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Risk Factors Associated with Human Infection during the 2006 West Nile Virus Outbreak in Davis, a Residential Community in Northern California

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Abstract

We collected a total of 15,329 mosquitoes during weekly sampling in Davis, CA, from April through mid-October 2006 at 21 trap sites uniformly spaced 1.5 km apart over an area of ~26 km². Of these mosquitoes, 1,355 pools of *Culex* spp. were tested by multiplex reverse transcriptase-polymerase chain reaction, of which 16 pools (1.2%) were positive for West Nile virus (WNV). A degree-day model with a developmental threshold of 14.3°C accurately predicted episodic WNV transmission after three extrinsic incubation periods after initial detection. Kriging interpolation delineated that *Culex tarsalis* were most abundant at traps near surrounding agriculture, whereas *Cx. pipiens* clustered within residential areas and greenbelt systems in the old portion of Davis. Spatial-temporal analyses were performed to test for clustering of locations of WNV-infected dead birds and traps with WNV-positive *Cx. tarsalis* and *Cx. pipiens*; human case incidence was mapped by census blocks. Significant multivariate spatial-temporal clustering was detected among WNV-infected dead birds and WNV-positive *Cx. tarsalis*, and a WNV-positive *Cx. pipiens* cluster overlapped areas with high incidences of confirmed human cases. Spatial analyses of WNV surveillance data may be an effective method to identify areas with an increased risk for human infection and to target control efforts to reduce the incidence of human disease.

INTRODUCTION

West Nile virus (WNV) is naturally maintained within *Culex* mosquito–avian transmission cycles.¹ When humans are incidentally infected with WNV, they can suffer from acute febrile illness and encephalitis, and in a small proportion of cases, death can occur.^{2,3} The invasion of North America by WNV in 1999 was followed by the largest mosquito-borne encephalitis epidemic in US history, with > 23,500 confirmed human cases and > 900 deaths reported through 2006.⁴ Many risk factors have been associated with increased human infection, including the presence of WNV-infected competent vectors,⁵ dead bird clusters,^{6,7} early season American crow (*Corvus brachyrhynchos*) deaths,⁸ dead crow densities of ≥ 0.1 dead

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crow/mi²,⁹ increased vegetation density,^{10,11} above-average summer temperatures,¹² and urban landscapes.¹³ These studies typically were conducted at a geographic scale of a county and state. Spatial analyses of risk factors should be confirmed at finer scale (i.e., the community level) to test previously observed patterns with more spatially explicit data. Results will improve understanding of factors associated with immediate risk for human WNV infection.

The spread of WNV across North America has been unprecedented¹⁴ and may have been facilitated by above average summer temperatures in temperate regions.¹² The introduction of WNV into new regions of the United States has typically been characterized by a low number of human cases during the initial year, followed by amplification to epidemic levels during the subsequent season. WNV invaded southern California in 2003,¹⁵ although activity remained confined south of the Tehachapi Mountains until 2004 when WNV invaded the rest of California.¹⁶ Sacramento County was the epicenter of WNV activity in California in 2005,¹⁷ with a case incidence of 14.5 per 100,000 (<http://westnile.ca.gov/>). Neighboring Yolo County, including the city of Davis, a moderately sized residential community in the Sacramento Valley, experienced considerable equine infection¹⁸ but a lower human case incidence of 7.1 per 100,000 (<http://westnile.ca.gov/>). The extensive establishment of WNV in northern California showed the ability of mosquito and avian populations to sustain and amplify WNV at temperate latitudes. However, few epidemiologic reports have documented the WNV outbreak in northern California.

In California, 12 mosquito species were able to transmit WNV under laboratory conditions,^{19–21} and 9 of these species were found naturally infected.^{16,22} The majority of WNV-positive mosquito pools were predominately *Culex tarsalis* and members of the *Cx. pipiens* complex—*quinquefasciatus* predominates in Southern California and *pipiens* in Northern California.^{16,22} *Cx. tarsalis* is the most abundant of these mosquitoes in rural areas of the Sacramento Valley, where historically it was the principle vector of western equine encephalomyelitis (WEEV) and St. Louis encephalitis (SLEV) viruses.²³ *Cx. tarsalis* exhibits opportunistic host selection by obtaining blood meals from both avian and mammal hosts,^{24, 25} larvae develop in newly created surface water,²⁶ and has been incriminated as an important vector of WNV in North America.²⁷ Dry ice or carbon dioxide-baited traps are used to measure vector abundance and collect specimens, which are tested for the presence of arboviruses.^{28, 29} *Cx. pipiens* is also common in the Sacramento Valley, but is considered to be principally ornithophilic,³⁰ whereas closely related *Cx. quinquefasciatus* feeds readily on both birds and mammals.²⁶ There is evidence of genetic introgression between these two species in the Central Valley,³¹ which may affect blood feeding behavior. Recently, for example, this species was found to feed repeatedly on horses.¹⁸ *Cx. pipiens* is an urban mosquito that prefers eutrophic water produced around households and other urban sources. Updraft traps baited with hay infusion attract ovipositing females and are useful in urban surveillance programs to trap *Cx. pipiens* to measure abundance and provide specimens to test for the presence of arboviruses.^{32,33} Although not yet critically evaluated, both *Cx. tarsalis* and *Cx. pipiens* are likely involved in the enzootic transmission of WNV among avian hosts and serve as bridge vectors transmitting the virus to humans and horses in northern California.

