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From the Editor's Desk

It wasn't too long ago in 2003 when Major Dhillon, to my surprise, nominated me as a candidate for the editorship of the Proceedings and Papers of the Mosquito and Vector Control Association of California (MVCAC). Having been serving as a reviewer for the Proceedings for several years and with some encouragement from Craig Downs, MVCAC President-Elect at the time, I soon settled down in my new job as the Proceedings Editor. With plenty of assistance from the MVCAC Office, especially Emily Young, together we made a determination to have the Proceedings made available to our membership in record time. There were also some print quality issues that we were able to iron out as well. Moreover, we found out that the Proceedings, without a valid ISSN, did not exist as a publication with the Library of Congress ever since it changed name to Proceedings and Papers of the MVCAC in 1995. The Proceedings and Papers of the California Mosquito Control Association had its first ISSN in 1951. By changing the name to the California Mosquito and Vector Control Association in 1976, a new ISSN was obtained. Changing the name to the Mosquito and Vector Control Association of California in 1995, however, the Proceedings went ISSN-less for almost a decade until we sought the current ISSN: 1554-4974 in 2005. As you might have noticed, we also added Proc. & Papers Mosq. Vector Control Assoc. Calif. at the bottom of each printed page, making it easier for readers to use the preferred abbreviation in citations. Last but not least, about two years ago, we unsuccessfully tried to change the name from Proceedings to Bulletin of the MVCAC to better serve the publication and its authorship

During the last four years of my editorship, I really enjoyed working with you all. It was a great learning experience for me to interact with you regarding your publications whether dealing with research, operations, or public relations matters. Without your cooperation and support, especially the MVCAC Office—Chris Voight and Emily Young, and our untiring, talented group of reviewers, namely Bruce Eldridge, Minoo Madon, Steve Schutz, Steven Su, Bill Walton, Jim Webb, and (late) Glenn Yoshimura, I could not have done it all by myself.

As you may know, earlier this year I accepted editorship of the Journal of the American Mosquito Control Association, a full service quarterly journal that requires more time and energy. Consequently, I have decided to step down as the Proceedings Editor by the year's end. I have been informed that Richard Meyer has already accepted the offer to be the new editor, effective January 2008. With Dick's qualifications and experience, I am confident, he will do a fine job.

Finally, I want thank the MVCAC and all those contributing authors who made our Proceedings possible every year.

With best wishes,

Lal Mian
Proceedings Editor

Dedication of the Seventy-Fifth Annual MVCAC Conference to Thomas D. "Tommy" Mulhern June 15, 1908 - August 12, 1993

Charles Myers

California Department of Health Services

The 75th Annual Conference is appropriately dedicated in the memory of Thomas Desmond "Tommy" Mulhern in recognition of his long and storied history of service to mosquito and vector control. Mr. Mulhern died in Morgan Hill, California on August 12, 1993, but his accomplishments and legacy of 50 years in mosquito control live on.

Tommy was born in Brooklyn, New York on June 15, 1908, and probably spent his youth "dodging trolleys." His association with mosquito control began in 1925 with a summer job as a mosquito inspector with the Monmouth County (New Jersey) Mosquito Extermination Commission. Starting in 1928, he worked with the New Jersey Agricultural Experiment Station, Department of Entomology, as a drainage engineer, administrative assistant and technical consultant in mosquito control until 1949. Tommy was well known for his involvement in the design and development of the New Jersey mosquito light trap and his advocacy of the use of traps to survey and evaluate mosquito populations. In addition, he managed the Sussex County (New Jersey) Mosquito Extermination Commission program from 1936 to 1945.

Tommy was also actively involved in the Eastern Association of Mosquito Control Workers from the time of its formation through its name change to the American Mosquito Control Association (AMCA) in 1944 and its incorporation as a non-profit in 1948. He maintained a close involvement with the AMCA throughout his professional life and for extensive periods between 1935 and 1985 served as its Secretary-Treasurer, Executive Secretary and Executive Director.

In 1949, Tommy came to California and began a long and productive career with the State Department of Health as a vector control specialist in Fresno, which housed a diverse and vibrant collection of mosquito research and control specialists. His duties included providing technical assistance, support and consultation to mosquito abatement districts throughout the state. Tommy was also the principal author and editor of the California Mosquito Control Association's training guide and program for technician certification. He also served as a trustee for the Fresno Mosquito Abatement District from 1974 to 1986.

It is truly fitting that this 75th Conference of the MVCAC in Fresno be dedicated to Tommy Mulhern, a man whose many contributions to mosquito control serve as an example to those who follow.

Symposium: West Nile Virus Invasion of California, Year 4 Introduction

William K. Reisen

Center for Vectorborne Diseases, University of California, Old Davis Rd, Davis, CA 95616

West Nile virus (WNV) activity, as indicted by the numbers of human cases reported to the Centers for Disease Control and Prevention, increased by 25% at the national level, as the virus resurged in the central plain states of Colorado and Nebraska and in the Gulf States of Texas, Louisiana and Mississippi, and invaded Idaho (Fig. 1). Increased activity at northern latitudes may have been driven by above normal temperatures throughout the entire NW USA. California, by comparison, had an almost a 70% reduction in the number of cases, but enzootic activity remained elevated, especially in rural portions of the Central Valley. Apparent subsidence was related to decreased transmission in the urban centers of the Los Angeles basin and Sacramento and cool temperatures in the San Francisco Bay area continuing to prevent amplification to epidemic levels. Foci of elevated activity were detected in Kern, Yolo and Butte counties.

The Arbovirus Research Unit of the Center for Vectorborne Diseases at the University of California, Davis, in collaboration with the Coachella Valley, Greater Los Angeles County, Kern, and Sacramento-Yolo Mosquito and Vector Control Districts and the California Department of Health Services have been studying the invasion of California by WNV collaboratively since 2003. At the 2004, 2005 and 2006 annual meetings of the Mosquito and Vector Control Association of California, we summarized our findings and described selected aspects of virus transmission. The current symposium describes our research during the fourth year of the invasion, focusing on factors enabling the success of this invading virus and allowing its persistence. This year we began exploring possible alternative transmission cycles without any positive findings.

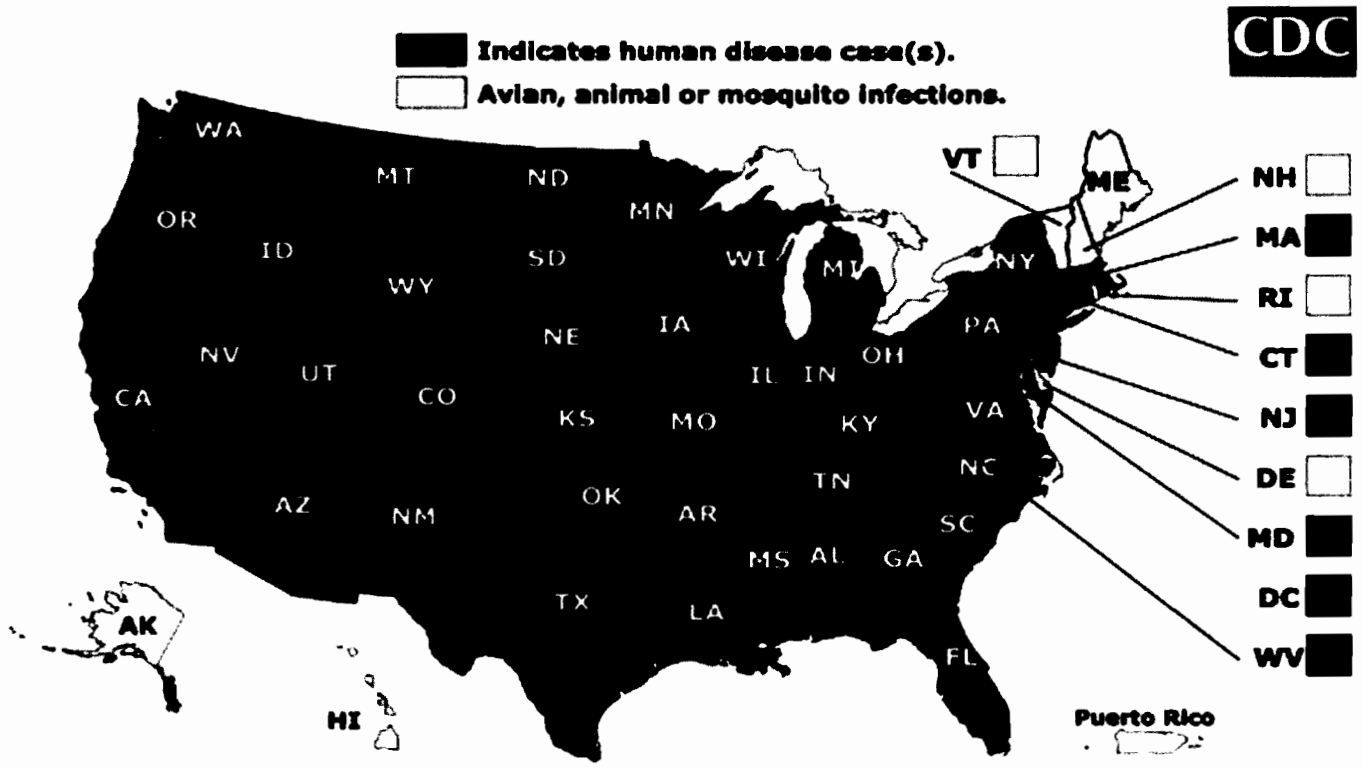

Figure 1. Distribution of human cases of West Nile virus in the United States during 2006 (http://www.cdc.gov/ncidod/dvbid/westnile/Mapsactivity/surv&control06Maps.htm)

The titles of the talks presented and the speakers are listed below.

1. William Reisen: Introduction
2. Sarah Wheeler: Are north bound migrants a source of encephalitis virus introduction into California?
3. Hugh Lothrop: Did early intervention at North Shore in Coachella Valley interrupt West Nile virus amplification?
4. Paul O'Connor: Focal transmission of West Nile virus in Los Angeles
5. Brian Carroll: West Nile virus in Kern County – Third consecutive epidemic year despite elevated avian seroprevalence
6. Stan Wright: Impact of avian 'herd immunity' on the force of West Nile virus transmission in the Sacramento County
7. Veronica Armijos: Ardeid nesting colonies as a source of West Nile virus amplification revisited – the impact of dry land
8. Chris Barker: Dynamics of California mosquito populations
9. Ying Fang: Is non-viremic transmission of West Nile virus really non-viremic?
10. William Reisen: *Ixodes pacificus* is not a competent vector of West Nile virus
11. Maureen Dannen: Improvements in surveillance processing during 2006 [10 min]
12. Keira Simmons: Proficiency panels – accuracy, specificity and sensitivity: implications for risk assessment
13. Bruce Eldridge: CalSurv: One-stop shopping for vectorborne disease surveillance in California
14. William Reisen: Summary

Acknowledgments

This research described in our symposium was funded by grants from the National Institutes of Allergy and Infectious Diseases, NIH, Centers for Disease Control and Prevention, Office of Global Programs, NOAA, Earth-Sun Science Applied Systems, NASA, California Department of Health Services, and the University-wide Mosquito Research Program. Additional funds, research space and logistical support were generously provided by the Coachella Valley MVCD, Greater Los Angeles Co VCD, Kern MVCD and the Sacramento/Yolo MVCD who hosted and were active participants in all field projects. Mosquito-pools were tested at the Center for Vectorborne Diseases [CVEC], University of California, Davis, by Maureen Dannen, Kiera Simmons and Andrew Chow under the supervision of Ying Fang and at Sac-Yolo MVCD by Kara Kelley using multiplex technology developed by Aaron Brault at CVEC. Dead birds were necropsied by the California Animal Health and Food Safety laboratories in San Bernardino and Davis with dead bird tissues tested at CVEC. Avian sera were tested by Kara Kelley [Sac/Yolo MVCD], Sandra Garcia and Ying Fang [CVEC]. Sentinel chicken sera were tested by the Viral and Rickettsial Diseases Laboratory of the CDHS under the direction of C. Cossen. Data on horse or human cases were provided by County Public Health Departments as well as by Cynthia Jean, CDHS. Data was managed through software developed by Bborie Park.

Migratory Birds and the Spread of Encephalitis Viruses in California: 10 Years of Data from the Coachella Valley

S. S. Wheeler, M.V. Armijos, S. Garcia, Y. Fang, W. K. Reisen

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INTRODUCTION

The Coachella Valley is a hot, arid valley located in the Colorado Desert of southeastern Riverside County, California. Portions of this area are heavily irrigated for agriculture, flooded for migratory waterfowl, managed for aquaculture or housing. High temperatures and a wide variety of mosquito breeding sites have lead to the persistence of several arboviruses, including western equine encephalomyelitis virus [WEE] (*Togaviridae: Alphavirus)* and Saint Louis encephalitis virus [SLE] (*Flaviviridae: Flavivirus*) which have persisted in the Coachella Valley most years (Reisen et al. 2000). West Nile virus [WNV] (*Flaviviridae: Flavivirus*), closely related to SLE, invaded the Coachella Valley in 2003 and has been detected every year since the first positive mosquito pool was collected in August 2003 (Lothrop et al. 2004, 2005, 2006). Since the emergence of WNV, SLE has not been detected in mosquito pools or sentinel chickens since 2004. Starting in 1996, wild birds from the Coachella Valley have been sampled for antibodies to WEE and SLE, and beginning in 2002 they also were screened for antibodies to WNV. During this period, 26,024 wild bird blood samples were tested. The primary goal of sampling these wild birds was to better understand which species were involved in the maintenance, amplification and spread of the above-mentioned viruses, and to learn more about these transmission cycles. In addition to the collection of serology data, representative avian species were experimentally infected to determine host competence (Reisen et al. 2003, Reisen et al. 2005)

During the spring migration (late April- early June), neotropical migratory birds concentrate on the northeastern edge of the Salton Sea. Due to the great speed (Hedenstrom et al. 1998) and distances (Ridgely 2005) that these birds fly, it was hypothesized that viremic birds may be able to introduce viruses while moving northward from southern overwintering grounds. Northward movement of viruses during spring migration may be one explanation for the repeated introduction of new SLE genotypes (Reisen et al. 2002) as well as the introduction of WNV into California through Imperial Valley (Reisen et al. 2004). To investigate the plausibility of this hypothesis, we reviewed 10 years of Coachella Valley serology data to determine the frequency of infection of neotropical migratory bird species entering and moving through California.

MATERIALS AND METHODS

Wild Bird Sampling

Migratory birds were captured while transiting two sites on the northeastern (35.511, -115.924) or northwestern (33.462, -116.061) shores of the Salton Sea during either spring or fall from 1996 through 2004. Only vernal migratory species were captured in 2005 and 2006. Insectivorous transients were collected primarily using mist nets, whereas granivorous birds were also captured in grain-baited traps. Mist nets were 10 m long, 2.5 m tall and had 32, 38 or 60mm mesh depending on the target species. Captured birds were banded with aluminum USGS bands, aged, sexed and a 0.1mL sample of blood was collected from the jugular vein with 28–gauge syringes. During 1996-2004, blood samples were added to 0.9 mL of 0.9% sodium chloride solution, clarified through centrifugation, and the serum stored at -70C. Wild bird sera were screened for antibodies to flavivirus (WNV and SLE) and WEE using an enzyme-linked immunosorbent assay [EIA], with positives confirmed by a plaque reduction neutralization test [PRNT] (Chiles et al. 1998). During 2005 and 2006, blood samples from migratory species were added to virus diluent (0.1mL of blood per 0.4mL of diluent) so that in addition to antibody screening, live virus could be detected by plaque assay using Vero cell culture (Chiles et al. 2004).

Experimental Infections

Experimental infections of Orange-crowned warblers (*Vermivora celata*), Common yellowthroats (*Geothlypis trichas*) and Yellow warblers (*Dendroica petechia*) were performed to better understand the viremia profiles of representative warbler species. Birds were caught by mist net in the Coachella Valley and Kern County, banded and pre-bled to assure that they were serologically negative to WNV, SLE and WEE. Birds were held in a mosquito proof, air conditioned facility and were fed live mealworms and finely ground trout food, and provided water *ad libitum*. Birds were inoculated subcutaneously in the cervial region with ≈1,000 plaque forming units (PFU) of NY99 strain of WNV. They were bled daily for 6-7d by jugular puncture (0.05 ml of blood taken by 28-gauge syringe and expelled into 0.4 ml of virus diluent). Additional birds inoculated with virus diluent were maintained as handling controls.

RESULTS

Of the 26,024 wild bird blood samples collected in the Coachella Valley, 3,071 were from neotropical migratory species that most commonly overwintered south of the Coachella Valley (Patten et al. 2003) and migrated through our study sites either in the spring or fall. Of the 3,071 samples collected, only one, from a Summer Tanager (*Piranga rubra*), tested positive for antibodies to WNV by both EIA and PRNT. The positive WEE and flavivirus EIA results that were negative by PRNT were considered unconfirmed. In addition to the 1 confirmed WNV infection, 3 unconfirmed flavivirus infections and 5 unconfirmed WEE infections were detected (Table 1). All birds tested by plaque assay for virus infection were negative.

All three experimentally infected warbler species (Orange-crowned, Yellow, and Common yellowthroat) produced peak viremias above 5 log10 PFU/mL (plaque forming units) that were considered to be sufficient to infect mosquitoes (Reisen et al. 2005), but varied in terms of peak viremia, viremia duration and mortality (Fig. 1). Orange-crowned warblers produced the highest viremia with the longest duration, but all individuals died of infection. On average, Yellow warblers produced a viremia that was over 5 log10 PFU/mL for 3 days and all individuals survived infection. The test infection done on the Common yellowthroat was confounded by small sample size (1 bird) but the infected bird followed the same pattern as the Yellow warblers.

Table 1: Migratory species with a positive serological result

Species	Test Result	Total
Nashville warbler *Vermivora ruficapilla*	Unconfirmed WEE	1
Violet-green swallow *Tachycineta thalassina*	Unconfirmed WEE	1
Warbling vireo *Vireo griseus*	Unconfirmed WEE	1
Wilson's warbler *Wilsonia pusilla*	Unconfirmed WEE	1
Western tanager *Piranga ludoviciana*	Unconfirmed WEE	1
MacGillvray's warbler *Oporornis tolmiei*	Unconfirmed Flavivirus	1
Yellow-breasted chat *Icteria virens*	Unconfirmed Flavivirus	1
Wilson's warbler *Wilsonia pusilla*	Unconfirmed Flavivirus	1
Summer tanager *Piranga rubra*	Confirmed WNV	1

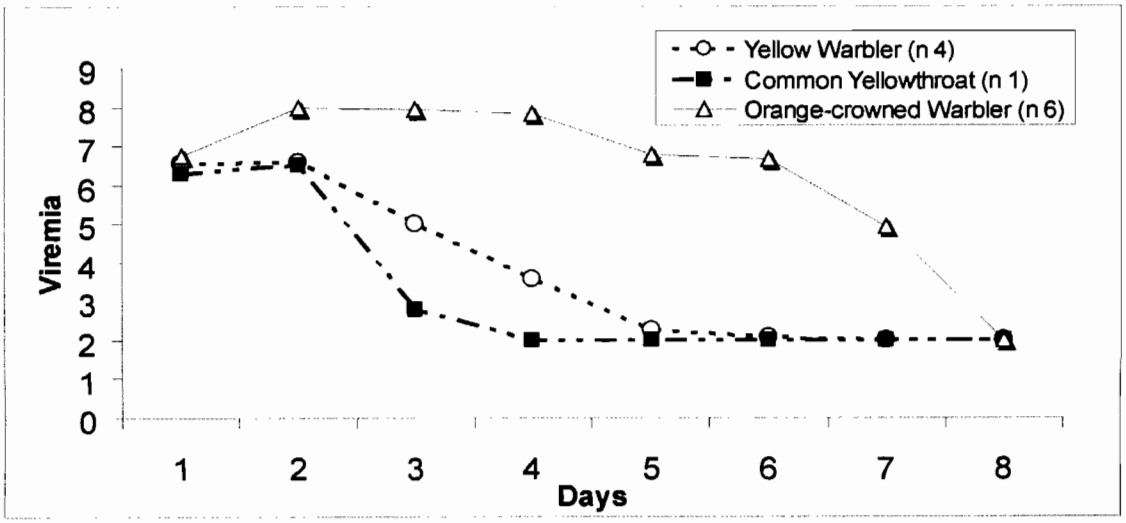

Figure 1: Viremia profile for three species of warbler. Viremia expressed as $\log_{10}$ plaque forming units per 1 mL.

DISCUSSION

Although the field collections from the Coachella Valley contained only one confirmed antibody positive (Summer tanager: WNV) and no positive viremia samples, the test infection data from the 3 infected warblers (Orange-crowned, Yellow, and Common yellowthroat) indicated that these species are competent WNV hosts. The lack of field findings could be due, in part, to the number of infected migrants being lower than our detection threshold. Although the window for detecting live virus is limited to <7 days (Komar et al. 2003, Reisen et al. 2005), we attempted to isolate virus for several reasons. First, the hot temperatures of the Coachella Valley allow for early season mosquito activity and virus transmission, making it possible for birds to be infected locally. Second, since not all species survive WNV infection, virus isolation would be the only way to detect infection other than from a fresh carcass, which is an unlikely scenario when working with small birds (6-30 grams) in rural areas (Ward et al. 2006).

Recent studies have indicated that WNV distribution has spread throughout Central and South America and therefore further efforts will be made during the spring migration of 2007 to try to detect birds infected or previously infected with WEE, SLE or WNV. While past data shows that this is not a common occurrence, we still feel that there is not enough negative data to rule out the possibility of northbound migrants disseminating these encephalitis viruses.

Acknowledgments

We would like to thank the Coachella Valley Mosquito and Vector Control District (CVMVCD) for logistical support and J. Lundstrom, R. Cusack, T. Chapin and H. Lothrop for assistance in the collection of field samples. Migratory bird range data provided by NatureServe in collaboration with Robert Ridgely, James Zook, The Nature Conservancy - Migratory Bird Program, Conservation International - CABS, World Wildlife Fund - US, and Environment Canada – WILDSPACE. This research was funded, in part, by Research Grants RO1-39483, RO1-AI47855, and AI55607 from the National Institute of Allergy and Infectious Diseases, NIH, the CVMVCD, and special funds for the Mosquito Research Program allocated annually through the Division of Agriculture and Natural Resources, University of California.

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Are Ardeid Colonies Nesting Over Dry Land a Source of West Nile virus Amplification?

Veronica Armijos[2], Sarah Wheeler[2], Ying Fang[2], Sandra Garcia[2], Stan Wright[3], Kara Kelley[3], William K. Reisen[1,2]

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Although West Nile virus (WNV) is now endemic throughout California, patterns of transmission tend to be clumped in time and space. Determination of what factors lead to sporadic virus amplification may be useful in planning control operations. Our research has been testing the hypothesis that aggregations of susceptible avian hosts that intersect vector mosquito populations may serve as foci for efficient virus amplification. The current research tested this hypothesis by measuring virus activity at two Ardeid colonies nesting in Yolo County California during 2006. Data from these colonies sampled from July to August 2006 were compared with data from a previous study of an Ardeid nesting colony conducted in 2004 at Rammer Lake in Imperial County. Results from Rammer Lake suggested that this large concentration of nesting birds was not a focus of WNV amplification (Reisen et al. 2005b).

METHODS

The Ardeid colonies in Yolo County were located near the city of Davis at the UC Davis Arboretum and on County Rd. 103. Nestlings found alive on the ground were collected by hand or net and a 0.1 ml blood sample was taken and expressed into 0.4 ml of virus diluent. Samples were clarified by centrifugation and frozen at -80C for later testing. Viremia was measured by standard plaque assay on Vero cells and antibody tested by an enzyme immunoassay [EIA]. All birds found dead were submitted for necropsy to the California Animal Health of Food Safety laboratory for necropsy. Kidney snips were tested for WNV RNA by real time RT-PCR at the Center for Vectorborne Diseases (CVEC). Additional living House sparrows (HOSP) were collected by mist net and tested for antibody using the same EIA mentioned above. Mosquitoes were collected weekly by 3 dry ice-baited and one gravid female traps, anesthetized by triethylamine, enumerated to species and counted into pools of <50 females each and frozen at -80C. Pools were tested for WNV, St. Louis encephalitis virus and western equine encephalomyelitis virus RNA by the Sac-Yolo MVCD and CVEC using a multiplex RT-PCR.

RESULTS AND DISCUSSION

A total of 151 blood samples were collected from Ardeid birds at both locations, of which WNV was isolated from 8 (5.2%) Black crowned night herons (BCNH). Four of the BCNH viremias were above 5 $\log_{10}$ plaque forming units [PFU]/ml, indicating they would be infectious for *Culex* mosquitoes (Reisen et al. 2005a). Antibodies to WNV were found in 11 (7.2%) BCNH and 4 (2.6%) Snowy egrets (SNEG). A total of 33 dead birds were collected, of which 3 (9%) BCNH, 2 (6%) SNEG, and 1 (3%) Great Egret (GREG) tested positive for WNV. In addition, 48 HOSP were tested for WNV antibodies by EIA, of which 3 (6.2%) were positive.

Culex tarsalis Coquillett was the most abundant species at both the County Rd. 103 (91% of 3,755 mosquitoes) and the UC Davis Arboretum (78% of 194) Ardeid colonies. Two *Cx. tarsalis* and 2 *Cx. pipiens* L. pools collected at County Road 103 and one *Cx. pipiens* pool from the UC Davis Arboretum tested positive for WNV RNA. However, *Cx tarsalis* infection rates from the colony at Country Rd. 103 (IR= 0.6) were not statistically different from a comparison site located ca. 5 to the SE at the Yolo By-Pass Wildlife Refuge (IR= 1.6) that lacked a colony of Ardeid birds.

Our interim results demonstrated extensive WNV activity at the ardeid nesting colony at Rd 103. Of the live and dead birds tested, infection and seroprevalence rates were highest in BCNH nestlings. Some of these nestlings had very elevated viremias [9.8 $\log_{10}$ PFU/ml] and therefore were considered extremely competent hosts. In agreement, infection was detected in both *Cx. tarsalis* and *Cx. pipiens* and several House sparrows were positive, perhaps indicating that transmission occurred to other bird species. Evidence of infection in this heronry situated within a Eucalyptus grove over dry land was considerably greater than observed previously at Ramer Lake in Imperial County where nests were situated over water. These observations supported our earlier study that indicated *Cx. tarsalis* quest infrequently over water (Lothrop and Reisen 2001).

Interestingly, the mosquito infection rates at the Rd 103 heronry were not different than observed in the nearby Yolo by-pass where nesting Ardeids were not observed. Therefore, at this point we cannot conclude that the elevated infection rates we observed in the Ardeid nestlings was the result of enhanced amplification at the heronry or simply reflected WNV activity associated with the 2006 outbreak centered in nearby Davis. Future research will continue to elucidate the role of communal birds in WNV amplification in California.

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Did Early Intervention at North Shore in the Coachella Valley Interrupt West Nile Virus Amplification?

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ABSTRACT: In response to high relative abundance in the area of North Shore and the early season appearance of West Nile virus [WNV] at this focus in 2004 and 2005, the Coachella Valley Mosquito and Vector Control District made ultra-low volume applications of Pyrenone® 25-5 mixed 1 to 2 with BVA Spray 13® oil by air for 30 days. Comparing the 2006 season with the 2 previous years, there was a large reduction in the extent and intensity of WNV activity detected.

INTRODUCTION

The Coachella valley extends from the northern shore of the Salton Sea to the foot of the San Bernardino Mountains (Fig. 1). Historically, mosquito-borne viruses including western equine encephalomyelitis (WEEV) and Saint Louis encephalitis (SLEV) have been first detected in surveillance at the shore of the Salton Sea in the area of the community of North Shore. Although the cause of this focal appearance has yet to be determined, this pattern has been repeated since 1991 when surveillance efforts were established to define the temporal and spatial distribution of these viruses in the Coachella Valley. This surveillance system has been monitoring West Nile virus (WNV) since its introduction in 2003 and the same early seasonal focus was observed in 2004, 2005 and 2006 near North Shore. This focal pattern of appearance and dispersal may provide the opportunity to disrupt virus dissemination at the beginning of the season because the focus lies at the extreme southwest corner of the valley with only a narrow band of wetlands connecting it to the rest of the valley. Previous attempts at this approach were conducted by the Coachella Valley Mosquito and Vector Control District (CVMVCD) using ground ultra-low volume (ULV) treatments with no observable epidemiological results.

SURVEILLANCE ASSETS

Surveillance in the Coachella Valley has been a collaborative effort by the CVMVCD and the University of California, Davis, Center for Vectorborne Disease Research. Surveillance assets include 17 gravid traps run weekly (Fig. 2), 58 CO2-baited CDC-style traps (CO2T) run biweekly on fixed stands, and 9 flocks of 10 chickens sampled biweekly (Fig. 3). An additional 3 flocks are maintained in Imperial County along the southern shore of the Salton Sea to monitor wetland habitats at or near wildlife refuges. CO2Ts primarily monitor *Culex tarsalis* Coquillett which is the

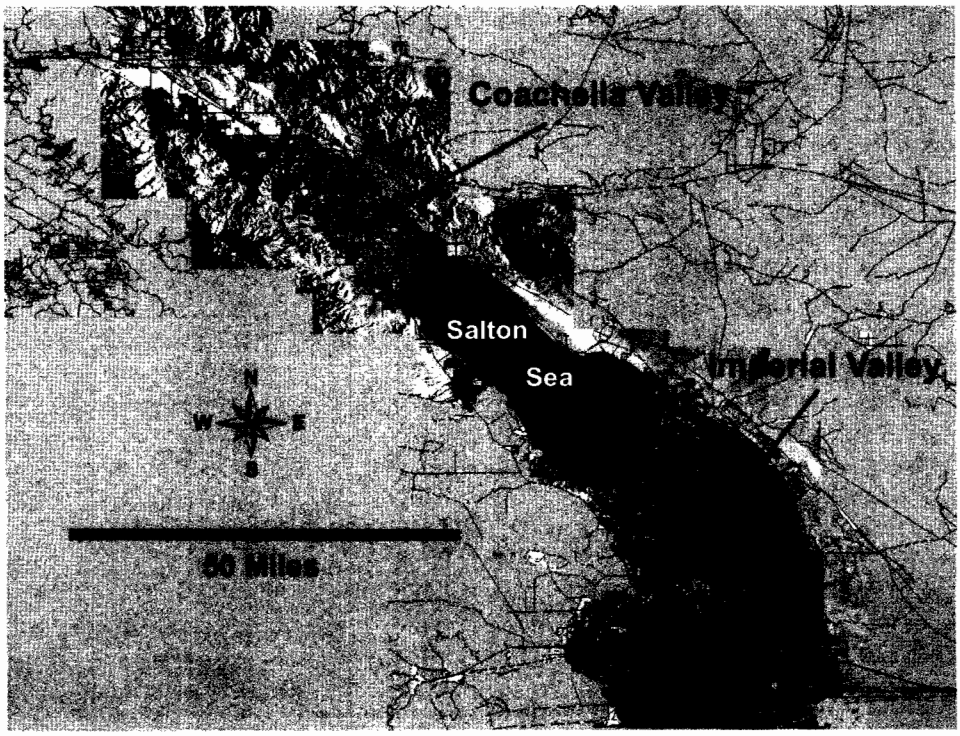

Figure 1. The Salton Sea basin showing the locations of the Coachella and Imperial Valleys.

predominant vector species in the lower valley, whereas gravid traps target *Culex quinquefasciatus* Say in the urban upper valley. Chicken flocks upland from the margin of the Salton Sea are accompanied by 2 CO2Ts each that have been relatively successful in collecting Cx. quinquefasciatus compared to gravid traps in the same areas. Additional CO2Ts are run during research projects, which locally enhance the sensitivity of the surveillance. A dead bird monitoring program is also active, but few dead birds have been positive for WNV, likely due to the sparse population of Corvids in the southwestern desert.

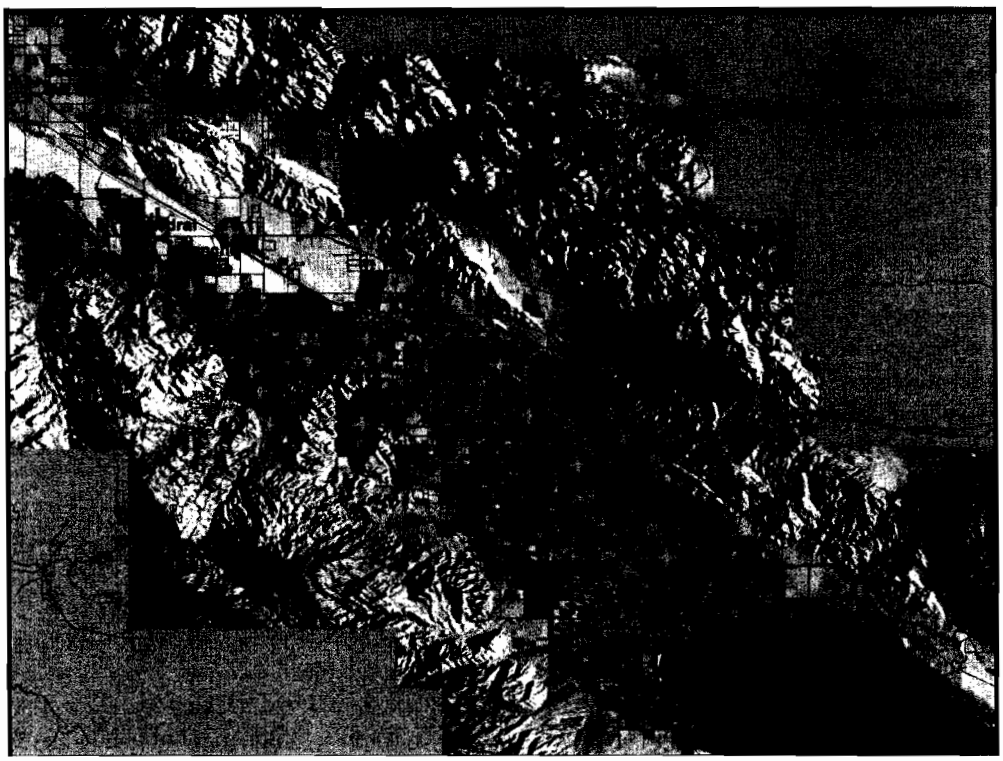

Figure 2. Gravid trap locations in the Coachella Valley, 2006.

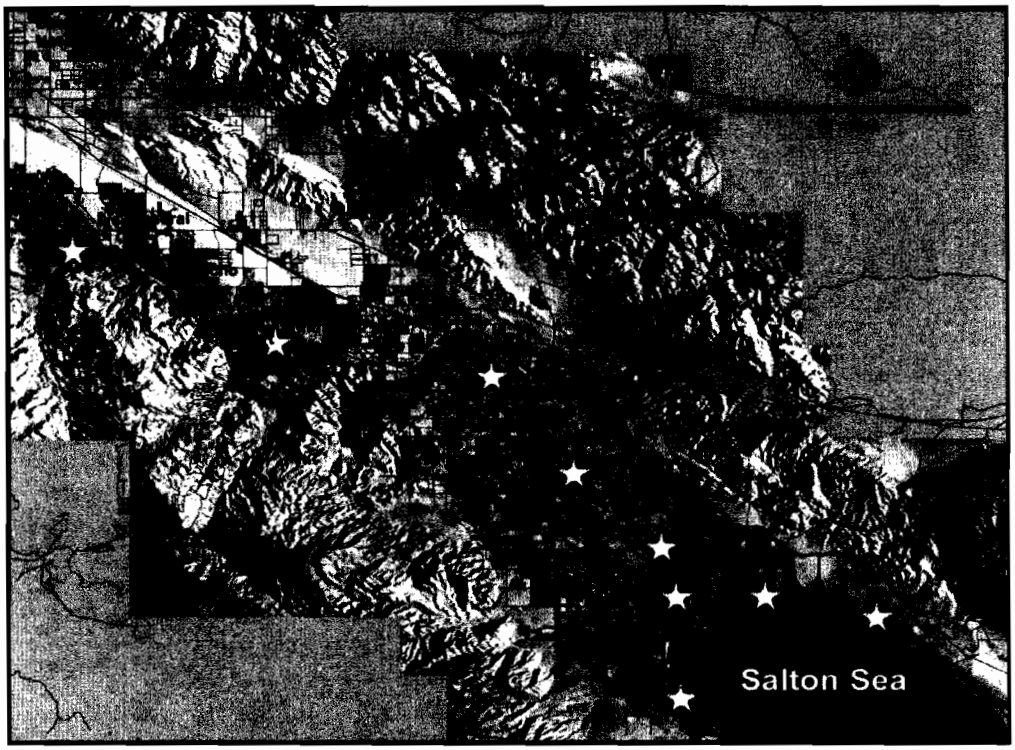

Figure 3. CO2-baited traps (triangles) and sentinel chicken flocks (stars) in the Coachella Valley, 2006.

SEASONAL VIRUS DISSEMINATION

Previously described seasonal patterns of dissemination of SLE and WEE in the Coachella Valley are shown in Fig 4. After the initial detection in mosquito pools or the chicken flock at North Shore in May to July, these viruses were disseminated along the shoreline and into the rest of the valley up to the region northeast of Indio. This pattern appeared to be driven by the abundance gradient of *Cx. tarsalis* as it declined northward away from the Salton Sea. Generally, the appearance of WEEV preceded SLEV, but SLEV continued later into the fall (Reisen et al, 1995).

In 2004 WNV dispersal followed the above pattern, but was detected earlier on 14 April and then remained mostly confined to the lower valley in *Cx. tarsalis*, although there were seroconversions at all surveillance flocks and 7 human cases in the upper valley by the end of September. In 2005 WNV was first detected 29 May, but rapidly transferred into the *Cx. quinquefasciatus* population in the urbanized upper valley. Midseason activity in the upper valley was widespread and had focal peaks of *Cx. quinquefasciatus* infection (MLE) (Biggerstaff, 2003) as high as 34 per 1,000 in one residential neighborhood. Despite widespread detection and high MLE in the upper valley, there were only 5 human cases reported. In the lower valley concurrent WNV activity was less during midseason with a MLE infection rate below 3. Late season activity, October through November, was mostly centered in the duck club region and associated with the fall flooding of duck ponds and subsequent increase in abundance of *Cx. tarsalis*.

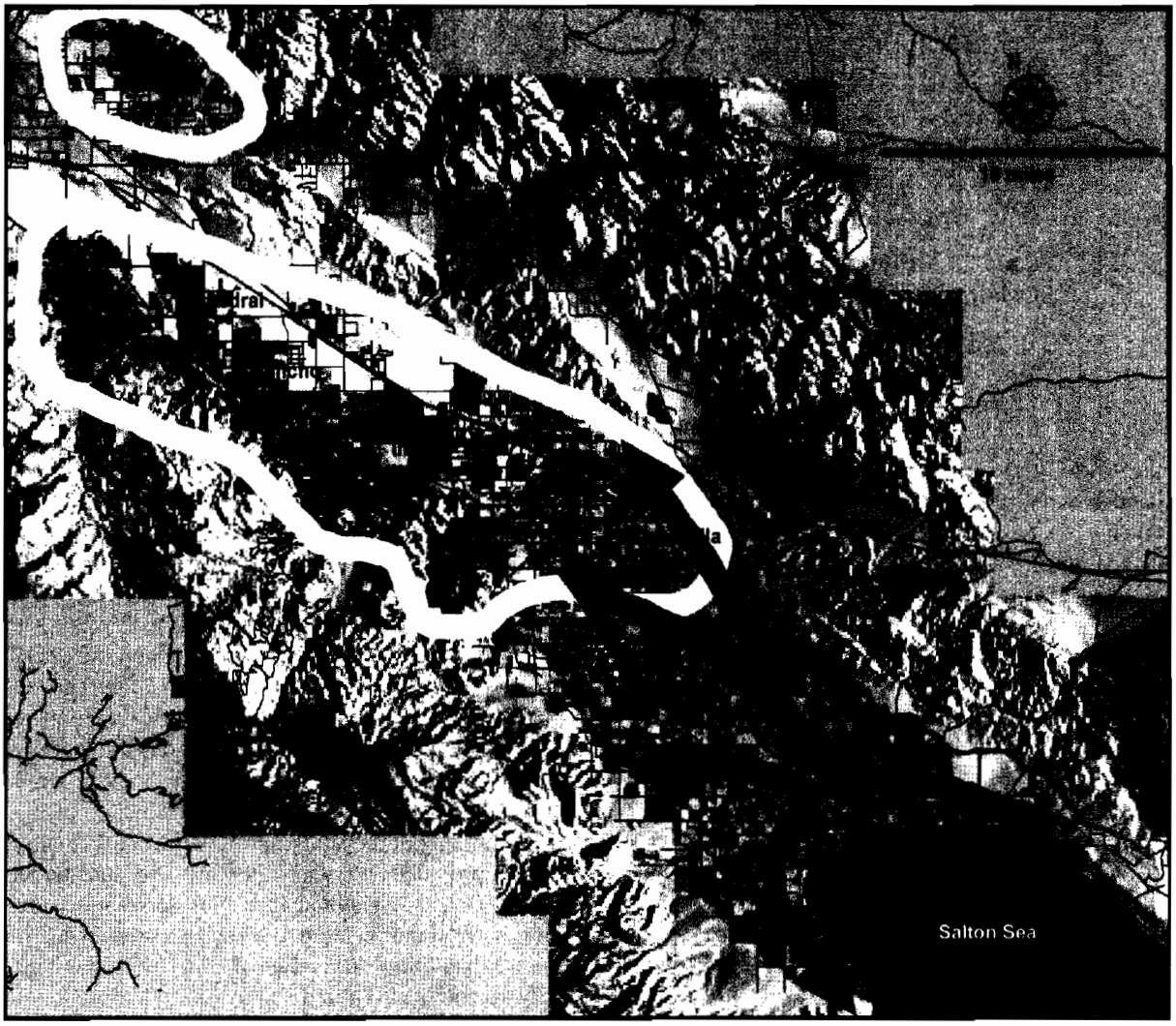

Figure 4. Pattern of WNV, SLEV, and WEEV amplification and dissemination from North Shore in *Culex tarsalis* (depicted by grey arrows). The areas of WNV infection in *Cx. quinquefasciatus* in urban zones are circumscribed by white perimeters.

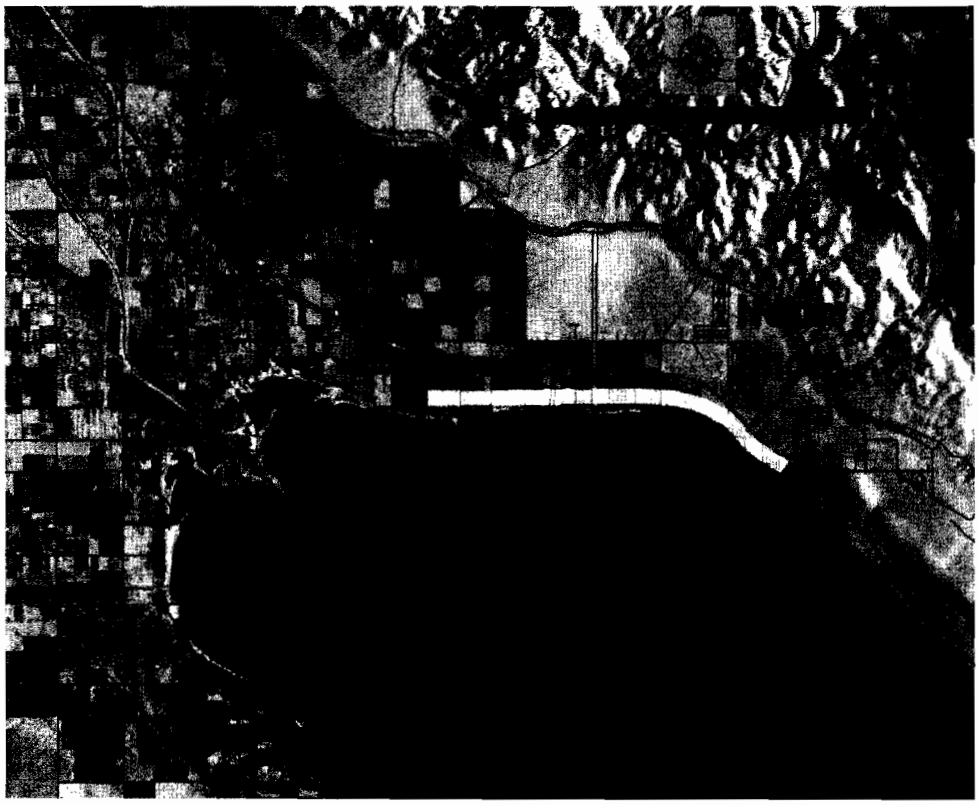

Figure 5. Area treated by ULV adulticides during 2006 along the northern shore shown in white.

TREATMENTS

In 2006 the CVMVCD anticipated the onset of WNV and planned aerial and ground treatments in the area of North Shore at the end of April (Fig. 5), based upon the potential for early season onset demonstrated during the previous 2 years. Results from mosquito pools tested just prior to treatment showed that treatments had begun just following the onset of virus activity, prompting the CVMVCD to extend the treatment area further west to stay ahead of the possible dissemination of WNV out of the treated area. Primary emphasis was placed on aerial ULV treatments along the shoreline with ground ULV filling in areas such as the Salton Sea State Park, to the southeast of North Shore. These treatments were continued for approximately 1 month to allow for surveillance results to confirm a cessation of virus activity. The next appearance of WNV, 1 pool of *Cx. quinquefasciatus* and 1 chicken seroconversion, was in Palm Desert at the end of May. The District responded with intensive larval control and ground ULV, because of the limited scope of the problem and no further virus was detected in the upper valley until September when 1 positive pool was collected in La Quinta. In the lower valley, WNV was detected in only 1 pool in July and 3 pools in August followed by 13 positive pools in September and early October. Again, those in August through October were primarily associated with duck club flooding and local increases in *Cx. tarsalis* abundance.

DISCUSSION

In assessing the impact of early season treatments on the amplification and dissemination of WNV in the Coachella Valley, we have included surveillance data from the Imperial Valley as a comparison (Fig. 1; Table 1). These data indicated that seasonal activity increased in the Imperial Valley during 2006 compared to contrasting decrease in the Coachella Valley. Since the two valleys are in proximity and similar in climate and seasonal mosquito-borne virus activity (Reisen et al, 1997), we speculated that these contrasting results for 2006 support our contention that early season treatments in and around the focus at North Shore were successful in interrupting the normal pattern of amplification and dissemination of WNV in the Coachella Valley. A careful understanding of the landscape ecology of arboviruses in Coachella Valley provided us with an early season target for focused intervention that resulted in a long term and widespread impact upon virus amplification during the subsequent season. These unique data show the value of long term ecological studies and early season intervention.

Acknowledgments

This work was a collaboration between the Coachella Valley Mosquito and Vector Control District and the University of

Table 1. Interannual summary of WNV activity in the Coachella (COAV) and Imperial (IMPR) valleys.

Region		2004		2005		2006	
COAV	Species	Cx. tars	Cx. quinq	Cx. tars	Cx. quinq	Cx. tars	Cx. quinq
	WNV positive pools	7.5%	1.7%	1.5%	8%	1.4%	0.2%
	Seroconversions	73		63		16	
IMPR	Species	Cx. tars	Cx. quinq	Cx. tars	Cx. quinq	Cx. tars	Cx. quinq
	WNV positive pools	8.3%	0%	3.3%	0%	5.8%	0%
	Seroconversions	56		47		55	

California, Davis, Center for Vectorborne Disease Research. Additional funding came from the National Institutes of Health and Centers for Disease Control. Testing of chicken sera was done by the California Dept. of Health Services. Mosquito pool testing was done by the UC Davis, Center for Vectorborne Diseases. Thanks are due to Patrick Miller and Marc Kensington (Center for Vectorborne Diseases, UC Davis), and Arturo Gutierrez (Coachella Valley MVCD) for technical assistance.

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West Nile Virus Foci in Greater Los Angeles County Vector Control District, 2003 – 2006

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ABSTRACT: West Nile virus (WNV), first detected within the boundaries of the Greater Los Angeles County Vector Control District (GLACVCD) in September 2003, has maintained varying intensities of transmission among mosquitoes, wild birds, equines, and humans through 2006. The current paper analyzes the trends in WNV distribution in GLACVCD, using WNV -positive dead birds, mosquitoes, and human cases. The temporal pattern of the WNV epidemic generally conformed to that observed elsewhere throughout the country and involved an initial introduction with some human cases the first season, explosive epidemic amplification the second season, and a subsidence to maintenance levels the third year. Although WNV activity declined substantially since the 2004 epidemic year, it continues to remain active in Los Angeles and should be considered endemic. Research continues to understand how the various ecological factors may reactivate "residual foci" or create new areas of focal amplification.

INTRODUCTION

In 2003 the Greater Los Angeles County Vector Control District [GLACVCD] joined a collaborative project with the Center for Vectorborne Diseases, University of California, Davis, and the California Department of Health Services to investigate the invasion of California by WNV. The current paper utilizes three indicators of WNV activity: WNV-positive dead birds, mosquito pools, and human cases, to illustrate changes in distribution and intensity of transmission from 2003 to 2006.

MATERIALS AND METHODS

Five primary monitoring sites were selected for weekly surveillance. Two of these sites were located in the northern part of the District in the San Fernando Valley at Encino and Griffith Park, and three sites were located in the southern half of the District in the Los Angeles Basin at Machado Lake, Rowland Heights, and Whittier Narrows. While both parts of the District are highly urbanized and similar in terms of residential and industrial densities and presence of parks and residual wetlands, they vary significantly in climate. The San Fernando Valley generally has hotter summers and colder winters than the Los Angeles Basin which is influenced by the cool onshore marine air flow. Annual rainfall in the two areas is similar.

Mosquitoes were collected at these primary sites on a biweekly basis using gravid and CO2-baited CDC traps to measure abundance and infection rates. At these same sites, blood samples were colleted biweekly from sentinel chickens and peridomestic wild birds to detect WNV antibodies (Wilson et. al. 2004). Dead birds were collected and submitted for testing as part of the California Department of Health Services' Dead Bird Surveillance Program. In addition to the primary sites, mosquitoes were collected monthly from approximately twenty-five transects in other areas of the District in order to assess abundance and virus activity outside of the primary study areas and to provide information for mosquito control activities.

The number of WN-positive dead birds and human cases were grouped spatially by zip code for each year. Infection rates were calculated by Maximum Likelihood Estimates (MLE/1000) for *Culex* quinquefasciatus Say pools during the eight-week period of peak transmission for each year. Mosquito pools were grouped by zip codes to obtain a sufficient number of samples for the calculation of MLEs (Biggerstaff, 2006).

RESULTS AND DISCUSSION

West Nile virus first invaded GLACVCD in September 2003. Nearly all of the WNV activity that year occurred in the southern half of the District in the Rio Hondo and San Gabriel River corridors. By year's end, 6 Cx. quinquefasciatus pools, 7 chickens, and 26 dead crows from this area tested positive for WNV. There was no indication of mosquito-borne virus activity in the San Fernando Valley until November 2003, when a pool of *Cx. quinquefasciatus* collected from a gravid trap tested positive for St. Louis encephalitis (SLE). In December, the first WNV positive dead crow was recovered in the San Fernando Valley. This was the first indication of the northward spread of WNV in the District (Wilson et. al. 2004).

In 2004 the WNV epizootic spread rapidly as evidenced by widespread WNV amplification throughout the District. A total of 616 dead birds reported by the public tested positive for WNV (Fig. 1). There also were 326 WNV-positive mosquito pools widely distributed throughout the District (Fig. 2). During peak transmission, many MLEs exceeded 15/1000, indicating intense transmission. There were 157 human cases in the District in 2004 that were widely distributed in both the Los Angeles Basin and the San Fernando Valley, with a single case in Santa Clarita (Fig. 3).

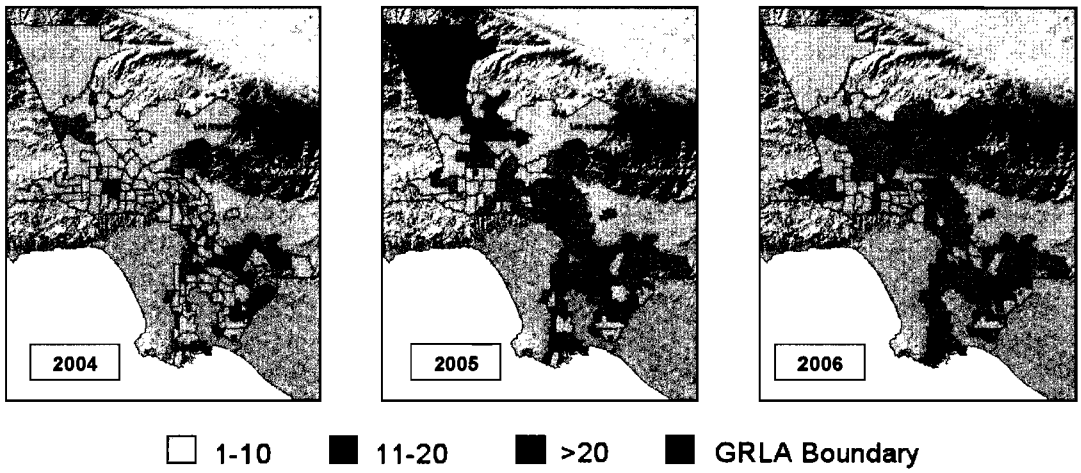

Figure 1. Decline in WNV positive dead birds recovered in GLACVCD, 2004-2006

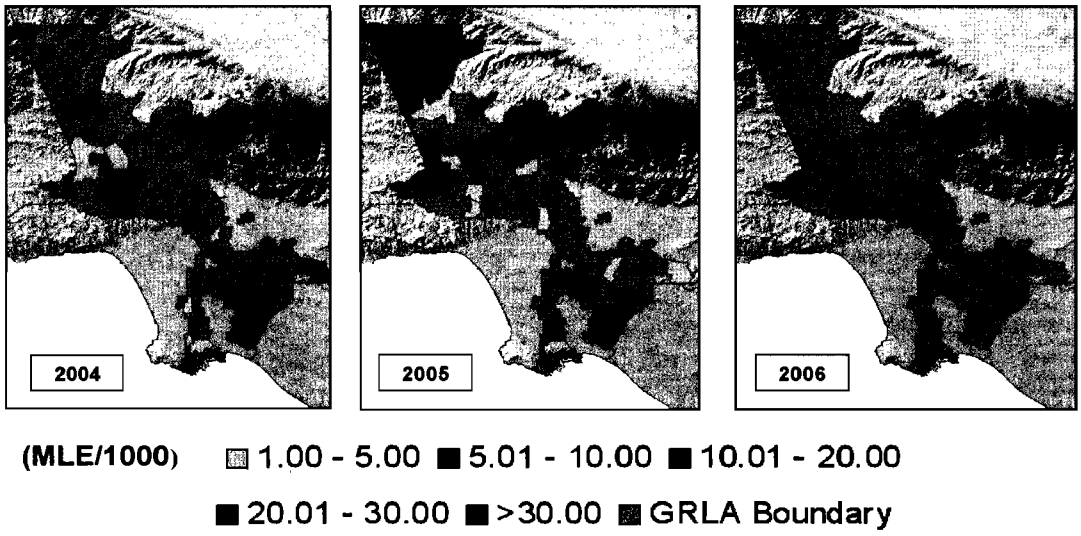

Figure 2. Decline in peak period activity of WNV positive mosquito pools

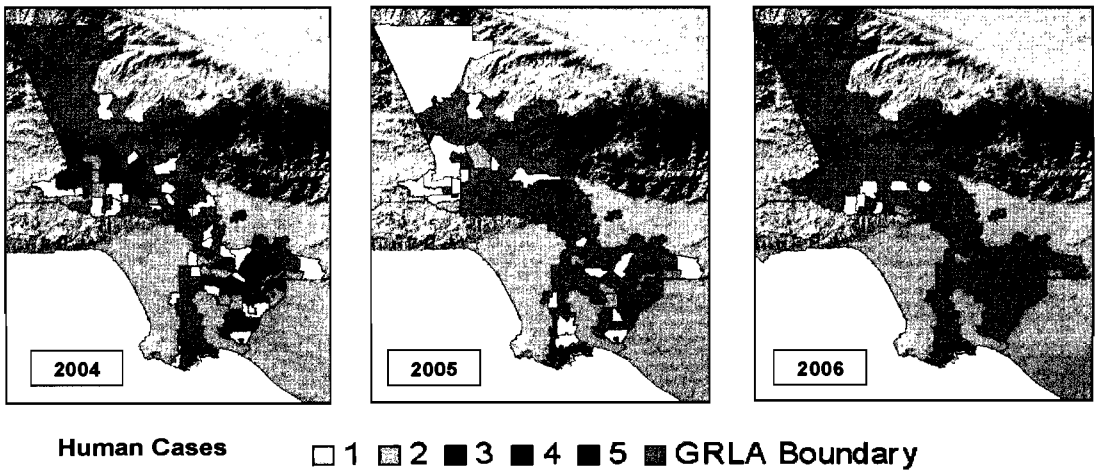

Figure 3. Decline in WNV human cases 2004-2006.

The use of zip codes as a unit of area for analysis was problematic. While zip codes are a convenient way to represent areas within the District, they have no intrinsic ecological basis. However, some zip codes or groups of zip codes may serendipitously circumscribe areas of ecological importance – for example wetlands, crow roosts, or concentrations of unmaintained swimming pools. A second disadvantage is that zip codes vary in size and may change through time. Third, when WNV positives are represented in figures by shading, a few positives in zip codes that cover large geographical areas encompass a large area and therefore may give a false impression of the extent of WNV distribution. Finally, some zip codes had small sample sizes, resulting in large confidence intervals for the calculated MIR. In spite of these caveats, using zip codes as the unit of scale worked well for the analyses presented in our paper.

Because of the wide extent of the 2004 WNV epizootic, gross analysis by zip code failed to detect distinct focal areas. However, spatial analysis of these data revealed clusters of dead corvids centered near American crow roosts in the cities of Northridge, Compton, and La Mirada, and two congruent clusters of human cases in La Puente and Cerritos (Reisen et al. 2004).

Surveillance efforts in 2005 detected fewer WNV-positive dead birds, mosquito pools and human cases as well as a contraction in the geographical distribution of positive indicators. The total number of WNV-positive dead birds recovered declined from 616 in 2004 to 148 in 2005. This decline was most notable in the central and southern part of the District, but there was an increase in the number of WNV-positive dead birds recovered in the northern San Fernando Valley and Santa Clarita (Fig. 1). The number of peak period WNV-positive mosquito pools declined from 346 in 2004 to 179 in 2005. A similar shift in the geographical distribution of positive mosquito pools was noted as was seen in the case of WNV-positive dead birds, where there was a decrease in the distribution of WNV-positive pools in the central and southern portions of the District, and an increase in the numbers and infection rates of mosquito pools in northern San Fernando Valley and Santa Clarita. Human cases declined from 157 in 2004 to 29 in 2005, with a similar shift in the geographical distribution of cases that was noted with WNV- positive dead birds and mosquito pools.

In 2006, the main focus of both WNV-positive pools and human cases was the southern portion of the San Fernando Valley (Fig. 2 & 3). The total number of WNV-positive dead birds declined from 148 in 2005 to 54 in 2006. The number of WNV-positive pools declined from 179 in 2005 to 77 in 2006, but MLEs remained relatively high. The human cases declined from 29 in 2005 to 10 in 2006.

With a decrease in infections during 2005 and 2006, foci delineated by zip codes became apparent. The general concurrence of human cases (Fig. 3) in areas where WNV-positive mosquito pools were detected (Fig. 2) highlighted the effectiveness of our surveillance program, and demonstrated the utility of transect sampling (Wilson et al. 2004). A similar agreement was seen among areas with WNV-positive dead birds and mosquito pools (Fig. 1 & 2) and human cases (Fig. 3) by the end of 2006.

These observations suggest the subsidence of WNV to endemic maintenance levels in GLACVCD. However, several mechanisms may serve to re-activate "residual foci" or create new foci, including the decline of immune status of peridomestic bird populations, increase in mosquito abundance, warm temperatures, or the creation of new mosquito sources. These conclusions should be considered preliminary as they are based on zip codes without spatial statistical analysis. We'll continue to examine these data to see if other factors, such as 'herd immunity' in birds, might enhance our understanding of the focal distribution of WNV during 2005 and 2006.

Acknowledgments

We would like to thank members of the Center for Vectorborne Diseases [CVEC] for their assistance: William K. Reisen for sampling design; Chris Barker and Bborie Park for database creation and management, and Ying Fang and the laboratory staff for mosquito pool, dead bird and wild bird sample processing, and the staff at the CDHS Dead Bird Surveillance Program and West Nile Virus Hotline.

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West Nile Virus Activity in Kern County During 2006

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ABSTRACT: West Nile virus (WNV) reappeared in Kern County in mid-May 2006 and was detected by all surveillance methods. Activity during 2006 was similar to that of previous years, with infections detected in 50 humans, 4 equines, 89 sentinel chickens, 24 dead birds, and 217 mosquito pools. There also were 811 seropositive wild birds, almost double the 412 positives detected in 2005. These data indicated that a marked increase in avian 'herd immunity' was not associated with a general subsidence in virus activity. During this third year of virus activity, WNV was found throughout Kern County on the floor of the Central Valley.

INTRODUCTION

West Nile virus (WNV) activity in California began in 2003 and was limited to six counties south of the Tehachapi Mountains (Hom et al. 2004). By June 2004 WNV activity had spread north of the Tehachapi Mountain range and into the Bakersfield area of Kern County (Takahashi et al. 2005). WNV quickly spread from there and by the end of the year it had been detected in every county of the state (Hom et al. 2005). During 2005 WNV activity was focused primarily within the city of Bakersfield (Carroll et al. 2006). The current paper discusses the reappearance of WNV in Kern County in 2006 and describes detection by various surveillance methods, spread through the county, and differences between 2006 and the previous WNV active years of 2004 and 2005.

MATERIALS AND METHODS

Background: Surveillance information was gathered by multiple agencies including five separate mosquito control agencies, the Kern County Department of Public Health, Edwards Air Force Base and the Arbovirus Field Station (AFS) of University of California, Davis (UCD). All of the data presented in this report was collected within the boundaries of the Kern Mosquito and Vector Control District (KMVCD), the largest district in the county covering 1,650 square miles. Other districts include the Delano Mosquito Abatement District, South Fork Mosquito Abatement District, West Side MVCD, and Antelope Valley MVCD. Sampling locations are shown in Fig. 1.

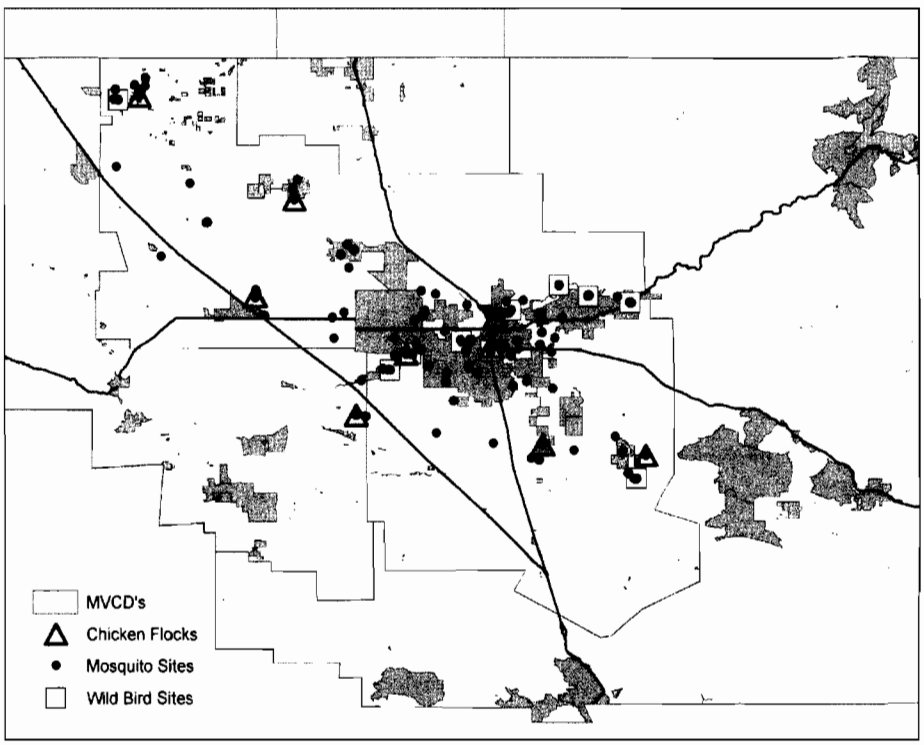

Figure 1. Surveillance sites in Kern County, 2006.

Dead Birds: Dead birds were reported by the public to the CDHS-VBDS hotline who forwarded pertinent information to the KMVCD for bird pickup. Birds were submitted to the California Animal Health and Food Safety (CAHFS) Central Laboratory at UCD for necropsy. Oral swabs and/or kidney tissue were sent to the UCD Center for Vector-borne Diseases (CVEC) laboratory for testing by reverse transcriptase-polymerase chain reaction (RT-PCR).

Mosquitoes: Mosquitoes were collected biweekly by dry ice baited CDC traps (Sudia and Chamberlain 1962) and by Reiter/Cummings gravid traps (Cummings 1992). Collections were identified by species and pooled into groups of ≤50 females each and then tested for viral RNA by CVEC using a multiplex RT-PCR that detects WNV as well as St Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) (Chiles et al. 2004).

Chickens: Sera were collected biweekly from 10 hens within each of 14 flocks. Individual blood samples were collected on strips of filter paper and then sent to CDHS Viral and Rickettsial Disease Laboratory (VRDL) for testing for IgG antibody by an indirect enzyme immunoassay (EIA) (Reisen et al. 1994). Positives were confirmed by indirect fluorescent antibody (IFA) and end-point plaque reduction neutralization tests (PRNT).

Free Ranging Birds: Birds were collected biweekly using mist nets and grain baited traps, banded and a blood sample taken [0.1 ml into 0.9 ml saline]. Samples were clarified by centrifugation and then screened for antibody by an EIA (Chiles and Reisen 1998), with positives confirmed and identified by PRNT. Sera confirmed as positive, but without a 4X difference between WNV and SLE end point titers were listed as unidentified Flavivirus.

Humans and Equines: Human and equine case information was provided by the Kern County Department of Public Health and by the California West Nile Virus Surveillance Information Center.

RESULTS

WNV was initially detected in a single *Culex tarsalis* pool collected on May 9th. There were no more positive indicators of WNV activity until June, when there were numerous mosquito pools and the first human case detected. It wasn't until July when the remaining surveillance indicators, including sentinel chickens, dead birds and equines, detected virus activity.

During 2006, 217 of 1,881 mosquito pools from Kern County tested positive for WNV (Table 1). From late February through early May, 2,812 *Culex eythrothorax* Dyar, 804 *Culex quinquefasciatus* Say, 1,511 *Culex tarsalis* Coquillett, and 1,485 *Aedes melanimon* Dyar were tested for virus infection in 183 pools, with negative findings. On 9 May a single Cx. *tarsalis* pool at the water recharge ponds on the western edge of Bakersfield tested positive for WNV. It took an additional 41 days for the virus to amplify and start spreading. From the initial positive pool until mid June there were 1,057 *Cx eythrothorax*, 4,973 *Cx quinquefasciatus,* 8,278 *Cx tarsalis*, and 1,783 *Ae melanimon* tested for virus infection in 387 pools, with negative findings. On 20 June one *Cx quinquefasciatus* pool collected from a gravid trap tested positive for WNV, on 22 June one *Cx. tarsalis* pool collected from a CDC trap tested positive for WNV, and on 23 June one *Cx quinquefasciatus* pool collected from a gravid trap tested positive for WNV. By the end of June the WNV had spread throughout the city of Bakersfield and the surrounding areas, producing 10 more positive mosquito pools. Of the six species of mosquitoes that were submitted for testing in 2006, WNV was detected from *Cx eythrothorax*, *Cx quinquefasciatus*, *Cx tarsalis*, and *Ae. melanimon.* There was only one *Ae. melanimon* and two *Cx eythrothorax* pools that tested positive and we felt that these species did not play a major role in virus amplification. *Culex quinquefasciatus* and *Cx tarsalis* were the major vectors for WNV transmission activity in 2006. Infection rates per 1,000 (MIRs/1000) for *Cx quinquefasciatus* and *Cx tarsalis* were ca. 1.0 during June, and then spiked in July. *Culex. tarsalis* MIRs quickly dropped from a peak of 10.8 in July to 6.8 in August and then to ca. 1.0 in September and October, whereas the MIRs of *Cx quinquefasciatus* stayed elevated for a longer period, reaching a peak of 13.3 during July and declining to 11.5 in August and 4.9 in September.

Table 1. Mosquito infection rates [IR] in Kern County, 2006

Species	Pools	Total Tested	WNV Positive	IR/1000
Aedes melanimon	238	10514	2	0.20
Culex eythrothorax	113	5068	4	0.79
*Cx. quinquefasciatus**	745	26954	117	4.34
Cx. tarsalis	772	33650	94	2.79
Culiseta incidens	2	11	0	0
Cs. inorata	11	138	0	0
Total	1881	76335	217	3.14

Marked viral amplification was detected in the later part of June (Fig. 2) and by the end of the month there were a total of 14 positive mosquito pools, 411 positive free ranging birds (YTD total), and one human case. There was a sharp increase in virus activity in July associated with rising summer temperatures and this activity remained at elevated levels through August. Virus activity declined in September and was almost completely absent by October. Seroprevalence rates in free-ranging birds remained elevated with 90 positives in October and 28 positives in November.

