Seroprevalence of West Nile Virus in Nonhuman Primates as Related to Mosquito Abundance at Two National Primate Research Centers

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West Nile virus (WNV) surfaced as an emerging infectious disease in the northeastern United States in 1999, gradually spread across the continent, and is now endemic throughout North America. Outdoor-housed nonhuman primates at the Tulane National Primate Research Center (TNPRC) in Louisiana were documented with a relatively high prevalence (36%) of antibodies to West Nile virus. We examined the prevalence of antibodies to WNV in a nonhuman primate population housed in outdoor colonies at the Yerkes National Primate Research Center Field Station located near Atlanta, Georgia. We screened rhesus macaques (*Macaca mulatta*) and sooty mangabeys (*Cercocebus atys*) that were at least 3 y old by serum neutralization for antibodies to WNV and confirmed these results by hemagglutination-inhibition assay. None of the 45 rhesus monkeys had antibodies to WNV, but 3 of the 45 mangabeys (6.6%) were positive by both serum neutralization and hemagglutination-inhibition tests. The ratio of seroprevalences in the TNPRC and Yerkes primate populations was similar to the ratio of WNV incidences in people in Louisiana and Georgia from 2002 to 2004. The difference in the exposure of nonhuman primates (and possibly humans) to WNV between these 2 regions is consistent with the difference in the abundance of mammal-biting WNV-infectious mosquitoes, which was 23 times lower near Yerkes than around TNPRC in 2003 and 33 times lower in 2004.

Abbreviations: HAI, hemagglutination-inhibition; NHP, nonhuman primate; SLEV, St Louis encephalitis virus; SN, serum neutralization; TNPRC, Tulane National Primate Research Center; UGA, University of Georgia; UTMB, University of Texas Medical Branch; WNV, West Nile virus

West Nile virus (WNV) is a member of the genus *Flavivirus* (family *Flaviviridae*), part of the Japanese encephalitis virus antigenic complex, which also includes St Louis encephalitis virus (SLEV). WNV was first documented in Uganda in 1937[21] and first appeared in the United States in New York in 1999.[11] Since its introduction into the United States, WNV has spread across the continental states and into South America. The number of human clinical cases of WNV (including West Nile neuroinvasive disease and WNV fever) in the United States peaked in 2003, with 9862 cases in 46 states reported to the Center for Disease Control. Although the overall number of reported WNV cases has declined since 2003, 2539 clinical cases were reported in 2004, 3000 in 2005 and 4052 in 2006,[6] indicating that WNV is now endemic in the United States.[10]

Domestic animals and wildlife may play a role in the transmission of WNV. Numerous species have been studied with regard to both the occurrence of clinical disease and the ability of wild-animal populations to serve as a reservoir for virus. Birds are well documented to be the primary hosts for WNV transmission.[29] Equines appear to be the principal nonhuman mammalian species to exhibit clinical disease from WNV, with a documented 15,000 cases of neurologic disease in North America since the introduction of WNV in 1999.[27] More than 300 vertebrate species of domestic and wild animals have been found infected with WNV,[13] but few mammals have been documented to maintain the level of viremia sufficient to infect mosquitoes[24] (that is, 10^5 plaque-forming units per milliliter).[25]

Experimental infections have been attempted in various exotic and domestic species, including cat, dog, pig, horse, monkey, lemur, and birds.[2,4,17,18,22,28] All of these species could be infected with virus, but most developed a low level of viremia, making them unlikely to perpetuate the disease in the field. Rhesus monkeys that are inoculated via the intradermal route exhibit a transient viremia with no clinical disease, whereas intracranial inoculation of the virus leads to WNV encephalitis.[12,15,17]

A spontaneous case of clinical West Nile encephalitis has been reported and involved a 25-y-old barbary macaque at the Toronto Zoo.[14] This animal presented with neurologic signs including ataxia, shaking, drooping lip, excessive salivation, nystagmus, and decreased awareness. The animal was euthanized due to the severity of clinical disease. The diagnosis of WNV was confirmed at necropsy, with positive immunohistochemistry in the cerebellum, midbrain, and hypothalamus.

The only published large-scale serologic survey for WNV in NHPs was performed in 2002 at the Tulane National Primate Research Center (TNPRC; Covington, LA). This survey followed a human epidemic of West Nile meningoencephalitis in southern Louisiana. Approximately 1700 serum samples from NHPs were evaluated for antibodies to WNV, and 36% were positive for immuglobulin M (IgM) to the virus.[16,17] This survey included rhesus macaques (*Macaca mulatta*), pigtail macaques (*Macaca*

nemestrina), and baboons (*Papio* spp). The prevalence of WNV antibodies varied between species, with baboons having the highest prevalence (51%) followed by rhesus macaques (39%) and pigtail macaques (20%).

