Turkish Journal of Medical Sciences

Volume 42 | Number 3 Article 10

1-1-2012

The seroprevalence of Rickettsia conorii in humans living in villages of Tokat Province in Turkey, where Crimean-Congo hemorrhagic fever virus is endemic, and epidemiological similarities of both infectious agents

TURABİ GÜNEŞ

ÖMER POYRAZ

MEHMET ATAŞ

NERGİZ HACER TURGUT

Follow this and additional works at: https://journals.tubitak.gov.tr/medical

 Part of the Medical Sciences Commons

Recommended Citation
GÜNEŞ, TURABİ; POYRAZ, ÖMER; ATAŞ, MEHMET; and TURGUT, NERGİZ HACER (2012) "The seroprevalence of Rickettsia conorii in humans living in villages of Tokat Province in Turkey, where Crimean-Congo hemorrhagic fever virus is endemic, and epidemiological similarities of both infectious agents," *Turkish Journal of Medical Sciences*: Vol. 42: No. 3, Article 10. https://doi.org/10.3906/sag-1102-1383
Available at: https://journals.tubitak.gov.tr/medical/vol42/iss3/10

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Medical Sciences by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

TÜBİTAK

Original Article

Turk J Med Sci
2012; 42 (3): 441-448
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1102-1383

The seroprevalence of *Rickettsia conorii* in humans living in villages of Tokat Province in Turkey, where Crimean-Congo hemorrhagic fever virus is endemic, and epidemiological similarities of both infectious agents

Turabi GÜNEŞ[1], Ömer POYRAZ[2], Mehmet ATAŞ[3], Nergiz Hacer TURGUT[1]

Aim: Tokat Province is an epicenter for Crimean-Congo hemorrhagic fever virus (CCHFV) in Turkey. The aim of this study was to investigate the seroprevalence of *Rickettsia conorii* and to clarify the epidemiological similarities between CCHFV and *R. conorii* in Tokat Province.

Materials and methods: The prevalence of antibodies reactive with *R. conorii* was examined by ELISA in 364 sera, 151 of which were seropositive for CCHFV.

Results: The overall prevalence of antibodies reactive with *R. conorii* was 36.81%. The prevalence of antibodies to *R. conorii* infection was higher in humans who showed CCHFV seropositivity than seronegativity, 52.32% and 25.82%, respectively (P = 0.001). A significant difference in seroprevalence was found between groups who had a history of tick bite and who did not, 41.52% and 29.29%, respectively (P = 0.019).

Conclusion: Our data show that people who are a risk group for CCHFV are likely to be a risk group for *R. conorii.*

Key words: *Rickettsia conorii*, Mediterranean spotted fever, seroprevalence, Crimean-Congo hemorrhagic fever, tick-borne infections, Turkey

Kırım Kongo hemorajik ateş virüsünün endemik olduğu Tokat'ın köylerinde yaşayan insanlarda *Rickettsia conorii* seroprevalansı ve her iki enfeksiyon etkeninin epidemiyolojik benzerlikleri

Amaç: Tokat, Kırım-Kongo Hemorajik Ateş Virüsü (CCHFV) yönünden yüksek derece endemik bir yöredir. Bu çalışmanın amacı, Tokat yöresinde *Rickettsia conorii*'nin seroprevalansını araştırmak ve CCHFV'ü ve *Rickettsia conorii* arasındaki epidemiyolojik benzerlikleri ortaya koymaktır.

Yöntem ve gereç: 151'i CCHFV'ü yönünden seropozitif olmak üzere, toplam 364 serum örneğinde ELISA yöntemi ile *R. conorii* ile reaktif antikorların prevalansı araştırılmıştır.