A total of 89 chickens from 9 flocks seroconverted to WNV during the 2006 surveillance season (Table 2). The first chicken infections occurred before 3 July, with 18 chickens from five flocks being confirmed. These flocks were in Arvin, southeast of Bakersfield, at three separate sites in metropolitan Bakersfield, and at the Kern National Wildlife Refuge, northwest of Bakersfield. By the end of July the virus had spread to 8 of the 9 flocks generating 42 more seroconversions. August and September showed a decreased number of seroconversions with 16 and 13, respectively. There were no seroconversions detected in October or November, because there were no replacement chickens available. There was one chicken flock on the northern edge of Bakersfield that never seroconverted.

The year ended with 50 laboratory confirmed human cases and no deaths in 2006. Forty-three of these cases were located in or around the metropolitan Bakersfield Area. The rest were located in small agrarian communities on the valley floor (Table 2).

The year ended with four confirmed positive WNV equine cases and no fatalities for 2006. All four of these cases were in the metropolitan Bakersfield area. This decrease in positive cases most likely was due to increases in vaccination of the equine population in Kern County.

In 2006, 24 out of 187 dead birds tested positive for WNV (Table 2). The most frequently reported dead bird species were Western scrub jays and American crows, with seven and six testing positive, respectively. Other Species included American robins (3), House finches (2), House sparrows (2), Barn owl (1), Black-headed grosbeak (1), Common raven (1) and unknown species (1). Twenty-two of the 24 positive dead birds were found in or around metropolitan Bakersfield (Table 2) and one each was found in the cities of Delano and Wasco. Since the dead bird program relies on the public to find and report the dead birds, most of the dead birds were found in metropolitan Bakersfield. A sparse human population and large numbers of scavengers most likely reduced the effectiveness of the dead bird program in rural areas.

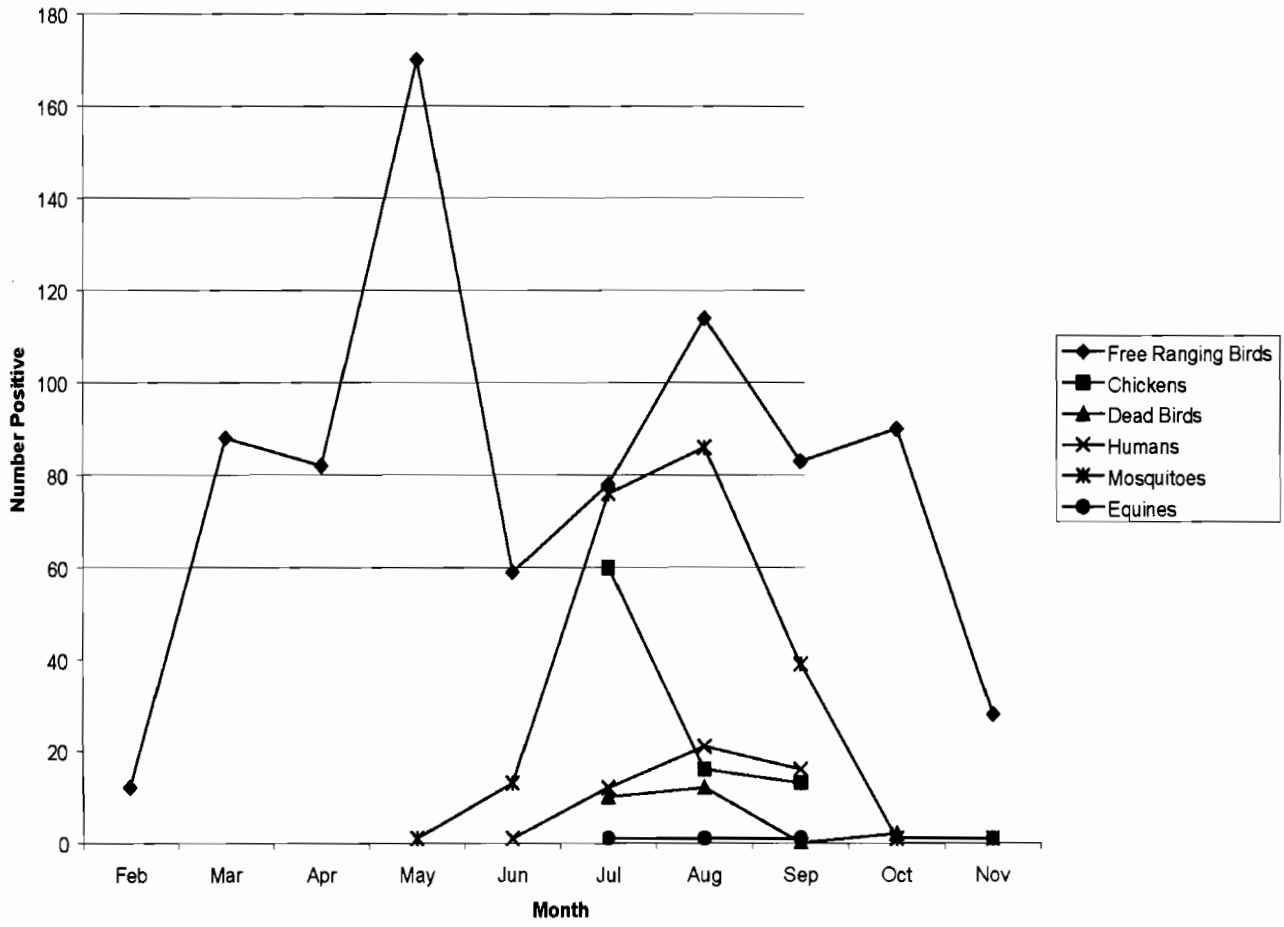

Figure 2. Positive Surveillance measures plotted as a function of month during 2006.

Table 2. Summary of positive surveillance results by cities within Kern County, 2006.

Arvin	17	25	1	0	0	14
Bakersfield	138	35	43	4	22	786
Buttonwillow	12	9	0	0	0	0
Delano	0	0	1	0	1	0
Lamont	6	0	1	0	0	0
Lost Hills	23	12	1	0	0	11
Shafter	18	0	1	0	0	0
Wasco	3	8	2	0	1	0
Total	217	89	50	4	24	811

The free-ranging bird seroprevalence program detected 811 EIA positives during 2006 that were represented by 15 species of birds (Table 3). Positivity rates ranged from 1% to 62%, depending upon species and residency status. As expected most of the transient migrants and winter residents had low seroprevalence rates compared to the year-round resident species.

DISCUSSION

Examining the seasonality of positive surveillance indicators revealed several patterns. First, the EIA positive free-ranging birds in February, March, and April most likely did not represent current transmission events and most likely were infected during 2005, because there were no other indications of virus activity during late winter and early spring 2006. Previous studies have shown that antibody in avian hosts is detectable for many months after the initial infection and therefore these birds could have been infected in the latter part of 2005. Second, as soon as mosquito pools became positive and increased in number, there was a concurrent increase in the number of chicken conversions, dead birds and human cases. This trend was similar during viral subsidence in late summer, i.e., decreased mosquito infection rates were followed closely by decreases in the remaining surveillance indicators. Third, there was a decrease in seroprevalence rates among wild birds in June and July, perhaps due to natural mortality among older immune birds and increases in the numbers of hatching year birds diluting the numbers with antibody.

Table 3. Species of free ranging birds testing positive for *Flavivirus*, Kern County, 2006.

Species Testing Positive	Total Tested	Total Positive	Percent Positive
Ash-throated flycatcher (Non-resident)	4	1	25.0
Bell's vireo (Non-Resident)	2	1	50.0
Bewick's wren (Resident)	11	2	18.2
Brown-headed cowbird (Resident)	70	4	5.7
California thrasher (Resident)	8	5	62.5
Loggerhead shrike (Resident)	5	1	20.0
Western kingbird (Non-Resident)	13	2	15.4
Golden-crowned sparrow (Non-Resident)	109	2	1.8
Song sparrow (Non-Resident)	119	4	3.4
White-crowned sparrow (Non-Resident)	959	12	1.3
Mourning dove (Semi-Resident)	298	117	39.3
California quail (Resident)	1026	446	43.5
House finch (Resident)	527	110	20.9
House sparrow (Resident)	443	18	4.1
Western scrub-jay (Resident)	150	86	57.3
Total	3744	811	21.7

There were differences in WNV activity between 2006 and previous virus active years, 2004 (Takahashi et al. 2005) and 2005 (Carroll et al. 2006). In 2004 WNV activity started in the southeastern corner of the valley and moved into Bakersfield and then to the west side of the valley. In 2005 WNV activity was appeared in the city of Bakersfield and then spread outward, finally affecting every surveillance site across the valley. In 2006 WNV was first detected in one mosquito pool in early May and then there was an absence of virus activity for six weeks until the third week of June when there were 13 mosquito positives from nine sites. These positives were spread all over the valley, from Arvin, southeast of Bakersfield to the Kern National Wildlife refuge, northwest of the refuge. It was not possible to determine if this apparent dispersal was the result of viral movement or delayed local amplification and detection related to temperature. Virus activity slowed in late September and finally subsided in October and November, when *Cx. tarsalis* entered diapause (Bellamy and Reeves 1963, Nelson 1964). By looking at the overall incidence and positivity rates, it appeared as if 2006 was similar to 2005 and 2004; however, the spatial and temporal patterns were quite different (Table 4). Although there were approximately the same number of human cases, sentinel chicken seroconversions, and mosquito pools positive all three years, the number of dead birds declined approximately 50% between 2005 and 2006 and the positive equine cases were reduced to almost zero. In addition to this, there was a large increase in seroprevalence among free ranging birds that may have initiated the decline of WNV activity in September. With almost all of these positives being resident species, host-seeking infectious mosquitoes likely had few antibody negative avian hosts to utilize in the transmission cycle. However, this carried over antibody or 'herd immunity' in the free-ranging birds did not seem to negatively impact on WNV activity in 2006. In summary, every surveillance site except one was affected by WNV in 2006. It will be very interesting to see where WNV reemerges in 2007 and if the continued high level of seroprevalence within the avian community will dampen vernal amplification.

Acknowledgments

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Table 4. Number of surveillance measures positive for WNV in Kern County, 2004 - 2006.

		2004	2005	2006
Human Cases		60	68	50
Sentinel chickens		101	121	89
Equine cases		46	26	4
Mosquito pools	Positive	214	235	217
	Total Tested	1367	1596	1868
Dead Birds	Positive	87	44	24
	Total Tested	159	240	187
Free ranging birds	Positive	157	412	811
	Total Tested	3400	3476	4036

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Avian Herd Immunity and WNV in Sacramento County

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ABSTRACT: West Nile Virus (WNV) was first detected in Sacramento County in the summer of 2004. To identify local avian reservoir species and to better understand the infection cycle in birds and mosquitoes, wild birds and *Culex* mosquitoes were sampled in rural and suburban habitats in Sacramento County during 2004 through 2006. Birds were captured according to Federal and State permit protocols using mist nets and various baited trap types. Each bird was banded, sampled, aged, and measured prior to release. Overall 32 species of birds in 17 families demonstrated antibody presence (Table 1). Initially (June 2004) antibody was detected in migrant summer resident birds of Sacramento County but has since become established within the local year-round resident bird population. The overall percent of antibody in these resident birds increased each year from 0.5% in 2004, to 2.1% in 2005 and 7.1% in 2006. The pattern of percent antibody increase was observed in WNV resistant as well as susceptible bird species. Two examples of resistant avian species include the Mourning dove and the Rock pigeon. Mourning dove antibody increased from 2.0% in 2005 to 38.0% in 2006 and Rock pigeon antibody increased from 10.7% in 2005 to 40.0% in 2006. The pattern in susceptible species such as the Western scrub-jay and House finch was similar. Western scrub-jay antibody increased from 2.6% in 2005 to 23.5% in 2006 and House finch antibody increased from 5.8% in 2005 to 10.6% in 2006. Noted in the House finch population was a shift in age profile of antibody presenting individuals. During the first year that WNV invaded Sacramento County (2004) all antibody positive House finches were Hatch-Year (HY) birds. In the epidemic year (2005), 77% of the antibody positive House finches were HY birds but in 2006 the age of antibody positive House finches shifted to 96% adult birds. The observed increase in avian antibody implies fewer susceptible and infectious birds to infect mosquitoes that lead to a fading of the epidemic. This was noted locally on the Stone Lakes National Wildlife Refuge and regionally in Sacramento County. For example House finches on the Refuge showed a 5.8% antibody while 3.6% of Refuge mosquito pools were WNV positive in 2005; House finch antibody increased to 10.6% while mosquito pools dropped to 1.4% in 2006. Regionally, in Sacramento County a similar pattern was observed. Overall avian antibody increased from 2.1% in 2005 to 7.1% in 2006 while positive mosquito pools decreased from 8.5% in 2005 to 1.4% in 2006. The concurrent increase in avian antibody and reduction in positive mosquito pools suggests that herd immunity provided a natural suppression of WNV transmission.

Table 1. Prevalence of antibodies against WNV in birds sampled in Sacramento County.

		Percent birds with antibodies to WNV (n)		
Common name	**Scientific name**	**2004**	**2005**	**2006**
Mallard	*Anas platyrhynchos*		1.4 (69)	7.5 (53)
Green heron	*Butorides virescens*			100 (1)
Rock pigeon	*Columba livia*	12.0 (109)	10.7 (178)	40 (93)
Mourning dove	*Zenaida macroura*		2.0 (15)	38.0 (71)
Sharp-shinned hawk	*Accipiter striatus*	50.0 (2)		
Cooper's hawk	*Accipiter cooperii*		25.0 (4)	100 (1)
Red-shouldered hawk	*Buteo lineatus*		75.0 (4)	
Red-tailed hawk	*Buteo jamaicensis*		100 (1)	
Western scrub-Jay	*Aphelocoma californica*	2.0 (51)	2.7 (37)	40.0 (15)
Brown-headed cowbird	*Molothrus ater*			5.4 (110)
Red-winged blackbird	*Agelaius phoeniceus*			1.7 (58)
Brewer's blackbird	*Euphagus cyanocephalus*			1.6 (60)
Lincoln's sparrow	*Melospiza lincolnii*		1.9 (53)	
Song sparrow	*Melospiza melodia*	0.5 (196)		3.4 (59)

>> Table 1. *(continued)*

		Percent birds with antibodies to WNV (n)		
Common name	**Scientific name**	**2004**	**2005**	**2006**
Black-headed grosbeak	*Pheucticus melanocephalus*		6.1 (66)	2.2 (44)
Cliff swallow	*Petrochelidon pyrrhonota*	2.4 (42)	1.0 (104)	1.6 (125)
Purple martin	*Progne subis*	10.0 (10)		
Black phoebe	*Sayornis nigricans*	1.1 (91)		
Wrentit	*Chamaea fasciata*			2.7 (74)
Ash-throated flycatcher	*Myiarchus cinerascens*	5.0 (20)		
Loggerhead shrike	*Lanius ludovicianus*			100 (1)
Northern mockingbird	*Mimus polyglottos*			50.0 (8)
American robin	*Turdus migratorius*			11.8 (17)
Ring-necked pheasant	*Phasianus colchicus*		17.0 (6)	
California quail	*Callipepla californica*		14.3 (7)	25.0 (4)
House finch	*Carpodacus mexicanus*	1.6 (317)	5.8 (468)	10.6 (292)
American goldfinch	*Carduelis tristis*	7.7 (13)		
Golden-crowned sparrow	*Zonotrichia atricapilla*	0.4 (220)	1.1 (281)	
White-crowned sparrow	*Zonotrichia leucophrys*		0.8 (127)	
Fox sparrow	*Passerella iliaca*	1.1 (91)		
Spotted towhee	*Pipilo maculatus*		0.7 (135)	
House sparrow	*Passer domesticus*		20.0 (5)	

Population Dynamics of *Culex tarsalis* in the Sacramento Valley of California

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ABSTRACT: *Culex tarsalis* is an important vector of arboviruses throughout the agricultural and urban areas of California. The association of this species with freshwater agricultural and palustrine breeding habitats has been described qualitatively, but quantification of the relationship between adult abundance and edaphic and meteorologic predictors is needed as a step toward development of a model to forecast mosquito abundance. In this study, Poisson regression was used to determine the association of *Cx. tarsalis* abundance with early-season predictors in the Sacramento Valley of California. Seasonally flooded agriculture and marshland were positively associated with *Cx. tarsalis* counts during the months in which they were flooded. Human population density was negatively associated with trap counts, probably because of the combined effects of competing light in urban areas and the preference of *Cx. tarsalis* for larval habitats normally found in rural areas. Low temperatures in January had positive and negative effects on trap counts during April and June, respectively, although there is some evidence from ongoing research over a longer time period that these associations are not universal within the geographic range of this study and might be the result of using a relatively short time series. Higher winter snowpack was associated with a delay in the *Cx. tarsalis* abundance peak probably caused by inundation of agricultural areas within floodwater channels and delayed rice planting during high-water years. Results from continued model development will be used eventually to forecast and map abundance of several mosquito species throughout the monitored areas of California.

INTRODUCTION

Culex tarsalis Coquillett is an important vector of arboviruses in California (Reeves 1990). Its abundance has been associated with activity of western equine encephalomyelitis and St. Louis encephalitis viruses (Olson et al. 1979), and more recently, *Cx. tarsalis* has been identified as a competent (Goddard et al. 2002, Reisen et al. 2005) and frequently infected (Hom et al. 2005, Reisen et al. 2004) vector of newly introduced West Nile virus. Although not the only factor affecting the transmission of arboviruses, vector abundance remains the most operationally measurable determinant of vectorial capacity.

Culex tarsalis breeds in standing freshwater, including large bodies such as agricultural sources or wildlife refuges (Bohart and Washino 1978), and annual abundance patterns for *Cx. tarsalis* vary among the ecological zones of California (Bohart and Washino 1978, Nelson 1971) and among sites within regions. A k-means clustering analysis for mean annual New Jersey light trap counts by half-month followed by mapping of the cluster means to which each site belonged revealed that the average annual pattern for each site was associated with land use surrounding the site (C.M. Barker, unpublished data). Sacramento Valley sites with sharp abundance peaks in July were in or near seasonally flooded agricultural areas, whereas sites with consistently low numbers of *Cx. tarsalis* were generally located in urban areas. Only three sites within this study region exhibited a late abundance peak during early fall, and all were adjacent to the Butte Sink duck clubs just north of the Sutter Buttes.

The objective of the current study was to test and quantify the associations of adult *Cx. tarsalis* female abundance with larval habitats and early-season meteorologic indicators over time as a step toward forecasting abundance prior to the annual virus transmission season.

MATERIALS AND METHODS

Study area and time period. The area of study was a 100-km x 50-km section of the Sacramento Valley in California (Fig. 1). This area includes several land use categories: seasonally flooded agriculture (rice fields), non-flooded agriculture (orchards and row crops), seasonally flooded marshes, and urban areas. New Jersey light traps (NJLTs) were operated by the Butte County, Colusa, Sacramento-Yolo, and Sutter-Yuba Mosquito and Vector Control Districts at 72 sites within the region during the study period from 1997-2000. This period was selected for this pilot study because it bracketed the 1997-1998 El Niño event which had been preceded by a warm, dry period during the spring of 1997 (Fig. 2). Mosquito abundance was measured by NJLTs collected weekly from April-October of each year. Collections were inconsistent during other months of the year and were excluded from analysis.

Mosquito counts. The mosquito collection data used in this study were obtained from historical archives maintained by the individual vector control agencies. Paper or electronic trap-by-trap mosquito collection records were obtained from each agency for the period from 1997-2000. For records that were in paper format, the collections were entered into a Microsoft Access 2000 database, and all records were imported into a Microsoft SQL Server 2000 database (Microsoft Corporation, Redmond, WA) prior to analysis. Total numbers of *Cx. tarsalis* females were divided by total numbers of trap-nights by month and site and multiplied by 30, resulting in a standardized number of mosquitoes per trap-month.

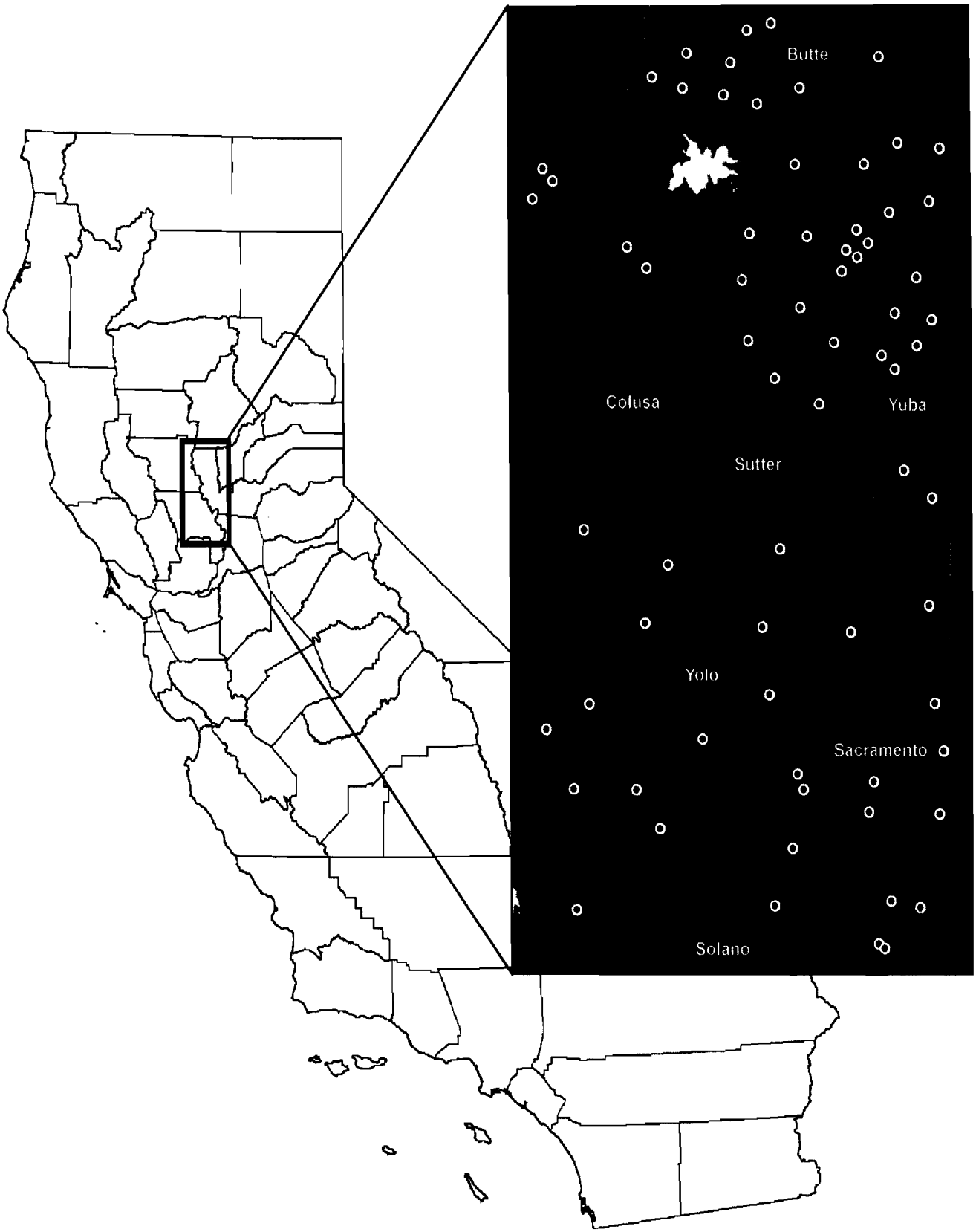

Fig. 1. Map showing the location of the study area in California and the locations of 72 New Jersey light traps operated between 1997 and 2000 (inset). County boundaries and names are indicated, and elevation is shown in grayscale.

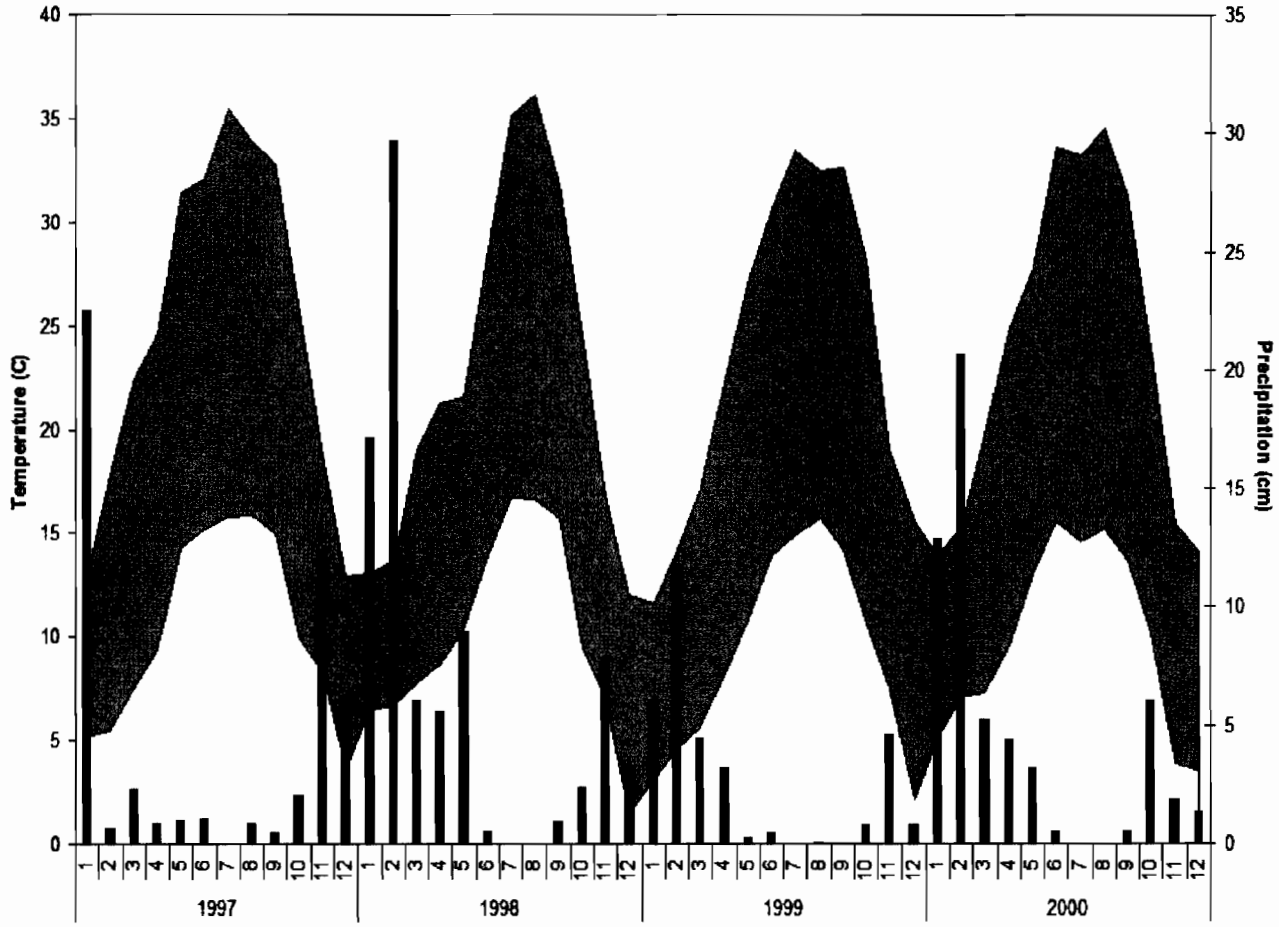

Fig. 2. Mean high and low temperatures (upper and lower bounds of the gray area) and total precipitation (black columns) by month for the 72 mosquito collection sites included in the study, 1997-2000.

Potential predictor variables. A summary of all potential predictors considered for inclusion in the models is presented in Table 1. Land cover and land use (LC/LU) data were extracted from the California Central Valley Wetlands and Riparian GIS dataset (California Department of Fish and Game 1997). The LC/LU classification in this dataset was based on a comparison of winter and summer Landsat Thematic Mapper satellite images acquired primarily from late 1992–1993, which allowed differentiation of seasonally flooded habitats from those flooded year-round. This was the most detailed LC/LU dataset available and was considered to be close enough to the study period (1997—2000) to be representative of the land uses during the trapping period. The proportion of the total area covered by each respective LCLU class was calculated within radii of 1, 2, ..., 10 km surrounding each trap site in ArcGIS 9.2 (Environmental Systems Research Institute (ESRI), Redlands, CA).

The human population data used in this study were published by the United States Census Bureau (USCB) for the 2000 census (http://www.census.gov). Land area values by census block were provided by personnel at the USCB. Using ArcGIS 9.2, these census and land area data were joined to USCB TIGER/Line shapefiles so that human population density in persons/km^2 could be mapped throughout California. After converting the shapefile to a 30-m resolution raster, the average population density was calculated within a 500-m buffer zone surrounding each trap site.

Mean monthly maximum and minimum temperatures and total precipitation were obtained as 4-km resolution grids from the PRISM group at Oregon State University (PRISM Group - Oregon State University 2006). Monthly values for each site were extracted from the 4-km grid data using ArcGIS 9.2. April 1 snow water equivalent at the Donner Summit station (Yuba River basin, Elevation: 2100m, Latitude: 39.310°N, Longitude: 120.338°W) in Placer County was included as an indicator of winter snowpack for the Central Valley (California Department of Water Resources 2006).

Data analysis. To test and quantify associations of mosquito counts with the potential predictors, a Bayesian Poisson regression model was fit to the monthly April-October trap counts for each year from 1997-2000. The model form was as follows:

$$\log(\square_{site,month}) = \square_0 + X_1\square_1 + \ldots + X_p\square_p + b_{site} + W_{site,month}$$

where $\square$ is the mean collection rate (mosquitoes per trap-month) for a given site and month, $\square_{1..,p}$ represent regression coefficients for each of the p fixed covariates, b is a sitewise conditional autoregressive (CAR) spatial random effect that allows the

Table 1. Summary of potential predictor variables for *Culex. tarsalis* collection rates.

Category	Variable	Description
Seasonal	Month	Dummy variables for each month (May-Oct) to flexibly account for seasonal changes in *Cx. tarsalis* abundance
Land use/ land cover	Seasonally flooded agriculture	Proportion of land within 2 km of the trap site that was occupied by flooded agriculture, typically rice
	Seasonally flooded marshes	Proportion of land within 2 km of the trap site that was occupied by marshlands and emergent vegetation
	Human population density	Average persons per km^2 within 500m of the trap site
Meteorological	Precipitation	Total precipitation (cm) by month, Jan-Apr
	Snowpack	Snow water equivalent on Apr 1 at Donner Summit
	Low temperatures	Average low temperature (°C) by month, Jan-Apr
	High temperatures	Average high temperature (°C) by month, Jan-Apr

estimated collection rate for each site to depend on those of neighboring sites (Banerjee et al. 2004), and W is a temporal random effect accounting for correlation of trap counts between months. A spatial neighborhood of 12.6 km was defined for the CAR spatial effect, based on the documented maximal *Cx. tarsalis* flight distance from a mark-release-recapture study in Kern County, CA (Reisen et al. 1992). Spatial weights were assigned as the inverse distance to neighboring traps to give closer traps greater influence on estimates than those farther away (Fig. 3). Models were fit with each of the potential predictor variables, and comparisons among models were based on significance of the regression coefficients and the Deviance Information Criterion (DIC, Spiegelhalter et al. 2002).

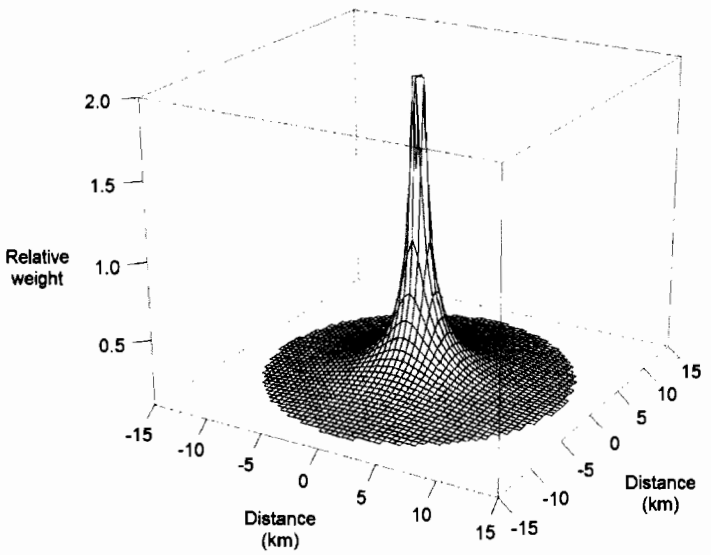

Fig. 3. Schematic showing the spatial weights (1/distance) for the conditional autoregressive prior within a 12.6-km radius of a given site.

RESULTS

Range of LC/LU influence on adult *Cx. tarsalis* abundance. To determine the range within which habitat has the greatest influence on adult female *Cx. tarsalis* abundance, models were fitted for seasonally flooded marshes summarized within each of 10 radii from 1,2,...,10 km. Radii from 1—6 km yielded very similar model fits, with a difference in DIC values < 1 between any two of the models. A radius of 2 km was chosen for all subsequent analyses because this was the maximum radius for which all sites had known LC/LU data. Radii > 2 km fell outside of the LC/LU layer for some sites and required imputation of the missing data.

Edaphic factors affecting *Cx. tarsalis* abundance. The LC/LU variables with the strongest association with *Cx. tarsalis* counts were seasonally flooded marshes, seasonally flooded agriculture, and human population density/km^2 (Fig. 4). The effect of marshland surrounding a site was limited to late summer and early fall, with approximately double or triple the number of *Cx. tarsalis* expected during September and October, respectively, for an area with 20% more of the surrounding area covered by marsh. Seasonally flooded agriculture had a consistently positive association with *Cx. tarsalis* abundance from May-September, with the strongest association occurring between June and August. Human population density was negatively associated with *Cx. tarsalis* counts, with collection rates in the most urban study sites expected to be approximately 25% of those in the most rural areas.

Meteorologic factors affecting *Cx. tarsalis* abundance. Models were fitted separately with mean monthly high and low temperatures and total precipitation during January—April, and April 1 snow water equivalent at Donner Summit as predictors of monthly *Cx. tarsalis* counts during the subsequent season. The counts were most strongly associated with January low temperatures and April 1 snowpack (Fig. 4). Warm January low temperatures were associated with an increase in April *Cx. tarsalis* abundance

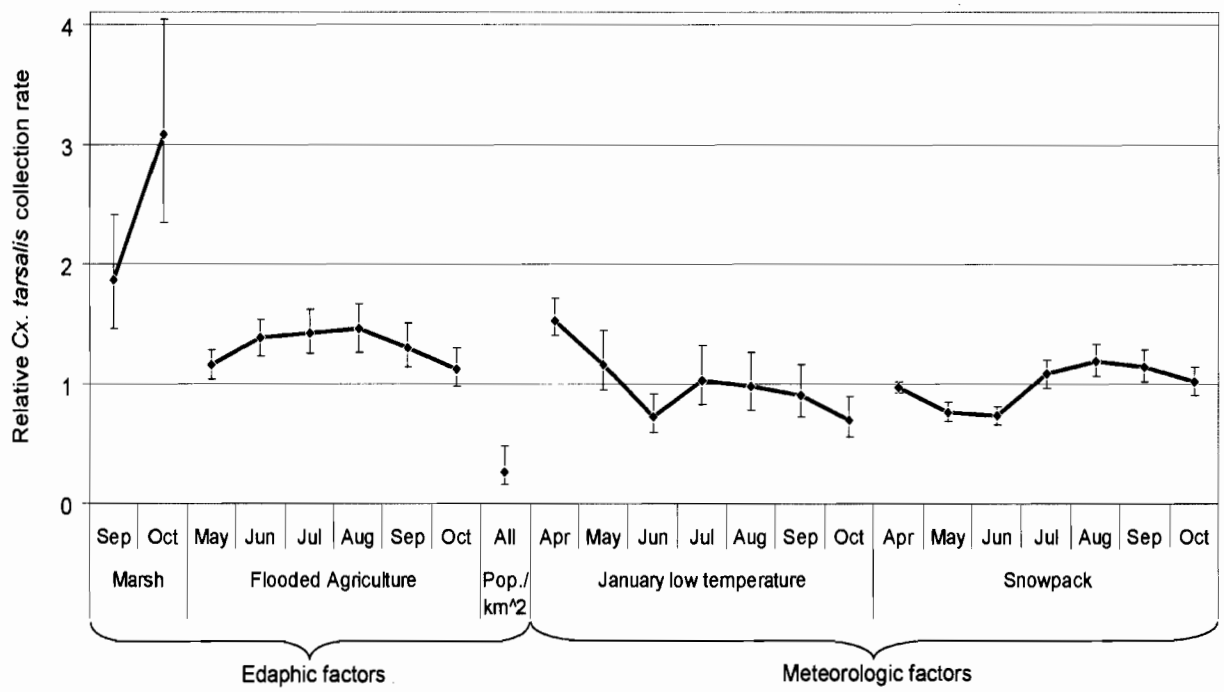

Fig. 4. Relative *Cx. tarsalis* collection rates and 95% probability intervals for each predictor variable and month, as applicable, from the final regression model. For seasonally flooded marsh and agriculture, rates are based on a change of 20% in the area covered by the respective land cover/land use class within 2 km of the trap sites. Rates for persons per mi^2 are based on a 4 standard deviation change in population density (i.e., very urban vs. very rural areas). Relative rates for temperature and snowpack are based on changes of 1°C and 10 cm, respectively. All relative rates represent a ratio of the rate for the higher category to the rate for the lower category, and intervals that exclude 1 are significant.

and a depression in counts during June, while increased April 1 snowpack was associated with fewer *Cx. tarsalis* during spring but greater numbers during late summer. Associations of *Cx. tarsalis* counts with other meteorologic variables were weaker, and in some cases, the other predictors were highly correlated with those presented here. Thus, they were not included in the final model.

Temporal dependence. Including random effects that accounted for correlations in mosquito counts over time permitted estimation of the strength of the connection between months during the collection season and over the winter from October through the start of the collection season the following April. Both measures of temporal association were significantly positive, and the month-to-month connection during the season was more than twice as strong as the over-winter connection.

DISCUSSION

This study agrees with previous studies (e.g., Bohart and Washino 1978, Reisen 1984, Reisen and Reeves 1990) showing that Cx.tarsalis adult female abundance patterns are closely associated with the agricultural and palustrine habitats in which this species breeds. The expected counts were greatest during periods coinciding with or immediately following peaks in the availability of aquatic breeding habitats.

Attraction of mosquitoes to NJLTs depends on the intensity of competing light from other sources (Barr 1963, Milby and Reeves 1989, Reisen et al. 1999), and human population density was included in the regression models as a surrogate for the level of background illumination. The negative association of *Cx. tarsalis* with human population density found in this study represents the combined effects of competing light and the preference of this species for freshwater breeding habitats typically found in rural areas.

The opposite associations of January low temperatures with April versus June, *Cx. tarsalis* abundance warrant further investigation. In particular, the positive effect of higher January temperatures on April *Cx. tarsalis* abundance is questionable and may be due to the use of a relatively short time period in this pilot study. Recent analyses on a broader temporal and spatial scale indicate that the association between January temperatures and April *Cx. tarsalis* abundance may be neutral--negative in the Sacramento Valley and that the effects of temperature and other predictors differ among ecological regions (C.M. Barker, unpublished data). These effects may be related to winter diapause in *Cx. tarsalis*, and they will be evaluated further in future studies.

High levels of snowpack are associated with a reduction in *Cx. tarsalis* during spring followed by an increase during summer. This seasonal shift in *Cx. tarsalis* abundance is the result of greater late-winter precipitation and snowmelt during high-snowpack years causing delayed schedules for planting and irrigating rice and other crops (Hill et al. 2006). During high-water years, leveed floodwater conveyance channels normally used for agriculture during spring are inundated with flowing water unsuitable for mosquito breeding, and planting of crops and associated creation of habitat for

immature mosquitoes are delayed until late spring when the precipitation and snowmelt waters have subsided (Yolo Bypass Working Group et al. 2001).

The random effects for connections in time indicated that higher-than-normal or lower-than-normal trap counts during a given month between April and September tend to carry forward into the following month. The slightly positive connection between October and the following April indicates that *Cx. tarsalis* abundance is not independent between years and knowledge of the size of the population going into diapause provides some indication of the abundance to be expected the following spring.

The model developed in this study will be expanded and modified as needed to include other mosquito species, time periods, and ecological regions throughout California with the goal of developing maps of predicted abundance of *Cx. tarsalis* and other species throughout California.

Acknowledgments

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Is Non-Viremic Transmission of West Nile virus by Culex Mosquitoes (Diptera: Culicidae) Non-Viremic?

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SUMMARY

Non-viremic transmission (NVT) of West Nile virus (*Flaviviridae, Flavivirus*, WNV) was demonstrated recently for mosquitoes using a *Culex quinquefasciatus* Say – laboratory mouse (*Mus musculus*) experimental model (Higgs et al. 2005). A summary of their experimental protocol is summarized in Figure 1. Blood samples from one experimental and several additional mice taken immediately after blood feeding lacked detectable virus, leading Higgs et al. (2005) to conclude that transmission was non-viremic; i.e., by their definition "before its propagation in the host and its appearance in the circulatory system." As we have generated artificial viremias in 2-day old chicks by direct injection of virus into the jugular vein (Mahmood et al. 2004), we felt that a low titered transient viremia could have been generated by highly competent mosquitoes that inoculate >10,000 plaque forming units (PFU) of virus during blood feeding. The purpose of our experiment was to confirm the recent results of Higgs' experiment using a natural *Culex* vector-WNV-avian host system by demonstrating intra- and interspecific transfer of WNV among co-feeding *Culex* and to assess the transient level of WNV in the vertebrate host circulatory system. Our protocol is summarized in Fig. 2.

In our study (Reisen et. al. 2006), inter- and intraspecific transfer of WNV occurred infrequently when donor *Culex tarsalis* Coquillett fed concurrently on House finches with recipient *Cx. quinquefasciatus* Say and *Cx. tarsalis*. Five out of 6 House finches had WNV in blood samples collected by jugular venipuncture 30 – 45 min post feeding, with titers ranging from 2.3 to 4.2 $\log_{10}$ PFU/ml. After 2 wks incubation at 26°C, 3 *Cx. quinquefasciatus* and 1 *Cx. tarsalis* of 230 blood fed recipients were infected, of which one *Cx. quinquefasciatus* was capable of transmission. Our data indicated that infectious female mosquitoes feeding on small vertebrates such as House finches create a nonpropagative viremia capable of infecting concurrently co-feeding females.

The non-propagative transfer of WNV through the circulatory system is dependent upon: 1) the quantity of virus expectorated by donor mosquitoes, 2) the blood volume of the vertebrate host, and 3) the oral susceptibility of the recipient mosquitoes. Our results suggest that females transferring >4 $\log_{10}$ PFU into a vertebrate host with a limited blood volume (here ca. 1 ml) produce a 'viremia' of sufficient titer to infect co-feeding females. The volume of virus expectorated by *Cx. tarsalis* from the cohort we used was assessed using a capillary tube assay (Aitken 1977) and was markedly variable

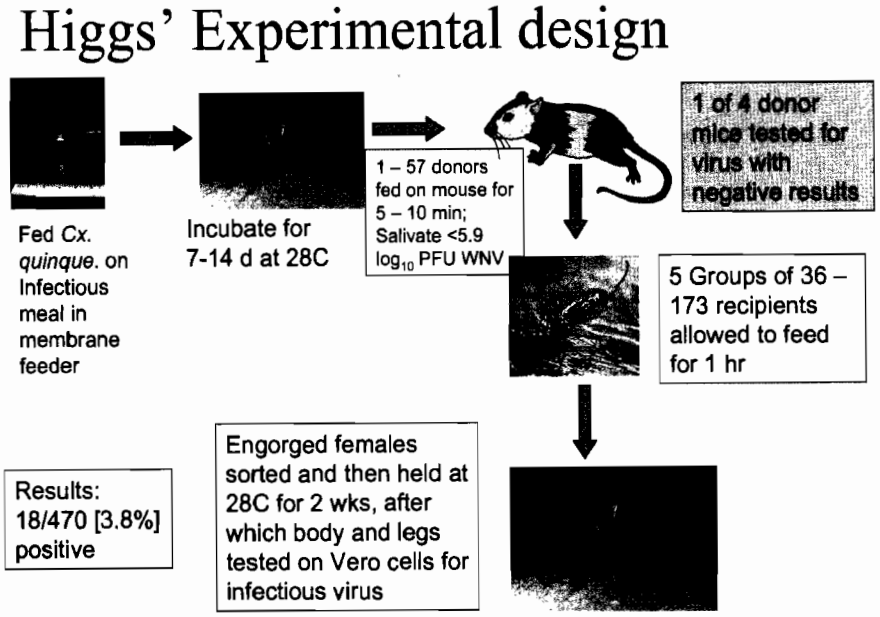

Figure 1.

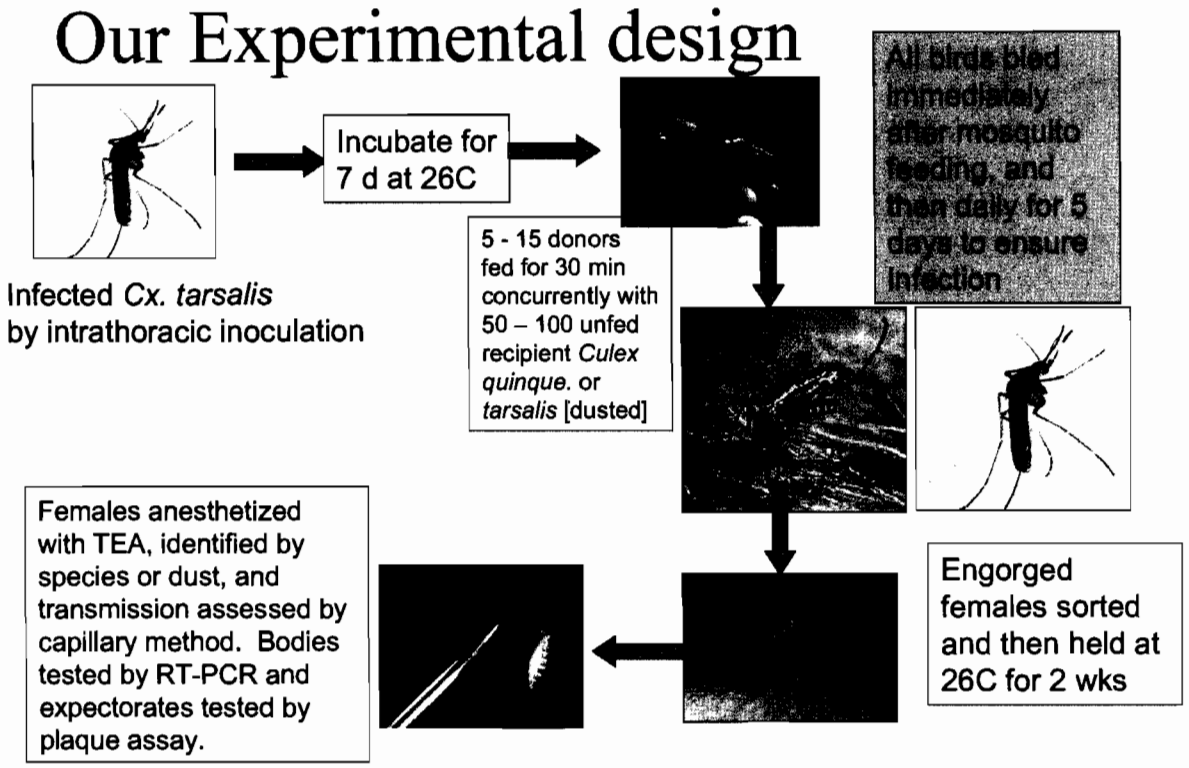

Figure 2.

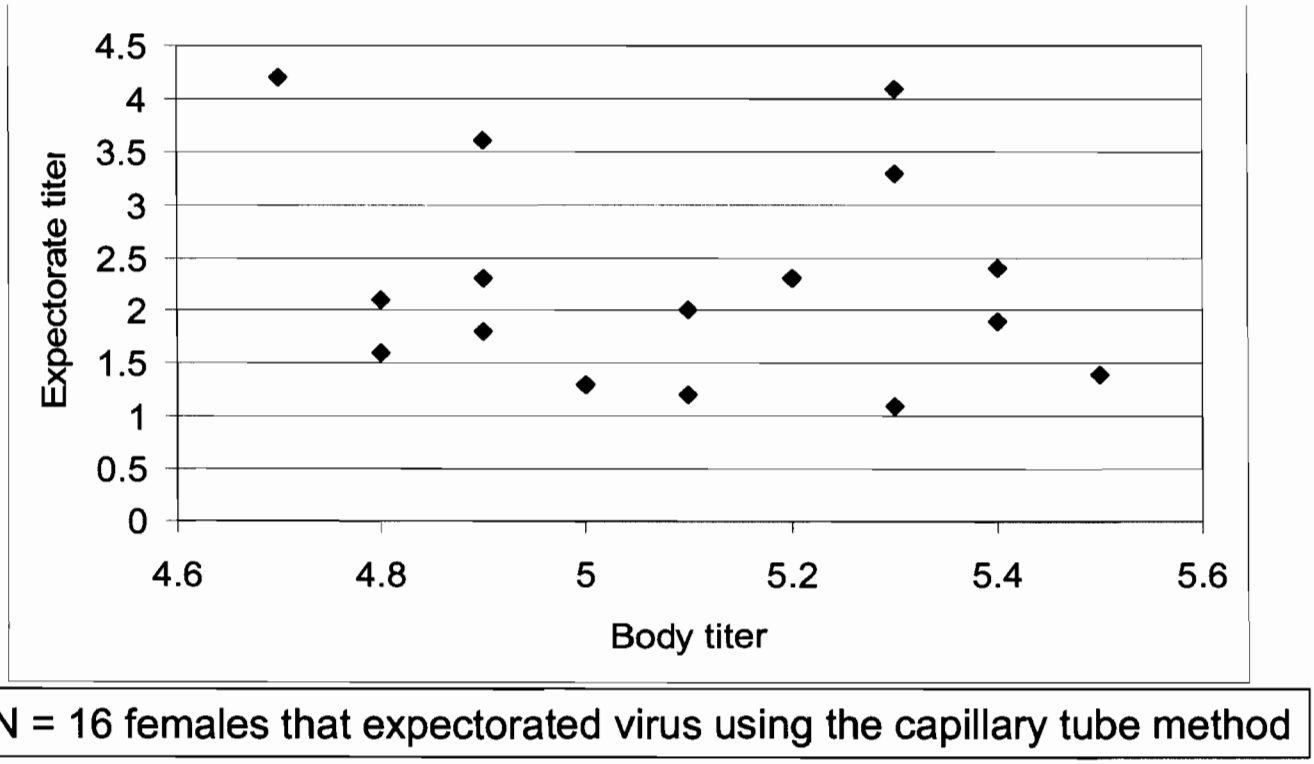

Figure 3. Titer of WNV in saliva samples plotted as a function of titer of WNV in whole mosquitoes in $\log_{10}$ PFU/mL.

(Fig. 3). The ability to infect recipient females is contingent upon the probability that the recipient female encounter sufficient virions within the blood meal to initiate a mesenteronal infection (Lord et al. 2005). Our mosquitoes apparently expectorated WNV directly into the circulatory system (rather than into the skin or pool feeding), and virus was detectible in the blood within 30 – 45 min after feeding.

If efficiency of recipient infection is related to the volume of virus in the vertebrate host circulatory system, then host blood volume is critical. Both the current and previous (Higgs et al. 2005) studies used small hosts (weight = ca. 20 – 25 g) with a total blood volume of ca. 1.0 – 1.5 ml. Smaller hosts such as passeriform nestlings have a smaller blood volume and potentially a higher concentration of virus, whereas progressively larger hosts such as American crows would circulate progressively less virus per ml of blood. The suggestion that large hosts such as horses that don't produce an elevated viremia or that have been vaccinated would now be important in WNV transmission (Higgs et al. 2005) obviously needs substantiation by further experimentation to assess the significance of both NVT and non-propagative transfer.

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Ixodes pacificus is Not a Competent Vector of West Nile Virus

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Ixodes pacificus Cooley & Kohls was evaluated as an experimental vector of West Nile virus (WNV) by testing the ability of this tick to become infected with the NY99 strain of WNV while feeding on viremic Song sparrows, to maintain the infection transstadially, and then to transmit WNV to recipient naïve Song sparrows or Western fence lizards during the nymphal stage. Our experimental protocol is summarized in Fig. 1. All Song sparrows initially inoculated subcutaneously with 130 plaque forming units (PFU) of WNV developed a viremia response that peaked at >7 $\log_{10}$ PFU/mL (Fig. 2A). The percentage of ticks positive for WNV RNA by RT-PCR decreased from 77% of 35 larvae (5 larvae per each of 7 Song sparrows) at day 6 after ticks were transferred to donor Song sparrows (day of detachment) to 23% of 35 nymphs at 59 days post-infestation (ca. 19 days after molting to the nymphal stage, but before blood fed on recipient hosts) (Fig. 2B,C). However, the percentage of ticks positive by RT-PCR from which infectious virus was recovered by Vero cell assay decreased from 59% on day 6 to 12% on day 59, even though there was no statistically significant decrease in the quantity of RNA estimated by Ct scores within positive ticks. Attempts to improve the sensitivity of these plaque assays by blind passage through C6/36 *Aedes albopictus* cell cultures were unsuccessful. These data indicated that ticks maintained viral RNA but not necessarily infectious virus over time.

Nymphs molting from larvae that fed on Song sparrows with peak viremias ranging from 7.2 – 8.5 $\log_{10}$ plaque forming units (PFU) per mL were used in transmission attempts (Fig. 2C). From 1 – 7 WNV RNA positive nymphal ticks engorged and detached from each of four recipient Song sparrows or Western fence lizards; however, subsequent blood samples from sparrows and lizards remained negative, indicating that transmission did not occur. An additional, 4 lizards inoculated with 1,500 PFU of WNV developed moderate viremias, ranging from 4.2 to 5.6 $\log_{10}$ PFU/mL. These and earlier data on Song sparrows indicated that the recipient host species were susceptible to WNV infection and should have become viremic if the nymphal ticks had transmitted WNV to them. Our data and previous negative studies with other hard tick species (Anderson et al. 2003) collectively indicated that ixodid ticks were not able to experimentally transmit WNV and therefore would not be important vectors in WNV transmission cycles.

Failure to detect transmission in the current and previous (Anderson et al. 2003) experiments indicated that ixodid ticks are not competent vectors. Interestingly, serosurveys have detected

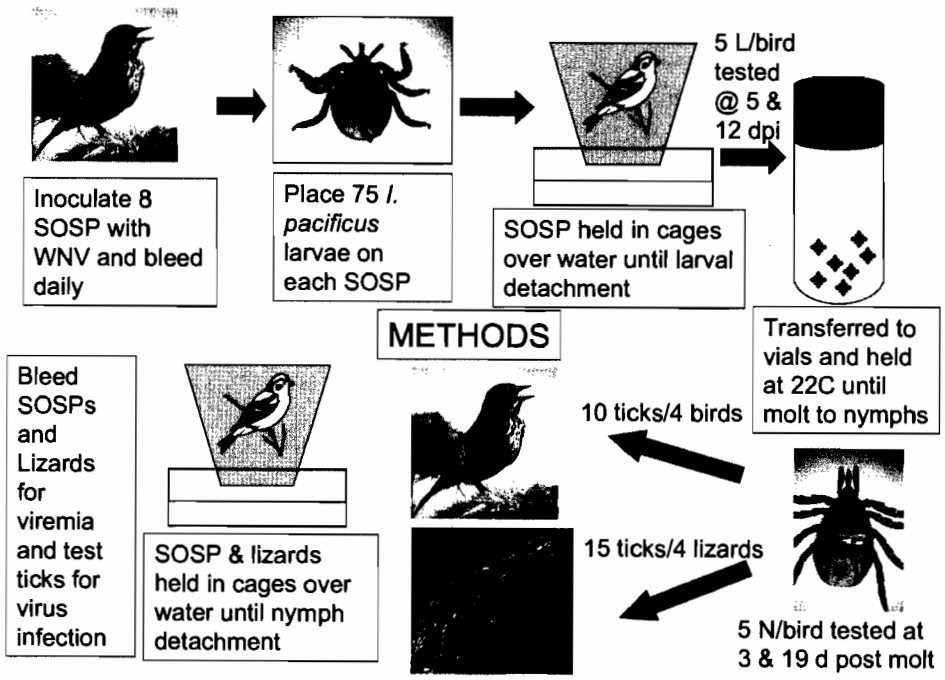

Figure 1. Experimental design to test the vector competence of *Ixodes pacificus* for WNV. L/bird or N/bird = larvae or nymphs tested per bird at 12 days post infestation [dpi]. SOSP = Song sparrow.

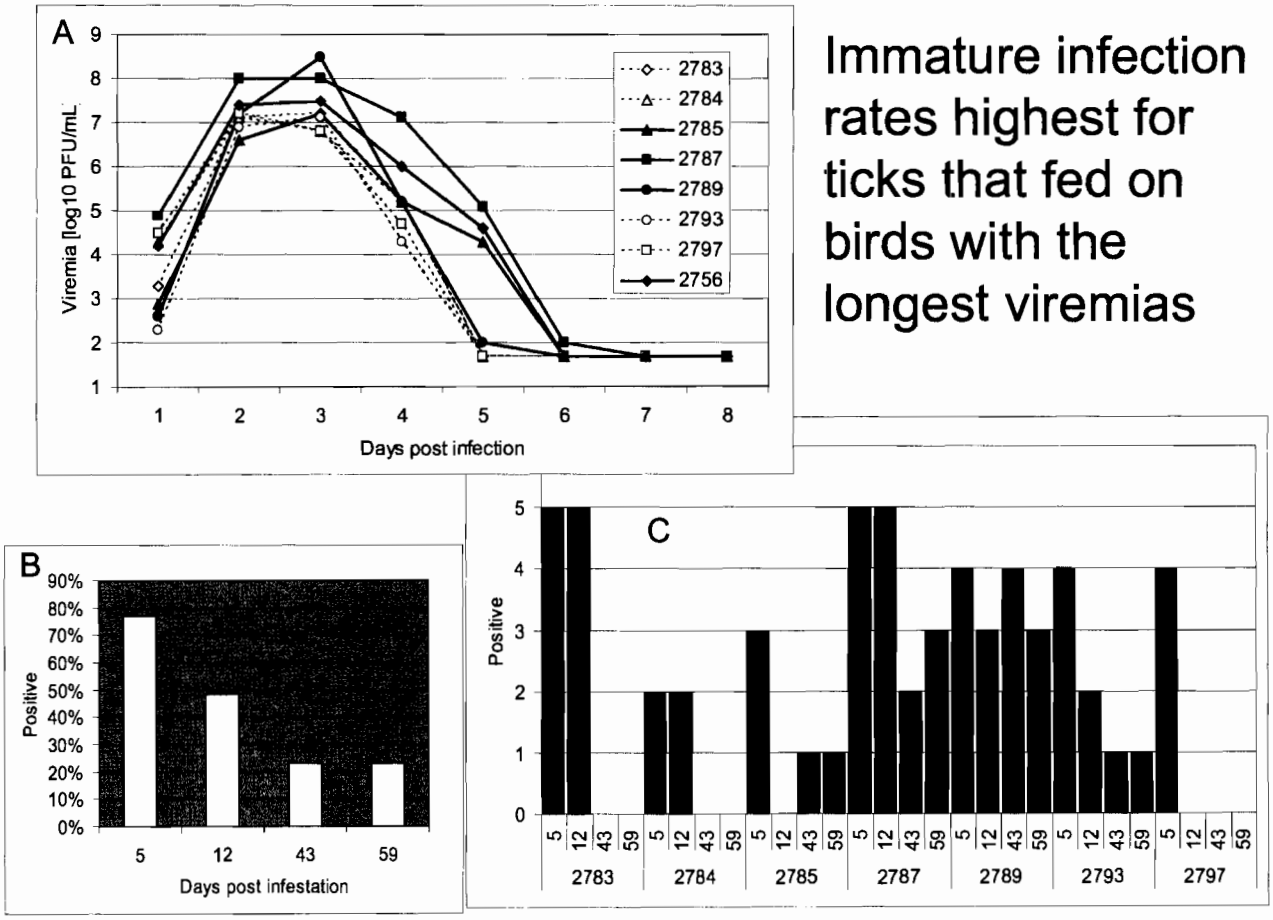

Figure 2. A) Viremia profile of Song sparrows inoculated with subcutaneously with 130 PFU of WNV; B) Percentage of 35 immature ticks positive for WNV RNA by RT-PCR; C) Number ticks positive for WNV RNA by RT-PCR on each Song sparrow on days 5 – 59 post infestation.

antibodies to WNV in a variety of small, medium and large mammals (Marfin et al. 2001; McLean et al. 2002; Komar 2003; Heinz-Taheny et al. 2004; Root et al. 2005; Dietrich et al. 2005; Farfan-Ale et al. 2006) leading several authors to propose a mammal-vector transmission cycle in parallel with the accepted bird-*Culex* maintenance and amplification cycle. Based on data available to date, ixodid ticks most likely are not the vector of WNV to these mammals.

Reptiles have been found positive for WNV or antibody in nature (Hayes 1989; Farfan-Ale et al. 2006) and have been found to produce modest or fairly elevated viremias following experimental infection (Klenk and Komar 2003; Klenk et al. 2004). The source of these infections remains obscure, because most *Culex* that feed on birds infrequently feed on poikilotherms (Tempelis 1975; Washino and Tempelis 1983; Apperson et al. 2002; Cupp et al. 2004; Apperson et al. 2004). Collectively, our data and those of others (Klenk and Komar 2003) strongly suggest that lizards probably are not an important component of WNV transmission cycles.

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Surveillance 2006: Overview, Changes and Improvements in Turnaround Time

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This presentation provides an overview of current molecular diagnostic procedures done at the Center for Vectorborne Diseases Arbovirus Research Unit [CVEC] in support of the California Encephalitis Virus Surveillance Program. Mosquito pools submitted to CVEC are processed through a protocol designed to ensure quality control, beginning with checking the mosquito pool numbers against data on the packing slips, entering data into the Surveillance Gateway, and continuing through the RNA extraction to final processing using the multiplex reverse transcriptase-polymerase chain reaction [RT-PCR]. Under the biosafety level 3 [BSL3] conditions, mosquito pools and tissue samples from dead birds are triturated during a Spex mixer mill, clarified by refrigerated centrifugation, and the RNA extracted using a robotic ABI 6700 unit or an ABI 6100 RNA preparation station. Mosquito pools are tested for West Nile virus [WNV], St Louis encephalitis [SLE] and western equine encephalomyelitis [WEE] viruses by multiplex RT-PCR, whereas bird tissues are tested only for WNV by a singleplex assay. Bird tissues were not tested for SLEV or WEE, because these viruses don't usually kill birds during acute infection. RT-PCR is performed using an ABI real time TaqMan platform.

Based on previous years' extensive confirmational testing, we abbreviated our testing and confirmation paradigm during 2006. Specimens which did not cross the critical cycle threshold (Ct) after 40 cycles were considered negative, whereas those with a Ct score <30 cycles were considered strongly positive. Both results were reported immediately without further testing. Specimens with Ct values >30 or <40 Ct were confirmed by singlex RT-PCR using conserved primer sets from the nonstructural region of the viral genome that generally were more specific but less sensitive. If positive by the second RT-PCR, these data were reported immediately. If negative, we then re-extracted the RNA from the original specimen and repeated the singleplex assay. If this was again positive, we considered the test confirmed and reported this specimen as positive. If negative by screening primer on this second attempt, the specimen was reported as negative. All results were entered and reported via the Surveillance Gateway, thereby precluding data re-entry and allowing autogenerated email notification to the submitting agency.

The abbreviated molecular testing paradigm and use of the Gateway has led to a significant improvement in turnaround time. During the 2006 arbovirus surveillance season 70% of mosquito pools were reported to the submitting agency within 48 hours or less; 83% were reported within 72 hours (Fig 1). Sample results reported after this time period were those few specimens with equivocal results that required retesting and/or re-extraction the following week. During the 2006 surveillance season, a total of 16,144 mosquito pools and 4,693 vertebrate tissues were tested. Of the 16,144 mosquito pools, 521 (3.2%) had a Ct score <30 and did not require confirmation. Of the 233 samples that had to be retested because the Ct score fell between 30 and 40, 156 (67%) were eventually confirmed and reported positive. These specimens presumably had lower amounts of virus and probably would have been missed by less sensitive assays such as VecTest. Of the 4,693 vertebrate tissues tested, 813 (17%) were positive and subjected to the same confirmational process as the mosquito pools.

The current system is very specific and sensitive and now has been refined to allow rapid throughput and reporting. However, this system will only detect those viruses for which the tests were developed. During 2007, we plan to expand our diagnostics to include plaque assays to capture endemic and potentially introduced arboviruses. Our ultimate plan is to improve our procedures to eventually be able to simultaneously test for these viruses using Luminex bead technology.

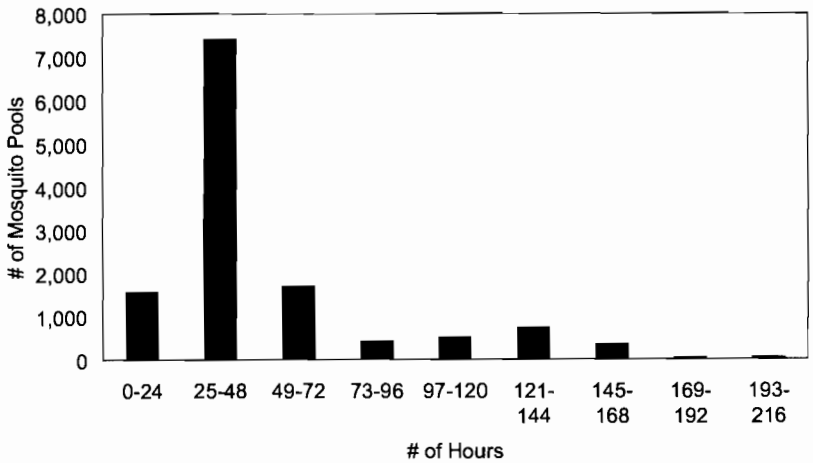

Figure 1. Number of mosquito pools plotted as a function of processing time in hours after receipt of the specimens at CVEC.

Proficiency Panels – Accuracy, Specificity, and Sensitivity Results With Implications for Risk Assessment

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ABSTRACT: The decentralization of West Nile virus [WNV] testing and the use of rapid, antigen-based assays has raised questions and concerns about the comparability and accuracy of data obtained in different laboratory settings. To facilitate a comparison, the Center for Vectorborne Diseases [CVEC] distributed a panel of ten-fold dilutions of killed NY-99 WNV to test the accuracy, specificity, and sensitivity of agency testing. CVEC reported detection sensitivity thresholds of 10 PFU [plaque forming units] for singleplex RT-PCR [reverse transcriptase-polymerase chain reaction]; 100 PFU for multiplex RT-PCR; 10,000 PFU for the RAMP test; and 100,000 PFU for the VecTest. These results were corroborated by 11 agencies reporting VecTest results, 9 agencies reporting RAMP results, and 4 of 6 agencies reporting RT-PCR results. The most variability in results was seen in the RT-PCR results and is most likely attributable to differences in RNA extraction systems and PCR platforms. To further assess the comparability of the rapid tests and RT-PCR assay, a parallel testing schedule was established with the Turlock Mosquito Abatement District where 1,042 mosquito pools were tested by both RAMP and RT-PCR. A 38% decrease in the detection of positive pools was found when RAMP was used over RT-PCR. The discrepancy created a doubling of the calculated MIR [minimum infection rate]/1,000 in the peak-season months of August and September when RT-PCR data was used. Using viral titer data from experimentally infected *Culex tarsalis* females held at five different temperatures and using the thresholds of detection for each of the testing modalities, it was predicted that RT-PCR would detect WNV within all mosquitoes at all temperatures by 4 days of ingestion of an infectious blood meal. Detection of viral antigen by RAMP would require an additional 2 days at a temperature of 22°C and 5 days at 18°C. The drastic differences in assay sensitivity and the effects of accurate detection on the assessment of risk make RT-PCR the most reliable method of WNV detection in the early season months and the most accurate tool for a timely agency response to pre-epidemic foci of viral amplification throughout the season.

INTRODUCTION

Recently several local agencies have initiated 'in house' testing for West Nile virus [WNV] using rapid antigen detection systems including RAMP [Response Biomedical Corp., Burnaby, BC Canada] and VecTest [Medical Analysis Systems, Inc., Camarillo, CA] as well as various kits developed for RNA extraction and RT-PCR using a variety of platforms. Decentralization of testing has raised questions concerning the comparability of results done by different persons in different laboratory settings. In 2005, the Center for Vectorborne Diseases Arbovirus Research Unit [CVEC] disseminated proficiency panels that consisted of several vials containing the same high titered concentration of killed virus to agencies doing RT-PCR. All agencies detected WNV, but this did not provide a quantitative assessment of laboratory capability needed to test mosquitoes and bird tissues. In 2006, CVEC repeated this evaluation using a 10-fold dilution series of killed NY-99 strain WNV to test the accuracy, specificity, and sensitivity of agency testing. The current paper summarizes results submitted for three assays by participating agencies and discusses the implications of assay sensitivity in surveillance. In addition we summarize the results of a field trial done in collaboration with Turlock Mosquito Abatement District [MAD].

