February 2005

RESEARCH STRATEGIES OF THE NATIONAL WILDLIFE RESEARCH CENTER TO CONTROL BOVINE TUBERCULOSIS IN WILDLIFE IN MICHIGAN, USA

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Am. J. Trop. Med. Hyg., 76(1), 2007, pp. 173–179

SEROLOGIC EVIDENCE OF WEST NILE VIRUS EXPOSURE IN NORTH AMERICAN MESOPREDATORS

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Abstract. Sera from 936 mammalian mesopredators (Virginia opossums, gray foxes, striped skunks, hooded skunks, raccoons, a bobcat, and a red fox) were collected during 2003 and 2004 in California, Arizona, Texas, Louisiana, Ohio, and Wyoming and screened for flavivirus-specific antibodies by an epitope-blocking enzyme-linked immunosobent assay (blocking ELISA). Serum samples positive for antibodies against flaviviruses were screened for West Nile virus (WNV)–specific antibodies by blocking ELISA and selectively confirmed with plaque-reduction neutralization tests. High prevalence rates were observed in raccoons (45.6%) and striped skunks (62.9%). The high WNV antibody prevalence noted in mesopredators, their peridomestic tendencies, and their overall pervasiveness make these species potentially useful sentinels for monitoring flaviviruses in defined areas.

INTRODUCTION

The introduction, outbreak, and subsequent spread of West Nile virus (WNV) into North America has been well documented.[1,2] This virus, a member of the Japanese encephalitis complex[3] (WNV; family Flaviviridae, genus *Flavivirus*), has been found to be active throughout the North American continent with surveys also demonstrating its presence in the Caribbean region.[4] Transmitted by mosquitoes, WNV infects a wide range of vertebrates.[5] The natural cycle involving the infection of reservoir-competent birds is the principal reason for the amplification and spread of the virus.[6] However, recent studies suggest that mammals, thought to be dead-end hosts, are not only exposed to WNV, but at least some may also serve as competent WNV reservoirs.[7–9]

Although serosurveys in mammals are uncommon and the extent of the potential host range of WNV in North America is largely unknown, evidence suggests that mammals are frequently exposed. Horses have been impacted by WNV infections and high morbidity and mortality rates have been observed.[10] Evidence of WNV exposure has also been observed in companion animals,[4] livestock,[5] and captive wildlife.[11] Wild mammals exposed to WNV include a little brown bat (*Myotis lucifugus*), a big brown bat (*Eptesicus fuscus*), an eastern chipmunk (*Tamias striatus*),[12] and a striped skunk (*Mephitis mephitis*),[13] as well as black bears (*Ursus americanus*),[14] eastern gray squirrels (*Sciurus carolinensis*),[12,15] fox squirrels (*Sciurus niger*),[15] and white-tailed deer.[16] Recently, Root and others observed high prevalence rates of antibodies to WNV among some mesopredators species (raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginianus*)).[15] Building upon these previous observations, we have focused exclusively on mesopredators in greater numbers to provide additional insights on the roles these species play in WNV transmission.

Because of their peridomestic habits, mesopredators might serve as useful sentinels for mosquito bridge vector activity and WNV exposure in mammals, such as humans. Although serosurveys yield little information about host competency, they can be used to document potential host range and may be used to identify sentinel wildlife species. On-going United States Department of Agriculture wildlife monitoring activities, such as wildlife rabies surveillance,[17] provide occasions to opportunistically survey for antibodies to WNV. Sera from various geographic locations in the United States were obtained in this manner, thus allowing us to assess mesopredators as WNV sentinel species across the United States.

