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Journal
Vector-Borne and Zoonotic Diseases, 15(2)

ISSN
1530-3667

Authors
Healy, Jessica M
Reisen, William K
Kramer, Vicki L
et al.

Publication Date
2015-02-01

DOI
10.1089/vbz.2014.1689

Peer reviewed

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 15, Number 2, 2015

DOI: 10.1089/vbz.2014.1689

Comparison of the Efficiency and Cost of West Nile Virus Surveillance Methods in California

Jessica M. Healy,[1] William K. Reisen,[1] Vicki L. Kramer,[2] Marc Fischer,[3] Nicole P. Lindsey,[3] Roger S. Nasci,[3]
Paula A. Macedo,[4] Gregory White,[5] Richard Takahashi,[6] La Khang,[6] and Christopher M. Barker[1,7]

Abstract

Surveillance systems for West Nile virus (WNV) combine several methods to determine the location and timing of viral amplification. The value of each surveillance method must be measured against its efficiency and costs to optimize integrated vector management and suppress WNV transmission to the human population. Here we extend previous comparisons of WNV surveillance methods by equitably comparing the most common methods after standardization on the basis of spatial sampling density and costs, and by estimating optimal levels of sampling effort for mosquito traps and sentinel chicken flocks. In general, testing for evidence of viral RNA in mosquitoes and public-reported dead birds resulted in detection of WNV approximately 2–5 weeks earlier than serological monitoring of sentinel chickens at equal spatial sampling density. For a fixed cost, testing of dead birds reported by the public was found to be the most cost effective of the methods, yielding the highest number of positive results per $1000. Increased spatial density of mosquito trapping was associated with more precise estimates of WNV infection prevalence in mosquitoes. Our findings also suggested that the most common chicken flock size of 10 birds could be reduced to six to seven without substantial reductions in timeliness or sensitivity. We conclude that a surveillance system that uses the testing of dead birds reported by the public complemented by strategically timed mosquito and chicken sampling as agency resources allow would detect viral activity efficiently in terms of effort and costs, so long as susceptible bird species that experience a high mortality rate from infection with WNV, such as corvids, are present in the area.

Key Words: West Nile virus—Surveillance methods—Cost effectiveness—California—Birds.

Introduction

West Nile virus (WNV) surveillance in mosquito vectors, avian hosts, and sentinel chickens is used to identify the location and timing of virus activity. The information gained through surveillance is used to guide vector control to prevent viral transmission to the human population, making early and efficient detection of the virus essential (Eldridge 1987, Gubler et al. 2000). A cost-effective WNV surveillance system would be one capable of reliably detecting the timing and location of virus activity while maximizing the information gained per unit of staff time or money expended.

Previous studies of existing surveillance programs have found that surveillance for WNV-positive mosquitoes and/or dead birds preceded WNV seroconversions in sentinel chickens and were more predictive of WNV disease risk in humans (Cherry et al. 2001, Patnaik et al. 2007, Unlu et al. 2009, Kwan et al. 2010). However, those comparisons did not account for differences in sampling effort or costs required for each method. One study conducted before the introduction of WNV in California found that testing mosquitoes or sentinel chickens incurred similar costs, although their effectiveness for arboviral detection was not evaluated (Scott et al. 2001).

Here, we extend these earlier studies to compare the timing and effectiveness of mosquito, dead bird, and sentinel

[1]Center for Vectorborne Diseases, Department of Pathology, Microbiology and Immunology, University of California, Davis, California.
[2]Vector-Borne Disease Section, California Department of Public Health, Sacramento, California.
[3]Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado.
[4]Sacramento–Yolo Mosquito and Vector Control District, Elk Grove, California.
[5]Coachella Valley Mosquito and Vector Control District, Indio, California.
[6]Kern Mosquito and Vector Control District, Bakersfield, California.
[7]Fogarty International Center, National Institutes of Health, Bethesda, Maryland.

chicken surveillance for detecting WNV activity after adjusting for effort or cost. For mosquitoes and sentinel chickens that are actively sampled, we also evaluated their performance for detecting WNV over a range of sampling effort (*i.e*., trap densities or flock sizes). Collectively, this information can be used by public health and vector control agencies to help determine the most cost-effective use of WNV surveillance resources.