Given the high prevalence of WNV antibodies in the NHP population at TNPRC, we elected to screen the population at the Yerkes National Primate Research Center (Atlanta, GA). Although Old World monkeys are not likely reservoir hosts for WNV, its prevalence in research animals is important because the infection has the potential to interfere with animal use in research. Because WNV is a member of the family *Flaviviridae*, cross-reaction might occur in studies examining other flavivirus infections, such as vaccine work with SLEV or Japanese Encephalitis. In addition, given the case report of WNV encephalitis in a NHP,[14] as well as evidence of seroprevalence in NHP populations, WNV could be considered as a differential diagnosis for NHPs with neurologic symptoms.

Materials and Methods

Animals. The animals sampled were maintained at the Yerkes Field Station located about 30 miles northeast of Atlanta. The Field Station sits on approximately 117 acres of wooded land, where breeding colonies of rhesus macaques, pigtail macaques, sooty mangabeys, and several groups of chimpanzees are maintained (Figure 1). Currently, approximately 2000 NHPs are group-housed in outdoor compounds enclosed by chain-link fencing and metal sheeting. The surrounding area is relatively suburban, with scattered housing along the perimeter.

Blood was collected from 45 India-origin rhesus monkeys (*Macaca mulatta*) and 48 sooty mangabeys (*Cercocebus atys*) while the animals were under ketamine HCl anesthesia for routine health screening, which included tuberculosis testing, parasite control, and physical exams. Samples were collected from 33 female and 12 male rhesus monkeys and 30 female and 17 male mangabeys. The animals sampled ranged from 3 to 27 y of age. All samples were collected between Fall 2004 and Spring 2006. The numbers of animals sampled reflected the sample size needed to achieve statistical significance according to estimated antibody prevalence in the NHP population in Georgia. This estimate was generated by comparing the incidences of WNV disease among humans in Georgia and Louisiana with that among NHPs at TNPRC and then extrapolating the prevalence in the NHP population at Yerkes from that information.

Samples were collected from animals housed in 7 different compounds that are geographically spread throughout the Field Station (Figure 1). Subjects were selected according to several criteria. To ensure that the animals were alive during the height of the 2003 WNV epidemic in Georgia, only animals 3 y of age and older were surveyed. All experimental procedures were performed under a protocol approved by the Emory University Institutional Laboratory Animal Care and Use Committee.

Serology. Blood samples were centrifuged and the sera frozen at –20 °C. Samples were shipped on ice to the University of Georgia (UGA) Veterinary Diagnostic and Investigational Laboratory for serology. Samples were heat-inactivated at 56 °C for 30 min to eliminate any active virus (for example, cercopithicine herpesvirus type I). Serum neutralization (SN) assays then were performed to screen for WNV antibodies. Briefly serum samples were added to 96-well microplates. Dilutions of WNV and Vero cells were added to sample wells, and samples were incubated for 72 h, when cytopathic effects were measured.

Because the laboratory at UGA had not previously performed

Figure 1. Aerial view of Yerkes National Primate Research Center. The asterisks denote compounds from which samples were collected. The blue asterisk represents the compound with the SLEV antibody-positive rhesus monkey. The green asterisk represents the compound with the WNV antibody-positive mangabeys.

serologic WNV testing on nonhuman primates, a subset of the samples (29 rhesus and 10 mangabeys) was sent for confirmatory testing to the University of Texas Medical Branch (UTMB), which had performed the WNV serology for the TNPRC study. These samples were validated by hemagglutination-inhibition (HAI) assay for both WNV and SLEV antigens, starting at a serum dilution of 1:10. Thereafter, samples initially were screened by SN at UGA, and any positive samples were shipped to UTMB for confirmation by HAI and discrimination between WNV and SLEV. Antigens for the HAI test were prepared from brains of newborn mice infected with the respective viruses by the sucrose-acetone extraction method.[20] Monkey sera were tested at serial 2-fold dilutions from 1:10 to 1:1280 at pH 6.6, with 4 U of antigen and a 1:200 dilution of goose erythrocytes.[23]

Mosquito surveillance. We attempted to understand the differences in antibody prevalence between the primates at Tulane and Yerkes by examining differences in mosquito surveillance data collected by the St Tammany Parish Mosquito Abatement District in Louisiana and by the Georgia Division of Public Health in Gwinnett County, GA. To analyze these data, we adapted a formula originally developed to predict risk of human infection and applied the adapted formula to nonhuman primates.[8] We assumed the risk of WNV infection to be proportional to the abundance of mammal-feeding West Nile virus-infectious mosquitoes, according to the following formula:

$$\text{Risk} = \Sigma_{i=1}^{n} A_i \bullet F_{m,i} \bullet P_i \bullet C_{v,i}$$

