

Coxiella burnetii in dogs and ticks from the Northeastern Anatolia region of Türkiye: serological and molecular findings

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Article Info	Abstract
Article history: Received: 11 July 2024 Accepted: 09 November 2024 Available online: 15 August 2025	<p>The Q fever is a zoonotic bacterial infection caused by an obligate intra-cellular bacterium, <i>Coxiella burnetii</i>. Members of the Canidae family (Mammalia), including dogs and foxes, are potential reservoirs of <i>C. burnetii</i>, which has a wide host range from mammals and birds to arthropods (primarily ticks). Infected dogs can transmit the disease to other animals and humans. This study aimed to investigate the presence of <i>C. burnetii</i> in dogs and ticks collected from infested dogs in the Kars, Ardahan, and Iğdir provinces of Türkiye by serological and molecular methods. Three hundred canine serum samples were analyzed for phase I and phase II <i>C. burnetii</i> antibodies using indirect enzyme-linked immunosorbent assay. Whole blood samples (n = 300) from the dogs sampled for sera and 184 ticks randomly collected from these dogs were also analyzed for <i>C. burnetii</i> with touch-down polymerase chain reaction. The ticks were classified according to the taxonomic characteristics. In result, 107 tick DNA samples collected from individual females and pooled males were evaluated. The <i>C. burnetii</i> was detected in 3.73% (of the tick samples). However, <i>C. burnetii</i> was not detected in any of the canine blood samples by polymerase chain reaction. Out of the 300 dogs, 18.33% presented antibodies against <i>C. burnetii</i> in their blood serum. When assessed for location, <i>C. burnetii</i> seropositivity was found to be significantly high especially in the Northeastern Anatolia region (18.33%). Study data highlighted the zoonotic risk of ticks, demonstrating that ticks on dogs can carry <i>C. burnetii</i>.</p>
Keywords: ELISA Dog PCR Q fever Tick	

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Introduction

The Q fever is a zoonotic disease, which is caused by *Coxiella burnetii* and occurs worldwide except in New Zealand and Antarctica. *Coxiella burnetii* is a Gram-negative, obligate intra-cellular bacterium.¹ This organism exhibits high resistance to external stress factors and is classified as a Category B biological warfare agent by the Centers for Disease Control and Prevention.² *Coxiella burnetii* has a broad range of hosts,² and domestic animals are considered the primary source of *C. burnetii* infections in humans. The Q fever in humans has also been observed following contact with dogs and cats.^{3,4} The main transmission route from these animals to humans is by inhaling contaminated aerosols shed through parturition secretions.⁵ The *C. burnetii* is one of the most highly infectious pathogens in the world. The inhalation of one to 10 organisms may lead to the development of human infections.⁶ Typically, Q fever occurs without any specific clinical signs in humans and animals.⁷

Vector-borne diseases, which are caused by a wide range of pathogens transmitted by arthropod vectors, are on the rise globally and threaten both animal and human health.⁸ Ticks can serve as vectors for major pathogens and may be dispersed by host animals across vast geographical regions.^{9,10} *Coxiella burnetii* is a zoonotic agent that can be transmitted by ticks.¹¹ Ticks, which are the vectors of *C. burnetii*, transmit the bacterium not only through fecal and salivary routes but also transstadially and transovarially.¹²⁻¹⁴ Many tick species, including those belonging to the *Ixodes*, *Haemaphysalis*, and *Dermacentor* genera, are susceptible to *C. burnetii* infection. The Q fever has been reported in species of ticks related to both humans and domestic animals.¹⁵⁻¹⁸

Dogs can become infected by the ingestion or inhalation of contaminated material.² Moreover, infection can occur *via* tick bites.^{14,19} While *C. burnetii* infections in dogs are usually asymptomatic, they result in environmental contamination both during and after parturition.²⁰ In some cases, clinical findings may also

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appear, such as fever, lethargy, anorexia, depression, incoordination, and seizures.²¹ The premature mortality of puppies has been reported in pregnant dogs.^{22,23} The presence of the bacterium in the reproductive system of healthy dogs poses a potential risk for human infection. Stray dogs or free-ranging dogs, which are not treated for infection and do not receive protective medication, are at an increased risk of encountering infected ticks. This is important, because dogs can act as carriers of the Q fever agent and spread it to new locations.²

The Q fever can be diagnosed by serological, molecular, and cell culture techniques.^{24,25} Molecular methods are preferred for the identification of *C. burnetii* because of their high sensitivity and specificity.²⁶⁻²⁸ Serological methods are also frequently used for the diagnosis of *C. burnetii* infections owing to their practicality. Although still preferred as a sensitive method for the diagnosis of Q fever, cell culture is less practical than enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR).²⁹

The aim of this study was to determine the presence of *C. burnetii* with touch-down PCR and ELISA in dogs, as well as ticks removed from infested dogs in the Northeastern Anatolia region of Türkiye. The study was carried out in the villages of the Kars, Ardahan, and Iğdir provinces located in this region, where dogs were previously diagnosed with tick-borne infections, and in foci of pasture farming with different geographical characteristics (altitude, climate, vegetation, tick population, etc.).

