Powassan virus (POWV) is a tick-borne virus (family Flaviviridae, genus Flavivirus) with recent and increasing prevalence. The only member of the tick-borne encephalitis (TBE) serogroup of flaviviruses endemic to North America, POWV is an emerging cause of human illness and death (1,2). Transmitted primarily by Ixodes spp. ticks and maintained in enzootic cycles involving small- to medium-size mammals, POWV exists as 2 genetically divergent and spatially distinct lineages that are serologically indistinguishable: lineage I, prototype POWV and lineage II, deer tick virus (DTV) (3,4). The 2 lineages are maintained in different vector and host species.

First discovered in eastern Canada, POWV is now known to also circulate in the northeastern United States and the Russian Far East and has been documented in the western United States and Canada in wildlife and human infections (5–9). Clinical signs range from self-limiting febrile illness to severe neurologic disruption and death (1,2). Transmitted primarily by Ixodes spp. ticks and maintained in enzootic cycles involving small- to medium-size mammals, POWV exists as 2 genetically divergent and spatially distinct lineages that are serologically indistinguishable: lineage I, prototype POWV and lineage II, deer tick virus (DTV) (3,4). The 2 lineages are maintained in different vector and host species.

The Study

Animals were collected in live traps and snap traps from sites in Siberia (2006), Alaska (2004–2005) and throughout the southwestern United States (2005–2007 (Table 1, Figure 1) under University of New Mexico Institutional Animal Care and Use Committee protocol 12–100764-MCC. Blood was collected on site during specimen processing. We screened blood samples from > 600 wild small-to-medium sized mammals representing 31 host species for POWV-specific antibodies.

Serum samples and supernatants were tested by using a strip immunoblot assay (SIA) with recombinant DTV envelope glycoprotein. Because POWV and DTV are serologically indistinguishable, this antigen binds antibodies specific for DTV, POWV, or other closely related viruses.

Antigen (10–50 µg/mL of DTV E-glycoprotein) was covalently bound to a nitrocellulose strip by using centrifugal filtration apparatuses. Samples were tested by using recombinant DTV E-glycoprotein (positive control), powdered DTV E-glycoprotein (negative control), mouse IgG (IgG-positive control), and purified DTV E-glycoprotein (test). Approximately 0.25 µg of DTV envelope glycoprotein was used per 2-mm test strip. Samples were tested at a 1:200 dilution, and antibody was detected by using an alkaline phosphatase–conjugated secondary antibody (goat antimouse IgG). Colorimetric intensity was assessed and
Table 1. Powassan virus seroprevalence in mammals captured in eastern Russia (Siberia), Alaska, and the southwestern United States*

