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RESEARCH ARTICLE

Molecular Characterization of Insect-Borne (Mosquito-Sandfly) Parasitic Pathogens of Dogs in Selected Regions of Northeast Anatolia, Türkiye

Nilgun Aydin[1*], Neslihan Olmez[1], Baris Sari[1], Zati Vatansever[1], Gencay Taskin Tasci[1], Semistan Kiziltepe[2] and Mesut Yigit[1]

[1]Kafkas University, Faculty of Veterinary Medicine, Department of Parasitology, TR-36100 Kars – Türkiye.[2]Iğdır University, Tuzluca Vocational School, Medical Services and Techniques Department, TR-76100 Iğdır- Türkiye
*Corresponding author: nlgnpaydin@gmail.com

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ABSTRACT

Canine and human health are at significant risk from insect-borne (sandfly and mosquito) zoonotic diseases such as leishmaniasis, dirofilariasis, and acanthocheilonemiasis. The objective of this study was to utilize PCR and sequencing on 500 asymptomatic canines in the provinces of Kars, Ardahan, and Iğdır in the Northeast Anatolia Region of Türkiye to examine the prevalence and molecular characterization of *Dirofilaria* spp., *Acanthocheilonema (A.) reconditum*, and *Leishmania* spp. Examination of dog blood samples from randomly selected focal areas was undertaken using the conventional PCR method. The molecular prevalence of these parasitic pathogens and the correlation between sex, age, breed and habitat of dogs were ascertained by employing the Pearson Chi-Square Test. The overall prevalence of *D. immitis* was 5% (25/500), while the PCR detected DNAs in dogs in Kars, Ardahan, and Iğdır at 6.08% (CI: 3.53-9.73), 4.03% (CI: 1.55-8.61), and 4.10% (CI: 1.73-8.27), respectively. Portions of the target genes amplified from positive samples were sequenced to confirm the PCR results. The outcomes were aligned with additional sequences in the NCBI database using BLAST search. Using the Bayesian Inference method with 2 million generations (MCMC criteria: 50,000 burn-in-length), phylogenetic trees were reconstructed using MrBayes v3.2.6. Furthermore, this study did not detect *Leishmania* spp., *D. repens* and *A. reconditum* DNAs. Knowing the existence and prevalence of insect-borne pathogens in dogs is most important and necessary for establishing a treatment protocol and determining protection and control measures.

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INTRODUCTION

Vector-borne zoonotic diseases, including dirofilariasis, leishmaniasis and acanthocheilonemiasis, constitute a substantial threat to global public and animal health. These infections, transmitted by vectors such as mosquitoes, sand flies and fleas, can cause severe morbidity and mortality, altering the course of public health and veterinary medicine (Brianti *et al.*, 2012; Aleem *et al.*, 2023; Touray *et al.*, 2023; Mohsin *et al.*, 2024). The epidemiology of these diseases is determined by vector ecology, environmental factors and host susceptibility, emphasising the necessity for regional studies to evaluate associated risks.

Dirofilariasis, a helminth-borne zoonotic disease caused by nematodes of the genus *Dirofilaria*, has a worldwide distribution. *Dirofilaria (D.) immitis* (heartworm) inhabits the right atrium and pulmonary arteries of dogs, causing clinical symptoms such as cough, ascites, and respiratory distress. The most severe form of the disease, known as Caval syndrome, has been observed to result in rapid mortality (Touray *et al.*, 2023). Conversely, *D. repens* predominantly causes subcutaneous infections in dogs and is the primary causative agent of human dirofilariasis, leading to the formation of nodular lesions in subcutaneous or ocular tissues (Saralı *et al.*, 2020). Mosquitoes transmit both species and the main vectors are *Aedes vexans* and *Culex pipiens* (Yıldırım *et al.*, 2010).

Acanthocheilonema (A.) reconditum, usually transmitted by fleas or lice, is another filarial nematode thought to be less pathogenic to canines. Despite it can cause subcutaneous infections, its clinical significance is often overshadowed by its ability to produce microfilaremia, which can be confused

with *D. immitis* infection during diagnostic screening (Rishniw *et al.,* 2006; Brianti *et al.,* 2012). However, more research is necessary to fully understand its role as a zoonotic pathogen, especially in endemic areas.