Materials and Methods

Field study area. Kars, Ardahan, and Iğdir are located in the Northeastern Anatolia region of Türkiye. This region is geographically important as it has borders with Armenia, Iran, Nakhchivan, and Georgia (Fig. 1), and its economy is largely dependent on animal husbandry, in particular pasture husbandry. A large number of dogs are also raised in the region to guard livestock herds.



Fig. 1. Geographical location of the place where the present study was conducted.

Collection of blood samples and ticks from dogs.

There is no data available regarding the number of sheepdogs in the study region. In this regard, a sampling strategy was devised to include animals, with the consent of their owners, from the population of available owned dogs. The ethical approval of the study was obtained from the Local Ethics Committee for Animal Experiments of Kafkas University, Kars, Türkiye (KAUHADYEK/2021/162). In this study, 5.00 mL canine blood samples were taken from the *vena cephalica antebrachii* into two separate tubes, one of which was K₂EDTA-coated tubes (BD Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, USA) for DNA isolation and the other contained a silica gel (for serological analysis). In total, 300 dogs were randomly selected from different foci for sampling. The sampled dogs were also examined for tick infestation, and the ticks on the dogs were recorded by collecting them into separate sterile tubes. A total of 184 ticks were collected and investigated. The foci, where the sampling was carried out, and the numbers of dogs and ticks are given in Table 1.

Identification of ticks. The tick species collected from the dogs were identified by stereo microscopy, based on the relevant diagnostic keys.^{30,31}

Serological investigation of phase I and phase II antibodies against *C. burnetii* in dog sera. Sera were obtained by the centrifugation of the canine blood samples at 300 rpm for 10 min. Serological analyses were performed for antibodies against the phase I and phase II forms of *C. burnetii*. For this purpose, a Q fever (*C. burnetii*) antibody test kit (IDscreen® Q fever indirect multi-species; IDvet, Grabels, France) was used according to the manufacturer's instructions. The results were read using an Epoch™ microplate spectrophotometer (BioTek Instruments Inc., Winooski, USA) at a wavelength of 450 nm. The result for each sample was expressed as a percentage of the ratio between the sample optical density (OD) and the positive control OD using the substrate/product percentage (SP%) formula, as shown below:

$$SP\% = \frac{OD \text{ sample} - OD \text{ negative control}}{OD \text{ positive control} - OD \text{ negative control}} \times 100$$

The SP% value of more than 80.00% was considered strongly positive, the SP% value between 50.00 and 80.00% was considered positive, the SP% value between 40.00 and 50.00% was considered doubtful, and the SP% value less than 40.00% was considered negative.

The DNA extraction from ticks and canine whole blood. The DNA was extracted from 300 canine whole blood samples and 107 pooled ticks for PCR. Before proceeding to the extraction process, grouping was carried out by separating the female and male ticks. While DNA was extracted individually from each female tick, a pooling method was applied for the male ticks (with the minimum of one and maximum of five ticks for the same tick species).

After being identified, the ticks were washed three times with 1.00 X phosphate-buffered saline, also washed with sterilized and deionized water, and dried with filter paper. They were then crushed in 1.50 mL sterile micro-centrifuge tubes.⁴ The PureLink™ Genomic DNA Mini Kit (ThermoFisher Scientific, Waltham, USA) was used for DNA extraction from both the tick and canine whole blood samples according to the manufacturer's instructions.

***Coxiella burnetii*-specific PCR.** A touch-down PCR was used to detect *C. burnetii* from the dog and tick samples.³² This PCR targets the amplification of the *IS1111A* transposase gene, which is 687 bp in length, using the Trans-I (5'-TAT GTA TCC ACC GTA GCC AGT C-3') and Trans-II (5'-CCC AAC AAC ACC TCC TTA TTC-3') primers.³² The PCR reaction was performed on 4.00 µL of each extracted DNA in a total volume of 25.00 µL. The final reaction mixture contained 2.00 mM of each primer, 200 mM of each dNTP, 3.00 mM MgCl₂, and 0.50 U of Taq DNA polymerase. The DNA amplification was performed using a thermal cycler (Bio-Rad, MJ Mini Gradient Thermal Cycler, Hercules, USA). Touch-down PCR was programmed as shown in Table 2. The amplified products were analyzed by 1.50% agarose gel electrophoresis. Bands, which were 687 bp in length, were considered as *C. burnetii*. Deionized distilled water and DNA obtained from the *C. burnetii*

Nine-Mile I strain were used as negative and positive controls, respectively.

