Ankara Üniv Vet Fak Derg, **60**, 263-268, 2013

Detection of *Coxiella burnetii* from ticks by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism*

Gülay ALTAY ÇAPIN, Zişan EMRE, Seyit CANPOLAT, Yusuf VATANSEVER, Ali DÜZGÜN

Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Department of Nuclear Applications on Animal Science, Ankara, Turkey.

Summary: For the detection of *Coxiella burnetii*, a total of 2472 ticks (1446 female, 1021 male and 5 nymphs) was collected from cattle and sheep of 38 provinces of Turkey. The ticks were pooled into groups of 1-7 ticks of the same province, species and gender for DNA extraction. Following DNA extraction, the groups were examined for the presence of *C. burnetii* DNA by using the primers CB1 and CB2. Six groups from the province of Denizli (13 groups of total 56 ticks), and one group from the province of Ankara (53 groups of total 160 ticks) were found to be positive for *C. burnetii*. The species of *Rhipicephalus turanicus, Rhipicephalus bursa* and *Hyalomma excavatum* were found to be infected with *C. burnetii.* The gender was not seem to have a role in transmission of the agent. The specificities of the PCR products were evaluated by the restriction fragment length polimorphism (RFLP) analysis. The positive PCR products were digested with the enzyme *Taq*I and four bands in order of 118, 57, 43 and 39 bp's were appeared such as seen in the positive control DNA (*C. burnetii* Nine Mile RSA493).

Key words: *Coxiella burnetii*, PCR, tick.

Coxiella burnetii'nin kenelerden Polimeraz Zincir Reaksiyonu ve Restriction Fragment Length Polymorphism ile saptanması

Özet: *Coxiella burnetii*'nin teşhisi amacıyla Türkiye'nin 38 ilinden toplam 2472 (1446 dişi, 1021 erkek, 5 nymph) adet kene toplandı. Keneler toplandıkları il, tür ve cinsiyetlerine göre 1-7 adedi bir araya getirilerek gruplandırıldı. Gruplar DNA ekstraksiyonu sonrasında CB1 ve CB2 primerleri kullanılarak PCR ile *C. burnetii*'nin varlığı yönünde incelendi. Denizli'ye ait 6 grup (Toplam 56 kene ve 13 grup) ile Ankara'ya ait bir grup (Toplam 160 kene ve 53 grup) *C. burnetii* yönünden pozitif bulundu. *Rhipicephalus turanicus, Rhipicephalus bursa* ve *Hyalomma excavatum* türlerinin *C. burnetii* taşıdıkları belirlendi. Cinsiyetin etkenin taşınmasında etkili olmadığı görüldü. PCR ürünlerinin spesifitesi restriction fragment length polimorphism (RFLP) analizi ile değerlendirildi. Pozitif PCR ürünleri *Taq*I enzimi ile kesilerek, *C. burnetii* Nine Mile RSA493 suşunda görüldüğü gibi, 118, 57, 43 ve 39 bp'lik 4 bant ortaya konuldu.

Anahtar sözcükler: *Coxiella burnetii,* kene, PCR.

Introduction

Coxiella burnetii, an obligate intracellular bacterium, is the causative agent of Q-fever in humans and animals. Domestic and wild mammals, birds and arthropods are known reservoirs of *C. burnetii* (1, 6, 26). In cattle, sheep and goats, which are the primary reservoirs of the agent, the infection is usually asymptomatic however, abortion may occur as a result of the infection (4, 14, 25). *C. burnetii* is resistant to many external physical and chemical environmental factors and has a long term ability to survive in the environment. These characteristics of the bacterium are the main obstacles in the control of the infection (1, 7).

Ticks are the vectors for *C. burnetii* in nature and transmit the organism not only by their feces or saliva horizontally but also transstadially and transovarially. Ticks' feces are probably the most common source of the *C. burnetii* in nature (21, 26). Up to 10^{10} organisms/gram can be recovered from feces of experimentally infected ticks (1). Transmission of *C. burnetii* to host animals is either directly via tick bites or indirectly through contact with infected excreta (1, 22). Ticks can be accepted as the indicators of the infection in nature for over 40 species have been found to be infected with *C. burnetii* (15). In ticks, *C. burnetii* can multiply to very high titers, remains viable during their entire life, and transmitted transovarially to next generations (1).

