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RESEARCH ARTICLE

Therapeutic efficacy of doxycycline with whole blood transfusion in management of thrombocytopenic ehrlichiosis in canines

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Abstract

The objective of present study was to evaluate the efficacy of doxycycline with whole blood transfusion in anemic and thrombocytopenic cases of canine ehrlichiosis. Clinical cases of thrombocytopenic ehrlichiosis in canines were diagnosed on the basis of reduced count of platelets and blood smear examination for morula stage in monocytes. Five cases of dogs (3-6 months) presented with wide spread petechiae and echimotic hemorrhage and other clinical signs were selected for study. These cases were kept as treatment group (Group-I) and five healthy dogs as control group (Group-II). Group-I was treated with specific therapy with oral doxycycline @10 mg/ kg body wt. twice daily for 20 days and whole blood transfusion was adopted as supportive therapy for anemia and thrombocytopenia. Five healthy dogs of same age group were kept as control (Group-II). Haematobiochemical profile of treatment group (Group-I) revealed significant ($p< 0.05$) reduction in Hb, PCV, TEC, platelets count, lymphocytes, total protein, albumin and globulin while significant ($p< 0.05$) increase was recorded in neutrophils, monocytes, serum ALT and AST at 0 day as compared to healthy control (Group-II). The infected dogs showed remarkable improvement in hemorrhagic tendency after whole blood transfusion and complete recovery after 20 days of therapy with doxycycline.

INTRODUCTION

Ehrlichiosis in canines also known as canine rickettsiosis, canine hemorrhagic fever, canine typhus, tracker dog disease, and tropical canine pancytopenia, is caused by rickettsial microorganism *Ehrlichia canis*, which is small pleomorphic gram negative coccoid bacteria belonging to family Ehrlichiaceae. The brown dog tick, *Rhipicephalus sanguineus* is the main vector which harbours *Ehrlichia* organism and transmits it to dogs (Melo et al., 2011; Mylonakis et al., 2004; Davoust et al., 2005). *Ehrlichia canis* is distributed worldwide in dog population and wild canids (Harrus et al., 1996; Dagnone et al., 2003). This microorganism enters the blood circulation by bite of brown dog ticks and parasitizes the monocytes and multiply intracellularly forming a mulberry-like structure called morula which is diagnostic feature for canine ehrlichiosis. Ehrlichiosis is a multisystemic disorder which may produce hemorrhage tendency, lymphadenopahty, splenomegaly, hepatomegaly, along with cardiac/renal disorders and myelosupression (Aysul, et al., 2012). The disease is clinically presented with varying grades of pyrexia, depression, anorexia, hemorrhagic tendencies and lymphadenopathies, anterior uveitis, chorioretinitis, papilledema, retinal hemorrhage, presence of retinal perivascular infiltrates, and bullous retinal detachment (Komnenou et al., 2007; Harrus et al., 2011).

Materials and methods

Animals and Treatment Regimen

Dogs irrespective of sex and breed of 3-6 months age group were presented at the referral veterinary polyclinic, IVRI, Izatnagar with the history of fever (103.4-105.1^{0}F), increased respiratory rate, heart rate, depression, anorexia, enlarged popliteal lymph nodes, ascites, melena and tick infestation on body with *Rhipicephulus*. On clinical examination, these cases revealed petechial hemorrhages in abdomen, inner sides of thigh and ear pinna, commissures of mouth and lips and around eyes. Five cases were found positive for ehrlichiosis and severe thrombocytopenia were selected for study and kept as treatment group (Group-I). At the time of presentation, Group- I animals were treated with oral doxycycline @ 10 mg/ kg body wt. twice daily for 20 days orally and whole blood transfusion was done. Whole blood was collected form healthy donors using anticoagulant heparin @ 625 IU/ 50 ml of blood. After major and minor cross matchings of donor and recipient, blood was transfused @ 20 ml/ kg body wt. @ 5-10 ml/kg/hr with continuous monitoring of adverse reactions. All cases were given supportive therapy of Paracetamol @ 10 mg/kg SOS, tab. Pantaprazole 20 mg once daily and syrup Silymarin @ 10 ml orally. Another five healthy dogs of same age group were kept as healthy control (Group-II).

