Prevalence of Rickettsia Species in Canadian Populations of Dermacentor andersoni and D. variabilis

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We determined the prevalence of rickettsiae in Dermacentor adults at 15 localities in Canada. Rickettsia rickettsii was not detected in any tick, whereas Rickettsia peacockii was present in 76% of Dermacentor andersoni adults and Rickettsia montanensis in 8% of Dermacentor variabilis adults. This host specificity was maintained in localities where both tick species occurred in sympatry.

Dermacentor andersoni and Dermacentor variabilis are vectors of Rickettsia rickettsii (6), the etiological agent of Rocky Mountain spotted fever (RMSF) in humans. RMSF has been a notifiable disease in the United States since the 1920s, with over 3,600 cases reported between 1997 and 2002 (9). Nonpathogenic rickettsiae have been also been reported for both tick species (3, 12, 13). The detection and identification of Rickettsia in ticks have greatly improved in accuracy and sensitivity since the advent of PCR-based techniques. The rickettsial cycle is maintained in amplification of a 381-bp fragment of Rickettsia gltA (22) from a single individual of each tick species that was not detected in any tick, whereas Rickettsia peacockii was present in 76% of Dermacentor andersoni adults and Rickettsia montanensis in 8% of Dermacentor variabilis adults. This host specificity was maintained in localities where both tick species occurred in sympatry.

The prevalence of infection in D. andersoni varied among localities (36 to 96%); the lowest prevalence was recorded within Danielson Provincial Park (Table 1). The prevalence of Rickettsia in D. variabilis was very low (0 to 8%) at most localities, except within Blackstrap Provincial Park, where 33% of ticks were Rickettsia positive (Table 1). There was heterogeneity in the prevalence of Rickettsia within Blackstrap Provincial Park with a significantly greater (P < 0.001) proportion of Rickettsia-infected D. variabilis individuals on the western side of Blackstrap Lake (39%; n = 115) than on the eastern side (4%; n = 26).

Genetic variation among the 452 gltA amplicons derived from Rickettsia-positive ticks was examined using single-strand conformation polymorphism (SSCP) analyses (10, 14). Two different SSCP banding patterns (i.e., profiles) were detected among samples: one profile (type I) was displayed by all D. andersoni individuals positive for Rickettsia, and the second (type II) was displayed only by D. variabilis individuals positive for Rickettsia (Fig. 1). The gltA sequences derived from 11 column-purified amplicons of type I were identical to each other and to the sequence for Rickettsia peacockii (GenBank accession number AF129885) (25). The eight type II gltA amplicons derived from Rickettsia-infected D. variabilis individuals were identical in nucleotide sequence to one another and to a sequence for Rickettsia montanensis (accession number U74756) (23). The presence of R. peacockii in D. andersoni adults and R. montanensis in D. variabilis adults was confirmed by the amplification and sequencing of a 532-bp fragment of ompA (22) from a single individual of each tick species that contained rickettsiae using primers Rr190.70p and Rr190.602n (22) and the same conditions used for gltA except that 30 amplification cycles were used. The ompA amplicon from D. andersoni was identical in sequence to that reported previously.
for R. peacockii (accession number U55821) (19). The omp4 amplicon from D. variabilis most closely matched the sequence for R. montanensis (accession number AY543682) (1), but it differed at a single nucleotide position. The results of a phylogenetic analysis showed that there was strong statistical support for the inclusion of the Rickettsia species from D. variabilis within the clade of R. montanensis (Fig. 2).

Our molecular analyses of 508 D. andersoni and 818 D. variabilis adults from 15 localities revealed the presence of R. peacockii in D. andersoni and R. montanensis in D. variabilis. This host-specificity was maintained at the seven localities where both tick species occurred in sympatry. These findings are consistent with the results of studies conducted in the United States, where R. peacockii has been reported only for D. andersoni (7, 19) and R. montanensis only for D. variabilis (1, 2, 12, 21). Philip and Casper (20) reported R. montanensis for D. andersoni from the western side of Bitterroot Valley (Montana), based on serotyping of rickettsiae from ticks. However, this probably represents a case of an incorrect identification of the rickettsiae. Philip and Casper (20) demonstrated that there were four serotypes within 106 rickettsial isolates from D. andersoni and attributed these to be R. rickettsii (9%), Rickettsia rhipicephali (44%), Rickettsia bellii (i.e., 369-C; 39%) and R. montanensis (i.e., “Rickettsia montana”; 8%). In contrast, Burgdorfer et al. (7) showed that R. peacockii occurs on the western side of Bitterroot Valley at a prevalence of 8 to 16%. It is, therefore, likely that the fourth rickettsial species detected by Philip and Casper (20) was not R. montanensis but R. peacockii, especially if the antibodies used in their assay were cross-reactive with both species. If this were the case, then R. montanensis would also represent a rickettsial species that is host specific for D. variabilis.

We only detected single-species rickettsial infections in both tick species. This is typical for Dermacentor spp. (1, 13, 27), except for the reports of a single D. variabilis adult from Ohio infected with R. bellii, R. montanensis, and R. rickettsii (8) and of a single Dermacentor occidentalis adult infected with R. bellii and R. rhipicephali (27). The prevalence of R. peacockii in D. andersoni at different localities (36 to 96%) was significantly greater than that for R. montanensis in D. variabilis (0 to 33%). This is likely due to the mode of transmission of R. peacockii, which is thought to be exclusively transovarial (i.e., from female ticks to their offspring) (7, 19). The prevalence of R. montanensis in D. variabilis at 12 of the 15 sites in the present study (0 to 8%) was similar to that for D. variabilis populations in Ohio (<0.1%) (21), Massachusetts (1%) (12), and Maryland (4%) (1). The relatively low prevalence of R. montanensis in ticks compared to that for R. peacockii suggests that horizontal transmission is required for the maintenance of this species in populations of D. variabilis. R. montanensis has been detected in mice (Peromyscus spp.) and voles (Microtus spp.) (18), hosts used by D. variabilis (4, 16), suggesting that small mammals may act as reservoirs for this species of Rickettsia.