The spatial distribution of most vector-borne pathogens is determined by the dispersion and requisite contact between competent vectors and vertebrate hosts that are involved in the transmission cycle,³⁴ which, in turn, are heterogeneously distributed depending on landscape patterns that delineate suitable habitat.¹⁰ Therefore, understanding the patterns of the avian hosts and mosquitoes in urban environments can identify focal areas of high risk for spill-over arbovirus transmission to humans. The ongoing invasion of WNV in Davis offered a unique opportunity to test the hypothesis that temporal and spatial measures of enzootic virus transmission maintenance and amplification delineate the risk of human infection in a residential community setting. Therefore, the objectives of our research were to determine the

association of the mosquito vectors and avian hosts in relation to human infection and identify the spatial and temporal patterns of WNV transmission during an outbreak in Davis, 1) to better understand the dynamics of transmission and 2) to predict risk of infection in humans, which in turn can be used to drive intervention strategies. To test our hypothesis we 1) identified environmental factors associated with the increased risk for enzootic WNV transmission, 2) sampled mosquitoes uniformly throughout an urban community to identify the distribution of vector species, 3) evaluated minimum and maximum temperature to measure the extrinsic incubation period of WNV (the time for an infected mosquito to become infective) within the mosquito species of interest, 4) evaluated the use of degree-day models as a predictive tool to forecast the initiation and subsidence of WNV transmission, and 5) identified significant temporal and geographical clustering of WNV-infected dead birds, mosquitoes, and human infection during the 2006 outbreak.

MATERIALS AND METHODS

Study Area

Davis, CA (38°32' N, 121°44' W), is an urban community in Yolo County, with a population of $\geq 64,400$ residents distributed over an area of $\sim 26 \text{ km}^2$ that is completely surrounded by irrigated agriculture, including row and fodder crops. A diverse greenbelt system of parks, golf courses, and bike paths is integrated within the residential areas. Davis was selected as our study site because of the unique geographic isolation of the urban setting, the occurrence of large populations of three species in the family *Corvidae* [American crows (*Corvus brachyrhynchos*), with a population size within Davis estimated at $> 10,000$,³⁵ western scrub-jays (*Aphelocoma californica*), and yellow-billed magpies (*Pica nuttalli*)].³⁶

Study Design for 2005

In 2005, an epidemic year for WNV transmission in Sacramento County, preliminary data were collected to determine whether any association existed between the abundance and infection rates of mosquito vectors in the genus *Culex* and communal roosting sites of American crows. Mosquitoes were collected at four sites weekly from 15 July to 23 September 2005 using dry ice (CO_2)-baited traps and gravid traps. The four sites were determined to be active crow roosts by following dawn and dusk flights of local crows for four nights. Study crows affixed with radio telemetry collars were confirmed to be present at these roost sites during midnight, which established that these sites were indeed used as overnight roosting sites (unpublished data). Although WNV was active at these confirmed roost sites, the number of crows present was limited during midsummer, and other corvid species such as western scrub jays were not clustered, indicating that a sampling design focused on crow roosts might not detect WNV transmission in other areas of Davis.

Study Design for 2006

In 2006, we changed our sampling design so we could determine whether mosquito abundance and WNV infection rates vary across urban, semi-urban, and agricultural landscapes in relation to WNV-infected dead birds and human cases. Our uniform sampling grid of 21 trap sites consisted of seven north-south transects of three trap locations each set 1.5 km apart by plotting a grid using ArcGIS versus 9.1 (ERSI, 2005) software. The distance of 1.5 km was selected to exceed the maximum nightly dispersal of 1 km/d for mosquitoes of the *Cx. pipiens* complex³⁷; however, this restriction on distance did not adjust for the maximum nightly flight range for *Cx. tarsalis*. Actual trap location at each of the 21 sites was dependent on resident consent and consisted of a variety of residential properties, agricultural interface, and greenbelt system of parks and golf courses. The geocoordinates for each trap site were recorded by a hand-held global positioning system device. All human cases reported during 2006 were confirmed to be infected with WNV by the Yolo County Health Department. Because of patient

confidentiality, only dates of onset and census blocks for each case were used in our analyses. Our research protocol was approved by the University of California Internal Review Board (IRB approval 200715100-1).

The Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) conducted routine integrated vector control to suppress mosquito populations throughout the season. When arbovirus response risk levels³⁸ reached epidemic levels, SYMVCD conducted aerial application of the insecticide pyrethrin synergized with piperonyl butoxide (EverGreen Crop Protection EC 60-6; McLaughlin Gormley King Company, Golden Valley, MN) sprayed in ultra-low volumes (0.0025 lb/acre) by a twin engine aircraft, flying at an approximate elevation of 95 m. The aerial spraying occurred on 8 and 9 August 2006 over Davis and the neighboring town of Woodland. *Cx. tarsalis* and *Cx. pipiens* from the Sacramento area were susceptible to pyrethrin insecticides when evaluated using the CDC bottle assay before application (SYMVCD, unpublished data).

