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Abstract. After the 2001 occurrence of West Nile virus (WNV) in Wisconsin (WI), we collected sera, during 2003–2006, from south-central WI mesopredators. We tested these sera to determine WNV antibody prevalence and geometric mean antibody titer (GMAT). Four-fold higher antibody prevalence and 2-fold higher GMAT in 2003–2004 indicated greater exposure of mesopredators to WNV during the apparent epizootic phase. The period 2005–2006 was likely the enzootic phase because WNV antibody prevalence fell to a level similar to other flaviviruses. Our results suggest that, in mesopredators, vector-borne transmission is the primary route of infection and WNV antibodies persist for <1 year. Mesopredators may be sensitive indicators of West Nile virus spill-over into humans and horses. Mesopredator serosurveys may complement dead crow surveillance by providing additional data for the timing of public health interventions. Research is needed to clarify the dynamics of WNV infection in these mammals and their role as potential WNV amplifiers.

West Nile virus (WNV) was first detected in the United States during 1999, and a surveillance system using dead American crows (Corvus brachyrhynchos) was established to detect the virus.1,2 During the next 5 years, the virus spread across the North American continent, with the most intense epidemic occurring during 2002 in the Great Lakes region, then shifting to the western plains and Rocky Mountains in 2003.3,4 West Nile virus was detected in Wisconsin (WI) from an American crow5 in 2001 and surveillance for WNV was initiated throughout the state, with 56 infected birds detected in four WI counties. During 2002, 543 avian, 144 equine, and 48 human WNV cases were detected in 66 of 72 WI counties (http://westnilemaps.usgs.gov). By 2003, WNV had been detected in 70 of 72 WI counties with most of the positive cases detected from mid-August through October (http://www.dnr.state.wi.us). The WI WNV epizootic appeared to peak in 2002 and then decline to enzootic levels in subsequent years (http://westnilemaps.usgs.gov).

We conducted a WNV seroprevalence study in mesopredators for 3 years (2003–2006) in south-central WI (Dane and Iowa counties). These counties were found to be positive for WNV beginning in 2002. The data collected from the fall of 2003 to the spring of 2004 likely represented an ongoing epizootic of WNV in WI and was previously reported.6 The purpose of this work is to evaluate the exposure of mesopredators in two likely enzootic WNV years (fall of 2004 to spring of 2006), compare those results with previously reported epizootic data in WI and other states, and consider whether these animals are a suitable indicator of WNV activity.

From October through April during 2004–2005 and 2005–2006, we obtained serum samples from 408 common mesopredators; 167 raccoons (Procyon lotor), 128 Virginia opossums (Didelphis virginiana), and 113 coyotes (Canis latrans) collected in the same manner used during 2003–2004 by trapping, shooting, or fresh road kill. These animals were collected in rural areas consisting of small woodlots, agricultural fields, and roadides. Serum samples were collected by absorption into Nobuto strips (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). They were prepared, stored, and tested for antibody at the U.S. Geological Survey, National Wildlife Health Center, Madison, Wisconsin.

The flaviviruses are divided into serologic complexes based on cross reactivity in serum neutralization tests.7 West Nile virus belongs to the Japanese encephalitis virus (JEV) antigenic complex along with JEV, Saint Louis encephalitis virus (SLEV), and others. In our serologic testing, antibody was determined to be WNV specific if the titer against WNV was ≥4-fold more than the titer against SLEV and vice versa. If a ≤4-fold SLEV and WNV titer difference was noted, the serum antibody was considered to be a result of non-specific exposure to a previously described, and cross-reacting or a yet to be recognized flavivirus (FV).8

Mesopredator WNV antibody prevalence during 2003–2006 (Table 1) was 10.5% (65/616) similar to FV antibody prevalence of 7.6% (47/616) and in agreement with previous studies further demonstrating that raccoons, coyotes, and opossums are included in the host range of WNV.8,9 We found no trend in WNV antibody prevalence by month of collection (χ² = 0.02, P = 0.89), species (χ² = 2.47, P = 0.29), or gender (χ² = 1.04, P = 0.31) of the mammals tested from our study area. However, our results suggest (χ² = 2.88, P = 0.09) a 1.4-fold (95% confidence interval [CI] = 0.94 to 2.05) higher rate of WNV antibody prevalence in juveniles than in adults in contrast to patterns reported in eastern gray squirrels (Sciurus carolinensis).10 There were no significant trends in FV antibody prevalence by month of collection (χ² = 0.01, P = 0.93), species (χ² = 0.97, P = 0.38), age (χ² = 0.53, P = 0.47), or gender (χ² = 0.01, P = 0.95) of the mammals tested.

