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## Prevalence of West Nile Virus Antibodies in a Breeding Population of American Kestrels (*Falco sparverius*) in Pennsylvania

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**ABSTRACT:** West Nile virus (WNV) has been identified in nearly 300 species of wild birds, including raptors, in North America since its introduction in New York City in 1999. American Kestrels (*Falco sparverius*) are susceptible to WNV infection, and the numbers of these birds have declined along the Atlantic coast in recent years. We examined the population biology and WNV exposure of kestrels breeding in the area surrounding Hawk Mountain Sanctuary in Kempton, Pennsylvania, USA. The reproductive biology of kestrels in this area was studied from 1992 until 2004. The number of kestrels breeding in nestboxes in 2004 was only 44% of the 6-yr mean observed prior to 1999. During the 2004 nesting season (study period: 8 June through 22 July 2004), adult kestrels were trapped near the site of their nestboxes. Blood samples were obtained, and serum antibodies specific to WNV were quantified using a plaque reduction neutralization test. Of 22 birds tested, 21 exhibited serum antibodies to WNV, suggesting that most (95%) of the adult kestrels in the population had been exposed to WNV.

**Key words:** American Kestrel, *Falco sparverius*, raptor, seroprevalence, West Nile virus.

Nearly 300 species of native and exotic birds have been reported to the Center for Disease Control and Prevention avian West Nile virus (WNV; family *Flaviviridae*) mortality database since the virus was introduced into North America in 1999 (CDC, 2006). Birds vary in their susceptibility to WNV infection and clinical course of infection; symptoms include neurologic disorders, anorexia and depression, rapid weight loss, and death (Fitzgerald et al., 2003; D'Agostino and Isaza, 2004; Wünschmann et al., 2005). Raptors are susceptible to the effects of the infection, and a number of recent studies have examined WNV-induced pathology in naturally and experimentally

infected raptors (Komar et al., 2003; Wünschmann et al., 2005; Gancz et al., 2006; Nemeth et al., 2006), but few studies have examined WNV prevalence in wild raptor populations (Stout et al., 2005).

The American Kestrel (*Falco sparverius*) is a small raptor that is widely distributed across North America. Kestrels, which nest in natural cavities, also nest in manmade nestboxes, and this has facilitated the study of their reproductive biology for a number of years (Katzner et al., 2005). As a result, population data are available for kestrels from both before and after the appearance of WNV. A reduction in kestrel numbers has been observed along the Atlantic Coast in recent years, and a number of possible explanations have been suggested for this decline. These include habitat loss, organophosphate poisoning, increased Cooper's Hawk (*Accipiter cooperii*) predation, loss of breeding space due to the decline of the Northern Flicker, and WNV infection (Sullivan and Wood, 2005). West Nile virus has been present in birds and mosquitoes in Pennsylvania since at least 2000 and is widespread in the state, as it was found in all counties of the commonwealth during 2003 (Pennsylvania WNV Surveillance Program, 2006). Our objective was to measure WNV exposure in the American Kestrels breeding in manmade nestboxes in the area surrounding Hawk Mountain Sanctuary (HMS).

The study site consisted of a 1,500 km<sup>2</sup> area surrounding HMS (Kempton, Pennsylvania, USA; 40°30'N, 75°50'W), with 140 kestrel nestboxes, mainly in agricultural areas. The kestrels nesting in this

area are partial migrants; some individuals remain in the area year-round. The HMS volunteers monitored activity at the nest-boxes as part of an ongoing study of the reproductive biology of the American kestrel from 1992 until 2004 (for details see Katzner et al., 2005).

Adult birds were trapped from 8 June through 22 July 2004, using either a bal-chatri snare trap baited with mice or with mist nests and an artificial owl set near the nestbox to elicit protective parental behavior. After capture, blood samples (0.6 ml) were taken from the jugular vein and handled as described (Komar et al., 2001). All birds were sexed, weighed to the nearest gram, banded with a uniquely numbered United States Geologic Survey aluminum leg band, and released. Exposure of kestrels to WNV was determined by testing serum samples using plaque reduction neutralization tests (PRNTs), similar to the method used in avian seroprevalence studies following the initial WNV outbreak in New York City (Komar et al., 2001). The serum samples were stored in cryovials at  $-20^{\circ}\text{C}$  until shipment to the New York State Animal Health Diagnostic Laboratory for analysis of WNV and St. Louis encephalitis virus (SLEV) antibodies by PRNT as follows. Serum samples were twofold serially diluted from 1:20 to 1:640 in a 1.0 ml volume of cell culture medium (CCM) (minimum essential medium with Earle's salts, Gibco-Invitrogen, Grand Island, New York, USA), 10% fetal bovine serum (FBS), and 10  $\mu\text{g/ml}$  ciprofloxacin hydrochloride (Bayer, Kankakee, Illinois, USA). Approximately 200 plaque-forming units of WNV were added in a volume of 0.1 ml CCM containing 10% guinea pig complement (Colorado Serum Company, Ft. Collins, Colorado, USA) to each dilution and incubated for 1 hr at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  incubator. Then 100  $\mu\text{l}$  of the virus-serum suspension was overlayed onto a confluent monolayer of Vero cells and incubated for another hour under the conditions described above. Cell mono-

