In Los Angeles, California, USA, 2 epidemics of West Nile virus (WNV) disease have occurred since WNV was recognized in 2003. To assess which measure of risk was most predictive of human cases, we compared 3 measures: the California Mosquito-Borne Virus Surveillance and Response Plan Assessment, the vector index, and the Dynamic Continuous-Area Space-Time system. A case–crossover study was performed by using symptom onset dates from 384 persons with WNV infection to determine their relative environmental exposure to high-risk conditions as measured by each method. Receiver-operating characteristic plots determined thresholds for each model, and the area under the curve was used to compare methods. We found that the best risk assessment model for human WNV cases included surveillance data from avian, mosquito, and climate sources.

West Nile virus (WNV; family Flaviviridae, genus Flavivirus) is amplified within a mosquito–bird cycle, with tangential transmission to equids and humans (1). Since the introduction of WNV into Los Angeles, California, USA, in 2003, our research (2–5) has focused on surveillance indicators for enzootic WNV transmission and prediction of human cases. The Greater Los Angeles County Vector Control District (GLACVCD) serves >6 million of the ≈10 million residents of Los Angeles County and conducts year-round surveillance for WNV activity (6). In addition to having a robust surveillance dataset, Los Angeles County is a suitable location for evaluating environmental risk because the large human population enables the sensitive detection of dead birds (7), increases opportunities for human–vector contact, and experienced 2 outbreaks during the study period (6).

We compared the predictive ability of 3 measures of human risk by using time-series graphs, sensitivity, specificity, positive predictive value (PPV), and concordance between human case onset and states of high risk based on enzootic transmission during 2004–2010. We believed that for operational decision support a successful risk measure should correctly 1) identify periods of low risk when few or no cases occur, 2) predict high or increased risk before human cases occur, and 3) identify periods of high risk concurrent with the occurrence of human cases.

The 3 measures of risk we compared were the California Mosquito-Borne Virus Risk Assessment (CMVRA), the vector index, and the Dynamic Continuous-Area Space-Time (DYCAST) system. The CMVRA (8) calculates risk on the basis of ranks of environmental variables for enzootic WNV transmission and is used by health agencies throughout California to measure risk. At its inception, the CMVRA was evaluated retrospectively for its ability to detect cases of Western equine encephalomyelitis virus (family Togaviridae, genus Alphavirus) and St. Louis encephalitis virus (family Flaviviridae, genus Flavivirus) in California during low-, medium- and high-risk seasons (9). Additional assessment of the ability of CMVRA to track WNV cases in Bakersfield, California, produced impressive results during 2004 and 2007 (10,11).

The second method was the vector index, an estimate of the number of infected mosquitoes collected per trap-night. This index successfully determined human risk in Colorado (12,13) and is used by the Colorado Department of Public Health and Environment (www.cdphe.state.co.us/dc/zoonosis/wvn/wnvsentinel.html).
The third method was the DYCAST (14) system, which provides an assessment of risk in time and space by using reports of dead birds from the California Department of Public Health Dead Bird Hotline. This risk estimate differs from the previous 2 in that the spatial scale is fine (0.44 km² grid cells), it is computationally more complex, and it does not rely on laboratory test results (15).

Understanding the characteristics of risk estimates to determine the best predictive measure for human cases is needed for several reasons. First, reducing the rate of false-positive results will reduce message fatigue associated with repeated false warnings of high-risk conditions. Second, increasing the proportion of high-risk areas correctly identified (sensitivity) can reduce the costs associated with emergency mosquito control by correctly focusing timely intervention. Third, a qualitative assessment of risk estimates that incorporates different variables for enzootic transmission enables understanding of the ability of different assemblages of surveillance data for predicting human risk. Overall, a better understanding of the tools used in decision support for emergency intervention can only improve the protection of human health.

Materials and Methods

The epidemiology of WNV in Los Angeles has been described in detail (6). Methods used for data collection for each risk assessment tool are summarized briefly below and in detail (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-1558-Techapp.pdf).

CMVRA

The CMVRA (8) calculated risk on the basis of average daily temperature, mosquito abundance and infection, counts of WNV RNA–positive dead birds, and sentinel chicken seroconversions over successive 2-week periods. Each variable was assigned to quintile ranks, and these categorical values were averaged to calculate a final risk estimate. Thresholds ≤2.5 were considered low-risk (normal season) conditions; those 2.6–4.0 were considered medium-risk (emergency planning) conditions; and those ≥4.1 were considered high-risk (epidemic) conditions.

