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RESEARCH ARTICLE

MOLECULAR INVESTIGATION OF CANINE HEPATOZOONOSIS IN BATMAN AND VAN PROVINCES OF TÜRKİYE

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ABSTRACT

Canine hepatozoonosis is a tick-borne protozoan disease spread by hard ticks of the Ixodidae family. Although this illness has been seen in numerous locations in Türkiye, its existence in the Batman and Van provinces has yet to be confirmed. The purpose of this research was to look into canine hepatozoonosis in stray dogs from two distinct areas in Türkiye using conventional polymerase chain reaction (PCR). Between 2019 and 2021, blood samples were collected from 197 stray dogs in Batman and Van provinces in Türkiye. A unique 486–520 bp segment of the 18S rRNA gene of *Hepatozoon* spp. was amplified using PCR. According to the PCR findings, none of the 197 stray dogs tested positive for *Hepatozoon* spp. This research offers epidemiological data on the prevalence of canine hepatozoonosis in Türkiye, which may be useful in future studies with larger sample sizes and dogs of varied origins.

Keywords: Batman, Canine hepatozoonosis, PCR, Stray dogs, Türkiye, Van

1. INTRODUCTION

In America, Latin America, Europe, Asia, and Africa, *Hepatozoon canis* and *H. americanum* cause canine hepatozoonosis, a tick-borne disease. *H. canis* is a more frequent species than *H. americanum* [1]. Hard ticks of the Ixodidae family transmit both species. Although *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus turanicus* are the main vectors of *H. canis*, other tick species, such as *Amblyomma ovale*, *Haemaphysalis longicornis*, *Haemaphysalis flava*, and *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus turanicus* have been reported to carry the parasite in recent years [2-8]. *A. maculatum* is the vector tick that transmits *H. americanum* [9]. Ticks operate as vectors, physically or physiologically transmitting various infections of parasitic, bacterial, or viral origin from one vertebrate host to another. Furthermore, certain infections are spread both vertically and horizontally. *H. canis* and *H. americanum* transfer their sprozoites to intermediate hosts by vector ingestion, which is a horizontal transmission mode [10]. In Türkiye, only three tick vector studies on canine hepatozoonosis in dogs have been undertaken [11, 12, 13]. According to these investigations, *R. sanguineus* sensu lato might be considered a possible *H. canis* vector in our country. In general, canine hepatozoonosis is asymptomatic. Although fever is a frequent symptom in both species, *H. americanum* infection has been associated with more serious clinical symptoms, such as lameness and mucopurulent eye discharge [14]. Canine hepatozoonosis has been recorded in a few clinical case investigations and epidemiological surveys in Türkiye, and has been linked to *Hepatozoon canis* and *Hepatozoon* sp. MF [1, 11, 13, 15-25]. Canine hepatozoonosis has been diagnosed using blood smears, serology, and molecular methods [17, 25, 26]. Of these, DNA-based approaches are the most preferred among them because of their remarkable sensitivity and specificity in distinguishing between *H. canis* and *H. americanum*. Previous research from several provinces of Türkiye, such as Diyarbakir,

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Kayseri, Konya, Karaman, Sivas, and Samsun, found canine hepatozoonosis. However, to the best of the authors' knowledge, canine hepatozoonosis has not been studied in Van province. It has yet to be discovered in Batman province. The purpose of this study was to use genetic approaches to evaluate the existence of *Hepatozoon* species in dogs residing in the Batman and Van province of Türkiye.

2. MATERIAL AND METHODS

2.1. Animals

During 2019–21, 197 reportedly asymptomatic stray dogs from municipal animal care and rehabilitation centers in Batman (97 sample) and Van (100 sample) provinces were investigated (Figure 1). Regulations define the responsibilities of these centers, which include actions for gathering and collecting stray animals, providing health treatment (including endo and ecto-parasitic applications), sterilization, and immunization. The age and gender of the animals were recorded (data not shown). Animal ethics, method and sampling approval was obtained from the University of Van Yuzuncu Yil Animal Ethics Committee (approval number: 2021/12-10). For DNA isolation, blood samples were collected from the cephalic veins and placed in sterile tubes containing EDTA.

