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Microscopic and PCR survey of *Hepatozoon* spp. and *Anaplasma phagocytophilum* in dogs from Antalya city of Türkiye*

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Summary

This study was planned and conducted to investigate the presence and prevalence of *Hepatozoon* spp., the causative agent of canine hepatozoonosis, as well as *Anaplasma phagocytophilum*, the causative agent of canine and human granulocytic anaplasmosis (CGA and HGA), in dogs in Antalya city of Türkiye. This region is characterized by intensive human and animal circulation and offers favorable living conditions for vector ticks. Blood samples collected from 120 randomly selected dogs were examined microscopically and molecularly by PCR to detect the presence of *Hepatozoon* spp. and *A. phagocytophilum*. As a result, the microscopic and molecular prevalences of *Hepatozoon* spp. were determined as 2.5% and 22.5%, respectively, while *A. phagocytophilum* was not detected through microscopic examination, and its molecular prevalence was found to be 3.33%. During the examination of the blood smears, *Babesia* spp. piroplasms were incidentally detected in two samples. For both *Hepatozoon* spp. and *A. phagocytophilum*, no statistically significant difference was found between the presence of infection and the parameters of age, gender, and breed. The literature review reveals that this is the first study investigating the presence and distribution of *Hepatozoon* spp. in dogs in the Antalya region, making it the first report both microscopically and molecularly. While there is a serological study using a rapid test kit for *A. phagocytophilum* in dogs in the Antalya region, this study is also the first microscopic and molecular screening, and the first molecular report of *A. phagocytophilum* in the area. In conclusion, it should not be overlooked that *Hepatozoon* spp., which is found at a significant prevalence in the region, poses a threat to animal health, while *A. phagocytophilum* presents a serious threat to both human and animal health. To effectively combat these pathogens, it is once again emphasized that comprehensive and result-oriented control and prevention programs must be established and promptly implemented based on relevant epidemiological data.

Keywords: ***Anaplasma phagocytophilum*****, Antalya, dogs,** ***Hepatozoon*** **spp., prevalence**

Türkiye has a significant population of stray dogs, and veterinary services such as antiparasitic treatments, vaccinations, and neutering for these dogs are carried out by municipal temporary animal care units. However, due to the dense population in some cities, it is a fact that these care units are unable to provide adequate care for all stray dogs. In recent years, there has been intense debate regarding the health and welfare of these homeless and street-dwelling dogs, as well as their impact on public health. These animals live in unhealthy and harsh conditions, occasionally exhibit aggressive behavior towards humans, and serve as reservoirs for zoonotic pathogens. Stray dogs, particularly those infested with various arthropods, suffer from CVBDs (canine vector-borne diseases), acting as reservoirs for these pathogens for both canine and human hosts, as well as mechanical carriers of infected vectors (37). CVBDs are especially important for the health and welfare of dogs and humans in countries with tropical climates. Studies on CVBDs have increased in the past few decades in various countries

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(23, 29, 31, 56, 57, 69, 72) and in Türkiye (2, 3, 28, 34, 41, 50, 67).

Hepatozoonosis, one of the most major blood diseases of dogs, is caused by the species *Hepatozoon canis* in the old world and *H. americanum* in the US (48). These apicomplexan protozoa are transmitted via ticks and infect the leukocytes, hemolymphatic tissues, muscles, and parenchymal organs of the vertebrate host (14, 17-19). The merogony and gametogony (the differentiation of sexual cells) stages of *H. canis* occur in intermediate hosts, such as dogs and other canids, while the syngamy (fertilization) and sporogony (formation of sporozoites through the asexual divisions of oocysts) stages occur in the definitive host, *Rhipicephalus sanguineus* (the brown dog tick). A unique and interesting feature of the life cycle of *H. canis* is that transmission to dogs occurs through the ingestion of a whole or fragmented mature tick containing mature oocysts (which contain infective sporozoites) (16, 17). The clinical signs of the disease can be asymptomatic with low parasitemia, while severe cases with high parasitemia may present with symptoms such as fever, lethargy, anemia, and weight loss, potentially leading to fatal outcomes (19).

