Spotted Fever Group Rickettsiae in Ticks, Germany

Cornelia Silaghi, Dietmar Hamel, Claudia Thiel, Kurt Pfister, and Martin Pfeffer

To explore increased risk for human *Rickettsia* spp. infection in Germany, we investigated recreational areas and renatured brown coal surface-mining sites (also used for recreation) for the presence of spotted fever group rickettsiae in ticks. *R. raoultii* (56.7%), *R. slovaca* (13.3%), and *R. helvetica* (>13.4%) were detected in the respective tick species.

*Rickettsia* species of the spotted fever group are causing emerging infectious diseases (1). Since 1977, *Rickettsia slovaca*, found in *Dermacentor marginatus* ticks, was the only known *Rickettsia* sp. in Germany until 2002, when the following were identified: *R. monacensis* and *R. helvetica* in *Ixodes ricinus* ticks, *Rickettsia* sp. RPA4 (now *R. raoultii*) in *D. reticulatus* ticks, *R. felis* in *Ctenocephalides felis* cat fleas, and *R. massiliae* in *I. ricinus* ticks (1,2). All of these species cause tick-borne rickettsioses in humans, including tick-borne lymphadenopathy (TIBOLA) (3–7). The aim of this study was to explore the interface between the vector tick and humans by investigating the presence of *Rickettsia* spp. in ticks at highly frequented recreational areas and renatured brown coal surface-mining sites that also are used for leisure.

The Study

Questing ticks were collected from vegetation by flagging in 3 regions in Germany (9 sites total) in March–September 2008 and April–October 2009 (Figure). Three sites, including renatured gravel pits and walking areas near villages and cities (A–C), were located in the federal state of Saarland. One site in southern Germany was in a natural alluvial forest north of Munich, popular for hiking and dog walking (D), and in East Germany (Saxony), 3 sites were former brown coal surface-mining areas near the city of Leipzig (E–G). Ongoing renaturation and flooding of the pit holes during past decades created a highly valuable recreational area with artificial lakes and surrounding meadows and forest (www.leipzigerneuseenland.de). Here, sampling was carried out around Lake Cospuden (436 ha, fully flooded for the past 10 years, 51.5 m deep; Figure). Further sampling sites were located on a renatured former waste disposal area (H), now a popular urban recreation area, and in an alluvial forest near a popular game park (I), both within Leipzig.

Ticks were screened for rickettsial DNA by using a PCR amplifying part of the *gltA* gene as described (8). Some tick samples positive for *Rickettsia* spp. were selected to amplify and determine the sequence of the outer membrane protein–coding genes *ompA* and *ompB* (9). New primers were developed for the first part of the *ompA* gene: OmpA-MMX1-for 5′-ACAAGCTTGAGGAAGCCTAGC-3′; OmpA-MMX1-rev 5′-TCTCCGCTCCTTTGGAAAAACTAT-3′.

Of the 3,076 ticks collected, 1,359 were identified as *D. reticulatus* ticks (542 males, 817 females, from all...
sequences of 24.1% (n = 205) of the ticks (772 nymphs in pools of up to 5). The rate of infection differed by tick species, sex of the tick, and collection site; for example, the infection rate was 20.5%–76.4% in female D. reticulatus ticks (Table). Sequencing of 24.1% (n = 205) of the gltA PCR-products verified the specific amplification of rickettsial DNA in 192 cases (93.6%); sequencing for the remaining 13 amplicons could not be determined because the sequencing result was of poor quality. In I. ricinus tick from site B, 87%–88% identity to R. asiatica, R. canadensis, and R. helvetica was identified, but amplification of ompA and ompB failed to further verify the species.

From each site, 1–6 positive tick samples were analyzed for the ompA and ompB genes. The ompB genes of R. raoultii (n = 27), R. helvetica (n = 8), and R. slovaca (n = 2), and ompA genes of R. raoultii (n = 25) and R. slovaca (n = 2) were 100% identical in the amplified part of the respective species, regardless of geographic origin. R. raoultii from our study showed 100% identity (ompA) and 99% identity (ompB) to R. raoultii strain Marne (GenBank accession nos. DQ365800 and DQ365797); for R. helvetica (ompB), 100% identity to GenBank entry AF123725; and for R. slovaca, 100% identity to GenBank entry AF123723 (ompB), and U83454 (ompA). R. raoultii, R. slovaca, and R. helvetica were found only in D. reticulatus, D. marginatus, and I. ricinus ticks, respectively. Sequences (ompA and ompB) from this study were deposited in GenBank (accession nos. HQ232215–HQ232278).