The summation is over all mosquito species i, and A_i is the abundance of species i, $F_{m,i}$ is the fraction of blood meals from mammals, P_i is the WNV infection prevalence, and $C_{v,i}$ is an index of vector competence (the fraction of WNV-infected mosquitoes of that species that will transmit virus in a subsequent bite).[8] We obtained mosquito abundance and WNV infection prevalence data from mosquito surveillance efforts in 2003 and 2004 at trapping locations within 10 miles of Yerkes and Tulane Primate Centers. Infection prevalence was calculated using maximum likelihood methods.[3] In Louisiana, *Cx. nigripalpus* and *Cx. salinarius* samples were often combined for testing and abundance determinations. Feeding behavior of *Cx quinquefasciatus* ($F_m = 0.11$) was based on a study in Ohio,[9] that of *Cx nigripalus* ($F_m = 0.57$) from a study in Florida,[7] and that of *Cx salinarius* ($F_m = 0.72$) from a study in New Jersey.[1] Vector competence data was based on data from populations in Florida for *Cx quinquefasciatus* ($C_V = 0.22$) and *Cx nigripalus* ($C_V = 0.12$) and Texas for *Cx salinarius* ($C_V = 0.36$).[19]

Statistical methods. We tested for differences in the prevalence of WNV between rhesus macaques at Yerkes and TNPRC using a chi-squared test using Minitab V12.1 (State College, PA). We tested

Table 1. Distribution of WNV and SLEV serology results by age and species

	Rhesus		Mangabey	
Age (y)	No. tested	No. positive	No. tested	No. positive
3–6	9	0	5	0
7–10	23	0	7	0
11–14	8	1 (SLEV)	16	2 (WNV)
≥15	5	0	20	1 (WNV)
Total	45	1	48	3

Table 2. Comparative incidence of human WNV in GA and LA between 2002 and 2004

	Human cases		Human incidence (# cases per 10^6 people)[a]		
	GA	LA	GA incidence	LA incidence	GA incidence/ LA incidence
2002	44	329	5.37	70.4	0.074
2003	50	124	6.11	20.8	0.22
2004	21	109	2.5	20.4	0.10
				Average	0.13

[a]Human incidence calculated as the number of WNV cases per year divided by population as provided from the 2000 US Census Bureau.[26]

Results

Of the 45 rhesus monkey serum samples that were tested by SN at the Veterinary Diagnostic Laboratory at UGA, only a single animal, a 13-y-old female monkey, was positive for flaviviral antibodies at a 1:8 dilution. This sample tested positive for SLEV on HAI at UTMB at a 1:10 dilution, for a prevalence of 2.2% (95% confidence interval, 0.1% to 11%). Therefore all 29 of the SN-negative samples were confirmed at UTMB to be negative for WNV, and the prevalence of WNV antibodies in rhesus macaques in this population was 0% (95% CI 0% to 6.4%). This prevalence was significantly different from that measured in the TNPRC rhesus macaque population (39.4%; 286 positive of 726 samples; $\chi^2 =$ 28.2; $P < 0.001$), even though the TNRPC animals were sampled in 2002 and had been exposed to WNV for only 2 y at most.

Of the 48 mangabeys tested by SN at UGA, 3 female monkeys (ages 11, 12, and 24 y) from the same compound tested positive (1 at 1:8 and 2 at 1:16) for flaviviral antibodies (Table 1). These samples then were tested at UTMB by HAI for discrimination between SLEV and WNV; all 3 samples were negative for SLEV but positive for WNV at a 1:80 dilution, for a prevalence of 6.3% (95% confidence interval, 1.3% to 17.2%). The proportions of mangabeys and macaques that were WNV antibody-positive did not differ significantly (Fisher exact test, $P = 0.24$). If data from both species are combined, the seroprevalence in the primate population at Yerkes was 3.2%, or approximately 9% of the seroprevalence of primates sampled at Tulane in 2002. This ratio of seroprevalences in NHPs at Yerkes and TNPRC is similar to the ratio of human WNV incidences in GA and LA (approximately 13%;[5, 26] Table 2).

The abundance of WNV-infectious mammal-biting mosquitoes was 23 times higher at TNPRC than at Yerkes in 2003 and 33 times higher in 2004, with no overlap in 95% confidence intervals (Table 3). Although the prevalence of WNV in *Cx quinquefasciatus* mosquitoes was similar between the 2 sites, 2 additional species of *Culex* mosquitoes, *Cx nigripalpus* and *Cx salinarius*, were present in high abundance at TNPRC and often were infected with WNV (Table 3). In addition, *Aedes* mosquitoes trapped near TNPRC were infected with WNV whereas those trapped near Yerkes were not (Table 3).

