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Research article

A RETROSPECTIVE EPIDEMIOLOGICAL STUDY: THE PREVALENCE OF *EHRLICHIA CANIS* AND *BABESIA VOGELI* IN DOGS IN THE AEGEAN REGION OF TURKEY

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Among tick-borne diseases, *Ehrlichia canis* and *Babesia* piroplasm cause important diseases in dogs where the distributions of the pathogen, vector and host overlap. The primary aim of the present study was to detect the prevalence of *Babesia* spp. and *E. canis* using PCR and reverse line blot (RLB) hybridization assay in a total of 379 samples comprising stray and owned dogs and to compare the diagnostic sensitivity of the two tests. Overall, 41.4% of dogs were infected with *B. vogeli* and/or *E. canis* as single (35.4%) and mixed (6.1%) infections. The majority of *Babesia* positive dogs (74.1%) were co-infected with *E. canis*. PCR detected a higher (P= 0.000) number of positivity in some provinces compared to RLB. To the best of our knowledge, these findings provide the first molecular evidence for the existence of *B. vogeli* in the Aegean Region, Turkey. The present study pinpoints the distribution and prevalence of *E. canis* and *B. vogeli* in the Aegean region of Turkey as of 2004 and as such establishes a baseline. This is of pivotal importance for future studies aimed to demonstrate changes in the dynamics of *E. canis* and *B. vogeli* infections in the region.

Key words: *Aegean region,* Babesia*,* Ehrlichia*, Dog, Prevalence, retrospective*, *Turkey*

INTRODUCTION

Ehrlichiosis and babesiosis are tick-borne diseases, caused by *Ehrlichia* and *Babesia* species*,* respectively, with a worldwide occurrence. These species are transmitted during blood feeding by infected ticks and the diseases threaten animal welfare and some also represent a concern to human public health and are considered as important tick-borne diseases in tropical and subtropical areas [1].

Ehrlichiae species are obligate intracellular organisms infecting the leukocytes of many vertebrates [2]. Dogs may be infected by several different *Ehrlichia* or *Anaplasma* species

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and among them, *E. canis* infects monocytes and cause canine monocytic erhlichiosis [3]. *E. canis* is transmitted through *R. sanguineus (sensu lato)* in tropical and subtropical regions as is the case for some *Babesia* spp.. Dogs infected with *E. canis* develop various clinical signs from asymptomatic to severe [3]. While dogs respond well to treatment during the acute stage of *E. canis* infection, it may be difficult to eliminate, and dogs become chronically infected, serving as reservoirs for the organism. When recrudescence occurs, dogs may become severely infected [4] and prognosis is poor during this stage [5].

Babesia parasites invade and proliferate in red blood cells (RBC) of their vertebrate hosts including carnivores, ruminants, equines, rodents and humans. Canine *Babesia* parasites are divided into to two morphologically distinct groups as large (3.0–5.0 μm) and small (1.5–2.5 μm) piroplasms [6]. The first group comprises *B. canis, B. vogeli* and *B. rossi* and more recently, an unnamed fourth "large" *Babesia* sp. (*coco*) has been identified in immunosuppressed dogs in the United States [7]. The second group, classified as small piroplasms, consists of *B. gibsoni* [1], *B. conradae* [8], and *B. vulpes* [9]. As they have different vector specificity, the distribution of *Babesia* spp. overlaps with the distribution of transmitting *Rhipicephalus*, *Dermacentor* and *Haemaphysalis* ticks [1,10]. *Babesia* spp. also differ in their antigenicity and pathogenicity to dogs [11,12] and the severity of the disease ranges from subclinical to severe, depending on the species of *Babesia,* immune status and the age of dogs as well as the presence of co-infections [6,7].