MATERIALS AND METHODS

Trapping sites. We obtained serum samples from mammals captured during spring, summer, and fall months in 2003 and 2004 from 18 rural study sites ranging in area from 9.1 km^2 to 35.0 km^2 in Arizona, California, Louisiana, Ohio, Wyoming and Texas (Figure 1). In Arizona, mammals were live-trapped on two sites (4/23/03–5/2/03): Fort Huachuca Army Base (AZF) in Cochise County and Las Cienegas National Conservation Area (AZL) in Pima County. The AZL study site was primarily composed of marshlands (cienegas) and cottonwood-willow riparian forests, and the AZF study site was primarily composed of sacaton grasslands, mesquite bosques, and semi-desert grasslands. In California, mammals were live-trapped on two sites (7/16/03–7/29/03): North Etna (CAN) and South Etna (CAS), both located in Siskiyou County. These sites were located at an elevation of approximately 915 meters on the Scott River flood plain. Study sites consisted of pastures with cattle or mixed agriculture (corn and alfalfa). In Louisiana, mammals were live-trapped at four locations: the Monroe North Site (MNS) and the Monroe South Site (MSS) located in Ouachita Parish (4/16/03–4/24/03 and 6/17/03–6/23/03), and the Lake Charles North site (LCN) and the Lake Charles South site (LCS), located in Calcasieu Parish (12/2/03–12/7/03). The Monroe sites were primarily agricultural (cotton, soybeans) interspersed with mature bottomland hardwoods, fallow fields, and urban areas. The Lake Charles sites consisted primarily of agricultural land (rice) with pasture and some mature bottomland hardwoods. In Ohio, raccoons were live-trapped on two sites; the U.S. National Aeronautics and Space Administration's Plum Brook Station (PBS) (5/6/03–10/16/03 and 3/30/04–10/21/04) and Old Woman Creek National Estuarine Research Reserve (OWC)

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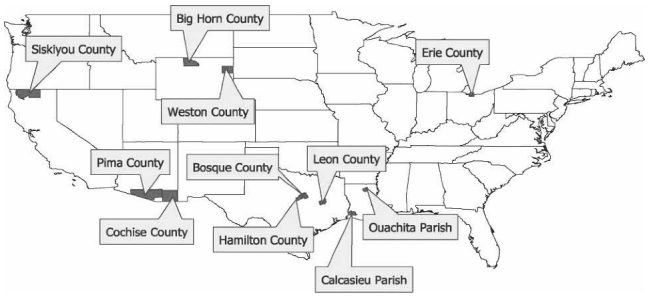

FIGURE 1. Mesopredator sampling site counties, 2003 and 2004.

(4/9/04–9/30/04), both located in Erie County. The PBS site, located on the Lake Erie coastal plane, consisted of herbaceous fields, shrublands and oak-dominated hardwood forest. Water on the site consisted of ephemeral sources (drainage ditches) and permanent creeks and ponds. The OWC site, also located on the Lake Erie coastal plane in Erie County 15 km east of PBS, comprises a hardwood and wetland estuary system (freshwater) surrounded by suburban and agricultural land. In Texas, mammals were collected on four sites (6/2/03–6/13/03): TXLM and TXCR, both in Leon County, TXRR in Hamilton County, and TXW4 in Bosque County. In Wyoming, mammals were sampled on four 4 sites: Upton (UPT) and Buffalo Creek (BUC), both located in Weston County (8/5/03–8/15/03), and Greybull East (GBE) and Greybull West (GBW), both located in Big Horn County (11/11/03–11/20/03). The UPT and BUC sites were located at an elevation of approximately 1,370 meters with study sites primarily consisting of sagebrush prairie with ephemeral creeks and artificial cattle reservoirs. The GBE and GBW sites, both with the Greybull River serving as a central transect, were in a mixed riparian, agricultural, and urban environment.

Grid trapping. In Ohio, trapping areas on PBS were randomly selected from within each of eight 1-km^2 grids, which represented the diversity of habitats in the study area, and were used throughout the trapping periods. Systematic rotation of traps ensured that every location was trapped at least twice in a trapping period. Traps were spaced at 250-meter intervals within each grid. Grids were grouped into four pairs such that the two areas comprising each pair were separated by ≥ 1 km at their closest point. At the OWC site, traps were placed subjectively, unlike those of the grid system established at PBS. At both sites, raccoons were sampled using live-catch traps (107 cm × 30 cm × 30 cm; Tomahawk Live Trap Co., Tomahawk, WI).