C. burnetii has been found in several tick species but there is a few reports on the role of ticks in the transmission of the pathogen to humans (1, 15). In

* This study was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK –VHAG 2100) and Turkish Atomic Energy Authority (TAEK)

humans, infection mostly take place after inhalation of contaminated aerosols, consumption of fresh dairy products, or contact with infected animals (24, 26).

Q-fever is endemic in Turkey and the mean seroprevalence rate in asymptomatic people is 15.2% (11). Seroprevalence of *C. burnetii* infection was found to be 10.5% in sheep and between 5.8-21.7% in cattle in Turkey (4, 19, 20, 28).

In Turkey, serology is the most common technique in the diagnosis of *C. burnetii* infection. There is a limited number of studies using PCR technique to detect the organism (2, 12, 18), and is no data on the tick species carrying *C. burnetii*.

Polymerase Chain Reaction (PCR) and PCR-based techniques are reported to be sensitive and specific for the detection of *C. burnetii* from tick samples (3, 17, 29, 31). *C. burnetii* were found in *Ixodes ricinus* (24, 29), *Dermacentor reticulatus* (24), *D. marginatus* (1, 23, 29), *Haemaphysalis concinna* (24, 29), *H. punctata* (24), *H. inermis* (24), *H. sulcata* (27), *H. longicornis* (13), *Rhipicephalus sanguineus* (3, 5, 22, 27), *R. turanicus* (3, 27), *Ambylomma variegatum* (16), *Hyalomma* spp. (5, 22) by using conventional (23) and PCR techniques (3, 5, 29, 31).

The aim of this study was to use PCR and restriction fragment length polymorphism (PCR-RFLP) techniques for the detection of *C. burnetii* from ticks collected from several provinces of Turkey.

Materials and Methods

Tick sampling and processing: A total of 2472 ticks (1446 female, 1021 male and 5 nymphs) was collected from 38 provinces of Turkey. The ticks were identified and sorted according to the province, species, sex and developmental stages. Later, ticks were gathered into groups of 1 to 7 ticks as to the provinces, species and gender for DNA extraction. The numbered tick groups were placed in aluminium foil and freezed in liquid nitrogen (-196 ºC). Frozen ticks were triturated thoroughly in a mortar. The total DNA from ticks was extracted using NucleoSpin Tissue kit (Macherey-Nagel GmbH, Düren, Germany). Maximum 50 mg triturated tick sample was transferred to an eppendorf tube and processed as described by the manufacturer. The DNA extracts were stored at -20º C until amplification.

The control of the DNA exctraction kit was performed with PCR using the primers 28 SR and 28 SF which detect 28S rRNA gene of ticks (9) (Table 1).

PCR amplification: PTC-100 thermal cycler (MJ Research, Watertown, MA) was used for DNA amplification. Amplification was performed in 30 µl volumes, containing 0.5 µl of 10 pmol of each primer (Table 1), 0.5 µl of dNTP Mix (10 mM, Fermentas), 3 µl 10xPCR buffer, 0.25 µl of *Taq* DNA polimerase (5 U/µl, Bioron), 2.4 µl of 25 mM $MgCl_2$, 1.8 µl DMSO (Fermentas), 3 µl of the DNA extract, and was made up to 30 µl with ddH_2O. In each test, a positive (Nine Mile RSA493) and a negative control (double distilled water) were used.

PCR was performed under the following conditions: denaturation at 94°C for 5 min, annealing at 52°C for 30 s, and extension at 72°C for 1 min for one cycle, and denaturation at 94°C for 45 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min for 34 cycles and a final cycle of denaturation at 94 ºC for 45 s, annealing at 50°C for 1 min, and extension at 72°C for 10 min.

The PCR products were separated on a 1.5% agarose gel stained with ethidium bromide. The DNA fragments were visualized by UV illumination.