Another significant blood disease is canine granulocytic anaplasmosis (CGA), which is a vector-borne disease commonly found in dogs worldwide. The disease is caused by *Anaplasma phagocytophilum*, a Gram-negative alpha-proteobacterium that belongs to the family Anaplasmataceae within the order Rickettsiales (25). The name *Anaplasma phagocytophilum* unifies three previously distinct pathogens – *Ehrlichia equi*, *Ehrlichia phagocytophila*, and an unnamed agent of human granulocytic ehrlichiosis (HGE) – due to their shared genetic, antigenic, and biological characteristics, representing a single species (33). As an obligatory intracellular pathogen, *A. phagocytophilum* primarily infects granulocytes, predominantly neutrophils but also eosinophils of various mammals, including humans, where it exists and reproduces in membrane-bound vesicles, forming microcolonies called morulae (25). Transmission occurs mainly through ticks, primarily of the *Ixodes* species and *Dermacentor silvarum* (21, 25). Clinical signs of CGA typically include lethargy, fever, and anorexia (68), while vomiting, diarrhea, petechiae, epistaxis, and lameness may occur less frequently (53). The zoonotic nature of *A. phagocytophilum* makes it a pathogen of public health significance in addition to its importance for animal health. In humans, anaplasmosis (HGA – Human Granulocytic Anaplasmosis) is an acute febrile illness caused by *A. phagocytophilum*, characterized by high fever, general myalgia, headache, and fatigue (13, 22).

For prevention and control of diseases effectively, it is crucial to obtain and interpret epidemiological data. Understanding the epidemiology of diseases requires knowledge of the prevalence of their causative agents and, if applicable, their intermediate hosts. Therefore, the present study aims to investigate the microscopic and molecular prevalence of *Hepatozoon* spp. and *A. phagocytophilum* in dogs from Antalya, which is a significant city in the Western Mediterranean Region of Türkiye, which offers suitable habitats for ticks due to its geographical and climatic characteristics, additionally, the region experiences high human and animal mobility, especially due to tourism activities, making it a critical area of focus (52, 73, 74).

Material and methods

The present study was approved by the Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee (05.10.2022/953). In addition, the owners of the dogs from which blood samples were taken were informed about the research and a signed "Informed Consent Form" was obtained. Moreover, permission for the study was also obtained from the Antalya Provincial Directorate of Agriculture and Forestry.

The material of the study consists of blood samples collected in EDTA tubes from the forearm veins (*vena cephalica antebrachii*) of 120 randomly selected dogs (owned and stray dogs housed in shelters that were brought to the Faculty's Animal Hospital and various private veterinary clinics in Antalya Center and/or other districts for various diseases) between November 2022 and January 2024 without considering factors such as age, sex, breed, housing conditions, or health status (Tab. 1).

Tab. 1. Distribution of sampled dogs according to age and sex

Age (year)			Sex		Total
0-3	3-6	6-10	Male	Female	
40	58	22	64	56	120

Blood smears were prepared for each sample, fixed in methanol, stained with 5% Giemsa solution and then examined under 100 × objective of light microscope (Olympus BX51).

DNA extraction was performed using a commercial kit (Promega Wizard® Genomic DNA Purification Kit, Madison/USA) following the protocol of the manufacturer.

One step conventional PCR was performed for the diagnosis of *Hepatozoon* spp. using the specific primers Hep-F(5'-ATACATGAGCAAAATCTCAAC-3') and Hep-R(5'-CTTATTATTCCATGCTGCAG-3') that amplify a conserved region of the 18S ssrRNA gene, which is 666 bp in size (48). For each PCR reaction, a 23 µl reaction mixture was prepared in an ependorf tube containing 2 µl of target DNA. The reaction mixture included 100 µM dNTP (deoxynucleoside triphosphates: dATP, dCTP, dGTP, dTTP, dUTP), 25 pmol forward and reverse primers (100 pmol/µl), 1.25 U Taq DNA Polymerase hot FIREPol 5 U/µl, 25 mM $MgCl_2$, 10 × PCR buffer (200 mM Tris-HCl, pH 8.3 (25°C); Nuclease Free Water (NFW). The PCR protocol was set as shown in Table 2.

Two-step Nested PCR protocol was performed for the diagnosis of *A. phagocytophilum*. The first step was performed using the ge3a (5' CACATGCAAGTCGAACGGAT TATTC 3') and ge10r (5'-TTCCGTTAAGAAGGATC-TAATCTCC-3') primer pair to amplify a 919 bp fragment

Tab. 2. Polymerase Chain Reaction conditions for *Hepatozoon* spp. and *A. phagocytophilum*

Species	Initial Denaturation	Denaturation – Annealing – Extension	Final Extention
Hepatozoon spp.	1× [12 min in 95°C]	35× [50 sec in 94°C; 50 sec in 50°C; 30 sec in 68°C]	1× [10 min in 68°C]
A. phagocytophilum	1× [5 min in 95°C]	35× [50 sec in 94°C; 50 sec in 55°C; 60 sec in 72°C]	1× [10 min in 72°C]

of the 16S subunit rRNA gene (58). The PCR reaction had a final volume of 25 µl and included 1.5 mM $MgCl_2$, 200 µM dNTPs, 1.5 U Hotstart Taq DNA polymerase, 25 µM of forward and reverse primers and 1 µl of template DNA. The PCR protocol was prepared as shown in Table 2 and the amplification was carried out using an automated thermal cycler. From the PCR products, 1 µl of the sample was used in a second-step PCR. The second-step PCR had a final volume of 50 µl and was prepared with the same component ratios as the first-step PCR. The species-specific nested PCR, which amplified a 546 bp fragment of the 16S subunit rRNA gene, was conducted under the same conditions using the ge9F (5'-AACGGATTATTCTTTATAGCTTGCT-3') and ge2 (5'-GGCAGTATTAAAAGCAGCTCCAGG-3') primer pairs (58).

