**Rickettsia helvetica in Dermacentor reticulatus Ticks**

Marinko Dobec, Dragutin Golubic, Volga Punda-Polic, Franz Kaeppeli, and Martin Sievers

We report on the molecular evidence that *Dermacentor reticulatus* ticks in Croatia are infected with *Rickettsia helvetica* (10%) or *Rickettsia slovaca* (2%) or co-infected with both species (1%). These findings expand the knowledge of the geographic distribution of *R. helvetica* and *D. reticulatus* ticks.

*Rickettsia helvetica* organisms were first isolated from *Ixodes ricinus* ticks in Switzerland and were considered to be a new nonpathogenic species of the spotted fever group (SFG) rickettsiae (1,2). Recently, *R. helvetica* was linked to acute perimyocarditis, unexplained febrile illness, and sarcoidosis in humans in Europe (3–5). It is generally accepted that *Dermacentor marginatus* is the main vector of *R. slovaca* and that *I. ricinus* is the main vector of *R. helvetica* (1,2,6).

Until now, only in the southern (Mediterranean) part of Croatia have *R. conorii*, *R. slovaca*, and *R. aeschlimannii* been detected in *Rhipicephalus sanguineus*, *D. marginatus*, and *Hyalomma marginatum* ticks, respectively (7). Human disease caused by *R. conorii* (Mediterranean spotted fever) has also been described in this region (8). No published reports of *R. helvetica* in Croatia are available. In a previous study, antibodies to SFG rickettsiae were found in dogs and *Borrelia* spp. ticks infected with *R. slovaca* were 99.8% identical to the corresponding *D. reticulatus* ITS2 sequence (S83081). The consensus sequence of the ITS2 spacer regions obtained from 13 *Dermacentor* spp. ticks infected with *R. helvetica* and *R. slovaca* were 99.8% identical to the corresponding *D. reticulatus* ITS2 sequence (S83080) and 85% identical to the corresponding *D. marginatus* sequence (S83081). The 646-bp ITS2 spacer fragment is sufficient to discriminate between the *Dermacentor* spp. The sequences of the ITS2 spacer regions obtained from 13 *Dermacentor* spp. ticks infected with *R. helvetica* and *R. slovaca* were 99.8% identical to the corresponding *D. reticulatus* ITS2 sequence (S83080) and 85% identical to the corresponding *D. marginatus* sequence (S83081). The 646-bp ITS2 spacer fragment is sufficient to discriminate between the *Dermacentor* spp. The consensus sequence of the ITS2 spacer regions obtained in this study was deposited at the European Microsynth AG (Balgrach, Switzerland) and our laboratory. All sequences were aligned with known sequences by using BLAST (http://blast.ncbi.nlm.nih.gov/blast.cgi).

The sequences of the ITS2 spacer regions obtained from 13 *Dermacentor* spp. ticks infected with *R. helvetica* and *R. slovaca* were 99.8% identical to the corresponding *D. reticulatus* ITS2 sequence (S83080) and 85% identical to the corresponding *D. marginatus* sequence (S83081). The 646-bp ITS2 spacer fragment is sufficient to discriminate between the *Dermacentor* spp. The consensus sequence of the ITS2 spacer regions obtained in this study was deposited at the European...
Molecular Biology Laboratory database under accession no. FM212280.

Results of the identification of *R. helvetica* and *R. slovaca* are shown in Table 2. The amplified *ompB* sequences of *R. helvetica* were 100% identical to the corresponding *ompB* gene of the *R. helvetica* strain C9P9 (AF123725), 92% identical to “Candidatus R. hoostraillii” (EF629536), 89% identical to *R. asiatica* (DQ110870), 84% identical to *R. rhipicephali* (AF123719), and 83.3% identical to *R. raoultii* (EU036984, DQ365798, DQ365797). The amplified *ompA* sequences were 100% identical to the corresponding *ompA* gene of *R. slovaca* and showed 2- to 12-bp differences to the corresponding *ompA* sequences of other *Rickettsia* spp.

