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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 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 citrate synthase (*gltA*) and the 190-kDa surface protein (*ompA*) genes have been used to distinguish among species of *Rickettsia* and to determine the prevalence of different rickettsiae in *D. andersoni* or *D. variabilis* adults within the United States, primarily at localities where these two tick species do not coexist (1, 13, 19, 27). Serological studies of the prevalence of *Rickettsia* in the United States are also based on an examination of ticks from allopatric populations (2, 20). Comparisons of the prevalence of rickettsiae in sympatric and allopatric populations of *D. andersoni* and *D. variabilis* would provide insight into the host specificity and transmission of *Rickettsia* species.

RMSF is not a reportable disease in Canada. As a consequence, little is known of the frequency of RMSF, except for a few published cases in Alberta between 1923 and 1943 (5, 11, 15). There is no detailed information of the distribution and prevalence of rickettsial species in Canada, even though *D. andersoni* and *D. variabilis* are relatively common (28). The geographic ranges of these tick species in Canada are largely allopatric, except for a zone of sympatry in central Saskatchewan (28). The aim of the present study was to determine the species of *Rickettsia* present and their relative prevalence in adult ticks from allopatric and sympatric populations of *D. andersoni* and *D. variabilis* in Canada.

Total genomic DNA (gDNA) was extracted and column purified (10) from 1,326 adult ticks collected in 2005 (May through July) and 2007 (April through June) from 15 localities (Table 1). The presence of rickettsiae in ticks was determined by amplification of a 381-bp fragment of *gltA* by PCR from the tick gDNA using primers *Rp*CS.877p and *Rp*CS.1258n (22) and the following conditions: 95°C for 5 min, followed by 25 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s and a final extension at 74°C for 5 min. The results of the PCR analyses revealed that a large proportion (76%) of *D. andersoni* adults were infected with *Rickettsia*, while relatively few (8%) *D. variabilis* adults tested positive for *Rickettsia* (Table 1). There was no significant difference in prevalence of *Rickettsia* in *D. andersoni* males (73%; $n = 205$) and females (79%; $n = 303$; $P = 0.128$), or in the prevalence of *Rickettsia* in *D. variabilis* males (9%; $n = 382$) and females (7%; $n = 436$; $P = 0.420$). 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 *Rr*190.70p and *Rr*190.602n (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

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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

Locality in Canada	Coordinates	No. of *D. andersoni* adults:		No. of *D. variabilis* adults:	
		Tested	Positive for *Rickettsia* (%)	Tested	Positive for *Rickettsia* (%)
Lethbridge, AB	49°44′N, 112°50′W	100	72 (72)		
Cypress Hills, AB	49°25′N, 110°15′W	61	37 (61)		
Saskatchewan Landing Provincial Park, SK	50°38′N, 107°57′W	101	97 (96)	100	0 (0)
Grasslands National Park, SK	49°13′N, 107°42′W	17	15 (88)	1	0 (0)
Buffalo Pound Provincial Park, SK	50°36′N, 105°25′W	35	30 (86)	100	2 (2)
Douglas Provincial Park, SK	51°02′N, 106°28′W	14	13 (93)	40	0 (0)
Danielson Provincial Park, SK	51°15′N, 106°49′W	61	22 (36)	100	0 (0)
Outlook, SK	51°28′N, 107°04′W	18	17 (94)	12	0 (0)
Harris, SK	51°42′N, 107°37′W	101	84 (83)	12	0 (0)
Saskatoon, SK	52°10′N, 106°36′W			38	0 (0)
Blackstrap Provincial Park, SK	51°47′N, 106°25′W			141	46 (33)
Bradwell, SK	51°54′N, 106°13′W			100	7 (7)
Wakaw, SK	52°36′N, 105°51′W			44	0 (0)
Minnedosa, MB	50°14′N, 99°50′W			100	8 (8)
Kenora, ON	49°45′N, 94°29′W			30	2 (7)

for *R. peacockii* (accession number U55821) (19). The *ompA* 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*.

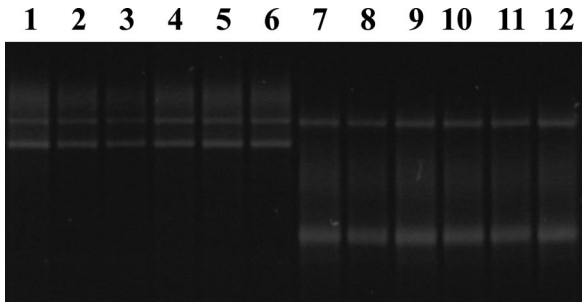

FIG. 1. SSCP analysis of *gltA* amplicons from total gDNA from *D. andersoni* (SSCP profile I) and *D. variabilis* (SSCP profile II). Lanes 1 to 6 and 7 to 12 contain *gltA* amplicons derived from single *D. andersoni* and *D. variabilis* individuals, respectively.

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.*

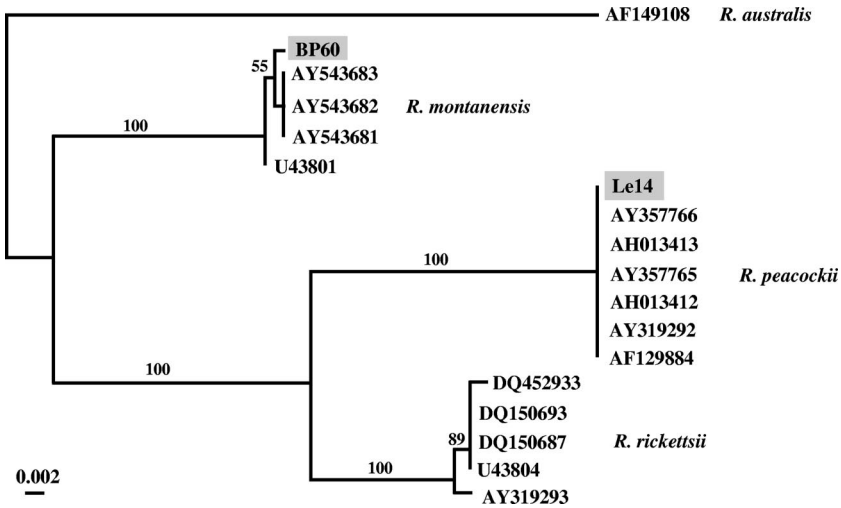

FIG. 2. A neighbor-joining tree depicting the relationships of the *ompA* sequences of *Rickettsia* from *D. andersoni* (Le14) and *D. variabilis* (BP60) obtained in the present study with those of *R. peacockii* (accession numbers AF129884, AH013412, AH013413, AY357765, and AY357766), *R. montanensis* (AY543681, AY543682, AY543683, and U43801), *Rickettsia australis* (AF149108), and *R. rickettsii* (AY319293, DQ452933, 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).

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 ovarial 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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