Original Research Article

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Molecular Diagnosis of *Babesia canis* Infection with Concurrent of Evans Syndrome in a Labrador Dog

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ABSTRACT

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Clinical case is five years old female Labrador dog presented to Small Animal Medicine unit of TVCC, VCRI, Orathanadu. History is dullness and anorexia two days. Recurrent tick infestations, anemic and thrombocytopenic crisis were regularly. Vaccination and deworming were regularly. Physical examination revealed dullness, pale mucosa, vital parameters were normal and ticks on interdigital space and in ears were noticed. Distended abdomen, hepatosplenomegaly and petechial hemorrhage on ventral abdomen were observed. Hematology revealed anemia and thrombocytopenia. Negative for blood born parasites in peripheral blood smear. Serum biochemical and urine analysis found to be normal. Serum and EDTA blood were submitted for molecular testing. Flow cytometry revealed Immune-mediated hemolytic anemia (IMHA) and Immune-mediated thrombocytopenia (IMTP). MAT negative for leptospira. *Babesia canis* positive by PCR method at 394bp. Haematology alteration was studied before and after therapy. Ultrasonography revealed hepatosplenomegaly. Based on PCR and flow cytometry the case was confirmed Evans syndrome with Concurrent *Babesia canis* in a Labrador dog. Dog treated with Inj. Diminazene aceturate @ 3.5mg/kg IM single dose, Inj. Ringers lactate @ 100 ml IV, Inj. Ascorbic acid @ 5 ml IV, Inj. Vitamin B complex @ 1ml IV, Inj. Prednisone @ 0.5mg/ kg IM, Tab. Doxycycline @ 10mg/kg PO and Syrup. Thrombup @ 15 ml BID PO. Dog showed clinical improvement as well as hematology parameters.

Introduction

Babesiosis is one among the deadly tick-borne hemaoprotozoan infection in the canines. Causative agents of canine babesiosis are *Babesia canis*, *B. gibsoni*, and *B. vogeli*, intracellular protozoan parasites of canine

erythrocytes (Breitschwert, 1984). *Babesia* infection causes autoimmune disorder like Immune-mediated haemolytic anaemia (IMHA) and immune mediated thrombocytopenia (ITP) in dogs and it may occur individually or concurrently. If they occur together they call it as Evans syndrome

(Norkus, 2012). Evans syndrome with Babesia in canines leads destruction of RBCs, platelets, oxidative stress and endothelial injury (Wingfield and Raffe, 2002; Di Cicco and Birkenheuer, 2012).

Case report

Five years old female Labrador was brought to the Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu with history of dullness and anorexia for the past two days. Recurrent tick infestations, anemic and thrombocytopenic crisis were reported 6 month back. Vaccination and deworming were regularly done. On general clinical examination revealed dull and depressed, Woebegone, pale mucus membrane and HR-102/minute, RR-45/minute and rectal temperature 38.9°C. On physical examination revealed distended abdomen, hepatosplenomegaly, Petechial hemorrhage on ventral abdomen, and ticks noticed on ear and interdigital space.

Samples collected: EDTA blood – CBC by Auto analyzer, Peripheral blood smear – Blood protozoal infection, Urinalysis, Ticks for identification. CBC revealed anemia and thrombocytopenia. No blood parasite could be detected through peripheral blood smear. No abnormalities could be detected in urinalysis. *Rhipicephalus sanguineus* tick was identification.

Since the animal was with intermittent tick infestation and anemia - Serum & EDTA

blood were collected and submitted for molecular testing to Zoonoses Research Lab and TRPVB Madhavaram, TANUVAS, Chennai for the diagnosis of Leptospirosis, Blood protozoal infection (*Babesiosis*, *Ehrlichiosis*, *Trypanosomiasis* and *Hepatozoonosis*) and autoimmune diseases (IMHA & IMTP) condition flow cytometry test for anti-platelets and anti-red blood cells antibodies were performed. Microscopic agglutination test (MAT) was negative for *Leptospira sp.* PCR was positive for *Babesia canis* at 394bp. IgM-anti-platelets and IgG-anti-red blood cells antibodies were detected and it confirms IMHA and IMTP (Figure 1 and Table 1). Based on the results of PCR and flow cytometry the case was confirmed as *Babesia canis* infection with concurrent of Evans syndrome in a Labrador dog.