Transect trapping. In all states except Ohio transect trapping was conducted in 2003. Typically, sites within a state were situated ≥ 5 km from each other and all sites contained a river or riparian zone that served as a central transect with two adjacent trapping transects placed approximately 1 km to either side of this transect for a total of three transects per site. Traps were typically spaced approximately 500 meters apart per transect. Mammals were sampled using live-catch Tomahawk traps (81 cm × 25 cm × 30 cm).

Mammal processing. Live-trapped mammals were immobilized with a ketamine:xylazine mixture and whole blood was collected by cardiac puncture, except for raccoons sampled in Ohio, which were bled by jugular venipuncture. Typically, whole blood was stored on ice until centrifugation for serum collection. Sera was temporarily stored at –20°C before and during transport (approximately 24 hours) and thereafter stored at –80°C.

Laboratory analyses. All assays were conducted at the National Wildlife Research Center. Mesopredator sera as well as appropriate negative and positive mammal control sera were tested to determine the presence of antibodies against WNV and other flaviviruses using epitope-blocking enzyme-linked immunosobent assays (blocking ELISAs) by the method of Blitvich and others.[18] Two monoclonal antibodies (MAbs) were used. The MAb 3.1112G (Chemicon International, Temecula, CA) is specific for the non-structural protein 1 (an NS1 epitope) of West Nile/Kunjin virus and was used to assay for WNV antibodies in serum samples. Horseradish peroxidase–labeled MAb 6B6C-1, which is specific for the flavivirus envelope protein (E protein epitopes), was used to assay for flavivirus exposure. Briefly, MAb bound to a 96-well microplate surface coated with viral antigen subsequently binds to peroxidase substrate molecules that upon color development exhibit a measurable optical density (OD). Optical densities were analyzed with a microplate reader and percent inhibition values were calculated $(100 - [(OD_{TS} - 0.05)/(OD_{CS} - 0.05)]\ 100$, where TS is test serum and CS is negative control serum). An inhibition value ≥ 30% was considered positive for antibodies to flaviviruses.

Samples with positive and negative ELISA results (inhibition values between 26% and 34%) for MAb 3.1112G were tested by plaque-reduction neutralization tests (PRNTs) for verification.[19] The PRNTs were performed using the attenuated recombinant vaccines ChimeriVax-WN and ChimeriVax-SLE (Acambis Inc., Cambridge, MA). These vaccines are the result of infectious clone technology in which genes encoding the pre-membrane and E structural proteins of yellow fever 17D vaccine virus are replaced with the corresponding genes of WNV or St. Louis encephalitis virus (SLEV), which produces a virion with the protein coat of WNV containing all antigenic determinants for neutralization. Thus, the recombinant vaccine replicates like yellow fever 17D and can be neutralized by neutralizing antibodies to WNV.[20] Chimerivax-SLE was used in these analyses because SLEV has been identified in several of our study areas and is known to cross-react with neutralizing antibodies to WNV.[21]

The PRNTs were performed using Vero cells. Sera were tested at dilutions of 1:10, 1:20, 1:40, 1:80, 1:160, and 1:320. Titers were expressed as the reciprocal of serum dilutions yielding ≥ 90% reduction in the number of plaques ($PRNT_{90}$). Sera was etiologically diagnosed as being positive for antibodies to WNV if the $PRNT_{90}$ WNV antibody titer was at least four-fold greater than that of its corresponding SLEV antibody titer,[19] it neutralized WNV plaque formation by at least 90% at a dilution ≥ 1:10, and it blocked the reaction of MAb 3.1112G by 30%. Similarly, sera was diagnosed as being positive for antibodies to SLEV if the $PRNT_{90}$ SLEV antibody titer was at least four-fold greater than that of its corresponding antibody titer to WNV, it neutralized SLEV plaque formation by at least 90% at a dilution ≥ 1:10, and it blocked the binding of MAb 6B6C-1, but not MAb 3.1112G, by ≥ 30%. Sera having positive $PRNT_{90}$ titers for both WNV and SLEV, sera reactive to either WNV or SLEV by $PRNT_{90}$ but negative by blocking ELISA, and sera reactive to either WNV or SLEV by blocking ELISA but negative by $PRNT_{90}$ were considered positive for antibodies to flavivirus. To be considered negative for antibodies to flavivirus, sera had to

