Role of Corvids in Epidemiology of West Nile Virus in Southern California

WILLIAM K. REISEN,[1] CHRISTOPHER M. BARKER, RYAN CARNEY,[2] HUGH D. LOTHROP, SARAH S. WHEELER, JENNIFER L. WILSON, MINOO B. MADON,[3] RICHARD TAKAHASHI,[4] BRIAN CARROLL, SANDRA GARCIA, YING FANG, MARZIEH SHAFII, NICOLE KAHL, SIRANOOSH ASHTARI, VICKI KRAMER,[5] CAROL GLASER,[6] AND CYNTHIA JEAN[6]

Center for Vectorborne Diseases, Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616

J. Med. Entomol. 43(2): 356–367 (2006)

ABSTRACT The invasion of different southern California landscapes by West Nile virus (WNV) and its subsequent amplification to epidemic levels during 2004 enabled us to study the impact of differing corvid populations in three biomes: the hot Colorado desert with few corvids (Coachella Valley), the southern San Joaquin Valley (Kern County) with large western scrub-jay but small American crow populations, and the cool maritime coast (Los Angeles) with a large clustered American crow population. Similar surveillance programs in all three areas monitored infection rates in mosquitoes, seroconversion rates in sentinel chickens, seroprevalence in wild birds, numbers of dead birds reported by the public, and the occurrence of human cases. Infection rates in *Culex tarsalis* Coquillett and sentinel chicken seroconversion rates were statistically similar among all three areas, indicating that highly competent mosquito hosts were capable of maintaining enzootic WNV transmission among less competent and widely distributed avian hosts, most likely house sparrows and house finches. In contrast, infection rates in *Culex pipiens quinquefasciatus* Say were statistically higher in Kern and Los Angeles counties with elevated corvid populations than in Coachella Valley with few corvids. Spatial analyses of dead corvids showed significant clusters near known American crow roosts in Los Angeles that were congruent with clusters of human cases. In this area, the incidence of human and *Cx. p. quinquefasciatus* infection was significantly greater within corvid clusters than without, indicating their importance in virus amplification and as a risk factor for human infection. In contrast the uniform dispersion by territorial western scrub-jays resulted in a high, but evenly distributed, incidence of human disease in Kern County.

KEY WORDS West Nile virus, American crow, western scrub-jay, *Culex tarsalis*, *Culex pipiens quinquefasciatus*

THE FAMILY CORVIDAE OF the order Passeriformes contains six species in California, of which American crows, *Corvus brachyrhynchus;* western scrub-jays, *Aphelocoma californica;* and common ravens, *Corvus corax*, are abundant in southern California at lower elevations historically affected by mosquito-borne encephalitis viruses (Reeves 1990). These species are highly susceptible to infection with West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) and produce elevated viremias during acute infection before death (Komar et al. 2003, Brault et al. 2004, Weingartl et al. 2004, Reisen et al. 2005). Rapid onset and frequent death of corvids because of WNV infection coupled with their large size has facilitated their use as a surveillance tool to track WNV during epidemics (Eidson et al. 2001a; Caffrey et al. 2003; Mostashari et al. 2003; Hom et al. 2004, 2005; Yaremych et al. 2004) and to map its movement westward across the United States (http://westnilemaps.usgs.gov/us_bird.html). However, other bird species such as house sparrows, *Passer domesticus*, and house finches, *Carpodacus mexicanus*, also develop elevated viremias during

The collection, banding and bleeding of wild birds were conducted under Protocol 11188 approved by the Animal Use and Care Administrative Advisory Committee (IACUC) of the University of California, Davis, California Resident Scientific Collection Permit 801009-02 by the State of California Department of Fish and Game, Federal Fish and Wildlife Permit No. MB082812-0, and Master Station Federal Bird Marking and Salvage Permit No. 22763 from the U.S. Geological Survey Bird Banding Laboratory. Special experimental use permits were obtained for bird collection on the Kern National Wildlife Refuge. Maintenance and bleeding of sentinel chickens was done under IACUC Protocol 11186.

[1] Corresponding author, e-mail: arbo123@pacbell.net.
[2] Vectorborne Disease Section, California Department of Health Services, Richmond, CA 94804.
[3] Greater Los Angeles County Vector Control District, Santa Fe Springs, CA 90670.
[4] Kern Mosquito and Vector Control District, Bakersfield, CA 93314.
[5] Vectorborne Disease Section, California Department of Health Services, Sacramento, CA 95899.
[6] Viral and Rickettsial Diseases Laboratory, California Department of Health Services, Richmond, CA 94804.

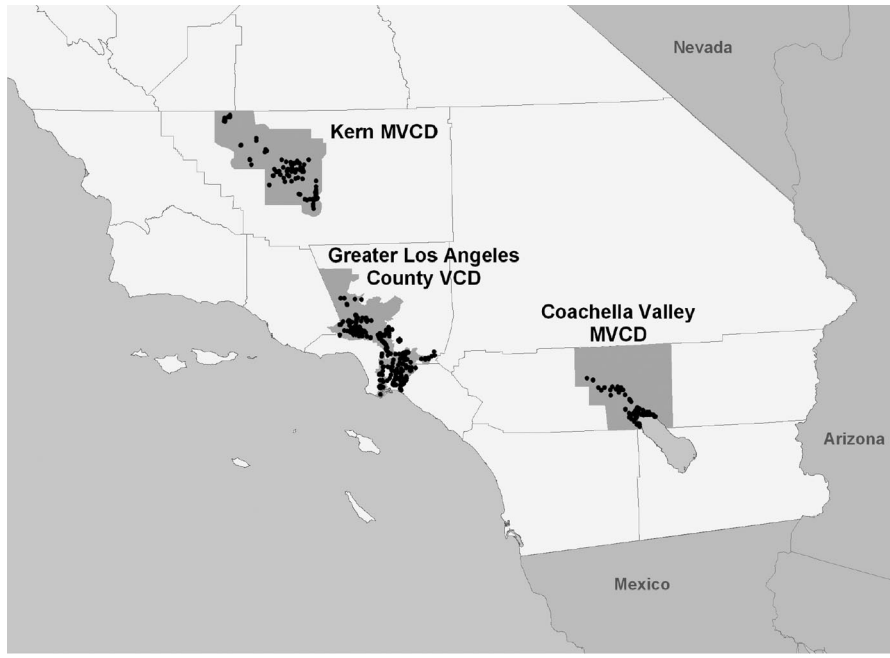

Fig. 1. Three study areas in southern California, with black dots indicating mosquito collection sites for virus testing in each area.

acute infection (Komar et al. 2003, Reisen et al. 2005) and have been considered to be important in WNV epidemiology because of their abundance and peridomestic distribution (Komar et al. 2001).

Vector competence studies of California mosquitoes have indicated that *Culex* species exhibit inter- and intraspecific variation in their susceptibility to infection and ability to transmit WNV, with *Culex stigmatosoma* Dyar most competent, followed by *Culex tarsalis* Coquillett, *Culex p. quinquefasciatus* Say, and *Culex erythrothorax* Dyar (Goddard et al. 2002, Reisen et al. 2005). Similar trends in vector competence were found for closely related St. Louis encephalitis virus (family *Flaviviridae*, genus *Flavivirus*, SLEV) during previous (Meyer et al. 1983, Hardy et al. 1985, Hardy and Reeves 1990) and recent studies (Reisen et al. 2005). With the exception of *Cx. erythrothorax* that feeds frequently on mammals (Tempelis and Washino 1967), these *Culex* species feed most frequently on avian hosts (Reisen and Reeves 1990) and therefore were infected frequently with WNV during 2003 (Reisen et al. 2004) and 2004 (Hom et al. 2005). Recently, we suggested that elevated WNV viremias in corvids might be important epidemiologically in driving WNV into *Culex* species and/or geographic populations that are moderately susceptible to infection (Reisen et al. 2005). However, corvid species vary markedly in their distribution in southern California, creating a natural experiment to evaluate their unique role as amplifying hosts. In the current article, we compare the intensity of enzootic and epidemic WNV transmission in the Coachella Valley essentially without corvids, in Los Angeles with extensive American crow populations, and in Kern County with moderate abundance of American crows and an elevated abundance of western scrub-jays to test the hypothesis that corvids function as critical amplifying hosts whose infection is necessary to effectively infect peridomestic *Cx. p. quinquefasciatus* that subsequently transmit virus to humans.