Details of sampling, laboratory testing, and risk calculation are summarized in the online Technical Appendix. In the current study, temperature data were aggregated from the National Aeronautics and Space Administration Terrestrial Observation and Prediction System (16) at a 1-km² scale for the GLACVCD jurisdiction. Abundance anomalies for Culex pipiens quinquefasciatus mosquitoes collected by gravid traps (6) were calculated by comparing current 2-week estimates to 5-year averages for the same period. WNV infection incidence in Cx. p. quinquefasciatus mosquitoes was calculated from mosquito pool data by using the Excel (Microsoft, Redmond, WA, USA) add-in developed by Biggerstaff (17). Dead birds reported by the public and testing positive for WNV RNA and sentinel chicken seroconversions were ranked according to frequency and scale of occurrence for the broad region (Los Angeles County) and the specific region (within GLACVCD jurisdiction). Reports of sentinel chicken seroconversions from Los Angeles County outside the GLACVCD boundary were found on the California West Nile virus Web site (www.westnile.ca.gov). Human cases, recorded by the Los Angeles County Department of Public Health, Acute Communicable Disease Control, were excluded from the current risk calculations because they were used as an outcome measure.

Vector Index

The vector index also was calculated for 2-week time steps by using abundance (numbers per gravid trap per night) and infection incidence for Cx. p. quinquefasciatus mosquitoes collected by gravid traps by using the bias-corrected maximum-likelihood estimate (6) (online Technical Appendix). Usually the species-specific maximum-likelihood estimate is multiplied by female mosquito abundance measured by CO₂ trap counts to yield an arbovirus equivalent of the entomologic inoculation rate in malaria epidemiology (18). Vector index estimates were stratified into frequency percentiles by using SAS version 9.1 software (SAS Institute Inc., Cary NC, USA), and the percentiles were assessed individually for their efficacy for predicting human cases.

DYCAST

For DYCAST, 0.44-km² grid cells were overlaid onto the Los Angeles County study area. There were 22,687 grid cells in Los Angeles County, but only 6,666 grid cells were within the GLACVCD boundary. We assessed the DYCAST risk estimates by using a predetermined Knox test significance threshold of ~0.10 = high risk. The Knox test statistically delineated significantly positive groups of grid cells into clusters or hot spots. Unlike the other 2 methods, the DYCAST model assessed risk on a daily basis, providing a time and location of high risk on the basis of the spatial grouping of the number of reports of dead birds; data were independent of a predetermined spatial allocation of sampling assets and laboratory diagnostics. To make this method comparable with the previous 2 methods, we selected the minimum DYCAST value by grid for each 2-week period. The DYCAST model then was assessed by using daily and 2-week aggregations.

Another unique feature of the DYCAST model is the spatial resolution. The other 2 methods provide an assessment of high-risk conditions that can be anywhere within the GLACVCD boundary, whereas DYCAST delineates high-risk conditions within a defined space.
Again, to make our assessments comparable, we aggregated DYCAST high- and low-risk cells spatially by week, up to the spatial limit imposed by the GLACVCD boundary (6,666 cells). The new spatial aggregates were compared with human case occurrence to determine an optimal number of grid cells needed to establish a high-risk area. This comparison was performed by constructing a receiver operator characteristic (ROC) curve of the plotted sensitivity versus 1 – specificity for all aggregated cell counts.

Reports of Human Cases
Reports of laboratory-confirmed human cases, compiled by the Acute Communicable Disease Control program of the Los Angeles County Department of Public Health and occurring within GLACVCD, included West Nile fever (WNF) and West Nile neuroinvasive disease (WNND) diagnoses and asymptomatic viremic blood donors. Onset dates for symptomatic persons were adjusted backward 10 days to account for the intrinsic incubation period (19,20). Seven blood donors with viremia later became symptomatic for WNV disease and were added as WNV cases; the mean time from donation to symptom onset was 6.2 days (SD ±6.14, median 3.5). To account for earlier detection, the infection dates for all viremic blood donors were adjusted backward 4 days (10 latent days minus 6 induction days). As reported for Los Angeles County (6), the percentage of WNND among all reported WNV infections increased significantly over time because of reduced physician requests for laboratory testing for febrile illness, thereby reducing the total number of human cases reported. In addition, unpublished data from elsewhere in California also indicate that relatively few persons hospitalized with neuroinvasive disease are tested for WNV, which possibly also indicate that relatively few persons hospitalized with WNV-associated human disease. Because mosquito and public health agencies respond to reports of human cases regardless of diagnosis, we chose to use these data in the current analyses. The Institutional Review Board at the University of California, Davis, approved protocols for using human surveillance data (approval no. 201018171-1).

