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Concurrent infection of *Babesia canis* and *Ehrlichia canis* in a Labrador Retriever Dog

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ABSTRACT: Vector-borne diseases (VBD) of canines persuade a hidden threat all over the globe and its prevalence is high in India due to the favorable hot and humid climatic conditions. Out of prevailing VBD, canine ehrlichiosis, babesiosis and hepatozoonosis are commonly reported, followed by anaplasmosis and trypanosomosis. In the present case report, an atypical case of co-infection with *Ehrlichia canis* and *Babesia canis* was observed in a two and half years old Labrador Retriever, female dog with the symptoms of anorexia, pale mucous membrane, transient fever, enlarged popliteal lymph node, distended abdomen, exercise intolerance, debility and severe panting, which was not responded to the earlier treatment of four doses of cefotaxime. Peripheral blood cytology revealed a mixed infection of *B. canis* and *E. canis*. Further, the pet was treated with deep intramuscular injection of a single dose of diminazene aceturate at 3.5mg/kg body weight followed by oral doxycycline at 10mg/kg for initial 10 days, later at 5 mg/kg was followed up to 20 days. One dose of Inj. Levamisole at 2mg/kg was administered subcutaneously on 10th-day post-therapy to boost the cell-mediated immunity. The recovery was good with babesiocidal, anti rickettsial drugs and supportive therapy. Re-examination of blood on 3, 14 and 30th days post-therapy revealed no parasitemia.

Keywords: *Babesia canis*, *Ehrlichia canis,* Anaemia, Doxycycline, Diminazine, Levamisole.

INTRODUCTION

Piroplasms are globally distributed; obligate intracellular hemotropic parasites of vertebrates from the genera *Babesia*, *Theileria* and *Cytauxozoon* (Allsopp and Allsopp 2006; Alvarado-Rybak *et al.*, 2016). The name piroplasm comes from the fact that, within erythrocytes, the parasites often appear as pear-shaped on Romanowsky stained cytological slides on microscopic examination (Uilenberg, 2006). Microscopic examination has played a central role in the early taxonomic classification of parasites in the genus *Babesia*, with these piroplasms, which primarily divided into 'large' and 'small' species based on the shape and size of their intra-erythrocytic stages, the *B. canis* and *B. gibsoni* were classified as large and small form respectively (Solano-Gallego and Baneth, 2011; Solano-Gallego *et al.*, 2016; Boozer and Macintire 2003).

Under the family Anaplasmataceae, *E. canis* is a gram-negative, obligatory intracellular bacterium, earlier classified under rickettsial organism (Unver *et al.*, 2006). It is highlyprevalent in tropical and subtropical areas, including India (Singla *et al.*, 2011). Dog, red fox, coyote and golden jackal are the essential reservoir of infection in nature (Neer 1998). Its distribution depends upon the epidemiology of the principal vector, *Rhipicephalus sanguineus* (brown dog tick), which transtadially transmits the disease to its canine host (Johnson *et al.*, 1998).

The life cycle consists of three intracellular phases: elementary body, initial bodies, and morulae (aggregate of initial bodies). The disease is characterized by three stages: acute, subclinical and chronic. Following an incubation period of 1-3 weeks, infected dogs may remain subclinical or present with nonspecific signs including fever, lethargy, lymphadenopathy, splenomegaly, lameness, oedema, bleeding disorders and mucopurulent ocular discharge. Bleeding disorders can include epistaxis, petechiae, ecchymoses, gingival bleeding and melena.

In India, *E. canis* infection has been reported from different parts by varied prevalence rates (Harikrishnan *et al.* 2009; Dhankar *et al.*, 2011; Lakshmanan *et al.*, 2011; Singla *et al.*, 2011; Das and Konar 2013; Bhadesiya and Raval 2015). Chemotherapeutic agents ranging from sulphonamides to imidocarb dipropionate

have been used with a variable success rate. However, a 28-day regimen of doxycycline was found to be most acceptable globally despite reports of persistent carrier status of dogs (Iqbal and Rikihisa 1994). Following an acute phase (2-4 weeks), clinical signs may resolve without treatment, and the dog could remain subclinically infected indefinitely or naturally clear the pathogen.

