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West Nile Virus Antibodies in Bats from New Jersey and New York

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ABSTRACT: Eighty-three serum samples were obtained from big brown (*Eptesicus fuscus*), little brown (*Myotis lucifugus*), and northern long-eared (*Myotis septentrionalis*) bats (Chiroptera: Vespertilionidae), from New Jersey and New York (USA) between July and October 2002. Samples were analyzed for neutralizing antibodies to West Nile virus (WNV) and St. Louis encephalitis (SLE) virus. One little brown bat and one northern long-eared bat tested positive for WNV neutralizing antibodies. No bats had antibodies to SLE virus. This was the first large-scale investigation of WNV infection in bats in New Jersey. Additional work is needed to determine the effects of WNV on bat populations.

Key words: Antibodies, bats, New Jersey, survey, West Nile virus.

West Nile virus (WNV) first appeared in the northeastern United States during the summer of 1999, in the New York metropolitan area, and it quickly spread through much of the continental United States (Lanciotti et al., 1999). In New Jersey (USA), the virus has been isolated from humans, birds, horses, a gray squirrel (*Sciurus carolinensis*), an eastern chipmunk (*Tamias striatus*), a striped skunk (*Mephitis mephitis*), and bats (Marfin et al., 2001). Little is known about the involvement of bats in WNV transmission cycle(s). We conducted a study in the summer of 2002 to expand on what is known about bats and WNV. Although WNV has been found in bats, there are no estimates of antibody prevalence.

Eighty-three bats were captured using nylon mesh mist nets and hand extraction from roosts (Jones et al., 1996). Bats were from Atlantic (39.5°N, 74.3°W; 40 bats), Morris (40.7°N, 74.4°W; 12 bats), and Passaic (41.1°N, 74.2°W; 18 bats) Counties in New Jersey and in Orange County (41.3°N, 74.6°W; 13 bats), New York. Bats were temporarily placed in cloth bags until they could be processed. All individuals were identified to species; lengths of ear, tragus, and forearm were collected; and the animals were weighed. Patterns of tail membrane and length of toe hair aided in identification (Jones et al., 1996). Sex and reproductive status were determined visually (Jones et al., 1996). Adults were distinguished from juveniles by observing calcification in the epiphyseal cartilage of the metacarpal-phalangeal joints (Anthony, 1988). All bats were banded with butt-lipped, numbered aluminum rings that were closed around each bat's forearm (Gey Band and Tag Company, Norristown, Pennsylvania, USA).

Bats were anesthetized with 0.1 ml of a 25:1 mixture of ketamine (5 mg/100 g) (Phoenix Pharmaceuticals, Inc., Belmont, California, USA) and xylazine (0.2 mg per 100 g) (Phoenix Pharmaceuticals) injected intraperitoneally with a 27 gauge, 12.7 mm needle (Leirs et al., 1982). Blood was collected using a 27-gauge syringe for cardiocentesis. The volume of blood collected was ~0.2 ml, depending on the weight of the bat. Blood was diluted in cryovial tubes that contained 0.5 ml of Barry Yeelah's solution (BA-1; Hank's M-199 salts, 1% bovine serum albumin, 350 mg/l sodium bicarbonate, 100 U/ml penicillin, 100 mg/l streptomycin, 1 mg/l fungizone in 0.05 M Tris, pH 7.6). Bats were returned to cloth bags to recover until they were capable of flight; they were released the night of capture. Blood samples were placed in a cooler for temporary storage; in the laboratory, they were frozen at −70 C until processing.

All neutralization, plaque, and titration assays were conducted at the Centers for Disease Control and Prevention Laboratories (CDC, Ft. Collins, Colorado, USA). For detection of WNV-neutralizing antibodies, 60 μl of diluted bat serum was mixed with 60 μl of WNV preparation

(200 plaque-forming units [pfu]/0.1 ml in BA-1) in a polypropylene 96-well plate. When performing the St. Louis encephalitis (SLE) virus neutralization, 60 μl of SLE virus preparation (200 pfu/0.1 ml) was used. The virus-serum mixtures were incubated at 37 C for 1 hr, to allow for virus neutralization. These mixtures were then tested using the Vero plaque assay.

Plaque assays were performed by using 100 μl of serum sample inoculated onto Vero cell monolayers in six-well plates and incubated for 1 hr at 37 C. The cells were then overlaid with 3 ml/well of 0.5% agarose in nutrient broth that contained antibiotics (Komar et al., 2002). A second overlay that contained neutral red dye for the visualization of plaques was added on day 2 for WNV cultures and on day 5 for SLE virus cultures. Plaques were counted on days 3 and 4 for WNV and on days 6 and 7 for SLE virus.

All samples that caused ≥90% neutralization, compared with serum-free controls, were serially diluted twofold from 1:10 to 1:320. Any specimen that had a 90% neutralization titer ($PRNT_{90}$) of at least 1:10 and had a titer for SLE virus–neutralizing antibodies of at least fourfold lower, was scored as positive for WNV-neutralizing antibodies (N. Komar, pers. comm.).

Blood was collected from big brown bats (*Eptesicus fuscus*), little brown bats (*Myotis lucifugus*), and northern long-eared bats (*Myotis septentrionalis*). Of 83 blood samples, two (2%) had (>90%) antibody neutralization of WNV at 1:10 dilution. An adult female northern long-eared bat captured in Passaic County on 8 October 2002 (Fig. 1) was seropositive. The other seropositive animal was an adult male little brown bat captured in Morris County on 16 September 2002 (Fig. 1). No bat serum samples had >90% neutralization for SLE virus.

The diet and habits of *Myotis* bats may contribute to infection by WNV and other mosquito-borne viruses. All are insectivores that feed on almost any small insects, including small beetles, small moths, flies, mosquitoes, and aquatic insects (Barclay and Bell, 1990). Through ingestion of virus-carrying mosquitoes, bats may be contracting WNV orally. The oral route of transmission might result in a low exposure to the virus and, thus, a weaker antibody response.

The two seropositive bats were caught on 16 September and 8 October 2002. This suggests the bats may have contracted the virus late in the season. There were 44 confirmed cases of avian WNV infection, five confirmed human cases of WNV infection, and 244 mosquito pools that contained WNV in counties surrounding our study area (Centers for Disease Control and Prevention, 2002a, b). With the rapid spread of WNV and increasing numbers of confirmed cases of WNV in mammals, late-season infection may be occurring elsewhere.

Recently, Komar et al. (2002) detected WNV in the oral cavities of birds. This could be significant for bat communities if the same transmission route is possible in mammals. Bats roost in large numbers (Altringham, 1996). Within the roost, social grooming takes place, and oral transmission could spread the virus throughout the roost.

These data provide evidence that some species of bats in New Jersey are exposed to WNV. The effect of WNV infection on bat populations is unknown. Individuals of the three species that we sampled have tested positive for WNV RNA according to other studies (Marfin et al., 2001). The extent to which bats contribute to the transmission of WNV is unknown; some small mammals, such as hamsters, develop infectious viremia (Xiao et al., 2001). The significance of WNV in bat species in New Jersey is unknown and will require further investigation.

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