Apart from a few human cases caused by *Babesia* spp. of dogs [13] and *E. canis* [14], they do not appear to pose a serious zoonotic risk. It is obvious that detecting dogs serving as carriers of *E.canis* and *Babesia* spp. will lead to a more accurate description of the distribution of these species. This is an important prerequisite for the implementation and improvement of control measures to reduce public health concerns related to these species. However, the detection of these parasites is difficult due mainly to the reduction in the number of *Babesia* piroplasms in circulating blood in long term and seasonal fluctuations in *E. canis* parasitaemia [15]. Occurrence of concurrent infections together with *Babesia* spp. and *E. canis* or with other haemoparasites results in a more complex situation [16-18].

A number of studies on canine babesiosis and monocytic erhlichiosis have been performed in Turkey**.** To date, two large *Babesia* species, namely *B. canis* and *B. vogeli* and one small *Babesia* species, *B. gibsoni* have been identified in dogs [16,17,19-23]. Canine monocytic erhlichiosis caused by *E. canis* was also reported in Turkey [16,17,24,25].

However, an accurate description of the prevalence and the distribution of *Babesia* spp. and *E. canis* in dogs is still lacking. Alterations in the dynamics of the infections throughout the years have been indicated for the Aegean region [26,27]. Therefore, the aim of the present study was to determine the prevalence of *Babesia* spp. and *E. canis* among stray and owned dogs in six different provinces located in the West Aegean Region of Turkey using a standard single PCR and nested PCR, respectively from a

retrospective perspective. RLB assay was performed for the differential detection of *Babesia* parasites at species level. The diagnostic sensitivity of the two tests was also compared.

MATERIALS AND METHOD

Ethical statement

An approval from an institutional Animal Ethics Committee was not required to collect samples from any animal species including dogs as of year 2004, during which all samples were collected for the present study. However, authors declare that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects".

Parasite material and sample collection

The present study was conducted within provinces comprising coastal regions located in Aydin, Izmir, Manisa and Mugla cities of the West Aegean region of Turkey. A total of 379 blood samples were collected from stray dogs captured and maintained in municipal shelters (n=327) as well as from owned dogs (n=52) that had been admitted to the Small Animal Clinics in Aydin during 2004. Blood samples were collected in EDTA tubes from randomly selected animals with different age groups in each shelter. DNA was extracted from 200 µl blood samples using the Promega Wizard Genomic DNA extraction kit (Madison, WI, USA) following the manufacturer's instructions. Extracted DNA was resuspended in 100 µl elution buffer and stored at -20°C until analyzed. The control DNA samples of *B. vogeli* and *E. canis* were isolated from naturally infected dog in Aydin, Turkey during previous studies. The control *B. gibsoni* DNA samples were kindly provided by Dr. A. Criado-Fornelio from Spain.

Standard PCR amplification of *Babesia* spp.

Collected blood samples were subjected to a standard PCR protocol to amplify a 454 bp region of 18S rRNA gene of known large *Babesia* spp. of dogs [28] using Can172F and Can626R primers set. Details of primer pairs are given in Table 1. The standard PCR was performed in a final volume of 25 µl consisting of 1× buffer (Promega, Madison, WI, USA), 2 mM $MgCl_2$ (Promega, Madison, WI, USA), 200 µm of each deoxynucleotide triphosphate (Promega, Madison, WI, USA), 25 pmol of each primer, 1.25 U of hot start polymerase (hot-start *Taq* polymerase (ThermoFisher Scientific, USA) and 2.5 µl template DNA. The reactions were performed in an automated DNA thermal cycler (Perkin-Elmer, Foster City, Calif.) for 40 cycles. Reactions consisted of an initial 5 min denaturation step at 95°C, followed by 35–40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 90 s. Final extension was performed at 72°C for 10 min, followed by a hold step at 4°C. Amplified DNA

was subjected to electrophoresis in a 1.5% agarose gel pre-stained with ethidium-bromide (10 µl/ml) in Tris-acetate-EDTA (TAE) buffer at 100 V and bands were visualized under UV light.