Materials and Methods

Study area and time period

We analyzed WNV surveillance data from three California mosquito control districts that represented diverse climates, land uses, and abundance patterns for vectors and hosts. The collaborating districts were the Coachella Valley Mosquito and Vector Control District (MVCD) in Riverside County, Kern MVCD in Kern County, and the Sacramento–Yolo MVCD in Sacramento and Yolo Counties (Fig. 1). We evaluated data from 2004 through 2012, which were the 9 years after WNV was first detected in California. For all years, analysis was limited to mid-April through October, the typical period for enzootic WNV activity in these areas.

Surveillance data

The WNV surveillance methods evaluated included collection and testing of mosquito vectors and dead birds, and serological monitoring of sentinel chickens. Mosquito traps and sentinel flocks were sampled either weekly or in alternate weeks, depending on the agency and method. Dates of specimen collection were used for all analyses because they provided the most objective comparisons. Due to the passive nature of dead bird surveillance, there was not a routine sampling schedule comparable to that of sentinel chickens and mosquitoes, and the date when each dead bird was reported by the public was used in our analyses. Surveillance data were reported to and stored in the CalSurv Gateway, a centralized data management system used by California mosquito control and public health agencies (Barker et al. 2010a).

FIG. 1. The locations of the three participating agencies: (**A**) Sacramento–Yolo Mosquito and Vector Control District, (**B**) Kern Mosquito and Vector Control District, and (**C**) the Coachella Valley Mosquito and Vector Control District.

Mosquitoes. Adult female mosquitoes were collected in CO_2-baited and gravid traps (Newhouse et al. 1966, Reiter 1987), anesthetized by triethylamine, and identified to species by mosquito control agency personnel. WNV testing was performed on *Culex tarsalis* and *Culex pipiens* complex mosquitoes, the primary WNV vectors in California. Females of these species were divided into pools of ≤ 50 mosquitoes and stored at $-80°C$ until they were tested using reverse transcription PCR to detect the presence of WNV RNA using previously published primers and probes (Lanciotti et al. 2000, Shi et al. 2001). Maximum likelihood estimates of the infection rate (Biggerstaff 2003) and the minimum infection rate per 1000 females (MIR) (California Department of Public Health et al. 2013) were calculated for the analyses.

Sentinel chickens. Flocks of seven to 10 immunologically naïve hens were placed at eight to 13 locations within each agency at the start of each surveillance season, after which the chickens were monitored for seroconversion every 1–2 weeks. A small blood sample from each bird was tested for the presence of anti-WNV immunoglobulin G (IgG) antibodies using an enzyme-linked immunosorbent assay (ELISA). Blood samples were tested at the California Department of Public Health (CDPH) or the mosquito control agency using identical methods. To distinguish possible cross-reactivity between WNV and St. Louis encephalitis virus antibodies, positive tests were confirmed through indirect fluorescent antibody tests, Western blots, or plaque reduction neutralization tests (PRNTs) (Patiris et al. 2008, California Department of Public Health et al. 2013). The proportion of chickens that seroconverted was calculated and used in the analyses.

Dead birds. Dead birds were reported by the public online or via a statewide toll-free hotline, and carcasses of birds that died within the past 24 h were picked up by mosquito control agency personnel. Dead birds were forwarded to the California Animal Health and Food Safety laboratory where either an oral swab (American crows) or kidney tissue (all other species) was sampled and then forwarded to the University of California Davis Center for Vectorborne Diseases for testing via RT-PCR (California Department of Public Health et al. 2013). The number of WNV-positive dead birds out of the total tested or the number of WNV-positive dead birds per human capita was calculated and used in our analyses. Because mosquito control agencies may discontinue collection of dead birds from certain zip codes during the progression of a season, calculations of positive dead birds per human capita were limited to zip codes with continued collection.

Comparison of timing and effectiveness of WNV detection

For this comparison, we eliminated biases toward surveillance methods sampled at higher spatiotemporal density by limiting the surveillance data set to pairings of regularly sampled sentinel flock sites—typically the least densely sampled method—and the single nearest mosquito trap that was operated in the same week. Sentinel flocks without a mosquito trap sampled within 10 km in a particular week were excluded from the analysis for that week. Matching and subsequent analyses were done separately for CO_2-baited and gravid mosquito traps because of inherent differences in the reproductive stages of the females captured (host-seeking vs. egg-laying, respectively) and trap placement (all habitats vs. urban-only).

Dead birds were detected passively through public reporting; consequently, ensuring spatial representation comparable to actively sampled mosquitoes and chickens was a challenge. We included all dead birds within the average distance from sentinel flocks to their respective nearest mosquito traps.

