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Molecular detection of canine monocytic ehrlichiosis and its management with whole blood transfusion: A case report

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Abstract
A three month old male Doberman pup was presented to the small animal medicine unit, VCC, RIVER, Puducherry with the history of inappetence, vomiting and severe tick infestation. Clinically, the pup was dull, conjuctival mucous membrane blanched with increased capillary refill time. On hemogram, Hb- 3.2 g/dl, PCV-11%, RBC-1.31 million/cmm with macrocytic hypochromic anemia. Molecular diagnosis with PCR of the blood was positive for *Ehrlichia canis.* Anemia was managed with whole blood transfusion and the underlying *Ehrlichiosis* was treated with oxytetracycline @ 10 mg/kg b.wt IV, prednisolone @ 1 mg/kg B.wt, pantoprazole @ 1 mg/kg b.wt for 3 days followed by oral doxycycline @ 5 mg/kg B.wt for next 21 days along with supportive therapy.

Keywords: PCR, *Ehrlichia canis*, blood transfusion, anemia, dog

Introduction
Canine Monocytic Ehrlichiosis (CME) is one of the most common tick borne disease caused by *Ehrlichia canis* which is an obligate pleomorphic intracellular rickettsial pathogen that resides as morulae in the monocytes and macrophages of dogs (Simpson, 1972) [9]. It is mainly transmitted by the bite of brown dog tick *Rhipicephalous sanguineus.* Previously canine ehrlichiosis has also been called as canine rickettsiosis, tropical canine pancytopenia, canine haemorrhagic fever, nairobi bleeding diorder, tracker dog disease, haemorrhagic fever and canine tick typhus. Disease occurs in three forms as acute, subclincal and chronic phase. Multisystemic nature of this disease leads to lymphadenopathy, splenomegaly, hepatomegaly, bleeding tendency, cardiac/renal disorders and myelosuppression (Ayusl *et al.*, 2012) [1]. Clincal signs of this disease includes anorexia, fcver, depression, lethargy, weight loss, lymphadenomegaly, splenomegaly and hemorrhagic tendencies. Hematological abnormalities comprise of thrombocytopenia, mild anemia and leukopenia during acute phase, mild thrombocytopenia in subclincal phase and pancytopenia in chronic phase. Biochemical alterations include hypoalbuminemia and hyperglobulinemia (Harrus *et al*., 1996) [4].

Materials and methods
A three month old male Doberman weighing 8.0 kg was presented to small animal medicine unit of Veterinary Clincal Complex, RIVER, Puducherry with the history of anorexia, dullnes, depression, vomiting and severe tick infestation. Regular deworming and vaccination was done. Clinical examination revealed blanched conjunctival and oral mucous membrane, rectal temperature-100.9°F, tachycardia (143 beats/min) and polypnoea. Peripheral blood smear was prepared and stained with leishman's stain for detection of tick borne pathogens under microscope. Blood and serum samples were collected for hematology, PCR (Table.1) and serum biochemistry respectively.

Table 1: Primer sequence for PCR analysis

Tick borne pathogen	Gene targeted	Primer sequences	Product size
E. canis	virB9	EcavB9 F:5'-CATTATCATTTCAATACGTAACTC-3' EcavB9 R:5'-TTTTGATTTTCTTCTGACATAGTG-3'	959 bp (Silva *et al*., 2012a) [10]

Treatment protocol

The treatment was started with Inj oxytetracycline @ 10 mg/kg IV, Inj prednisolone @ 1 mg/kg IM, Inj pantoprazole @ 1 mg/kg IV, Inj tribivet 1 ml IV for 3 days followed by Tab Doxycycline @ 10 mg/kg PO sid, Tab Dompan @ 1 mg/kg PO sid and oral Iron supplements @ 5 ml PO bid daily for 28 days. Management of anemia by whole blood transfusion @ 20 ml/kg body weight was carried out by collecting blood from a suitable donor on the same day of presentation (Fig.1).

