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Türk Hijyen ve Deneysel Biyoloji Dergisi

Do companion animals represent a potential risk factor for Q fever transmission to humans in Kastamonu?

Kastamonu'da evcil hayvanlar, Q ateşinin insanlara bulaşında potansiyel bir risk faktörü oluşturuyor mu?

Elçin GÜNAYDIN[1] (ID), Yağmur Dilara YILMAZ[2] (ID), Çağatay SALUM[3] (ID), Pınar MURSALOĞLU KAYNAR[4] (ID), Gülşen GONCAGÜL[5] (ID)

ABSTRACT

Objective: A better understanding of the role of cats and dogs in the epidemiology of Q fever is necessary within the framework of the "One Health" approach. The first step in this process should be to investigate the presence of Q fever in dogs and cats. This study aimed to examine *Coxiella burnetii* DNA (deoxyribonucleic acid) in blood samples collected from randomly selected cats and dogs in close contact with their owners in Kastamonu.

Methods: Fifty-two blood samples were randomly collected from two groups of companion animals in Kastamonu between late March and September 2024, a period considered favorable for tick infestations. The animals comprised 32 dogs (29 stray dogs and three owned dogs) and 20 cats (12 stray cats and eight owned cats). Aseptically collected venous blood samples from pets in EDTA (ethylenediaminetetraacetic acid)-containing tubes were used for DNA extraction and blood smear preparation. *C. burnetii* was examined in the

ÖZET

Amaç: "Tek Sağlık" yaklaşımı çerçevesinde, Q ateşinin epidemiyolojisinde kedi ve köpeklerin rolünün daha iyi anlaşılması gereklidir. Bu süreçte ilk adım, köpek ve kedilerde Q ateşinin varlığının araştırılması olmalıdır. Bu çalışma, Kastamonu'da sahipleriyle yakın temas halinde bulunan, rastgele seçilmiş kedi ve köpeklerden alınan kan örneklerinde *Coxiella burnetii* DNA (deoksiribo nükleik asit)'sını incelemeyi amaçlamıştır.

Yöntem: Kene enfestasyonları için uygun kabul edilen bir dönem olan Mart ayı sonu ile Eylül 2024 arasında, Kastamonu'da iki evcil hayvan grubundan rastgele elli iki kan örneği toplandı. Hayvanlar 32 köpekten (29 sahipsiz köpek ve üç sahipli köpek) ve 20 kediden (12 sahipsiz kedi ve sekiz sahipli kedi) oluşmaktaydı. EDTA (etilendiamintetraasetik asit) içeren tüplere evcil hayvanlardan aseptik olarak alınan venöz kan örnekleri, DNA ekstraksiyonu ve kan yayması hazırlanması için kullanıldı. Ekstraktlarda *C. burnetii*, *C.*

[1]Hatay Mustafa Kemal University, Veterinary Faculty, Hatay, Türkiye
[2]Kastamonu University, Veterinary Faculty, Kastamonu, Türkiye
[3]Kastamonu University, Veterinary Faculty, Department of Physiology, Kastamonu, Türkiye
[4]Ankara Medipol University, Faculty of Pharmacy, Ankara, Türkiye
[5]Bursa Uludag University, Menann Pasinli Equine Vocational School, Bursa, Türkiye

İletişim / Corresponding Author : Elçin GÜNAYDIN
Hatay Mustafa Kemal Üniversitesi, Veteriner Fakültesi Hatay - Türkiye
E-posta / E-mail : elcin_gunaydin@hotmail.com

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extracts by touchdown polymerase chain reaction (PCR) using primers complementary to the IS1111 fragment, a transposon-like repetitive region of *C. burnetii*. The expected amplicon size was 687 bp.

Results: Three of the 52 examined blood samples (5.7%) were found to harbor *C. burnetii* DNA. The proportions of cats and dogs positive for *C. burnetii* DNA among all samples were 3.84% and 1.92%, respectively. The positivity rates among cats and dogs were 10% and 3.12%, respectively. Blood smears were negative. No significant correlation was detected between the variables in the study group.

Conclusion: Companion animals in close contact with humans in the urbanized region of Kastamonu showed a 5.7% positivity rate, although the sample size in the present study was limited. Therefore, more comprehensive studies including both companion animals and their owners should be planned to evaluate whether cats and dogs pose a risk for the transmission of Q fever to humans.