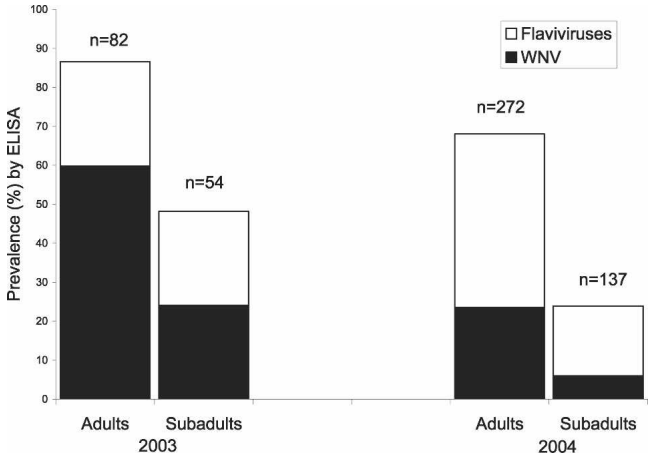

FIGURE 2. Prevalence of antibodies against flaviviruses and West Nile virus (WNV) in adult and subadult raccoons sampled in Ohio, 2003 and 2004. ELISA = enzyme-linked immunosorbent assay.

show no reactivity to flaviviruses by $PRNT_{90}$ and by blocking ELISA.

RESULTS

Sera from raccoons (n = 647), striped skunks (n = 179), hooded skunks (*Mephitis macroura*) (n = 4), Virginia opossums (n = 98), gray foxes (*Urocyon cinereoargenteus*) (n = 6), a bobcat (*felis rufus*) (n = 1), and a red fox (*Vulpes vulpes*) (n = 1) were analyzed. The most detailed monitoring for antibodies against flaviviruses and WNV focused on raccoons sampled in Ohio during 2003 and 2004.

Ohio monitoring efforts. During 2003, 86.6% of adult raccoons and 48.1% of subadult raccoons showed evidence of exposure to flaviviruses, as determined by detectable antibodies to MAb 6B6C-1 (Figure 2). Exposure to WNV, as determined by detectable antibodies to MAb 3.1112G, was 59.8% and 24.1% for adult and subadult raccoons, respectively. During 2004, 68.0% of adults and 23.4% of subadult raccoons showed evidence of exposure to flaviviruses, and 23.5% of adults and 5.8% of subadults showed evidence of exposure to WNV.