Within each year for each surveillance site, we used the earliest week of virus detection to assess the degree of early warning provided by chicken flocks, mosquito testing, or dead birds. Similarly, the week of peak activity in each flock (proportion seroconverted), trap (MIR), and dead birds (number of positives per human capita) within a year was compared as a second measure of timing. Statistical comparisons of the weeks of first and peak activity between equalized flocks, traps, and positive dead birds were based on the nonparametric Wilcoxon signed rank test, which was used due to the ordinal data type. In addition, we compared the effectiveness of virus detection between the methods by calculating the proportion of positive surveillance weeks (virus activity detected by any method in a week) detected by each surveillance method.

Comparison of cost effectiveness

The effectiveness of traps, flocks, and dead birds was compared over the entire surveillance data set in terms of the costs of information gained. Average costs of obtaining samples per single unit of each method (sentinel flock, mosquito trap, or dead bird) were provided from the three participating mosquito control agencies and combined to produce an estimate of typical costs for each method. The averaged costs for collections and maintenance included that of routine supplies for mosquito traps (*e.g.*, dry ice attractant) and sentinel chicken flocks (feed and water), as well as the distributed cost per tested dead bird of maintaining a statewide reporting hotline (total costs for the hotline/number of birds tested statewide) and the average cost of local personnel time to drive to each location and collect and process samples in the field or laboratory. Testing costs included the materials for testing or the cost charged for external testing of samples, depending on the approach of the particular agency. We calculated the total costs per unit for each agency, and then averaged for each method to yield a final cost to be used in the analysis. For each surveillance method, the per-unit costs were multiplied by the number of surveillance units tested, yielding a total cost for each week. We regarded positive samples as the spatial indicators of WNV activity (the objective of surveillance), so we compared methods over time based on the average number of positives per \$1000 spent (number of positive units/total calculated cost in dollars * 1000). Results were stratified by vector control agency to determine whether differences among agencies significantly changed the results; the paired Student *t*-test was used for comparisons.

Estimating optimal trapping densities

The number of mosquitoes captured for virus testing increases with the number of traps operated, typically yielding more pools of mosquitoes to be tested. The optimal trapping density for mosquito surveillance was considered to be the density that yielded the least uncertainty in estimates of infection prevalence, where uncertainty was estimated by the widths of 95% confidence intervals (CIs) (Biggerstaff 2008) for each week (weeks 23–40) in each agency. For this analysis, the surveillance data set was limited to the mosquito collection data. Each agency was subdivided into 6- × 6-mile (93 km^2) grid cells corresponding to Public Land Survey System townships (Barker et al. 2010b), and the number of traps in each township each week was divided by the area to obtain a spatial density. Because the number of traps operated varied markedly among agencies and over time, this resulted in a range of trapping densities that we compared to identify an optimal density. Trap types were evaluated separately because gravid traps typically are located only in urban settings whereas CO_2-baited traps are placed in a broad range of habitats from rural to urban settings. The Student *t*-test with the Bonferroni correction for multiple comparisons was used at each density to determine if there was a significant difference in uncertainty between CO_2-baited and gravid trap types.

Estimating optimal flock sizes

We estimated optimal size for sentinel chicken flocks through random subsetting of the sentinel chickens within flocks, followed by a comparison of the sensitivity and timing of viral antibody detection between full and reduced flocks. Subsetting was done randomly within each flock and year, selecting two to seven chickens among the total number available in the flock, resulting in simulated flocks of varying smaller size. When a chicken seroconverted or died, it was replaced randomly by one of the remaining chickens in the flock to sustain the two to seven chicken flock size throughout the surveillance year. Flocks were included only if the full size of the flock exceeded the target size for the reduced flock to allow for random selection of chickens (*e.g.*, for a full flock of seven chickens, reduced flocks were limited to six or fewer chickens). We used the week of first seroconversion to compare the timing of WNV antibody detection between the simulated and real flocks. Paired Student *t*-tests were used to test for statistically significant differences in the weeks of first seroconversions between the simulated and real flocks. Sensitivity at each flock size was evaluated as the proportion of reduced flocks with seroconversions out of the number of full flocks with seroconversions.

R statistical software was used for all analyses (R Core Team 2012), except for the calculation of maximum likelihood estimates of infection rates in mosquitoes that were done using a Microsoft Excel add-in (Biggerstaff 2008).

