Rickettsia peacockii sp. nov., a New Species Infecting Wood Ticks, Dermacentor andersoni, in Western Montana†

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Rickettsia peacockii, a new species of spotted fever group rickettsiae, was identified from Rocky Mountain wood ticks (Dermacentor andersoni) collected in the Sapphire Mountain Range on the eastern side of Bitterroot Valley, Montana. DNA from R. peacockii Skalkaho7 (T = type strain) in naturally infected tick tissue was amplified by a PCR assay with primer sets derived from subelemental 16S ribosomal DNA (rDNA), rickettsial citrate synthase, and 190-kDa surface antigen (rOmpA) genes. Partial 16S rDNA and rOmpA gene sequences exhibited levels of similarity of 99.7 and 93.2%, respectively, with the sequences of the spotted fever agent Rickettsia rickettsii. By using Giménez staining, fluorescent antibody tests, a PCR assay, and a restriction fragment length polymorphism analysis, 76 of 115 female ticks (minimal field infection rate, 66.1%) collected between 1992 and 1995 were found to be infected. The organism is passed transstadially and transovarially (minimal vertical transmission rate, 73.3%), and infections are localized in ovarian tissues. Attempts to cultivate R. peacockii were unsuccessful.

Rickettsiae are gram-negative bacteria that are maintained in nature by replication in cells of small mammals and arthropods (5, 7, 23, 32, 47). At least 23 Rickettsia species (alpha subdivision of the Proteobacteria, family Rickettsiaceae) have been or are being described (40, 43), while undoubtedly more remain to be discovered. Of these species, Rickettsia rickettsii, the etiological agent of Rocky Mountain spotted fever (RMSF), is the most significant public health threat in the western hemisphere. Human RMSF cases were first recognized in 1873 in Bitterroot Valley in western Montana (34). Through a series of classic experiments begun in 1906, H. T. Ricketts and coworkers clearly established that the etiology of RMSF involves R. rickettsii in Rocky Mountain wood ticks (Dermacentor andersoni) (39). Transmission to humans occurs principally through bites of infected ticks. Between 1981 and 1992, 4,217 confirmed human RMSF cases (minimum case/fatality ratio, 4.0%) were reported in the United States (9).

Since the pioneering work of Ricketts, several other species of rickettsiae have been recognized in Bitterroot Valley. A survey of 3,705 adult D. andersoni ticks collected in the valley during 1977 revealed four distinct serotypes of hemocyte-associated rickettsiae, which have been described as R. bellii (33). Remarkably, only 2 of the 309 isolates found came from the eastern side of the valley, whereas both of these isolates were nonvirulent R. rhipicephali strains (33). With only one exception, spotted fever group (SFG) rickettsiae virulent for guinea pigs have never been recovered from east side wood ticks (32). These data correspond to the RMSF disease focusality in the valley, where most human cases result from exposure to west side ticks (8, 32). In 1992, a PCR analysis of hemolymph samples from 226 west side D. andersoni adult ticks revealed that 4.0% of the samples were infected with one of three species of previously described rickettsiae (14). No dual rickettsial infections were reported in either the 1977 tick survey or the 1992 tick survey (14, 33).

Despite the low prevalence of hemocyte-associated rickettsiae in east side wood ticks, examination of the tissues of these ticks revealed that a high proportion are infected with rickettsiae. Parker and Spencer determined that 56% of wood ticks collected during 1925 from the eastern side of Bitterroot Valley harbored nonvirulent rickettsiae (29). In 1981, Burgdorfer et al. found that a nonvirulent rickettsia was transstadially and transovarially maintained in approximately 70% of the east side wood ticks examined but was uncommon in west side ticks (8). This organism was designated the east side (ES) agent. The ES agent has not been found in hemocytes, and, given the high natural infection rate, it seems likely that the ES agent went undetected in the 1977 and 1992 tick hemolymph surveys. Burgdorfer et al. postulated that the ES agent interfered with the stable maintenance of virulent rickettsiae in nature, thereby providing a plausible explanation for the focal distribution of RMSF in Bitterroot Valley (8). Unfortunately, the inability to cultivate the ES agent and distinguish it from other rickettsiae precluded further study at the time.

