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A Survey for West Nile Virus in Bats from Illinois

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ABSTRACT: A blocking enzyme-linked immunosorbent assay was used to test 97 serum samples from big brown bats (*Eptesicus fuscus*) captured in six counties in Illinois between May 2002 and February 2004 for West Nile virus (WNV) antibodies. One female big brown bat tested positive for WNV antibodies. Samples of kidney, liver, and heart tissue were collected from 312 bats of seven species that were submitted to the Illinois (USA) Department of Public Health or the Illinois Department of Agriculture diagnostic laboratories between January 2001 and December 2003. Tissue samples were tested for WNV using TaqMan reverse transcriptase polymerase chain reaction and all were negative. Prevalence of WNV antibodies in the bats (1%) was lower than previously reported for other flaviviruses, but similar to the prevalence (2%) of WNV antibodies reported in bats from New Jersey and New York, USA. Additional research is needed to determine potential impact of WNV infections on bats and to determine whether they play a role in the WNV transmission cycle.

Key words: Antibodies, bats, Chiroptera, Illinois, survey, West Nile Virus.

Since 1999, when West Nile virus (WNV) was introduced into the northeastern USA, it has spread across the continental USA and has caused >14,000 human cases (CDC, 2003). In Illinois, USA, WNV was first detected in 2001, and the number of human cases peaked in 2002 (Illinois Department of Public Health, 2005). Mosquitoes are the primary vectors for WNV, and wild birds are the principal hosts (Hubalek and Halouzka, 1999; Campbell et al., 2002; McLean et al., 2002). Evidence of WNV infections in a variety of vertebrates (Steele et al., 2000; Komar et al., 2001; Ludwig et al., 2002; Lichtensteiger et al., 2003; Steinman et al., 2003; Heinz-Taheny et al., 2004) suggests that WNV is widespread in wildlife, and other vertebrate hosts might serve as an overwintering and maintenance reservoir for WNV.

A role for bats in the maintenance, dispersal, and natural history of arboviruses has been suggested (Fontanelli et al., 1989; Geevarghese and Banjeree, 1990). In Israel, 8% of surveyed fruit bats (*Rousettus aegypticus*) tested positive for WNV antibodies (Akov and Goldwasser, 1966). WNV infection was reported in four live big brown bats (*Eptesicus fuscus*) and two dead little brown bats (*Myotis lucifugus*) in New York in 2000 (CDC, 2000). One little brown bat and one northern long-eared bat (*M. septentrionalis*) tested positive for WNV antibodies in New Jersey, USA, in 2002 (Pilipski et al., 2004). Other flaviviruses closely related to WNV also infect bats (Constantine, 1970; Geevarghese and Banjeree, 1990). In Ohio, nearly 10% of the big brown bats and little brown bats tested were positive for Saint Louis encephalitis virus (SLEV) antibodies (Herbold et al., 1983).

When bats hibernate, their metabolic rate drops considerably, reducing the rate at which bats can produce antibodies (Sulkin et al., 1963; McNab, 1982). Hibernating bats may remain viremic with flaviviruses such as SLEV during their dormant season (Herbold et al., 1983) and may serve as a source of virus transmission and amplification in the spring. For this reason, Sulkin and Allen (1974) suggested that hibernating and migrating bats might be involved in arbovirus overwintering or reintroduction into a particular area.

Blood samples were collected from live big brown bats in Illinois to estimate the prevalence of antibodies to WNV. We selected big brown bats as the focus of our

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study because they often live in proximity to humans (Kurta and Baker, 1990), they hibernate in Illinois (Hoffmeister, 1989), and their large size allows for the collection of a sufficient volume of blood for testing without posing a risk to the health of the bat. To increase our sample size, we also collected tissue samples from bats that were submitted for rabies testing to the Illinois Department of Public Health (IDPH) and Illinois Department of Agriculture (IDA) for WNV.

Blood was collected from 97 live big brown bats that were either captured by hand in hibernacula or with mist nets. Bats were collected in Champaign (40.385°N, 87.975°W; $n=6$) and Vermillion (39.970°N, 87.579°W; $n=3$) counties during the summers of 2002 and 2003; in Edgar County (39.611°N, 87.696°W) during July 2002 ($n=11$) and July 2003 ($n=7$); at the LeRoy Oakes Forest Preserve (41.927°N, 88.347°W; $n=7$) and the Paul Wolff Forest Preserve (42.067°N, 88.367°W; $n=2$), Kane County, once each in July 2003; from a privately owned barn in Momence, Kane County (41.167°N, 87.663°W; $n=17$), and from Guthrie Cave, Union County (37.567°N, 89.220°W; $n=4$), in November 2002; and from Magazine Mine, UNIMIN Corporation, Alexander County (37.327°N, 89.259°W; $n=40$), in February 2004. Additional details and descriptions of sites are in Bunde (2004).