Table 1. Numbers and Percentage of West Nile Virus–Positive Samples by Surveillance Method and District, 2004–2012

	Chickens flocks[a]		*Mosquito traps*[b]		*Dead birds*	
District	*No. positive/tested*	*(%)*	*No. positive/tested*	*(%)*	*No. positive/tested*	*(%)*
Coachella Valley	227/1370	(17)	362/7102	(5)	13/79	(16)
Kern	281/1047	(27)	11333/6306	(21)	302/963	(31)
Sacramento-Yolo	82/1850	(4)	892/13518	(5)	1543/4382	(35)
All districts	590/4267	(14)	2587/26926	(10)	1858/5424	(34)

[a]A flock of 7–10 chickens tested in a week of a year.
[b]A mosquito trap tested in a week of a year.

Results

Surveillance data

The surveillance data set included 73 sentinel chicken flocks with an average of 8.5 chickens per flock, 575 regularly sampled mosquito traps (sampled ≥ 10 surveillance weeks over the entire study period), and an average of 603 dead birds tested annually from the three participating vector control agencies. The levels of virus activity detected by surveillance varied between agencies. Overall, Sacramento–Yolo MVCD sampled most intensively, but Kern MVCD had comparatively high proportions of positive samples, indicating higher virus activity relative to the other agencies (Table 1). The number of birds tested was highly variable among the agencies due to the low abundance of bird species susceptible to WNV mortality in Coachella Valley MVCD and the strong public relations campaign to encourage reporting of dead birds in Sacramento–Yolo MVCD.

Comparison of timing and effectiveness of WNV detection

After limiting the data to the surveillance methods matched in time and space, the average distance from flocks to their nearest mosquito trap was 1.5 km (5^{th}–95^{th} percentile: 0–6.66 km), and this average distance was used spatially to define the area for inclusion of dead birds around a flock. Overall, viral activity was detected in 62 out of 64 the matched surveillance locations and in 524 out of total 2812 surveillance-weeks (a surveillance location in a week of the study period). Of the 524 surveillance-weeks in which positives were observed, mosquito traps most frequently detected early season activity (April–June), detecting 71% of positive weeks; however, in the peak (July–August) and late season (September–October) sentinel flocks detected WNV most frequently with 62 and 72% of positive weeks detected in each time period, respectively (Table 2). When two surveillance methods were combined, 93% of positive surveillance weeks were detected by either mosquito traps or sentinel flocks, whereas 71% were detected by either sentinel flocks or dead birds, and 54% were detected by either mosquito traps or dead birds.

Dead birds and mosquitoes tended to detect WNV earlier than sentinel chickens, although results for dead birds were strongly influenced by results from Sacramento–Yolo MVCD due to their promotion of public reporting mechanisms and high abundance of susceptible corvid species in urban areas compared to the other agencies. Mosquito testing detected the onset of virus activity a median of 2 weeks earlier than sentinel flocks ($p < 0.001$) and detected peak activity 3 weeks before sentinel flocks ($p < 0.001$). Similarly, dead birds detected virus onset 5 weeks before sentinel flocks ($p = 0.005$) and peak activity 3 weeks earlier than sentinel flocks ($p < 0.001$). Mosquito and dead bird testing were not significantly different in the median week of onset or peak of activity ($p = 0.73$ and $p = 0.98$, respectively). When we stratified the results by mosquito trap type, the lead time in detection of peak activity ahead of sentinel flocks decreased from 3 to 2 weeks for gravid traps ($p < 0.001$), but remained the same for the onset of activity; both measures remained unchanged for CO_2-baited traps.

Comparison of cost effectiveness

The average total cost per surveillance unit per week was $65 for dead birds, $72 for mosquito traps, and $111 for sentinel flocks (Table 3). The majority of the per-unit cost in sentinel flocks and mosquito traps was attributed to laboratory testing of samples, whereas in dead birds the combined costs of collection, maintenance of a reporting hotline, and shipping were the largest contributors to overall cost per unit. For $1000 per week, our cost estimates imply that a mosquito control agency could sample 15 dead birds, 13 mosquito traps, or nine chicken flocks (flock size of eight to nine chickens).

Table 2. Numbers and Percentages of West Nile Virus–Positive Surveillance Weeks Detected by Each Surveillance Method for Each Part of the Season, 2004–2012

	Chickens flocks		*Mosquito traps*		*Dead birds*	
Season part	*No. positive/tested*	*(%)*	*No. positive/tested*	*(%)*	*No. positive/tested*	*(%)*
Early (April–June)	22/58	(38)	41/58	(71)	8/58	(14)
Peak (July–August)	188/303	(62)	147/303	(49)	33/303	(11)
Late (September–October)	117/163	(72)	52/163	(32)	14/163	(9)

Table 3. Estimated Weekly Costs per Surveillance Unit for Sentinel Chicken Flocks, Mosquito Traps, and Dead Birds during the Study Period, 2004–2012[a]

