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Serosurvey for West Nile Virus Antibodies in Steller's Jays (*Cyanocitta stelleri*) Captured in Coastal California, USA

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ABSTRACT: West Nile virus (WNV) was first detected in New York in 1999 and, during its expansion across the continental US, southern Canada, and Mexico, members of the Corvidae (ravens, crows, magpies, and jays) were frequently infected and highly susceptible to the virus. As part of a behavioral study of Steller's Jays (*Cyanocitta stelleri*) conducted from 2011–14 in the coastal California counties of San Mateo and Santa Cruz, 380 Steller's Jays were captured and tested for antibodies to WNV. Using the wild bird immunoglobulin G enzyme linked immunoassay, we failed to detect antibodies to WNV, indicating either that there was no previous exposure to the virus or that exposed birds had died.

Key words: *Cyanocitta stelleri*, immunoassay, Steller's Jay, West Nile virus.

Immediately following the introduction of West Nile virus (WNV) into the New York, US area, significant mortality was recognized in birds of the family Corvidae (Steele et al. 2000) and, subsequently, population declines were recorded in corvids (Ladeau et al. 2007; Wheeler et al. 2009). In North America, the Corvidae include ravens, crows, magpies, and jays and in the southwestern US the jays include the Steller's Jay (*Cyanocitta stelleri*) at higher elevations and the Pinyon Jay (*Gymnorhinus cyanocephalus*) and the California Scrub Jay (*Aphelocoma californica*) at lower elevations. Challenge studies with WNV show a range of susceptibility among corvids. American Crows (*Corvus brachyrhynchos*) are more susceptible than are Fish Crows (*Corvus ossifragus*; Komar et al. 2003). Blue Jays (*Cyanocitta cristata*; Komar et al. 2003; Weingartl et al. 2004) and California Scrub Jays are also highly susceptible to WNV (Reisen et al. 2005). Despite evidence of the high susceptibility of corvids to WNV, antibodies to WNV have been confirmed in wild crows, demonstrating that some naturally infected corvids survive infection and develop detectable antibodies to the virus (Wilcox et al. 2007; Reed et al. 2009). All species of jays commonly found in California have been reported as dying from WNV in surveillance conducted by the California Department of Health (Foss et al. 2015). Similar to crows, WNV antibodies have been detected in Blue Jays (Gleiser et al. 2007; Dusek et al. 2009) and California Scrub Jays (Reisen and Wheeler 2016), providing evidence that some naturally exposed jays also survive infection with the virus.

As part of a behavioral ecology study of Steller's Jays conducted at sites in the coastal mountains of San Mateo and Santa Cruz California counties from 2011–14, we tested Steller's Jays for antibodies to WNV. While California Department of Health surveillance for WNV indicated the virus was detected in dead birds in the counties in which the birds were captured, and mosquito vectors known to transmit the virus were also recorded in those counties, WNV antibodies were not detected in any Steller's Jay sample.

Steller's Jays were captured during the breeding season (April through August) using live traps (model 1045, Havahart®, Woodstream Corp., Lititz, Pennsylvania, USA) and mistnets (Avinet, Dryden, New York, USA). Sampling was conducted in state parks from 2011 to 2014 in Santa Cruz County, California and from 2012 to 2014 in San Mateo County, California. We broadcasted jay vocalizations (Vigallon and Marzluff 2005) and placed shelled peanuts near mistnets to attract jays. Birds were marked with a unique leg band and after-hatch-year (AHY) jays were distinguished from hatch-year (HY) jays based on

TABLE 1. The numbers of hatch-year (HY) and after-hatch-year (AHY) Steller's Jays (*Cyanocitta stelleri*) captured and sampled in Santa Cruz and San Mateo counties, California, USA, for detection of West Nile virus antibodies by enzyme linked immunoassay, 2011–14.

	Location			
	Santa Cruz County		San Mateo County	
	Age		Age	
Year	AHY	HY	AHY	HY
2011	105	15	0	0
2012	15	2	4	2
2013[a]	107	26	12	0
2014	67	15	3	0

[a] An additional 10 AHY and 3 HY birds were captured in 2013, but insufficient sera were available for testing.

gape coloration, plumage pattern, the shape of rectrices, and the level of covert contrast (Pyle 1987). We defined AHY jays as those known to have hatched before the calendar year of banding and HY jays as those known to have hatched during the calendar year of banding. The sex was determined through observations of sex-specific vocalizations and tail-length measurements (Pyle 1987) and were later confirmed using polymerase chain reaction methods on DNA extracted from blood samples (Griffiths et al. 1998). Approximately 150 μL of blood was collected from the brachial vein into capillary tubes, transferred into conical tubes, and allowed to clot. Serum was collected following centrifugation and stored at –20 C. All federal, state, and local permits were secured prior to field work, and the project was approved by the University of Wisconsin Madison Animal Use and Care Committee (IACUC-A01424-0-01-10).