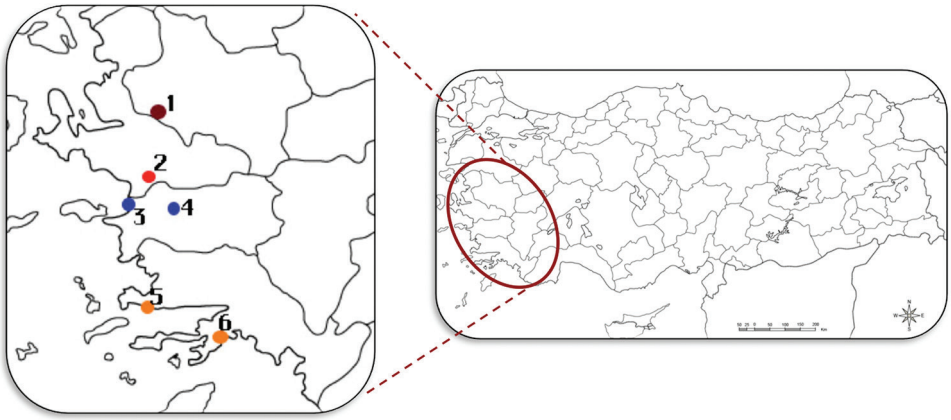

Figure 1. A map illustrating the geographical location of sampling sites. Numbers; 1-6 given on the map indicates Manisa (1; Centrum), Izmir (2; Selcuk), Aydin (3; Kusadasi and 4; Centrum), Mugla (5; Bodrum and 6; Marmaris) provinces where the samples were collected.

Table 1. Primers used for standard/nested PCR protocols

Target gene	Primer_ ID	Sequences[a,b]	Specificity	Amplicon size (bp)	References
18S ssu rRNA	RLB_F2 RLB_R2	F; GACACAGGGAGGTAGTGACAAG R; 5'-biotin-CTAAGAATTTCACCTCTGACAGT	All *Theileria* and *Babesia* spp.	460–540	Oura et al. 2004
18S ssu rRNA	Can172 Can626	F; GTTTATTAGTTTGAAACCCGC R; GAACTCGAAAAAGCCAAACGA	*Babesia* spp.	454	Inokuma et al 2004
16S ssu rRNA	ECC ECB	F; AGAACGAACGCTGGCGGCAAGC R; CGTATTACCGCGGCTGCTGGCA	All *Ehrlichia* spp.*	478	Dawson et al., 1996
16S ssu rRNA	Ecan5 HE3	F; CAATTATTTATAGCCTCTGGCTATAGGA R; TATAGGTACCGTCATTATCTTCCCTAT	*E. canis***	389	Murphy et al. 1998; Anderson et al., 1992

*indicates outer primers used in the first round of nested PCR of *E. canis*.
**indicates inner primers used in the second round of nested PCR of of *E. canis*.
[a] Primer sequences are given in 5'-3' direction.
[b] 'F' and 'R' indicates forward and reverse primers, respectively.

Reverse Line Blot assay for the detection of Babesia parasites at species level

All samples were further tested using the RLB assay in order to identify the underlying *Babesia* parasites at species level. The V4 hypervariable region of the 18S and V1 hypervariable region of the 18S rRNA gene of all *Theileria* and *Babesia* species were amplified by PCR prior to RLB. PCR was performed under the previously described

conditions [29] using primers given in Table 1. Then, 20 µl of biotin-labeled PCR products were then screened by RLB assay as previously described [30, 31]. Oligonucleotide probes used in RLB assay contained an N-terminal-C_6 amino linker (Isogen, Germany). Sequences of and oligonucleotides are listed in Table 2.

Table 2. Sequences and specificity of oligonucleotide probes used for RLB assay

Prob names*	Oligonucleotide prob sequences[a]	Concentration of each probe (pmol)	Specificity	References
T/B catchall[c]	TAATGGTTAATAGGARCRGTTG	50	All *Theileria* and *Babesia* species	Matjila et al., 2004
B. rossi	CGGTTTGTTGCCTTTGTG	200	*B. rossi*	
B. vogeli	AGCGTGTTCGAGTTTGCC	400	*B. vogeli*	
B. canis	TGCGTTGACGGTTTGAC	400	*B. canis*	
B. gibsoni	TACTTGCCTTGTCTGGTTT	900	*B. gibsoni*	
T. annulata	CCTCTGGGGTCTGTGCA	100	*T. annulata*	Oura et al. 2004
B. bovis	CAGGTTTCGCCTGTATAATTGAG	200	*B. bovis*	

[a]Oligonucleotide probes with 'R' indicates A or G bases in that position.
*Sequences are given in 5'-3' direction.