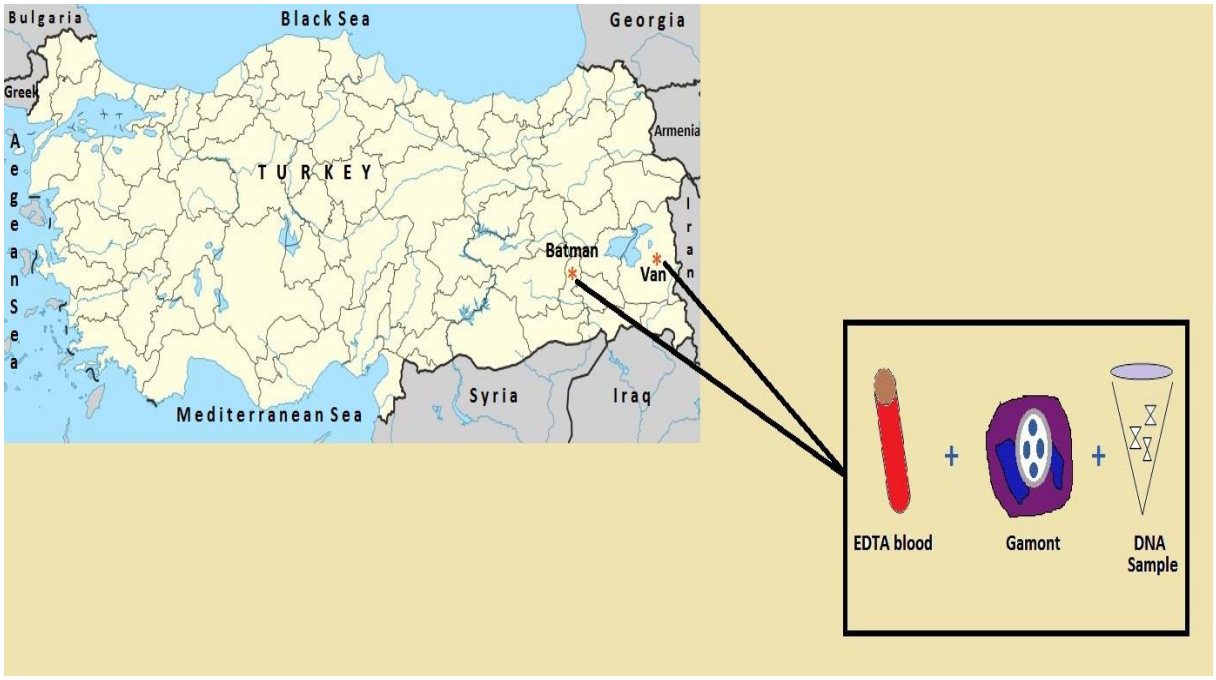

Figure 1. Map of Türkiye, locations Batman and Van. (https://tr.pinterest.com)

2.2. DNA Extraction and PCR

Using the Blood Genomic DNA Isolation Kit (Cat no: MG-GDNA-01-250, Hibrigen, Türkiye), total genomic DNA was extracted from 200 µl blood samples according to the manufacturer's instructions. Until used, genomic DNA was stored at -20 °C. The BJ1 (5′-GTCTTGTAATTGGAATGATGG-3′) and BN2 (5′-TAGTTTATGGTTAGGACTACG-3') primers were used to amplify the 486-520 bp region of the 18S rRNA gene of *Hepatozoon* spp. using a PCR technique [27]. The peculiarity of these primers is that they are specific for all *Babesia* species and can amplify blood parasites, such as *Hepatozoon* spp., *Hemolivia mauritanica*, and *Theileria* spp [23, 24, 28]. The PCR was performed in a final volume of 25 µL, which included 7.5 µL of DNase-and RNase-free sterile distilled water (Biobasic, Canada), 10 L of 5X MyTaq Reaction buffer, 1 µL of each primer (20 pmol), 5 µL of

template DNA (100-200 ng), and 0.5 µL of Taq DNA polymerase (1.25 IU) (MBI Fermentas, Lithuania).The PCR conditions were as follows: 5 min at 95 °C(initial denaturation), 35 cycles of 60 s at 94 °C, 60 s at 55 °C, 2 min at 72 °C, and finally 5 min at 72 °C(final extension) [27]. The PCR products were separated on 1.5% agarose gels, stained with ethidium bromide, and photographed using an UV transilluminator.

