



## Investigation of *Babesia* spp. Prevalence in Dogs in Diyarbakir, Türkiye



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**C**ANINE babesiosis is a disease that occurs by the transmission of *Babesia* spp. through ticks. The animal material of the study consisted of 100 dogs of different ages, sexes and breeds which brought to Dicle University Veterinary Faculty Animal Hospital between July – August 2022 with complaints of lethargy, apathy, hematuria, and which had fever, pale mucous membranes, anemia symptoms, and muscle tremors on clinical examination. Blood samples were taken from 100 dogs for the diagnosis of *Babesia* spp. species and with the samples collected blood smears were prepared on a microscope slide and air dried, stained with the Wright-Giemsa method and microscopic analyses were made. In addition, a polymerase chain reaction was performed. None of the samples from Canine babesiosis suspected dogs yielded positive polymerase chain reaction results. In conclusion, studies to be repeated at different time intervals in the Diyarbakir region will be helpful in determining the prevalence of Canine babesiosis.

**Keywords:** *Babesia*, Diyarbakir, Dog, Prevalence.

### Introduction

Dogs can be infested with many different infectious and zoonotic ectoparasites. Apart from infectious disease transmission, these ectoparasites also cause direct harmful effects such as irritation, itching, anemia, toxicity, hypersensitivity, and allergic reactions [1]. Diseases caused by tick-borne agents worldwide and seen in dogs include Anaplasmosis, Babesiosis, Ehrlichiosis, Lyme borreliosis, Rickettsiosis, Hepatozoonosis, Haemoplasmosis and tick-borne encephalitis [2]. Canine babesiosis (CB) is caused by the transmission of *Babesia* spp., which is a protozoan that settles in the erythrocytes, through ticks [3, 4]. It has been reported that there are 12 different *Babesia* spp. worldwide that may cause CB. In addition, different researchers [4, 5] stated that the

most common subdivided *Babesia* subspecies are *Babesia gibsoni* (*B. gibsoni*) and *Babesia canis* (*B. canis*). *B. canis* is 4-5 µm in size, in pear-shaped pairs of merozoites divided into two in a single pyroplasma or erythrocyte [6]. *B. canis* has three different subtypes, such as *B. canis canis*, *B. canis vogeli* (*B. vogeli*), *B. canis rossii* due to its differences in genetic structure revealed in recent years as well as geographical, pathogenicity, antigenic structure, and vector differences [7]. The incubation period following natural infection varies between 10 days and 3 weeks in CB [8]. Season, geographical region, the immunity of the host, and the other underlying diseases are the most important factors that determine the clinical severity of the disease [9]. The most frequent clinical findings are fever, anorexia, dehydration, lameness, and anemia-related symptoms, anemia

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can be regenerative or non-regenerative [10]. Neutropenia, bilirubinemia, bilirubinuria and pigmenturia are among the laboratory findings that can be seen [11]. The most common diagnostic technique for CB is the examination of blood smear under the microscope after Wright-Giemsa staining. Pear, oval or ring-shaped forms of the agent may be detected in infected erythrocytes however, in the case of low parasitemia, infected cells may not be detected by microscopic analyses [12]. In addition, serological tests such as indirect fluorescent antibody test, enzyme linked immunosorbent assay and molecular diagnostics including polymerase chain reaction (PCR) are also useful for diagnosis [8]. In the treatment of CB, the aim is to eliminate the parasite, anemia and metabolic disorders that may occur [13]. Imidocarb dipropionate and diminazene aceturate are frequently preferred as antiparasitic drugs [14]. Canine babesiosis is a worldwide spread tick-borne infection and is also widespread the dogs in Türkiye. Researchers reported the prevalence of *B. canis* in several countries, such as the USA [15], Hungary [16], Serbia, Nigeria [17], Poland [18] and Türkiye [8] as 0.19%, 61.4%, 96%, 17.3%, 58.2% and 10.4%, respectively.

In the Diyarbakir region, Ozubek *et al.* [19] conducted studies to determine the prevalence of *Babesia* spp. in ticks, but no literature was found by the authors such as investigating the prevalence of the CB in the Diyarbakir region. Since anemia is one of the symptoms frequently encountered during CB, the current study aimed to investigate the prevalence of *B. canis*, especially in dogs with anemia symptoms.

## **Materials and Methods**

### *Ethical Approval*

26.05.2022 dated and numbered E-77707679-020-292348 ethical approval of the current study was obtained from the Local Ethics Committee for Animal Experiments.