Some dogs, however, will go on to develop chronic canine monocytic ehrlichiosis (CME). It is still not clear why some dogs progress to the chronic phase, but possible reasons include co-infections, *E. canis* strain virulence, or the dog's immune status. Some reports suggest a defective cell-mediated immune response may have a significant role in determining the course of disease (Nyindo *et al*., 1980). Dogs with chronic CME typically show pancytopenia, anaemia, thrombocytopenia and neutropenia due to *E. canis* induced suppression of hematopoietic stem cells (Mylonakis *et al*., 2011). Dogs with acute CME can transiently develop milder pancytopenia. Bone marrow cytology or histology in dogs with chronic CME (myelosuppression) will have a marked reduction in hematopoietic tissue (Mylonakis *et al*., 2011).

In endemic areas, infection with multiple tick-borne pathogens is possible in individual animals, especially secondary to a heavy tick infestation (Shaw *et al*., 2001). A single tick species can act as a vector for multiple pathogens, and simultaneous infection with different organisms is possible (Schouls *et al*., 1999; Shaw *et al*., 2001). In the present case report, a critical canine piroplasm, *Babesia canis*, was reported along with a gram-negative bacterium, *Ehrlichia canis*. Many vector-borne blood parasites illustrate similar symptoms of rising temperature, anaemia, enlargement of peripheral lymph nodes etc. Severe thrombocytopenia is a common finding in canine babesiosis (Kettner *et al*., 2003; Scheepers *et al*., 2011), and similarly, thrombocytopenia and leukopenia are the common haematological abnormalities in both the acute and chronic phase of canine ehrlichiosis (Kelly 2000; Shipov *et al*., 2008).

The majority of veterinary practitioners in India have reported diagnosing canine ehrlichiosis by leukopenia, while half of the clinicians based their diagnosis on the presence of thrombocytopenia (Collett, 2000). The present case report is comprehensive about the clinical symptoms, Haemogram and therapeutic management of a Labrador Retriever dog infected with concurrent infection of both canine babesiosis and ehrlichiosis.

Case history: An adult female, two and half years Labrador Retriever dog was presented at a private veterinary clinic, Vijayawada, Krishna district, Andhra Pradesh, with a clinical history of severe tick infestation, anorexia, high fever (105.6°F), severe panting, not responded to the cefotaxime therapy of earlier four days. Significant weakness and lethargy were noticed on the day of admission. Vaccination and deworming history were regular, and nothing was due.

MATERIAL AND METHODS

A drop of peripheral blood was collected from the ear vein, and a wet blood film (WBF) examination was carried out to detect microfilaria and *Trypanosoma* sp. To detect intracellular parasites, a thin blood smear was made in a clean glass slide, air-dried, and the smear was stained with Leishman's stain (Coles 1986), later examined under oil immersion objective (x100). Complete blood profile viz. hemoglobulin (Hb), Packed cell volume (PCV), Total erythrocyte Count (TEC), Total leukocyte count (TLC) and Differential leukocyte count (DLC) was performed manually as per the standard protocol (Beverly and Katherine, 2003). Platelet count was carried out using blood analyzer.

RESULT

On wet film examination, both trypanosome and microfilaria were found negative; the faecal examination also did not reveal any ova of parasitic importance. Leishman's stained blood Smear of the dog revealed mixed infection of pear-shaped proplasm of *Babesia canis* infected RBC and inclusions of developing morulae of *Ehrlichia canis* in monocyte. The large paired pear-shaped piroplasm (merozoites) of *B. canis* was ranged between 3.37–4.12 × 1.6–1.93 µm length and width. Few red blood cells were infected with single, large, pear-shaped merozoites with the size of 4.82 × 1.6 µm (Fig. 1).