Expense	*Chicken flocks*	*Mosquito traps*	*Dead birds*
Field processing			
Maintenance	$9	$4	$3
Collections	$26	$17	$22
Lab processing			
Preparation of samples	$3	$6	$7
Shipping[b]	$1	$5	$18
Lab testing per unit[c]	$72	$40	$15
Average weekly cost per unit	$111	$72	$65

[a]Costs were calculated within each agency, then reconciled to yield an estimate of typical costs per line item.
[b]Cost per shipment divided by the number of samples per shipment multiplied by the number of samples per trap or flock.
[c]Average cost per test multiplied by the average number of samples tested per surveillance unit.

Dead birds were the most cost-effective indicator of WNV occurrence throughout a season yielding, on average, approximately three more positives per $1000 than both sentinel flocks and the combination of CO_2-baited and gravid mosquito traps (Fig. 2). Stratifying the results by vector control agency showed a strong influence of Sacramento—Yolo and Kern MVCDs, which have higher numbers of reported dead birds (Table 4). In Coachella Valley MVCD, very few dead birds were detected during the study period, but in terms of cost, both sentinel flocks and dead birds were more cost effective than mosquito traps, producing approximately one more positive per $1000 ($p < 0.001$ and $p = 0.05$, respectively). Sentinel flocks did not differ in cost effectiveness compared to mosquito traps in Kern ($p = 0.13$) or Sacramento–Yolo ($p = 0.72$) MVCDs, or compared to dead birds in Coachella Valley MVCD ($p = 0.56$). The overall results for mosquito traps and sentinel flocks varied seasonally, with mosquito traps yielding slightly more positives during late spring and sentinel flocks yielding more positives per $1000 during the latter part of the season (Fig. 2).

Estimating optimal trapping densities

Observed trap densities ranged from one to 12 traps per 100 km^2, and the uncertainty in the estimated infection prevalence (width of 95% CIs) for both CO_2-baited and gravid traps showed a gradual decreasing trend at higher densities, with the uncertainty reaching a minimum at the highest observed density of approximately 12 traps per 100 km^2 (Fig. 3). In gravid traps, there was little variation in precision at low densities from one to four traps per 100 km^2. At each of the observed densities, CO_2-baited traps had greater precision than gravid traps ($p < 0.001$).

Estimating optimal flock sizes

At seven chickens per flock, sensitivity remained high at 91% and there was no difference in the average week of first detection of virus activity between the reduced and full flocks ($p = 0.06$) (Table 5). A further reduction to six chickens resulted in similar sensitivity, and the difference in first detection of virus activity remained < 0.5 week, which was of little practical importance in relation to the typical sampling interval of 1–2 weeks. Reductions in flock size below four chickens markedly affected sensitivity and delayed the first detection of virus activity by approximately 2 weeks.

Discussion

We compared the most widely used methods of enzootic WNV surveillance to identify those that provide the greatest return on investment, either in terms of spatial allocation of resources or financial costs. Each surveillance method provides unique information about viral activity in the vector and host populations; dead birds inform control agencies about the location and magnitude of virus activity, testing of