The *C. burnetii* Nine Mile RSA493 strain was provided from Prof. Dr. Habil G. Baljer (Institut für Hygiene und Infektionskrankheiten der Tiere de Justus-Liebig-Universitat Giessen). The strain was used at 3.3 x 10^8 particles/ml concentration in 0.9 % saline solution. Bacteria had been heat inactivated at 100ºC, 30 min in water bath. The isolate was treated with proteinase K overnigth and subsequently heated for 15 min at 100 ºC to inactivate the proteinase K.

RFLP: The specificity of the amplification was evaluated by restriction fragment length polimorphism analysis of the PCR products. The CB1 and CB2 products were digested with the enzyme *Taq*I (Fermentas) as described by the manufacturer. Restriction fragments were examined by electrophoresis on a 3.5% low-melting agarose gel. Samples were compered with fragments of *Coxiella burnetii* Nine Mile RSA493 strain.

Table 1. Primer sequences used in the study
Tablo 1. Çalışmada kullanılan primerler

Gene	Primer	Oligonucleotide sequence (5'-3')	Fragment length (bp)	Reference
Superoxide dismutase	CB1[a]	5'-ACT CAA CGC ACT GGA ACC GC-3'	257	30
	CB2[a]	5'-TAG CTG AAG CCA ATT CGC C-3'		
28S rRNA	28 SF[b]	5'-GAC TCT AGT CTG ACT CTG TG-3'	449	9
	28 SR[b]	5'-GCC ACA AGC CAG TTA TCC C-3'		

[a] Primers CB1 and CB2 were derived from the *C. burnetii* superoxide dismutase gene
[b] Primer 28 SF and 28 SR were 28S rRNA gene sequence of *Haemaphysalis*, *Rhipicephalus* and *Ixodes.*

Results

The distribution of ticks collected from 38 provinces of Turkey was presented in Table 2.

Table 2.Distribution of ticks according to species and gender.
Tablo 2. Kenelerin tür ve cinslerine göre dağılımı.

Species	Total (%)	Female	Male
Rhipicephalus turanicus	612 (24,8)	324	288
Rhipicephalus sanguineus	294 (11,9)	137	157
Rhipicephalus bursa	1021 (41,3)	700	321
Hyalomma anatolicum	133 (5,4)	40	93
Hyalomma excavatum	104 (4,2)	38	66
Hyalomma detritum	143 (5,8)	92	51
Hyalomma marginatum	29 (1,2)	17	12
Dermacentor niveus	36 (1,5)	22	14
Dermacentor marginatus	13 (0,5)	6	7
Haemaphysalis sulcata	11 (0,4)	10	1
Haemaphysalis punctata	24 (0,9)	15	9
Ixodes ricinus	36 (1,5)	34	2
Ixodes hexagonus	3 (0,1)	3	-
Boophilus annulatus	8 (0,3)	8	-
Nymph (Rhipicephalus)	5 (0,2)		

The control of DNA exctraction kit was performed with PCR using 28 SR and 28 SF primers which detect 28S rRNA gene of ticks. Visualization of a fragment of 449 bp proved that 28S rRNA gene is extracted.

Fifty six ticks collected from Denizli region were divided into 13 groups according to species and gender. Six groups of these were found positive for *C. burnetii* by PCR. From Ankara region one group of 53 groups of collected 160 ticks was detected positive by PCR. For internal quality control, positive and negative controls were used in each assay to receive the band of 257 bp of reference strain of Nine Mile (Figure 1).

The species and gender distribution of *C. burnetii* infected ticks was given in Table 3. The results have shown that the gender is not effective on the transmission of infection.

Table 3. The species and gender distribution of *C. burnetii* infected ticks by PCR.
Tablo 3. PCR ile *C. burnetii* pozitif bulunmuş kenelerin tür ve cins dağılımı.

Province	Species	Gender	Number of ticks in the group
Denizli			
Group No. 302	*Rhipicephalus bursa*	Female	5
Group No. 303	*Rhipicephalus bursa*	Female	5
Group No. 304	*Rhipicephalus bursa*	Female	5
Group No. 305	*Rhipicephalus bursa*	Female	4
Group No. 306	*Rhipicephalus bursa*	Male	5
Group No. 307	*Rhipicephalus turanicus*	Male	2
Ankara			
Group No. 516	*Hyalomma excavatum*	Male	1

The specificity of the amplification was confirmed by restriction analysis of the PCR products. The PCR products were digested with the enzyme *Taq*I and the fragments of 118 bp and 57 bp were viewed clearly. The fragments of 43 and 39 bp were obtained as a thick band as they close to each other (Figure 2). Samples were compared with the fragments of *C. burnetii* Nine Mile RSA493 strain.

