West Nile Virus Infection in Nonhuman Primate Breeding Colony, Concurrent with Human Epidemic, Southern Louisiana

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During the summer of 2002, an epidemic of West Nile meningoencephalitis occurred in southern Louisiana. Following the outbreak, blood samples were collected from 1,692 captive rhesus monkeys (*Macaca mulatta*), pigtail macaques (*M. nemestrina*), and baboons (*Papio* spp.) that were permanently housed outdoors at a nonhuman primate breeding facility in St. Tammany Parish, Louisiana. The serum samples were examined for antibodies to West Nile virus (WNV). Overall, 36% of the captive nonhuman primates had WNV antibodies; comparison of these samples with banked serum samples from previous blood collections indicated that the animals were infected subclinically from February to August 2002. WNV activity was demonstrated in surveillance at the nonhuman primate-breeding colony and in the neighboring community during this same period. The high infection rate in this captive nonhuman primate population illustrates the intensity of WNV transmission that can occur silently in nature among other susceptible vertebrates during epidemic periods.

Since its first appearance in New York City in 1999, West Nile virus (WNV) has spread rapidly across the North American continent, infecting a wide range of avian and mammalian species (1,2). Within 3 years of its initial appearance, WNV activity was reported in 44 of the 48 states that comprise the continental United States (2). During 2002, a total of 3,389 human cases and ~15,000 equine cases of WNV-associated illness were reported by the Centers for Disease Control and Prevention (CDC) and the U.S. Department of Agriculture (USDA), respectively (2). In the same year, a total of 14,122 WNV-infected dead birds, representing 94 different avian species, were reported (2). Despite the increasing number of reported West Nile cases and the wide vertebrate host range of this emerging flavivirus, surprisingly little information is available on the true prevalence of WNV infection among humans and animals living within newly epidemic regions in North America. This paucity of information is partly due to the method in which WNV infections are recorded: only recognized cases of clinical illness or death are usually reported.

On the basis of retrospective serosurveys conducted in New York City in 1999 and 2000, symptomatic illness develops in approximately 20% of persons infected with WNV and approximately 1 in 150 human infections results in meningoencephalitis, the most commonly reported form of WNV-associated illness (1,3). Serosurveys among equines living in WNV-endemic areas, as well as results of WNV experimental infections of horses, indicate that most equine infections are inapparent or result in only mild clinical illness (4–7). Most reported WNV-associated bird deaths in the United States have been in crows and blue jays (2,8), two species with a relatively high case-fatality rate; however, many other bird species are naturally infected with the virus and have few or no deaths (8–11). Because the current WNV surveillance systems in the United States are largely disease- and death-based (cases of human meningoencephalitis, equine encephalitis, dead birds), the actual prevalence of WNV infection among humans and other susceptible vertebrate hosts and the intensity of virus transmission are underestimated.

During 2002, the state of Louisiana reported 319 human cases of WNV-associated illness; 71% of these cases had WNV meningoencephalitis (2). Most of the West Nile cases were in the southeastern portion of the state. St. Tammany Parish, which is located in that region of the state, was a focus of intense WNV activity in 2002 and

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recorded 40 human cases (12). The Tulane National Primate Research Center (TNPRC) is located in St. Tammany Parish and houses large outdoor breeding colonies of baboons and macaques. The availability of sequential serum samples from these animals during a period of known epidemic WNV activity prompted us to examine some of the animals for serologic evidence of recent WNV infection. The results of these studies are reported here and indicate that approximately 36% of the nonhuman primates tested from the breeding colony were naturally infected with WNV during the 2002 transmission season.

Methods

Study Site

TNPRC is located on 500 acres of land in Covington, St. Tammany Parish, Louisiana, approximately 56 km north of New Orleans. TNPRC has approximately 5,000 nonhuman primates in its research and breeding populations. Approximately 4,000 of these animals are housed in outdoor social groups for breeding. The animal care program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and is registered with the USDA as a class B animal dealer and as a research institution.