Bulgular: *R. conorii* ile reaktif antikorların genel prevalansı % 36,81 olarak saptanmıştır. *R. conorii* enfeksiyonuna karşı oluşmuş antikor prevalansı, CCHFV seropozitifliği olanlarda, seronegatiflere göre daha yüksek tespit edilmiş olup sırasıyla % 52,32 ve % 25,82 oranlarında saptanmıştır (P = 0,001). Kene ısırığı öyküsü olanlarda % 41,52 ve olmayanlarda % 29,29 oranında görülmesiyle, gruplar arasında seroprevalans bakımından anlamlı bir farklılık bulunmuştur (P = 0,019).

Received: 16.02.2011 – Accepted: 05.05.2011

[1] Vocational School of Health Services, Cumhuriyet University, Sivas - TURKEY
[2] Department of Microbiology, Faculty of Medicine, Cumhuriyet University, Sivas - TURKEY
[3] Public Health Laboratory, Sivas Health Directorate, Sivas - TURKEY
Correspondence: *Turabi GÜNEŞ, Vocational School of Health Services, Cumhuriyet University, Sivas - TURKEY*
E-mail: turabigunes@hotmail.com

Support: The study was funded by the Scientific Research Projects Commission (CUBAP) of Cumhuriyet University, Sivas, Turkey (SHMYO-005).

Sonuç: Bulgularımız CCHFV yönünden risk grubundan olan insanların *R. conorii* yönünden de risk grubundan olmasının muhtemel olduğunu göstermiştir.

Anahtar sözcükler: *Rickettsia conorii*, Akdeniz benekli ateşi, seroprevalans, Kırım kongo hemorajik ateşi, kene-kaynaklı enfeksiyonlar, Türkiye

Introduction

Tick-borne rickettsioses are caused by obligate intracellular bacteria belonging to the spotted fever group (SFG) of the genus *Rickettsia* within the family *Rickettsiaceae* in the order *Rickettsiales*. Spotted fever rickettsia (SFR) is among the oldest known vector-borne diseases. In 1906, it was stated that the Rocky Mountain spotted fever (RMSF) caused by *Rickettsia rickettsii* and also in 1925 the Mediterranean spotted fever (MSF) caused by *Rickettsia conorii* are related with tick bite (1,2).

MSF, also known as Boutonneuse fever, is a zoonosis caused by *R. conorii* and transmitted by the brown dog tick, *Rhipicephalus sanguineus*. MSF occurs throughout southern Europe, Africa, and in Western to Central Asia (3). *R. conorii* infects and multiplies in almost all organs of ticks, in particular the salivary glands, which enables the rickettsiae to be transmitted to vertebrate hosts during feeding (1,2). In 80% of patients there was a previous dog contact history. Subsequent to tick bite, after 6-10 days, symptoms such as high fever, headache and myalgia appear. Approximately 3 days after the occurrence of high fever, maculopapular rash appears in 95% of patients (3).

The diagnosis of infections is made through serological tests, immunodetection of organisms in blood samples and tissues, isolation of organisms in cell culture, and identification of organisms by molecular-based methods (1,2). In serology and epidemiologic research, indirect immunofluorescence (IFA) is the most commonly used technique, but it can be replaced by enzyme-linked immunosorbent assay (ELISA) with similar sensitivity and specificity results (4,5).

R. sanguineus ticks are found in all regions of Turkey (6). The presence of MSF cases has been shown on a molecular basis in Turkey (7), and with seroepidemiological studies it has been shown that this agent can lead to an important health problem (8). In recent years, in Tokat Province, Turkey, especially people living in villages are exposed to tick bites and CCHF cases are frequently seen in this region (9-11). The aim of this study was to examine the prevalence of antibodies reactive with *R. conorii* in humans from rural villages, which is endemic for CCHFV, in Tokat Province and to clarify epidemiological similarities between CCHFV and *R. conorii*.