Nested PCR amplification of *E. canis*

A nested PCR approach was used to screen for the presence of *E. canis* in all 379 samples. Details of primers used during the first and second rounds of the nested PCR are given in Table 1. Primers ECC and ECB were used to amplify a 478 bp region of 18S rRNA of all *Ehrlichia* spp. [32] in the first round of nested PCR. First round PCR reactions were performed in a final volume of 25 µl containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 250 µM of each deoxynucleotide triphosphate, hot start polymerase (hot-start *Taq* polymerase (ThermoFisher Scientific, USA), 10 µM of forward and reverse outer primers (**Table 1**) and 2 µl of template DNA. The amplified PCR products of each sample obtained in the first round of PCR were diluted (1:5) with sterilized deionized water. For the second round of nested PCR, forward Ecan5 and reverse HE3 primer set was used to amplify a 389 bp region of 18S rRNA of *E. canis* [4, 33]. Second round PCR was performed under the conditions described above using 1 µl of diluted first round PCR product as a template.

Sequence analyses

In order to confirm the specificity of the PCR products, amplicons generated using *Babesia* spp. and *E. canis* specific primers were sequenced. Sequencing was performed through a commercial sequencing service (Iontek, Turkey).

Statistical analyses

The rate of positivity obtained by PCR and RLB assays among different provinces, different age groups and between stray and owned dogs were compared by the Chi-square test (SPSS 15.00 software program). *P*-values <0.05 were considered to be significant. Agreement between PCR and RLB assay assessing the presence of *Babesia* spp. was calculated using the kappa (κ) measure of agreement test; $\kappa < 0$ indicates no agreement, while a κ-value between 0.81 and 0.99 indicates almost perfect agreement. A κ-value between 0.41 and 0.60 indicates a moderate level of agreement [34, 35].

RESULTS

Prevalence of single and mixed infections with *E. canis* and *Babesia* spp. detected by PCR

A total of 379 samples were screened with single and nested PCRs for the presence of *Babesia* spp. and *E. canis*, respectively. PCR revealed that, of 379 samples 134 (35.4%) had single infections, whereas 23 (6.1%) had mixed infections. Overall, 157 (41.4%) of 379 dogs were infected with either *E. canis* and/or *Babesia* spp. The prevalence and distribution of single and mixed infections detected by PCR are given in Table 3. The most abundant species was *E. canis* with a prevalence of 39.3% (149/379). *E. canis* was detected in all provinces with a significant difference in the prevalence among provinces (P = 0.000). The highest infection rate was found in Bodrum (84.3%), while the lowest was in Aydin (25%). The standard PCR detected a total of 31 infections with *Babesia* spp. both as a single (2.1%) and mixed infection (6.1%) in all provinces, except Marmaris. The prevalence of *Babesia* spp. infections among provinces was also statistically significant (P = 0.000). The highest prevalence was found in Bodrum (19.6%) followed by Selcuk (18.5%) and Kusadasi (7.8%). The lowest prevalence was in central Aydin (2.7%) (Table 3). None of the samples collected from Marmaris were positive for *Babesia* spp. as determined by PCR. Among owned dogs (n=52), 14 were *E.canis* positive, while only a single dog was positive for *Babesia* spp. The number of dogs infected with *Babesia* spp. and *E. canis* in Aydin region was significantly higher (P= 0.000) in stray dogs than owned dogs. A total of 23 (6.1%) dogs were co-infected with *Babesia* spp. and *E. canis*. Of a total of 31 dogs infected with *Babesia* spp., 23 (74.2%) were co-infected with *E. canis*. This rate dropped down and 15.4% (23/149) of *E. canis* positive dogs were co-infected with *Babesia* spp. The prevalence of mixed infections detected by PCR was significantly different among sampling sites (P= 0.000). The highest prevalence was found in Bodrum (17.7%), followed by Selcuk (12.3%) with the lowest prevalence in central Aydin (1.8%).