PROFICIENCY PANEL

Methods: West Nile Virus, NY-99 strain, was grown in Vero cell culture, harvested, titrated by standard plaque assay, and frozen at -80°C. The virus was later diluted in CVEC's virus diluent to create a 10-fold dilution series starting at 6 $\log_{10}$PFU [plaque forming units]/0.1 ml and ending with 1 $\log_{10}$PFU/0.1 ml. A negative control consisting solely of the mosquito diluent was included in the panel. All of the dilutions and the control were inactivated by the addition of the detergent Triton X-100 at a concentration of 0.5%. Samples were then incubated overnight at 4°C, and subsequently tested negative by plaque assay for the presence of viable virus. The panel consisting of 7 number coded vials was tested blind at CVEC by VecTest, RAMP, and/or RT-PCR. Both singleplex and multiplex RT-PCR were performed on RNA extracted using the QIAmp Viral RNA Kit [Qiagen, Valencia, CA]. Samples then were aliquotted, number coded, frozen at -80°C, and then shipped on dry ice to requesting agencies where they were tested blind.

Results: CVEC reported thresholds of detection of 10 PFU for singleplex RT-PCR; 100 PFU for multiplex RT-PCR; 10,000 PFU for the RAMP test; and 100,000 PFU for the VecTest [Figure 1]. The multiplex RT-PCR assay was slightly less sensitive than the

1: CVEC RESULTS	Vial number and Test Results						
Test type							
log_{10}PFU/0.1 mL*							
VecTest [scored by 2 people]	0	0	0	0	1	2	3
RAMP [units]	0	0	0	27.8	276.2	640	640
RT-PCR [TaqMan Ct values]**	>40	36.9	33.3	30	26.7	20.6	15.5
RT-PCR [TaqMan Ct values]***	>40	>40	34.9	30.2	26.8	21.1	16.12

*Original virus dilution; virus inactivated by 0.5% Triton X-100 overnight incubation in 4°C
**QIAmp viral RNA kit, ABI 7900 TaqMan singlex for WNV
***QIAmp viral RNA kit, ABI 7900 TaqMan multiplex for WNV, WEEV, SLEV

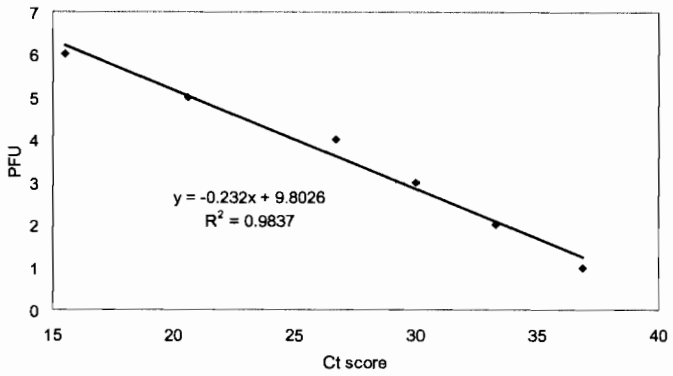


Figure 1: CVEC Proficiency Panel Results and regression of virus titer [log_{10} PFU/0.1 mL] as a function of RT-PCR Ct value.

singleplex RT-PCR assay, but this assay also tests for the presence of western equine encephalomyelitis and St. Louis encephalitis viruses and was more sensitive than either of the rapid antigen detection methods. Eleven agencies reported VecTest results, 9 reported RAMP test results, and 6 agencies reported RT-PCR results [Figure 2].

The Proficiency Panel results were specific, accurate, and consistent among agencies, but varied widely in sensitivity. VecTest results were very consistent with no positives being found in samples with less than or equal to 4 log_{10}PFU/0.1 mL. This test was also very specific but not very sensitive. The RAMP tests were similarly consistent; however, results for the 3 log_{10}PFU/0.1 mL

2: VecTest [n = 11 agencies]	7	1	3	5	6	4	2
log_{10}PFU/0.1 mL*	0	1	2	3	4	5	6
Mean	0.0	0.0	0.0	0.0	0.9	2.1	3.0
Max	0.0	0.0	0.5	0.0	2.0	3.0	3.0
Min	0.0	0.0	0.0	0.0	0.5	1.5	3.0

3: RAMP [n = 9 agencies]	7	1	3	5	6	4	2
log_{10}PFU/0.1 mL*	0	1	2	3	4	5	6
Mean	0.0	0.0	1.0	39.6	376.7	640.0	640.0
Max	0.0	0.0	3.2	64.2	640.0	640.0	640.0
Min	0.0	0.0	0.0	16.0	219.6	640.0	640.0

4:RT-PCR [n = 6]	7	1	3	5	6	4	2
log_{10}PFU/0.1 mL*	0	1	2	3	4	5	6
Mean	40.0	39.2	38.6	36.0	32.8	29.3	22.3
Max	40.0	40.0	40.0	40.0	40.0	33.8	25.9
Min	40.0	35.3	32.9	30.4	26.1	23.7	18.4

Problem results

Figure 2: RAMP, VecTest, and RT-PCR results for local agency testing of Proficiency Panel. Presented are VecTest results scored 0 – 3 for color intensity, RAMP units from the machine ranging from 0 – 640, and RT-PCR TaqMan Ct scores ranging from 0 – 40.

sample at the limit of sensitivity were problematic. The mean was 39.6, but ranged from 18.0 to 64.2 RAMP units. Depending upon the cut-off value (a positive being ≥30 units for mosquito pools or ≥50 units for corvid swab samples), there could have been quite a few false negatives detected by this method. Unexpectedly, the method with the most variability was the RT-PCR assay [Figure 3]. Four RT-PCR results were comparable to those obtained at CVEC, whereas others showed sensitivity below that of the RAMP test. Most likely, the wide range in results was due to differences in the RNA extraction protocols and/or PCR platforms used.

OPERATIONAL COMPARISON WITH THE TURLOCK MAD

Methods: To assess the comparability of the RAMP and RT-PCR assays in the detection of WNV in field mosquitoes, CVEC and the Turlock Mosquito and Abatement District [Turlock MAD] established a parallel testing program during the 2006 surveillance season. Pools were ground in CVEC diluent and an aliquot tested by RAMP via the Response Biomedical protocol. Remainder of the pool was sent to CVEC where 1.5 mL of CVEC mosquito diluent was added and the RNA extraction and RT-PCR performed.

Results: A total of 1,042 pools were tested in parallel [Figure 4]. Of these pools, 26 positive pools and 996 negative spools were in agreement after testing by both RAMP and RT-PCR. However, there were 16 RAMP negative pools that tested positive by RT-PCR and 4 RAMP positive pools that tested negative by RT-PCR. This showed a 38% loss in the detection of positive pools and a corresponding decrease in the calculated monthly MIR/1000. For the month of July, the calculated MIR/1000 was identical for samples tested by the two methods. However, in the peak-season month of August, the MIR changed from approximately 4.8 using RAMP results to 8.7 using RT-PCR results. This near-doubling of

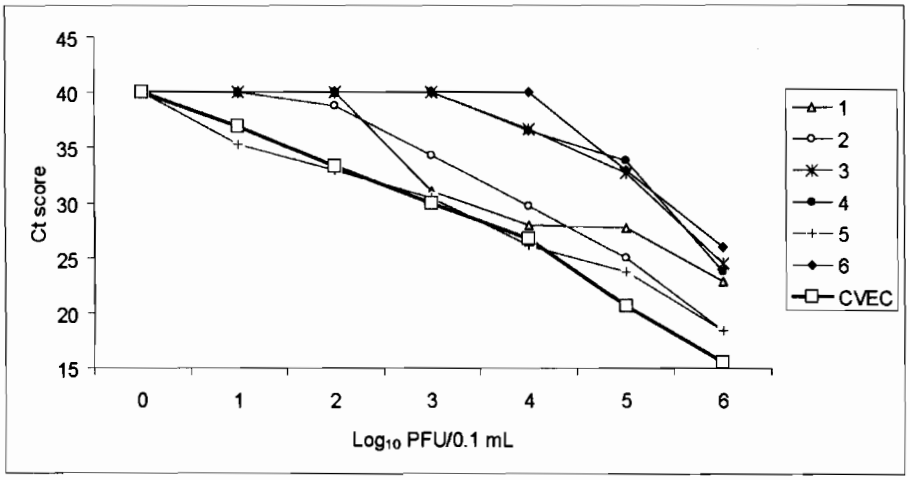
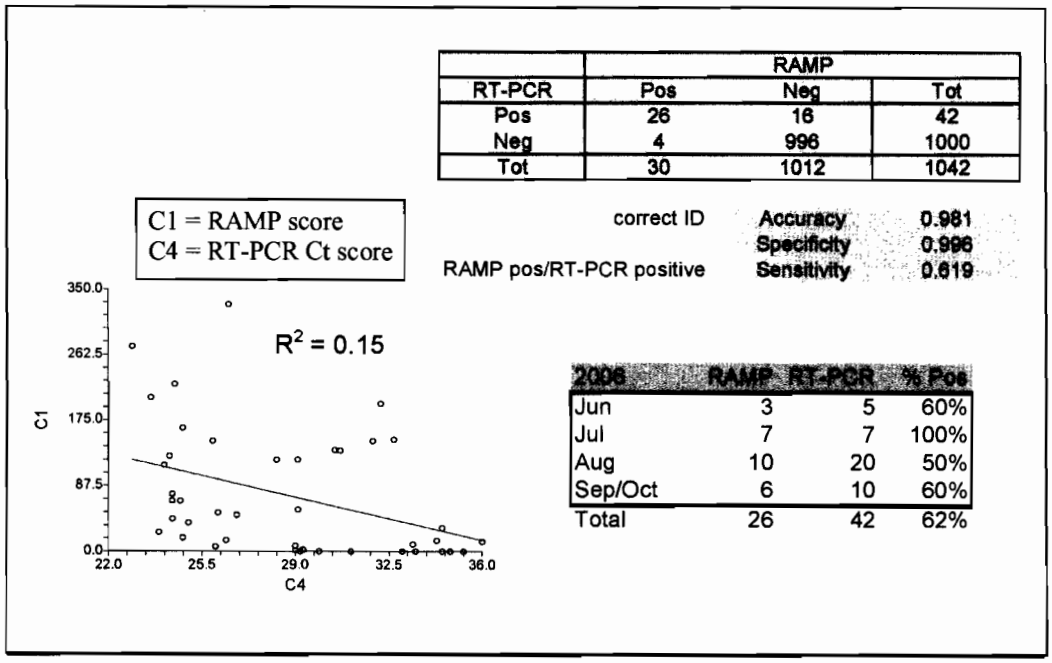


Figure 3: Singleplex RT-PCR results of Proficiency Panel testing from CVEC and 6 reporting agencies, Ct scores plotted as a function of virus titer in $\log_{10}$PFU/0.1 mL.



	RAMP		
RT-PCR	Pos	Neg	Tot
Pos	26	16	42
Neg	4	996	1000
Tot	30	1012	1042

correct ID	Accuracy	0.981
	Specificity	0.996
RAMP pos/RT-PCR positive	Sensitivity	0.619

2006	RAMP	RT-PCR	% Pos
Jun	3	5	60%
Jul	7	7	100%
Aug	10	20	50%
Sep/Oct	6	10	60%
Total	26	42	62%

Figure 4: Operational Comparison with the Turlock MAD, 2006, n = 1,042 mosquito pools tested by both RAMP and RT-PCR assays.

the calculated MIR/1000 was also observed in the month of September when the RAMP and RT-PCR results produced values of 1.0 and 2.1, respectively.

DETECTION SENSITIVITY: ANTIGEN VS. RNA

The assays in question all detected the presence of WNV but differed in what part of the virus was detected. The RAMP and VecTest assays detected the presence of specifically-recognized antigens. Antigens were comprised of protein or polysaccharide and detected according to their shape or conformation. A change in the antigen conformation may be introduced if the samples are heated or not refrigerated properly. As a result, improper storage of a sample may destroy the conformation of any antigen present, thus its detection by these modalities.

In contrast, the RT-PCR detects the presence of RNA which may remain stable for up to a week without maintenance of a cold-chain (Turrell et al. 2002). In a similar study, CVEC compared RT-PCR Ct [critical threshold] values for the Proficiency Panel heated at 56°C or held at 4°C for 30 minutes. Upon RNA extraction and amplification, the discrepancy in holding temperatures created a Ct differential of ≤0.8. This represents less than a one-third $\log_{10}$ PFU difference between the samples subjected to heat and those maintained within a cold-chain. These data suggests that the more sensitive RT-PCR assay may also confer the practical benefit, in comparison to the antigen detection methods, of allowing agencies to collect and ship mosquito pools at ambient temperatures, effectively reducing the cost and danger of shipping/handling copious amounts of dry ice.

IMPLICATIONS FOR RISK ASSESMENT

While the Proficiency Panel results for each of the testing modalities were specific, accurate, and consistent (with the exception of the aforementioned RT-PCR results) between CVEC and the reporting agencies, there still exists a significant discrepancy in sensitivity among the assays. This dramatic difference in sensitivity between the detection of antigen and that of RNA must be taken into consideration when assessing infection rates within mosquito populations [Figure 5]. Cool weather slows virus growth within infected mosquitoes such that more mosquitoes containing less virus will be collected, thereby delaying detection by less sensitive methods. As demonstrated in a 2006 field comparison with Turlock MAD, there was a 38% loss in the detection of WNV positive pools when the RAMP test was used instead of the RT-PCR assay. The decrease in detected positive pools decreased estimates of the MIR/1000, the level of risk for human infection, and therefore the accuracy and timeliness of agency response with mosquito abatement measures.

The growth of WNV within experimentally infected *Culex*

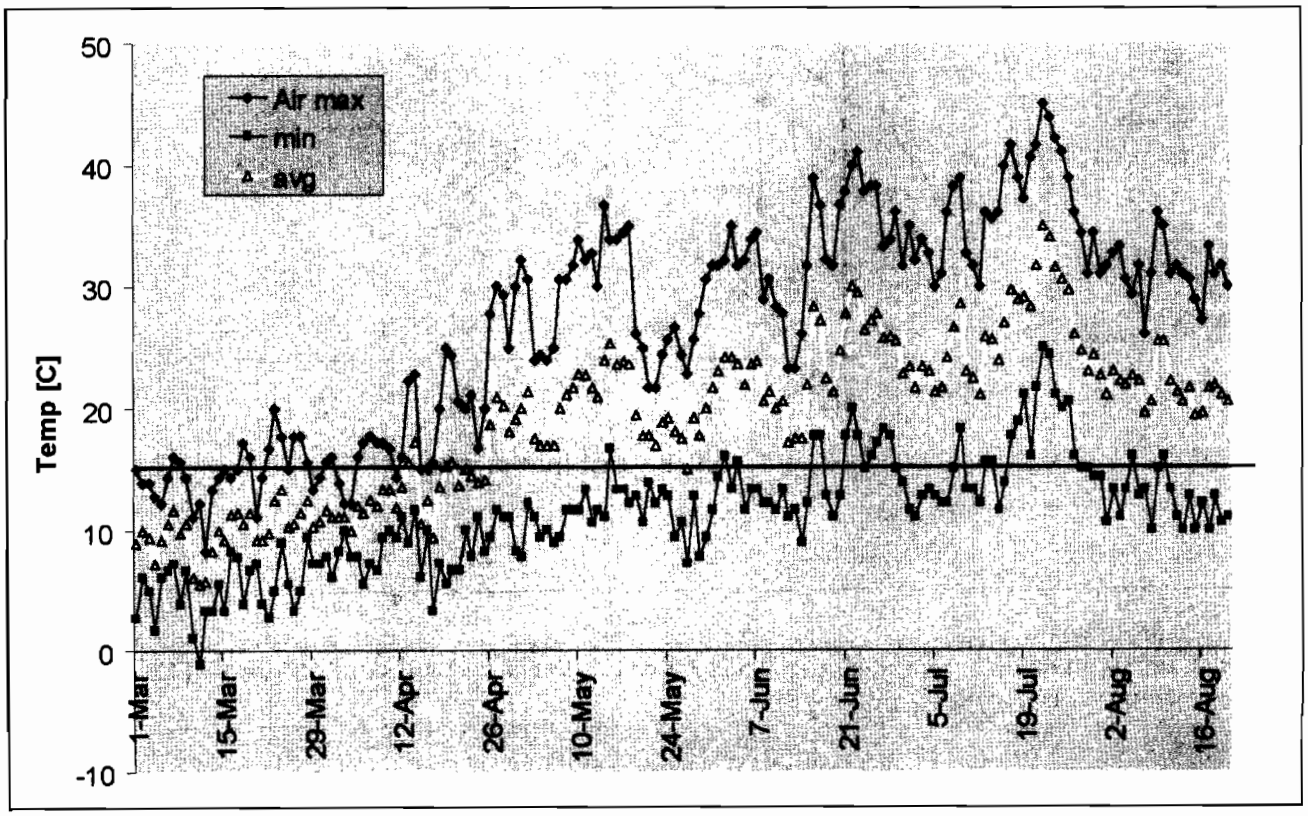


Figure 5: Maximum, minimum, and average air temperature at Davis, CA plotted with the WNV zero-growth threshold of 14.7°C.

tarsalis Coquillett females held at five different temperatures was measured in the laboratory [Figure 6]. Assay sensitivity thresholds in the figure show when infected females could be detected by the three assays. The RT-PCR would detect WNV within all mosquitoes at all temperatures by 4 days after their infectious blood meal or after the first gonotrophic cycle. It would take an additional 2 days at a constant temperature of 22°C for viral antigen to be detected by RAMP and an additional 5 days for detection at 18°C. By the time that WNV positive pools would be detected by RAMP, mosquitoes would already have acquired a transmissible WNV titer.

While the antigen-based rapid tests are quick and convenient with a fast turn-around time and no requirement of Biosafety Level 3 facilities, they lack sensitivity in the early detection of WNV at sub-transmissible levels. The multiplex RT-PCR assay can detect as little as 100 PFUs and therefore provides accurate, sensitive results, independent of a cold-chain, early in the surveillance season as well as being useful in detecting foci of viral amplification before they reach epidemic levels throughout the season.

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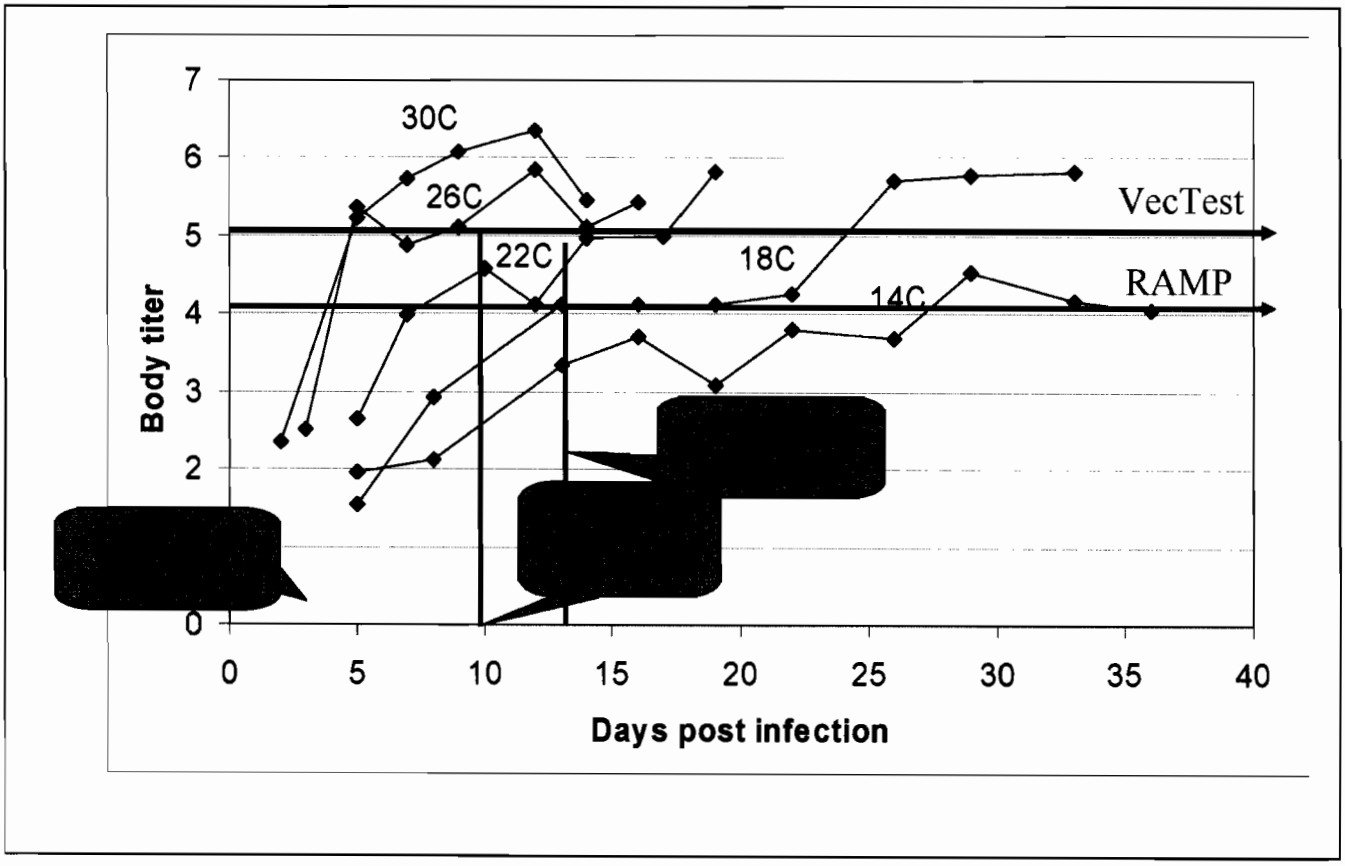

Figure 6: Quantity of WNV in $\log_{10}$ PFU in *Culex. tarsalis* females plotted as a function of days when held at 5 temperatures (Reisen et al. 2006). Shown as horizontal lines are limits for virus detection by VecTest, RAMP and RT-PCR assays [based on current proficiency panel data].

CalSurv: One-stop Shopping for Vectorborne Disease Surveillance

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For the 2007 vectorborne disease surveillance season an entirely new system for data reporting via the Internet has been implemented. The system is the California Vectorborne Disease Surveillance System or CalSurv for short. CalSurv is jointly managed by the Mosquito and Vector Control Association of California, representing more than 50 mosquito and vector control agencies in California, the California Department of Public Health, and the Environmental Assessment and Information Technology Program at the University of California, Davis.

The objectives of CalSurv are:

1. The integration of web-based reporting activities for vectorborne disease surveillance of California mosquito and vector control agencies, the California Department of Public Health, and the University of California,
2. To provide a single interface for access to all surveillance-related websites in California, and
3. To strengthen visibility of surveillance for vectorborne diseases other than mosquitoborne diseases e.g., plague, Lyme disease, relapsing fever, Hantavirus pulmonary syndrome and others (Fig. 1).

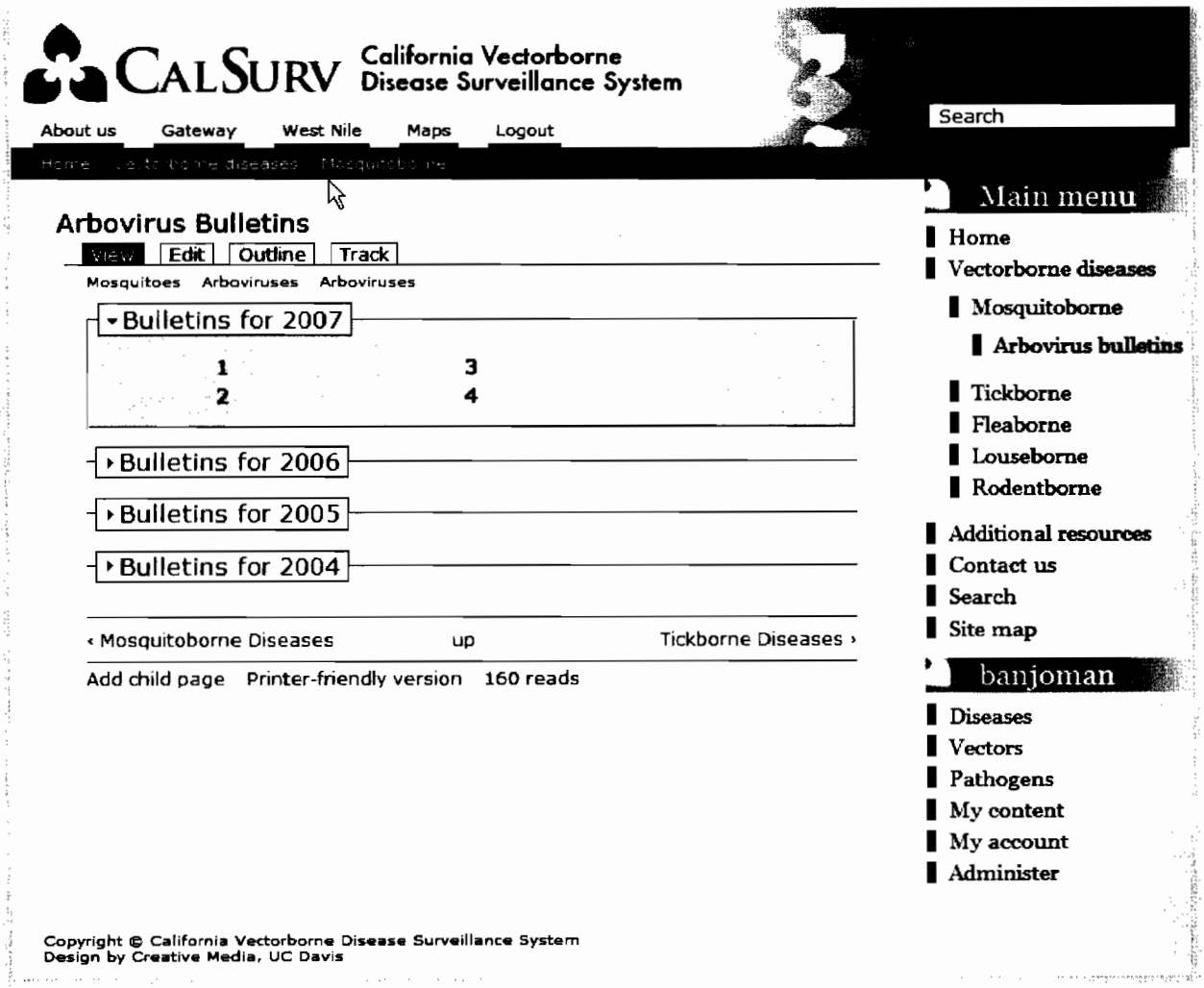

Figure 1. Example of a CalSurv website page.

CalSurv is based on a new technology known as a content management system. This system permits individual content managers from a variety of locations and agencies to manage content components over the Internet. For example, a biologist in Elk Grove may have responsibility for plague surveillance, another in Richmond responsibility for malaria surveillance. The CMS we are using is called Drupal. A content manager may incorporate graphics, tables, text, and other web components into his/her portion of the website, and control visual elements such as colors and fonts. If surveillance data are available in databases, interactive maps can also be incorporated and customized.

CalSurv replaces the former surveillance website <vector.ucdavis.edu>. The content at this site has been transferred to CalSurv, and users will be directed to CalSurv from this old address.

For 2007, there will be 3 surveillance websites, each with a different purpose and for a different user audience. CalSurv (The California Vectorborne Disease Surveillance website) will serve as the entry portal for all of these websites, although the other 3 also may be reached directly by users. CalSurv will encompass all vectorborne diseases, whereas the others will emphasize mosquitoborne viral diseases (Table 1).

Table 1. Surveillance websites for California vectorborne diseases.

Website	Purpose	URL
California Surveillance	Public and agency information on California vectorborne diseases	www.calsurv.org
Gateway	MAD management of mosquitoborne disease surveillance	gateway.calsurv.org
Surveillance maps	Direct access to all surveillance map resources	maps.calsurv.org
West Nile virus	Public and agency information on WN virus	westnile.ca.gov

The California Surveillance Gateway, designed and coded by Bborie Park, is now in its second year. This website is intended for the use of mosquito abatement agencies to manage their arbovirus surveillance programs. It provides many tools for data collection and forms management, and new tools for data input and analysis, such as calculation of mosquito viral infection rates, geocoding of addresses, bulk uploading of locally maintained surveillance data, calculation of mosquito abundance indices, and comparisons among mosquito species. It also provides interactive mapping for district surveillance data.

The California West Nile virus website has been completely re-designed by Ervic Aquino of the Vector-borne Disease Section of CDHS. This attractive site is designed primarily for the use of the media and the general public, but also provides considerable information on many aspects of West Nile virus for mosquito and vector control districts.

Interactive maps for displaying of surveillance data have continued to evolve. Last year, a combination of ArcIMS (Environmental Systems Research Institute, Redlands, CA) and Geocortex (Latitiude Geographics Group, Vancouver, BC) were used. This year, Google Maps and Google Earth are being explored as alternatives. Also under consideration is the use of animations for the visualization of surveillance events over time.

Although much has been accomplished over the past several years, improvements can be expected to occur which will provide efficient, rapid, and cost-effective means of surveillance data reporting and analysis.

Acknowledgements

The progress described in this report was made possible by the hard work and support of many agencies: the Environmental Assessment and Information Technology Program of the Center for Vectorborne Diseases, UC Davis; the Vector-borne Disease Section and the Viral and Rickettsial Disease Laboratory Branch, California Department of Health Services; and the Mosquito and Vector Control Association of California. Direct financial support was provided by grants from the National Oceanic and Atmospheric Administration (NOAA) and National Aeronautics and Space Administration (NASA). Integration of weather data was made possible by collaboration of the Scripps Institution of Oceanography, UC San Diego.

The continuing support and encouragement of many California mosquito and vector control agencies has helped immeasurably. Their investment of time and resources to make and process the actual surveillance collections is paramount. Finally, the financial support provided by a few individual abatement agencies to what was known as the Model Surveillance Program was of enormous significance. Without this support, CalSurv probably never would have been developed.

Invasion of California by West Nile Virus, Year 4 Summary and Predictions

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INTRODUCTION

Patterns of West Nile virus [WNV] transmission continue to vary markedly among our four study areas as well as throughout California. West Nile virus activity was detected first in California during 2006 at a narrow strip of marsh near the town of North Shore in Coachella Valley in southeastern California [see below]. Our attempts to identify the mechanism of persistence here continue to be unsuccessful, although several possibilities have been considered (Reisen et al. 2006). Other than warm vernal temperatures, the only unique attribute of this area is the consistent concentration of transient northbound Neotropical migrant birds during spring. During 2006, over 500 migrants were collected at the North Shore marsh site and tested for both virus infection and antibody with negative results. In addition, we reviewed our historical data on antibody samples taken from transient vernal migrants and found a similar absence of infection for western equine encephalomyelitis [WEE] and St Louis encephalitis [SLE] antibody. Collectively, these data indicate that Neotropical warblers as other associated transients seem to be rarely involved in encephalitis virus transmission cycles in either their summer or winter ranges and therefore may be a poor or, at best, an infrequent vehicle for the northward dissemination of viruses, even though they are competent hosts.

REGIONAL ECOLOGY

During 2006, WNV positive *Cx. tarsalis* Coquillett pools were detected in April [maximum likelihood infection rate (IR) = 5.56 per 1,000 *Culex tarsalis*] along the north shore of the Salton Sea. These early positive findings triggered an intensive control response by the Coachella Valley Mosquito and Vector Control District from April through June that eliminated virus activity in this area until August. In addition, the overall intensity of virus activity throughout the Coachella Valley was markedly reduced compared to 2005 and no human cases were detected. These data especially were promising, because it appeared that intensive early season control directed at a relatively small and confined overwintering refugia or amplification site successfully interrupted WNV transmission until late summer. During 2006 WNV continued to be transmitted at a low enzootic level throughout most of the Los Angeles basin, with the exception of the inland valleys to the NW. WNV was active here at a high level during 2005 (Wilson et al. 2006) that persisted through 2006. Elevated avian seroprevalence seems to be one factor limiting vernal amplification, especially at monitoring sites to the SE. In contrast, activity in Kern County during 2006 was remarkably similar to 2005 (Carroll et al. 2006), with an elevated human case incidence of 6.4 per 100,000. The overall seroprevalence rate in wild birds in Kern County during 2006 was 21% in all species, 57% in Western scrub-jays, the most important corvid species in and around Bakersfield, and 20% in House finches. Interestingly, some winter residents including Golden crowned sparrows [1.8%] and White crowned sparrows [1.3%] were seropositive, perhaps reflecting the continuation of transmission into the fall, when these species move south into Kern County. Avian antibody rates were also very high in the Sacramento-Yolo area during 2006, the year after the 2005 epidemic. Some interesting trends in House finch populations included a decline in relative abundance [birds/mist net hour] and an increase in seroprevalence, especially among after hatching year birds. Lower transmission during 2006 apparently resulted in lower seroprevalence rates among House finches collected from the same habitats sampled during 2005. Although not evident in Kern County, these data seemed to indicate that WNV subsidence was associated with increasing 'herd immunity' in peridomestic passerines.

Frequent infections were detected in several species of herons nesting communally within a Eucalyptus grove near Davis, Yolo County. Evidence of infection in nestling Black-crowned night herons included virus detection in tissues taken at necropsy and in blood samples from surviving chicks, and antibody positive sera collected from night herons and Snowy egrets. These data contrasted our findings at a similar colony in Imperial County where nesting over water at the Finney-Ramer Wildlife Refuge was associated with limited virus infection (Reisen et al. 2005). Further studies will be necessary to understand the importance of this nesting colony near Davis in local WNV amplification.

LABORATORY STUDIES

Research concurrent with our field studies has continued to examine possible transmission mechanisms that may have enabled the amplification and maintenance of WNV in California. Recently non-viremic transmission of WNV by *Culex* mosquitoes was demonstrated using a mouse system (Higgs et al. 2005). Inter- and intra-specific non-viremic transmission of WNV between *Culex*

tarsalis and *Cx. quinquefasciatus* Say and *Cx. tarsalis* was demonstrated using *Cx. tarsalis* as donor and House finches as blood meal hosts. Unexpectedly, all House finches fed upon by one or more infectious *Cx. tarsalis* became viremic within 30-45 min of donor feeding, with titers as high as 4.3 $\log_{10}$ PFU (plaque forming units)/ml. These data indicated that some virus was expectorated directly into the circulatory system and produced an immediate non-propagative viremia, similar to the artificial viremias described earlier (Weaver et al. 1991, Mahmood et al. 2004). Therefore, blood volume would seem critical and non-propagative transmission probably restricted to small hosts with a small blood volume to dilute the virus released by the blood-feeding donor mosquito.

During field serosurveys, mammals frequently have been found positive leading some researchers to consider the possible role of Ixodid ticks in transmission (Komar 2000, Bentler et al. 2007). Although Ixodid ticks were capable of transstadial transmission, infected nymphs did not transmit the virus to recipient hamsters or mice (Anderson et al. 2003). We had similar results shown for *Ixodes pacificus*. Although larval ticks became infected feeding on viremic Song sparrows and transtadial transmission to the nymphal stage was documented frequently, these infected nymphs did not transmit WNV to recipient Song sparrow or Western fence lizard hosts. Collectively, these data indicate that ixodid ticks probably are not important in WNV transmission and that mammalian infection is due to transmission by *Culex* mosquitoes.

SURVEILLANCE AND RISK

Monitoring mosquito abundance through a carefully designed trapping program provides a rapid measure of mosquito population size, potential frequency of vertebrate host contact and therefore the risk of pathogen dissemination. In a preliminary study, the abundance of *Cx. tarsalis* during summers from 1997-2000 in the Sacramento Valley was related to remotely sensed landscape features and antecedent measures of temperature, rainfall and snow pack at Donner summit. Seasonally flooded agriculture and marshland were positively associated with *Cx. tarsalis* counts during the months in which they were flooded. Human population density was negatively associated with trap counts, probably because of the combined effects of competing light in urban areas and the preference of *Cx. tarsalis* for larval habitats normally found in rural areas. January low temperatures had positive and negative effects on trap counts during April and June, respectively, although there is some evidence from ongoing research over a longer time period that these associations are not universal and might be the result of using a relatively short term data series. Higher winter snowpack was associated with a delay in the *Cx. tarsalis* abundance peak and probably was caused by inundation of agricultural areas within floodwater channels and delayed rice planting during high-water years. Planned model development will forecast and map abundance of several mosquito species throughout California.

The current diagnostic paradigm and the molecular methods used to test mosquito pools and avian tissues at the Center for Vectorborne Diseases [CVEC] were reviewed. Quality controls and assay comparisons have now allowed us to limit confirmations and retesting to problematic samples with high critical threshold values [Ct scores indicating low amounts of virus]. In combination with new web-based data management using the Surveillance Gateway, turn-around-time has been reduced markedly, with 70% of the test results now reported to the submitting agency within 48 h of specimen receipt at CVEC. Specimens taking longer were either received late in the week such as Friday or produced problematic results requiring re-extraction of the RNA and retesting to resolve infection status during the following week.

The development and dissemination of antigen-based assays such as RAMP and VecTest have stimulated some local agencies to test their own mosquito pools and/or oral swabs from corvids instead of submitting specimens to CVEC. In addition, six local agencies have indicated they may begin using singleplex RT-PCR to test for WNV in birds and mosquitoes using a variety of RNA and RT-PCR systems and PCR platforms. During 2006 a proficiency panel consisting of a 10-fold dilution series of killed WNV and a negative control was distributed to participating agencies. RAMP [n = 9] and VecTest [n = 11] produced consistent results that were specific and accurate, but were less sensitive than RT-PCR. Unexpectedly, RT-PCR results varied markedly among agencies [n = 6]; three agencies were in good agreement with CVEC results, whereas three were far less sensitive. Variation here was related to differences in test reagents and testing platforms, but varied considerably among agencies. An operational comparison of pools collected by Turlock MAD and processed using both RAMP and RT-PCR, indicated a 39% loss in sensitivity and an underestimate of monthly infection rates using RAMP data. Cool temperatures as experienced during spring would slow virus growth in the mosquito and delay detection of viral infection by antigen assays compared to RT-PCR that can detect virus after a single gonotrophic cycle. Clearly the use of different assays with varying levels of sensitivity precludes the direct comparison of results among neighboring agencies. Decentralization of testing and the use of different assay systems by different agencies has become problematic for cohesive state-wide risk assessment, especially during the critical vernal amplification period.

For the 2007 surveillance season a new content management system for data reporting via the Internet and information exchange has been implemented. The California Vectorborne Disease Surveillance System, or CalSurv for short, is jointly managed by the California Mosquito and Vector Control Association, the California Department of Health Services, and the Environmental Assessment and Information Technology Program at CVEC. The objectives of CalSurv include: the integration of web-based reporting activities for vectorborne disease surveillance of California mosquito and vector control districts, the California Department of Health Services, and the University of California; providing a single interface for access to all surveillance-related websites in California; and to strengthen visibility of surveillance for vectorborne diseases other than mosquitoborne diseases such as plague. The ultimate goal is to have a single place to find information on the current status of all vectorborne diseases in California.

2007 AND BEYOND

Based on our research and the collective experience of California agencies with WNV over the past 4 years, three major factors have emerged as being related to the occurrence of WNV outbreaks: vector abundance, peridomestic passerine herd immunity, and climate anomalies such as increased temperature or rainfall. As WNV becomes endemic, *Cx. tarsalis* and *Cx. pipiens* complex mosquitoes remain responsible for most maintenance, amplification and tangential transmission in rural and urban landscapes, respectively, with few other species naturally infected. Other species found with high infection rates such as *Cx. stigmatosoma* and *Cx. thriambus* are primarily ornithophagic and focally distributed thereby limiting their importance in statewide epidemic transmission.

Most likely the ongoing WNV epidemic will continue through 2007, with few horse cases because of widespread immunization but with continued moderate numbers of human cases throughout the state. Some epidemiological thoughts include:

Intense focal enzootic transmission in peridomestic landscapes with high mosquito infection rates will continue to be associated with very low mosquito abundance levels, perhaps increasing the importance using of sentinel chickens or other birds to track WNV.

Focal outbreaks may occur during periods of hot weather as WNV tracks pockets of largely non-immune avian populations. Incidence of human disease within these outbreaks may be elevated, especially in rural communities, but the overall state incidence will remain low.

Statewide, periurban corvid epiornitics may continue to subside due to depopulation and associated increases in peridomestic passerine herd immunity, thereby decreasing the value of dead bird reports and increasing the need for bird necropsy and testing to ensure that clusters of bird death can be associated with WNV amplification. Reduced reporting and death associated with WNV may compromise the current DYCAST paradigm

A warm, dry winter, continued evidence of horizontal transmission and dwindling avian immunity in southern California and eastern Los Angeles may lead to the resurgence of human cases.

An increasing global population, frequent and rapid travel, and increased globalization of commerce set the stage for the rapid transport of infectious agents, including those transmitted by vectors. Therefore, WNV probably will not be the last arbovirus to invade California. However, current surveillance diagnostics focus specifically on this virus and will not detect other agents. In 2007 testing of mosquito pools collected at probable portals of entry will be extended to detect additional arboviruses using a combination of cell culture and molecular methods. This is the beginning of an effort to expand and enhance surveillance to also examine the genetic structure of endemic or emerging viruses to assess their epidemic potential. Studies on the vector competence of California mosquitoes for emerging viruses such as Chikungunya that have a strong potential for introduction will be included as part of a plan to prepare California for the next introduction.

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Surveillance for Mosquito-Borne Encephalitis Virus Activity in California, 2006

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The California arbovirus surveillance program is a cooperative effort of the Mosquito and Vector Control Association of California (MVCAC), local mosquito and vector control agencies, the Center for Vectorborne Diseases (CVEC), School of Veterinary Medicine, University of California at Davis and the California Animal Health and Food Safety Laboratory (CAHFS), the California Department of Food and Agriculture (CDFA), the California Department of Health Services' (CDHS) Division of Communicable Disease Control, including the Vector-Borne Disease Section (VBDS), the Veterinary Public Health Section (VPHS), and the Viral and Rickettsial Disease Laboratory (VRDL), county and local public health departments, physicians and veterinarians throughout California.

In 2006, the surveillance program elements included the following:

1. Diagnostic testing of specimens from human patients exhibiting symptoms of encephalitis, aseptic meningitis, acute flaccid paralysis, or with unexplained febrile illness of more than 7 days;
2. Diagnostic testing of specimens from horses exhibiting clinical signs of viral neurologic disease compatible with western equine encephalomyelitis virus (WEE), West Nile virus (WNV), and other arboviruses as appropriate;
3. Monitoring mosquito abundance and testing for the presence of St. Louis encephalitis virus (SLE), WEE, and WNV; testing for other arboviruses, as appropriate;
4. Serological monitoring of sentinel chickens for SLE, WEE, and WNV antibodies;
5. Surveillance and diagnostic testing of dead birds, especially crows and other birds in the family Corvidae, and tree squirrels for infection with WNV;
6. Weekly reporting in the CDHS Arbovirus Surveillance Bulletin of arbovirus test results in California and arbovirus activity throughout the United States;
7. Posting of WNV information twice weekly on the California WNV website: www.westnile.ca.gov, and the California Vectorborne Disease Surveillance System: http://vector.ucdavis.edu/arbo.html, including test results, reports, maps, and public education materials;
8. Identifying reported dead bird clusters using the WNV Dynamic Continuous- Area Space-Time (DYCAST) model to identify areas of peak WNV activity;
9. Data management and reporting through the California Surveillance Gateway, a web application used by local agencies, CDHS, CVEC, and VRDL.

A summary of West Nile virus positive surveillance elements by county is in Table 1.

HUMAN DISEASE SURVEILLANCE

In 2002, VRDL initiated a regional public health laboratory network consisting of VRDL and 33 local county public health laboratories to enhance human WNV testing and surveillance efforts in California. Local laboratories tested for WNV using an IgM or IgG immunofluorescent assay (IFA) and/or an IgM enzyme immunoassay (EIA). Specimens with inconclusive results were forwarded to VRDL for further testing or confirmation with a plaque reduction neutralization test (PRNT). Additional WNV infections were identified through testing performed at blood donation centers.

In 2006, specimens from 1,583 individuals were tested for WNV infection at VRDL. The first case in 2006 was a 62-year-old male from Butte County who developed West Nile fever symptoms on June 1st. . In total, 292 human WNV infections were identified among residents of 36 counties in California, a 69% decrease from 2005. Twenty-eight of the 292 WNV infections were detected in blood donors, 14 of whom later developed clinical symptoms consistent with West Nile fever.

Of the 278 WNV cases (symptomatic infections, Figure 1), 190 (68%) were classified as West Nile fever, 83 (30%) were identified as neuroinvasive disease cases (i.e. encephalitis, meningitis, or acute flaccid paralysis), and 5 (2%) were of unknown clinical presentation. Males represented 179 (64%) of 278 cases. The median age for all cases for whom data were available was 49 years (range: 8-86 years). The median age for West Nile fever and neuroinvasive cases was 47 (range: 8-86) and 53 years (range: 14-86 years), respectively. The median age of the seven WNV-associated fatalities was 82 years (range: 47-86 years).

EQUINE SURVEILLANCE

Serum or brain tissue specimens from 622 horses displaying neurological signs were submitted to the CAHFS and CVEC for arboviral testing. WNV infection was detected in 58 horses from 23 counties (Figure 2), of which 24 (41%) died or were euthanized. Five of the 58 infected horses were currently vaccinated with the WNV vaccine, 2 did not complete the recommended vaccine dosage schedule, 44 were unvaccinated, and vaccination history was unknown for 7.

Table 1. Summary of WNV-Positive Surveillance Elements in California, 2006

County	Human*	Horse	Dead Bird	Mosquito Pool	Sentinel Chicken	Other
Alameda	1	0	41	9	0	2
Alpine	0	0	0	0	0	0
Amador	0	0	2	0	0	0
Butte	31	0	40	1	49	1
Calaveras	0	0	2	2	0	0
Colusa	4	0	9	0	1	0
Contra Costa	8	1	93	18	25	18
Del Norte	0	0	0	0	0	0
El Dorado	2	0	19	2	0	0
Fresno	11	5	75	40	37	0
Glenn	12	0	35	1	10	0
Humboldt	0	0	2	0	0	0
Imperial	1	0	0	14	57	0
Inyo	0	0	8	3	0	0
Kern	49	4	24	230	100	0
Kings	1	0	13	34	25	0
Lake	2	2	8	12	6	0
Lassen	0	7	3	3	0	0
Los Angeles	13	0	98	78	38	1
Madera	0	0	3	4	9	0
Marin	1	1	9	2	2	1
Mariposa	0	0	1	0	0	0
Mendocino	0	2	7	0	0	0
Merced	4	3	41	8	15	0
Modoc	2	2	2	0	0	0
Mono	1	0	1	0	0	0
Monterey	0	0	5	0	0	0
Napa	1	0	8	0	0	0
Nevada	1	0	4	0	0	0
Orange	6	0	49	14	0	0
Placer	8	1	13	23	11	0
Plumas	0	0	0	0	0	0
Riverside	4	0	3	31	27	0
Sacramento	15	1	89	34	13	0
San Benito	0	0	1	0	0	0
San Bernardino	3	0	33	25	26	3
San Diego	1	3	26	0	0	0
San Francisco	0	0	3	0	0	0
San Joaquin	8	2	47	40	19	0
San Luis Obispo	1	5	14	0	0	0
San Mateo	0	0	7	0	0	2
Santa Barbara	0	2	19	1	0	0
Santa Clara	5	1	224	8	1	2
Santa Cruz	0	0	7	0	0	0
Shasta	4	4	89	7	4	2
Sierra	0	0	0	0	0	0
Siskiyou	0	0	1	0	0	0
Solano	8	0	21	1	22	0
Sonoma	0	1	23	1	1	0
Stanislaus	11	3	77	51	45	0
Sutter	12	1	2	55	36	0
Tehama	6	2	12	0	3	0
Trinity	0	0	1	0	0	0
Tulare	6	3	14	8	22	0
Tuolumne	0	0	1	0	0	0
Ventura	3	0	62	2	1	0
Yolo	27	0	54	64	26	0
Yuba	5	2	1	6	9	0
State Totals	**278**	**58**	**1,446**	**832**	**640**	**32**

*Does not include asymptomatic infections

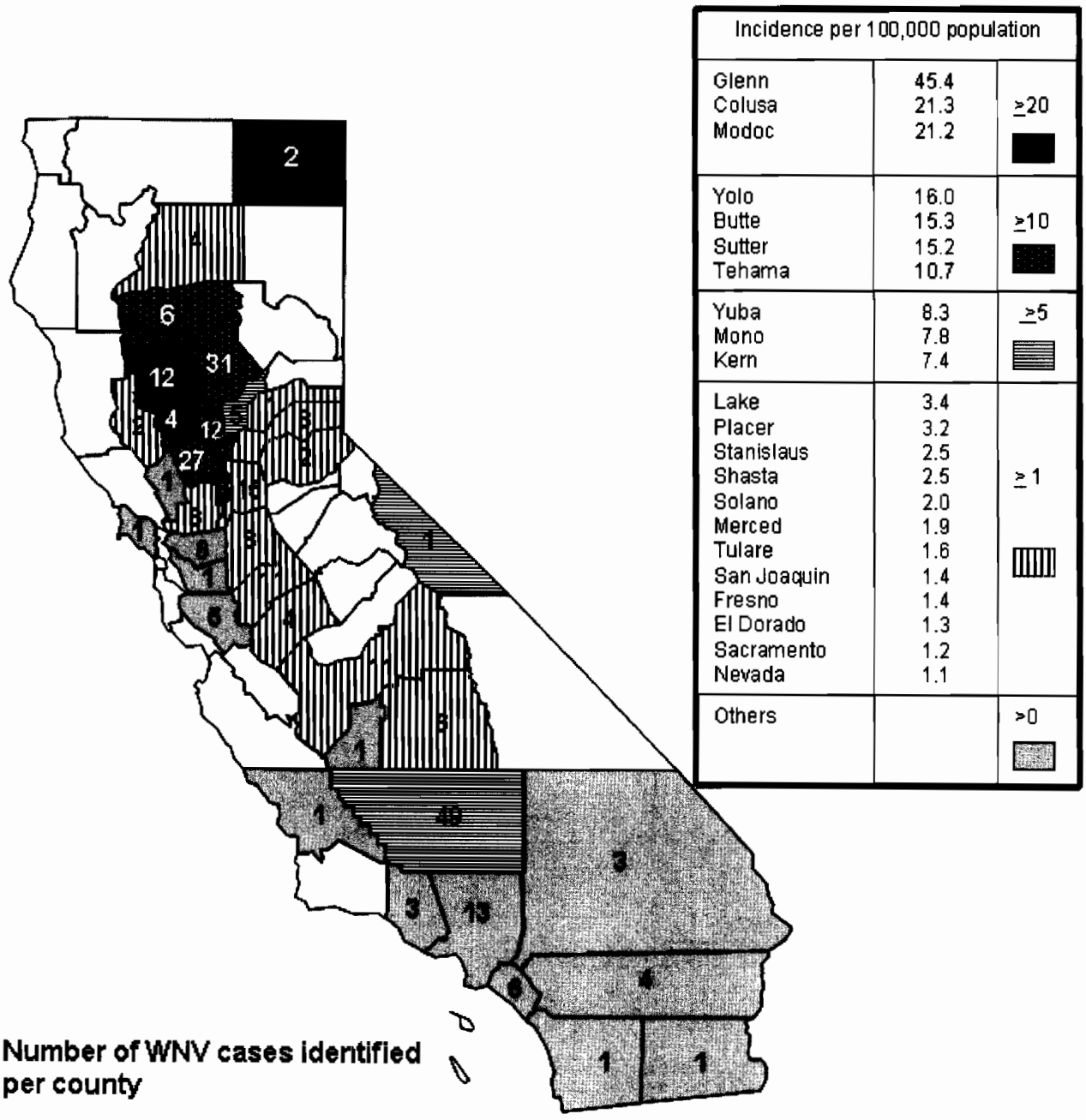

Figure 1. Human cases of West Nile virus infection, California 2006.

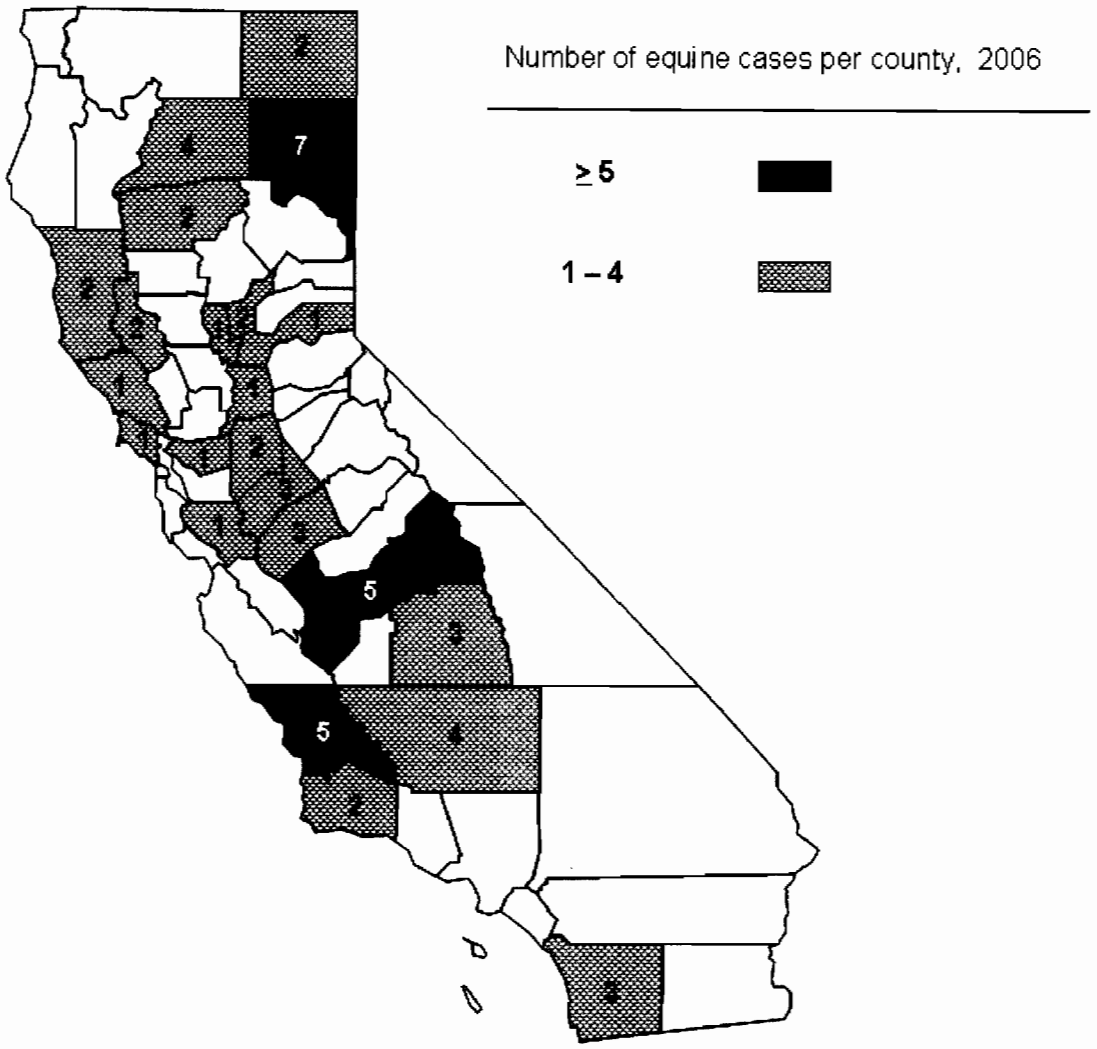

Figure 2. Equine cases of West Nile virus infection, California 2006.

ADULT MOSQUITO SURVEILLANCE AND TESTING

From April to November, statewide adult mosquito abundance was monitored weekly by 41 local agencies from 32 counties contributing trap collection data to the VBDS weekly adult mosquito occurrence reports (AMOR). Local agencies maintained 587 New Jersey light traps (36 agencies), over 500 carbon dioxide-baited traps (26 agencies), and over 250 gravid-baited traps (16 agencies). The weekly AMOR reports and the accompanying 5-year average AMOR summaries were used by agencies to compare mosquito abundance with neighboring districts, measure the effectiveness of their larval control programs, help identify unknown breeding sources, and establish thresholds as part of the state response plan.

Laboratory testing of mosquito pools was performed at CVEC. Nine local agencies conducted in-house testing. Fifty-one agencies in 40 counties collected a total of 694,087 mosquitoes (21,711 pools) that were tested by reverse transcriptase polymerase chain reaction (RT-PCR) for SLE, WEE, and WNV viral RNA (Table 2). An additional 111,942 mosquitoes (3,902 pools) were tested for only WNV by local agencies using a commercial rapid assay- RAMP® (Response Biomedical Corp., Burnaby, British Columbia).

WNV was detected in 832 of 25,613 mosquito pools from 33 counties (Table 3 -4 & Figure 3). In 2006, WNV was first detected from 4 pools of *Culex tarsalis* collected on April 13 in Riverside County. The last detection of WNV in mosquitoes in 2006 was from a pool of *Cx. quinquefasciatus* collected on December 8 in Los Angeles County. WNV was identified from 5 *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cx. tarsalis)* and 3 other species (*Aedes melanimon, Anopheles freeborni*, *Culiseta incidens*).

WEE virus was detected in 18 mosquito pools (17 *Cx. tarsalis*, 1 *Cx. quinquefasciatus*) from Kern County (Table 2). The first and last WEE positive pools were collected on July 11 and September 21, respectively. SLE virus was not detected in mosquito pools in 2006.

Table 2. Mosquitoes and sentinel chickens tested for SLE*, WEE, and WNV, California 2006†

County	Agency	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	WEE + pools	No. flocks	No. chickens	No. sera tested	WNV + sera	WEE + sera
Alameda	Alameda Co. MAD	14,001	397	9	0	3	21	307	0	0
Alameda	Alameda Co. Vector Control	240	5	0	0	0	0	0	0	0
Alpine		0	0	0	0	0	0	0	0	0
Amador	Amador Co. Dept. Agriculture	103	10	0	0	0	0	0	0	0
Butte	Butte Co. MVCD	1,223	31	1	0	7	77	1,056	49	0
Calaveras	Saddle Creek Comm. Serv	187	7	1	0	1	10	128	0	0
Colusa	Colusa MAD	0	0	0	0	1	10	141	1	0
Contra Costa	Contra Costa MVCD	24,220	520	11	0	5	50	763	25	0
Del Norte		0	0	0	0	0	0	0	0	0
El Dorado	El Dorado Co. Vector Control	516	12	2	0	0	0	0	0	0
Fresno	Consolidated MAD	13,479	382	37	0	6	60	839	23	0
Fresno	Fresno MVCD	2,206	53	2	0	2	20	250	12	0
Fresno	Fresno Westside MAD	2,994	69	1	0	2	20	303	2	0
Glenn	Glenn Co. MVCD	1,700	34	1	0	1	13	178	10	0
Humboldt		0	0	0	0	0	0	0	0	0
Imperial	Coachella Valley MVCD	10,091	217	14	0	3	30	430	38	0
Imperial	Imperial Valley VCD	4,035	96	0	0	4	40	431	19	0
Inyo	Owens Valley MAP	3,751	75	3	0	0	0	0	0	0
Kern	Antelope Valley MVCD	1,375	29	0	0	0	0	0	0	0
Kern	Delano MAD	0	0	0	0	1	10	153	8	0
Kern	Kern MVCD	30,125	719	120	5	9	90	1,278	89	9
Kern	South Fork MAD	0	0	0	0	1	10	129	0	0
Kern	UCD Field Station	43,843	1109	88	13	0	0	0	0	0
Kern	Westside MVCD	4,611	107	22	0	3	30	460	3	2
Kings	Consolidated MAD	204	8	2	0	0	0	0	0	0
Kings	Kings MAD	14,842	410	32	0	4	40	464	25	0
Lake	Lake Co. VCD	14,186	351	12	0	2	20	305	6	0
Lassen	Lassen Co. Dept. Agriculture	513	13	3	0	0	0	0	0	0
Los Angeles	Antelope Valley MVCD	1,073	30	0	0	8	48	614	12	0
Los Angeles	Greater Los Angeles Co. VCD	54,075	1,588	78	0	7	70	1,234	19	0
Los Angeles	Long Beach EH	8,922	280	0	0	3	30	503	0	0
Los Angeles	Los Angeles Co. West VCD	8,922	261	0	0	20	116	2,101[b]	6	0
Los Angeles	San Gabriel Valley MVCD	0	0	0	0	11	46	919[b]	1	0
Madera	Consolidated MAD	160	4	0	0	0	0	0	0	0
Madera	Fresno Westside MAD	38	1	0	0	0	0	0	0	0
Madera	Madera Co. MVCD	2,090	42	4	0	2	20	200	9	0
Madera	Merced Co. MAD	38	2	0	0	0	0	0	0	0
Marin	Marin-Sonoma MVCD	244	11	2	0	2	20	278[b]	2	0
Mariposa		0	0	0	0	0	0	0	0	0
Mendocino		0	0	0	0	0	0	0	0	0

*No mosquito pools or sentinel chickens were positive for SLE in 2006
†Unless noted otherwise, testing was performed by CVEC (mosquitoes) and VRDL (chickens)
[a]Tested by local agency
[b]Tested by local agency or VRDL

>>Table 2. Mosquitoes and sentinel chickens tested for SLE*, WEE, and WNV, California 2006† (*continued*)

County	Agency	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	WEE + pools	No. flocks	No. chickens	No. sera tested	WNV + sera	WEE + sera
Merced	Merced Co. MAD	6,197	245	4	0	8	48	698	15	0
Merced	Turlock MAD	9,653	270	4	0	0	0	0	0	0
Modoc		0	0	0	0	0	0	0	0	0
Mono		0	0	0	0	0	0	0	0	0
Monterey	North Salinas MAD	0	0	0	0	2	20	330	0	0
Napa	Napa Co. MAD	0	0	0	0	3	30	420	0	0
Nevada		27	2	0	0	2	19	323	0	0
Orange	Orange Co. VCD	36,283	1,040	14	0	1	10	170	0	0
Placer	Placer Co. MVCD	13,757	468	23	0	6	36	546[b]	11	0
Plumas		0	0	0	0	0	0	0	0	0
Riverside	Coachella Valley MVCD	100,011	2,629	24	0	9	90	1,467	15	2
Riverside	Northwest MVCD	8,797	249	1	0	6	60	906	10	0
Riverside	Riverside Co. EH	30,583	719	6	0	6	66	1,015	2	0
Sacramento	Sacramento-Yolo MVCD	47,685[a]	2,640	34	0	5	50	924[b]	13	0
San Benito		0	0	0	0	0	0	0	0	0
San Bernadino	San Bernardino Co. VCP	17,056	485	13	0	10	100	1,968	20	0
San Bernardino	West Valley MVCD	749	49	1	0	7	28	509	6	0
San Diego	San Diego Co. Dept of Health	5,022	110	0	0	4	40	647	0	0
San Francisco	Presidio Trust	20	1	0	0	0	0	0	0	0
San Joaquin	San Joaquin Co. MVCD	1,085	29	6	0	5	50	637[b]	19	0
San Luis Obispo	San Luis Obispo Co. EH	590	12	0	0	0	0	0	0	0
San Mateo	San Mateo Co. MAD	4,285	103	0	0	2	20	289	0	0
Santa Barbara	Santa Barbara Coastal VCD	15,229	352	1	0	5	53	806	0	0
Santa Clara	Santa Clara Co. VCD	0	0	0	0	5	50	655	1	0
Santa Cruz	Santa Cruz Co. MVCD	85	6	0	0	2	20	341	0	0
Shasta	Burney Basin MAD	0	0	0	0	2	20	202	1	0
Shasta	Shasta MVCD	3,751	101	7	0	5	55	708	3	0
Sierra		0	0	0	0	0	0	0	0	0
Siskiyou		0	0	0	0	0	0	0	0	0
Solano	Solano Co. MAD	1,607	36	0	0	3	36	451	22	0
Solano	Sacramento-Yolo MVCD	843[a]	91	1	0	0	0	0	0	0
Sonoma	Marin-Sonoma MVCD	159	4	1	0	4	40	519[b]	1	0
Stanislaus	East Side MAD	6,342	12	7	0	2	16	210	7	0
Stanislaus	Turlock MAD	40,993	1,553	43	0	7	84	1,163	38	0
Sutter	Sutter-Yuba MVCD	20,380	459	55	0	5	50	562	36	0
Tehama	Tehama Co. MVCD	0	0	0	0	3	30	305	3	0
Trinity		0	0	0	0	0	0	0	0	0
Tulare	Delano MAD	0	0	0	0	1	10	151	5	0
Tulare	Delta VCD	5,088	133	8	0	3	30	364	2	0

*No mosquito pools or sentinel chickens were positive for SLE in 2006

†Unless noted otherwise, testing was performed by CVEC (mosquitoes) and VRDL (chickens)

[a]Tested by local agency

[b]Tested by local agency or VRDL

>>Table 2. Mosquitoes and sentinel chickens tested for SLE*, WEE, and WNV, California 2006† (*continued*)

County	Agency	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	WEE + pools	No. flocks	No. chickens	No. sera tested	WNV + sera	WEE + sera
Tulare	Tulare MAD	0	0	0	0	2	20	297	15	0
Tulare	Kings MAD	293	9	0	0	0	0	0	0	0
Tuolumne		0	0	0	0	0	0	0	0	0
Ventura	City of Moorpark	0	0	0	0	1	5	70	1	0
Ventura	Orange Co. VCD	146	8	0	0	0	0	0	0	0
Ventura	Ventura Co. EH	1,451	41	2	0	4	40	678	0	0
Yolo	Sacramento-Yolo MVCD	51,013[a]	2,958	64	0	5	50	947[b]	26	0
Yuba	Sutter-Yuba MVCD	900	24	6	0	2	20	259	9	0
Total		**694,087**	**21,711**	**770**	**18**	**245**	**2,197**	**33,001**	**640**	**13**

*No mosquito pools or sentinel chickens were positive for SLE in 2006
†Unless noted otherwise, testing was performed by CVEC (mosquitoes) and VRDL (chickens)
[a]Tested by local agency
[b]Tested by local agency or VRDL

Table 3. Mosquito pools (Culex spp.) tested for WNV, 2006.