Haemato-biochemical profile

Blood sample was collected from cephalic vein in EDTA vial for complete blood count (CBC) and serum was harvested for biochemical analysis on day 0, day 10 and day 20. A thin smear was prepared for confirmation of ehrlichiosis stained with Gimesa stain and examined microscopically for presence of morula in monocytes. The determination of Hb and PCV was done within two hours of collection. However, serum samples were stored at -20°C for further analysis. Haemoglobin concentration (g/dl) in the blood was estimated by cyano-methaemoglobin method (Vankampen and Zinglstra, 1961**).** Packed cell volume (PCV %) was determined by capillary micro-haematocrit method (Coles, 1980). Total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC), and total platelets count was performed using EDTA blood with the standard protocols.

Serum samples stored in deep freeze at –20^{0}C were used for estimation of serum alanine amino transferase (ALT) and serum aspartate amino transferase (AST) by Reitman and Frankel method (1957), Serum total protein (TP) by modified biuret method and albumin by Dumas method (Varley *et al.,* 1980). Serum globulin was estimated by subtracting albumin from total protein. Estimation of blood urea nitrogen (BUN) was done by diacetylmonosamine method and serum creatinine by Jaffe's alkaline picrate method (Marsh et al., 1965). Data was analyzed using paired 't' test and one way ANOVA with the help of statistical software package SPSS 16.0. The values of $p < 0.05$ were considered statistically significant.

Results

On the basis of examination of thin blood smear stained with Giemsa stain, all five cases were found to be positive for morula stage of *Ehrlichia canis* in monocytes. Comparison of mean hematological values between two groups has been presented in table 1. There were significantly (p<0.05) reduced levels of Hb, PCV, TEC, platelets count and lymphocyte while significantly increased levels of neutrophils and monocytes were noticed in *E. canis* infected dogs (Group I) as compared to healthy dogs (Group II) before instituting therapy. On day 20 significant (p<0.05) elevation was noticed in the levels of Hb, PCV and TEC, platelets count and lymphocytes and reduction in neutrophils and monocytes which was nonsignificant when compared with the healthy control (Group-II) after therapy with doxycycline and blood transfusion.

Comparison of mean biochemical values of serum between two groups has been presented in table 2. Among serum biochemical markers, significantly (p<0.05) increased levels of ALT, AST and significant reduction of total protein, albumin and globulin were observed before initiation of treatment as compared to healthy dogs. On day 20, after treatment with doxycycline, significant (p<0.05) reduction was noticed in the levels of ALT, AST while elevation in the levels of total protein, albumin and globulins which was comparable to healthy control (Group-II). No significant changes were observed in the levels of BUN and serum creatinine. All the infected animals were recovered fully at the end of therapy and found to be negative for *E. canis*. No significant variations were observed in the blood parameters of the healthy dogs throughout the study period.

Table.1–Hematological changes of dogs in infected (Group I) and control healthy dogs (Group II) before initiation of treatment and thereafter at post treatment periods

Parameter	Group	0 Day	10 day	20 day
Hemoglobin (Hb) g/dl	Group-I	$5.60\pm0.26^{a,A}$	$8.02\pm0.35^{b,A}$	$14.08\pm0.42^{c,A}$
	Group-II	$12.60\pm0.57^{a,B}$	$13.30\pm0.5^{ab,B}$	$14.88\pm0.40^{b,A}$
Packed cell volume (PCV) (%)	Group-I	$18.36\pm0.35^{a,A}$	$25.56\pm0.87^{b,A}$	$42.96\pm1.26^{c,A}$
	Group-II	$40.84\pm1.34^{a,B}$	$42.80\pm0.73^{a,B}$	$42.90\pm0.59^{a,A}$

