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The molecular prevalence of *Borrelia burgdorferi*, Babesia spp., and *Anaplasma* spp. in shelter dogs of the Thrace Region in Turkey

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Abstract: The study aimed to update the molecular prevalence of some tick-borne pathogens (*Borrelia burgdorferi*, *Babesia* spp., and *Anaplasma* spp.) in the shelter dogs of the Thrace Region, Turkey. The study was carried out on 450 dogs from 7 pet shelters. The individual data of the dogs were recorded, and blood samples were collected in tubes with anticoagulants (EDTA). Then, individual PCR protocols were applied to all samples for the three infective agents. PCR test results recorded for *B. burgdorferi* is 38.22% (n = 172), 24.22% (n = 109) for *Babesia* spp., and 21.6% (n = 97) for *Anaplasma* spp. The positivity of dogs with at least one pathogen was 56.22% (n = 253). Only one pathogen positivity rate was determined in positive samples as 56.92% (n = 144). The positivity was determined 33.99% (n = 86) for two pathogens and 9.09% (n = 23) for three pathogens. The coexistence of the two pathogens was statistically significant ($p < 0.01$). The effect of sex and age was not statistically significant in the agent positivity ($p < 0.01$, $p < 0.05$). Among tested three pathogens, only the positivity of *B. burgdorferi* ($p = 0.155$) was statistically significant compared with the prevalence of the others ($p < 0.01$). As a result, pathogens transmitted by ticks in shelter dogs of the Thrace region were simultaneously investigated and detected for the first time. Results revealed that shelter dogs pose a hidden risk for animal and human health in the region and so the necessity to plan systematic epidemiological studies about tick-borne zoonose pathogens more frequently.

Key words: *Borrelia burgdorferi*, *Babesia*, *Anaplasma*, shelter dogs, One Health, Thrace

1. Introduction

The increased notifications of tick-borne diseases threaten the global "One Health" measures. One Health approach supports earlier detection and understanding of zoonotic health problems, enabling a timelier and effective response to public health threats at the human-animal-environment interface. *Borrelia burgdorferi* (*B. burgdorferi*) is the etiologic agent of tick-borne Lyme disease that poses potential risks to human and pet health. The dogs may remain *B. burgdorferi*-infected for at least 17 months, but only 5% to 10% of the Lyme-positive cases are clinically symptomatic. Therefore, the symptomatic diagnosis is not a preferred method alone for Lyme confirmation. Due to the absence of specific clinical evidence in all cases, molecular diagnostic techniques are advantageous to conventional methods [1-4].

Some *Babesia* species transmitted to dogs by vector ticks are zoonotic. The canine babesiosis has symptomatic forms such as subclinical, acute, or chronic, which differs in species [3, 5]. Molecular diagnosis is highly sensitive to canine babesiosis regardless of symptoms and clinical signs [2, 5, 6]. Another tick-borne zoonotic genus is the *Anaplasma* spp., which causes different infections in humans, dogs, horses, cattle, sheep, and goats [3]. While most dogs infected with various *Anaplasma* species can remain asymptomatic and look healthy, especially in endemic areas, self-limiting can end the infection in some cases [7].

Thrace of Turkey and its borderline EU member countries have common public health concerns about tick-borne zoonosis. Unqualified animal shelters can gather pathogens, vectors, and hosts together, so animal shelters' role in transmitting infectious diseases cannot be ignored [8]. The prevalence of tick-borne infections are reported higher in shelter dogs than in owned dogs [8-10]. The updated information on simultaneous molecular diagnosis of shelter origin zoonosis in the Thrace of Turkey is insufficient. Among the tick-borne zoonotic pathogens, *B. burgdorferi*, *Babesia* spp., and *Anaplasma* spp. were previously reported in Turkey, Greece, and

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Bulgaria [11-18]. This research refers to the first molecular detection of *B. burgdorferi*, *Babesia* spp., and *Anaplasma* spp. simultaneously in Thrace of Turkey shelter dogs. Evaluation of the results has provided comprehensive, up-to-date data required in preventive veterinary medicine and biosecurity.

2. Materials and methods

2.1. Animal materials and sampling

Blood samples were obtained from dogs from "Municipal Animal Rehabilitation Centers" in Tekirdağ-Süleymanpaşa, Tekirdağ-Çorlu, Çanakkale-Gelibolu, Kırklareli-Lüleburgaz, Kırklareli-Center, Edirne-Center, and İstanbul-Kısırkaya located in the Thrace Region of Turkey. Shelter selection was done to represent the sample of the Thrace Region provinces. Approximately 10% of the dog populations in the areas under the control of the shelters were sampled. The blood samples were taken via venipuncture of "*Vena cephalica antebrachii*" into anticoagulant tubes and transferred to the laboratory at 4 °C. The tubes were centrifuged for 10 min at 2000 rpm, and leukocyte samples were stored at –80 °C for molecular tests.