Key Words: Q fever, PCR, cat, dog, hematological assessment

burnetii'nin transpozon benzeri tekrarlayan bir bölgesi olan IS1111 fragmentine komplementer primerler kullanılarak kademeli sıcaklık düşürme PCR polimeraz zincir reaksiyonu (PCR) ile incelendi. Beklenen amplikon büyüklüğü 687 bp idi.

Bulgular: İncelenen 52 kan örneğinin üçünde (%5,7) *C. burnetii* DNA'sı bulunduğu saptandı. Tüm örnekler içinde *C. burnetii* DNA'sı pozitif olan kedi ve köpek oranları sırasıyla %3,84 ve %1,92 idi. Kediler ve köpekler arasındaki pozitiflik oranları sırasıyla %10 ve %3,12 idi. Kan yaymaları negatifti. Çalışma grubundaki değişkenler arasında anlamlı bir korelasyon saptanmadı.

Sonuç: Mevcut çalışmada örneklem büyüklüğü sınırlı olmasına rağmen, Kastamonu'nun kentleşmiş bölgesinde insanlarla yakın temas halinde bulunan evcil hayvanlarda %5,7 pozitiflik oranı gösterildi. Bu nedenle, kedilerin ve köpeklerin Q ateşinin insanlara bulaşında bir risk oluşturup oluşturmadığını değerlendirmek için hem evcil hayvanları hem de sahiplerini içeren daha kapsamlı çalışmalar planlanmalıdır.

Anahtar Kelimeler: Q fever, PZR, kedi, köpek, hematolojik değerlendirme

INTRODUCTION

C. burnetii is mainly associated with farm animals, particularly ruminants, in which it causes reproductive disorders. The presence of large numbers of bacteria, mainly in the placenta, birth fluids, and milk, creates a high risk of infection for workers, veterinarians, and owners who come into contact with infected animals and their products (1,2). Therefore, the number of human cases is often correlated with the local livestock population. Moreover, raw milk and unpasteurized dairy products are risk factors for consumers (2).

Apart from livestock, companion animals, especially dogs and cats kept in close contact with their owners, are known to be important reservoirs of *C. burnetii* during urban Q fever outbreaks (2-6). However, data on the spread of *C. burnetii* in domestic and/or stray cat and dog populations remain limited. Therefore, a better understanding of the role of cats and dogs in the epidemiology of Q fever is necessary within the "One Health" framework (7, 8). It has been suggested that dogs and cats may become infected through tick bites, consumption of placenta or milk from infected livestock, consumption of raw meat, inhalation of aerosolized bacteria in the environment, or predation on contaminated animal species (6,9).

In particular, certain groups of dogs, such as hunting dogs, stray dogs, and shepherd dogs, may

be exposed to this infection during outbreaks in ruminants. Exposed barn cats and farm dogs have also been reported to seroconvert, and some fluids, such as vaginal secretions, have been suggested to test positive by molecular methods (10). Infected pets may pose a potential threat to human and livestock health because infection in pets is often completely asymptomatic (11). Human Q fever outbreaks linked to pets have often resulted from contact with cats or dogs during or shortly after parturition, particularly in cases involving dystocia, stillbirths, and neonatal mortality (12). Detections of *Coxiella* spp. have also been reported after normal birth (13). However, the presence of *C. burnetii* DNA in pet fluids appears to be sporadic, and many studies have suggested limited bacterial shedding (1). This study aimed to determine the presence of *C. burnetii* in randomly collected blood samples from cats and dogs and to assess the early phase of infection. In addition, it investigated whether companion animals may represent a potential risk for humans living in Kastamonu.

MATERIAL and METHOD

Fifty-two blood samples were randomly collected from two different groups of companion animals in Kastamonu between the end of March 2024 and September 2024. The study population comprised 32 dogs (29 stray dogs and 3 owned dogs) and 20 cats (12 stray cats and eight owned cats). Of the 32 dog blood samples, 18 were collected from female dogs, including one owned dog and 17 stray dogs, while 14 were collected from male dogs, including two owned dogs and 12 stray dogs. Of the 20 cat blood samples, 14 were collected from female cats, including nine stray cats and five owned cats. Among the six male cats, three were owned cats and three were stray cats. The blood samples collected from dogs and cats are summarized in detail in Table 1. No information was available for stray dogs and cats. Information on owned cats and dogs is presented in Tables 2 and 3.

Under aseptic conditions, approximately 4 mL of venous blood was collected from the cephalic or saphenous vein of each dog and cat and transferred into labeled EDTA anticoagulant tubes. After one drop of each blood sample was used immediately to prepare blood smears, the remaining EDTA-anticoagulated blood samples were stored at −20 °C until DNA extraction.