Collection and Testing of Public Reported Dead Birds

In collaboration with the California Department of Health Services dead bird hotline, all dead birds reported by Davis residents were collected by us and sent to the California Animal Health and Food Safety laboratory for necropsy. All dead birds that were submitted without evidence of decay were tested. Kidney samples or oral swabs were sent to the Arbovirus Research Unit of the Center for Vectorborne Diseases (CVEC) laboratory at the University of California, Davis, for WNV testing by singleplex reverse transcriptase-polymerase chain reaction (RT-PCR).³⁹ Coordinates of the addresses for all reported dead birds that were tested for WNV infection were recorded by species and by date reported and included in spatial and temporal analyses. Dead birds with negative test results were identified as control birds, and dead birds with positive test results were identified as case birds.

Collection and Processing of Adult Mosquitoes

At each of the 21 sites in Davis, one dry ice-baited and one gravid trap were operated one night per week from April to mid-October. The mosquitoes were anesthetized with triethylamine, identified and enumerated to species, and pooled by species per trap type per site. Pools of ≤ 50 females were tested by a multiplex RT-PCR, which can detect 10 plaque forming units (PFUs) per 0.1 mL for WNV, SLEV, and WEEV RNA.^{40,41} Infection rates and 95% confidence intervals (CIs) from each trap site and cumulative infection rates, at weekly and monthly intervals, were calculated using maximum likelihood estimates.⁴²

Weather Variables

Daily minimum and maximum temperatures, precipitation, soil temperature, and wind speed were recorded at a weather station located in Davis (38°32' N, 121°47' W) and downloaded from the California Integrated Pest Management project website (<http://www.ipm.ucdavis.edu>). Accumulated degree-days (or heat index for WNV replication in a mosquito) were calculated from the date of the first detection of WNV activity in 2006 to model the extrinsic incubation periods associated with transmission cycles during the epidemic (<http://www.ipm.ucdavis.edu>). The minimum temperature for the degree-day accumulation was set at 14.3°C, which was the minimum threshold for WNV replication in laboratory studies.¹²

Statistical Analyses

Data were analyzed using Minitab version 13.1 (Minitab Software, State College, PA) and R program version 2.3.1 (The R-Development Core Team, <http://www.cran.r-project.org>). Data were checked for normality using the Kolmogorov-Smirnov test for normality. Abundance

data were not normally distributed. Approximate normality was achieved by $\ln(x + 1)$ transformation and back transformed to obtain the geometric mean of the number of female *Culex* spp. collected per trap night. The Mann-Whitney *U* test, a non-parametric test, was used to determine if the differences in mosquito abundance because of the aerial adulticide application were statistically significant.

A Yolo County land cover layer was downloaded from <http://frap.cdf.ca.gov/data.html> and mapped using ArcGIS versus 9.1 (ERSI, 2005) software. Coordinates of each trap site were recorded and plotted on the map. Mosquito collection dates and test results for each mosquito pool were recorded, and dispersion of WNV was monitored by using Tracking Analyst, an ArcGIS extension.

First-order measure of spatial intensity was calculated using a spatial scan statistic method (SatScan 7.0 software, Kulldorf, M. and Information Mgmt. Services, 2004. Available from <http://satscan.org>) using a retrospective space-time Bernoulli probability model to detect significant spatial-temporal clustering of WNV-infected dead birds. This technique delineated clusters of positive dead birds by comparing the locations of the control bird coordinates, which were locations at which dead birds that tested negative for WNV infection were reported during the same time period. The spatial scan statistic tests the statistical likelihood of the distribution of the cases in relation to the controls using a Bernoulli probability distribution through a Monte Carlo simulation repeated 999 times. Similar spatial-temporal cluster analyses were conducted using the coordinates of the WNV-positive *Cx. pipiens* and *Cx. tarsalis* pools compared with the coordinates of all of the negative pools. Significant spatial-temporal clusters were mapped using ArcGIS.

A local Moran I test for spatial autocorrelation was performed using SSTAT v 4.70 (Carpenter, University of California, Davis, CA) as a second-order measurement for spatial dependency of the *Cx. tarsalis* and *Cx. pipiens* abundance on the trap sites of the uniform trapping grid.⁴³ Spatial autocorrelation was expected because of the underlying natural heterogeneity of mosquito distributions. Interpolation method of ordinary kriging, which takes into account spatial autocorrelation, was used to describe the spatial distribution of *Cx. tarsalis* and *Cx. pipiens* to detect areas with high abundance using the ArcGIS Spatial Analyst software extension. The validity of the variogram models was evaluated using the mean prediction error closest to 0 for an unbiased model and the root mean square standardized error closest to 1 to create best fit kriging surfaces. The validity of the kriging surfaces was confirmed separately for *Cx. pipiens* and *Cx. tarsalis* abundance outputs to adjust for the differences in flight patterns and dispersal behaviors between these two species.