Methods and Materials

Study Areas. Information was compiled for study areas within three climate domains of California: 1) Coachella Valley in the Colorado Desert, 2) Los Angeles in the maritime southern California coast, and 3) Kern County in the southern Central Valley (Fig. 1). The ecology of these areas and the epidemiology of recent SLEV outbreaks have been described previously (Reeves 1990; Reisen et al. 1992a, b, 2002). Information and maps depicting bird counts were obtained from Christmas bird counts compiled by the Audubon Society for 2002–2003 (<http://audubon2.org/birds/cbc/h/map.html>). These maps depict bird abundance and distribution before any depopulation associated with the 2004 epiornitic, and therefore show the previous receptivity of these areas for WNV introduction.

Active Surveillance. During 2004, each study area contained replicated monitoring sites with comparable biweekly enzootic sampling programs, that included replicated CDC dry ice-baited (Newhouse et al. 1966) and/or gravid female (Cummings 1992) traps to monitor mosquito abundance and infection, and flocks of 10 sentinel chickens to monitor transmission.

Mosquitoes were returned alive to field laboratories, anesthetized by triethylamine, enumerated by species, and grouped into pools of ≤50 females each, stored frozen at −80°C and then shipped on dry ice to the Center for Vectorborne Diseases Arbovirus Laboratory (CVEC) at the University of California, Davis, for testing. Sentinel chickens were bled by lancet prick of the comb onto filter paper strips (Reisen et al. 1993) that were shipped to the Viral and Rickettsial Diseases Laboratory (VRDL) at the California Department of Health Services (CDHS) in Richmond, CA, for testing (Reisen et al. 1994). Free-ranging wild birds were collected at replicated sites by using grain-baited ground and modified crow traps (McClure 1984) and by mist netting (Coachella and Kern) to measure the intensity of infection within the enzootic host populations. Birds were identified to species, aged, banded, bled by jugular puncture (0.1 cc taken by 28-gauge needle into 0.9 cc of saline), and released at the site of capture (Reisen et al. 2000). Sera were frozen and then shipped to CVEC for testing.

Passive Surveillance. Dead birds reported by the public to the Dead Bird Hotline at CDHS and collected by local agency staff were shipped to a regional California Animal Health and Food Safety (CAHFS) laboratory for necropsy. Kidney snips or swabs or oral swabs then were shipped frozen in virus diluent to CVEC for testing. Equine and human cases were diagnosed by local health providers, and selected serum and tissue samples were submitted for testing to CAHFS, county health, and/or the CDHS laboratories.

Diagnostics. Pools of mosquitoes were disrupted using a mixer mill and RNA extracted using an ABI robotic system (Shi et al. 2001). RNA then was tested simultaneously for WNV, SLEV, and western equine encephalomyelitis virus (family *Togaviridae*, genus *Alphavirus*, WEEV) using a multiplex reverse transcription-polymerase chain reaction (RT-PCR) system combining primers that detect genotypes historically circulating within California (Chiles et al. 2004). Positives initially were confirmed by virus isolation using an in situ enzyme immunoassay (EIA) and/or a second primer set. Sentinel chicken sera were screened for IgG by an EIA and confirmed by a plaque reduction neutralization test (PRNT) (Reisen et al. 1994, Hom et al. 2005). Wild bird sera were screened for *Alphavirus* or *Flavivirus* antigen-reactive antibody by an EIA (Chiles and Reisen 1998) and then confirmed or identified specifically by PRNTs. Endpoint titers >4× those of the competing virus were considered diagnostic. Dead bird tissues were tested for WNV by using a singleplex RT-PCR with the same primer sets used for mosquito pools.

Spatial Analysis. Zip code points and polygon layers and 2003 zip code-level human population estimates were obtained from the Environmental Systems Research Institute (ESRI) Data & Maps 2004 data set that was included with ArcView 9.0 (ESRI, Redlands, CA). Data on human cases summarized by zip codes were obtained from the Division of Communicable Disease Control, CDHS. Dead bird records were obtained from the Vector-borne Disease Section, CDHS, through the Dead Bird Surveillance Program (McCaughey et al. 2003). Geocoded mosquito pool records with virus test results for 2004 were acquired from the Greater Los Angeles County Vector Control District (VCD).

The distributions of dead corvids confirmed as being infected with WNV and cases of human WNV illness were used to delineate dispersion patterns of the 2004 epiornitic and epidemic within southern California, which included the areas that had the largest numbers of dead corvids and human cases in California (Hom et al. 2005). These records were examined at the zip code level to identify areas with clustering, if any, beyond that expected by random chance. The spatial scan statistic (Kuldorff and Nargawalla 1995, Kuldorff 1997) used in SaTScan software version 5.1 (http://www.satscan.org) uses case and control series and an underlying Bernoulli probability model to detect dead corvid and human disease clusters separately. This model was applied recently in New York City to identify clusters of dead bird reports as an early warning system for WNV disease in humans (Mostashari et al. 2003). The control series for dead bird and human data consisted of zip codes without WNV-positive dead birds or human cases, respectively. SaTScan imposed a range of circular windows from zero width to a width that included up to 10% of the data points around each data point, and evaluated whether, for any given circle, there was a larger number of case points than would be expected based on the model (Kuldorff 1997). We scanned for areas with high case point densities (clusters) for 9,999 Monte Carlo iterations. To limit the degree of cluster overlap, all cluster centers were required to be outside of other identified clusters. All significant clusters identified from the dead bird data were mapped based on their centers and radii using ArcView.

Human incidence of WNV-related illness was calculated for comparison inside and outside of significant ($\alpha = 0.05$) corvid clusters. Also, maximum likelihood estimates and 95% CL for *Cx. p. quinquefasciatus* infection rates inside and outside significant dead corvid clusters were calculated using the Pooled Infection Rate version 2.0 add-in (Biggerstaff 2003) for Microsoft Excel (Microsoft, Redmond, WA).

Results

Avian Abundance. Based on previous avian host competence studies (Komar et al. 2003, Reisen et al. 2005) and their abundance, American crows, western scrub-jays, house finches, and house sparrows were considered to be important hosts for WNV in southern California. Their distribution and abundance based on Christmas bird counts made during 2002–2003 before the 2004 WNV epiornitic indicated that American crows were abundant in Los Angeles, and western scrub-jays were abundant in Los Angeles and Kern counties, whereas house finches and house sparrows were abundant throughout all three areas (Fig. 2). Similar patterns were evident from the examination of