Analysis
Time-series graphs of the CMVRA and the vector index were plotted with human cases to depict which attained high-risk thresholds before human cases occurred. A true or false-positive finding was a time period identified as high risk during which ≥1 or 0 human infections occurred, respectively. A true-negative period was a period identified as low risk and during which no human infections occurred; conversely, false-negative periods were identified as low-risk periods when human infections occurred. Sensitivity was calculated as the proportion of high-risk periods correctly identified; specificity was the proportion of low-risk periods correctly identified (21). The PPV, likelihood ratio positive, and likelihood ratio negative were calculated as measures of relative precision (22).

ROC curves were plotted to define optimum response thresholds. The area under the curve (AUC) was calculated to compare the 2 methods. ROC and AUC calculations were performed by using SAS version 9.1 and the Macro %ROC (http://support.sas.com/kb/25/addl/fusion25017_5_roc.sas.txt).

With the above analyses providing information about the accuracy of each risk assessment, a separate case-crossover study was performed by using the known onset information to create an estimate of the relative risk of acquiring WNV during high-risk periods. Illness onset dates for case-patients and asymptomatic viremic blood donors were lagged backward as described above. Mantel-Haenszel relative risks were calculated to determine whether high-risk values were significantly associated with human infection (23–25). Mantel-Haenszel relative risks were calculated by using the proportion of high-risk periods before estimated infection as the expected frequency of exposure and the concordance odds of disease transmission occurring during a high-risk period by each model and threshold. Data aggregation and zonal statistics were performed by using PostgreSQL 8.3.7 and PostGIS 1.3.1.

Results

CMVRA
Risk estimates (Figure 1, panel A) consistently reached emergency planning thresholds (threshold ≥2.6) before human case detection. In 2004, epidemic thresholds (≥4.1) were reached by mid-August (Table 1) after 39 human cases had been reported. During the second epidemic in 2008, risk assessments reached epidemic thresholds after 8 human cases were identified. Using the epidemic threshold, we identified 13 true-positive intervals, 0 false-positive intervals, 151 true-negative intervals, and 28 false-negative intervals. Estimates using this method were driven by ranks for environmental conditions and infections in dead birds, followed by mosquito infection rates and abundance. Antecedent sentinel chicken seroconversions consistently ranked lowest on the 5-point scale until human cases occurred because they were temporally concordant (26).

Using the emergency planning threshold, we identified 40 true-positive intervals, 28 false-positive intervals, 123 true-negative intervals, and 1 false-negative interval. Although there were more false-positive intervals, they represented high-risk periods before the onset of human cases because the threshold reached ≥2.6 at least 2 weeks before human cases occurred in all study years except 2008.
Predicting West Nile Disease

(Table 1). On the basis of the advance warning that this risk estimate provided and the increase in sensitivity (Table 2), the 2.6 threshold was a better threshold for epidemic prediction.

We calculated sensitivity and specificity separately for each study year by using the 2.6 emergency planning threshold (Table 3). Use of this test validity revealed that sensitivity, i.e., correctly identified high-risk periods, dipped in 2005, whereas specificity, i.e., proportion of correctly identified low-risk periods, was lowest in 2006 and 2008.

**Vector Index**

Vector index estimates (Figure 1, panel B) were calculated biweekly for the entire study period for Cx. *p. quinquefasciatus* mosquito collections and were driven exclusively by mosquito infection incidence. Using the 65th percentile (0.018) as the threshold, we identified 38 true-positive, 37 false-positive, 116 true-negative, and 1 false-negative intervals. The frequency distribution of the vector index was highly right skewed and could not be evaluated at lower percentiles because all other percentiles were 0. The vector index increased and remained >0.095 (85th percentile) 4 weeks before the onset of human cases in 2004, 2009, and 2010 and 2 weeks before case onset in 2005 and 2006 (Figure 1, panel B). The sensitivity and specificity of the vector index, calculated annually (Table 3), demonstrated that sensitivity was lower than for the CMVRA in all study years except 2009 and 2010, with the lowest value (0.500) in 2007. The specificity of the vector index was consistently better than that of the CMVRA, except for 2009 and 2010, when only 2 human cases occurred.