Statistical analyses. The SPSS Software (version 26.0; IBM Corp., Armonk, USA) was used for statistical analysis. The chi-squared test was used to compare the results between the provinces. The *p*-values < 0.05 were considered statistically significant.

Results

Sample distributions. Blood samples were collected from 114 dogs from three different foci in Iğdir province, and 147 *Rhipicephalus sanguineus* ticks were collected from 46 of these dogs. Blood samples were collected from 55 dogs from three different foci in Ardahan province, and 9 *Ixodes* spp. nymphs were collected from one of these dogs. Blood samples were collected from 131 dogs from three different foci in Kars province and 23 *Dermacentor reticulatus* and five *Haemaphysalis parva* ticks were collected from 11 of these dogs (Table 1).

Serological findings. Out of the 300 canine serum samples, 16 (5.33%) samples were found to be doubtful, 20 (6.66%) samples were positive, and 35 (11.66%) samples were strongly positive for antibodies against *C. burnetii*. Totally, 55 (18.33%) of the 300 dog sera were

Table 1. The distribution of the foci, and dogs and ticks sampled in the present study using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR).

Provinces	No. of dogs (infested with ticks)	Distribution of ticks	Results of ELISA in dogs				<i>p</i> -value	Result of PCR in tick		
			Doubtful	Positive	Strong positive	Negative				
Iğdir	Center	65(21)	16 <i>R. sanguineus</i>	2	0	1	62	<i>p</i> < 0.05 ^a	0	
	Aralik	23(10)	62 <i>R. sanguineus</i>	2	5	7	9		4	
	Tuzluca	26(15)	69 <i>R. sanguineus</i>	3	2	7	14		0	
Total	114(46)	147	7	7	15	85	<i>p</i> > 0.05^b	0		
Ardahan	Center	9(0)	0	2	5	2	<i>p</i> < 0.05 ^{ac}	0		
	Posof	21(1)	9 <i>Ixodes</i> spp. (nymph)	3	0	0		18	0	
	Çıldır	25(0)	0	3	7	6		9	0	
Total	55(1)	9	6	9	11	29	0			
Kars	Center	83(10)	3 <i>H. parva</i> 21 <i>D. reticulatus</i>	3	4	9	67	<i>p</i> > 0.05 ^b	0	
	Akyaka	25(1)	2 <i>H. parva</i> 2 <i>D. reticulatus</i>	0	0	0	25		<i>p</i> < 0.05 ^c	0
	Selim	23(0)	0	0	0	0	23		0	
Total	131(11)	28	3	4	9	115	<i>p</i> < 0.05^d	4		

^a *p*-value between Iğdir and Ardahan (*p* < 0.05); ^b *p*-value between Iğdir and Kars (*p* > 0.05); ^c *p*-value between Kars and Ardahan (*p* < 0.05); ^d *p*-value among Iğdir, Ardahan, and Kars (*p* < 0.05).

Table 2. Touch-down polymerase chain reaction thermal conditions for *Coxiella burnetii*.

Reaction stages	Temperature (°C)	Time	Cycles
Initial denaturation	95	2 min	1
Denaturation	94	30 sec	
Annealing	From 61.00 - 66.00 °C (the temperature was reduced 1.00 °C in each subsequent cycle)		
Extension	72.00	1 min	5
Denaturation	94.00	30 sec	
Annealing	61.00	1 min	40
Extension	72.00	1 min	
Final extension	72.00	10 min	

determined to be positive for antibodies against *C. burnetii*. The distribution of the seropositive samples by the provinces was as follows: 22 (40.00%) were from Iğdir, 20 (36.36%) were from Ardahan, and 13 (23.63%) were from Kars. The highest ELISA seropositivity rates were determined in the dogs from Aralık, Çildir, and the centre of Kars. Statistical analyses were performed separately for Kars, Ardahan, and Iğdir. For each province, the results were categorized as *C. burnetii*-ELISA-strongly positive, *C. burnetii*-ELISA-positive, *C. burnetii*-ELISA-doubtful, and *C. burnetii*-ELISA-negative. The differences between the results belonging to three of the study locations (a, c, and d) were statistically significant (Table 1).

