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# Short Report

# Serologic Evidence of West Nile Virus and St. Louis Encephalitis Virus Infections in White-Tailed Deer (*Odocoileus virginianus*) from New Jersey, 2001

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## ABSTRACT

**Serum samples from 689 hunter-killed white-tailed deer (*Odocoileus virginianus*) collected during the 2001 fall hunting season in New Jersey were tested for neutralizing antibodies to West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) by plaque-reduction neutralization tests. WNV-neutralizing antibodies were detected in six (0.9%) of the samples, and SLEV-neutralizing antibodies were found in 11 (1.6%) of the samples. We provide the first report of WNV infection in white-tailed deer. Key Words: West Nile virus—St. Louis encephalitis virus—Serosurvey—White-tailed deer.** Vector-Borne Zoonotic Dis. 4, 379–383.

## INTRODUCTION

West Nile virus (WNV; Flaviviridae: *flavivirus*) is a mosquito-transmitted pathogen of humans, equines and wildlife that has extended rapidly across the United States since its initial 1999 introduction in the New York City metropolitan area (Komar 2003). The antigenically related St. Louis encephalitis virus (SLEV; Flaviviridae: *flavivirus*) is also a mosquito-borne human pathogen. Both WNV and SLEV are maintained primarily in an enzootic cycle between *Culex* mosquitoes and avian amplifying hosts (Mitchell et al. 1980). Mammals, including humans, are considered dead-end hosts that may become periodically infected during epizootics. However, due to low viremia of short duration, mammals do not generally play an amplifying role in the transmission cycles of these viruses. Bridge vector mosquitoes that feed indiscriminately on both avian and mammalian hosts are generally responsible for infecting mammals. West Nile virus infections in several candidate bridge vector species have been reported in New Jersey (Centers for Disease Control and Prevention 2001), and previous host preference studies have shown that the majority of the mammalian blood meals taken by these mosquitoes were derived from the abundant white-tailed deer (*Odocoileus virginianus*) (Crans 1964, Apperson et al. 2003). Previous serological investigations have detected SLEV antibodies in the sera of white-tailed deer from New York (Whitney et al. 1969), North Dakota (Hoff et al. 1973), Texas, Wisconsin and Wyoming (Trainer and Hanson 1969). However, no data is available for SLEV infection in deer from New Jersey, or WNV infection in deer endemic to the new world. Therefore, the objective of this

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study was to determine the prevalence of WNV and SLEV antibodies in white-tailed deer populations in New Jersey.

## MATERIALS AND METHODS

In collaboration with the New Jersey Division of Fish and Wildlife, whole blood samples were collected from hunter-killed deer at designated check stations throughout New Jersey from September 1, 2001 to December 12, 2001. Sterile disposable pipettes were used to extract 4–5 mL of blood from freshly killed and field-dressed white-tailed deer. The blood was deposited into sterile serum separator tubes with gel clot activators and transported to the laboratory for centrifugation and processing. In the laboratory, blood samples were centrifuged for 15 min at $1{,}800 \times G$, and the resulting serum was stored at $-70$°C until assayed. The relative ages of the deer sampled were determined by tooth eruption and wear pattern (Taber 1963). The majority of New Jersey deer are born in late May and early June; therefore, at the time of sampling, age classes were recorded in 6-month (0.5 year) increments, and three categories of age were used for statistical analysis: unknown; fawns and yearlings (0.5–1.5 years); and adults (>2.5 years).

Serum samples were assayed for neutralizing antibodies to WNV and SLEV by plaque-reduction neutralization test (PRNT), as previously described (Komar et al. 2001). Due to the fact that cross-reactions are common between these two very similar flaviviruses (Calisher et al. 1989), a fourfold greater reciprocal 90% neutralization titer to one of these viruses was needed to affirm that virus as the causative agent. A plaque reduction of 90% or more was considered positive, with 90% plaque reduction neutralization titer being the greatest serum dilution exhibiting ≥90% reduction of plaques relative to a serum-free control. If a fourfold titer difference could not be determined between the two arboviruses, then the sample was designated as an undifferentiated flavivirus infection. The data were analyzed by the Fisher exact test.

## RESULTS

A total of 689 serum samples were collected from deer from 12 New Jersey counties. WNV-neutralizing antibodies were detected in six (0.9%), and SLEV-neutralizing antibodies were detected in 11 (1.6%) of the samples tested. Nineteen (2.8%) additional samples were designated as positive for an undifferentiated flavivirus infection, due to the fact that a fourfold increase was not apparent between WNV and SLEV (Table 1). Reciprocal 90% neutralization titers ranged from 40 to ≥320 for WNV-neu-