**DYCAST**

Positive DYCAST cells were observed before human case occurrence in 5 of the 7 study years (Table 1). Counts of positive DYCAST grid cells compared with human case onset is presented in Figure 1, panel C. The DYCAST risk estimate, calculated by grouping the biweekly estimates, was used in the yearly comparisons of sensitivity and specificity (Table 3). Temporal changes in sensitivity and specificity showed the impact of reduced reporting of dead birds over time because the values for both measures of validity were highest in 2004 and declined to 0 or near 0 in all subsequent years.

**Human Case Reports**

A total of 389 cases of WNV disease were reported during the study period. Of these, 14 reports were missing onset date information and were not used to evaluate the risk estimates.

**Analysis**

The proportion of high-risk intervals correctly identified (sensitivity) was greatest in the CMVRA when the 2.6 emergency planning threshold was used (Table 2). The vector index provided the second highest sensitivity by using values just >0 (65th percentile). The greatest specificity, i.e., proportion of low-risk intervals correctly identified, was observed in the CMVRA at the epidemic threshold of 4.1, followed by the vector index at the 95th
Table 1. First dates for risk assessment thresholds and onset of human West Nile disease, Los Angeles, California, USA, 2004–2010*

| Model               | Threshold                  | Year     | Threshold met | First case |
|---------------------|----------------------------|----------|---------------|------------|
| CMVRA               | 2.6, emergency planning    | 2004     | Apr 30       | Jun 21     |
|                     |                            | 2005     | Jun 30       | Jul 5      |
|                     |                            | 2006     | Jul 31       | Jul 10     |
|                     |                            | 2007     | Jul 15       | Jul 20     |
|                     |                            | 2008     | Jun 15       | Jun 24     |
|                     |                            | 2009     | Jul 15       | Aug 18     |
|                     |                            | 2010     | Jun 30       | Sep 14     |
|                     | 4.1, epidemic               | 2004     | Aug 15       | Jun 21     |
|                     |                            | 2005     | Jul 31       | Jul 5      |
|                     |                            | 2006     | Aug 31       | Jul 10     |
|                     |                            | 2007     | Sep 15       | Jul 20     |
|                     |                            | 2008     | Jul 31       | Jun 24     |
|                     |                            | 2009     | Not observed | Aug 18     |
|                     |                            | 2010     | Not observed | Sep 14     |
| Vector index        | >0.018, 65th percentile    | 2004     | Apr 15       | Jun 21     |
|                     |                            | 2005     | Jun 15       | Jul 5      |
|                     |                            | 2006     | May 15       | Jul 10     |
|                     |                            | 2007     | May 15       | Jul 20     |
|                     |                            | 2008     | May 30       | Jun 24     |
|                     |                            | 2009     | Jul 15       | Aug 18     |
|                     |                            | 2010     | Jul 15       | Sep 14     |
|                     | >0.069, 80th percentile    | 2004     | Apr 15       | Jun 21     |
|                     |                            | 2005     | Jun 30       | Jul 5      |
|                     |                            | 2006     | Aug 15       | Jul 10     |
|                     |                            | 2007     | Jul 31       | Jul 20     |
|                     |                            | 2008     | Jul 15       | Jun 24     |
|                     |                            | 2009     | Aug 15       | Aug 18     |
|                     |                            | 2010     | Jul 31       | Sep 14     |
| DYCAST              | Daily                      | 2004     | May 4        | Jun 21     |
|                     |                            | 2005     | Jun 12       | Jul 5      |
|                     |                            | 2006     | Oct 4        | Jul 10     |
|                     |                            | 2007     | Aug 13       | Jul 20     |
|                     |                            | 2008     | Jun 4        | Jun 24     |
|                     |                            | 2009     | Jun 20       | Aug 18     |
|                     |                            | 2010     | Apr 5        | Sep 14     |
|                     | Weekly, wk. no.            | 2004     | 18           | 26         |
|                     |                            | 2005     | 24           | 28         |
|                     |                            | 2006     | 40           | 28         |
|                     |                            | 2007     | 33           | 29         |
|                     |                            | 2008     | 23           | 26         |
|                     |                            | 2009     | 24           | 34         |
|                     |                            | 2010     | 19           | 37         |

*CMVRA, California Mosquito-Borne Risk Assessment; DYCAST, Dynamic Continuous-Area Space-Time system.

percentile; the PPV followed this finding. The likelihood ratio positive, i.e., the likelihood that a high-risk condition was identified correctly when a human case occurred, was greatest for the vector index at the 95th percentile. The likelihood ratio negative, i.e., how much the odds of a human case decrease during low-risk conditions, was lowest in the emergency planning threshold of the CMVRA.

