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Case Report

DIAGNOSIS AND TREATMENT OF CANINE MONOCYTIC EHRLICHIOSIS IN A BOXER BREED OF DOG – A CASE REPORT

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Abstract: Canine monocytic ehrlichiosis (CME) is one of the most common tick borne disease among dog population of Ludhiana, Punjab and is caused by *Ehrlichia canis*. The present report deals with diagnosis and treatment of canine monocytic ehrlichiosis in a Boxer breed of dog presented at small animal clinic, TVCC, Ludhiana with a history of pyrexia, anorexia, weight loss, weakness, tick infestation, severe epistaxsis, corneal edema with opacity, swollen prefemoral lymphnodes and melena. Peripheral blood smear examination revealed intracytoplasmic morulae of *Ehrlichia canis*. Hematobiochemical parameters were analyzed. Confirmative diagnosis was done by both serology and molecular technique. Therapeutic management included doxycycline @ 10 mg/kg orally for a period of 21 days until blood smear becomes negative and till complete clinical recovery along with supportive therapy. Dog recovered uneventfully after 21 days of post treatment. Results suggests that continuous monitoring, specific and appropriate supportive therapy as well as owner's compliance are key factors in reversing clinical signs and elimination of the infection from blood in CME affected dog.

Keywords: Canine monocytic ehrlichiosis, *Rhipicephalus sanguineus*, *Ehrlichia canis*, doxycycline.

Introduction

Tickborne haemoprotozoan infections are frequently encountered across tropical subtropical regions, where *Rhipicephalus sanguineus*, brown dog tick acts as important vector for many diseases in dogs (Badesiya et al. 2014). Canine monocytic ehrlichiosis mainly seen in three forms. Acute form followed by subclinical and chronic forms. Disease is mainly characterized by fever, anorexia, weakness, epistaxis, lymphadenopathy, tick infestation, and ocular changes (Hernandez et al. 2012). The present investigation deals with the feasible diagnostic technique in detecting *Ehrlichia canis* infection in a 5 year old male boxer dog and also deals with the hematobiochemical changes, treatment, clinical recovery by use of specific and supportive therapy over a period of 21 days.

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Materials and Methods

A 5 year old Boxer male dog was brought to the TVCC, small animal clinics, GADVASU, Ludhiana, Punjab with the history of anorexia, tick infestation and epistaxsis. Upon clinical examination the body temperature noted was 104.6° F, congested mucus membrane, weakness, ticks on the body, oozing of blood from both the nostrils (Figure 1). Blood sample was collected for blood smear examination (Leishman staining technique), hematology (ADVIA® 2120 Hematology system) and PCR (primary and nested). Serum samples were separated and used for biochemical (Johnson & Johnson VITROS 750Xrc) and serological tests (ImmunoComb® canine ehrlichia antibody test kit). Finally the dog was put under treatment over a period of 21 days with the oral doxycycline @ 10mg/kg body weight along with the supportive therapy for early clinical recovery and until blood smear and PCR results become negative. PCR was employed on day 0, day 15 and day 21 to check the role of doxycycline in elimination of infection from blood.

Results and Discussion

On day 0, Leishman stained thin blood smear examination revealed morula in the cytoplasm of monocyte with severe thrombocytopenia and regenerative anemia (Figure 2). Microscopic examination of morula in the present case report was agreeing with finding of Dhankar *et al.* (2011) observed morula stage in the monocytes in 23 out of 203 dogs in Haryana and Delhi States. Genomic DNA was isolated from blood and subjected to both primary and nested PCR by amplifying a portion of 16S rRNA gene of *E. canis* as described by Murphy *et al.* (1998) and dog was found to be positive by both primary and nested PCR and produced an amplicon of desired size 478bp and 386bp respectively (Figure 3). This study showed a confirmative molecular diagnosis of the disease by using specific primers for PCR for canine ehrlichiosis was agreeing with the findings of Milanjeet *et al.* (2014) and Lakshmanan *et al.* (2007) both targeted 16S rRNA gene and produced amplicon of desired size as above mentioned. Further affected dog sera was subjected to ImmunoComb® canine ehrlichia antibody test and showed high positive reaction to *E. canis* with a titre of 1:320 - 1280 (Figure 4). Similarly shah *et al.* (2010) diagnosed 10 cases using Immunocomb dot-ELISA kit, a rapid test kit used to diagnose the present case. In the present study biochemical analysis revealed hypoalbuminemia, hyperglobulinemia and increase in the values of ALT, ALKP, creatinine and decrease in albumin and globulin ratio. The biochemical alterations were agreeing with the findings of Eljadar (2010) observed hypoalbuminemia, hyperglobulinemia and increase in the values of liver enzymes. The animal was treated with