RESULTS

2005 Results

In 2005, the Sacramento County WNV human case incidence was 14.5 per 100,000, whereas the incidence in neighboring Yolo County was 7.1 per 100,000 (<http://westnile.ca.gov/>); the case incidence for Davis was 6.2 cases per 100,000. All four human cases in Davis occurred within 12 days from 28 July to 9 August 2005 (Figure 1). An additional asymptomatic infection was detected during routine screening of blood donors on 1 September but was not included as a clinical case in the epidemic curve. During 2005, a total of 1,174 mosquitoes were collected at the four crow roosts over 12 weeks from 15 July to 23 September. Six WNV-positive mosquito pools were collected at two of the four roost sites. At one site, a public park, three gravid *Cx. pipiens* pools were collected on 12 and 19 August and 9 September, when the infection rate was 14.61 per 1,000 (95% CI, 3.95, 41.92) and one WNV-positive *Cx. tarsalis* pool was collected on 15 July, when the infection rate was 3.44 (95% CI, 0.20, 17.47). At a second site, the City Hall in the center of Davis, one positive *Cx. tarsalis* pool was collected

on 15 July when the infection rate was 6.54 (95% CI, 0.40, 32.65), and the second pool was collected on 29 July and consisted of gravid *Cx. pipiens*; the minimum infection rate was 13.34 (95% CI, 0.88, 64.33). A total of 12 (32%) reported dead birds were positive for WNV in 2005 of 38 that were tested. The majority (75%) of the WNV-infected dead birds were corvids; five American crows (42%), three yellow-billed magpies (25%), and one western scrub-jay (8%).

2006 Results

In 2006, even though our mosquito sampling began in April and we collected and submitted all of the reported dead birds considered suitable for testing, we did not detect WNV activity until 28 June, when a WNV-infected dead crow was collected in west Davis. The date of onset for the first human case reported from Davis occurred 4 days later. The onset of WNV activity was temporally associated with a warming period when evening temperatures remained above the minimum required for virus replication within *Culex* mosquitoes (Figure 2). Although the 2006 human case incidence in Sacramento County decreased to 1.23 per 100,000, the Yolo County human case incidence increased to 15.42 per 100,000 (<http://westnile.ca.gov/>). The epidemic curve for these cases is plotted by the reported week of onset with the 2005 cases (Figure 1). The 2006 reported dates of onset are plotted with the recovery dates for each WNV-infected dead bird and dates of collection for each WNV-positive mosquito, the episodic peaks of activity that are associated with the completion of an extrinsic incubation period are indicated with arrows (Figure 3). The census blocks of the cases were plotted with the WNV-positive mosquito trapping sites and were used to determine the raw annualized incidence per block (Figure 4). Case incidence by census block ranged from 0 to 200 per 1,000, showing the high degree of variation among blocks and the effects of small population size and low case numbers on our estimates. Because of the occurrence of asymptomatic and unreported infections and patient confidentiality, a cluster analysis was not conducted on these data. Significant clusters of WNV-positive mosquito pools and WNV-infected dead bird clusters were included in Figure 4 to show the temporal-spatial association of the WNV-positive gravid *Cx. pipiens* cluster to the distribution of the human cases.

Overall, for 2006, 114 dead birds reported from Davis were collected and tested, of which 37 (32.5%) were infected with WNV. The majority of these WNV-infected dead birds were corvids: American crow ($N = 17$, 46%), western scrub-jay ($N = 7$, 19%), and yellow-billed magpie ($N = 9$, 24%). Although we increased our dead bird recovery effort in 2006, the proportion of WNV-infected dead birds out of the total tested (32%) was identical during 2005 and 2006 and not significantly different ($P = 0.92$) when tested to detect a difference in two proportions. In July, 25 WNV-infected dead birds were collected in Davis, at a high density of 2.5 infected dead birds per square mile, far exceeding the 0.1 per square mile risk threshold.⁴⁴ Eleven more WNV-infected dead birds were collected in August and one in September. A cluster analysis, of all WNV-infected dead birds, using a Bernoulli probability model, detected a significant ($P = 0.016$) spatial-temporal cluster of eight WNV-infected dead birds with a cluster radius 7.0 km occurring between 26 July and 8 August, with the optimal temporal cluster size restricted to 12% (2 weeks) of the period when WNV-infected dead birds were collected (Figure 4). Results of a purely spatial cluster analysis were not significant because of the widespread distribution of the WNV-infected dead birds throughout the study area by the end of the season. Because crow or magpie populations did not communally roost at this time of the year, dead birds were more evenly dispersed.⁴⁵

A total of 15,329 mosquitoes were collected within 21 CO₂-baited traps over 617 trap nights, and 1,704 mosquitoes were collected within 21 gravid traps during 590 trap nights (Table 1). CO₂-baited trap collections consisted predominately of female *Cx. tarsalis* (83.1%), whereas the gravid trap collections consisted predominately of female *Cx. pipiens* (61.9%; Table 1). A

total of 1,355 mosquito pools were tested, of which 16 were positive for WNV (Table 2); none were positive for WEEV or SLEV.

