

## *Babesia canis* caused clinical babesiosis in a female Shih Tzu dog

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### Abstract

A 2-year-old female Shih Tzu dog was submitted with the history of anorexia and depression for one week and no prior surgery. Fever and pale mucosa were noticed in physical examination. Microscopic examination of the Giemsa-stained blood smear disclosed large form of *Babesia* and single to four pear-shaped merozoites within erythrocytes (RBCs). Regenerative anemia characterized by a marked reticulocytosis, significant intra-vascular hemolysis, nucleated RBCs, left-shifted neutrophils, thrombocytopenia, azotemia, high serum creatinine and urea concentrations were recorded following hemato-biochemical analysis. Abundant bilirubin crystals and abnormal reddish color after centrifugation were observed in urinalysis. Molecular analysis was performed using specific primers for detection of *Babesia canis*. Diminazene aceturate, ciprofloxacin, ivermectin and phosphorus-vitamin B12 were prescribed and the clinical signs improved after four days. Two months follow-up showed no recurrence. Such studies would significantly contribute to the development of appropriate preventive strategies and successful treatment. This communication reports a clinical case of canine babesiosis caused by *B. canis* in a female Shih Tzu dog.

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### Introduction

Canine babesiosis is a common and clinically significant tick-borne disease caused by obligate hematozoan parasites of the genus *Babesia*. Lethargy, anorexia, fever, jaundice, hemolytic anemia, hemoglobinuria/bilirubinuria, weight loss and sometimes death are the most common encountered clinical signs of the acute infection.<sup>1</sup> According to the morphological characteristics, the causative agents of canine babesiosis can routinely be divided into two distinct classes including the large *Babesia* measuring 3.00 - 5.00 µm known as *Babesia canis* which mainly appears to be pyriform, and the smaller counterpart with the size of 1.00 - 3.00 µm, known as *B. gibsoni* which mainly appears to be signet ring form.<sup>2,3</sup> Diagnosis of canine babesiosis can be achieved by microscopic examination of Giemsa-stained blood smears, serological tests and molecular methods such as polymerase chain reaction (PCR). The main drawbacks for

serological assays and microscopic detection of *Babesia* spp. are cross-reactions, low sensitivity and difficulty of differentiating between the involved species. The PCR presents a higher sensitivity and specificity than traditional diagnostic methods to detect babesial infection at the subspecies level differentiation.<sup>3,4</sup> The present case report describes a new case of the infection by *B. canis* in a dog suffering from hemolytic anemia.

### Case Description

In September 2020, a 2-year-old female Shih Tzu dog weighing 12.00 kg from Tehran, located in the north of the central plateau of Iran, was admitted by a private veterinary hospital. The main complaint was anorexia and depression for one week without history of prior surgery. Pale mucous membrane, mild dehydration, 40.00 °C body temperature, 135 beat/min heart rate and 23 breaths per min respiratory rate were recorded in

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physical examination. The blood samples were collected into plain and EDTA-containing tubes (Kendall Co., Mansfield, USA) for hemato-biochemical and molecular examinations. The Giemsa-stained blood smears were prepared for detection of pathogens under a light microscope (SZ61; Olympus Corp., Tokyo, Japan). Then, based on the obtained results from the microscopic examination, molecular assays were performed as below.