	Cx erythrothorax		*Cx pipiens*		*Cx quinquefasciatus*		*Cx stigmatosoma*		*Cx tarsalis*		Other *Culex* spp.	
County	pools	WNV +	pools	WNV +	pools	WNV +	pools	WNV +	pools	WNV +	pools	WNV +
Alameda	84	0	213	7					102	2	1	0
Amador									10	0		
Butte			3	0					22	1		
Calaveras*			7	0			3	0	17	2		
Contra Costa*	89	0	160	7					573	11		
El Dorado									4	1		
Fresno	16	0			260	29	6	2	200	9	1	0
Glenn									30	1		
Imperial	26	0	1	0	6	0			266	14	6	0
Inyo									75	3		
Kern	111	3			764	111			838	114		
Kings	13	0			155	11			250	23		
Lake	15	0					20	2	299	10		
Lassen									13	3		
Los Angeles*	23	0			1813	78	92	0	208	0	14	0
Madera	1	0	41	4	1	0			6	0		
Marin*	5	0	56	0			3	0	48	2		
Merced	8	0	187	3					309	5	1	0
Napa*			2	0					8	0		
Nevada	1	0							1	0		
Orange	76	0			851	13	33	1	53	0		
Placer	7	0	139	6			8	0	197	16	8	0
Riverside	364	3	1	0	1107	3	22	0	2018	25	38	0
Sacramento	89	0	1210	9			42	0	1005	25	1	0
San Bernardino*	154	0			890	12	49	0	136	13		
San Diego	32	0			28	0	2	0	47	0		
San Francisco									1	0		
San Joaquin*	1	0	613	18					1050	22		
San Luis Obispo	12	0							0	0		
San Mateo	24	0	72	0					5	0		
Santa Barbara	158	0			36	0	13	0	102	1	1	0
Santa Clara*			99	7					68	1		
Santa Cruz			4	0					2	0		
Shasta	1	0	61	3			3	0	25	4	9	0
Solano			46	0					75	1		
Sonoma*	1	0	84	0			12	0	95	1		
Stanislaus*	13	0	828	38	1	0	10	0	819	13	2	0
Sutter			13	0					423	55		
Tulare	1	0			98	8	1	0	42	0		
Ventura	16	1	2	0	8	1	1	0	16	0	2	0
Yolo			1131	17			109	1	1630	46		
Yuba			1	0					23	6		
Total	**1,341**	**7**	**4,974**	**119**	**6,018**	**266**	**429**	**6**	**11,111**	**430**	**84**	**0**

*Includes pools tested by RT-PCR and additional pools tested by RAMP at local agency
RAMP®, commercial antigen test

Table 4. Mosquito pools (*Aedes spp., Anopheles* spp., *Coquillettidia perturbans, Culiseta* spp, *Psorophora signipennis)* tested for WNV, 2006

Mosquito Species	*Ae dorsalis*	*Ae melanimon*	*Ae nigromaculis*	*Ae squamiger*	*Ae sierrensis*	*Ae vexans*	*Ae washinoi*	Other *Aedes*	*Aedes* spp. Subtotal	*An franciscanus*	*An freeborni*	*An hermsi*	*Anopheles* spp. Subtotal	*Coquillettida perturbans*	*Cs incidens*	*Cs inornata*	*Cs particeps*	*Ps signipennis*	unknown	Other spp. Subtotal
County																				
Alameda									0	1	1		2							0
Butte		5							5				0		1					1
Contra Costa*		35				27			62				0							0
El Dorado								2	2				0		6[a]					6
Fresno		11			1	1			13				0		7		1			8
Glenn		1							1		3		3							0
Imperial	1					5			6	1			1			1				1
Kern		238[b]							238				0		2	11				13
Lake					15				15				0	2						2
Los Angeles*									0	2		9	11		97	1				98
Marin*	27						2		29				0							0
Merced		7							7				0			3				3
Napa*									0				0			1				1
Orange									0			27	27							0
Placer		23	1			2		3	29		75[a]		75	1	3	1				5
Riverside						12			12			31	31			4				4
Sacramento		135	4		12	72	18	1	242				0		17	5			29	51
San Bernardino*					1	10			11	5		4	9		27	5		2		34
San Diego									0				0		1					1
San Joaquin*	2	215			3	136	3		359				0		1					1
San Mateo									0				0				2			2
Santa Barbara							12		12	7		9	16		7		7			14
Santa Clara*				1					1				0							0
Shasta									0				0	1			1			2
Solano		6							6				0							0
Sonoma*	1				6		7		14				0		2		2			4
Stanislaus*		26							26				0		4	15			6	25
Sutter		16							16		7		7							0
Ventura					1		1		2				0		1		1			2
Yolo		56	4		4	4	3	2	73		1		1		4	3			7	14
Total	31	774	9	1	43	269	46	8	**1,181**	16	87	80	**183**	4	180	50	14	2	42	**292**

*Includes pools tested by RT-PCR and additional pools tested by RAMP at local agency.
RAMP®, commercial antigen test
[a]One pool positive for WNV
[b]Two pools positive for WNV

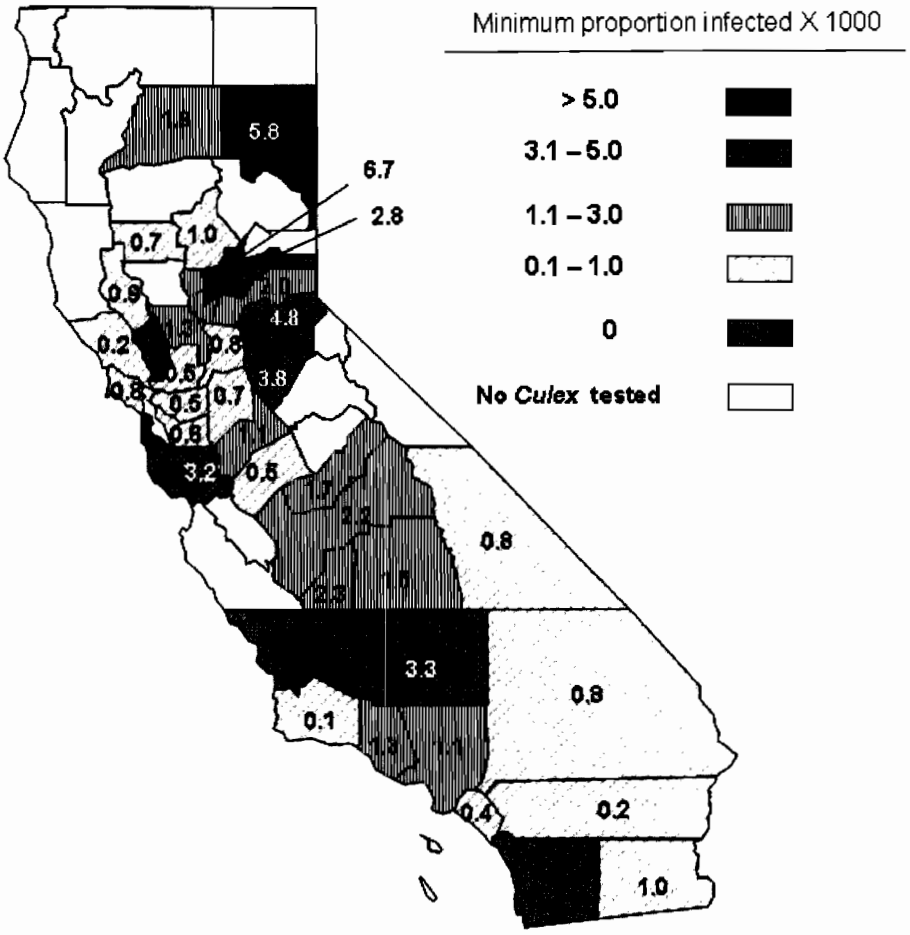

Figure 3. West Nile virus in *Culex* spp., California, 2006. Minimum proportion = number of positive pools/number of mosquitoes tested x 1000.

SENTINEL CHICKEN SEROSURVEILLANCE

Fifty-two local mosquito and vector control agencies in 39 counties maintained 245 sentinel chicken flocks (Table 2). From April through November, blood samples were collected from chickens every other week and tested for antibodies to SLE, WNV, and WEE using an EIA and IFA. Detection of flavivirus or WEE antibody was confirmed with western-blot and a neutralization test. In areas where SLE has never been documented, flavivirus positive chickens from the same flock where at least 2 WNV confirmed positive chickens had been identified were assumed to be infected with WNV and confirmatory testing was not performed.

VRDL and four local mosquito and vector control agencies tested 33,001 chicken sera samples for antibodies to SLE, WEE, and WNV (Table 2). A total of 640 seroconversions to WNV were detected among 122 flocks from 29 counties (Table 2 & Figure 4). The first and last WNV seroconversions were detected among chickens located in Los Angeles County on May 3 and November 14, respectively. In addition, there were 13 unconfirmed flavivirus seroconversions detected in chickens from five counties: Kings (2), Madera (3), Placer (5), San Bernardino (1), and Stanislaus (2). These data are not included in Table 2.

A total of 13 WEE seroconversions were detected among 5 sentinel flocks from 2 counties: Kern (11) and Riverside (2) (Table 2). The first WEE seroconversion was detected in Riverside County on July 24, and the last seroconversion was detected in Kern County on October 9. No SLE seroconversions were detected in 2006.

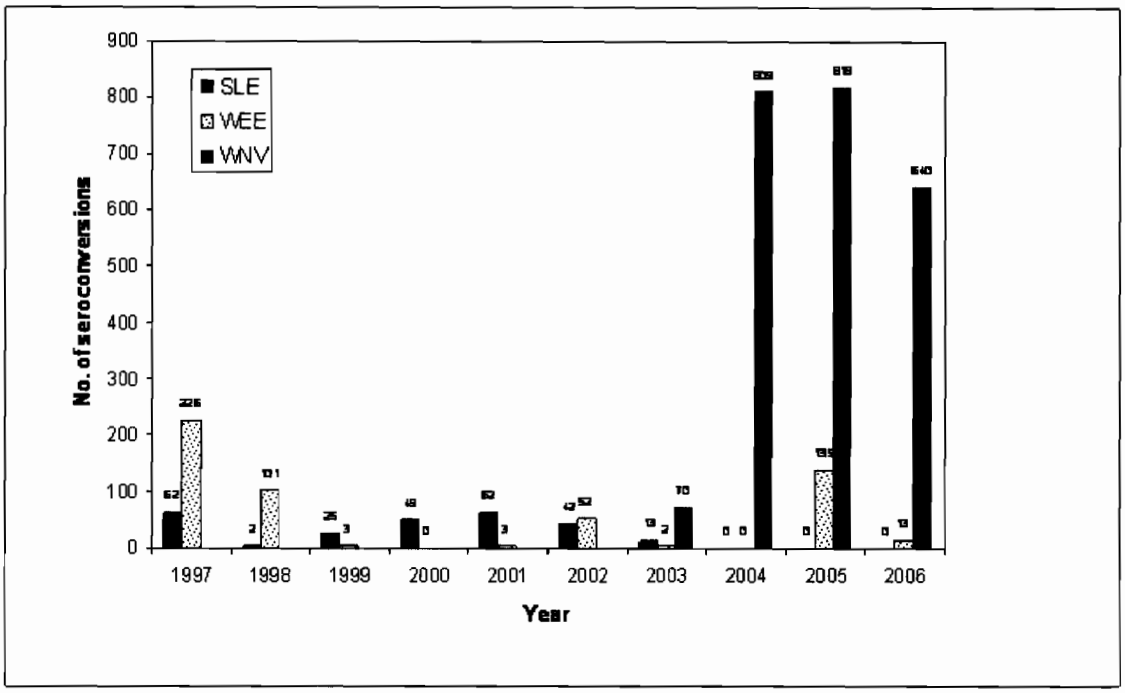

Figure 4. SLE, WEE, and WN virus sentinel chicken seroconversions 1997-2006

DEAD BIRD AND TREE SQUIRREL SURVEILLANCE FOR WEST NILE VIRUS

Established in 2000, the WNV dead bird surveillance program is a collaborative program between CDHS and over 130 local agencies. In 2006, the WNV Hotline operated seven days a week from 8am to 5pm. Staff fielded 53,752 calls in English and Spanish and obtained 46,345 reports of dead birds from 58 counties. The peak months were July and August (9,400 calls each) Fig. 5. Of the 6,535 carcasses deemed suitable for testing, WNV was detected in 1,446 carcasses from 53 counties (Table 5).

Carcasses were tested at CVEC by RT-PCR, or at local agency labs by immunohistochemistry or rapid immunodiagnostic assays. Since 2004, local agencies in California have screened birds for WNV using two commercially available rapid immunodiagnostic assays – RAMP and VecTest (Medical Analysis Systems Inc., Camarillo, CA). In 2006, 23 local agencies tested 863 birds by VecTest and 10 agencies tested 319 birds by RAMP. Of the 1,106 corvids that tested positive for WNV in 2006 (Table 5), 471 were tested by VecTest and 176 were tested by RAMP. Positive rapid test results from corvids were considered definitive and no further testing was required for reporting purposes. Birds that tested negative by either rapid assay were sent to CVEC for confirmation by RT-PCR.

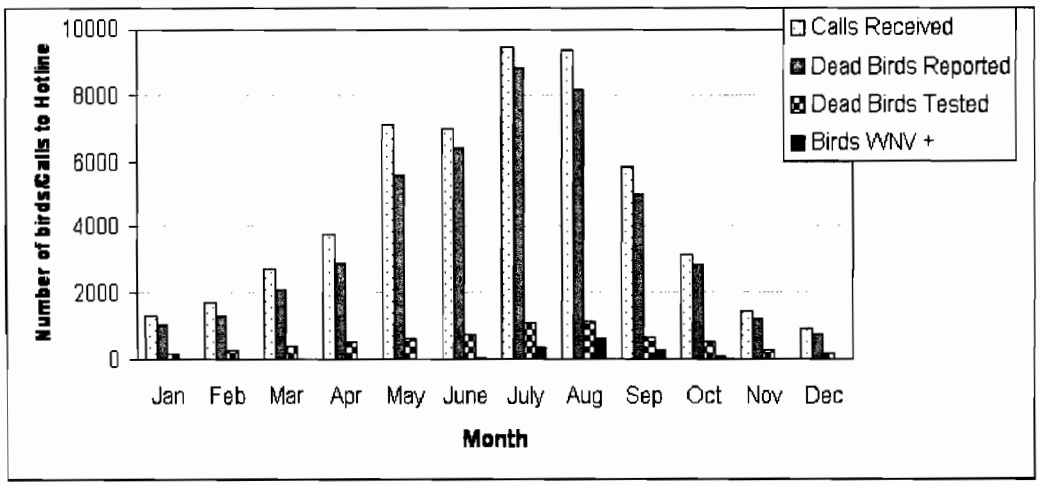

Figure 5. Calls and reports to the CDHS West Nile virus hotline, 2006

Table 5. Dead birds and tree squirrels reported, tested, and positive for West Nile virus, 2006.

	Corvid[a]			Non-Corvids			Tree Squirrels[b]		
County	Reported	Tested	Positive	Reported	Tested	Positive	Reported	Tested	Positive
Alameda*	379	73	27	1297	194	14	24	11	2
Alpine	1	1	0	5	1	0	0		
Amador	19	3	1	162	25	1	0		
Butte	388	55	35	465	53	5	13	3	1
Calaveras	31	2	1	234	34	1	1	0	
Colusa	27	9	9	23	5	0	0		
Contra Costa*	866	138	74	2619	249	19	83	40	18
Del Norte	2	1	0	18	6	0	0		
El Dorado	178	27	12	599	107	7	8	0	
Fresno*	914	111	49	1988	213	26	8	2	0
Glenn*	106	43	34	67	28	1	0		
Humboldt	37	7	0	102	32	2	0		
Imperial	5	0		32	4	0	0		
Inyo	29	8	7	77	17	1	0		
Kern	148	23	14	782	95	10	1	0	
Kings	105	17	9	231	33	4	2	0	
Lake*	93	14	8	188	19	0	1	0	
Lassen	14	1	0	48	11	3	0		
Los Angeles*	768	197	62	1772	451	36	18	8	1
Madera	65	10	2	177	41	1	0		
Marin*	340	27	8	515	17	1	14	2	1
Mariposa	6	0		69	12	1	0		
Mendocino	56	16	5	130	28	2	1	1	0
Merced	284	47	36	396	40	5	0		
Modoc	3	2	1	24	6	1	0		
Mono	10	1	1	54	4	0	0		
Monterey*	131	17	1	349	71	4	1	1	0
Napa*	86	17	8	154	15	0	5	1	0
Nevada	44	4	1	308	55	3	10	2	0
Orange*	309	104	34	762	144	15	0		
Placer*	199	18	6	1226	99	7	14	4	0
Plumas	17	2	0	51	10	0	1	0	
Riverside	156	17	1	740	95	2	2	0	
Sacramento	1151	137	63	3678	287	26	33	5	0
San Benito	17	5	1	78	16	0	0		
San Bernardino*	345	47	20	961	143	13	29	5	3
San Diego*	481	216	14	496	175	12	0		
San Francisco	56	7	0	242	42	3	0		
San Joaquin*	910	64	41	1121	69	6	2	0	
San Luis Obispo	92	16	8	422	79	6	0		
San Mateo	184	36	1	816	122	6	55	29	2
Santa Barbara	170	27	15	318	55	4	1	0	
Santa Clara*	1261	350	215	2043	69	9	46	15	2
Santa Cruz	66	8	1	345	69	6	2	1	0
Shasta*	246	107	86	344	47	3	5	4	2
Sierra	2	1	0	3	2	0	0		
Siskiyou	3	1	0	16	6	1	0		
Solano*	454	45	16	975	45	5	1	0	
Sonoma*	506	67	18	902	53	5	8	1	0
Stanislaus*	629	98	56	994	114	21	1	0	
Sutter	131	5	1	153	9	1	0		
Tehama*	64	18	9	129	22	3	3	0	
Trinity	6	1	1	22	5	0	0		
Tulare	218	34	9	585	92	5	0		
Tuolumne	15	3	0	115	25	1	2	2	0
Unknown	10	0		61	0		0		
Ventura	501	90	44	649	139	18	2	1	0
Yolo	862	115	40	797	120	14	1	0	
Yuba	56	2	1	128	4	0	0		
Totals	**14,252**	**2,512**	**1,106**	**32,057**	**4,023**	**340**	**398**	**138**	**32**

*Includes birds tested at CVEC or by local agency

[a]Family Corvidae includes crows and ravens (*Corvus* spp.), magpies (*Pica* spp.), and jays (*Aphelocoma californica, Cyanocitta stelleri, Gymnorhinus cyanocephalus*).

[b]Includes fox (*Sciurus niger*), eastern gray (*S. carolinensis*), and western gray (*S. griseus*) squirrels.

Tree squirrels have been included as a WNV surveillance element since 2004, based upon evidence they were susceptible to WNV mortality and could provide information on localized WNV transmission. Dead tree squirrels were reported to the California WNV hotline and suitable carcasses were tested at CVEC. In 2006, 32 of 138 tree squirrels (23.2%) tested positive from 9 counties (Table 5). These included 20 fox squirrels (*Sciurus niger*), 4 western gray squirrels (*S. griseus*), 1 eastern gray squirrel (*S. carolinensis*), and 7 squirrels of undetermined species. In addition, a collaborative study was initiated between CDHS, CVEC, and the Lindsey Wildlife Museum to determine WNV viremia and antibody levels in sick tree squirrels. Virus was detected in blood from 7 of 33 (21.2%) tree squirrels tested by Vero cell plaque assay (average viremia of positive squirrels = 4.14 $\log_{10}$ PFU (plaque forming units)/ml; range: 3.0-5.2). Of the remaining 26 tree squirrels negative for viremia, 14 (53.9%) had $PRNT_{80}$ titers $\geq$1:20 indicating that they had survived acute infection.

In 2006, CDHS developed daily maps for the entire State that identified areas with clustered dead bird reports. Local agencies used the maps to help focus surveillance and public education activities, and to help establish priority areas for mosquito control. These maps were generated by analyzing the incidence in space and time of dead bird reports with the WNV DYCAST model developed in cooperation with the Center for Advanced Research of Spatial Information (CARSI) at Hunter College, City University of New York. Maps were made available on the Surveillance Gateway (CalSurv) website, and a real-time alert system was instituted to provide counties with custom reports about WNV transmission. A majority of local agencies reported that they used DYCAST maps to assist in decision-making processes for mosquito larviciding (81%, 35 out of 43 local agencies) and adulticiding (71%, 30 out of 42 local agencies).

PUBLIC EDUCATION AND REPORTS

The "Fight the Bite" WNV prevention campaign was adopted by CDHS in 2004 from the Colorado Department of Health and Environment and continues to be the main theme for prevention activities. In 2006, CDHS distributed "Fight the Bite" educational materials in both English and Spanish to public health and vector control agencies in all 58 California counties. Local agencies customized the materials and distributed nearly 150,000 wallet cards, 6,000 posters, 30,000 bookmarks, 120,000 West Nile virus, and 40,000 "West Nile Virus For Seniors" brochures throughout the State. The "Fight the Bite" materials were also made available in 6 other languages: Tagalog, Vietnamese, Hmong, Lao, Cambodian, and Russian. Press releases, media advisories, and events were used extensively to inform the public on the spread of WNV throughout the state and personal protection measures. To promote consistent public messages about WNV, bi-weekly conference calls were held with local health department risk communication coordinators.

Throughout the year, CDHS published weekly bulletins reporting statewide arbovirus surveillance data and national WNV activity. Surveillance bulletins were distributed to local, state, and federal public health agencies and universities in California, posted on the California West Nile virus website (www.westnile.ca.gov) and on the California Vector-Borne Disease Surveillance System (http://vector.ucdavis.edu/arbo.html). The WNV website provided information to the public on WNV prevention, and averaged 1,000 individual visits a day during peak season. The website also contained an online submission form for reporting dead birds directly to the WNV hotline. The site posted up-to-date county specific information on WNV activity and provided comparison surveillance data from 2004 and 2005, both which were used extensively by the media. Reports, educational materials, and presentations were also made available for local agencies.

WEST NILE VIRUS IN THE UNITED STATES

In 2006, 48 states and the District of Columbia (DC) reported WNV activity. A total of 4,256 human cases were reported to the Centers for Disease Control and Prevention from 43 states and DC. Of the 4,256 cases, 1,449 (34%) were defined as neuroinvasive disease cases (WNND), 2,636 (62%) as West Nile fever cases, and 171 (4%) were of unknown clinical presentation. There were 165 reported WNV-associated fatalities. Idaho reported the highest incidence of human cases, with 77.03 cases per 100,000.

Non-human WNV activity reported included the following: 35 states reported 1,104 veterinary WNV cases (1,069 horses, 33 squirrels, 2 other species); 44 states reported 4,105 WNV positive dead birds; 40 states and DC reported 11,898 WNV positive mosquito pools; and 14 states reported 917 WNV positive sentinel chickens.

Acknowledgments

The authors gratefully acknowledge the cooperation and assistance of the local mosquito and vector control agencies in the collection and submission of samples for testing and their financial support to the testing laboratories; the local public health laboratories which tested samples; the many physicians and veterinarians who submitted specimens from clinical cases, and the valuable contributions of the staffs of MVCAC, CARSI, CVEC (especially Maureen Dannen, Keira Simmons, and Andrew Chow), CAHFS (especially Jacquelyn Parker), VRDL (especially Carol Glaser), CDFA Animal Health Branch, the Infectious Disease Branch (especially Ben Sun), and VBDS (especially Renji Hu, Mark Novak, Jamie Riggs-Nagy, Ervic Aquino, Rachel Owens, and the WNV Hotline staff) of CDHS.

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When Was West Nile Virus in Contra Costa County? Tracking the 'Hot Zone'

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ABSTRACT: Evidence of West Nile virus (WNV) transmission in Contra Costa County during the 2006 season was detected almost exclusively during periods when the overnight low temperatures stayed at or above 55° F. This is close to the developmental threshold of 57.7° F identified by Reisen et al. Minimum infection rates in *Culex* species and numbers of positive dead birds peaked during a heat wave in late July and early August, which also coincided with the onset of human cases and sentinel chicken seroconversions. Transmission peaked earlier in the season in the eastern part of the county where average temperatures were somewhat higher. Differences in the spatial and temporal patterns of WNV activity in 2005 and 2006 appear to be associated with microclimate variation.

INTRODUCTION

Since temperature is an important determinant of both mosquito developmental rates and the extrinsic incubation period of West Nile virus (WNV) (Reisen et al. 2006), microclimate may play a significant role in both the geographic and temporal incidence of WNV activity. Contra Costa County's proximity to the San Francisco Bay creates a large microclimate variation across a fairly small geographic span, with eastern inland areas as much as 20° F warmer than western coastal areas during July and August, dividing the county roughly into three distinct microclimate regions (Fig. 1). During 2005, the first year in which we saw significant levels of West Nile virus transmission, the majority of human and equine cases occurred in the warmer eastern region of the county. Although the numbers of dead bird reports to the statewide WNV hotline were higher in the more densely populated central area of the county, we found that the incidence of reports per capita was also higher in the east (Schutz et al. 2006). We hypothesized that these differences in intensity of transmission were related to higher summer temperatures in the inland areas of the County.

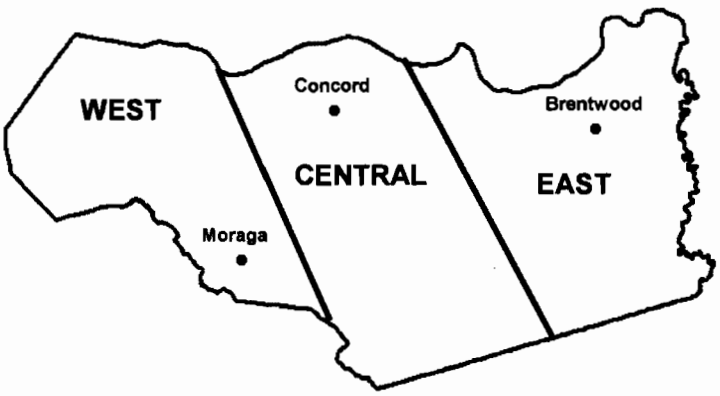

Figure 1. Outline of Contra Costa County showing major microclimate regions

Our objective in the present study was to compare the spatial and temporal distribution of WNV activity in Contra Costa County in 2006 with the distribution observed in 2005, and determine whether any differences could be attributed to changes in microclimate.

MATERIALS AND METHODS

Information on the dates and locations of dead bird and squirrel reports were compiled and reported to us weekly by the staff of the California Department of Health Services West Nile Virus hotline. A subset (approximately 10%) of the dead birds and squirrels reported to the hotline were collected by District personnel and either tested for WNV in-house using the RAMP® rapid test (in the case of corvids), submitted to the California Animal Health and Food Safety Laboratory for necropsy and PCR testing (all other birds), or both (all squirrels and RAMP negative corvids). Mosquitoes for virus testing were collected in dry-ice baited EVS traps; between 40 and 90 of these traps were set weekly at a combination of fixed and non-fixed locations. Pooled mosquitoes from fixed trap locations were submitted to the UC Davis Center for Vectorborne Disease for PCR testing, while samples from non-fixed locations were tested in-house using the RAMP® test. As mosquito populations and workload permitted, we tested a combined total of approximately 20-30 pools per week during the height of the WNV transmission season (July through October). Minimum infection rates by species and week were calculated using the bias-corrected maximum likelihood method (Biggerstaff 2006). Sentinel chicken serum samples were collected biweekly from five flocks of ten chickens each and tested at the CDHS Viral and Rickettsial Disease Laboratory in Richmond, CA. Information on the location of human cases was provided by the Contra Costa County Department of Health Services, and equine case locations by the California Department of Food and Agriculture. Locations of all positive surveillance indicators were mapped with ArcGis® 9.2 (ESRI 2006).

Average monthly high and low temperatures for representative cities from each of three regions of Contra Costa County (Moraga, Concord and Brentwood, representing west, central and east county respectively) were obtained from the California Irrigation

Management Information System (CIMIS) (California Department of Water Resources 2005). Monthly climate maps from the Western Regional Climate Center California Climate Data Archive (2005) were used to identify specific microclimate zones within the county.

RESULTS

The numbers of positive West Nile virus indicators were fairly similar in 2005 and 2006, with the exception of a higher overall minimum infection rate (MIR) in mosquitoes tested in 2006 and a lower number of equine cases (Table 1). However, despite the similarity in numbers, examination of the geographic distribution of virus activity during the two seasons revealed some important differences. In 2005, the majority of human and equine cases and positive mosquito pools occurred in the eastern part of the county (Fig. 2a), whereas in 2006 they were concentrated in the central area (Fig. 2b). The highest density of positive dead birds and squirrels occurred in the central area in both years; however, as the authors have previously noted (Schutz et al. 2006), this is at least in part due to higher human population density in the central county corridor, and hence a higher likelihood of dead birds being reported and submitted for testing.

Although the first indication of WNV transmission in both years was a positive dead bird reported on 29 June, the temporal distribution of virus activity was clearly different. In 2005, the numbers of positive birds reported per week increased gradually, peaking the week of August 19th (Fig. 3a), while in 2006 the increase was much steeper and the peak occurred two weeks earlier (Fig. 3b). In both years, WNV activity commenced and increased as weekly average low temperatures (measured at the district office, located in Concord) exceeded 55° F and decreased as low temperatures dropped back below 55°F. In 2005, low temperatures increased and decreased gradually over the course of the summer, peaking in mid-July and staying below 60°F. However, there was a pronounced heat wave in late July and early August 2006 where average low temperatures increased sharply and stayed high for several weeks. This coincided with very sharp and almost immediate increases in MIR in *Culex tarsalis* Coquillett and *Cx. pipiens* L., sentinel chicken seroconversions, positive dead birds, and the first onset of human cases. As the heat wave subsided and overnight low temperatures declined, positive surveillance indicators declined as well. In both seasons, we saw little or no evidence of transmission once overnight low temperatures remained below 55°F, although in 2005 we did continue to sporadically find WNV-positive dead birds until mid-November.

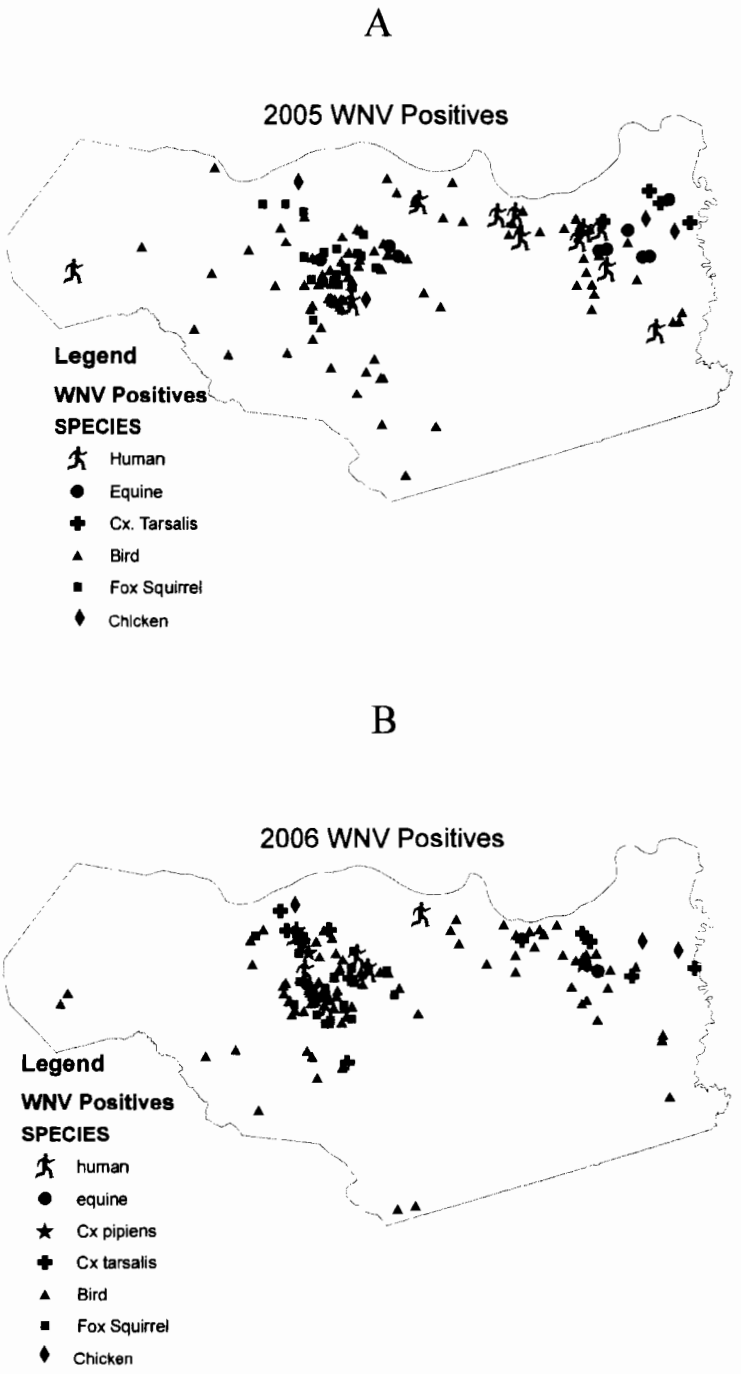

Figure 2. Map of Contra Costa County showing locations of positive West Nile virus indicators in 2005 (a) and 2006 (b).

Table 1. Numbers of WNV positive indicators reported in Contra Costa County in 2005 and 2006. Numbers in parentheses are percent positive of total tested. * indicates county-wide average minimum infection rate (bias-corrected maximum likelihood estimate) per 1,000.

	Dead birds	Squirrels	Mosquito pools	Sentinel chickens	Human	Equine
2006	92 (24%)	19 (46%)	20 (0.44*)	24 (48%)	8	1
2005	94 (18%)	25 (58%)	4 (0.14*)	18 (36%)	11	10

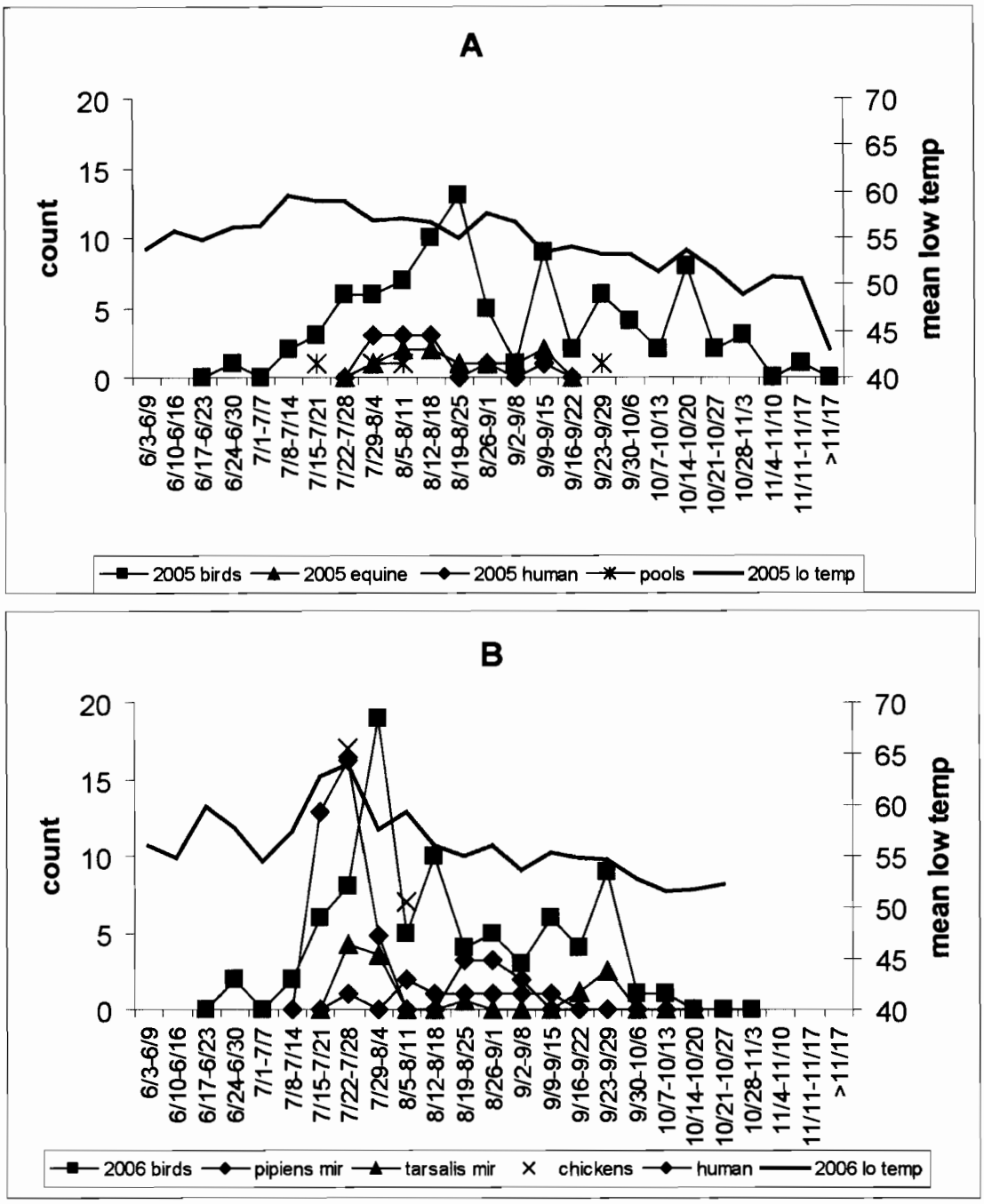

Figure 3. West Nile virus positive indicators (left y axis) and average low temperature (right y axis) by week in 2005 (a) and 2006 (b). Note that sentinel chicken seroconversions were back-dated to probable infection date (estimated as one week prior to blood collection date). Human and equine cases are reported dates of onset. Weekly minimum mosquito infection rates were not calculated for 2005 due to the low number of positive pools (4).

Since it appeared that microclimate was associated with the observed differences in temporal distribution of WNV activity, we also examined its role in spatial distribution by plotting weekly totals dead bird reports and average monthly low temperatures for each of our three microclimate regions (weekly temperature data were not available for all three regions). In 2005, overnight low temperatures were highest in east county, slightly lower in central county, and considerably lower in west county from May through August. In September, however, this pattern changed and central county was warmest (Fig. 4a). An examination of weekly dead bird report totals showed that counts for east and central county ran roughly parallel until September, when they dropped off sharply in east county while declining more gradually in central county. Activity in west county remained very low throughout the season (Fig. 4b). In contrast, during 2006 the 'reversal' in east and central county low temperatures occurred a month earlier and this was concurrent with decreased dead bird reports in east county and an increase in reports from central county (Fig. 5a,b). In both years, average monthly low temperatures barely exceeded 55°F in west county and we had few dead bird reports or positive indicators of transmission in that region. Linear regression of monthly dead bird report totals vs. monthly average low temperatures for each of the three regions (Fig. 6) showed a strong correlation (r^2= 0.38, $p<0.001$).

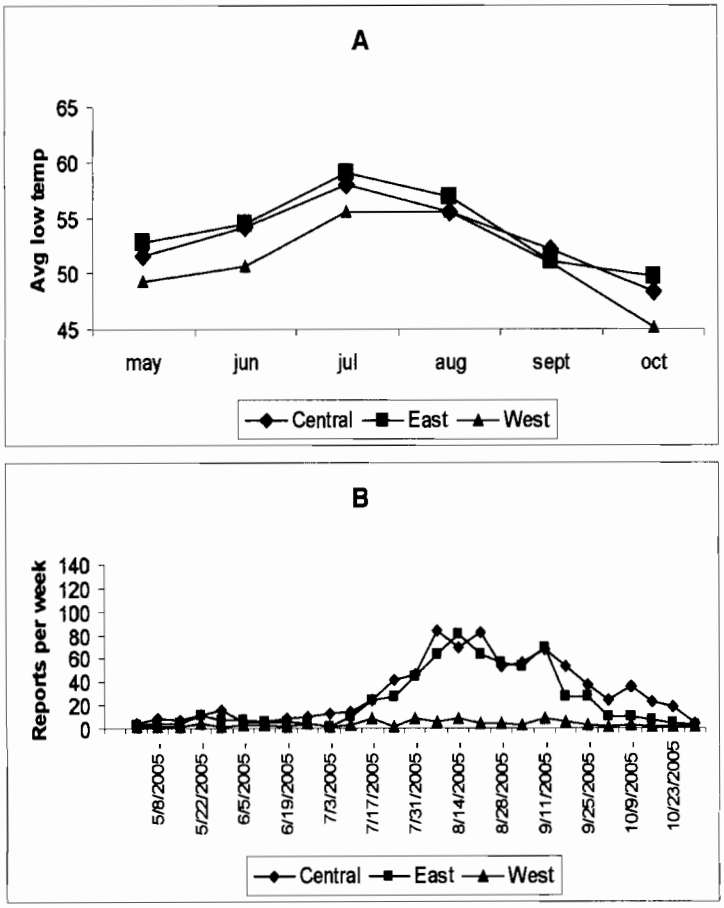

Figure 4. Average monthly minimum temperatures (a) and weekly dead bird report totals (b) for three microclimate regions of Contra Costa County in 2005.

DISCUSSION

Based on our observations during two years of high WNV activity, it appears that microclimate variation can explain both spatial and temporal differences in patterns of virus transmission within Contra Costa County. Specifically, overnight low temperatures above approximately 55°F appear to trigger and maintain WNV transmission. This temperature is very close to the viral developmental threshold of 57.7° F reported by Reisen et al. (2006). Temperatures remaining above this threshold for extended periods may cause significant increases in viral load in vector mosquitoes, driving up transmission rates and 'jump starting' the transmission cycle. A cursory examination of statewide WNV incidence and average low temperatures, as well as discussions with other vector ecologists (T. Su, S. Bearden, pers. comm.) suggest that microclimate may explain larger scale patterns as well.

The authors recognize that this study is largely observational and that factors other than microclimate, such as ongoing mosquito control operations, may also have affected WNV transmission rates. Also, the lack of availability of weekly or daily temperature data from all three microclimate regions of the County made thorough comparisons or statistical analyses difficult. However, the observed patterns do appear to be consistent with observations being made independently by other investigators. We believe that

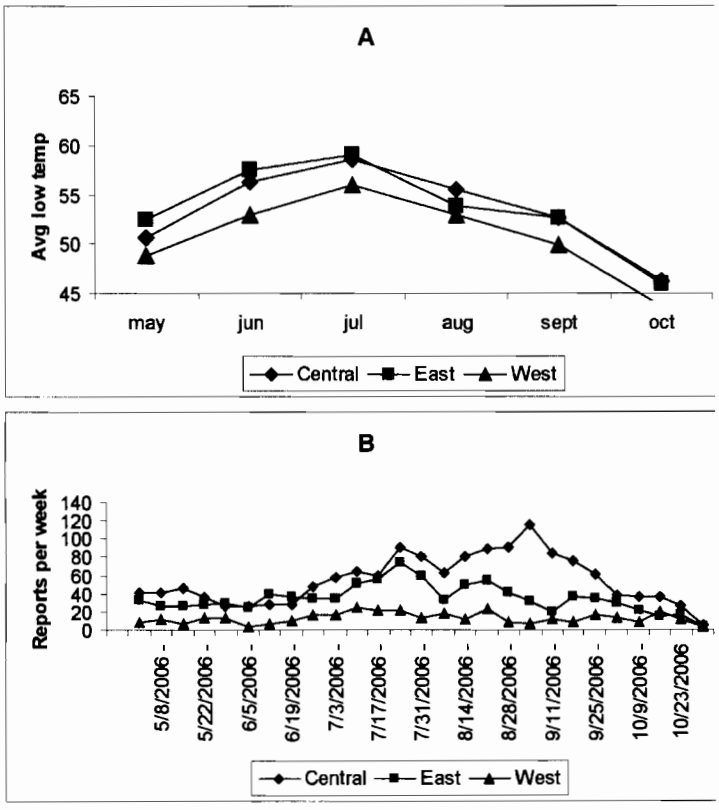

Figure 5. Average monthly minimum temperatures (a) and weekly dead bird report totals (b) for three microclimate regions of Contra Costa County in 2006.

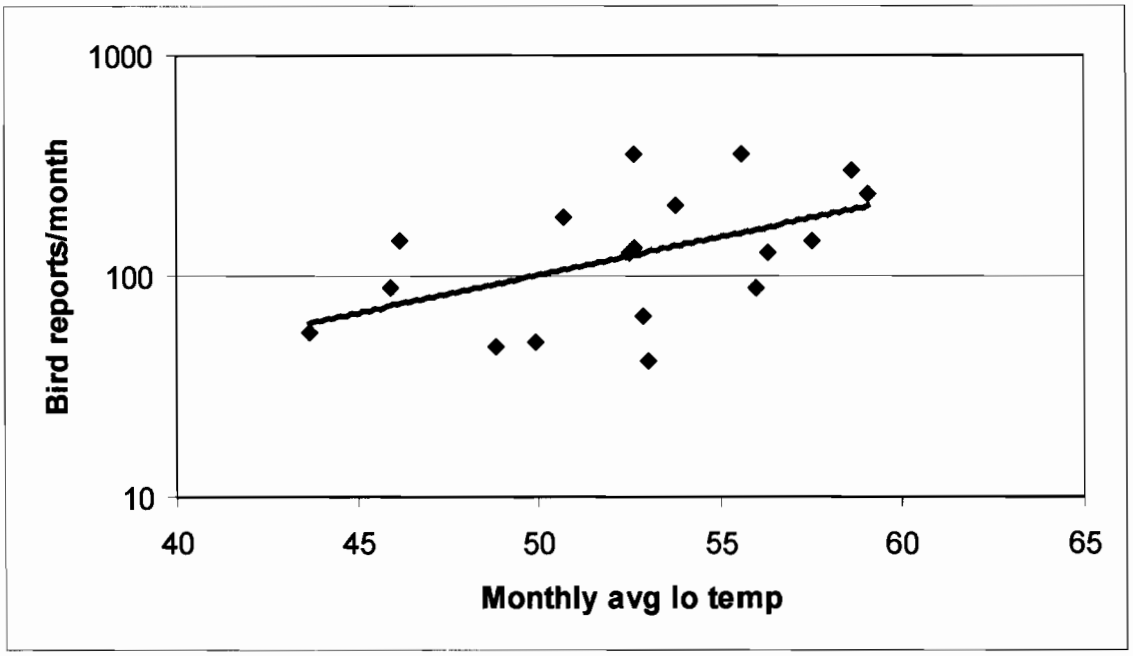

Figure 6. Log (monthly dead bird report totals) vs. average monthly minimum temperatures) for three microclimate regions of Contra Costa County in 2006.

microclimate is a strong determinant of WNV transmission, possibly an important determinant of human case risk, and should be incorporated into existing risk-assessment models to provide both increased spatial and temporal resolution.

Acknowledgments

We wish to acknowledge the staff of the West Nile virus hotline for quickly and efficiently compiling and transmitting dead bird report information, CDFA and Contra Costa Health Services for providing information on equine and human cases, the UC Davis Center for Vectorborne Disease for testing dead birds and mosquito pools and the DHS Viral and Rickettsial Disease laboratory for testing sentinel chicken serum samples. We are also grateful to our dedicated crew of vector control inspectors, technicians and aides for helping to expand and enhance our surveillance program.

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A Preliminary Evaluation of Mosquito Attractiveness for Bird-Baited Traps

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ABSTRACT: To better understand the host-seeking nature of *Culex* mosquitoes we compared the trap attractiveness of our standard CO_2-baited trap with that of a bird-baited trap (BBT). The Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) routinely operate CO_2-baited traps as part of the standard encephalitis virus surveillance (EVS) program. In our bird- baited traps we used the Rock Pigeon, *Columba livia*, to attract mosquitoes that were then drawn into a collection bag just as the standard CO_2-baited trap, which utilizes dry ice. Eight traps were operated at the same sites overnight each week. Our results indicate that greater quantities of *Culex* mosquitoes were attracted to the BBTs than the CO_2-baited traps in both rural and suburban sites. The diversity of mammal feeding mosquitoes was greater in the CO_2-baited traps as expected.

INTRODUCTION

West Nile virus (WNV) was initially detected in North America in 1999 and rapidly spread westward throughout the country. With its first appearance in southern California in 2002, WNV spread to northern California in 2004 (CDHS 2007). The virus is transmitted largely by *Culex* mosquitoes and maintained in wild birds (Gibbs et al. 2005).

Currently, the standard methods for collecting mosquitoes for the encephalitis virus surveillance (EVS) program in Sacramento County are through the use of CO_2-baited traps and gravid traps. In northern California, the primary enzootic vectors of WNV appear to be two ornithophilic species, *Culex tarsalis* Coquillett and *Culex pipiens* L. (Goddard et al. 2002). These species were the target of our bird-baited traps.

Bird-baited traps have been used to monitor WNV vectors. In one study conducted in New York, host-seeking mosquitoes were trapped in bird-baited traps set at ground level and in the canopy level by using chickens (*Gallus gallus domesticus*) and house sparrows (*Passer domesticus* L.) as attractants (Darbro et al. 2006). The most abundant mosquitoes captured in the bird-baited traps were *Cx. restuans* Theobald and *Cx. pipiens*, representing 88% of the total mosquito catch. In addition, Deegan et al. (2005) conducted a study in New York City using pigeons as sentinels for surveillance of WNV. Pigeons were placed in modified lard-can traps as bait to attract and capture mosquitoes. The pigeon-baited traps were placed in three classes of canopy cover at two elevations. Results indicated that 99.8% of mosquitoes collected were from the genus *Culex*. In our study we placed BBTs and CO_2-baited traps in suburban and rural sites and compared the number of *Culex* species collected during the season (June through September 2006).

MATERIALS AND METHODS

Sampling of mosquitoes using both CO_2-baited traps and BBTs was conducted from June to September 2006. Four sites were selected in Sacramento County; two in rural locations and two in suburban locations. The rural locations consisted of one sylvan site, designated rural A, the Stone Lakes National Wildlife Refuge (SLNWR), (38° 24□ 32N 121°14□ 22W), and an agricultural site, designated rural B, (38° 22□ 19N 121°29□ 22W). The suburban locations consisted of one commercial site, the SYMVCD, designated suburban A, (38° 25□ 31N 121°22□ 59W) and one residential site, designated suburban B, (38° 39□ 39N 121°26□ 49W).

Rock Pigeons, *C. livia*, were utilized as attractants in the BBTs. The pigeons were captured at the SYMVCD property using a standard baited drop trap, where the pigeons are lured in by bait and the trap is manually dropped to capture the birds. The handling and care of birds followed the protocol established by the Ornithological Council Guidelines for the use of wild birds in research (Gaunt et al. 1997). Each pigeon was pre-screened for WNV antibodies via an Enzyme-Linked Immunosorbent Assay (ELISA). The WNV antibody free birds were used as attractants in the BBTs. Each individual bird was identified using uniquely numbered leg bands. Pigeons were housed at SYMVCD and provided with food and water as needed.

All BBTs and CO_2-baited traps were placed in the same locations overnight each week. Both traps were configured in the same manner. A motor driven fan was placed under the bait (either CO_2 or pigeon) which forces the host-seeking mosquitoes into a collection basket below. The pigeon was held secure above the fan in a wire cage and provided sufficient food and water for the overnight period. All traps were collected the following morning after they were set the day before. Collected mosquitoes were identified to species and recorded according to trap type, date and location.

RESULTS

Table 1 and Figures 1-2 present comparisons between numbers of mosquitoes collected in the BBTs and the CO_2-baited traps set out in rural and suburban locations of Sacramento County during June through September 2006. Our results indicate that throughout the season, BBTs captured significantly higher numbers and higher percentage of *Cx. tarsalis* and *Cx. pipiens,* than did the standard CO_2-baited traps. Excluding all other species, the percentage of *Cx. tarsalis* collected by the BBTs in suburban site A was 78 %, and suburban site B was 90%. In comparison with the CO_2-baited traps, we collected 22% in suburban site A and 10 % in suburban site B.

Table 1. Total number of *Cx. tarsalis* and *Cx. pipiens* trapped per site.

	Cx. tarsalis		*Cx. pipiens*	
	BBT	CO_2	BBT	CO_2
JUNE				
Suburban A	175	49	53	6
Suburban B	82	5	175	51
Rural A	21	13	1	1
Rural B	172	42	98	12
JULY				
Suburban A	131	19	15	11
Suburban B	2	4	20	11
Rural A	77	177	20	29
Rural B	138	33	95	28
AUGUST				
Suburban A	64	37	78	25
Suburban B	7	1	14	9
Rural A	102	37	51	16
Rural B	108	48	69	50
SEPTEMBER				
Suburban A	138	35	112	28
Suburban B	25	3	27	10
Rural A	153	168	155	112
Rural B	173	64	320	193

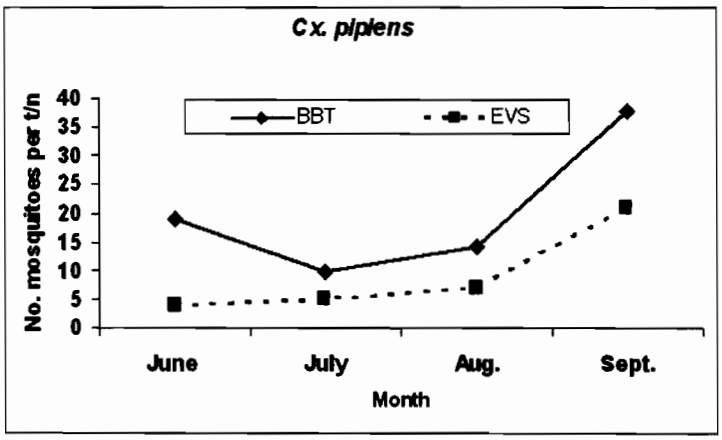

Figure 1. Comparison of the efficiencies of bird-baited traps (BBT) and CO_2 -baited traps in collection of *Culex pipiens* in Sacramento County during June through September 2006.

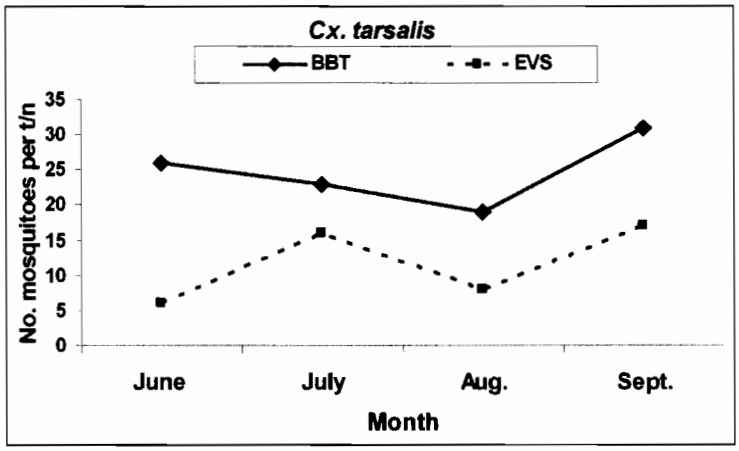

Figure 2. Comparison of the efficiencies of bird-baited traps (BBT) and CO_2-baited traps in collection of *Culex tarsalis* in Sacramento County throughout June through September 2006.

For *Cx. pipiens*, we collected 79% in the BBTs in suburban site A and 74% in suburban site B. In comparison with the CO_2-baited traps we collected 21% in suburban site A, and 26% in suburban site B. For *Cx. tarsalis*, our rural sites, A and B, we collected 47% and 76% for BBTs, respectively. The CO_2 -baited traps for rural sites A and B collected 53% and 24%, respectively. For *Cx. pipiens*, the BBTs collected 59% in rural site A, and 67% in rural site B, compared to the CO_2-baited traps where we collected 41% in rural site A, and 33% in rural site B. From June to September, BBTs were more efficient than CO_2-baited traps in collecting *Cx. tarsalis* and *Cx. pipiens* per trap-night (t/n) (Figure 1, 2). Overall, BBTs collected 24.75 *Cx. tarsalis* per t/n, and the CO_2 -baited traps captured 11.75 per t/n. The BBTs collected 20.25 *Cx. pipiens* per t/n and the CO_2 collected 9.25 per t/n.

Although the BBTs collected the species of interest, the CO_2-baited traps still captured a greater variety of species, including *Cx. erythrothorax* Dyar, *Aedes melanimon* Dyar, *Aedes vexans* (Meijen), *Aedes nigromaculis* (Ludlow), *Culiseta inornata* (Williston), *Culiseta incidens* (Thomsom), *Anopheles freeborni* (Aitken), and *Anopheles franciscanus* (McCracken). Overall, the CO_2-baited traps collected 2.03 per t/n of these mosquito species, whereas, the BBTs collected only one of these other species mentioned above throughout the whole season (June through September 2006).

DISCUSSION

By developing new traps to attract mosquito vectors of WNV it is possible to increase our probability for the early detection of the disease. Moreover, as *Cx. pipiens* and *Cx. tarsalis* are largely ornithophilic and are the primary vectors of WNV the use of BBTs will aid in the collection of significant numbers of these target mosquitoes and potentially increase our overall detection of avian viruses.

Our BBTs captured greater numbers of both *Cx. tarsalis* and

Cx. pipiens than did the standard CO_2-baited traps. This is likely due to the increased attraction to the actual host (bird) rather than the dry ice used to create CO_2. The use of BBTs can effectively focus our surveillance on these species. This data indicates that BBTs are more attractive to host-seeking ornithophilic mosquito species in both rural and suburban sites in Sacramento County. Therefore, the use of BBTs can more effectively indicate the range of host-seeking populations of these WNV vectors. More replications of this study are necessary to be able to further compare rural and urban/suburban sites. We plan to continue our comparison of BBTs with the CO_2-baited traps by expanding the geographic range and habitats. In addition we plan to compare both BBTs and CO_2-baited traps with mammal-baited traps and perhaps other species of birds.

Acknowledgments

The authors wish to thank the laboratory and fisheries staff at Sacramento-Yolo MVCD: Katy Parise, Kara Kelley, Woody Schon, Veronica Armijos, Leslie Shama, Conlin Reis, Marilou Thomas and Jenny Johnson for helping set up traps, counting and identifying mosquitoes, testing the mosquitoes and birds for WNV, capturing the pigeons utilized in the bird-baited traps and reviewing the paper. Thanks are also extended to Tom Harvey and the staff at Stone Lakes National Wildlife Refuge.

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West Nile Virus Surveillance in Fresno County, California: 2004-2006

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ABSTRACT: West Nile Virus (WNV) has been detected in Fresno County during each year from 2004 through 2006. Evidence of viral activity was revealed through surveillance programs conducted by the County's mosquito control agencies (Districts) with assistance from other local and state departments. Detection of WNV was accomplished through the collection and testing of dead birds, mosquitoes, and sentinel chicken blood samples. Additional data of WNV occurrence was obtained from equine and human cases confirmed by the California Department of Food and Agriculture (CDFA) and the Fresno County Department of Environmental Health, respectively. Dead birds collected by District personnel were submitted to the local California Animal Health and Food Safety Laboratory (CAHFS) for testing. Adult mosquito pools collected from gravid and CO_2-baited traps were submitted to the University of California at Davis, Center for Vectorborne Diseases (CVEC) for testing. Blood samples obtained from sentinel chickens were submitted to the Viral Rickettsial Disease Laboratory (VRDL) of the California Department of Health Services for testing. The data indicated varying degrees of sensitivity in the detection of WNV among the surveillance programs, WNV was first detected from dead birds, followed by mosquito pools, human and equine cases, and sentinel chickens in each of the 3 years.

INTRODUCTION

Fresno County (the County) is located near the center of California's San Joaquin Valley and covers an area of 5,962 square miles (FedStats). The Coast Range foothills, which form the County's western boundary, reach a height of over 4,000 feet while some peaks along the crest of the Sierra Nevada, the County's eastern boundary, exceed 14,000 feet. The valley floor in between is fifty to sixty miles wide and ranges between 200 and 400 feet in elevation in most areas. About 900,000 people live in the County with over 60% of the residents in the cities of Fresno and Clovis (CDFDRU 2006).

Mosquito abatement programs are organized into four independent districts within the County. From east to west, they are the Consolidated Mosquito Abatement District (CMAD), the Fresno Mosquito and Vector Control District (FMVCD), the Fresno Westside Mosquito Abatement District (FWMAD), and the Coalinga-Huron Mosquito Abatement District (CHMAD). CMAD also provides services in a small portion of Kings County. The entire geographical area in which services are provided comprises a variety of ecotypes, including urban, suburban, rural residential, irrigated pastures and alfalfa, orchards, vineyards, row crops, dairies, riparian watercourses, and foothill woodlands.

Elements of the Districts' surveillance programs designed to detect West Nile Virus (WNV) include collecting mosquitoes, dead wild birds, and sentinel chicken blood. Dead birds are tested at the California Animal Health and Food Safety Laboratory (CAHFS), mosquito pools at the University of California Davis, Center for Vectorborne Diseases (CVEC), and chicken blood samples at the California Department of Health Services, Viral Rickettsial Disease Laboratory (VRDL). Additional data on WNV occurrence are obtained from equine and human cases confirmed by the California Department of Food and Agriculture and the Fresno County Environmental Health Department, respectively.

WNV was first discovered in the County during the summer of 2004. Viral infections were detected sequentially in wild birds, mosquito pools, humans, horses, and sentinel chickens. WNV activity increased substantially in 2005 from its level in 2004, detected in dead birds before the onset of spring and in mosquitoes during mid-spring. In 2006, viral activity was first confirmed in early June and decreased dramatically from the previous year but not equally in all surveillance categories. The extent of WNV activity from 2004 to 2006 is illustrated in Figure 1.

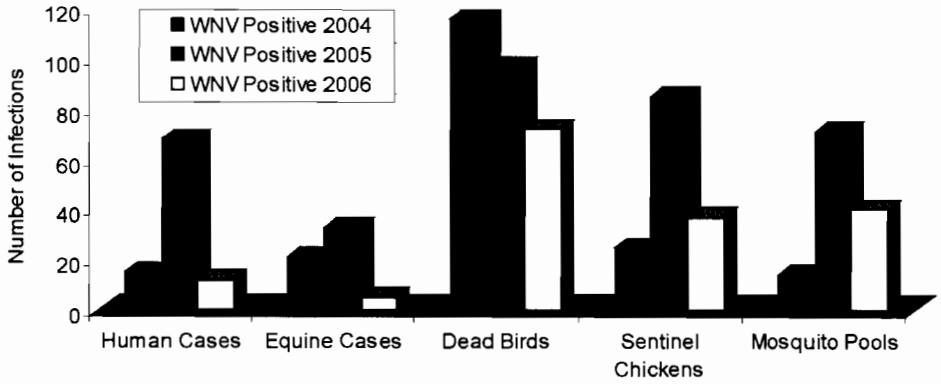

Figure 1. Evidence of WNV in Fresno County from 2004 to 2006.

SURVEILLANCE PROGRAMS CONDUCTED BY THE DISTRICTS

Dead Birds: Dead birds suitable for testing were collected and sent to the local CAHFS facility. Additionally, many of the crows and jays (Family *Corvidae*) were tested using two commercial antigen-based immunochromatic assays—VecTest® and RAMP®—in order to provide the Districts with immediate results. Most birds were eventually sent to the CAHFS laboratory in Davis for confirmation by RT-PCR. Figure 2 compares the dead birds that tested positive for WNV from 2004 to 2006.

By mid-August of 2005, the collection of dead birds was substantially reduced in most of the County when it was evident that 80% of submitted specimens were infected. This accounts for the greater number of infected birds during the corresponding time period in 2004 when the Districts continued to collect birds. The Districts also collected birds continuously during 2006.

Sentinel Chickens: Ten flocks of chickens were maintained within the County as part of the California statewide encephalitis virus surveillance program. A total of 25 chickens in five of the flocks became infected with WNV during August through November, 2004 (Figure 3). During 2005, 85 chickens within all ten flocks became infected with the virus, the earliest seroconversions occurring in July. Although the number of infected chickens in 2006 decreased by over 50% (37 seroconversions) from the 2005 level, the infections commenced earlier than in either of the previous two years, eventually occurring within nine of the flocks and exceeding the amount of activity from 2004.

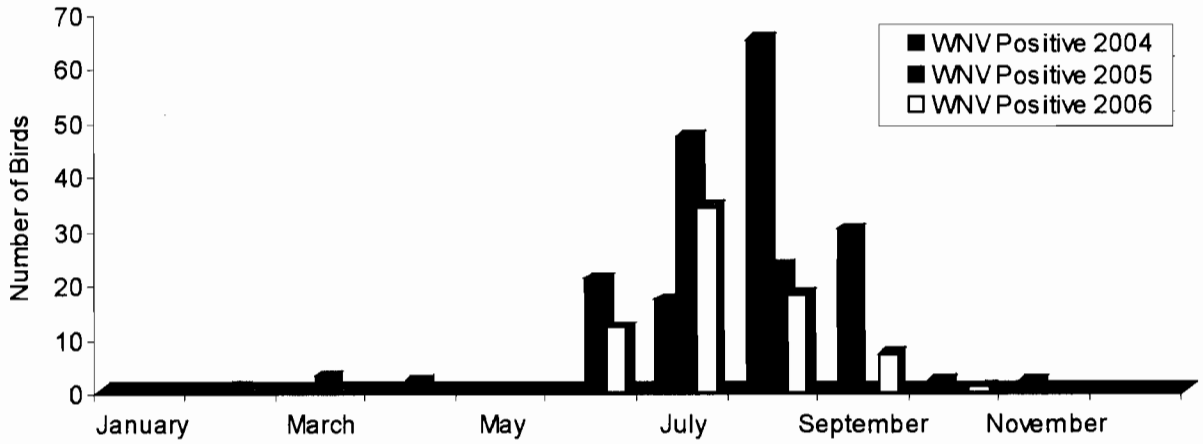

Figure 2. Comparison of dead birds tested positive for WNV in Fresno County from 2004 to 2006.

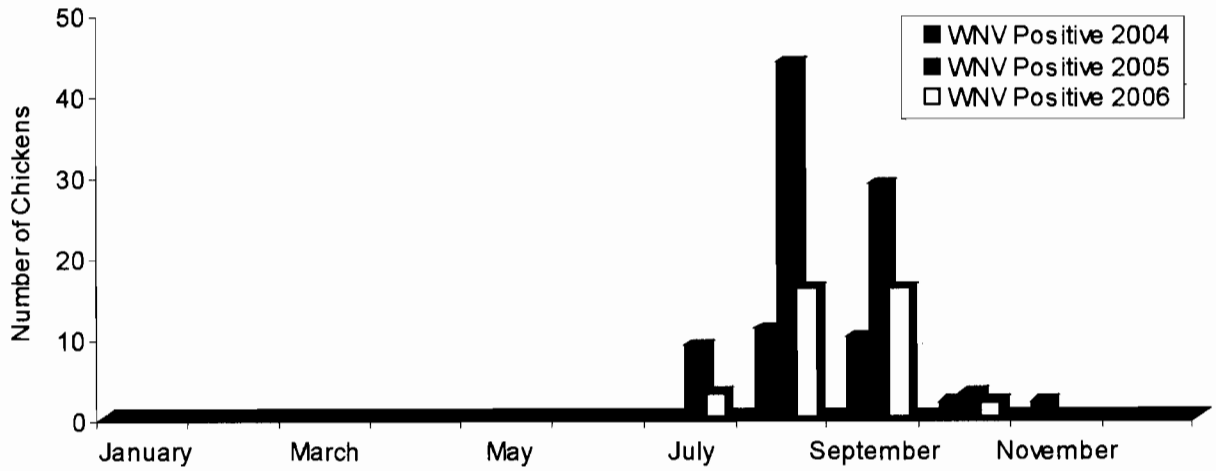

Figure 3. Sentinel chickens sero positive for WNV in Fresno County from 2004 to 2006.

Mosquitoes: Mosquitoes tested for WNV were captured with carbon dioxide-baited (CO_2) and gravid traps. Infected mosquitoes were much more widely distributed throughout the County in 2005 and 2006 than in 2004. The highest number of infections occurred in July of each year, and pools submitted in July and August of 2006 had substantially higher isolations of virus compared to the same months in 2004 (Figure 4).

Data on virus isolation from different mosquito species are presented in Table 1. Of the 221 mosquito pools submitted in 2004, 14 (6.3%) tested positive for WNV. In 2005, 71 out of 457 pools (15.5%) were found positive, and 40 out of 525 pools submitted in 2006 tested positive (7.6%). WNV was isolated only from *Culex quinquefasciatus* in 2004. In 2005 and 2006, the virus was detected in *Cx. quinquefasciatus*, *Cx. tarsalis*, and *Cx. stigmatosoma*.

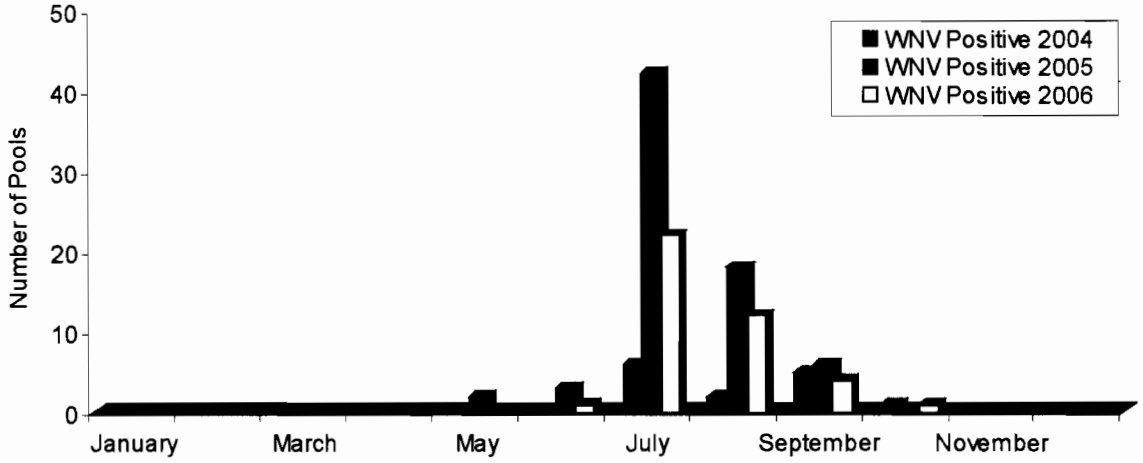

Figure 4. Mosquito pools tested positive for WNV in Fresno County from 2004 to 2006.

Table 1. Data on WNV occurrence in mosquito pools of different species in Fresno County from 2004 to 2006.

	Pools in 2004			Pools in 2005			Pools in 2006		
Mosquito Species	**Submitted**	**WNV+**	**%+**	**Submitted**	**WNV+**	**%+**	**Submitted**	**WNV+**	**%+**
Ae. melanimon	4	0	0	11	0	0	12	0	0
Ae. sierrensis							1	0	0
Ae. vexans				1	0	0	1	0	0
An. freeborni				1	0	0			
Cx. erythrothorax	7	0	0	16	0	0	16	0	0
Cx. quinquefasciatus	96	14	14.6	219	45	20.5	267	33	12.4
Cx. stigmatosoma	0	0	0	4	3	75	5	2	40
Cx. tarsalis	113	0	0	200	39	19.5	213	14	6.6
Cx. thriambus							1	0	0
Cs. incidens	1	0	0	5	0	0	8	0	0
Cs. particeps							1	0	0

SUPPLEMENTARY SURVEILLANCE INFORMATION

Human Cases: The Districts were informed of human infections of WNV by the County Environmental Health Department and from the California Department of Health Services. The highest number of infections occurred in August of each year, and there were slightly more cases in that month during 2006 compared to the same month in 2004 (Figure 5).

The County reported 15 confirmed human infections and no fatalities in 2004. The infection/fatality ratio increased to 68/2 in 2005 and decreased to 12/1 in 2006 (Table 2). The median age remained virtually the same in all three years, and the incidence of disease per 100,000 people was lowest in 2006.