Total erythrocyte count (10^6/ mm^3)	Group-I	$2.86\pm0.10^{a,A}$	$4.19\pm0.29^{b,A}$	$6.60\pm0.23^{c,A}$
	Group-II	$5.38\pm0.22^{a,B}$	$5.58\pm0.28^{a,A}$	$6.42\pm0.11^{b,A}$
Platelets count (lacs/ mm^3)	Group-I	$0.48\pm0.05^{a,A}$	$1.42\pm0.12^{b,A}$	$4.29\pm0.21^{c,A}$
	Group-II	$4.35\pm0.62^{a,B}$	$4.71\pm0.59^{a,B}$	$4.81\pm0.21^{a,A}$
Total leucocyte count (10^3/ mm^3)	Group-I	$15.20\pm2.27^{a,A}$	$10.23\pm0.90^{ab,A}$	$9.22\pm0.75^{b,A}$
	Group-II	$13.77\pm0.95^{a,A}$	$13.89\pm0.22^{a,A}$	$8.15\pm0.41^{b,A}$
Neutrophil (%)	Group-I	$81.61\pm1.16^{a,A}$	$73.41\pm0.92^{b,A}$	$66.64\pm2.24^{c,A}$
	Group-II	$70.60\pm1.53^{a,B}$	$71.00\pm0.71^{a,A}$	$68.20\pm0.66^{a,A}$
Lymphocyte (%)	Group-I	$13.00\pm0.45^{a,A}$	$23.40\pm0.87^{b,A}$	$32.20\pm2.26^{c,A}$
	Group-II	$27.40\pm1.81^{a,B}$	$26.20\pm1.20^{a,A}$	$29.40\pm0.62^{a,A}$
Monocyte (%)	Group-I	$2.80\pm0.37^{a,A}$	$1.00\pm0.00^{b,A}$	$0.40\pm0.24^{b,A}$
	Group-II	$1.00\pm0.31^{a,A}$	$1.00\pm0.31^{a,A}$	$0.60\pm0.24^{a,A}$

Value are expressed as mean±SEM. Values with superscripts (a, b) differ significantly (p<0.05) from day 0 of treatment in same group. Values with superscripts (A, B) differ significantly (p<0.05) between groups

Table.2–Serum biochemical changes of dogs in infected (Group I) and control healthy dogs (Group II) before initiation of treatment and thereafter at post treatment periods

Parameter	Group	0 Day	10 day	20 days
ALT (IU/L)	Group-I	$91.40\pm15.30^{a,A}$	$62.80\pm9.05^{ab,A}$	$34.40\pm2.01^{b,A}$
	Group-II	$20.00\pm2.30^{a,A}$	$27.20\pm3.28^{a,A}$	$25.80\pm0.86^{a,A}$
AST (IU/L)	Group-I	$80.00\pm16.50^{a,A}$	$40.00\pm1.58^{b,A}$	$34.00\pm2.00^{b,A}$
	Group-II	$35.80\pm5.36^{a,A}$	$34.20\pm3.68^{a,A}$	$37.21\pm3.94^{a,A}$
Total protein (g/dl)	Group-I	$4.98\pm0.12^{a,A}$	$5.92\pm0.08^{b,A}$	$6.78\pm0.11^{c,A}$
	Group-II	$6.80\pm0.14^{a,B}$	$6.84\pm0.07^{a,B}$	$7.00\pm0.06^{a,A}$
Albumin (g/dl)	Group-I	$2.00\pm0.07^{a,A}$	$2.56\pm0.07^{b,A}$	$3.00\pm0.07^{a,A}$
	Group-II	$2.80\pm0.09^{a,B}$	$2.91\pm0.07^{ab,B}$	$3.18\pm0.08^{b,A}$
Globulins (g/dl)	Group-I	$3.00\pm0.05^{a,A}$	$3.32\pm0.07^{b,A}$	$3.78\pm0.15^{c,A}$
	Group-II	$4.00\pm0.15^{a,B}$	$3.94\pm0.07^{a,B}$	$3.90\pm0.10^{a,A}$
Blood urea nitrogen (mg/dl)	Group-I	$16.2\pm2.25^{a,A}$	$13.00\pm1.93^{a,A}$	$13.00\pm1.75^{a,A}$
	Group-II	$11.8\pm1.06^{a,A}$	$14.80\pm1.43^{a,A}$	$13.00\pm0.70^{a,A}$
Serum creatinine (mg/dl)	Group-I	$0.88\pm0.05^{a,A}$	$0.82\pm0.02^{a,A}$	$0.78\pm0.03^{ab,A}$
	Group-II	$0.74\pm0.06^{a,A}$	$0.88\pm0.05^{a,A}$	$0.74\pm0.02^{a,A}$