The data obtained from the present study was analysed using Minitab 16 Statistical Software. The Chi-Square test was performed to analyse the association among age, sex, breed parameters and PCR positivity. Differences for which the P-value was less than 0.05 were considered statistically significant during comparisons for each parameter within itself.

Results and discussion

As a result of the microscopic examination of blood smears prepared from the blood samples, *Hepatozoon* spp. gamonts (Fig. 1) were detected in three samples, while no *A. phagocytophilum* was observed in any of the samples (Tab. 3). However, during the examination of the blood smears, *Babesia* spp. piroplasms (Fig. 2) were incidentally detected in two samples. When compared with the PCR results, it was found that the samples in which *Hepatozoon* spp. and *Babesia* spp. were microscopically observed were also PCR-positive for *Hepatozoon* spp. However, although the samples in which *Babesia spp.* were detected microscopically PCR-positive for *Hepatozoon* spp., they were microscopically negative for *Hepatozoon* spp.

Tab. 3. Microscopical examination results

Species	Number and rate of positive dogs
Hepatozoon spp.	3/120 (2.5%)
A. phagocytophilum	0/120 (0%)
Babesia spp.	2/120 (1.66%)

According to the conventional PCR assay, the molecular prevalence of *Hepatozoon* spp. was

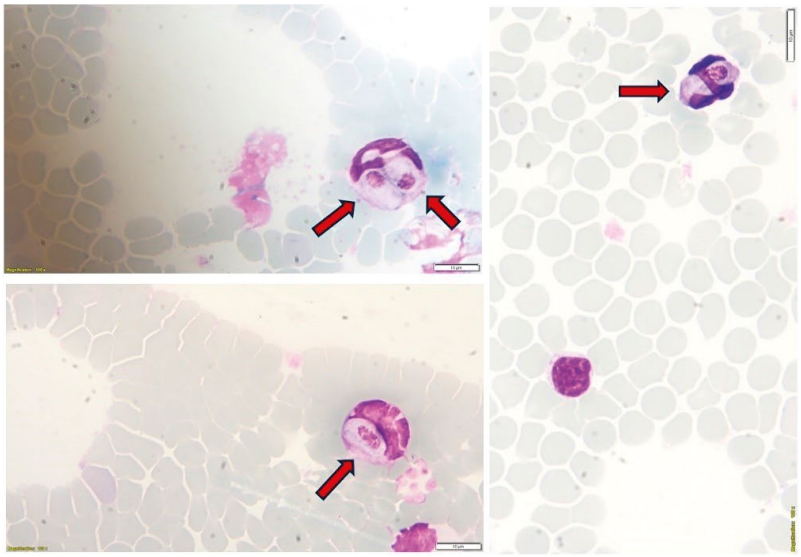

Fig. 1. *Hepatozoon* spp. gamonts (red arrows)
Explanations: objective: 100 ×, scale bar: 10 µm

