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Eastern Equine Encephalitis in Moose (*Alces americanus*) in Northeastern Vermont

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ABSTRACT: During fall 2010, 21 moose (*Alces americanus*) sera collected in northeastern Vermont were screened for eastern equine encephalitis virus (EEEV) antibodies using plaque reduction neutralization tests. Six (29%) were antibody positive. This is the first evidence of EEEV activity in Vermont, and the second report of EEEV antibodies in moose.

Serologic evidence suggests that moose (*Alces americanus*) are exposed to a wide variety of arboviruses endemic to North America (e.g., Trainer and Jochim 1969; Zarnke et al., 1983). However, only one study (Carstensen et al., 2007) has reported evidence that moose are infected by eastern equine encephalitis virus (EEEV). Although EEEV has been isolated or detected in all surrounding states and provinces (Morris, 1988; Armstrong et al., 2008), EEEV activity has not been detected in Vermont.

In 2009, we demonstrated that screening serum of free-ranging white-tailed deer (*Odocoileus virginianus*) was a sensitive method for detecting EEEV activity and that serosurveys of deer could be used as a tool to map EEEV activity (Mutebi et al., 2011). The Vermont Department of Health; the Vermont Agency of Agriculture, Food and Markets; and the US Centers for Disease Control and Prevention (CDC) conducted serosurveys of Vermont white-tailed deer from 6 October 2010 to 14 November 2010 and collected 489 serum samples (Berl et al., unpubl. data). Additionally, in northeastern Vermont, during these serosurveys, blood samples were collected from 21 harvested moose carcasses. Our objective was to screen the moose sera for EEEV antibodies.

On 16 October 2010, moose blood samples were collected from moose carcasses brought for harvest registration in Essex, Lamoille, Caledonia, and Orleans counties (Fig. 1, Table 1). Blood samples were collected from pools in body cavities using disposable plastic pipettes. The samples were collected into 7.5-mL Vacutainer tubes, stored on ice for 24–48 hr, and centrifuged (Beckman AccuSpin FR, Beckman Coulter, Inc., Brea, California, USA) at 80 × G for 15 min to separate the serum at the Vermont Agency of Agriculture Laboratory. Sera were frozen at −20 C and shipped on dry ice to the CDC laboratories in Fort Collins, Colorado, for antibody screening.

Serum samples diluted 1:10 were screened for EEEV-neutralizing antibodies by plaque-reduction neutralization tests (PRNTs; Beaty et al., 1995). Positive samples were retested and titrated in duplicate for confirmation. Serum samples were considered positive for EEEV antibodies if they neutralized 80% of a challenge dose of ≈100 plaque-forming units of EEE-Sindbis chimeric virus (Wang et al., 2007). To ensure that neutralization was specific to EEEV and not resulting from antibody cross-reactivity, samples with low neutralizing titers were screened for Highlands J virus antibodies. The Highlands J virus strain used for these PRNTs was MW8-5AD, which was isolated from a mosquito pool in Maryland in 1968, obtained

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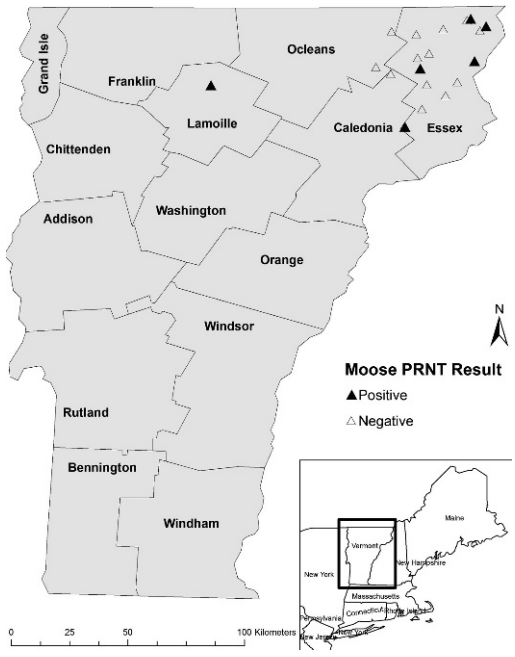

FIGURE 1. Distribution of eastern equine encephalitis virus antibody-positive and antibody-negative moose (*Alces americanus*) collected in Vermont, USA, 2010.

from the CDC virus bank in Fort Collins.

