Seroprevalence of Powassan Virus in New England Deer, 1979–2010

Robert A. Nofchissey, Eleanor R. Deardorff, Tia M. Blevins, Michael Anishchenko, Angela Bosco-Lauth, Erica Berl, Charles Lubelczyk, John-Paul Mutebi, Aaron C. Brault, Gregory D. Ebel,* and Louis A. Magnarelli

University of New Mexico School of Medicine, Albuquerque, New Mexico; Center for Vector Biology and Zoonotic Diseases, The Connecticut Agricultural Experiment Station, New Haven, Connecticut; Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado; Vermont Department of Public Health, Burlington, Vermont; Maine Medical Center Research Institute, Vector-borne Disease Laboratory, South Portland, Maine

Abstract. Powassan virus and its subtype, deer tick virus, are closely related tick-borne flaviviruses that circulate in North America. The incidence of human infection by these agents appears to have increased in recent years. To define exposure patterns among white-tailed deer, potentially useful sentinels that are frequently parasitized by ticks, we screened serum samples collected during 1979–2010 in Connecticut, Maine, and Vermont for neutralizing antibody by using a novel recombinant deer tick virus–West Nile virus chimeric virus. Evidence of exposure was detected in all three states. Overall our results demonstrate that seroprevalence is variable in time and space, suggesting that risk of exposure to Powassan virus is similarly variable.

INTRODUCTION

Powassan virus (POWV; *Flaviviridae*, *Flavivirus*) is the sole North American representative of the tick-borne encephalitis serocomplex of flaviviruses. The virus was initially described as a tick-transmitted human pathogen by McLean and Donahue in the 1950s.¹ A subtype of POWV, frequently termed deer tick virus (DTV), was first identified in 1995² and has subsequently been recognized as a human pathogen.³ Because it is associated with the aggressively human-biting blacklegged or deer ticks (*Ixodes scapularis*),^{4,5} this virus has been considered to pose a more significant threat to public health than the prototype virus, POWV, that is associated mainly with the relatively host-specific ticks *Ixodes cookei* and *I. marxi*.^{6,7}

Recent studies have suggested that the incidence of human POWV infection is increasing in the United States,⁸ raising the possibility that POWV, like other members of the deer tick-associated guild of emerging zoonoses (Lyme disease, human babesiosis, and human granulocytic anaplasmosis), constitutes a mounting threat in regions where it is enzootic and where *I. scapularis* ticks are abundant. Paradoxically, molecular epidemiologic studies of POWV have failed to document significant increases in the size of the virus population.⁹ White-tailed deer (*Odocoileus virginianus*) are heavily parasitized by *I. scapularis* ticks and may thus serve as a useful sentinel for assessing relative enzootic activity of DTV over time.¹⁰ We therefore assessed neutralizing antibodies against DTV/POWV from deer serum samples obtained in a region to which this virus is enzootic and *I. scapularis* are abundant to determine whether increased intensity of enzootic transmission could be correlated with the apparent increase in human infections.⁸

MATERIALS AND METHODS

Serum collection. Serum samples were collected from hunter-killed deer in Connecticut, Maine, and Vermont. Whole-blood samples were obtained from the body cavities of deer killed during fall hunting seasons or by venipuncture

and processed as reported.^{11,12} A total of 266 deer were sampled from Connecticut during 1979–2009. Three hundred twenty-six deer were sampled from Maine and 487 were sampled from Vermont in 2010.

Serologic testing. Serum samples were heat-inactivated at 56°C for 30 minutes before testing and screened for neutralizing antibody by using a plaque-reduction neutralization testing (PRNT) with DTV–West Nile virus (WNV) chimeric virus (DTV-prME/WNV) consisting of the premembrane (prM) and envelope (E) structural proteins of DTV and the capsid and nonstructural coding sequences and untranslated regions of WNV. Cross-neutralization studies indicated that antisera raised against DTV and POWV efficiently neutralized the chimeric DTV-prME/WNV chimeric virus but not WNV, and that antisera raised against WNV did not efficiently neutralize the DTV-prME/WNV (Table 1). Use of the chimeric DTV-prME/WNV assay virus enabled PRNT testing to be conducted on African green monkey kidney (Vero) cells according to standard procedures¹³ using a 90% neutralization cutoff value to be considered positive.

Because of strong cross-reactivity between antibodies generated against DTV and POWV,¹⁴ our serologic assay was incapable of differentiating between these two agents. Accordingly, results are presented as DTV/POWV neutralization. Endpoint neutralization titers were determined for all positive samples. Deer tick virus neutralizing antibody–positive samples collected after 1999 were also assayed for neutralizing antibodies to WNV as described above to rule out the possibility of serologic cross-reactivity between the two agents. A ≥ 4 -fold difference in homologous to heterologous endpoint titers between DTV/POWV and WNV was required for virus type classification of serum samples. In addition, specimens with a DTV/POWV PRNT₉₀ titer of 1:10 but that failed to neutralize WNV were considered DTV/POWV positive, given the likely flavivirus exposures of deer and the significant one-way differences in neutralization that have been detected between strains of DTV/POWV. Samples that neutralized both viruses within a 4-fold dilution range are presented as indeterminate flavivirus positive results.