Discriminatory ability, as measured by the AUC, was greatest for the CMVRA (0.982), followed by the vector index (0.845) (Figure 2). Ideal response level cutoffs for the CMVRA as indicated in the ROC plots would be 1.8 and 2.6. The ideal response level for the vector index was more difficult to identify because of the obvious tradeoff between the sensitivity and specificity as evidenced in the ROC plot. The DYCAST cell aggregates performed no better than chance with an AUC of 0.468, with worst performance occurring when a single positive cell was used to assess risk.

A case-crossover study was conducted for all cases and asymptomatic blood donors with a known illness onset or donation date. The relative risk, i.e., risk for WNV infection given exposure to high-risk conditions, was greatest when detected by the CMVRA by using the emergency planning threshold (Table 2).

Discussion

Since the introduction of WNV into the United States in 1999, WNF and WNND have caused at least 31,365 illnesses and 1,250 deaths (27). Once considered to be a mild influenza-like illness, WNF is now understood to be an acute viral infection, often followed by months of illness associated with depression, altered moods,
Table 2. Comparison of CMVRA, vector index, and DYCAST for predicting risk for West Nile disease by the calculation threshold applied, validation method, and associated risk, Los Angeles, California, USA, 2004–2010*

| Model    | Sensitivity | Specificity | PPV | LRP | LRN | Mantel-Haenszel RR (95% CI) |
|----------|--------------|-------------|-----|-----|-----|----------------------------|
| CMVRA    |              |             |     |     |     |                            |
| 2.6      | 0.976        | 0.815       | 0.588 | 5.261 | 0.03 | 403.453 (70.506–2,308.659) |
| 4.1      | 0.317        | 1           | 1    | UND | 0.683 | 38.255 (29.425–49.736)    |

Vector index (percentile)

|          | Sensitivity | Specificity | PPV | LRP | LRN | Mantel-Haenszel RR (95% CI) |
|----------|-------------|-------------|-----|-----|-----|----------------------------|
| 0.018 (65) | 0.974 | 0.758 | 0.507 | 4.029 | 0.034 | 25.251 (18.120–35.033) |
| 0.041 (75) | 0.846 | 0.902 | 0.688 | 8.631 | 0.171 | 25.383 (18.350–35.112) |
| 0.096 (85) | 0.564 | 0.954 | 0.759 | 12.33 | 0.457 | 24.284 (17.503–33.692) |
| 0.276 (95) | 0.246 | 0.993 | 0.909 | 36.231 | 0.748 | 23.253 (16.878–32.036) |

DYCAST

|          | Sensitivity | Specificity | PPV | LRP | LRN | Mantel-Haenszel RR (95% CI) |
|----------|-------------|-------------|-----|-----|-----|----------------------------|
| Daily    | 0.268       | 0.165       | <0.001 | 0.321 | 4.443 | 10.112 (7.367–13.880) |
| Biweekly | 0.361       | 0.045       | 0.006 | 0.378 | 14.242 | 9.756 (7.764–12.258) |

*CMVRA, California Mosquito-Borne Virus Risk Assessment; DYCAST, Dynamic Continuous-Area Space-Time system; PPV, positive predictive value; LRP, likelihood ratio positive; LRN, likelihood ratio negative; RR, relative risk; UND, undefined due to the high specificity.

Table 3. Comparison of the sensitivity and specificity of CMVRA calculated at the emergency planning threshold of 2.6, the vector index calculated at the 80th percentile, and DYCAST risk estimates aggregated weekly for detecting risk for West Nile disease, Los Angeles, California, USA*

| Model    | 2004 Sen | 2005 Sen | 2006 Sen | 2007 Sen | 2008 Sen | 2009 Sen | 2010 Sen |
|----------|----------|----------|----------|----------|----------|----------|----------|
| CMVRA    | 1        | 0.667    | 0.857    | 0.647    | 1        | 0.556    | 0.778    | 0.9      | 0.571    | 1        | 0.857    | 1        | 0.913    |
| Vector index | 0.778   | 0.867    | 0.714    | 0.941    | 0.667    | 1        | 0.5      | 0.8       | 1        | 0.714    | 1        | 0.652    |
| DYCAST   | 0.517    | 0.268    | 0.034    | 0.143    | 0        | 0        | 0.063    | 0        | 0.013    | 0        | 0        | 0        |