### *Animals*

The animal material of the study consisted of 100 dogs of different ages, sexes and breeds which brought to Dicle University Veterinary Faculty Animal Hospital between July – August 2022 with the complaints of lethargy, apathy, hematuria, and which had fever, pale mucous membranes, anemia symptoms, and muscle tremors on clinical examination.

### *Blood samples*

Blood samples were collected from vena cephalica antebrachi into Ethylenediamine Tetraacetic Acid (EDTA) tubes from 100 dogs included the study. Blood smears made with the samples collected and each blood smear was prepared on a microscope slide and air dried, stained with Wright-Giemsa method and microscopic analyses were made. The blood smear was examined under low power (100X magnification) using an optical microscope (Nikon, YS100, Japan) to check for *Babesia* spp. in erythrocytes. Samples taken for DNA extraction were kept at -20°C until analyses were performed.

### *DNA extraction and amplification*

DNA extraction was performed as specified by the manufacturer using a commercial kit (Thermo Scientific GeneJET Genomic DNA Purification Kit) using 200µl of blood samples taken from animals into EDTA tubes. The amplification of the 18S ribosomal RNA gene of *Babesia* spp. was evaluated using the primary pair combinations of BAB1/BAB3 (*B. canis canis*), BAB1/BAB4 (*B. canis vogeli*) and BAB1/BAB5 (*B. canis rossi*) [20]. PCR reactions were performed under final extension conditions in a total volume of 50 µL containing 10xPCR buffer, 2 mM MgCl<sub>2</sub>, 0.2 mM dNTP, 20 pmol of each primer and 2 U Taq DNA polymerase, and for 2 minutes at 94°C, first denaturation 35 cycles at 30 seconds at 94°C, 55°C for 30 seconds and 72°C for 20 seconds and 72°C for 5 minutes.

## **Results and Discussion**

Dogs with prominent symptoms of CB such as anemia, anorexia, high fever and cachexia were included in the study and although *Babesia* spp. suspicious samples were detected in microscopic analyses, none of the samples from CB suspected dogs yielded positive PCR results belonging to *B. canis* and its subspecies.

Anemia is a symptom defined as a decrease in the percentage of red blood cells [21]. It is caused by intravascular and extravascular hemolysis as a result of the breakdown of erythrocytes [22]. Since erythrocytes are the cells most abundant in the blood and involved in the transport of respiratory gases, they are necessary for the continuity of respiration [23]. Various genetic and acquired diseases, mechanical causes, and parasitic diseases can cause anemia or manifest themselves with hemolysis [21, 23].

Dogs are often suffering from various infectious agents especially tick-borne infestations caused by *Babesia* spp. [24]. The clinical severity of the disease varies according to the types that cause the disease [25]. The principal vector of *B. canis* is *Rhipicephalus sanguineus* which occurs throughout the world and the species of the genus *Dermacentor* have also been incriminated as vectors of CB [2]. Three subspecies of *B. canis* are known and have been identified in many countries around the world. However, it is impossible to distinguish between these subspecies by direct examination of blood smears therefore, molecular diagnostic methods should be preferred [26]. In this study, PCR analyses were performed for the detection of *B. canis canis*, *B. canis rossi* and *B. canis vogeli* as the researchers reported [26].

Various researchers reported that the most frequent clinical findings of CB are anemia, loss of appetite, dehydration, lethargy, pale mucous membranes, and fever [3, 6, 8, 15-18, 27]. The symptoms of the dogs included in this study were consisted with the findings of abovementioned studies [3, 6, 8, 15-18, 27].

In Asia, Europe, Africa, America and also in Türkiye *Babesia* spp. prevalence is reported. In the studies, *Babesia* spp. prevalence was determined by Muguiro et al. [3] 1508/435 (28.8%) in Hong Kong, Földvari et al. [16] in Hungary 39/44 (88.64%), Davitkov et al. [17] 58/60 (96.67%) in Serbia, Wezyk et al. [18] 82/140 (58.57%) in Poland, Okubanjo et al. [27] in Nigeria, 26/150 (17.33%), Barash et al. [15] reported that they detected 18/9367 (0.19%) in the USA and Kırılı [8] 40/383 (10.44%) in Türkiye. Contrary to the researchers [3, 8, 15-18, 27] reported in the present study, *Babesia* spp. negative was detected.

### Conclusions

In conclusion, although *B. canis* was detected in ticks in the Diyarbakir region [19], the presence of *Babesia* spp. was not detected in dogs with anemia symptoms in the current study. It was concluded that clinically detected anemia in dogs included in the study may be related to other underlying diseases or conditions, and studies to be repeated at different time intervals in the Diyarbakir region will be helpful in determining the prevalence of CB.

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### Conflict of Interest

No conflict of interest was declared by the authors.

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