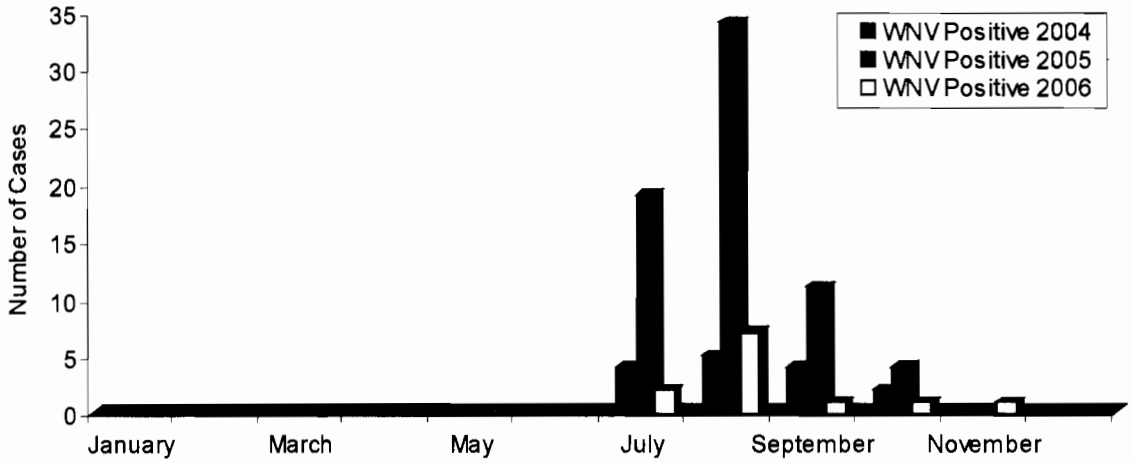

Figure 5. Human cases of WNV in Fresno County from 2004 to 2006.

Table 2. Demographic data on human WNV cases in Fresno County from 2004 to 2006.

	2004	2005	2006
WNV Reported Cases	15	68	12
West Nile Fever	1	41	6
West Nile Neuroinvasive Disease	3	15	4
Asymptomatic Blood Donor	4	8	1
Unknown Clinical Presence	7	4	1
Fatalities	0	2	1
Age Range	30 - 74	6 - 93	24 - 82
Median Age	51	53	53.5
Male / Female	13 / 2	34 / 34	9 / 3
Incidence / 100,000	1.88	8.50	1.40

Equine Cases: The Districts were informed of equine infections of WNV by the CDFA. The highest number of infections occurred in August of each year, and the number of total cases in 2006 was greatly reduced from the two previous years (Figure 6). The case/fatality (dead or euthanized) ratio for 2004 through 2006 was 21/6, 33/12 and 5/0, respectively.

DISCUSSION AND CONCLUSIONS

WNV activity in Fresno County was first confirmed in 2004, increased substantially in 2005, and decreased significantly in 2006. In each year, the first evidence of WNV was first discovered in dead wild birds. Viral isolations from mosquito pools preceded human cases of WNV except in 2004 when viral activity appeared at virtually the same time in both categories. Evidence of equine WNV followed human cases in each year, and sentinel chicken infections were the last of the surveillance elements to be confirmed.

By the middle of August in each year, approximately 80% of dead birds tested were infected with WNV. In 2004 and 2005, Western scrub jays represented about half of the infected birds while American crows represented about one-third of the total. In 2006, nearly 40% of the infected wild birds were jays while several other species had slightly higher totals than in the previous two years.

Frequency of WNV infection in mosquito pools appeared highest in July of each year. Surveillance information compiled by the Districts combined with data analysis from the Department of Health Services suggests that both the minimum infection rate of WNV in mosquitoes and the human case incidence within the County increased substantially in 2005 from 2004. Many more mosquitoes were captured and submitted for testing in 2005 and 2006, infected pools were more widely dispersed throughout the County, and specimens were more widely distributed between both gravid and CO_2-baited traps. Almost all the positive pools were processed from gravid traps in 2004 in which WNV was isolated only from *Culex quinquefasciatus*. In 2005 and 2006, the virus was isolated from pools of *Cx. tarsalis* and *Cx. stigmatosoma*, in addition to *Cx. quinquefasciatus*.

Sentinel chicken infections of WNV occurred in five of the ten flocks in the County during 2004. The number of infected chickens more than tripled in 2005, with seroconversions occurring in all flocks. The total infections in 2006 exceeded the level in 2004 and were spread among nine flocks. Additionally, chicken seroconversions occurred weeks earlier in each successive year. Although the sentinel chickens became infected with WNV after evidence of the virus was discovered in other hosts, the data suggests that the virus greatly amplified in 2005 and maintained a relatively high level in 2006.

WNV infections in horses increased significantly from 2005 over 2004, with twice as many fatalities in 2005. The dramatic decrease in equine cases in 2006, with no fatalities, is possibly due to more horses being vaccinated in addition to the overall decline in viral activity throughout the County.

The substantially higher human caseload of WNV in 2005 over 2004 followed by the dramatic case decrease in 2006 is further evidence of viral amplification and decline in Fresno County during the three-year period. This data is consistent with that of all the other surveillance categories.

Acknowledgments

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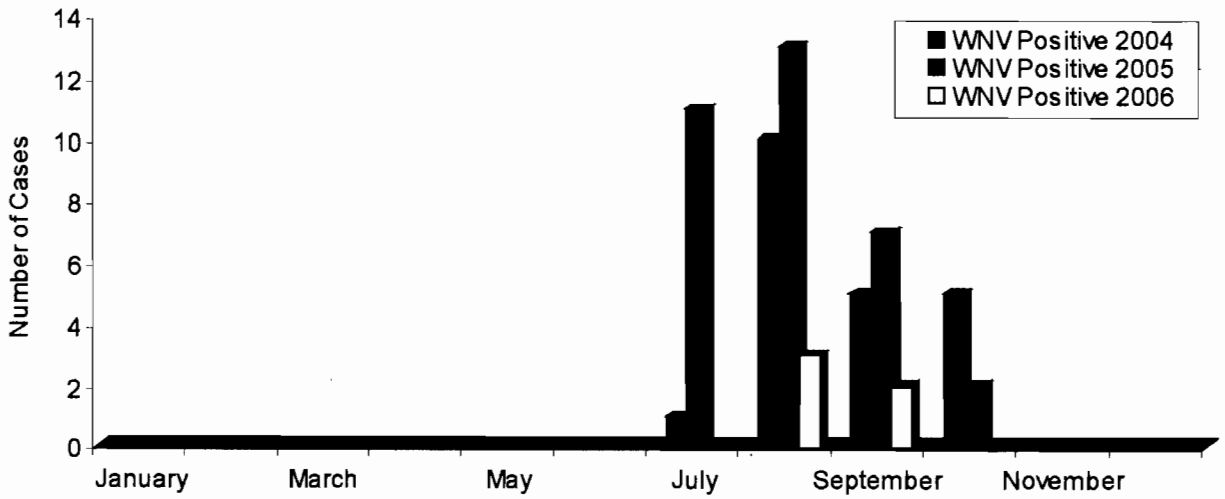

Figure 6. Equine cases of WNV in Fresno County from 2004 to 2006.

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Evaluation of West Nile Virus Activity in Orange County, California During 2006

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ABSTRACT: The Orange County Vector Control District (OCVCD) continued its arbovirus surveillance program in 2006 by collecting and pooling mosquitoes, testing avian blood samples drawn from free-ranging wild birds and sentinel chickens, as well as testing dead birds collected from various animal control agencies and the public. Evidence of West Nile virus (WNV) infection was detected in mosquito pools (14 of 1,028), wild birds (67 of 4,876), and dead birds (49 of 250). No sentinel chickens in a flock of 10 birds tested positive for WNV antibodies. Six non-fatal human cases of WNV infection were reported in the county during 2006. *Culex quinquefasciatus* was the most abundantly trapped mosquito, accounting for the majority of submitted pools (900 of 1,028) and positive pools (13 of 14). House finches (*Carpodacus mexicanus*) and house sparrows (*Passer domesticus*) comprised all of the WNV-seropositive free-ranging wild birds (67 of 67), while American crows (*Corvus brachyrhynchos*) made up most of the positive dead birds (34 of 49). The minimum infection rate (MIR) in *Cx. quinquefasciatus* was comparatively less in 2006 than 2005 or 2004 (0.7 vs. 1.6 and 3.7, respectively). Additionally, the WNV-seropositive rate in the sampled wild bird population (1.5% vs. 5.2% and 5.2%, respectively), percent of WNV-positive dead birds (69.4% vs. 80.2% and 89.2%, respectively), and the number of reported human cases of WNV infection (6 vs. 17 and 64, respectively) were lower in 2006 than 2005 or 2004. However, seasonal MIRs (~ 7.0) and avian seroprevalence levels (~ 5.0%) remained relatively high at several locations, indicating persistent, focal WNV transmission. Polymerase chain reaction (PCR) analysis of products of the *cytochrome b* gene in blood meals from 179 *Cx. quinquefasciatus* females indicated that mourning doves (*Zenaida macroura* L.) and house finches were the preferred hosts ($P < 0.01$) for this mosquito, comprising 43.6% (78 of 179) and 33.5% (60 of 179), respectively, of the blood meals. Contrastingly, American crows accounted for only 2.2% (4 of 179) of the blood meals. The role of mourning doves in modulating WNV infection in the free-ranging wild bird population is not well understood and warrants further investigation.

INTRODUCTION

The Orange County Vector Control District (District) encompasses approximately 789 square miles (all of Orange County), and approximately 3.1 million residents reside within its borders (US Census Bureau 2006). Most of the District consists of urban/suburban habitats with a variety of residential mosquito-breeding sources: improperly maintained swimming pools and ponds, debris-choked drainage channels, and other man-made habitats. Interspersed within this development are several natural, mosquito-producing fresh and salt-water wetlands. Four important vectors of the West Nile virus (WNV), *Culex tarsalis* Coquillett, *Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar, and *Culex erythrothorax* Theobald (Goddard et al. 2002, Reisen et al. 2005) are collected routinely in the county (Gruwell et al. 1988). The District employed an integrated arboviral disease surveillance system throughout the year, comprised of avian serosurveillance (sentinel chickens and wild birds), testing dead birds and mosquitoes, and monitoring veterinarian and physician reports for WNV infections in animals and humans.

MATERIALS AND METHODS

Mosquito surveillance. Mosquitoes were collected weekly from a total of 50 - 60 traps throughout the District, combining CDC/CO_2 - style, host-seeking EVS traps (Rohe and Fall 1979) and Reiter/Cummings gravid female ovipositional traps (Cummings 1992). Blood-fed mosquitoes were collected in CO_2-baited traps, gravid traps, and aspirated at known mosquito resting sites, and at locations of service requests.

Culex quinquefasciatus made up the largest component of the specimens collected (25,130 of 31,624) (Table 1). Of 1,028 mosquito pools submitted for arbovirus testing by mutiplex reverse transcriptase-polymerase chain reaction (RT-PCR) by the Center for Vector-borne Diseases (CEVC) at the University of California, Davis, 14 tested WNV-positive. *Culex quinquefasciatus* comprised the majority of these (13 of 14); nine of the 13 positive pools for this species were collected at Craig Park, a suburban regional park in the city of Fullerton. MIRs for the months of May – November of each year from 2004 – 2006 are shown in Figure 1.

Blood-fed mosquitoes were quickly sorted from the collections, identified to species and placed into sequentially numbered vials unique for each site and date, held in a low-temperature freezer at -80° C., and sent on dry-ice to the Connecticut Agricultural Experimental Station (CAES) for PCR testing to identify the sources of the blood meals (Molaei et al. 2006). Most blood-fed *Cx. quinquefasciatus* were isolated from gravid trap collections (246/293) and came from sites representative of the county as a whole. All blood-fed *Cx. tarsalis* (24/24) and *Cx. erythrothorax* (61/61) females were collected in CO_2-baited traps from wetland

Table 1: Comparison of mosquito collection data and minimum infection rates (MIR) by species for peak activity months, May – November, 2004 – 2006.

	Total Mosquitoes			WNV Positive Pools			Minimum Infection Rate (MIR)		
Species	**2004**	**2005**	**2006**	**2004**	**2005**	**2006**	**2004**	**2005**	**2006**
Culex quinquefasciatus	29,214	44,989	25,130	150	93	13	5.1	2.1	0.5
Cx. erythrothorax	8,936	9,172	3,229	1	1	0	0.1	0.1	0.0
Cx. tarsalis	4,633	7,927	1,602	4	7	0	0.9	0.9	0.0
Cx. stigmatosoma	879	4,950	644	6	11	1	6.8	2.2	1.6
Other	5,498	4,047	1,019	0	0	0	0.0	0.0	0.0
	49,160	**71,085**	**31,624**	**161**	**112**	**14**	**3.3**	**1.6**	**0.4**

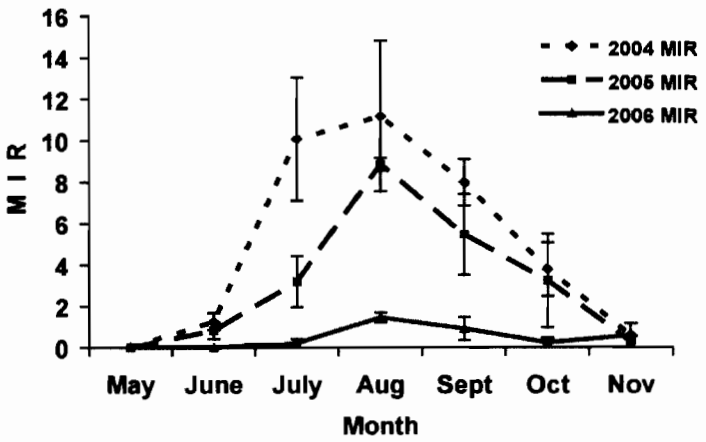

Figure 1. Annual comparisons of seasonal monthly minimum infection rates (MIR) in *Cx. quinquefasciatus*, 2004 – 2006. A factorial ANOVA yielded a significant effect of year, $F(2,63) = 20.26$, $P < .001$ and month, $F(6,63) = 12.38$, $P = <.001$, on Minimum Infection Rate (MIR). However, the interaction effect was significant $F(12,63) = 3.08$, $P < .01$, indicating that the temporal (month) effect was greater for 2004 and 2005 than 2006 during July, August, September and October.

locations. Results were obtained on blood meals taken by 179 *Cx. quinquefasciatus* (179 of 293 submitted, or 61.1%), 29 *Cx. erythrothorax* (29 of 61 submitted, or 47.5%), and 11 *Cx. tarsalis* (11 of 24 submitted, or 45.8%) females (Table 2).

Sentinel chickens. The District maintained one sentinel chicken flock of 10 chickens near a *Cx. tarsalis* - producing freshwater marsh at the San Joaquin Wildlife Sanctuary in Irvine. Blood samples from the chickens were tested biweekly for SLE, WEE, and WNV antibodies by the California Department of Health Services' Viral and Rickettsial Diseases Laboratory (CDHS/VRDL) by enzymatic immunoassay (EIA) (Reisen et al.1994) from April - November and blocking ELISA at the District (Hall 1995, Jozan et al. 2003). None of the sentinel chickens tested positive for exposure to any arbovirus.

Wild bird serosurveillance. The District's wild bird serosurveillance program focused primarily on two abundant peridomestic passerines, house sparrows (*Passer domesticus* L.) and house finches (*Carpodacus mexicanus* Say). Birds were trapped in 12 modified Australian Crow traps (McClure 1984) at sites also used to sample the adult mosquito population. Eight trap sites were located in riparian corridors or wetland areas surrounded by suburban development. House finches were abundant at these riparian/wetland sites, while house sparrows were collected almost exclusively at two sites located in urbanized communities with few open areas. Near-equal mixes of house sparrows and house finches were seen at only two locations.

Birds were sampled at each site on alternate weeks (6 sites/ week). Newly captured birds were banded, aged, sexed (if possible), bled and released. Blood samples (0.2-ml) were taken from the jugular vein with a 1.0-ml syringe and a 28-gauge needle, dispensed into a 1.8-ml field diluent solution (Gruwell et al. 1988), kept cool and processed at the District's laboratory by EIA for SLE and WEE antibodies and blocking ELISA for evidence of WNV infection.

Of the 3,392 house finches sampled in 2006, 60 birds (1.8%) tested positive for WNV antibodies; seven (0.6%) of the 1,249

Table 2. Blood meal sources for *Cx. quinquefasciatus, Cx. tarsalis, and Cx. erythrothorax.*

Blood-meal Source	*Cx. quinque* No.	Percent	*Cx. tarsalis* No.	Percent	*Cx. erythro* No.	Percent
Mourning dove	76	42.5	4	36.4		
House finch	60	33.5	5	45.4	2	6.9
American robin	14	7.8				
American crow	4	2.2	1	9.1		
House sparrow	4	2.2				
Song sparrow	2	1.1				
Western bluebird	2	1.1				
Calif. thrasher	2	1.1				
Green heron	1	0.6			1	3.5
Blue-throated hummingbird	1	0.6				
House wren	1	0.6				
Western tanager	1	0.6				
Turkey	1	0.6				
Mallard					2	6.9
Black-crowned night Heron					2	6.9
Wood rat					7	24.1
Roof rat					8	27.5
Coyote					1	3.5
Domestic cat	5	2.8				
Human	3	1.7	1	9.1	1	3.5
Opossum	1	0.6			3	10.3
Cottontail rabbit	1	0.6			2	6.9
Totals	179	100	11	100	29	100

house sparrow samples and no birds of other species were WNV-positive during the year (Table 3). Antibody-positive birds were detected in every month of 2006 (Figure 2), except September.

Dead bird surveillance. Dead birds were collected from the public via dead bird call-ins and through cooperation with various animal control agencies. Of the 400 birds collected, only 250 were suitable for testing, and 49 of these were found positive for WNV antigen by immunohistochemistry (Steele et al. 2000). Rates of WNV-positive dead birds declined from 58.6% (253/452) in 2004 and 45.4% (302/665) in 2005 to 19.6% (49/250) during 2007 (Table 4). The proportion of WNV-positive non-corvids to total WNV-positives dead birds varied from 19.8% (50/253) in 2004 to 10.3% (31/302) in 2005, and rose to 30.6% (15/49) in 2006.

Climatological data. Temperature and precipitation data were obtained through the National Oceanic and Atmospheric Administration (NOAA) to determine potential differences in temperature patterns between 2004, 2005 and 2006. There were no apparent differences among mean monthly temperatures for 2004, 2005, and 2006 (Figure 3), although June – July of 2006 were several degrees above normal (Figure 4).

Table 3: Wild bird data and WNV-seroconversion rates by species, 2004 – 2006.

	Total Blood Samples			No. WNV Positive			Percent Positive		
Species	**2004**	**2005**	**2006**	**2004**	**2005**	**2006**	**2004**	**2005**	**2006**
House finch	2,293	2,718	3,392	120	140	60	5.2%	5.2%	1.8%
House sparrow	612	1,075	1,249	29	42	7	4.7%	3.9%	0.6%
Other	984	364	235	11	6	0	1.1%	1.6%	0.0%
	3,889	**4,157**	**4,876**	**160**	**188**	**67**	**4.1%**	**4.5%**	**1.4%**

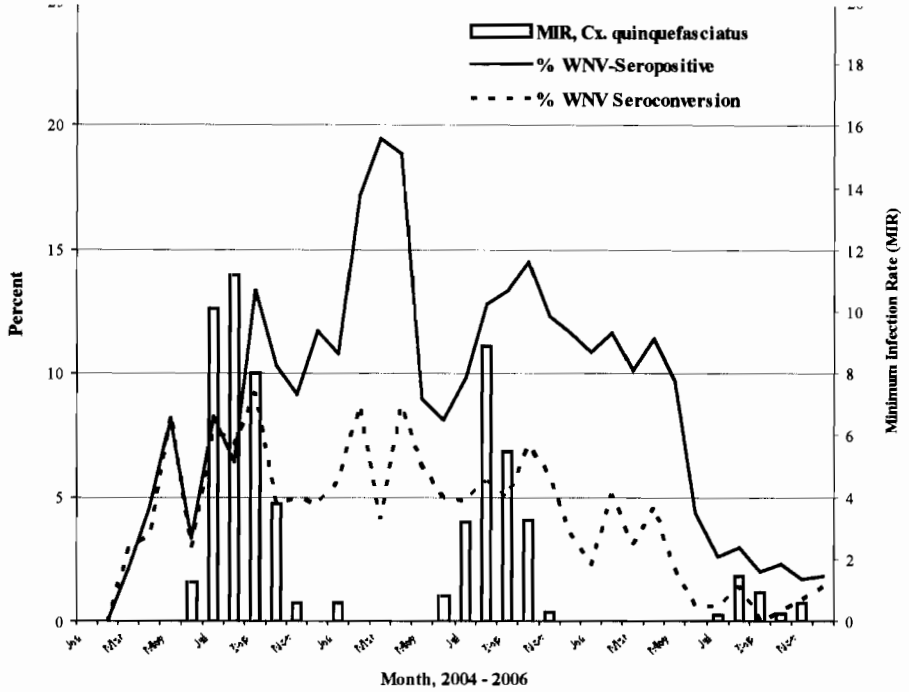

Figure 2. WNV-seroprevalence and seroconversion rates in free-ranging house sparrows and house finches and minimum infection rates (MIR) in *Cx. quinquefasciatus* for each month from 2004 – 2006.

Table 4. Numbers of WNV-positive dead birds / total tested per month, 2004 – 2006.

Year	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Totals	Percent
2004	0/8	0/5	0/14	2/18	4/20	43/81	62/91	60/72	58/71	14/29	7/14	3/9	**253/432**	**58.6%**
2005	1/20	2/26	6/46	2/22	28/92	99/168	42/80	79/101	28/46	10/35	3/15	2/14	**302/665**	**45.4%**
2006	0/9	1/7	3/18	6/19	4/22	5/32	2/26	12/36	8/22	3/30	4/19	1/10	**49/250**	**19.6%**

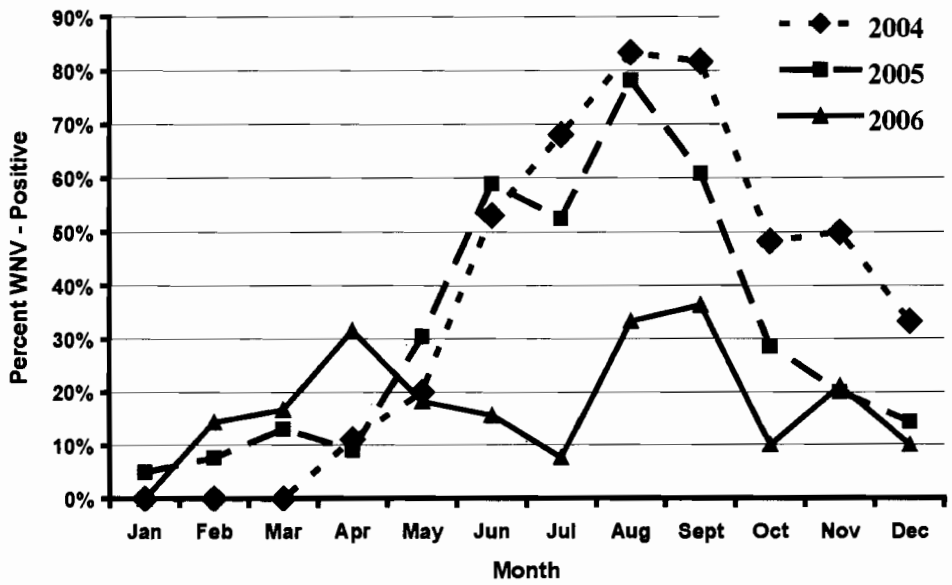

Figure 3. Comparison of monthly WNV-positive dead bird rates, 2004 – 2005.

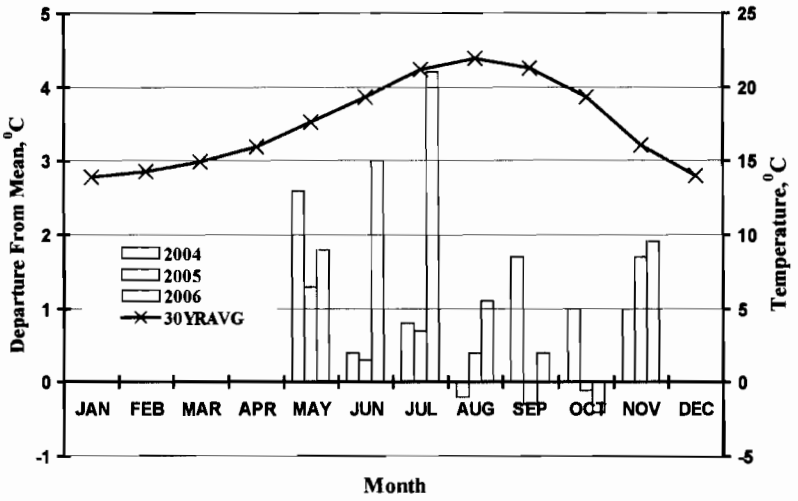

Figure 4. Temperature comparisons (°C), showing monthly departures from historical means for the months of May – November, 2004 – 2005.

RESULTS AND DISCUSSION

Data from all three arboviral surveillance systems demonstrated a significant decline in detectable WNV activity in Orange County during 2006 compared to the two previous years. Similar declines in the WNV Minimum Infection Rate (MIR) in the mosquito population and levels of WNV-positive dead birds were observed in adjoining southern California counties in 2006 (Riverside, San Bernandino, Los Angeles, and San Diego) (http://westnile.ca.gov) along with similar reductions in WNV-seropositive rates in free-ranging wild birds of Los Angeles and Riverside counties (Madon, Williams, and Hess, personal communication 2006).

The WNV MIR for *Cx. quinquefasciatus*, Orange County's primary WNV vector (Schwedes et al. 2005, Weir et al. 2006), peaked at 1.5/1,000 in August during 2006, much lower than comparable levels of 11.2/1,000 and 8.9/1,000 for 2004 and 2005, respectively (Figure 1). The highest temporal focal MIR for *Cx. quinquefasciatus* reached 7.02/1,000 at Craig Park in Fullerton during a six-week period in July – August 2006, where 9 of the District's yearly total of 14 positive pools were found (No mosquito pools tested positive for either St. Louis encephalitis or western equine encephalomyelitis viruses).

Unlike 2004 and 2005, WNV-seropositive rates in free-ranging wild birds did not rise during the summer/fall months of 2006 (Figure 2), and only four (4 of 1,635, or 0.25%) immunologically-naïve, antibody-positive immature wild birds were found during May – October 2006. Additionally, no wild birds (0/302) were found seropositive in September 2006 (Figure 2). Previously, approximately 12% of hatching-year birds were seropositive by October in 2004 and 2005, and up to 25% of wild birds were seropositive at active foci during the fall months of these years (Schwedes et al. 2005).

The WNV-positive dead birds were found in every month except January during 2006, but overall monthly numbers and rates declined substantially for the year compared to 2004 – 2005 (Figure 3). The decline in dead bird reports from the public, through which the District collects many of its dead bird samples, may partially be caused by a lack of public interest. The perceived threat of this disease has waned from media attention, despite the District's efforts to involve the residents of the county in WNV surveillance. Hence, some of the reduction in numbers may reflect causes other than biological factors.

The detection of WNV antibody-positive wild birds had foreshadowed the detection of WNV in mosquitoes and dead birds in 2004, ultimately indicating the emergence of multiple WNV transmission foci throughout Orange County (Schwedes et al. 2005). Reduced infection levels in wild birds, however, may have indicated antibody persistence in adult passerine birds as the result of infection to WNV from the previous year (Schell et al. 2006). Antibody persistence and herd immunity in wild bird populations may have dampened WNV transmission for the second half of 2005 through 2006.

The results of the mosquito blood meal data may shed light on the relative importance of mosquito host selection on developing herd immunity in the wild bird population of Orange County. The PCR-based methods of blood meal identification allow for direct estimates of vector contact with different avian species, which can be used to evaluate potential amplification hosts and clarify the role of these hosts in the epizootiology and ecology of arboviruses (Molaei et al. 2006). Apperson et al. (2002) found in New York City that *Cx. pipiens* did not randomly feed on birds species based upon their abundance as predicted by breeding bird surveys. Birds from the orders Columbiaformes and Passeriformes were found to be the primary hosts of *Cx. pipiens* –complex mosquitoes during outbreaks of SLE in the Midwest (McLean and Bowen 1980). The host-seeking behavior of *Cx. quinquefasciatus* appears to follow a similar pattern in Orange County: PCR analysis of 179 blood-fed *Cx. quinquefasciatus* females indicated that collectively, mourning doves (*Zenaida macroura* L.) and house finches were the preferred hosts ($P < 0.01$) for this mosquito (Figure 4); these two avian species comprised 43.6% (78 of 179) and 33.5% (60 of 179), respectively, of

identified blood meals. Contrastingly, American crows (*Corvus brachyrhynchos*) accounted for only 2.2% (4 of 179) of the blood meals (Table 2). Blood meal numbers for *Cx. tarsalis* and *Cx. erythrothorax* were too small for analysis; however, of these small numbers, *Cx. tarsalis* was found to have fed primarily off mourning doves and house finches, while *Cx. erythrothorax* sought mainly mammals (Table 2).

In a series of artificial infection experiments, Komar et al. (2003) and Reisen et al. (2005) obtained similar results, finding that adult mourning doves survived WNV infection and produced a moderate viremia around 3 – 5.5 $\log_{10}$ plaque forming units per milliliter (PFU/mL) for 3 - 5 days. WNV seroprevalence studies of free-ranging wild birds in Illinois (Beveroth et al. 2006), Kern (Carroll et al. 2006), and Sacramento and Yolo (Wright et al. 2006) counties have shown that mourning doves are abundant and have relatively high seropositive rates (14% – 40%), indicating frequent exposure to mosquitoes and high survivability when infected. Herd immunity in mourning doves may play a significant role in attenuating WNV amplification in Orange County, when considering mourning dove abundance, longevity, seroprevalence levels, and the host-seeking preferences of *Cx. quinquefasciatus* (Figure 5).

From June through September, six human cases (no deaths) were reported in 2006, a dramatic decline from the 64 cases (4 deaths) reported in 2004 and a modest reduction from the 17 cases in 2005. The first human case occurred in June, approximately one month before the first WNV-positive mosquito pool. Prior to the first known human infection, avian serosurveillance in free-ranging house sparrows and house finches and testing of dead birds had demonstrated reduced, but continuous WNV transmission throughout 2006 in the county, suggesting a persistent, low-level risk to the public. Overall, fewer human cases may have been the result of a combination of wild bird immunity, lower mosquito infection rates, better mosquito control and public awareness of disease prevention.

CONCLUSION

Year-round arbovirus surveillance in 2006 continued to demonstrate that WNV is endemic in the suburban *Cx. quinquefasciatus*-peridomestic small bird cycle of Orange County and remains a threat to the public. Although detectable WNV activity declined throughout the county during 2006 compared to the two previous years, seasonal MIRs (~ 7.0) and avian antibody seroprevalence levels (~ 5.0%) remained relatively high at several foci. Future WNV abundance is likely to undergo yearly oscillations, decreasing and increasing with changes in avian immunity and shifts in mosquito numbers and species composition. The relative roles of vertical transmission, vector competency, and host preferences in the mosquito population, coupled with the roles of chronic infections and the attenuation of WNV amplification via herd immunity in avian reservoirs, along with the inability to discriminate between old and new infections in these hosts, remain to be determined for predicting the occurrence of WNV epizootics in Orange County.

Acknowledgments

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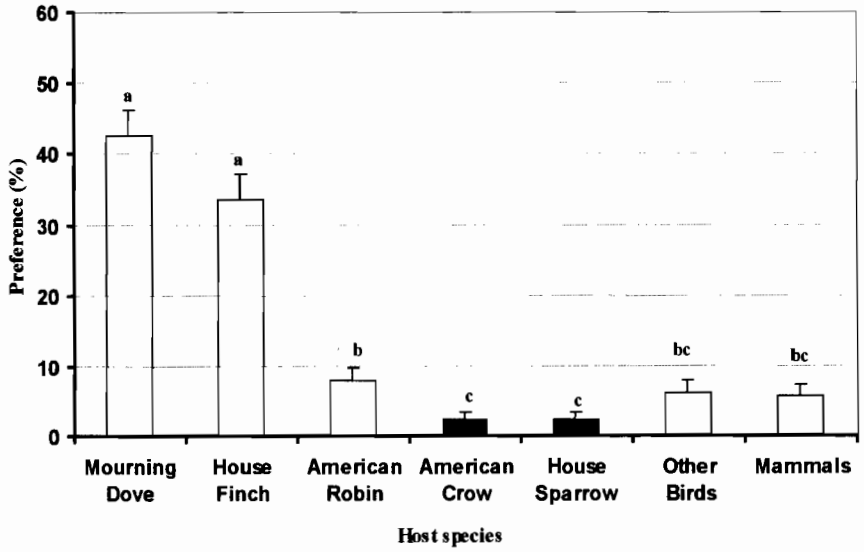

Figure 5. Host preferences for *Cx. quinquefasciatus*. Different letters indicate significant differences among various host species at 0.05 levels by Chi-square test. $X^2 = 36.1$, $P < 0.01$ for differences between mourning doves and house finches versus other sources (N = 179).

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Evaluation of an Avidity Test for Detection of Early/Current West Nile virus Transmission in Avian Populations

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ABSTRACT: The avidity (or functional affinity), which refers to the multivalent binding between antibody and antigen, reflects the strength of an immune response during the process of infection and the development of long-term immunity. Avidity determinations of viral antibodies in human sera have thus been performed since the late 1980's, with the goal of separating current ongoing infections from older ones. The techniques have been applied to various viruses since 1984, and more recently to Tick-borne Encephalitis virus, Dengue, and West Nile virus (WNV). The relevance of such tests for discriminating recent from old infection in avian sera has not been evaluated.

A blocking ELISA was performed on untreated and urea-treated sera to test two sets of specimens for avidity: 1) Sequential sera collected every two weeks from 16 WNV- positive passerine birds selected from a population of 29 individuals confined to a mosquito-proof cage for 1 to 21 months. 2) Six weekly serum collections from experimentally infected song sparrows, provided by the University of California, School of Veterinary Medicine. An inhibition ratio and an avidity index ratio were calculated for each serum (12 experimental and 425 caged birds). In experimental birds the avidity started to rise significantly after the third week but the sample was too small to make any kind of extrapolation. The avidity results obtained from the 16 caged birds over time did not show any pattern suggestive of an early immune response. The distribution of avidity values was scattered, erratic, and inconsistent from one bird to another; and there was no statistical significance when the averaged tests were compared as a function of the sampling week. Such results do not corroborate the results from avidity tests performed on mammalian sera. This difference may result from the unique quality of the avian immune response which may not mature with a distinct and strong affinity. It is also possible that the blocking ELISA, as used in this study, needs to be improved by modulating such parameters as pH and temperature. At this point the test will not help discriminate between current and old infections. Thus, further testing strategies will have to be pursued.

INTRODUCTION

For more than 20 years, the routine antibody testing of wild birds has been part of the arbovirus surveillance routine at the Orange County Vector Control District, along with the detection of virus from mosquitoes and sick/moribund crows. On the average 3,500 to 4,000 specimens were collected yearly. House finches and house sparrows trapped weekly at 12 fixed locations were bled, banded, and released. Although the rate of recapture can reach 35%, it is rare to obtain a negative and a positive specimen less than one or two months apart. Thus, it is impossible to assess the exact time of West Nile virus (WNV) or St.Louis encephalitis (SLE) sero-conversion. Such information would be paramount to our understanding of ongoing transmission and might help redefine our vector control strategies. To compound this issue, the occurrence of antibodies in an area where two or more flaviviruses coexist, may be misleading since the first immune response detected might be due to the first infecting virus (antigenic sin) and not to the current infection. The final diagnosis is further complicated by the existence of cross-reactions often observed with conventional ELISA, hemagglutination-inhibition and even neutralization tests.

The immune reaction, which results from the formation of an homologous or heterologous antigen (AG)-antibody (AB) complex, is a dynamic event that evolves over time and involves the participation of different epitopes and even molecular configurations characteristic of each virus. The quality of immunity may be reflected by the antibody titer, which will rise following infection and may plateau with age. The strength of the multivalent AG-AB bond, defined as avidity (or functional avidity), can be measured and provides a better qualitative assessment of the immune response. Typically the avidity of the AG-AB complex following a primary infection will be low, whereas, old and past infections will exhibit a very high avidity. The measurement of avidity has thus been used since 1984 for the diagnosis of new or current human infections with a variety of infectious diseases, notably mononucleosis, rotavirus, hepatitis C, rubella, mumps, influenza, EBV and cytomegalo-virus to name a few (Schubert et al. 1998, Baccard-Long□re et al. 2001) and even parasites (Picher et al. 2000, Marcipar et al. 2001, Lecolier and Pucheu 1993). Studies of avidity with arboviruses are recent, and have been limited to tick-borne encephalitis (Gassman et al. 1997), Dengue (de Souza et al. 2004) and West Nile (Prince et al. 2005, Levett et al. 2005, Fox et al.

2006). In most instances, an avidity index of less than 40% might be presumptive of a recent infection (less than 20 days), whereas, an index between 40% and 60% might indicate exposure within 45 days following exposure. One study undertaken in deer mice experimentally infected with Sin Nombre Hanta virus shows similar results (Safronetz et al. 2006).

This is a first attempt at measuring the avidity of West Nile (WN) antibodies to distinguish between recent and old or recurrent infections in field collections of avian specimens.

MATERIALS AND METHODS

Avidity test

The determination of WN antibodies was made by blocking ELISA, using the test developed at the University of Queensland, Australia (Hall 1995) and evaluated at the District (Jozan et al. 2003). This study was performed using a whole cell lyzate prepared from the 1999 New York strain, and two monoclonal antibodies reacting respectively against a broad spectrum of flavivirus (3H6) and against the NS1 West Nile epitope (31112G).

The Kunjin virus (KUN), genetically indistinguishable from West Nile, was chosen as the antigen of choice for the avidity test, because it provided better specificity. This recombinant antigen was prepared from lysates of SF9 *fall army worm* cells infected with a recombinant baculovirus expressing the NS1 protein, derived from the KUN virus strain MRM 61C sequence (Clark, Heise-Seabrook, Nisbet, and Hall, unpublished results, Kim Pham, to be published). The anti-KUN NS1 antibodies are reported as West Nile antibodies to simplify this presentation.

For the purpose of avidity testing the following modifications were made: After overnight incubation of the antigen at 4° C, test sera diluted 1:20 to 1:320 were added to two plates in duplicate and incubated for two hours at 32° C. At the end of the incubation period, the plates were washed twice in PBS-Tween. To one plate 0.1ml of PBS pH 7.5 was added and 0.1ml of 7 Molar Urea in PBS was added to the duplicate plate. Both plates were incubated at room temperature for six minutes, and then rinsed three times in PBS-Tween. The test was then carried on as usual, with the addition of anti-WN NS1 monoclonal 31112G for one hour, followed by four rinses, then anti-mouse peroxidase conjugate for one hour, six more rinses and addition of substrate ABTS. Percent of inhibition of mab for each test sample was calculated using the formula (100- [(TS-B/TC-B) x 100]) where TS-B is the test sample value minus mock and TC-B is the negative serum control value minus mock.

Optical densities were read with a microplate spectrophotometer reader at dual wave lengths of 414 and 492. Results were transferred to an excel spread sheet for final calculations.

The measure of avidity was calculated in two ways: a) the percent inhibition obtained by usual blocking ELISA was designated as inhibition A and percent inhibition obtained after treatment of serum with urea was designated as inhibition B. The inhibition ration B/A will be close or equal to 1 if there is no reduction in antibody titer after urea treatment, and this would indicate an avidity between 60-100%; b) for the avidity index, the optical density of the serum tested with blocking ELISA was designated ODA and ODB. The ratio ODB/ODA used in conventional ELISA test had to be reversed because with blocking ELISA a low OD indicates a positive test and vice versa. Thus, ODA/ODB will be close to 1 if there is no change after urea treatment.

Test Sera

Experimentally infected sparrows (courtesy Aaron Brault, University of California Davis): Two song sparrows were inoculated with the 1999 New York strain West Nile virus (0.1 ml subcutaneous). Blood samples were taken weekly for six weeks following inoculation and stored at –70° C.

Captive house finches and sparrows: Between January and August 2005, 29 birds which had tested positive for the first time in the field were transferred to a mosquito-proof cage and bled every two weeks for 21 months (Schell et al. 2006). The duration of anti NS1 antibodies is shown on Table 1; antibodies persisted for what

Table 1. Distribution of West Nile antibody positive and negative samples for 29 birds confined to mosquito-proof cage and bled every two weeks.

Band #	Duration (months)	# Positive samples	# Negative samples	Total # samples
03-0832	1	0	3	3
05-0698*	2	3	3	6
08-2316*	2	3	0	3
03-0859	3	3	3	6
04-1478*	3	5	3	8
01-0707	3	0	7	7
04-1272	4	0	9	9
03-0858	4	9	1	10
05-0444	5	0	11	11
05-0745	5	11	2	13
05-0509	5	1	8	9
05-0546*	6	9	6	15
05-0443*	7	12	3	15
03-0932*	11	3	26	29
04-1491*	11	15	10	25
07-0882	13	1	18	19
01-0655	15	0	30	30
05-0606	15	1	32	33
01-0624	16	2	33	35
03-0702*	16	6	29	35
03-0891*	17	21	15	36
06-0384	18	0	41	41
05-0424	18	2	35	37
03-0510*	18	22	19	41
03-0812*	18	7	34	41
03-0699*	19	37	5	42
05-0616*	18	27	13	40
04-1287*	19	14	29	43
03-0577*	21	23	23	46
Total		237	451	688

* selected for avidity analysis

could be considered the lifetime of this particular avian species (up to 23 months). The median titer was 1:320 at the beginning of the study but steadily decreased to 1:20 in the latest months of observation. There was a consistent pattern of sera becoming negative for a few weeks then reverting to positive, often with a high titer. A total of 16 birds was selected from this population, representing 5 of 13 birds confined for 2 to 7 months (38% of total sample) and 10 of 16 birds confined for 11 to 21 months (63%), each sampled group providing, respectively, 47 and 378 serum samples for testing. This selection was done on the basis of multiple positive sera, duration of observation and existence of recaptures as early as 2004, prior to inclusion into the study. An additional bird was added to the study because it was consistently recaptured and provided a field control.

Statistical analysis (S. Su, West Valley Mosquito and Vector Control District): "Avidity values" from the 425 samples tested were averaged as a function of sampling week and compared by using one-factor ANOVA at a P value of 0.05. This analysis covered only 80 weeks of observation (40 sampling weeks after first positive capture) since only five birds remained up to 100 weeks, with an occasional positive sample.

RESULTS

Experimentally infected song sparrows:

Figure 1: There was no antibody detected in the first week following inoculation. At dilution 1:20 the avidity ratio (inhibition with urea/without urea) rose from week 2 to 6. It reached 0.8 for Song sparrow 2778 at week 6 and 0.5 for song sparrow 2779 at week 5, dropping drastically at week 6 for this particular bird.

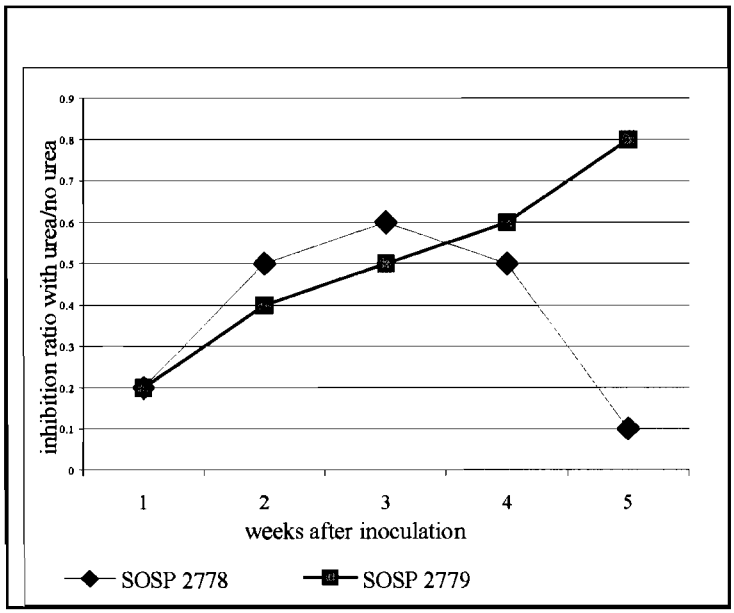

Figure 1. Inhibition ratio per week following West Nile virus inoculation of two song sparrows (A. Brault, UC Davis): IR Ratio = % inhibition after urea treatment / % inhibition of untreated serum.

Figure 2 shows antibody titer before and after treatment with urea for six weeks following inoculation in Song sparrow 2778. A serum was considered positive when the percent inhibition reached 30% or above (chosen threshold). No antibodies were detected in the first week. The antibody titer was 1:20 at week 2 and 1:160 at week 3, 4 and 5. After urea treatment, the titer falls to less than 1:20 for week 2, 3, and 4 and 1:40 at week 5. The interval between graphs of treated versus untreated sera at each dilution narrows at week 5 and disappears at week 6 when the titer drops to 1:40 after urea treatment.

Caged birds:

Figure 3 shows the antibody duration of 29 WNV positive birds during 21 months of confinement in a mosquito-proof cage. The median titer of antibodies is as high as 1:320 in the first three months of observation, and then gradually decreases over time. The occurrence of negative tests was noticeable throughout for each bird under study, with two or three antibody negative sera followed by one or more positive and a rebound in titer. There was no correlation between these particular occurrences and the month of collection or a particular individual.

Figure 4 represents the statistical analysis of avidity results for all 16 samples for 73 weeks following the first diagnosis of WNV antibodies. Results were expressed as avidity index (AI) (Fig. 4-a) and as inhibition ratio (IR) (Fig. 4-b). The distribution of values is erratic and does not relate to the length of time following apparent exposure, because high avidity values were found early in the study and low values 20 months later. There was no statistical significance at $P>0.05$. The range of values for a given week was similarly very irregular, with wide unexpected spikes. The inhibition ratio (IR) or the avidity index (AI) proved to be interchangeable since the expressed results were similarly inconsistent and without statistical significance.

DISCUSSION

The main purpose of the above study was to assess the usefulness of the avidity test to discriminate between recent and past transmission of WNV, as reflected by the presence of antibodies in wild passerines recaptured over time and subsequently confined up to 21 months in a mosquito-proof cage. A preliminary study in two song sparrows, experimentally infected with the virus, showed that the avidity did not rise before the third week following inoculation. This observation tends to corroborate the findings in some studies of mammalian sera, but such a small sample does not tell much about this avian species nor about variation within other species or families. It was anticipated that the serial collections from 16 birds every two weeks following the first positive diagnosis on the field specimen would similarly show low avidity at the beginning of confinement and an increased avidity over 60% at the end of the study. Yet, the differences in avidity index or inhibition ratio were not significant, and both low and high values were distributed with no particular pattern at each sampling point during the 21 months of observation. When these birds were found positive for the first time upon recapture, there was no way to know when the primary exposure took place and if reinfection had occurred as well. Only two birds, recaptured within 4 and 5 weeks, respectively,

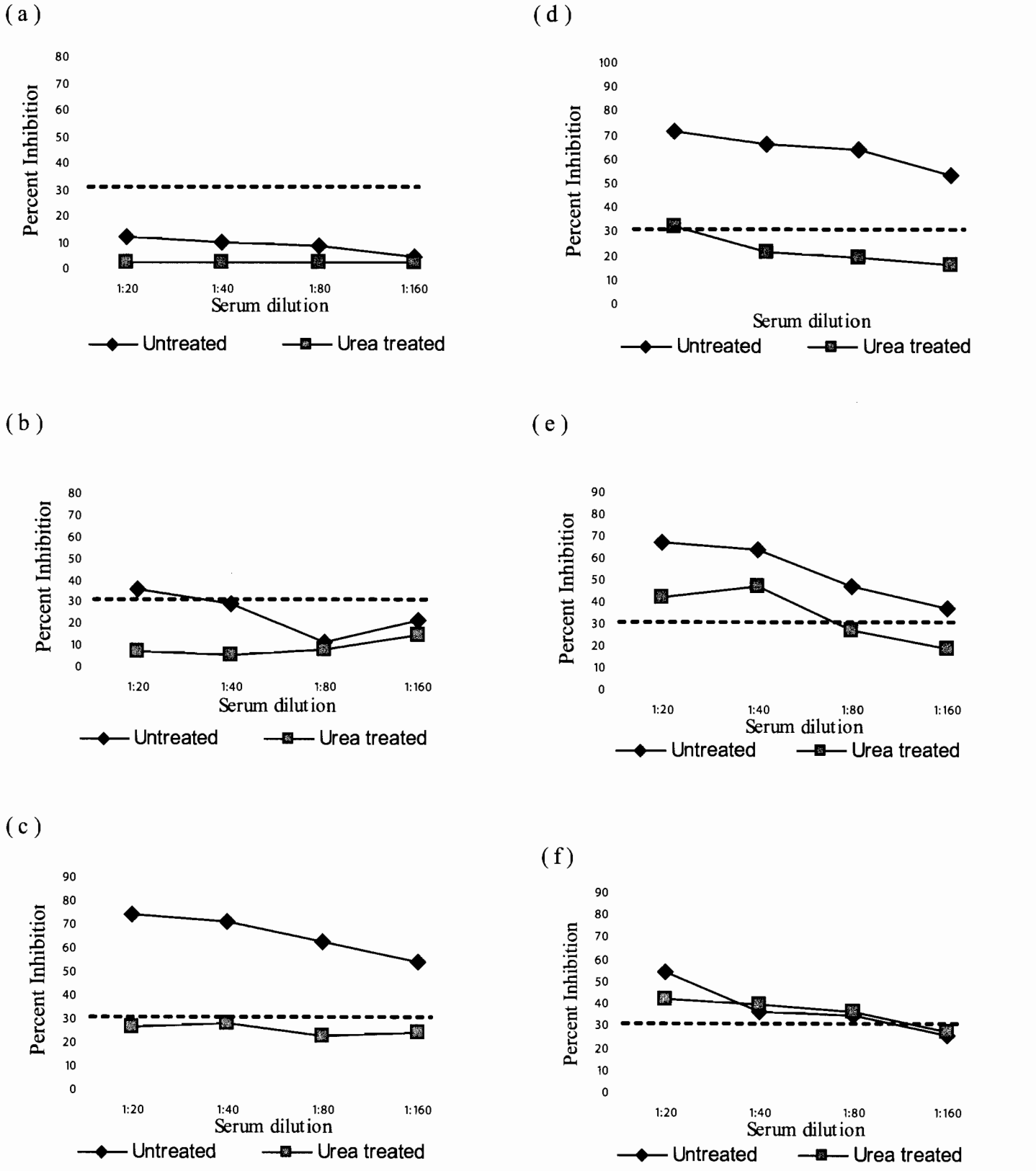

Figure 2: Comparison of West Nile antibody titer in one experimentally infected song sparrow before and after treatment with 7M urea per week following inoculation. (a) week 1, (b) week 2, (c) week 3, (d) week 4, (e) week 5, (f) week 6. The positive threshold was set at 30 percent inhibition.

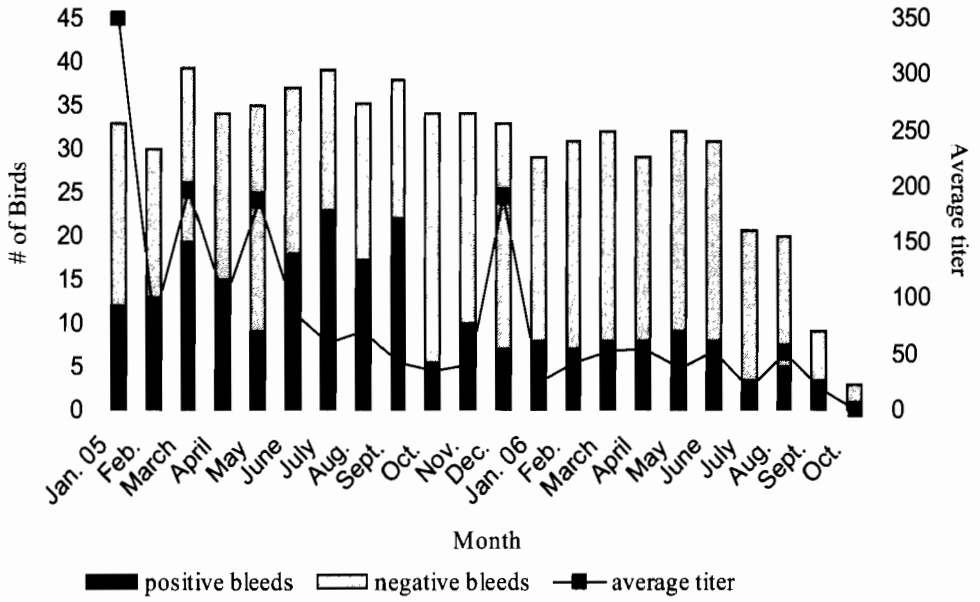

Figure 3- Duration and titer of West Nile antibodies in 29 positive house finches and sparrows confined to a mosquito-proof cage for 21 months (January 2005-October 2006).

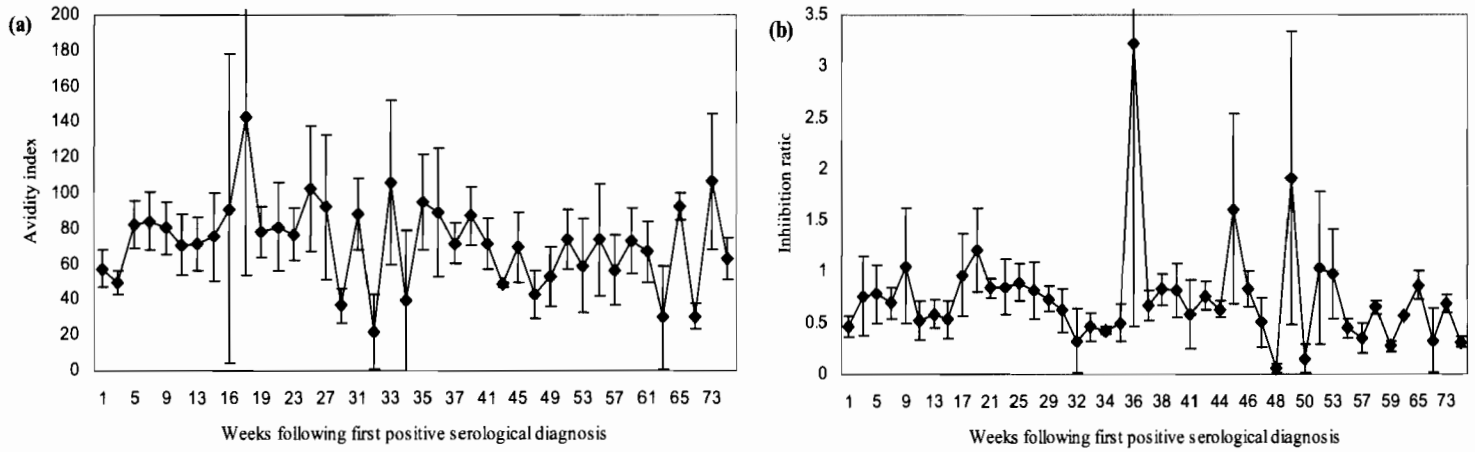

Figure 4: Statistical comparison of avidity test values for 16 birds, per week following the first specimen detected as WN positive. 4a) values expressed as avidity index (OD of untreated serum / OD of treated serum). 4b) Values expressed as inhibition ratio (percent inhibition of untreated serum / percent inhibition of treated serum).

showed a sero-conversion. In both cases the avidity of the first positive specimen was 40% and 42%, respectively. Bird 03-932 died within two months and one spike of 100% avidity was observed. In bird 04-1287 the avidity increased inconsistently, reaching its highest value at week 52, 57 and 77 following first positive result (Table 2). These erratic results might reflect the innate difference between mammalian and avian immunoglobulin. The main and ancestral avian IgY immunoglobulin differs from its IgG counterpart. There is no hinge linking light and heavy chains (Diagram 1) and an extra domain CV2 appears on the constant section of the heavy chain. Thus, the immune reaction might lack in flexibility and in its ability to agglutinate, precipitate, and even fix the complement. Perhaps, as suggested by some immunologists (E. Gould, E. Westaway personal communication, Warr et al. 1995, Carlender 2002, Klimovitch 2002), due to evolutionary constraints the immune antigen-antibody reaction of birds and reptiles does not rise to proper maturity as we conceive it in a mammalian system and therefore the ability to develop affinity might be minimal or unpredictable. This could explain the erratic results obtained in a random population, and it may not be possible to recognize any significant differences between current and old infection in field collections of naturally infected birds despite the results obtained with only two experimental birds.

Table 2. WN antibody titer, Inhibition Ratio and Avidity Index in two birds with apparent sero-conversion within five weeks.

Lab.Number	Date Collection	Titer before treatment	Inhibition Ratio	Avidity Index
Specimen 03-932				
OCV06-1404	6/8/05			
OCV05-2159 +	7/19/05	1:40	0.58	43
OCV05-2653	8/16/05	≥1:160	0.6	100
OCV05-2824	8/31/05	1:20	1.1	18
OCV05-2996	9/14/05	1:40	0.62	47
18 Negative specimens				
End of study: 8 weeks following the first positive				
Specimen 04-1287				
OCV04-0744	4/28/2004			
OCV04-1514	6/2/2004			
OCV04-2069	7/20/2004			
OCV04-2400	8/3/2004			
OCV04-2576	8/17/2004			
04OCV-2834	9/14/2004	≥1:160	0.47	40
04OCV-3082	10/13/2004	≥1:160	0.62	49
04OCV-3643	12/10/2004	≥1:160	0.7	51
OCV05-0044	1/5/05	≥1:160	0.53	29
OCV05-0320	2/4/05	1:80	0.39	36
OCV05-0420	2/16/05	≥1:160	0.11	57
OCV05-0516	3/2/05	≥1:160	0.3	36
OCV05-0622	3/6/05	≥1:160	0.3	33
OCV05-0667	3/28/05	1:80		
OCV05-0799	4/12/05	1:20	0.01	48
OCV05-2234	7/21/05	1:20	0.2	45
OCV05-3010	9/14/05	1:40	0.8	74
OCV05-3132	9/30/05	1:40	0.6	81
OCV06-0601	3/1/06	1:20	0.2	66
End of study: 92 weeks following the first positive				

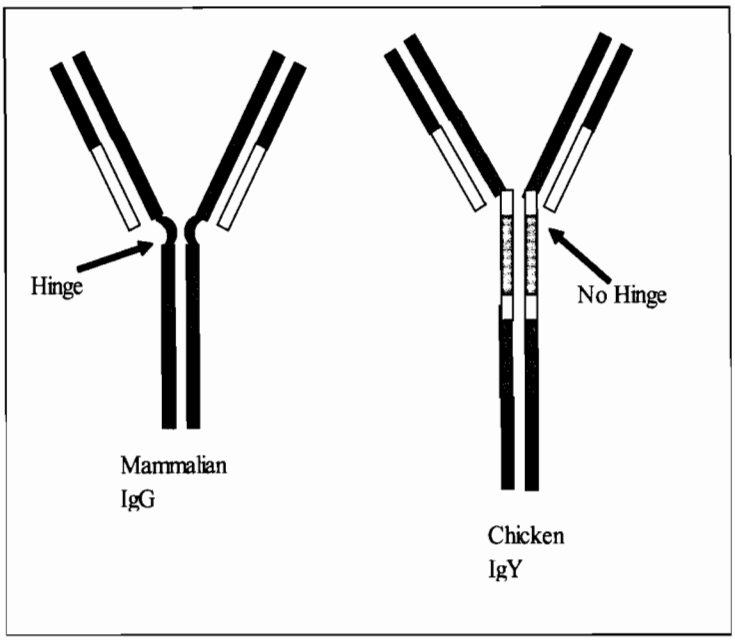

Diagram 1: Morphological differences between mammalian and avian immunoglobulins. The chicken IgY is given as an example. Characteristics differs in duck, and possibly other avian species

The duration of antibodies in finches and sparrows was long lasting, as much as 21 months for some birds, but it was interrupted consistently by an apparent loss of antibody, which had no correlation with season or particular birds. More negative results were observed in the last six months of the study at a time when the titer was also tapering off as the bird reaches the end of its lifetime expectancy. This alternation of negative and positive results is puzzling and tends to indicate that the finding of a negative bird during the transmission season may obscure the true incidence of the virus. Therefore, a larger collection sample would be necessary to minimize such occurrence.

Another problem in testing avian sera might rest within the parameters used for the blocking ELISA. The test could be improved by modulating both pH and temperature, as they may be of particular importance (Slaght et al. 1979, Lucyna 2005). The evaluation of avidity, either with the classical avidity index or the inhibition ratio, may not be sensitive enough to exemplify clear differences or define a threshold of avidity to ascertain a recent/ current infection. This question has been raised by others, who designed their own mathematical schemes (Korhonen et al. 1999, Kneitz et al. 2004).

In conclusion this preliminary study did not provide the expected results that avidity tests in human and mammalian sera have given for many years with many different types of viruses. The refinement of the technique and its application to more experimental birds may prove to be the only venue available to decide whether or not this test could provide relevant information during active circulation of flaviviruses in the field.

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West Nile Virus in Orange County: Stepping into Endemicity

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ABSTRACT: West Nile Virus (WNV) emerged in the United States in the fall of 1999. In 2000, the Orange County Vector Control District (OCVCD) initiated a comprehensive and integrated surveillance program consisting of three components, each linked to an aspect of the biology of WNV: avian serosurveillance (sentinel chickens and free-ranging wild birds), dead bird viral assay by immunohistochemistry and mosquito viral surveillance by PCR. The subsequent location mapping of these components over the past 4 years indicates a probable change in WNV epidemiology from epidemic to endemic. The question now arises, how will these changes affect the future WNV surveillance efforts of OCVCD in Orange County? By careful analysis of the WNV enzootic lifecycle, it will be shown that surveillance emphasis must now be shifted to "endemic foci." By defining the geophysical parameters of "endemic foci" and searching for areas that display these parameters, a plan of future surveillance can be constructed. Evidence will be presented on defining and illustrating one such "endemic foci."

INTRODUCTION

According to the U.S. Census Bureau, Orange County has a total area of 948 sq. mi. Surface water accounts for 159 sq. mi., while 789 sq. mi of it is land. The county is composed of a variety of ecotypes, including urban, suburban, riparian flood channels and coastal mountains. Extensive acreage is devoted to parks and open-space for outdoor recreation. The southern part of the County still includes large, relatively undeveloped sections of coastal sage scrub habitat. In addition to man-made mosquito breeding habitats, such as drainage channels etc., there are several natural mosquito producing fresh and saltwater wetlands within the county, especially in coastal areas. Based on our four-year data on mosquito surveillance and WNV enzootic cycle, the present report provides an account of the endemic foci of WNV in Orange County.

MATERIALS AND METHODS

Mosquito Surveillance

Mosquitoes were collected weekly from sites throughout the county. A combination of CDC/CO_2–style, host-seeking EVS traps (Rohe and Fall 1979) and Reiter/Cummings gravid female, ovipositional traps (Cummings 1992) were used to collect mosquitoes. Collected mosquito pools were identified to species, counted and submitted for arbovirus testing by multiplex reverse transcriptase-polymerase chain reaction (RT-PCR).

Avian Serosurveillance

Wild bird serosurveillance consisted of the capture of free-ranging wild birds in modified Australian crow traps, set throughout the county and baited with fresh water and wild bird seeds (McClure 1984). Birds were banded, identified to species, sexed and 0.2 ml of blood was collected from the jugular vein. Serum from blood samples were subsequently tested for antibodies against WNV by hemagglutination inhibition (HAI) (Gruwell et al. 1988) and blocking ELISA assays (Jozan et al. 2003) in the OCVCD microbiology laboratory.

One sentinel chicken flock located along the San Diego Creek watershed in Central Orange County was bled bi-weekly throughout the year, and blood samples were tested for SLE, WEE, and WNV antibodies at the California Department of Health Services and the OCVCD Laboratory.

Dead Bird surveillance

Dead bird surveillance consists of sampling dead birds throughout the county for the presence of WNV. Birds were collected by the staff of OCVCD, with the help of the public, animal rehabilitation groups and animal control agencies. Necropsies were conducted by a veterinary pathologist on carcasses deemed suitable for WNV testing and at least liver, spleen and kidney were collected and fixed in 10% buffered-formalin. Following fixation, tissues were embedded in paraffin, sectioned at 6 μm and mounted on charged slides. Slides were then stained by a WNV specific immunohistochemistry (IHC) procedure to detect WNV envelope protein (Jozan et al. 2003).

RESULTS

Orange County and the WNV experience

In September 2003, West Nile Virus was first detected at low levels in birds collected by two free-ranging wild bird traps (Fig. 1); 5 positive House finches (*Carpodacus mexicanus*) of 3424 wild birds collected and sampled. In addition, the dead bird surveillance program identified WNV in 3 birds during the month of October, 2 of which were American crows. WNV was not detected in any mosquito pools.

During 2004, WNV activity in Orange County was clearly epidemic (Fig. 2), being distributed predominantly in the highly-populated northern-half of the county. High numbers of free-ranging wild birds, dead birds and mosquitoes tested positive for WNV or antibodies to it. WNV infection was also detected in humans in the county.

In 2005, the WNV epidemic continued in the same county-wide distribution as 2004 (Fig. 3). However, there was an unusually large cluster of activity in the Coastal and inland marshes, which was probably the result of a tremendous increase in *Culex tarsalis* Coquillett reproduction, following torrential rains in the fall and winter of 2004-2005.

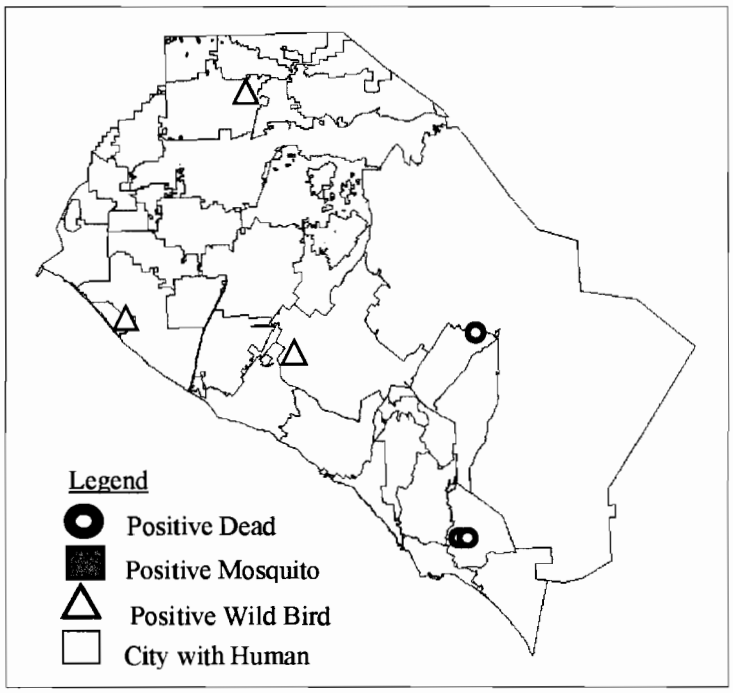

Figure 1. Map of Orange County depicting locations of positive West Nile Virus detection in dead and free-ranging wild birds during 2003.

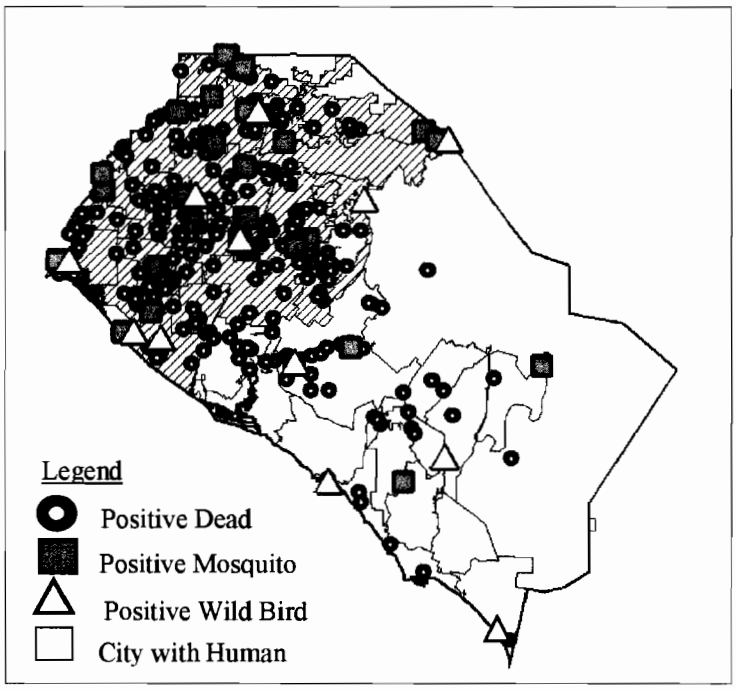

Figure 2. Map of Orange County depicting locations of positive West Nile Virus detection in dead and free-ranging wild birds and mosquito pools during 2004.

