Short Report: Stable Prevalence of Powassan Virus in *Ixodes scapularis* in a Northern Wisconsin Focus

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Abstract. Deer tick virus (DTV), a variant of Powassan virus (POWV), appears to be maintained in nature in an enzootic cycle between *Ixodes scapularis* ticks and small mammals. Although POWV infection of human beings is rare, a recent report suggests increasing incidence and the possibility that POWV may be an emerging tick-borne zoonosis. Therefore, we assessed the long-term stability of the POWV transmission cycle in northwestern Wisconsin. Adult *I. scapularis* and *Dermacentor variabilis* were collected from Hayward and Spooner, Wisconsin, screened for infection by reverse transcriptase polymerase chain reaction (RT-PCR), and virus was isolated. Seventeen of 1,335 (1.3%) of *I. scapularis* and 0 of 222 (0%) of *D. variabilis* ticks were infected. All isolated virus belonged to the DTV genotype of POWV. These findings suggest stable transmission of POWV in this focus over ten years and highlight the potential for this agent to emerge as a public health concern.

Viruses of the tick-borne encephalitis (TBE) complex (Flaviviridae: flavivirus) are distributed across Eurasia and North America and are a causative agent of severe central nervous infection in humans.\(^1\) Powassan virus (POWV), originally isolated from a fatal case of encephalitis in Ontario, Canada in 1958, is the sole member of the TBE complex circulating in North America.\(^2\) The natural transmission cycle of POWV includes the enzootic transmission between *Ixodes cookei* and *Ixodes marxi* and medium-sized woodland rodents, mainly sciurid rodents and carnivores.\(^3,4\) *Ixodes cookei* and *I. marxi* are generally highly host-specific and rarely attack human beings, although in some cases *I. cookei* in particular may frequently feed on human beings.\(^5,6\) This highly host-specific feeding behavior seems to explain the low incidence of human POWV cases in North America; 27 cases were reported between 1958 and 1998 (0.7 cases/year).\(^7\) Recently, however, an apparent increase in incidence has been reported: nine POWV cases occurred between 1999 and 2005 (1.3 cases/year).\(^8,9\) The POWV cases occurred in north-central Wisconsin in 2003\(^9\) and 2006 (http://diseasemaps.usgs.gov/2006/pow_us_human.html). The serologic detection of several additional cases (Kramer LD, personal communication) suggests that current incidence may be higher than the 1.3 cases per year reported. Thus, recent data suggest that the incidence of human infection by POWV is increasing. Deer tick virus (DTV), is a serologically indistinguishable genotype of POWV that is maintained in nature by *I. scapularis*.\(^10-12\) DTV has been detected in the northeastern and upper Midwestern United States, where deer ticks are abundant, and POWV cases are most common. *Ixodes scapularis* are competent vectors of POWV\(^15\) and transmit virus within 15 minutes of attachment to hosts.\(^16\) It may be that the reported increase in incidence is partly attributable to DTV infections transmitted by the aggressively human-biting *I. scapularis*. Therefore, we sought to determine whether DTV is stably maintained in a transmission cycle involving *I. scapularis*. Ticks were collected from sites originally visited in the late 1990s from which DTV-infected ticks were collected. In addition, we determined the prevalence of DTV in *Dermacentor variabilis* ticks because of their opportunistic feeding behavior and reports of their ability to transmit POWV.\(^17\)

Adult *I. scapularis* and *D. variabilis* ticks were collected from three sites in Hayward and Spooner, Wisconsin in October of 2007 and May of 2008 by dragging a 1 m\(^2\) flannel cloth over emergent vegetation. Live ticks were transported back to the laboratory and sorted by site and sex. Ticks were screened for infection according to methods described previously.\(^11\) Briefly, *I. scapularis* ticks were individually crushed with a sterilized glass pestle in 50 μL of phosphate buffered saline (PBS) with 20% fetal calf serum (FCS). Pools of 2 to 10 tick homogenates, 10 μL from each tick, were combined and RNA extracted using the RNasy Protect Mini-Kit according to the manufacturer’s protocol (Qiagen, Valencia, CA), and eluted in 50 μL of ddH\(_2\)O. *Dermacentor variabilis* were pooled in 500 μL of PBS with 20% FCS along with a single ball bearing and homogenized using a mixer mill (Retch, Haan, Germany). RNA was extracted from tick homogenates as mentioned previously. Reverse transcriptase-polymerase chain reaction (RT-PCR) was conducted using Superscript III One-Step RT-PCR with Platinum Taq (Invitrogen, Carlsbad, CA) using the following parameters: a 30-minute RT step at 50°C followed by 94°C for 2 minutes and 40 cycles of 94°C for 15 seconds, 56°C for 30 seconds and 68°C for 2 minutes followed by a 5-minute final extension at 68°C. Primers used in these studies are similar in sensitivity to those published previously (data not shown). The forward primer was designed to correspond to DTV position 1274 located within the E-glycoprotein (5′-GTGCAAGCTTGAATGCAGGAAAG-3′). The reverse primer corresponded to DTV position 3180 within the NS1 gene (5′-GAACGGGGCCCAGCGAGGTGAC-3′). Amplicons were visualized on a 1% agarose gel by ethidium bromide staining and subsequently cloned into the pCR Script Amp vector (Stratagene, Cedar Creek, TX). For *I. scapularis*, individual ticks from positive pools were screened by the same method. Cloned fragments were sequenced using M13 forward and reverse primers by the University of New Mexico DNA Research Services. Virus isolation was attempted on RT-PCR-positive ticks using baby hamster kidney (BHK) cells. Confluent BHK monolayers

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were grown in six well tissue culture plates at 37°C at 5% CO₂. Fifty microliters of the clarified supernatants of RT-PCR positive tick homogenate were applied to monolayers. After 1 hour adsorption, appropriate maintenance medium was applied and cells were returned to the incubator. Cultures were inspected daily for the presence of cytopathic effects (CPE) and positive cultures were harvested. The identity of the isolated virus was confirmed by RT-PCR as described previously.

