SHORT COMMUNICATIONS

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VARIATION OF WEST NILE VIRUS ANTIBODY PREVALENCE IN MIGRATING AND WINTERING HAWKS IN CENTRAL CALIFORNIA

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Abstract. To assess the extent of West Nile virus (WNV) exposure of migrating (Marin Headlands) and wintering (Central Valley) hawks in California, plasma from 271 Red-tailed Hawks (*Buteo jamaicensis*), 19 Red-shouldered Hawks (*B. lineatus*), and 30 Cooper's Hawks (*Accipiter cooperii*) was tested for WNV antibodies during the winter of 2004–2005. WNV antibodies were found in 5% of migrating and 15% of wintering Red-tailed Hawks, 20% of migrating and 58% of wintering Red-shouldered Hawks, and 13% of migrating Cooper's Hawks. No individuals demonstrated visible signs of WNV illness. Red-tailed Hawks that tested positive for WNV antibodies displayed no difference from Red-tailed Hawks without WNV antibodies in weight to wing chord ratio or white blood cell counts. In the Central Valley, WNV antibodies were significantly more prevalent in Red-shouldered Hawks than in Red-tailed Hawks. Significantly more Red-tailed Hawks sampled on wintering grounds tested positive for WNV antibodies than Red-tailed Hawks sampled during migration.

Key words: antibody, arbovirus, Flavivirus*, hematology, raptor, Red-tailed Hawk, West Nile virus.*

Variación en la Prevalencia de Anticuerpos del Virus del Nilo Occidental en Halcones Migratorios e Invernales en California Central

Resumen. Con el fin de determinar el grado de exposición al virus del Nilo occidental (VNO) de halcones migratorios (en los Cabos de Marín) y halcones invernales en California (Valle Central), se analizaron plasma de 271 *Buteo jamaicensis*, 19 *Buteo lineatus* y 30 *Accipiter cooperii* para encontrar anticuerpos del VNO durante el invierno de 2004–2005. Se encontraron anticuerpos del VNO en el 5% de los individuos migratorios y en el 15% de los individuos invernales de B. *jamaicensis*, en el 20% de los individuos migratorios y en el 58% de los individuos invernales de *B. lineatus* y en el 13% de los individuos migratorios de *A. cooperii*. Ningún halcón mostró señales de la enfermedad del VNO. Los individuos de *B. jamaicensis* que resultaron positivos para anticuerpos del VNO no mostraron diferencias con los individuos de *B. jamaicensis* que no presentaron anticuerpos en el cociente entre el peso y el largo del ala o en el conteo sanguíneo de glóbulos blancos. En el Valle Central, la prevalencia de los anticuerpos del VNO fue significativamente mayor en *B. lineatus* que en *B. jamaicensis*. El número de individuos de *B. jamaicensis* que resultaron positivos para anticuerpos del VNO fue significativamente mayor en los sitios de invernada que durante la migración.

West Nile virus (WNV) is a mosquito-borne flavivirus (family Flaviviridae) native to the Old World (Hayes 1989) that was first introduced into North America in 1999 (Asnis et al. 1999, Nash et al. 2001) and arrived in the Imperial Valley of California in July 2003 (Reisen et al. 2004, Centers for Disease Control 2005). WNV amplified to epidemic levels in southern California before dispersing to every county in the state by the end of 2004 (Hom et al. 2005). Several families of birds appear to be particularly susceptible to the invading strain of WNV, including Corvidae and Accipitridae (Komar 2003). In raptors, clinical pathology has provided evidence of WNV-

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induced mortality in both owls (Fitzgerald et al. 2003) and diurnal raptors (Wünschmann et al. 2004). Wünschmann et al. (2004) found evidence of WNV-induced mortality in Red-tailed Hawk (*Buteo jamaicensis*) and Cooper's Hawk (*Accipiter cooperii*) carcasses collected in Minnesota. In contrast, Stout et al. (2005) presented evidence of widespread antibody prevalence in apparently healthy Wisconsin populations of these same raptor species. Investigation of additional wild raptor populations may help to determine prevalence of WNV infection and identify potentially adverse effects.

