

Prevalence and Effects of West Nile Virus on Wild American Kestrel (*Falco sparverius*) Populations in Colorado

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Abstract. To assess the potential impacts of West Nile virus (WNV) on a wild population of free-ranging raptors, we investigated the prevalence and effects of WNV on American Kestrels (*Falco sparverius*) breeding along the Front Range of the Rocky Mountains in northern Colorado. We monitored kestrel nesting activity at 131 nest boxes from March to August 2004. Of 81 nest attempts, we obtained samples from 111 adults and 250 young. We did not detect WNV in sera; however, 97.3% (108/111) of adults tested positive for WNV neutralizing antibodies. In contrast, 10.0% (23/240) of chicks tested positive for WNV neutralizing antibodies, which possibly represented passive transfer of maternal antibodies. Clutch size, hatching, and fledging success in our study did not differ from that previously reported for this species, suggesting that previous WNV exposure in kestrels did not have an effect on reproductive parameters measured in the breeding population we studied in 2004.

Key Words: American Kestrel, Colorado, *Falco sparverius*, nesting, raptor, reproductive success, West Nile virus.

La Prevalencia y los Efectos del Virus del Nilo Occidental en Poblaciones del Cernícalo Americano (*Falco sparverius*) en Colorado

Resumen. Para determinar los impactos potenciales del virus del Nilo Occidental (VNO) en una población de aves rapaces silvestres, estudiamos la prevalencia y los efectos del VNO en cernícalos americanos (*Falco sparverius*) que nidifican a lo largo del rango frontal de las montañas Rocosas en el norte de Colorado. Monitoreamos la actividad de nidificación en 131 cajas nido de marzo a agosto de 2004. De 81 intentos de nidificación, obtuvimos muestras de 111 adultos y 250 juveniles. No se detectó VNO en el suero; sin embargo, el 97.3% (108/111) de los adultos fueron positivos para los anticuerpos de neutralización del VNO. Por el contrario, el 10.0% (23/240) de los polluelos fueron positivos para los anticuerpos de neutralización del VNO, lo cuál pudo representar la transmisión pasiva de los anticuerpos a través de la madre. El tamaño de la nidada, la eclosión, y el éxito de los volantones durante el estudio no fue diferente a lo que se ha reportado previamente para esta especie, sugiriendo que la exposición previa al VNO en cernícalos americanos no tuvo un efecto sobre los parámetros de reproducción que se midieron en la población reproductiva estudiada en 2004.

Palabras Clave: Cernícalo Americano, Colorado, éxito reproductivo, *Falco sparverius*, nidificación, rapaz, virus del Nilo Occidental.

Dusek, R. J., W. M. Iko, and E. K. Hofmeister. 2012. Prevalence and effects of West Nile virus on wild American Kestrel (*Falco sparverius*) populations in Colorado. Pp. 45–54 in E. Paul (editor). Emerging avian disease. Studies in Avian Biology (vol. 42), University of California Press, Berkeley, CA.

The detection of West Nile virus (WNV) in North America raised considerable concern about its effects on wild bird populations. Reports provide evidence that some species have been heavily impacted, including American Crow (*Corvus brachyrhynchos*; Caffrey et al. 2003, Yaremych et al. 2004) and Greater Sage-Grouse (*Centrocercus urophasianus*; Naugle et al. 2004). Other studies analyzing long-term population monitoring data suggest that while some species may be negatively impacted by WNV, others may be unaffected (LaDeau et al. 2007).

Raptors are among the bird groups that are susceptible to WNV. West Nile virus has been detected in at least 34 North American raptor species since its arrival in the Western Hemisphere in 1999 (Nemeth et al. 2006a). While WNV causes morbidity and mortality in numerous species of North American birds, particularly corvids, a health risk to raptors was first documented in 2002. Beginning in the summer and fall of 2002, submissions of sick raptors to rehabilitation centers in the eastern and midwestern U.S. increased substantially (Wünschmann et al. 2004, Joyner et al. 2006, Saito et al. 2007). Investigation into a subset of these submissions concluded that approximately 70% were directly or likely due to WNV infection (Joyner et al. 2006, Saito et al. 2007). In contrast, experimental infections rarely have produced clinical signs of disease or death (Komar et al. 2003; Nemeth et al. 2006a, 2006b). Additionally, WNV antibodies have also been reported from a number of apparently healthy free-living raptors, providing further evidence of their ability to survive infection with this virus (Banet-Noach et al. 2004, Stout et al. 2005, Hull et al. 2006).

