

## Serologic Evidence of West Nile Virus Infection in Black Bears (*Ursus americanus*) from New Jersey

Ary Farajollahi,<sup>1,4</sup> Nicholas A. Panella,<sup>2</sup> Patrick Carr,<sup>3</sup> Wayne Crans,<sup>1</sup> Kelcey Burgess,<sup>3</sup> and Nicholas Komar<sup>2</sup> <sup>1</sup>Department of Entomology, Rutgers University, New Brunswick, New Jersey 08901-1190, USA; <sup>2</sup>Centers for Disease Control and Prevention, Arbovirus Diseases Branch, Fort Collins, Colorado 80522, USA; <sup>3</sup>New Jersey Division of Fish and Wildlife, Hampton, New Jersey 08827, USA; <sup>4</sup>Corresponding author (email: farajoll@rci.rutgers.edu)

**ABSTRACT:** Serum samples obtained from 51 free-ranging black bears (*Ursus americanus*) in northwestern New Jersey in February and March 2002 were analyzed for neutralizing antibodies to West Nile virus (WNV) and St. Louis encephalitis virus. Three (6%) of the black bears tested positive for WNV-neutralizing antibodies. One additional sample was positive for flavivirus-neutralizing antibodies but could not be differentiated for a specific virus type. This is the first report of WNV infection in black bears.

**Key words:** Black bear, serosurvey, *Ursus americanus*, West Nile virus.

West Nile virus (WNV; family *Flaviviridae*) appeared for the first time in northeastern USA during the summer of 1999 (Lanciotti et al., 1999). St. Louis encephalitis virus (SLEV), a closely related flavivirus found throughout North America, has been shown to infect black bears (*Ursus americanus*) from Idaho (Binninger et al., 1980) and Florida (Dunbar et al., 1998), although it is not known whether the infection produces disease in these animals. Because the role of wild mammals in transmission cycles of these mosquito-borne flaviviruses is not fully understood, and no data are available regarding WNV infection in black bears, we evaluated WNV seroprevalence in a population of bears in the northeastern USA.

A total of 51 blood samples were obtained for this study, all of which were collected in northwestern New Jersey from 3 February 2002 to 27 March 2002 (40°44' to 41°20'N, 75°10' to 74°20'W). Thirty-seven of the captured bears were adult females, six were yearling females, three were adult males, and two were yearling males. The study was undertaken during the hibernation period, and most bears

were sampled at the den sites by using a dart gun or jab pole to sedate the animal prior to bleeding (DAN-INJECT, Wildlife Pharmaceuticals, Fort Collins, Colorado, USA). A mixture of ketamine hydrochloride (Ketaset®, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) at a dose of 5.0 mg/kg and xylazine hydrochloride (Rompun®, Mobay Corporation Animal Health Division, Shawnee, Kansas, USA) at a dose of 2.0 mg/kg was used as the anesthetic (Addison and Kolenosky, 1979). All bears were released at the capture location after they recovered from anesthesia. Whole blood (4–5 ml) was collected from the femoral vein using 21-gauge needles and deposited in sterile serum separator tubes with gel clot activators. Samples were allowed to clot in the field and then refrigerated for 12–48 hr prior to processing. In the laboratory, whole blood samples were centrifuged for 15 min at 1,800×G, and assayed for neutralizing antibodies to WNV and SLEV by plaque-reduction neutralization test, as previously described (Komar et al., 2001). Because cross-reaction between these two viruses occurs (Calisher et al., 1989), a four-fold greater reciprocal 90% neutralization titer to one of these viruses was considered sufficient to implicate that virus as the etiologic agent.

West Nile virus-neutralizing antibodies were detected in three (6%) of the 51 bears sampled (Table 1). Reciprocal 90% neutralization titers were 160 to ≥320 for WNV-neutralizing antibody positive bears, and in all cases, were at least fourfold higher than SLEV-neutralizing antibody titers. The WNV-neutralizing antibody positive samples came from adult females that

TABLE 1. Neutralizing antibody response to West Nile virus and St. Louis encephalitis virus detected in black bears during February and March 2002 by New Jersey county.