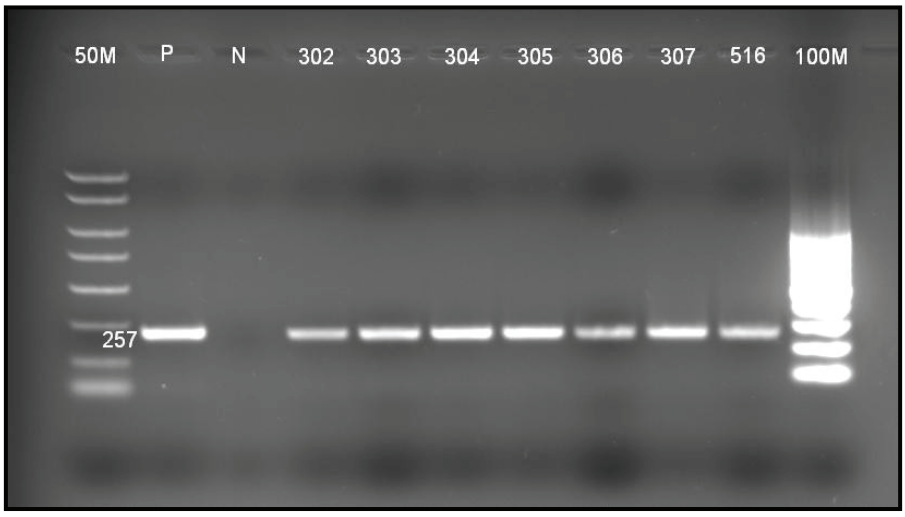

Figure 1. Positive PCR products with primer pair CB1 and CB2.
50M: Marker; P: *Coxiella burnetii* Nine Mile RSA493 strain; N: Negative control; 302-307: Positive samples from Denizli; 516: Positive sample from Ankara; 100M: Marker.
Şekil 1. CB1 ve CB2 primer çifti ile elde edilen pozitif PCR sonuçları
50M: Marker; P: *Coxiella burnetii* Nine Mile RSA493 suşu; N: Negatif kontrol; 302-307: Pozitif örnekler (Denizli); 516: Pozitif örnek (Ankara); 100M: Marker.

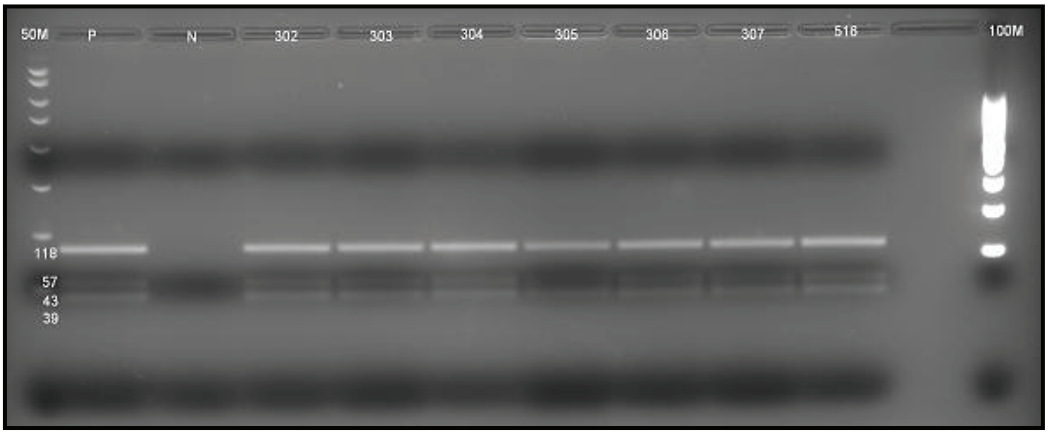

Figure 2. Restriction fragment length polymorphism analysis of the 257-bp amplification products.
50 M: Molecular size markers; P: *Coxiella burnetii* Nine Mile RSA493 strain; N: Negative control; 302-307: Positive samples from Denizli; 516: Positive sample from Ankara; 100M: Marker.
Figure 2. 257-bp amplifikasyon ürünlerinin restriksiyon endonükleaz profil analizleri sonucu.
50 M:marker; P: *C. burnetii* Nine Mile RSA493 suşu; N: Negatif kontrol; 302-307: Pozitif örnekler (Denizli); 516: Pozitif örnek(Ankara); 100M: Marker.