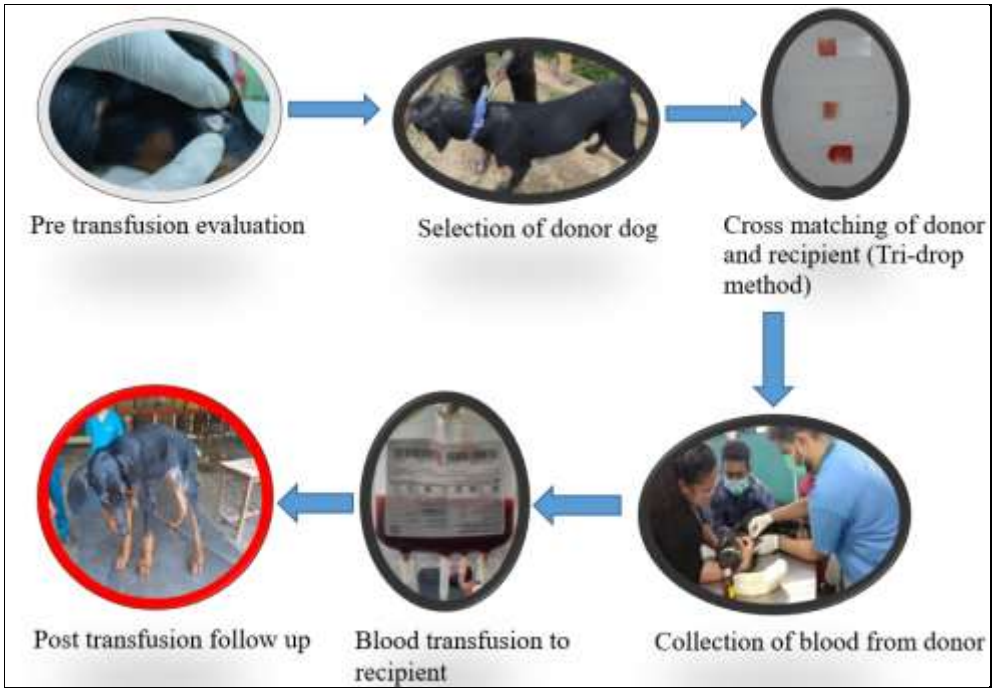

Fig 1: Whole blood transfusion

Management of adverse reaction

Within four hours of blood transfusion, the pup started showing haematemesis within an interval of 30 min for four times. The pup was administered Inj Adrenaline (1:1000) @ 0.02 mg/kg IV with NS, Inj Prednisolone @ 1 mg/kg, Inj Chlorpheniramine maleate @ 3 mg/kg b.wt, Inj Pantoprazole @ 1 mg/kg, Inj Ondansetron @ 0.2 mg/kg. No further adverse reactions was noticed after the treatment.

Results

Haematological parameters revealed marked anemia and leucocytosis. Biochemical analysis revealed elevated ALP, hypoprotenemia, hypoalbuminemia, normal BUN and Creatinine levels (Table 1). The stained blood smear was negative for tick borne pathogens. Polymerase chain reaction on whole blood revealed positive for *Ehrlichia canis* antigens. On the basis of history, clinical signs and PCR analysis (Fig.2) the condition was diagnosed as Canine Monocytic Ehrlichiosis.

Table 1: Hemato-biochemical values before transfusion

Haematological Parameters		**Biochemical parameters**	
Parameter	**Values**	**Parameter**	**Values**
Haemoglobin (g/dl)	3.2	Liver function tests	
RBC Count (million/cmm)	1.31	AST (U/L)	18
Packed cell volume (%)	11	ALT (U/L)	14
WBC (10^3 cells/cmm)	28.3	ALP (U/L)	195
Neutrophils (%)	65	Total protein (g/dl)	3.1
Lymphocytes (%)	28	Albumin (g/dl)	1.5
Monocytes (%)	05	Globulin (g/dl)	1.6
Eosinophils (%)	02	A:G Ratio	0.94
Basophils (%)	01		
Platelets (10^3/cmm)	293	Kidney function tests	
MCV (fl)	83.5	BUN (mg/dl)	13.55
MCH (pg/ml)	24.3	Creatinine (mg/dl)	0.13
MCHC (g/dl)	29.1		
RDW (%)	25.1		

