megakaryocytic cells. Non regenerative anemia is a consequence of liberal replication of *E. canis* in the bone marrow and inhibition of outcome of colony forming units in the process of erythropoiesis and megakaryopoiesis. Generalized vasculitis (Pick *et al.*, 2000) has been testified in *E. canis* infections accompanying to production of IL-1 which may play a vital role in marginalization and adhesion of leucocytes to the vascular wall and subsequently accumulation of cells in inflammation foci. The dogs infected with *E. canis* shows marked neutrophilia on day of presentation which could attributed to underlying co-infection (Agnihotri *et al.*, 2012). Neutrophils produce a trypsin-like enzyme by which they digest dead tissue and bacteria, so phagocytic action of neutrophils may thus be correlated with their increased number (Yadav *et al.*, 2017). The mean TLC, TEC, Hb, PCV and Lymphocyte concentration were significantly improved on day 7 and then day 14 from pretreatment value. Among the three treatment groups, significant changes were observed in TLC, TEC, Hb and PCV on day 14 in *E. canis* infected dogs receiving conventional treatment along with polyherbal formulation (Group-3). Neutrophil concentration reveals significant decrease in its concentration on day 7 and day 14 in all treatment groups as compare to healthy

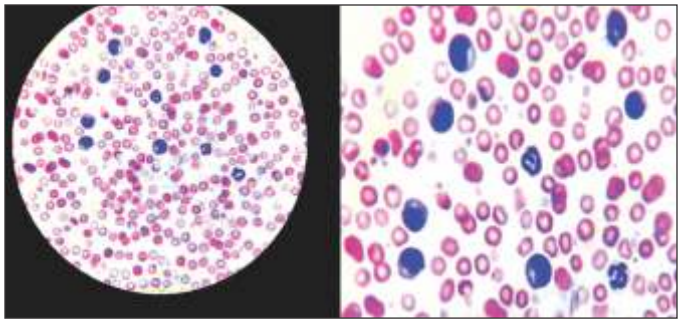

Fig. 2. Morula in blood smear of Dog infected with *Canine Ehrlichiosis*

control (Table 1 & 2).

Statistically, no significant changes were observed in mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and monocyte concentration on day 0, 7 and 14 in all treatment groups as compare to healthy control (Table 3 & 4). Statistical analysis revealed that the mean platelets concentration in all the treatment groups were significantly lower than that of control group on the day of presentation (Table 7).

Thrombocytopenia is considered to be the most common and reliable hematological abnormality of dogs naturally or experimentally ill with *E.canis*. The mechanism leading to thrombocytopenia is not rather clear while the main role looks to be played by autoimmunological processes (Herring and Michael, 2012), decreased production (chronic phase) (Harrus *et al.*, 1997), increased platelet consumption (Bulla *et al.*, 2004), splenic sequestration (Lakkawar *et al.*, 2003), intravascular disseminated coagulopathy, decreased circulating half-life of platelets during acute phase of infection, reduced adhesiveness of platelets due to antiplatelet antibody and platelet aggregation, augmented concentrations of circulating platelet migration -inhibition factor. Throughout, the *E. canis* infection antibodies against glycoprotein of the dog's own platelets are produced which leads to their malfunction. Platelet survival time decreased from a mean of 9 days to 4 days, within 2- 4days of infection with *E. canis*. A platelet migration inhibition factor is also estimated to play role in enhancing platelet sequestration and stasis, primary to reduced peripheral blood platelet counts. There was significant increase in the mean platelets concentration on day 7 followed by on day 14 post-treatment in all the treatment groups with highest recovery in 3rd group followed by 2nd group. Therefore, in terms of improvement in all treatment groups of dogs, best recovery was seen in group which was treated with conventional treatment combined with polyherbal platelet booster containing extracts of *Carica papaya*, *Tinospora cordiofolia*, *Withinia somnifera* and *Asparagus racemosus*.