Discussion

Coxiella burnetii, an obligate Gram negative intracellular bacterium, is the causative agent of Q fever. Maurin and Raoult (15) indicated that Q fever, a rarely notifiable zoonotic disease of worldwide distribution, should be considered a public health problem. In humans, *C. burnetii* infection is usually asymptomatic. However Q fever may lead to serious complications such as meningoencephalitis, myocarditis or endocarditis (1, 15).

In the enzootic cycle, ticks and vertebrates are important components. As a divers range of tick species have been found to be infected with *C. burnetii*, ticks are the susceptible host for Q fever and potentially spread *C. burnetii*. For that reason ticks serve as the indicators of the infection in nature (21).

The possibility of studying *C. burnetii* strains by molecular biological techniques has improved genetic characterization of the bacterium and contributed to the research area of vectors and reservoirs of the organism (15, 32).

PCR have facilitated the sensitive and specific detection of *C. burnetii* in several materials in comparing with the serological techniques in which the diagnosis is mostly retrospective and limited (33). PCR also enables to work on field materials fixed in alcohol or formaldehyde (10, 15). PCR-RFLP is an advised method for the detection and identification of *C. burnetii* from materials of human and animal origin (29, 30, 32). The results of this study confirmed that PCR-RFLP was a reliable combined technique for the detection of *C. burnetii* in tick species.

The isocitrate dehydrogenase gene (17), the superoxide dismutase gene (14, 32) and chromosomal transposon-like repetitive region (5, 8, 18) of *C. burnetii* were used as target genes in PCR assays and reported to be successful for the identification of *C. burnetii* strains in different materials.

Masala et al.(14) and Stein and Raoult (32) were used PCR assay with CB1 and CB2 primers targeting superoxide dismutase gene for the detection of *C. burnetii* in different materials. In this study, the PCR using CB1 and CB2 primers derived from the superoxide dismutase gene was found to be a reliable technique for the detection of *C. burnetii* from ticks.

In this study, pools of ticks were used for DNA extraction which give the possibility of testing large numbers of ticks collected in the field. PCR-RFLP technique showed that *R. turanicus, R. bursa* and *H. excavatum* were positive for *C. burnetii.* Seven *C. burnetii* positive samples gave identical profiles with Nine Mile reference strain in the RFLP. These results confirm the previously reported results (3, 5). It was found that the gender had no effect on the transmission of *C. burnetii.*

In previous studies, *C. burnetii* was reported to be found in the ticks *I. ricinus, D. marginatus* and *H. punctata* (24, 29). In this study, *C. burnetii* was not detected in these species. These ticks were few in the field (*D. marginatus* 0.5%, *I. ricinus* 1.5% and *H. punctata* 0.9%) and this may effect the presence of infection.

The profiles of the strains isolated from Q fever patients in France (32) and Greece (30) were reported to be identical with the reference *C. burnetii* Nine Mile strain from ticks originating from USA. In this study, profiles of the seven positive samples were also found to be similar to the reference Nine Mile RSA493 strain by RFLP.

In conclusion, we demonstrated the presence of *C. burnetii* in *Rhipicephalus turanicus, Rhipicephalus bursa* and *Hyalomma excavatum* ticks by PCR-RFLP. It is once

indicated that the combination of PCR-RFLP is a faster and reliable technique for the detection of *C.burnetii* from ticks. This was the first molecular detection of *C. burnetii* in ticks in Turkey. However, the role of ticks in the epidemiology of Q fever needs to be further investigated.