Table 1. Neutralizing Antibody Response to WNV and SLEV Detected in White-Tailed Deer During September to December 2001, by County

| *County (NJ)* | *Total tested (%)* | *Number virus antibody positive (% [95% CI])* | | |
|---|---|---|---|---|
| | | *WNV* | *SLEV* | *FLAV* |
| Atlantic | 18 (2.6) | 0 | 0 | 0 |
| Burlington | 61 (8.9) | 0 | 0 | 1 (1.6 [0.1–7.6]) |
| Camden | 1 (0.2) | 0 | 0 | 0 |
| Hunterdon | 176 (25.5) | 1 (0.6 [0.1–2.7]) | 2 (1.1 [0.2–3.5]) | 9 (5.1 [2.7–8.7]) |
| Mercer | 43 (6.2) | 1 (2.3 [0.1–10.6]) | 2 (4.7 [0.8–13.9]) | 0 |
| Middlesex | 21 (3.0) | 0 | 0 | 1 (4.8 [0.3–20.6]) |
| Monmouth | 24 (3.5) | 0 | 0 | 1 (4.2 [0.2–18.3]) |
| Morris | 18 (2.6) | 1 (5.6 [0.3–23.8]) | 0 | 0 |
| Ocean | 1 (0.2) | 0 | 0 | 0 |
| Somerset | 22 (3.2) | 0 | 0 | 0 |
| Sussex | 173 (25.1) | 2 (1.2 [0.2–3.6]) | 3 (1.7 [0.5–4.4]) | 4 (2.3 [0.8–5.2]) |
| Warren | 131 (19.0) | 1 (0.8 [0.1–3.6]) | 4 (3.1 [1.1–6.9]) | 3 (2.3 [0.6–5.8]) |
| Totals | 689 (100) | 6 (0.9 [0.4–1.7]) | 11 (1.6 [0.9–2.6]) | 19 (2.8 [1.8–4.0]) |

WNV, West Nile virus; SLEV, Saint Louis encephalitis virus; FLAV, undifferentiated flavivirus.

tralizing antibody positive deer, and from 20 to 1280 for SLEV-neutralizing antibody positive deer (Table 2).

Gender and age of the deer were tested for association with flavivirus infection (Table 2). Of 218 females, three (1.4% [0.4–3.5]) were positive for WNV antibodies compared to three (0.66% [0.2–1.7]) of 458 males ($p = 0.2$). Eight (3.7% [1.8–6.5]) females were positive for SLEV antibodies compared to three (0.66% [0.2–1.7]) males ($p = 0.007$). Combining all flavivirus antibody-positive deer, there were 19 (8.7% [5.8–12.5]) females compared to 17 (3.7% [2.4–5.5]) males ($p = 0.01$). Significant associations for age categories could not be made for either WNV ($p = 0.067$) or SLEV ($p = 0.251$) or all flavivirus infections ($p = 0.85$).

TABLE 2. SUMMARY OF SEROLOGIC DATA FOR WHITE-TAILED DEER IN NEW JERSEY, 2001

| | | | *$PRNT_{90}$ titer* | | |
|---|---|---|---|---|---|
| *Deer* | *Sex* | *Age* | *WNV* | *SLEV* | *Diagnosis* |
| NJ-29 | M | 3.5 | <10 | 10 | FLAV |
| NJ-30 | M | 3.5 | <10 | 10 | FLAV |
| NJ-33 | F | 1.5 | 160 | 80 | FLAV |
| NJ-38 | F | 0.5 | <10 | 80 | SLEV |
| NJ-45 | M | 0.5 | ≥320 | 20 | WNV |
| NJ-66 | M | 1.5 | ≥320 | 80 | WNV |
| NJ-67 | M | 0.5 | <10 | 40 | SLEV |
| NJ-70 | F | 1.5 | 160 | <10 | WNV |
| NJ-74 | F | 0.5 | ≥320 | 160 | FLAV |
| NJ-101 | F | 1.5 | 320 | 20 | WNV |
| NJ-158 | F | 1.5 | <10 | 10 | FLAV |
| NJ-199 | M | 1.5 | <10 | 10 | FLAV |
| NJ-214 | M | 1.5 | 10 | <10 | FLAV |
| NJ-218 | F | 3.5 | <10 | 80 | SLEV |
| NJ-308 | F | 1.5 | <10 | 10 | FLAV |
| NJ-376 | M | 2.5 | <10 | 10 | FLAV |
| NJ-443 | M | 0.5 | 40 | <10 | WNV |
| NJ-448 | M | 2.5 | 10 | 40 | SLEV |
| NJ-453 | F | 1.5 | <10 | 10 | FLAV |
| NJ-461 | M | 2.5 | 80 | 320 | SLEV |
| NJ-470 | M | 1.5 | <10 | 10 | FLAV |
| NJ-488 | M | 1.5 | <10 | 10 | FLAV |
| NJ-491 | M | 2.5 | <10 | 10 | FLAV |
| NJ-534 | F | 0.5 | 10 | 20 | FLAV |
| NJ-564 | F | 2.5 | 160 | 640 | SLEV |
| NJ-566 | F | 1.5 | 320 | 1280 | SLEV |
| NJ-569 | M | 2.5 | <10 | 10 | FLAV |
| NJ-600 | F | 1.5 | <10 | 80 | SLEV |
| NJ-641 | F | 0.5 | <10 | 20 | SLEV |
| NJ-654 | F | 0.5 | 40 | <10 | WNV |
| NJ-666 | F | 3.5 | 10 | 40 | SLEV |
| NJ-699 | M | 0.5 | 10 | 10 | FLAV |
| NJ-670 | F | 0.5 | 20 | ≥320 | SLEV |
| NJ-705 | F | 0.5 | 20 | 40 | FLAV |
| NJ-718 | F | 1.5 | 40 | 80 | FLAV |
| NJ-728 | M | 2.5 | 10 | <10 | FLAV |

PRNT, plaque reduction neutralization test; WNV, West Nile virus; SLEV, Saint Louis encephalitis virus; FLAV, undifferentiated flavivirus; $PRNT_{90}$, reciprocal 90% plaque reduction neutralization titer.