The age, race, sex, pregnancy, lactation, vaccination, and antiparasitic treatment records were gathered from shelter archives. The clinical symptoms observed in the sampling were recorded. Animals that did not show clinical symptoms during sampling were recorded as healthy, while symptomatic animals were recorded separately. Accordingly, the health status of the sampled animals was divided into two categories, namely, sick and healthy. The sick animals were further divided into subgroups according to their symptoms: fever, depression, kennel cough, tick infestation, scabies, and cachexia.

The research was performed under the permission of Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee No. 2017 / 03 - 4.

2.2. Nucleic acid extraction

As per the manufacturer's protocol, a commercial extraction kit (Thermo Scientific GeneJET DNA Purification Kit, USA) was employed to obtain DNA from the blood samples. Accordingly, 400 µL of lysis solution and 20 µL of proteinase K were added to the blood sample and the mixture was incubated for 10 min at 56 °C. The samples were transferred to mini spin columns and washed. The samples were placed in a fresh 1.5-mL Eppendorf tube, and elution was performed using 200 µL of nuclease-free water. The DNA samples were stored at 20 °C for use in polymerase chain reaction (PCR) analysis.

2.3. Polymerase chain reaction analysis

Different PCR protocols were applied for the three agents investigated within the scope of the research. BioRad T100 thermal cycler device was used for PCR analysis. Optimization trials were performed with different primer pairs targeting different gene regions, and suitable PCR conditions and protocols were determined. Positive control DNA was used for PCR optimization.

The Taq polymerase enzyme to be used in PCR analysis was a preoptimized supermix (Evagreen super mix, BioRad, USA) containing original buffer mixture, dNTP mixture, $MgCl_2$ ratio, and next-generation content that eliminates PCR inhibitors. The mixture quantities were adjusted according to the manufacturer's recommendations.

The relevant genes specific to each pathogen were selected from the literature, and primer pairs synthesized accordingly were used in PCR analysis. Universal primer pairs targeting the 16S rRNA gene (A1: 5-′CGGGATCCCGAGTTTGCCGGGACTTYTTCT -3′ and A2: 5′ - GGAATTCAGAGGGATCMTGGYTCAG - 3′) were used for *Anaplasma* spp. identification [19]. Universal primer pairs targeting the 18S rRNA gene (B1 : 5′ - TGACACAGGGGTAGTGACA - 3′ and B2 : 5′ - CAGGACATCTAAGGGCATCA - 3′) were used for *Babesia* spp. identification [20]. Lastly, primer pairs targeting the 5S rRNA and 23S rRNA gene (BB1 : 5′ - CTGCGAGTTCGCGGGAGA - 3′, BB2 : - 5′ - TCCTAGGCATTCACCATA -3′) were used for *Borrelia burgdorferi* identification [21]. The PCR products were visualized on agar gels. For all PCR applications, 10X Taq buffer, 3 mM $MgCl_2$, 2U Taq polymerase, 2 µM forward and reverse primers, and 200 nmol dNTP, and water were used with a total volume of 30 µL. Different reaction conditions were applied for each primer. Five minutes of predenaturation at 95 °C, 40 cycles of 1 min at 95 °C, 1 min at 50–57 °C, and 1 min at 70 °C, and finally 7 min at 70 °C were standard in all PCR applications.

2.4. Agar gel electrophoresis

Agar gel electrophoresis was used to visualize specific DNA regions replicated as PCR products. For this purpose, 1.5%–2% agarose (Sigma, America) was mixed in 0.5X TAE buffer solution, and 5 µL/mL EtBr (Invitrogen, America) was added to the mix. After the PCR products were loaded onto the gel, 8 volt/cm current was given, and the gels were run for 20–30 min. DNA bands were visualized under ultraviolet light with the help of a gel transilluminator (WiseDoc).

2.5. Statistical analysis

Statistical analysis was conducted to investigate any significant relationship between the shelter records and the laboratory data obtained using PCR analysis. SPSS package program (version 18.0) was used for data analysis. Chi-squared test and correlation analysis were used to investigate the relationship between the variables. $p < 0.05$ and $p < 0.01$ were accepted as statistically significant.

3. Results

Within the scope of the present research, a total of 450 dogs were sampled from shelters in seven different provinces and districts in the Thrace region. The demographic characteristics and clinical status of the sampled dogs are summarized in Table 1.

3.1. Sampled animals

Of the 450 dogs sampled, 41.8% (n = 188) were male and 58.2% (n = 262) were female (Table 1). During the field efforts, sampling was performed on dogs of different age groups while also considering the differences between shelters. The age of the sampled animals ranged between 2 and 156 months, and the mean age was 38.55 months. The weight of the sampled animals varied between 2 and 55 kg, and the mean weight was 21.68 kg. Furthermore, 349 (77.6%) dogs were recorded as a crossbreed.