On day 0, Haemogram revealed severe anaemia with reduction of all erythrogram values viz. haemoglobin (6.2g/dl), packed cell volume (23%) and total erythrocyte count (3.51×10^6/uL). Relative leukopenia (4850/ µl), thrombocytopenia (0.73 lakhs/µl) and monocytosis (11%) were also observed (Table 1). Following the single dose of deep intramuscular injection of diminazene aceturate at 3.5mg/kg body weight, a drastic improvement was noticed. Six hours of post diminazene therapy, doxycycline was infused intravenously at 10mg/kg, followed by oral doxycycline at 10mg/kg for subsequent 7 days, later at 5 mg/kg up to 20 days. Although with the supplementation of oral hematinic (dexorange, 5 ml oil) and probiotic: *Saccharomyces boulardii* (Econorm sachet, 100mg oid), the animal was found with marginal anaemia until 14^{th} day of post-therapy and hence one dose of Inj. Levamisole (2mg/kg) was administered to enhance cell-mediated immunity. Finally, the erythrogram values got stabilized steadily on the 30^{th}-day post-therapy. The state of leucopenia and thrombocytopenia was also reached the normal range within 72 hours of medication.

Two erythrocytes (*black arrow*) containing paired pear-shaped merozoites measuring 3.37–4.12 µm in length and 1.6–1.93 µm in width; one erythrocyte (*white arrowhead*) containing one pear-shaped merozoite measuring 4.82 × 1.6µm.

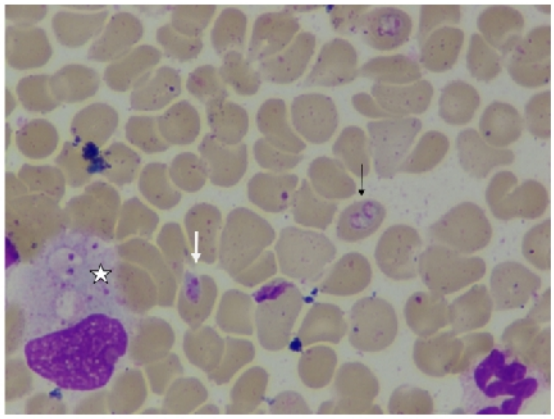

Fig. 1. Leishman's Stained Blood Smear of Dog (100x) showed mixed infection with pear-shaped proplasm of *Babesia canis* infected RBC (Arrow) and morulae of *Ehrlichia canis* infected monocyte (Star).

Two erythrocytes (*black arrow*) contain paired pear-shaped merozoites measuring 3.37–4.12 µm in length and 1.6–1.93 µm in width; one erythrocyte (*white arrowhead*) containing one pear-shaped merozoite measuring 4.82 × 1.6µm.

Table 1: Hemogram values of *B. canis* and *E. canis* infected Labrador Retriever.

Complete Blood Parameters	Day 0 Before treatment	Day 3 Post-treatment (PT)	Day 14- PT	Day 30- PT
Hb(g/dl)	6.2	8.8	11.6	14.2
PCV (%)	23.0	25.0	36.0	44.0
TEC (10^6/µL)	3.51	4.83	6.04	7.31
TLC (10^3/µl)	4850	7050	10150	12950
DLC: Neutrophils (%)	74	71	75	71
Lymphocytes (%)	09	17	21	27
Monocytes (%)	11	08	03	02
Eosinophils (%)	04	04	01	0
Basophil (%)	02	0	0	0
Platelet count (lakhs/µl)*	0.73	0.92	1.34	1.92

Hb, PCV, TLC, DLC were performed manually; Platelet count (lakhs/µl) was performed by an auto-analyzer

