West Nile and St. Louis Encephalitis Virus Antibody Seroconversion, Prevalence, and Persistence in Naturally Infected Pig-Tailed Macaques (Macaca nemestrina)

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Pig-tailed macaques (Macaca nemestrina) naturally infected with West Nile virus were monitored from 1999 to 2005 to determine virus-specific antibody seroconversion, prevalence, and persistence. Antibodies persisted for up to 36 months, as detected by epitope-blocking enzyme-linked immunosorbent and hemagglutination inhibition assays. Exposure to cocirculating St. Louis encephalitis virus was evaluated by Western blotting and immunofluorescence assays.

The seroepidemiology of wild macaque populations demonstrates a natural seroconversion to flaviruses including West Nile virus (WNV) and Japanese encephalitis virus (10, 15). Across species, there is a wide variation in WNV antibody persistence. Immunoglobulin M antibodies may persist less than 3 weeks in fowl, a sentinel species, and up to 500 days in humans, a dead-end host (11, 19, 24). Domestic pigs have remained seropositive (hemagglutination inhibition assay [HAI] antibodies) and immune to both WNV and Japanese encephalitis virus for more than 3 years (7, 9). In some cases, antibody persistence in the form of a recurrent, but intermittent, appearance of specific plaque reduction neutralization test (PRNT) antibodies after a single exposure to virus has been attributed to a host immune reactivation of persistent virus (13, 21). Neutralizing antibody persistence for more than 5 months due to a single experimental inoculation of macaques (intracerebral or subcutaneous) without recurrence has been documented (16, 17).

West Nile virus emerged in Louisiana during the spring of 2002, with endemic establishment and year-round regional virus activity reported during the winter months of 2003 to 2004 (12, 23). WNV and St. Louis encephalitis virus (SLEV) share a close phylogenetic relationship and elicit cross-reactive antibodies (5). Both viruses are maintained in similar transmission cycles with mosquito vectors (mainly Culex spp.), avian reservoirs, and amplification hosts. St. Louis encephalitis virus has been endemically established in the southern United States for many decades. Just prior to the emergence of WNV in Louisiana, a regional outbreak of SLEV took place (fall of 2001); SLEV and WNV now cocirculate within the region. Serologic distinction between flaviruses is hampered by the extensive homologies of viral structural proteins. West Nile virus infection exhibits low pathogenicity compared to many other members of the flavivirus family while producing broad-spectrum immunity (14). Sequential infections with one or more flaviruses elicit strong cross-reactive anamnestic responses, which may confer immunity (22).

The Washington National Primate Research Center (WaNPRC) and the Tulane National Primate Research Center (TNPRC) house animals in the same outdoor breeding cohorts. The WNV seroprevalence in TNPRC animals was shown to be greater than 30% (18). We therefore aimed to assess the WNV exposure level among WaNPRC animals through serologic techniques (enzyme-linked immunosorbent assay [ELISA], immunofluorescence assays [IFAs], PRNT, and HAI). Since repeated exposures, persistent antibody response, and exposure to cocirculating flaviruses can complicate the interpretation of serologic results, we also aimed to establish methods to discern between WNV and SLEV infections.

Of the cross-reactive viral structural proteins, the envelope (E) protein is the most immunogenic (25). The E protein is highly conserved among flaviruses and elicits an antibody response with relatively low specificity. Cross-reactivity is confounding to assays that depend to a great extent on antibodies to the E protein, for example, HAI. This may be overcome, at least partially, by employing assays based on nonstructural (NS) proteins, which elicit more virus-specific immune responses.

A colony serosurvey was performed using a WNV epitope-blocking ELISA validated in multiple avian and mammalian species including the pig-tailed macaque (Macaca nemestrina) (3, 4, 8). ELISAs were performed using monoclonal antibody 3.112G (Chemicon International, Inc., Temecula, CA), which is specific for the NS1 protein of Kunjin virus, a subtype of WNV (7, 20). Immunoassays were performed with banked (−70°C and −20°C) plasma samples collected from 1999 to
2005 for viral screening from approximately 700 WaNPRC pig-tailed macaques housed at the TNPRC in Covington, LA. Negative serum samples were obtained from WaNPRC animals born and housed indoors in Seattle, Washington. Western blot analysis was performed using infected Vero cell lysates (Chimeravax SLEV and WNV; Acambis Int., Cambridge, MA) as previously described (2). Immunofluorescence assays (PanBio, Inc., Columbia, MD) were performed according to the manufacturer’s specifications. Viral screening was performed under a general husbandry protocol approved by the University of Washington Institutional Animal Care and Use Committee. Both the WaNPRC and the TNPRC are AAALAC-accredited facilities. Hemagglutination inhibition assays were performed at the University of Texas Medical Branch using a previously published protocol (6). Previously obtained PRNT (Arthropod-Borne Infectious Disease Laboratory, Colorado State University) and HAI (University of Texas Medical Branch) data were used for correlation analysis (8).

Plasma samples collected at 6-month intervals from 1999 to 2005 were tested by ELISA. Samples from 2002 and 2003 were tested by HAI. No WNV-specific antibodies were detected in samples from 1999, 2000, or 2001. WNV antibodies were demonstrated in colony animals from 2002 to 2005.

The distribution of seroconversion and maintained titers among seropositive animals is summarized in Fig. 1. Seroconversion rates are a close approximation, as a small number of
The specificity of each assay is increased. In lieu of PRNTs, a Western blot assay may be used for comparison of responses to two or more viruses. Using this approach, Western blots for WNV and SLEV were compared with WNV PRNT titers, ELISA titers, and both SLEV and WNV IFA. West Nile virus antibody titers measured by ELISA correlated with PRNT titers with 93% sensitivity and 100% specificity (8). Western blot banding patterns were found to correlate with the NS1 ELISA (88% concordance), PRNT (76% concordance), and IFA and HAI detect cross-reactive antibodies (WNV and SLEV). NS1 ELISA, Western blotting, and PRNT are specific for WNV.

Cohort chosen to represent a range of HAI and NS1 ELISA titers.

Western blot banding pattern is a measurement of the relative signal strength of the E protein (50 kDa).
IFA (66% concordance) data, demonstrating the ability of Western blots to distinguish between WNV and SLEV (Table 1).

In 2002, 63 animals were positive by HAI and negative by ELISA, suggesting exposure to a cocirculating flavivirus. In no case did an HAI-negative animal test positive for WNV by ELISA. Discordant samples were evaluated by Western blot assay. Four of the 63 animals positive by HAI and negative by ELISA (in 2002) were found to have strong SLEV-specific reactivity; the remaining animals (59/63 animals) demonstrated an SLEV band intensity equal to or less than that for WNV.

When developing flaviviral monitoring programs for macaques in zoos and primate centers with outdoor facilities, complications in data interpretation from persistent antibody titers must be considered. Persistent titers may be related to continued flaviviral environmental exposure and/or the presence of cocirculating flaviviruses. We have demonstrated that macaques can maintain WNV seropositivity by ELISA for more than 36 months with continual environmental exposure. Western blots demonstrated repeated flavivirus exposure and discerned between WNV and SLEV exposures.

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