Materials and methods

Study area

Tokat is located in the inner part of the Middle Black Sea region of Turkey (Figure 1). The climate of Tokat features a transition between the climate of the Black Sea region and the climate of the Central Anatolia. Tokat Province has lands within the Kelkit valley and has appropriate vegetation cover for wild and domestic animals, which can be hosts for ticks (12). This region has also been considered an epicenter for CCHFV epidemics (9,10).

Collecting blood samples

The statistical parameters used to calculate the sample size were at a 95% confidence level, and error limits were of ±5% for the total population of 500,000. The prior estimated rate of seroprevalence for *R. conorii* was 20%, based on seroprevalence data from previous studies (8), and the estimated sample size required was 246. In the current study, 364 serum samples, of which 151 were seropositive and 213 seronegative for CCHFV, were used. The 294 of the 364 serum samples were provided from our previous study in which we had investigated the seroprevalence of CCHFV in 644 serum samples (10), from 43 villages (throughout 9 districts of Tokat Province), which were endemic for CCHFV (Figure 1). The remaining 70 of the 364 serum samples were provided from 70 patients (from the same villages) who were treated for CCHFV at the Medical School Hospital of Cumhuriyet University.

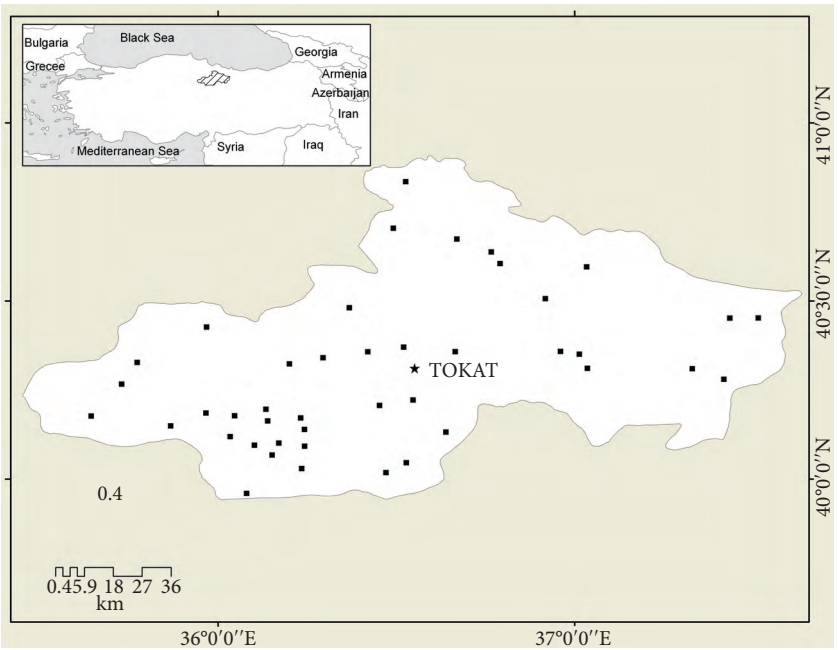

Figure 1. Geographic location of Tokat and the areas where blood samples were collected from humans.

Eighty-one of the 644 stock serum samples were seropositive for CCHFV, and 563 of them were seronegative. To be used in this study, 213 seronegative sera in terms of CCHFV were randomly selected from 563 seronegative stock serum samples. Because the size of the sample is limited, random selection was not used in the selection of the 81 seropositive samples to CCHFV or in the selection of the 70 samples from patients who were treated for CCHFV.

Serologic tests

Three hundred and sixty-four serum samples, of which 151 were positive (Group 1) and 213 negative (Group 2) for CCHFV, were included in the study to investigate *R. conorii*. Because 294 serum samples were known in terms of CCHFV serology (10), there was no need for investigation of antibodies against CCHFV in these sera. To justify the presence of CCHFV antibodies, the remaining 70 serum samples collected from patients who were treated for CCHF at the Medical School Hospital of Cumhuriyet University were tested for antibodies of CCHFV using immunoglobulin G (IgG) ELISA kits (Vector-Best; Kolsovo Novosibirsk, Russia). The presence of antibodies against *R. conorii* (Strain: *Moroccan*) was determined using ELISA (Vircell Microbiologists, Granada, Spain). Both tests were performed according to the manufacturers' instructions and optical densities (OD) of samples were measured at 450 nm using an EL 312 Microplate Bio-kinetics Reader (Bio-Tek Instruments, Inc., Winooski, Vermont, USA).