References

1. **Aitken, I.D., Bögel, K., Cracea, E. et al.** (1987) Q Fever in Europe: Current aspects of aetiology, epidemiology, human infection, diagnosis and therapy. Infection, **15**,323-327.
2. **Akgün, E., Yılmaz, M., Pınarbaşı, E**. (2006) Q-fever şüphesi olan hasta serum ve kan örneklerinde nested-PCR yöntemiyle *Coxiella burnetii*'nin saptanması. Cumhuriyet Üniv Tıp Fak Derg, **28**, 50-54.
3. **Bernasconi, M.V., Casati, S., Peter, O., Piffaretti, J.C.** (2002) Rhipicephalus ticks infected with *Rickettsia* and *Coxiella* in Southern Switzerland (Canton Ticino). Infect Genet Evol, **2**,111-120.
4. **Çetinkaya, B., Kalender, H., Ertaş, H.B. et al.** (2000) Seroprevalence of coxiellosis in cattle, sheep and people in the east of Turkey. Vet Rec, **146,** 131-136.
5. **Fard, S.R.N. and Khalili, M.** (2011) PCR detection of *Coxiella burnetii* in ticks collected from sheep and goats in southeast Iran. Iran J Arthropod-Borne Dis, **5**,1-6.
6. **Fournier, P.E., Marrie, T.J., Raoult, D.** (1998) Diagnosis of Q-Fever. J Clin Microbiol, **36**, 1823-1834.
7. **Higgins, J.A., Azad, A.F.** (1995) Use of polymerase chain reaction to detect bacteria in arthropods: a review. J Med Entomol, **32**, 213-222.
8. **Houver, A.T., Vodkin, M.H., Williams, J.C.** (1992) A *Coxiella burnetii* repeated DNA element resembling a bacterial insertion sequence. J Bacteriol, **174**, 5540-5548.
9. **Inokuma, H., Yoshizaki, Y., Shimada, Y. et al.** (2003) Epidemiological survey of *Babesia* species in Japan performed with specimens from ticks collected from dogs and detection of new *Babesia* DNA closely related to *Babesia odocoilei* and *Babesia divergens* DNA. J Clin Microbiol, **41**, 3494-3498.
10. **Kenneth, L.G., Schrumpf, M.E., Karstens, R.H. et al.** (1994) DNA typing of *Rickettsiae* in naturally infected ticks using polymerase chain reaction/restriction fragment length polymorphism system. Am J Trop Med Hyg, **50**, 247-260.
11. **Kılıç, S ve Çelebi, B.** (2008) Türkiye'de *C. burnetii*'nin epidemiyolojisi (2. Bölüm). Türk Hij Den Biyol Derg, **65** S3, 21-31.
12. **Kırkan, Ş., Kaya, O., Tekbıyık, S. and Parın, U.** (2008) Detection of *Coxiella burnetii* in cattle by PCR. Turk J Vet Anim Sci, **32**, 215-220.
13. **Lee, J.H., Park, H.S., Jang, W.J. et al.** (2004) Identification of the *Coxiella sp*. detected from *Haemaphysalis longicornis* ticks in Korea. Microbiol Immunol, **48**,125-130.
14. **Masala, G., Porcu, R., Sana, G. et al.** (2004) Occurence, distribution, and role in abortion of *Coxiella burnetii* in sheep and goats in Sardinia, Italy. Vet Microbiol, **99**, 301-302.
15. **Maurin, M. and Raoult, D.** (1999) Q Fever. Clin Microbiol Rev, **12**, 518-553.
16. **Mediannikov, O., Fenollar, F., Socolovschi, C. et al.** (2010) *Coxiella burnetii* in Humans and ticks in rural Senegal. PLOS Negl Trop Dis, **4**, e654, doi:10.1371/journal.pntd.0000654
17. **Nguyen, S.V. and Hirai, K.** (1999) Differentiation of *Coxiella burnetii* isolates by sequence determination and PCR-restriction fragment length polymorphism analysis of isocitrate dehydrogenase gene. FEMS Microbiol Lett, **180**, 249-254.
18. **Öngör, H., Çetinkaya, B., Karahan, M. et al.** (2004) Detection of *Coxiella burnetii* by immunomagnetic separation-PCR in the milk of sheep in Turkey. Vet Rec, **154**, 570-572.
