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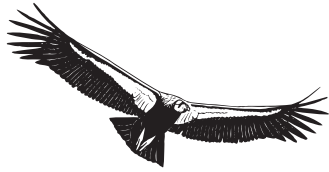
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SHORT COMMUNICATIONS

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WEST NILE VIRUS ANTIBODY SURVEILLANCE IN THREE SIERRA NEVADA RAPTORS OF CONSERVATION CONCERN

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Abstract. West Nile virus (WNV) infection has caused high levels of mortality in North American hawks and owls. To investigate the extent of infection among raptors of conservation concern in the Sierra Nevada, we tested 62 Northern Goshawks (*Accipiter gentilis*), 209 Spotted Owls (*Strix occidentalis*), and 22 Great Gray Owls (*Strix nebulosa*) for WNV antibodies during the summers of 2004 to 2007 and compared our results with avian WNV mortalities detected by the California Department of Public Health. We detected no antibodies to WNV among the individuals tested. During the same period WNV RNA was detected in dead birds from 26 species in the Sierra Nevada region. These results suggest that the populations we studied were not exposed, that the level of WNV infection was so low as to be undetectable by our sampling scheme, or that the mortality rate from WNV was high enough to leave no surviving individuals; there is no independent evidence of the last alternative.

Key words: *Accipiter gentilis*, antibody, Flavivirus, Sierra Nevada, *Strix nebulosa*, *Strix occidentalis*, West Nile virus.

Monitoreo de Anticuerpos del Virus del Nilo Oeste en Tres Rapaces con Categoría de Conservación Preocupante en la Sierra Nevada

Resumen. La infección con el Virus del Nilo Oeste (VNO) ha causado altos niveles de mortalidad en las águilas y lechuzas de América del Norte. Para investigar la magnitud de la infección entre las rapaces con categoría de conservación preocupante en la Sierra Nevada, evaluamos los anticuerpos para el VNO en 62 individuos de *Accipiter gentilis*, 209 de *Strix occidentalis occidentalis* y 22 de *Strix nebulosa* durante los veranos de 2004 al 2007 y comparamos nuestros resultados con los de mortalidad de aves por VNO detectados por el Departamento de Salud Pública de California. No detectamos anticuerpos del VNO entre

los individuos evaluados. Durante el mismo período, se detectó ARN del VNO en aves muertas pertenecientes a 26 especies de la región de la Sierra Nevada. Estos resultados sugieren que las poblaciones que estudiamos no estuvieron expuestas, que el nivel de infección con VNO fue tan bajo como para pasar inadvertido por nuestro esquema de muestreo, o que la tasa de mortalidad por VNO fue suficientemente alta como para no dejar individuos sobrevivientes; no hay evidencia independiente de la última alternativa.

West Nile virus (WNV), a mosquito-borne flavivirus, was first detected in eastern North America during the summer of 1999 (Asnis et al. 1999, Nash et al. 2001) and spread rapidly across the continent, arriving in California in 2003 (Reisen et al. 2004). As WNV spread, it caused significant morbidity and mortality in naïve native birds, particularly of the families Accipitridae, Strigidae, and Corvidae (Komar 2003, Marra et al. 2004). While free-ranging individuals of some of these species respond to WNV with antibodies (Stout et al. 2005, Hull et al. 2006, Crosbie et al. 2008), WNV infection appears to cause near 100% mortality in captive individuals of several species (McLean et al. 2001, Komar et al. 2003, Gancz et al. 2004). In California, birds' susceptibility to WNV varies by species, with several population declines associated with WNV infection, notably in the family Corvidae (Wheeler et al. 2009, Smallwood and Nakamoto 2009).

In the Sierra Nevada region of California, populations of the Northern Goshawk (*Accipiter gentilis*), Spotted Owl (*Strix occidentalis*), and Great Gray Owl (*S. nebulosa*) are of conservation concern (Winter 1980, Seamans et al. 2001, Boyce et al. 2006), and WNV may pose a significant threat to these populations. Among Northern Goshawks naturally infected with WNV, significant lesions of the heart and central nervous system have been documented on post-mortem examination and histopathology (Wünschmann et al. 2005). Among Great Gray Owls, WNV may cause significant hepatic and splenic necrosis, and infection frequently results in sudden death (Gancz et al. 2004, Lopes et al. 2007).