Statistical analysis

A chi-squared test and a binary logistic regression analysis were used to evaluate risk factors associated with seroprevalence of *R. conorii*, including age, sex, tick bite, and tick contacts. Phi correlation and Spearman's rank correlation tests (Spearman's rho) were used to examine the relationship between parameters. Statistical analyses were performed with SPSS, Ver. 17 (SPSS, Inc., Chicago, IL, USA) and statistical significance was defined as a 2-tailed P value ≤0.05.

Results

All of the serologic findings of the current study are presented in Tables 1 and 2. Of the 364 high-risk individuals, whose mean age was 44.93 years (SD ± 17.48), 134 (36.81%) were positive for IgG against

Table 1. Demographics and seroprevalence of *R. conorii.*

Risk factor category	n	Positive (%)	X^2 (P)	Odds ratio (95% CI)
Total	364	134 (36.81)		
Gender				
Female	177	55 (31.07)	4.80 (0.027)	1.62 (1.05-2.50)
Male	187	79 (42.25)		
History of tick bite				
No	140	41 (29.29)	5.54 (0.019)	1.71 (1.09-2.69)
Yes	224	93 (41.52)		
Tick removal from the animals				
No	117	45 (38.46)	0.20 (0.654)	0.90 (0.57-1.42)
Yes	247	89 (36.03)		
Occupational groups				
Farming and animal husbandry	318	120 (37.74)		
Only farmer	22	8 (36.36)	1.56 (0.459)	0.78 (0.51-1.19)
Retired, students, etc.	24	6 (25.00)		
Age, 45				
Age ≤ 45 years	177	36 (20.34)	40.20 (0.001)	4.31 (2.71-6.87)
Age > 45 years	187	98 (52.41)		
Age groups (means)				
9-20 (15.26)	42	4 (9.52)		
21-30 (25.59)	41	7 (17.07)		
31-40 (35.68)	62	13 (20.97)	51.73 (0.001)	1.65 (1.42-1.91)
41-50 (45.62)	66	25 (37.88)		
51-60 (55.61)	75	38 (50.66)		
61-70 (64.35)	54	32 (59.26)		
71-80 (74.79)	24	15 (62.50))		
Co-seroprevalence				
R. conorii and CCHFV	644	49 (7.61)		

CI = Confidence interval

R. conorii. Fifty-five (31.07%) of the 177 female participants and 79 (42.25%) of 187 male participants were seropositive for *R. conorii* (X^2 = 4.80, P = 0.027, Odds ratio [OR] = 1.62). Co-seroprevalence with CCHFV and *R. conorii* was detected in 49 (7.61%) of the 644 participants who had been previously investigated for CCHFV serology.

The seroprevalence of the antibodies reactive with *R. conorii* was higher in the older age groups than in the younger age groups (X^2 = 51.73, P = 0.001, OR = 1.65) (Table 1, Figure 2). A statistically significant correlation was observed between the means of age groups and the prevalence of antibodies to *R. conorii* in age groups (Spearman's rho = 0.98, P = 0.001), and also the scatter plot curve between them shows the direction of correlation (Figure 3).

Although a significant difference in the prevalence of antibody to *R. conorii* was found between groups in terms of history of tick bite (41.52% and 29.29%, respectively) (X^2 = 5.54, P = 0.019, OR = 1.71), a statistical difference was not seen between people who removed the ticks by hand and with people who had no contact with ticks (36.03% and 38.46%, respectively) (X^2 = 0.20, P = 0.654, OR= 0.90).