In six shelters, a total of 301 dogs (66.88%) were registered as receiving internal and external parasitic treatments within the scope of antiparasitic treatment. Antiparasitic treatment was applied to almost all animals sampled in İstanbul and Lüleburgaz, while this rate varied in other shelters. No information on antiparasitic treatment could be obtained for animals sampled in Gallipoli.

The vaccination status was evaluated under two separate categories of polyvalent and rabies vaccines. Two shelters had no vaccination information. A total of 28 (6.22%) dogs had received polyvalent vaccines in two shelters (İstanbul and Edirne) within the last 12 months of sampling.

In this study, 77.6% (n = 349) of the sampled animals were recorded as healthy, and 22.4% (n = 101) were recorded as sick (Table 2). The sick animals ranged from 0.9% to 29.7% in the different shelters. The highest number of sick animals was recorded in the Edirne shelter. The most common clinical symptoms in the 101 animals recorded as sick were scabies, with a rate of 46.5% (n = 47), and kennel cough, with a rate of 39.6% (n = 40). The other clinical findings were fever, depression, tick infestation, and cachexia, with rates of 4.9% (n = 5), 2.9% (n = 3), 8.9% (n = 9), and 7.9% (n = 8), respectively. More than one clinical symptom was observed in some of the sick animals.

3.2. Polymerase chain reaction results

PCR tests explicitly conducted for the three pathogens were visualized using gel electrophoresis (Figures 1–3).

According to the results of the PCR tests, the rate of positivity was 38.22% for *B. burgdorferi* (n = 172), 24.22% for *Babesia* spp. (n = 109), and 21.55% for *Anaplasma* spp. (n = 97) (Table 3). The positivity rates for tick-borne pathogens varied among the seven shelters. *B. burgdorferi* positivity, which was the highest among the pathogens investigated, ranged from 17.39% to 60% in the sampled shelters. *Babesia* spp. positivity varied between 5% and 42.85%. *Anaplasma* spp. positivity ranged from 2% to 31.42% in the sampled shelters.

The percentage of the sampled animals that were positive for at least one of the pathogens was 56.22% (n = 253). This rate varied among the shelters. Single/multiple positivity rates varied for the three pathogens investigated among the different shelters. In the positive samples, the rate of positivity for only one pathogen was 56.92% (n = 144), the rate of positivity for two pathogens was 33.99% (n = 86), and the rate of positivity for all three pathogens was 9.09% (n = 23).

Among the positive animals for only one pathogen (n = 144), *B. burgdorferi* was the most common pathogen, with a rate of 33.20% (n = 84). The positivity rate for only *Babesia* spp. was 13.44% (n = 34), and the rate of positivity for only *Anaplasma* spp. was 10.28% (n = 26). Among the positive animals for two pathogens (n = 86, 33.99%), *Babesia* spp./*B. burgdorferi* positivity was observed in

Table 1. General condition of the sampled animals by shelters*.

Shelter number	Sampling site	Sex		Vaccination status		Health status		Parasitic treatment	
		Male	Female	Polyvalent	Rabies	Healthy	Patient	Internal	External
1	İstanbul	101	106	23	184	187	20	207	207
2	Edirne	27	53	5	18	50	30	19	19
3	Lüleburgaz	13	37	0	27	39	11	49	49
4	Kırklareli	17	6	0	9	12	11	11	11
5	Çorlu	13	22	0	9	22	13	10	10
6	Gelibolu	3	17	0	0	19	1	0	0
7	Süleymanpaşa	14	21	0	0	20	15	5	5
Total (%)		188 (41.8)	262 (58.2)	28 (6.2)	247 (54.88)	349 (77.55)	101 (22.44)	301 (66.88)	301 (66.88)

**Vaccination and parasitic treatment data are provided according to shelter records (CI: 95%).*

Table 2. Distribution of clinical appearance of animals registered as sick in shelters*.