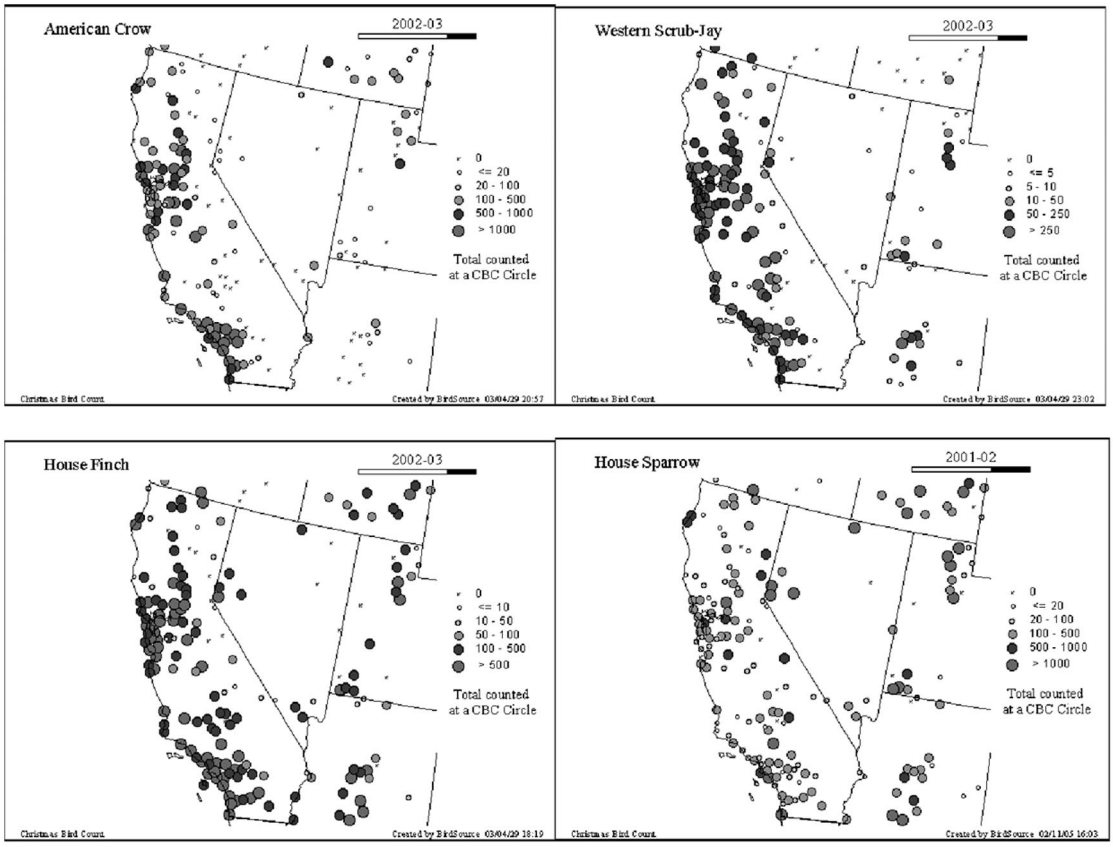

Fig. 2. National Audubon Society Christmas bird counts for American crows, western scrub-jays, house finches, and house sparrows for 2002–2003.

maps produced from the summer Breeding Bird Survey conducted by the U.S. Geological Survey (Sauer et al. 2004).

Mosquito Infection. WNV mosquito infection rates were summarized from April through September (weeks 18–40) 2004 when WNV was active (Table 1). Overall, 162,299 female mosquitoes were tested for virus by RT-PCR in 4,366 pools, of which 601 were positive for WNV. All pools also were tested for WEEV and SLEV with negative findings. Numbers of mosquitoes pooled were related to their abundance in CDC and gravid traps. Infection rates peaked at 6.8 per 1000 for *Cx. tarsalis* during June in Coachella Valley; at 7.7 and 8.9 per 1000 for *Cx. p. quinquefasciatus* and *Cx. tarsalis*, respectively, in Kern County during August; and at 15.0 per 1000 for *Cx. p. quinquefasciatus* in Los Angeles during August, indicating that our data were collected during peak WNV activity in these areas. Infection rates for host-seeking *Cx. tarsalis* were similar statistically in all three areas (inspection of 95% CLs in Table 1), whereas *Cx. p. quinquefasciatus* infection rates at CDC traps were significantly lower than *Cx. tarsalis* in the Coachella Valley, but similar in Kern and Los Angeles counties. Infection rates for *Cx. p. quinquefasciatus* from Kern and Los Angeles counties were significantly higher for females collected by gravid traps than by CDC traps, because this method collected females that were physiologically older (i.e., gravid) than host-seeking females and that had ingested at least one bloodmeal that potentially was infectious before capture. Too few *Cx. p. quinquefasciatus* were collected by gravid traps in Coachella Valley for valid comparison. *Cx. stigmatosoma* and *Cx. thriambus* were tested infrequently, but they had elevated infection rates. Mammal-feeding species such as *Aedes melanimon* Dyar and *Anopheles hermsi* Barr & Guptavanji were rarely infected.

Sentinel Chickens. All chicken flocks in our study areas had one or more seroconversions, including flocks maintained at hot, dry upland Palm Springs in Coachella Valley and heavily populated urban Los Angeles (Table 2). Positive chickens were replaced after seroconversion and therefore four flocks had >10 conversions per flock, including 19 near the small town of Arvin in Kern County. Coachella Valley had the greatest coefficient of variation among seroconversions per flock (range 1–16; coefficient of variation = 3.3), whereas Greater Los Angeles had the lowest (range 5–10, coefficient of variation = 0.5). There was no significant difference among the three study areas in the number of seroconversions per flock

Table 1. Mosquito infection rates (95% CL in parentheses) during weeks 18–40 2004 calculated by the maximum likelihood method (Biggerstaff 2003) at three study areas in southern California

Mosquito species	Trap type[a]	No. pools	Total females	WNV positive	Infection rate	95% CL
			Coachella Valley			
Cx. erythrothorax	cdc	22	1,036	0		
Cx. p. quinquefasciatus	cdc	148	3,597	4	1.12	(0.36, 2.68)
Cx. tarsalis	cdc	638	24,097	71	3.14	(2.48, 3.94)
Total		808	28,730	75		
			Kern County			
Cx. p. quinquefasciatus	grav	303	11,329	80	8.51	(6.79, 10.57)
Cx. p. quinquefasciatus	cdc	182	7,240	30	4.57	(3.14, 6.44)
Cx. stigmatosoma	cdc	2	60	0		
Cx. tarsalis	cdc	431	18,045	70	4.27	(3.36, 5.37)
Cx. thriambus	cdc	3	57	1	13.49	(1.12, 71.51)
Ae. melanimon	cdc	180	8,255	1	0.15	(0.01, 0.75)
Total		1,101	44,986	182		
			Greater Los Angeles County VCD			
Cx. p. quinquefasciatus	grav	1,156	41,695	249	6.73	(5.94, 7.60)
Cx. p. quinquefasciatus	cdc	440	15,829	66	4.48	(3.50, 5.66)
Cx. stigmatosoma	grav	38	709	6	8.76	(3.71, 17.89)
Cx. stigmatosoma	cdc	35	660	0		
Cx. tarsalis	grav	6	55	1	17.65	(1.10, 86.12)
Cx. tarsalis	cdc	155	4,572	15	3.46	(2.02, 5.56)
Cx. erythrothorax	grav	3	55	0		
Cx. erythrothorax	cdc	408	19,228	4	0.21	(0.07, 0.50)
Cx. restuans	grav	1	15	0		
Cx. restuans	cdc	1	11	0		
Cx. thriambus	grav	6	124	0		
Cx. thriambus	cdc	16	610	2	3.44	(0.62, 11.51)
An. hermsi	grav	3	44	0		
An. hermsi	cdc	41	1,346	1	0.74	(0.04, 3.57)
Cs. incidens	grav	49	941	0		
Cs. incidens	cdc	85	2,372	0		
Cs. inornata	cdc	7	170	0		
Cs. particeps	cdc	7	147	0		
Total		2,457	88,583	344		

[a] cdc, dry ice-baited CDC style traps; grav, gravid female traps.

when tested by a one-way analysis of variance (ANOVA) ($P > 0.05$).