County	Total tested	Number virus antibody positive (% [95% CI])			WNV PRNT <sub>90</sub> <sup>d</sup>
		WNV <sup>a</sup>	SLEV <sup>b</sup>	FLAV <sup>c</sup>	
Morris	3	0	0	0	
Passaic	2	0	0	0	
Sussex	33	2 (6.1 [1.1–17.9])	0	1 (3.0 [0.2–13.6]) <sup>e</sup>	1:160, ≥1:320
Warren	13	1 (7.7 [0.4–31.6])	0	0	≥1:320
Totals	51	3 (5.9 [1.6–14.5])	0	1 (2.0 [0.1–9.0])	

<sup>a</sup> WNV = West Nile virus.

<sup>b</sup> SLEV = Saint Louis encephalitis virus.

<sup>c</sup> FLAV = undifferentiated flavivirus.

<sup>d</sup> PRNT<sub>90</sub> = 90% plaque reduction neutralization titer (greatest serum dilution exhibiting ≥90% reduction of plaques relative to serum-free control).

<sup>e</sup> For this specimen, the WNV PRNT<sub>90</sub> was <1:10, and the SLEV PRNT<sub>90</sub> was 1:10.

had two cubs each. Two of the bears were sampled from Sussex County (Franklin and Sandyston Townships), while the third was sampled in Warren County (Harmony Township). One additional sample, negative for WNV-neutralizing antibodies, had a low 90% neutralization titer (1:10) for SLEV-neutralizing antibodies, but insufficient sample was available to rule out cross-reaction with other flaviviruses (such as Powassan). Thus, it was designated as an undifferentiated flavivirus infection. This serum sample was obtained from an adult female with three cubs in Sussex County (Vernon Township).

These data provide evidence that black bears in New Jersey are exposed to WNV infection. West Nile virus transmission activity in the counties where these bears were sampled had been documented in the summer of 2001 through New Jersey's WNV Vector Surveillance Program, which tests mosquito specimens and dead crows (*Corvus brachyrhynchos*) (Centers for Disease Control and Prevention, 2001). The antibody positive bears all seemed to be in normal health and showed no signs of sickness or disease indicating that at least some bears survive WNV infection. It remains to be seen whether some bears may develop illness due to WNV infection and whether bears develop adequate viremia to infect mosquitoes thus playing a role in the transmission cycle. West Nile

virus fatalities have been encountered among several species of free-ranging mammals in the northeastern USA, including gray squirrel (*Sciurus carolinensis*), eastern chipmunk (*Tamias striatus*), striped skunk (*Mephitis mephitis*), big brown bat (*Eptesicus fuscus*), and little brown bat (*Myotis lucifugas*) (Marfin et al., 2001). Experimental infection studies of horses (Schmidt and El Mansoury, 1963; Bunning et al., 2002) and dogs (Blackburn et al., 1989) have shown that these large mammals develop very low levels of viremia that are insufficient to infect significant numbers of mosquitoes. However, smaller mammals such as hamsters develop infectious-level viremias (Xiao et al., 2001). The significance of WNV infection in black bears and other free-ranging mammals in the USA is unknown at this time and will require further investigation.

We thank T. McBride, C. Sliker, B. Longcor, M. Madonia, H. Lemasters, R. Shramko, A. Ostroff, K. Guild, A. Byrd, D. Roberts, and K. Mackenzie of the New Jersey Division of Fish and Wildlife for assisting in blood collections. L. Reed is thanked for help and advice on this manuscript.

This is New Jersey Agricultural Experiment Station publication D-08114-05-02 supported by State funds with partial support from the New Jersey State Mosquito Control Association.