Table 3. Distribution of single and mixed infections detected by PCR among provinces tested (no. of positive samples/no. of collected samples)

Region		Single infections						Mixed infections			Total[b]	
		E. canis		P-value	*Babesia*		P-value	*E. canis* / *Babesia*		P-value		
		(+)	(%)		(+)	(%)		(+)	(%)		(+)	(%)
Aydin												
	Kuşadası	18/77	23.4		2/77	5.2		2/77	2.6			
	Centrum	25/111	22.5		1/111	0.9		2/111	1.8		50/188	26.6
Izmir												
	Selçuk	22/65	33.8		4/65	6.15		8/65	12.3		34/65	52.3
Manisa				0.000*			0.000*			0.000*		
	Centrum	13/24	54.1		-/24	-		2/24	8.3		15/24	62.5
Mugla												
	Marmaris	14/51	27.5		-/51	-		-/51	-			
	Bodrum	34/51	66.7		1/51	2		9/51	17.7		58/102	56.9
Overall Total[a]		126/379	33.2		8/379	2.1		23/379	6.1		157/379	41.4

*P-values considered as statistically significant (P < 0.05) based on the Chi-square test.
([a]) overall total number of dogs infected solely with *E. canis*, *Babesia* spp., and mixed with *E. canis* / *Babesia* spp.
([b]) total infection rate of single and mixed infection in each sampling site and overall sampling sites.

Comparison of *Babesia* spp. single-PCR and RLB hybridisation assay

RLB assay revealed that all samples positive for *Theileria/Babesia* genus were also positive for *B. vogeli*, with a prevalence of 6.3% (24/379). The highest prevalence of *B. vogeli* detected by RLB was in Bodrum (2.6%) with a significant difference (P = 0.000) among provinces. The prevalence of *B. vogeli* in Selcuk was 1.6%. Only one animal was positive for *B. vogeli* by RLB in Aydin Centrum with a prevalence of 0.3%. PCR analyses using *Babesia* spp. primers detected a significantly higher (P= 0.000) number of *Babesia* spp. (31/379) infections in Izmir and Aydin provinces compared to the RLB assay. No significant differences were observed in samples collected from Manisa and Mugla (data not shown). None of the samples was infected with *Theileria annulata* and *B. bovis*, indicating the absence of any possible cross species infections between cattle and stray dogs.

In Marmaris, two dogs determined to be negative with PCR were infected with *B. vogeli* by the RLB assay. Besides, a total of nine PCR positive samples were demonstrated to be negative with the RLB hybridization assay. The majority of PCR-positive, yet RLB-negative samples produced very weak bands on the agarose gel. The kappa values between PCR and RLB tests for *B. vogeli* was higher than a moderate agreement ($\varkappa$-value >0.60) indicating nearly a perfect level of agreement ($\varkappa$ = 0.74) between the two assays (Table 4).

Table 4. Comparison of *Babesia* spp. infections detected by PCR and RLB results

		PCR		Total	P-value	Measurement of agreement[a]	
		Positive	Negative			Kappa value	SD (95% CI)
RLB	Positive	22	2	24		0.79	0.06
	Negative	9	346	355			
Total		31	348	379	0.000*		

**P*-values considered as statistically significant (P < 0.05) based on the Chi-square test.
[a]Agreement expressed as kappa value when comparing PCR and RLB tests for *B. canis* two-by-two.

Sequence analysis

The specificity of the single and nested PCRs was confirmed by sequencing PCR amplicons generated using *Babesia* spp. and *E. canis* specific primer pairs. When compared with the reference sequences in the NCBI database, the 454 bp *Babesia* spp. product showed 99% identity with 18S rRNA genes of *B. vogeli* (Okinawa strain) [GenBank accession numbers: MH100716-22] isolate as well as with other clones in the database. Additionally, the 389 bp PCR product of *E. canis* showed 96.8% identity with 16S rRNA genes of *E. canis* isolates [GenBank accession numbers: KJ995838, KY434112, KX165358] in the database (data not shown).