The results of the present study also showed that the other rickettsial species recorded in D. andersoni and/or D. variabilis in the United States (i.e., the pathogenic R. rickettsii [6] and the nonpathogenic R. bellii and R. rhipicephali [13]) were not detected in any of the 1,326 ticks tested. The prevalence of R. rickettsii in D. andersoni adults in the Bitterroot Valley of Montana varies from 1.5 to 5% (6), while infections of R. rickettsii in D. variabilis range from 0.1% in Ohio (21) to 8.6% in Maryland (24). The lack of detection of R. rickettsii in D.

### TABLE 1. Localities and coordinates of the collection sites of D. andersoni and D. variabilis adults within Canada and the number of ticks that were positive for infection with Rickettsia using PCR analyses of the gltA gene

<table>
<thead>
<tr>
<th>Locality in Canada</th>
<th>Coordinates</th>
<th>No. of D. andersoni adults:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tested</td>
</tr>
<tr>
<td>Lethbridge, AB</td>
<td>49°44’N, 112°50’W</td>
<td>100</td>
</tr>
<tr>
<td>Cypress Hills, AB</td>
<td>49°25’N, 110°15’W</td>
<td>61</td>
</tr>
<tr>
<td>Saskatchewan Landing Provincial Park, SK</td>
<td>50°38’N, 107°57’W</td>
<td>101</td>
</tr>
<tr>
<td>Grasslands National Park, SK</td>
<td>49°13’N, 107°42’W</td>
<td>17</td>
</tr>
<tr>
<td>Buffalo Pound Provincial Park, SK</td>
<td>50°36’N, 105°25’W</td>
<td>35</td>
</tr>
<tr>
<td>Douglas Provincial Park, SK</td>
<td>51°02’N, 106°28’W</td>
<td>14</td>
</tr>
<tr>
<td>Danielson Provincial Park, SK</td>
<td>51°15’N, 106°49’W</td>
<td>61</td>
</tr>
<tr>
<td>Outlook, SK</td>
<td>51°28’N, 107°04’W</td>
<td>18</td>
</tr>
<tr>
<td>Harris, SK</td>
<td>51°42’N, 107°37’W</td>
<td>101</td>
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<tr>
<td>Saskatoon, SK</td>
<td>52°10’N, 106°36’W</td>
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</tr>
<tr>
<td>Blackstrap Provincial Park, SK</td>
<td>51°47’N, 106°25’W</td>
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</tr>
<tr>
<td>Bradwell, SK</td>
<td>51°14’N, 106°13’W</td>
<td>100</td>
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<tr>
<td>Wakaw, SK</td>
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<td>44</td>
</tr>
<tr>
<td>Minnedosa, MB</td>
<td>50°14’N, 99°50’W</td>
<td>100</td>
</tr>
<tr>
<td>Kenora, ON</td>
<td>49°45’N, 94°29’W</td>
<td>30</td>
</tr>
</tbody>
</table>

FIG. 1. SSCP analysis of gltA amplicons from total gDNA from D. andersoni (SSCP profile I) and D. variabilis (SSCP profile II). Lanes 1 to 6 and 7 to 12 contain gltA amplicons derived from single D. andersoni and D. variabilis individuals, respectively.
The apparent absence of the Canadian localities we examined would not account for the relatively low prevalence with respect to D. andersoni (7, 19). Only 8 to 16% of D. andersoni on the western side of Bitterroot Valley are infected with R. peacockii (7, 19), whereas the prevalence is 70 to 80% for ticks on the eastern side (7, 19), which is equivalent to the average prevalence of R. peacockii in D. andersoni in the present study (76%). It has also been shown that establishment of R. rickettsii (accession numbers AF149108, and R. rickettsii (AY319293, DQ459233, DQ150693, DQ150687, and U43804) derived from GenBank. The numbers above the branches in the tree indicate the statistical support following bootstrap analyses (1,000 iterations) for each clade. R. australis was used to root the tree (26).

D. andersoni from the nine localities in Canada may be associated with the relatively high proportion of ticks infected with R. peacockii. Although R. peacockii is closely related to R. rickettsii (19), it appears to be nonpathogenic to D. andersoni and has no effect on the fecundity of infected females (18). The greater incidence of RMSF on the western side of Bitterroot Valley compared to the eastern side of the valley has been shown to be associated with a significantly lower prevalence of R. peacockii (7, 19). Only 8 to 16% of D. andersoni on the western side of the Bitterroot Valley are infected with R. peacockii (7), whereas the prevalence is 70 to 80% for ticks on the eastern side (7, 19), which is equivalent to the average prevalence of R. peacockii in D. andersoni in the present study (76%). It has also been shown that establishment of R. rickettsii in the ovarian tissues of D. andersoni is prevented by an “interference phenomenon” when ticks are already infected with R. peacockii (7). D. variabilis adults infected with R. montanensis are also known to prevent the establishment of R. rickettsii (17). Thus, R. peacockii and R. montanensis have epidemiological significance with respect to R. rickettsii because of a negative effect on its enzootic maintenance. However, the relatively low prevalence of D. variabilis adults infected with R. montanensis in 13 of the Canadian localities we examined would not account for the apparent absence of R. rickettsii. Therefore, other factors must be responsible for this.

Nucleotide sequence accession numbers. The sequences of the gltA and ompA genes for representative samples have been deposited in GenBank under accession numbers FM883668 to FM883671.

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REFERENCES