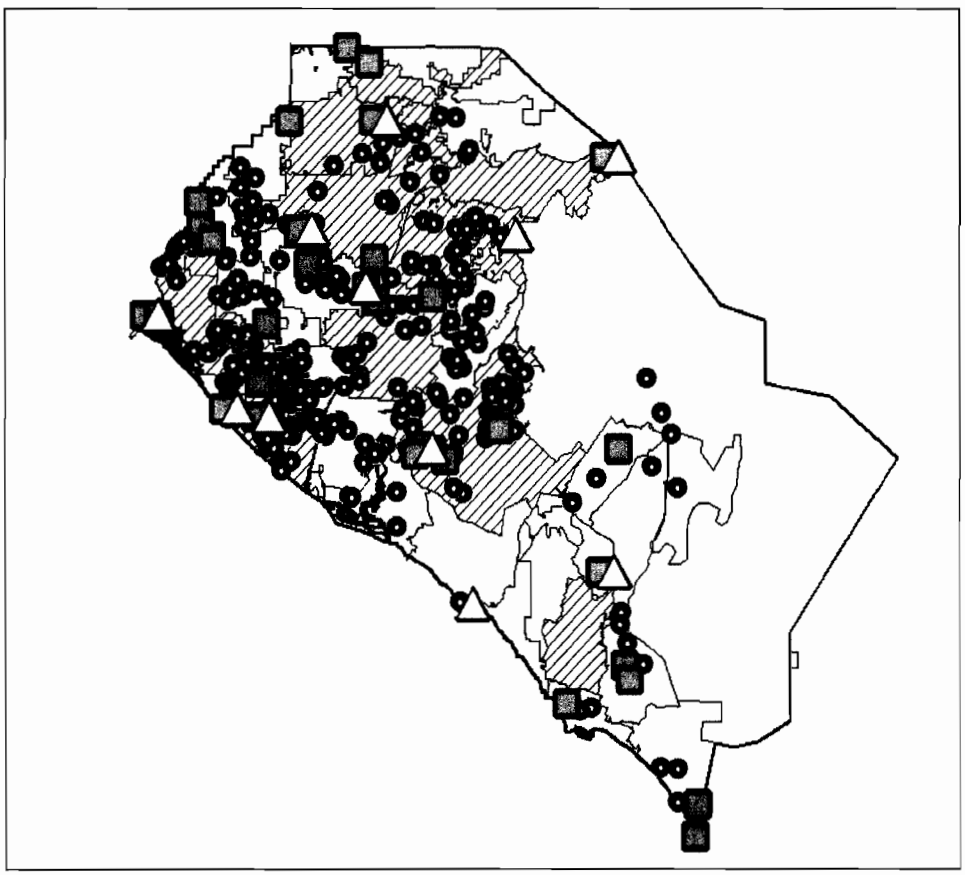

Figure 3. Map of Orange County depicting locations of positive West Nile Virus detection in dead and free-ranging wild birds and mosquito pools during 2005.

In 2006, there was a dramatic reduction in the numbers of WNV positive dead birds and mosquito pools (Fig 4). However, seropositive, free-ranging wild birds were still found in the same general county distribution as noted in 2005 (Fig. 3).

Overall, during 2004-2006, *Culex quinquefasciatus* Say was the most abundant mosquito trapped, accounting for 257 out of 288 positive pools. House finches had the highest sero-positive rate (3.9%) of avian species sampled and were the most frequently collected wild birds (8,403 of 12,922). Corvids (American crow, Common raven, Scrub jay) made up the majority of WNV positive dead birds (508 of 604).

Ted Craig Regional Park: A WNV "endemic focus"

Craig Park was one of the first sites to show WNV activity by all three methods of the OCVCD integrated surveillance plan (free-ranging wild birds, dead birds and mosquitoes) (Schwedes et al. 2005). Located in northern Orange County, in the suburban city of Fullerton, Craig Park encompasses 124 acres of open space with a small pond, two year-round creeks and a large variety of mature trees.

Low levels of WNV activity were detected in wild birds at the Craig Park site late in 2003 (Figure 5). The highest levels of WNV activity occurred in the summer of 2004 (Figure 5). The MIR (Minimum Infection Rates) for *Cx. quinquefasciatus* peaked at 20 for July and August and positive mosquito pools were detected from July to October.

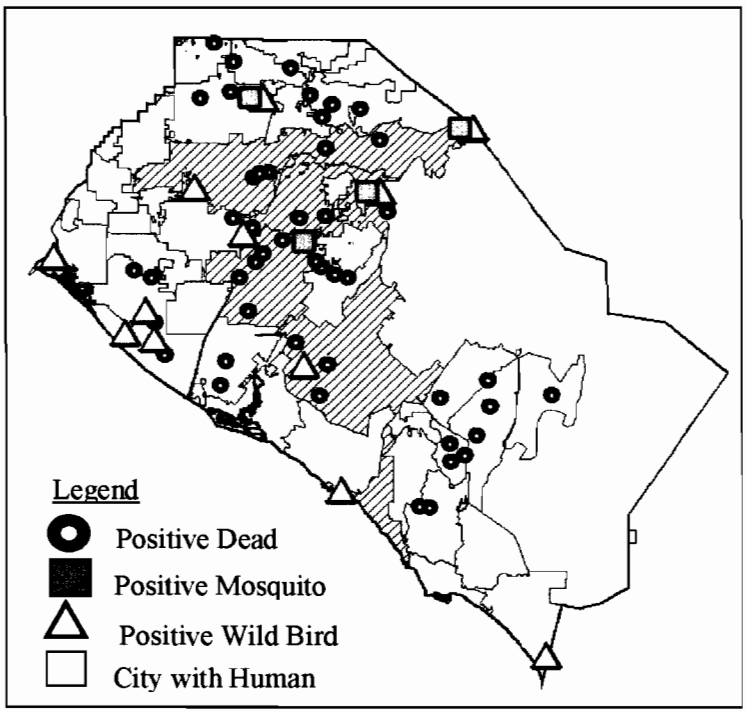

Figure 4. Map of Orange County depicting locations of positive West Nile Virus detection in dead and free-ranging wild birds and mosquito pools during 2006.

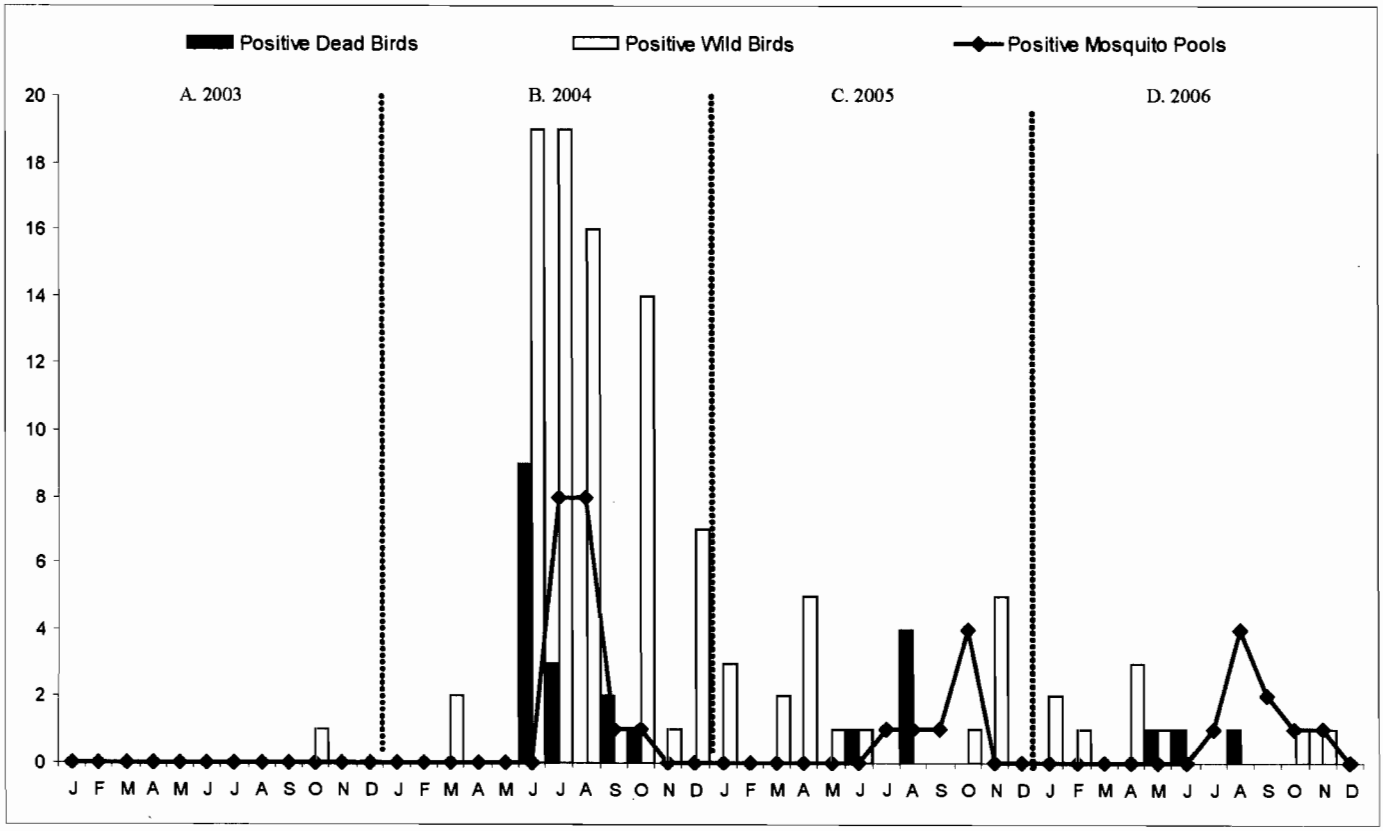

Figure 5. Mortality numbers of WNV positive dead birds (within 2 mile radius), free-ranging wild birds and mosquito pools at Ted Craig Regional Park, 2003-2006.

In 2005, the number of positive wild birds detected at the park decreased (Figure 5). The MIR for *Cx. quinquefasciatus* reached its highest levels of 16.4 in October. Positive mosquito pools were detected during the same months as in 2004. Interestingly, WNV antibodies were not detected in 78 free-ranging wild birds bled between July and September but a few WNV-positive dead birds were detected during those months within a 2 mile radius from the park.

WNV continued to be detected through 2006 in much lower levels (Figure 5). The MIR for *Cx. quinquefasciatus* peaked at 9.1 during August. Again, WNV antibodies were not detected in 154 free-ranging wild birds bled between July and September. However, positive mosquito pools and a few positive dead birds were detected during those months.

DISCUSSION

Considering the trends of WNV activity detected by the OCVCD integrated WNV surveillance plan since 2003, it is not completely certain that WNV activity in Orange County can be predicted. However, it certainly appears that WNV is in transition from epidemic to endemic activity and thus, future effective surveillance sites will be relegated to "endemic foci". Our task will be to find and define these endemic foci. In other words, we must locate areas were WNV activity has been continuously detected since 2003, i.e. locations where the WNV enzootic cycle continues to be maintained (Fig. 6).

Data collected at Craig Park during the last four years illustrated the general oscillating pattern typical of an epidemic-endemic cycle (Figure 5). By definition, an epidemic disease is one that appears at higher rates than "expected." After it runs its course it may either dwindle out or persist at varied levels and locations, in other words, it becomes endemic. However, this endemicity may diminish in magnitude to the point where it is barely detectable or even give rise to periodic epidemics. It should be noted that there is a substantially higher disease frequency during an epidemic as opposed to lower more stable levels when the disease becomes endemic (Figure 5).

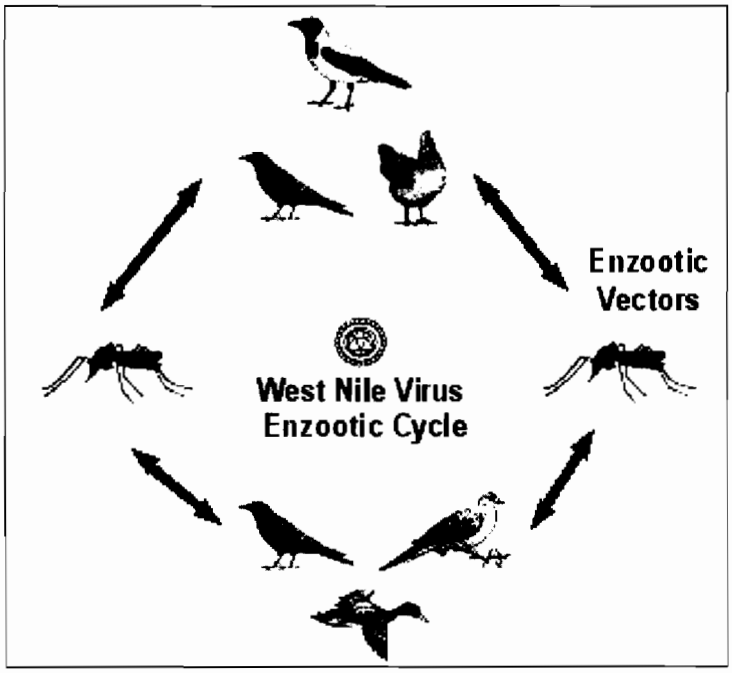

Figure 6. West Nile virus Enzootic Cycle

Now that we have identified Craig Park as an endemic focus, the next step is to determine the factors that make it suitable for the maintenance of a WNV enzootic cycle (Fig. 6). In other words, we need to define the biological and geophysical parameters at this site. It is important to establish a detailed profile of the temperature, rainfall and topography patterns of the site, as well as the number of vectors and hosts in the WNV cycle (Fig. 6). Once defined, these parameters will be used to identify additional endemic foci, allowing for the "optimization" of future surveillance plans.

Acknowledgments

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Murine Typhus in Southern California: Epidemiologic Investigation Update

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ABSTRACT: In June of 2006 Long Beach Department of Health and Human Services received notification that a person living in the city had become ill with murine typhus. This case drew particular attention from Long Beach DHHS, California DHS, and the CDC RZB being it was the first case of murine typhus reported in the city. By the end of summer, ten human cases of murine typhus were reported in Southern Los Angeles County and Orange County. Surveillance conducted around case patient homes yielded blood, tissue, and flea (849) specimens from opossums (11) and roof rats (20) that were submitted to CDC for PCR detection of *R. typhi* and *R. felis*. As of February 2007, four opossum blood specimens were negative, twelve roof rats were negative, and eight of thirty five *Ctenocephalides felis* fleas collected from three opossums were found positive for a *Rickettsia felis-like* organism by nested PCR assays. The remaining opossum, roof rat, and flea specimens will be tested in 2007.

INTRODUCTION

Every year since 1919, human cases of murine typhus have been reported to California Department of Health Services (CDHS) from local health jurisdictions in Southern California. Over the last fourteen years, typhus cases were primarily reported from residents of the foothill regions of Los Angeles County, where a suburban transmission cycle involving opossums, feral cats, and fleas occurs. A new cluster of typhus cases, herein referred to as the "Long Beach" cluster, appeared in southern Los Angeles County and Orange County in 2006, consisting of ten human cases reported from the following public health jurisdictions; Long Beach Department of Health and Human Services (Long Beach DHHS) 6 cases, Orange County Health Care Agency (OCHCA) 3 cases, and an unincorporated area covered by Los Angeles County Public Health (LACDPH) adjacent to Long Beach. The only confirmed typhus case from this geographic area was reported to CDHS in 1947 when exposure was attributed to the rat-flea-rat, urban transmission cycle of the disease. OCHCA reports no historical human or animal cases of typhus in this new focus, with no human case of typhus in the jurisdiction since 1994. In all, 21 cases of murine typhus were identified among residents of Los Angeles and Orange Counties in 2006. Ten cases are geographically assigned to the "Long Beach" cluster, with the other eleven occurring primarily in the foothill regions of Angeles National Forest. This report summarizes findings from the field investigation of cases occurring in the "Long Beach" cluster.

MATERIALS AND METHODS

Diagnosis of human cases was based initially on symptomology and a raised IgG or IgM titer from a Rickettsia IFA panel tested by commercial diagnostic laboratories associated with the hospital where the patient initially presented. When possible, laboratory diagnostics were confirmed by Los Angeles Public Health Laboratory.

The Centers for Disease Control and Prevention, Rickettsial Zoonoses Branch (CDC RZB) agreed to test animal and flea specimens by nested PCR assays for the presence of *Rickettsia typhi* and *R. felis*. Surveillance of opossums and roof rats around case patient homes and neighborhoods was conducted by Long Beach DHHS Vector Management Program (DHHS VMP), Los Angeles County Public Health Vector Management Program (LACDPH VMP), and Orange County Vector Control District (OCVCD). Whole blood samples were taken via cardiac puncture, with fleas and other ecotoparasites removed by brushing. Animals collected by OCVCD were necropsied, and brain, spleen, and liver tissues were collected for future testing.

RESULTS

Of the ten typhus cases reported from this area, eight were hospitalized from 3 to 9 days. Nine cases presented to an emergency room and one case presented to an urgent care facility. Eight females and two males, ages ranging from 4 to 81, were afflicted during the months of May (2), September (1), October (4), and December (3). Symptoms included high fever, rash, headaches, fatigue, nausea and vomiting. Two of ten cases were confirmed by paired Rickettsial IFA panels, at Los Angeles Public Health Laboratories. Eight cases showed elevated IgG and/or IgM titers in the acute phase of illness.

Human cases were interviewed to determine potential exposure routes to fleas (Table 1). Surveillance conducted around case patient homes yielded blood, tissue, and flea (849) specimens from opossums (11) and roof rats (20) that were submitted to CDC for PCR detection of *R. typhi* and *R. felis*. As of February 2007, four opossum blood specimens were negative, twelve roof rats were negative, and eight of thirty five *Ctenocephalides felis* fleas collected from three opossums were found positive for a *Rickettsia felis-like* organism by nested PCR assays. The remaining opossum, roof rat, and flea specimens will be tested in 2007.

The flea index on opossums was substantial (average of 110 fleas/animal, range10-220+), and comprised four species; *C. felis* (742), *Pulex irritans* (105), *Diamanus montanus* (2), and *Echidnaphaga gallinecea* (1). The flea load indicates that the flea population in the environment surrounding case patient homes is high. Surveillance is ongoing in case patient neighborhoods and environmental flea control plans are in development for 2007.

Table 1. Month of Illness Onset and Presence of Animals and Fleas Around the Homes of Murine Typhus Case Patients in the "Long Beach" Cluster, 2006

Case	Month of Illness Onset	Pet Cat or Dog	Dead Opossum in Yard	Opossum in Yard	Feral Cats in Yard	Rodents In Yard	Flea Bites
LB1	May	YES	YES	YES	NO	NO	denies
LB2	May	YES	YES	YES	YES	YES	denies
LB3	Sept	YES	NO	YES	YES	YES	YES
LB4	Oct	NO	n/a	n/a	n/a	n/a	YES
LB5	Oct	YES	NO	NO	n/a	YES	YES
LB6	Oct	YES	NO	YES	n/a	YES	YES
OC1	Dec	YES	NO	YES	YES	YES	denies
OC2	Dec	YES	NO	NO	NO	NO	denies
OC3	Dec	YES	NO	NO	NO	NO	denies
LA	Oct	YES	NO	YES	NO	YES	denies

LB= Long Beach DHHS
OC= Orange County HCA
LA= Los Angeles DPH

DISCUSSION

Human Cases/Seasonality/Flea Bites

No laboratory diagnostic tests are currently available to diagnoses human cases of murine typhus during the acute phase of illness. No national case definition exists for murine typhus, with no agreement between laboratories on standardized antigens and conjugates used to test for *R. typhi* or *R.felis* infections. CDHS considers paired Rickettsia IFA panels showing a four fold rise in IgG or IgM titers, taken during the acute and convalescent phase of illness adequate to confirm a diagnosis of murine typhus. Obtaining a convalescent serum often poses a challenge for physicians and local health departments since the majority of patients are discharged from the hospital and convalescing at home at the time the convalescent serum should be collected. The CDC RZB is currently developing a PCR diagnostic test for the acute phase of murine typhus and CDHS is planning to participate in the development of the test in 2007.

The epidemiologic breakdown of cases by gender, age, and symptomology is similar to what has been described from other murine typhus outbreaks in Southern California and Texas, with the majority of infections found in female homemakers and small children owning cats and dogs (Adams et. al. 1970). The majority of murine typhus cases do not report flea exposure, although exposure to infected flea feces or tissues is the primary means of human infection. Interestingly, two of three Orange Co. cases shared a residence during onset of illness, implying a similar exposure route, yet both denied flea exposure and no reservoir hosts were captured in the neighborhood.

The seasonality of illness onset parallels known flea activity patterns in the area (Figure 1). The self reported presence of dead opossums in the yards of two case patients is of note, as is the number of cases owning a pet cat. The diversity of periodomestic animals in case patient yards implies ample harborage and food sources for opossums, roof rats, and feral cats in the neighborhood.

Test Results

Sixteen animals collected around the homes of case patients tested negative for *R. felis and R. typhi* organisms by PCR. This result is expected, since previous studies show low titers of circulating rickettsiae in blood, as compared to tissues that are more typical sites for the persistence of the bacteria (Williams et. al. 1992). Fleas collected from some of these animals were found positive for a *R. felis-like* organism (Eremeeva, CDC RZB personal communication), supporting previous research showing no correlation between infected fleas and seropositive opossums (Boostroom, et al 2002). The CDC RZB is planning to amplify other genes from these samples to see if better differentiation of these rickettsiae is possible. At this time it is not known if the *R. felis-like* organism is the same organism causing human disease. Since only small subsets of collected animals have been tested, infection prevalence of periodomestic animals living in the area of the "Long Beach" cluster can not be determined at this time.

Animal Surveillance, Flea Species and Index

Opossums are known to have a small home area (0.01-0.1 miles) and opossum density in urban areas has been estimated at 75

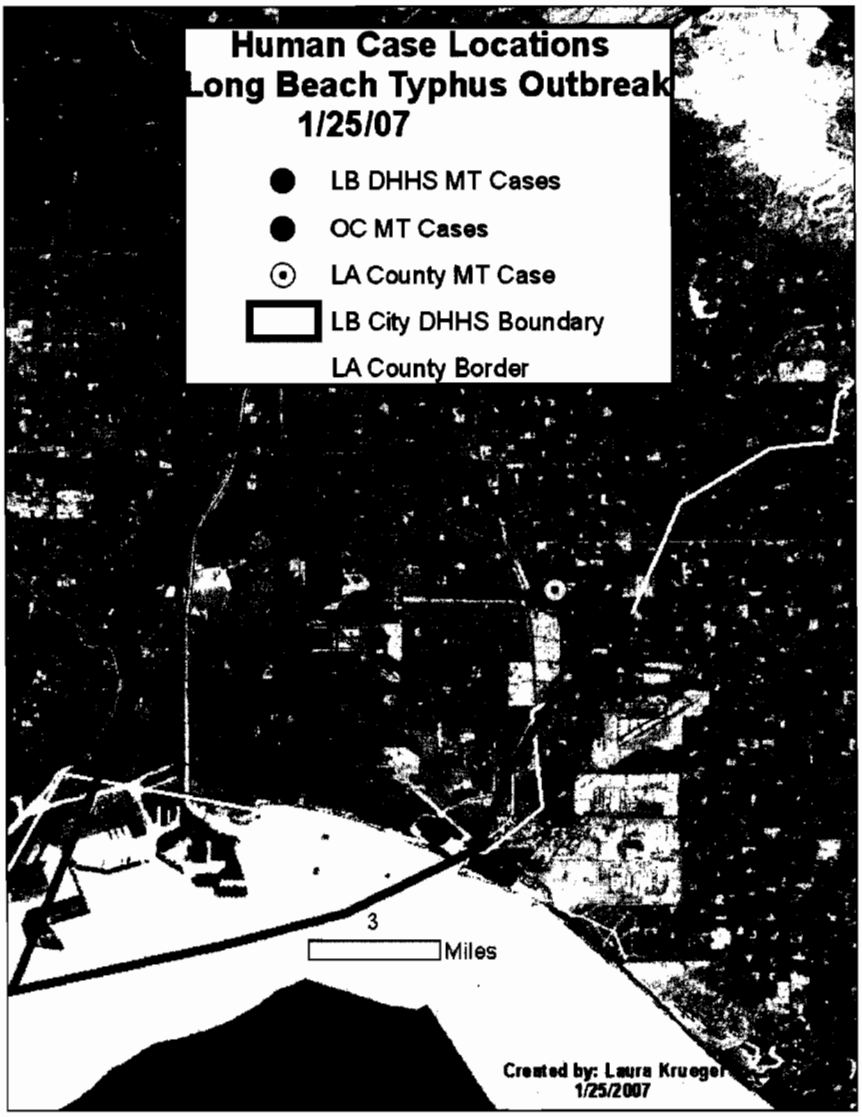

Figure 1: Distribution of human cases of murine typhus in the vicinity of Long Beach 2006.

opossums/square mile (Boostroom et. al. 2002). Seven opossums were captured and removed by OCVCD from a case patient's neighbor's backyard, implying estimates of opossum density per square mile in an urban environment could be much higher, or that opossum density is clustered in areas with substantial food and harborage. All opossums necropsied by OCVCD were underweight, with around half infested with lung flukes and stomach worms (Dr. Evans, OCVCD, personal communication). Opossums are known to be heavily infested with the cat flea, *C. felis,* in Southern California. The flea load on opossums collected in the geographical area of the Long Beach cluster indicates a substantial flea population in the environment surrounding case patients' homes.

The presence of feral cats was noted near homes of almost all cases (Table 1).

Although surveillance on feral cats was not conducted as part of the Long Beach field investigation, their importance as possible reservoir hosts for Rickettsiae should not be overlooked. Previous studies of feral cats in Los Angeles Co. found 90% with demonstrated antibody titers (Sorvillo et. al. 1993).

The scarcity of fleas and low prevalence of Rickettsiae in roof rats suggest they play a minor role in maintaining and transmitting the illness to humans (Adams et. al. 1970). Norway rats are found in neighborhoods south of PCH in Long Beach (Lamar Rush, LB DHHS, personal communication,), however none have been collected in the vicinity of case patients' homes and no confirmed activity was noted by vector control personnel. Incidental reports of Norway rats in sewers by Department of Water and Power workers in Long Beach have not been substantiated. Furthermore, no *Xenoposllya cheposis* fleas were removed from opossums collected in neighborhoods where Norway rats are assumed present, suggesting that the urban cycle of typhus transmission is not driving the Long Beach epidemic.

Surveillance is ongoing in case patient neighborhoods and environmental flea control plans are in development for 2007. Previous reports estimate that undiagnosed cases of murine typhus surpass reported cases by a factor of 4 to 1, suggesting there may be many more cases associated with the Long Beach epidemic than reported to health officials in 2006 (Traub, et al. 1978). As murine typhus is a wildlife disease, and no wildlife management has taken place, cases of the disease are expected to continue in 2007.

Acknowledgements

All personnel at the following organizations who conducted animal and flea surveillance around case patient homes: Long Beach DHHS VMP, Long Beach Animal Control, Los Angeles County PHD VMP, and Orange County VCD. The CDC ZRB, especially Marina Eremeeva, for providing laboratory testing of animal and flea specimens. Many thanks to Michael Rood, Joe Ramirez, Lamar Rush, John Holguin, Steve Bennett, Robert Cummings, Curtis Fritz, Renjie Hu, and Dick Davis for helpful suggestions over the course of the investigation.

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Where Did You Come From, Where Did You Go, Where Did You Come From *Culex. erythrothorax*, oh!

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ABSTRACT. A mark-recapture study was performed to determine if *Culex erythrothorax* from Lake Merced could cross the natural barriers and enter residential areas of San Mateo County , potentially posing a public health risk. Lake Merced is a freshwater lake in San Francisco just north of the San Mateo-San Francisco County border. The lake is lined with tules and is a larval sources for the development of *Cx. erythrothorax* Dyar. Mosquitoes were collected in $C0_2$ traps and marked with fluorescent dust. Of the 5400 marked mosquitoes, 36 (0.7%) were recaptured. 94% (34/36) of the recaptures were found along the perimeter of the lake. Only 2 *Cx. erythrothorax* crossed into San Mateo County but most remained in the vegetation surrounding the Lake Merced.

INTRODUCTION

Lake Merced is a freshwater lake on the southernmost tip of San Francisco County bordering San Mateo County (Figure 1). The rim of the lake is surrounded by tules. The proximity of tule marsh to a large population of migrating birds and recreating humans makes the perimeter of Lake Merced ideal habitat for the tule mosquito, *Culex erythrothorax* Dyar. These mosquitoes are opportunistic and vicious day and night biters that prefer birds and/ or mammals for blood-meals (Tempelis 1975). Mark-capture release studies in a southern California wetland and coastal lake revealed *Cx. erythrothorax* adults remain mostly within a 0.5 to 1.0 km range of their larval source but could be found at a maximum of 2.0 km from these sites (Walton et al 1999, Tietze et al. 2003).

An increase in service requests from the Westlake District of Daly City (San Mateo County) south and southeast of Lake Merced, in October of 2006 sparked the question: are mosquitoes from Lake Merced entering this neighborhood? More specifically can mosquitoes cross the barriers presented by the two wide roads, John Muir Drive and Lake Merced Blvd, surrounding the lake? A preliminary mark-capture-release study was performed to determine whether or not mosquitoes from Lake Merced were entering neighboring communities and if so, how far they could disperse.

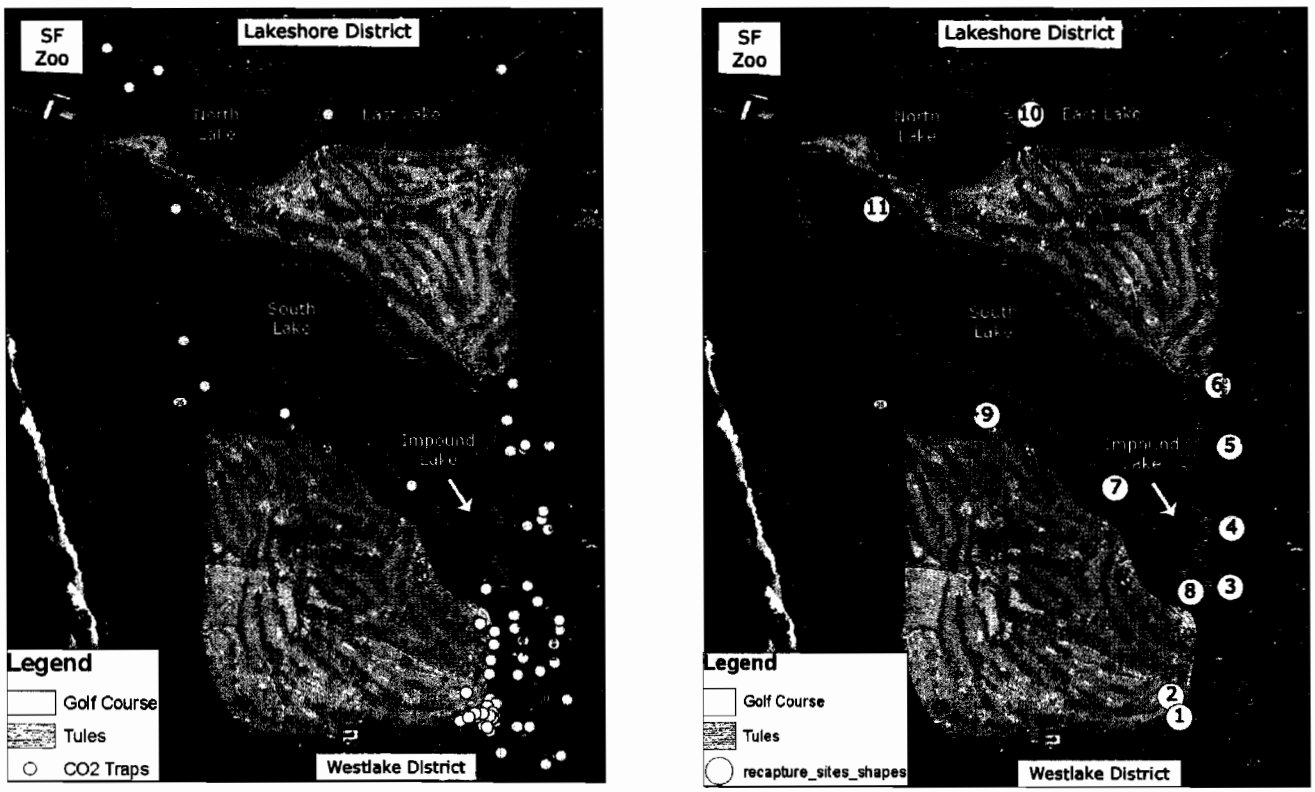

Figure 1. A) A map of the Lake Merced area including the four lakes, the Westlake and Lakeshore districts, and the golf courses. All trap sites used in the experiment are highlighted. B) Trap sites are numbered where marked mosquitoes were recaptured.

MATERIALS AND METHODS

Site Description

Lake Merced is made up of four sections: the north, east, south, and impound lakes (Figure 1). North and east lakes are connected by a small channel, covered by a foot bridge, yet separated from south and impound lakes. The latter lakes are separated by a culvert but can theoretically flow together if the water level of the lakes rises (SNRA 2006). Of the 388 acres natural areasof Lake Merced, 63% are open water and 37% are vegetated. Wetlands comprise 30% of the vegetation extending roughly 7 to 50 meters around the perimeter of all four lakes (Figure 1). California bulrush (*Scirpus californicus*) surrounds roughly 75% of the water. Swamp knotweed (*Polygonum amphibium*) is the next most abundant component of the marshland followed by smaller areas of cattails (*Typha latifolia*), rush meadow (*Juncus lesueurii*), and giant vetch (*Vicia gigantea*) (SNRA 2006).

Lake Merced is bordered by residential areas along the north, east, and south. Northwest of the lake is the San Francisco Zoo. North of the lake is the Lakeshore District of San Francisco, the Olympic Country Club is southwest of Impound Lake, and the Westlake District of San Mateo County lies south and southeast of impound lake (Figure 1).

Collection and Marking:

Culex erythrothorax adults were collected using three carbon dioxide traps at site 7 (Table 1). This site was selected because traps set in the tules at this location, during the past two years, have collected over 1000 mosquitoes per trap-night. Mosquitoes were collected in a modified ice cream container suspended below carbon dioxide-baited traps (Figure 2A). Traps were left for 24 hours.

Traps were collected and containers were visually divided into 8 sections to estimate the total number of mosquitoes. Mosquitoes in each container were enumerated and the totals were averaged (Table 2). Mosquitoes were marked by inserting a mucus trap (Figure 2B) filled with fluorescent dust into a hole cut out of the side of the ice cream container. Fluorescent dust was blown into the trap. Once dusted, the mosquitoes were released from the containers.

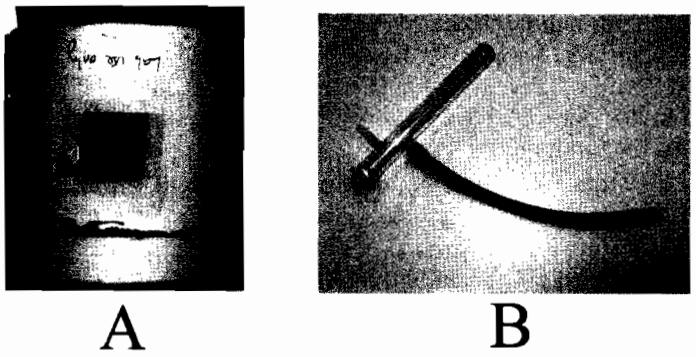

Figure 2: Marking equipment. A: Modified ice cream trap. B: Mucus trap.

Prior to dusting, 20-30 carbon dioxide traps were positioned around Lake Merced to collect marked mosquitoes. Mosquitoes were collected 24 hours post-marking. Ice and nets were changed and mosquitoes were collected again after 48 hours. Mosquitoes collected were brought to the laboratory, sedated with triethylamine, counted, identified under a black light. Marked mosquitoes were viewed under a stereoscope to determine which color were they marked with.

Marking was performed weekly for five consecutive weeks using different colors as indicated in Table 2. Mosquitoes were dusted pink for the first two weeks, green on the third week, yellow on week 4, and red on week 5. Traps were set for three weeks following the marking portion of the study. Trapping continued until nightly temperatures reached and stayed below freezing, when no mosquitoes were collected from traps for a week.

Recapture rates were determined using the following formula:

[(# mosquitoes recaptured/total marked/released) X 100]

Distance between the mark and release sites were calculated using measurements made in ArcView.

RESULTS

Roughly 5,400 mosquitoes were marked and released at Lake Merced between October 18 and November 13, 2006. Of these, 36 (0.7%) *Cx. erythrothorax* were recaptured. These mosquitoes were found in the tules throughout south, north, east, and impound lakes as well as a few sites east of impound lake (Figure 1B). Other

Table 1. Description of recapture sites and their distance from points of release.

Site #	Distance (km) from Release Site	Description
1	1	S of impound lake
2	0.9	SE of impound lake
3	0.6	E of Impound lake
4	0.4	E of Impound lake
5	0.4	E of Impound lake
6	0.5	At E impound lake
7	0	Collection / Release Point - W Impound lake
8	0.5	S point of Impound Lake
9	0.5	At W Impound Lake
10	1.7	At junction of N and S Lakes
11	1.5	At channel between N and E lakes

Table 2. Number of mosquitoes marked, recaptured, and percent recovery by trial.

Trial #	Date	Color	# Marked	# Recaptured	% Recovery
1	10/18/2006	Pink	1100	28	1.2
2	10/25/2006	Pink	1300		
3	10/31/2006	Green	1100	6	0.5
4	11/7/2006	Yellow	1000	5	0.5
5	11/13/2006	Red	900	2	0.2
		Grand Total	5400	41	0.8

species recaptured included *Culiseta. incidens* (Thomson) (1 / 5400), one *Cs. inornata* Williston) (1 / 5400), and three *Cx. pipiens* L. (3 / 5400). These were found east and south of impound lake.

In the first trial, *Cx. erythrothorax* were recaptured at a rate of 1.0% (23/2400) (Table 3A). The maximum dispersal distance was 1.7 km from the release point, across Lake Merced Blvd in two locations east of impound lake (Figure 1B). Five of 2,400 (0.2%) mosquitoes marked were species, other than *Cx. erythrothorax*, that were recaptured. Averaging trap data for the five previous years, *Cx. erythrothorax* made up 88% of mosquitoes trapped at the collection site, number 7. This is equal to the percentage of *Cx. erythrothorax* recaptured, 88% (36/41). Other species marked and recaptured during trial 1 were *Cx. pipiens*, *Cs. incidens*, and *Cs. inornata* found south and east of impound lake. It is interesting to note species other than *Cx. erythrothorax* were marked at Lake Merced and traveled throughout adjacent neighborhoods.

Culex. erythrothorax in trial 2 (Table 3B) were recaptured at divergent sites around the lake, east and west of impound lake and at the channel between north and east lakes. Six of 1100 marked mosquitoes were recaptured at a rate of 0.5% within a range of 0.5 to 1.7 km from the release site. The 60% mosquitoes recaptured in trial 3 (Table 3C) were from site 11, the channel between north and east lakes, 1.7 km from the release site. *Culex erythrothorax* were recaptured at a rate of 0.5% (5/1000). The recapture rate in trial 4 (Table 3D) was 0.2% (2/900). Both of the recaptured mosquitoes were found at the original collection site.

Table 3. Number of *Cx. erythrothorax* recaptured by site in four trials—A: Trial 1, B: Trial 2, C: Trial 3, and D: Trial 4.

A: Trial 1

3				1			**1**
4	1						**1**
8	9	6					**15**
10			2	2			**4**
11				1		1	**2**
Total	**10**	**6**	**2**	**4**		**1**	**23**

B: Trial 2

6			1		**1**
9	1	2			**3**
10			2		**2**
Total	**1**	**2**	**3**		**6**

C: Trial 3

7			2	**2**
11	1	2		**3**
Total	**1**	**2**	**2**	**5**

D: Trial 4

7	2	**2**
Total	**2**	**2**

DISCUSSION

Culex erythrothorax stay close to tule marshland, which provides them optimal conditions for survival. The maximum dispersal distance of a marked *Cx. erythrothorax* was 1.7km, similar to distances noted by Walton et al. (1999) and Tietze et al. (2003). Two marked *Cx. erythrothorax* were found in the Westlake district. However, 74% (238/312) of mosquitoes found in the Westlake District were not *Cx. erythrothorax* (Figure 1B); 36% (112/312) and 34% (105/312) of the mosquitoes trapped in the Westlake District were *Cs. incidens* and *Cx. Pipiens,* respectively. These probably originated from known breeding sites nearby, such as catch basins or fishponds.

The majority, 94% (34/36), of tule mosquitoes recaptured were very close to the perimeter of the lake. The fact that the mosquitoes were found across from impound lake and at East Lake in three of four trials suggests the possibility that the *Cx. erythrothorax* of Lake Merced makes up one distinct population and utilize the extensive wetland habitat circumnavigating the lake for dispersal. Based on data from this preliminary study, *Cx. erythrothorax* from Lake Merced can leave the lake to travel into neighboring residential areas, but rarely do. This is in agreement with average trap data from 2001-2005; 93% of *Cx. erythrothorax* collected in that area were trapped along the perimeter of the lake and 7% were collected in the Westlake District. Wind speed and direction and temperature changed greatly throughout this experiment and possibly had an influence on rate and direction of dispersal, as it did in a study by Reisen et al. (2003). Additionally, the number of mosquitoes marked and subsequently recaptured could be increased if the study was performed at the end of September through October, when the population size is greater.

Further studies will be performed to determine if *Cx. erythrothorax* from the Lake are getting into the Lakeshore neighborhood and into the San Francisco zoo. By adjusting seasonality, more mosquitoes can be collected in all of these areas to gain a better understanding of the mosquito population and dispersal at this urban lake bordering San Mateo County.

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Brown Dog Tick: A Potential Vector for Rocky Mountain Spotted Fever in California

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ABSTRACT: Rocky Mountain spotted fever (RMSF) is a tick-borne human illness. The causative agent of this disease is *Rickettsia rickettsii*. In the United States, *Dermancentor andersoni* and *Dermacentor variabilis* are the primary vectors of RMSF. The brown dog tick, *Rhipicephalus sanguineus*, is found worldwide with a predominantly urban distribution. This tick species is mainly an important blood sucking nuisance of dogs, but can be found on other mammals as well, including humans. Recent studies have identified *R. rickettsii* DNA in *R. sanguineus* collected in the southwestern United States. Here we report the first detection of *R. rickettsii* DNA from adult *R.. sanguineus* ticks collected in southern California and discuss the potential role of this tick in transmitting RMSF in an urban environment.

INTRODUCTION

Rocky Mountain spotted fever (RMSF) is a tick-borne human illness that was first described in the US in the 1890s. The causative agent of this disease is *Rickettsia rickettsii*. Although *Amblyomma americanum, Haemaphysalis leporispalustris,* and *Dermacentor parumapertus* have been implicated as potential vectors of RMSF, only two tick species, *Dermancentor andersoni* and *Dermacentor variabilis*, are known to transmit *R. rickettsii* (Azad and Beard 1998). The first human case of RMSF in California was diagnosed in 1903 (Rotramel et al., 1976). In the following 35 years, 188 cases were reported and nearly all were from the Modoc Plateau area of northeast California where *D. andersoni* occurred. Subsequently, sporadic cases of RMSF in California were also diagnosed outside the distribution of *D. andersoni*, suggesting that other ticks might be involved in transmission of the disease (Rar et al., 1976, Lane et al., 1981).

The brown dog tick, *Rhipicephalus sanguineus*, is found worldwide with a predominantly urban distribution. This tick species is mainly an important blood sucking nuisance of dogs, but can be found on other mammals as well, including humans (Furman and Loomis 1984, Carpenter et al., 1990). Severe tick infestations can occur in and around homes and may persist there for years (Lord 2001). In California, *R. sanguineus* adults have been collected year-round, especially in areas where dogs are present (Furman and Loomis 1984). This tick may also have potential for harboring disease-causing pathogens. Studies have shown that New World *R. sanguineus* are associated with *Ehrlichia canis*, the causative agent of canine ehrlichiosis, *Babesia canis*, the causative agent of canine babesiosis, and *E. platys,* whose pathogenicity is not fully understood (Burgdorfer et al. 1975, Walker et al. 2000). In addition, *R. rickettsii* DNA was recently detected in *R. sanguineus* collected in Arizona (Demma et al., 2005).

MATERIALS AND METHODS

In response to a tick infestation complaint from a resident in the city of Riverside, Riverside County, questing adult *R. sanguineus* ticks were collected by direct removal from blades of turfgrass and adjacent concrete walkways at the suburban residence on July 26, 2005. The ticks were preserved in 70% ethanol and sent to the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia for rickettsial pathogens testing.

DNA extractions were performed for each submitted tick and all reactions were done using a *Taq*PCR Master Mix kit (QIAGEN) and an Eppendorf Master Cycler (Eppenddorf, Westbury, NY). Polymerase chain reaction (PCR) assays for the 17-kDa antigen gene of spotted fever group rickettsiae, the rOmpA gene for *Rickettsia* species identification, the variable number tandem repeat region B for characterization of *R. rickettsii* at the genotype level were used. Primers used were all synthesized by the CDC (Eremeeva 2006a and b).

RESULTS AND DISCUSSIONS

A total of 62 adult ticks were tested for the presence of *R. rickettsii* DNA. Nested PCR testing results indicated that one male *R. sanguineus* (1.6%) contained *R. rickettsii* DNA for the 17-kDa antigen. This tick was also positive with the seminested PCR for a rOmpA fragment. The *R. rickettsii* DNA found in the tick, however, differed from strains found in Arizona. Our finding represented the first detection of *R. rickettsii* DNA from adult *R. sanguineus* ticks collected in California. The 2003-2004 outbreak investigation of RMSF in Arizona was the first to provide molecular evidence implicating *R. sanguineus* in the transmission of *R. rickettsii* in the USA (Demma et al., 2005). We further extended the area where *R. sanguineus* may be involved in *R. rickettsii* transmission, although more studies are needed.

Acknowledgments

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Efficacy of Deltamethrin (Suspend) on Density of *Dermacentor* Ticks Along a Recreational Trail in Coastal California

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ABSTRACT: The insecticide Deltamethrin (Suspend) was evaluated for its ability to control *Dermacentor occidentalis* along a recreational trail in San Mateo County. Suspend was applied to a 3 foot swath on the uphill side of a 0.37 mile section of the trail. An adjacent 0.27 mile section was left untreated. Both sections were sampled at 3 days post-treatment and at weekly intervals thereafter for 5 weeks to determine the length of residual control. Application of Suspend resulted in 99% reduction in tick density at 3 days post-treatment and complete elimination of ticks at 1 week. Five weeks after application, the treated area was still 100% free of ticks.

INTRODUCTION

The Pacific Coast tick (*Dermacentor occidentalis* Marx) is one of three species commonly encountered by humans on recreational trails along the central coast of California. In San Mateo County, adults of this species reach peak densities in May and June. This tick is a potential vector of tularemia and Colorado tick fever (Furman and Loomis 1984, Schmid et al. 1983). It has been implicated in the transmission of Rocky Mountain spotted fever to humans in California and spotted fever group rickettsia have been isolated from it in this state (Lane et al. 1981, Philip et al. 1981, Schmid et al. 1983, Rotramel et al. 1976).

In 2006, the San Mateo County Mosquito Abatement District began testing methods for controlling ticks. The District has conducted surveys for ticks and tick-borne disease for several years and has received numerous requests from local residents for information on tick control. In the spring of 2006, the San Mateo County Parks Department requested information about controlling ticks along public trails. Methods of control have been reviewed by Stafford (2004). However, some of the materials used for tick control have lost registration and there have been few studies published on tick control in Calilfornia habitats. To the authors' knowledge, the only published study on tick control along trails in California is that of Monsen et al. (1999) which demonstrated good control of *Ixodes pacificus* Cooley & Kohls, the western black-legged tick, in the Sierran foothill with carbaryl and diazinon. The present study was conducted to assess the feasibility of control along trails with commercially available materials. A liquid formulation of deltamethrin (Suspend®) was applied to a highly trafficked trail in a county park in coastal San Mateo County. Percent reduction in tick population and length of residual control were measured.

MATERIALS AND METHODS

Study Site- The test was conducted in the San Pedro Valley County Park, located in the foothills of the Santa Cruz Mountains near the town of Pacifica. The park covers 1,150 acres containing seven trails in a wide variety of habitats and receives approximately 130,000 visitors per year. The trial was conducted on Wheeler Ranch Road, a level paved trail bordered by high grasses and patches of chaparral. This trail lies at sea level in a valley at the base of the mountains. The site was chosen for its accessibility, high visitor use, and high density of ticks. The trail was separated into two sections, one treated and one untreated. The treated section measured 0.37 miles the untreated section 0.27 miles. A 25-foot buffer was left between the treated and untreated sections of trail to prevent the possibility contamination by drift during the application.

Deltamethrin Application- On April 28, 2006, a liquid formulation of Suspend SC Insecticide (deltamethrin 4.75) was applied to the uphill side of the treated section of trail at a rate of 1.5 oz./gal. The material was applied along the edge of trail in a three foot swath with a backpack sprayer.

Tick Sampling- Immediately before treatment, both the treated and untreated sections were sampled to determine density of adult ticks. A white flannel flag (1 m x 1 m) was dragged across vegetation on the edge of the trail to collect questing ticks. Every twenty paces the flag was inspected, attached ticks were counted and then placed back on the vegetation. Both treated and untreated sections were sampled three days after treatment and then once weekly for seven weeks. Separate flags were used to sample treated and untreated sections to avoid any possibility of cross-contamination.

Analysis- The efficacy of Suspend was evaluated by calculating percent control of adult ticks using the formula modified by Mount et al. (1996). The formula for percent control = 100 - (T/U x 100), where T = the post treatment mean divided by the pretreatment mean in the treated plots and U= the post treatment mean divided by the pretreatment mean in the untreated plots.

RESULTS

Prior to treatment, the density of ticks along the treated and untreated section was 4.61 ticks per 100 ft and 9.33 ticks per 100 ft, respectively. 97% of the ticks were *D. occidentalis* adults, 2% were *I. pacificus* and 1% was *D. variables*. Three days after treatment, a density of 0.2 ticks per 100 ft (98% control) of *D. occidentalis* were found on the treated section. On the untreated section there were

9.47 *D. occidentalis* ticks per 100 ft three days after treatment. One week post-treatment, there were no ticks present on the treated section of trail, while tick density in the untreated section was 2.72 ticks per 100 ft. No ticks were detected in the treated section (100% control) during the following seven weeks of sampling (Figure 1). By the seventh week, 0.17 ticks per 100 feet were present in untreated site and sampling was discontinued.

DISCUSSION

Application of Suspend at this site achieved 100% of *D. occidentalis*. Complete control lasted for 7 weeks in the treated site. Sampling was terminated after density of ticks on the untreated section declined to less than 0.5 ticks per 100 ft. The decrease of ticks over time at the untreated site is believed to be due to seasonal decline. However, it is possible that the handling of ticks while removing them from the flag and replacing them on the vegetation at the site affected their survival. The seasonal population dynamics of *D. occidentalis* at this site will be investigated further in future studies.

This preliminary trial suggests that control of ticks with applications of deltamethrin is feasible in targeted areas. A single application gave 98% control at three days, and 100% control after 1 week. Control lasted for at least seven weeks. The degree and duration of control achieved here is similar to that reported by Monsen et al. (1999) after application of chlorpyrifos. By confining the application to areas where people are most at risk of coming into contact with ticks, the amount of pesticide used can be minimized. This reduces the impact of pesticide applications on non-target species. Although deltamethrin is a broad-spectrum insecticide, insects were still present near the trail and flying insects were observed on the vegetation in the treated area throughout the sampling interval.

This was a preliminary study and further work will be done testing the material on a larger number of sites. The present site will be sampled again in April, one year after the initial application, to determine whether the control achieved decreases tick populations the following season. Monsen et al. (1999) showed a reduction in overall density of *I. pacificus* on the treated area the following year. Future studies will also look at the efficacy and length of residual control on *I. pacificus*, a vector of the Lyme disease spirochete in San Mateo County. It is not known whether the duration of control achieved in this study would also occur during the rainy season.

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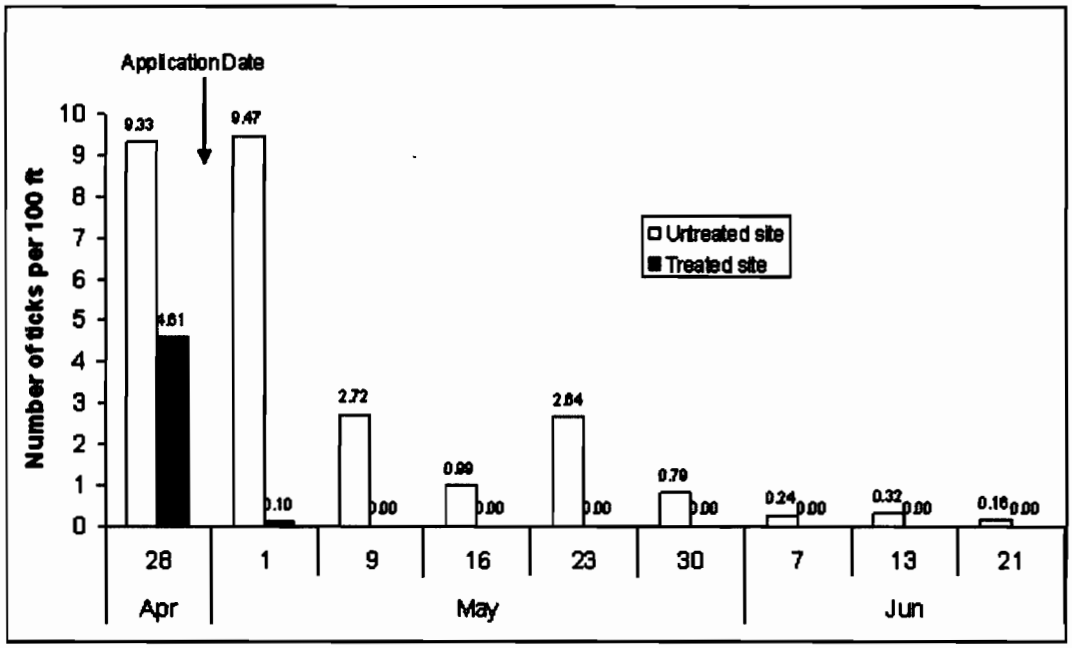

Figure 2. Comparison of tick density at treated and untreated sites along Wheeler Ranch Road when sampled at weekly intervals after application of Suspend® (Deltamethrin).

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The Irresistible Stench of Stormwater: How Far Will *Culex* Mosquitoes Fly to Reach it?

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ABSTRACT: In southern California, *Culex quinquefasciatus* is the dominant mosquito species that breeds in below-ground habitats created by stormwater management systems. Preventing adult mosquitoes from accessing these below-ground sources using physical barriers would potentially provide a sustainable and cost effective alternative to routine insecticide treatment. Conveyance pipes may prevent mosquito entry at certain critical distances. In this study, we evaluated the persistence of gravid female *Cx. quinquefasciatus* to reach an irresistible source of standing water through simulated stormwater systems incorporating conveyance pipes of different lengths and configurations. Results suggest increasing pipe length and decreasing pipe diameter does have a negative effect on oviposition and revealed that gravid females will travel a distance of at least 24 m through a small diameter (10 cm) pipe.

INTRODUCTION

Management of stormwater runoff in urban areas necessitates a complex system of above- and below-ground conveyance structures. Due to their propensity to accumulate debris, hold standing water, and maintain relatively stable air temperatures, below-ground stormwater systems can facilitate the widespread production of *Culex* mosquitoes (Hazelrigg and Pelsue 1980, Strickman and Lang 1986, Su et al. 2003). The size, complexity, and limited access of below-ground municipal storm sewer systems have historically limited the options for mosquito control to insecticides (Hedeen 1961, Mulligan and Schaefer 1981, Dhillon et al. 1984, Kluh et al. 2006). Recent studies have demonstrated that physical barriers to mosquito entry can be successful and may represent a cost effective, long-term solution to mosquito breeding in certain small structures (Caltrans 2004). However, in-line barriers such as flapper valves are susceptible to debris accumulation, which may create gaps for mosquito entry. A non-intrusive mosquito proofing option for conveyance pipes is needed.

The structure of breeding sources can affect oviposition site selection of mosquitoes (Subra 1981, Rey et al. 2006), thus the distance and configuration of conveyance pipes utilized within stormwater systems may have deterrent effects at certain critical lengths. Knowledge of these potential limitations could be valuable to both stormwater designers and vector control professionals to guide the design and construction of new stormwater systems to minimize or exclude mosquito production. The objective of this study was to determine the critical length of conveyance pipe of a small diameter needed to prevent mosquito oviposition in a simulated stormwater management device.

MATERIALS AND METHODS

An above-ground, modular system (ovitrap) baited with alfalfa infusion was built to simulate an enclosed stormwater management device with a single entry and a single exit conveyance pipe (Fig. 1). The dimensions and configurations of pipe tested were evaluated

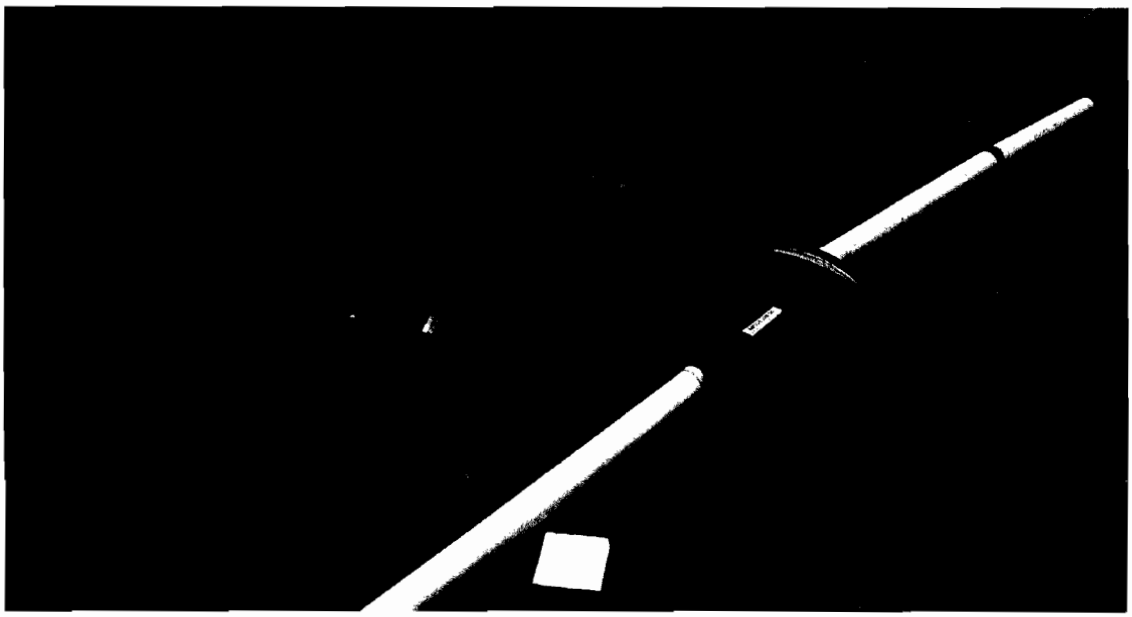

Figure. 1. Simulated stormwater system with interchangeable pipe attachments.

by comparing the number of *Culex quinquefasciatus* egg rafts collected daily from the infusion. To test pipe length effect, three trials consecutively testing lengths of 0, 90, and 270 cm were run with daily collections of egg rafts. Concurrently pipe diameters of 1.3, 5, and 10 cm were compared within each of three sixteen day trials. These small pipe diameters were chosen because they could be manipulated easily and purchased inexpensively. To determine the critical length of pipe that could successfully prevent mosquitoes from ovipositing, an ovitrap was fitted with 3 m of 10 cm diameter pipe. When at least 1 egg raft was found, additional pipe was added to the ovitrap using plastic couplings to double the length. Subsequent lengths up to 24.4 m were sequentially evaluated. Additional trials at this length tested a variety of pipe configurations using 90° elbow couplings. These configurations included; 1) a vertical "S" shape, 2) the same design lain horizontally, 3) a vertical "U" shape, and 4) and a vertical loop.

RESULTS AND DISCUSSION

It is well known that *Cx. quinquefasciatus* readily oviposit in complete darkness (Clements 1963) and larvae are commonly found in such habitats provided by stormwater management structures; however, the capacity to which gravid females travel within these environments to reach breeding sources had not previously been documented. Increasing pipe length, decreasing diameter, and the addition of various in-line configurations were found to reduce, but not prevent oviposition. A mean of 0.53 egg rafts per day was found during all trials at the 24.4 meter length. Findings suggest that oviposition could continue at distances far beyond 24 m within systems containing pipes of larger diameters, or with distances broken up by access points such as manhole covers and grates. Additionally, this work supports the assumptions of vector control professionals regarding movements of Cx. quinquefasciatus in below-ground storm sewers. Our results emphatically demonstrate the persistence of egg-laying females to reach breeding sites and the importance of routine monitoring of stormwater systems. Future studies should be focused on development of non-intrusive mechanical barriers, elimination of permanent standing water (such as in sumps and basins), and the efficacy of insecticide treatment.

Aknowledgments

This study was supported by contract funding from the California Department of Transportation. We thank Richard Gordon and Wally Jordan of District 7, California Department of Transportation, for kindly allowing their maintenance station facility to be used for this study and Curtis L. Fritz and Vicki L. Kramer for their valuable input in the development and analysis of this study.

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An Evaluation of the Aerial Spraying Conducted in Response to West Nile Virus Activity in Yolo County

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ABSTRACT: The first locally acquired human cases of West Nile virus in 2006 in Yolo County, CA, were confirmed on July 27 for two adults, one in Davis and one in Woodland, with onset dates between July 16 and 22. At the same time, mosquito infection rates for those cities increased substantially, and the decision was made by the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) to develop a strategy to address the affected areas and to conduct aerial adulticide applications. Due to weather conditions, the applications were postponed one week and conducted on August 8 and 9, 2006. We evaluated pre- and post-spray mosquito abundance inside and outside (control) the sprayed areas using encephalitis virus surveillance (EVS) and gravid traps. Infection rates were calculated before and after spraying. Preliminary results showed an overall decrease in infection rates, mosquito abundance, and number of locally acquired human cases after the spraying.

West Nile virus (Family Flaviridae, genus *Flavivirus*, WNV) was first detected in the United States in 1999 in New York City. Since then it spread throughout the country and reached California in the summer of 2003 (Reisen et al. 2004). In 2004, WNV amplified to epidemic levels and dispersed to all 58 counties in the state, including Sacramento and Yolo Counties, where it was associated with low level transmission to humans and horses (Armijos et al. 2005, Hom et al. 2005). There was a severe outbreak in Sacramento County in 2005, with 183 human cases and 40 equine cases (Elnaiem et al. 2006).

The Sacramento and Yolo Mosquito and Vector Control District (SYMVCD) conducts surveillance and control of mosquitoes in Sacramento and Yolo Counties. The District monitors adult mosquito abundance with American light traps, gravid female traps, and Mosquito Magnet Traps® (SYMVCD 2005a). In 2006, we monitored a total of 6 traps at 3 sites in the city of Woodland (2 of each type), and 3 traps in Davis (1 of each type). Additional intensive trapping was conducted in the city of Davis as a collaborative effort between the Center for Vectorborne Diseases at the University of California – Davis and SYMVCD (data not shown here). We also used encephalitis virus surveillance (EVS) traps baited with CO_2 and gravid female traps at additional locations in Davis and Woodland in response to positive dead birds to capture live mosquitoes to test for WNV. The first evidence of active WNV transmission in Yolo County was obtained with the detection of WNV in a dead crow picked up on June 28. Additional evidence of activity was obtained on July 06, with the detection of the virus in two positive pools of *Culex pipiens* L., collected on July 06 from Davis. WNV continued to amplify during the month of July, and minimum infection rates (MIR) were calculated on a weekly basis. In our surveillance program we only had one sentinel chicken flock in that area, located between the cities of Woodland and Davis. The first detection of WNV antibodies in the sentinel chickens was obtained on July 18, when one of the ten chickens showed antibody to the virus. On July 27, we received notification of the first human cases of WNV in Davis and Woodland, both with a reported probable onset between July 16 and 22, 2006.

Despite all efforts from the SYMVCD's public information program and from the intensive integrated pest management (IPM) program, which included environmental management, larviciding and biological control, WNV reached epidemic levels in the cities of Woodland and Davis. Following the response plan established to monitor and control mosquito-borne viruses (Kramer 2005) and the District's mosquito and mosquito-borne disease management plan (SYMVCD 2005b), on July 31, 2006, the decision was made to conduct aerial spraying of a pyrethrin insecticide over the cities of Woodland and Davis. The aerial spraying was scheduled to take place on the nights of August 3 and 4, 2006; however, weather conditions were not favorable and the adulticiding was postponed until the nights of August 8 and 9, 2006. A twin engine aircraft was used to spray the insecticide Evergreen® EC-60-6 (6% pyrethrin and 60% piperonyl butoxide (PBO); MGK, Minneapolis, USA), over 51 km^2 in Woodland and approximately 59km^2 in Davis each day. The aircraft speed was 150 miles/hour (130 knots), release altitude was approximately 91m (300'), wind speed was between 8 and 11.5 miles/hour (7-10 knots), and the temperature was 27-28°C.

In order to evaluate the success of the aerial spraying on mosquito populations in the spray zone, the District, in collaboration with the Center for Vectorborne Diseases at the University of California – Davis, conducted trapping of mosquitoes 2 days before and 2 days after the spray events. We established twenty-one trapping sites in Davis, which were established at the beginning of the 2006 WNV transmission season, and were set along seven North-South transects approximately 1.5 km apart, and nine trapping sites in Woodland. The trapping sites consisted of one EVS trap baited with CO_2 and one gravid female trap per site (Figure 1 A and B). Twelve trapping sites were established outside of the spray zones and served as controls (Figure 1 C). Mosquito abundance was measured before and after the spray events (Figure 2). Percent control was estimated by Mulla's formula (Mulla et al. 1971)(Table 1).