In this study, we identified the ES agent as a unique member of the genus Rickettsia which infects D. andersoni ticks. Staining, indirect fluorescent antibody (IFA) tests, and a PCR assay of tick tissues revealed that this organism is an apparent endosymbiont that can be distinguished from previously described rickettsiae. The results of DNA sequencing and a restriction fragment length polymorphism (RFLP) analysis, techniques which have been successful in helping to characterize rickettsiae and other bacteria associated with hematophagous arthropods (1, 3, 10, 14, 20, 38, 41, 46), indicated that the organism is a unique SFG rickettsial species. The sequence results suggest that strain Skalkaho7 (T = type strain) either lacks or has a truncated form of the major rickettsial 190-kDa surface antigen (rOmpA). Unsuccessful attempts to cultivate the bacterium are also described below.

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† This paper is dedicated to Mort G. Peacock in recognition of his lifelong contributions to rickettsiology, inimitable cell culture talents, and help provided to us and many others in studying rickettsiae.
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TABLE 1. SFG rickettsial strains used to characterize R. peacockii
Skalkaho and their GenBank nucleotide sequence accession numbers.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nucleotide sequence accession no.</th>
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<tr>
<td>Rickettsia peacockii Skalkaho</td>
<td>U55821-d</td>
</tr>
<tr>
<td>Rickettsia peacockii Rd</td>
<td>U55820-d</td>
</tr>
<tr>
<td>Rickettsia rickettsi HLP</td>
<td>U55823-d</td>
</tr>
<tr>
<td>Rickettsia conori Malinch</td>
<td>NA</td>
</tr>
<tr>
<td>Rickettsia japonica YM</td>
<td>U10208</td>
</tr>
<tr>
<td>Rickettsia slovaca 13-B</td>
<td>U43795</td>
</tr>
<tr>
<td>Rickettsia slovaca ESS-25</td>
<td>U43808</td>
</tr>
<tr>
<td>Rickettsia montana MA-6</td>
<td>U55822</td>
</tr>
<tr>
<td>Rickettsia montana ATCC VR-611</td>
<td>NA</td>
</tr>
<tr>
<td>Rickettsia rihipchell 5-7-6</td>
<td>U43803</td>
</tr>
<tr>
<td>Rickettsia rihipchell BSTW</td>
<td>NA</td>
</tr>
<tr>
<td>Rickettsia sibirica 246</td>
<td>U43807</td>
</tr>
<tr>
<td>Rickettsia sibirica 246</td>
<td>U43807</td>
</tr>
<tr>
<td>Rickettsia sibirica 246</td>
<td>NA</td>
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</tbody>
</table>

- The sequences compared to positions 91 through 602 and positions 29 through 1185 on the forward strand of the R. rickettsii R OmpA and 16S rDNA genes, respectively.
- SFG rickettsial strain used in RFLP analyses.
- The partial rOmpA gene sequences of R. peacockii generated from four infected ticks (laboratory colony tick RML-795 and field-collected ticks KG-392, VR-611, and AC-1000), which were stained separately with monoclonal antibody (MAb) 13-2 to rOmpA and MAbs 14-13 and 13-5 to rOmpA as described previously (2). Samples were then stained with fluorescein isothiocyanate-labeled rabbit anti-mouse serum. Each conjugate was diluted 1:50 in sterile DABH2O, incubated for 30 min at 27°C, and then washed three times in phosphate-buffered saline. Cell-cultured R. rickettsii R and rickettsia-free tick ovarian tissue were included in IFA tests as positive and negative controls, respectively. For comparison, R. rickettsii R and five other rickettsial strains were stained with MAb. IFA slide preparations were examined with a fluorescence microscope under oil immersion (magnification, x1,000) for evidence of rickettsiae.

MORPHOLOGICAL STUDIES. Tick ovarian tissues from infected (RML-295 and RML-795) and uninfected (RML-195) females were examined for rickettsiae with a transmission electron microscope (model CM-10; Philips) (24). The tissues were fixed overnight in 4% paraformaldehyde-2.5% glutaraldehyde in 100 mM sodium cacodylate buffer (pH 7.4). Cells were postfixed in 0.5% osmium tetroxide-0.8% potassium ferriyanide and then in 1% aqueous tannic acid and stained overnight en bloc in 1% aqueous uranyl acetate. Samples were dehydrated through a graded ethanol series and embedded in Spurr's sections were cut with an ultramicrotome (model RMC MT-7000), stained with 1% aqueous uranyl acetate and Reynolds's lead citrate, and observed at 80 kV.