Free-Ranging Avian Serology. Overall, 874 (8.8%) of 9,940 wild bird sera from 37 species collected by mist netting or grain-baited traps tested positive for antibodies against *Flavivirus* antigen (Table 3). Of these sera, 866 were retested by PRNT, of which 83% were confirmed to be WNV or SLEV (Table 3); the remainder yielded equivocal results or were negative. *Flavivirus* seroprevalence rates for 2004 of >10% were detected for species in the orders Galliformes (California quail, 11% and Gambel's quail, 14%) and Columbiformes (common ground dove, 27% and rock dove or pigeon, 24%) that were low to moderately competent hosts (Reisen et al. 2005). Other commonly infected species included the house finch (11%), house sparrow (14%), and western scrub-jay (14%), all of which are species that succumb to infection, produce an elevated viremia, and have been shown to be moderately to highly competent hosts (Komar et al. 2003, Reisen et al. 2005). Overall, the percentage of sera positive by EIA varied significantly among the three study areas ($\chi^2 = 79.8, P < 0.001$), being highest in Los Angeles (14%; $n = 2,940$). The percentage positive in Coachella Valley (8%) was greater ($\chi^2 = 37.8, P < 0.001$) than in Kern County (5%), perhaps reflecting the presence of WNV infection in birds in Coachella Valley during both 2003 (Reisen et al. 2004) and 2004. Interestingly, by September 2004, seroprevalence peaked at comparable levels ($\chi^2 = 0.07, P = 0.8$) in both Coachella (14%; $n = 246$) and Kern (13%; $n = 187$), but it remained significantly less than in Los Angeles where seroprevalence remained >40% during weeks 30–44. Because sampling methods and species diversity differed markedly among the three study areas, we compared seroprevalence rates for house

Table 2. Number of sentinel chickens and flocks seroconverting within each area of southern California (lower and upper 95% CL)

Study area	No. flocks	No. positive	No. seroconversions	Seroconversion/ flock	Lower limit	Upper limit
Coachella Valley	10	10	73	7.3	3.5	10.4
Kern County	9	9	90	10.0	7.0	13.0
Los Angeles	6	6	47	7.8	4.8	9.6

Table 3. Avian seroprevalence in Kern County and Coachella Valley, 2004

Species	Coachella Valley			Los Angeles			Kern County		
	Tested	Positive[a]	%	Tested	Positive[a]	%	Tested	Positive[a]	%
California quail	4	0	0.0	0			458	53	11.6
Common ground dove	98	27	27.6	0			3	0	0.0
Gambel's quail	669	96	14.3	0			0		
House finch	191	13	6.8	1,210	150	12.4	340	25	7.4
House sparrow	337	13	3.9	1,396	229	16.4	57	0	0.0
Mourning dove	827	55	6.7	79	17	21.5	130	14	10.8
Rock pigeon	137	59	43.1	67	11	16.4	153	14	9.2
Western scrub-jay	0			0			161	22	13.7
Subtotal	2,263	263	11.6	2,752	407	14.8	1,302	128	9.8
Other species	1,304	38	2.7	188	2	1.1	2,131	36	1.6
Total	3,567	301	8.4	2,940	409	13.9	3,433	164	4.7
SLEV		12			0			0	
WNV		196			373			128	
Total PRNT		301			401			164	

[a] Antibodies against *Flavivirus* antigen tested by EIA. Positives retested by PRNT were positive for SLEV, WNV, or were negative or equivocal.

finches, house sparrows, mourning doves, and rock pigeons, species that were collected in all areas (Table 3). In agreement with the overall analyses mentioned above, seroprevalence was significantly greatest for house finches ($\chi^2 = 10.6$, df = 2, $P = 0.005$), house sparrows ($\chi^2 = 45.8$, df = 2, $P < 0.001$), and mourning doves ($\chi^2 = 22.1$, df = 2, $P < 0.001$) collected in Los Angeles. In contrast, seroprevalence in rock pigeons was greatest in Coachella Valley ($\chi^2 = 48.5$, df = 2, $P < 0.001$); however, these data may have been upwardly biased because most birds were collected by grain-baited traps at one ranch near the Salton Sea, whereas birds from Los Angeles and Kern counties were taken from numerous sites as part of pigeon removal programs.

Dead Birds. Of the dead birds reported by the public that were submitted for virus testing, a total of six of 21, 840 of 1,153, and 87 of 159 tested positive for WNV from Coachella Valley, Los Angeles, and Kern County, respectively (Table 4). Dead bird species were dominated by corvids, and the numbers of positives reflected their relative abundance (Fig. 2) as well as the size of the human population (Table 5) to report them within the three study areas.

Passive Case Detection. A total of 840 human cases (WN fever plus WN neuroinvasive disease) were detected in California by medical providers and confirmed serologically by the CDHS or local health departments (Table 5). Human case incidence seemed to be associated with elevated infection rates in peridomestic *Cx. p. quinquefasciatus*, being lowest in the Coachella Valley and highest in Kern County.

Spatial Analysis. Six significant ($\alpha = 0.05$) clusters of zip codes with dead corvids and four significant clusters of WNV-attributed human cases were identified within the study area by the spatial scan procedures in SaTScan (Table 6). These clusters covered the southeastern portions of the Los Angeles basin and the inland valleys near Riverside, but did not include the highly urbanized central area of Los Angeles, southwestern Los Angeles County, the Coachella Valley, or Kern County (Fig. 3). One dead corvid cluster was resolved in the northwestern portion of the Los Angeles basin, centered in Northridge.

The clusters of corvids confirmed to die from WNV infection (Fig. 3) seemed to be associated with moderate to high corvid abundance (Fig. 2) and with dense human populations (Fig. 4) in southern California. Cluster size varied, ranging in radius from 9.8 to 26.8 km, and encompassed two of the known large communal roosts in the Los Angeles area (Fig. 3). All four identified human case clusters were congruent with dead corvid clusters (Fig. 5). The Riverside human case and dead corvid clusters were centered at the same zip code, whereas other human case clusters were closely aligned spatially with dead corvid clusters. Interestingly, human cluster dimensions were similar to American crow dimensions, ranging in radius from 15 to 28 km (Table 7). Collectively, these cluster dimensions most likely reflected the size of

Table 4. Summary of dead birds positive for WNV in three areas of California

Species	Coachella Valley	Los Angeles	Kern County
American crow	1	783	26
Western scrub-jay	0	25	28
Common raven	0	10	3
Raptor	1	5	11
House finch	0	5	0
House sparrow	1	4	3
Other species	3	58	16
Total	6	840	87

Table 5. Incidence of WNV human cases in California during 2004

Area	Population size (in 1000s)[a]	WNV cases[b]	Incidence/ 100,000
California	33,871	819	2.42
Coachella Valley	336	7	2.08
Los Angeles County	9,519	327	3.44
Kern County	662	60	9.06

[a] Based on 2,000 census figures.
[b] Through Dec. 15, 2004, West Nile fever and neurological disease as well as infections detected by blood banks included.

Table 6. Characteristics of WN-positive zip code clusters for dead corvids within southern California, including the central city, zip code, and the radius

City	Zip code	Latitude	Longitude	Radius (km)	No. Zip codes in cluster	*P* value[a]
West Covina	91790	34.0671	117.9376	18.40	47	0.0001
Riverside	92509	34.0033	117.4461	26.86	49	0.0001
Northridge	91324	34.2384	118.5502	15.02	31	0.0001
La Mirada	90638	33.9024	118.0091	15.88	51	0.0002
Redlands	92373	34.0014	117.1522	22.55	29	0.0281
Compton	90221	33.8860	118.2057	9.84	24	0.0303

Listed clusters were significant at the $\alpha = 0.05$ level.

[a] Based on 9,999 Monte Carlo simulations testing against the null hypothesis of a random spatial distribution of WN-position dead birds.

foraging areas used by American crows about communal roosts such as that identified in Fig. 3.

Within the study areas, the cumulative incidence of human WNV-attributed illness was greater within the dead bird clusters than without (Fig. 5). Overall, 75% (440/586) of human cases occurred within dead bird clusters, even though only 41% of the human population resided within areas circumscribed by these clusters. The incidence of human cases inside and outside dead corvid clusters was 5.90 and 1.38 per 100,000, respectively. Likewise, the *Cx. p. quinquefasciatus* infection rate (95% CL) was significantly greater at traps located within dead corvid clusters (7.86 [6.98, 8.83]) than without (2.42 [1.75, 3.27] infections per 1000 females tested, respectively).