The center's breeding colony is situated on approximately 100 acres of land and comprises 223 outdoor holding compounds, which consist of corrals and corncribs. Corrals are constructed of chain link with earthen floors composed of clay and topsoil (Figures 1 and 2). Most corrals have grass coverage. Corncribs are constructed of galvanized metal and are completely covered on top, open with wire on the sides, and have cement flooring. All corrals and corncribs have perching, which is sheltered. The corrals have additional shelter in the form of walled and covered areas, concrete culverts, and calf huts to allow all of the animals to have shelter in inclement weather.

Because the regional climate is mild, animals in the breeding colony are housed outdoors throughout the year. Animals are provided a commercial nonhuman primate diet, fed once a day, and supplemented with fruit 3 days per week. Additionally, foraging feed (cracked corn, seed) is spread throughout the corrals. Water from the Center's artesian well is available ad libitum through an automated Lixit system (Napa, CA).

The breeding colonies housed at the center consist of the TNPRC Conventional Breeding Colony and the TNPRC Specific Pathogen Free Colony, both of which consist of rhesus monkeys (*Macaca mulatta*). TNPRC also houses the breeding colonies for the Washington National Primate Research Center (WaNPRC), which consists of both baboon (*Papio* spp.) colonies and Specific Pathogen Free pigtail macaques (*M. nemestrina*).

Figure 1. Aerial view of the nonhuman primate outdoor holding compounds at the Tulane National Primate Research Center. Approximately 4,000 animals are housed here in social groups for breeding.

Every animal at the TNPRC has a unique identifying number tattooed on the animal's chest or medial thigh. A secondary form of identification is also used in breeding colony animals and consists of neck chains and tags, dye marks, or subcutaneous microchip transponders. Animals are observed for illness twice a day, and ill animals are moved to indoor clinical areas for diagnosis and treatment on a daily basis. All animals in the breeding colony are routinely examined a minimum of twice annually as part of the preventive medicine program.

Sampling Technique

The animals sampled for this study were from the TNPRC SPF colony and the WaNPRC SPF and baboon colonies. At the time of inventory, physical examination, anthelmintic treatment, pregnancy determination, tattooing, and blood sample collection were performed as part of the semiannual preventive medicine program. All inventory procedures were performed in ketamine HCl (10 mg/kg IM) anesthetized animals. Blood was collected from the

Figure 2. Inside view of one of the outdoor holding corrals at the Tulane National Primate Research Center.

femoral vein after preparation of the overlying skin with alcohol. Twenty-two–gauge needles and a Vacutainer collection system were used for blood collection. All procedures performed on animals were reviewed and approved by the Tulane University Institutional Animal Care and Use Committee.

Serologic Studies

After blood collection, serum samples were removed and frozen at –20°C. These samples were subsequently transported on dry ice to the University of Texas Medical Branch, where all serologic testing was done. Initially, all primate sera were screened by hemagglutination-inhibition (HI) test against dengue-1 (DENV-1), DENV-2, yellow fever (YFV), St. Louis encephalitis (SLEV), and West Nile (WNV) antigens. The antigens were prepared from brains of newborn mice injected intracerebrally with each of the flaviviruses; infected brains were treated by the sucrose-acetone extraction method (13,14). Primate serum samples were tested by HI at serial twofold dilutions from 1:20 to 1:5120 at pH 6.6 (WNV and SLEV) or 6.4 (YFV and DENV) with 4 U of antigen and a 1:200 dilution of goose erythrocytes by using established protocols (13,14).

A subsample of the WNV antibody–positive samples by HI test was also examined by complement fixation (CF) and plaque reduction neutralization test (PRNT). CF tests were performed by a microtechnique (13) with two full units of guinea pig complement and antigen titers of ≥1:32. Titers were recorded as the highest serum dilutions giving +3 or +4 fixation of complement on a scale of 0 to +4.