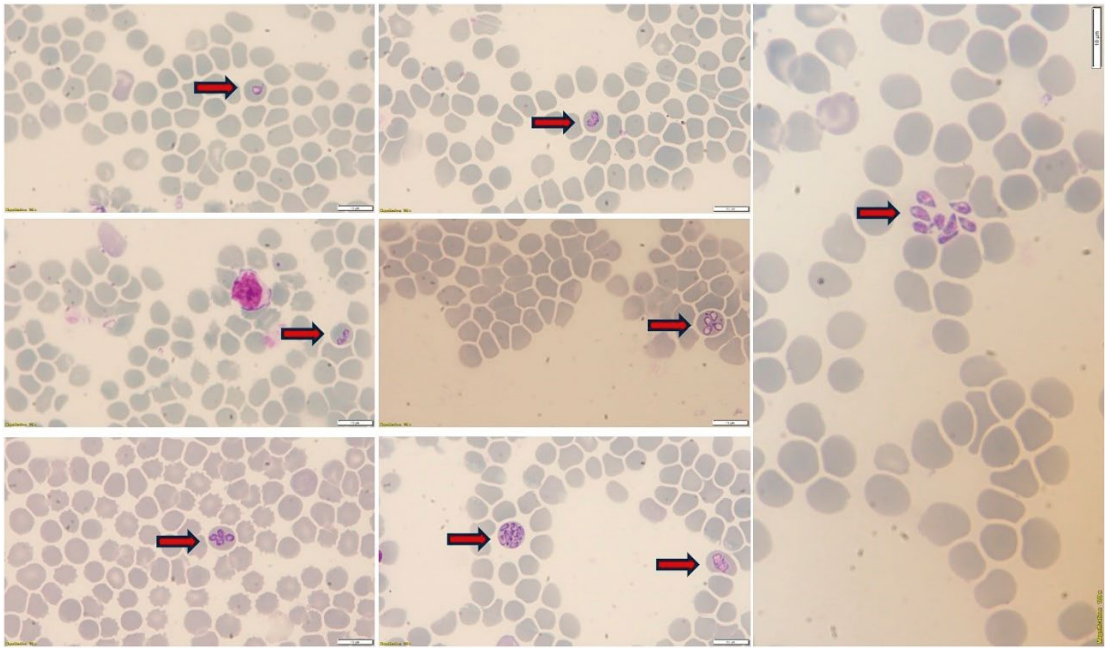

Fig. 2. *Babesia* spp. piroplasms (red arrows)
Explanations: objective: 100 ×, scale bar: 10 µm

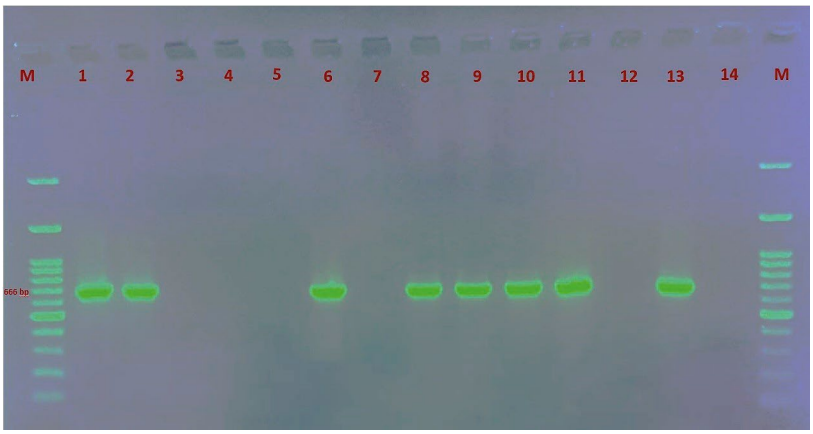

Fig. 3. Agarose gel electrophoresis image of conventional PCR products of some positive samples for *Hepatozoon* spp.
Explanations: M – 100 bp molecular weight marker; 1, 2, 6, 8, 9, 10, 11 – positive samples; 3, 4, 5, 7, 12 – negative samples; 13 – positive control; 14 – negative control

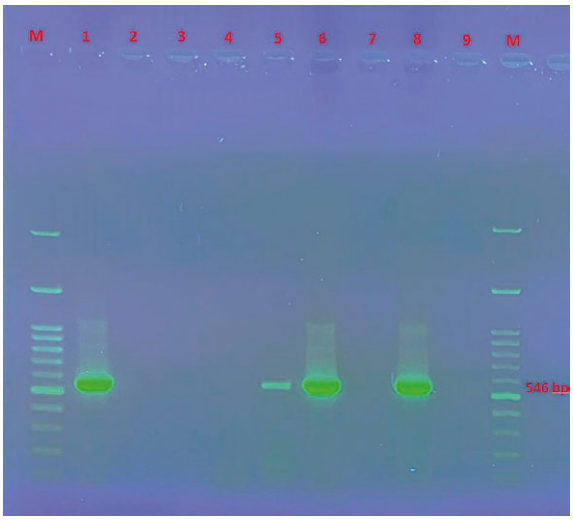

Fig. 4. Agarose gel electophoresis image of second step nested PCR products of some positive samples for *A. phagocytophilum*
Explanations: M – 100 bp molecular weight marker; 1, 5, 6 – positive samples; 2, 3, 4, 7 – negative samples; 8 – positive control; 9 – negative control

found to be 22.5% among the examined dog blood samples. Agarose gel electrophoresis image of conventional PCR products of some positive samples for *Hepatozoon* spp. is presented in Figure 3. A rate of 18.75% was observed in female dogs and 26.78% in male dogs; however, there was no statistically significant difference in the positivity rates between genders (Tab. 3). The distribution of *Hepatozoon* spp. positivity according to age groups is shown in Table 4. No statistically significant difference was found in positivity rates between the age groups. As shown in Table 4, no statistically significant difference was observed in the analysis of positive animals among mixed breed and shepherd dogs, which had the highest percentages. However, for breeds other than mixed and shepherd dogs, the sample sizes were insufficient for statistical analysis, and therefore, a P-value could not be obtained using the Chi-square test.