3. RESULTS

According to the PCR data, none of the 197 stray dogs tested positive for *Hepatozoon* spp. This study's positive control sample was amplified properly (Figure 2). All dogs were treated for ectoparasites.

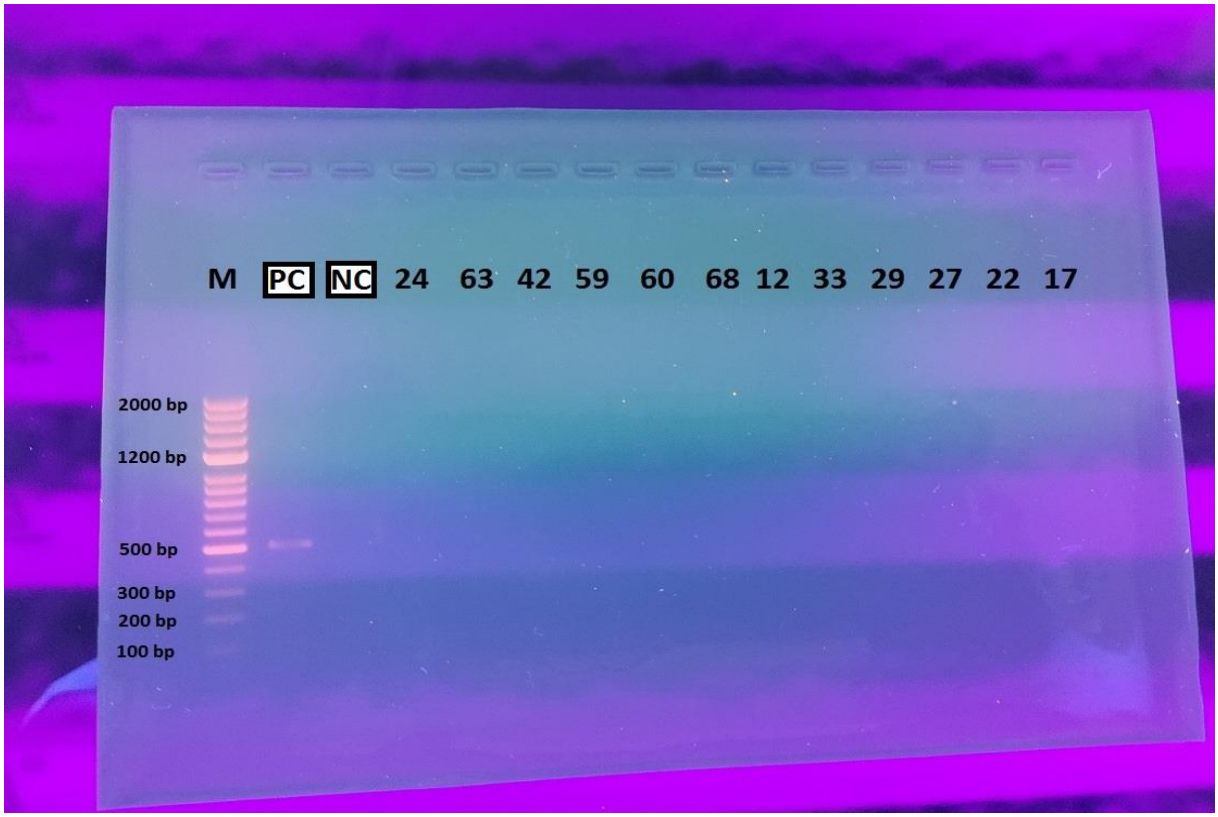

Figure 2. The single-PCR products of *Hepatozoon* species. M: Marker 24/63/42/59/60/68/12/33/29/27/22/17: samples, PC: Positive control, NC: Negative control