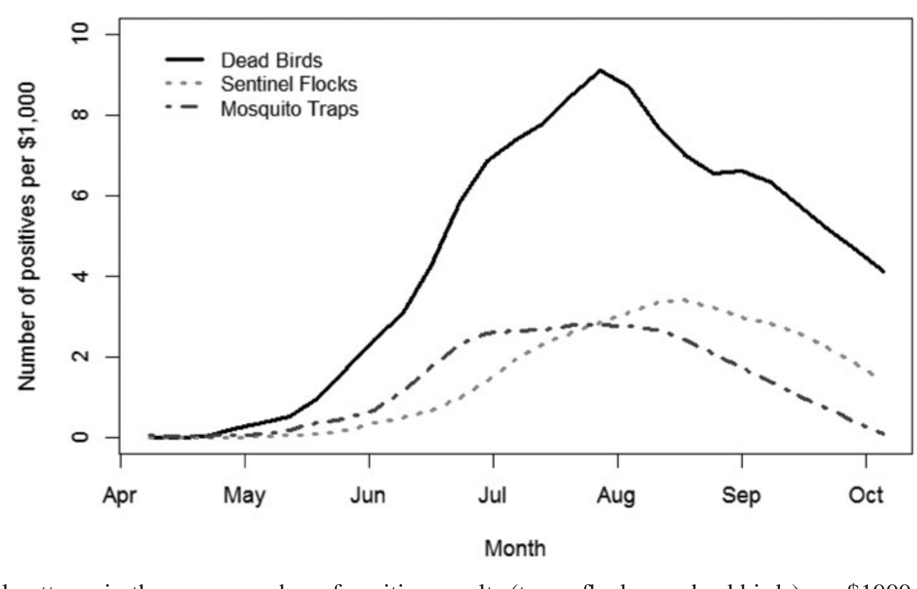

FIG. 2. Seasonal patterns in the mean number of positive results (traps, flocks, or dead birds) per $1000 spent on testing for each surveillance type. Averages are the result of smoothing using locally weighted regression to reduce the noise in the data.

Table 4. Average Number of Positive Surveillance Units per $1000 by Surveillance Method and District, 2004–2012

	Chicken flocks		Mosquito traps		Dead birds	
District	*No.*	*(95% CI)*	*No.*	*(95% CI)*	*No.*	*(95% CI)*
Coachella Valley	1.7	(1.3, 2.0)	0.7	(0.6, 0.8)	1.9	(0.7, 3.2)
Kern	2.6	(2.1, 3.1)	2.7	(2.3, 3.1)	4.8	(3.9, 5.7)
Sacramento-Yolo	0.8	(0.5, 1.1)	0.8	(0.6, 0.9)	4.8	(4.1, 5.4)
All districts	1.6	(1.4, 1.8)	1.4	(1.2, 1.5)	4.4	(4.0, 5.0)

mosquitoes provides estimates of the proportion of infected vectors in a region, and seroconversion in sentinel flocks indicates the frequency and location of viral transmission between testing intervals.

Our results for the timeliness of detection showed that mosquitoes and dead birds generally detected virus activity earlier than nearby sentinel chicken flocks when sampling frequency and spatial density were equalized. Our findings agree with those of several studies, which showed infection in mosquitoes and WNV-positive dead birds preceded sentinel chicken seroconversion by 1–2 weeks (Cherry et al. 2001, Patnaik et al. 2007, Unlu et al. 2009, Kwan et al. 2010). Part of this lag in detection by sentinel chickens may be due to the delay between the infectious mosquito bite and the time when antibodies become detectable by ELISA in the sentinel chickens, which can be 7–10 days after the infectious bite (Senne et al. 2000, Patiris et al. 2008). Additionally, when chickens are bled every other week, detection of infection could be delayed by >3 weeks, depending on the exact timing of the transmission event in relation to the sampling schedule. Nevertheless, our results support the notion that mosquito and dead bird testing data will precede sentinel seroconversion data, even when the spatial sampling effort is equal, and this lead time can stimulate mosquito control application in an attempt to interrupt virus amplification before transmission to the human population occurs.

In contrast to the timing results, sentinel chickens detected virus activity significantly more often than both dead birds and mosquito testing. This apparent advantage of sentinel flocks over mosquito traps was predominantly in the typical period of peak activity, July and August, through late summer, which may have resulted in part from the delay from infection to seroconversion in sentinel chickens. Chickens also were available for mosquito bites every night, unlike traps that were operated for only a single night per 1–2 weeks. This means that chickens could have continued to receive bites from infectious female mosquitoes as the mosquito population aged and declined and trapping became less productive. Limiting dead birds to areas near chicken flocks for the purposes of our study may have biased our results

