**Rickettsia slovaca and R. raoultii in Tick-borne Rickettsioses**

Philippe Parola, Clarisse Rovery, Jean Marc Rolain, Philippe Brouqui, Bernard Davoust, and Didier Raoult

Tick-borne lymphadenopathy (TIBOLA), also called Dermacentor-borne necrosis erythema and lymphadenopathy (DEBONEL), is defined as the association of a tick bite, an inoculation eschar on the scalp, and cervical adenopathies. We identified the etiologic agent for 65% of 86 patients with TIBOLA/DEBONEL as either *Rickettsia slovaca* (49/86, 57%) or *R. raoultii* (7/86, 8%).

In 1968, *Rickettsia slovaca*, a spotted fever group (SFG) rickettsia, was isolated from *Dermacentor marginatus* ticks in the former Czechoslovakia before being detected in *D. marginatus* or *D. reticulatus* ticks throughout Europe (Figure 1) (1). In 1997, *R. slovaca* was described as a human pathogen and an agent of tick-borne lymphadenopathy (TIBOLA) (2). This syndrome, also called Dermacentor-borne necrosis erythema and lymphadenopathy (DEBONEL), is defined as the association of a tick bite, an inoculation eschar on the scalp, and cervical lymphadenopathies (3).

Since 1999, several rickettsial genotypes, called DnS14, DnS28, and RpA4, have been detected in *Dermacentor* spp. ticks throughout Europe (Figure 1). Isolates have been obtained and shown to belong to a unique new SFG rickettsia species named *R. raoultii* (4). In 2002, *R. raoultii* DNA was detected in a *D. marginatus* tick taken from the scalp of a patient in whom TIBOLA/DEBONEL developed in France (4). Moreover, DNA of what is now known to be *R. raoultii* has been found in the blood of 1 patient with TIBOLA/DEBONEL (5). The goal of this study was to identify the rickettsial agents in patients with TIBOLA/DEBONEL symptoms and in those who had an isolated tick bite on the scalp without any symptoms from whom samples (serum, skin biopsy, or ticks harvested from the scalp) were received at our laboratory from January 2002 through December 2007. Epidemiologic and clinical data were collected retrospectively. The study was approved by the ethics committee of the Medicine School of Marseille under reference 08-008.

Immunoglobulin (Ig) G and IgM titers against rickettsial antigens were estimated by microimmunofluorescent assay; results were verified by Western blot and cross-absorption studies (3). Ticks found on persons and skin biopsy specimens were cultured on human embryonic...
lung cells (6). These samples were also used to amplify and identify outer membrane protein A–encoding gene fragments of rickettsiae by PCR (3). Also, the so-called suicide PCR-assay was used with acute-phase serum samples (1).

Among 98 study patients, 86 were classified as TIBOLA/DEBONEL patients. Twelve (12.2%) patients made up the second group with an isolated tick bite. All but 1 patient, who was bitten in Belgium, were bitten in France. Tick bites more frequently occurred from February through May (50/86, 58.1%). Because of results of serologic techniques, we could conclude that 66 (84.6%) of 78 TIBOLA/DEBONEL patients with obtained serum specimens had a recent rickettsial disease. Western blot and cross-absorption analyses enabled detection of antibodies specifically directed against R. slovaca and R. raoultii in 34 and 4 patients, respectively (online Appendix Table, available from www.cdc.gov/EID/content/15/7/1105-appT.htm).

Two patients who were infected with R. slovaca were found to be co-infected with Coxiella burnetii in an acute form of Q fever. Serologic testing was performed in 12 patients with isolated tick bites, and results were negative in all cases. A total of 19 skin biopsy specimens were obtained from 12 patients with isolated tick bites and 7 from patients with secondary or tertiary lymphadenopathy. The suicide PCR on acute-phase serum samples identified a total of 80 R. slovaca infections in 34 and 4 patients, respectively (online Appendix Table, available from www.cdc.gov/EID/content/15/7/1105-appT.htm).

All DNA sequences obtained showed 100% identity with R. raoultii or R. slovaca, excluding the coexistence of several rickettsiae in the corresponding samples. According to our investigations, 49 (57%) of 86 patients with TIBOLA/DEBONEL had probable or certain R. slovaca infections, and 7 (8%) of 86 had probable R. raoultii infections. The characteristics of these patients are shown in the Table.

Conclusions

We report 86 patients with TIBOLA/DEBONEL; this group includes 14 patients whose conditions had been preliminarily reported (7). We also describe several cases caused by the emerging pathogen R. raoultii (4), including patients with indirect molecular evidence of infection because the pathogen was detected in the ticks that had bitten them. Original findings also include facial edema as a new clinical feature in TIBOLA/DEBONEL, and the report of the second patient co-infected with R. slovaca and C. burnetii (8). Because acute Q fever, a worldwide zoonosis, may be asymptomatic, we recommend that patients infected with tick-borne pathogens also undergo testing for concurrent infections with C. burnetii.

No TIBOLA/DEBONEL cases were recorded during the warmest summer months; peak incidence occurred during March–May and during September–November, linked with the activity of Dermacentor ticks in Europe (Figure 1) (9). However, to date, we have no explanation for the finding that children and women are at higher risk for TIBOLA/DEBONEL or why D. marginatus and D. reticulatus ticks prefer to bite persons on the scalp. A possible explanation could be that Dermacentor ticks usually bite hairy domestic and wild animals and the longer hair of women and children may attract them.