Discussion

Research and surveillance measures in California have begun to resolve the components of parallel rural and urban enzootic WNV transmission cycles. Highly competent mosquito vectors seem to maintain modest transmission levels among widely distributed and moderately competent passeriform species. However,

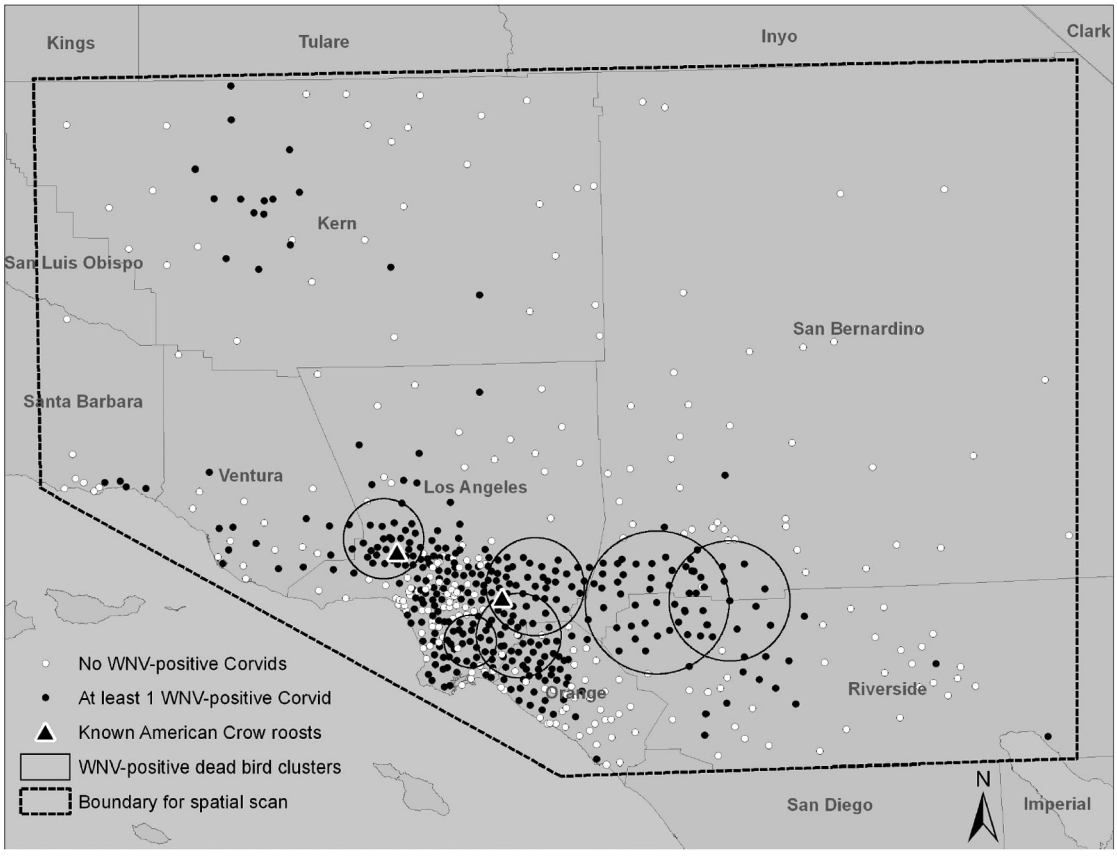

Fig. 3. Map showing the boundary for the spatial scan and zip code centroids with (black circles) and without (white circles) at least one dead corvid during 2004. Significant clusters of zip codes with dead corvids are shown as hollow circles.

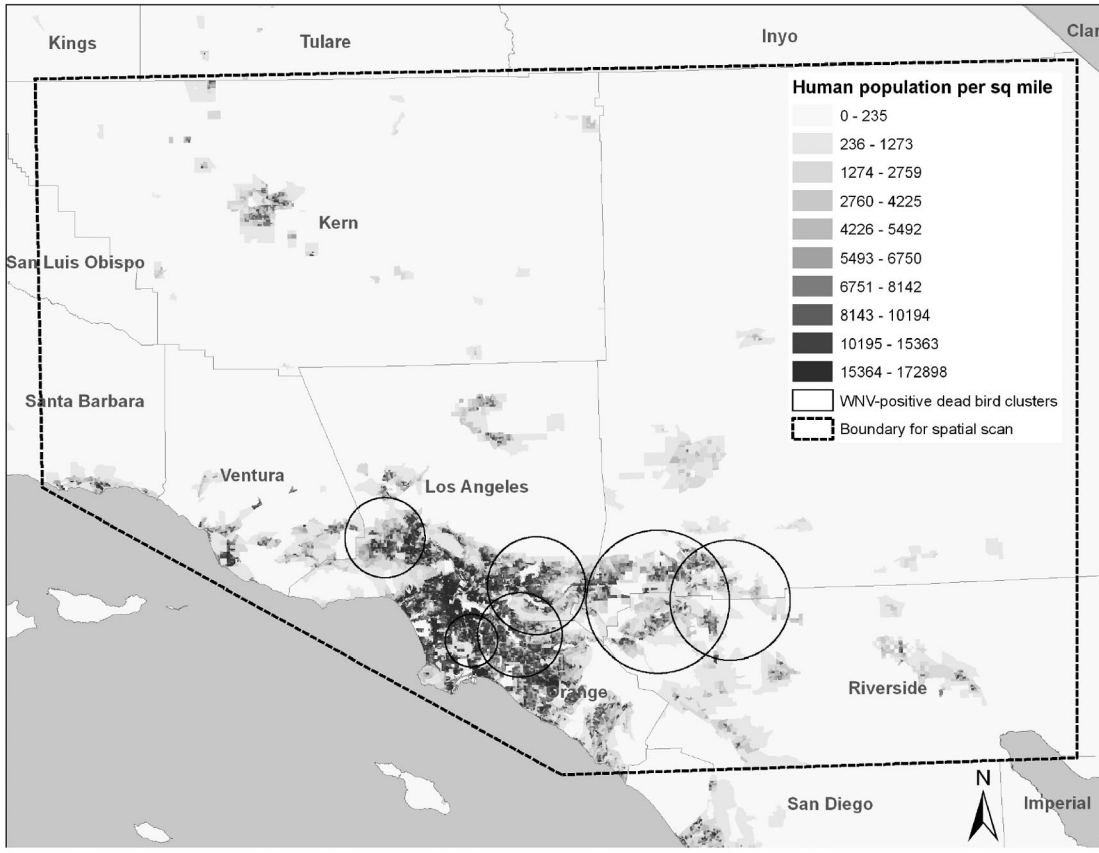

Fig. 4. Map of the human population density by U.S. census block group within the study area.

corvids seem necessary to amplify WNV and increase transmission rates to epidemic levels, because their extremely elevated viremias effectively drive WNV into moderately competent peridomestic mosquito and then human populations. The distribution of different corvid species over the differing landscapes of California are determined by their abundance and roosting/foraging behavior that, in turn, may delineate the distribution of human risk for WNV infection.

Two active surveillance measures of enzootic WNV activity did not vary significantly among our three study areas in southern California, despite marked differences in the abundance and distribution of corvids. *Cx. tarsalis* infection rates during 2004 remained remarkably similar among the three study areas as indicated by overlapping 95% CL, even though abundance at dry ice-baited traps (data not shown) and the number of positive pools were less in urban Los Angeles than in rural Coachella Valley or Kern County. Similarly, seroconversion rates within flocks of sentinel chickens did not vary significantly among the three areas, perhaps indicating that C*x. tarsalis* may be important in transmitting virus to sentinel chickens. In the eastern United States where *Cx. p. pipiens* L. is the primary vector (Kilpatrick 2005) and seeks bloodmeals within forest canopy (Anderson et al. 2004), sentinel chickens positioned at ground level have been a poor indicator of WNV activity (Cherry et al. 2001). Baseline WNV enzootic activity in southern California transmitted by highly competent *Cx. tarsalis* populations was comparable among varying landscapes (desert, maritime coast, inland agriculture), proceeded at similar rates with or without abundant corvid populations, and probably used moderately competent passeriform species as primary hosts, especially house finches and house sparrows. WNV amplification within these rural cycles potentially served as a source of virus to infect mosquitoes and birds in periurban landscapes, most likely by postfledging movements by hatching year birds.