Leishmaniasis is a neglected tropical disease and a growing global public health problem caused by protozoa of the genus *Leishmania*. In Türkiye, the primary causes of the visceral and cutaneous forms are *L. infantum* and *L. tropica*, which are transmitted by sand flies (*Phlebotomus* spp.) (Karakuş *et al.,* 2019; Özbel *et al.,* 2022). With clinical manifestations ranging from asymptomatic infections to severe systemic disease, including renal failure and skin lesions, dogs are a significant reservoir for the parasite and serve as an important reservoir (Sarı *et al.,* 2015; Balıkçı *et al.,* 2023).

Dirofilariasis, leishmaniasis, and *A. reconditum* have been studied in various parts of Türkiye (Güven *et al.,* 2017; Düzlü *et al.,* 2020; Özbel *et al.,* 2022). However, in the Northeastern Anatolian region, particularly the provinces of Kars, Ardahan, and Iğdır, there is a lack of data on the prevalence, vector ecology, and epidemiological aspects of these infections. Thus, the aim of this study was to fill this gap by investigating the prevalence, molecular features and epidemiological aspects of acanthocheilonemiasis, leishmaniasis and dirofilariasis in dogs in Northeastern Anatolia.

MATERIALS AND METHODS

Study locations: Between September 2022 and September 2023, a cross-sectional survey was carried out in the Eastern Anatolian provinces of Kars (40°45′5″N, 42°99′7″E), Ardahan (42°70′2″N, 41°11′3″E), and Iğdır (39°89′4″N, 43°94′2″E) (Fig. 1). These regions are bordered by Georgia, Armenia, Iran and the Autonomous Republic of Nakhchivan. While Iğdır has a temperate climate influenced by a microclimate created by its soil structure, Kars and Ardahan have cold winters and cool, rainy summers. Extensive pastures that support livestock farming, a major economic activity, characterize the region. In rural areas, dogs are primarily kept for protecting livestock, whereas urban and semi-urban areas host a significant population of stray dogs, which are often inadequately protected against diseases.

Collection of blood samples: Blood samples were collected from clinically healthy 500 dogs across 42 villages, which were randomly selected to represent the three provinces included in the study from September 2022 to September 2023. A random sampling method was employed for selecting 42 villages across the three provinces. In each selected village, a convenience sampling approach was used to collect blood samples from clinically healthy dogs. The dogs were chosen based on availability during field visits, without any specific selection criteria such as breed or age. Additional data, including demographic and clinical information, were gathered through direct observation and interviews with dog owners. Blood tubes were transported to the Department of Parasitology Laboratory, Faculty of Veterinary Medicine, Kafkas University, Türkiye, and stored at -20°C freezer until use for molecular analysis. No clinical signs were revealed in any of the dogs in the first inspection, and all of the characteristics (location, sex, breed and age) were recorded as either healthy or asymptomatic.

DNA extraction and PCR amplification: Following the manufacturer's instructions, 200µL of blood samples were utilized to isolate genomic DNA using a commercial kit (EcoPURE Blood Genomic DNA Kit, Cat. No: E1075, Türkiye). Until amplifications, genomic DNAs were stored at -20°C. Using primer pairs designated for leishmaniasis (*Leishmania* spp.) and filariasis (*D. immitis, D. repens* and *A. reconditum*), all samples were investigated by conventional PCR analysis. A mixture of 7.5µL nuclease-free water, 12.5µL master mix (ThermoScientific, ABD), 1.25µL each of the forward and reverse primers (Sentebiolab, Türkiye), and 2.5µL of template DNA was utilized in the reaction. The PCR products were visualized by staining Ethidium bromide under ultraviolet light and analyzed on a 1.5% agarose gel using 0.5X TAE buffer. Sequences, specificity, target gene, product length and PCR type for the primers were demonstrated in Table 1.

Sequencing: Sequence analyses were carried out to verify the outcomes of PCR and to contrast them with the other consensus sequences that are listed in GenBank. A commercial company, Triogen Biyoteknoloji, Istanbul, Türkiye, used specific primers to perform bidirectional Sanger sequencing. The sequenced samples were analyzed by transferring them to Geneious® 9.1.8 software. Low-quality reads of the sequences were trimmed to within 0.05 margin of error with the Trim Ends plugin of Geneious software. At the end of the process, the chromatograms of the trimmed sequences were reviewed again, and forward and reverse reads were assembled de novo to obtain consensus sequences. For samples where contigs could not be obtained, forward reads and reverse complements of reverse reads were aligned with the MAFFT alignment tool to obtain consensus sequences.

