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

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Dermacentor nuttallii from Siberia, Rhipicephalus sanguineus from Crimea, and *Rh. pumilio* from the Astrakhan region were infected with *Rickettsia sibirica* (12%), *R. conorii* (8%), and the Astrakhan fever agent (3%), respectively. Three new *Rickettsiae* of the *R. massiliae* genogroup were identified in ticks by 16S rDNA, gltA, and *ompA* sequencing.

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

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 *Rickettsia* in *Rh. pumilio* (Astrakhan fever agent and RpA4 genotype); two *Rickettsia* from *D. nuttallii* in Siberia (*Rickettsia sibirica* and DnS14 and DnS 28 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 *Rickettsia*.

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 *Rickettsia* 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

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**Table. Ticks infected in regions of the former Soviet Union**

| Tick species          | Location               | No. positive ticks/total examined | % infected ticks | Rickettsia species                |
|-----------------------|------------------------|-----------------------------------|------------------|----------------------------------|
| *Rhipicephalus pumilio* | Astrakhan region       | 2/65                              | 3                | Astrakhan fever agent            |
| *Rh. pumilio*         | Astrakhan region       | 1/65                              | 1.5              | RpA4 genotype                    |
| *Dermacentor nutallii* | Siberia                | 12/101                            | 12               | *Rickettsia sibirica*            |
| *D. nutallii*         | Siberia                | 4/101                             | 4                | DnS14 and DnS28 genotypes        |
| *Rh. sanguineus*      | Crimea                 | 3/37                              | 8                | *R. conorii* (Malish strain)     |
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 \textit{Rickettsiae} occur in areas endemic for \textit{R. rickettsii}, the agent of Rocky Mountain spotted fever (7). Similarly, in Mediterranean countries, several recently described \textit{Rickettsiae} have been found in ticks of the \textit{Rh. sanguineus} complex in the regions endemic for Mediterranean spotted fever caused by \textit{R. conorii} (6). The effects of the presence of different \textit{Rickettsiae} on the prevalence of infection rates of ticks with individual \textit{Rickettsiae} and on the epidemiology of infections in humans have yet to be determined. \textit{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.

\begin{acknowledgments}
We thank P.J. Kelly and E. Birtles for reviewing the article.

Dr. Rydkina is a senior researcher at the Gamalaya Institute in Moscow. She was trained as a postdoctoral fellow in Marseille, France. Her main interest is in rickettsial diseases.
\end{acknowledgments}
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