Table 1. Haematological alterations in various groups of dogs suffering from CME

Groups	TLC ($x10^3/\mu L$)			TEC ($x10^6/\mu L$)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	9.61 ± 1.07^{bA}	9.81 ± 1.07^{bA}	10.25 ± 1.17^{bA}	6.59 ± 0.30^{cA}	6.83 ± 0.29^{dA}	7.10 ± 0.16^{cA}
G1	5.12 ± 0.32^{aA}	6.92 ± 0.45^{aB}	7.56 ± 0.29^{Bb}	2.58 ± 0.12^{aA}	3.47 ± 0.13^{aB}	4.38 ± 0.08^{aC}
G2	5.45 ± 0.35^{aA}	7.37 ± 0.62^{aB}	8.69 ± 0.41^{abB}	3.16 ± 0.18^{abA}	4.90 ± 0.20^{bB}	5.38 ± 0.45^{bB}
G3	5.80 ± 0.38^{aA}	8.33 ± 0.61^{abB}	9.83 ± 0.36^{bC}	3.77 ± 0.32^{bA}	5.99 ± 0.24^{cB}	6.31 ± 0.28^{cB}

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant. Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant.

Table 2. Haematological alterations in various groups of dogs suffering from CME

Groups	Hb (gm/dl)			PCV (%)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	15.06 ± 0.67^{bA}	15.32 ± 0.68^{cA}	15.84 ± 0.63^{cA}	42.76 ± 2.64^{bA}	43.11 ± 2.65^{bA}	43.46 ± 2.66^{cA}
G1	5.57 ± 0.54^{aA}	8.06 ± 0.42^{aB}	10.28 ± 0.21^{aC}	22.97 ± 0.60^{aA}	28.71 ± 0.43^{aB}	33.17 ± 0.82^{aC}
G2	6.51 ± 0.41^{aA}	9.60 ± 0.38^{bB}	11.12 ± 0.22^{aC}	23.96 ± 0.51^{aA}	31.46 ± 0.88^{aB}	34.84 ± 0.42^{abC}
G3	7.14 ± 0.53^{Aa}	10.46 ± 0.20^{bB}	13.15 ± 0.48^{bC}	24.67 ± 0.36^{aA}	32.32 ± 0.35^{aB}	38.80 ± 0.49^{bC}

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant. Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant.

Table 3. Haematological alterations in various groups of dogs suffering from CME

Groups	MCV (fL)			MCH (pg)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	67.65±1.67	68.18±1.64	68.70±1.57	33.80±0.59	34.01±0.61	34.05±0.58
G1	67.60±0.35	67.54±0.25	67.88±0.29	33.78±0.29	34.28±0.48	34.48±0.96
G2	67.52±0.52	67.96±0.37	68.23±0.33	34.05±0.68	34.57±0.81	35.08±0.98
G3	67.84±0.70	68.09±0.61	68.11±0.91	34.58±0.71	34.83±0.29	34.95±1.10

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant. Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant.

Table 4. Haematological alterations in various groups of dogs suffering from CME

Groups	MCHC (gm/dL)		
	Day 0	Day 7	Day 14
Healthy (Control)	34.30±0.59	34.02±0.61	34.05±0.58
G1	33.64±0.59	34.08±0.99	33.96±0.88
G2	34.39±1.04	34.12±0.88	34.63±1.18
G3	34.46±0.92	34.81±1.36	34.95±1.56

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant. Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant

Changes in Serum Biochemistry

In present study, there was significant increase in ALT, AST, ALP on day 0 in all treatment groups (Group-1, Group-2 & Group-3) of dogs in comparison with control. There was significant decrease in the ALT, AST, ALP on day 7^{th} and 14^{th} after the treatment in all the treatment groups (Table 10 & 12). ALT is a liver specific enzyme in dogs an increase may follow hepatic necrosis or milder revocable impairment in which hepatocytes become leaky but do not die. Augmented levels of SGPT and SGOT are indicative of hepatic dysfunction leading to hypoproteinemia in dogs with ehrlichiosis (Agnihotri *et al.*, 2012). Liver pathology accompanying with experimental *E. canis* infection without overt clinical disease has been documented as a portal infiltration of lymphocytes, plasma cells and macrophages resulting in marked distortion of the surrounding acinar architecture.