Shelter number	Total number of sick animals *n (%)*	Clinical appearance *n (%)*					
		Fever	Depression	Kennel cough	Tick	Scabies	Cachexia
1	20 (19.80)	3	3	1	2	13	1
2	30 (29.70)	2	-	16	6	7	5
3	11 (10.89)	-	-	9	-	2	-
4	11 (10.89)	-	-	4	1	7	-
5	13 (12.87)	-	-	10	-	4	-
6	1 (0.99)	-	-	-	-	-	1
7	15 (14.85)	-	-	-	-	14	1
Total	101 (22.44)	5 (4.95)	3 (2.97)	40 (39.60)	9 (8.91)	47 (46.53)	8(7.92)

**Some sick animals exhibited more than one clinical manifestation.*

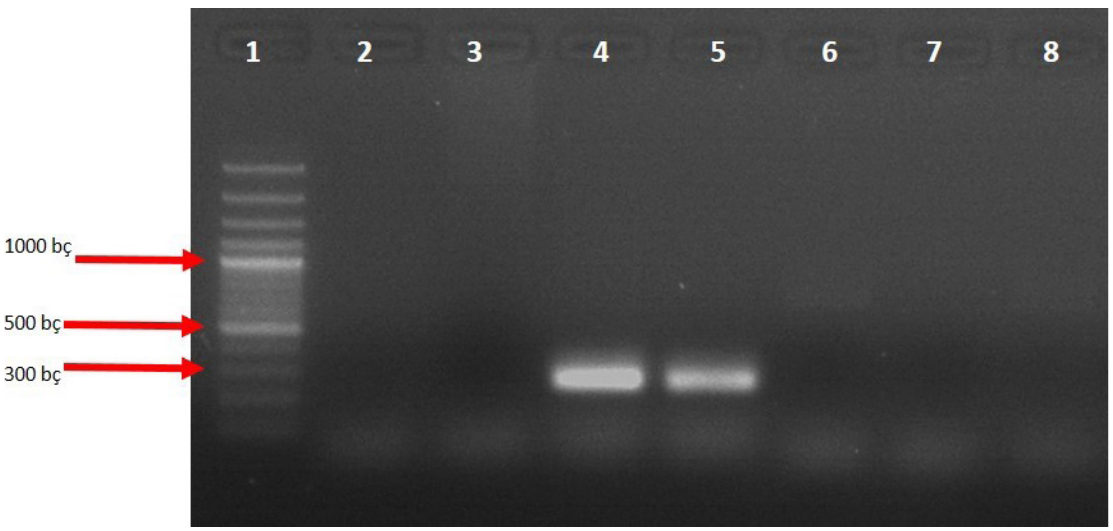

Figure 1. PCR results for *Anaplasma* spp. visualized using gel electrophoresis 1: DNA ladder, 4, 5: positive samples, 2, 3, 6, 7, 8: negative samples.

16.21% (n = 41), *Anaplasma* spp./*B. burgdorferi* positivity was observed in 13.83% (n = 35), and *Anaplasma* spp./*Babesia* spp. positivity was observed in 3.95% (n = 10) of the animals. *B. burgdorferi* positivity with either of the other two pathogens was seen in 30.03% (76/253) of the animals. Furthermore, 9.09% of the animals were positive for all three pathogens (n = 23). The positivity for the two pathogens was statistically significant ($p < 0.01$).

When the shelters were evaluated in terms of positivity for the pathogens, the positivity rates for at least one pathogen varied between 25% and 77.14%. When pathogen positivity was examined concerning sex, it was found that the positivity rates were higher in female animals than in male animals. When evaluated separately for each pathogen, 70.1% of *Anaplasma* spp.-positive animals, 63.3% of *Babesia* spp.-positive animals, and 59.88% of *B. burgdorferi*-positive animals were female (Table 4). The age distribution of the sampled animals according to pathogen positivity is shown in Table 4. According to the relevant literature, pup and adult ages were evaluated under five different age categories. Positivity for all three pathogens was seen in different age groups, but the rates were higher in animals under 60 months. However, no significant difference was found in the positivity rates concerning sex and age.

When the clinical appearance of the positive animals in PCR analysis was evaluated, it was found that four animals from İstanbul and Edirne with symptoms of "fever" were

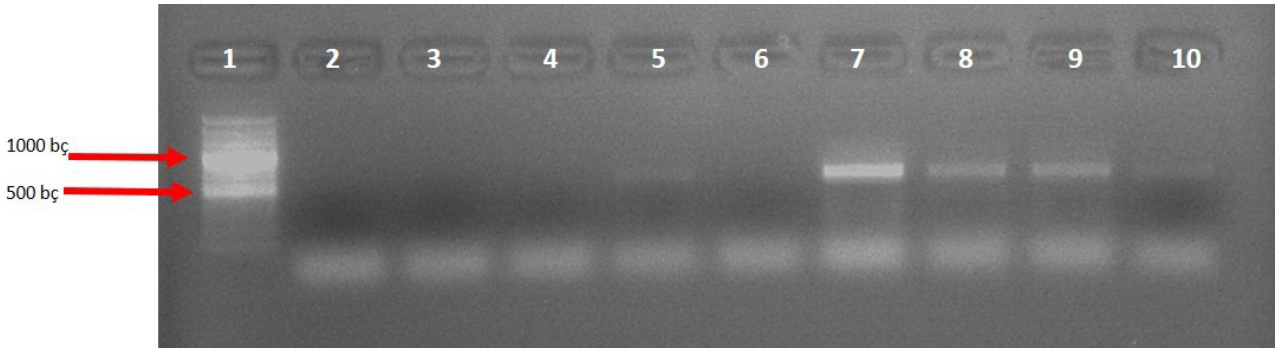

Figure 2. PCR results for *Babesia* spp. visualized using gel electrophoresis 1: DNA ladder, 10: positive control, 5, 7, 8, 9: positive samples, 2, 3, 4: negative samples.