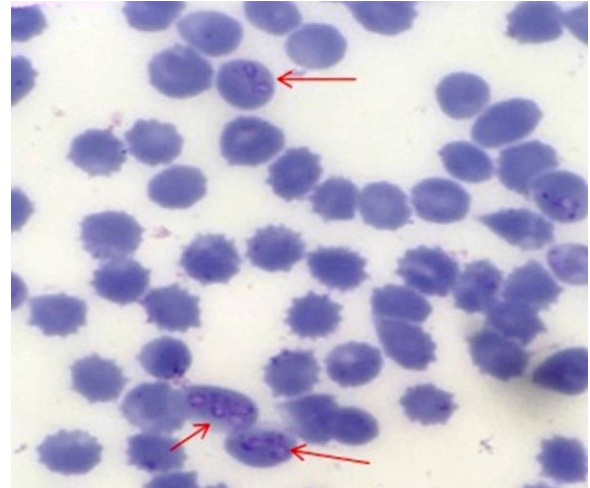
The DNA from the blood sample was extracted using a DNA extraction kit (MBST, Tehran, Iran). Specific PCR assay for canine babesiosis was performed according to the previously described method for detection and differentiation of three *B. canis* subspecies and *B. gibsoni* based on 18S rRNA gene sequence by semi-nested PCR technique. For the PCR, an outer primer pair (455-479F and 793-772R) was designed which would amplify an approximately 340 bp fragment from *B. canis* subsp. that spanned a hypervariable region of the 18S rRNA gene.<sup>5</sup> The PCR assay was conducted in a final reaction volume of 20.00  $\mu$ L containing 1X PCR premixed (Cinnagen, Tehran, Iran), 6.00  $\mu$ L ddH<sub>2</sub>O, 10.00 pmol of each primers and 2.00  $\mu$ L of DNA template using an automatic DNA thermal cycler (CP2-003; Corbett Research, Sydney, Australia) with the first denaturation at 94.00 °C for 3 min followed by 35 cycles consisted of a denaturing step of 10 sec at 94.00 °C, an annealing step of 20 sec at 58.00 °C and an extension step of 35 sec at 72.00 °C. Finally, the reaction was terminated with an extension step of 5 min at 72.00 °C. The positive control for *B. canis* was kindly provided by Dr. Afshari (Department of Parasitic Vaccines Research and Production, Razi Vaccine and Serum Research institute, Karaj, Iran). Distilled water was used as a negative control.

Hematological and biochemical assessments were performed using automatic cell counter (Exigo, Stockholm, Sweden) and automated biochemistry analyzer (BT1500, Biotechnica, Rome, Italy). The study was approved by the Ethics Committee of Faculty of Veterinary Medicine, Urmia University, Urmia, Iran (IR-UU-AEC- 3/1113/DA).

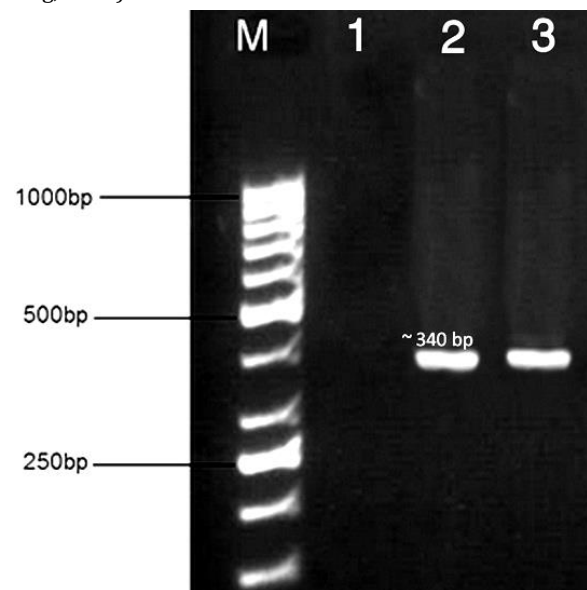
## Results

Microscopic examination revealed single to four pear-shaped intra-erythrocytic merozoites, often occurring in pairs and measuring 4.00–5.00  $\mu$ m, typical of the large form *Babesia* which characteristically pointed one end and round other. Therefore, canine babesiosis due to a large form of the parasite (i.e., *B. canis*) was diagnosed (Fig. 1). The parasitemia was determined to be 2.70%.<sup>6</sup>

The molecular analysis confirmed infection with *B. canis* (an expected 340 bp). Meanwhile, no PCR amplification products were observed in a negative control (Fig. 2).



**Fig. 1.** Blood smear from a 2-year-old female Shih Tzu dog showing merozoites of large *Babesia* spp (arrows), (Giemsa staining, 100 $\times$ ).



**Fig. 2.** Polymerase chain reaction analysis with primers 455-479F and 793-772R specific for 18S rRNA gene of *Babesia canis* (Lane 3). Lane M (50 bp): Molecular weight marker; Lane 1: Negative (i.e., no-DNA) control; Lane 2: Positive control.