In addition to direct mortality brought about by WNV infection, the possibility exists of longer-term effects brought about from infection. Humans with more severe WNV illness can experience fatigue, depression, poor physical health, weakness, and aching that can last for months, and in more severe cases, lifelong neurologic deficits (Rao et al. 2005, Carson et al. 2006, Hayes and Gubler 2006). In birds, this issue is much less understood. Nemeth et al. (2006a) reported a naturally infected Great Horned Owl (*Bubo virginianus*) with mild clinical signs for more than 5 mo while receiving care. Without more detailed studies on free-living and captive raptors, the impacts of this virus relative to raptor populations cannot be completely understood.

To better understand the impacts of WNV on free-living raptors, we initiated a study in 2004 on American Kestrels (*Falco sparverius*) in Colorado. American Kestrels are a common North American raptor with a breeding range that includes much of the continent (Smallwood and Bird 2002). In 2003 in Colorado, kestrels found dead frequently tested positive for WNV (Nemeth et al. 2007) and have previously been reported with WNV antibodies (Medica et al. 2007). However, the short-term and long-term effects of WNV on kestrel populations, including overall survivorship and reproductive success in the wild, are not well understood. In this study, we investigated the prevalence of WNV in a population of American Kestrels; we measured their reproductive success and compared that against baseline data for this species to assess the impacts of (WNV) on a wild population of free-ranging raptors.

METHODS

Nest boxes were monitored from March to August 2004 at multiple sites along the Front Range of the Rocky Mountains in Colorado, from the Denver metropolitan area and north of Fort Collins to the Wyoming border. In the Denver metropolitan area we sampled birds from the following locations: Rocky Mountain Arsenal National Wildlife Refuge (39°49'N, 104°51'W), Barr Lake State Park (39°56'N, 104°45'W), Cherry Creek State Park (39°37'N, 104°50'W), York Street Ponds (39°49'N, 104°57'W), Denver Metro Wastewater Reclamation District (39°48'N, 104°57'W), Riverside Cemetery (39°47'N, 104°57'W), and Aurora Reservoir (39°36'N, 104°39'W). In Northern Colorado we sampled birds at Meadow Springs Ranch (40°54'N, 104°57'W), Rawhide Power Plant (40°51'N, 105°01'W), and in Wellington, Colorado (40°53'N, 105°01'W).

We monitored nest boxes every 10–14 d throughout the breeding season for evidence of nesting activity and to trap and sample adult and nestling kestrels from the box. In addition, we used bal-chatri traps to catch adult birds near nest boxes (McClure 1984, Iko 1991). Nests were checked for activity by closing off the nest box hole and climbing to the box to check for presence of nest cup, eggs, young, or adult kestrels. All adult kestrels were banded with an individually numbered U.S. Geological Survey (USGS)

aluminum band and a unique color band combination. Chicks were initially banded with a temporary color band that was removed and replaced with a permanent aluminum USGS band prior to fledging. We also obtained at least one blood sample from all adults and chicks. After sample collection and banding, kestrels were placed back in the box and the nest box hole covered for up to 2 min before reopening. Kestrels captured using a bal-chatri trap were directly released.

At each nest visit the number of eggs or chicks present was recorded. Clutch size was the highest count of eggs made prior to hatching. If hatching did not occur, the nest was recorded as abandoned and that clutch was not incorporated into calculation of mean clutch size. Brood size was determined by direct count of hatched chicks. Fledging success was determined by direct count of chicks that left the nest. If a chick was absent from the nest and known to be ≥ 28 d post-hatching and no evidence of chick remains was found in or around the nest, the chick was considered to have fledged. We compared the reproductive parameters from Denver metropolitan area and northern Colorado using Systat 12 (Systat Software Inc., Chicago, IL).