| Region               | Species                          | Common name                  | No. positive/no. tested (%) | 95% CI     |
|----------------------|----------------------------------|------------------------------|----------------------------|------------|
| Siberia, Russia      | Lepus timidus                    | Mountain hare                | 0/1 (0)                    | 0–79.35    |
|                      | Microtus gregalis               | Narrow-headed vole           | 0/2 (0)                    | 0–65.76    |
|                      | Microtus oeconomus              | Tundra vole                  | 0/12 (0)                   | 0–24.25    |
|                      | Mustela emerina                 | Stoat                        | 0/1 (0)                    | 0–79.35    |
|                      | Myodes rufocanus                | Gray red-backed vole         | 0/8 (0)                    | 0–39.03    |
|                      | Myodes rutilus                  | Northern red-backed vole     | 6/79 (7.6)                 | 3.52–15.59 |
|                      | Myopus schisticolor             | Wood lemming                 | 0/2 (0)                    | 0–65.76    |
|                      | Sciurus vulgaris                | Tuft-eared squirrel          | 0/2 (0)                    | 0–65.76    |
|                      | Spermophilus undulatus           | Long-tailed ground squirrel  | 0/1 (0)                    | 0–79.35    |
|                      | Tamias sibiricus                | Siberian chipmunk            | 0/5 (0)                    | 0–43.45    |
| Total                | NA                              | NA                           | 6/111 (5.4)                | NA         |
| Central Alaska       | Microtus oeconomus              | Tundra vole                  | 0/5 (0)                    | 0–43.45    |
|                      | Mustela vison                   | American mink                | 0/2 (0)                    | 0–65.76    |
|                      | Myodes rutilus                  | Northern red-backed vole     | 14/243 (5.8)               | 3.46–9.44  |
|                      | Sorex cinereus                  | Cinerue shrew                | 0/8 (0)                    | 0–32.44    |
|                      | Sorex hoyi                      | Pygmy shrew                  | 0/1 (0)                    | 0–79.35    |
|                      | Sorex monticolus                | Montane shrew                | 0/6 (0)                    | 0–39.03    |
|                      | Sorex tundrensisis              | Tundra shrew                 | 0/2 (0)                    | 0–65.76    |
|                      | Tamiasciurus husdonicus         | Red Squirrel                 | 0/2 (0)                    | 0–56.15    |
| Total                | NA                              | NA                           | 6/89 (6.7)                 | 3.13–13.93 |
| Southern Alaska      | Myodes gapper                   | Southern red-backed vole     | 20/359 (5.6)               | NA         |
| Total                | NA                              | NA                           | 20/359 (5.6)               | NA         |
| Southwestern USA     | Dipodomys merriami              | Merriam's kangaroo rat       | 0/15 (0)                   | 0–20.39    |
|                      | Dipodomys ordii                 | Ord's kangaroo rat           | 0/1 (0)                    | 0–79.35    |
|                      | Mus musculus                    | House mouse                  | 0/4 (0)                    | 0–48.99    |
|                      | Neotoma albipila                | White-throated woodrat       | 0/10 (0)                   | 0–27.75    |
|                      | Neotoma cinerea                 | Bushy-tailed woodrat         | 0/4 (0)                    | 0–48.99    |
|                      | Notiosorex crawfordi            | Desert shrew                 | 0/1 (0)                    | 0–79.35    |
|                      | Onychomys arenicola             | Meam's grasshopper mouse     | 0/14 (0)                   | 0–21.53    |
|                      | Perognathus flavus              | Silky pocket mouse           | 0/3 (0)                    | 0–50.75    |
|                      | Peromyscus boyli                | Brush mouse                  | 0/6 (0)                    | 0–39.03    |
|                      | Peromyscus eremicus             | Cactus mouse                 | 0/19 (0)                   | 0–16.82    |
|                      | Peromyscus maniculatus          | Deer mouse                   | 2/33 (6.0)                 | 1.68–19.61 |
|                      | Peromyscus leucopus             | White-footed mouse           | 0/22 (0)                   | 0–14.87    |
|                      | Peromyscus truei                | Piñon mouse                  | 2/9 (22.2)                 | 6.32–54.74 |
|                      | Sigmodon hispidus               | Hispid cotton rat            | 0/3 (0)                    | 0–56.15    |
| Total                | NA                              | NA                           | 4/144 (2.8)                | NA         |

*NA, not applicable.

DTV envelope glycoprotein–positive results were compared with 3+ and 1+ IgG control bands.

In Siberia and central Alaska, antibodies reacting with DTV antigen were detected exclusively in northern red-backed voles (*Myodes rutilus*) (6.2%) (Table 1). In southern Alaska, DTV-reactive antibodies were detected in the only species tested, the southern red-backed vole (*M. gapperi*) (6.7%). In the southwestern United States, DTV-reactive antibodies were found in New Mexico in 2 *Peromyscus* species mice: the piñon mouse (*P. truei*) and the deer mouse (*P. maniculatus*) (22.2% and 6.0%, respectively) that were collected sympatrically. The deer mouse is of particular interest because it is the primary host of Sin Nombre virus, the etiologic agent of hantavirus cardiopulmonary syndrome in North America (13).

To identify the virus responsible for serologic reactivity, we collected ticks (*Ixodes angustus*) from coastal southeastern Alaska (61.3210°N, 145.3030°W; 59.2459°N, 135.1753°W; and 55.8717°N, 132.3481°W) in 2009 from captured mammals (Table 2). Reverse transcription PCR was performed for ticks and tissues from seronegative animals collected proximally to seropositive animals and thus potentially in the acute stage of infection. No viral RNA was detected in ticks or in seronegative rodent tissue.