Discussion

The results of this serosurvey indicate that the prevalence of WNV in the NHP population at Yerkes National Primate Research Center Field Station was approximately 3% overall. This rate is far lower than the 36% prevalence documented at the TNPRC and was unanticipated. These results prompted us to search for factors that might affect the prevalence of disease in this population. To rule out laboratory error, results were confirmed at a second laboratory with established NHP WNV tests.

Mosquito surveillance in the areas surrounding Yerkes and Tulane showed that the abundance of mosquitoes differed substantially. Our estimated risk of WNV infection was nearly 30-fold higher for mammals (including primates and humans) in Louisiana near TNPRC than in Georgia near Yerkes. Although these data are limited to trapping stations within 10 miles of the 2 field stations, if similar differences occur in mosquito abundance and prevalence in other areas of the 2 states, this may partly explain the difference in human incidence as well as the difference in the prevalence of non-human primates.

Although the ratio of WNV antibody prevalence of the 2 NHP populations was roughly 10:1, the primates at TNPRC were sampled in 2002, and the prevalence in these long-lived animals likely would have been higher had they also been sampled in 2005. In addition, WNV was present in mammalophilic *Aedes* mosquitoes at TNPRC, and their presence contributed approximately 25% of the risk of transmission of WNV to mammals, including primates and humans. Therefore, in this case, the ratio of human disease incidence in GA to that in LA during the height of WNV infection served as an index of antibody prevalence in NHPs.

The TNPRC survey examined rhesus macaques, pigtail macaques, and baboons for antibodies to WNV and identified a higher rate of infection in baboons than macaques. We speculated that African and Asian species of NHPs could have different infection rates and therefore screened both macaques (Asian)

Table 3. Risk of WNV transmission (95% confidence interval) in LA and GA estimated by use of local mosquito surveillance data (± 1 standard error)

Yerkes (Gwinnett County, GA)	Mosquito species	A	P	F_m	C_v	Risk	Total risk
2003	Cx quinquefasciatus	16.0 ± 6.3	0.0077 ± 0.0042	0.11 ± 0.04	0.22 ± 0.097	0.0030 (0–0.0096)	0.0030 (0–0.0096)
2004	Cx quinquefasciatus	10.7 ± 0.9	0.0060 ± 0.002	0.11 ± 0.04	0.22 ± 0.097	0.0015 (0–0.0043)	0.0015 (0–0.0043)
TNPRC (St Tamminy Parish, LA)	**Mosquito species**	**A**	**P**	**Fm**	**Cv**	**Risk**	**Total risk**
2003	Cx quinquefasciatus	23.0 ± 19.4	0.0067[a] ± 0.0027	0.11 ± 0.041	0.22 ± 0.097	0.0037 (0–0.016)	0.0669 (0.02–0.14)
	Cx salinarius + Cx nigripalpus	500.3 ± 140	0.0006[a] ± 0.0002	0.65 ± 0.063	0.24 ± 0.077	0.0468 (0.007–0.12)	
	Ae vexans	52.3 ± 16.3	0.0020 ± 0.0009	0.94 ± 0.04	0.17 ± 0.089	0.0164 (0–0.05)	
2004	Cx quinquefasciatus	0.8 ± 4.0	0.0067[a] ± 0.0027	0.11 ± 0.041	0.22 ± 0.097	0.0001 (0–0.0002)	0.0514 (0.015–0.11)
	Cx salinarius + Cx nigripalpus	409.7 ± 92	0.0006[a] ± 0.0002	0.65 ± 0.063	0.24 ± 0.077	0.0384 (0.007–0.093)	
	Aedes spp	23.4[b] ± 5.2	0.0029 ± 0.0019	0.75[b] ± 0.06	0.25[b] ± 0.16	0.0129[b] (0–0.04)	

A, host seeking abundance; P, prevalence of WNV infection; F_m, fraction of feedings from mammals; C_v, fraction of infected mosquitoes that are infectious.
Estimated risk was calculated as described in Methods.
[a]Prevalence estimate based on combined data from 2003 and 2004.
[b]WNV-positive mosquito pools included mixed *Aedes* species, so prevalence and abundance estimates were obtained from all trapped and tested *Aedes* spp.

and mangabeys (African). Our results showed no infection in the macaques and a low rate of infection in the mangabeys. Given the higher antibody levels found in the African species at Yerkes (mangabeys) and TNPRC (baboons), further exploration of possible differences in infection rates and disease susceptibility between other African and Asian species may be warranted.

Our results suggest that exposure of nonhuman primates to WNV varies significantly in different regions of the United States. This difference appears to be related to differences in the composition, abundance, and prevalence of mosquito communities. Our results imply that mosquito surveillance data and human disease incidence may be useful in predicting the exposure of mammals to WNV and in planning control strategies to minimize the effect of this disease. In addition, our results indicate that NHPs in geographic proximity to humans may serve to predict WNV exposure in humans.

Acknowledgments

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