Approximately 1.0 ml of whole blood was collected by jugular or brachial venipuncture from adult birds and transferred to a labeled centrifuge tube with no additives. In some individual adult kestrels, we obtained blood samples on more than one trapping occasion. However, only results from the first blood sample were included in this study. For chicks, blood samples were similarly collected, but of variable volume so that samples did not exceed 1.0% of body weight. We attempted to serially sample kestrel chicks approximately every 10–14 days. Small volume blood samples (≤ 0.2 ml) were diluted by putting the sample in a cryovial or centrifuge tube containing 0.5–1.0 ml BA-1 diluent (M199 medium with Hank's salts and Tris HCl [with 7.5% sodium bicarbonate] 20% bovine serum albumin, 20% fetal bovine serum, Penicillin-Streptomycin, 100X, and Fungizone) immediately after collection. All samples were stored with frozen ice packs until processed in the laboratory. For processing, blood samples were separated by centrifugation and sera frozen to -80°C . Serum was later shipped to the USGS National Wildlife Health Center (NWHC) on dry ice, where they remained frozen at -80°C until testing. Serum samples were tested for the presence of specific WNV neutralizing antibodies and determination

of reciprocal antibody titers by plaque reduction neutralization test (PRNT; Beaty et al. 1995). West Nile virus antibody positive samples were also tested for specific St. Louis encephalitis virus (SLE) neutralizing antibodies and reciprocal titer determination by PRNT (Beaty et al. 1995). Serum samples with $\geq 90\%$ neutralization of WNV were considered antibody positive. When a serum sample was positive for both WNV and SLE neutralizing antibodies, a four-fold increase in titer of one virus over the other distinguished that virus as the causative etiologic agent of infection resulting in the antibody development. Serum was also tested for WNV by standard plaque assay (Beaty et al. 1995). Appropriate serum, cell, and WNV test dose controls were included in the test.

RESULTS

Of monitored nest boxes, 56% (74/131) were used by kestrels, resulting in 81 nesting attempts—72 first nesting attempts and nine renesting attempts. Two of the first nests were lost from further analysis because one box was knocked down and one box could no longer be checked. Of the remaining first-nest attempts, 74.3% (52/70) successfully fledged at least one young. From these boxes, a total of 111 adults (67 females and 44 males) were captured and sampled for WNV. From the 81 nesting attempts, 260 young hatched, including 103 females, 115 males, and 42 where the sex was unknown because they died or fledged before sex could be determined. Of those that hatched, 224 (86.2%) young successfully fledged.

We obtained serum for all 111 adults and tested for active viral infections and for specific WNV neutralizing antibody. We obtained serum for 241 chicks and tested 240 for WNV specific neutralizing antibody and 224 samples for active viral infections. We were able to obtain an additional 163 samples from recaptured chicks that were also tested for WNV antibody ($n = 162$) and viral infections ($n = 103$). No infectious WNV was detected in 439 (representing 336 individuals) serum samples tested, while all positive control samples yielded virus in the expected quantity. We tested 513 serum samples (representing 351 individuals) for WNV neutralizing antibodies, with 100% (67/67) of the adult females, 93.2% (41/44) of the adult males, and 10.0% (23/240) of chicks testing positive. For adult birds, reciprocal specific WNV neutralizing antibody titers ranged

TABLE 4.1
Specific West Nile virus neutralizing reciprocal antibody titers in American Kestrels
(Falco sparverius), Colorado.

Test subjects	Titer frequency at first capture								
	Neg	20	40	80	160	320	640	1,280	2,560
Adult female (<i>n</i> = 67)	0	1	2	4	25	18	13	3	1
Adult male (<i>n</i> = 44)	3	1	5	9	11	9	5	1	
Chick male (<i>n</i> = 113)	101	8	2		2				
Chick female (<i>n</i> = 101)	92	5	3		1				
Chick unknown (<i>n</i> = 26)	24	2							

from 20 to 2,560, whereas for chicks the range was 20 to 160 (Table 4.1). The chicks testing positive for WNV antibodies represented nine (15.5%, *n* = 58) different nests where chicks were sampled (Table 4.2).