**Conclusions**

Although we used a DTV antigen because of its technical convenience, we do not believe that DTV per se is present in these rodent populations. POWV is present throughout western United States and western Canada. However, the virus responsible for the observed seropositivity in Alaska is unknown. The most likely candidate is POWV but without an isolate or sequence data, tickborne encephalitis virus or other Eurasian flavivirus cannot be ruled out, and we cannot rule out the possibility that the virus is a flavivirus with no known vector. The utility of the SIA is partially based on known cross-reactivity of flaviviruses because it enables detection of divergent lineages. Determination of endpoint antibody titers and confirmation of POWV specificity by plaque-reduction neutralization tests were not possible because of freezer failure.

Because few wild rodent antibodies are commercially available, our methodology used anti-Mus secondary
antibody, which may have varying sensitivity against the 31 species tested. Thus, low-level reactivity may have been missed. However, the prevalence of antibodies detected by SIA in our study is consistent with that reported from known POWV transmission foci (14).

These serologic results enable us to conclude that ≥1 flaviviruses antigenically similar to DTV circulate in Siberia, Alaska, and the southwestern United States (Table 1). Transmission appears to involve Myodes spp. voles in northern regions and Peromyscus mice in southern regions. Considerable overlap in the geographic ranges of these species may provide continuous populations of competent amplifying hosts from Mexico (P. maniculatus and P. truei) to Siberia (M. rutilus) (Figure 2). The seropositivity in Siberia may be from introduced POWV, native TBE virus, or other related virus. Viral RNA sequence is necessary to delineate the viral species that are circulating among M. rutilus in Siberia. Additional host species may be involved; considering the small sample for the current study, seropositivity rates and distributions, although consistent with expectations, may be considerably refined with increased sampling (Table 1). The incidence and host association of Ix. angustus ticks were similar to those of a previous report (15), and further vectorial studies are warranted.

Our findings augment knowledge of distribution of TBE serogroup flavivirus in the Nearctic and will guide further studies of New World TBE serogroup flavivirus ecology. Future work will focus on acquisition of viral isolates and nucleic acid sequences from Myodes spp. voles in Alaska and Siberia and from Peromyscus spp. mice in the southwestern United States.

Table 2. Ticks collected from trapped mammals in southeastern Alaska, USA, June–July 2009, and tested by reverse transcription PCR for flavivirus RNA*

| Host species            | No. | Adult males | Adult females | Nymphs | Lavae | Total | Average infestation |
|-------------------------|-----|-------------|---------------|--------|-------|-------|---------------------|
| Microtus longicaudus    | 2   | 0           | 1             | 1      | 0     | 2     | 1.0                 |
| Microtus pennsylvanicus| 1   | 0           | 0             | 0      | 0     | 1     | 1.0                 |
| Myodes gapperi          | 18  | 1           | 17            | 33     | 4     | 55    | 3.1                 |
| Myodes rutilus          | 12  | 0           | 5             | 9      | 2     | 16    | 1.3                 |
| Peromyscus keenii       | 21  | 2           | 16            | 33     | 26    | 77    | 3.7                 |
| Peromyscus maniculatus  | 5   | 0           | 2             | 3      | 3     | 5     | 1.0                 |
| Sorex cinereus          | 3   | 0           | 3             | 12     | 0     | 15    | 5.0                 |
| Sorex monticolus        | 10  | 0           | 0             | 18     | 22    | 40    | 4.0                 |
| Synaptomys borealis     | 1   | 0           | 0             | 10     | 0     | 10    | 10.0                |
| Tamiasciurus hudsonicus | 6   | 0           | 8             | 2      | 2     | 12    | 2.0                 |
| Total                   | 79  | 3           | 52            | 122    | 56    | 233   | 2.9                 |

*Several individual ticks (1 adult male, 3 adult females, and 12 nymphs) were not tested by reverse transcription PCR because of desiccation during storage. No larvae were tested. Infestation rate was calculated by dividing the total number of ticks by the total number of individuals for each mammalian species.
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Dr Deardorff is a postdoctoral researcher in the Biology Department of the University of New Mexico, Albuquerque, New Mexico. Her research interests include emerging zoonotic viral disease and host switching in changing environments.

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Address for correspondence: Eleanor R. Deardorff, Biology Department and Museum of Southwestern Biology, University of New Mexico, MS C03-2020 Biology, 1 University of New Mexico, Albuquerque, NM 87131, USA; email: edeardor@unm.edu