Two locations in California where raptors can be easily captured and surveyed for WNV antibodies are a concentration point north of San Francisco (Binford 1979, Behr 2003) through which thousands of raptors pass during fall migration and the Central Valley, which supports one of the highest concentrations of wintering Red-tailed Hawks in North America (Johnsgard 1990, Preston and Beane 1993). This study investigated whether rates of WNV exposure differed among raptor species and between sampling regions, and whether past WNV infection adversely affected general health. We surveyed for antibodies against WNV in Red-tailed Hawks, Red-shouldered Hawks (*Buteo lineatus*), and Cooper's Hawks during 2004–2005, following an epidemic of WNV in southern California (Hom et al. 2005).

METHODS

SAMPLE COLLECTION

Plasma specimens were collected from hawks captured using bow-net and dho-ghaza traps and mist nets (Clark 1970, Bloom 1987) at the Golden Gate Raptor Observatory in the Marin Headlands (37°50′N, 122°30′W) during fall migration between 15 August and 19 December 2004. Wintering raptors were captured for sampling in the northern Central Valley (38°5′–39°57′N, 121°22′–122°17′W) from 26 November 2004 to 6 March 2005 using bal-chatris placed along roadsides (Berger and Mueller 1959, Bloom 1987, Bub 1991). Sample collection took place after the peak of WNV activity in California, allowing adequate time for raptors to develop antibodies following WNV infection (U.S. Geological Survey 2005). For all individuals we recorded species, age, weight, and wing chord (Pyle 1997). Raptors were banded with U.S. Geological Survey leg bands, a blood sample was taken by medial metatarsal venipuncture, and a general health assessment was performed before release at the site of capture.

SEROLOGY

Plasma samples were screened for antibodies by an enzyme-linked immunosorbent assay using *Flavivirus* and western equine encephalomyelitis antigens (Chiles and Reisen 1998). *Flavivirus*-positive samples were retested using plaque reduction neutralization tests (PRNT) to verify neutralizing antibody presence and to distinguish between WNV and St. Louis encephalitis virus antibodies (Chiles and Reisen 1998). Separation of the latter two viral infections was based upon a fourfold or greater difference in endpoint PRNT titers.

ASSESSMENT OF RELATIVE HEALTH

We used the ratio of weight to wing chord as a measure of general health. A significant decrease in this ratio in individuals positive for WNV antibodies would be suggestive of a detrimental effect resulting from WNV infection. Total white blood cell (WBC) and differential WBC counts (the numbers of different kinds of white blood cells in the blood) of heterophils, lymphocytes, basophils, monocytes, and eosinophils were used as a second estimate of general health. An increased total WBC count may indicate an inflammatory response, elevated levels of heterophils, lymphocytes, and basophils may be associated with infectious agents and antigenic stimulation, and an increase in monocytes and eosinophils may result from tissue necrosis (Campbell 1997). Total and differential WBC counts in Red-tailed Hawks were estimated from blood smears (Campbell 1995).

STATISTICAL ANALYSES

We used a Pearson chi-square test to assess differences in WNV antibody prevalence among Red-tailed Hawks, Red-shouldered Hawks, and Cooper's Hawks in the Marin Headlands. Fisher's exact tests were used to test for differences between Red-tailed Hawks and Red-shouldered Hawks and between juvenile and adult age classes in the Central Valley. Differences in WNV antibody prevalence in Red-tailed Hawks between regions were tested using a Yates corrected chi-square for 2×2 tables. We used the general linear model in SYSTAT version 9 (SPSS 1998) to compare the slopes and intercepts of the weight to wing chord regressions for the Marin Headlands and Central Valley Red-tailed Hawks to determine whether the two regions could be analyzed together or separately. Weight was used as the dependent variable with wing chord and location as independent variables and the interaction term between location and wing chord used to determine if the slopes were different between regions. Following comparison between regions, an effect of WNV antibody status on the slope and intercept of the regression was similarly investigated. Total WBC, heterophil, lymphocyte, basophil, monocyte, and eosinophil distributions were tested for normality using a two-tailed Kolmogorov-Smirnov goodness-of-fit test in SYSTAT. Non-normal distributions were natural log-transformed prior to further analysis. A two-tailed *t*-test was used to test WBC counts between WNV-positive and WNV-negative Red-tailed Hawks. WBC counts are reported as mean $\pm$ SD. Statistical significance was assumed at $P = 0.05$.