Nested-PCR analysis revealed that DNA of *A. phagocytophilum* was detected in four of the 120 dog blood samples examined, resulting in a species-level positivity rate of 3.33%. As a result of agarose gel electrophoresis of first-step PCR products, no band was observed in any of the samples, including the positive control. In contrast, as shown in Figure 4, the gel electrophoresis images of the second-step PCR products revealed bands in the expected 546 bp region. When examining the relationship between the gender and *A. phagocytophilum* PCR positivity, rates of 3.12% and 3.57% were found in female and male dogs, respectively and no statistically significant difference detected in positivity rates between genders (Tab. 5). The distribution of *A. phagocytophilum* positivity according to age groups is shown in Table 5. There was no statistically significant difference in positivity between the 0-3 and 3-6 years age groups. Since no positive cases were found in dogs older than six years, statistical analysis could not be performed for this age group. As shown in Table 5, the relationship between breeds and *A. phagocytophilum* positivity reveals a positivity rate of 2.70% in mixed-breed dogs and 9.52% in shepherd dogs, while

Tab. 4. Distribution of *Hepatozoon* spp. according to gender, age and breed

	Number of examined dogs	Number of positive dogs	Rate of positive dogs	P	P^2
Gender					
female	64	12	18.75%	0.293^{NS}	
male	56	15	26.78%		
Age (Year)					
0-3	40	8	20.00%	0.775^{NS}	0.512^{NS}
3-6	58	13	22.41%		
6-10	22	6	27.27%	0.648^{NS}	
Breed					
mix breed	74	16	21.62%	0.269^{NS}	
shepherd dog	21	7	33.33%		
pointer	6	1	16.66%		
pitbull	5	0	0		
golden retriever	5	1	20.00%		
terrier	3	1	33.33%		
labrador	3	1	33.33%		
German shepherd dog	3	0	0		

Explanations: NS – non significant ($P > 0.05$); * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; P^2 – for 0-3 and 6-10 age groups

Tab. 5. Distribution of *A. phagocytophilum* according to gender, age and breed

	Number of examined dogs	Number of positive dogs	Rate of positive dogs	P
Gender				
female	64	2	3.12%	0.920^{NS}
male	56	2	3.57%	
Age (Year)				
0-3	40	1	2.5%	0.511^{NS}
3-6	58	3	5.17%	
6-10	22	0	0	
Breed				
mix breed	74	2	2.70%	
shepherd dog	21	2	9.52%	
pointer	6	0	0	
pitbull	5	0	0	
golden retriever	5	0	0	
terrier	3	0	0	
labrador	3	0	0	
German shepherd dog	3	0	0	

Explanations: NS – non significant (P > 0.05), * – P < 0.05, ** – P < 0.01, *** – P < 0.001

Tab. 6. Comparison of microscopical examination and PCR results for both parasites

Species	Positivity in microscopy	Positivity in PCR	P
***Hepatozoon* spp.**	2.5% (3/120)	22.5% (27/120)	0.000***
A. phagocytophilum	0%	3.33% (4/120)	0.044*

Explanations: NS – non significant (P > 0.05), * – P < 0.05, ** – P < 0.01, *** – P < 0.001

no positivity was detected in other breeds. However, due to the insufficient number of positive cases across different breeds, statistical analysis could not be performed, and no P-value was obtained using the Chi-square test.

In the present study, the microscopic and molecular prevalence rates of *Hepatozoon* spp. were found to be 2.5% and 22.5%, respectively. For *A. phagocytophilum*, no positivity was detected by the microscopic method, while a molecular prevalence of 3.33% was identified through PCR. Statistically significant differences were observed between the results of two methods in the detection of both parasites (Tab. 6).