DISCUSSION

Among tick-borne pathogens in dogs, canine babesiosis and canine monocytic ehrlichiosis caused by *E. canis* and *Babesia* spp. are globally distributed and well-known diseases in tropical and subtropical regions [6,11]. However, studies aimed to determine the prevalence and the distribution of *E. canis* and *B. vogeli* among dogs in Turkey are few in number and infections caused by these parasites are rather neglected.

Ehrlichia canis is a pathogenic rickettsial organism causing canine monocytic erhlichiosis in dogs [3,36] with a world-wide distribution in tropical and subtropical regions [37]. Diagnosis of the disease caused by *E. canis*, could be challenging due to the variable spectrum of the disease and seasonal fluctuations in the parasitaemia [15,36]. Conventionally, microscopy has been used to detect the organism in Giemsa stained peripheral blood mononuclear cells. However, this methodology is difficult and time consuming. Furthermore, the success rate is as low as four percent during the acute phase, which drops to even lower levels during the chronic phase of the disease [37] In the present study, a more sensitive nested PCR approach was used to reveal the presence of *E. canis* in carrier animals [16,17,24,25] and ticks [38] from different regions in Turkey with a prevalence rate ranging from 4.9 – 27.5%. The present study demonstrates that among the species examined *E. canis* was the most abundant species (39.3%) in all provinces with a significant difference in the prevalence (P = 0.000).

Babesia spp. such as *B. vogeli* [19,21,22], *B. canis* [16, 17, 20], *B. gibsoni* [22], *B. vulpes* [39] and *B. rossi* [39,40] were recently reported either in dogs, ticks or wild animals in different parts of Turkey. The present study demonstrated that *B. vogeli* was the only *Babesia* spp. detected among dogs sampled in the Aegean region of Turkey in 2004. To the best of our knowledge, this study provides the first molecular evidence for the existence of *B. vogeli* in the Aegean region of Turkey. The absence of other *Babesia* spp. in dogs examined in the present study could be either due to the lack of transmitting vector ticks of the related parasite within the sampling sites, differences in the breeding purposes (like fighting or shepherd etc.) of sampled animals and/ or the lack of maintenance of some wildlife *Babesia* spp. in domesticated dogs at the time of sampling. The prevalence of *B. vogeli* among different geographical regions was reported to range from 0.4 to 3.8% [19,21,41]. The overall prevalence of *B. vogeli* detected in the present study (8.7%) was higher than those recently reported for other parts of Turkey Altered infection dynamics of due to the global warming, shifting use of the landscape, the increase in the number of wild animals, and spreading of transmitting vectors by wild birds and wild animals could be responsible for different prevalences observed in different regions as demonstrated to be the case for canine babesiosis in Europe [26,27]. Differences in the characteristics of study populations and/or the methodology used could be another contributing factor for varying prevalences observed among different regions. It should also be noted that the structure and management of the shelters, as well as the presence of a relatively high number of dogs present in a shelter affects the number of positive dogs. In fact, if the disease control measures are not well maintained, dog shelters could turn into places where the vector ticks can easily reach dogs, resulting in similar parasitic loads for each dog [42]. Our findings demonstrating the presence of only *B. vogeli* among other *Babesia* spp. could be due to the fact that *R. sanguineus (s.l.),* the transmitting vector ticks of *B. vogeli,* is the most widespread tick species of dogs in the Aegean region [43].

Significant differences observed in the prevalence rates of *E. canis* and *B. vogeli* among sheltered and owned dogs as well as differences in the overall prevalence of *E. canis* ($P = 0.000$) in some sampling sites could be attributed, at least in part, to the high capacity of this tick species to survive and hibernate indoors [11,44] at the time of sampling. In contrast to previous reports [16], the present study demonstrated that none of the samples was infected with *Theileria annulata* and *B. bovis*, indicating the absence of any possible cross species infections between cattle and stray dogs.