Ultrasonographic examination of the abdomen revealed hepatosplenomegaly. Treatment initiated with Inj. Diminazene aceturate @ 3.5mg/kg IM single dose, Tab. Doxycycline @10mg/kg SID PO for 15 days, Tab. Prednisolone started @ 0.5mg/kg PO for 30 days, @ 0.25mg/kg PO for 15 days followed by alternate days as tapering. Supportive with syrup. Thrombup @ 15 ml BID PO for 30 days and syrup. Astymin @ 15ml BID PO for 30 days. Haematology alteration was studied before and after therapy. Animal showed clinical improvement like alertness, food intake and improvement in hematology values were also noticed (Table 2).

Table.1 Flow cytometry of *Babesia canis* dog with IMHA and IMTP

	IMHA	IMTP
Unstained	1.46	2.07
IgG	1023.94	10688.84
IgM	607.82	3022.33

Table.2 Changes of blood values in *Babesia canis* dog with IMHA and IMTP

Parameter	Day 1	Day 4	Day 30	Day 45
Hb (g/dl)	5.9	6.4	8.6	8.8
PCV (%)	17.63	19.70	25.16	26.76
RBC ($\times 10^6/\mu\text{L}$)	2.70	2.99	4.05	4.30
WBC ($\times 10^3/\mu\text{L}$)	6.75	6.56	8.99	7.68
PT($\times 10^4/\mu\text{L}$)	8.1	12.1	15.9	22.4

Figure.1 Flowcytometric evaluation of anti-platelets and anti-red blood cells antibodies

B. canis is a large intraerythrocyte piroplasm which causes hemolytic crisis and Immune-mediated hemolytic anemia (IMHA) occur secondary to the inappropriate production of antierythrocyte membrane antibodies (Archer and Mackin, 2013). Nalubamba *et al.*, (2015) recorded babesiosis in forty-five percent of the dogs were infested with ticks mainly of the genus *Rhipicephalus*. Thrombocytopenia and hematologic abnormalities were occurs in babesiosis as alone or together and mostly the bleeding disorder or coagulopathy are associated with DIC and immune-mediated platelet destruction (Tuttle *et al.*, 2003; Taboada and Lobetti, 2006).

The common clinical signs of babesiosis are anorexia, fever, depression, lethargy, pale mucosae, splenomegaly, poor body condition, and thrombocytopenia (Taboada and Lobetti, 2006). Sarma *et al.*, (2016) observed in his study hepato-splenomegaly in dogs naturally infected babesiosis by ultrasonographically.

PCR assay is the high specificity and sensitivity test to confirm the *Babesia spp* infection in dogs (Di Ciccio and Birkenheuer, 2012) by detecting low levels of parasitemia, recognizing subclinical infections and monitoring response to therapy (Boozer and Macintire, 2015). It correlated with the

present cases *B. canis* was positive in PCR assay and negative in peripheral blood smear.

Flow cytometry are considered to be more rapid, cost-effective, sensitive methods to determine erythrocytes-bound immunoglobulins (Quigley *et al.*, 2001) and Platelet bound immunoglobulins (Dircks *et al.*, 2009). Norton and Roberts (2006) reported Evans syndrome is a pathological condition, defined by the combination (either simultaneously or sequentially) of IMT and IMHA in the absence of known underlying etiology. Evans syndrome have been documented in dogs by Michimoto *et al.*, (2004), Goggs *et al.*, (2008) and Giannuzzi *et al.*, (2014).

Treatment given in the present case was correlated with (Ayoob *et al.*, 2010; Sarma *et al.*, 2014). Diminazene aceturate or Imidocarb dipropionate are very much effective for the elimination of *Babesia canis* from the host (Schoeman, 2009) and in the present case Diminazene aceturate @ 3.5 mg/kg IM showed better recovery. Prednisolone is a one of the potent immuno suppressive drug that reduce phagocytosis of RBCs, decrease production of cytokines and immunoglobulin IgG and which is commonly used in autoimmune disorders like IMHA and IMTP in dogs (Breton, 2015)

In conclusions, etiology based treatment along with adjuvant therapy is always helps in early and complete recovery. Molecular diagnostic tool like PCR is essential to rule out tick born infection in dogs those which are intermittent or persistent tick infestation and even though negative in the routine peripheral blood smear examination. Persistent subclinical tick born infection may prone for the development of Immune mediated diseases like IMHA and IMTP. Flow cytometry helped in the diagnosis of IMHA and IMTP in dogs.

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