4. DISCUSSION

Babesiosis, anaplasmosis, dirofilariosis, hepatozoonosis, bartonellosis, ehrlichiosis, borreliosis, and leishmaniosis are the most commonly diagnosed canine vector-borne illnesses in dogs [29, 30]. These infections are spread by blood-feeding arthropods such as ticks, fleas, mosquitoes, and sand flies [31]. Ticks and fleas are the most common arthropods in dog, cat, and human vector-borne diseases [32]. *Hepatozoon* spp. were not discovered in any of the stray dogs in our investigation. However, the existence and prevalence of *Hepatozoon* spp. in dogs in several provinces of Türkiye has been reported. The prevalence of canine hepatozoonosis in dogs in Türkiye ranges between 0.5 and 54.3% [13, 17, 18, 20, 22-25]. Bolukbas et al. [20] and Aslantas et al. [24] reported a *H. canis* PCR prevalence of 0.5% in shelter dogs in Samsun and Hatay. According to Aktas and Ozubek et al. [13], *H. canis* was found in 54.3% of stray dogs in a Diyarbakir shelter. They stated that all canines in the Samsun research were administered anti-endo and anti-ectoparasitic medications. All of the dogs in

the Hatay study were clinically healthy and free of ectoparasites. However, Aktas et al. [19] in Diyarbakir stated that no tick or flea management therapy was provided to the animals. In a recent survey conducted in five different parts of Sivas province, Ulas was the most prevalent region for canine hepatozoonosis (67.85%). The prevalence of canine hepatozoonosis was found to be quite low in another part of the province (Susehri) (3.84%). The difference in infection rates between studies could be due to the use of ectoparasiticides on a regular basis.

Batman province, which is located in the Tigris Section of the Southeastern Anatolia Region, has a continental climate. The summers are hot and dry, while the winters are mild and rainy [33]. The province of Van is located in the Upper Murat-Van Section of the Eastern Anatolia Region, in the closed basin of Lake Van. Van experiences a continental climate, and the winters are cold and long [34]. To date, no research on canine hepatozoonosis has been published from Türkiye's Van province. In Batman province, however, a study on canine hepatozoonosis was conducted [24]. According to the findings, none of the 50 canines tested positive for *Hepatozoon* DNA. *Hepatozoon* infection was not discovered in our current investigation, which is consistent with earlier research. The existence of *H. canis* has been confirmed in research conducted in several provinces in the Eastern and Southeastern Anatolia areas. For example, Aktas and Ozubek [13] found a PCR frequency of 54.3% for *H. canis* in Diyarbakir (the nearest city to Batman). In a study conducted in Erzurum, 377 kilometers from Van, Aktas et al. [19] discovered a 42.8% *H. canis* PCR infection rate. Guven et al. [22]; however, discovered a lower *H. canis* prevalence of 5.3% in the same area. These differences between our results and previous studies in the same area could be explained by different sampling periods and animal origins, as well as the spread of arthropod vectors in different parts of Türkiye [35, 36]. In regions where *R. sanguineus* sensu lato is the major tick species, *Hepatozoon canis* is often seen. In research done in the Eastern Anatolia Region, the presence of *R. sanguineus* s.l. in the province of Van was also documented [37]. The existence of the vector tick species in the Van province increases the risk of infection, but in our present investigation, we found no positive findings for this species. There has been no research on the prevalence and distribution of related tick species in the Batman province. We hypothesize that this somewhat validates the study's negative findings. Furthermore, hepatozoonosis may have a different transmission mechanism than tick-borne infections, such as *Theileria*, *Babesia*, and *Anaplasma* [31, 38, 39]. This may have a direct impact on the prevalence and occurrence of these diseases.

5. CONCLUSION

In our study, dogs in Batman and Van provinces were not infected with *Hepatozoon* spp. According to earlier investigations, two genotypes (*H. canis* and *Hepatozoon* spp. MF) have been discovered in dogs in Türkiye [1, 22, 25]. More research is needed to better understand the pathogenicity and frequency of emerging new genotypes. Additionally, studies using hunting dogs, sheepdogs, or wild canids are essential to determine the parasite's existence.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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AUTHOR CONTRIBUTION

B.O designed and carried out the experiments. B.O and M.S.D researched literature, analyzed the data and wrote the manuscript. S.A edited the language and helped with the review process of the final manuscript.

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