One of the most remarkable findings of this work is the proportional importance of R. slovaca in TIBOLA/DEBONEL patients, compared with R. raoultii. In 2006, Ibarra...
of ticks removed from TIBOLA/DEBONEL patients, we found that 12 of 19 ticks harbored 
R. raoultii more significantly associated with TIBOLA/DEBONEL patients than 
R. slovaca, whereas only 3 of 19 harbored R. raoultii (p = 0.047). In the patients with 
asymptomatic tick bites, from whom 9 ticks were obtained, all ticks positive for PCR harbored R. raoultii.

Moreover, R. raoultii seems to be more highly prevalent in D. marginatus and D. reticulatus ticks in nature than is R. slovaca. Although comparing field surveys of ticks is difficult because of the sampling methods, the sizes of the samples, and the potential PCR inhibitors, R. raoultii has been more frequently detected in D. marginatus ticks than has R. slovaca. In southeastern Spain, 73% of 101 D. marginatus ticks were infected by R. raoultii and 27% by R. slovaca (11). Similar differences have been shown in Germany, Portugal, the Netherlands, and Spain (12–15). Although interpreting these data definitively is difficult, the recurrence of similar published results by different teams suggests that exposure to R. raoultii through the bite of a Dermacentor spp. tick is likely more frequent than exposure to R. slovaca. However, more cases of R. slovaca infection have been recorded, which suggests that R. raoultii is less pathogenic.

TIBOLA/DEBONEL is a newly recognized disease, and its incidence is likely underestimated. In our laboratory, TIBOLA/DEBONEL is the most frequently reported rickettsial disease, except during the dry summer period. Doxycycline remains the treatment of choice, with new macrolides as alternative treatments (1). Although we report 6 more cases of R. raoultii infection in addition to the 2 recently reported (4, 5), this Dermacentor-borne rickettsia seems to be less pathogenic than R. slovaca.

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Dr Parola is associate professor of infectious diseases and tropical medicine at the Medical School of Marseille, France. His research interests focus on vector-borne infectious tropical diseases, travel medicine, and medical entomology.

References


Table. Characteristics of TIBOLA/DEBONEL patients with certain or probable Rickettsia slovaca infection compared with patients with certain or probable R. raoultii infection*


Address for correspondence: Didier Raoult, URMITE, Unité des Rickettsies, CNRS-IRD UMR 6236, Faculté de Médecine, 27, blvd Jean Moulin, 13385 Marseille, France; email: didier.raoult@gmail.com

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Appendix Table. Results of microbiologic investigations regarding rickettsial diseases of patients with TIBOLA/DEBONEL or tick-bite*

<table>
<thead>
<tr>
<th>Patients</th>
<th>Serologic assays (MIF assay and WB)†</th>
<th>Suicide PCR‡ of acute-phase serum sample</th>
<th>Standard PCR and/or culture of cutaneous biopsy specimens</th>
<th>Tick study</th>
<th>All methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. pos/</td>
<td>No. tested</td>
<td>Rs</td>
<td>Rr</td>
<td>ND</td>
<td>No. tested</td>
</tr>
<tr>
<td>With TIBOLA/DEBONEL (n = 86)</td>
<td>66/78</td>
<td>34</td>
<td>4</td>
<td>28</td>
<td>36</td>
</tr>
<tr>
<td>Had isolated tick bite (n = 12)</td>
<td>0/5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5</td>
</tr>
</tbody>
</table>

*TIBOLA, tick-borne lymphadenopathy; DEBONEL, Dermacentor-borne necrosis erythema and lymphadenopathy; MIF, microimmunofluorescent; WB, Western blot; pos, positive; Rs, Rickettsia slovaca specific diagnosis or identification; Rr, Rickettsia raoultii specific diagnosis or identification; ND, specific rickettsia involved was not determined; neg, negative.

†MIF assay using 7 spotted fever group rickettsial antigens (R. conorii, R. aeschlimannii, R. massiliae, R. helvetica, R. slovaca, R. raoultii, R. felis), a typhus group antigen, R. typhi, and Coxiella burnetii. For rickettsial antigens, titers >64 for immunoglobulin (Ig) G and >32 for IgM, as well as seroconversion or a 4-fold increase in antibody titers from early to late serum specimens, were considered MIF positive. When cross-reactions were noted among several rickettsial antigens, a rickettsial antigen was considered to indicate presence of the infectious agent if titers of IgG and/or IgM antibody against this antigen were at least 2-fold higher than titers of IgG and/or IgM antibody against other rickettsial antigens. When titers differed by ± 1 standard dilution, WB assays and cross absorption studies were performed.

‡Nested PCR in which both the template DNA fragment and the primers are used only once. (Primers available on request.) All positive PCR amplicons were sequenced with the primers used for PCR, for precise identification of the infecting Rickettsia species. As negative controls, introduced for every 5 patient specimens, we used cardiac valve biopsy specimens obtained from patients who had undergone a valvular replacement for a degenerative disease. No positive controls were used.

§Sample from patient negative by serologic assays.

¶Includes 3 samples from patients negative by serologic assays.

#Includes 10 samples from patients negative by serologic assays, culture, and molecular methods.

**p<0.05 (by Mantel-Haenszel test) between these 2 groups.

††Including 2 cases with coinfection with C. burnetii, the agent of Q fever. The first case occurred in the patient described above with neurologic symptoms (diagnosed on the basis of an anti–phase II IgG titer of 100 and an anti–phase II IgM titer of 50). The second case-patient was a pregnant woman with no symptoms other than the TIBOLA/DEBONEL (diagnosed on the basis of an anti–phase II IgG titer of 400 and an anti–phase II IgM titer of 100).

‡‡p<0.05 (by Mantel-Haenszel test) between these 2 groups.