RESULTS

SAMPLE COLLECTION

In the Marin Headlands 164 Red-tailed Hawks (156 juvenile, eight adult), five Red-shouldered Hawks (all juvenile), and 30 Cooper's Hawks (28 juvenile and two adult) were sampled. In the Central Valley 107 Red-tailed Hawks (59 juvenile and 48 adult) and 12

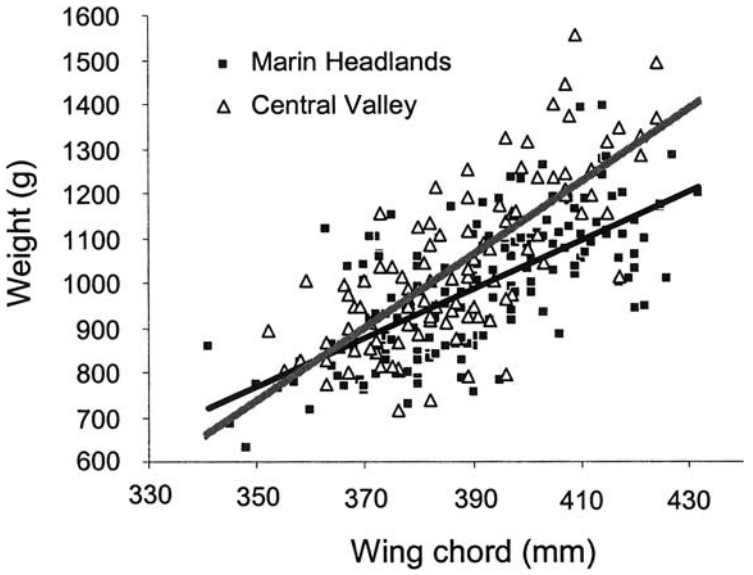

FIGURE 1. Weight versus wing chord regression for Marin Headlands (migrating; black line) and Central Valley (wintering; gray line) Red-tailed Hawks measured in 2004–2005. The slopes of the regressions are significantly different between the two regions.

Red-shouldered Hawks (seven juvenile and five adult) were sampled.

SEROLOGY

Of raptors sampled in the Marin Headlands, 5% (eight juvenile and one adult) of Red-tailed Hawks, 20% of Red-shouldered Hawks, and 13% (three juvenile, one adult) of Cooper's Hawks tested positive for WNV antibodies. In the Central Valley, 15% (10 juvenile and six adult) of Red-tailed Hawks and 58% (four juvenile, three adult) of Red-shouldered Hawks were WNV antibody positive. No hawks tested positive for antibodies to either St. Louis encephalitis virus or western equine encephalomyelitis, which is consistent with statewide surveillance results showing low or no activity of these viruses during 2003 and 2004 (Hom et al. 2004, 2005). No significant differences in antibody prevalence were found between juveniles and adults in the Marin Headlands (Red-tailed Hawks: $P = 0.31$; Cooper's Hawks: $P = 0.24$) or in the Central Valley (Red-tailed Hawks: $\chi^2_1 = 0.1$, $P = 0.71$; Red-shouldered Hawks: $P = 0.44$). In the Marin Headlands, no significant difference in WNV antibody prevalence was observed among species ($\chi^2_2 = 3.7$, $P = 0.16$). In the Central Valley, Red-shouldered Hawks had a significantly greater prevalence of WNV antibodies than Red-tailed Hawks ($P < 0.001$). A significantly greater proportion of Red-tailed Hawks were positive for WNV antibodies in the Central Valley than in the Marin Headlands ($\chi^2_1 = 5.2$, $P = 0.02$).