Daily minimum and maximum temperatures for Davis for January to October 2006 were plotted along with the infection rates for both *Cx. tarsalis* and *Cx. pipiens* (Figure 2). WNV infection in the mosquitoes was not detectable until nightly temperatures remained above the minimum threshold of 14.3°C for viral replication in the mosquitoes. We focused our analyses on night minimal temperatures, because *Cx. tarsalis* and *Cx. pipiens* egress from diurnal resting refugia after dusk⁴⁶ and coincidental to frequent low temperatures associated with the inland movement of cool maritime air from the coast. A degree-day model was initiated from the first date WNV activity was detected in Davis (the collection date of the first reported WNV-infected dead bird), with the minimum temperature set at 14.3°C and 109 degree-days required for each extrinsic incubation period.¹² This model predicted the initial increase in WNV transmission and the subsequent episodic peaks of the epidemic through two additional cycles of an extrinsic incubation period (Table 3; Figures 2 and 3).

Most WNV activity occurred during July and August, with 14 of 15 confirmed human cases in 2006 reported within an 8-week period. Seven cases were reported in July, and the incidence reached 10.9 cases per 100,000 by the end of July. Seven additional cases were reported in August (Figure 1). WNV infection rate in gravid *Cx. pipiens* collected from the gravid traps also was highest in July at 29.91 per 1,000 (95% CI, 11.32, 65.05; Figure 5). Peak infection rate in host-seeking *Cx. pipiens* occurred in August at 9.30 per 1,000 (95% CI, 2.46, 24.94), and no WNV-positive *Cx. pipiens* pools were collected in September when abundance was low (Figure 5). Infection rate of *Cx. tarsalis* from CO₂-baited traps peaked in August at 2.61 per 1,000 (95% CI, 0.86, 6.24); however, WNV-positive pools were also collected in September (Figure 5).

Local Moran I test for randomly distributed data showed evidence of spatial clustering for *Cx. tarsalis* CO₂ trap abundance ($P = 0.014$) and *Cx. pipiens* gravid trap abundance ($P = 0.009$) but not *Cx. pipiens* CO₂ trap abundance ($P = 0.67$). A significant ($P = 0.001$) spatial-temporal cluster of the WNV-positive gravid *Cx. pipiens* was detected with a radius of 3.2 km occurring between 5 and 20 July (Figure 4). A significant ($P = 0.007$) spatial-temporal clustering of *Cx. tarsalis* included three WNV-positive pools collected with CO₂ traps occurring between 26 July and 1 August, all at site 1, situated on the northwest corner of the sampling grid (Figure 4). Because of the observed spatial overlapping of the significant WNV-infected dead bird cluster and WNV-positive *Cx. tarsalis* cluster, a multivariate scan test using both data sets was conducted. A significant ($P = 0.002$) spatial-temporal cluster was detected, further strengthening the association of these two WNV indicators. The kriging of the mosquito abundance data showed that the distribution of the two species varied spatially. *Cx. tarsalis* abundance was highest at the periphery of Davis adjacent to agricultural habitats (Figure 6), whereas *Cx. pipiens* abundance clustered within Davis and was associated with residential housing and park land (Figure 7).

Enzootic virus activity and the occurrence of human cases throughout Davis by the end of July led to an emergency response by the SYMVCD to conduct aerial ULV applications of Evergreen over the cities of Davis and Woodland on 8 and 9 August. Although mosquito abundance declined after the spray, the decrease was marginally significant for *Cx. pipiens* (one-sided Mann-Whitney test statistic, $W = 471$; $P = 0.05$) and *Cx. tarsalis* (one-sided Mann-Whitney test statistic, $W = 459$; $P = 0.10$). Although WNV activity peaked by mid-July, low levels of WNV activity were detected into September. One additional human case was reported in December. However, the date of onset for this case was not determined.

DISCUSSION

Using extensive mosquito and dead bird surveillance as well as GIS and spatial statistics, we studied the spatial and temporal distribution of WNV during the introduction and epidemic phases of the invasion into Davis, CA, and were able to identify significant risk factors for human infection. An epizootic with high avian mortality and high equine incidence of infection¹⁸ was associated with a moderate human incidence of human disease in 2005. Avian mortality associated with WNV-positive *Cx. tarsalis* was likely the first evidence of WNV activity in Davis during 2006.⁴⁷ Our results indicate, however, that high human incidence in 2006 was accompanied by significant spatial and temporal clustering of WNV-positive *Cx. pipiens*, which was not detected by our sampling protocols in 2005.

For our study, the urban landscape completely surrounded by agriculture provided a unique opportunity to characterize a WNV outbreak in an isolated community with minimal outside influences to confound the factors involved in WNV enzootic and epidemic transmission. It was necessary to quantify associations among vectors, avian hosts, and the human cases spatially and temporally to understand the interplay of risk factors on the dynamics of WNV amplification leading to the outbreak. In 2005, the temporal clustering of the four human cases in Davis was preceded by WNV-positive *Cx. tarsalis* pools that were collected at crow roosts. Previous studies have shown spatial and temporal associations of human infection and higher than normal die-offs among American crows.^{8,48,49} The associated mosquito species, their abundance, and infection rates were not evaluated, however, in these studies. Our results from 2005 suggested that mosquito WNV activity intermittently was high at two of four roost sites and was evidence for increased risk for human infection based on the California risk assessment model.³⁸ It should be noted that mosquito trapping was not conducted at areas throughout the urban landscape (i.e., in places that did not have established roosts); thus, we could not determine an association across all habitats in the city. Therefore, our 2006 sampling efforts were modified to account for this detection bias.