A literature review revealed that several studies have documented the occurrence of *H. canis* in dogs in Türkiye (49, 67, 75, 76). Some others have been conducted to investigate the prevalence of *Hepatozoon canis* in dogs, providing regional microscopic and/or molecular prevalence values. Upon reviewing these studies, it is observed that the microscopic prevalence of canine hepatozoonosis in Türkiye ranges between 0% and 10.6% (3, 24, 50, 64). The 2.5% microscopic prevalence found in this study seems to be in line with the findings of studies conducted across Türkiye. Molecular diagnostic studies have revealed varying prevalence rates of *H. canis* across Türkiye. According to the results of these studies, molecular prevalence rates for canine hepatozoonosis ranging from 0% to 54.3% have been reported from various regions of Türkiye (1-3, 7, 9, 12, 24, 27, 34, 38, 41, 42, 50, 61, 64). In Antalya, no previous epidemiological study on canine hepatozoonosis had been conducted, making this study the first to report *Hepatozoon* spp. prevalence and epidemiology in dogs from the region. The findings showed a high *Hepatozoon* spp. positivity rate of 22.5%, which is consistent with some previous studies (3, 42, 50) but higher than others (7, 9, 12, 24, 27, 34, 41, 61). However, the rate was lower compared to studies by Aktaş and Özübek (1), Orkun et al. (64), and Erol et al. (38). The variation in prevalence across regions can be attributed to factors such as the distribution of the parasite, the population of intermediate and definitive hosts, animal and human movements, vector control strategies, environmental factors like temperature and climate change, sample size, sampling season, and diagnostic methods used. In this study, the subtropical climate of Antalya, which provides a favorable environment for vector arthropods, along with the high population of stray dogs in municipal shelters, likely contributed to the high prevalence observed. In terms of tick control, environmental insecticide applications are generally not recommended due to their limited effectiveness against ticks, environmental pollution, disruption of ecological balance, and high costs (6). Ethical and sustainable solutions, such as intensive neutering programs, antiparasitic treatments, and fostering/adoption of stray dogs, remain the best strategies to prevent ectoparasite infestations and control these pathogens.

One of the most important factors in the epidemiology of canine hepatozoonosis is the prevalence of tick vectors. Some studies have investigated not only the dogs but also the tick species infesting these dogs and whether they are infected with *Hepatozoon* spp. or *H. canis*. For example, Kıral et al. (51) reported that they collected *Rhipicephalus sanguineus* from *H. canis*-infected dogs, but no ticks were found on healthy dogs. Aktaş et al. (7) found that 20.58% of *R. sanguineus* pools were infected with *Hepatozoon spp.*, with an infection rate of 4.9% per 100 ticks. In a similar study, the prevalence of *H. canis* in *R. sanguineus* collected from two different climate zones was found to be 7.10% (5). Bölükbaş et al. (24) examined 200 dogs for tick infestation, where 0.5% tested positive for *Hepatozoon spp.* by PCR, but no ticks were found on these dogs. There are also studies regarding

the distribution of tick species. In a review by Aydın and Bakırcı (11) on the distribution of tick species in Türkiye, it is noted that in the Mediterranean region, which includes the southwestern coast of Antalya, vectors of *H. canis* such as *R. sanguineus* and *R. turanicus* are present. Another study (52) revealed that *R. sanguineus* (n = 619, 45.01%) and *R. turanicus* (n = 521, 37.89%) were found in all five sampled regions of Antalya and were the most common tick species identified, both overall and among those infesting dogs. Although this data is from about 10 years ago, the prevalence of *R. sanguineus* and *R. turanicus* in Antalya, as reported by Koç et al. (52), supports the significant 22.5% prevalence of *Hepatozoon* spp. found in this study. While some of the dogs from which blood samples were collected were quickly inspected for tick infestation, detailed examinations were not performed due to time constraints, and some of the blood samples were collected by shelter and private clinic staff, meaning no data on the prevalence of the pathogen in ticks was obtained. However, it is important to note that for more meaningful epidemiological results, the prevalence of tick infestation and the distribution of the pathogen in ticks should also be determined.