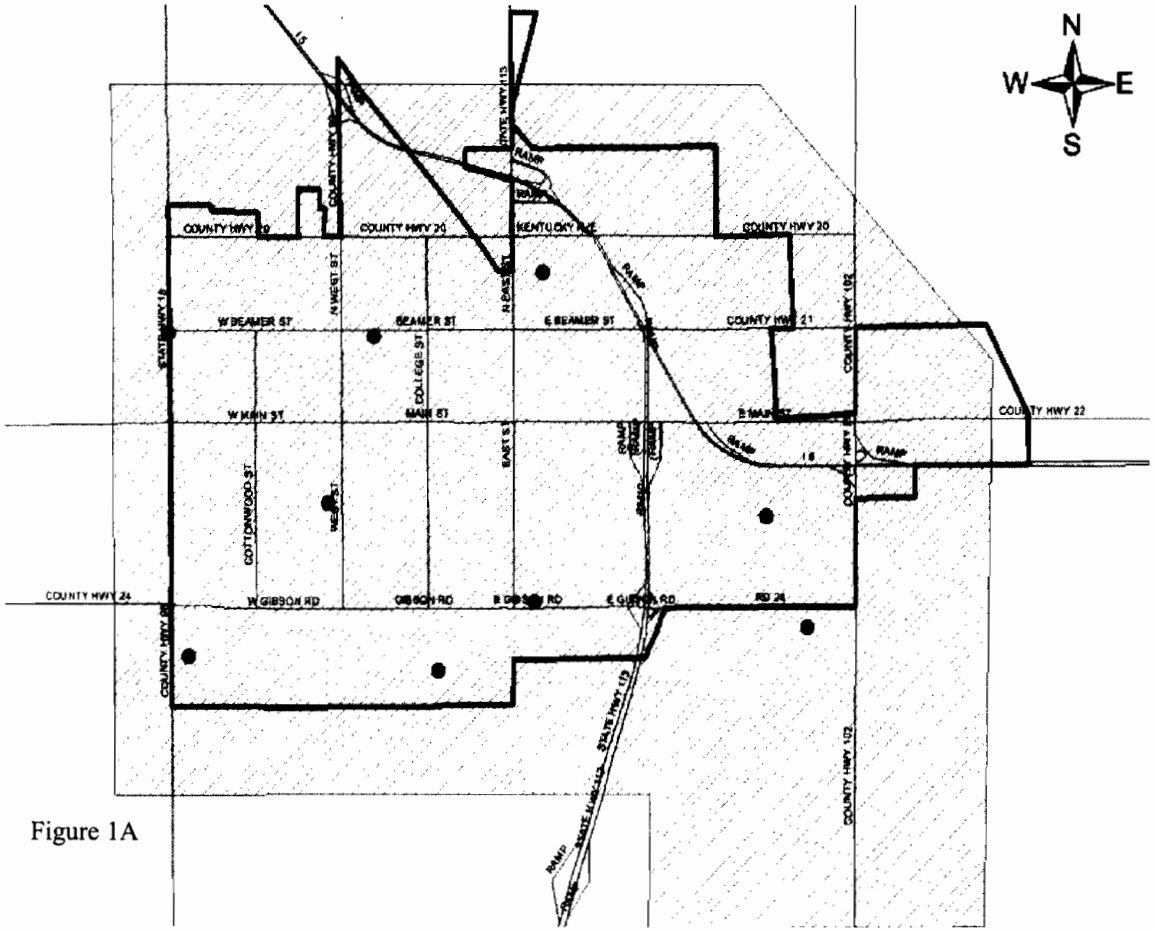

Figure 1A

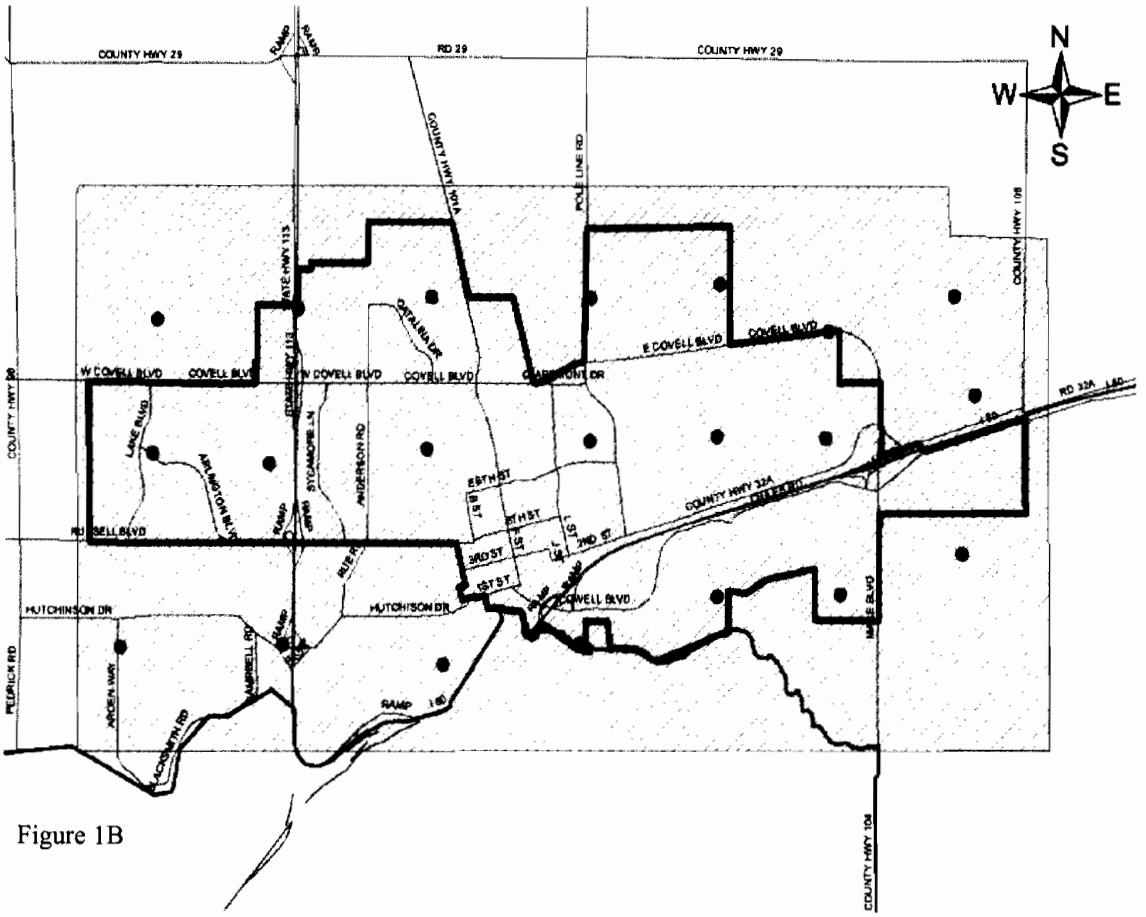

Figure 1B

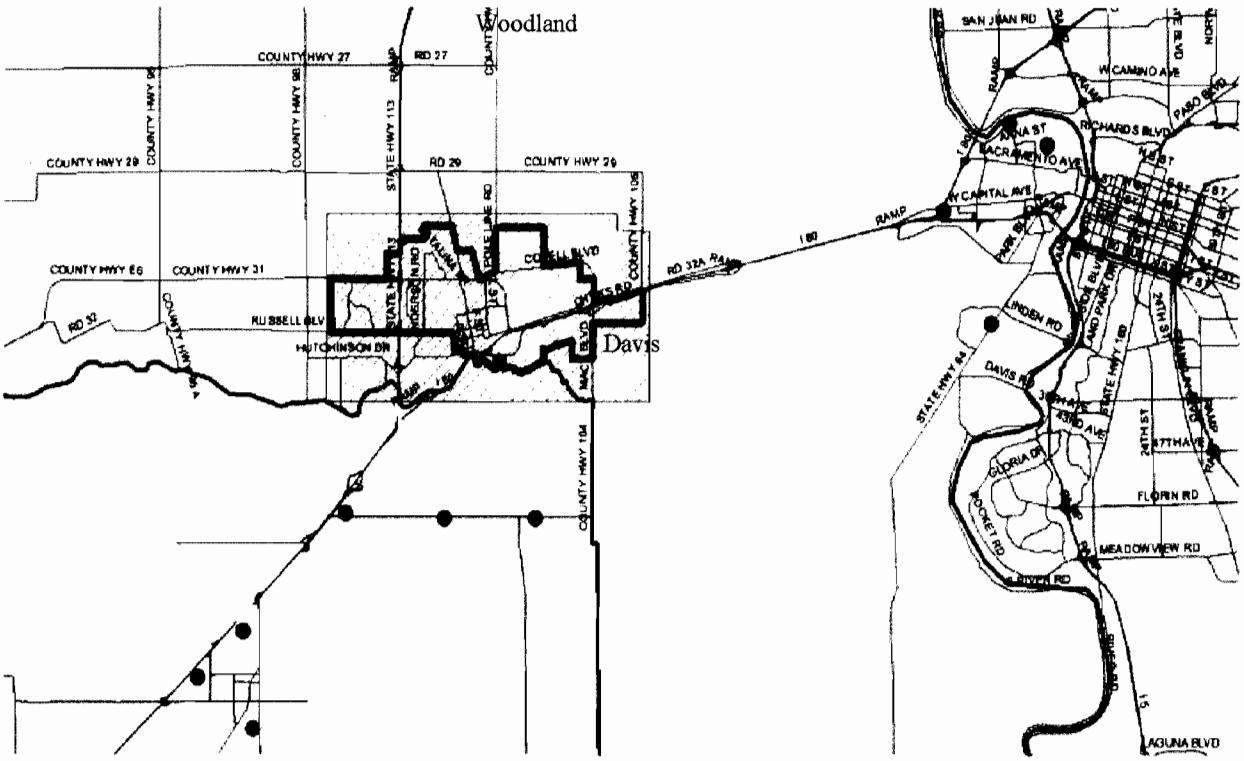

Figure 1C

Figures 1A-C. Maps of the cities of Woodland (A), Davis (B), and control outside Woodland and Davis (C). Outline represents city limits, box represents spray zone, and dots represent trap locations.

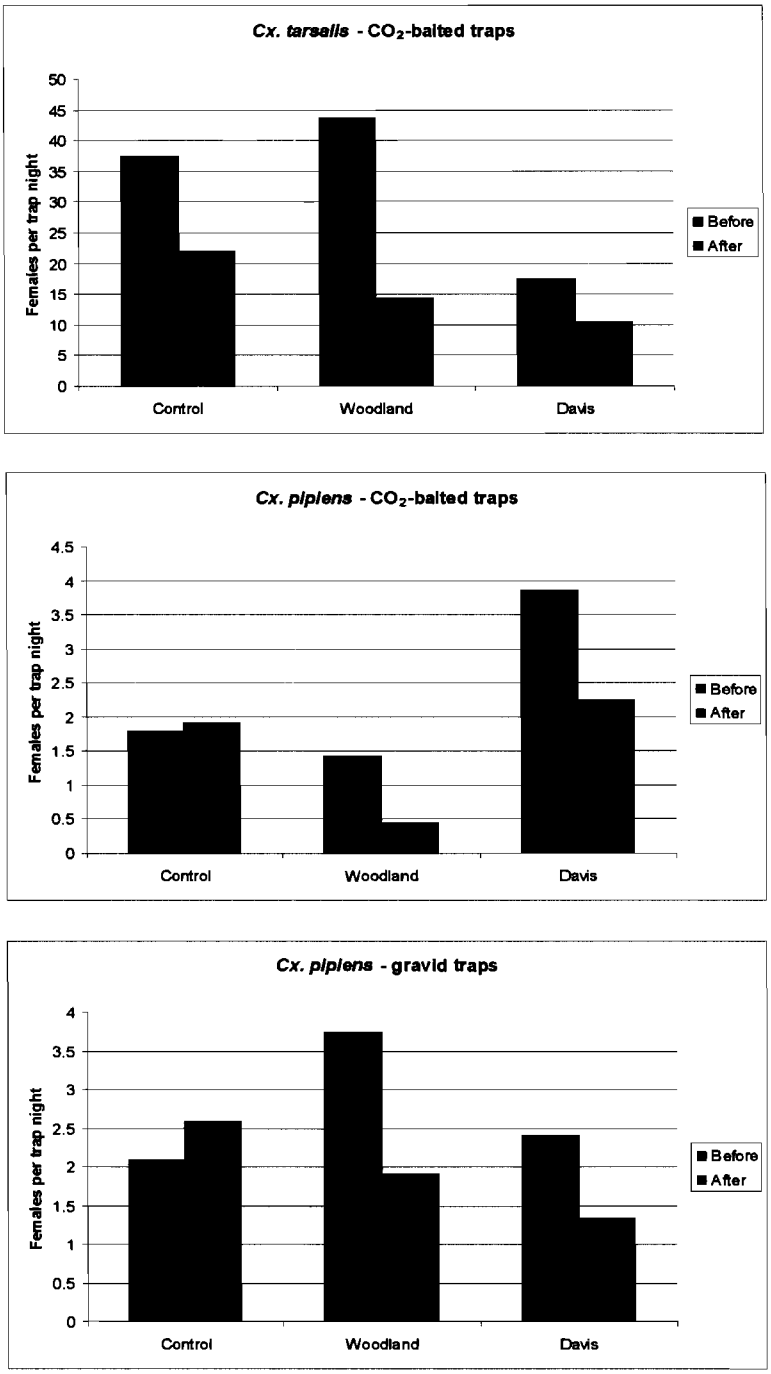

Figure 2. Adult female mosquito abundance per trap night in CO_2-baited (*Cx. pipiens* and *Cx. tarsalis*) and gravid female traps (*Cx. pipiens*) 2 days before and 2 days after the adulticide spraying events conducted in the cities of Woodland and Davis, California, 2006.

Table .1 Percent control from adult mosquito abundance data collected 2 days before and 2 days after the spraying events conducted in the cities of Woodland and Davis, California, 2006.

Location	Mosquito species	Trap type	% Control
Woodland	*Cx. tarsalis*	EVS – CO_2	46.8%
	Cx. pipiens	EVS – CO_2	77.7%
		Gravid female	70.2%
Davis	*Cx. tarsalis*	EVS – CO_2	25.6%
	Cx. pipiens	EVS – CO_2	58.0%
		Gravid female	46.2%

Adult mosquito abundance continued to be monitored with American light traps, gravid female traps, and Mosquito Magnet Traps® throughout the year. The number of females of *Cx. pipiens* and *Cx. tarsalis* Coquillett captured in these traps is shown in Figure 3. Minimum infection rates were also calculated on a weekly basis throughout the year. After the aerial spraying we noticed a decrease in the number of female mosquitoes collected in these traps, as well as in the number of positive mosquito pools and minimum infection rates (Figures 3 and 4).

Aerial ULV applications appeared to interrupt epidemic transmission. Only one of 7 and 2 of 15 human cases reported for the cities of Woodland and Davis possibly became infected after

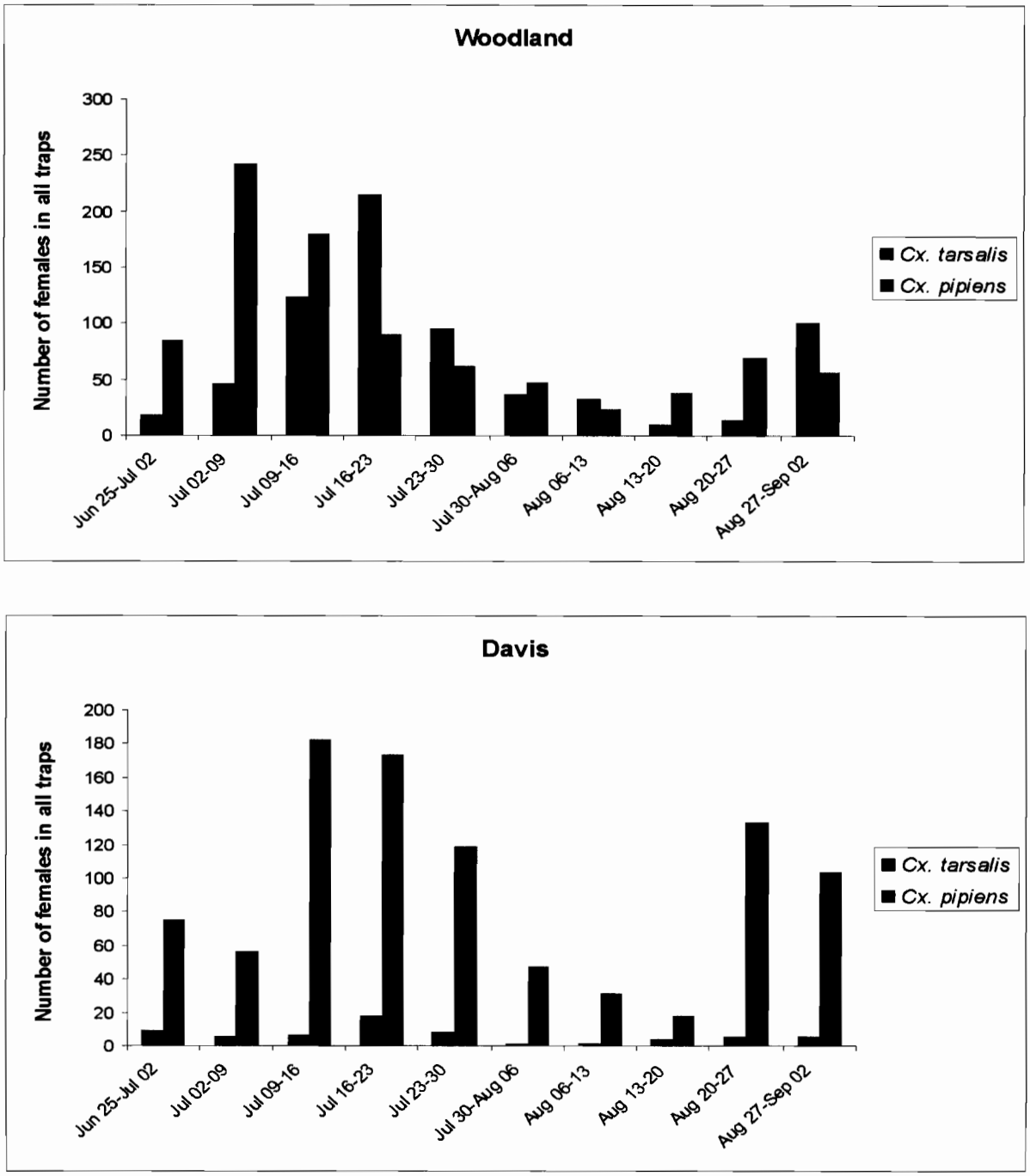

Figure 3. Adult female mosquito abundance in all traps (American light traps, gravid female traps, and Mosquito Magnet Traps®) in Woodland and Davis, California. 2006.

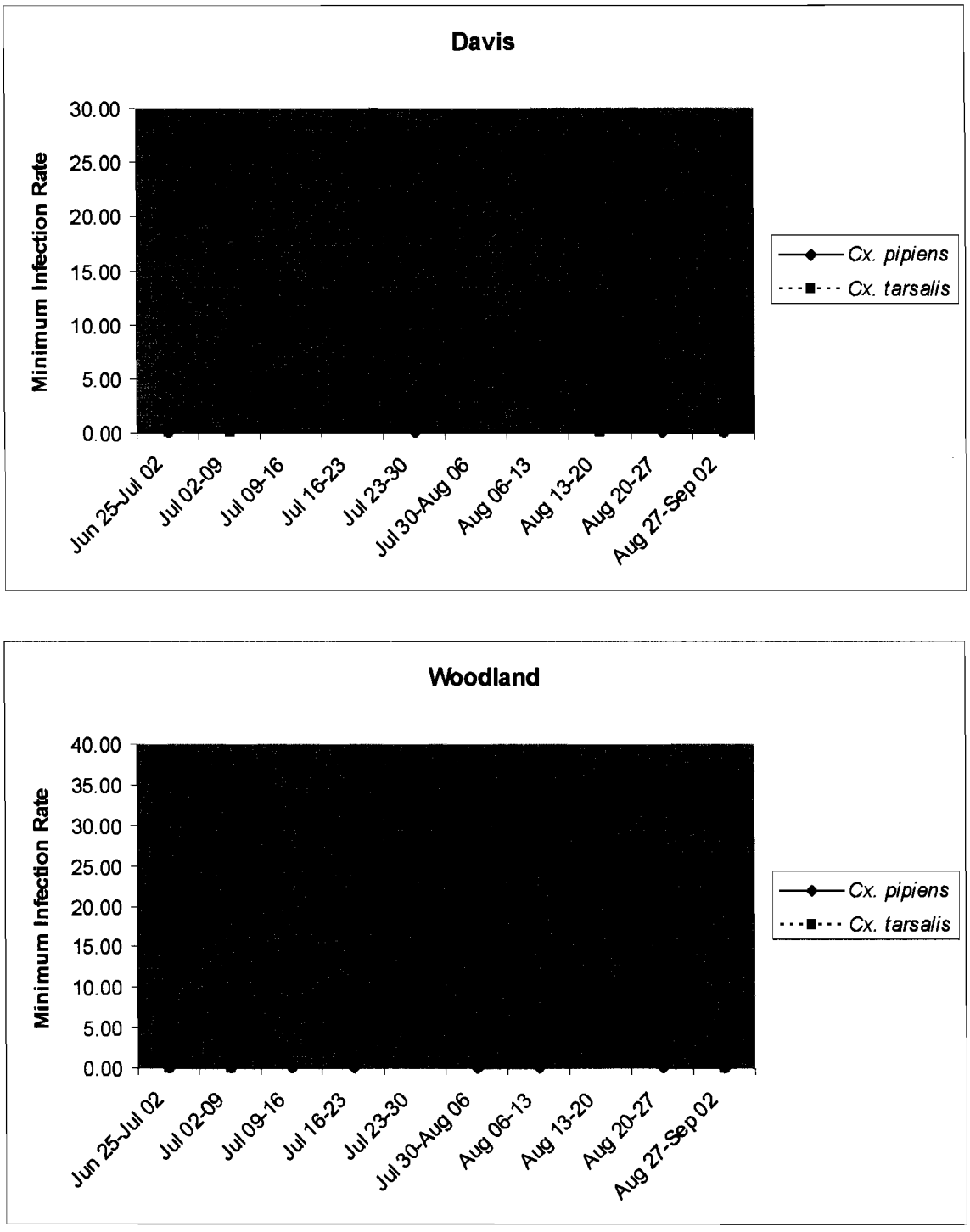

Figure 4. Minimum infection rates for *Cx. pipiens* and *Cx. tarsalis* in Woodland and Davis, California, 2006.

the aerial application, respectively. Date of onset of symptoms after infection can vary from 2 to 14 days in humans (CDC 2004) and therefore even these three cases may have been contracted prior to aerial application and experienced a delay in the onset of clinical symptoms.

In our assessment, the aerial application of Evergreen on two successive nights was successful in temporarily reducing the number of adult mosquitoes in Davis and Woodland and interrupted epidemic transmission. We believe this emergency control would have been more successful if we had intervened two or three weeks earlier and based our decision solely on enzootic amplification. Pre-emptive emergency adulticiding may have prevented some, if not most of the human infection. As shown in figure 5, on July 14, 2006, the minimum infection rate for *Cx. tarsalis* in Woodland was greater than 5, which is the epidemic threshold in the State Response Plan and is used by many mosquito and vector control agencies and districts in the U.S. (Kramer 2005). At the same time, the peak abundance in traps had been reached (Figure 3), showing not only that there was an increase in the number of mosquitoes, but also WNV infection in mosquitoes from that area. On the same trapping week of July 16 to 23, 2006, we observed WNV activity in the area where our chicken flock is located, verifying that transmission was occurring, which was further proved by the subsequent onset of human cases. Minimum infection rates reached 29.85 for *Cx. pipiens* in Woodland the following week. In Davis, the minimum infection rate for *Cx. pipiens* was greater than 5 on July 07, 2006, and was greater than 10 by the week of July 16-23, when *Cx. pipiens* abundance was also high (Figure 3).

These results show the importance of following abundance data and minimum infection rates when trying to prevent disease transmission by mosquitoes. The current and our previous evaluation in 2005 showed clearly that there is a serious delay in gathering and analyzing information on virus amplification and tangential transmission to humans. Therefore, it may be prudent to preemptively adulticide based on enzootic amplification rather than wait for the onset of human illness to be detected and reported by medical providers. By this time, transmission to humans is well underway and many have been infected, but not yet displaying clinical symptoms. The State of California Response Plan, which is based on a variety of environmental factors, enzootic indicators and the occurrence of human cases (Kramer 2005), may not reach Epidemic Response levels without human cases and therefore support for intervention will be difficult to support to that public sector that does not desire treatment.

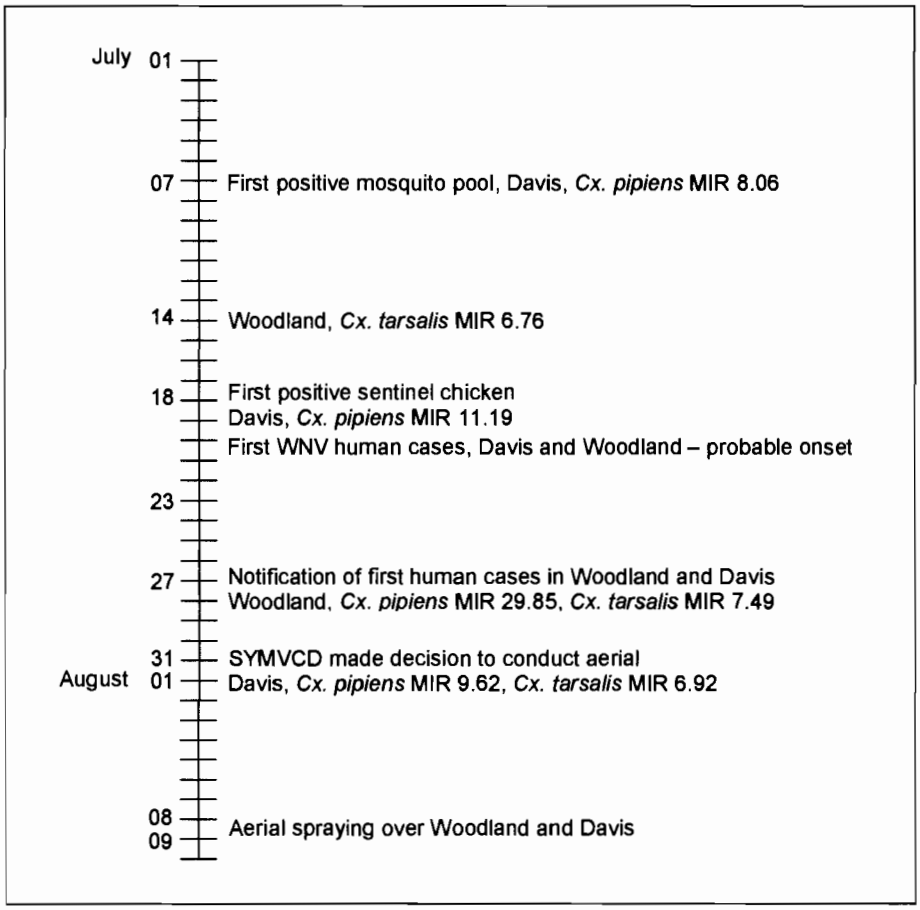

Figure 5. Timeline of events from July 01, 2006 to August 09, 2006 in Woodland and Davis, California.

Acknowledgments

We thank the laboratory technicians from SYMVCD and CVEC at UC-Davis for the great help in collecting and processing all samples presented in this study. We also thank Rhonda Laffey for her help with all the maps presented in this study.

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An Evaluation of Trailside Mowing as a Control Method for *Dermacentor* Ticks in San Mateo County, California

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ABSTRACT: The effect of mowing trailside vegetation on the density of adult *Dermacentor occidentalis* and *Dermacentor variabilis* ticks was evaluated in June and July 2006 in San Mateo County, CA. Trailside grass and vegetation was mowed for fire control along the public-use Sawyer Camp Trail on June 27 and 28, 2006. Tick surveys were conducted on a weekly basis from June 26 through July 20, 2007, on three ½-mile sections of the Sawyer Camp Trail, as well as on three ½-mile sections on un-mowed trails, one from the San Antonio Trail, and two from Edgewood Park. At each site, ticks were flagged in a 1m swath from the edge of the trail, counted, and replaced into the environment every 20 paces (approximately 50 feet), and a maximum trailside grass height measurement was taken every 100 paces. A total of 1319 ticks were counted, *D. occidentalis,* (1069 coll.) were most abundant, followed by 238 *D. variabilis.* The 12 remaining ticks were nymphs or adult *Ixodes pacificus,* and were not included in this analysis. An independent *t*-test (□ = 0.025) determined that the densities of *D. occidentalis* and *D. variabilis* were not significantly decreased by mowing. There was no significant difference between the mean density of *D. occidentalis* in mowed and un-mowed areas (Sig. = 0.235). Although there was a significant difference found between the mean density of *D. variabilis* in mowed and un-mowed areas (Sig. = 0.013), further analysis determined that the mowed areas held the higher density of *D.variabilis.* Additionally, the potential relationship between measured grass height and the presence or absence of ticks in measure sections was examined. Canonical correlation analysis (□ = 0.025) showed no significant relationship between grass height and the presence or absence of either species of tick (Sig.= 0.085) This study concludes that trailside mowing is not a feasible control method for *Dermacentor* ticks.

Biology and Control of the Invasive European Paper Wasp (*Polistes dominulus*) in San Mateo County, California

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ABSTRACT: European paper wasps, *Polistes dominulus* (Christ), are native to Europe, along the Mediterranean to China, and are one of the most common *Polistes* species in those areas. In 1978 its occurrence in North America was first documented in Massachusetts, USA. By 1995 they were common throughout the northeastern U.S., and by 2000 were widespread in Washington, Oregon and California. There is no evidence of continuous westward spread, suggesting possible independent introductions. *Polistes. dominulus* tend to produce numerous large colonies in close proximity to each other on or within structures. Large nest size may be partly because nests are sometimes re-used and expanded the next year by new queens. Evidence suggests that *P. dominulus* are out-competing and replacing native paper wasp species. Because of less competition among neighboring nests and being general predators, there is greater potential of seeing an impact on insect populations. Native *Polistes* show much greater dietary specialization (i.e., lepidoptera larvae) and have smaller colonies. In 2006, the San Mateo County Mosquito Abatement District initiated a limited control program to destroy individual nests encountered by residents. Service requests yielded 128 locations where at least one European paper wasp nest was found; only 4 were native nests. Eighty-four percent of service requests were during the months of July through September. Although species were not identified prior to this project, only 35 paper wasp calls were received in 2005. Paper wasps are not attracted to artificial lures or poison baits, therefore, nests were knocked down by water spray, commercial wasp spray, or treated with insecticidal dust. Access to nests was a limiting factor in treatment. Care was taken to remove nests and/or kill remaining adults to avoid nest rebuild.

Sound Bites & Spiel: Making the Most of Media Interviews

Deborah Bass

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Some people believe the function of public relations is to "spin" the truth. In reality, the function of public relations is to change people's behavior. Public relations professionals persuade key audiences to take action. They persuade them to *dump* standing water, *change* their perspective, or *support* mosquito and vector control districts, for example.

Most people believe that interviewing with the media simply means answering a reporter's question; however, interviewing consists of more than that. It's using the media as an opportunity to flex our organization's power to change peoples' behavior. We can do that by simply telling our story. Last year, the Contra Costa Mosquito and Vector Control District "told the story" over 250 times in interviews and communications with the media. We made the media work for us by using a foolproof formula for crafting sound bites and using other successful strategies.

In telling our stories and working with the media, we have the power to garner *free* and *priceless* communication - literally. Every media interview we participate in, if we could actually purchase a story in that news hour, is worth thousands upon thousands of dollars. Therefore, making the media a priority in our work day is a sound business decision. Think about the opportunity, the potential, of every story to go beyond the obvious answering questions, conveying instructions, or simply delivering facts. Each story gives us the opportunity to:

- Brand our districts
- Create awareness
- Build credibility
- Instill confidence
- Illustrate our professional and expert qualities

We have the power and the opportunity to communicate, but how do we do this in just over a minute - the average air time of a story? Sound bites. Short, concise statements we make when we interview with the media. Talking in sound bites is the most efficient way to ensure that our messages are received by the public. If they are spoken properly and in the right amount of time, then we won't get edited by reporters who are just trying to do their job.

CRAFTING SOUND BITES

Figure 1 is a simple formula for developing proper sound bites. Take a piece of paper and write down questions that you expect to get asked or are fearful of getting asked by your constituents. For example, "How come you can't notify the public sooner when you spray?" or worse, "My mother died from West Nile virus. How could YOU let this happen?"

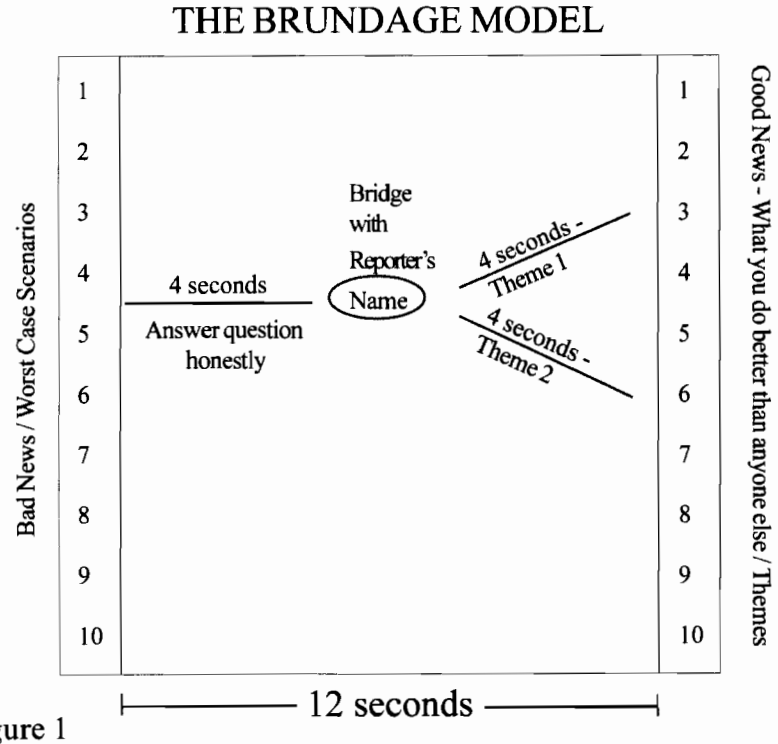

Figure 1

Develop your answers *before* a crisis takes place. It takes more time than you might imagine to find the words for a four-second answer. That's the goal: a four-second answer. When developing your answers, be sure to answer in a positive manner and not in a negative one. Talk about what it is you do and not what you *don't* do. For example, don't answer with "It's not like we.....". Don't waste your precious time talking about what you *don't* do and concentrate on what it is that you, in fact, *do*. To use an overused, but valuable example, take Richard Nixon's famous words "I'm not a crook!" If you didn't think of him as a crook before he spoke these words, you may now. Perhaps Mr. Nixon could have said "I have always maintained my position with integrity and dedication." A listener is more likely to form a positive opinion of Nixon through the connection he has made using the words "Nixon", "integrity", and "dedication", instead of drawing a negative connection through the words "Nixon" and "crook."

On the right side of your paper, write down the good news-what you do best, your good attributes. We call these "themes." For example, one theme might be "all of our technicians are trained and certified" or "all of the materials we use are approved by the environmental protection agency." Each theme should also be about four seconds in duration and there is generally time to say two of them in a typical sound bite. When you are asked a question, you simply answer honestly, bridge to your first theme and then to your second theme. The entire sound bite should be about 10-15 seconds. This is the correct amount of time that a reporter will use for his or her story in order to fit it into a typical news segment. If you don't deliver a sound bite that's within this time frame, reporters will have no choice but to edit your spiel into something they can use. This runs the risk of them, not knowing your business as well as you do, taking your words out of context or worse, changing your meaning. If you give them a proper sound bite, then that portion of their job is finished and you can rest assured that you will ultimately *say* on the air, what you *said* in the interview.

THE ANATOMY OF A SOUND BITE

In one example, a reporter asked me, "How many birds have been called in so far this year?" Okay, not that difficult a question, but at the time, which was early in the season, our constituents were getting discouraged because they were calling in dead birds as we had instructed them to and for a variety of reasons, many of the birds were not getting picked up and tested. But we still needed them to call in the birds to the dead bird hotline because we needed those reports for our surveillance and control efforts. Instead of simply answering the question, I used the opportunity to get *our* message across. I answered the question honestly: "We've had over 1000 birds so far this year called in..." I then added one theme: "Not all birds are candidates to be picked up and tested..." And then another: "...however, those reports alone are crucial information to our control efforts." This sound bite was a total of 14 seconds, well within the proper time frame. Ultimately my message was delivered with no misquotes or misinformation: what I actually said was aired.

It's okay to repeat your themes for each question during the interview. For example, when the reporter asks you a question, simply answer and then say your two themes. If they ask you another question, then answer and repeat those themes (or other themes if you wish, but themes nonetheless). Answering in this manner feels awkward at first and will take some getting used to; however, while the reporter may interview you for five or 10 minutes, they are more than likely only going to use one sound bite. If you repeat your message with each answer and that's all you say, then that's what they use. You are flexing your power to deliver *your* message.

Ensure that everyone at your agency has your current themes – including your board members. It takes repetition to change behavior. Studies prove that people need to hear a message 11 times before they actually take action.

FOSTERING GREAT RELATIONSHIPS WITH THE MEDIA

Make it a point to keep the media a priority in your busy schedule. If they call you at 4:30 a.m. for an interview for their 5:00 a.m. show, honor their request. They will reward you in a variety of ways in the future. Ask yourself: "How willing am I to reap the rewards of working with the media?" Treat reporters and photojournalists with respect by accommodating them with not only your time and preparedness, but also by ensuring that they are comfortable. Offer them the use of your restrooms, your water cooler, and your vending machine. When on location, drive home the point of wearing mosquito repellents by providing the very product you recommend. Doing so brings credibility to your recommendations and cements your commitment to protecting public health.

Make their job easy to cover your story. Give them maps, directions, and safe recommendations of where to park since you know your locations better than they do. Ensuring the reporters have everything they need saves you countless phone calls if they become lost or worried that they are not going to get their footage or interview. Provide pesticide label sheets and up-to-date statistics on West Nile virus cases/spraying - you know they are going to ask you for them anyway.

EXCEED EXPECTATIONS BY SUPPLYING RAW FOOTAGE

Exceed reporter's expectations by giving them b-roll. For a small investment, a 30-second television story can be turned into a three or four-minute story. B-roll is a professional, air-quality taping of the various aspects of your agency and programs. It should include a variety of footage, such as: personnel in the field performing their work; footage of the agency's grounds and portions of the office building; the entrance sign; laboratory tests being performed; scientists at work; mosquitoes; and anything else your audience needs to adequately understand your agency and programs. Include footage that reporters have taped before since you know it's what they will need. You then have the say over the perfect footage. It's not staged and you get to review and distribute exactly what you want your audience to see. You have the power to keep only the footage you want and the ability to ensure that when a story is told about your agency, that it is *your* agency that is being

depicted. It also helps to ensure that the television station isn't using outdated, incorrect, or another agency's footage. Ensure that your audience views *your* technicians, following *your* protocols and building *your* brand. It also allows you to be everywhere at one time.

Keep the b-roll with you always. Stories are created in the field, rarely in the studio. Pulling a BETA or DV format out of your bag in a remote location will seal the deal on having a great working relationship with the reporter and the photojournalist. Frankly, it will shock and thrill them. You will distribute b-roll to the same stations repeatedly and may question the cost effectiveness of providing such a service; however, paying $35.00 for a broadcast news story is a bargain. What other communication vehicle do you know of that will illustrate your agency exactly as you wish for less?

In 2006, the Contra Costa Mosquito & Vector Control District and or local health department held a media conference to announce the county's first human death from West Nile virus. Interviewing with each and every reporter in the field was not logistically possible and so b-roll was distributed to every television reporter at the conference. Rave reviews poured in for this professional and accessible resource. In addition to conveying the facts of the circumstances, we used the event to tell our story with visuals illustrating *our* technicians, following *our* protocols, branding *our* agency, and ensuring that *our* message was disseminated: priceless communication.

There is more to working with the media than simply answering questions: it's telling the story to change people's behavior. We can do that by utilizing successful public relations strategies, building solid relationships with media personnel, and crafting solid sound bites to convey *our* messages.

Understanding and Accommodating People with Chemical Sensitivities, Allergies, and Asthma

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According to the California Department of Health Services' Behavior Risk Factor Survey of 1995, individuals with allergic responses or unusual sensitivities to everyday chemicals account for 15.9% of the people surveyed (Neutra et al. 1999). The figure on chemical sensitivities for Marin County was a little higher, 17% (Marin County Department of Health and Human Services 2001). Many doctors and some state surveys show Multiple Chemical Sensitivity (MCS) prevalence to be one third of the population. Based on the author's own experience in the health field and consulting with not-for-profit organizations helping people with chemical sensitivities, one third of the population has some degree of chemical sensitivity.

Multiple chemical sensitivity was defined by consensus in 1999 (Donnay et al., 1999). Marked by multiple symptoms affecting multiple organs, MCS is a chronic condition that recurs reproducibly in response to low levels of exposure (lower than previously tolerated) to multiple unrelated chemicals, and improves or resolves when incitants are removed.

As a fundamental breakdown in natural tolerance, the process involves 2 step — an initiating toxic exposure, or a series of low level exposures, followed by newly acquired intolerances that trigger multi-system symptoms (Miller 1996).

According to Heuser (2000), the symptoms range from cognitive to intestinal, such as impaired cognitive and memory functions, word finding problems, intermittent confusion and disorientation, changes in behavior and mood, seizure like events, sleep disorders, decreased libido and potency, recurrent flu-like symptoms, fatigue, exhaustion, malaise, headaches, chronic pain, skin rashes, and gastrointestinal complaints. One of the difficulties with diagnosis is that patients react differently to various chemicals

In his recent book, Pall (2007) described a mechanism he discovered that not only clarifies MCS, but also Fibromyalgia and chronic fatigue syndrome, and allergies and asthma. Initiated by diverse stressors such as viral and bacterial, trauma, and exposures to organic solvents and pesticides (including pyrethroids), a vicious cycle develops called by its initials — NO/ONOO. It involves elevated levels of nitric oxide and its oxidant product, peroxynitrite. The complex cycle leads to inflammation and reduced energy production in cells — which means symptoms can vary from day to day, exposure to exposure, and person to person. This ongoing inflammation also increases atherosclerosis and risk of Parkinson's, Alzheimer's, osteoporosis, arthritis, etc.

Doctors can be puzzled by diseases and syndromes with multiple symptoms. When these patients are given drugs, they often become worse because of their sensitivity to chemicals.

Treatment to down regulate the NO/ONOO cycle involves vitamins, minerals, and other supplements. However, the main protection and relief is by avoiding exposure to a wide range of chemicals. For example, nitric oxide inhibits the metabolism of chemicals via cytochrome P-450, which may lead to increased sensitivity to chemicals. Thus, someone can become 100x to 1000x more sensitive to chemicals. Further more, the P-450 system regulates the metabolism of estrogen and testosterone, and certain medications.

According to Haley (1999), genetic predisposition for MCS may involve altered biotransformation of environmental chemicals. These findings parallel others' observation of a link between PONI heterozygosity and neurological symptoms in Gulf War Syndrome — the military's name for multiple chemical sensitivities (Haley, 1999). Veterans with these genes produced protein less able to metabolize pesticides and toxins, and had greater prevalence of neurological symptoms including chemical sensitivity.

Asthmatics are reported to have elevated nitric oxide levels that increase to much higher levels during asthmatic attacks. According to Pall (2007), the overall evidence is substantial that asthma may be NO/ONOO Cycle Lung Disease. This offers hope for asthmatics, as well as people with MCS, as they can use the Pall/Zeim protocol designed to down regulate the NO/ONOO cycle.

Chemical sensitivities is recognized by 25 Federal and 28 State authorities including: Army, Congress, Environmental Protection Agency, National Institutes of Health, Health and Human Services, Housing and Urban Development (HUD), Social Security Administration, Department of Justice which oversees Americans With Disabilities Act (ADA), and the Forest Service; California Attorney General's Office, California Department of Health Services, and the state Senate.

Vector control agencies are experiencing individuals and groups requesting not to have their homes sprayed with chemicals used to control pests. As noted above, avoidance is the main protection for people who already know they are made ill by exposure to chemicals. People who are disabled by their chemical sensitivities have a right to accommodation under ADA. It is also important to realize that for people who are unknowingly on the verge of becoming chemically sensitive, exposure to pesticides can be the tipping point for them.

In 1993, the Environmental Illness Task Force of the California Senate Subcommittee on the Rights of the Disabled reported on issues and solutions, which has helped shape accommodation guidelines. Barker (2007), who presented at the MVCAC 2007 conference, gave a first hand account of living with MCS and the importance of working with pest control operators to find the most appropriate accommodations for people with MCS. For example, after Barker had been exposed to pesticides on a camping trip, recovery was possible after 20 long years, due in large by moving

to and living at Ecology House, HUD housing for people with MCS in Marin County.

In January 2007, a Summit on Environmental Challenges to Reproductive Health and Fertility, convened by University of California San Francisco and the Collaborative for Health and Environment, revealed that disease sensitivity and gender bending from chemical exposure can occur in the womb at parts per trillion. Avoiding exposure at these extremely minute amounts is difficult, making it even more important to be vigilant.

That's why integrated pest management is so important. By using exclusionary and other tactics learned from observing life cycles, we can significantly reduce pests before considering resorting to chemicals that can compromise health and safety. It is important for pest control operators to keep current with innovative, non- and low toxic methods and products. Also, simple but not well known things like running a small fan in the patio or by the back door can keep away mosquitoes. And the public is not really aware of the polymer that can be put in tree crotches to soak up trapped water so mosquitoes can't breed there.

The Precautionary Principle offers sensible guidelines to approaching vector control situations. The City of San Francisco defines this approach as: take anticipatory action to prevent harm; decisions to be transparent, participatory, and informed by the best available information; right to know complete and accurate information; burden is on the proponent of action; examine a full range of alternatives, including doing nothing; and consider the full range of costs, including costs outside the initial price, such as health effects on staff and public.

It is hoped that all involved in mosquito and vector control and management will consider the information presented in this paper, adopt the precautionary principle approach to their work, and recognize that people with chemical sensitivities need special protection and accommodation.

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Green Pool Surveillance: You Can't Buy Public Relations Better Than This!

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The San Gabriel Valley Mosquito & Vector Control District entered into a program of joint benefit with the Foothill Air Support Team (FAST). The Pasadena Police Department's Air Operations Section provides regional helicopter support in various San Gabriel Valley cities through the FAST program. Pilots from the program were concerned about the number of green pools they spotted from the air during the epidemic year of 2004 and greatly desired to assist in the battle against West Nile virus.

In spring of 2005, the District conducted their first highly successful aerial green pool surveillance program dubbed "Operation Dirty Water." Helicopters equipped with Global Positioning Systems (GPS) coupled with the Los Angeles County Tax Assessor's Geographical Information System (Figure 1) flew over the six contracted FAST cities. Due to its terrific success, the program was repeated in 2006 under the code name "Operation Big Green," and was expanded to include all 23 cities in the District's jurisdiction.

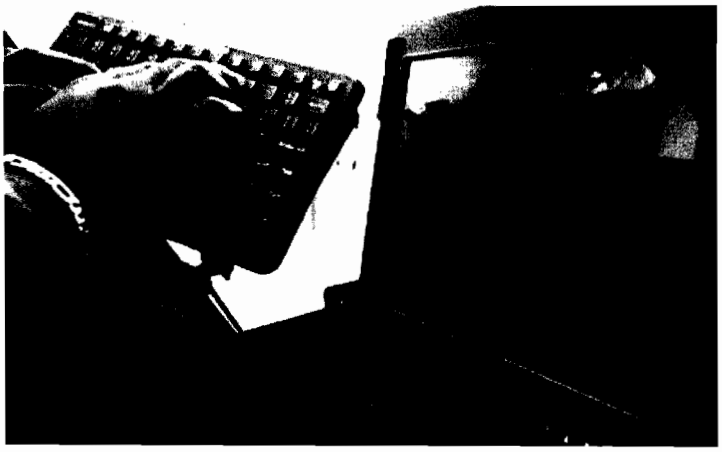

Figure 1. Onboard GPS & GIS equipment pinpointed addresses of green pools

In 2005, the Pasadena Police Department offered these services at no cost as a benefit to their contract cities and to generate positive public relations for their FAST program. With the expansion to include additional cities in 2006, the District was offered the services for the low rate of $250/hour – the same charged to contract cities for air support time.

Pilots used the onboard equipment to identify addresses while District staff photographed the green pools from the air. Equipped with conclusive evidence, vector control technicians worked with residents who were eager to promptly correct their swimming pools/spas instead of risking potential fines of $1,000 per day plus the cost of abatement.

PROGRAM BENEFITS

The success of this program was three-fold:

1. Identifying and eliminating mosquitoes in these hidden urban sources undoubtedly reduced both the summer mosquito populations and the risk of mosquito-borne disease to our District's residents. To evaluate the impact of this program, we looked at the number of pools in a 'typical' neighborhood. Ten pools were spotted in one photo, two (20%) were green. Based on a typical green pool surveyed, we estimated that even with mosquito survival and reproduction rates of 10%, over one million biting adult female mosquitoes could be produced over four weeks. If this were extrapolated to the number of pools found breeding in 2006 alone, this program easily eliminated billions of mosquito larvae during the critical spring season – over 100 million of which could have become biting (and potentially disease-transmitting) female mosquitoes.

Table 1. Summary of green pool helicopter surveillance results - 2005 and 2006

Area surveyed	Number of pools found	Pools new to database	% of pools breeding mosquitoes*
2005 (64 mi^2 surveyed)	101	88%	16%
2006 (250 mi^2 surveyed)	172	87%	21%*

Additionally, the number of green pools found per square mile decreased by 43% from 2005 to 2006 (Table 1). The vast majority of pools spotted each year were new to our database indicating that those pools identified in the past were continuing to be properly maintained and that this effort is instrumental in identifying new breeding sources each year.

2. *Although fewer pools were likely found (compared to fixed-wing aircraft surveillance methods), those pools could be rapidly corrected due to instantaneous address identification and supporting photography.* Our technicians were on the ground the next day knocking on doors and treating sources. Some residents even reported expecting us as they'd heard the helicopters overhead or had seen their house on TV the day before.

3. Many of the pools potentially missed during the surveillance flights were identified by residents self-reporting or reporting neighboring pools. Calls to the District peaked both years during the months we flew (Figure 2) even though mosquito reproduction and West Nile virus (WNV) activity were both low in

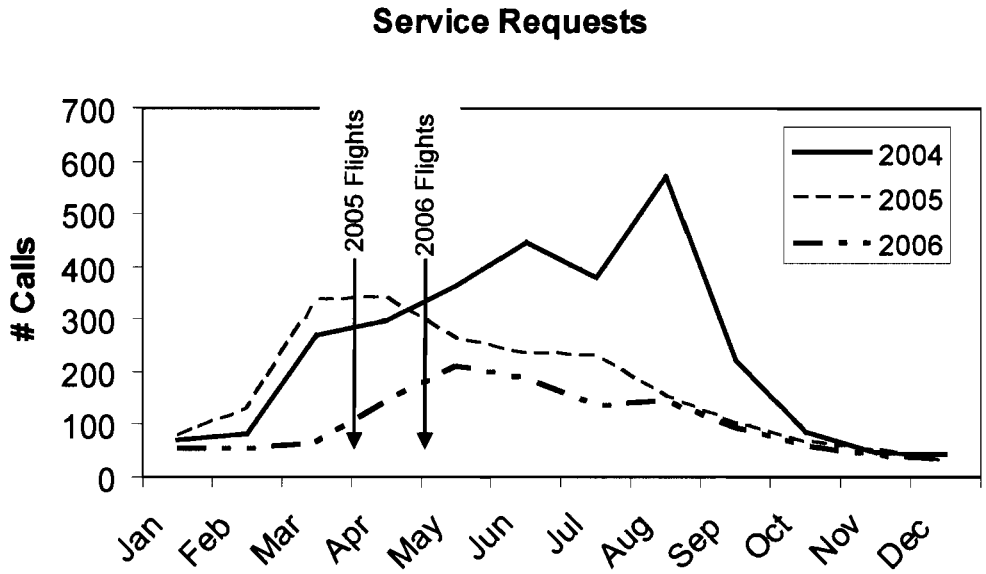

Figure 2. Surveillance flights in April of 2005 and May of 2006 correlated with increased service request calls to the District.

the San Gabriel Valley. This is a key point; reduced disease activity in 2005 and 2006 resulted in lax attitudes among residents who assumed WNV was no longer a threat.

VALUE OF PUBLIC RELATIONS

In both 2005 and 2006, the District and FAST team members held a joint press conference at the heliport in Altadena, California. Undoubtedly, the success of this media campaign rests in large part on the tremendous support provided by Pasadena Police Department and members of the FAST team. Pasadena's Air Operations staff provided outstanding photo opportunities for the media, and offered rides in a secondary helicopter so reporters could get unique footage. Pilots were available for interviews, and the media was given access to the inside of the surveillance helicopters to photograph the equipment. While on one particular flight, a radio reporter was allowed to record radio traffic communications between himself and the pilot giving listeners a truly unique experience.

This surveillance program alone generated 34 interviews resulting in print, radio, and TV broadcast coverage from local to national level media outlets which lasted for weeks reminding the public that WNV was not a transitory issue.

Public relations professionals strive to relate key messages to the public. During this surveillance program, our District's message was: 1) improperly maintained pools and other sources of standing water pose risks by breeding mosquitoes; 2) residents must do their part and keep their pools clean; and 3) WNV is here to stay and precautions must be taken year-round. Ironically, the key messages the media preferred to deliver were: 1) vector control districts are watching and 2) when we find you, you could face fines of up to $1,000 per day. While we are not typically in favor of heavy handed messaging, this slant proved far more successful than our typical "awareness and prevention" approach.

Evaluating the benefits of a public relations campaign and the value of media coverage is difficult. Neilsonmedia.com provides a simple yet illustrative formula that calculates the efficacy of a public relations program as the cost per thousand impressions, or the CPM.

$$CPM = \frac{\text{Cost of Media X 1,000}}{\text{\# Impressions}}$$

For comparisons, we used this formula to calculate the CPM for the highly successful joint WipeOut West Nile Virus campaign utilized in Southern California from 2004-2006, our District's 4-page newspaper tabloid that went to every household, and finally, the value of a 2:20 min. network news story on WNV which ran in 2006 (Table 2).

Table 2. Efficacy of various WNV outreach efforts calculated as the cost per thousand impressions. The cost of the Helicopter Flights was the cost for the surveillance program, not the cost to purchase airtime necessary to reach the estimated 2 million viewers.

	Reach	Cost	CPM
WipeOut WNV (2004)	23 million	$207,000	9
WipeOut WNV (2005)	22 million	$133,000	6
Newspaper Tabloid	.5 million	$ 33,000	62
KNBC-TV News (2:20)	.25 million	$ 3,000	12
2006 Helicopter Flights	2 million	$ 3,600	1.8

Several districts and county health offices in the Southern California Region contributed funds to partner with the Greater Los Angeles County Vector Control District between 2004 and 2006 in the WipeOut West Nile Virus Campaign. While this campaign did purchase radio and some print ads each year, the program focused on community partnering to distribute information to a huge number of residents (target population of 17 million)

through various means as economically as possible. The CPM for this campaign calculated out to a very respectable 9 and 6 for 2004 and 2005 respectively. Newspaper tabloids delivered to every household in our service area were expensive by comparison (CPM = 62) but literally placed WNV materials into every home which was especially critical in 2004.

Using the reported value and reach of a 2 minute 20 second newscast on WNV from 2006, we see that the CPM for this typical TV network news story was 12. Identifying the reach for the news coverage related to *Operation Big Green* in 2006 was impossible thus a rough (and likely highly underestimated) number of 2 million was used resulting in an impressive CPM of 1.75.

DISCUSSION

Reaching the greatest number of people with the most effective message as efficiently, i.e., cheaply as possible is the challenge of every District's outreach program. Additionally, agencies must find ways to keep the message newsworthy while still conveying important information.

The use of helicopters to survey our District for green swimming pools began as a surveillance program. In retrospect, it was likely more effective as a public relations tool having the often-desired but rarely accomplished effect of changing the behavior of the population. For this reason, it was easy to justify using money budgeted for public relations to fund this surveillance effort.

Probably the most significant, if not disheartening lesson we learned is that people are much more motivated by potential fines than they are by potential risks to their health. In light of the impact a single case of highly preventable WNV can have on an individual and their family, we will continue to utilize this motivation if it achieves the desired results. A better $3,600 was never spent.

Impact of Climate Variation and Adult Mosquito Control on the West Nile Virus Epidemic in Davis, California During 2006

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ABSTRACT: Climate variation and vector control can greatly impact arbovirus transmission cycles. The objective of our study was to investigate the impact of temperature and aerial application of adulticide on West Nile virus (WNV) enzootic transmission during an epidemic in Davis, California. WNV transmission activity was determined by calculating infection rates of *Culex* spp. collected using a uniform mosquito trapping grid and dead bird surveillance program. Emergency aerial adulticiding was conducted over Davis after human cases were detected. Both *Cx. pipiens* and *Cx. tarsalis* abundance decreased slightly after the adulticiding and WNV transmission decreased. A degree-day model was also calculated and the decrease in WNV activity was concurrent with nightly ambient temperatures dropping below the experimentally determined minimum threshold for WNV replication. Vector abundance remains an important measure to evaluate the efficacy of vector control strategies and is an important contributor to the force of arboviral transmission. However, integrating additional environmental cues in risk assessment guidelines may expedite the initiation of emergency control.

INTRODUCTION

During arbovirus epidemics vector control strategies and effectiveness are often of great interest and can even be legally disputed (Hodge and O'Conell 2005). In California response plans have been established to monitor and control vectors of endemic and emerging arboviruses such as western equine encephalomyelitis virus (WEEV) and West Nile virus (WNV) (Kramer 2005). Throughout California, there are at least 5 species of *Culex* mosquitoes that are involved in the transmission of WNV and are of interest in the statewide arbovirus surveillance and control program. *Culex tarsalis*, a highly competent vector of WNV and *Culex pipiens*, a moderately competent WNV vector (Goddard et al. 2002), are the 2 most abundant of these *Culex* species in the Sacramento Valley. During the 2005 WNV transmission season, Sacramento County was the epicenter of the California WNV epidemic with 122 WNV positive mosquito pools, of which 69% were *Cx. pipiens* and 30% were *Cx. tarsalis*. The annual human case incidence was 14.5 per 100,000 (http://westnile.ca.gov/maps_data.htm). In neighboring Yolo County there were 28 WNV positive mosquito pools, of which 50% were *Cx. pipiens* and 43% were *Cx. tarsalis* (http://westnile.ca.gov/maps_data.htm). Although it is important to determine the abundance and infection rates of the vectors involved in an arbovirus transmission cycle, it is also essential to understand the effects of climate variation and vector control on the patterns of transmission in time and space. The objective of our study was to investigate the impact of temperature and aerial application of adulticide on WNV enzootic transmission during an epidemic.

Above average summer temperatures at northern latitudes have been implicated in the dispersal and amplification of WNV throughout North America (Peterson et al. 2003, Reisen et al. 2006). Estimates of the daily survivorship of *Cx. tarsalis* collected from the foothills of the Sacramento Valley during August range between 0.84 - 0.86 (Reisen et al. 1990) and survivorship of *Culex* species from Southern California is 0.9 (Reisen et al. 1991). With the summer heat, a *Culex* mosquito can develop from egg to adult within 7-9 days and survive up to 3-4 weeks (Reeves et al. 1994; Reisen et al. 1990). However, during warm summer days (≥25°C) only 5% of female *Cx. tarsalis* survive the approximate 7-14 day extrinsic incubation period and take enough blood meals to be involved in an arbovirus epidemic (Reeves et al. 1994). Therefore, it is difficult to estimate from abundance alone the entomological abundance threshold required for an arbovirus epidemic to occur.

Experimental infection studies of *Cx. tarsalis* with WNV determined that the extrinsic incubation period required for viral replication was 109 degree days above the minimum threshold of 14.3°C (Reisen et al. 2006). Below this threshold WNV does not replicate and therefore disseminate within the mosquito vector. It is outdoor, ambient temperatures that determine both the rate at which the virus replicates and the rate at which mosquitoes age (Reisen et al. 2006), yet there is evidence that resting adult mosquitoes occupy protected environments that have higher thermal temperatures thus decreasing the extrinsic incubation period and increasing the chance for viral replication and transmission (Meyer et al. 1990). The success of WNV to emerge and become endemic in widely varying environmental conditions and varying temperate regions has been discussed (Dohm et al. 2002; Komar 2003; Reisen et al. 2006). Yet actively measuring nightly ambient temperature is not widely used as a predictive parameter for initiating control strategies unless other surveillance indicators of WNV activity have already occurred (Barker et al. 2003; Kramer 2005). The application of mosquito adulticides is effective at interrupting arboviral transmission. The efficacy of these control measures is best when applied during the earliest stages of an epidemic; however, predicting when an epidemic will occur is difficult and may be compounded by rapidly changing environmental factors such as temperature.

MATERIALS AND METHODS

A uniform mosquito trapping grid was established in Davis, California, a residential community in Yolo County with a population of over 64,000 residents in an area of approximately 26 km^2. Carbon dioxide-baited and gravid traps were operated at 21 sites spaced approximately 1.5 km apart, 1 night per week from April to October 2006. Additionally the Sacramento-Yolo Mosquito and Vector Control District [SYMVCD] continued trapping at their established surveillance sites. All mosquitoes were enumerated by species per trap and trap type. All female *Culex* were pooled by species in pools of ≤ 50 individuals and tested for WNV, SLEV and WEEV infection by a multiplex RT-PCR. To quantify the transmission activity of WNV among vertebrate hosts, all dead birds reported by Davis residents were collected and sent to the California Animal Health and Food Safety laboratory for necropsy. Kidney snips or swabs were sent to the Center for Vectorborne Diseases laboratory for viral testing by RT-PCR. Daily temperatures were downloaded and degree-days were calculated from the California IPM project website (http://www.ipm.ucdavis.edu/). Routine vector control measures were conducted by the SYMVCD. Domestic source reduction and larvicide applications commenced in and around Davis before the beginning of the WNV transmission season to keep the adult mosquito populations low. Emergency adulticide applications were made over Davis in response to surveillance indicators and the occurrence of human cases. An ultra-low concentration (0.0025 lbs/acre) of a pyrethrin and piperonyl butoxide adulticide formulation (Evergreen® Crop Protection EC 60-6, McLaughlin Gormley King Company, Golden Valley, MN) was applied on August 8 and 9 by a twin engine aircraft, flying at an approximate elevation of 95 m. To determine the effectiveness of the application within Davis mosquito abundance measured was before and after the application and was compared to concurrent abundance data from areas that were not sprayed. Two cages (Townzen et al. 1973) with sentinel adult *Cx. tarsalis* mosquitoes were set in exposed and protected microenvironments at each of the 21 surveillance sites on both nights when spraying occurred.

RESULTS AND DISCUSSION

Even with intensive mosquito surveillance the initial introduction of WNV into Davis in 2006 was not detected until an infected bird was reported on 28 June. If WNV was first brought into the area by an infected bird, it is likely the transmission cycle would not have been able to continue without the nightly ambient temperatures remaining above the minimum threshold for WNV replication. Nightly ambient temperatures first exceeded 14.3°C during the first week in June; however it was only sustained for 2 days. June 21st was the first night after which nightly ambient temperatures exceeded 14.3°C for an extended 10 consecutive days. WNV activity was first detected in Davis when a WNV-infected crow was reported on June 28th. WNV-positive mosquito pools were collected from 2 of the 21 grid sites approximately 1 week later. A total of 78.2 degree days had accumulated from the date of the first WNV-infected dead bird to the first WNV-positive mosquito pool. Interestingly, a small cluster of WNV-infected birds occurred after approximately 119 accumulated degree days or approximately 1 extrinsic incubation period (the required minimum degree days for extrinsic incubation to occur is estimated to be 109 degree days) (Reisen et al. 2006). The peak of the Davis WNV epidemic occurred after 217 degree days, or approximately 2 extrinsic incubation periods or transmission events, had elapsed since the first indicator of WNV activity occurred in the area (Figure 1). By mid-July WNV activity reached epidemic levels and in following the California State Mosquito-borne Virus Surveillance

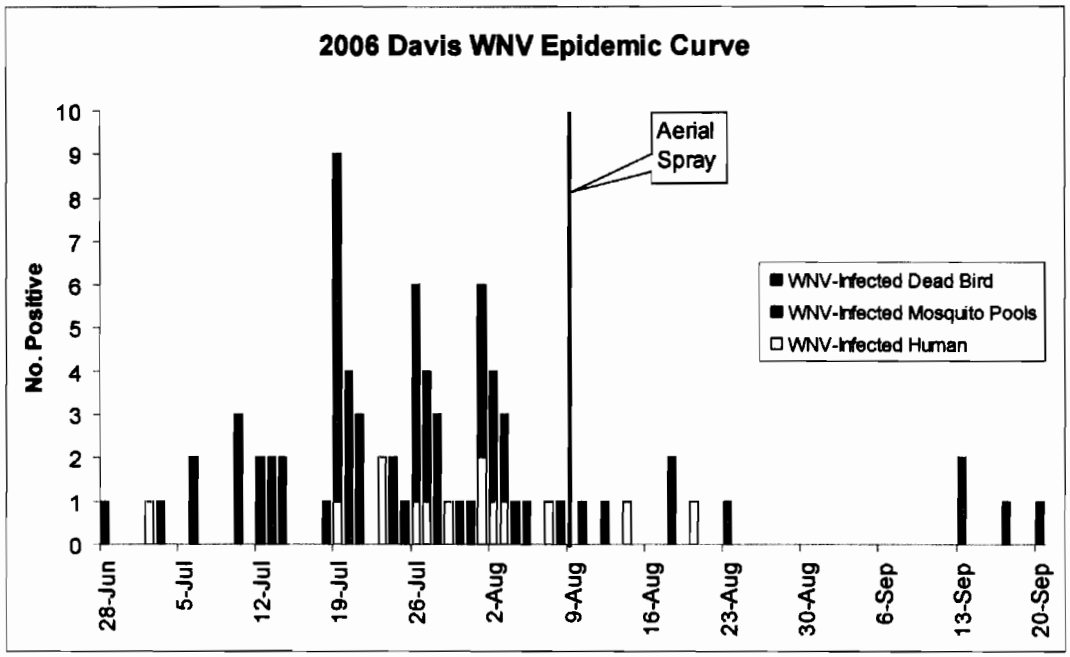

Figure 1. West Nile virus epidemic curve for Davis, California 2006 with aerial adulticide application timing indicated. Data include WNV-infected dead birds, WNV-infected mosquitoes and clinical human cases of WNV-infection.

and Response Plan (Kramer 2005), aerial adulticide applications were done on 8 and 9 August 06. Mortality rates in the sentinel mosquitoes ranged from 10-100% dependent on the amount of canopy cover and orientation of the cages to the dispersal of the adulticide (Table 1). The percent control, estimated by Mulla's formula (Mulla et al. 1971), within 2 days after the second application was 58% and 73% for host-seeking and gravid *Cx. pipiens* respectively (Table 2). Host-seeking *Cx. tarsalis* populations were reduced by 26% (Table 2). Differences between these *Culex* species may relate to the location of their larval developmental sites, with *Cx. pipiens* originating from urban sites within Davis and *Cx. tarsalis* immigrating into Davis from rural sites.

Table 1. Mortality of sentinel adult *Cx. tarsalis* in cages, set around Davis, California to determine efficacy of aerial adulticide application during August 8 & August 9 2006.

Survivorship of Sentinel Mosquitoes			
August 8, 2006	Morning Count (# Down)	Total # Mosquitoes	% Mortality
Under Canopy	46	473	9.7%
Out of Canopy	244	504	48.4%
Total	290	977	29.7%
August 9, 2006	Morning Count (# Down)	Total # Mosquitoes	% Mortality
Under Canopy	97	506	19.2%
Out of Canopy	304	535	56.8%
Total	401	1041	38.5%

After the aerial adulticide application a decrease in *Cx. pipiens* abundance occurred; however, this decrease was not significant (Figure 2). As indicated in Figure 3, outliers exist in the data; therefore a non-parametric test was performed. A one-sided Mann-Whitney test was performed on the medians of the CO_2 trap collections before and after the spraying occurred with a resulting test statistic of W = 471 and p-value = 0.05. A reduction in WNV infection rates in the *Cx. pipiens* population was observed; however, when this was adjusted for sampling effort and the minimum infection rate was used with the assumption of one positive mosquito per positive pool, the difference of the proportion of WNV positive mosquitoes was not significantly different before and after the spraying occurred (Fisher's exact test p-value =0.56). Additionally, *Cx. tarsalis* populations did not significantly decrease; Mann-Whitney test statistic W= 459, p-value=0.10. Figures 4 & 5 illustrate the median abundance of *Cx. tarsalis* before and after spray. The number of dead WNV-infected birds reported per week by the public decreased from 25 in July to 10 after the peak of the epidemic (Figure 1).

Table 2. Percent control calculations (Mulla et al. 1971) from adult mosquito abundance data collected 2 consecutive days prior and for 2 days post aerial adulticide application in the residential community of Davis, California and from control sites in surrounding agricultural areas.

DAVIS	CO_2-Baited		Gravid
	Cx. tarsalis Females/trap/night	*Cx. pipiens* Females/trap/night	*Cx. pipiens* Females/trap/night
Pre Count 1	10.2	1.6	1.7
Pre Count 2	7.4	2.3	0.8
Average	8.8	1.9	1.2
Post Count 1	5.4	1.0	0.4
Post Count 2	5.1	1.3	1.0
Average	5.2	1.1	0.7
CONTROL SITES	*Cx. tarsalis* Females/trap/night	*Cx. pipiens* Females/trap/night	*Cx. pipiens* Females/trap/night
Pre Count 1	20.6	0.4	1.3
Pre Count 2	25.2	1.2	0.8
Average	22.9	0.8	1.1
Post Count 1	21.6	1.7	2.3
Post Count 2	15.1	0.5	2.2
Average	18.3	1.1	1.7
% Control	25.6	58.0	73.1

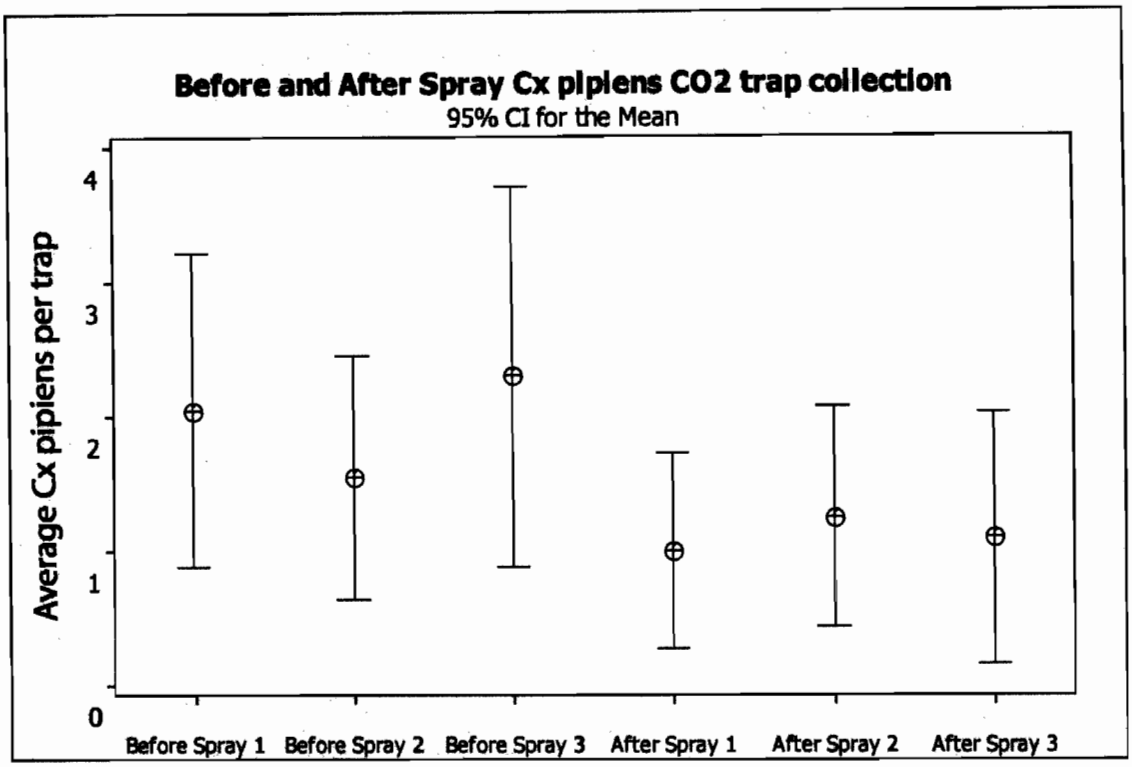

Figure 2. Interval Plot of the average number of female *Cx. pipiens* collected from CO_2 traps before and after the application of the aerial adulticide in Davis, California 2006. Data are the mean number of females collected per trap per trap night with 95% confidence intervals.