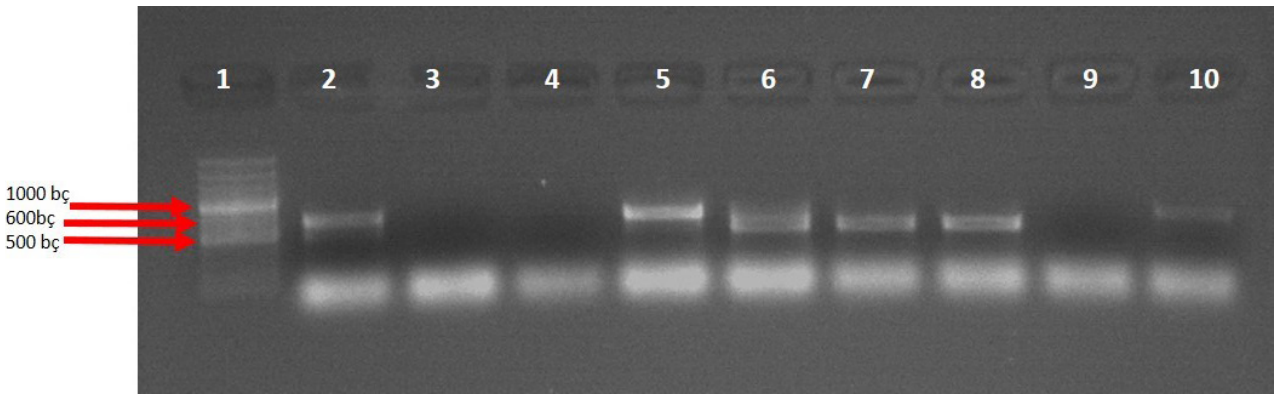

Figure 3. PCR results for *B. burgdorferi* visualized using gel electrophoresis 1: DNA ladder, 2: positive control, 3: negative control, 5, 6, 7, 8, 10: positive samples, 4, 9: negative samples.

Table 3. Positivity rates of the pathogens by shelters.

Shelter number	Total number of sampled animals *n (%)*	*Anaplasma* spp. *n (%)*	*Babesia* spp. *n (%)*	*B. burgdorferi* *n (%)*
1	207 (46.00)	48 (23.18)	45 (21.73)	72 (34.78)
2	80 (17.77)	16 (20.00)	27 (33.75)	32 (40.00)
3	50 (11.11)	8 (2.00)	12 (24.00)	20 (40.00)
4	23 (5.11)	4 (17.39)	6 (26.08)	4 (17.39)
5	35 (7.77)	7 (20.00)	15 (42.85)	18 (51.42)
6	20 (4.44)	3 (15.00)	1 (5.00)	5 (25.00)
7	35 (7.77)	11 (31.42)	3 (8.57)	21 (60.00)
Total	450 (100)	97 (21.55)	109 (24.22)	172 (38.22)

positive for at least one pathogen. Among the animals with symptoms of "depression," only three animals from İstanbul were positive for *B. burgdorferi*, and one animal from İstanbul was positive for *Anaplasma* spp. Moreover, 60% of the animals with "kennel cough" symptoms were positive for at least one pathogen (Table 5). *B. burgdorferi* was the most common pathogen in pathogen-positive animals with clinical symptoms. Compared with the other pathogens, *B. burgdorferi* was more common in samples recorded as having symptoms of "cachexia, scabies, and tick infestation" (Table 5). The symptomatic cases for positive results were only shown in *B. burgdorferi* infection, and it was found to be statistically significant ($p < 0.01$). When the vaccination status of PCR-positive animals was

Table 4. Distribution of polymerase chain reaction positivity by age and sex.

Pathogens	Number of positive animals *n*	Sex *n (%)*		Age *n (%)*				
		Male	Female	<12 months	12–<36 months	36–<60 months	60–<84 months	≥84 months
Anaplasma spp.	97	29 (29.9)	68 (70.1)	24 (24.7)	25 (25.8)	32 (32.9)	9 (9.3)	7 (7.2)
Babesia spp.	109	40 (36.7)	69 (63.3)	24 (22.0)	29 (26.6)	30 (27.5)	12 (11.0)	14 12.8)
B. burgdorferi	172	69 (40.3)	103 (59.8)	43 (25.0)	37 (21.5)	58 (33.7)	18 (10.5)	16 (9.3)

Table 5. Distribution of polymerase chain reaction results by clinical appearance and vaccination status of the animals.