MayGrunwald-Giemsa Staining: A drop of blood was placed on a slide, and smears were prepared using a coverslip, repeated at least three times, and air-dried. The smears were stained as described by Konuk (14). The presence of the agent within erythrocytes was investigated under a light microscope at 100X magnification on the same day.

DNA Extraction: DNA was extracted from blood samples using a commercial DNA isolation kit (Blood Genomic DNA Kit, EcoSpin, EcoBGD-50X) according to the manufacturer's instructions. The extracted DNA samples were stored at −20 °C until use.

Table 1. Sample size of the blood collected from dogs and cats

	Dogs		Cats	
Gender	**Female**	**Male**	**Female**	**Male**
Owned	1	2	5	3
Stray	17	12	9	3
Total	18	14	14	6
General Total	**32**		**20**	

Table 2. The information about owned dogs

Number of samples	Gender	Age (Year)	Race	Residing Place
1	Male	1	Shepherd	Home
1	Male	1.5	Kangal	Back yard
1	Female	13	German Shepherd	Home

Table 3. The information about owned cats

Number of samples	Gender	Age (Year)	Race
1	Female	1	Yellow cat
2	Male	3	Tabby cat
1	Female	2	Tabby cat
1	Female	2	Scottish
1	Female	1.5	Tabby cat
1	Female	2.5	Tabby cat
1	Male	1.5	Tabby cat

Primers: The primers previously described by Hoover et al. (15) were used for the detection of *C. burnetii* by touchdown PCR. The primer sequences were as follows: Trans-1 forward, 5′-TAT GTATCC ACC GTA GCC AGT C-3′; and Trans-2 reverse, 5′-CCCAAC AAC ACC TCC TTA TTC-3′. These primers are complementary to the IS1111 fragment, a transposon-like repetitive region of *C. burnetii*. The expected amplicon size was 687 bp.

Touchdown PCR: Each reaction was performed in a final volume of 25 µL, consisting of 22 µL of reaction mixture and 3 µL of template DNA. The reaction mixture contained 2.5 µL of 10X PCR buffer without $MgCl_2$, 0.5 µL of dNTPs (10 mM), 1.5 µL of MgCl2 (25 mM), 1 µL of each primer (10 pmol/µL), 0.25 µL of Taq DNA polymerase (5 U/µL) (Fermantas; EP 402), and 15.25 µL of deionized water. The cycling conditions were as follows: initial denaturation at 95 °C for 2 min; followed by five cycles of denaturation at 94 °C for 30 s, annealing at 66–61 °C for 1 min, with the temperature decreased by 1 °C in each consecutive cycle, and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min. Touchdown PCR was performed using a thermal cycler (Arktik, Thermo Scientific).

Agarose Gel Electrophoresis: PCR products were electrophoresed on a 1.5% agarose gel in TBE (Tris-borate-EDTA) buffer containing 0.5 µg/mL ethidium bromide at 100 V for 45 min and visualized under UV light.

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as frequencies and percentages (n, %). The distribution of PCR results (positive/negative) across different categorical variables, including species (cat/dog), sex (female/male), and living status (stray/owned), was evaluated using Pearson's chi-

square test and Fisher's exact test.

Fisher's exact test was specifically used when the expected cell counts in contingency tables were less than 5. A p-value of less than 0.05 (p <0.05) was considered statistically significant for all analyses.

This study was approved by the Animal Experiments Local Ethics Committee of Kastamonu University Rectorate (Date: 10.11.2023 and Number: 9-42).

RESULTS

Three of the 52 examined blood samples (5.7%) were found to harbor *C. burnetii* DNA. The proportions of cats and dogs positive for *C. burnetii* DNA among all samples were 3.84% and 1.92%, respectively. The positivity rates among cats and dogs were 10% and 3.12%, respectively. Two of the three positive blood samples belonged to female stray cats, while the remaining positive sample belonged to a male household dog kept in the backyard of a house. The remaining blood samples were negative for *C. burnetii* DNA. Examination of the blood smears revealed normal erythrocyte morphology, and no pathogen was detected. No association was determined between the variables in the study group.