Species, sex, age class (juvenile or adult), and weight were recorded for each bat and a 50–100 μl blood sample was then taken from a vein in the interfemoral membrane; bats were not anesthetized. Blood was collected in a 100-μl heparinized capillary tube, immediately transferred to a centrifuge tube, and placed in a cooler on dry ice, followed by centrifugation to separate the sera. Samples were stored at −80 C until analysis. Bats sampled from hibernacula were placed in a bag with hand warmers to rouse them from torpor before blood was collected. All bats were released at their capture sites after bleeding had stopped and their condition appeared stable.

Sera were tested for WNV antibodies using blocking enzyme-linked immunosorbent assays (ELISAs), using a 1:10 serum dilution and monoclonal antibodies 2B2 and 6B6C-1 following Blitvich et al. (2003). The 2B2 flavivirus-specific monoclonal antibody (MAb) reacts with WNV and Koutango virus (Blitvich et al., 2003). The 6B6C-1 MAb reacts broadly with many flaviviruses, including WNV and SLE virus (Blitvich et al., 2003). We used parallel testing to provide a more sensitive diagnostic strategy resulting in a higher negative predictive value (Smith, 1995). Bats that tested negative in both tests were considered seronegative. Only one bat, an adult female from the maternity roost at LeRoy Oakes Forest Preserve in Kane County, tested positive for antibodies to WNV. This bat tested positive to both the 6B6C-1 MAb and the 2B2 Mab. We did not test for virus in these samples.

We necropsied 312 bats submitted to the IDPH and the IDA from 35 counties between 9 January 2001 and 10 December 2003 (Bunde, 2004). Most samples came from Cook County ($n=85$ bats), followed by Winnebago ($n=73$), Will ($n=27$ bats), and McHenry ($n=21$) counties. From 1 to 15 bats were available from other counties. Seven species of bats were included in this analysis: 258 big brown bats, 27 red bats (*Lasiurus borealis*), 20 silver-haired bats (*Lasionycteris noctivagans*), four hoary bats (*Lasiurus cinereus*), one little brown bat, one evening bat (*Nycticeus humeralis*), and one eastern pipistrelle (*Pipistrellus subflavus*).

Samples of kidney, liver, and heart tissue were individually tested for WNV by TaqMan reverse transcriptase-polymerase chain reaction (RT-PCR) following Lanciotti et al. (2000). A North American WNV strain (NY99) was used as a positive control. Brain tissue, which was removed as part of rabies testing, was not available for this study, but kidney, liver, and heart tissue have been used to test bats for

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arboviruses in previous studies (Rueger et al., 1966; Sulkin and Allen, 1974; Herbold et al., 1983). All bat tissues tested negative for WNV.

Bats may be involved in the epidemiology of WNV either as amplifying hosts or as a virus reservoir allowing for WNV maintenance during the winter. Reduced metabolic activity during hibernation may prolong the period of WNV infection. The clustering behavior of bats in maternity colonies also has been hypothesized to be conducive to transmission of viruses via arthropod vectors (Main, 1979). However, in this study, antibodies were detected in only one bat, and all attempts to detect WNV by RT-PCR were negative. Herbold et al. (1983) reported antibodies to SLEV in 9% of 390 live bats tested, but, similar to results from this study, SLEV antigen was not detected from >1,000 tissue samples.

Although WNV antibodies can be detected from bats using cELISA methods, the sensitivity and specificity of these tests for bats is unknown (Blitvich, pers. comm.). However, the low prevalence of antibodies detected in this study are consistent with previous reports from bats. Akov and Goldwasser (1966) used a hemagglutination inhibition test in their survey that reported three of 37 (8%) fruit bats tested positive for WNV in Israel, although none of six pipistrelles (*Pipistrellus* sp.) tested positive in their study. Pilipski et al. (2004) reported that only two of 83 (2%) bats from New York and New Jersey tested positive for WNV antibodies by plaque-reduction neutralization test.

The number of bats sampled in hibernacula in this study ($n=44$) was too small to critically test hypotheses about the role of hibernating bats in the WNV transmission cycle, and our results should be considered as preliminary. Because big brown bats hibernate in buildings in urban areas and because our survey was conducted during years when WNV was entering Illinois, additional work is recommended.

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