Establishing a fixed uniform mosquito sampling scheme was critical to spatially relate WNV risk factors to human infections. Although our intense 2006 trapping scheme is not feasible for local mosquito control agencies to maintain for long time periods because of cost and labor, it was useful in determining the spatial distribution of future sampling efforts and areas that require focused surveillance to provide an early warning of future outbreaks. As expected, we observed significant spatial autocorrelation within *Cx. tarsalis* CO₂-baited trap abundance and within *Cx. pipiens* gravid trap abundance. The difference in abundance estimations from different trap types shows the effectiveness of using different trap types to monitor population density of different mosquito species. Our sampling results from 2006 indicated that the distribution of WNV was not random throughout an urban landscape. Instead, it was clustered in both time and space and may have been dependent on the distribution of the mosquito species. The significant spatial-temporal distribution of WNV, as determined from the cluster analyses, may have been influenced by environmental factors affecting the heterogeneity of the vectors, as determined from the kriging, such as agricultural land use for *Cx. tarsalis* and residential and park systems for *Cx. pipiens*. The distance of 1.5 km between the sampling sites may have influenced the kriging predictions for *Cx. tarsalis* because the distance was set in response to the dispersal patterns of *Cx. pipiens*; however, *Cx. tarsalis* abundance was greatest around the perimeter of the trapping grid. Therefore, abundance was not dependent on the distance between trap sites within the urban area. A method to evaluate the accuracy of the prediction would be to change the distances between the trapping sites during future sampling or to conduct a cross-validation of the kriging prediction by systematically removing point data and confirming that the predictions remained accurate.⁵⁰

The presence of spatially distinct spatial-temporal clustering of *Cx. tarsalis* and *Cx. pipiens* is indicative of the differences in the habitats each species occupies and the differences in the risk that each species pose for human infection. Results from multivariate cluster analysis show spatial overlap in WNV activity. The association of WNV-positive *Cx. tarsalis* and WNV-infected dead birds, as determined from the multivariate analysis, may have been a critical factor during the initiation of the outbreak.⁴⁷ We similarly detected overlap of *Cx. pipiens* clusters with a high incidence of human cases within census block groups. It is possible that we would have detected a significant spatial-temporal multivariate cluster of WNV-positive *Cx. pipiens* and human cases if the distribution of all of the human cases (symptomatic and asymptomatic) were determined and available to us to conduct those kinds of analyses.

The exact temporal association between WNV infectious mosquitoes and human infection is difficult to assess. It is difficult to determine the length of time from the first indication of WNV activity to the peak risk of human infection because of the varying temperature dependent duration of the extrinsic incubation period in the mosquito, the wide range (3–14 days) of the intrinsic incubation period in the human host,⁵¹ and the low sensitivity of mosquito sampling where considerable virus amplification must occur before activity is detectable.¹⁸ We were able to approximate this time period, however, using a degree-day model. By measuring accumulated degree-days, we calculated the approximate length of time it took for the completion of the extrinsic incubation period in the infected mosquito cohort and documented the subsequent transmission to avian and human hosts during the 2006 outbreak.^{12,52} When the first detection of WNV in a dead crow was used as the initiation of WNV amplification, the degree-day model accurately determined two episodic peaks of WNV transmission that occurred through the completion of three cycles of extrinsic incubation. Interestingly, if the onset of WNV activity for the 2006 outbreak was the first publicly reported WNV-infected dead crow, it is likely that the amount of WNV activity would not have been as intense if temperatures did not concurrently increase. WNV activity was not detected in Davis until nightly temperatures remained above the virus developmental threshold of 14.3°C. Research studying the regional stability of viral genotypes, virus over wintering strategies, and the role of migrating birds in the northward movement of virus should be continued to better understand the yearly initiation of WNV transmission in temperate latitudes.

Our results provide important key risk factors for vector control programs to monitor, especially in northern latitudes. Our findings show that warm nightly temperatures combined with finding WNV-positive *Cx. pipiens* within residential landscapes are indicative of a high risk for human infection and the need for immediately initiating emergency adult control efforts, targeted around the locations with evidence of viral activity, to interrupt transmission before substantial human infection occurs. During our study, WNV transmission reached epidemic emergency response levels even though mosquito abundance remained relatively low. This shows how WNV can be amplified at low entomologic thresholds (e.g., mosquito densities) and why risk of arbovirus epidemics cannot be predicted by mosquito abundance alone. Although subsidence in WNV transmission followed the emergency aerial adulticide applications, this was associated with a concurrent decrease in nightly temperatures.⁵² We suspect that natural environmental factors, combined with vector control efforts, limited WNV transmission during the remainder of the season. Research on the timing of vector control measures should be continued to refine our understanding of the most effective vector interventions strategies.

