Prevalence of *Rickettsia rickettsii* infection in dogs from the urban and rural areas of western Turkey

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**Summary:** In this study, rickettsial infections in dogs living in rural and urban areas were evaluated in the Western Turkey. Dog sera has been tested indirect immunofluorescence test (IFA) by containing *Rickettsia rickettsii* antigen. Dog blood sera samples were obtained from 250 dogs (50 dogs from urban area and 200 dogs from rural area) covering the two-year period between March 2009 - March 2011. For each sera, the antigen reactive with *Rickettsia rickettsii* constituent the end point titers were determined. A total of 250 dog sera samples were tested. Dog sera samples were found 54.0% (135/250) seropositive and 43.2% (108/250) were found seronegative for the *Rickettsia rickettsii*. However, of the 2.8% (7/250 dog sera samples were found to be suspicious for the *Rickettsia rickettsii* at ≥ 1:64 - 1:256 titers. In this study, 7 (14.0%) dogs in urban areas were found to be seropositive and 128 (64.0%) dogs in rural areas were found to be seropositive in the ≥ 1:256 titer with the IFA test. In conclusion, titers of antibodies against *Rickettsia rickettsii* obtained from rural and urban dogs in Western Turkey appeared to be remarkable and more research needs to be done on this subject reveals.

Key words: Dog, IFA, *Rickettsia rickettsii*.

**Bati Türkiye’de kırsal ve kentsel alanlarında yaşayan köpeklerde *Rickettsia rickettsii* infeksiyonunun prevalansı**


Anahtar sözcükler: IFA, köpek, *Rickettsia rickettsii*.

**Introduction**

Rickettsiae are Gram-negative, obligate intracellular bacteria in the family Rickettsiaceae and order Rickettsiales. The genus *Rickettsia* is distributed worldwide, with some species maintained in nature and transmitted to vertebrate hosts by arthropod vectors (21).

The genus *Rickettsia* has been classically divided into two groups, the typhus group (TG) and the spotted fever group (SFG), which have ticks as their major reservoirs (17).

The spotted fever group (SFG) unites a phylogenetically well-defined clade of Rickettsiae that are distinct from other species and that have a life cycle involving arthropods, mainly ticks (18). The SFG includes a number of pathogenic organisms that cause so-called tick-borne (TB) rickettsioses in humans. *Rickettsia rickettsii* (Rocky Mountain spotted fever, RMSF) and *R. conorii conorii* (Mediterranean spotted fever, MSF) are very important strains (19).

Ticks are usually thought to be the main reservoir and vectors of SFG Rickettsiae in nature, due to the ability of Rickettsiae to survive perpetually in ticks and to be transmitted transstadially and transovarially (19).

In the case of *R. rickettsii* and other spotted fever group rickettsiae, transfer of the infection from adult female to her eggs (transovarial transmission) supports infection of larval ticks, which is maintained past molting in the subsequent stages. Dogs are frequently exposed to ticks, and evidence of current or past infection in dogs can be used to determine whether there is a risk of
infection with rickettsial tick-borne disease agents in a given geographic area (2, 4, 6, 16). All tick-borne pathogens are distributed focally within the ecologic landscape. Therefore documentation of infection in a dog, which presumably is more often in contact with ticks than humans, should prompt veterinary professionals to warn owners of an increased risk of tick-borne diseases (14).

Infection with R. rickettsii, the causative agent of RMSF, induces an extremely severe, potentially fatal disease in people and in dogs. Indeed, among the tick-borne diseases in the Americans, RMSF is the most severe, and can result in an acute-onset illness accompanied by a rapid course of disease and a high fatality rate. Mortality rates have been estimated at 20% in people in the absence of appropriate antibiotic treatment and 5% in patients who receive antibiotics. Canine patients present with fever, lethargy, vomiting and anorexia. As infection progresses, additional signs can develop in dogs, including ocular lesions, bleeding disorders, joint pain and neurologic abnormalities. Fatalities can occur in both people and dogs, particularly if appropriate anti-rickettsial treatment is delayed or never instituted (14). Dogs are usually subclinically infected, although more over disease has recently been reported (23).

The indirect immunofluorescence assay (IFA) is currently the test of choice for serologic diagnosis of rickettsial infection in humans and animals (10). In dogs, Rocky Mountain spotted fever is often diagnosed retrospectively by serology. A fourfold rise in titer in acute and convalescent samples taken more than three weeks apart is diagnostic. A single high titer may also be suggestive. Cross-reactions are seen with other spotted fever group Rickettsia. The indirect fluorescent antibody test is the most commonly used serologic test (25).