It was observed that no statistically significant difference was found in *Hepatozoon* spp. positivity among gender, age and breeds. Most studies conducted in Türkiye have reached similar conclusions regarding the lack of predisposition based on sex and age for canine hepatozoonosis (1, 3, 7, 27, 38, 42). Although these data might suggest that the sex, age or breed is not a predisposing factor for infection, it is important not to overlook other factors such as the prevalence of tick vectors, the percentage of ticks carrying the pathogen, exposure to tick infestations, physiological conditions like pregnancy and lactation, and the use of steroid medications. Because there are also studies that suggest the opposite or discuss certain predisposing factors. The literature on canine hepatozoonosis prevalence in the dog population shows conflicting data. Aktaş et al. (7) reported no statistically significant difference based on sex and age groups, however, they found a significant relationship with tick infestation. Another study by Aktaş et al. (3) showed that the positivity rate in adult dogs was significantly higher than in young dogs, suggesting that this could be due to longer exposure to tick vectors. Some researchers (3) found a statistically significant lower positivity rate in owned dogs (10.4%) compared to stray (26.3%) and shelter dogs (25.7%). Guo et al. (41) similarly found a significantly higher prevalence of *H. canis* in shepherd dogs (11.1%) compared to house (3.6%) and guard dogs (1.4%), though there was no significant difference between house and guard dogs. Chisu et al. (26) found that *Hepatozoon* spp. prevalence was higher in female dogs (23%) compared to males (10%). This result contradicted another study that reported no sex predisposition for hepatozoonosis (55), but Chisu et al. (26) suggested that this discrepancy might be due to differences in tick exposure. The same study (26) found that *H. canis* prevalence was lower in dogs aged 5-10 years (14%) compared to those aged 1-5 years (20%) and over 10 years (29%). Pacifico et al. (66) found a significantly higher prevalence in medium and long-haired dogs, attributing this to the ease with which hard ticks can attach to such dogs and remain undetected, as was also mentioned in a previous study (46). Some studies (55, 66, 70) found no significant relationship between *H. canis* prevalence and age, while others (3, 39) reported significantly higher infection rates in adult dogs than in younger dogs. Pacifico et al. (66) noted that male dogs might have higher environmental exposure to tick-borne diseases due to their tendency to roam freely, which could explain why some studies find higher positivity rates in males. Pacifico et al. (66) also emphasized that breed is an important factor in *H. canis* infection in hunting dogs, suggesting that hounds, which come into close contact with wild mammals, may be more exposed to infected ticks than pointing dogs used in bird hunting. In the present study, only two of the 120 sampled dogs were owned pets, while the remaining 118 were stray animals temporarily housed in shelters for protection and treatment. All infected dogs were among the stray population. Although the vast difference in numbers between owned (n = 2) and stray dogs (n = 118) precludes statistical comparison, it can be expected that owned dogs, which are more likely to receive regular antiparasitic treatments and are less exposed to ticks than stray and shelter dogs, would have a lower infection rate. Pacifico et al. (66), referencing several epidemiological studies on *H. canis* in Europe (19, 30, 65) noted that the infections prevalence is generally related to seasonality and the distribution of suspected vector ticks. Indeed, local cases of *H. canis* are commonly reported in areas endemic to *R. sanguineus* (3, 10, 15, 36). However, in recent years, *H. canis* has also been detected in dogs in regions where *R. sanguineus* is not present (47, 60) and is often associated with *H. canis* infections in foxes and other wild carnivores (45, 59). Studies on the epidemiology of canine hepatozoonosis indicate that many factors that are intertwined in a complex network affect its spread, including geography, climate, vector density, and dogs' exposure to tick infestation. To obtain reliable data, it is essential to conduct research in different seasons, using as large a sample size as possible, involving both domestic and wild canids, and collecting ticks from these animals. It is also important to evaluate environmental factors and animal movements together to better understand the infection's dynamics.

Another important blood disease, canine granulocytic anaplasmosis (CGA), is a vector-borne disease that is commonly found in dogs worldwide. Studies have been conducted in Türkiye and globally to understand the epidemiology of the disease and the distribution

of its causative agents. For example, in the Aegean region 52.0% (49) and 40% (67) *A. Phagocytophilum* molecular positivities has been reported in dogs by different studies. In Sinop, 30.1% seropositivity found in dogs (43). A study in the Kayseri region reported a molecular prevalence of 7.8% in dogs using real-time PCR (34). Another study conducted in the Thrace region detected a molecular prevalence of 4% in dogs using nested PCR (28). Similarly, *A. phagocytophilum* prevalence was reported as 3.1% in stray dogs from Batman province using PCR (62). In a more recent study in the Thrace region 21.6% molecular prevalence were detected in dogs (8). Another study from Afyon, using rapid test kits, found a prevalence of 1.25% in dogs (20). In Antalya, where this study was conducted, a previous study (54) reported a prevalence of 0.44% in dogs using rapid test kits, while the present study detected a molecular prevalence of 3.33% using nested-PCR. The molecular prevalence reported in this study is similar to the results reported by Düzlü et al. (34), Çetinkaya et al. (28), and Oğuz and Değer (62), but significantly lower than the results from Karagenç et al. (49), Paşa et al. (67), and Altuğ et al. (8).

A review of the literature reveals several studies conducted in Türkiye that have investigated the presence of pathogens in vector ticks. For example, using PCR and DNA sequencing, the prevalences of *A. phagocytophilum* in *Ixodes ricinus* ticks were found to be 2.7% in Istanbul and 17.5% in Kırklareli (71). Another study from the Thrace region reported a 3.93% rate of *A. phagocytophilum* in a pool of *I. ricinus* collected from dogs and examined via PCR (28). In the Antalya region, where the present study was conducted, a study that typed ticks collected from goats and sheep in rural areas found that 80.4% were *I. ricinus,* the main vector of *A. phagocytophilum* (74). Another study in Antalya on ticks collected from goats reported the presence of *I. ricinus* (29.3%), *Rhipicephalus bursa* (46.0%), and *Haemaphysalis parva* (6%) – the latter two being other vectors of *A. phagocytophilum* (73). Also in a separate study on tick species collected from dogs in Antalya (52), *I. ricinus* was not found; however, *H. parva* (0.8%), as well as *R. sanguineus* (45.01%) and *R. turanicus* (37.89%), vectors of *A. platys*, were reported in high numbers. Due to time and budget constraints, no ticks were collected from the sampled dogs in this study, nor was the presence of the pathogen in ticks investigated. As in the epidemiology of other CVBDs (canine vector-borne diseases), various factors such as geographical and climatic conditions, the distribution of vector arthropods, their status as pathogen carriers, vertebrate host populations, and uncontrolled human and animal movements play significant roles in the epidemiology of anaplasmosis. It is evident that different results reported from different regions are influenced by these factors. To make more accurate epidemiological inferences, it is essential to consider these factors in future studies.