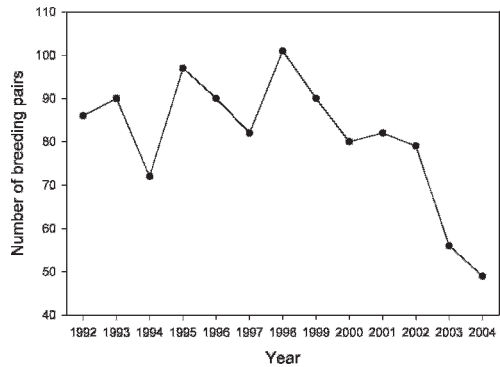


FIGURE 1. Number of breeding pairs of kestrels using HMS nestboxes, which has decreased to approximately 44% of the 6-yr mean observed prior to 1999.

layers were overlayed with CCM containing 2% FBS and 1% low melting point agarose (Invitrogen, Carlsbad, California, USA). Assays were incubated for 3 days as described above. Monolayers were stained overnight by adding three drops of CCM containing 3 mg neutral red/ml (Gibco). Plaques were counted on day 4. Wells were scored positively for neutralization if the number of plaques was less than or equal to the average plaque count at a 1:10 dilution of input virus.

The relationships between WNV antibody titers, body weight (males and females analyzed separately to account for sexual dimorphism), and date of sampling were determined by Pearson correlation. Antibody titers were compared between males and females by *t*-test. Antibody titers of  $\geq 640$  were assumed to be equal to 640 for statistical analysis. All analyses were performed using SPSS (SPSS Inc., Chicago, Illinois, USA), and a *P* value  $\leq 0.05$  was considered significant.

The numbers of breeding pairs vary from year to year, and a sharp decline in population numbers began after 1999 (Fig. 1). As a result, the number of nesting pairs in 2004 was only 44% of the mean number of pairs observed from 1992 to 1998. Nest productivity (number of eggs laid and nestlings fledged) has remained

TABLE 1. Prevalence of antibodies to West Nile virus in American Kestrels nesting near Hawk Mountain Sanctuary, Kempton, Pennsylvania, USA.

Sample	No. negative $\leq 40$	No. with titers of				
		40	80	160	320	$\geq 640$
Male	1	1	1	3	2	0
Female	0	2	1	7	2	2

relatively constant during this time (data not shown).

Twenty-two kestrel blood samples were obtained from 18 of the 49 nestbox sites where birds were observed during 2004 (at two sites both male and female adults were captured). This corresponds to 22% of the population of birds using the nestboxes during the 2004 breeding season. The average weights for the females and males in the study population (males  $110.5 \pm 7.5$  g,  $n=8$ ; females  $125.2 \pm 10.4$  g,  $n=14$ ) were within normal ranges for this species (Smallwood and Bird, 2002).

West Nile virus PRNT analysis resulted in positive antibody titers ranging from 40 to 640 for females and 40 to 320 for males (Table 1). One male tested negative for WNV antibodies. WNV antibody titers did not differ significantly between female and male birds and were not significantly correlated to body weight or sampling date ( $P>0.05$ ). All samples were negative for antibodies to SLEV, indicating that these birds were not exposed to SLEV and that there was no cross-reactivity with SLEV in the PRNT assay.

Ninety-five percent of the adult kestrels we sampled had detectable levels of antibodies. This suggests a high level of exposure to WNV in kestrels nesting at our study site. Seroprevalence studies have been conducted to measure WNV exposure and antibody production in a number of bird species in the United States (Komar et al., 2001; Ringia et al., 2004) and one previous study has measured seroprevalence in raptors (Stout et al., 2005). Data from these studies indicate that WNV seroprevalence in birds is generally less than 50% and is usually

closer to 20% of the population. One notable exception is Cooper's Hawks in Wisconsin, which had a seroprevalence of 88% (Stout et al., 2005). One possibility is that these birds are infected not only by mosquitoes, but also by consuming infected prey. Oral transmission of WNV to carnivores including kestrels has been experimentally documented (Nemeth et al., 2006), and feeding on infected animals has been suggested as an important route of transmission in birds of prey (Garmendia et al., 2000). Many small mammals are seropositive for WNV, including in Pennsylvania (Root et al., 2005), and some species of small birds are thought to serve as reservoirs of WNV (Komar et al., 2003). These animals are common food sources for kestrels (Smallwood and Bird, 2002) and may serve as a source of infection.

Experimentally infected kestrels have not died from WNV, but it has been suggested that the long-lasting effects of the infection could result in death in the wild (Nemeth, 2006). There is evidence that WNV infection may result in high mortality in this species in the wild, as one third of kestrels found dead in upstate New York tested positive for WNV (Chu et al., 2003). The decline in the kestrel populations along the Atlantic coast began years before WNV first occurred in North America (Sullivan and Wood, 2005), suggesting that much of the decline could be due to increased predation or other factors. However, the precipitous decline in kestrel numbers since 1999, the high exposure to WNV indicated by the seroprevalence in this study, and the potential mortality from WNV infection (Chu et al., 2003) suggest that WNV could be

a contributing factor that is hastening the decline. Additional studies to determine the source and timing of infection (during migration or overwintering) may help to determine the dynamics of WNV infection in American Kestrels.

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