oral doxycycline @ 10mg/kg body weight, over a period of 21 days along with supportive treatment, ranitidine at the dose rate of 2 mg/kg P.O bid were given daily as H_2 Blocker, liver suppliment (Hepamust® Pet mankind Pvt. Ltd. New Delhi) and multivitamins (Multistar pet® Pet mankind Pvt. Ltd. New Delhi) were administered for 15 days. Adrenaline (local application) to control nasal bleeding and Inj. Adrenochrome @ 5-10mg/kg body weight I/M till reduction of epistaxis. Cypermethrin containing shampoo for bathing for ticks control. Advised owner to come for the check up at 15th day and 21st day post treatment. Blood sample was collected on 0, 15, 21 days for both PCR and hematology to check the clinical improvement in dog and status of infection in blood by PCR. The dog was clinically improved and blood smear was negative for morula on 15 day post treatment but shown positive result by nested-PCR. So, treatment was continued upto 21st day and routine check was done. Dog was completely recovered, normal apatite, absence of epistaxsis (Figure 5) and case was negative by nested PCR on day 21. The hematobiochemical changes on day 0 pre-treatment and days 15 and 21 post-treatment as been shown in Table 1. Similar study was made by Perea et al. (2009) diagnosed *E. canis* affected cases and dogs were treated with oral doxycycline. Banet et al. (2009) mentioned even the conjunctivas of all dogs were positive even after 12 days of oral doxycycline therapy was demonstrated by real-time PCR.

Conclusion

PCR is the most sensitive and specific diagnostic test whereas blood smear examination and serology can support to get a confirmative diagnosis of canine monocytic ehrlichiosis. Oral doxycycline for a period of 21 days can be followed as better protocol for treating canine ehrlichiosis. Clinical and hematobiochemical recovery can be seen just after one week of doxycycline therapy whereas elimination of *E. canis* infection from blood can be assessed by PCR and detect the role of 21 day therapy with oral doxycycline in affected dogs. Oral doxycycline can be used as a specific drug in treating canine monocytic ehrlichiosis.

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Table 1. Hematobiochemical findings before and after therapy

Parameters	Day 0 Post treatment	Day 15 Pre treatment	Day 21 Pre treatment
Hb (g/dL)	7.4	8.2	11.2
TEC ($\times 10^6$/µL)	3.06	4.1	5.2
PCV (%)	19	24.5	32.8
TLC ($\times 10^3$/µL)	24.98	20.52	12.6
Neutrophil (%)	96.00	78.00	68.00
Lymphocytes (%)	08.00	12.00	20.00
Eosinophils (%)	01.00	01.00	00
Basophils (%)	00	00	00
Monocytes (%)	06.00	01.00	01.00

Platelet count (×10^5/µL)	0.98	1.85	2.1
Total bilirubin (mg/dL)	0.6	0.6	0.7
AST (U/L)	42	42	41
ALT (U/L)	68	62	55
ALKP (U/L)	181	136	106
Total protein (g/dL)	7.2	5.7	5.3
Albumin (g/dL)	1.6	2.1	3.2
Globulin (g/dL)	5.6	3.6	2.1
A:G ratio	0.28	0.58	1.1
BUN (mg/dL)	23	23	22
Creatinine (mg/dL)	1.1	1.2	1.1

Fig. 1. Boxer breed of dog showing epistaxis before treatment

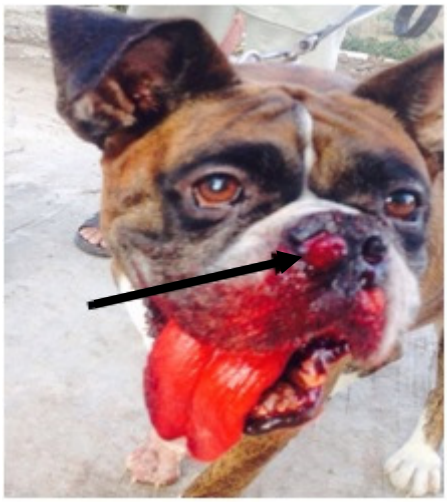
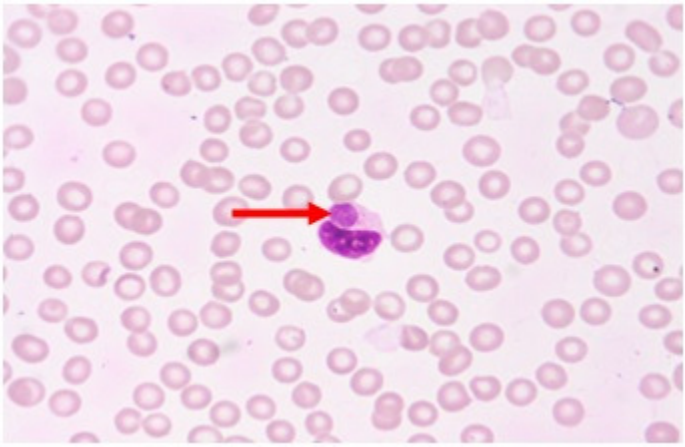