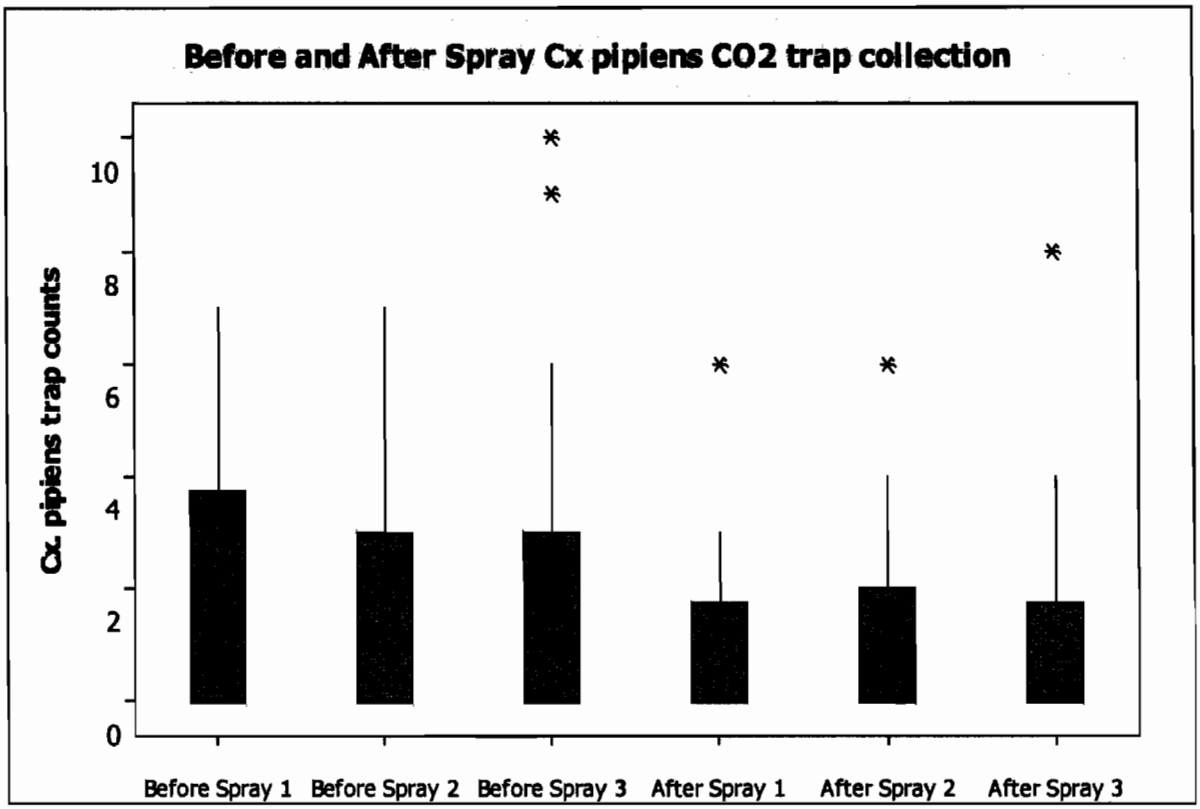

Figure 3. Boxplot of *Cx. pipiens* females from 21 CO_2 traps per 3 collection nights before and after the application of the aerial adulticide. The median and outliers are indicated.

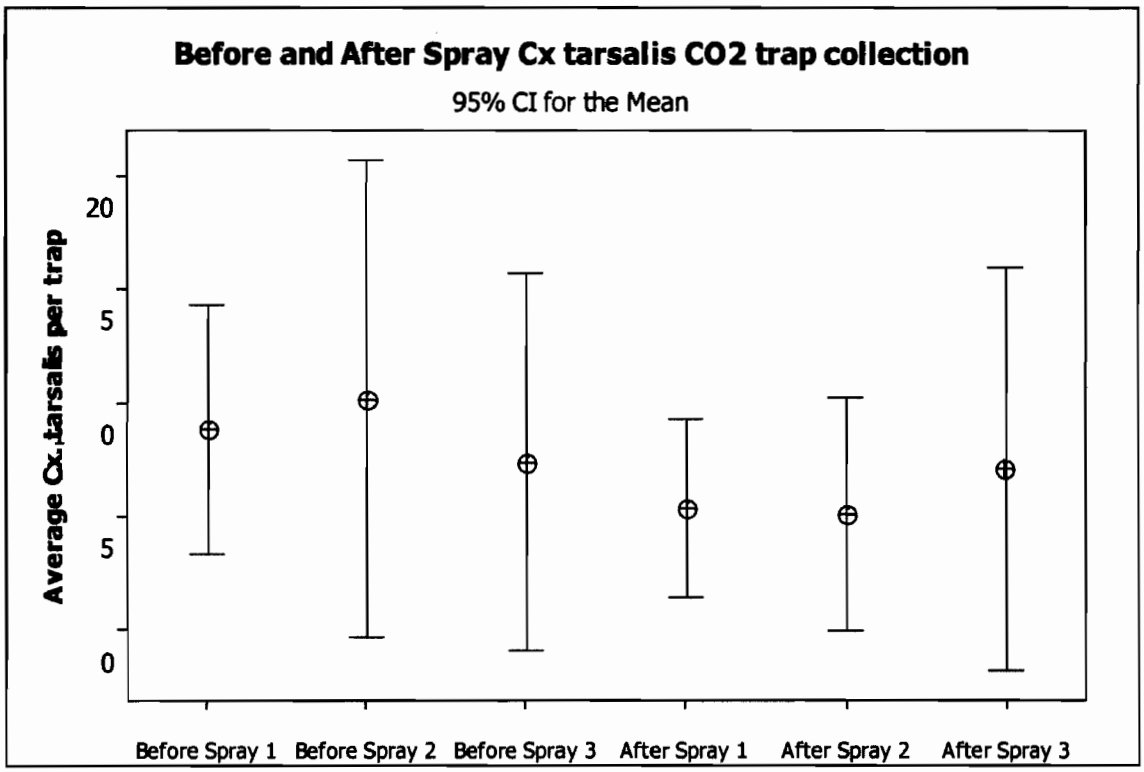

Figure 4. Interval Plot of the average number of female *Cx. tarsalis* collected from CO_2 traps before and after the application of the aerial adulticide in Davis, California 2006. Data are the mean number of females collected per trap per trap night with 95% confidence intervals.

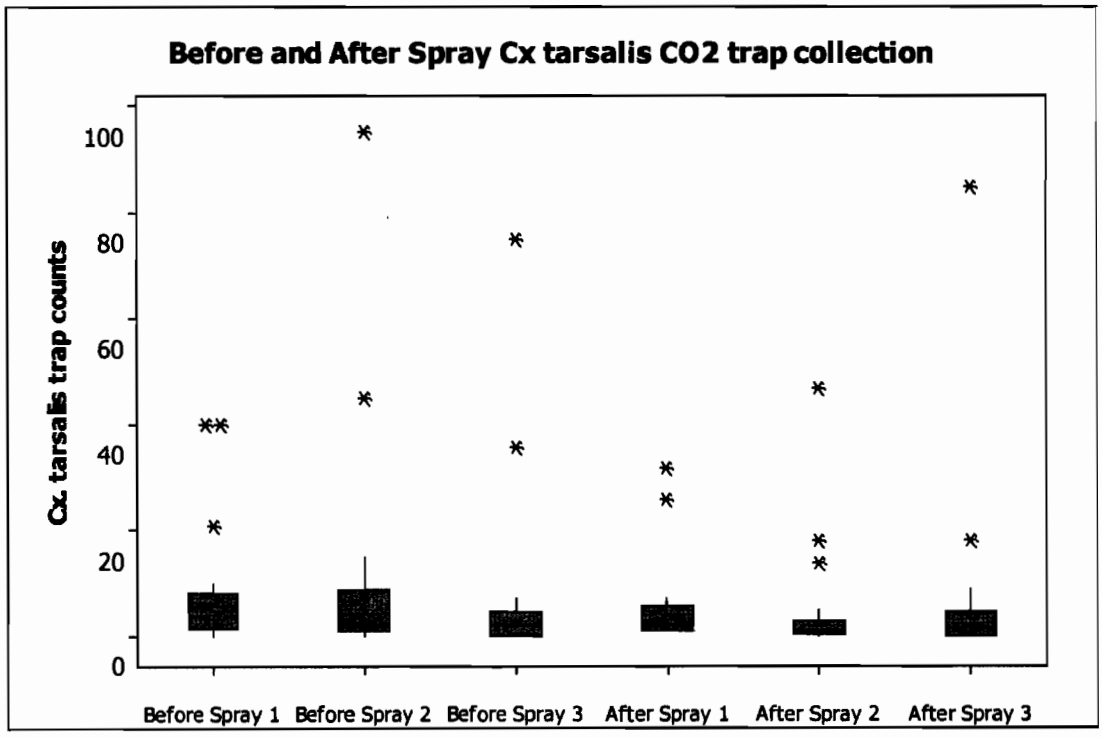

Figure 5. Boxplot of *Cx. tarsalis* females from 21 CO_2 traps per 3 collection nights before and after the application of the aerial adulticide. The median and outliers are indicated.

Evening temperatures cooled concurrently perhaps contributing to the cessation of transmission. In July, 17 of 31 nights were warmer than the minimum threshold for virus replication. After adulticide applications, by August 11, nightly ambient temperatures dropped below the 14.3°C threshold for 20 consecutive nights. Therefore, independent analysis of the effects of aerial adulticide application and temperature was impossible in this situation. WNV-positive *Cx. tarsalis* were detected again in mid-September at sites set around the periphery of Davis. This is likely due to WNV-infected mosquitoes immigrating into the residential areas from the surrounding agricultural areas. WNV did not amplify in Davis at this time, there was only one WNV-infected dead bird reported and no new human cases (Figure 1). The nightly ambient temperatures were not conducive for viral replication; of the following 17 nights, only 2 remained above the minimum threshold.

Vector abundance remains an important measure to evaluate the efficacy of vector control strategies and is an important contributor to the force of arboviral transmission. However, integrating additional environmental cues in risk assessment guidelines may expedite the initiation of emergency control. California has many widely different ecological zones with varying levels of surveillance and control efforts making vector control strategies difficult to generalize statewide. Vector control agencies must consider logistical factors as well the level of surveillance needed to truly approximate the amount of circulating virus. Arbovirus transmission may still occur when vector abundance is low making it is almost impossible to predict the levels of surveillance necessary to quantify the true infection rates. Using risk models to predict arbovirus activity and integrating multiple arbovirus transmission factors, such as using environmental measures during arbovirus outbreaks, as tools for making control decisions, will continue to be essential for effective vector control.

Acknowledgments

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Mosquito and Fly Control Research by the USDA-ARS Center for Medical, Agriculture and Veterinary Entomology (CMAVE) in the Deployed War-Fighter Protection (DWFP) Program

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ABSTRACT: Despite existing measures to prevent and control arthropod-borne diseases in military units, these diseases continue to be serious threats to deployed troops. Due to a shrinking list of safe, cost-effective pesticides for control of disease vectors, new and improved toxicants and methods for delivery are needed by the armed forces. Since 2004, the USDA Agricultural Research Service (ARS) has participated in the Department of Defense (DOD) - sponsored Deployed War-Fighter Protection (DWFP) program to identify and develop new tools for combating pest and vector species that impact deployed war-fighters. Ongoing research at the USDA-ARS Center for Medical, Agricultural and Veterinary Entomology (CMAVE) laboratory involves the discovery, evaluation, development, and optimization of: a) new pesticides effective against mosquitoes and flies; b) new personal protection products effective in preventing mosquito and fly bites, and c) new application and personal protection methodologies and strategies. Products of this research are designed to protect military troops from mosquito and fly borne diseases but will be also be available to International and U.S. Public Health agencies and Mosquito and Vector Control Districts to prevent disease transmission. Here we describe a brief summary of the DWFP mosquito control research conducted at the CMAVE.

INTRODUCTION

Arthropod-borne diseases pose a significant threat that historically has seriously affected military operations. The largest outbreak of imported malaria since Vietnam occurred in U.S. Marine Corps personnel returning from Somalia in 1993 (Newton et al. 1994), and in August 2003 a significant proportion of Joint Task Force personnel inserted into Liberia (80 out of 290 who had been ashore) experienced symptoms of malaria (Smith and Hooper 2005). It has been demonstrated that elevated mosquito populations also inhibit human activities, diminish the productivity of livestock and reduce the effectiveness of US military personnel (Chretien et al. 2005). For these reasons the U.S. military has been a leader in research on preventing these diseases. Scientists in the U.S. Department of Agriculture (USDA) have played an important role in supporting this disease research, specifically in designing novel surveillance and control methodologies for arthropod vectors (Core et al. 2005). Starting in 1942 the USDA was involved in creating delousing operations that saved thousands of U.S. troops and more than 25 million people worldwide from lice-borne typhus. The first formal collaboration between the Department of Defense (DOD) and the USDA was instituted by General George C. Marshall in 1944 and most recently in 2004 the USDA has started a new DOD program, called the Deployed War-Fighter Protection program (DWFP) to develop a new generation of tools to protect the U.S. military from disease-transmitting arthropods.

DWFP RELATED RESEARCH IN THE MOSQUITO AND FLY RESEARCH UNIT, CENTER FOR MEDICAL, AGRICULTURAL AND VETERINARY ENTOMOLOGY

The Mosquito and Fly Research Unit (MFRU) of the Center for Medical, Agricultural and Veterinary in Gainesville, Florida has a long history of cooperating with the DOD to provide solutions to specific needs. The earliest predecessor of the MFRU was a medical entomology unit created during World War II in Orlando, Florida to develop methods for stopping transmission of insect-borne disease. At this lab, DDT was demonstrated to kill lice that transmit epidemic typhus and fleas that transmit plague. The earliest plans for delousing were put into effect in Sicily, where it has been credited with preventing an epidemic of typhus. In the 1960s, the lab developed DEET as an effective broad-spectrum repellent for biting insects to protect troops from arthropod-borne diseases. In the 1960's-70s, ultra-low volume (ULV) pesticide systems were developed to provide an area-wide protection of troops from disease vectors. This technique greatly reduces the amount of pesticide dispersed in outdoor applications. From the 1960-1980's, the lab was a pioneer in development of permethrin-treated military uniforms and bed nets that repel ticks and kill disease-carrying mosquitoes, saving millions of people from malaria. In the 1980s, research at the lab lead to the introduction of QuickStrike™ baited with fly-pheromone (Z)-9-tricosene plus nithiazine insecticide, and its addition to the DOD Standard Pesticide List. From 1990 to the present, the lab has been addressing the need to develop more effective permethrin treatment of uniforms to protect Marines from disease vector mosquitoes and ongoing work is directed to determining the best treatment and methods of binding permethrin to permanent press US Marine Corps uniforms and nettings.

The mission of the MFRU is to develop novel technologies for detection and population monitoring, repellents for the protection of humans and animals from biting and filth breeding flies, and effective chemical, biological, and genetic control technologies and

integrated management strategies for insects and arthropods of medical and veterinary importance. The mission is primarily in support of the Departments of Agriculture and Defense; however, results of research undertaken at CMAVE have application to programs of animal and public health in international, national, state, and local agriculture and public health government agencies, private industry, and the general public. The medical and veterinary entomology staff of MFRU consists of 10 permanent scientists, 8 postdoctoral/visiting scientists/collaborators, and approximately 30 technical/support personnel. The laboratory facility is modern and well equipped and comprises approximately 45,000 square feet of space.

Currently in the DWFP program the emphasis is on identifying and testing new classes of pesticides aimed at disease vectors, new tools for pesticide application suited to the military environment, and new methods for personal protection. The objective of the DWFP program in the MFRU is to develop novel control methods to protect deployed military personnel from vectors as follows:

- Discover, evaluate and develop new candidate adulticides effective against mosquitoes and flies
- Discover, evaluate and develop new candidate chemicals effective in preventing mosquitoes and flies from biting deployed personnel
- Optimize use of candidate chemicals for mosquito/fly control and personal protection to serve military needs
- Devise and develop "attract and kill" management systems for mosquitoes and flies
- Discover, evaluate and develop new personal protection strategies

The primary DWFP Research Areas in the MFRU include:

1. Novel insecticide chemistries or formulations
   - New compounds
   - Native plants compounds
   - Physiological responses to new compounds
2. Personal protection
   - Revaluation of "old" repellents
   - Sustained release repellents
   - Spatial repellent in military tents
   - Repellent-treated uniforms, fabrics and tent materials
   - Sand fly protection
   - Fly traps and baits
   - Repellents and inhibitors against infected mosquitoes
3. Application technology
   - Pesticides on natural barriers
   - Barrier treatments in *Anopheles* habitats
   - Barrier treatments in desert habitat
   - Repellents, inhibitors, and barrier treatments in sub-Saharan habitat
   - Repellents, inhibitors, and barrier treatments in humid tropical habitat
   - Development of portable devices for detection and quantification of insecticides, repellents and inhibitors
   - Electrostatic and other sprayers
   - Thermal fog machines
   - Insecticide-treated visual targets for flies
   - Mosquito coils
4. General support for DWFP
   - New insecticidal compounds – University of Florida, Gainesville Florida
   - New insecticidal neuropeptides and Application methods – Areawide Pest Management Research Unit, College Station, Texas
   - Application methods – Navy Entomology Center of Excellence, Jacksonville, Florida
   - New toxicants and repellents – Chemicals Affecting Insect Behavior Laboratory, Beltsville, Maryland
   - Sand fly rearing – Walter Reed Army Institute of Research, Washington DC

As one example of the research being conducted in the DWFP program of the MFRU, Pridgeon et al. (2007) examined the structure-insecticidal activity relationships of 33 piperidines against adult female *Aedes aegypti*. On the basis of 24 hour LD_{50} values after topical application, the most toxic compound was 2-ethyl-piperidine. The toxicities of piperidine derivatives were significantly decreased when a benzyl moiety was attached to the carbon of the piperidine ring. The toxicity order of three moieties attached to the carbon of the piperidine ring was ethyl- > methyl- > benzyl-derivatives. These preliminary results will be useful in guiding further piperidine ring modifications in the development of potential new insecticides.

SUMMARY

The scope of DWFP program research at the MFRU in CMAVE is entirely within the scope of USDA-ARS National Program 104, Veterinary, Medical, and Urban Entomology Research, and addresses the goals of program including: efforts to provide new and improved knowledge of mosquito behavior, host-pathogen interactions, and neural and sensory pathways; repellents; and biological and chemical control strategies. The primary customer of this research is the DOD and the products of this research are designed to protect deployed military troops from the vectors of mosquito and fly borne disease; however, the products of this research will also be available to the international and U.S. Public Health agencies and Mosquito and Vector Control districts to prevent disease transmission in the General Public.

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GIS Early-Warning System for Vectors of Rift Valley Fever: Anomaly Analysis of Climate-Population Associations

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ABSTRACT: A critical component of predicting the risk of transmission of mosquito-borne viruses is knowing the status of vector populations. Mosquito control agencies have good systems for measuring mosquito populations at county or district levels, but these data are not synthesized to regional or national levels – a strategic disadvantage in the face of emerging human and animal disease threats such as Rift Valley fever, dengue, or chikungunya. We are creating a Geographic Information System (GIS) of mosquito surveillance records compiled from mosquito and vector control districts and public health agencies throughout the U.S. This GIS of historic and current surveillance data will be used to develop an understanding of the effects of climate on mosquito distribution, timing, and abundance. The principal goal of the project is to provide early warning and flag areas of the U.S. at risk of unusually large mosquito populations based on historical climate-population associations. The project is initially focused on potential vectors of Rift Valley fever in the U.S., but since we include trap data for all mosquito species the GIS will be informative for vectors of nearly any emerging or established mosquito-borne disease threat. At local and national levels the spatial information product of this GIS is an important resource for the public and animal health communities, particularly in coordinating and targeting vector control, vaccines, diagnostics, and educational resources during routine, emergency, or disaster situations. By modeling U.S. vector population data in the context of RVF activity and movement worldwide, we may be able to enhance surveillance and control measures at critical areas of the U.S. and avert an RVF event. Here we discuss one early phase of the model, that of developing effective ways to compare climate and population data by calculating anomalies.

INTRODUCTION

Rift Valley fever (RVF) virus is a mosquito-borne zoonotic hemorrhagic virus endemic to much of sub-Saharan Africa (Megan and Bailey 1989). RVF epizootics and epidemics can bring about large health and economic impacts in Africa including mortality and abortion in domestic animals, and significant morbidity and mortality in humans. In 2000 the west coast of the Arabian Peninsula experienced an outbreak of RVF (DoD-GEIS website), which demonstrated the ability of the pathogen to move outside of its endemic region. Given the complexity and speed of international commerce, and given that laboratory studies of U.S. mosquitoes suggest that the ecological infrastructure exists for RVF to be transmitted in the U.S. (Gargan et al. 1988), there is a need for a system that could alert areas of the U.S. of the potential for RVF transmission. The recent 2006-2007 outbreak of RVF in east Africa was predicted by climate-population modeling (Anyamba et al. 2006) and highlights the potential for similar predictive models to be used to minimize the risk of RVF in the U.S. Specifically, a predictive model could estimate the distribution and abundance of populations of potential mosquito vectors of RVF in the U.S. Combining knowledge of the status of RVF outbreaks in Africa, likely pathways of arrival of infected mosquito vectors such as ports and air terminals, and the geographic arrangements of U.S. mosquito vectors, human populations, and susceptible wildlife and livestock populations, the system could flag areas at high risk of RVF transmission. Spatially targeted early warning of RVF transmission risk would allow efficient distribution of mosquito control measures, vaccines, diagnostics, and information. We are in the early phases of developing a vector early-warning model for the U.S. which exploits historical relationships between mosquito population dynamics and climate dynamics. This work is being conducted in a GIS platform using remotely-sensed climate data from NOAA satellites and long-term mosquito population surveillance records gathered from mosquito control and public health agencies throughout the U.S.

THE VECTOR EARLY-WARNING SYSTEM

The detailed methodology of the RVF vector early-warning GIS is described elsewhere (Linthicum et al. 2007). In summary, the core of the GIS is an extensive database of georeferenced mosquito population surveillance records from several regions of the U.S. Although mosquito population surveillance does not sample all areas, and sampling methodology is heterogeneous, many data sets span 20 or more years and provide images of population change which can inform predictive models. Population data are normalized using a trap-night index averaged by month. Although additional environmental and land-use data will eventually be incorporated, the current GIS analyses compare mosquito population data to the Normalized Difference Vegetation Index (NDVI) 25-year data set gathered by NOAA satellites and processed

by NASA-Goddard Space Flight Center (Tucker et al. 2005). NDVI is particularly useful for study of population-climate associations since the index couples climate and vegetation dynamics in a single "greenness" index. This index saves multiple analyses between populations and component environmental parameters which otherwise may on their own be misleading.

ANOMALY DATA AND PRELIMINARY FINDINGS

Figure 1 shows co-plots of monthly mosquito surveillance data and NDVI for a county in Florida over 20 years. These data happen to document the replacement of *Aedes aegypti* L. by *Ae. albopictus* (Skuse) in that county. Population data are not available for four periods, 1985, 1987, 1998 and 2001, but the gradual extinction of *Ae. aegypti* can be clearly seen, along with seasonal variations over the years in populations of both species. In this figure NDVI data are displayed in two forms. The upper dashed curve shows mean monthly raw NDVI values for that county across the sample period. Observe that the curve rises and falls annually, tracking vegetation changes from winter to summer to winter; the higher the NDVI value the greener the landscape. The lower solid curve shows mean monthly anomaly NDVI values for that county across the sample period. The anomaly curve oscillates above and below a zero line, which is a line of no difference against a 25-year mean for that month. Anomaly values above zero indicate a wetter- and greener-than-average month; values below zero indicate a dryer- and browner-than-average month. NDVI anomalies capture important patterns in climate that are difficult to detect from raw data. Given the tight link between mosquitoes and precipitation, and the tight link between NDVI and precipitation, the NDVI anomaly values should relate to population levels. In the simplest terms, the vector early warning model will function using algorithms derived from these historical NDVI-population associations. However, the complexity of the model will evolve as we consider the mode of action of climate on a population of a mosquito species.

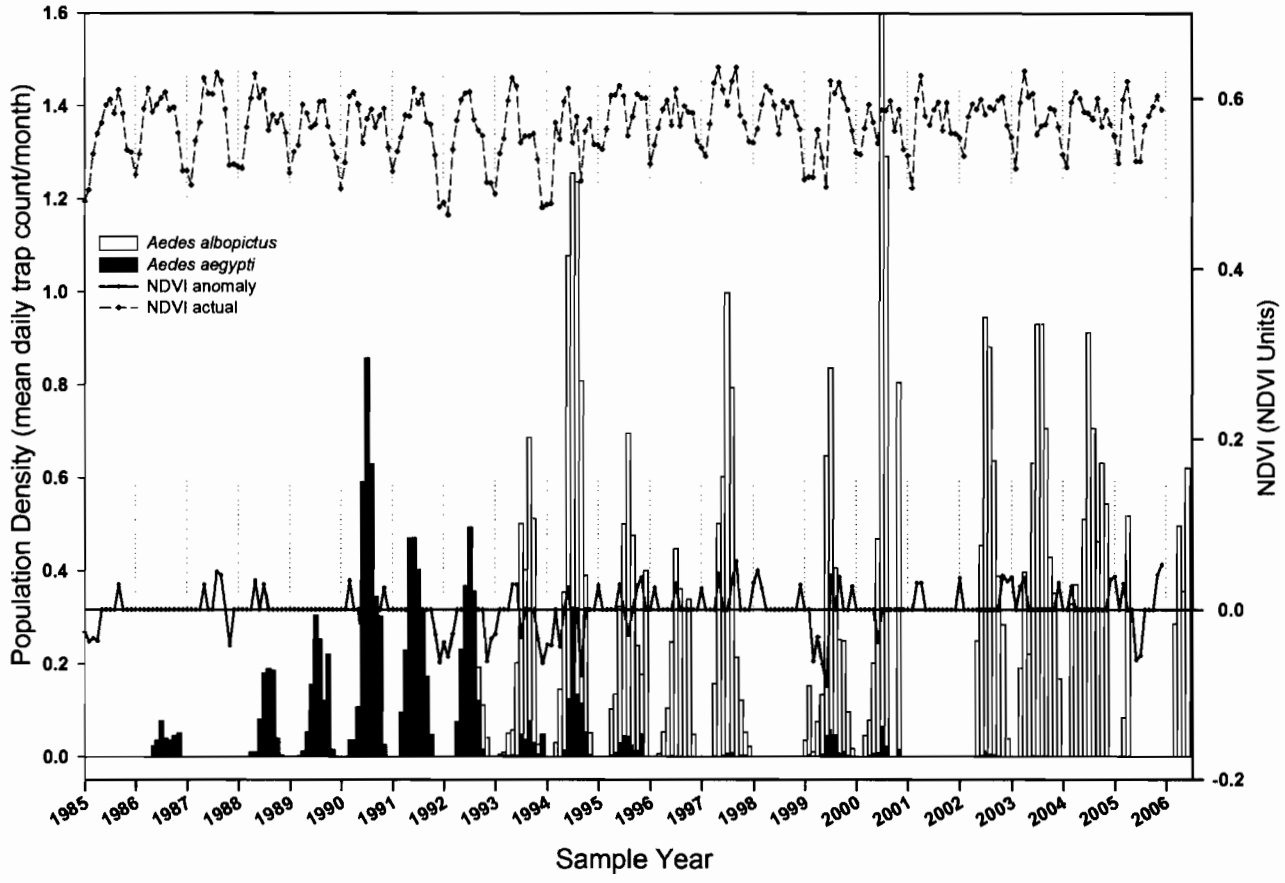

Figure 1. Population dynamics of *Ae. aegypti* (gray bars) and *Ae. albopictus* (white bars) over a 20-year period in a Florida county. Climate data are shown as raw NDVI values (dashed line) and anomaly NDVI values (solid line). NDVI anomaly values oscillate above and below a zero line, which indicates no change from a 25-year mean.

Figure 2 shows a subset of the data plotted in Figure 1, namely, a series of values from all June samples of populations and climate from 1986 through 2006. This is a different kind of plot since all data are excluded except June data, and the rises and falls in NDVI are replaced by flatter curves: most Junes are comparable to other Junes in this county with respect to vegetation greenness. This is also a different kind of plot since there are two sets of raw NDVI values and two sets of anomaly NDVI values, i.e., not just NDVI data from June, but NDVI from May as well. By plotting data from consecutive months together for each year we can see that some are very similar (such as in 1993) and some are very different (such as in 1990), and these attributes of adjacent months have implications for mosquito populations. For instance, sustained greenness (i.e., sustained moisture) or sustained brownness (i.e., sustained dryness) should have different effects on later population samples. Similarly, adjacent months with heterogeneous values should bring about sudden increases or decreases in populations in the later sample. Another important difference in the population plot in Figure 2 as compared to Figure 1 is that population values are anomalies relative to the mean 20-year sample for June. Much like with NDVI, increases and decreases in raw population values can be misleading or confusing, but anomalies provide an instant quantitative index of the population.

The next question with analysis of climate anomalies and population anomalies is what mode of action of climate is associated with large positive population anomalies. For example, the 1990, 1991, and 1992 population anomalies in Figure 2 appear to be related to consecutive monthly anomalies that have magnitude differences. Further analysis will be performed on this and many other data sets arranged in such anomaly plots to derive algorithms

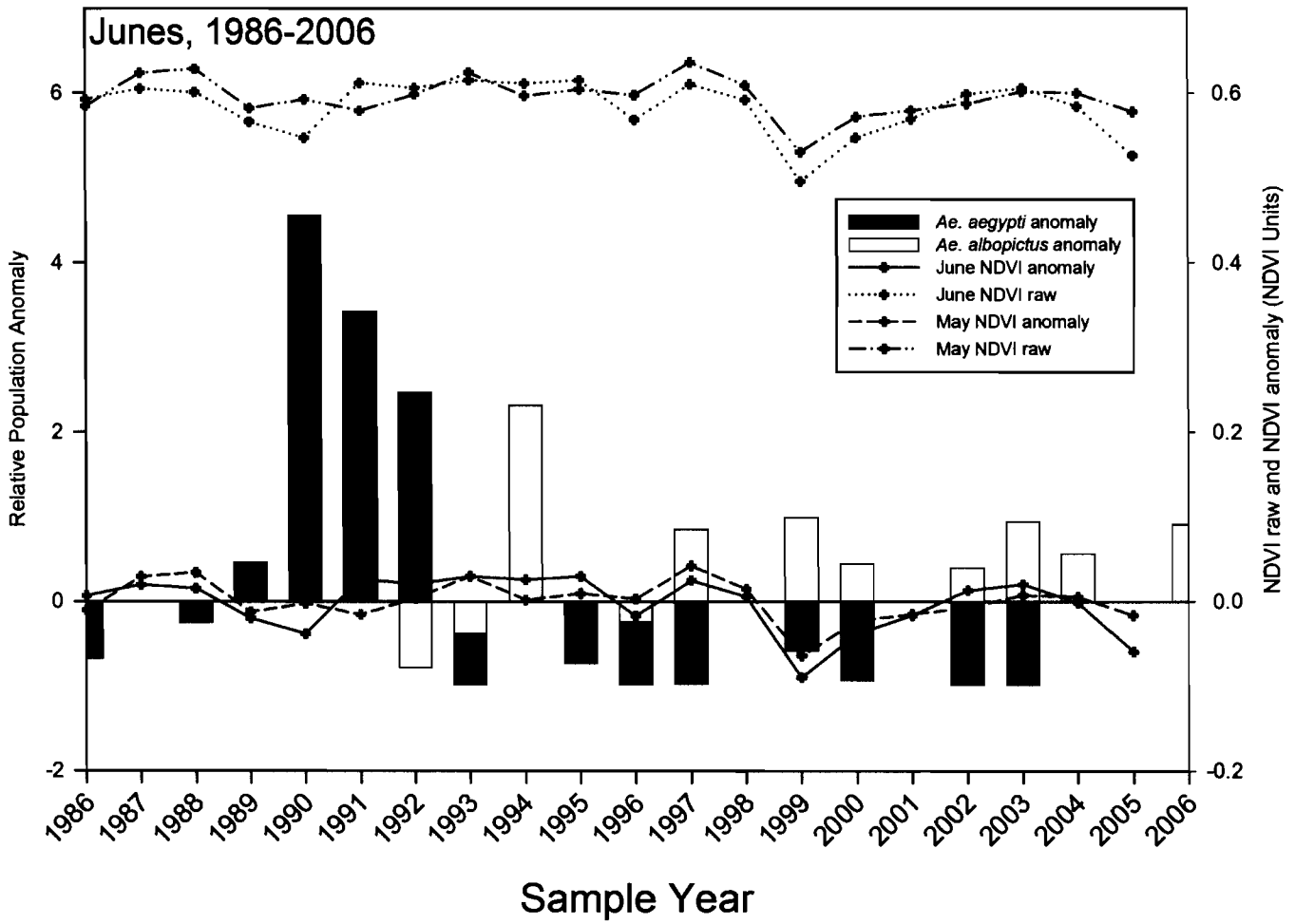

Figure 2. *Ae. aegypti* (gray bars) and *Ae. albopictus* (white bars) population anomalies for June over a 20-year period in a Florida county. Climate data for May and June of each year are shown as raw NDVI values (dashed/dotted and dotted lines, respectively) and anomaly NDVI values (dashed and solid lines, respectively). Population and NDVI anomaly values oscillate above and below a zero line, which indicates no change from 20-year and 25-year means, respectively.

that capture more attributes of these consecutive NDVI anomalies, such as the relative importance of magnitude differences when both are below zero or above zero, or on either side of zero. Rules will be derived and algorithms will be available that will forecast population dynamics, given climate. Climate-mosquito population work described elsewhere (Britch et al. *Military Medicine, in review*) suggests these algorithms will likely differ with ecological regions of the U.S. In the case of species competing for similar habitat, as with the two container-breeding species shown in Figures 1 and 2, ecological interactions among species in the mosquito community in a region will also be taken into account.

SUMMARY

Emerging mosquito-borne pathogen threats such as Rift Valley fever are of great concern, but there are steps we can take to strategically protect the U.S. against disease transmission. Although much is known regarding the total distributions of U.S. mosquitoes, less is known about their temporal and spatial heterogeneity within these distributional polygons. High quality satellite climate data and abundant records of mosquito populations exist and are being analyzed in a GIS. We found that examining climate and population anomalies from long-term means reveals potentially powerful predictive associations. These associations will be the driving core of a GIS designed to spatially predict unusually large populations of mosquitoes using near real-time climate data. With an understanding of the environmental forces that drive the distribution, timing, and abundance of mosquitoes in the U.S. we may design GIS-based early warning systems. These systems could monitor the environment and flag regions at high risk of mosquito-borne pathogen transmission and more effectively guide the distribution of surveillance and control measures.

Acknowledgements

We thank the people from the multitude of mosquito and vector control districts and military and civilian public health agencies throughout the U.S. who have kindly and enthusiastically participated in this research by providing data and expertise. We also thank our colleagues at CMAVE and D.A. Strickman of ARS National Program Staff for valuable insight and discussions.

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Mosquito Abundance and Arbovirus Surveillance in Northwestern Riverside County, California in 2006

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ABSTRACT: The Northwest Mosquito and Vector Control District continued its arbovirus surveillance program in 2006. Based on the total number of mosquitoes (35,980) collected in encephalitis virus surveillance traps and the New Jersey light traps, the most abundant species was *Culex erythrothorax* (67.9%), followed by *Cx. quiquefasciatus* (14.7%), *Cx. tarsalis* (5.3%), *Cx. stigmatosoma* (3.7%), and *Anopheles hermsi* (2.4%). Other species that followed in small numbers, in descending order, included *Culiseta incidens* (1.9%), *Cx. thriambus* (1.8%), *Cs. inornata* (1.6%), *Cs. particeps* (0.5%), *Ae. washinoi* (0.1%), and *An. franciscanus* (0.1%). Based on the seasonal distribution, mosquito activity sustained throughout the year with different species peaking at different times. Of the 249 mosquito pools representing different species, only one pool of *Cx. erythrothorax* tested positive for the West Nile virus (WNV). Among the 76 birds distributed over six flock locations, only 9 birds from 5 different flocks tested positive for WNV. There was also one live House finch (*Carpodacus mexicanus*) and one dead American crow (*Corvus brachyrhynchos*) positive for WNV. Although mosquito abundance was higher than previous years, arbovirus activity declined significantly in 2006.

INTRODUCTION

The Northwest Mosquito and Vector Control District (NWMVCD) has provided arbovirus surveillance and mosquito and vector control services in the cities of Corona, Norco, Lake Elsinore, parts of the city of Riverside and several adjoining unincorporated communities for over 45 years. The NWMVCD service area encompasses approximately 615 km^2 with over 400,000 residents. Before the invasion of the West Nile virus (WNV), arbovirus surveillance focused on St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE). With the first detection of WNV in *Culex tarsalis* Coquillett in Imperial County, California in 2003 (Reisen et al. 2004) and its spread to the Coachella Valley, Riverside County, WNV activity in NWMVCD was first reported from a live House finch, *Carpodacus mexicanus* Say, in the Canyon Crest area in the city of Riverside on September 22, 2003, followed by first WNV isolation from a dead America crow, *Corvus brachyrhynchos* Say, a month later (Wisniewska-Rosales et al. 2003). In the following year, the WNV activity was reported in 45 dead birds, 15 live wild birds, 56 sentinel chickens, and 23 mosquito pools in NWMVCD with 72 horse infections and 116 human cases county-wide (Wisniewska-Rosales and Williams 2004). In 2005, there were 8 dead birds, 7 live wild birds, 6 mosquito pools, and 43 sentinel chickens were positive for WNV with only 2 horse infections and 2 human case (GW unpublished data).

As a result of increased WNV activity in previous years, the District intensified its efforts in both arbovirus surveillance and mosquito control operations in 2006. Whereas our operational activities focused on source reduction and larvicidal treatments, occasional use of adulticides was carried out to protect the health of our constituents. The present paper highlights arbovirus surveillance efforts with data generated on mosquito abundance in the NWMVCD areas in 2006.

MATERIALS AND METHODS

The general methodology and equipments used, same as described by Wisniewska-Rosales and Williams (2004), were as follows:

Mosquito surveillance:

For monitoring adult mosquito populations, mosquitoes were collected by using New Jersey light traps (NJLTs) (Mulhern, 1942) operated at 12 fixed locations throughout the District (Fig. 1). The traps were set at 3 urban, 6 suburban and 3 rural habitats as described by Mian and Reed (2002) and were checked weekly during April through October and biweekly from January through March and in November and December. The traps were equipped with 25-watt incandescent light bulbs and placed approximately 2.4 m above ground level. The mosquitoes trapped were counted and sorted according to sex and species, and were submitted to the California Department of Health Services as part of the state-wide adult mosquito occurrence report. Data on mosquito abundance in 2006 were compared with previous three years, 2002-2004, for significant differences, using the *Chi*-square test (PSI-Plot 1993).

For arbovirus surveillance, host-seeking female mosquitoes were collected by using carbon dioxide-baited encephalitis virus surveillance traps (EVSTs) without light or rain shields (Cummings and Meyer, 1999). Each trap was operated at an approximate height of 1.25 m and CO_2 was presented in a 3.7-liter Styrofoam®-insulated bucket with 4 to 5 openings at the bottom (diameter: 4 mm). The openings were located approximately18 cm above trap entry.

A total of 25 trap locations were selected to best monitor mosquito-infested areas within the District (Fig. 1). The traps operated from dusk to dawn at different locations were set on alternating weeks so that each location was sampled once every two

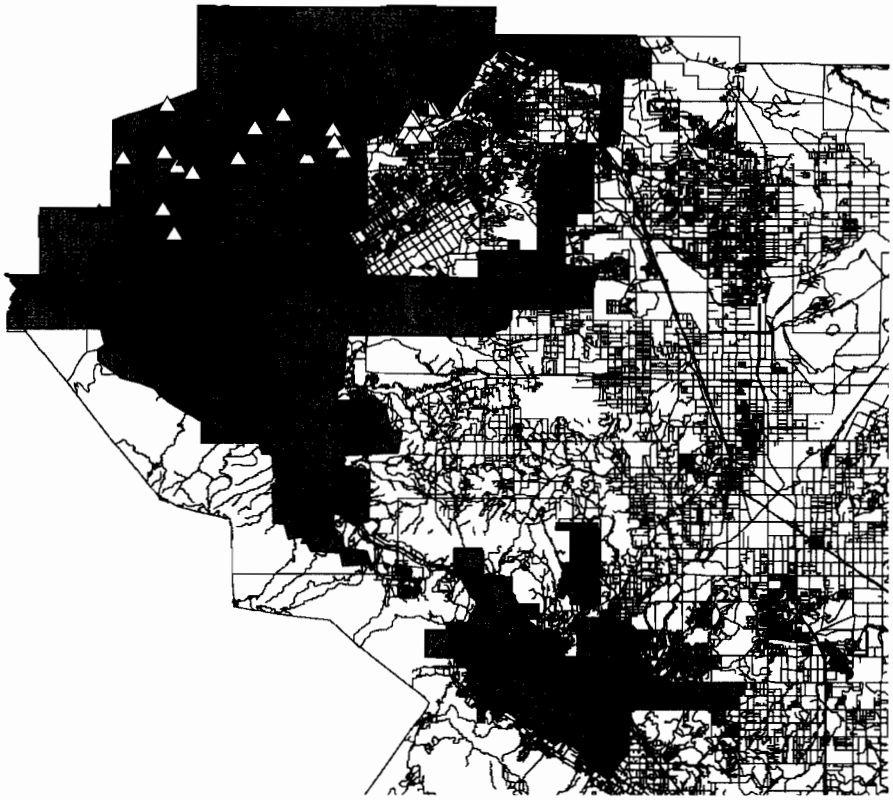

Figure 1. Districtuion of wild bird traps, sentinel chicken flocks, New Jersey (NJ) light traps and encephalitis virus surveillance (EVS) traps in 2006.

weeks. Traps were deployed weekly May through October. Due to much lower mosquito abundance, they were used biweekly in January, February, November and December. Beginning July 2006, 20 additional locations along the Santa Ana River (Fig. 1) were monitored weekly with EVSTs.

Mosquitoes collected in EVSTs were anesthetized with triethylamine and sorted by species and sex. Pools of 12 to 50 mosquitoes were then shipped on dry ice overnight to the University of California at Davis Center for Vector-Borne Diseases (CVEC) for testing for arboviruses, SLE, WEE, WNV, and CE (California encephalitis) (Chiles et al. 2004). Female *Culex erythrothorax* Dyar, *Cx. quinquefasciatus* Say, *Cx. stigmatosoma* Dyar and *Cx. tarsalis* were included in the samples submitted.

Arbovirus surveillance

Six sentinel chicken flocks of 10 white leghorn birds each were maintained at different locations throughout the District (Fig. 1). Blood samples were collected biweekly from each chicken January through December. The samples were absorbed on filter-paper strips, air dried and submitted to the California Department of Health Services—Viral and Rickettsial Laboratory (CDHS-VRDL) for testing by enzymatic immunoassay (Reisen et al. 1994).

For arbovirus surveillance in wild birds, four modified Australian crow traps (McClure, 1984) were used in Corona, Norco, Canyon Crest and Lake Elsinore (Fig. 1) during 2006. The traps were baited with wild bird seed (Golden State Commodities, P.O. Box 458, Oakdale, CA 95361) and water to attract House finches and House sparrows, *Passer domesticus* L. The traps were checked twice a week for birds. The birds attracted into the traps were identified to species and sex, banded, bled and released at the site. We also collected and tested blood samples of Brown-headed cowbirds, *Molothrus ater* Boddaert, obtained from modified Australian crow traps operated by the Least Bells Vireo Conservation Project of the Santa Ana Watershed Authority (SAWA) and by the Orange County Water District (OCWD). Bird blood samples (0.1 - 0.2 ml from each bird) were collected from the jugular vein with a 1-ml insulin syringe fitted with a 28 gauge, ½ inch hypodermic needle. Each sample, dissolved in 0.9 ml of 0.75% bovine serum albumin/ PBS (phosphate-buffered saline) diluent, was submitted to the Orange County Vector Control District Laboratory for SLE and WEE antibody testing by serum hemagglutination inhibition as described by Gruwell at al. (2000). The samples were also tested for antibodies to the West Nile virus by a blocking enzyme-linked immunosorbent assays (ELISA)

(Jozan et al. 2003).

As part of the California Department of Health Services (CDHS) Dead Bird Surveillance Program, dead birds reported to the District were picked up and submitted to the California Animal Health and Food Safety (CAHFS) Laboratory in San Bernardino for testing for WNV.

RESULTS AND DISCUSSION

Mosquito Surveillance

In 2006, a total of 35,980 adult mosquitoes were collected in EVSTs and NJLTs combined (Table 1). The most abundant species collected was *Cx. erythrothorax* accounting for 67.9% (24,430), followed distantly by *Cx. quiquefasciatus* (14.7%), *Cx. tarsalis* (5.3%), *Cx. stigmatosoma* (3.7%), and *An hermsi* Barr & Guptvanji (2.4%). Other species that followed in small numbers, in descending order, included *Culiseta incidens* (Thomson) (1.9%), *Cx. thriambus* Dyar (1.8%), *Cs. inornata* Williston (1.6%), *Cs. particeps* (Adams) (0.5%), *Ae. washinoi* Lanzaro & Eldridge (0.1%), and *An. franciscanus* MacCracken (0.1%). The three species, *Cx. eryhtrothorax*, *Cx. quinquefasciatus* and *Cx. tarsalis*, have been holding the top three rankings, especially in data from EVSTs. For example, *Cx. erythrothorax* ranked top in 2004, but was preceded by *Cx. quinquefasciatus* in 2002 and 2003 (Wisniewska-Rosales and Williams 2005). Comparing mosquito abundance data of 2006 with 3-year (2002-2004) combined data, all species showed significantly higher numbers in 2006 in both EVSTs and NJLTs (Table 2). *Culex thriambus* found in 2006, was not reported in earlier years, 2002-2004 (Wisniewska-Rosales et al. 2004, Wisniewska-Rosales and Williams 2005).

Table 1. Mosquito abundance by species caught in encephalitis virus surveillance traps (EVSTs) and New Jersey light traps (NJLTs) in Northwestern Riverside County in 2006.

Species	EVSTs	NJLTs	Total	%
Aedes washinoi	21	28	49	0.1
Anopheles franciscanus	20	11	31	0.1
An. hermsi	822	42	864	2.4
Culex erythrothorax	23956	474	24430	67.9
Cx. quinquefasciatus	4746	530	5276	14.7
Cx. stigmatosoma	1059	291	1350	3.7
Cx. tarsalis	1814	110	1924	5.3
Cx. thriambus	515	116	631	1.8
Culiseta incidens	681	11	692	1.9
Cs. inornata	280	280	560	1.6
Cs. particeps	155	18	173	0.5
Total	34069	1911	35980	100

Table 2. Mean abundance of mosquitoes/trap-night in 2006 in comparison with 2002-2004 data[1].

Species	EVSTs			NJLTs		
	3-years[2]	2006	Chi-sq.	3-years	2006	Chi-sq.
Aedes washinoi	0.02	0.44	0.38	0.00	1.00	na
Anopheles franciscanus	0.00	0.41	na	0.04	1.00	0.88
An. hermsi	0.76	17.12	15.12*	0.00	1.50	na
Culex erythrothorax	15.11	499.08	455.52*	0.06	1.41	1.23
Cx. quinquefasciatus	22.32	98.90	48.32*	0.13	1.58	1.22
Cx. stigmatosoma	3.26	22.06	13.95*	0.10	0.86	0.60
Cx. tarsalis	13.18	37.80	11.88*	0.15	0.33	0.06
Cx. thriambus	na	10.73	na	na	0.34	na
Culiseta incidens	0.10	14.17	13.87*	0.05	0.83	0.69
Cs. inornata	0.15	5.83	5.39*	0.01	0.03	0.01
Cs. particeps	0.19	3.23	2.70	0.02	0.05	0.12
Total	55.09	709.77	560.37*	0.56	8.93	7.33*

[1]mosquitoes collected in encephalitis virus surveillance traps (EVSTs) and New Jersey light traps (NJLTs).
[2] 2002—2004. *significant (P=0.05), na—not available.

Based on mosquito responses to different trap types using carbon dioxide or light, the seasonal abundance is illustrated by trap type. The data from NJLTs (Fig. 2) show activity of *Cx. erythrothorax* from March though December with peaks in May and July. Similarly, *Cx. quinquefasciatus* that appeared late in May, continued into December with a small peak in September. In general mosquito numbers caught in NJLTs were smaller than those of EVSTs.

Data from EVSTs showed mosquito activity throughout 2006 with spikes in February, May, August and November (Fig. 3). *Culex erythrothorax* had distinct peaks in February, April, August and November, whereas *Cx. quiquefasciatus* peaked in February, June, September and November. *Culex tarsalis* had relatively smaller peaks in May, August and October, whereas *Culex thriambus* was abundant in August and November only.

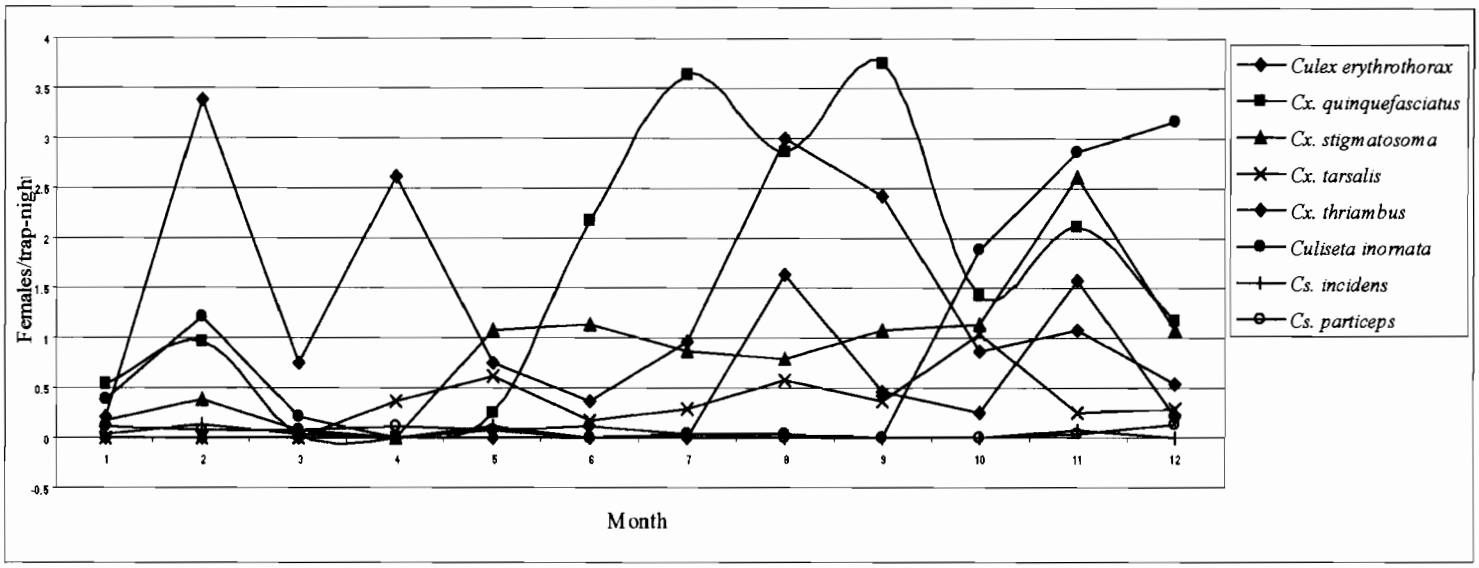

Figure 2. Temporal distribution of adult mosquitoes collected in New Jersey light traps in Northwestern Riverside County, CA in 2006.

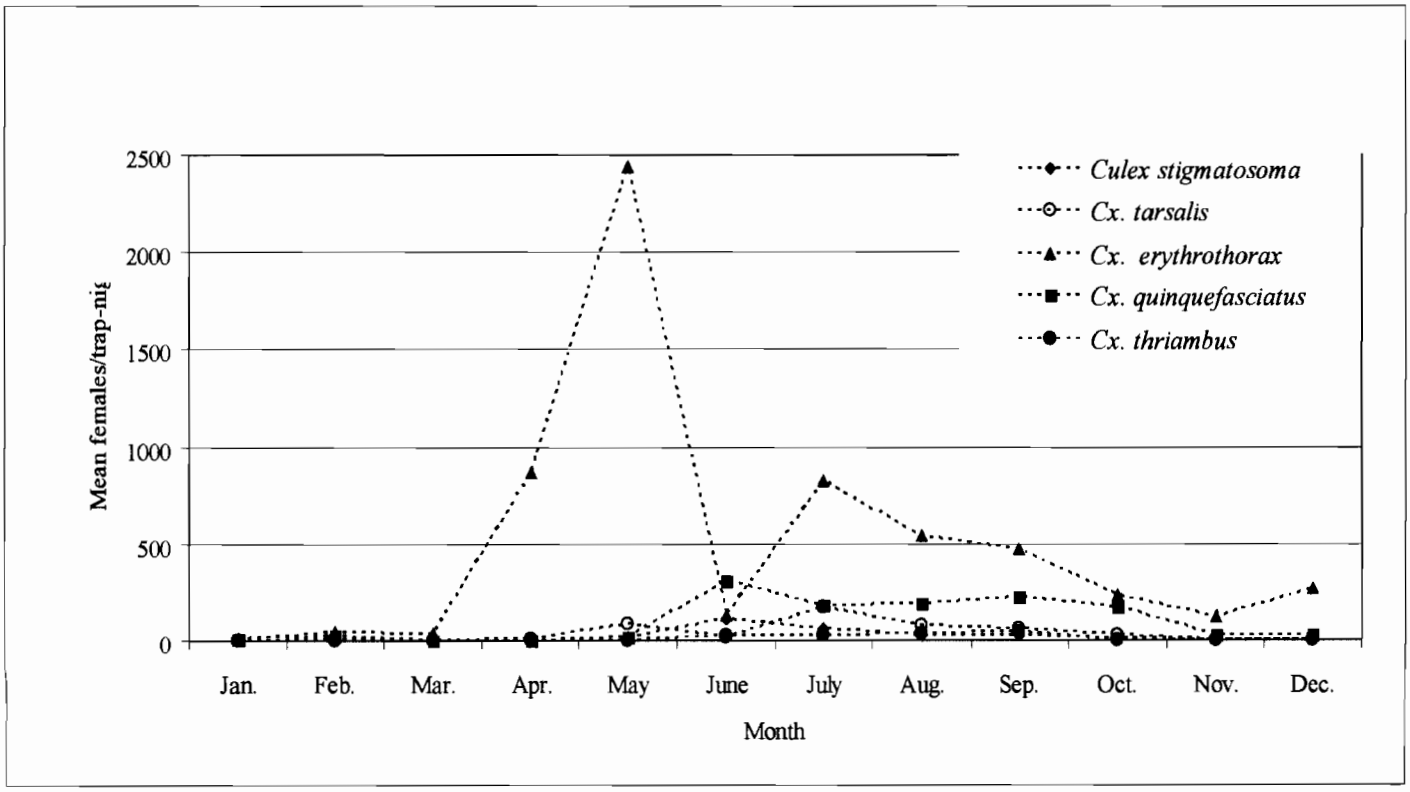

Figure 3. Temporal distribution of female mosquitoes collected in encephalitis virus surveillance traps in 2006.

Arbovirus surveillance

In 2006, a total of 249 mosquito pools (8,842 mosquitoes) were tested for arbovirus activity (Table 3). These included 129 pools of *Cx. erythrothorax*, 47 *Cx. quinquefasciatus*, 32 *An. hermsi*, 27 Cx. *tarsalis*, 9 *Cx. thriambus*, 6 *Cx. stigmatosoma*, and one *Cs.inornata*. Only one pool of *Cx. erythrothorax* tested positive for WNV in 2006. This is a significant decline in arbovirus activity compared to previous years. We had 23 WNV-positive pools in 2004 (Wisniewska-Rosales and Williams 2005) and 6 WNV positives in 2005 (unpublished data).

As shown in Table 4, arbovirus activity in sentinel chickens was detected in 9 birds belonging to 5 different flocks. Virus activity was detected in late August and continued into the first week of October. In 2004, we had 56 sentinel chickens positive for WNV (Wisniewska-Rosales and Williams (2005). Virus activity in live wild birds was detected early when a House finch tested positive for WNV on June 5, 2006 (Table 5). Similarly, of the dead birds tested, only one American crow, tested positive for WNV on August 30, 2006.

Table 3. Number of mosquito pools (mosquitoes) by species[1] submitted for arbovirus testing[2] in 2006.

Month	*Cxe*	*Cxq*	*Cxs*	*Cxt*	*Cxth*	*Csino*	*Anh*	Total
Jan	0	0	0	0	0	0	0	0
Feb	3(150)	2(47)	0	0	0	0	0	5(197)
Mar	1(21)	0	0	0	0	0	0	1(21)
Apr	6(300)	0	0	0	0	0	0	6(300)
May	12(482)	0	0	2(52)	0	0	1(22)	15(556)
Jun	0	0	0	0	0	0	0	0
Jul	14(635)	4(131)	3(50)	10(280)	2(47)	0	7(147)	40(1290)
Aug	29(1115)*	7(205)	1(16)	6(133)	5(103)	0	7(169)	55(1741)
Sep	26(1163)	18(606)	1(14)	6(191)	2(79)	0	6(164)	59(2217)
Oct	12(463)	12(417)	0	3(43)	0	0	2(32)	29(955)
Nov	3(123)	3(69)	0	0	0	0	3(100)	9(292)
Dec	21(981)	1(50)	1(17)	0	0	1(12)	6(183)	30(1273)
Total	129(5433)	47(1525)	6(97)	27(1972)	9(229)	1(12)	32(849)	249(8842)

[1]Species included: *Anh—Anopheles hermsi, Cxe—Culex erythrothorax, Cxq—Cx. quinquefasciatus, Cxs—Cx. stigmatosoma, Cxt—Cx. tarsalis, Cxth—Cx. thriambus, Csino—Culiseta inornata.*

[2] Tested for St. Louis encephalitis (SLE), western equine encephalomyelitis (WEE), West Nile (WNV) and California encephalitis (CE) viruses.

*One pool positive for WNV.

Table 4. Arbovirus activity in sentinel chicken flocks stationed at various locations in Northwestern Riverside County in 2006.

Date	Flock	Bird band#	Virus*
08/22/06	Mockingbird Canyon	2522	WNV
09/05/06	Temescal Canyon Fire Station	2533	Flav, WNV
		2535	Flav, WNV
	Rancho Jurupa Park	2483	Flav, WNV
		2486	Flav, WNV
10/03/06		2480	WNV
	Elsinore Vly Munic. Water District	2490	WNV
10/04/06	Corona Airport	2505	WNV
		2507	WNV

*Flav—Flavivirus (e.g., St. Louis encephalitis), WNV—West Nile virus

Table 5. West Nile virus (WNV) surveillance in wild birds caught in bird surveillance traps in 2006.

Month	No. blood samples tested by bird species[1]			Total
	Molothrus ater[2]	*Carpodacus mexicanus*	*Passer domesticus*	
Jan	24	0	0	24
Feb	0	0	0	0
Mar	0	0	0	0
Apr	11	0	0	11
May	26	10	0	36
Jun	12	13+	2	27
Jul	10	9	0	19
Aug	0	19	6	25
Sep	0	0	0	0
Oct	20	1	15	36
Nov	0	16	4	20
Dec	0	12	6	18
Total	103	80	33	216

[1]In multiple recaptures, birds captured multiple times were counted once. In single recaptures, birds captured multiple times are counted each time.
[2]For Brown-headed cowbirds recaptures refer to multiple bleedings of sentinel birds in cages.
+One tested positive for the WNV.

The foregoing data on mosquito abundance and arbovirus surveillance in 2006, clearly show a declining trend in arbovirus activity, compared with data in 2004 and 2005, although mosquito numbers were significantly higher in 2006. In 2004, we had 23 positive mosquito pools, 56 sentinel chickens, 15 live wild birds, and 45 dead birds, all tested positive for WNV (Wisniewska-Rosales and Williams 2005). These numbers were down in 2005 with 2 horses, 7 live wild birds, 8 dead birds, 6 mosquito pools, and 43 sentinel chickens, all positive for WNV. In 2006, we only had one live bird, one dead bird, one mosquito pool, and 9 sentinel chickens positive for WNV. With only one human case in 2006, 2004 and 2005 had 19 and 2 cases, respectively. It is too early to conclude, however, the declining trend could be due to several factors, including enhanced vector control services, better public awareness and possibly bird immunity.

Acknowledgments

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The History of Plague in California: 1900 – 1949
Addendum: The 411 Human Plague Cases by Year, Month, Locality, Sex and Outcome

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This addendum was the first part of Table 1, as described in the third sentence of the ABSTRACT (Hitchcock et al. 2006): "The paper also lists all of the 411 cases of human plague in the state by year, month, locality, sex and outcome for the first 50 years." Table 1 of the publication was the summary by year, of those 411 human plague cases.

The addendum provides detailed monthly totals, including locality, thus providing the dynamics of the several epidemics, as well as, a chronological presentation of the human plague cases reported from the 42 localities within the State of California from 1900-1949. An astrix (*) represents the first report of human plague for a given locality.

These Data have been extracted and modified from an appendix in Link (1955), in which he listed all of the 523 individual cases of human plague for the entire United States of America from 1900-1951.

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Table 1. Chronological list of human cases of plague by locale, gender and fate in California, 1900-1949.

YEAR	MON	LOCALITY	♂			♀			♂/♀?			Total		
			F	R	T	F	R	T	F	R	T	F	R	T
1900	FEB	San Francisco *	1	0	1							1	0	1
	MAR		3	0	3							3	0	3
	APR		1	0	1							1	0	1
	MAY		3	0	3	2	0	2				5	0	5
	JUN		2	0	2							2	0	2
	JUL		1	0	1							1	0	1
	AUG		2	0	2							2	0	2
	SEP		1	0	1							1	0	1
	OCT		2	0	2							2	0	2
	DEC		1	0	1							1	0	1
1901	JAN	San Francisco	7	0	7	1	0	1				8	0	8
	FEB		2	0	2	1	0	1				3	0	3
	MAR		1	0	1							1	0	1
	APR		1	0	1							1	0	1
	JUL		1	0	1	3	1	4				4	1	5
	AUG		1	0	1	1	0	1				2	0	2
	SEP		4	0	4	1	0	1	1	0	1	6	0	6
	OCT		2	0	2				2	0	2	4	0	4
	NOV		1	0	1							1	0	1
	DEC								0	1	1	0	1	1
1902	JAN		1	0	1							1	0	1
	APR		1	0	1							1	0	1

>> Table 1. (*continued*)

YEAR	MON	LOCALITY	♂			♀			♂/♀?			Total		
	MAY		4	0	4							4	0	4
	AUG		8	0	8	1	0	1				9	0	9
	SEP		8	0	8	3	0	3				11	0	11
	OCT		7	0	7	1	0	1				8	0	8
	NOV		3	0	3							3	0	3
	DEC		1	0	1							1	0	1
1903	MAR	San Francisco				1	0	1				1	0	1
	JUN		1	0	1							1	0	1
	JUL		3	0	3	1	0	1				4	0	4
	AUG	Pacheco *	1	0	1							1	0	1
		San Francisco	1	0	1							1	0	1
	SEP	Pinole *	1	0	1							1	0	1
		San Francisco	1	0	1							1	0	1
		San Ramon *	1	0	1							1	0	1
	OCT	San Francisco	4	0	4	1	0	1	1	0	1	6	0	6
	NOV					3	0	3				3	0	3
1904	JAN	San Francisco	2	0	2	1	0	1				3	0	3
	FEB	Concord *				1	0	1				1	0	1
		San Francisco	2	0	2	2	1	3				4	1	5
1905		N/A												
1906	APR	Oakland *	0	1	1							0	1	1
1907	MAY	San Francisco	1	0	1							1	0	1
	AUG	Richmond *				1	0	1				1	0	1
		San Francisco	9	7	16	1	3	4				10	10	20
1907	SEP	Oakland	1	0	1	1	0	1				2	0	2
		San Francisco	21	20	41	8	12	20				29	32	61
	OCT	Oakland	2	3	5	2	1	3				4	4	8
		San Francisco	14	10	24	6	1	7				20	11	31
	NOV	Berkeley *	1	0	1							1	0	1
		Oakland	1	1	2	0	1	1				1	2	3
		Port Richmond *	0	1	1							0	1	1
		San Francisco	6	20	26	6	9	15				12	29	41
	DEC	Oakland	1	1	2							1	1	2
		Port Richmond	1	0	1							1	0	1
		San Francisco	5	4	9	1	1	2				6	5	11
1908	JAN	Oakland	0	1	1							0	1	1
		San Francisco	0	1	1							0	1	1
		Stege *	1	0	1							1	0	1
	FEB	Oakland				1	0	1				1	0	1
	MAR	San Francisco	0	1	1							0	1	1
	JUL	Briones Valley *				1	0	1				1	0	1
		Concord	1	0	1							1	0	1
		Oakland	1	0	1							1	0	1

>> Table 1. (*continued*)

YEAR	MON	LOCALITY	♂			♀			♂/♀?			Total		
	AUG	Los Angeles *	0	1	1							0	1	1
1909	JUN	Alameda *	1	0	1							1	0	1
	JUL	Sunol *	1	1	2							1	1	2
	OCT	Oakland	0	1	1							0	1	1
1910	JUN	Hollister *	1	0	1							1	0	1
	AUG	Coyote *				0	1	1				0	1	1
1911	APR	Modesto *	0	1	1							0	1	1
	JUL	Lafayette *	1	0	1							1	0	1
		Modesto	0	1	1							0	1	1
	AUG	Oakland	0	1	1							0	1	1
	SEP	Ripon *	0	1	1							0	1	1
1912		N/A												
1913	JUN	San Juan Baptista *				1	0	1				1	0	1
	SEP	Pittsburg *	1	0	1							1	0	1
1914	MAY	Walnut Creek *	0	1	1							0	1	1
1915	JUL	Concorde	1	0	1							1	0	1
1916-1918		N/A												
1919	AUG	Oakland	5	0	5	0	1	1				5	1	6
	SEP		5	0	5	3	0	3				8	0	8
1920	APR	Hayward *				1	0	1				1	0	1
1921	JAN	San Juan Baptista	1	0	1							1	0	1
	JUN	Bitter Root Valley *	0	1	1							0	1	1
1922	JUN	Oakland	1	0	1							1	0	1
	JUL	Soquel *	0	1	1							0	1	1
1923	AUG	Pacific Grove *				0	1	1				0	1	1
1924	OCT	Los Angeles	17	2	19	10	1	11				27	3	30
	NOV		5	0	5	2	1	3				7	1	8
1925	JAN	Los Angeles	1	0	1	0	1	1				1	1	2
1926		N/A												
1927	JUL	Clayton *	1	0	1							1	0	1
1928	JAN	Santa Cruz *	0	1	1							0	1	1
	JUL	Monterey *	1	0	1							1	0	1

>> Table 1. (*continued*)

YEAR	MON	LOCALITY	♂			♀			♂/♀?			Total		
	AUG	Santa Ynez *				1	0	1				1	0	1
1929-1932		N/A												
1933	AUG	Whittier *	1	0	1							1	0	1
1934	JUN	Posy Creek *	1	0	1							1	0	1
1935		N/A												
1936	APR	Santa Rosa *	0	1	1							0	1	1
	JUN	San Simeon *	0	1	1							0	1	1
	JUL	San Bernardino *	0	1	1							0	1	1
		Lake Tahoe *				0	1	1				0	1	1
1937	AUG	Huntington Lake *				1	0	1				1	0	1
1938-1940		N/A												
1941	JUN	Montague *	1	0	1							1	0	1
	AUG	Mt. Shasta City *	1	0	1							1	0	1
1942	NOV	Yreka *				1	0	1				1	0	1
1943	AUG	Ft. Jones *	0	1	1							0	1	1
1944	MAY	San Francisco	0	1	1							0	1	1
1945-1946		N/A												
1947	JUN	Alturas *	1	0	1							1	0	1
1948-1949		N/A												

*indicating first case by locality
F--fatal, R--recovered, T--total, N/A—not available

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