ASSESSMENT OF RELATIVE HEALTH

The slopes of the weight versus wing chord regressions for Red-tailed Hawks in the Central Valley and the Marin Headlands were significantly different ($F_{1,261} = 11.4$, $P < 0.01$; Fig. 1), therefore the two regions were analyzed separately. In the Central Valley, there were no significant differences between age classes in slope ($F_{1,99} = 0.01$, $P = 0.92$) or intercept ($F_{1,99} = 0.01$, $P = 0.93$). Slopes ($F_{1,158} = 0.3$, $P = 0.58$ and $F_{1,100} = 0.9$, $P = 0.42$) and intercepts ($F_{1,159} = 1.1$, $P = 0.29$ and $F_{1,99} = 0.7$, $P = 0.33$) of the weight to wing chord regressions were not significantly different between individuals that were positive or negative for WNV antibodies in either study area.

The distributions for total WBC count, heterophils, lymphocytes, basophils, monocytes, and eosinophils did not conform to normality ($P = 0.09$, 0.03, 0.001, 0.01, 0.003, and 0.04, respectively); following natural log transformation these distributions were not significantly different from normal. There were no significant differences in total WBC count, heterophils, lymphocytes, basophils, monocytes, or eosinophils between WNV-positive and WNV-negative hawks (Table 1).

DISCUSSION

Antibodies to WNV were found in all raptor species sampled in both geographic regions. The presence of WNV antibodies in outwardly healthy wild raptors indicates that many individuals survived WNV infection. No statistical differences were found in the proportions of individuals positive for WNV antibodies among species tested in the Marin Headlands. In the Central Valley, a significant difference in the prevalence of individuals with WNV antibodies was found between Red-tailed Hawks (16 of 107) and Red-shouldered Hawks (seven out of 12). In addition

TABLE 1. Range and mean total white blood cell counts (WBC) and the numbers of different kinds of white blood cells in the blood of Red-tailed Hawks sampled for West Nile virus during migration and winter in California, 2004–2005. No significant differences were found between West Nile virus (WNV) negative and WNV-positive individuals.

	WNV negative (n = 102)		WNV positive (n = 12)		
Cell type	Range	Mean ± SD	Range	Mean ± SD	P
Total WBC	7000–41 500	16 768 ± 6100	8500–32 000	18 050 ± 7000	0.60
Heterophils	2300–21 995	6842 ± 3500	3132–16 414	7861 ± 3760	0.33
Lymphocytes	2220–18 774	6778 ± 3222	1751–22 400	7510 ± 5555	0.92
Basophils	0–1132	164 ± 186	0–549	167 ± 193	0.96
Monocytes	119–7810	1198 ± 1134	146–2264	868 ± 663	0.37
Eosinophils	351–8550	1723 ± 1203	721–2830	1644 ± 587	0.56

to the differences in sample size, there are several possible biological mechanisms that may explain this observed difference, including varying exposure to WNV vectors due to differences in roosting habitat (Meyer et al. 1991, Preston and Beane 1993, Crocoll 1994), differing rates of exposure based on infection rate in prey species (Komar et al. 2003), and potentially different rates of WNV-induced mortality between species, which may have removed more WNV-positive Red-tailed Hawks from the population prior to sampling. Further investigation is required to distinguish among these possibilities.

The difference in antibody prevalence in Red-tailed Hawks from Marin Headlands and the Central Valley may be partially explained by dissimilar regions of origin. Band recovery data from the Golden Gate Raptor Observatory suggest that the majority of Red-tailed Hawks captured in the Marin Headlands breed north of San Francisco Bay and into British Columbia (AH, unpubl. data), a region that has experienced relatively low WNV activity. In contrast, wintering birds in the Central Valley breed in these northern regions as well as in southern California, where high WNV activity occurred during the summer of 2004 (Bloom 1985, Hom et al. 2005). Similar infection rates between juvenile and adult birds indicate that most infections probably occurred during the summer of 2004.

