**Rickettsia conorii** in Humans and Dogs: A Seroepidemiologic Survey of Two Rural Villages in Israel

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**Abstract.** The prevalence of IgG-antibodies reactive with an Israeli strain of *Rickettsia conorii* (Israeli strain 487), the agent of Israeli spotted fever, was examined in humans and dogs from two rural villages in Israel where the disease has been reported in humans. Sixty-nine of 85 (81%) canine sera and 14 of 136 (10%) of human sera had anti-*R. conorii* antibodies. No direct association could be made between seropositivity of people and ownership of a seropositive dog. This study indicates that exposure to spotted fever group rickettsiae was highly prevalent among dogs compared with humans in the two villages examined, probably reflecting a greater exposure rate of canines to the tick vector. These results support a previous suggestion that canine serology could be a sensitive indicator for the presence and magnitude of human exposure to *R. conorii*.

**INTRODUCTION**

Mediterranean spotted fever (MSF), also known as Boutonneuse fever and Israeli spotted fever (ISF), is a zoonosis caused by *Rickettsia conorii* and transmitted by the brown dog tick, *Rhipicephalus sanguineus*.1–3 The disease is widely distributed throughout the Mediterranean basin and the Middle East.4 Based on multi-locus sequence typing, an Israeli isolate was recently proposed as a subspecies, named *R. conorii* subsp. *israelensis*.5 Predominant clinical signs of MSF include fever, headache, rash, myositis, myalgia, and arthralgia.4,6 Appearance of an eschar at the tick bite site is also a common finding in MSF; however, this has rarely been reported in Israeli MSF cases.7

*Rhipicephalus sanguineus* ticks feeding on dogs and hedgehogs in Israel have been found to harbor *R. conorii*,7 but the role of vertebrate reservoirs in maintaining zoonotic foci has yet to be clarified. A survey in Israel indicated that dogs have the highest rate of exposure among domesticated animals. Wild merions and hedgehogs also are frequently seropositive.8 The prevalence of antibodies to spotted fever group (SFG) rickettsiae in dogs in several countries of the Mediterranean basin has also been shown to be high4,8–11; however, whether exposure leads to clinical manifestations in dogs has yet to be determined. Dogs that were experimentally inoculated with a Zimbabwean strain of *R. conorii* had asymptomatic infection associated with seroconversion and intermittent rickettsaemia,12 whereas a febrile acute illness has recently been associated with *R. conorii* infection in Sicilian dogs.13

The goal of this study was to examine the prevalence of antibodies reactive with *R. conorii* in humans and dogs from two rural villages in Israel where human clinical cases have been diagnosed and to look for a possible association between human and canine exposure. In addition, some epidemiologic characteristics of MSF in these two endemic foci were studied.

**MATERIALS AND METHODS**

**Sera.** Serum samples were collected from 136 people and 85 dogs from the villages of Klil and Nataf, located in northern and central Israel, respectively. Sera used were initially collected for a leishmaniasis serosurvey in these two villages during the summer of 1998. Sera were stored at −70°C until tested for this study. All persons included in this serologic study (or their parents) signed an informed consent form agreeing to participate. Sex and age were recorded for all humans and dogs included in the study. The breed of the dogs was also recorded.

**Serology.** Sera were tested for the presence of IgG antibodies reactive with *R. conorii* by the indirect immunofluorescent antibody (IFA) test. The IFA test was performed on rickettsial spotted fever antigen slides. *R. conorii* (Israeli strain 487) was cultivated in yolk sac of chicken embryos and partially purified by differential centrifugation. Slides containing 10 wells were overlaid with *R. conorii* antigen and inactivated in formalin. Test sera diluted at 1:100 in phosphate-buffered saline (PBS) were placed in each well, incubated for 30 minutes at 37°C in a humid chamber, and thereafter rinsed in PBS. A second incubation was carried out with rabbit anti-dog IgG-fluorescein isothiocyanate (FITC) conjugate solution (Sigma, St. Louis, MO) diluted at 1:100 in PBS for the canine sera, and with goat anti-human IgG-FITC conjugate solution (Sigma) diluted at 1:100 in PBS for the human sera. Slides were dried and examined under a fluorescence-microscope. All tests were done in duplicate. Titer of IgG antibodies to *R. conorii* ≥ 1:100 were considered positive. Positive sera were further diluted until an endpoint titer was reached. In addition, all positive human sera were tested for the presence of IgM antibodies using anti-human IgM-FITC μ-specific conjugate (Sigma) as the secondary antibody, as described above. Positive control sera from both humans and dogs were obtained from the “Israel Institute for Biological Research” in Ness Ziona.

**Statistical analysis.** Differences in the distributions of seropositivity between sexes, age groups (0–20, 20–40, > 40 years for humans and 0–1, 1–7, and > 7 years for dogs) and dog breeds (purebred, mixed breeds) were tested using the χ² test. Significant difference was considered when P ≤ 0.05.
RESULTS

**Humans.** Fourteen of 136 (10%) human sera tested had IgM reactive with *R. conorii* antigens at a 1:100 titer. Seroprevalence was similar in Kili and Nataf villages and not significantly different between males and females and between the different age groups (Table 1). None of the seropositive humans had a history of MSF. Of these 14 seropositives, 10 were dog owners at the time of testing. Among them, six owners had dogs with anti-*R. conorii* antibodies and one owner had a seronegative dog. No samples were available from the dogs of three *R. conorii*–positive owners. Thirteen of the 14 (93%) IgG-seropositive human sera were also positive for IgM at 1:100 dilution, and 4 of the latter 13 had an IgM titer of ≥ 1:400.