Pathogens	Total *n*	Vaccination status *n (%)*				Health status *n (%)*						
		Polyvalent vaccine administered		Not vaccinated		Healthy	Patient					
		Healthy	Sick	Healthy	Sick		Fever	Depression	Kennel cough	Tick	Scabies	Cachexia
Anaplasma spp.	97	6 (6.18)	4 (4.12)	68 (70.1)	19 (19.58)	74 (76.28)	4 (4.12)	1 (1.03)	6 (6.18)	2 (2.06)	10 (10.3)	2 (2.06)
Babesia spp.	109	6 (5.5)	1 (0.9)	76 (69.72)	26 (23.85)	82 (75.22)	1 (0.9)	-	10 (9.17)	1 (0.9)	14 (12.84)	4 (3.66)
B. burgdorferi	172	7 (4.06)	4 (2.32)	109 (63.37)	52 (30.23)	116 (67.44)	3 (1.74)	3 (1.74)	20 (11.62)	12 (6.97)	21 (12.2)	3 (1.74)

evaluated, the pathogen positivity rates were higher in healthy and sick animals without vaccination (Table 5). When the effect of vaccination on pathogen positivity was evaluated, no significant relationship was found between vaccination status and *Babesia* spp. and *Anaplasma* spp. positivity. However, a very weak positive correlation was found between vaccination and *B. burgdorferi* positivity ($p = 0.19$).

4. Discussion

Tick-borne pathogens, which are common in human and veterinary medicine, are difficult to prevent and control. Combating these pathogens is possible with the cooperation of veterinarians and human physicians, by evaluating the current data in the concept of "One Health" [22]. In this context, tick-borne pathogens *Anaplasma* spp., *Babesia* spp., and *B. burgdorferi* were investigated simultaneously for the first time in shelter dogs from the Thrace Region (Table 1) and the results of this study were compared with previous data. Accordingly, the results of previous studies to identify single and multiple infections differ considerably between the regions and countries studied. Multiple positivity rates about pathogens are the subject of this study have been reported in a wide range of 0.17%–71.89% [23-28].The number of dogs found positive for at least one pathogen is 253. The one, two, and three pathogen positivity was 56.92% (n = 144), 33.99% (n = 86), 9.09% (n = 23), respectively, in the study. This finding is in the previously reported range of 0.17%–71.89% for these pathogens by various researchers [23-28]. The present results are similar to the cooccurrence of two and three pathogens in previous studies [13, 17, 24, 28]. Multiple positivity results of *D. immitis*, *Ehrlichia* spp., *Anaplasma* spp., and *B. burgdorferi* were 5.3% in Greece just near the study area [13], which is considerably higher than the multiple positivity in this study. Current results are similar to reports from Bulgaria about *A. phagocytophilum*, *B. canis*, *B. burgdorferi*, *E. canis*, *D. immitis*, in which multiple positivity was 31.14% [17].

Various rates of multiple pathogen positivity have been reported in Turkey dogs so far. In a study evaluating *A. platys*, *B. canis*, *T. annulata*, and *E. canis* multiple pathogen positivity was 0.26% [9]. In a study evaluating *Anaplasma* spp. and *Ehrlichia* spp. in shelter dogs of the Thrace Region, it was 3.0% [29]. In a study in which eight tick-borne pathogens including *Babesia* spp. and *Anaplasma*

spp. were evaluated in Konya, it was 1.04% [30]. In a study in Kayseri where *Babesia* spp., *A. phagocytophilum*, *H. canis*, and *E. canis* were evaluated, it was 5.25% [31]. In a study in which *D. immitis*, *B. burgdorferi*, *E. canis*, and *A. phagocytophilum* were evaluated in Antalya, it was 0.44% [32]. In a study in which *B. burgdorferi* and *A. phagocytophilum* were evaluated in Sinop, it was 20.43% [33]. In a study in Diyarbakır where *Babesia* spp., *Theileria* spp., *Hepatozoon* spp. were evaluated, it was 3.20% [34]. In a study evaluating *Babesia* spp., *Leishmania* spp., *Hepatozoon* spp., *D. immitis*, *D. repens*, *A. reconditum*, *E. canis*, and *A. platys* in shelter dogs in Erzurum, it was 5.26% [35]. Multiple positivity rates obtained in this study were similar to the study evaluating *B. burgdorferi* and *A. phagocytophilum* in Sinop [33], yet it was considerably higher than the positivity rates of tick-borne dog pathogens in Turkey.