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Our primary objective in this study was to test for immunological exposure to WNV in wild populations of the Northern Goshawk, Spotted Owl, and Great Gray Owl in the Sierra Nevada of California. The samples we collected from Spotted Owls were associated with long-term research to investigate and monitor demographic trends, providing an opportunity for future research into the relationship between WNV prevalence and population trends. The samples from Northern Goshawks and Great Gray Owls were collected as part of short-term projects from 2004 to 2007. Our secondary objective was to compare serology results with WNV mortalities in Sierra Nevada birds documented as part of WNV surveillance.

METHODS

SAMPLE COLLECTION

For testing for antibodies to WNV, we sampled plasma from 62 Northern Goshawks, 209 Spotted Owls, and 22 Great Gray Owls. We captured birds with mist nets, noose poles, and pan traps (Bub 1991) at three study sites in the northern and central Sierra Nevada during the breeding seasons of 2004, 2005, 2006, and 2007 (Table 1). We banded all individuals with U.S. Geological Survey leg bands and released them at the site of capture. We drew approximately 0.5 mL of whole blood via venipuncture of the medial metatarsal vein and separated plasma from whole blood by centrifugation. We stored plasma samples at -20°C before submitting them for testing.

We sampled blood from Northern Goshawks and Spotted Owls at elevations from 800 to 2150 m in the northern Sierra Nevada in the Plumas and Lassen national forests, Plumas and Lassen counties. We sampled Great Gray Owls at elevations from 700 to 2600 m in Yosemite National Park and the Stanislaus National Forest in the central Sierra Nevada, in Calaveras, Tuolumne, and Mariposa counties. In our third and southernmost study area, at elevations 300–3100 m in the Sierra National Forest and Sequoia and King's Canyon national parks, Fresno and Tulare counties, we sampled only Spotted Owls. Vegetation in all three areas is broadly similar, consisting of forest and woodland vegetation types characteristic of the western Sierra Nevada. Lower-elevation habitats consist of oak woodland dominated by Blue Oak (*Quercus douglasii*), Interior Live Oak (*Q. wislizenii*), Canyon Live Oak (*Q. chrysolepis*), and Gray Pine (*Pinus sabiniana*) ranging into pine–oak woodlands dominated by Ponderosa Pine (*Pinus ponderosa*) and Black Oak (*Quercus kelloggii*). Middle elevations are covered with Sierran montane mixed-conifer forest consisting of Ponderosa Pine, Black Oak, White Fir (*Abies concolor*), Sugar Pine (*P. lambertiana*), Incense Cedar (*Calocedrus decurrens*), and Douglas Fir (*Pseudotsuga menziesii*), which grades into upper montane forest consisting of Red Fir (*Abies magnifica*), Lodgepole Pine (*P. contorta*), Jeffrey Pine (*P. jeffreyi*), and Western White Pine (*P. monticola*). Wet and

dry meadows, annual and perennial grasslands, chaparral, and riparian vegetation are interspersed throughout these forests and woodlands (Kuchler 1977).

SEROLOGY

We tested plasma samples by an enzyme-linked immunosorbent assay (EIA) by using flavivirus and crude antigen of western equine encephalomyelitis. We considered samples with a mean optical density $>2\times$ the negative control well positive (Chiles and Reisen 1998). We confirmed all results positive for flavivirus by the EIA with a plaque-reduction neutralization test (PRNT); this test distinguishes between WNV and Saint Louis encephalitis and provides antibody endpoint titers. We distinguished WNV infection from Saint Louis encephalitis on the basis of a $\geq 4\times$ difference in endpoint PRNT titers.

RECORDS OF TESTS POSITIVE FOR WNV RNA

We obtained records of dead birds testing positive for WNV RNA from 2004 to 2007 from the California Department of Public Health WNV surveillance program. The California Department of Public Health tests carcasses of free-ranging birds, reported by the public, that are estimated to be less than 24 hr old and in good condition. The procedure testing for WNV involves sending a sample of kidney tissue to the Center for Vectorborne Diseases at the University of California, Davis, where the presence of viral RNA specific to WNV was tested by a real-time reverse-transcription polymerase chain reaction (RT-PCR) assay (Reisen et al. 2004) with published primers (Lanciotti et al. 2000). California Department of Public Health testing also attempted to isolate the virus from pooled organs of RNA-positive birds by means of a plaque assay on Vero cell culture (Reisen et al. 2004). We retrieved records of dead birds positive for WNV RNA from the California Department of Public Health for 14 Sierra Nevada counties (Alpine, Amador, Calaveras, El Dorado, Fresno, Lassen, Madera, Mariposa, Mono, Nevada, Placer, Plumas, Sierra, and Tuolumne). We used information on cross streets from the California Department of Public Health data and a GIS layer for the Sierra Nevada to determine the elevation for each record. We searched the California Department of Public Health data for records of birds positive for WNV RNA at elevations >900 m, which broadly overlap our study areas.