Antibody prevalence patterns in the central and western United States. A total of 391 mammals, belonging to 7 species, were captured in Arizona, California, Louisiana, Texas, and Wyoming (Table 1). In Arizona, prevalence of antibodies to flaviviruses ranged from 44.4% at AZF to 80.0% at AZL for all animals combined, with an overall mean of 57.1%. Striped skunks and hooded skunks showed evidence of antibodies to flavivirus. Results of the epitope-blocking ELISA using WNV specific MAb 3.1112G indicated that no antibodies to WNV were present in the Arizona mammals sampled. In California, prevalence of antibodies to flaviviruses ranged from 35.1% at CAN to 66.7% at CAS, with an overall mean of 52.0%. Striped skunks, raccoons, Virginia opossums, and a bobcat displayed evidence of antibodies to flavivirus. No antibodies to WNV were detected in the California mammals sampled. In Louisiana where striped skunks, raccoons, and Virginia opossums showed evidence of antibodies to flavivirus, prevalences ranged from 0.0% at LCN to 46.9% at MSS, with an overall mean of 31.0%. The presence of antibodies to WNV was observed in Virginia opossums and a raccoon. The overall prevalence of antibodies to WNV in Louisiana mammals sampled was 12.0% and site-specific prevalences ranged from 0.0% at LCN to 16.7% at MNS. In Texas, prevalence of antibodies to flaviviruses ranged from 16.0% at TXW4 to 62.5% at TXRR, with an overall mean of 28.9%. All species sampled, striped skunks, raccoons, Virginia opossums, and a gray fox, showed evidence of antibodies to flavivirus. Prevalence of antibodies to WNV in Virginia opossums was observed. The overall prevalence of antibodies to WNV in Texas mammals sampled was 2.6% and site-specific prevalences ranged from 0% at TXCR and TXRR to 4.5% at TXLM. Wyoming mesopredators displayed prevalences of antibodies to flaviviruses ranging from 46.7% at UPT to 93.0% at GBW, with an overall mean of 73.0%. Of the three species sampled in Wyoming, all showed evidence of antibodies to flavivirus. These were striped skunks, raccoons, and a Virginia opossum. Results of the epitope-blocking ELISA using WNV-specific MAb 3.1112G indicated the presence of antibodies to WNV in all these species. The overall prevalence of antibodies to WNV in Wyoming mammals sampled was 50.8% and site-specific prevalences ranged from 46.7% at UPT and to 69.0% at GBE.

TABLE 1
Summary of seroprevalence for flaviviruses and West Nile virus (WNV) in mesopredators, 2003

Species	Arizona			California			Louisiana			Texas			Wyoming		
	Flavi.* (%)	WNV† (%)	No.	Flavi.* (%)	WNV (%)	No.	Flavi.* (%)	WNV (%)	No.	Flavi.* (%)	WNV (%)	No.	Flavi.* (%)	WNV (%)	No.
Didelphis virginianus (Virginia opossum)	0	0	1	70	0	10	38	17	65	24	10	21	100	100	1
Vulpes vulpes (red fox)				0	0	1									
Urocyon cinereoargenteus (gray fox)										17	0	6			
Procyon lotor (raccoon)				65	0	20	30	10	10	35	0	40	84	16	32
Mephitis mephitis (striped skunk)	56	0	9	42	0	47	12	0	25	22	0	9	68	63	89
Mephitis macroura (hooded skunks)	75	0	4												
Felis rufus (bobcat)				100	0	1									
All species	57		14	52	0	79	31	12	100	29	3	76	73	51	122

* Monoclonal antibody (MAb) 6B6C-1 detects antibodies to all flaviviruses.
† MAb 3.1112G is specific in its ability to detect antibodies against WNV.

Plaque-reduction neutralization test. Positive WNV antibody results were based upon traditional PRNT serology (e.g., $PRNT_{90}$ WNV antibody titer at least four-fold greater than that of its corresponding SLEV titer and plaque formation neutralized at a defined serum dilution of ≥ 1:10). Raccoon (n = 63), striped skunk (n = 16) and opossum (n = 2) samples were tested by PRNT. The $PRNT_{90}$ results are shown in Table 2. Of 80 mesopredators positive for antibodies to flavivirus by epitope-blocking ELISA, 20 had sufficient titers (40–320) to indicate exposures to SLEV (i.e., $PRNT_{90}$ SLEV antibody titer at least four-fold greater than that of its corresponding WNV titer and plaque formation was neutralized at a defined serum dilution of ≥ 1:10), and 47 mesopredators positive for antibodies to WNV by blocking ELISA were confirmed as WNV antibody positive by $PRNT_{90}$ with titers of 40 to 320. Twelve sera samples could not be categorized as either WNV antibody positive or SLEV antibody positive and were therefore considered positive for antibodies to flavivirus. One sample showed no reactivity to flaviviruses by blocking ELISA and $PRNT_{90}$ and thus was considered negative for antibodies to flavivirus.