In summary, *R. helvetica* DNA was detected in 10 of 100 *D. reticulatus* ticks, and the pathogen loads ranged from 380 to 1,700 copies per tick. *R. slovaca* DNA was found in 2 of 100 *D. reticulatus* ticks with copy numbers of 400 and 460. One *D. reticulatus* tick was co-infected with *R. helvetica* (410 copies) and *R. slovaca* (20,000 copies). No *Borrelia burgdorferi* DNA was found in *D. reticulatus* ticks.

**Conclusions**

Scientific literature supports the premise that *D. marginatus* ticks are the main vector of *R. slovaca* and that *I. ricinus* ticks are the main vector of *R. helvetica* (1,2,6). *R. slovaca* was also detected in *D. reticulatus* ticks (6,14). Additionally, the DNA of *R. raoultii* strain Marne, which is well separated from *R. helvetica* according to phylogenetic analyses of 16S rDNA sequences, was also detected in *D. reticulatus* ticks (14,15).

Previous studies showed that *D. marginatus* ticks are common in Croatia, and *R. slovaca* was identified in 36.8% of *D. marginatus* ticks collected in the southern part of the country (7). Further, *R. helvetica* as well as *D. reticulatus* ticks have never been detected in Croatia. In our study, 2% of *D. reticulatus* ticks were infected by *R. slovaca*, 10% were positive for *R. helvetica*, and 1% (1 tick) was co-infected by both pathogens. Our findings may explain the high seroprevalence (20.7%) of SFG antibodies in dogs detected in a previous study in the continental part of Croatia that is *R. conorii* free (9). This study suggests that these antibodies to SFG rickettsiae are presumably related to *R. helvetica* and *R. slovaca* infections, which can be transmitted by the same tick vector.

Because *D. reticulatus* is the second most common tick species occurring in all 16 counties of neighboring Hungary, we believe our findings point to an enlargement of its distribution area (10). Visual identification of *Dermacentor* spp. ticks has traditionally been confirmed on the basis of morphologic features. Because *D. marginatus* and *D. reticulatus* exhibit overlapping phenotypes, this means of identification can be very difficult (12). Therefore, we cannot exclude the possibility that *D. reticulatus* ticks were frequently misinterpreted as *D. marginatus*. Our study

**Table 1. Primers and probes designed for real-time PCR**

<table>
<thead>
<tr>
<th>Species</th>
<th>Primers and probe</th>
<th>Sequence (5' → 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rickettsia helvetica</em></td>
<td><em>ompB</em> forward</td>
<td>GATTTCGAGGGGAATAATTCC</td>
</tr>
<tr>
<td></td>
<td><em>ompB</em> reverse</td>
<td>GCTACCTGCATTACCTACAG</td>
</tr>
<tr>
<td></td>
<td><em>ompB</em> probe†</td>
<td>ACTCTACTGCTACAAATGTTGCTACAG</td>
</tr>
<tr>
<td><em>R. slovaca</em></td>
<td><em>ompA</em> forward</td>
<td>GTAGATGTTGGAATATATTG</td>
</tr>
<tr>
<td></td>
<td><em>ompA</em> reverse</td>
<td>CTCCTGTTATAATCGAACCAAC</td>
</tr>
<tr>
<td></td>
<td><em>ompA</em> probe†</td>
<td>CAGCAGGAGTATTACCTACCCCTCC</td>
</tr>
<tr>
<td><em>Dermacentor</em> spp.</td>
<td>ITS_forward</td>
<td>GTGCCGTCGCGACTGCTTTAG</td>
</tr>
<tr>
<td></td>
<td>ITS_reverse</td>
<td>ACGCGCGACTACGCGGAATG</td>
</tr>
</tbody>
</table>

† Amplified products: 162 bp (*R. helvetica*); 228 bp (*R. slovaca*); 646 bp (*D. reticulatus*); standard curve: slope, Y-intercept and correlation coefficient: −3.634, 40.129, 0.9937 (*R. helvetica*); −0.23, 42.82, 0.9942 (*R. slovaca*); ITS, internal transcribed spacer.