19. **Özgür, N.Y., Haköksüz, M., Yılmaz, H. et al.** (1997) İnfertilite sorunu olan dişi sığırlarda ve insanlarda *Coxiella burnetii* antikorlarının ELISA testi ile belirlenmesi ve seroprevalansının saptanması. Pendik Vet Mikrobiyol Derg, **28**, 207-217.
20. **Özyer, M., Miroğlu, M., Köksal, F.** (1990) Çukurova bölgesinde yaşayan insan ve hayvanlarda Q fever infeksiyonu insidansının komplement fiksasyon testi ile araştırılması. Pendik Hay Hast Merk Araşt Enst Derg, **21**, 28-39.
21. **Parola, P. and Raoult, D.** (2001) Tick-borne bacterial diseases emerging in Europe. Clin Microbiol Infect, **7**, 80-83.
22. **Psaroulaki, A., Ragiadakou, D., Kouris, G. et al.** (2006) Ticks, tick-borne *Rickettsiae*, and *Coxiella burnetii* in the Greek Island of Cephalonia. Ann N Y Acad Sci, **1078**, 389-399.
23. **Rehacek, J.** (1987) Epidemiology and significance of Q Fever in Czechoslovakia. Zentralbl Bakteriol Microbiol Hyg A, **267**,16-19.
24. **Rehacek, J, Urvölgyi, J., Kocianova, E. et al.** (1991) Extensive examination of different tick species for infestation with *Coxiella burnetii* in Slovakia. Eur J Epidemiol, **7**, 299-303.
25. **Reinthaler, F.F., Mascher, F., Sixl, W., Arbesser, C.H**. (1988) Incidence of Q Fever among cattle, sheep and goats in the Upper Nile Province in Southern Sudan. Vet Rec, **122**, 137.
26. **Rolain, J.M., Gouriet, F., Brouqui, P. et al.** (2005) Concomitant or consecutive infection with *Coxiella burnetii* and tickborne diseases. Clin Infect Dis, **40**, 82-88.
27. **Satta, G., Chisu, V., Cabras, P. et al.** (2011) Pathogens and symbionts in ticks: A survey on tick species distribution and presence of tick-transmitted micro-organisms in Sardinia, Italy. J Med Microbiol, **60**, 63-68.
28. **Seyitoğlu, Ş., Özkurt, Z., Dinler, U. et al.** (2006) The seroprevalence of coxiellosis in farmers and cattle in Erzurum district in Turkey. Turk J Vet Anim Sci, **30**, 71-75.
29. **Spitalska, E., Kocianova, E**. (2003) Detection of *Coxiella burnetii* in ticks collected in Slovakia and Hungary. Eur J Epidemiol, **18**, 263-266.
30. **Spyridaki, I., Gikas, A., Kofteridis, D., Psauroulaki, A. and Tselentis, Y**. (1998) Q fever in the Greek island of Crete: Detection, isolation and molecular identification of eight strains of *Coxiella burnetii* from clinical samples. J Clin Microbiol, **36**, 2063-2067.

31. **Spyridaki, J., Psaroulaki, A., Loukaides, F. et al.** (2002) Isolation of *Coxiella burnetii* by a centrifugation shell-vial assay from ticks collected in Cyprus: detection by nested polymerase chain reaction (PCR) and by PCR-restriction fragment length polymorphism analyses. Am J Trop Hyg, **66,** 86-90.
32. **Stein, A., Raoult, D.** (1992) Detection of *Coxiella burnetii* by DNA amplification using polymerase chain reaction. J Clin Microbiol, **30**, 2462-2466.
33. **Zhang, G.Q., Hotta, A., Mizutani, M. et al.** (1998) Direct identification of *Coxiella burnetii* plasmids in human sera by nested PCR. J Clin Microbiol, **36**, 2210-2213.

Geliş tarihi: 15.01.2013 / Kabul tarihi: 05.06.2013

Address for correspondence
Gülay Altay Çapın
Sarayköy Nükleer Araştırma ve Eğitim Merkezi
Nükleer Teknikler Bölümü/Hayvancılık Birimi
Saray Mah. Atom Cad. No.27
06983 Kazan, Ankara, Turkey
e-mail: gulayaltay24@hotmail.com