**Dogs.** Overall, 81% (69 of 85) of the canine sera tested had IgG-antibodies reactive with *R. conorii* antigens at a titer of 1:100. The seroprevalence was significantly higher (*P* = 0.0052) for dogs in Nataf than in Kili (Table 2). The seroprevalence was similar for males and females but significantly higher in purebred dogs (*P* = 0.0241). No difference in exposure to *R. conorii* was found between dogs of different age groups.

The distribution of IgG-antibody titers among the seropositive dogs was as follows: one dog was positive at 1:100, whereas 50 of 69 dogs (72.5%) reached a 1:400 titer and 18 of 69 dogs (26%) reached a titer of 1:800.

**DISCUSSION**

This study indicates that ~10% of humans living in the two villages have been exposed to SFG rickettsiae. No significant difference in seroprevalence was found between the different age groups. Israeli spotted fever is an important pediatric disease, with a reported mean annual incidence of 11.5 cases per 100,000 Israeli children 0–9 years of age compared with 1.6 cases per 100,000 for Israelis 15–44 years of age. The fact that the seroprevalence was not significantly higher in children might suggest equal exposure to SFG rickettsiae among all age groups, with children manifesting clinical infection more frequently than adults. However, these results should be interpreted with caution because of the relatively small number of participants in the study. Interestingly, a relatively high prevalence of anti-SFG rickettsiae antibodies was found among people in the two villages, whereas only two human clinical cases had been diagnosed in these two villages during the 2 years preceeding the sera collection. The presence of SFG antibodies without apparent clinical manifestations suggests that most *R. conorii* exposures in this study were asymptomatic or accompanied by mild or non-specific symptoms that were not associated with MSF at the time of occurrence. IgG antibodies persist for several years post-MSF in humans. The presence of anti-*R. conorii* IgM in 13 of the 14 seropositive people in this study suggests recent exposure to the rickettsia. However, this assumption requires further testing because no data are available on the persistence of anti-SFG IgM in humans.

The high seroprevalence of dogs (81%) in this study indicates that dogs were heavily exposed to SFG rickettsiae in the two rural villages examined. These results corroborate a previous study that found that 82–84% of dogs from Israeli villages where human outbreaks of MSF occurred were seropositive. In addition, 58% of dogs in Israel that were suspected as having a canine tick-borne disease were seropositive to *R. conorii*. A significant difference in seroprevalence was found between the two villages. This may reflect differences in the abundance of tick vectors or variations in the infection rates of ticks between the villages located in two distant geographical regions. The high prevalence of antibodies to SFG rickettsia in canines in endemic foci in Mediterranean countries and the recent new association of *R. conorii* with clinical manifestations in three dogs from Sicily warrant future study to elucidate the possible role of this pathogen in causing a clinical disease in canines.

The prevalence of antibodies reactive with *R. conorii* was much higher in dogs than humans from the same village. The higher seroprevalence in canines compared with humans in endemic areas is in agreement with previous studies, probably reflecting the higher exposure rate of canines to the tick vector. Our results and those of the two latter studies suggest that canine serology could be a useful and sensitive indicator for the presence and extent of MSF at the village or municipality level. Similarly, the use of canine serology was recently suggested for this purpose for Brazilian spotted fever caused by *Rickettsia rickettsii*. The seroprevalence in this study seemed to be high for both

<table>
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<th>Variable</th>
<th>Number of dogs</th>
<th>Positive (1:100 dilution)</th>
<th>Percent positive</th>
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<td></td>
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<tr>
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<tr>
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<td>11</td>
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</tr>
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<tr>
<td>0–20</td>
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<td>9</td>
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<tr>
<td>&gt; 40</td>
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<tr>
<td>Total</td>
<td>136</td>
<td>14</td>
<td>10.3</td>
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</tbody>
</table>

**Table 1**

Prevalence of IgG seroreactivity to *R. conorii* among 136 people in two villages in Israel.
the canines and humans in the villages studied. Although *R. conorii* has been isolated and genetically characterized from people and ticks in Israel,²² serologic cross-reactivity among SFG rickettsia is common,²² thus it is possible that some of the dogs and humans included in this study were exposed to SFG rickettsia other than *R. conorii*. *Rickettsia felis* DNA was recently detected in fleas in Israel; however, no human cases of spotted fever caused by *R. felis* has been reported in Israel.²³ To the best of our knowledge, *R. felis* is the only SFG rickettsia other than *R. conorii* that has been detected in Israel to date. The prevalence and clinical importance of *R. felis* in humans and dogs in Israel has yet to be determined.

The epidemiology of MSF and the role of potential animal reservoir hosts have not yet been clarified. Of particular interest is the role of canines in the epidemiology of the disease. Dogs often live in close proximity to humans and often share the same living space. Dogs may possibly be infected by MSF rickettsiae and act as reservoirs for tick infection or act strictly as hosts for *R. sanguineus* ticks harboring the bacteria. The high seroprevalence among dogs in the two villages and the lack of information on some of the dogs belonging to the seropositive people made the attempt to associate human infection with owning a seropositive dog difficult. No clear association was found between serologic evidence of human exposure to *R. conorii* and ownership of a seropositive dog. Because the major route of infection with *R. conorii* is transmission by a vector tick, the presence of infected ticks in the vicinity of humans is probably the most important risk factor for human infection. This must be borne in mind by public health officials and veterinary practitioners when advising dog owners on the importance of prophylactic treatments against ticks.

In conclusion, this study showed that exposure to SFG rickettsiae was highly prevalent among dogs compared with humans in the two rural villages examined. Our results suggest that canines might serve as sentinels for MSF in endemic regions. Further studies are warranted to investigate the risk factors for infection with *R. conorii* in humans and dogs and to ascertain the association of anti-*R. conorii* antibodies and/or the presence of the pathogen or its DNA in canines with clinical manifestations in this species.

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