Table 4. Distribution and statistical comparison of PCR results according to species, gender, and living status of animals

Variable	PCR Positive n (%)	PCR Negative n (%)	Total	Pearson x2 (p)	Fisher's Exact (p)
Animals					
Dogs	1 (3.1%)	31 (96.9%)	32	0.301	0.551
Cats	2 (10.0%)	18 (90.0%)	20		
Gender					
Female	2 (6.3%)	30 (93.8%)	32	0.851	1.000
Male	1 (5.0%)	19 (95.0%)	20		
Living Status					
Stray	2 (4.9%)	39 (95.1%)	41	0.595	0.518
Owned	1 (9.1%)	10 (90.9%)	11		

DISCUSSION

Global studies conducted in cats have demonstrated exposure to *C. burnetii* at rates of 14.2% in Japan (16), 9% in the United States (17), 13% in Zimbabwe, 2% in South Africa (18), 4.9% in Türkiye (19), and 13.1% in Australia (13). Similar surveys in dogs have reported *C. burnetii* exposure rates of 26.1% in Australia (13), 66% in the United States (17), 59.3% in Bulgaria (20), 12% in Croatia (21), 9.8% in France (22), and 1% in Italy (23). No anti-*C. burnetii* antibodies were detected in either dogs or cats in Canada (24) or China (25). However, all of these studies were based on serological responses. Serological tests may not detect specific anti-*C. burnetii* antibodies during the early stage of infection (22,23). Therefore, we designed our study to detect the early stage of infection during the tick infestation period, when the favorable climatic and ecological conditions of Kastamonu support the development of several tick

species between April and September. Moreover, the study period coincided with the peak birth season. Considering the potential spread of *Coxiella* spores mainly through the placenta, birth fluids, milk, and resistant spores in the environment, this period may increase the likelihood of detecting *C. burnetii* DNA in blood samples during the early stage of infection.

The positivity rate of 1.92% determined in dog samples in the present study was lower than those reported in previous studies (23,26-29). Studies investigating the presence of *C. burnetii* DNA in whole blood samples of dogs by PCR have reported rates of 6.4% in Iraq (26), 10% in Zambia (27), 2.7% in southern Italy (23), 10% in Algeria (28), and 11% in Iran (29). Although we expected to detect *C. burnetii* in the 29 stray dogs that had not received antiparasitic treatment, *C. burnetii* DNA was detected in only one of the 32 dog blood samples, which belonged to a male owned dog kept in the backyard of a house. Commenting on the potential reasons for detecting the agent in this male dog would be unreliable because of the limited dog sample size. Nevertheless, a dog kept in the backyard of a house may come into contact with livestock, wildlife, and ticks, which could favor exposure to *C. burnetii* (30). As previously emphasized, the sampling period coincided with the tick infestation period in Kastamonu. The negative results obtained from dogs kept indoors were expected, because animals in urban areas are less exposed to livestock, wildlife, and ticks than those in rural areas (31).

Comparatively, studies focusing on the molecular detection of Q fever in cats are scarce worldwide. Similar to the results obtained from dog samples, the positivity rate of 3.84% in the present study was lower than the rates reported in domestic cats in Iran (11.5%) and stray cats in Iran (15.3%) (32), stray cats in Italy (29.4%) (33), pet cats in Egypt (7.5%) (34), and pet cats in the United States (8.5%) (35). However, it was higher than the rates reported in studies conducted in Spain and Australia, both of which reported 0.00% positivity (13,36). The detection of the agent in whole blood samples from stray cats in the current study was not attributed primarily to ticks, because cats usually live in environments where ticks are less common, they do not frequently come into contact with wildlife areas, and they may remove ticks during self-grooming (37). Supporting this interpretation, a study conducted in northern Italy reported that ticks collected from cats with *C. burnetii* bacteremia at a rate of 29.4% were PCR-negative for *C. burnetii* (38). Therefore, considering that the stray cats lived on the street, the possible route of Q fever contamination was attributed to predation on rodents, which are considered important reservoirs of the agent (37). In addition, birds, known as potential vectors of *C. burnetii*, may represent a potential risk factor for stray cats because of their habitual hunting activity (38). Environmental contamination with avian feces may also predispose cats to Q fever, as the study period between April and September coincided with the migratory season (39).

In conclusion, although the sample size of the present study was limited, the detection of the early phase of infection in whole blood samples from companion animals in close contact with their owners suggests that cats and dogs may pose a potential risk for Q fever transmission to humans in Kastamonu. Further studies should aim to examine samples collected from both companion animals and their owners to determine whether these animals may serve as potential reservoirs of Q fever in urbanized areas.

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ETHICS COMMITTEE APPROVAL

* This study was approved by the Animal Experiments Local Ethics Committee of Kastamonu University Rectorate (Date: 10.11.2023 and Number: 9-42).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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