RESULTS

We found no antibodies to WNV among Northern Goshawks, Spotted Owls, or Great Gray Owls over our 4-yr investigation. The California Department of Public Health had records of 89 WNV-positive dead birds of 26 species at elevations >900 m in 10 Sierra Nevada counties reported during the span of our study (Table 2).

DISCUSSION

While WNV infection in Sierra Nevada avifauna was documented during the study period, we found no antibody response in the populations of the Northern Goshawk, Spotted Owl, or Great Gray Owl we studied. These results were somewhat unexpected, as the California Department of Public Health's reports of dead birds testing positive for WNV RNA included a single Northern Goshawk, one Swainson's Hawk (*Buteo swainsoni*), and four Red-tailed Hawks (*B. jamaicensis*). WNV RNA in these and 23 other avian species suggests that WNV was present in the Sierra Nevada region during our study.

TABLE 1. Numbers of plasma samples tested by year from the Northern Goshawk, Spotted Owl, and Great Gray Owl in the Sierra Nevada, California.

	2004	2005	2006	2007
Northern Goshawk	16	34	12	0
Spotted Owl	103	61	20	25
Southern study area	42	0	10	17
Northern study area	61	61	11	8
Great Gray Owl	0	1	2	19

TABLE 2. Numbers of dead birds (26 species) that tested positive for West Nile virus (WNV) RNA during the summers of 2003–2007. The presence of viral RNA specific to WNV was tested with a real-time assay using a reverse-transcription polymerase chain reaction. Data are from the California Department of Public Health Dead Bird Surveillance Program.

Species	<i>n</i>	Counties
California Quail (<i>Callipepla californica</i>)	2	Alpine
Northern Goshawk (<i>Accipiter gentilis</i>)	1	Plumas
Swainson's Hawk (<i>Buteo swainsoni</i>)	1	Lassen
Red-tailed Hawk (<i>Buteo jamaicensis</i>)	4	Lassen, Nevada, Sierra
Red-breasted Sapsucker (<i>Sphyrapicus ruber</i>)	1	Plumas
Steller's Jay (<i>Cyanocitta stelleri</i>)	30	Alpine, El Dorado, Lassen, Mono, Nevada, Plumas, Sierra
Western Scrub-Jay (<i>Aphelocoma californica</i>)	5	Amador, El Dorado, Lassen
Pinyon Jay (<i>Gymnorhinus cyanocephalus</i>)	1	Mono
Black-billed Magpie (<i>Pica hudsonia</i>)	3	Lassen, Sierra
Common Raven (<i>Corvus corax</i>)	1	El Dorado
Barn Swallow (<i>Hirundo rustica</i>)	2	El Dorado, Plumas
Pygmy Nuthatch (<i>Sitta pygmaea</i>)	1	Mono
American Robin (<i>Turdus migratorius</i>)	9	El Dorado, Lassen, Plumas, Sierra
European Starling (<i>Sturnus vulgaris</i>)	2	Lassen
Yellow-rumped Warbler (<i>Dendroica coronata</i>)	1	El Dorado
Wilson's Warbler (<i>Wilsonia pusilla</i>)	2	Plumas
Western Tanager (<i>Piranga ludoviciana</i>)	3	El Dorado, Plumas, Sierra
Fox Sparrow (<i>Passerella iliaca</i>)	2	Calaveras, Plumas
Golden-crowned Sparrow (<i>Zonotrichia atricapilla</i>)	2	Plumas
Black-headed Grosbeak (<i>Pheucticus melanocephalus</i>)	2	Plumas, Sierra
Brewer's Blackbird (<i>Euphagus cyanocephalus</i>)	3	Mono, Plumas
House Finch (<i>Carpodacus mexicanus</i>)	3	Lassen, Plumas
Lesser Goldfinch (<i>Carduelis psaltria</i>)	4	Lassen, Plumas, Sierra
American Goldfinch (<i>Carduelis tristis</i>)	1	Lassen
Evening Grosbeak (<i>Coccothraustes vespertinus</i>)	2	El Dorado, Lassen
House Sparrow (<i>Passer domesticus</i>)	1	Plumas

The primary route of WNV infection is through the bite of infected mosquitoes, particularly of the genus *Culex* (Bernard and Kramer 2001, Kilpatrick et al. 2006). In California, experimental and field investigations suggest that *C. tarsalis*, *C. stigmatosoma*, *C. erythrothorax*, and *C. pipiens* are the most efficient vectors (Goddard et al. 2002, 2003). The range of these, and other, mosquito species extends into the Sierra Nevada and overlaps with both the dead birds collected by California Department of Public Health and the antibody-negative populations of raptors we examined (Bohart and Washino 1978). In spite of overlap with vector-competent mosquitoes, we did not detect WNV antibodies in any individuals examined.