Polymerase chain reaction findings. Out of the 107 pooled tick DNAs, one pool consisting of the DNAs of three male *R. sanguineus* and three female *R. sanguineus* ticks was found to be positive with trans-PCR for *C. burnetii* DNA. In total, four (3.73%) out of the 107 tick DNAs were found to be positive for *C. burnetii* using PCR (Fig. 2). All of the canine blood samples were found to be negative for *C. burnetii* DNA. The dogs, from which the ticks infected with *C. burnetii* were collected, were serologically and molecularly negative.

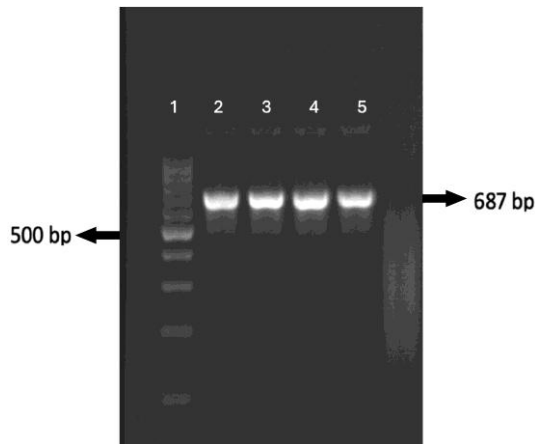


Fig. 2. Gel electrophoresis of amplified products of trans-polymerase chain reaction. Lane 1: Molecular size markers (100-bp DNA ladder; Invitrogen, Waltham, USA); Lane 2: Positive control; Lanes 3-5: Field samples; Lane 6: Negative control.

Discussion

Coxiella burnetii has the potential to infect humans and domestic and wild animals. Given the zoonotic nature of the pathogen, farm animals are the most important source for transmitting the disease to humans.³³ In a meta-analysis conducted by Kilbas *et al.*³⁴ on *C. burnetii* in animals (cattle, sheep, and goats) and humans in Türkiye, the pooled prevalence rates were reported as 13.49 and 22.78%, respectively. This suggests a possible transmission between animals and humans. It is essential that *C. burnetii* is studied in both humans and animals and that the results are evaluated in the context of the One Health

approach. When evaluated from this perspective, the importance of the present study conducted in the Northeastern Anatolia region becomes better understood.

Ticks, which act as vectors for infectious agents, are reported as being carriers of *C. burnetii*.¹¹ Studies have shown that many tick species can be infected with Q fever.^{2,11,28,35,36} Ticks can spread the agent both transstadially and transovarially.¹¹ The infection can also occur after getting bitten by a tick or during the crushing of ticks.^{14,37}

Arthropod-borne infections are more common in some regions as a result of global climate changes.¹ Thus, *C. burnetii* has gained more attention in the field of health lately.²⁶ Different prevalence rates have been reported in ticks in different countries. In a study conducted in Italy, *C. burnetii* positivity was determined to be 0.90% in *R. sanguineus* ticks, especially preferring dogs as hosts.³⁸ *Coxiella burnetii* was found in one out of eight (12.50%) pooled ticks, which were identified as *R. sanguineus* and collected from dogs in Iran.¹¹ The presence of *C. burnetii* was investigated by trans-PCR in ticks on sheep and goats in Iran, and a positivity rate of 11.42% was determined.³⁹ In Egypt, *C. burnetii* DNA was detected at a rate of 4.16% from *R. sanguineus* ticks collected from domestic dogs.⁴⁰ Ybañez has determined a positivity rate of 5.50% in 164 ticks collected from 36 dogs by amplifying the partial 16S ribosomal RNA gene region using the nested PCR.⁴¹ In Japan, 261 ticks were tested for *C. burnetii*, but no positive results were found.⁴²

Although some serological and molecular studies have been conducted in Türkiye regarding Q fever in different animal species, previous research on ticks is scarce. The PCR positivity rates of 46.15% (6/13 groups) and 1.88% (1/53 groups) were reported in tick groups collected from the Denizli and Ankara provinces of Türkiye, respectively.³⁶ In another study, 350 *R. sanguineus* ticks were collected from 85 dogs in the Siirt province and *C. burnetii* DNA was detected in 0.85% of the samples by nested PCR.⁴³ In the present study, *C. burnetii* was identified from four (3.73%) of the 107 pooled tick DNA samples. Comparison with previous studies revealed that the findings of this study were similar to those reported by Loftis *et al.*⁴⁰ Ybañez,⁴¹ and Aslan and Ayyildiz,⁴³ but differed from those reported by Khalili *et al.*¹¹ and Fard and Khalili, by a lower positivity rate.³⁹ The ticks collected from the seropositive dogs did not yield positive results for the presence of *C. burnetii*. This suggests that even if there is transmission through ticks, the collected ticks were not those that had transmitted the infection to the seropositive dogs as engorged ticks fall off the host into the substrate in order to molt into the next life stage or to lay eggs during their life cycle. As in the majority of previous studies, the presence of *C. burnetii* in *R. sanguineus* ticks was also determined in this study. While there are some indications that ticks have a part to play

in the epidemiology of *C. burnetii*, additional research is necessary to establish the vector capacity of *R. sanguineus* for transmitting this bacterium.