Twenty-one moose serum samples were collected from four counties in northeastern Vermont; 17 samples (81%) were from Essex County, one (5%) was from Lamoille County, one (5%) was from Caledonia County, and two (10%) were from Orleans County (Fig. 1). Six (29%) samples were positive for EEEV antibodies (Table 1). Two of the positive animals were calves, suggesting recent infections (Table 1). In Essex County, five of the 17 (28%; Table 1) moose sera had EEEV antibodies and were collected in different townships (Table 1, Fig. 1). Sample sizes from the other counties were too small for robust estimates of EEEV activity, but the single moose sample collected from Lamoille County was EEEV antibody positive (Table 1).

The percentage of moose with serologic evidence of EEEV infection in Vermont

TABLE 1. Plaque reduction neutralization test (PRNT) results of eastern equine encephalitis virus antibodies for 21 positive moose (*Alces americanus*) sera collected in Vermont, USA, 2010. The six positive sera are highlighted in bold print.

Moose no.	Township	County	Sex[a]	Weight (kg)[b]	Age (yr)	Serum $PRNT_{80}$
1	Newark	Caledonia	F	305.7	7	<10
2	Averill	Essex	F	249.5	5	<10
3	Averill	Essex	M	329.3	4	<10
4	Lewis	Essex	M	360.2	8	<10
5	**Averill**	**Essex**	**M**	**122.9**	**<1**	**80**
6	Avery's	Essex	F	181.9	1	<10
7	**Bloomfield**	**Essex**	**M**	**284.4**	**3**	**20**
8	Brighton	Essex	M	372.9	7	<10
9	Brighton	Essex	F	174.6	1	<10
10	Brighton	Essex	M	254.9	2	<10
11	**Brighton**	**Essex**	**M**	**286.2**	**2**	**20**
12	East Haven	Essex	M	193.7	1	<10
13	Ferdinand	Essex	M	199.1	1	<10
14	Ferdinand	Essex	F	172.8	3	<10
15	**Lemington**	**Essex**	**M**	**288.9**	**4**	**640**
16	Lemington	Essex	F	136.5	4	<10
17	Brunswik	Essex	M	324.3	6	<10
18	**Victory**	**Essex**	**M**	**290.8**	**3**	**≥2560**
19	**Eden**	**Lamoille**	**F**	**97.52**	**<1**	**160**
20	Westmore	Orleans	M	236.8	1	<10
21	Morgan	Orleans	M	260.8	2	<10

[a] M = male; F = female.

[b] Dressed weight.

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(29%) is among the highest reported for wild ungulate populations (range 0.5–31%; Bigler et al., 1975; Hoff et al., 1973; Tate et al., 2005; Mutebi et al., 2011). Recently, EEEV antibodies were detected in moose in northern Maine (Lubelcyck et al., unpubl. data), suggesting that moose are widely exposed to EEEV in northern New England. Carstensen et al. (2007) reported 4% EEEV antibody–positive moose sera in northwestern Minnesota, suggesting that moose are exposed to EEEV in the northern midwestern US. Moose commonly graze on hydrophytes in wooded wetlands, marshes, and swamps, which are breeding sites for the EEEV enzootic mosquito vector *Culiseta* (*Climacura*) *melanura* (Coquillett) and the bridge vectors *Coquillettidia* (*Coquillettidia*) *perturbans* (Walker), *Aedes* (*Ochlerotatus*) *canadensis* (Theobald), *Aedes* (*Ochlerotatus*) *sollicitans* (Walker), which may increase the chances of exposure to EEEV. Blood meal analysis has not detected moose DNA in engorged field-collected *Culiseta melanura* (Molaei et al., 2006), but studies have not been conducted in areas with large moose populations such as northern New England.

Moose have home ranges of usually 20–30 km^2 (Leptich and Gilbert 1989; Morris 2007; Van Dyke et al., 1995); therefore, the presence of EEEV antibodies in moose populations suggests localized EEEV transmission and that EEEV is endemic in Vermont. Additionally, the white-tailed deer serosurveys conducted in 2010, Berl et al. (unpubl.) detected EEEV antibodies in free-ranging deer from large areas of the state suggesting widespread EEEV activity in Vermont. The outbreak of EEEV on an emu (*Dromaius novaehollandiae*) farm in Rutland County, Vermont, in September 2011 (Berl et al. unpubl.) provides additional documentation of EEEV activity in Vermont.

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