B. burgdorferi-induced Lyme disease poses a global risk to human and pet health [3-4]. Molecular diagnostic studies on *B. burgdorferi* are limited. In different studies, the molecular prevalence of *B. burgdorferi* has been reported to be 1.27% in healthy sled dogs in Poland [36], 0.8% in sheltered and owned dogs in Portugal [23], 18.5% in a population consisting primarily of sheltered dogs in Egypt [37], and 0.0% in Greece [14], in Romania [38], and in the United States [26]. A previous molecular diagnostic study conducted on a limited number of dogs in Turkey reported no positive results [39]. In the present research, *B. burgdorferi* was the pathogen with the highest molecular prevalence (Table 3).

Aside from the Thrace region, the serological prevalence of *B. burgdorferi* in Turkey has been reported to be between 23.2% and 27.75% in owned, healthy dogs with clinical symptoms brought to the Uludağ University Veterinary Faculty Clinic [40], 28% in owned, healthy dogs in Sinop Province [33], 0.65% in sick dogs with owners in the Aegean Region [41], and 0.0% in studies using SNAP tests [32, 42].

The serological prevalence of *B. burgdorferi* has been reported to be 0.1%–22.9% in Greece [13, 15, 16] and 2.4% [17] and 1.7% [18] in Bulgaria. One study in Romania reported seronegative results [38], while another reported 6.52% positivity [43]. Moreover, 0.69% positivity has been documented in Croatia [24]. In Serbia, one study stated 24.7% positivity in dogs with owners, sheltered, and hunting dogs, and the positivity rate was the highest in sheltered dogs (31.2%) [10]. The seroprevalence in Germany was 30.19% [25]. Seronegativity was recorded in sheltered dogs in Italy [27]. In other countries, in Iran, *B. burgdorferi* positivity was 9.52% in healthy-looking dogs [44], and 0.34% in owned and sheltered dogs in Costa Rica [28]. Seronegativity was observed in a study in Taiwan [45]. Furthermore, 36% *B. burgdorferi* positivity was noted in the United States in a population consisting of 88% of healthy dogs with owners and 12% of dogs with *Anaplasma* or *Borrelia* clinical findings [46]. In the United States, *B. burgdorferi* positivity was 1.0% in healthy-looking dogs and 3.4% in dogs with clinical manifestations [26]. *B. burgdorferi* positivity was reported to be 9.7% in healthy-looking dogs with owners in Brazil [47], 71.4% in 70 sheltered dogs, and 30 dogs with owners in Egypt [37], and 0% in Malaysian sheltered dogs [8]. Except for some studies conducted in Egypt [37] and the United States [46], *B. burgdorferi* dog positivity has been found higher in the present research than other serological and PCR studies, including those conducted in Turkey's borderline countries.

Babesia spp. PCR positivity in sheltered and stray dogs in Turkey has been observed to be between 0.13% and 23.3%. There are no studies with representative data on shelters, which provides a clear picture of the prevalence of *Babesia* spp. in the Thrace Region. In these studies, the positivity rates were 23.3% in Kayseri [31], 4.6% in Diyarbakir [34], 0.13% in the sampled population from Elazığ, Diyarbakır, Erzurum, Ankara, and Nevşehir [9], 5.3% in Erzurum [35], 2.1% in Konya [30], 2.7% in some provinces of the Mediterranean and South-eastern Anatolian regions [39], and 8.7% in the Aegean Region [48]. In the present research, *Babesia* spp. PCR positivity was comparable to the previously detected rates in Kayseri in a similar population [31]. However, the *Babesia* spp. positivity was considerably higher than the rates obtained in other regions of Turkey.

Babesia spp. PCR positivity in dogs has been reported between 0.1% and 87.8% in Europe [6]. In Greece, which borders the Thrace region in which the present research was conducted, the molecular prevalence of *Babesia* spp. in sheltered dogs has been observed to be 0.5% [14], and the serological prevalence of *Babesia* spp. has been found to be 16.2% in dogs in Bulgaria [17]. The positivity rate obtained in the present research is within the positivity range in Europe, but higher than the rates in Greece [14] and Bulgaria [17].

The *Babesia* spp. molecular prevalence is 0% in stray dogs of Iraq [49], 0.99% in shelter dogs of Iran and Pakistan [50], 54% in sheepdogs in Pakistan [51], 30% in stray dogs of Saudi Arabia [52], and 15% in shelter dogs of Japan [53]. The current *Babesia* spp. positivity result is lower than in Pakistan [51] and Saudi Arabia [52]. However, it is considerably higher than the positivity rates observed in similar populations in borderline countries such as Iraq [49] and Iran [50], indicating that *Babesia* spp. may pose significant risks for human and animal health in Turkey.