PRNT on the serum samples was performed by a previously described technique (13) in 24-well, Vero microplate cultures, using a fixed virus inoculum (~100 PFU) against varying serum dilutions (1:10 to 1:20,480). For PRNT, the Egypt 101 strain of WNV (13) was used. Virus plaques were read on day 4; ≥90% plaque reduction was used as the endpoint.

Mosquito Surveillance

Because WNV activity had occurred in Louisiana in 2001, an initial survey was conducted on the grounds of the TNPRC from January 29 to April 5, 2002, to determine the potential for WNV transmission to the captive nonhuman primates. Larval mosquito sites were identified by using standard dipping measures to determine larvae and pupae density. At this time, some adult mosquitoes were also collected by using modified dry ice-baited CDC light traps, although assay for WNV was not performed. Results of this survey were used to properly target control strategies to problem areas within the corral facility. Strategies used during this period included larval source reduction (maintenance of ditches and proper drainage) and application of a mosquito growth regulator (Altosid) (Wellmark International, Schaumburg, IL). A subsequent partnership forged with the local mosquito control agency (St. Tammany Mosquito Control) enabled aerial applications of the chemical pesticide Dibrom (AMVAC, Commerce, CA). Throughout the season, seven aerial applications of Dibrom were sprayed at 0.75 oz per acre. A second survey of adult mosquitoes was conducted from July 29 to October 31, 2002.

Two types of traps were used in the mosquito surveillance program. Modified dry ice-baited CDC light traps (Clarke Environmental Mosquito Mgmt., Inc., Roselle, IL) to collect host-seeking mosquitoes were set 1 m from the ground. Gravid traps (John W. Hock, Gainesville, FL) targeting primarily gravid *Culex quinquefasciatus* were placed on the ground in a shaded area and filled with 1 to 2 inches of water and fish emulsion. In this study, one light trap and one gravid trap were placed at four sites around the perimeter of the nonhuman primate breeding corrals and the main campus. Mosquito traps were positioned weekly and operated for 12 hours each collection period. Upon collection, the mosquitoes were immobilized on dry ice and then stored at –20°C. The mosquitoes were later placed on a chill table and identified to species (15).

Results

Serosurvey on Captive Primates

A total of 1,692 primates in the breeding colony at TNPRC were sampled from August to November 2002. The species breakdown of the animals sampled was as follows: 726 rhesus macaques, 563 pigtail macaques, and 403 baboons. All serum samples from these animals were screened by HI test against DENV-1, DENV-2, YFV, SLEV, and WNV antigens; a subset of the WNV antibody positives also were tested by CF and PRNT. The results of serologic tests are summarized in Tables 1–5.

A total of 726 *M. mulatta* were bled between August and November 2002 and tested by HI test for WNV antibodies; 286 (39.39%) of the serum samples were positive

Table 1. Prevalence of West Nile virus hemagglutination inhibition (HI) antibodies by age group among captive rhesus macaques in the breeding colony at the Tulane National Primate Research Center by age group, August–November 2002

Age group (y)	No. tested	No. positive	% positive
0 to <1	8	1	12.50
1 to <2	293	96	32.76
2 to <4	349	141	40.40
4 to <6	61	43	70.49
6 to <8	3	1	33.33
8 to <10	7	3	42.86
10 to <12	2	1	50.00
12 to <14	2	0	0.00
≥14	1	0	0.00
Total	726	286	39.39

Table 2. Results of hemagglutination inhibition (HI) tests on blood samples collected in August 2002 from eight West Nile-seropositive rhesus macaques living outdoors at the Tulane National Primate Research Center[a]

Animal number (mo bled)	HI antibody titer				
	DENV-1	DENV-2	YFV	SLEV	WNV
CK62	1:40[b]	1:20	1:20	1:160	1:320
CL07	1:40	1:20	1:20	1:160	1:320
CL11	1:40	1:40	1:20	1:160	1:640
CL80	1:40	1:40	1:40	1:160	1:640
CL93	1:40	1:40	1:40	1:160	1:640
CM11	1:20	1:20	0	1:20	1:80
CP90	1:40	1:20	1:20	1:40	1:160
CR36	1:40	1:20	1:20	1:80	1:320