Nesting success for first nests was not significantly different for any of the parameters measured between Denver metropolitan area and northern Colorado sites (Table 4.3; clutch size, $t = -1.58$, $P = 0.121$; brood size, $t = -0.06$, $P = 0.963$; number of fledglings, $t = -0.75$, $P = 0.458$). For all first nesting attempts, mean clutch size was 4.8 (*n* = 58, $SD = 0.49$), mean brood size 4.3 (*n* = 58, $SD = 0.99$), and mean number of fledglings per successful nest 3.77 (*n* = 57, $SD = 1.58$). Nest abandonment of first nests after at least one egg was laid was 25.7% (18/70).

We recorded renesting attempts in nine nest boxes. In those boxes we captured five banded females that had previously attempted nesting (four in their original box and one in a new box not previously used by any birds in 2004); we did not capture the adult female in two of the boxes; and for the two other boxes it was the second nest recorded for the box but with a new, previously unbanded, adult female. Of the five banded females that renested, four fledged at least one nestling; in three cases this represented a second successful nest. The four that successfully renested had WNV neutralizing antibody titers of 160, 160, 320, and 640; and for the one that did not successfully renest, the titer was 160. The two adult females that represented a second nest in a box previously occupied by another female were

unsuccessful nesters and had titers of 320 and 1,280.

DISCUSSION

Our study took place in the spring of 2004, one year following an epizootic and epidemic WNV season in Colorado. We documented a high prevalence of specific WNV neutralizing antibodies in kestrels, but we detected no evidence of ongoing WNV transmission. The high WNV seroprevalence among breeding adult kestrels was likely at least in part a result of the extensive WNV transmission in Colorado in 2003. While WNV was initially detected in Colorado in 2002, in 2003 Colorado had the highest number of WNV human cases in the United States (U.S. Centers for Disease Control, <http://www.cdc.gov/ncidod/dvbid/westnile/surv&control.htm>). The first WNV case detected in Colorado in 2004 was on 30 May in a human, suggesting that limited transmission was occurring among wild birds and mosquitoes previous to this date (ProMED-Mail 2004). West Nile virus antibody persistence is poorly studied but has been documented to persist in wild-caught captive Rock Pigeons (*Columba livia*) for >1 yr and in Fish Crows (*Corvus ossifragus*) for at least 1 yr (Gibbs et al. 2005, Yabsley et al. 2007).

American Kestrels found dead have been tested for WNV as part of annual state WNV surveillance programs. In 2000, 57% (*n* = 14) of kestrel carcasses in New York tested positive for WNV (Bernard et al. 2001). In 2003 in Colorado, 43% (*n* = 42) of kestrel carcasses tested positive for

TABLE 4.2
Relationship of American Kestrel (Falco sparverius) adult- and chick-specific West Nile virus neutralizing antibody titers for family groups with seropositive chicks, Colorado, 2004.

Nest	Adults			Chicks (first capture)				Chicks (second capture)			
	Sex	Titer	Date	Sex	Date	Weight (grams)	Titer	Date	Weight (grams)	Titer	
BL04	F	320	23-Jun	F	17-May	119	20	26-May	131	Neg	
	M	180	16-Apr	F	17-May	118	160	26-May	143	Neg	
				M	17-May	89	20	26-May	123	Neg	
				M	17-May	104	NR	26-May	125	Neg	
				M	17-May	99	NR	26-May	121	Neg	
CC05	F	320	22-Apr	F	1-Jun	95	20	15-Jun	144	Neg	
	M	80	22-Apr	F	1-Jun	83	Neg	15-Jun	132	Neg	
				M	1-Jun	77	20	15-Jun	126	Neg	
				M	1-Jun	79	160	15-Jun	128	Neg	
				M	1-Jun	65	Neg	15-Jun	118	Neg	
CC10	F	640	1-Jun	U	12-Jul	97	20				
	M	320	29-Jun	U	12-Jul	79	20				
				U	12-Jul	87	Neg				
				U	12-Jul	89	Neg				
MS70	F	160	27-May	F	8-Jul	59	40	15-Jul	111	Neg	
	M	40	15-Jun	M	8-Jul	62	20	15-Jul	99	Neg	
				M	8-Jul	47	40	15-Jul	95	Neg	

TABLE 4.2 (continued)

TABLE 4.2 (CONTINUED)