Table 1: Features of the primers used in this study: sequences, specificity, target gene, product length, and PCR type

Primer	Primers (5′–3′)	Specificity	Gene (Target sequence)	Target amplikon length (bp)	PCR Type and sequencing	Reference
DIDR-F1	AGTGCGAATTGCAGACGCATTGAG	*D. immitis*	5.8S-ITS2-28S	542	Conventional	Rishniw *et al.*
DIDR-R1	AGCGGGTAATCACGACTGAGTTGA	*A.reconditum*		578	PCR, Sanger	(2006)
		D. repens		484	sequencing	
DR-COI-F1	AGTGTAGAGGGTCAGCCTGAGTTA	*D. immitis*	COI	203	Conventional	
DR-COI-R1	ACAGGCACTGACAATACCAAT				PCR	
AR COI-F1	AGTGTTGAGGGACAGCCAGAATTG	*A. reconditum*	COI	208	Conventional	
AR COI-R1	CCAAAACTGGAACAGACAAAACAAGC				PCR	
DR COI-F1	AGTGTTGATGGTCAACCTGAATTA	*D. repens*	COI	209	Conventional	
DR COI-R1	GCCAAAACAGGAACAGATAAAACT				PCR	
R221	GGTTCCTTTCCTGATTTACG	*Leishmania* spp.	ssr RNA	603	Conventional	Van Eys *et al.*
R332	GGCCGGTAAAGGCCGAATAG				PCR	(1992)
RV1	CTTTTCTGGTCCCGCGGGTAGG	*L.infantum/donovani*	LT1	145	Conventional	le Fichoux *et al.* 1999
RV2	CCACCTGGCCTATTTTACACCA	*komplex*			PCR	Gao *et al.* 2015