Comparisons of weight versus wing chord ratio and WBC counts between WNV-positive and WNV-negative Red-tailed Hawks suggest the WNV-positive individuals were in good health with no lingering effects of WNV infection (but see Marra et al. 2004). The significant difference in weight versus wing chord ratio between Red-tailed Hawks sampled in the Marin Headlands and the Central Valley might be the result of the cumulative stress of migration compared to the more sedentary behavior of wintering birds.

As WNV spreads throughout the Pacific Coast region, raptor populations will continue to be at risk of exposure. Further investigation of region of origin for migrating and wintering raptors as well as determination of relative mortality rates may allow further inference to be drawn about species- and population-specific risks.

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LITERATURE CITED

ASNIS, D., R. CONETTA, G. WALDMAN, A. TEIXEIRA, T. MCNAMARA, AND A. FINA. 1999. Outbreak of West Nile-like viral encephalitis, New York, 1999. Morbidity and Mortality Weekly Report 48:845–849.

BEHR, T. 2003. 2002 hawk watch report. Pacific Raptor Report 24:6–10.

BERGER, D. D., AND H. C. MUELLER. 1959. The balchatri: a trap for the birds of prey. Bird-Banding 30:18–26.

BINFORD, L. 1979. Fall migration of diurnal raptors at Point Diablo, California. Western Birds 10:1–16.

BLOOM, P. H. 1985. Raptor movements in California, p. 313–323. *In* M. Harwood [ED.], Proceedings of the hawk migration conference IV. Hawk Migration Association of North America, Kempton, PA.

BLOOM, P. H. 1987. Capturing and handling raptors, p. 99–123. *In* B. A. Giron Pendleton, B. A. Millsap, K. W. Kline, and D. M. Bird [EDS.], Raptor management techniques manual. National Wildlife Federation, Washington, DC.

BUB, H. 1991. Bird trapping and bird banding: a handbook for trapping methods all over the world. Translated by F. Hamerstrom and K.

Wuertz-Schaefer. Cornell University Press, Ithaca, NY.

CAMPBELL, T. 1995. Avian hematology and cytology. 2nd ed. Iowa State Press, Ames, IA.

CAMPBELL, T. 1997. Hematology, p. 88–99. *In* B. W. Ritchie, G. J. Harrison, and L. R. Harrison [EDS.], Avian medicine: principles and application. Abridged. Wingers Publishing, Inc., Lake Worth, FL.

Centers for Disease Control [ONLINE]. 2005. Centers for Disease Control West Nile virus home page. <http://www.cdc.gov/ncidod/dvbid/westnile/> (15 June 2005).

CHILES, R. E., AND W. K. REISEN. 1998. A new enzyme immunoassay to detect antibodies to arboviruses in the blood of wild birds. Journal of Vector Ecology 23:123–135.

CLARK, W. S. 1970. Migration trapping of hawks (and owls) at Cape May, NJ, third year. Eastern Bird Banding Association News 33:181–189.

CROCOLL, S. T. 1994. Red-shouldered Hawk (*Buteo lineatus*). *In* A. Poole and F. Gill [EDS.], The birds of North America, No. 107. The Academy of Natural Sciences, Philadelphia, PA, and The American Ornithologists' Union, Washington, DC.

FITZGERALD, S. D., J. S. PATTERSON, M. KIUPEL, H. A. SIMMONS, S. D. GRIMES, C. F. SARVER, R. M. FULTON, B. A. STEFICEK, T. M. COOLEY, J. P. MASSEY, AND J. G. SIKARSKIE. 2003. Clinical and pathologic features of West Nile virus infection in native North American owls (Family Strigidae). Avian Diseases 47:602–610.

HAYES, C. G. 1989. West Nile fever, p. 59–88. *In* T. P. Monath [ED.], The arboviruses: epidemiology and ecology. CRC Press, Boca Raton, FL.

HOM, A., A. HOUCHIN, K. MCCAUGHEY, V. L. KRAMER, R. E. CHILES, W. K. REISEN, E. TU, C. GLASER, C. COSSEN, E. BAYLISS, B. F. ELDRIDGE, B. SUN, K. PADGETT, L. WOODS, L. MARCUS, L. T. HUI, M. CASTRO, AND S. HUSTED. 2004. Surveillance for mosquito-borne encephalitis activity and human disease, including West Nile virus, in California, 2003. Proceedings of the Mosquito and Vector Control Association of California 72:48–54.