Nest	Adults			Chicks (first capture)				Chicks (second capture)			
	Sex	Titer	Date	Sex	Date	Weight (grams)	Titer	Date	Weight (grams)	Titer	
MS99	F	160	3-May	F	10-Jun	126	20				
	M	80	17-May	F	10-Jun	122	Neg				
				M	10-Jun	109	20				
				M	10-Jun	114	20				
				M	10-Jun	118	Neg				
RMA19NW	F	80	20-Jul	M	4-Aug	82	20	11-Aug	116	Neg	
	M	160	6-Jul	M	4-Aug	75	20	11-Aug	119	Neg	
RMA29SE	F	320	28-Apr	F	24-May	118	20	7-Jun	134	Neg	
				F	24-May	104	40	7-Jun	134	Neg	
				M	24-May	96	40	7-Jun	120	Neg	
				M	24-May	108	Neg	7-Jun	112	Neg	
				M	24-May	74	Neg	7-Jun	114	Neg	
RMA35NW	F	320	27-Apr	F	20-May	81	40	7-Jun	120	Neg	
	M	80	13-Apr	F	20-May	92	Neg	7-Jun	137	Neg	
				F	20-May	95	Neg	7-Jun	134	Neg	
RP40				M	20-May	70	160	7-Jun	110	Neg	
				M	20-May	91	Neg	7-Jun	120	Neg	
	F	160	21-Apr	F	26-May	101	20	9-Jun	131	Neg	
	M	640	5-May	F	26-May	79	Neg	9-Jun	123	Neg	
			F	26-May	97	Neg	9-Jun	139	Neg		
			M	26-May	95	20	9-Jun	127	Neg		

NOTE: Titer results expressed as reciprocal titers. Neg = negative. NR = no result (chick not sampled).

TABLE 4.3
American Kestrel (Falco sparverius) nesting success at Denver metropolitan area and northern Colorado during the 2004 breeding season.

Location	n	Clutch size		Brood size		n fledged chicks	
		\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
Denver metropolitan area	37	4.9	0.49	4.3	1.1	3.9	1.52
Northern Colorado	21	4.7	0.46	4.3	0.78	3.6	1.70 ^a

^an = 20.

WNV (Nemeth et al. 2007). However, little information exists on the numbers of free-ranging kestrels that survive WNV infection. Our data indicate that many wild American Kestrels in Colorado survived infection and developed WNV antibodies. Survival of American Kestrels following infection with WNV is also supported by past research. Our results are similar to those reported in a small breeding population of kestrels in Pennsylvania, where 95% (21/22) of American Kestrels were seropositive for WNV (Medica et al. 2007). Kestrels that were experimentally infected with WNV survived with no clinical signs; however, the sample size was small (Nemeth et al. 2006a).

In addition to assessing WNV among breeding adults, we monitored their nestlings for infectious WNV and WNV neutralizing antibodies. In contrast to the high rate of WNV seroprevalence in adults, we detected low seroprevalence rates in chicks. Due to the lack of evidence of recent WNV infection of individuals within the breeding season (e.g., antibodies only detected in relatively young chicks, no seronegative chicks showed subsequent evidence of seroconversion, no chick first identified as positive was still positive at its second capture, and lack of detection of viremia), antibodies in chicks were likely maternally derived. The observation of higher WNV seroprevalence rates in adults versus their offspring has been corroborated by previous studies. Eighty-eight percent of adult Cooper's Hawks (*Accipiter cooperii*) in southeast Wisconsin were seropositive for WNV but only 2.1% of chicks were seropositive (Stout et al. 2005). In addition, 9.2% of nestling Red-tailed Hawks (*Buteo jamaicensis*) and 12% of nestling Great Horned Owls had detectable WNV antibodies within the same study area (Stout et al. 2005). In serial sampling of individual kestrels in our study, we found none of the chicks that were sero-