[a]DENV, dengue virus; YFV, yellow fever virus; SLEV, St. Louis encephalitis virus; WNV, West Nile virus.
[b]Highest positive serum dilution

as shown in Table 1. Of the total, 462 were female, and 264 were male; the prevalence of WNV antibodies by sex was 42.2% and 34.5%, respectively. Table 1 summarizes the WNV antibody rates by age group. The lowest WNV antibody rate was in the 0 to <1 age group and the highest was in the 4 to <6 age group.

All serum samples were tested in HI tests against DENV-1, DENV-2, YFV, SLEV, and WNV antigens. Although cross-reactions were observed with these other flavivirus antigens, in all cases the HI antibody titers were highest with WNV antigen. Table 2 illustrates the results obtained with eight HI-positive rhesus serum samples collected in August 2002. The pattern of the HI antibody response in these animals was characteristic of a single flavivirus infection with WNV (16,17).

A subsample of 40 WNV antibody-positive rhesus sera from August were also examined by CF and PRNT to confirm the HI results. The CF and PRNT results on the same eight HI positive serum samples are summarized in Table 3. Results of the latter tests confirmed that the nonhuman primates had been infected with WNV.

To determine more precisely when the animals had been infected with WNV, serum samples collected 6 months earlier (February or March 2002) from 40 of the antibody-positive rhesus macaques were recovered from the serum bank and examined by HI test together with serum samples collected from the same animals in August or September. All of the samples from February to March 2002 were WNV antibody-negative, whereas, serum samples from the same animals from August or September 2002 were WNV antibody-positive. These data indicate that the primates were infected from February to September 2002.

We compared the prevalence of WNV antibodies in 712 captive rhesus macaques by their cage location within the 100-acre breeding colony. The resulting data (not shown) indicated that the risk for infection was similar regardless of cage location.

Table 4 summarizes HI test results on 563 pigtail macaques (*M. nemestrina*) from the TNPRC breeding colony by age group. Overall, 20.25% of these animals had WNV antibodies. The antibody rates among the various age groups were similar. The antibody prevalence by cage location was compared; and as observed with the rhesus macaques, the antibody rates among cage groups were not markedly different.

Table 5 shows the prevalence of WNV antibodies by age group among 403 captive baboons (*Papio* spp.) bled from August to November 2002 at the TNPRC. Overall, the prevalence of WNV antibodies among the baboons was higher (51.36%) compared to the rhesus (39.39%) and pigtail (20.25%) macaques. Except for the 0 to <1 group, the

Table 3. Results of complement-fixation (CF) and plaque reduction neutralization (PRNT) tests done on sera of eight WNV-seropositive rhesus macaques bled in August 2002 at the Tulane National Primate Research Center[a]

Animal no.	CF antibody titer					PRNT antibody titer with WNV
	Viral antigen					
	DENV-1	DENV-2	YFV	SLEV	WNV	
CK62	0	0	0	16/8[b]	128/≥32	1:320[c]
CL07	0	0	0	8/8	64/≥32	1:160
CL11	0	0	0	8/8	64/≥32	1:1280
CL80	0	0	0	16/8	128/≥32	1:80
CL93	0	0	0	32/8	128/≥32	1:320
CM11	0	0	0	0	16/≥32	1:160
CP90	0	0	0	0	32/≥32	1:160
CR36	0	0	0	8/8	64/≥32	1:320

[a]Viral antigens used in CF test: DENV, dengue virus; YFV, yellow fever virus; SLEV, St. Louis encephalitis virus; WNV, West Nile virus.
[b]CF titers expressed as reciprocal of highest antibody titer/reciprocal of antigen titer.
[c]PRN titers expressed as highest serum dilution producing ≥90% plaque reduction.