In CGA, as in many other diseases, understanding the predisposing factors and their degree of influence is important for control measures. In this study, no statistically significant difference was found between age and gender concerning *A. phagocytophilum* positivity. An analysis of different breeds could not be conducted due to insufficient data. Among similar studies conducted in Türkiye, only Altuğ et al. (8) examined the relationship between *A. phagocytophilum* positivity and gender and age and no correlation was found among gender or age groups. Similarly, a study conducted in Iran found no significant difference between age, gender, and *A. phagocytophilum* positivity (44). In a study conducted in Italy, (35) no significant difference was found between genders among hunting dogs, but they reported significantly higher seropositivity in dogs aged 6-10 years. The researchers attributed this to the possibility that this age group had been exposed to environments infested with *A. phagocytophilum*-infected ticks for longer than younger animals, and the lower prevalence observed in older dogs (over 10 years) could be related to a smaller sample size, as older dogs are less frequently used as hunting dogs (35). It is difficult to directly attribute age, gender, and breed as predisposing factors for *A. phagocytophilum* infection and epidemiology, highlighting the need for further detailed studies on this topic. However, it is worth considering that male dogs may experience higher environmental exposure to tick-borne diseases due to their tendency for free-roaming behavior (66). The ability of hard ticks to attach more easily and remain undetected on medium-to-long-haired animals (46) and the longer potential tick exposure duration in older animals (3) are also factors that should not be overlooked. Additionally, as mentioned in the canine hepatozoonosis section, only two of the 120 dogs sampled in our study were owned pets, while the remaining 118 were stray animals. All dogs that tested positive for *A. phagocytophilum* by PCR were among these stray dogs. The stark difference in the numbers of owned (n = 2) versus stray dogs (n = 118) does not allow for meaningful statistical comparison. Nonetheless, it is likely that owned dogs receive regular antiparasitic treatments for protection and therapy, and their chances of tick exposure are lower compared to stray dogs that lack regular care and live outdoors. Consequently, the higher infection rate in stray and/or outdoor dogs is an expected outcome.

Since *A. phagocytophilum* is also a zoonotic pathogen, it affects public health, making the evaluation of human cases epidemiologically significant. Numerous HGA cases have been reported from various regions of the world (32). In Türkiye, *A. phagocytophilum* has been identified in *I. ricinus* ticks collected from humans (4), and cases of HGA have been reported (40, 63). Güneş et al. (40) found 10.62% seropositivity in Sinop and 5.77% in Tokat. In a study examining hard ticks collected from humans (4), adult *I. ricinus*

(17.0%) and *Ixodes* spp. nymphs (22.2%) were found to be infected with *A. phagocytophilum*. In a study conducted in Antalya (63), the seropositivity rate was 8% in humans. The 3.33% molecular prevalence found in dogs in our study marks the first detection of *A. phagocytophilum* DNA in dogs in this city. When considered alongside the data on HGA and vector tick prevalence from Türkiye and Antalya, it is evident that *A. Phagocytophilum* poses a threat not only to animal health but also to public health in Antalya, with circulation of the pathogen in dogs, ticks, and humans. In regions with high tick populations, this can be a significant public health concern. Therefore, assessing human cases, in addition to animal cases, is crucial from an epidemiological perspective to understand the spread of the disease and develop effective control strategies. To draw more robust epidemiological conclusions, it would be beneficial to investigate the distribution of *A. phagocytophilum* in humans, dogs, and vector ticks with a larger sample size within the same study (One Health Concept). To protect dogs and the public from *A. phagocytophilum*, measures should include identifying and treating infected animals to eliminate their carrier status, synchronized tick control during periods of tick activity, daily self-examinations for ticks by high-risk individuals and occupational groups, reducing the stray animal population to acceptable levels through sound policies, and controlling animal and human movement with quarantine measures when necessary.

Finally, the accidental microscopic detection of *Babesia* spp. at a rate of 1.66% indicates the need for further epidemiological studies on blood parasites, particularly *Babesia* species, in dogs in the region. Therefore, the next study is planned to focus on canine *Babesia* species.

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