Concurrent infection of *Hepatozoon canis* and *Babesia gibsoni* in a non-descript dog and its therapeutic management

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Abstract

A two year old male non-descript dog with clinical signs of anorexia, debility, pyrexia, icteric mucous membrane, lymphadenopathy and hepatomegaly was presented at District Veterinary Centre, Palakkad, Kerala. Blood smear examination revealed animal positive for *Hepatozoon canis* and *Babesia gibsoni*. Hemato-biochemical findings were anemia, thrombocytopenia, neutrophilic leukocytosis, hypoalbuminemia, hyperglobulinemia, hyperbilirubinemia and elevated levels of alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine and alkaline phosphatase. The animal was treated with Diminazine aceturate (3.5 mg/kg body weight, IM) on first day of presentation, Imidocarb dipropionate (6.6 mg/kg body weight, SC) on second day of presentation, and lymphadenopathy by impairing host immune response. The animal had an uneventful recovery.

Keywords: *Hepatozoon canis*, *Babesia gibsoni*, doxycycline, hematinsics

Introduction

The genus Hepatozoon belongs to phylum Apicomplexa that infect birds, reptiles, mammals and amphibians of all continents with tropical and subtropical climate [1]. In dogs, *Hepatozoon canis* and *Hepatozoon americanum* are the two different species of protozoa that can cause hepatoplenal malformation. The distribution of *Hepatozoon canis* has been reported in Asian countries and *Rhipicephalus sanguineus* tick species is responsible for its transmission, while *Hepatozoon americanum* is restricted to United States and it is transmitted by tick species *Amblyomma maculatum* [2]. Dogs become infected with *Hepatozoon canis* by ingesting adult *Rhipicephalus sanguineus* with sporozoite within the hemocoel [3]. Concurrent infection of *Hepatozoon canis* with other pathogens like *Babesia* spp, *Anaplasma* spp, *Ehrlichia canis* and *Mycoplasma hemocanis* are possible as single tick may harbor multiple pathogens [4-7]. Infection with this pathogen usually manifested as mild or subclinical. High parasitic load as well as concurrent infection with other pathogens leads to lethargy, anemia, fever, weight loss and lymphadenopathy by impairing host immune response [7, 8]. First scientific report on hepatoplenal malformation in India was made by Bentley, who published “a preliminary note upon a leucocytozoan of the dog” in 1905 [1]. Increasing prevalence of natural infection with *Babesia gibsoni* was detected among canine population of Kerala [9]. However, few scientific reports of hepatoplenal malformation in dogs are available in Kerala either detected by conventional methods like microscopic examination of blood or buffy coat smear or molecular diagnostic techniques such as PCR [4, 10-12]. The present paper discuss about microscopic detection of *Hepatozoon canis* concurrent with *Babesia gibsoni* in a non-descript dog and its therapeutic management.

Materials and Methods

The present case was brought in the outpatient ward of District Veterinary Centre, Palakkad, Kerala with clinical signs like anorexia, debility, pyrexia (105°F), icteric conjunctival and buccal mucous membrane (Fig. 1&2), enlarged popliteal lymph nodes, hepatomegaly and history of tick infestation.
Thin blood smear was prepared from ear tip, fixed in absolute methanol for 3 minutes and stained with Geimsa stain 1 in 10 dilution for 45 minutes for light microscopic examination. Around 2ml of blood was collected in EDTA vial for hematology analysis. Hemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count and platelet count were estimated. Serum sample collected from animal was processed for total protein, serum albumin, serum globulin, total bilirubin, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Abdominal ultrasonography was also performed. Serum sample was also subjected to rapid immunochromatography assay for detection of Leptospira specific IgM antibody. Blood smear was examined after one month of therapy.

Results and Discussion

Blood smear examination revealed characteristic gelatin capsule shaped gamonts of *Hepatozoon canis* within the cytoplasm of neutrophils and piroplasms of *Babesia gibsoni* (Fig.3&4). Approximately 50% of neutrophils were parasitized with gamonts of *Hepatozoon canis*. Neutrophil left shift was another key finding of blood picture examination. Animal was negative for rapid immunochromatography assay for detection of Leptospira specific IgM antibody. On abdominal ultrasonography, enlargement of hepatic lobes beyond costal arches was noticed. Anemia, neutrophilic leucocytosis, thrombocytopenia, hypoalbuminemia, hyperglobulinemia, hyperbilirubinemia, elevated levels of AST, ALT, ALP, BUN and creatinine are the major hematobiochemical alterations observed in this case and depicted in Table 1&2. Based on the above finding, the case was diagnosed as concurrent infection with *Hepatozoon canis* and *Babesia gibsoni*.