## LITERATURE CITED

- ADDISON, E. M., AND G. B. KOLENOSKY. 1979. Use of ketamine hydrochloride and xylazine hydrochloride to immobilize black bears (*Ursus americanus*). *Journal of Wildlife Diseases* 15: 253–258.
- BINNINGER, C. E., J. J. BEECHAM, L. A. THOMAS, AND L. D. WINWARD. 1980. A serologic survey for selected infectious diseases of black bears in Idaho. *Journal of Wildlife Diseases* 16: 423–430.
- BLACKBURN, N. K., F. REYERS, W. L. BERRY, AND A. J. SHEPHERD. 1989. Susceptibility of dogs to West Nile virus: A survey and pathogenicity trial. *Journal of Comparative Pathology* 100: 59–66.
- BUNNING, M. L., R. A. BOWEN, C. B. CROPP, K. G. SULLIVAN, B. S. DAVIS, N. KOMAR, M. S. GODSEY, D. BAKER, D. L. HETTLER, D. A. HOLMES, B. J. BIGGERSTAFF, AND C. J. MITCHELL. 2002. Experimental infection of horses with West Nile virus. *Emerging Infectious Diseases* 8: 380–386.
- CALISHER, C. H., N. KARABATSOS, J. M. DALRYMPLE, R. E. SHOPE, J. S. PORTERFIELD, E. G. WESTAWAY, AND W. E. BRANDT. 1989. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. *Journal of General Virology* 70: 37–43.
- CENTERS FOR DISEASE CONTROL AND PREVENTION. 2001. Weekly update: West Nile Virus activity—United States, November 14–20, 2001. *Morbidity and Mortality Weekly Report* 50: 1061–1062.
- DUNBAR, M. R., M. W. CUNNINGHAM, AND J. C. ROOF. 1998. Seroprevalence of selected disease agents from free-ranging black bears in Florida. *Journal of Wildlife Diseases* 34: 612–619.
- KOMAR, N., N. A. PANELLA, AND E. BOYCE. 2001. Exposure of domestic mammals to West Nile virus during an outbreak of human encephalitis, New York City, 1999. *Emerging Infectious Diseases* 7: 736–738.
- LANCIOTTI, R. S., J. T. ROEHRIG, V. DEUBE, J. SMITH, M. PARKER, K. STEELE, B. CRISE, K. E. VOLPE, M. B. CRABTREE, J. H. SCHERRET, R. A. HALL, J. S. MACKENZIE, C. B. CROPP, B. PANIGRAHY, E. OSTLUND, B. SCHMITT, M. MALKINSON, C. BANET, J. WEISSMAN, N. KOMAR, H. M. SAVAGE, W. STONE, T. MCNAMARA, AND D. J. GUBLER. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286: 2333–2337.
- MARFIN, A. A., L. R. PETERSEN, M. EIDSON, J. MILLER, J. HADLE, C. FARELLO, B. WERNER, G. L. CAMPBELL, M. LAYTON, P. SMITH, E. BRESNITZ, M. CARTTER, J. SCALETTA, G. OBIRI, M. BUNNING, R. C. CRAVEN, J. T. ROEHRIG, K. G. JULIAN, S. R. HINTEN, D. J. GUBLER, AND THE ARBONET COOPERATIVE SURVEILLANCE GROUP. 2001. Widespread West Nile virus activity, Eastern United States, 2000. *Emerging Infectious Diseases* 7: 730–735.
- SCHMIDT, J. R., AND H. K. EL MANSOURY. 1963. Natural and experimental infection of Egyptian equines with West Nile virus. *Annals of Tropical Medicine and Parasitology* 57: 415–427.
- XIAO, S., H. GUZMAN, H. ZHANG, A. P. TRAVASSOS DA ROSA, AND R. B. TESH. 2001. West Nile virus infection in the golden hamster (*Mesocricetus auratus*): A model for West Nile encephalitis. *Emerging Infectious Diseases* 7: 714–721.

Received for publication 16 October 2002.