*CMVRA, California Mosquito-borne Virus Risk Assessment; DYCAST, Dynamic Continuous-Area Space-Time system; Sen, sensitivity; Spe, specificity.
Angeles, where Cx. p. quinquefaciatus mosquitoes are the primary vectors and temperatures generally permit viral amplification. The estimates of the vector index increased before case occurrence in 5 of the 7 years. The sensitivity and specificity were comparable with those of the CMVRA, but the likelihood ratio positive was the greatest of all risk estimates. The likelihood ratio negative was better than that of the DYCAST but not as good as that of the CMVRA. Therefore, the vector index was moderate at predicting high-risk periods and very good at predicting low-risk periods. The measure of risk associated with a high-risk value, assessed by the Mantel-Haenszel relative risk, was also better than the DYCAST risk estimate but not as good as either CMVRA threshold.

The DYCAST risk estimate was useful in years with amplified enzootic transmission, when dead birds were considered the primary WNV surveillance indicator (437–39). However, after the initial epidemic, WNV activity has been progressively more difficult to predict by using DYCAST because of reduced reporting to the California Dead Bird Hotline. Whether this decrease resulted from truly decreased numbers of dead birds as bird populations became progressively more resistant to infection or to public apathy/decreased awareness was not possible to ascertain. Losing time precision by aggregating estimates clearly increased measures of validity, which considering the uncertainty regarding time between WNV exposure and disease onset seemed appropriate to improve predictive power. The sensitivity of the weekly DYCAST risk estimate was similar to that of the CMVRA, but the specificity, PPV, likelihood ratio positive, and likelihood ratio negative were all uniformly worse than the other 2 methods, even when aggregated spatially. Additionally, the measure of relative risk associated with risk estimates was less than that of the CMVRA and the vector index.

In conclusion, critical decisions on intervention by using risk estimates require knowledge of the strengths and weaknesses of the selected method to respond in an adequate and timely manner to prevent human cases while reducing unnecessary response and costs associated with falsely identified high-risk periods. The goals we set for a good WNV risk estimate were a balance of these attributes and were achieved best in urban and suburban Los Angeles by the CMVRA by using the 2.6 epidemic planning threshold. In light of this finding, an evaluation of the CMVRA should be done in other ecologic settings with transmission driven by other vector species to determine whether the threshold should be adjusted to provide better antecedent estimates of human risk.

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Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 18, No. 8, August 2012
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Comparison of Enzootic Risk Measures for Predicting West Nile Disease, Los Angeles, California, USA, 2004–2010

Technical Appendix

Details of Data Collection, Analysis, and Calculation of Risk by Using the CMVRA

The risk calculation form, downloaded from the CMVRA (http://westnile.ca.gov/downloads.php?download_id=2321&filename=2012%20CA%20Response%20Plan%205-8-12.pdf), is shown in Technical Appendix Tables 1 and 2. Each of 5 factors were assigned a risk level increasing from 1 (low) to 5 (high) based on data accumulated by the GLACVCD during the previous 2-week time step. These values then were arithmetically averaged and risk assessed based as “low” or normal season, “epidemic planning” with increasing trends in some/all factors, and “epidemic” with most factors >4. Although each of the 5 factors have their own variance about the estimates, it was not possible to readily combine these into an estimate error about the arithmetic average of the rank values. Standard error calculated for this mean was proportional to increasing ranks and therefore increased with increasing risk. The overall risk model was designed to be adapted to local conditions in a large state with markedly different ecologic conditions, vector populations, and control agency budgets. Data from Los Angeles were gathered by the GLACVCD as described previously (1) and detailed in general below:

1. Weather. Because there is little rain during the transmission season, only temperature is considered. Escalating risk is based on the decreasing duration of the extrinsic incubation period of WNV in Cx. tarsalis as a function of temperature (2), where 14.3°C is the threshold for virus growth. Here, antecedent warm temperatures reduce the age at which vector transmission may occur and defines elevated risk.
2. Vector abundance. There are 2 primary vector species in California, and risk can be calculated separately for each; however, only *Cx. p. quinquefasciatus* is abundant in Los Angeles (1), and data here were restricted to this species. Risk was determined by counts of *Cx. p. quinquefasciatus* females in gravid traps (3), transformed by \( \ln [y+1] \) to normalize the distribution, averaged among traps and then backtransformed. These geometric means are compared with means calculated for the same time period over the previous 5 years, expressed as a percentage anomaly, and assigned to a category of escalating risk from 1 to 5 (Technical Appendix Tables 1, 2).