DISCUSSION

High prevalence rates of antibodies to WNV were previously detected in mesopredators (e.g., opossums and raccoons), albeit from small sample sizes.[15] We focused on a wider variety of mesopredator species and examined larger sample sizes. High flavivirus prevalence rates suggest mesopredators are commonly exposed to one or more flaviviruses. Data indicating the occurrence of flaviviruses not identified as WNV or SLEV are of interest. Several flaviviruses, such as those assigned to the Modoc antigenic complex (Cowbone Ridge, Sal Vieja, San Perlita, and Modoc viruses) were all first isolated in mammals (rodents from Florida and the western United States).[22] Although little fieldwork has been conducted on mesopredator-associated flaviviruses, some mesopredators positive for antibodies to flavivirus sampled in this study could have been exposed to one or more of the Modoc antigenic complex viruses or to an undescribed flavivirus.

In the states studied, the presence of antibodies to WNV in mesopredators during 2003 and 2004 reflected the overall presence of WNV across the country. In California, no serologic evidence of WNV was reported to the Centers for Disease Control and Prevention (CDC) until late August 2003[23] (California mesopredator sampling concluded in late July). Mosquito[24] and avian[25] samples submitted in counties immediately adjacent to the California study site (e.g., Del Norte, Humboldt, and, Shasta Counties) did not indicate the presence of WNV in the region during 2003. Likewise, there were no reports of WNV in Arizona at the time mesopredators were sampled (mid-spring). Evidence of WNV was not observed in Arizona until early August 2003 (WNV-positive mosquito pools).[26] No WNV-seropositive mesopredators were collected in Arizona or California.

WNV was well established in Texas by the summer of 2003.[27] Of the 76 mesopredators sampled, 2.6% (all Virginia opossums) had antibodies to WNV. Similarly, in Louisiana, where the first human case of WNV-related disease was reported to the CDC in 2001,[28] mesopredators sampled in 2003 showed a prevalence rate of 12.0% for antibodies to WNV. In Wyoming, reports of WNV in humans began to appear in early August 2003.[26] The presence of antibodies to WNV in striped skunks coincides with these reports. Mesopredator sampling was carried out in early to mid-August and mid-November in 2003, and the resulting high prevalence rates are striking.

The CDC began receiving reports from Ohio of WNV in humans in August 2002.[29] Therefore, the presence of antibodies to WNV in mesopredator samples from Ohio (2003 and 2004) was not unexpected. The number of human WNV-related cases reported in Ohio in 2003 (n = 108)[30] was greater than the number reported in 2004 (n = 12).[31] Prevalence rates for antibodies to WNV in raccoons sampled in Ohio appear to reflect this trend. Specifically, 45.6% of our 2003 sample tested positive for antibodies to WNV, in contrast to 17.6% in 2004. Using antibody prevalence for subadults only (because antibody prevalence in subadults reflects exposure in naive individuals), apparent transmission of WNV in raccoons was lower in 2004 relative to 2003 (5.8% versus 24.1%, respectively; $z = 2.96$, $P = 0.003$). However, the apparent transmission of other, unknown or unidentified flaviviruses was similar for those years, (24.1% in 2004 versus 17.5% in 2003 $z = 0.98$, $P = 0.325$).