**Conclusions**

In Germany, the most common tick is I. ricinus; D. reticulatus ticks have a focal distribution, and D. marginatus ticks have been described on only a few occasions because the latter require warm and dry habitats (2,10,11). Climate change and structural landscape changes have been discussed as reasons for the creation of new tick habitats (12). Brown coal surface-mining sites of the former German Democratic Republic undergo extensive renaturation, thus providing new biotopes for many plant and animal species, including ticks.

In Germany, R. slovaca was first described in 1977 (2) and again recently (11). Even though the sample size in the present study was small, comparable prevalence rates were detected (13%). R. raoultii was first detected in Russia and has recently been described as a new species (13). The average infection rate of R. raoultii in this study was 56.7% and, in the renatured brown coal surface-mining sites, ≈80.1%. The latter rate is high in comparison with results of previous studies (11,12). R. helvetica prevalence in I. ricinus ticks was similar to results of other studies in Germany (8).

Our results confirm the presence of these rickettsial pathogens in Germany. In addition, we identified previously unknown areas where Rickettsia spp. are endemic. This finding is of major concern to public health: both R. slovaca and R. raoultii can cause TIBOLA, even though R. slovaca is considered to be more pathogenic (7,14). A case of TIBOLA caused by R. slovaca was identified in a human patient in an R. slovaca–endemic area in western Germany (11). The pathogenicity of R. helvetica has not been fully clarified, but serologic evidence shows human infections in

| Table. Rickettsia spp. found in ticks from different sites, Germany, 2008–2009* |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **State (region)** | **Site†** | **Ixodes ricinus ticks** | **Dermacentor reticulatus ticks** | **D. marginatus ticks** |
| | | Male | Female | Nymph‡ | Male | Female | Male | Female |
| **Saarland (West)** | A | 1/6 (16.6) | 0/6 (0) | 1–3/8 (12.5–37.5) | 5/14 (35.7) | 7/14 (50) | NA | NA |
| B | 8/26 (30.8) | 6/35 (17.1) | 3–7/14 (21.4–50.0) | 67/128 (52.3) | 71/153 (46.4) | 1/8 (12.5) | 1/7 (14.3) |
| C | 2/4 (50.0) | 0/2 (0) | 2–7/14 (14.3–50.0) | 9/35 (25.7) | 8/39 (20.5) | NA | NA |
| **Bavaria (South)** | D | 11/58 (19.0) | 8/42 (19.0) | 6/28 (21.4) | 11/40 (27.5) | 30/95 (31.6) | NA | NA |
| **Saxony (East)** | E | 5/56 (8.9) | 10/45 (22.2) | 0/6 (0) | 144/190 (75.8) | 236/309 (76.4) | NA | NA |
| F | 1/4 (25.0) | 1/6 (16.6) | 2–10/10 (20.0–100) | 17/21 (80.1) | 21/29 (72.4) | NA | NA |
| G | 0/2 (0) | 1/6 (16.6) | 1–5/13 (7.7–38.4) | 30/80 (37.5) | 50/111 (45.0) | NA | NA |
| H | 8/53 (15.1) | 4/74 (5.4) | 4–11/35 (11.4–31.4) | 10/16 (62.5) | 33/48 (68.1) | NA | NA |
| I | 7/104 (6.7) | 5/81 (6.2) | 1/34 (2.9) | NA | NA | NA | NA |
| **Total** | A–I | 43/313 (13.7) | 35/297 (11.7) | 20–50/162 (12.3–30.1) | 293/524 (55.5) | 457/798 (57.1) | 1/8 (12.5) | 1/7 (14.3) |

*NA, not available.
†For exact location of site, see Figure.
‡In the regions Saarland and Saxony, some of the nymphs were placed in pools of up to 5 specimens; range of positive ticks indicates the possible minimum and maximum number of ticks positive for Rickettsia spp. in a pool.
France (3), and DNA of R. helvetica was recently identified in a patient in Sweden who had meningitis (15).

Renaturation of industrial sites specifically provides new areas for human recreation and, simultaneously, new habitats for many plant and animal species. Previously nonexistent opportunities for intensive contact between vector ticks and humans are now available. Thus, human-made habitats may lead to increased emerging diseases, especially in tick-borne rickettsioses, because renaturation areas may form favorable biotopes for enhanced human-vector interactions.

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Dr Silaghi is a veterinary parasitologist and researcher at the Veterinary Faculty (Comparative Tropical Medicine and Parasitology) of the Ludwig-Maximilians-Universität in Munich. Her research activities include the epidemiology, public health aspects, and molecular analysis of tick-borne pathogens.

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Address for correspondence: Cornelia Silaghi, Ludwig-Maximilians-Universität, Chair of Comparative Tropical Medicine and Parasitology, Leopoldstr. 5, 80802 Munich, Germany; email: cornelia.silaghi@tropa.vetmed.uni-muenchen.de