In present study there was significant decrease in total protein on day 0 in all treatment groups (Group-1, Group-2 & Group-3) of dogs in comparison with control (Table 13). The hypoalbuminemia in CME might be the consequence of peripheral loss of albumin to edematous inflammatory fluids as a consequence of increased vascular permeability (Woody and Hoskins 1991), blood loss or diminished protein production due to concurrent liver disease, hyperglobulinemia-related down-regulation

Table 5. Haematological alterations in various groups of dogs suffering from CME

Groups	Monocytes (%)			Eosinophil (%)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	4.70±0.55	4.89±0.55	4.97±0.54	2.75±0.35	2.70±0.33	2.63±0.48
G1	4.56±0.23	4.94±0.33	5.03±0.37	2.74±0.03	2.75±0.19	2.67±0.20
G2	4.67±0.26	4.65±0.17	4.74±0.17	2.77±0.16	2.74±0.20	2.43±0.19
G3	4.82±0.45	4.60±0.43	4.78±0.43	2.76±0.40	2.58±0.27	2.59±0.42

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant

Table 6. Haematological alterations in various groups of dogs suffering from CME

Groups	Neutrophil (%)			Lymphocyte (%)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	78.83 ± 1.47^{aA}	79.51 ± 1.52^{aA}	81.58 ± 1.18^{bA}	14.71 ± 1.87^{bA}	15.10±1.89cA	15.45 ± 1.88^{bA}
G1	82.64 ± 0.40^{bB}	81.05 ± 0.48^{aAB}	80.30 ± 0.86^{bA}	5.58 ± 0.30^{aA}	8.21 ± 0.41^{aB}	10.94 ± 0.37^{aC}
G2	83.36 ± 0.52^{bB}	80.90 ± 1.02^{aAB}	78.92 ± 0.96^{abA}	6.03 ± 0.20^{aA}	9.84 ± 0.18^{abB}	12.41 ± 0.54^{abC}
G3	86.17 ± 0.43^{cB}	79.71 ± 1.71^{aA}	76.76 ± 0.57^{aA}	6.79 ± 0.35^{aA}	11.91 ± 0.55^{bB}	14.24 ± 0.97^{abC}

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant

Table 7. Haematological alterations in various groups of dogs suffering from CME

Groups	Platelet ($x10^5/\mu L$)			PCT (%)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	2.93 ± 0.30^{bA}	2.97 ± 0.30^{cA}	3.00 ± 0.29^{cA}	0.29 ± 0.02^{bA}	0.30 ± 0.02^{aA}	0.57 ± 0.10^{Bb}
G1	0.48 ± 0.01^{aA}	1.00 ± 0.04^{aB}	1.56 ± 0.01^{aC}	0.03 ± 0.00^{aA}	0.16 ± 0.05^{aB}	0.26 ± 0.03^{aB}
G2	0.54 ± 0.05^{aA}	1.30 ± 0.03^{bB}	2.22 ± 0.10^{bC}	0.03 ± 0.00^{aA}	0.24 ± 0.11^{aAB}	0.43 ± 0.05^{abB}
G3	0.67 ± 0.01^{aA}	1.99 ± 0.02^{bB}	2.70 ± 0.05^{cC}	0.05 ± 0.00^{aA}	0.32 ± 0.07^{aB}	0.51 ± 0.08^{bC}

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant

Table 8. Haematological alterations in various groups of dogs suffering from CME

Groups	MPV (fL)			PDW (%)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	6.82 ± 0.07^{aA}	6.94 ± 0.08^{aA}	6.39 ± 0.21^{aA}	15.90±0.38	15.66±0.39	15.40±0.41
G1	6.83 ± 0.05^{aA}	6.61 ± 0.07^{aB}	6.36 ± 0.06^{aC}	14.75±0.77	15.71±0.64	15.48±0.80
G2	6.83 ± 0.28^{aA}	6.60 ± 0.21^{aA}	6.07 ± 0.17^{aA}	14.95±0.64	15.40±0.60	15.41±0.63
G3	6.77 ± 0.21^{aA}	6.66 ± 0.22^{aA}	6.31 ± 0.14^{aA}	15.78±0.61	15.07±0.54	15.61±0.67