If exposure to flaviviruses had no effect on survivorship or capture probability and exposure was generally continuous throughout the spring and summer, it is expected that the antibody prevalence should be cumulative and should in-

TABLE 2
Summary of serologically positive mesopredators tested by PRNT*

Percentage inhibition by blocking ELISA using MAb 3.1112G (blocking ELISA result)†	No. WNV antibody-positive by $PRNT_{90}$ (titer range)‡	No. SLEV antibody-positive by $PRNT_{90}$ (titer range)§	Results		
			WNV antibody positive	SLEV antibody positive	Undetermined flavivirus antibody positive
10–19 (negative)	0	1 (> 320)	0/1	1/1	0/1
20–29 (negative)	0	20 (40–320)	0/27	20/27	7/27
30–39 (positive)	30 (40–> 320)	0	30/35	0/35	5/35
40–49 (positive)	2 (160)	0	2/2	0/2	0/2
50–59 (positive)	3 (80–160)	0	3/3	0/3	0/3
60–69 (positive)	4 (80–160)	0	4/4	0/4	0/4
70–79 (positive)	3 (160)	0	3/3	0/3	0/3
80–89 (positive)	4 (40–> 320)	0	4/4	0/4	0/4
90–99 (positive)	1 (> 320)	0	1/1	0/1	0/1

* PRNT = plaque-reduction neutralization test; ELISA = enzyme-linked immunosorbent assay; MAb = monoclonal antibody; WNV = West Nile virus; SLEV = St. Louis encephalitis virus.
† Samples with percentage inhibitions values ≥ 30 for MAb 6B6C–1 (flavivirus antibody specific). MAb 3.1112G is WNV antibody specific.
‡ Serum was etiologically diagnosed as WNV antibody positive if the $PRNT_{90}$ WNV antibody titer was at least four-fold greater than that of the SLEV antibody titer.
§ Serum was etiologically diagnosed as SLEV antibody positive if the $PRNT_{90}$ WNV antibody titer was at least four-fold greater than that of the WNV antibody titer.

crease as a function of time. This was not the case for subadult raccoons in Ohio (Figure 3). Perhaps exposure to flaviviruses was not continuous throughout spring and summer or perhaps flavivirus exposure did affect survivorship or capture probability or both. Also, if serum antibody levels waned as the year progressed, an accumulation of antibodies in the raccoon population may have not been perceptible.

Animals sampled in Wyoming had a WNV antibody prevalence rate of 50.8% among all species sampled, and a 62.9% WNV antibody prevalence rate among striped skunks. An affinity for arboreal habitats that would place a mesopredator in close proximity to arthropod vectors of WNV might help explain high WNV antibody prevalence rates. Both raccoons and opossums are known to use trees; however, striped skunks are poor climbers.[32] Still, association with wetland areas could bring skunks into frequent contact with arthropod vectors. McLean and others isolated SLEV from the widespread North American tick *Dermacentor variabilis* removed from a raccoon and, in the same study, neutralizing antibodies against SLEV were detected in another raccoon, two Virginia opossums, and 10 rodents from the same area identifying a possible tick-mesopredator pathway for SLEV.[33] It is possible that WNV could follow a similar pathway in mesopredators although WNV vector competency in *D. variabilis* appears to be unlikely. West Nile virus has also been recovered from other ticks[34–36] and has been transmitted under laboratory conditions,[37] raising speculation that these hematophagous arthropods could be implicated in the trafficking and survival of WNV.[33] Additional research is needed to determine the role of alternative WNV vectors, such as ticks, especially in historically arid regions where flavivirus transmission by mosquitoes would not be expected to readily occur.

Studies with raptors[38] and cats[39] showed evidence of seroconversion after consumption of WNV-infected animals. Thus, Root and others have suggested that partially carnivorous habits in mesopredators could make them susceptible to an additional predator-to-prey mode of transmission.[15] A similar oral route has also been observed in golden hamsters (*Mesocricetus auratus*).[40] If mesopredators were to consume WNV-infected prey or carcasses with sufficient virus, this mode of transmission could lead to increased WNV prevalence rates in these species. Striped skunks, for example, are known to consume bird eggs, mice, shrews, small rabbits, and small reptiles.[41] It is possible that skunks, and perhaps other mesopredators, could become infected through the consumption of viremic prey species or other food, including dead birds (e.g., corvids) with high WNV viremias. Of note, greater sage grouse (*Centrocercus urophasianus*) populations in Wyoming were significantly impacted by WNV in 2003, resulting in 25% decreases in survival in four populations.[42] Mesopredators encountering sick or dead birds could potentially have been exposed to WNV. However, antibody prevalence rates among skunk prey species remain undetermined, and additional surveillance is warranted.