RESULTS

Overall, eighty-four (32%) of 266 serum specimens collected in Connecticut demonstrated DTV/POWV-specific

* Address correspondence to Gregory D. Ebel, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, 1690 Campus Delivery, Fort Collins, CO 80523. E-mail: gregory.ebel@colostate.edu

TABLE 1

Neutralization of DTV-prME/WNV by antibodies to POW, DTV and WNV*

Virus	PRNT ₉₀ antibody titers		
	DTV-SPO	POW-LB	WNV
DTV-SPO	160	320	< 10
POW-LB	320	1,280	< 10
WNV-NY99	< 10	< 10	640
DTV-prME/WNV	160	1,280	< 10

*DTV = deer tick virus; prME = premembrane; WNV = West Nile virus; POW = Powassan; PRNT₉₀ = 90% plaque-reduction neutralization test.

neutralizing antibodies. The reciprocal endpoint titers from these samples ranged from 10 to 160. A total of 85% of positive samples had endpoint titers > 1:10. No samples exhibited WNV-specific neutralizing antibodies (Table 2). One serum sample collected in 2002 demonstrated neutralizing antibodies against both agents at similar endpoint dilutions (≤ 4 -fold difference) and was classified as indeterminate flavivirus-positive. Examination of the proportions of positive serum samples by year of collection showed variable prevalence depending on year of collection (Table 2 and Figure 1).

To assess geographic variation in prevalence estimates, reactivity to flaviviruses was also examined among white-tailed deer collected in northern New England (Vermont and Maine). Prevalence of serologic reactivity to DTV/POW in these specimens was approximately 12% (reciprocal endpoints range from 10 to 80, and 25–30% of positive samples had endpoints > 1:10). West Nile virus-specific neutralization was detected in 1% and 3% of samples collected Maine and Vermont, respectively. Indeterminate flavivirus neutralization was also detected in samples from these states (Table 3).

DISCUSSION

A guild of tick-transmitted pathogens that includes the agents of Lyme disease, human babesiosis, and human granulocytic anaplasmosis has emerged in recent decades in North America. The emergence of these pathogens has been driven by dramatic expansions in the populations of *I. scapularis*, which is correlated with similarly dramatic expansions in populations

TABLE 2

Prevalence of antibodies to POWV/DTV in serum samples from white-tailed deer in Connecticut, 1979–2009*

Year	No. tested	POW, no. positive (%)	WNV, no. positive (%)*	Indeterminate (flavivirus, no. positive)
1979	25	1 (4)	ND	ND
1980	24	2 (8)	ND	ND
1984	25	2 (8)	ND	ND
1985	25	4 (16)	ND	ND
1989	25	6 (24)	ND	ND
1990	25	6 (24)	ND	ND
1992	7	0 (0)	ND	ND
1996	4	3 (75)	ND	ND
2000	2	0 (0)	0 (0)	0
2001	24	15 (63)	0 (0)	0
2002	24	6 (25)	0 (0)	1
2003	15	8 (53)	0 (0)	0
2004	8	3 (38)	0 (0)	0
2005	17	14 (82)	0 (0)	0
2006	5	4 (80)	0 (0)	0
2009	11	10 (91)	0 (0)	0

*POWV = Powassan virus; DTV = deer tick virus; WNV = West Nile virus; ND = not done.

FIGURE 1. Prevalence of deer-tick virus/Powassan virus antibodies in white-tailed deer, Connecticut, 1979–2009. Points indicate prevalence during each sampling year, vertical bars indicate Bayesian 95% confidence limits on the point estimate. Solid line indicates linear regression line fitted to the data (slope = 0.02416 ± 0.005958 , $P = 0.0012$).

of white-tailed deer, the main host for adult deer ticks. Recent apparent increases in the incidence of DTV/POWV led us to question whether deer, which have been used as sentinels for monitoring the intensity of zoonotic transmission of several other arthropod-borne zoonoses, may be similarly useful as sentinels for DTV/POWV.

First, we analyzed deer serum samples collected in Connecticut to assess the prevalence of neutralizing antibodies in hunter killed deer. Deer serum samples were collected from 54 towns and 1 city in Connecticut over a period of 30 years. Seropositive deer were collected from 18 municipalities in all eight counties of Connecticut, and the highest prevalence observed in southern counties. On the basis of these findings and recent literature,¹⁵ deer appear to be useful sentinels for serologically monitoring DTV/POWV activity.

