Case Report

Peritoneal Effusion in a Dog due to *Babesia gibsoni* Infection

Suresh Gonde,¹ Sushma Chhabra,^{1,2} L. D. Singla,¹ and B. K. Bansal¹

¹College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141004, India

²Department of Veterinary Medicine, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141004, India

Correspondence should be addressed to Sushma Chhabra; chhabrasushma@rediffmail.com

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A five-year-old male Labrador was presented to Teaching Veterinary Clinics of GADVASU with a primary complaint of distended abdomen, fever, and anorexia. The dog was found to be dull with elevated rectal temperature (104°F), heart rate (148 per minute), and respiration rate (58 per minute). Blood smear examination and PCR assay revealed that dog was positive for *Babesia gibsoni*. Elevated bilirubin, alanine amino transferase (ALT), alkaline phosphatase (ALP), creatinine, blood urea nitrogen (BUN), total leucocyte count, hypoalbuminaemia, and hypoproteinaemia were haematobiochemical alterations. Radiography and ultrasonography showed ground glass appearance and anechoic area of abdomen, respectively.

1. Introduction

Canine babesiosis is one of the most important life-threatening tick borne haemoprotozoan diseases of dogs caused by intraerythrocytic protozoan parasites of the genus *Babesia* which are reported worldwide and in various parts of India including Punjab state [1, 2]. The variable prevalence of both *B. canis* and *B. gibsoni* has been observed in India (0.66 to 21.7%) and in Ludhiana the prevalence was recorded as 5.26 per cent [3]. The pathogenesis of canine babesiosis varies in different regions [4], possibly due to variation in the pathogenicity of different strains of *Babesia* species in various ecological conditions [5]. The severity of babesiosis is related to the extent of parasite replication in the host's red blood cells with subsequent cell lysis. A wide variety of clinical signs like anorexia, lethargy, icterus, vomiting, and marked loss of body condition have been observed [6, 7] along with variable clinicopathologic abnormalities including haemoglobinuria, hypoglycemia, acid-base disturbances, azotemia, and elevations in the levels of liver enzymes [8]. Further, *B. gibsoni* causes regenerative hemolytic anemia and thrombocytopenia [9]. Spherocytosis and positive direct Coombs' test results suggest an immunomediante component. Thrombocytopenia is a frequent finding. In the present communication we describe the clinico-haematobiochemical, radiographic,

and ultrasonographic observations in a very rare case of peritoneal effusion due to the pathogenic effect of *Babesia gibsoni* infection.

2. Case Description

A five-year-old male Labrador dog was presented to the Veterinary Teaching Hospital of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, July, 2013, with abdominal distension and persistent anorexia for the last two days. The dog had shown poor body condition. During the physical examination, the dog was pyretic (104°F), with heart rate of 148 beats per minute and a respiration rate of 58 breaths per minute. Mucous membranes were pale. Ticks were removed from the dog and were identified as *Rhipicephalus sanguineus*.

Blood samples were submitted for hematologic and serum biochemical analysis.

2.1. Hematologic and Biochemical Analysis. Hematologic and biochemical analysis revealed moderate to severe anaemia with elevated bilirubin, alanine amino transferase (ALT), alkaline phosphatase (ALP), creatinine, blood urea nitrogen (BUN), total leucocyte count, hypoalbuminaemia, and hypoproteinaemia were haematobiochemical alterations (Table 1).

TABLE 1: Haematobiochemical parameters in the dog infected with *Babesia gibsoni*.

Parameters	Reference range*	Infected dog
Hb (g/dL)	12–18	6
TLC ($10^3/\mu\text{L}$)	6–17	16
TEC ($10^6/\mu\text{L}$)	5.5–8.5	3.1
PCV (%)	35–55	18.4
MCV (fL)	60–70	59.4
MCH (pg)	19.5–24.5	19.4
MCHC (g/dL)	30–36	32.6
Platelet ($10^5/\mu\text{L}$)	2–9	1.58
Neutrophils (%)	60–70	72
Lymphocytes (%)	12–30	26
Eosinophils (%)	2–10	02
Albumin (g/dL)	2.6–4	1
Total protein (g/dL)	5.5–7.5	3.2
Total bilirubin (mg/dL)	0.1–0.6	1.8
ALT (U/L)	8.2–57	313
ALKP (U/L)	10.6–101	563
BUN (mg/dL)	8.8–26	30
Creatinine (mg/dL)	0.5–1.6	1.2
Blood glucose (mg/dL)	62–108	104

* Kahn et al. [15].