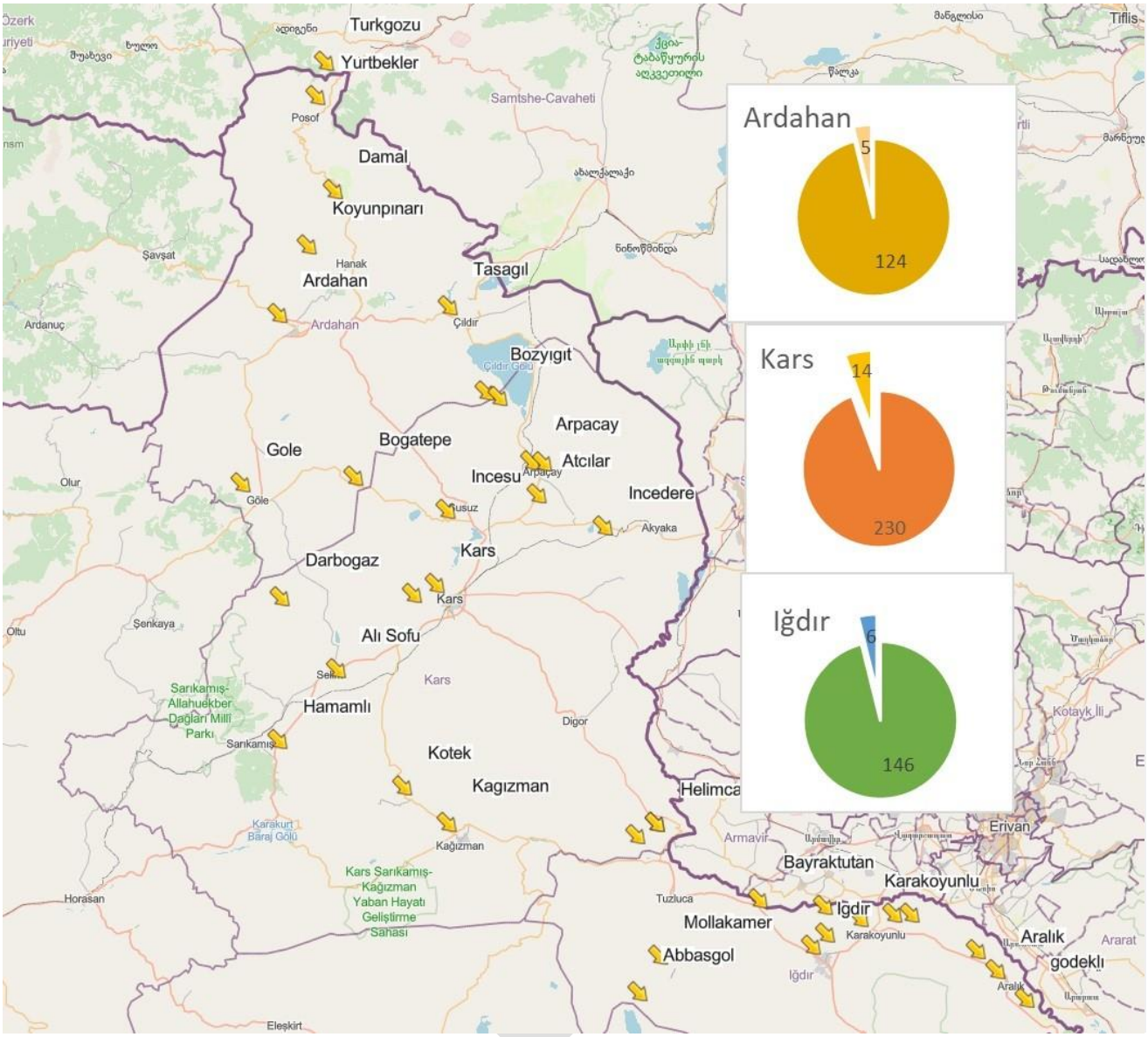

Fig. 1: The overall infection rates of *D. immitis* in the study area.

Phylogenetic analysis: Sequences were trimmed with 0.05 error probability via Geneious® 9.1.8 (Kearse *et al*., 2012). Trimmed forward and reverse sequences were assembled and consensus contigs were obtained. For phylogenetic analysis, the 5.8S rRNA-ITS2 sequences of *D. immitis* available in GenBank (Fig. 2 and 3) were retrieved and aligned with the sequence obtained in this study (PQ273743) using the MAFFT multiple sequence alignment tool (Katoh *et al.*, 2002). The alignment was cleaned with Gblocks 0.91b (Castresana, 2000). For the 5.8S rRNA-ITS2 data set, the most suitable base substitution model was identified as HKY+G using the Bayesian Information Criterion in jModeltest 2.1.10 (Darriba *et al*., 2012). Phylogenetic trees were reconstructed using the Bayesian Inference (BI) method with 2 million generations (MCMC criteria: 50,000 burn-in-length), performed in MrBayes v3.2.6 (Ronquist *et al.,* 2012). *Dirofilaria (D). repens* 5.8S rRNA-ITS2 sequence (MN200338) was used as an outgroup.

Statistical analysis: The molecular prevalence of the parasitic pathogens, as well as the correlations between sex, age, breed, and habitat of the dogs, were assessed using the Pearson chi-square test. Statistical analysis was performed using IBM SPSS Statistics version 26.0 and the R package "prevalence" (version 0.2.0). The prevalence values were estimated using the R package prevalence (version 0.2.0.) created by Devleesschauwer *et al.*, (2013). As in the package, Jeffreys confidence intervals were used to calculate apparent prevalence (AP) in the R package. The following model was created using the package and it was used to calculate true prevalence (TP) in a Bayesian framework with perfect test assumption and a uniform prior beta distribution: Model {x ~ dbin (AP, n) AP <- SE *TP + (1-SP) *(1-TP), SE<-1, SP<-1, TP ~ dbeta (1, 1)}.

RESULTS

The overall prevalence of *Dirofilaria* spp. was determined to be 5% (25/500) in an analysis of 500 dog blood samples using genus-specific primers. Meanwhile, *D. immitis* DNA was detected in seven

samples (1.4%) with species-specific primers. However, *Leishmania* spp., *D. repens*, *A. reconditum* DNAs were not detected in this study. The infection was detected in all 20 villages, representing the 3 provinces included in this study (Table 2, Fig. 1).

Parts of the target genes amplified from positive samples were sequenced to verify the PCR results. The results were aligned with other sequences in the NCBI database using BLAST search. Obtained *D. immitis* 5.8S rRNA-ITS2 sequences were deposited to the GenBank with accession numbers PQ273743 (346 bp), PQ273777 (249 bp), PQ276579 (149 bp), PQ276580 (135 bp) and PQ276581 (242 bp).