We considered specimens that neutralized DTV/POWV at a 1:10 dilution positive if they did not neutralize WNV at the same dilution, even though a 4-fold difference in titer was not observed. Our rationale for this is that although DTV and POWV are closely related serologically, cross-neutralization studies (Nofchissey RA and others, unpublished data), including one presented herein, have documented evidence of one-way differences in neutralization efficiency. Therefore, excluding these samples from those deemed positive may underestimate the true prevalence of infection in these hosts. Interestingly, endpoint titers tended to be higher in samples from Connecticut than samples from Maine and Vermont. Because DTV is well established in Connecticut, and prototype POWV is likely present in Maine and Vermont, the difference in endpoints might be related to the identity of the virus circulating in Connecticut compared with that circulating in Maine

TABLE 3

Comparison of prevalence of neutralizing antibodies to flaviviruses in Connecticut, Maine, and Vermont, 1979–2010*

State	Year of collection	No. tested	% Positive (95% CI) for neutralizing antibodies		
			DTV/POW	WNV	Indeterminate
Connecticut	1979–2009	266	32 (26–37)	0 (0–1)	1 (0–2)
Maine	2010	326	13 (9–17)	3 (2–6)	6 (4–9)
Vermont	2010	487	11 (8–14)	1 (0–2)	1 (0–2)

*CI = confidence interval; DTV = deer tick virus; POW = Powassan; WNV = West Nile virus.

and Vermont. Nonetheless, our current serologic tools do not enable us to conclude this suggestion with certainty.

A small proportion of specimens tested reacted more strongly to WNV than to DTV/POWV, or reacted approximately equally to both flaviviruses. These findings suggest that deer are exposed to other enzootic flaviviruses. In some cases, this finding clearly indicates the presence of WNV. This finding is consistent with the intense seasonal transmission of WNV and the feeding behavior of the main *Culex* vectors of WNV. Although most *Cx. pipiens* feed on birds, some of these mosquitoes have been found to have fed on mammals, including deer.¹⁶ Therefore, it is not surprising that a fraction of deer in an enzootic focus of WNV would be exposed to WNV. Overall, these results suggest that DTV/POWV is likely the most common flavivirus infecting deer in New England.

Deer collected in Connecticut tended to have higher levels of exposure compared with deer collected in either Vermont or Maine. This finding appears to be related to the relative abundance of *I. scapularis*, which use deer as hosts for the primary reproductive stage.¹⁰ Although cases of POWV have been reported in both Maine and Vermont,⁸ where *I. cookei* and *I. marxi* are widely distributed¹⁷ and are reported to bite humans,¹⁸ *I. scapularis* is found in greater abundance in southern New England than in northern New England. *Ixodes scapularis* has only become well established in Maine relatively recently, and Lyme disease risk in Connecticut much higher than in either Maine or Vermont.¹⁹ Therefore, our data on prevalence of DTV/POWV in three states in New England is consistent with risk estimates for other deer tick-borne infections.

In summary, our data add to the body of literature on DTV/POWV exposure among sentinel mammals in a region where human infection may occur, and highlight the risk posed by this relatively understudied pathogen. Recent increases in the deer populations in the northeastern and upper midwestern United States and associated increases in the range and abundance of deer ticks, coupled with the increasing incidence of human infection with deer tick-borne agents suggest that the current burden of tick-borne flaviviruses may be due mainly to DTV and not POWV. Furthermore, because several recent reports have documented severe cases of infection by DTV/POWV,^{3,20,21} our results suggest that tick-borne flaviviruses should be considered in the differential diagnosis for persons who might have a history of tick exposure and neurologic symptoms.

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Authors' addresses: Robert A. Nofchissey, Department of Medicine, University of New Mexico, Albuquerque, NM, E-mail: rnofchissey@salud.unm.edu. Eleanor R. Deardorff, Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM, and Department of Experimental Pathology, University of Texas Medical Branch, Galveston, TX, E-mail: edeardorff@salud.unm.edu.

Tia M. Blevins and Louis A. Magnarelli, Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven, CT, E-mails: tia.blevins@ct.gov and louis.magnarelli@ct.gov. Michael Anishchenko, Angela Bosco-Lauth, John-Paul Mutebi, and Aaron C. Brault, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, E-mails: iot5@cdc.gov, mopargal@rams.colostate.edu, john-paul.mutebi@cdc.hhs.gov, and acbrault1@mac.com. Erica Berl, Department of Infectious Disease Epidemiology, Vermont Department of Health, Burlington, VT, E-mail: erica.berl@state.vt.us. Charles Lubelczyk, Vector-Borne Disease Laboratory, Maine Medical Center Research Institute, South Portland, ME, E-mail: lubelc@mmc.org. Gregory D. Ebel, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, E-mail: gregory.ebel@colostate.edu.

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