Chronic hepatic insufficiency in case of babesiosis could lead to hypoalbuminaemia [10]. Haematological parameters suggested that the dog might have blood parasites or other relevant infectious diseases. Pathogenesis of anemia appears to involve nonhemolytic and hemolytic mechanisms. Hemolysis may involve proteases produced by the invading parasite, an immune reaction to parasitized cells, and/or oxidative damage to erythrocytes. MCV, MCH, and MCHC values were just at borderline of the normal range (Table 1) indicating that anemia was not due to iron deficiency but MCV and MCHC frequently fail to correctly differentiate the various patterns of anemia [9]. The most common abnormality was thrombocytopenia. The reason for thrombocytopenia in babesiosis could be due to platelet sequestration in the spleen or immune mediated platelet destruction and development of disseminated intravascular coagulation [11].

2.2. Parasitological Examination. So to distinguish between these possibilities, the microscopic examination of Giemsa-stained thin blood smears prepared from the ear margin was carried out [12] under oil immersion (100x) lens. Examination revealed the presence of ring shaped, oval, parachute, and comma-like organisms, about 1–3 μm in diameter in the erythrocytes (Figure 1). On the basis of the size of the intracellular parasites in this case, the possibility that the dog has been infected with small *Babesia* spp., especially with *B. gibsoni*, was considered. The degree of parasitemia, calculated as the percentage of infected red blood cells by counting 1000 red blood cells, was 10.5%. Ticks present on

FIGURE 1: Photomicrograph of Giemsa-stained thin blood smear of the dog showing regenerative anemia and small circular shaped trophozoites of *Babesia gibsoni* in erythrocytes $\times 100$.

the dog were identified as *Rhipicephalus sanguineus*. The fact that *B. gibsoni* cannot be distinguished from other canine small babesial isolates by microscopy prompted us to perform PCR for final diagnosis using *B. gibsoni* specific primer [13].

2.3. DNA Extraction. DNA from whole blood (300 μL) was extracted using the DNA purification kit (QIAGEN, GmbH, Germany) according to the manufacturer's instructions.

A primer set including forward primer (Gib599 F): 5'CTCGGCTACTTGCCTTGTC3' and reverse primer (Gib1270R): 5'GCCGAAACTGAAATAACGGC3' was used to amplify a 671 bp fragment of the 18S rRNA gene region specific to *B. gibsoni* [13]. The PCR mix consisted of 1X molar concentration of 12.5 μL master mix (1X QIAGEN PCR buffer, 2.5 units of Taq DNA polymerase, 200 μM of each dNTP, and 1.5 mM MgCl_2), 1.5 μL of 10 pmol each of the respective primers, and 5 μL of template as DNA source.

After an initial denaturation at 95°C for 5 min, 30 cycles of denaturation (94°C for 45 sec), annealing (57°C for 1 min), and extension (72°C for 1 min) were conducted and the final extension was performed at 72°C for 8 min. A negative sample control (canine blood DNA only) and a negative DNA control (Milli-Q water in a substitute of DNA) were included in the PCR reaction. The PCR products were run on 1.5% agarose gel and stained with ethidium bromide. The size of the amplified PCR product was 671 bp (Figure 2).

2.4. Peritoneal Fluid Analysis. Based on abdominal distension ascites was suspected. A peritoneal tap was performed and 2 mL of clear peritoneal fluid was collected. Peritoneal fluid typically was examined for color, turbidity, total protein, and albumin concentration. The fluid was transparent and clear. Total protein and albumin were 0.4 g/dL and 0.2 g/dL, respectively, indicating hypoproteinaemia (0.4 g/dL) and hypoalbuminaemia (0.2 g/dL).

2.5. Radiography and Ultrasonography. Radiography and ultrasonography showed ground glass appearance and anechoic area of the abdomen, respectively (Figures 3 and 4). The

FIGURE 2: *Babesia gibsoni* species specific PCR assay. Lane M: GeneRuler 100 bp Ladder; lane 1: positive sample; lane 2: negative sample control; lane 3: negative DNA control.

FIGURE 3: USG of abdomen showing anechoic area indicating free flowing fluid in abdomen.

case was thus confirmed as being ascites. Though most common clinical signs of babesiosis include anorexia, lethargy, recurrent fever, pale mucus membrane, and emesis [14], our findings revealed that it is important not to neglect babesiosis in differential diagnosis of ascites. Dogs with ascites should be subjected to classical parasitological or molecular diagnosis to rule out babesiosis. *Babesia* organisms infect the erythrocytes of dogs, leading to hemolytic anemia. Infection with *B. gibsoni* is known to cause more severe clinical signs than infection with large *Babesia* spp. and may result in multiple organ dysfunction syndromes [13]. As the dog was severely anaemic with other severe abnormalities in parameters related to vital organs it died within two days of treatment.

3. Conclusion

To the best of our knowledge, this is the first case report of peritoneal effusion in a dog associated with *B. gibsoni* infection diagnosed by microscopic examination and polymerase

FIGURE 4: X-ray of abdomen showing ground glass appearance indicating free fluid in abdominal cavity.

chain reaction (PCR) in combination with peritoneal fluid analysis, radiography, and ultrasonography.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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