‡ GenBank accession nos. EU622810, DQ490502, DQ490501, DQ490500, DQ49049, DQ49048, DQ49047, DQ49046, DQ49045, DQ49030, DQ49029, Q469027, Q469054, Q469053, and U43808.

§ DNA of *B. burgdorferi* DSM 4680 and *B. afzelii* DSM 10508 were used as positive controls.

**Table 2. Identification of *Dermacentor*, *Rickettsia*, and *Borrelia* species by molecular methods**

<table>
<thead>
<tr>
<th>Species</th>
<th>Identification method</th>
<th>Targeting sequence</th>
<th>Confirmation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dermacentor</em> spp.</td>
<td>PCR</td>
<td>ITS2 (646 bp)</td>
<td>Sequencing</td>
<td>13/13 (100%) positive,* 99.8% identical to <em>D. reticulatus</em> ITS2 sequence (S83080)</td>
</tr>
<tr>
<td><em>R. helvetica</em></td>
<td>Real-time PCR</td>
<td><em>ompB</em> (162 bp)</td>
<td>Sequencing</td>
<td>11/100 (11%) positive;† 100% identical to <em>R. helvetica</em> strain C9P9 (AF123725)</td>
</tr>
<tr>
<td><em>R. slovaca</em></td>
<td>Real-time PCR</td>
<td><em>ompA</em> (228 bp)</td>
<td>Sequencing</td>
<td>3/100 (3%) positive;‡ 100% identical to <em>R. slovaca</em> strains‡</td>
</tr>
<tr>
<td><em>B. burgdorferi</em></td>
<td>Real-time PCR</td>
<td>flaA (p41)</td>
<td>Not done (PCR negative)</td>
<td>0/100 positive; not detected in <em>Dermacentor</em> spp. ticks§</td>
</tr>
</tbody>
</table>

* Only infected ticks (13 ticks) were identified by molecular methods (PCR and sequencing).
† GenBank accession nos. EU622810, DQ490502, DQ490501, DQ490500, DQ49049, DQ49048, DQ49047, DQ49046, DQ49045, DQ49030, DQ49029, Q469027, DQ49054, DQ49053, and U43808.
‡ DNA of *B. burgdorferi* DSM 4680 and *B. afzelii* DSM 10508 were used as positive controls.
shows that the identification problem can be solved through use of molecular biology techniques.

We provide molecular evidence of the existence of *D. reticulatus* ticks in Croatia. Our results expand the knowledge of *R. helvetica* hosts. *D. reticulatus* ticks occur at far more sites than previously known and thus have probably expanded their habitats. Our data point out the need for further studies on the epidemiology of *R. helvetica* and other SFG rickettsiae in Croatia as well as their association with infections in humans and animals.

Acknowledgments

We are grateful to Mark Jaeggi, Priska Keller, Tobias Wermelinger, Andreas Steffen Stein, and Alexandra Schauerte for their help during this study.

This work was supported by grants from the Commission of Technology and Innovation of the Federal Office for Professional Education and Technology, Bern, Switzerland, and from the Ministry of Science, Education and Technology of the Republic of Croatia (No. 216-0481153-1148).

Dr Dobec is associate professor of medical microbiology at the University of Split, School of Medicine, Split, Croatia; scientific director of the Institute Virion, Ruschlikon, Switzerland; and head of the laboratory for infectious diseases and immunology at medica, Medizinische Laboratorien Dr F. Kaeppeli, Zurich, Switzerland. His research activities address tick-borne infections and zoonoses.

References


Address for correspondence: Marinko Dobec, medica, Medizinische Laboratorien Dr F. Kaeppeli, Wolfbachstrasse 17, CH-8024 Zurich, Switzerland; email: m.dobec@medica-labor.ch