The most extended sequence, PQ273743, was used in phylogenetic analyses since the sequences obtained shared the same consensus except for a single nucleotide (Fig. 2). 5.8S rRNA-ITS2 gene region sequence of *D. immitis* with accession number PQ273743 (isolate Turkiye-KAU23-Halikisla4) exhibited close similarity to sequences from Chile, Brazil, Russia, and Iran in the BI tree (Fig. 3). The clade containing the Turkiye-KAU23-Halikisla4 sequence was separated from the main branch of the BI tree with a high posterior probability (0.996). The identity within the clade exhibited a range of 98.4% to 100% sequence similarity with the Turkey-KAU23-Halikisla4 sequence. The Iranian sequences (JX889634 and JN084166) within this clade formed a sub-branch and were relatively less similar to the Turkiye-KAU23-Halikisla4 sequence, with identity of 99.2% and 98.4%, respectively. On the other hand, the *D. immitis* 5.8S rRNA-ITS2 sequences from Bangkok, Thailand, were found to be separated from the Portuguese sequences with high posterior probability (0.999).

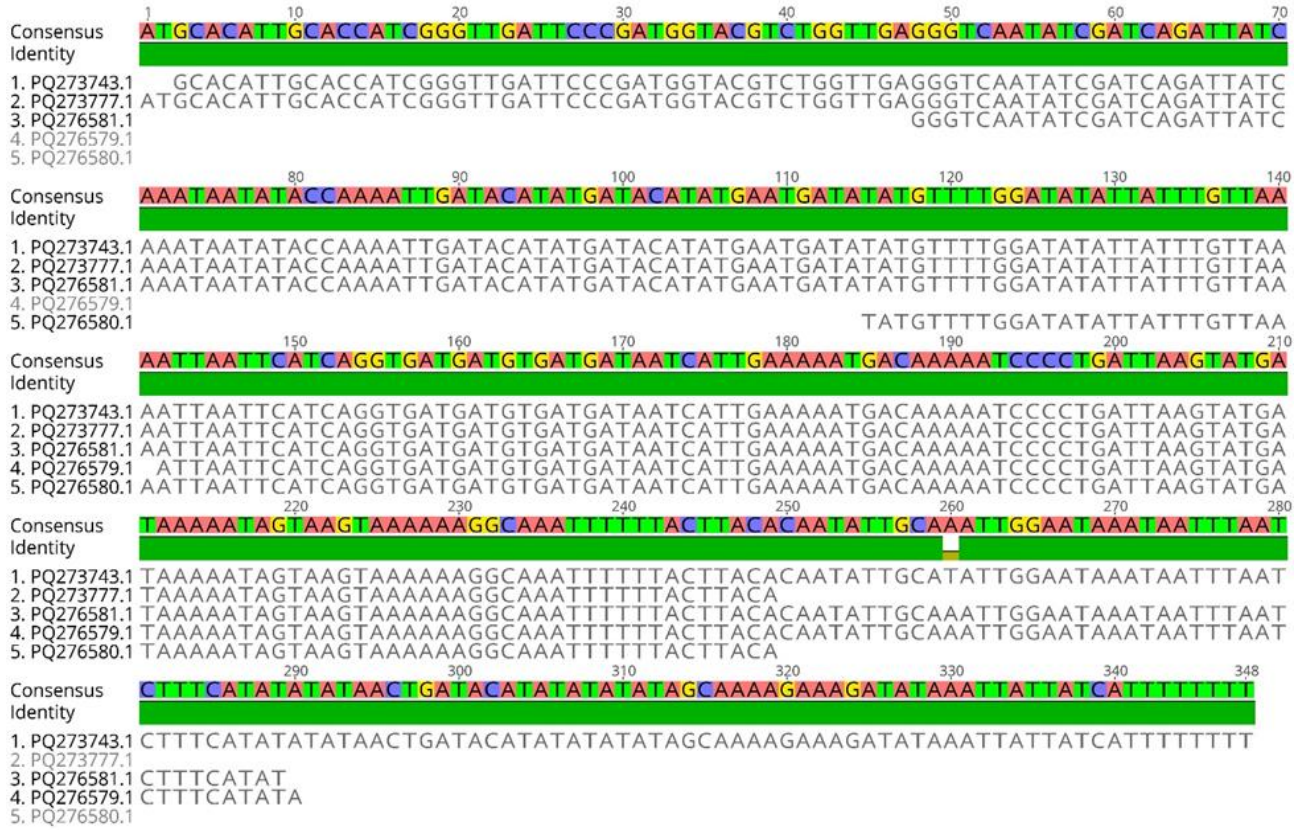

Fig. 2: An alignment image of the nucleotide sequences, identified by accession numbers PQ273743 (346 bp), PQ273777 (249 bp), PQ276579 (149 bp), PQ276580 (135 bp), and PQ276581 (242 bp), as generated through the MAFFT algorithm, is presented.