The aim of the present study is to investigate the prevalence of the Rickettsia rickettsii in dogs obtained from Western Turkey.

Materials and Methods

Materials: Dog blood sera samples were obtained from 250 dogs, living in urban (50 dogs) and rural (200 dogs) areas located in Western Turkey within 2 years of period from March 2009 to March 2011. Sampling was carried out in Aydın (n=90), İzmir (n=80) and Muğla (n=80) provinces. Samples were collected from Aydın, İzmir, and Muğla provinces were distributed focally within the ecologic landscape. Therefore documentation of infection in a dog, which presumably is more often in contact with ticks than humans, should prompt veterinary professionals to warn owners of an increased risk of tick-borne diseases (14).

Table 1. The sampling provinces and distribution of dog breeds.

<table>
<thead>
<tr>
<th>Dog Breeds</th>
<th>Sampling Provinces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aydın R</td>
</tr>
<tr>
<td>German Shepherd</td>
<td>3</td>
</tr>
<tr>
<td>Kangal</td>
<td>12</td>
</tr>
<tr>
<td>Pointer</td>
<td>2</td>
</tr>
<tr>
<td>Setter</td>
<td>2</td>
</tr>
<tr>
<td>Doberman</td>
<td>1</td>
</tr>
<tr>
<td>Terrier</td>
<td>3</td>
</tr>
<tr>
<td>Mongrel (n=123)</td>
<td>52</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>75</td>
</tr>
</tbody>
</table>

R: Rural area, U: Urban Area

Results

In this study, a total of 250 dogs sera samples collected from Aydın, İzmir and Muğla provinces were examined by using IFA for Rickettsia rickettsii antigens. Overall, 54.0% (135/250) of the dogs were seropositive and 43.2% (108/250) of the dogs were seronegative for Rickettsia rickettsii. In addition 2.8% (7/250) of the dogs with titer ≥1:64 <1:256 were found to be suspected for Rickettsia rickettsii (Table 2).
In this study, we found 7 (14.0%) urban area dogs were seropositive with a titer of ≥1:256, and 128 (64.0%) rural area dogs seropositive with a titer of ≥1:256 for *Rickettsia rickettsii* by IFA test (Table 3).

The results of IFA test for *Rickettsia rickettsii* in dog breeds according to the provinces investigated are given at Table 4.

Table 2. Interpreting the results of *Rickettsia rickettsii* by IFA test (Focus Diagnostics, Rickettsia IFA IgG, IF0100G)
Tablo 2. IFA test ile *Rickettsia rickettsii* sonucunun değerlendirilmesi

<table>
<thead>
<tr>
<th>IgG Antibody Titer</th>
<th>Titer Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1:64</td>
<td>No antibody detected</td>
</tr>
<tr>
<td>≥1:64 - &lt;1:256</td>
<td>Single IgG sera endpoint titer ≥1:64 - &lt;1:256 are suggestive of infection at an undetermined time and may be indicative of either past infection or early response to a recent infection</td>
</tr>
<tr>
<td>≥1:256</td>
<td>IgG sera endpoint titers of 1:256 and greater are considered presumptive evidence of recent or current infection by organisms of the appropriate Rickettsial antigen group</td>
</tr>
</tbody>
</table>

Table 3. The antibody titers by IFA for *Rickettsia rickettsii* antigen in the dogs
Tablo 3. Köpeklerde *Rickettsia rickettsii* antijelerine karşı oluşan IFA antikor titreleri

<table>
<thead>
<tr>
<th>Animal Sera</th>
<th>Number of seropositive animals according to IFA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban Area Dogs</td>
<td>&lt;1:64</td>
</tr>
<tr>
<td>Rural Area Dogs</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4. Results of positive IFA test for *Rickettsia rickettsii* in dogs
Tablo 4. Köpeklerde *Rickettsia rickettsii* için pozitif IFA test sonuçları

<table>
<thead>
<tr>
<th>Dog Breeds</th>
<th>Aydın</th>
<th>İzmir</th>
<th>Muğla</th>
</tr>
</thead>
<tbody>
<tr>
<td>German Shepherd (n=18)</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kangal (n=35)</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pointer (n=20)</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Setter (n=22)</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Doberman (n=12)</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Terrier (n=20)</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Mongrel (n=123)</td>
<td>41</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>55</strong></td>
<td><strong>2</strong></td>
<td><strong>36</strong></td>
</tr>
</tbody>
</table>

R: Rural area, U: Urban Area

Discussion and Conclusion
The frequency of the serological positivity for *R. rickettsii* differs in various parts of the world. According to a research carried out in Southern Brasil, *R. rickettsii* positivity was found to be as 33.7% in dogs (22). However, in another study carried out in Minas Gerais, Brazil, it was 81.3% in dogs (26). In the study presented here, we found the serologic evidence of spotted fever group rickettsial infection in 54.0% of dogs examined. This difference might be related with various endemicity of the different areas and the frequent contact between humans, wild animals and domestic animals.