Table 4. Prevalence of West Nile virus hemagglutination inhibition (HI) antibodies among captive pigtail macaques at the Tulane National Primate Research Center by age group, August–November 2003

Age group (y)	No. tested	No. positive	% positive
0 to <1	49	5	10.20
1 to <2	72	12	16.67
2 to <4	126	25	19.84
4 to <6	94	24	25.53
6 to <8	78	22	28.21
8 to <10	93	14	15.05
10 to <12	33	6	18.18
12 to <14	6	2	33.33
≥14	12	4	33.33
Total	563	114	20.25

WNV antibody rates among the various age groups were similar. The baboons are housed in three contiguous large breeding enclosures. The higher density of animals in the baboon cage may make them more attractive to host-seeking mosquitoes and might account for the higher infection rate among this species.

Mosquito Surveillance Studies

A second survey of adult mosquitoes was made in the breeding colony at TNPRC from July 29 to October 31, 2002, to determine the vector abundance and species composition, after the intensive mosquito control effort in St. Tammany Parish in response to the human WNV epidemic. Mosquito collections were made 1 night each week. A total of 718 adult mosquitoes were collected during this period; the species composition is shown in Table 6. Virus isolation was attempted by culture in Vero cells, but all mosquito pools yielded negative results. Mosquitoes of the genus *Culex* comprised 40.9% of the total collected. Because of Tropical Storm Isidore and Hurricane Lili, which inundated south Louisiana with rain in September and October 2002, relatively large populations of flood water mosquitoes (i.e. *Aedes vexans, Ochlerotatus infirmatus, Psorophora ferox*) were present in collections made during those months.

Discussion

The results of our serosurvey indicate that approximately 36% of the primates housed outdoors at TNPRC were infected with WNV during the spring or summer of 2002. This timing coincides with the observed pattern of WNV activity documented in the Covington area during surveillance by the St. Tammany Mosquito Control, Louisiana State Department of Health, and CDC (12). The presence of WNV in 2002 was first detected in the Covington area with sentinel chicken seroconversions on June 6 and June 10. After these events, a pool of *Cx. quinquefasciatus* from a gravid trap at the TNPRC breeding corrals tested positive for the virus on June 11 (testing done at Louisiana State University, Baton Rouge). The following week (June 13–21), a dead crow, three horses, and five blue jays in Covington also tested positive for WNV. Viral testing of birds was suspended in the parish after 8 WNV-infected dead birds were reported.

Despite the number of nonhuman primates infected with WNV during the summer of 2002, no compatible clinical illness or neurologic disease was observed in any of the animals. In addition, no changes were noted in monthly data on illness and death derived from the breeding colony during the time of seroconversion or thereafter. Animals at TNPRC are observed twice a day for illness, and ill animals are moved indoors to a clinical area for diagnosis and treatment. Although antibodies to WNV have been reported before in wild nonhuman primates (18), relatively little is known about the pathogenesis of natural WNV infection in nonhuman primates. The available data (19–23) suggest that intracerebral or intranasal inoculation of rhesus and cynomolgus (*Macaca fascicularis*) monkeys may result in encephalitis and death, whereas intravenous or subcutaneous inoculation of these monkeys generally results in an asymptomatic infection or mild febrile illness.

In view of the frequency of WNV infection observed among nonhuman primates in the breeding colonies at TNPRC, one obvious question is whether these animals could serve as amplifying hosts of the virus to infect biting mosquitoes. We recently completed an experiment in which 5 seronegative rhesus monkeys were inoculated subcutaneously with a 1999 New York strain of WNV. The animals were bled daily to determine the level and duration of viremia. Clinical signs of illness did not develop in any animal, and the viremia was of short duration (1–5 days) and did not exceed $10^{2.0}$ infectious units per mL (23). This level of viremia is much lower than that reported for many avian species (11) and is below the titer considered infectious for *Cx. quinquefasciatus* (11,24), the presumed major vector of WNV in Louisiana.