In marked contrast, infection rates in *Cx. p. quinquefasciatus* were highest in urban Los Angeles and Kern County, areas with elevated infections within American crow and/or western scrub-jay populations as indicated by the numbers of dead birds reported by the public (Hom et al. 2005). In addition to being reported dead by the public, western scrub-jays in Kern County also had elevated seroprevalence rates by the end of the summer (August; 28%; $n = 29$), even though most infected individuals succumb after experimental infection (Reisen et al. 2005). We have not been able to collect American crows to determine whether any individuals survived natural infection such as reported previously (Yaremych et al. 2004);

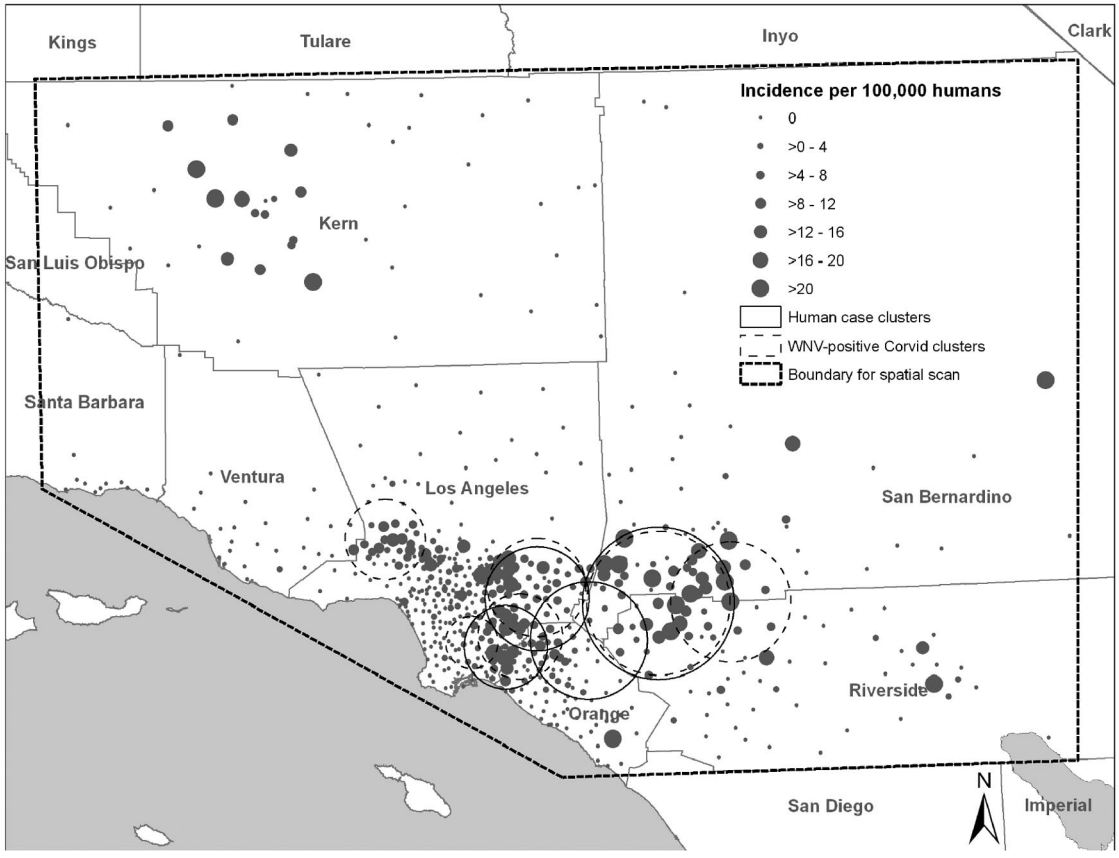

Fig. 5. Map of the human case and dead corvid clusters with the WNV-attributed human case incidence per 100,000 shown as a proportionally sized point for each zip code centroid.

however, in the laboratory 100% of infected individuals die after experimental infection (Komar et al. 2003, Brault et al. 2004). During most of the year, American crow populations roost communally, which probably explained why clusters of dead corvids were grouped in neighborhoods around roosts within Los Angeles, Riverside, and San Bernardino counties, but not in Kern County or Coachella Valley, where there were no large American crow roosts (Fig. 3). In the Bakersfield area of Kern County, corvid populations were dominated by western scrub-jays, a territorial species evenly distributed in small family groups among suburban housing and adjacent riparian habitat. This species comprised almost half of the WNV-positive dead birds reported by the public in Kern County. Western scrub-jays produce viremias during acute infection that are sufficiently elevated to infect *Cx. p. quinquefasciatus* (Reisen et al. 2005), although lower than reported for American crows (Komar et al. 2003, Brault et al. 2005). Interestingly, maximum seroprevalence rates among free-ranging birds during September in Coachella Valley and Kern County were comparable, but significantly lower than observed for Los Angeles where *Cx. p. quinquefasciatus* was the most frequently infected mosquito. Possibly, elevated transmission rates at American crow roosts resulted in

Table 7. Characteristics of zip code-level clusters of WNV-attributed human illness within the southern California study area, including the central city, zip code, and the radius

City	Zip code	Latitude	Longitude	Radius (km)	No. Zip codes in cluster	*P* value[a]
La Puente	91744	34.0289	117.9373	19.51	59	0.0001
Riverside	92509	34.0033	117.4461	28.43	57	0.0001
Cerritos	90703	33.8680	118.0686	15.60	57	0.0003
Yorba Linda	92887	33.8839	117.7317	21.96	48	0.0146

Listed clusters were significant at the $\alpha = 0.05$ level.

[a] Based on 9,999 Monte Carlo simulations testing against the null hypothesis of a random spatial distribution of human WNF and WNND cases.

WNV-infected sick birds succumbing to illness during foraging flights to surrounding neighborhoods, where they possibly infected local *Cx. p. quinquefasciatus*, which then infected peridomestic bird species such as house sparrows and house finches as well as humans (Nasci et al. 2002).

The incidence of human cases and infection in *Cx. p. quinquefasciatus* in Los Angeles were significantly higher within clusters of dead corvids than outside of these statistically defined areas. Elsewhere some zip codes had high human case incidence, but these were isolated or created artificially by a single human case occurring within a relatively small human population, e.g., Mojave Desert in San Bernardino County. The exception to this occurred in Kern County, where the human case incidence was high for several zip codes in and around the City of Bakersfield. Although the dead corvids or human cases were not significantly clustered at the $\alpha = 0.05$ level, a cluster of human cases centered in zip code 93301 in Bakersfield along the Kern River was nearly significant ($P = 0.08$). A number of zip codes near Bakersfield also had dead corvids (Fig. 3); however, these included multiple western scrub-jays, were not strongly clumped, and were widely distributed in suburban and urban Bakersfield.

We recognize that imposing circular boundaries on clusters, although necessitated by the SaTScan software, is somewhat arbitrary and that actual aggregation patterns may conform to landscape features and may not be circular in shape. However, circular boundaries were useful in depicting the radius of clustering about known American crow roosts (Fig. 3). To avoid having very large circular "clusters" containing inhomogeneous densities of WNV-positive zip codes, we set the upper limit for allowable cluster size at 10% of all data points. The total number of zip code points within our study was 599, so the maximum possible number of zip codes included in any cluster was 59. None of the significant clusters reported contained >51 zip codes, so we feel that the restriction to 10% of the data points was justified and not overly restrictive.