3. Vector infection. Female *Cx. p. quinquefasciatus* from the above traps were pooled into lots of <50 females each, stored at −80°C, and shipped on dry ice to the Center for Vectorborne Diseases, where they were tested for WNV RNA by qRT-PCR by using an ABI 7900 platform (Applied Biosystems, Foster City, CA, USA) and primers and probes described by Lanciotti et al. (4). Pools also were tested concurrently for WEEV and SLEV, but were negative during the current study period. Infection rates per 1,000 for each 2-week time step were calculated by the bias corrected maximum likelihood estimate [MLE] by using the Excel spreadsheet add-in described by Biggerstaff (5) and available from the CDC West Nile virus website. MLE estimates were ranked 1–5 (Technical Appendix Tables 1, 2) and assigned an escalating risk value based on previous field studies in California.

4. Sentinel chickens. Flocks of 7 sentinel hens were deployed at 7 locations throughout the GLACVCD in March–April and then bled at 2-week intervals until replaced the following season or when >5 seroconverted. Serum was tested for evidence of previous WNV infection by an enzyme immunoassay with positives confirmed by Western blot or plaque reduction neutralization test (6). Risk was based on the spatial distribution and number of seroconversions detected during the 2-week time step (Technical Appendix Tables 1, 2), with the “broad region” Los Angeles County and the “specific region” the GLACVCD jurisdiction.
5. Dead bird reporting and testing. Many species of California birds die due to WNV (7), usually within 5–7 days of infection (8,9), thereby providing a measure of recent transmission. In Los Angeles, large populations of American crows have suffered severe die-offs and these have been provided a useful measure of WNV activity (1,10). Dead or dying birds were reported to the California Dead Bird Hotline by the public, collected by GLACVCD personnel, and shipped to the California Animal Health and Food Safety laboratory for necropsy under BSL-3 conditions. Oral swabs and kidney samples then were sent to Center for Vectorborne Diseases where they were tested by qRT-PCR for WNV RNA as described above for mosquitoes. Risk was based on the geographic distribution and numbers of WNV-positive dead birds (Technical Appendix Tables 1, 2).

In the calculation example below, the 2-week average daily temperature was warm (77°F), Cx. p. quinquefasciatus females were moderately abundant, averaging 28 females per gravid trap per night (280% above the 5-year average for the same time period), and 10 pools consisting of 50 females each were tested and 3 were WNV positive. The resulting MLE estimate of 6.69 had a broad 95% CI of 1.8–18.8 but was ranked as 5. In addition, 4 chickens in 3 flocks seroconverted, and 8 American crows and 2 house finches tested positive of 26 submitted for testing—all were collected within the GLACVCD boundaries. This resulted in a risk score of 4.4, placing the GLACVCD at epidemic level of risk, indicating an ongoing epidemic and the probable occurrence of human cases.

| Factor       | Factor value | Calculation   | Risk score |
|--------------|--------------|---------------|------------|
| 1. Temp      |              | none          | 4          |
| 2. Abundance | 28 F/TN      | 280% = 28/10  | 4          |
| 3. Infection | 3 WNV+/10    | 6.69 (1.8-18.8) | 5          |
| 4. Sentinels | 4 in 3 flocks| none          | 4          |
| 5. Dead birds| 10 WNV+      | none          | 5          |