Table 2. Demographics and seroprevalence of *R. conorii* in people who had CCHFV antibody and those who did not.

Risk factor category	Group 1*		Group 2†		X^2 (P)‡	Odds (95% CI)#
	n	Positive (%)	n	Positive (%)		
Total	151	79 (52.32)	213	55 (25.82)	25.54 (0.001)	3.15 (2.02-4.91)
Gender						
Female	73	33 (45.21)	104	22 (21.15)	10.49 (0.001	3.08 (1.60-5.94)
Male	78	46 (58.97)	109	33 (30.28)	14.19 (0.001)	3.31 (1.80-6.09)
History of tick bite						
No	34	16 (47.06)	106	25 (23.59)	5.76 (0.016)	2.88 (1.28-6.47)
Yes	117	63 (53.85)	107	30 (28.04)	14.28 (0.001)	2..99 (1.72-5.23)
Tick removal from the animals						
No	48	23 (47.92)	69	22 (31.88)	2.43 (0.118)	1.97 (0.92-4.20)
Yes	103	56 (54.37)	144	33 (22.92)	24.43 (0.001)	4.00 (2.32-6.94)
Occupational groups						
Farming and animal husbandry	141	73 (51.77)	177	47 (26.55)	20.18 (0.001)	2.97 (1.86-4.75)
Only farmer	6	4 (66.67)	16	4 (25.00)	(0.137)	6.00 (0.78-46.14)
Retired, students, etc.	4	2 (50.00)	20	4 (20.00)	(0.251)	4.00 (0.42-37.78)
Age, 45						
Age ≤ 45 y	54	19 (35.19)	123	17 (13.82)	10.57 (0.001)	3.39 (1.59-7.22)
Age > 45 y	97	60 (61.86)	90	38 (42.22)	7.21 (0.007)	2.22 (1.24-3.99)

* = Seropositive people for CCHFV, † = Seronegative people for CCHFV, ‡, #. = Groups 1 and 2 were compared with each other, CI = Confidence interval.

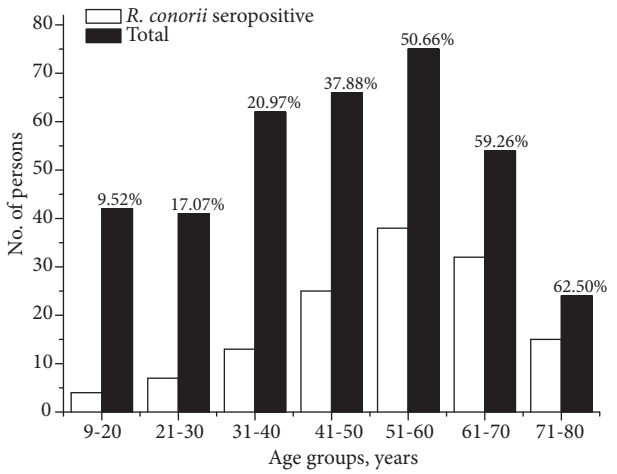

Figure 2. Distribution of prevalence of antibodies against *R. conorii* by age groups.

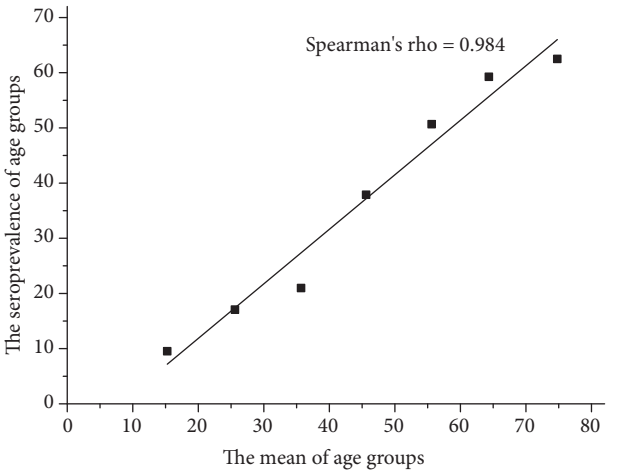

Figure 3. Scatterplot curve between mean of age groups and seroprevalence of age groups for *R. conorii*.