Hemogram revealed moderate normocytic normochromic regenerative anemia characterized by a marked reticulocytosis. Additionally, decreased levels of packed cell volume, erythrocyte (RBC) count, thrombocyte and hemoglobin were noticed.<sup>7</sup> Moreover, increased nucleated RBCs (four per 100 white blood cells) and immature neutrophils were also recorded. The appearance of plasma was indicative of significant intra-vascular hemolysis (hemoglobinemia). Further biochemical analyses of the sample disclosed azotemia as well as elevated creatinine and urea concentrations (Table 1). After centrifugation of the freshly collected urine sample, reddish color was still evident and several bilirubin crystals precipitated.

**Table 1.** The hemato-biochemical parameters measured in this study.

Parameter	Level	Reference interval <sup>7</sup>
Reticulocyte ( $\times 10^3 \mu\text{L}^{-1}$ )	87.00	> 70.00
PCV (%)	36.00	37.00 - 55.00
RBC ( $\times 10^6 \mu\text{L}^{-1}$ )	5.20	5.50 - 8.50
Hemoglobin (g dL <sup>-1</sup> )	12.10	12.00 - 18.00
Band (cells $\mu\text{L}^{-1}$ )	1,113	0 - 300
Thrombocyte ( $\times 10^3 \mu\text{L}^{-1}$ )	25.00	200 - 500
BUN (mg dL <sup>-1</sup> )	104	10.00 - 35.00
Creatinine (mg dL <sup>-1</sup> )	6.47	0.50 - 1.50
Urea (mg dL <sup>-1</sup> )	221.80	15.00 - 55.00

PCV: Packed cell volume; RBC: Red blood cell; Band: Band neutrophils; BUN: Blood urea nitrogen.

Subcutaneous injections of diminazene aceturate (Aburaihan Co., Tehran, Iran; 3.50 mg kg<sup>-1</sup>; repeated after a week) and ivermectin (Razak Co. Tehran, Iran; 200  $\mu\text{g}$  kg<sup>-1</sup>; single dose) were prescribed. Ciprofloxacin (Farabi Pharmaceutical Co. Isfahan, Iran; 20.00 mg kg<sup>-1</sup>, q24hr) was also administered orally for 7 days. Furthermore, a single dose of phosphorus-vitamin B12 (Aburaihan Co., Tehran, Iran; 0.50 mL kg<sup>-1</sup>) was injected as a supportive care, subcutaneously. The clinical signs improved after four days and two months follow-up showed no recurrence.

## Discussion

The first effort to describe canine babesiosis in Iran was made in 1973. In that study, 155 dogs and one fox were included from the north of Iran and *B. canis* was found in only one splenectomized dog. The fox was also identified to be infected with *B. gibsoni*.<sup>8</sup> Since then, sporadic cases of this disease were reported from Tabriz and Ahvaz provinces in Iran.<sup>9,10</sup> However, all of the aforementioned studies have employed traditional microscopic approach to detect *Babesia* spp. In previous studies in Iran, molecular tests employing PCR were used and prevalence of *B. canis* infection varied from 0.35% to 182% in different regions of the country.<sup>11-14</sup> However, this is the new case of infection by *B. canis* along with recording of clinical findings as well as successful treatment in a dog from Iran.

The clinical manifestations of *B. canis* infection are moderate to severe and the severity of the disease depends on the species of *Babesia* causing infection and other factors such as immune status and the host age. The clinical signs observed in this study were comparable to those reported by Wang *et al.* from China.<sup>2</sup> Unfortunately, the only study conducted at subspecies level did not perform microscopic examination and determine the level of parasitemia.<sup>15</sup> Therefore, the clinical and laboratory findings of the present report cannot be compared to the previous ones. This shortcoming highlights the importance of novel molecular assays combination with the traditional methods.

However, the observed clinical signs and hemato-biochemical alterations in the current study are in agreement with other studies. The present anemia may be attributed to mechanical damage, autoimmune phenomena, erythrophagocytosis and vasoactive molecules such as kallikrein release.<sup>5,8,12</sup> The observed thrombocytopenia in this study can be attributed to supersession of anti-thrombin III activity which is an important enzyme in regulation of anti-coagulant pathway.<sup>15</sup>

In conclusion, the findings recorded here strongly indicated that *B. canis* should be considered in the differential diagnosis of diseases characterized by anemia and pyrexia. Besides, diminazene aceturate might be effective in the treatment of *B. canis* in dog, however; similar studies should urgently be conducted throughout the country to recommend appropriate preventive measures and successful treatments.

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## Conflict of interest

The authors declare no conflict of interest.

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