Our study documented the potential importance of elevated titers in viremic corvids in infecting moderately competent peridomestic *Cx. p. quinquefasicatus* and thereby delineating areas of high virus transmission risk to humans as well as peridomestic passerine birds. Competent vectors such as *Cx. tarsalis* and *Cx. stigmatosoma* seemed to be capable of maintaining a modest level of transmission among peridomestic and rural bird populations with or without corvids. *Cx. stigmatosoma* may be especially important in urban Los Angeles, because this species is a highly susceptible to WNV infection (Goddard et al. 2002, Reisen et al. 2005). Although surveillance for this species is limited by the difficulty of collecting adults, immature stages frequently are found in and around residential communities, indicating adult abundance probably is underestimated by trapping (Reisen et al. 1990). Periurban American crow roosts served as sites for viral amplification to epidemic levels, and birds infected there died within clusters with a 9–26-km radius. One to 2 d before death, American crows and western scrub-jays become lethargic and have viremias in excess of 10^9 plaque-forming units per ml of blood (Brault et al. 2005, Reisen et al. 2005). During this viremic period, American crows often fail to return to communal roosting sites (Weingartl et al. 2004), resulting in an introduction of virus into peridomestic habitats, infecting local mosquitoes and then birds (Nasci et al. 2002) and humans (Eidson et al. 2001b). Our data supported this scenario in Los Angeles. Clusters of dead corvids delineated areas with significantly higher *Cx. p. quinquefasciatus* infection rates and incidence of human cases compared with areas outside these clusters.

In North America, the invading WNV currently benefits from producing high viremias in corvids and other bird species that enhances amplification and dispersal. From the standpoint of the virus, this relationship seems to be the serendipitous result of virus introduction into an environment with highly susceptible vertebrate host species, rather than an end point in host–parasite evolution (Levin 1996). Elsewhere, antibody-positive corvids frequently may be found in nature (Hayes et al. 1982, Hayes 2001), indicating that these birds are fed upon by infectious mosquitoes yet survive infection. Although antibody-positive American crows have been reported in North America (Yaremych et al. 2004), survival after infection seems rare and may not be sufficient to lead to the evolution of resistant populations. Conversely, viral attentuation has been reported (Davis et al. 2004), and it may be that the emergence of less virulent strains rather than the emergence of host resistance may allow for the eventual coexistence of American crows with WNV.

Acknowledgments

We thank the staff of CVEC, Barbara Cahoon-Young and Thuan Ho for laboratory diagnostics, Bruce Eldridge and Steve Lewis for data management, Marc Kensington, Pat Miller and Marc Palmer for fieldwork in Coachella Valley, and Vincent Martinez and Scott Hallam for fieldwork in Kern County. We also thank the Coachella Valley Mosquito and Vector Control District (MVCD) (Donald Gomsi and Branka Lothrop), Greater Los Angeles County VCD (Jack Hazelrigg, Paul O'Connor, Jacquie Spoehel, Susanne Kluh, and Saeed Tabatabaeepour), and Kern MVCD (Robert Quiring) for fiscal, logistical, and personnel support without which this research would not have been possible. The Dead Bird Surveillance Program was managed by the VBDS of the CDHS and funded, in part, by the Centers for Disease Control and Prevention, CDC. Dead Bird Surveillance Program necropsies were done by the California Animal Health and Food Safety Laboratory system under the direction of Leslie Woods. Our research was funded, in part, by Research Grants 5RO1-AI55607 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, the Climate Variability and Human Health program, Office of Global Programs, National Oceanic & Atmospheric Administration, funds for enhanced surveillance provided by the CDC, and special funds from the Mosquito Research Program allocated annually through the Division of Agriculture and Natural Resources, University of California.

References Cited

Anderson, J. F., T. G. Andreadis, A. J. Main, and D. L. Kline. 2004. Prevalence of West Nile virus in tree canopy-inhabiting *Culex pipiens* and associated mosquitoes. Am J. Trop. Med. Hyg. 71: 112–119.

Biggerstaff, B. J. 2003. Pooled infection rate. http://www.cdc.gov/ncidod/dvbid/westnile/software.htm. Centers for Disease Control and Prevention, Ft. Collins, CO.

Brault, A. C., S. A. Langevin, R. Bowen, N. A. Panella, B. J. Biggerstaff, B. R. Miller, and N. Komar. 2005. Differential virulence of West Nile strains for American crows. Emerg. Infect. Dis. 10: 2161–2168.

Brault, A. C., S. A. Langevin, R. A. Bowen, N. A. Panella, B. Biggerstaff, B. R. Miller, and K. Nichols. 2004. Differential virulence of West Nile strains for American crows. Emerg. Infect. Dis. 10: 2161–2168.

Caffrey, C. L., T. J. Weston, and S.C.R. Smith. 2003. High mortality among marked crows subsequent to the arrival of West Nile virus. Wildl. Soc. Bull. 31: 870–872.

Cherry, B., S. C. Trock, A. Glaser, L. Kramer, G. D. Ebel, C. Glaser, and J. R. Miller. 2001. Sentinel chickens as a surveillance tool for West Nile virus in New York City, 2000. Ann. N.Y. Acad. Sci. 951: 343–346.

Chiles, R. E., E. N. Green, Y. Fang, W. K. Reisen, J. D. Edman, and A. C. Brault. 2004. Surveillance for arboviruses in California mosquito pools: current and future protocols. Proc. Mosq. Vector Control Assoc. Calif. 72: 15–17.

Chiles, R. E., and W. K. Reisen. 1998. A new enzyme immunoassay to detect antibodies to arboviruses in the blood of wild birds. J. Vector Ecol. 23: 123–135.

Cummings, R. F. 1992. Design and use of a modified Reiter gravid mosquito trap for mosquito-borne encephalitis surveillance in Los Angeles County, California. Proc. Mosq. Vector Control Assoc. Calif. 60: 170–176.

Davis, C. T., D. W. Beasley, H. Guzman, M. Siirin, R. E. Parsons, R. B. Tesh, and A. D. Barrett. 2004. Emergence of attenuated West Nile virus variants in Texas, 2003. Virology 330: 342–350.

Eidson, M., N. Komar, F. Sorhage, R. Nelson, T. Talbot, F. Mostashari, and R. McLean. 2001a. Crow deaths as a sentinel surveillance system for West Nile virus in the northeastern United States, 1999. Emerg. Infect. Dis. 7: 615–620.

Eidson, M., J. Miller, L. Kramer, B. Cherry, Y. Hagiwara, E. N. Ostlund, R. L. Crom, D. D. Pedersen, D. J. Johnson, W. O. Williams, et al. 2001b. Dead crow densities and human cases of West Nile virus, New York State, 2000. Emerg. Infect. Dis. 7: 662–669.

Goddard, L., A. Roth, W. K. Reisen, and T. W. Scott. 2002. Vector competence of California mosquitoes for West Nile virus. Emerg. Infect. Dis. 8: 1385–1391.

Hardy, J. L., S. B. Presser, R. P. Meyer, W. K. Reisen, L. D. Kramer, and A. V. Vorndam. 1985. Comparison of a 1984 Los Angeles strain of SLE virus with earlier California strains of SLE virus: mouse virulence, chicken viremogenic, RNA oligonucleotide and vector competence characteristics. Proc. Calif. Mosq. Vector Control Assoc. 53: 10–15.

Hardy, J. L., and W. C. Reeves. 1990. Experimental studies on infection in vectors, pp. 145–250. *In* W. C. Reeves [ed.], Epidemiology and control of mosquito-borne arboviruses in California, 1943–1987. Calif. Mosq. Vector Control Assoc., Sacramento, CA.

Hayes, C. G. 2001. West Nile virus: Uganda, 1937, to New York City, 1999. Ann. N.Y. Acad. Sci. 951: 25–37.