Value are expressed as mean±SEM. Values with superscripts (a, b) differ significantly (p<0.05) from day 0 of treatment in same group. Values with superscripts (A, B) differ significantly (p<0.05) between groups.

Discussion

Canine eherlichiosis is acute, subclinical or chronic disease which is manifested as multisystemic forms. *E. canis* can infect all breeds of dogs but the German shepherd breed appears to be more susceptible, showing the more severe form of the disease with a higher morbidity and mortality compared to other breeds (Nyindo et al., 1980). No age or gender predilection has been established. Disease manifestations may be affected by the pathogenicity of different *E. canis* strains and co-infections with other arthropod-borne pathogens such as *Babesia canis vogeli* and *Hepatozoon canis* which are transmitted by the same vector (Gal et al., 2007). The acute disease is characterized by a high fever, depression, lethargy, anorexia, lymphadenomegaly, splenomegaly and hemorrhagic tendencies. The latter are usually exhibited by dermal petechiae and echymoses, and epistaxis. In initial incubatory period the diagnosis of the disease poses challenge due to its no or minimum clinical manifestations. Canine ehrlichiosis should be suspected when a compatible history (living in or travel to an endemic region, previous tick exposure), typical clinical signs and characteristic hematological and biochemical abnormalities are present.

Among all selected clinical cases showed microcytic hypochromic anemia, severely decreased platelets count, and decrease in haematocrit values which were significant findings. After whole blood transfusion and therapeutic regimen followed, dogs showed remarkable improvement in hemorrhagic tendencies. The findings of present study were similar to Waner et al., (1995) who reported, significant thrombocytopenia develops in experimentally infected dogs in approximately 1 week post-infection, and is most severe from Days 17 to 25. Thrombocytopenia is immune-mediated platelet destruction which may develop as a primary disorder or in

association with other disease and poorly understood (Abeygunawardena et al., 1990). Whole blood transfusion improves the erythrocyte count, PCV and platelets. Reduction in monocyte and neutrophils was recorded after few days of therapy which can be explained by antimicrobial doxycycline clearing up the infection of *E. canis*. Harrus et al., (2004) reported that most dogs recover from acute and subclinical disease when treated with appropriate and adequate dosages of doxycycline or other tetracyclines which are the first line of drugs for ehrlichiosis.

Serum biochemical parameter such as ALT and AST were slightly high at 0 day but found nonsignificant as these varies in normal range of species which indicated non significant hepatopathy in these cases. The results of present study were in acordance with Harrus et al., (2011). There was significant increase in serum total protein, albumin and globulins. Our findings were concurrent with Harrus et al., (1996) who reported that serum protein electrophoretic pattern of the infected dogs had a significant hypoalbuminaemia, hyperglobulinaemia and hypergammaglobulinaemia compared to the control dogs. BUN and serum creatinine values showed no alteration in both groups during the disease progression. The dogs recovered completely and found to be negative for ehrlichiosis 20 days post therapy by blood smear examination and also hematol-biochemical parameters were in the normal range of species.

Conclusion

The animals showed substantial improvement in condition after 2-3 days, started taking food. Doxycycline is the drug of choice for canine ehrlichiosis and whole blood transfusion can be adopted as a supportive therapy for severely anemic and thrombocytopenic cases. The clinical recovery can be observed within 2-3 day of therapy and regimen needs to be continued for 21 days for clearing up parasitemia.

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Competing interests

The authors declare that they have no competing interests.

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