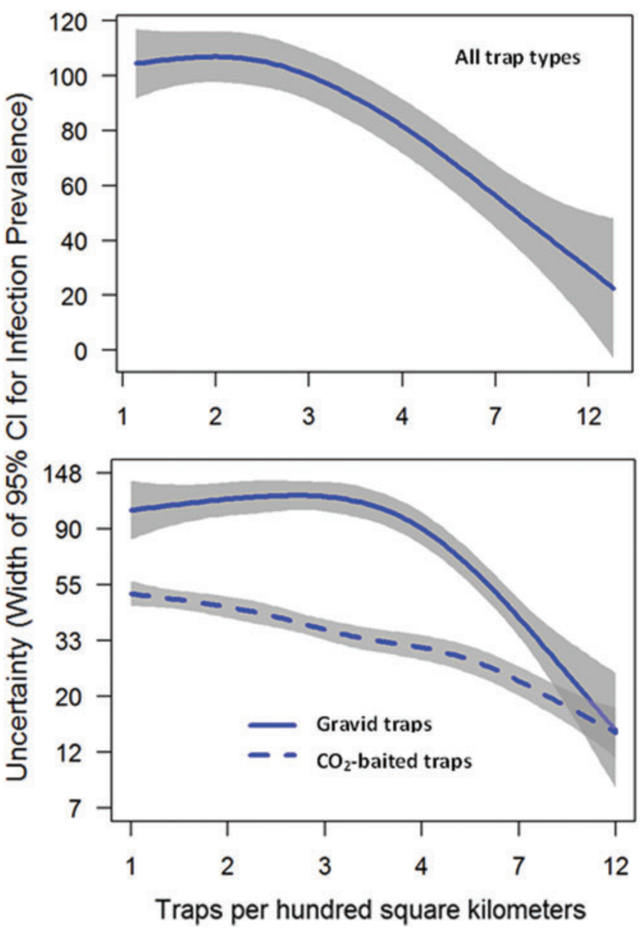

FIG. 3. Uncertainty of West Nile virus (WNV) prevalence estimates in relation to trapping density. Uncertainty was calculated for each surveillance week as the width of 95% confidence intervals (CIs) for infection prevalence in female *Cx. tarsalis* and *Cx. pipiens* complex mosquitoes captured by all trap types combined, or gravid or CO_2-baited traps alone. The lines and gray areas represent the means and 95% CIs using a generalized additive model to depict overall trend in uncertainty. Color images available online at www.liebertpub.com/vbz

Table 5. Timing and Sensitivity of Varying Numbers of Sentinel Chickens per Flock for First Detection of West Nile Virus Activity in the Three Surveillance Districts, 2004–2012

	Delay in WNV first detection in weeks			
Chickens per flock	*Mean*	*(95% CI)*	p *value*	*Sensitivity*[a]
7	0.1	(−0.004, 0.2)	0.06	91%
6	0.2	(0.1, 0.4)	0.02	88%
5	0.7	(0.3, 1.1)	<0.01	80%
4	0.8	(0.5, 1,1)	<0.01	75%
3	1.8	(1.3, 2.4)	<0.01	60%
2	2.4	(1.7, 3.1)	<0.01	46%

[a]Probability of detecting a WNV seroconversion when a seroconversion occurred in the full flock.

toward chickens because dead birds typically are collected throughout an agency and often in urban areas that may not have a nearby chicken flock. In a previous study, it was similarly concluded that sentinel chickens detect arboviral activity frequently when other methods do not (Reisen et al. 2000), although that study focused on periods of moderate to low activity of arboviruses prior to the invasion by WNV.

Among the methods we studied, testing of dead birds was most cost effective in detection of virus activity. In Coachella Valley where there are few bird species susceptible to mortality by WNV infection (Reisen et al. 2006a), testing of dead birds remained cost effective, although the number of positives detected per \$1000 was less than half of that seen in Kern and Sacramento–Yolo MVCDs. The similarity in cost effectiveness between Kern and Sacramento–Yolo MVCDs may be explained by the presence of susceptible birds combined with the relatively high level of virus activity in one agency (Kern) and a strong public awareness campaign in the agency with more moderate virus activity (Sacramento–Yolo). In these two agencies, dead bird surveillance was clearly the most cost effective of the three surveillance methods. In Coachella Valley, both the testing of dead birds and of sentinel flocks produced slightly better results than mosquito testing. Sentinel flocks produced more positives per \$1000 than mosquito trapping in the late season in all agencies, which is likely due to the delay in seroconversions in sentinel chickens. Passive surveillance systems, including testing of dead birds reported by the public, previously were found to be cost effective for WNV surveillance. The passive system of reporting of equine cases by sentinel veterinarians in France was found to be a cost-effective and efficient method of early detection of virus activity when compared to sentinel horses, sentinel chickens, and mosquito testing by using mathematical modeling to simulate data for low activity and endemic and epidemic situations (Chevalier et al. 2011). Although equines frequently are vaccinated in California (American Association of Equine Practitioners 2012) and, therefore, our horse population has not experienced the recent epizootics seen in France (Hayes et al. 2005), our results for areas with a susceptible bird population agree that passive surveillance is the most cost-effective system for WNV surveillance.