Serum samples were heat inactivated at 56 C for 30 min and were tested for WNV antibodies using the WNV enzyme immunoassay (EIA) for wild bird immunoglobulin G (IgG; Ebel et al. 2002), with slight modifications as previously described (Hofmeister et al. 2016). Bound WNV antibody was detected with goat anti-wild bird IgG (Bethyl Laboratories, Montgomery, Texas, USA), which has been shown to bind to corvid IgG (Ebel et al. 2002). A negative control included blue jay sera previously found negative for antibodies to WNV by the plaque reduction neutralization test and a positive control included sera from Chukar Partridge (*Alectoris chukar*) experimentally challenged with WNV at the US Geological Survey National Wildlife Health Center

A total of 373 serum samples was obtained from 386 Steller's Jays captured from 2011–14 in San Mateo and Santa Cruz counties (Table 1). Antibodies to WNV were not detected in any Steller's Jay serum sample by the EIA. Positive and negative control sera performed as expected on the EIA. Surveillance for WNV by the California Department of Health showed that the virus was active in mosquitoes, birds, or humans in both counties from 2011–14, with WNV more active in 2012 and in 2013 when WNV was reported in six and 22 dead birds, respectively (California Department of Health 2011, 2012, 2013, 2014). However, compared to the surrounding counties, which are lower in elevation and have a higher human population, the WNV activity (based on mosquito, human cases, and dead bird WNV surveillance) in San Mateo and Santa Cruz counties was low. For serology to validly detect exposure to infectious disease, four conditions must be met: an opportunity for exposure to the agent, the development of specific antibodies to the agent, survival to the point of blood sampling, and persistence of specific antibodies to the point of sample collection. Additionally, the serologic assay must have the appropriate sensitivity and specificity to detect agent-specific antibodies in the sample (Gilbert et al. 2013).

Several mosquito species known to transmit WNV have been reported in the counties in which Steller's Jays were sampled (Meyer 2003). *Culex pipiens*, *Culex stigmatosoma*, and *Culex tarsalis*, all orthnithophilic feeders, are found at elevations where birds were sampled and are competent vectors for WNV. In contrast, *Culex erythrothorax*, while an opportunistic feeder and competent host for WNV (Goddard et al. 2002; Turell et al. 2005), is primarily active at lower elevations in California. It is likely that mosquito sampling

occurred in more suburban and urban areas of the counties rather than at remote sites. The density of human populations in urban sites potentially results in the increased detection and reporting of dead birds as compared to rural sites (Ward et al. 2006).

Prediction of the survival of WNV-infected Steller's Jays is difficult and there are no reports of experimental WNV studies conducted in the species. While corvids in North America are known to be highly susceptible to WNV, based on field observation and experimental studies, WNV antibody has been detected in apparently healthy wild-caught crows (Wilcox et al. 2007; Reed et al. 2009), Blue Jays (Gleiser et al. 2007; Dusek et al. 2009), and California Scrub Jays (Reisen and Wheeler 2016), indicating some infected corvids can survive infection. Nevertheless, from 2003–12 WNV was detected in 132 of 410 (32%) dead Steller's Jays tested in 18 California counties (Foss et al. 2015). Assuming the Steller's Jays captured over the four study years were from a large population, that exposed birds survived and developed WNV antibodies, and that the EIA was $\geq$90% sensitive, then the current study had a 95% confidence for detecting WNV antibodies at a 0.5–1% prevalence (Sergeant 2016). However, because survival of WNV in Steller's Jays is likely to be $<$50%, the confidence for detection of antibodies to WNV in our sample was $<$95%. We conclude that Steller's Jays captured in this region of California were either unexposed to WNV or that Steller's Jays are more susceptible to the virus than are either Blue Jays or California Scrub Jays, and that all affected birds died from the infection.

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LITERATURE CITED

California Department of Health. 2011. *2011 WNV activity by county*. http://www.westnile.ca.gov.case_counts.php?year=2011&option=print. Accessed January 2017.

California Department of Health. 2012. *2012 WNV activity by county*. http://www.westnile.ca.gov.case_counts.php?year=2012&option=print. Accessed January 2017.

California Department of Health. 2013. *2013 WNV activity by county*. http://www.westnile.ca.gov.case_counts.php?year=2013&option=print. Accessed January 2017.

California Department of Health. 2014. *2014 WNV activity by county*. http://www.westnile.ca.gov.case_counts.php?year=2014&option=print. Accessed January 2017.