Furthermore, a statistical difference was not observed among professional groups for the distribution of positive antibody against *R. conorii* (X^2 = 1.56, P = 0.459).

In 79 of 151 (52.32%) people with positive antibody against CCHFV (Group 1) and in 55 of 213 (25.82%) people with no CCHFV antibody (Group 2), antibodies against *R. conorii* were detected (X^2

= 25.54; P = 0.001, OR = 3.15). The mean ages of Groups 1 and 2 were 49.97 (SD ± 16.54) and 41.35 (SD ± 17.28), respectively. History of tick bite was recorded for 117 of 151 people (77.48%) in Group 1 and for 107 of 213 people (50.23%) in Group 2 (X^2 = 27.72, P = 0.001). In people with history of tick bite, a higher seroprevalence of antibodies reactive with *R. conorii* was detected in Group 1 compared to Group 2 (53.85% and 28.04%, respectively) (X^2 = 14.28, P = 0.001, OR = 2.99). In addition, in people with no previous tick bite, a higher seroprevalence of antibodies reactive with *R. conorii* was detected in Group 1 compared to Group 2 (47.06% and 23.59%, respectively) (X^2 = 5.76, P = 0.016, OR = 2.88). Additionally, for other parameters (such as gender, occupational groups, being over or under 45 years of age) a higher seroprevalence of antibodies reactive with *R. conorii* was detected in Group 1 compared to Group 2 (Table 2).

Discussion

In the Mediterranean region, the incidence of MSF is generally estimated from 1 to 50 cases per 100,000 (annually), depending on the geographical area (13-17). In Turkey, MSF case reports are extremely limited and most of them are in the Marmara region (7). Some authors suggest that there are 7 times more MSF cases than officially reported (15,16). Seroepidemiological findings also support this concept. In Europe and in countries with a Mediterranean shore, the prevalence of antibodies against *R. conorii* in individuals living in cities is usually between 2% and 30% (13,18-20). However, in people who live in rural areas, it is seen at a higher level (10%-60%) (19-22). In 1998, Vural et al. found *R. conorii* antibody in 13.27% of humans in the rural areas of Antalya district, which is located in the Mediterranean region of Turkey (8). The present study is a seroepidemiological proof that this region, which is endemic for CCHFV, can also be an endemic region for MSF. For all that, the cross serologic reaction may be between *R. conorii* and other *Rickettsia* spp. Since the results obtained by the tests (*R. conorii*-IgG ELISA) have not been confirmed by additional tests and false positive reactions are not ruled out, these findings were evaluated as possible results.

The antibody prevalence to *R. conorii,* which was higher in men compared to women, may be due to men dealing with farming and ranching activities at a higher level in Tokat region.

Because the long-term persistence of detectable antibodies in SFG rickettsial infections is usual (23), long-lasting antibody titers may be one of the reasons for the high prevalence of antibody to *R. conorii* in older age groups. In this region, the length of farming and ranching throughout the day increases with age and this may cause the increment of possible tick bite exposure. In addition, older individuals, who have lower educational level than younger individuals, may show lower sensitivity about personal protection against tick bites.

In the present study, a higher seroprevalence in individuals who had been exposed to tick bite than those who had no previous tick bite history was expected because *R. conorii* is transmitted by ticks. However, in this study in individuals with no previous tick bite history, *R. conorii* seroprevalence was also observed to be considerably high. The nymph and larvae forms of ticks can also be infected by *R. conorii* (1). In those without a history of tick bite, one of the reasons for seropositivity could be unnoticed tick bites, egg nymphs, and larvae, which are small and feed for shorter times than adults.