positive on the first sampling remained positive on subsequent samplings, which is very strong evidence of maternal antibody transfer (Hahn et al. 2006), and also suggests that detection of antibodies in these chicks may be dependent on how early after hatching chicks are sampled. Maternal antibodies were undetectable in most domestic chicken (*Gallus gallus domesticus*) chicks derived from WNV seropositive hens by 28 d post-hatch (Nemeth and Bowen 2007). In addition, hen sera and egg yolks had similar antibody titers at the time of egg laying, but by 1 d post-hatching, chick serum antibody titers had at least a four-fold (and up to 32-fold) reduction below that of their hens, indicating a sharp drop in detectable titers, which continued through 14 d post-hatching (Nemeth and Bowen 2007). Therefore, detection of maternal antibody transfer among free-living raptors may depend on early sampling of chicks. Stout et al. (2005) sampled chicks beginning at 10 d post-hatching, while we attempted to sample chicks at approximately 7 d post-hatching, when maternal antibodies had potentially waned below detectable levels. Even though maternal antibodies may have been undetectable, they could potentially still offer some level of protection if a chick were infected with WNV, as observed by Nemeth and Bowen (2007) in chickens. At 42 d post-hatching in seven experimentally infected chicks that had previously shown maternal antibodies, three failed to become viremic and the remaining four had viremias of later onset and lower peak levels than their seronegative counterparts (Nemeth and Bowen 2007).

Measurements of reproductive success in American Kestrels are variable depending on geographic location of the population; however, throughout the range of American Kestrels, average clutch size is 4.6 eggs, mean brood size

is 3.5 young, and mean number of fledglings is 3.3 (Smallwood and Bird 2002). Except that our mean number of fledglings was slightly higher (3.7), our findings were similar. In studies on the Cooper's Hawk, Red-tailed Hawk, and Great Horned Owl, WNV did not appear to have a detectable impact on population numbers (Stout et al. 2005). In contrast, Medica et al. (2007) suggested that WNV could be a contributing factor in an observed loss of breeding kestrels in their study area. Compared with the findings of Smallwood and Bird (2002), our study population of American Kestrels did not appear to be negatively impacted by WNV. Additionally, three successful double broodings were recorded in our study population in 2004, supporting the notion that the reproductive efforts of some breeding adults were not hindered by previous WNV exposure (Toland 1985, Smallwood and Bird 2002).

While we observed many healthy seropositive kestrels in the wild, increases in submissions of symptomatic raptors including kestrels and other species diagnosed with WNV have been documented in rehabilitation facilities (Joyner et al. 2006, Saito et al. 2007). Unfortunately, it is difficult to determine the exact reasons for this dichotomy. These observations may represent individuals with an increased susceptibility to this disease (Stout et al. 2005). An increased susceptibility may be the result of a number of factors, including age, co-infection with other disease agents, genetic variability, and species differences. Other reasons may include variable doses of WNV because not all mosquitoes will inject the same quantity of virus into a bird due to interrupted feeding, or, if kestrels are becoming infected with WNV via ingestion of infected prey (Komar et al. 2003), virus levels in prey may vary. In addition, awareness has been heightened by concern about WNV, and the public may be more likely to notify a rehabilitation center when an apparently diseased bird is observed. State surveillance programs have also actively solicited reports of dead birds, especially corvids and raptors. In the end, it is difficult to determine whether the increases noted are actually due to an increase in the number of sick and dead raptors due to WNV infection or simply an increase in awareness and concern in human observers. Although our study may not fully address this dichotomy, these results provide a better understanding of WNV impacts on free-living, breeding populations of

American Kestrels. However, longer-term monitoring of free-ranging bird populations is needed to assess how WNV may impact the overall population in both the short and long term.

ACKNOWLEDGMENTS

We would like to thank our staff and collaborators on this project: H. Mohan, I. LeVan, J. Berven, and A. Skeen; S. Gillihan (Rocky Mountain Bird Observatory), N. Ronan (Rocky Mountain Arsenal National Wildlife Refuge), R. Ryder (Colorado State University), B. Petersen (USDA APHIS), J. Sherpelz and G. Kratz (Rocky Mountain Raptor Program, Colorado State University), W. Andelt (Colorado State University), J. Arington (Cherry Creek State Park), M. Bonnum (Aurora Reservoir), C. Dougal (Riverside Cemetery), D. Meyer (City of Ft. Collins), M. O'Brien (Poudre River Power Authority), R. Rivers (Barr Lake State Park), and F. Smylie (Wellington, CO). We would also like to thank K. Griffin, L. Karwal, and M. Lund for laboratory analysis of samples. C. Franson and N. Nemeth provided helpful comments on earlier versions of this manuscript.

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