Higher densities of mosquito traps resulted in more precise estimates of infection prevalence in both trap types. This trend continued through the highest densities in our study, so we were not able to identify a target density above which gains in precision would not be practically important. However, our estimates of uncertainty at each spatial density can inform the selection of an optimal density to obtain the desired precision within each trap type. Our study focused on density-based comparisons within each trap type, and comparison of precision between trap types was not our goal. The greater precision in CO_2 traps was due to the fact that they collected more mosquitoes on average than gravid traps. Gravid traps attract mostly females that have taken a previous blood meal, potentially increasing the per-capita probability of detecting WNV. However, the near-elimination of nulliparous females from collections often results in smaller numbers of total females collected. CO_2 traps frequently collect larger numbers of *Cx. tarsalis* and *Cx. pipiens* complex females, resulting in increased precision, but many of the females collected are nulliparous, meaning that their probability of having been infected with WNV is generally lower than that of gravid females. These additional considerations should be combined with our study's estimates of precision to determine which traps are better for WNV surveillance in a particular agency.

During the past two decades, most sentinel chicken flocks have included seven to ten chickens per flock (Reisen et al. 1992a, Reisen et al. 2000, Cherry et al. 2001, Scott et al. 2001, Reisen et al. 2004, Kwan et al. 2010), with a flock size of 10 chickens recommended by the CDPH (California Department of Public Health et al. 2013). This was preceded by a period through the early 1990s when 25 chickens was the typical flock size. The current flock size of 10 is widely accepted as suitable for detecting viral antibody and quantifying activity during the surveillance season, but there was a lack of evidence on whether there is a more efficient size. Although a larger flock size is likely to be more attractive to mosquitoes due to the larger amount of CO_2 and other odors emitted by more birds, our findings suggest that six to seven chickens per flock will capture approximately the same spatiotemporal information on WNV detection and timing as the number currently used, with seven chickens retaining the highest sensitivity of 91% (Reeves 1953). Reducing flocks to this size could possibly allow for additional flocks to be used at the same cost, resulting in better spatial coverage within each agency.

The performance of WNV surveillance programs is influenced by their local context. For example, socioeconomic factors have a strong effect on the resources available, either through property values that affect the overall tax base or the public will to allocate an adequate portion of tax monies to surveillance and vector control. Dead bird programs also perform best in urban areas where people are likely to notice the birds and be aware of reporting mechanisms (Mostashari et al. 2003, Patnaik et al. 2007). Local land use and other ecological factors can also affect WNV transmission in several ways, including the abundance and contact rates of mosquito vectors and avian hosts (Reisen et al. 1992b, Lothrop and Reisen 2001, Reisen et al. 2006a, Barker et al. 2009), which in turn could affect the requisite surveillance effort. We could not capture all possible local contexts in our study, but we chose representative collaborating agencies with a large degree of heterogeneity within and among the three study areas in terms of average income, population density, rural and urban land uses, avian diversity, and the typical incidence of human WNV disease.

Conclusion

In summary, we have shown that public-reported dead birds efficiently detect virus activity, given that susceptible bird species are present in the area. This method provided early virus detection and was most cost effective throughout a season. In areas where susceptible birds are not abundant, high-density testing of the primary mosquito vectors in the early season, possibly combined with testing of sentinel flocks of six to seven chickens in the late season would effectively detect early and continued virus activity in a timely manner and minimize costs. Given that each surveillance method provides unique information about viral activity in the vector and host populations, an ideal surveillance system would combine: (1) passive detection of dead birds throughout each season as a cost-effective means for assessing the spatial extent of viral activity, (2) early-season testing of the mosquito vectors to detect temporal changes in the intensity of viral activity, and (3) late-season testing of sentinel chickens to define the end of the transmission season. The results of this work can be interpreted within the ecological contexts of individual mosquito and vector control agencies to tailor surveillance efforts and resources to maximize detection of virus activity for a specified budget.

Acknowledgments

We thank the Sacramento–Yolo, Kern, and Coachella Valley Mosquito and Vector Control Districts for their collection of the data used for this research. In particular, we want to add an additional note of appreciation to Richard Takahashi, who conducted enhanced surveillance at Kern MVCD for two years of the study. We also thank Bborie Park for data management, Kerry Padgett and Leslie Foss for their help with dead bird data, and the California Department of Public Health and the US Centers for Disease Control and Prevention for their support and funding, as well as the National Institute of Allergy and Infectious Diseases of the National Institutes of Health for their support of J. Healy under award number F31AI108189. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. C.M. Barker also acknowledges support from the Research and Policy for Infectious Disease Dynamics (RAPIDD) program of the Science & Technology Directorate, Department of Homeland Security and Fogarty International Center, National Institutes of Health.

Author Disclosure Statement

The authors have no competing financial interests to declare.

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Address correspondence to:
Christopher M. Barker
Center for Vector Borne Diseases
University of California, Davis
Davis, CA 95616

E-mail: cmbarker@ucdavis.edu