For *R. conorii* seropositivity, the finding of no difference between people who removed the ticks by hand and with people who had no contact with ticks shows that cutaneous contact with ticks does not have much effect on the transmission to humans for this agent. Even if such an infection occurs, it is probably very rare. To the best of our knowledge, there is no report in the literature regarding such a transmission.

In the current study, because the seroprevalence of *R. conorii* was found higher in Group 1 than in Group 2 in terms of all parameters (Table 2), an epidemiological similarity was presented between CCHFV and *R. conorii*. The fact that individuals with antibodies to CCHFV are in a high risk group for tick borne infections is one of the major reasons for the epidemiological similarity between them. Especially the tick bite rate and mean age being higher in Group 1 compared to Group 2 is one of the major factors of this similarity. Besides tick bite and age, other factors, such as education level and habitual attitude

before and after tick bite, may play a role for the epidemiological similarity.

H. marginatum, R. bursa, and *R. turanicus* ticks, apart from *R. sanguineus,* may also play a significant role as enzootic vectors of MSF (24,25). It is reported that *H. marginatum*, which is the primary vector for CCHFV, and *R. bursa* ticks were encountered as the most dominant species within the ticks collected from animals in Tokat (26). Observing the ticks having a role in the widespread enzootic transmission of both infection agents may be another reason for the epidemiological similarity between both infectious agents.

Prevalence of the tick-borne infections in humans living in certain geographical areas is determined by several factors, such as density of the vector ticks, the prevalence of infectious agents in ticks, geographical structure and climate, and the human activities leading to high tick-human contact (1,9). High prevalence of CCHF and MSF cases in Tokat Province could be a combinatorial effect of these factors. Consequently, our data confirm the fact that rickettsiosis could be a significant health problem in many parts of Turkey and the study shows that people who are in a risk group for CCHF, are likely to be in a risk group for *R. conorii*.

Acknowledgments

The study was funded by Scientific Research Projects Commission (CUBAP) of Cumhuriyet University, Sivas, Turkey (SHMYO-005). We would like to thank Zübeyde Güneş for her help for taking blood from the people living in rural areas of Sinop and Tokat during the study and Zati Vatansever for his suggestions to improve the manuscript as well as the technical support he provided.

