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Successful therapeutic management of canine ehrlichiosis with aid of blood transfusion: A case report

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Abstract
Ehrlichiosis is a globally distributed canine vector- borne disease transmitted by the brown dog tick, *Rhipicephalus sanguineus*. A 45- day- old male husky dog weighing 2 kg is presented with the history of tick infestation, inappetence, fever, lethargy, anemia and melena. Clinical examination revealed elevated rectal temperature of 104.6^0 F, increased respiratory rate and pale mucous membrane. Ehrlichiosis was diagnosed in a dog from blood smear examination with Giemsa staining, *Ehrlichia* morulae were noticed in lymphocytes and also confirmed by rapid test kit. Complete blood picture revealed a decrease in red blood cell count, platelet count and low hemoglobin concentration compared to the normal range. The dog was treated with doxycycline at a dose rate of 10 mg/kg body weight intravenously with DNS slowly, Imidocarb was given in two doses with an interval of 14 days at a dose rate of 6.6 mg/kg body weight subcutaneously, followed by supportive drugs. Blood transfusion was done on the first day of treatment. After 21 days of therapy, the dog was completely recovered from the ehrlichiosis.

Keywords: Ehrlichiosis, *Rhipicephalus sanguineus*, morulae

Introduction
Ehrlichiosis is a tick-borne disease caused by *Ehrlichia canis*, a gram-negative obligate intracellular bacterium, and transmitted by the brown dog tick *Rhipicephalus sanguineus*. Canine ehrlichiosis is caused by three different *Ehrlichia* species namely *E. canis*, *E. chaffeensis and E. ewingii*. The disease is also known as canine typhus, canine hemorrhagic fever, tracker dog disease, idiopathic hemorrhagic syndrome and tropical canine pancytopenia (Ristic and Holland, 1993) [1]. The incidence of canine ehrlichiosis in India was reported for the first time by Mudaliar in a Spaniel dog of eight months old. *Ehrlichia canis* is naturally spread by the tick Rhipicephalus sanguineous both intrastadially and transstadially, but not transovarially (Sainz *et al.*, 2015) [2]. After an incubation period of 8-20 days, the course of *E. canis* infection, can be sequentially divided into acute, subclinical and chronic phases (Syaputra *et al*., 2020) [3].
Immunocompetent dogs may eliminate the infection during the acute or subclinical phases (Breitschwerdt *et al.*, 1998; Harrus *et al*., 1998) [4, 5], but some will eventually develop the chronic phase, characterized by bone marrow aplasia, peripheral blood pancytopenia and high mortality due to septicemia and/or severe bleeding (Mylonakis *et al.*, 2004) [6]. There are many clinical signs of the disease, but the most prevalent ones are depression, lethargy, weight loss, anorexia, pyrexia, lymph adenomegaly, splenomegaly, and bleeding tendencies. The most common and consistent hematological abnormality of dogs infected with *E. canis* naturally or experimentally is thought to be thrombocytopenia (Waner *et al.*, 1999) [7]. Mechanisms assumed to be involved in the pathogenesis of thrombocytopenia in the acute phase of the disease include increased platelet consumption due to inflammatory changes in blood vessel endothelium, increased splenic sequestration of platelets, and immunologic destruction or injury resulting in a significantly decreased platelet life span (Troy and Forrester, 1990) [8].
Ehrlichia spp. infect primarily leukocytes, forming intracytoplasmic, membrane-bound bacterial aggregates, called morulae (Sainz *et al.*, 2015) [2]. Demonstration of *Ehrlichia* spp. morulae in monocytes, macrophages and lymphocytes in Romanowsky type stained smears from buffy coat and less frequently lymph node, BM, spleen, liver and cerebrospinal fluid

smears is helpful in establishing a definitive diagnosis of acute CME (Harrus *et al.*, 2012; Harrus and Waner, 2011) [9, 10]. Though tetracycline and oxytetracycline have been considered to be the initial drugs of choice, doxycycline and minocycline were also used as drugs of choice (Chandran *et al.*, 2021) [11]. For up to five months following engorgement, the disease can be spread by ticks. The infection can also be transmitted through blood transfusions. The acute cases occur mostly in summer because of the greatest activity of the tick vector during that period (Sharma *et al*., 2010) [12].

Case History and Observations

A 45-day-old male husky dog weighing 2 kg is presented to the veterinary clinic, Vijayawada with the history of tick infestation, inappetence, fever, lethargy, anemia and melena. Clinical examination revealed elevated rectal temperature of 104.6^0 F, increased respiratory rate and pale mucous membranes (Figure 4). superficial lymph nodes especially the popliteal lymph node were found to be enlarged in size.

Materials and Methods

The blood was collected in an EDTA vial for studying the complete blood picture (CBP). Giemsa stain was used in the thin blood smear method to examine the blood sample (Bhatia *et al.*, 2010) [13]. The stained blood smear was screened for hemoprotozoa under the light microscope. Hematological analysis was carried out by using automatic hematological analyzer. Fecal sample is also examined for parasitic ova under the light microscope.