The spatial determinants or other factors that impeded the movement of WNV between Sacramento and Yolo Counties in 2005 are unknown. Our results showed the dynamic epidemiology of WNV in North America and difficulties in predicting the occurrence and amplification transmission of WNV and spill-over transmission to people in neighboring counties. The use of GIS and spatial statistics in tracking outbreaks and focusing intervention on focal areas of WNV transmission has been shown in this study and should be continued in

WNV surveillance. Real-time analysis of surveillance data is needed to forecast precisely when and where enzootic activity is likely to amplify to levels where tangential transmission is eminent and emergency control necessary. WNV surveillance in northern California should include monitoring early seasonal changes in temperatures and sampling at sites with WNV-infected dead birds within urban landscapes.

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Figure 1. Clinically diagnosed human WNV cases from 2005 to 2006 by reported week of onset for Davis, CA. The date of onset was not determined for the case reported in December 2006.

Figure 2.

Daily minimum and maximum temperatures for Davis, CA, 2006, with minimum temperature threshold for viral replication to occur at 14.3°C indicated with the thick horizontal line. Minimum temperatures remaining above the minimum threshold preceded WNV infection in *Culex* mosquitoes.

Figure 3.

Epidemic curve showing clinical human WNV cases by date of onset and WNV-infected dead birds by date reported in Davis, CA, 2006. The three arrows indicate the dates of the completion of an EIP determined from a degree-day model associated with an increase in the occurrence of human cases, WNV-positive mosquitoes, and WNV-infected dead birds.

Figure 4.

Map of Davis, CA, showing WNV-positive mosquito trapping sites and the census block of the 2006 human WNV cases* with the raw annualized incidence rates per 1,000 at risk and overlapping sections of significant spatial-temporal clusters of WNV-infected dead birds, WNV-positive *Cx. tarsalis*, and WNV-positive gravid *Cx. pipiens*. *Point locations of the cases were randomly plotted within the confirmed census block to protect individual privacy but used to show how many cases occurred within each positive census block.

Figure 5. Weekly mean* abundance and WNV infection rate estimates for (A) *Cx. pipiens* at gravid and CO₂ traps and (B) *Cx. tarsalis* at CO₂ traps collected in Davis, CA, 2006. *Geometric mean calculated as back transformed mean of $\ln(x + 1)$.

Figure 6. Distribution of *Cx. tarsalis* as predicted using a kriging interpolation model on the mean abundance of females collected with carbon dioxide-baited traps at 21 trapping sites throughout Davis, CA, for 2006. Dots represent trap locations.

Figure 7. Distribution of *Cx. pipiens* as predicted using a kriging interpolation model on the median abundance of females collected with gravid traps at 21 trapping sites throughout Davis, CA, for 2006. Dots represent trap locations.

Table 1

Mosquito species collected in carbon dioxide-baited and gravid traps at 21 sites in Davis, CA, during April to October 2006

Species	CO ₂ -baited		Gravid	
	Male [no. (%)]	Female [no. (%)]	Male [no. (%)]	Female [no. (%)]
<i>Aedes melanimon</i>		129 (0.9)		4 (0.3)
<i>Aedes sierrensis</i>	207 (81.2)	210 (1.4)	27 (7.5)	4 (0.3)
<i>Aedes washinoi</i>		1 (< 0.1)		
<i>Anopheles franciscanus</i>		3 (< 0.1)		2 (0.2)
<i>Anopheles freeborni</i>	4 (1.6)	160 (1.1)	42 (11.7)	51 (3.8)
<i>Culiseta incidens</i>		132 (0.9)	19 (5.3)	52 (3.9)
<i>Culiseta inornata</i>	1 (0.4)	135 (0.9)	23 (6.4)	6 (0.4)
<i>Culex pipiens</i>	18 (7)	1,561 (10.3)	124 (34.4)	1,055 (78.5)
<i>Culex stigmatosoma</i>		1 (< 0.1)		42 (3.1)
<i>Culex tarsalis</i>	25 (9.8)	12,742 (84.5)	125 (34.7)	128 (9.5)
Total	255	15,074	360	1,344

Table 2

Female *Cx. tarsalis* and *Cx. pipiens* abundance (back transformed geometric mean per trap night, tn) and WNV infection rates at 21 sites along seven north to south oriented west to east transects in Davis, CA, during 2006