Table 2: Infection rates with *D. immitis* in Kars, Ardahan and Iğdır provinces vary by age, breed, sex and habitat

Risk Factors		Kars					Ardahan					Iğdır				
		N	Pos	P	Pearson χ2	MLE%/CI	N	Pos	P	Pearson χ2	MLE%/CI	N	Pos	P	Pearson χ2	MLE%/CI
Age	≤1	66	3	0.737	1.265705	4.45/1.29-11.63	33	4	0.038*	8.396129	12.12/4.23-26.29	26	1	0.875	0.69356	3.84/0.41-16.60
	2	39	2			5.12/1.08-15.44	23	0			0/0-7.92	30	1			3.33/0.36-14.54
	3	42	2			4.76/1.00-14.41	24	1			4.16/0.45-17.86	39	1			2.56/0.27/11.36
	4≥	83	7			8.43/3.85/15.84	44	0			0/0-4.24	51	3			5.88/1.68-14.87
Breed	Kangalx	88	4	0.736	0.612819	4.54/1.55-10.44	69	4	0.451619	1.589831	5.79/1.98-13.19	52	5	0.043*	6.305466	9.61/3.76-19.79
	Kafkasx	54	4			7.40/2.55-16.65	32	1			3.12/1.33-13.69	70	1			1.42/0.15-6.48
	Mix	88	6			6.81/2.89-13.51	23	0			0/0-7.92	24	0			0/0-7.61
Sex	Male	152	10	0.663095	0.189786	6.57/3.43-11.35	72	4	0.310265	1.029548	5.55/1.98-13.19	90	3	0.549	0.358793	3.33/0.94-8.62
	Female	78	4			5.12/1.75-11.73	52	1			1.92/0.20-8.63	56	3			5.35/1.53-13.61
Habitat	Rural	196	12	0.956892	0.002922	6.12/3.39-10.13	118	5	0.60676	0.26492	4.23/1.63-9.03	71	5	0.082422	3.016485	7.04/2.73-14.74
	Shelter	34	2			5.88/1.24-17.55	6	0			0/0-26.41	75	1			1.33/0.14-6.06
Total		230	14			6.08/3.53-9.73	124	5			4.03/1.55-8.61	146	6			4.10/1.73-8.27

*A p-value of less than 0.05 is statistically significant.

Fig. 3: BI phylogenetic tree constructed using the 5.8S rRNA-ITS2 gene region sequences of *D. immitis*. BI tree topography obtained as a result of MAFFT alignment.

DISCUSSION

Filaroids are parasitic nematodes with a global distribution, particularly in tropical and subtropical regions where their vectors thrive. Among them, *D. immitis* and *D. repens* are the most clinically significant species due to their impacts on both humans and dogs (Brianti *et al.,* 2023; Esteban-Mendoza *et al.,* 2024). These parasites are transmitted by mosquitoes of the Culicidae family (Simon *et al.,* 2012; Otranto *et al.,* 2013; Touray *et al.,* 2023). Another common filaroid species, *A. reconditum*, is less pathogenic and uses fleas or lice as intermediate hosts (Otranto *et al.,* 2013). Because of climatic and environmental changes, dirofilariasis has been spreading in both intensity and geographic distribution, with wild reservoirs and mosquito populations growing even in areas that were thought to be infection-free (Güven *et al.,* 2017).

Depending on the region, the prevalence of dirofilariasis in Türkiye varies from 0.2% to 46.2% (Ağaoğlu *et al.,* 2000; Düzlü *et al.,* 2020). However, Ardahan province has not previously performed molecular detection of *D. immitis* and *Leishmania* spp. in dogs. Using molecular and serological techniques, previous research in

Kars and Iğdır has reported the existence of these species (Taşçı and Kılıç, 2012; Sarı *et al.,* 2015; Demirci *et al.,* 2021; Ayvazoğlu *et al.,* 2022). The molecular prevalence of *D. immitis* in the current study was 5%, which is much lower than the 25% prevalence found in previous studies in Kars and Iğdır (Taşçı and Kılıç, 2012). This variation may be due to factors such as clinically healthy animals in the sample, random sampling design, challenges in microfilariae detection due to timing, and low mosquito vector density influenced by local climate.