The existence of mixed infections together with *E. canis* and other *Anaplasma* spp. were reported in Turkey [16,17] in the past few years. The present study revealed concurrent occurrence of *B. vogeli* and *E. canis* in dogs with a prevalence rate of 6.7%. Mixed infections detected in the present study were very common among dogs (74.2%) that were positive for *Babesia* spp. (Table 3). The influence of concurrent infections in the course of prognosis of infected animals was indicated previously [18, 36]. However, in the present study, any possible effects of simultaneous infections on the clinical profile of each co-infected dog were not investigated.

In the present study, the efficacy of RLB assay and PCR was compared in terms of their sensitivity in detecting *Babesia* spp. of dogs. Results demonstrated that PCR was more sensitive than the RLB assay. This is in agreement to previous observations [29,45]. It is well established that the sensitivity and specificity of any PCR assay is affected by so many factors, like primers and other ingredients used during the amplification phase of target DNA [46]. Therefore, different sensitivities detected in the present study between PCR and RLB assays could be due to any of these factors. However, the relatively high kappa agreement ($\varkappa = 0.74$) between the two tests suggests that RLB assay should still be considered a reliable assay in diagnosing *Babesia* spp. especially in possible cases of mixed infections [21,29].

The prevalence of single and mixed infections with *E. canis* and *B. vogeli* observed in the present study was significantly higher compared to mixed infection rates reported in recent years [16,17]. Further studies should be performed to determine possible causes of altered dynamics of infections. In conclusion, the present study pinpoints the distribution and prevalence of *E. canis* and *B. vogeli* in the Aegean region of Turkey as of 2004 and as such establishes a baseline. This is of pivotal importance for future studies aimed to demonstrate changes in the dynamics of *E. canis* and *B. vogeli* infections in the region.

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Authors' contributions

HBB, GKP, MH and TK carried out the sample collection and participated in drafting of the manuscript. TK and HBB interpreted data, performed the statistical analysis and participated in the critical writing/revision of the publication. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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RETROSPEKTIVNA EPIZOOTIOLOŠKA STUDIJA: PREVALENCIJA *EHRLICHIA CANIS* I *BABESIA VOGELI* KOD PASA U EGEJSKOJ REGIJI TURSKE

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Od svih oboljenja koja se prenose krpeljima, *Ehrlichia canis* i *Babesia* piroplazmoza izazivaju značajna oboljenja pasa kod kojih se nalazi preklapanje distribucije uzročnika, vektora i prijemčive vrste tj. domaćina. Primarni cilj ove studije je bio da se proceni prevalencija *Babesia* spp i *E. canis* upotrebom PCR metode i reverzne linijske blot hibridizacije (RLB) kod ukupno 379 uzoraka koji su poticali od pasa lutalica i vlasničkih pasa. Istovremeno, obavljeno je poređenje dijgnostičke osetljivosti ove dve dijagnostičke metode. Ukupno je 41,4% pasa bilo inficirano sa *B. vogeli* i/ili *E. canis* pri čemu je kod 35,4% pasa uočena mešana infekcija, a kod 6,1% pasa se radilo samo o jednom uzročniku. Većina pasa sa babeziozom (74,1%) bilo je inficirano i sa *E. canis*. PCR metodom je dijagnostikovan (P=0,000) veći broj pozitivnih životinja u nekim provincijama u poređenju sa rezultatima analize RLB metodom. Prema našem saznanju, ovi nalazi obezbeđuju po prvi put molekularni dokaz prisustva *B. vogeli* u Egejskoj regiji u Turskoj. Ova studija ukazuje na distribuciju i prevalenciju *E. canis* i *B. vogeli* u Egejskoj regiji Turske u periodu od 2004. godine i kao takva, daje početne vrednosti prevalencije. Ovo je od velikog značaja za buduća istraživanja koja bi imala za cij da procene promene u dinamici *E. canis* i *B. vogeli* infekcija u regionu.