We collected a total of 1,335 *I. scapularis* and 222 *D. variabilis* at three collection sites during 14 person-hours of sampling (Table 1). Thus, an average of 111 ticks was collected per hour, including ~95 *I. scapularis* and 16 *D. variabilis* adults per hour. Although similar measures of tick abundance have not been widely reported for the northcentral United States, the infestation sampled for this study appears to be relatively intense.

**TABLE 1**

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Species</th>
<th>Number collected in:</th>
<th>Prevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><em>I. scapularis</em></td>
<td>86 286 372</td>
<td>1.1 (0.3, 2.8)</td>
</tr>
<tr>
<td></td>
<td><em>D. variabilis</em></td>
<td>0 128 128</td>
<td>0.0 (0.0, 3.5)</td>
</tr>
<tr>
<td>B</td>
<td><em>I. scapularis</em></td>
<td>213 643 856</td>
<td>1.4 (0.8, 2.5)</td>
</tr>
<tr>
<td></td>
<td><em>D. variabilis</em></td>
<td>0 46 46</td>
<td>0.0 (0.0, 9.2)</td>
</tr>
<tr>
<td>C</td>
<td><em>I. scapularis</em></td>
<td>ND 107 107</td>
<td>0.9 (0.0, 5.6)</td>
</tr>
<tr>
<td></td>
<td><em>D. variabilis</em></td>
<td>ND 48 48</td>
<td>0.0 (0.0, 8.9)</td>
</tr>
<tr>
<td>Total</td>
<td><em>I. scapularis</em></td>
<td>299 1,036 1,335</td>
<td>1.3 (0.8, 2.1)</td>
</tr>
<tr>
<td></td>
<td><em>D. variabilis</em></td>
<td>0 222 222</td>
<td>0.0 (0.0, 2.1)</td>
</tr>
</tbody>
</table>

CI = confidence interval; ND = not determined.

Analysis of the ticks for the presence of viral RNA revealed that a total of 17 *I. scapularis* and zero *D. variabilis* ticks were infected. Virus was isolated from all RNA positive ticks. Positive ticks included both male and female individuals, and individuals collected from all sites sampled during this study. The prevalence of infection was similar (*P > 0.05*) between male and female ticks, all sites sampled, and each sampling period. Phylogenetic analyses of a 257 nucleotide fragment of the envelope (E) coding sequence amplified directly from individual ticks indicated that, as expected, on the basis of previous observations, the isolates belonged to the deer tick virus lineage of POWV (Figure 1). Thus, the prevalence of DTV infection in adult *I. scapularis* in this upper Midwestern focus is 1.3%. This estimate of DTV prevalence in adult ticks is identical to that reported previously for ticks collected from this focus of transmission between 1997–1999, when 10 of 792 were found infected (1.3%, 95% confidence interval [CI] = 0.6–2.3), and similar to reported prevalence from other enzootic sites in the northeastern United States. These findings suggest the stability of DTV perpetuation near Spooner, Wisconsin, and support the observation that *I. scapularis* ticks may be important enzootic vectors of this agent. No infection was detected in *D. variabilis*. However, only 222 were tested, and the 95% CI includes the point estimate of prevalence obtained for *Ixodes* ticks collected from the same focus (Table 1). Our collection sites and methods were targeted toward sampling *Ixodes* ticks, with *Dermacentor* ticks being collected incidentally. Additional studies targeting *Dermacentor* ticks are required to define their role in enzootic perpetuation of this agent.
These findings suggest that deer tick-borne POWV may present a public health risk to human residents of and visitors to infested sites. Paradoxically, despite the apparent increase in the incidence of human infection, the proportion of ticks infected remained constant between the 1990s and the present. The increased geographic distribution of deer ticks and concomitant increases in their populations might produce a greater number of infected ticks overall, or might facilitate spillover from cryptic enzootic foci, which might produce additional human POWV infections. It may be that a proportion of the recent increase in the recognition of POWV encephalitis in North America9 is attributable to the emergence of DTV. Ongoing studies will therefore more completely characterize the transmission cycle and molecular epidemiology of DTV within this focus. Importantly, infection by viruses within the TBE serologic complex results in a wide range of clinical outcomes, ranging from asymptomatic to severe18; DTV may be less pathogenic than prototypical POWV, as has been suggested previously.10,19 Experimental studies are required to evaluate this hypothesis. In the absence of this data, clinicians should consider tick-borne flavivirus infection in patients presenting 1) with neurologic symptoms and 2) residence or travel to tick-infested sites.

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