Hayes, C. G., S. Baqar, A. Ahmed, M. A. Chowdhry, and W. K. Reisen. 1982. West Nile virus in Pakistan. 1. Sero-epidemiological studies in Punjab Province. Trans. R. Soc. Trop. Med. Hyg. 76: 431–436.

Hom, A., A. Houchin, K. McCaughey, V. L. Kramer, R. E. Chiles, W. K. Reisen, E. Tu, C. Glaser, C. Cossen, E. Baylis, et al. 2004. Surveillance for mosquito-borne encephalitis activity and human disease, including West Nile virus in California, 2003. Proc. Mosq. Vector Control Assoc. Calif. 72: 48–54.

Hom, A., L. Marcus, V. L. Kramer, B. Cahoon-Youang, C. Glaser, C. Cossen, E. Baylis, C. Jean, E. Tu, B. F. Eldridge, et al. 2005. Surveillance for mosquito-borne encephalitis virus activity and human disease, including West Nile virus, in California, 2004. Proc. Mosq. Vector Control Assoc. Calif. 73: 66–77.

Kilpatrick, A. M. 2005. West nile virus risk assessment and the bridge vector paradigm. Emerg. Infect. Dis. 11: 425–429.

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. Emerg. Infect. Dis. 9: 311–322.

Komar, N., N. A. Panella, J. E. Burns, S. W. Dusza, T. M. Mascarenhas, and T. O. Talbot. 2001. Serologic evidence for West Nile virus infection in birds in the New York City vicinity during an outbreak in 1999. Emerg. Infect. Dis. 7: 621–625.

Kuldorff, M. 1997. A spatial scan statistic. Communications in Stat.: Theory and Methods. 26: 1481–1496.

Kuldorff, M., and M. Nargawalla. 1995. Spatial disease clusters: detection and inference. Stat. Med. 14: 799–810.

Levin, B. R. 1996. The evolution and maintenance of virulence in microparasites. Emerg. Infect. Dis. 2: 93–102.

McCaughey, K., S. Q. Miles, L. Woods, R. E. Chiles, A. Hom, V. L. Kramer, M. Jay-Russel, B. Sun, W. K. Reisen, T. W. Scott, L. T. Hui, D. B. Steinlein, M. Castro, A. Houchin, and S. Husted. 2003. The California West Nile virus dead bird surveillance program. Proc. Mosq. Vector Control Assoc. Calif. 71: 38–43.

McClure, H. E. 1984. Bird banding. Boxwood Press, Pacific Grove, CA.

Meyer, R. P., J. L. Hardy, and S. B. Presser. 1983. Comparative vector competence of *Culex tarsalis* and *Culex quinquefasciatus* from the Coachella, Imperial, and San Joaquin Valleys of California for St. Louis encephalitis virus. Am. J. Trop. Med. Hyg. 32: 305–311.

Mostashari, F., M. Kulldorff, J. J. Hartman, J. R. Miller, and V. Kulasekera. 2003. Dead bird clusters as an early warning system for West Nile virus activity. Emerg. Infect. Dis. 9: 641–646.

Nasci, R. S., N. Komar, A. A. Marfin, G. V. Ludwig, L. D. Kramer, T. J. Daniels, R. C. Falco, S. R. Campbell, K. Brookes, K. L. Gottfried, et al. 2002. Detection of West Nile Virus-infected mosquitoes and seropositive juvenile birds in the vicinity of virus-positive dead birds. Am. J. Trop. Med. Hyg. 67: 492–496.

Newhouse, V. F., R. W. Chamberlain, J. G. Johnston, Jr., and W. D. Sudia. 1966. Use of dry ice to increase mosquito catches of the CDC miniature light trap. Mosq. News 26: 30–35.

Reeves, W. C. 1990. Epidemiology and control of mosquito-borne arboviruses in California, 1943–1987. Calif. Mosq. Vector Control Assoc., Sacramento, CA.

Reisen, W. K., and W. C. Reeves. 1990. Bionomics and ecology of *Culex tarsalis* and other potential mosquito vector species, pp. 254–329. *In* W. C. Reeves [ed.], Epidemiology and control of mosquito-borne arboviruses in Cali-

fornia, 1943–1987. Calif. Mosq. Vector Control Assoc., Sacramento, CA.

Reisen, W. K., R. P. Meyer, C. H. Tempelis, and J. J. Spoehel. 1990. Mosquito abundance and bionomics in residential communities in Orange and Los Angeles counties, California. J. Med. Entomol. 27: 356–367.

Reisen, W. K., R. P. Meyer, M. M. Milby, S. B. Presser, R. W. Emmons, J. L. Hardy, and W. C. Reeves. 1992a. Ecological observations on the 1989 outbreak of St. Louis encephalitis virus in the southern San Joaquin Valley of California. J. Med. Entomol. 29: 472–482.

Reisen, W. K., M. M. Milby, S. B. Presser, and J. L. Hardy. 1992b. Ecology of mosquitoes and St. Louis encephalitis virus in the Los Angeles Basin of California, 1987–1990. J. Med. Entomol. 29: 582–598.

Reisen, W. K., J. Lin, S. B. Presser, B. Enge, and J. L. Hardy. 1993. Evaluation of new methods for sampling sentinel chickens for antibodies to WEE and SLE viruses. Proc. Calif. Mosq. Vector Control Assoc. 61: 33–36.

Reisen, W. K., S. B. Presser, J. Lin, B. Enge, J. L. Hardy, and R. W. Emmons. 1994. Viremia and serological responses in adult chickens infected with western equine encephalomyelitis and St. Louis encephalitis viruses. J. Am. Mosq. Control Assoc. 10: 549–555.

Reisen, W. K., J. O. Lundstrom, T. W. Scott, B. F. Eldridge, R. E. Chiles, R. Cusack, V. M. Martinez, H. D. Lothrop, D. Gutierrez, S. Wright, et al. 2000. Patterns of avian seroprevalence to western equine encephalomyelitis and St. Louis encephalitis viruses in California, USA. J. Med. Entomol. 37: 507–527.

Reisen, W. K., H. D. Lothrop, R. E. Chiles, R. Cusack, E.-G. N. Green, Y. Fang, and M. Kensington. 2002. Persistence and amplification of St. Louis encephalitis virus in the Coachella Valley of California, 2000–2001. J. Med. Entomol. 39: 793–805.

Reisen, W. K., H. D. Lothrop, R. E. Chiles, M. B. Madon, C. Cossen, L. Woods, S. Husted, V. L. Kramer, and J. D. Edman. 2004. Invasion of California by West Nile Virus. Emerg. Infect. Dis. 10: 1369–1378.

Reisen, W. K., Y. Fang, and V. M. Martinez. 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.

Sauer, J. R., J. E. Hines, and J. Fallon. 2004. The North American breeding bird survey, results and analysis 1966–2003, version 2004. 1. USGS Patuxent Wildlife Research Center, Laurel, MD.

Shi, P. Y., E. B. Kauffman, P. Ren, A. Felton, J. H. Tai, A. P. DuPuis, S. A. Jones, K. A. Ngo, D. C. Nicholas, J. Maffei, et al. 2001. High-throughput detection of West Nile virus RNA. J. Clin. Microbiol. 39: 1264–1271.

Tempelis, C. H., and R. K. Washino. 1967. Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley, California, with notes on other species. J. Med. Entomol. 4: 315–318.

Weingartl, H. M., J. L. Neufeld, J. Copps, and P. Marszal. 2004. Experimental West Nile virus infection in blue jays (*Cyanocitta cristata*) and crows (*Corvus brachyrhynchos*). Vet. Pathol. 41: 362–370.

Yaremych, S. A., R. E. Warner, P. C. Mankin, J. D. Brawn, A. Raim, and R. Novak. 2004. West Nile virus and high death rate in American crows. Emerg. Infect. Dis. 10: 709–711.

Received 9 July 2005; accepted 26 October 2005.