Previous research suggests that a secondary route of WNV infection is through consumption of infected prey (Komar et al. 2003) and possibly feces (Kipp et al. 2006), making the absence of an indication of WNV infection in the Northern Goshawk particularly notable. In the Sierra Nevada, Northern Goshawks prey on both small mammals and medium-sized birds; Steller's Jays (*Cyanocitta stelleri*) and American Robins (*Turdus migratorius*) are common prey, and both of these species were commonly found dead and tested positive for WNV RNA. This diet contrasts with that of the Spotted Owl and Great Gray Owl, which feed primarily on mammalian prey (Bull and Duncan 1993, Gutiérrez et al. 1995).

Our not detecting WNV antibodies may indicate that the populations we studied had little or no exposure or that the rate of infection was so low as to be undetected by our sampling scheme. Limited sampling may miss rare infections; however we sampled between 10% and 60% of the population of the three species

within our study areas, indicating an absence of WNV infection. Using the same methods, and sampling a much smaller proportion of the total population, Hull et al. (2006) found that 5%–20% of migrating and 15%–58% of wintering Cooper's Hawks (*Accipiter cooperii*), Red-shouldered Hawks (*B. lineatus*), and Red-tailed Hawks from the central coast and Central Valley of California tested positive for WNV antibodies during 2004 and 2005 (Hull et al. 2006).

Alternatively, we may have failed to detect antibodies because of extremely high rates of WNV mortality. If mortality rates due to WNV infection are high, few if any individuals would be expected to survive long enough to mount a detectable immune response. Such high rates of WNV-induced mortality could present a serious threat to the persistence of these populations. In *C. pipiens* and *C. tarsalis*, however, ambient temperature has been associated with WNV amplification, suggesting the relatively cool temperatures in the Sierra Nevada, as compared with the Central Valley of California, may slow WNV transmission and make epidemic disease outbreaks less likely (Dohn et al. 2002, Reisen et al. 2006). While we found 89 records of birds positive for WNV RNA in the California Department of Public Health data from 2004 to 2007, 9120 birds positive for WNV RNA were reported statewide. The majority of these were reported from low elevations in the Central Valley and southern California. This pattern may indicate a lower incidence of WNV in the Sierra Nevada due to lower ambient temperature, a reporting bias toward populated areas, or a combination of the two factors.

Whether absence of infection or extremely high mortality is the underlying cause of the nonappearance of WNV antibodies

in our study cannot be determined with the current WNV antibody data and reinforces the need for additional research in conjunction with monitoring of seroprevalence (Walker et al. 2007, Wheeler et al. 2009). Long-term demographic data from our study area are available for the Spotted Owl and can be examined for evidence of declines in apparent annual survival rates that coincide with the spread of WNV into the Sierra Nevada. There is no current detailed demographic monitoring of the Northern Goshawk or Great Gray Owl with which survival rates could be estimated. During the course of our study, each species' site occupancy remained high, suggesting that if the rate of mortality from WNV is indeed high, the proportion of the population infected annually may be small. Since data on survival rates are lacking, these conclusions should be tempered. In territorial species, continuous occupancy of a site can mask declines in survival if recruitment of floaters is sufficient to replace loss of the territorial individuals, particularly in the short term.

To determine actual rates of WNV infection in these species, future research should focus on estimating survival rates, identifying causes of mortality to determine if WNV infection is resulting in mortality, and whether this potential added source of mortality is a significant threat to a population. Though requiring a consistent annual commitment to logistics and funding, long-term demographic studies provide a framework of baseline information for assessing associations among ecological factors and environmental stressors on the demographic and population trends of focal species of conservation concern. We recommend that demographic studies of the Spotted Owl be continued and that carefully designed demographic studies of the Northern Goshawk and Great Gray Owl be initiated. Long-term demographic studies of these focal species will be of increasing future value for assessing the effects of invasive species (e.g., Barred Owl, *Strix varia*) and exotic infectious diseases (e.g., WNV), as these types of stressors are likely to increase in the future.

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