Isolation and cultivation attempts. Ticks (ovarian tissues stained with Skalkaho7) were triturated with glass grinders in 5 ml of 4% sucrose-phosphate-glutamate buffer. Next, 0.25 ml of the resulting suspension was inoculated into tripotentially into six standard cell cultures (MDV, PCE, mouse fibroblast, and mouse histiocytoma cell lines) inoculated with Skalkaho7 or four strains of Rickettsia (Rickettsia slovaca, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rickettsia peacockii, Rickettsia rickettsii, Rickettsia peacockii, Rickettsia rickettsii, and Rickettsia peacockii) and five other strains (Rick...
rOmpA gene. These primer sets have been useful for detecting and identifying rickettsiae in arthropods (3, 37, 48), including wood ticks (12, 14).

A PCR assay, in which a eubacterial 16S ribosomal DNA (rDNA) conserved gene primer set (27) was used, was then conducted with tick ovarian tissues which had already tested positive for rickettsial infection as determined by Gimenez staining, two separate IFA tests, and PCRs performed with both the rickettsial CS gene and rOmpA gene primer sets. The 16S rDNA gene primer set (5'GCT TTA AAC CAT GTC GAA G [Escherichia coli positions 601 to 612] and 5'-CCATTGGT AGACCGCGT [E. coli positions 1242 to 1227]) was modified for use in our study by switching a single base on the forward primer. This primer set amplifies an 1,157-bp fragment which corresponds to positions 29 through 1185 in the on forward strand of the R. ricketsii 16S rDNA gene.

Prior to the PCR assay, individual tick tissue samples suspended in 200 l of BHI were triturated in disposable tubes with matching pestle pestles (Kontes, Vineland, N.J.). The triturated samples were then diluted with 10 l of proteinase K (120 pg/ml), incubated for 1 h at 37°C, and heated to 95°C for 10 min to inactivate the proteinase K. A 5-ml portion of each suspension was then used in the PCR assay. The assays were performed with a GeneAmp PCR reagent kit in 0.5-ml reaction tubes as recommended by the manufacturer (Perkin-Elmer Cetus). In the IFA tests a polyclonal antibody to r. ricketsii R was used, and in the PCR assays both rickettsial CS and rOmpA gene primer sets were used.

Five ovarian tissue samples that were negative for rickettsiae as determined by Gimenez staining and IFA tests were positive as determined by PCR assays. The organisms in these samples and 35 other samples were identified as R. peacockii by an RFLP analysis. All tissues that were positive for rickettsiae gave amplification products with both primer sets.

A number of positive samples as determined by Gimenez staining/number of positive samples as determined by IFA tests/number of positive samples as determined by PCR assays (number of samples tested). NT, not tested.
TABLE 3. Reactivity of *R. peacockii* Skalkaho\(^{a}\) and related rickettsiae with MAbs to rOmpA and rOmpB

<table>
<thead>
<tr>
<th>Strain</th>
<th>rOmpB MAb 13-2</th>
<th>rOmpA MAb 13-5</th>
<th>rOmpA MAb 14-13</th>
</tr>
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<tbody>
<tr>
<td><em>R. peacockii</em> Skalkaho(^{a})</td>
<td>+(^{b})</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>R. rickettsii</em> R</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>R. rickettsii</em> HLP</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>R. conorii</em> Malish 7</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>R. montana</em> M5-6</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>R. rhipicephali</em> 8STW</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WB8-2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^{a}\) Infected ovari al tissues from laboratory colony ticks (RML-295 and RML-795) and field-collected ticks (SK-594 and SK-295) were tested and elicited identical reactivities.

\(^{b}\) +, positive; -, negative.

The reactivities of Skalkaho\(^{a}\) and several other SFG rickettsial strains to MAbs are summarized in Table 3. Both heat-sensitive MAb 14-13 and heat-resistant MAb 13-5 to rOmpA failed to bind to Skalkaho\(^{a}\), while at least one of the two MAbs bound strongly to all other rickettsial strains tested (Table 3). Conversely, MAb 13-2 to rOmpB bound strongly to Skalkaho\(^{a}\).

tests gave PCR amplification products whose RFLP profiles were consistent with those of Skalkaho\(^{a}\).