References

1. Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev 2005; 18: 719-56.
2. Parola P and Raould D. Tick and tickborne bacterial diseases in humans: An emerging infectious threat. Clin Infect Dis 2001; 32: 897-928.
3. Buckingham SC. Tick-borne infections in children. Pediatr Drugs 2005; 7: 163-176.
4. Keysary A, Strenger C. Use of enzyme-linked immunosorbent assay techniques with cross-reacting human sera in diagnosis of murine typhus and spotted fever. J Clin Microbiol 1997; 35: 1034-5.
5. La Scola B, Raoult D. Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. J Clin Microbiol 1997; 35: 2715-27.
6. Aydin L, Bakirci S. Geographical distribution of ticks in Turkey. Parasitol Res 2007; 101: 63-66.
7. Kuloglu F, Rolain JM, Fournier PE, Akata F, Tugrul M, Raoult D. First isolation of Rickettsia conorii from human in the Trakya region (European part) of Turkey. Eur J Clin Microbiol Infect Dis 2004; 23: 609-14.
8. Vural T, Ergin C, Sayin F. Investigation of Rickettsia conorii antibodies in the Antalya area. Infection 1998; 26: 170-72.
9. Vatansever Z, Uzun R, Estrada-Pena A, Ergonul O. Crimean-Congo hemorrhagic fever in Turkey. In: Ergonul O, Whitehouse CA, eds. Crimean-Congo hemorrhagic fever: a global perspective. Amsterdam: Springer; 2007.
10. Gunes T, Engin A, Poyraz O, Elaldi N, Kaya S, Dokmetas I et al. Crimean-Congo hemorrhagic fever virus in high-risk population, Turkey. Emerg Infect Dis 2009; 15: 461-4.
11. Gunes T. Crimean-Congo Hemorrhagic Fever. Mikrobiyol Bul 2006; 40: 279-87.
12. Karaer F, Kılınç M, Kutbay HG. The woody vegetation of Kelkit Valley. Tr J of Botany 1999; 23: 319-44.
13. Raoult D, Dupont HT, Chicheportiche C, Peter O, Gilot B, Drancourt M. Mediterranean spotted fever in Marseille, France: correlation between prevalence of hospitalized patients, seroepidemiology, and prevalence of infected ticks in three different areas. Am J Trop Med Hyg 1993; 48: 249-56.
14. Aharonowitz G, Koton S, Segal S, Anis E, Green MS. Epidemiological characteristics of spotted fever in Israel over 26 years. Clin Infect Dis 1999; 29: 1321-2.
15. Raoult D, Weiller PJ, Chagnon A, Chaudet H, Gallais H, Casanova P. Mediterranean spotted fever: clinical, laboratory and epidemiological features of 199 cases. Am J Trop Med Hyg 1986; 35: 845-50.
16. Sousa R, Nobrega SD, Bacellar F, Torgal J. Mediterranean spotted fever in Portugal: risk factors for fatal outcome in 105 hospitalized patients. Ann N Y Acad Sci 2003; 990: 285-94.
17. Punda-Polic V, Luksic B, Capkun V. Epidemiological features of Mediterranean spotted fever, murine typhus, and Q fever in Split-Dalmatia County (Croatia), 1982-2002. Epidemiol Infect 2008; 136: 972-9.

18. Meskini M, Beati L, Benslimane A, Raoult D. Seroepidemiology of rickettsial infections in Morocco. Eur J Epidemiol 1995; 11: 655-60.

19. Daniel SA, Manika K, Arvanmdou M, Antoniadis A. Prevalence of Rickettsia conorii and Rickettsia typhi infections in the population of northern Greece. Am J Trop Med Hyg 2002; 66: 76-9.

20. Punda-Polic V, Klismanic Z, Capkun V. Prevalence of antibodies to spotted fever group rickettsiae in the region of Split (southern Croatia). Eur J Epidemiol 2003; 18: 451-5.

21. Babalis T, Dupont HT, Tselentis Y, Chatzichristodoulou C, Raoult D. Rickettsia conorii in Greece: comparison of a microimmunofluorescence assay and western blotting for seroepidemiology. Am J Trop Med Hyg 1993; 48: 784-92.

22. Harrus S, Lior Y, Ephros M, Grisaru-Soen G, Keysary A, Strenger C et al. Rickettsia conorii in humans and dogs: a seroepidemiologic survey of two rural villages in Israel. Am J Trop Med Hyg 2007; 77: 133-5.

23. Mansueto S, Vitale G, Bentivegna M, Tringali G, Di Leo R. Persistence of antibodies to Rickettsia conorii after an acute attack of boutonneuse fever. J Infect Dis 1985; 151: 377.

24. Guberman D, Mumcuoglu KY, Keysary A, Ioffe-Uspensky I, Miller J, Galun R. Prevalence of spotted fever group rickettsiae in ticks from southern Israel. J Med Entomol 1996; 33: 979-82.

25. Psaroulaki A, Loukaidis F, Hadjichristodoulou C, Tselentis Y. Detection and identification of the aetiological agent of Mediterranean spotted fever (MSF) in two genera of ticks in Cyprus. Trans R Soc Trop Med Hyg 1999; 93: 597-8.

26. Tonbak S, Aktas M, Altay K, Azkur AK, Kalkan A, Bolat Y et al. Crimean-Congo Hemorrhagic Fever Virus: genetic analysis and tick survey in Turkey. J Clin Microbiol 2006; 44: 4120-4.