Average 4.4
### Technical Appendix Table 2. Risk calculation form details

| WNV Surveillance Factor | Assessment Value | Benchmark | Assigned Value |
|------------------------|------------------|-----------|---------------|
| **1. Environmental Conditions**<br>High-risk environmental conditions include above-normal temperatures with or without above-normal rainfall, runoff, or snowpack. Weather data link: http://ipm.ucdavis.edu | 1 | Avg daily temperature during prior 2 weeks ≤ 56 °F |  |
| | 2 | Avg daily temperature during prior 2 weeks 57 – 65 °F |  |
| | 3 | Avg daily temperature during prior 2 weeks 66 – 72 °F |  |
| | 4 | Avg daily temperature during prior 2 weeks 73 – 79 °F |  |
| | 5 | Avg daily temperature during prior 2 weeks > 79 °F |  |
| **2. Adult *Culex tarsalis* and *Cx. pipiens* complex relative abundance*<br>Determined by trapping adults, enumerating them by species, and comparing numbers to those previously documented for an area for the prior 2-week period. | 1 | Vector abundance well below average (≤ 50%) | Cx tars Cx pip |
| | 2 | Vector abundance below average (51 - 90%) |  |
| | 3 | Vector abundance average (91 - 150%) |  |
| | 4 | Vector abundance above average (151 - 300%) |  |
| | 5 | Vector abundance well above average (> 300%) |  |
| **3. Virus infection rate in *Culex tarsalis* and *Cx. pipiens* complex mosquitoes*<br>Tested in pools of 50. Test results expressed as minimum infection rate per 1,000 female mosquitoes tested (MIR) for the prior 2-week period. | 1 | MIR = 0 |  |
| | 2 | MIR = 0.1 - 1.0 |  |
| | 3 | MIR = 1.1 - 2.0 |  |
| | 4 | MIR = 2.1 - 5.0 |  |
| | 5 | MIR > 5.0 |  |
| **4. Sentinel chicken seroconversion**<br>Number of chickens in a flock that develop antibodies to WNV during the prior 2-week period. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 10 chickens per flock. | 1 | No seroconversions in broad region |  |
| | 2 | One or more seroconversions in broad region |  |
| | 3 | One or two seroconversions in a single flock in specific region |  |
| | 4 | More than two seroconversions in a single flock or two flocks with one or two seroconversions in specific region |  |
| | 5 | More than two seroconversions per flock in multiple flocks in specific region |  |
| **5. Dead bird infection**<br>Number of birds that have tested positive (recent infections only) for WNV during the prior 3-month period. This longer time period reduces the impact of zip code closures during periods of increased WNV transmission. | 1 | No positive dead birds in broad region |  |
| | 2 | One or more positive dead birds in broad region |  |
| | 3 | One positive dead bird in specific region |  |
| | 4 | Two to five positive dead birds in specific region |  |
| | 5 | More than five positive dead birds in specific region |  |
| **6. Human cases**<br>Do not include this factor in calculations if no cases are detected in region. | 3 | One or more human infections in broad region |  |
| | 4 | One human infection in specific region |  |
| | 5 | More than one human infection in specific region |  |

**Response Level / Average Rating:**
- Normal Season (1.0 to 2.5)
- Emergency Planning (2.6 to 4.0)
- Epidemic (4.1 to 5.0)

| | TOTAL | AVERAGE |
|------------------------|---------|---------|
| Cx tars | Cx pip |         |

*Calculation of separate risk values for *Cx. tarsalis* and the *Cx. pipiens* complex may be useful if their spatial distributions (e.g., rural vs. urban) differ within the assessment area.

### Vector Index

The vector index calculations used in this paper were developed by Roger Nasci, Research Entomologist for the National Center for Infectious Disease at CDC ([www.cdphe.state.co.us/dc/zoonosis/wnv/Nasci_VectorIndexPoster.pdf](www.cdphe.state.co.us/dc/zoonosis/wnv/Nasci_VectorIndexPoster.pdf)) (Technical Appendix Table 3). This simple metric multiplies the mosquito infection rate by the mosquito abundance.
As with the CMVRA, the data were aggregated into the same 2-week previous interval for calculation.

| Factor           | Factor value |
|------------------|--------------|
| Abundance        | 28 F/TN      |
| Infection rate   | 6.69/1,000   |
| Calculation      | 0.187        |

**DYCAST**

The DYCAST estimates were achieved by geocoding dead bird reports for the state of California. A 0.5-mile grid was then superimposed on the state, and the dead bird reports were assigned to the center of each grid cell. Knox space–time interaction tests were performed to determine whether the reported birds were “close” in both dimensions. The Knox test creates pairs of bird reports and assigns a value of 0 if the distance between the 2 reports in the pair is greater than the critical distance, 0.40 km ($t_{ij}$), (1 if within the critical distance) and a 0 if the time between reports is greater than the critical time of 3 days ($s_{ij}$) (1 if within). The test statistic is the summation, over all bird pairs, of the products of $t_{ij}$ and $s_{ij}$, and is compared with a random spatiotemporal distribution of reports. A Knox test p value of >0.1 was considered low risk, and a p value of ≤0.10 was considered high risk. The minimum number of birds required for calculation of the statistic was 15 per cell, and the results were calculated daily (11).

In our analyses, we assessed the DYCAST daily estimates as well as 2-week aggregates where we selected the minimum p value over the same 2-week periods as the CMVRA and vector index. To assess the spatial accuracy we aggregated high- or low-risk cells up to the limit of the GLACVCD boundary.

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