HOM, A., L. MARCUS, V. L. KRAMER, B. CAHOON-YOUNG, C. GLASER, C. COSSEN, E. BAYLIS, C. JEAN, E. TU, B. F. ELDRIDGE, R. CARNEY, K. PADGETT, B. SUN, W. K. REISEN, L. WOODS, AND S. HUSTED. 2005. Surveillance for mosquito-borne encephalitis activity and human disease, including West Nile virus, in California, 2004. Proceedings of the Mosquito and Vector Control Association of California 73:66–72.

JOHNSGARD, P. A. 1990. Hawks, eagles, and falcons of North America: biology and natural history. Smithsonian Institution Press, Washington, DC.

KOMAR, N. 2003. West Nile virus: epidemiology and ecology in North America. Advances in Virus Research 61:185–234.

KOMAR, N., S. LANGEVIN, S. HINTEN, N. NEMETH, E. EDWARDS, D. HETTLER, B. DAVIS, R. BOWEN, AND M. BUNNING. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerging Infectious Diseases 9:311–322.

MARRA, P. P., S. GRIFFING, C. CAFFREY, A. M. KILPATRICK, R. MCLEAN, C. BRAND, E. SAITO, A. P. DUPUIS, L. KRAMER, AND R. NOVAK. 2004. West Nile virus and wildlife. BioScience 54:393–402.

MEYER, R. P., W. K. REISEN, AND M. M. MILBY. 1991. Influence of vegetation on CO_2 trap effectiveness for sampling mosquitoes in the Sierra Nevada foothills of Kern County, California. Journal of the American Mosquito Control Association 7:471–475.

NASH, D., F. MOSTASHARI, A. FINE, J. MILLER, D. O'LEARY, K. MURRAY, A. HUANG, A. ROSENBERG, A. GREENBERG, M. SHERMAN, S. WONG, G. CAMPBELL, J. ROEHIG, D. GUBLER, W. SHIEH, P. SMITH, AND M. LAYTON. 2001. The outbreak of West Nile virus infection in the New York City area in 1999. New England Journal of Medicine 344:1807–1814.

PRESTON, C. R., AND R. D. BEANE. 1993. Red-tailed Hawk (*Buteo jamaicensis*). *In* A. Poole and F. Gill [EDS.], The birds of North America, No. 52. The Academy of Natural Sciences, Philadelphia, PA, and The American Ornithologists' Union, Washington, DC.

PYLE, P. 1997. Identification guide to North American birds, part I. Slate Creek Press, Bolinas, CA.

REISEN, W., H. LOTHROP, R. CHILES, M. MADON, C. COSSEN, L. WOODS, S. HUSTED, V. KRAMER, AND J. EDMAN. 2004. West Nile virus in California. Emerging Infectious Diseases 10: 1369–1378.

STOUT, W. E., A. G. CASSINI, J. K. MEECE, J. M. PAPP, R. N. ROSENFIELD, AND K. D. REED. 2005. Serologic evidence of West Nile virus infection in three wild raptor populations. Avian Diseases 49:371–375.

SPSS. 1998. SYSTAT version 9 user's guide. SPSS, Inc., Chicago.

U.S. GEOLOGICAL SURVEY [ONLINE]. 2005. U.S. Geological Survey West Nile virus maps, 2004. <http://westnilemaps.usgs.gov/2004/ca_bird.html> (15 June 2005).

WÜNSCHMANN, A., J. SHIVERS, J. BENDER, L. CARROLL, S. FULLER, M. SAGGESE, A. VAN WETTERE, AND P. REDIG. 2004. Pathologic findings in Red-tailed Hawks (*Buteo jamaicensis*) and Cooper's Hawks (*Accipiter cooperi*) [sic] naturally infected with West Nile virus. Avian Diseases 48:570–580.