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant

Table 9. Coagulation profile in various groups of dogs suffering from CME

Groups	PT (sec)			aPTT (sec)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	8.98±0.17	8.97±0.16	8.96±0.16	12.70 ± 0.07^{aA}	12.74 ± 0.06^{aA}	12.86 ± 0.01^{aA}
G1	8.79±0.18	9.18±0.02	9.62±0.27	23.79 ± 0.26^{bC}	21.23 ± 0.62^{cB}	16.28 ± 0.17^{dA}
G2	8.91±0.17	9.16±0.00	8.72±0.19	25.57 ± 0.94^{bC}	17.49 ± 0.34^{bB}	14.34 ± 0.19^{cA}
G3	9.15±0.01	8.92±0.11	8.82±0.20	23.98 ± 0.20^{cB}	14.05 ± 0.88^{aA}	13.42 ± 0.19^{bA}

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant.
Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant

Table 10. Biochemical alterations in various groups of dogs suffering from CME

Groups	ALT (IU/L)			AST (IU/L)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	32.44 ± 2.65^{aA}	33.55 ± 2.70^{aA}	34.06 ± 2.69^{aA}	18.91 ± 1.29^{bA}	19.86 ± 1.27^{bA}	20.36 ± 1.35^{bA}
G1	79.95 ± 0.48^{bC}	54.97 ± 0.46^{dB}	47.55 ± 1.72^{cA}	78.01 ± 1.91^{aC}	65.50 ± 1.54^{aB}	49.01 ± 0.42^{aA}
G2	82.58 ± 0.36^{bC}	49.49 ± 1.65^{cB}	40.13 ± 0.73^{bA}	80.01 ± 1.44^{aC}	60.83 ± 3.13^{aB}	42.33 ± 2.06^{aA}
G3	83.79 ± 0.22^{bC}	38.98 ± 1.53^{bB}	30.74 ± 1.17^{aA}	77.50 ± 0.43^{aC}	52.16 ± 3.36^{aB}	30.67 ± 1.20^{aA}

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant. Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant

Table 11. Biochemical alterations in various groups of dogs suffering from CME

Groups	BUN (mg/dl)			Creatinine (mg/dl)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	17.58 ± 0.99^{aA}	18.46 ± 1.02^{aA}	19.24 ± 1.00^{aA}	0.68 ± 0.09^{aA}	0.66 ± 0.07^{aA}	0.685 ± 0.08^{aA}
G1	42.16 ± 1.13^{bC}	35.16 ± 1.14^{cB}	30.04 ± 1.29^{cA}	0.83 ± 0.10^{aA}	0.55 ± 0.07^{aA}	0.47 ± 0.05^{aA}
G2	45.00 ± 2.42^{bC}	36.17 ± 2.55^{cB}	27.00 ± 1.88^{bcA}	0.64 ± 0.10^{aA}	0.53 ± 0.09^{aA}	0.39 ± 0.10^{aA}
G3	40.83 ± 1.37^{bC}	28.83 ± 1.38^{bB}	22.86 ± 1.56^{abA}	0.67 ± 0.14^{aA}	0.49 ± 0.12^{aA}	0.37 ± 0.10^{aA}

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant. Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant

Table 12. Biochemical alterations in various groups of dogs suffering from CME

Groups	ALP (IU/L)			Total Protein (gm/dl)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	34.45 ± 0.93^{aA}	36.43 ± 0.96^{aA}	37.15 ± 0.97^{aA}	6.58 ± 0.17^{cA}	6.59 ± 0.18^{bA}	6.60 ± 0.19^{bA}
G1	88.00 ± 4.53^{bC}	68.01 ± 4.53^{cB}	48.16 ± 2.02^{bA}	4.68 ± 0.21^{abA}	5.03 ± 0.49^{aAB}	6.02 ± 0.33^{aB}
G2	89.33 ± 2.87^{bC}	59.33 ± 2.87^{bcB}	44.67 ± 2.65^{abA}	4.85 ± 0.22^{bA}	6.09 ± 0.20^{bB}	6.61 ± 0.20^{abB}
G3	92.16 ± 3.54^{bC}	53.66 ± 4.56^{bB}	39.83 ± 4.31^{abA}	4.07 ± 0.30^{aA}	6.48 ± 0.42^{bB}	7.02 ± 0.31^{bB}

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant. Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant

Table 13. Biochemical alterations in various groups of dogs suffering from CME

Groups	Albumin (gm/dl)			Globulin (mg/dl)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Healthy (Control)	2.82 ± 0.14^{bA}	2.92 ± 0.14^{cA}	3.01 ± 0.14^{cA}	3.35 ± 0.24^{bA}	3.55 ± 0.24^{aA}	3.75 ± 0.24^{aA}
G1	1.39 ± 0.10^{aA}	1.48 ± 0.10^{aA}	1.58 ± 0.12^{aA}	2.61 ± 0.05^{aA}	3.17 ± 0.11^{aB}	3.46 ± 0.19^{aB}
G2	1.50 ± 0.05^{aA}	2.47 ± 0.08^{bB}	2.55 ± 0.07^{bB}	3.10 ± 0.35^{abA}	3.29 ± 0.27^{aB}	3.61 ± 0.27^{aB}
G3	1.63 ± 0.04^{aA}	2.55 ± 0.07^{bB}	2.69 ± 0.06^{bB}	2.70 ± 0.15^{abA}	3.38 ± 0.15^{aB}	3.73 ± 0.10^{aB}

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant. Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant

Table 14. C-PMIF alterations in various groups of dogs suffering from CME

Groups	C-PMIF		
	Day 0	Day 7	Day 14
Healthy (Control)	60.81 ± 2.07^{bA}	60.61 ± 1.66^{aA}	60.80 ± 2.54^{aA}
G1	51.28 ± 3.64^{aA}	57.85 ± 1.00^{aAB}	62.77 ± 2.57^{abB}
G2	48.66 ± 2.13^{aA}	58.94 ± 0.53^{aB}	67.09 ± 0.71^{bcC}
G3	47.39 ± 2.63^{aA}	60.07 ± 0.54^{aB}	72.34 ± 0.84^{Cc}

Mean with different superscript (a, b, c, d) in columns are differing significantly in between the groups, otherwise non-significant. Mean with different superscript (A, B, C, D) in rows are differing significantly in between the intervals, otherwise non-significant.

of albumin synthesis, intralobular necrosis secondary to hemorrhage, anemic hypoxia or secondary bacterial sepsis (Harrus *et al.*,1999). There was significant increase in the total protein on day 7th and day 14th after the treatment in all the treatment groups. Present study showed significant decrease in albumin on day 0 in all treatment groups (Group-1, Group-2 & Group-3) of dogs in comparison with control. There was significant increase in the albumin on day 7th and day14th after the treatment in all the treatment groups. Test of significance showed that the globulin of all the treatment groups were significantly lower than that of control group on the day of presentation. However, there

was no significant change in the serum globulin on day 7 and day 14 post-treatment in all the treatment groups. A significant interaction was found between treatments and days for BUN concentration but such interaction was not found for serum creatinine concentration (Table 11). Present investigation showed significant increase in BUN in all treatment groups (Group-1, Group-2 & Group-3) in comparison with control. The presence of inflammatory infiltrates rich in lymphocytes might be responsible for immuno-pathogenesis of renal lesion in dogs with CME. Due to membrano-proliferative glomerulopathy, interstitial nephritis and may also be due to glomerulonephritis with or without deposition of immune-complexes (Agnihotri *et al.*, 2012), which raises the urea and creatinine level in body. There was a significant decrease in the BUN on day 7th and day 14th after treatment in all the treatment groups. Among the all treatment groups, significant reduction in BUN concentration was observed on day 14 in group 3rd which is in pact with healthy control group. A non-significant interaction was observed between treatments groups in the concentration of serum creatinine, however a significant reduction in serum creatinine was observed on day 7 and day 14 with maximum reduction in group-3.

Present investigation reveals that non-significant interaction was found between different treatments groups for C-PIMF concentration. C-PMIF concentration was reduced in all treatment groups (Group-1, Group-2 & Group-3) on day 0 comparison with control (Table 14) However, there was significant increase in PMIF concentration in all treatment groups (Group-1, Group-2 & Group-3) on day 7th in all the treatment groups.

CONCLUSION

The study shows that nested PCR is a reliable diagnostic method for *Ehrlichia canis* infection. Infected dogs showed serious blood and chemical imbalances, pointing to the widespread nature of the disease. The treatment resulted in significant clinical improvement. The best recovery occurred with a combination of traditional treatment and a polyherbal preparation. These results suggest the potential benefits of using integrative therapy to treat canine monocytic ehrlichiosis.

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