Table 5. Prevalence of West Nile virus hemagglutination inhibition (HI) antibodies among captive baboons at the Tulane National Primate Research Center by age group, August–November 2002

Age group (y)	No. tested	No. positive	% positive
0 to <1	57	14	24.56
1 to <2	60	23	38.33
2 to <4	76	39	51.32
4 to <6	47	33	70.21
6 to <8	36	27	75.00
8 to <10	25	17	68.00
10 to <12	39	61	41.03
12 to <14	29	19	65.52
≥14	34	19	55.88
Total	403	207	51.36

The results of our serosurvey illustrate the intensity of WNV transmission that can occur during periods of epidemic activity. To date, most of the studies of WNV ecology in North America have focused on humans, equines, and a few susceptible bird species. However, WNV, like its close relative Japanese encephalitis virus (25), has an extremely wide host range among vertebrates (2,26–28). The role of some of these other species in the amplification and maintenance of WNV in North America remains to be determined.

The overall prevalence of WNV antibodies (36%) among the 1,692 primates sampled at TNPRC is comparable to WNV infection rates reported in bird populations elsewhere in the United States after human epidemics of West Nile encephalitis. In a retrospective serosurvey in New York City after the 1999 outbreak, Komar et al. (9) reported that 33% of 430 birds sampled had WNV antibodies. Likewise, a retrospective serosurvey at the Bronx Zoo (the epicenter of the 1999 outbreak) found that 34% of captive and wild birds had antibodies (27). During 2001, the Florida Department of Health recorded annual seroconversion rates as high as 54% and 57% among sentinel chickens in some counties where West Nile human and equine cases occurred (8). In a retrospective survey of wild birds in the Houston metropolitan area in the fall and winter of 2002 (WNV was first detected in Texas in the summer of 2002), 34% of 218 bird sera tested had WNV antibodies (RS Tesh and A.P.A. Travassos da Rosa, unpub. data). These infection rates have occurred in affected regions after WNV was introduced. Whether this high level of virus transmission will continue to occur annually remains to be determined.