Site	Female <i>Cx. tarsalis</i>					Female <i>Cx. pipiens</i>				
	CO ₂ -trap total (mean/tn)	Gravid trap total (mean/ tn)	WNV +ve pools (total tested)	Infection rate per 1,000 (95% CI)	CO ₂ -trap total (mean/tn)	Gravid trap total (mean/tn)	WNV +ve pools (total tested)	Infection rate per 1,000 (95% CI)		
1	1,080 (6.8)	12 (0.3)	6 (47)	5.33 (2.24, 10.98)	38 (1.1)	19 (0.6)	0 (20)	—		
2	142 (1.7)	2 (0.04)	0 (25)	—	64 (1.4)	31 (1.0)	1 (40)	8.20 (0.48, 38.77)		
3	238 (2.6)	4 (0.1)	0 (28)	—	169 (3.8)	62 (1.1)	0 (39)	—		
4	203 (2.8)	3 (0.1)	0 (27)	—	52 (1.3)	144 (3.1)	1 (36)	5.03 (0.30, 24.14)		
5	74 (1.3)	0	0 (20)	—	86 (1.6)	90 (1.7)	0 (39)	—		
6	79 (1.5)	5 (0.1)	0 (18)	—	13 (0.5)	26 (0.9)	0 (20)	—		
7	477 (5.1)	0	0 (30)	—	138 (2.7)	114 (2.1)	1 (45)	3.89 (0.23, 18.56)		
8	75 (1.4)	2 (0.01)	0 (22)	—	87 (1.8)	86 (2.0)	1 (45)	5.69 (0.33, 27.28)		
9	113 (1.8)	0	0 (23)	—	169 (2.5)	41 (1.2)	0 (43)	—		
10	252 (3.4)	6 (0.3)	0 (32)	—	23 (0.9)	93 (1.7)	0 (32)	—		
11	281 (3.3)	25 (0.7)	1 (35)	3.40 (0.19, 17.26)	82 (1.9)	122 (2.4)	1 (44)	5.08 (0.30, 24.42)		
12	78 (1.6)	3 (0.1)	0 (20)	—	16 (0.6)	35 (1.0)	0 (24)	—		
13	1,166 (8.3)	3 (0.1)	1 (47)	0.9 (0.05, 4.40)	44 (1.3)	22 (0.7)	1 (24)	15.29 (0.88, 72.82)		
14	375 (3.9)	0	0 (25)	—	193 (2.9)	36 (1.1)	1 (37)	4.10 (0.25, 19.51)		
15	547 (7.7)	1 (0.03)	1 (31)	1.80 (0.11, 8.70)	146 (2.5)	21 (0.7)	0 (36)	—		
16	551 (4.1)	30 (1.0)	0 (28)	—	4 (0.2)	1 (0.1)	0 (4)	—		
17	170 (3.1)	2 (0.1)	0 (19)	—	68 (1.4)	43 (1.2)	1 (26)	8.81 (0.52, 41.81)		
18	614 (5.9)	0	1 (31)	1.58 (0.09, 7.60)	68 (1.6)	10 (0.4)	0 (22)	—		
19	3307 (23.2)	16 (0.5)	2 (88)	0.61 (0.11, 1.99)	28 (0.9)	8 (0.3)	0 (11)	—		
20	831 (10.0)	8 (0.4)	0 (32)	—	21 (0.7)	36 (0.9)	0 (14)	—		
21	1989 (18.4)	6 (0.2)	1 (60)	0.50 (0.03, 2.44)	52 (1.3)	15 (0.6)	0 (26)	—		

Table 3

Degree-day model^{*} through three extrinsic incubation periods[†] starting from the date WNV activity was first detected

Date	Air temperatures (°C) degree-days			
	Minimum	Maximum	Daily	Accumulated
28-Jun	17.2	32.8	10.7	10.7
29-Jun	14.4	35.0	10.4	21.1
30-Jun	15.0	30.0	8.2	29.3
1-Jul	12.2	33.9	9.0	38.3
2-Jul	13.3	31.1	8.0	46.3
3-Jul	12.8	33.3	8.9	55.3
4-Jul	13.3	32.2	8.6	63.8
5-Jul	12.8	29.4	7.0	70.8
6-Jul	12.2	30.6	7.4	78.2
7-Jul	12.2	33.9	9.0	87.2
8-Jul	13.3	37.2	11.0	98.3 [‡]
9-Jul	17.2	37.8	13.2	111.5 [‡]
10-Jul	12.8	31.7	8.1	119.6
11-Jul	12.8	31.1	7.8	127.4
12-Jul	12.2	29.4	6.8	134.3
13-Jul	14.4	34.4	10.1	144.4
14-Jul	14.4	35.0	10.4	154.8
15-Jul	11.7	35.6	9.7	164.5
16-Jul	12.8	39.4	12.0	176.4
17-Jul	17.8	40.0	14.6	191.0
18-Jul	18.3	37.8	13.8	204.8 [§]
19-Jul	18.9	36.1	13.2	218.0 [§]
20-Jul	15.0	38.9	12.7	230.6
21-Jul	21.7	40.0	16.6	247.2
22-Jul	25.0	45.0	20.7	267.9
23-Jul	24.4	43.9	19.9	287.7
24-Jul	20.0	41.1	16.3	304.0
25-Jul	18.3	40.0	14.9	318.8 [¶]
26-Jul	18.9	37.8	14.1	332.9 [¶]
27-Jul	16.1	35.6	11.6	344.4

The first EIP, completed on 8 July, was temporally associated with the first cluster of WNV transmission in birds and humans, and the completion of the second and third EIP on 19 and 26 July, respectively, was temporally associated with the two additional peaks of WNV activity.

^{*} Minimum temperature of 14.3°C set for degree-days to accumulate.

[†] 109 degree-day accumulation per extrinsic incubation period.¹²

[‡] End of first EIP.

[§] End of second EIP.

[¶] End of third EIP.