The animal was treated with Diminazine aceturate at 3.5 mg/kg body weight, IM, on the first day of presentation. Imidocarb dipropionate at 6.6 mg/kg body weight, SC, on second day of presentation and repeated at 14 day interval. Doxycycline at 5 mg/kg body weight, PO, BID for 21 days along with Ranitidine at 0.5 mg/kg body weight. Apart from this oral hematins and hepatoprotectants were given for 30 days.

### Table 1: Hematological parameters

| Parameter      | Value  | Reference range |
|----------------|--------|-----------------|
| Hb (g/dl)      | 8.6    | 10-16           |
| PCV (%)        | 26     | 30-50           |
| (TEC) Millions/mm$^3$ | 2.8 | 5-8 |
| (TLC) cells/mm$^3$ | 46800 | 6000-16000 |
| Neutrophils (%) | 94     | 60-70           |
| Lymphocytes (%) | 5      | 15-30           |
| Monocytes (%)  | 1      | 3-8             |
| Platelets (lakhs/mm$^3$) | 1.22 | 2-8.5 |

### Table 2: Serum biochemistry parameters

| Parameter      | Value  | Reference range |
|----------------|--------|-----------------|
| AST (U/L)      | 92     | 10-62           |
| ALT (U/L)      | 128.68 | 25-92           |
| ALP (U/L)      | 223.02 | 10-94           |
| Total Protein (g/dl) | 5.84 | 5.4-7.5 |
| Albumin (g/dl) | 1.03   | 2.5-4.0        |
| Globulin (g/dl) | 4.81  | 2.5-4.4        |
| Total bilirubin (mg/dl) | 16.43 | 0-0.6 |
| BUN (mg/dl)    | 41.95  | 8-28            |
| Creatinine (mg/dl) | 2.18 | 0.5-1.7       |
Anemia observed in this case could be due to direct mechanical damage caused by Babesia gibsoni on infected RBCs as well as antibody mediated cytotoxic destruction of circulating RBCs. Thrombocytopenia may be associated with an immune mediated phenomenon involving the binding of IgG to the platelet surface following by its removal or release of inflammatory mediators during RBC lysis in case of babesiosis or myelosuppression in hepatozoonosis or a combination of all these factors. Neutrophilic leucocytosis and neutrophil left shift in the present case may be due to inflammatory response to tissue invasion (liver, lung, kidney) and merogony by Hepatozoon canis, which can be exacerbated by secondary bacterial infection and co-infection with Babesia gibsoni. Increased serum AST, ALT, BUN and creatinine levels in this case may be due to merogony of Hepatozoon canis in vital organs like liver and kidney and associated hepatitis and glomerulonephritis. Elevated levels of serum ALP have an association with abnormal function of biliary system. Hypoalbuminemia may be due to severe liver damage caused by both protozoa since the site of albumin synthesis is liver. Hyperbilirubinemia in this case was resulted from both intravascular and extravascular hemolysis due to Babesia gibsoni. Combination therapy with imidocarb dipropionate and doxycycline was found successful for treatment of Hepatozoon canis according to many researchers. In the present case animal was successfully treated with combination therapy including Diminazine aceturate, Imidocarb dipropionate and Doxycycline. After initiation of treatment, animal started feed intake slowly and the temperature had dropped to normal. Blood smear examination after one month of therapy showed negative result for Hepatozoon canis and Babesia gibsoni and the animal recovered.

Conclusion

The hot humid tropical climate of Kerala favours survival of Rhipicephalus sanguineus making high prevalence of canine tick born diseases in the region. But incidence of canine hepatozoonosis is rarely reported. This may be either due to the nature of parasitemia or due to adoption of diagnostic methods. Diagnosis by blood smear examination may not be sensitive. PCR is the best diagnostic assay especially when the infections are subclinical with low parasitemia rates.

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