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References

1. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. Ann Intern Med 2002;137:173–9.
2. Centers for Disease Control and Prevention. Provisional surveillance summary of the West Nile virus epidemic-United States, January–November 2002. MMWR Morb Mortal Wkly Rep 2002;51:1129–33.
3. Mostashari F, Bunning ML, Kitsutani PT, Singer DA, Nash D, Cooper MJ, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. Lancet 2001;358:261–4.
4. Autorino GL, Battisti A, Deubel V, Ferrari G, Forletta R, Giovannini A, et al. West Nile virus epidemic in horses, Tuscany Region, Italy. Emerg Infect Dis 2002;8:1372–8.
5. Schmidt JR, El Mansoury HK. Natural and experimental infection of Egyptian equines with West Nile virus. Ann Trop Med Parasitol 1963;57:415–27.
6. Bunning ML, Bowen RA, Cropp B, Sullivan KG, Davis BS, Komar N, et al. Experimental infection of horses with West Nile virus. Emerg Infect Dis 2002;8:380–6.
7. Ostlung EN, Crom RL, Pedersen DD, Johnson DJ, Williams WO, Schmitt BJ. Equine West Nile encephalitis, United States. Emerg Infect Dis 2001;7:665–9.
8. Blackmore CGM, Stark LM, Jeter WC, Oliveri RL, Brooks, RG, Conti LA, et al. Surveillance results from the first West Nile virus transmission season in Florida, 2001. Am J Trop Med Hyg 2003;69:141–50.
9. Komar N, Panella NA, Burns JE, Dusza SW, Mascarenhas TM, Talbot TO. Serologic evidence for West Nile virus infection in birds in the New York City vicinity during an outbreak in 1999. Emerg Infect Dis 2001;7:621–5.
10. Ludwig GV, Calle PP, Mangiafico JA, Raphael BL, Danner DK, Hile JA, et al. An outbreak of West Nile virus in a New York City captive wildlife population. Am J Trop Med Hyg 2002;67:67–75.
11. Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, et al. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Dis 2003;9:311–22.
12. Centers for Disease Control and Prevention. West Nile activity—United States, July 31–August 7, 2002, and Louisiana, January 1–August 7, 2002. MMWR Morb Mortal Wkly Rep 2002;51:681–3.
13. Tesh RB, Arroyo J, Travassos APA, Guzman H, Xiao S-Y, Monath TP. Efficacy of killed virus vaccine, live attenuated chimeric virus vaccine, and passive immunization for prevention of West Nile virus encephalitis in hamster model. Emerg Infect Dis 2002;8:1392–7.
14. Beaty BJ, Calisher CH, Shope RE. Arboviruses. In: Lennette EH, Lennette DA, Lennette ET, editors. Diagnostic procedures for viral, rickettsial and chlamydial infections. 7th ed. Washington: American Public Health Association; 1995. p. 189–212.
15. Darsie RF, Morris CD. Keys to the adult females and fourth instar larvae of the mosquitoes of Florida (Diptera; Culicidae). Technical Bulletin of Florida Mosquito Control Association, Vol. 1. Fort Myers (FL): Florida Mosquito Control Association; 2000.
16. Casals J. The arthropod-borne group of animal viruses. Trans NY Acad Sci 1957;19:219–35.
17. Tesh RB, Travassos da Rosa APA, Guzman H, Araujo TP, Xiao SY. Immunization with heterologous flaviviruses protective against fatal West Nile encephalitis. Emerg Infect Dis 2002;8:245–51.
18. Hayes CG. West Nile fever. In: Monath TP, editor. The arboviruses: epidemiology and ecology, Vol. 5. Boca Raton (FL): CRC Press; 1989. p. 59–88.
19. Smithburn KC, Hughes TP, Burke AW, Paul JH. A neurotropic virus isolated from the blood of a native of Uganda. Am J Trop Med 1940;20:471–92.
20. Nathanson N, Davis M, Thind IS, Price WH. Histological studies of the monkey neurovirulence of group B arboviruses. Am J Epidemiol 1966;84:524–31.
21. Manuelidis EE. Neuropathology of experimental West Nile virus infection in monkeys. J Neuropath Exp Neurol 1956;15:488–60.
22. Pogodina VV, Froloba MP, Malenko GV, Fokina GI, Koreshkova GV, Kiseleva LL, et al. Study on West Nile virus persistence in monkeys. Arch Virol 1983;75:71–86.
23. Ratterree MS, Gutierrez RA, Travassos da Rosa APA, Dille BJ, Beasley DWC, Bohm RP, et al. Experimental infection of rhesus monkeys with West Nile virus; level and duration of viremia and kinetics of the antibody response following infection. J Infect Dis. In press 2003.

24. Sardelis MR, Turell MJ, Dohm DJ, O'Guinn ML. Vector competence of selected North American *Culex* and *Coquillettidia* for West Nile virus. Emerg Infect Dis 2001;7:1018–22.
25. Endy TP, Nisalak A. Japanese encephalitis virus: ecology and epidemiology. Curr Top Microbiol Immunol 2002;267:11–48.
26. Komar N. West Nile viral encephalitis. Rev Sci Tech 2000;19: 166–76.
27. Ludwig GV, Calle PP, Mangiafico JA, Raphael BL, Danner DK, Hile JA, et al. An outbreak of West Nile virus in a New York City captive wildlife population. Am J Trop Med Hyg 2002;67:67–75.
28. Miller DL, Mauel MJ, Baldwin C, Burtle G, Ingram D, Hines ME, et al. West Nile virus in farmed alligators. Emerg Infect Dis 2003; 9:794–9.

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