We found that WNV antibody prevalence was 4.4-fold higher (χ² = 30.5, P < 0.001) at 22% (45/208) in 2003–2004 than in the subsequent 2 years (5% = 20/408). Our results also suggest that FV antibody prevalence was nearly 2-fold higher (χ² = 5.13, P = 0.08) in 2003–2004 (11% = 23/208), compared with the following 2 years (6% = 24/408). During 2003–2004, the animals we sampled were more likely (P > 0.01) to be exposed to WNV (22%) than to FV (11%). Our data revealed that overall WNV antibody prevalence was lower and stable during 2004–2005 (5%) and 2005–2006 (5%), and similar to FV antibody prevalence (5% and 7%, respectively) indicating that the

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animals were as likely to be exposed to WNV as FV. Similar temporal patterns in WNV antibody prevalence were noted among birds in Illinois from 2001–2004 and white-tailed deer in Iowa from 1999–2003. Lillibridge and others reported that WNV antibody prevalence (56.5%) was much higher than SLEV (3.9%) among stray dogs sampled 1 year after WNV appeared in Harris County, Texas. In addition, they reported a WNV antibody prevalence of 31.2% and a much lower SLEV antibody prevalence (1.4%) among birds in Harris County, Texas. Data for subsequent years were not reported.

We also compared the geometric mean antibody titers (GMAT) of WNV and FV during our study (Table 2). The WNV GMAT in 2003–2004 was 2-fold higher at 186.6 (F\textsubscript{2.57} = 2.57, \(P = 0.04\)) compared with 72.5 and 89.0 in 2004–2005 and 2005–2006, respectively. It appeared that opossums (F\textsubscript{2.57} = 2.63, \(P = 0.08\)) had higher WNV titers than raccoons or coyotes. There were no trends in WNV GMAT for the month of collection (F\textsubscript{1.39} = 1.39, \(P = 0.94\)), or gender (F\textsubscript{1.57} = 1.57, \(P = 0.85\)). In contrast, FV GMAT was 62.9 in 2003–2004, 49.2 in 2004–2005, and 59.4 in 2005–2006. The level of activity may depend upon a number of factors, such as the frequency of susceptible animals in the mesopredator population, infection rates, and population levels of the vectors, titer of transmitted virus, viremia levels in infected mesopredators, and consequences of infection on the mesopredator population.

Our results also indicate that WNV antibodies persist for at least 3 months in opossums and for at least 6 months in raccoons and coyotes. We assume that WI mesopredators, like wild birds, could be exposed to vector-borne WNV until the hard frosts of October and to exposure by ingestion at other times. West Nile virus antibody was detected in opossums obtained in December and raccoons and coyotes obtained in March. It has been reported that WNV antibody was maintained for 36 months in pig-tailed macaques (Macaca nemestrina), domestic pigs for more than 3 years, humans for ≥ 2 years, and New York horses for at least 15 months. The length of WNV antibody persistence is difficult to determine from our data because collections were limited by the trapping and hunting seasons, and animals were not serially sampled. Our data strongly suggest that specific WNV antibodies in raccoons, opossums, and coyotes do not last substantially longer than 1 year, because we found that specific antibody prevalence significantly declined in 2004–2005 and 2005–2006. We recommend further research to determine the persistence of WNV antibody in these and other mammalian species.

Our study likely started during the WNV epizootic period in WI and other Midwestern states. Prevalence then declined to apparent enzootic levels by 2004–2006. The FV antibody prevalence and GMAT appeared to remain at enzootic levels during 2003–2006 suggesting consistent cross reaction between WNV and FV. Because our serosurvey data in mesopredators are similar to the apparent epizootic and enzootic cycle of WNV in Wisconsin and other Midwestern states, this group of animals should be included in WNV surveillance to provide additional information for public health interventions.

Wild birds are an important WNV reservoir and current surveillance methods, using dead crows and other corvids, provide a reliable method for early detection of infectious levels of vector-borne WNV circulating in an area. Similarity in antibody prevalence among mesopredators with divergent feeding habits suggest a common source of infection, such as vector-borne transmission, which is also shared with birds, humans, and horses. Unlike avian sentinels, WNV antibody prevalence and titers in mesopredators may help quantify the risk of WNV spillover into humans and horses because mesopredators cannot alter their behavior to reduce exposure (humans)

### Table 2

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*No positives.
or be vaccinated (horses). In addition, other routes of infection among mesopredators, including predation and scavenging, deserve further investigation.

To facilitate the interpretation of seroprevalence data, further research is needed on the time period for WNV seroconversion, the persistence of WNV titers in mesopredators, the levels and longevity of WNV viremia, the effect of WNV on these species, possible variations in WNV prevalence between urban and rural landscapes, and whether these species could be used to evaluate how ecologic factors such as climate patterns and reservoir dynamics influence WNV transmission. Of particular interest is the potential role these common mammals play, as WNV amplification hosts, in close association with humans in both urban and suburban environments.

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