New *Rickettsiae* in Ticks Collected in Territories of the Former Soviet Union

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Rickettsiae are obligate intracellular gram-negative bacteria associated with arthropod vectors, ticks, mites, and insects that, while feeding, can transmit *Rickettsiae* to animals and humans. The rickettsioses have characteristic clinical features, including fever, headache, maculopapular eruption, and sometimes eschar formation (primary lesion). The number of representatives of the genus *Rickettsia* has increased over the last decades as a result of improved cell culture isolation and agent identification techniques (1). Sequence comparison of gene coding for citrate synthase (*gltA*) (2), rOmpA outer membrane protein (*ompA*) (3), and 16S rRNA (4) has become the most reliable method of identifying *Rickettsiae* (5-8). We describe polymerase chain reaction (PCR) amplification and sequence determination to identify *Rickettsiae* in naturally infected ixodid ticks in three regions of Russia endemic for tickborne rickettsioses.

*Rhipicephalus pumilio* ticks (65 adults) were collected in 1996 from dogs in the Astrakhan region. *Dermacentor nuttalli* ticks (101 adults) were collected in 1994 in the village of Verhnyi Kouus, the Altay Mountains, Siberia. In 1997, *R. sanguineus* ticks (2 adults and 35 nymphs) were collected in the town of Saki, Crimea region, from dogs whose owners had serologic evidence of Mediterranean spotted fever (Figure 1). The ticks were kept at room temperature before being washed in iodized alcohol (10 minutes) just before testing, rinsed in distilled water, and dried on sterile filter paper. DNA was extracted from ticks by using the QIAmp Tissue Kit (QIAGEN, Hilden, Germany). Rickettsial DNA was detected by PCR with primers specific for *Rickettsiae*: RpCS.877p-RpCS.1273r, which amplify a 396-bp fragment of *gltA* (2), and Rr190.70p-190-701 (3), which amplify a fragment of *ompA* from 629 to 632 bp. For all positive ticks, 587 to 590 bp of *ompA* were sequenced by using the ABI PRISM Dye

Figure 1. Areas from which ticks in the study were collected.

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Terminator Cycle Sequencing Kit with Amplitap Polymerase FS (PE Applied Biosystems, Warrington WA1 4SR, UK). Sequences were analyzed with the Applied Biosystem 377 automatic sequencing system. For newly detected genotypes, sequences of 16S rRNA encoding gene, gltA, and ompA were determined as previously described (2-4) (see Figure 2 for GenBank codes).

We detected two different Rickettsiae in *Rh. pumilio* (Astrakhan fever agent and RpA4 genotype); two Rickettsiae from *D. nuttallii* in Siberia (*Rickettsia sibirica* and DnS14 and DnS28 genotypes); and *R. conorii* from *Rh. sanguineus* ticks in Crimea (Table).

Our results confirm previous data of high epidemic activity of the Altay focus for North Asian tick typhus and the crucial role of *D. nuttallii* as a reservoir of *R. sibirica* infection (9). Our results are also consistent with those of a study in 1991 based on hemolymph testing (10), in which 3.2% of ticks from the Astrakhan region were demonstrated to be infected with *Rickettsiae*.

An outbreak of Mediterranean spotted fever due to infection with *R. conorii* occurred in Crimea from 1947 to 1957. Only sporadic cases of the disease were reported (11) until 1995, when the incidence of Mediterranean spotted fever increased in central Crimea, with 40 cases in 1996 and more than 70 in 1997. Most cases occurred in the summer, when the *Rh. sanguineus* nymphs (principal vectors of *R. conorii*) (1) were active. Our results, showing that 8% of the *Rh. sanguineus* studied contained *R. conorii* DNA, provide further evidence of the Mediterranean spotted fever outbreak in the region. To date, only the *R. conorii* strain M-1, isolated in the territories of the former Soviet Union (the Black Sea coast of Georgia), has been genetically characterized. This strain is genetically distinct from the other strains of *R. conorii*, i.e., Indian tick typhus and the Moroccan and Malish strains (3). Our detection of the *R. conorii* strain identical to the Malish strain is the first evidence of the genetic heterogeneity of *R. conorii* in the region.

The *ompA* sequences obtained from PCR-amplified products were different from those described for the known *Rickettsiae* for one DNA sample extracted from *Rh. pumilio* from the Astrakhan region (RpA4) and four DNA samples from *D. nuttallii* collected in Siberia (DnS14, DnS28, DnS79, DnS94). The sequences for the samples from *D. nuttallii* (DnS28, DnS79, and DnS94) were identical but differed from those of DnS14 and *Rh. pumilio* RpA4/2.

The three new rickettsial agents were closely related and branched with members of the *R. massiliae* group, together with *R. rhipicephali*, Bar 29, *R. aeschlimannii*, and *R. montanensis* (Figure 2). Comparison of the sequences obtained by using the program BLAST demonstrated that they also differed from those of the Cadiz agent characterized from *Ixodes ricinus* in Spain (6), those of the Cooley genotype characterized from *I. scapularis* (5), MOA and WB-8-2 isolated from *Amblyomma americanum* and *I. scapularis*, respectively (8), and *R. peacockii* (7) in the United States.

The pathogenicity of the members of the *R. massiliae* group is unknown, and their main reservoirs are regarded as ticks of the genus *Rhipicephalus* for *R. massiliae* and Bar 29. *R. aeschlimannii* has been isolated from *Hyalomma marginatum* and *R. montanensis* from ticks of the genus *Dermacentor*. *R. rhipicephali* has been demonstrated in ticks of the genus *Dermacentor* and in *Rh. sanguineus* (1). The similarity of gltA, ompA, and 16S rRNA gene sequences indicates that these three new

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Location</th>
<th>No. positive ticks/total examined</th>
<th>% infected ticks</th>
<th>Rickettsial species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhipicephalus pumilio</em></td>
<td>Astrakhan region</td>
<td>2/65</td>
<td>3</td>
<td>Astrakhan fever agent</td>
</tr>
<tr>
<td><em>Rh. pumilio</em></td>
<td>Astrakhan region</td>
<td>1/65</td>
<td>1.5</td>
<td>RpA4 genotype</td>
</tr>
<tr>
<td><em>Dermacentor nutallii</em></td>
<td>Siberia</td>
<td>12/101</td>
<td>12</td>
<td><em>Rickettsia sibirica</em></td>
</tr>
<tr>
<td><em>D. nuttallii</em></td>
<td>Siberia</td>
<td>4/101</td>
<td>4</td>
<td>DnS14 and DnS28 genotypes</td>
</tr>
<tr>
<td><em>Rh. sanguineus</em></td>
<td>Crimea</td>
<td>3/37</td>
<td>8</td>
<td><em>R. conorii</em> (Malish strain)</td>
</tr>
</tbody>
</table>
agents are close to each other (from 99.7% to 99.9%) and could constitute a new rickettsial species.

In the United States, various tickborne Rickettsiae occur in areas endemic for R. rickettsii, the agent of Rocky Mountain spotted fever (7). Similarly, in Mediterranean countries, several recently described Rickettsiae have been found in ticks of the Rh. sanguineus complex in the regions endemic for Mediterranean spotted fever caused by R. conorii (6). The effects of the presence of different Rickettsiae on the prevalence of infection rates of ticks with individual Rickettsiae and on the epidemiology of infections in humans have yet to be determined. R. sibirica and the Astrakhan fever agent are prevalent in Siberia and the Astrakhan region, respectively, but the pathogenicity of the new rickettsial genotypes has yet to be investigated.

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