DISCUSSION

Canine babesiosis and ehrlichiosis are the important tick-borne infections prevailing all over the globe, resulting in severe clinical diseases (Rautenbach 1991; Collett, 2000). Published data on the prevalence of co-infection with multiple tick-borne pathogens in canines of Andhra Pradesh is minimal (Reddy *et al*., 2016). Our study exhibited in detail the mixed infection of canine babesiosis and ehrlichiosis in a Labrador Retriever dog. The morphological characteristics of *B. canis* and *E. canis* were observed in this study agreeing with Saravanan *et al.* (2014); Waner *et al*. (1999), respectively. The severe reduction of erythrogram values was also correlated with the study of Sainz *et al*. (2015), who observed the severe reduction of haemoglobin in canines infected with ehrlichiosis and babesiosis.

In the present case report, anaemia mediated hypoxia and probable tissue damage could have led the animal to elicit symptoms of high temperature, debility, enlarged popliteal lymph node and severe panting. A similar type of clinical symptoms was noticed by Zvorc *et al.* (2010), and he stated that the anaemia results in hypoxia and tissue damage were the cause to release the inflammatory mediators resulting in vascular endothelial damage. The two primary syndromes produced by Babesia, including hemolytic anaemia and multiple organ dysfunction syndromes (MODS), are the consequences of systemic inflammatory response syndrome (Jadhav *et al.*, 2011). Though we did not

encounter the typical haemolytic anaemia in the presented Labrador Retriever, Rafael (2007) affirmed that haemolytic anaemia is the hallmark sign and can be intravascular, extravascular, or both. *Babesia* initiates a mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes, and anaemia may be more dependent on the host immune response than on the direct destruction of RBC by the piroplasm (Boozer and Macintire, 2003). Anaemia and thrombocytopenia, the mostcommon hematologic alterations of *B. canis* and *E. canis* infection associated in this study, got concordance with the report of Schetters *et al*. (2009). Although the treatment could remove both the blood parasites within 72 hours of medication, reduction in haemoglobin and anaemia was obtained only on the 14th day of post-therapy.

As a routine, the babesiosis was treated with a single intramuscular injection of diminazene aceturate at the dose of 3.5 mg/kg was partial consonant with the report of Birkenheuer *et al*. (1999), where they utilized 5mg/kg. Similarly, the concurrent infection of *E. canis* and *B. canis* was treated successfully with a combination of diminazene aceturate, parenteral tetracycline and oral doxycycline drugs supported by haematinics, agreed with that of Miller *et al*. (2005); Greene *et al*. (2012); Sainz *et al*. (2015). Even though we administered levamisole at 2mg/kg body weight subcutaneously on the 14th-day post-treatment to induce cell-mediated immunity (CMI), we did not estimate the role of various cytokines involved in promoting the CMI. Chethan *et al*. (2019) used levamisole as an immunomodulator to stimulate certain antiviral immune markers. They measured the concentrations of interferon-γ (IFN-γ), nitric oxide (NOx), and total immunoglobulin G (IgG), found that the levamisole prevented the gut injury, faecal consistency and dehydration scores in rotavirus type A (RVA) infected piglet diarrhoea.

CONCLUSION

The gold standard traditional microscopic examination of peripheral blood smears in correlation with hemogram is consistently an effective method for diagnosing and treating vector-borne blood parasites at the field level. However, the technique is time-consuming and less sensitive in detecting carriers. Mixed infection of different haemoparasites in dogs are common in India, which warrants awareness among field veterinarians, and this could be due to repeated exposure of the infected dogs to ticks infected with different parasites. Further, in the current study, the recovery was good with the routinely used babesicidal (diminazene) and anti rickettsial drug (Doxycycline) along with immune-modulatory (Levamisole) and other supportive therapy. In this conventional study, we did not pursue the detection of genomic DNA of *Ehrlichia* and *Babesia* for the species level confirmation.

FUTURE SCOPE

Updating the basic skills in microscopy and hemogram would be an asset for a veterinary physician to effectively diagnose blood parasites of livestock and pet animals at the field level.

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Conflict of interest. Nil.

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