This study found no DNA of *D. repens* or *A. reconditum*, highlighting the need for more research on their epidemiology in the area. Globally, *Dirofilaria* species are recognized as a major cause of severe disease in domestic and wild canids (Kravchenko *et al.,* 2016) and represent a significant public health concern (Genchi and Kramer, 2017). Alkaline phosphatase staining and the modified Knott test are two methods that are frequently used to detect microfilariae in filarial infections (Rishniw *et al.,* 2006). Recently, molecular methods have become essential for the identification of filarial species (Taşçı and Kılıç, 2012; Güven *et al.,* 2017; Aydın *et al.,* 2020; Esteban-Mendoza *et al.,* 2024).

Kars had the highest infection intensity (6.08%), followed by Iğdır (4.1%) and Ardahan (4.03%) in the current study. This rate is associated with the density of infection and causing vector flies, according to an analysis of earlier research done in the areas (Taşçı and Kılıç, 2012; Demirci *et al.,* 2021). At the same time, previous studies have found that infection rates were high both serologically (Taşçı and Kılıç, 2012; Sarı *et al.,* 2013; Ayvazoğlu *et al.,* 2022) and molecularly (Taşçı and Kılıç, 2012). The high infection rates are thought to be influenced by the timing of blood collection.

The study region, dog breed, number of dogs, vector population and diagnostic techniques can all account for differences in prevalence rates (Güven *et al.,* 2017). Our study assessed age, breed, sex and habitat as risk factors. As a result, the risk factors vary according to the difference in the number of samples taken in these 3 provinces. Seropositivity has increased (Öge *et al.,* 2003; Yıldırım *et al.,* 2007; Çetinkaya *et al.,* 2016), but the infection rate of *D. immitis* in dogs has not changed with age (Razi *et al.,* 2010; Güven *et al.,* 2017). This phenomenon can be explained by the fact that older dogs are more exposed to this parasite than younger puppies (Öge *et al.,* 2003). In Kars and Iğdır, the rate of *D. immitis* was observed to increase in correlation with the age of the dogs and the vector population. Dogs aged one year (12.12%) had the highest infection rate in Ardahan. This is because all age groups are not equally distributed.

According to some research, there is no breed-specific predisposition to dirofilariasis, meaning that all dog breeds are equally susceptible to infection (Razi *et al.,* 2010). However, other studies suggest that large-breed dogs might be more susceptible to the disease than small-breed dogs (Yıldırım *et al.,* 2007; Yaman *et al.,* 2009). CaucasianX was detected in Kars (7.40%), KangalX in Ardahan (5.79%) and Iğdır (9.61%) when evaluating the intensity of infection in dog breeds. As a result, it is observed that, depending on population density, the rate of infection is higher in the breeds raised intensively in the region.

The analysis of *D. immitis* distribution by sex revealed a higher prevalence in male dogs; however, the difference in prevalence between male and female dogs was not statistically significant. However, it was determined that the parasite was more common in male dogs (Taşçı and Kılıç, 2012; Demir and Aktaş, 2020). It is thought that the higher infection rate in males (Kars 6.57%, Ardahan 5.55%, and Iğdır 3.33%) is due to the low number of female dogs examined and the fact that male dogs in these regions are generally kept for more extended periods of time as guards in herds and homes and therefore are exposed to mosquito attacks for a longer time.

Compared to indoor, owned, and urban dogs, the prevalence of *D. immitis* was found to be higher than that of outdoor, stray, and suburban dogs (Yaman *et al.,* 2009; Oi *et al.,* 2014; Çetinkaya *et al.,* 2016). In contrast, in the present study, the infection rate is higher in rural dogs (Kars 6.12%, Ardahan 4.23%, and Iğdır 7.04%). The lower infection rate among shelter dogs was probably caused by compliance with Türkiye's Animal Protection Law (No. 5199), which requires that stray dogs be given antiparasitic medications when they are brought into shelters. Infection rates were found to be 0% in Ardahan, 1.33% in Iğdır, and 5.88% in Kars. In addition to being advised about heartworm screening and the use of the proper medications for preventative treatment, dog owners should bring their pets for routine veterinary examinations.