## DISCUSSION

The detection of neutralizing antibodies to WNV marks the first evidence of this infection in new world deer. These data also represent the first evidence of natural infection of New Jersey white-tailed deer to SLEV. Even though SLEV is endemic to North America and has been studied extensively for a number of years, the ecological role of wild mammals in this disease cycle is still unclear, and it is unknown if infections in ungulates produce disease in these animals (McLean and Bowen 1980). WNV may behave similarly, and infected deer may not show any signs of illness. Although several species of North American mammals have succumbed to WNV infection since the 1999 introduction (Marfin et al. 2001), experimental infection studies with horses (Bunning et al. 2001) and dogs (Blackburn et al. 1989) have shown that these mammals are incidental hosts that develop levels of viremia that are too low to infect subsequently feeding mosquitoes.

Arbovirus activity in the counties where the deer were sampled had been documented in the summer of 2001 through New Jersey's Vector Surveillance Program (Centers for Disease Control and Prevention 2001). The program tests corvids and mosquito pools for the presence of WNV viral RNA through Taqman RT-PCR techniques; however, no tests have been conducted for SLEV. In the counties where WNV-neutralizing antibody-positive deer were found, the vector surveillance program in 2001 detected WNV in pools of *Aedes vexans* (Meigen), *Anopheles quadrimaculatus* Say, *Culex pipiens* Linnaeus, *Cx. restuans* Theobald, *Coquillettidia perturbans* (Walker), *Ochlerotatus japonicus* (Theobald), and *Oc. trivittatus* (Coquillett). Only two of the above (*Cx. pipiens* and *Cx. restuans*) are predominantly ornithophilic, while the rest are mainly mammal feeders and, at least in New Jersey, derive a large propor-

tion of their blood-meals from white-tailed deer (Crans 1964, Apperson et al. 2003). More recently in northwestern New Jersey, WNV-neutralizing antibodies were also detected from free-ranging black bears at a rate of 6%; none of these were seropositive for SLEV-neutralizing antibodies (Farajollahi et al. 2003). Further investigations that scrutinize the possible role of wild mammals in the ecological cycle of these flaviviruses are needed, particularly where large numbers of those animals occur and where the suitable vectors are present. Given the very low levels of exposure of white-tailed deer to WNV and SLEV after the 2001 arbovirus transmission season, it is unlikely that deer served as important amplifying hosts in the transmission cycle.

Ages of the deer were collected in order to evaluate whether some of the arbovirus infections that we detected were recent (yearlings would have been infected during the 2001 arbovirus transmission season) and whether there was evidence for enzootic transmission rather than epizootic transmission. Enzootic transmission would be characterized by increasing seroprevalence with increasing age, because risk of transmission would occur each year, and the seroprevalence would accumulate in the older animals. However, insufficient sample sizes precluded us from making this determination. Similar numbers of WNV-positive and SLEV-positive yearlings would indicate however that risk of infection from either virus was about the same in 2001. It is unlikely that the high titers seen in these yearlings (Table 2) could be explained by maternal antibody inherited from does infected in previous years.

Through serologic testing, we have presented indirect evidence of arbovirus infections in white-tailed deer. Although the PRNT assay is widely recognized as the most reliable serologic test for flavivirus infections, cross-reactions are possible. The test algorithm we use should eliminate most cross-reactions. Nonetheless, flaviviruses are notoriously cross-reactive (Calisher et al. 1989), and secondary flavivirus infections can lead to "original antigenic sin" in which a titer to an old flavivirus infection may be abnormally high as a result of an infection with a heterologous virus (Inouye et al. 1984). Thus a new WNV infection may result in a higher SLE titer than the WNV titer, provided that the animal was previously infected with SLEV or possibly another flavivirus. The high proportion of undifferentiated flavivirus infections suggests the possibility that yet another flavivirus may be circulating in New Jersey. Powassan or deer tick virus would be candidate tick-borne flaviviruses infecting deer in New Jersey, although these have not yet been reported.

In summary, we have provided serologic evidence for WNV and SLEV infections in white-tailed deer from New Jersey. This is the first published report of WNV-neutralizing antibodies being detected in an ungulate in North America, and the first report of SLEV-neutralizing antibodies being detected in New Jersey deer. The significance of WNV infection in white-tailed deer is unknown at this time and further research is warranted to determine if these animals develop sickness and can become viremic as a result of the infection, or whether WNV and SLEV infections in deer may be useful surveillance indicators for predicting future risk of flavivirus infections in humans.

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