Anaplasma spp. PCR positivity in sheltered and stray dogs in Turkey has been documented to range between 0.0% and 32.4%. In these studies, *A. phagocytophilum*

positivity has been stated to be 7.8% in Kayseri [31] and 3.1% in Batman [54]. Furthermore, *A. phagocytophilum* positivity has been observed to be 4% and *Anaplasma platys* positivity to be 6% in the Thrace region [29], *A. platys* positivity has been stated to be 0.5% in Elazığ, Diyarbakır, Erzurum, Ankara, and Nevşehir provinces [9], 32.4% in sheltered dogs in Diyarbakır [55], and 0% in Erzurum [35] and Konya [30]. Except for the rate reported in Diyarbakır [55], *Anaplasma* positivity obtained in the present research is higher than the previously determined positivity rates in the Thrace region having a similar population [29] as well as other regions of Turkey [9, 30, 31, 35, 54].

The molecular positivity of *Anaplasma* spp. in dogs is 0.0%–14.0% in some European Countries, 0.0%–9.5% in the Americas, 0.0%–57.3% in Asia, and 0.0%–2.1% in Africa [7]. *Anaplasma* spp. positivity has been documented to be 0.5% in sheltered and stray dogs in Greece [14], 2.75% in Romania [56], 1.3% in Hungary [56], 1.4% in Italy [27], and 1.9% in Portugal [23]. *Anaplasma* positivity has been recorded to be 1.0% in sheltered and stray dogs in Iraq [49], 6.4% in Iran and Pakistan [50], 57.1% in Saudi Arabia [52], and 1.70% in Costa Rica [28]. In the present study, the rate of *Anaplasma* spp. positivity identified using PCR analysis was 21.6% (n = 97) (Table 3). The *Anaplasma* spp. positivity obtained in the present research is lower than that reported in Asia, 57.3% in Iran [57], %39.5% in Jordan [58], and 57.1% in Saudi Arabia [52]. The canine *Anaplasma* spp. positivity is higher than previous results both globally and in neighbouring countries such as Greece [14], Iran [50], and Iraq [49].

According to previous studies, there is no significant relationship between pathogen positivity and sex [9, 13, 23, 24, 33-35, 43-45, 55]. Similar to the studies mentioned above, no significant relationship was found between pathogen positivity and sex in the present research (Table 4; $p < 0.05$). However, contrary to our findings, studies report that tick-borne diseases, including those caused by *B. burgdorferi*, *Babesia* spp., *and Anaplasma* spp. are more common in males than in females [14].

Studies investigating diseases transmitted by ticks used different age categories such as young, adult, and old. In the present research, the dogs were evaluated under five different age categories (Table 4) and, consistent with the literature, age had no significant effect on pathogen positivity ($p > 0.05$) [13, 23, 28, 33-35, 40, 43, 45, 47, 55]. The age categories in the present research were also consistent with other studies evaluating similar age groups [13, 23, 35, 43]. However, it has been reported that pathogen positivity increases with adult animals [9, 14, 59] or advancing age [36, 44].

In the present research in which symptomatic and asymptomatic dogs were randomly sampled (Table 2), the effect of clinical appearance on pathogen positivity was significant only for *B. burgdorferi* ($p = 0.155$) ($p < 0.01$). This result is consistent with the literature on *B. burgdorferi* [14, 40, 46]. Symptoms of "cachexia, skin lesions (such as scabies), and tick infestation" were more common in dogs positive for Lyme disease when compared with the other agents (*Anaplasma* spp. and *Babesia* spp.) (Table 5). The symptomatic findings obtained in this research have also been reported previously in relation to the positivity of tick-transmitted diseases in dogs [14, 36, 38, 45, 47].

Although some of the dogs sampled in the present research had received polyvalent vaccines, no records were obtained for *B. burgdorferi* vaccination in the sampled population. However, a very weak positive correlation was found between *B. burgdorferi* positivity and polyvalent vaccination status ($p = 0.19$). Consistent with several previous studies [40, 43, 44], polyvalent vaccination status could not be associated with pathogen positivity due to its lack of protection for *B. burgdorferi*.

5. Conclusion

The increased global incidence of tick-borne diseases is easily associated with abnormal climatical and ecological data with ascending mobility in human and animal populations. At this point, the necessity to update previous results more frequently is also increased. In the present research, the first simultaneous molecular diagnosis of some tick-borne zoonotic agents directly related to the "One Health" concept in the dogs of Thrace region, Turkey. Based on the results, high PCR positivity rates for tick-borne zoonotic pathogens were observed in shelter dogs, which spend most of their lives on the streets and are only housed in shelters for a certain period. Because these dogs have contact with people, the results are essential from animal and public health perspectives. Therefore, preventive measures, including vector and reservoir management, should be implemented for different pathogens transmitted by vectors. The Thrace region, which mediates the circulation of human and animal populations between Europe and Asia, is a critical bio-transit point for "One Health".

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