The Q fever is endemic in Türkiye. However, there are not enough published data about the presence of *C. burnetii* in domestic animals, such as dogs.^{7,44} Dogs can be infected by inhalation, the ingestion of contaminated food, and tick infestation. These animals, particularly during or after birth, have the potential to spread the infection into the environment, as well as to other animals and humans.⁷ The seroprevalence of *C. burnetii* in dogs has been reported to range between 0.00 - 35.00%.^{4,27,45} In a seroprevalence study conducted in Iraq, *C. burnetii* was reported in 5.50% of feral dogs.¹⁹ Hildebrandt *et al.* also found 21.80% seropositivity in dogs.¹⁵ In a study conducted by Orr *et al.* in Australia, anti-*C. burnetii* IgG was found at a rate of 18.30% in pig-hunting dogs.⁴⁶ In studies conducted on dogs and cats by Anastácio *et al.* in Portugal, the seroprevalence rate of *C. burnetii* was determined as 12.60% in 2012 and 1.70% in 2021 in dogs, and 17.20% in 2012 and 0.00% in 2021 in cats.⁴ Hornok *et al.* determined a positivity rate of 20.30% in stray dogs by ELISA and a single sample analysis by PCR.²⁶ In a study conducted in the Netherlands, *C. burnetii* DNA was detected in four (7.40%) out of 54 canine placenta tissues.⁴⁷ In a study conducted by Razaei *et al.*, *C. burnetii* was detected in 12.10 and 20.00% of canine uterine and vaginal swab samples by nested trans-PCR, respectively.⁴⁸

In the present study, *C. burnetii* could not be determined by PCR from any of the 300 canine blood samples. Out of the 300 canine blood serum samples tested by ELISA, 16 (5.33%) samples were doubtful, 20 (6.66%) samples were positive, and 35 (11.66%) samples were strongly positive. A total of 55 (18.33%) of the 300 dogs tested were found to be positive. The seropositivity rate determined in dogs in the present study is significantly high compared to the previous data reported from the world and Türkiye. Whole blood samples taken from the same dogs were examined molecularly and no positivity was detected. This can be explained by the dogs having acquired the infection before (not coinciding with the bacteremia period); so, no positive results were detected in the blood samples by PCR. Comparison with other studies reporting positive PCR results,^{27,47} showed that these studies had revealed positivity in samples from the placenta and uterus. When comparing the seroprevalence of the infection between locations, it was confirmed that there was a significant difference between a, c and d, but this difference was not significant in b. This suggests that varying factors, including nutrition of dogs and their contact with other animals and the environment, may play a role in the emergence of differences between locations. Further investigation into these factors is required to better understand and address the disparities in infection prevalence.

Studies have been conducted on *C. burnetii* in the Northeastern Anatolia region. These studies have shown the presence of *C. burnetii* in both humans and farm animals, such as cattle and sheep.^{43,49} However, while research on ticks and dogs in Türkiye is scarce, no study has yet been conducted to concurrently investigate dogs and their infesting ticks. This is the first study of this kind in the region.

In the current study, the presence of *C. burnetii* in ticks and dogs was determined by serological and molecular methods. As there is currently no routine vaccination program implemented against *C. burnetii* in Türkiye, it is assumed that the dogs, which tested positive in this study, were naturally infected. The results of the present study provide evidence of the exposure of dogs in the Kars, Ardahan, and Iğdir provinces to *C. burnetii* infection. Thus, previous studies indicating the presence of *C. burnetii* in other animal species in the region are supported by this study. The *C. burnetii* seropositivity may be attributed to dogs being housed outdoors (due to contact with farm animals, wildlife, and ticks) and being fed a raw diet. This study establishes a correlation between the infection detected in dogs and ticks. In this study, the presence of *C. burnetii* in ticks suggests that ticks may be potential vectors and may play a role in the epidemiology of the disease. However, further research is necessary to definitively establish the role of ticks in transmitting this agent. The feeding of ticks on dogs poses a risk of infection for other dogs and dog owners, and this risk should not be ignored.

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Conflict of interest

The authors declare that they have no conflict of interest.

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