E. canis/Anaplasma/Babesia Ab Test Kit was used for diagnosis. One drop of whole blood is placed in each well followed by three drops of diluent in the same wells. The presence of two color bands ("T" and "C") within the result window, indicates a positive result. Based on history, clinical and laboratory findings it was diagnosed as a case of canine ehrlichiosis.

Results and Discussion

Hematological analysis revealed anemia with decreased levels of hemoglobin and red blood cell count, thrombocytopenia and lymphocytosis (Table 1). These results were in accordance with the findings of Waner *et al*., 1999 [7]; Meneses, 1997 [14]. Upon Giemsa staining and microscopic examination of blood smear, a light blue-stained morulae was observed in the lymphocytes (Figure 1) (Harrus and Waner, 2011) [10]. The *E. canis*/Anaplasma/ Babesia antibody rapid test showed positive results for *E. canis* antibody (Figure 2), so the dog was diagnosed with canine ehrlichiosis (Syaputra *et al.*, 2020) [3]. Fecal sample is negative for parasitic ova.

Dog was treated with doxycycline at a dose rate 10 mg/kg and clindamycin @ 11 mg/kg body weight intravenously with DNS slowly for 3 days, from the 4th day onwards both were given orally twice daily for 21 days. Similar results were observed by Shrestha & Karmacharya, 2016[15]; Qurollo *et al.*, 2013 [16]; Sainz *et al*., 2015 [2] and Neer *et al.*, 2002 [17]. Metronidazole 15 mg/kg body weight was administered intravenously for 3 days, and then it was given orally for 21 days, Imidocarb was given in two doses with an interval of 14 days at a dose rate of 6.6 mg/kg body weight subcutaneously (Chandran *et al.*, 2021) [11], Advaplat 5 ml orally 3 times a day, aRBC 1ml orally twice daily, Livoferol 2 ml orally twice a day. Blood transfusion (60 ml) was done on the first day of treatment (Figure 3). After 3 weeks of treatment, the dog showed marked improvement in clinical condition (Figure 5). Haematological values were found to be within a normal range and peripheral blood smear was negative for *E. canis* on the 21st day of post-treatment.

Table 1: Hematological findings of infected dog

Hematology	Infected dog	Reference range
Hemoglobin (g/dl)	2.9	12-18
Packed Cell Volume (%)	17	45-55
Total erythrocyte count (x 10^6/µl)	0.93	5.5-8.5
Total Leucocyte Count (x 10^3/µl)	27.8	6-17
Differential Leucocyte Count (%)		
Neutrophils (%)	51	60-77
Eosinophils (%)	4	2-10
Basophils (%)	0	00-01
Lymphocyte (%)	38	12-30
Monocytes (%)	7	3-10
Mean Corpuscular Volume (fl)	76	60-77
Mean Corpuscular Hemoglobin (pg)	31.1	19.5-24.5
Mean Corpuscular Hemoglobin Concentration (gm %)	40.7	32-36
Platelet count (cells/cumm)	1,21,000	150,000-350,000

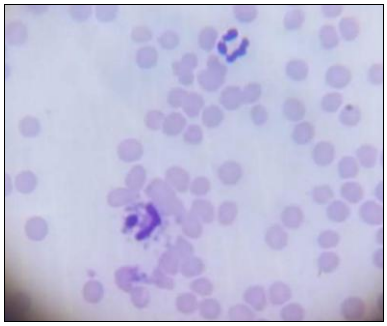

Fig 1: *Ehrlichia canis* morulae in a lymphocyte

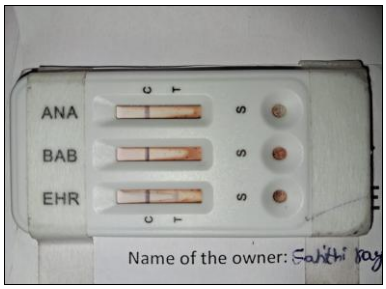

Fig 2: *E. canis*/Anaplasma/Babesia Ab Test Kit revealed positive *E. canis* Ab.

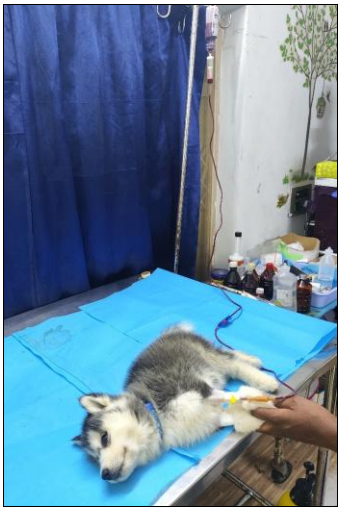

Fig 3: Blood transfusion in Dog

Fig 4: Pale mucous membranes before treatment

Fig 5: Mucous membranes after recovery

Conclusion

Canine ehrlichiosis is caused by gram-negative, obligate intracellular, pleomorphic bacteria of the genus *Ehrlichia*. Blood transfusion, use of Doxycycline, along with supportive drugs had a significant impact in recovery of the animal. Dogs that have recovered and cleared of the infection do not acquire permanent immunity and may become re-infected. Therefore, tick control with careful manual removal or by applying appropriate acaricides on a year-round basis, is the single most important measure for the prevention of *E. canis* infection**.**

Conflict of Interest

Not available

Financial Support

Not available

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