Table 2: Hemato-biochemical changes before and after transfusion

Parameters	0th day	14th day	28th day
Hemoglobin (g/dl)	3.2	7.6	9.28
Packed cell volume (%)	11	28	38
WBC (10^3/cu.mm)	28.3	8.1	10.5
PlateletS (10^3/cmm)	293	153	243
Neutrophils (%)	65	64	62
Lymphocytes (%)	28	36	38
Monocytes %	05	00	00
Eosinophils %	02	00	00
Total protein (g/dl)	3.1	3.9	4.74

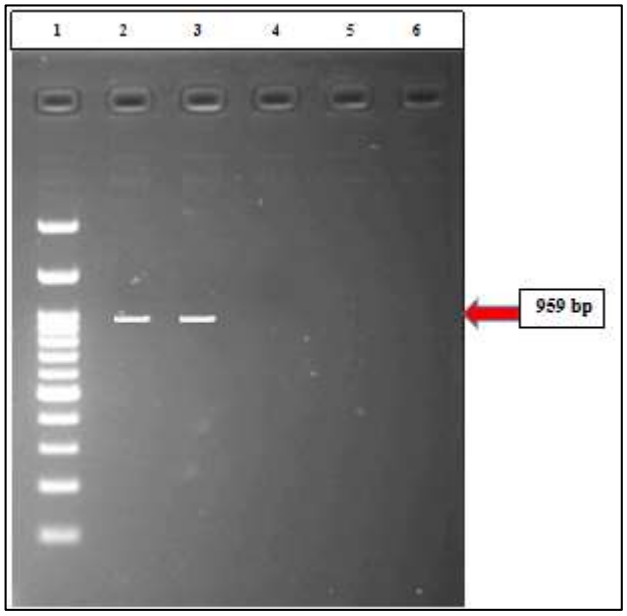

Fig 2: Gel electrophoresis result for the PCR of *E.canis*, Lane 1=100bp ladder; Lane 2=Positive control; Lane 3=Positive sample; Lane 4, 5, 6=Negative control

Discussion

Thrombocytopenia, leucopenia and anemia were mostly encountered in CME (Hoskins, 1991) [7]. Low hemogram in *Ehrlichiosis* is because of blood loss due to thrombocytopenia, transient suppression of bone marrow and immune mediated destruction of red blood cells (Buhles *et al.*, 1974) [2]. The pathogenesis of thrombocytopenia is due to Anti-platelet antibodies produced in CME (Harrus *et al*., 1997) [5]. Hypoproteinemia and hypoalbuminemia could be due to reduced hepatic function and / or due to decreased protein intake due to anorexia.

Though microscopic examination of leishman's stained blood smear is routinely used for the detection of tick borne pathogens, it requires heavy load of the pathogens for detection. So molecular detection using PCR is the best technique for detection of tick borne pathogens in blood (Rucksaken *et al*., 2019) [8].

Whole blood transfusion improves the hemoglobin, erythrocyte count and PCV (Table 2). Reduction in total leucocyte count was recorded on 14th and 28th day which is due to antimicrobial activity of doxycycline in eliminating the pathogens. Most dogs recover from acute and subclinical disease when treated with adequate doses of doxycycline or other tetracyclines which are the first line of drugs for *Ehrlichiosis* reported by Harrus *et al.*, (2004) [6] which was in accordance with the present study.

There are more incidence of immunological mechanisms involved in the pathogenesis of disease. So immunosuppresive doses of prednisolone in treatment of CME was used. Anemia is a major sign in Canine Monocytic Ehrlichiosis, whole blood transfusion and haematinics was given as ancillary treatment.

In the present case, transfusion reactions are due to the immune responses staged against the antigens found on the RBC membranes, the RBCs were destroyed in the vascular space (acute hemolytic reaction) due to foreign antigens, so the reaction falls under the category of acute immunologic reactions (Chiaramonte, 2004) [3]. Adverse transfusion reactions can be avoided by selecting healthy donors, performing cross matching, proper collection methods, storage and administration of only required components.

Conclusion

The animal showed over all improvement. PCR is considered more sensitive diagnostic tool in the detection of Canine Monocytic Ehrlichiosis than blood smear examination. Doxycycline is the drug of choice for canine *Ehrlichiosis* and was administered for 28 days for clearing up parasitemia. Whole blood transfusion was adopted as ancillary treatment for severe anemia. Adverse blood transfusion reactions was managed with Adrenaline, corticosteroids and fluid therapy.

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