Fig. 2. Morula in the cytoplasm of monocyte with severe thrombocytopenia



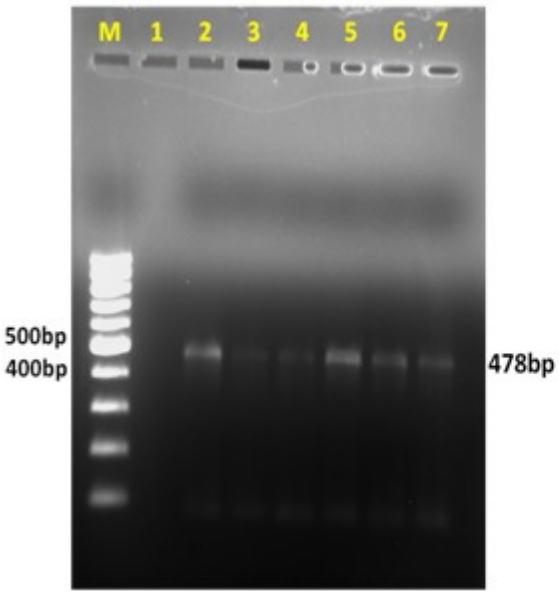


Fig. 3. Standardization of primary PCR (E-PCR) assay
Lane M: Generuler™ 100bp Ladder
Lane 1: Negative control
Lane 2: Positive control
Lane 3 to 7: Samples positive for *E. canis* by blood smear Examination

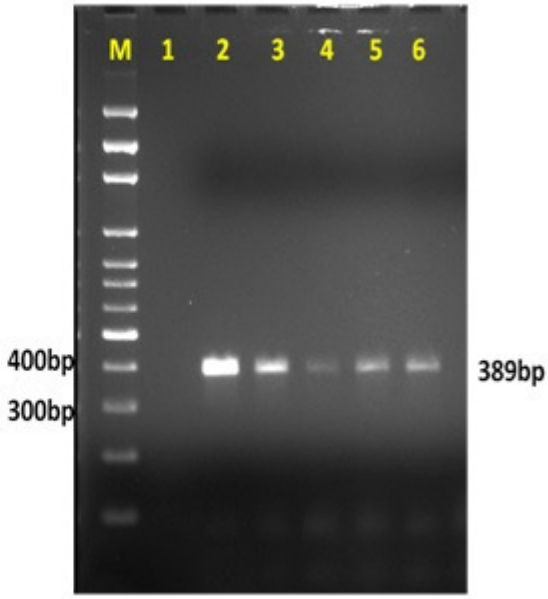


Fig. 3. Standardization of nested PCR (Ec-PCR) assay
Lane M: Generuler™ 100bp Ladder
Lane 1: Negative control Lane 2: Positive control
Lane 3 to 6 samples positive for *E. canis* by blood smear examination

Fig. 4. The developed spots represent different concentrations of serum antibodies and the upper spot represents a known positive control indirect immunofluorescent antibody test (IFA) pretititrated serum sample of 1:80.

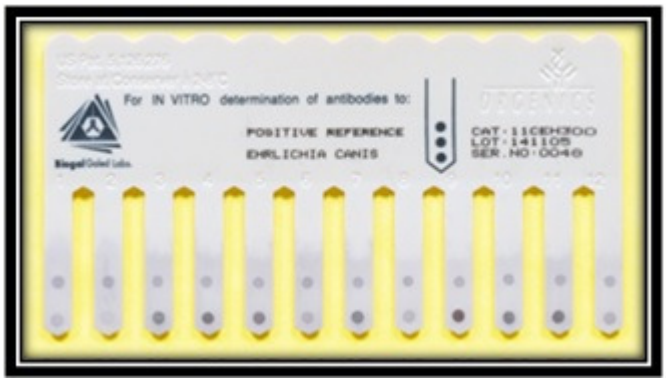


Fig. 5. Boxer breed of dog recovered after treatment