Based on the literature search that we carried out, there is no literature regarding to the detection of SFG Rickettsiaae dogs especially for *R. rickettsii*, in Turkey. In a study conducted on human blood samples in Black Sea Region of Turkey (24), it was reported that Spotted fever group anti-Rickettsia IgG antibody seropositivity were detected in 68/580 (11.7%). In another case, *Rickettsia rickettsii* infection, causing septic shock associated adult respiratory distress syndrome in 72-year-old woman was reported (13). In these two researches given above, all samples were positive for IgM and IgG against *Rickettsia rickettsii* antibodies by the microimmunofloresence technique. These results show that SFG infections exist in our country.

We consider that Western Turkey might be an endemic area, because rural local areas are intense for the presence of ticks. The tick-borne diseases are frequently seen in the dogs found in the rural areas; however the number researches are poor for tick-borne diseases in these regions. The higher prevalence (64%) found among rural dogs in this study was possibly linked to the higher diversity of ticks that these dogs were exposed to, in contrast to the urban area where very low prevalence (14%) was established. In a BSF-endemic area in the state of São Paulo, where the tick vector (*Amblyomma aureolatum*) commonly infest dogs, 64% (16/25) of the dogs contained reactive antibodies to *Rickettsia spp.* (20).

The present study examined canine sera against *Rickettsia rickettsii* in dogs in the Western Turkey. According to the literatures, three other *Rickettsia* species (*R. rickettsii*, *R. parkeri*, and *R. felis*) are known to occur in regions of Brazil and other South American countries (5, 7, 9, 15). We have detected *R. rickettsii* antibodies in canine sera and found seropositivity against *Rickettsia rickettsii*. This result shows the presence of SFG Rickettsia species in Western Turkey.

In southeastern Brazil, *R. rickettsii* is the most important human rickettsial agent, which causes a highly lethal, acute exanematic febrile disease locally called as Brazilian spotted fever (BSF). Lethality rates of BSF in southern Brazil are between 18–30% (1, 3). The high
prevalence (64.0%) of *Rickettsia rickettsii* obtained in dogs with no clinical signs in the rural areas of Western Turkey may indicate the public health risk for human population who has close contact with dogs.

IFA is the gold standard method for serological diagnosis of rickettsial infections (10) but it presents some cross reactivity. Antibody titers against *R. rickettsii* are solely a proof of infection by a SFG rickettsia.

According to the research carried out in Minas Gerais, Brazil, a high prevalence was also found among dogs, 81.3% of them had antibody titers of 64 and 128 (26). This prevalence value is higher than others previously reported for dogs from BSF-endemic areas in Minas Gerais (13.68%) and Sao Paulo (25 to 66%) (8, 11, 12). In this case, dogs can be responsible for bringing infected ticks to the home environment and increasing the risk of human infection. Besides, according to the research made in São Paulo, Brasil, 10 sera reacted positive to *R. rickettsii* among 164 rural dogs, and 1 sera reacted positive to *R. rickettsii* among 153 urban dogs in Monte Negro (9). This result shows correlation with the results of our study also. Because in our study, 54.0% (135/250) of the dogs were seropositive and 43.2% (108/250) of the dogs were seronegative for *Rickettsia rickettsii*. In addition, 2.8% (7/250) of the dogs with titer ≥1:64 -<1:256 were found to be suspected for *Rickettsia rickettsii*. Besides, 7 (14.0%) urban area dogs were seropositive with titer ≥1:256 and 128 (64.0%) rural area dogs seropositive with titer ≥1:256 of *Rickettsia rickettsii* by IFA test.

In conclusion, the present study showed that the antibody titers against *Rickettsia rickettsii* agent in rural and urban dogs obtained in Western Turkey were at considerable levels. This situation may have great importance for public health hazard. The high prevalence of antibody titers in rural dogs shows that the Western Turkey is endemic by tick population and the exposure of disease to humans should also be evaluated in further studies. Our study might challenge to epidemiological researches in the future by the presence and prevalence of rickettsial diseases both in humans and other companion animals.

References


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