Dusek RJ, McLean RG, Kramer LD, Ubico SR, Dupis AP II, Ebel GD, Guptill SC. 2009. Prevalence of West Nile virus in migratory birds during spring and fall migration. *Am J Trop Med Hyg* 81:1151–1158.

Ebel GD, Dupuis AP II, Nicholas D, Young D, Maffei J, Kramer LD. 2002. Detection by enzyme-linked immunosorbent assay of antibodies to West Nile virus in birds. *Emerg Infect Dis* 8:979–982.

Foss L, Padgett K, Reisen WK, Kjemtrup A, Ogawa J, Kramer V. 2015. West Nile virus-related trends in avian mortality in California, USA, 2003–12. *J Wildl Dis* 51:576–588.

Gilbert AT, Fooks AR, Hayman DTS, Horton DL, Muller T, Plowright R, Peel AJ, Bowen R, Wood JLN, Mills J, et al. 2013. Deciphering serology to understand the ecology of infectious diseases in wildlife. *Ecohealth* 10:298–313.

Gleiser RM, Mackay AJ, Roy A, Yates MM, Vaeth RH, Faget GM, Folsom AE, Augustine WF, Wells RA, Perich MJ. 2007. West Nile virus surveillance in East Baton Rouge Parish, Louisiana. *J Am Mosq Control Assoc* 23:29–36.

Goddard LB, Roth AE, Reisen WK, Scott TW. 2002. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis* 8:1385–1391.

Griffiths R, Double MC, Orr K, Dawson RJG. 1998. A DNA test to sex most birds. *Mol Ecol* 7:1071–1075.

Hofmeister EK, Jankowski MD, Goldberg D, Franson JC. 2016. Survey for West Nile virus antibodies in wild ducks, 2004–06, USA. *J Wildl Dis* 52:354–363.

Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Bunning DM. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis* 9:311–322.

Ladeau SL, Kilpatrick AM, Marra PP. 2007. West Nile virus emergence and large-scale declines of North American bird populations. *Nature* 447:710–713.

Meyer R. 2003. *Regional guide to the common mosquitoes of California, I. Coastal California*. Mosquito and Vector Control Association of California, Sacramento, California, 40 pp.

Pyle P. 1987. *Identification guide to North American passerines: A compendium of information on identifying, ageing, and sexing passerines in the hand*. Slate Creek Press, Bolinas, California, 278 pp.

Reed LM, Johansson MA, Panella N, McLean R, Creekmore T, Puelle R, Komar N. 2009. Declining mortality in American crow (*Corvus brachyrhynchos*) following natural West Nile virus infection. *Avian Dis* 53:458–461.

Reisen WK, Fang Y, Martinez VM. 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis Encephalitis virus transmission. *J Med Entomol* 42: 367–375.

Reisen WK, Wheeler SS. 2016. Surveys for antibodies against mosquitoborne encephalitis viruses in California birds, 1996–2013. *Vector Borne Zoonotic Dis* 16:264–282.

Sergeant E. 2016. Epitools epidemiological calculators. *AusVet Animal Heath Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease*. http://epitools.ausvet.com.au. Accessed December 2016.

Steele KE, Linn MJ, Schoepp RJ, Komar N, Geisbert TW, Manduca RM, Calle PP, Raphael BL, Clippinger TL, Larsen T, et al. 2000. Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. *Vet Pathol* 37:208–224.

Turell MJ, Dohm DJ, Sardelis MR, O'Guinn ML, Andreadis TG, Blow JA. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *J Med Entomol* 42:57–62.

Vigallon SM, Marzluff JM. 2005. Is nest predation by Steller's Jays (*Cyanocitta stelleri*) incidental or the result of a specialized search strategy? *Auk* 122:36–49.

Ward MR, Stallknecht DE, Willis J, Conroy MJ, Davidson WR. 2006. Wild bird mortality and West Nile virus surveillance: Biases associated with detection, reporting, and carcass persistence. *J Wildl Dis* 42:92–106.

Weingartl HM, Neufeld JL, Copps J, Arszal PM. 2004. Experimental West Nile virus infection in blue jays (*Cyanocitta cristata*) and crows (*Corvus brachyrhynchos*). *Vet Pathol* 41:362–370.

Wilcox BR, Yabsley MJ, Ellis AE, Stallknecht DE, Gibbs SEJ. 2007. West Nile virus antibody prevalence in American crows (*Corvus brachyrhynchos*) and fish crows (*Corvus ossifragus*) in Georgia, U. S. A. *Avian Dis* 51:125–128.

Wheeler SS, Barker CM, Fang Y, Armijos MV, Carroll BD, Husted S, Johnson WO, Reisen WK. 2009. Differential impact of West Nile virus on California birds. *Condor* 111:1–20.

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