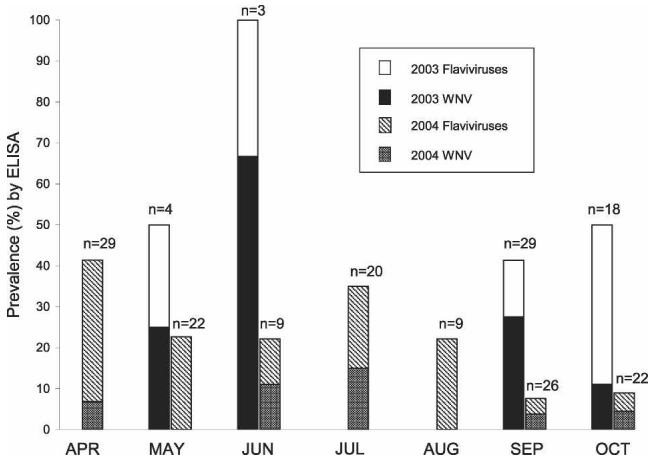

FIGURE 3. Prevalence of antibodies against flaviviruses and West Nile virus (WNV) in subadult raccoons sampled in Ohio 2003 and 2004. ELISA = enzyme-linked immunosorbent assay.

Life history characteristics may have also played a role in the high WNV antibody prevalence rates. Striped skunks aggregate over winter in natural dens or in other human-made spaces and live in family groups (females with young) through the summer.[41] If striped skunks are capable of shedding WNV in sufficient quantities, gregariousness could facilitate horizontal transmission and play a role in virus transmission. It has been demonstrated that golden hamsters develop chronic renal infections and are capable of shedding WNV in urine for up to five months after infection.[43] It has also been demonstrated that crows (*Corvus brachyrhynchos*), in regular close contact with WNV-infected crows, succumb to WNV infection.[44] More recently, Klenk and others provided evidence of a fecal to oral WNV transmission route within American alligators *(Alligator mississippiensis*) in a population-dense experimental environment.[45]

Serologic evidence of flaviviruses was found in all study areas. Among the striped skunks, opossums, and raccoons studied from a variety of geographic areas, high antibody prevalence rates were noted. Compared with flaviviruses, prevalence rates for WNV were reduced, suggesting the possibility of exposure to one or more different and perhaps unknown flavivirus. Isolation and description of unknown flaviviruses in mesopredator species, as well as an understanding of the vectors involved, could shed light on the uncertainty generated by using the flavivirus-generic MAb 6B6C-1 in the epitope-blocking ELISA. We have provided evidence of WNV exposure in seven species of mesopredators from North America. Although our findings varied across states and among sites, antibody prevalences mirrored a number of independent indicators of WNV activity. These data suggest that mesopredator species hold promise as surveillance animals of WNV activity. Since mesopredators are widespread and their ecologies are diverse, surveillance using these species could be conducted across wide geographic ranges and ecologic niches in North America. Although these data provide a better understanding of exposure to WNV, more information on the nature of the disease and the transmission dynamics in these species is needed.

Received May 22, 2006. Accepted for publication September 6, 2006.

Acknowledgments: We thank Heather Sullivan (National Wildlife Research Center) for technical assistance in the laboratory, Craig Acres and Michael Pipas (Wyoming Wildlife Services), David Ruid (Louisiana Wildlife Services), Patrick Smith (California Wildlife Services), Denise Ruffino (Texas Wildlife Services), and Chad Heuser (Arizona Wildlife Services) for field and logistical assistance. The comments and suggestions from two anonymous reviewers improved earlier versions of this manuscript.

Financial support: This study was supported by the United States Department of Agriculture.

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