The levels of sequence similarity between the new organism and other rickettsiae were comparable to the levels of similarity observed among the SFG relatives of Skalkaho\(^{a}\) (Table 4). The 16s rDNA sequence of Skalkaho\(^{a}\) was most similar (level of similarity, 99.7%) to the sequences of *R. rickettsii* R (three base differences) and *Rickettsia slovaca* 13-B (four base differences) (Table 4). For comparison, *R. rickettsii* R also exhibits 99.7% similarity to *Rickettsia conorii* Malish 7 (Table 4). The partial 16S rDNA sequence of Skalkaho\(^{a}\) generated from a PCR fragment amplified from a tick collected in 1994 was identical to the sequence of a cloned PCR product amplified from a tick collected in 1995. For the aligned sequences, the base signatures for Skalkaho\(^{a}\) were T and C at positions 474 and 532, respectively, on the forward strand of the 16S rDNA gene sequence. Furthermore, the four partial rOmpA gene sequences generated for the bacterium from three separate ticks collected in 1992, 1992, and 1995, as well as a laboratory colony tick, were identical. The rOmpA gene sequence of Skalkaho\(^{a}\) was most similar (93.0%) to the sequence of *R. slovaca* 13-B (Table 5). A lower level of similarity (91.6%) was observed with *R. rickettsii* HLP and *R. conorii* strains (Table 5). For comparison, the rOmpA gene sequence of *R. slovaca* 13-B exhibits 93.0% similarity to the *Rickettsia japonica* YM sequence, while the *R. rickettsii* R sequence exhibits 93.2
The ability to detect and differentiate Skalkaho by molecular techniques provides a promising approach for investigating the cycling, behavior, and interaction of rickettsiae in nature. In particular, the low incidence of RMSF on the eastern side of Bitterroot Valley has never been adequately explained; however, given its abundance there, it seems plausible that Skalkaho may be responsible for this phenomenon (8). In ongoing studies addressing this hypothesis, workers are trying to determine the incidence of Skalkaho on the western side of the valley and whether massive infections of this rickettsia in tick ovarial tissue inhibit transovarial transmission of virulent Rickettsia. Another potential limiting factor of Skalkaho is the mammalian reservoir of suitable RMSF-amplifying hosts on the eastern side may be resistant to rickettsial infection due to prior exposure and seroconversion to Skalkaho. Considering the high level and stable maintenance of Skalkaho in ticks, this bacterium may be beneficial to ovarial cells or may bias ticks toward infection, as has been demonstrated for low- and high-virulence strains of Rickettsia (35, 36) and for distinctly related SFG rickettsiae (11, 13), but no interference was observed for Skalkaho since guinea pigs inoculated with triturates of wood ticks harboring the ES agent remained susceptible to infection with Rickettsia (8, 29). Studies to determine the capacity of the organism to be transmitted to mammals during tick feeding were inconclusive (8).

### DISCUSSION

Considering the high level and stable maintenance of Skalkaho in ticks, this bacterium may be beneficial to ovarial cells or may bias ticks toward infection, as has been demonstrated with some arthropod endosymbionts, such as the AB agent, a...
rickettsial species associated with male killing in the ladybird beetle (48), and other members of the \textit{Rickettsiaceae} (26, 45). If this is true, the result would be selection for a greater percentage of \textit{D. andersoni} tick ovarian cells infected with Skalkaho\(^5\). The biological properties which the organism may share with other rickettsia-like endosymbionts (44, 48) remain to be determined.

**Description of \textit{Rickettsia peacockii} sp. nov.** We propose the name \textit{Rickettsia peacockii} (pea.co’ck’i.i. M.L. gen. n. peacockii of Peacock, named after rickettsiologist M. G. Peacock) for a new SFG rickettsial species which maintains natural infections in wood ticks via transstadial and transovarial transmission. Although \textit{R. peacockii} could not be maintained in laboratory tissue culture, there is precedent for naming and describing uncultivable prokaryotes (1, 22). A type strain (Skalkaho) was characterized directly from an infected \textit{D. andersoni} tick (SK-594) which was used to generate 16S rDNA and rOmpA gene sequences (Tables 4 and 5), RFLP profiles (Fig. 2), and IFA reactivity data (Table 3) and to establish an infected laboratory tick colony (RML-795).

The localization of \textit{R. peacockii} Skalkaho\(^5\) in tick ovarian tissue, our inability to maintain infections in mammalian systems, the apparent lack or truncation of the rOmpA surface antigen, and the gene sequence data indicate that this fastidious microorganism is a unique rickettsial species. With its stable maintenance in ticks, \textit{R. peacockii} Skalkaho\(^5\) appears to be an endosymbiont whose biology contrasts with the biology of other rickettsiae. Continued studies on the organism should contribute to our understanding of rickettsia-vector-mammalian host dynamics and the mechanisms which virulent rickettsiae employ to infect and ultimately damage host cells.

**ACKNOWLEDGMENTS**

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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