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 GÜNEŞ¹, Ömer POYRAZ², Mehmet ATAŞ³, Nergiz Hacer TURGUT¹

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
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.
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 CCHFV 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
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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 1. Demographics and seroprevalence of R. conorii.

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

Cl = Confidence interval
Furthermore, a statistical difference was not observed among professional groups for the distribution of positive antibody against R. conorii (X² = 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 (X² = 25.54, P = 0.001).
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= 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 (X² = 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) (X² = 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) (X² = 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.*

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**References**