According to PCR, the prevalence of *D. immitis* was 15.5% in Brazil (Trancoso *et al.,* 2020), 8.0% in Mexico (Torres-Chable *et al.,* 2018), 3.27% in Texas (Sobotyk *et al.,* 2022), 2.3-4.02% in Iran (Bamorovat *et al.,* 2017; Pedram *et al.,* 2019), 1% in Colombia (Esteban-Mendoza *et al.,* 2024), 0.6-25% in Türkiye (Taşçı and Kılıç, 2012; Şimşek and Çiftçi, 2016), 0.29% in Kyrgyzstan (Aydın *et al.,* 2020), and 0.0% in Cape Verde (Marcos *et al.,* 2017). In contrast to these findings, Taşçı and Kılıç (2012), Torres-Chable *et al.* (2018) and Trancoso *et al.* (2020) reported better results using PCR. The prevalence of dogs in Kars, Ardahan, and Iğdır provinces was comparatively low at 5%. Environmental factors, particularly climate and ecological aspects, impact mosquito populations, and this has been related to the occurrence of dirofilariosis. Compared to other molecular prevalences (Çetinkaya *et al.,* 2016; Şimşek and Çiftçi, 2016; Güven *et al.,* 2017) in Türkiye, a not-too- low prevalence of 5% was found in our study. It is thought that this is due to the climate, time and season of blood collection, mosquito population and activity status, number, age, and clinical health of the dogs from which blood was collected. Furthermore, postmortem examinations and other diagnostic techniques should be used in future investigations.

Leishmania parasites can also infect dogs, wolves, foxes, and jackals. These animals can serve as reservoirs for the parasite. The agent is usually seen in visceral and cutaneous forms in dogs. Less than 40% of infected dogs have clinical signs, and dogs without symptoms are equally contagious to sandflies (Karakuş *et al*., 2019; Özbel *et al.,* 2022; Dantas-Torres, 2024). This disease is endemic in all southern Europe countries and can cause both cutaneous and visceral infections (Touray *et al.,* 2023). The prevalence in blood samples ranged between 0 and 18.5% in dogs in Türkiye by PCR (Bölükbaş *et al.,* 2016; Düzlü *et al*. 2020; Pekağırbaş *et al.,* 2022).

No *Leishmania* species could be found in our molecular investigation, despite *Leishmania* seropositivity being previously detected in the region (Sarı *et al.*, 2015). This situation includes the presence of anti-*Leishmania* antibodies that persist for a long time even after the elimination of PCR-detectable *Leishmania* DNA (Ikonomopoulos *et al.*, 2003), the utilization of blood material, which has a relatively low sensitivity compared to other biological materials in the detection of infection by PCR (Töz *et al.*, 2009), or the detection of low levels of parasitemia. It is thought to be connected with insufficient PCR sensitivity. Although *A. reconditum* (formerly *Dipetalonema (D.) reconditum*) microfilariae were detected in previous years (Taşan, 1984; Toparlak *et al.*, 2005), *A. reconditum* DNA could not be detected in this study and other studies (Güven *et al.*, 2017; Aslantaş *et al.*, 2020).

When phylogenetic relationships of *D. immitis* are assessed using the 5.8S rRNA-ITS2 gene region, samples from Thailand are separated from those from other locations by significant genetic differences. Although the sequences from different locations show partial genetic divergence, the extent of this separation is insufficient for a detailed phylogenetic assessment. Therefore, to draw definitive conclusions about the phylogenetic relationships of *D. immitis* based on geographic localization, it is necessary to analyze more suitable genetic regions for intraspecific phylogenetic relationships, such as complete mitogenome analyses. However, despite the limitations of the 5.8S rRNA-ITS2 gene region in resolving phylogenetic relationships, it is noteworthy that the Thailand samples show a clear topological structure in the BI analysis.

Conclusion: Vector-borne diseases and their associated vectors continue to pose significant challenges, with their prevalence increasing due to factors such as climate change, the development of resistance caused by the improper use of medications, genetic adaptations in pathogens, and unregulated human and animal movement. *Dirofilaria* found to be an important parasite of dog in Türkiye. The age and breed of the dog is a significant risk factor influencing prevalence in the area. The results of this study might contribute to prevention and control measures against the spread of dirofilariasis. Knowing the existence and prevalence of vector-borne pathogens in dogs is critical and necessary for establishing a treatment protocol and determining protection and control measures. The effective control and prevention of such infections and diseases necessitate a multidisciplinary approach, integrating health, research, sociology, economics, governance, and public collaboration.

Authors contribution: NA, NO, BS, ZV and GTT designed the study and conducted the experiment. SK and MY collection of blood samples. Each author evaluated the data, made significant intellectual revisions to the manuscript, and gave their final approval.

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