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February 1 through 4, 2004

Held in Sacramento, California

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PROCEEDINGS AND PAPERS

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Conference Dedication in Memory of Ernest (Ernie) Edward Lusk 1926 - 2003

William C. Hazeleur

Shasta Mosquito and Vector Control District, P.O. Box 990331, Redding, CA 96099

The 72nd Annual Conference of the MVCAC is dedicated in memory of Ernest (Ernie) Edward Lusk in recognition of his many years of service to mosquito and vector control. He passed away at Mercy Hospital in Redding, California on May 20, 2003. Ernie was born October 6, 1926 in Chico, California. He served honorably in the United States Navy during World War II. Later in life, he was a member of the Redding Elks Lodge 1073 and the Redding Moose Lodge 1006. Ernie had many different interests and accomplishments, as evidenced by his colorful career. He was the manager of the Los Molinos and Corning mosquito abatement districts, where he met and eventually married his wife, Lois. They shared 47 years together as husband and wife. He spent part of his professional life employed as a chemical salesperson . . . as well as a school teacher in Sacramento!

He worked for the California Department of Health, Vector Control unit in the early 1960's and retired in 1990. Ernie spent countless hours in the field of mosquito and vector biology, where he made many significant contributions. This included development of effective strategies for mosquito and fly control and plague detection. He was an important contributor to the understanding of several vector-borne diseases. In 1989, Ernie Lusk was awarded Honorary Membership by the California Mosquito and Vector Control Association, now called the Mosquito and Vector Control Association of California (MVCAC). He was an active member of the Society for Vector Ecology (SOVE). Ernie Lusk's professional journey eventually led him to the Shasta Mosquito and Vector Control District, where he served with distinction on its Board of Trustees, most recently as Past President. Ernie Lusk's research contributions, coupled with his genuine personality and contagious sense of humor, made him a deeply admired and highly respected man who will be long remembered and admired by all who knew him.

Invasion of Southern California by West Nile Virus: Introduction

William K. Reisen

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West Nile virus (WNV) invaded the New York area in 1999 and subsequently has spread rapidly westward across the United States. This continuing epidemic has resulted in unprecedented human, equine and wildlife illness and death (Table 1) and is the largest WNV epidemic ever recognized globally (Work 1971, Hayes 1989, Petersen et al. 2003). Compared to most of the United States, California may be uniquely prepared to monitor and contain WNV, because well-funded surveillance and mosquito control programs have been in place for >50 years to combat local mosquito, malaria and endemic mosquito-borne encephalitis viruses problems (Reeves et al. 1990). These abatement programs cover more than 59,000 sq. mi. and protect more than 88% of California residents. The construction of homes without screened porches or swimming pools attests to the efficacy of this program. The invasion of WNV into California and its anticipated amplification during the next few years will provide a unique and rigorous test of how well an integrated vector management approach to mosquito control can protect the residents of California from mosquito-borne disease.

WNV invaded the irrigated desert regions of southeastern California during the summer of 2003 and then spread to the densely populated urban centers of Los Angeles and eventually San Diego, with minimal human or horse involvement (Table 1). The purpose of our symposium is to describe in detail this introduction and present what has been learned about WNV ecology and epidemiology and the complications created for laboratory diagnostics and surveillance.

Speakers and their titles will include:

- W. K. Reisen, University of California, Davis: Introduction
- H. D. Lothrop, University of California, Davis: Findings in Imperial and Coachella Valleys
- J. Wilson & M. Madon, Greater Los Angeles MVCD: Findings in Greater Los Angeles
- K. Fujioka, San Gabriel Valley MVCD: San Gabriel Valley
- S. Wheeler, University of California, Davis: Infections of birds in Coachella Valley
- L. Baylis & C. Cossen, Department of Health Services: Sentinel chicken serology
- R. Chiles & E. N. Green, University of California, Davis: Mosquito pool testing
- W. K. Reisen, University of California, Davis: Mosquito and avian host competence
- B. Lothrop, Coachella Valley MVCD: Risk assessment and control response
- W. K. Reisen, University of California, Davis: Concluding remarks

Table 1. Comparison between WNV activity for the USA and California, 2003.

Nation Wide (MMWR, Jan 2004)

- 8,912 human cases (2,641 neurological), 241 deaths
- 4,146 horse cases, 30 dog infections
- 11,350 dead birds
- 1,377 sentinel chicken seroconversions
- 7,725 positive mosquito pools

California (Arbovirus Surveillance Bulletin #33)

- 3 humans (fever not reported), 0 deaths
- 1 horse
- 89 dead birds (no raptors)
- 70 sentinel chicken seroconversions (13 SLE, 2 WEE)
- 32 positive mosquito pools (4 SLE, 1 WEE, 5 CE)

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The Invasion of California by West Nile Virus, 2003: Imperial and Coachella Valleys

Hugh D. Lothrop, Marc Kensington and William K. Reisen

Arbovirus Research Unit, Center for Vector-borne Disease Research, School of Veterinary Medicine, University of California, Davis, CA 95616

INTRODUCTION

Arbovirus surveillance in the Coachella and Imperial Valleys is a collaborative effort by the California Department of Health Services (DHS), University of California Davis Center for Vectorborne Diseases (CVEC), the Coachella Valley Mosquito and Vector Control District (CVMVCD), and the Imperial County Health Department, Vector Control. The two valleys lie within one basin in southeastern California and are linked and divided by the Salton Sea (Fig.1).

The Coachella Valley is located north of the Salton Sea and oriented northwest to southeast. Residential communities predominate in the north, whereas the south is primarily irrigated agriculture including row crops, citrus, grapes, and dates as well as approximately 350 hectares of seasonal managed wetland for ducks. Salt marshes along the margin of the Salton Sea have historically been productive sources for *Culex tarsalis* and continue to be the foci of annual arbovirus transmission (Reisen et al. 1995).

The Imperial Valley is more than twice the size of the Coachella Valley, with residential communities scattered throughout. The majority of agriculture is row and hay crops with a few hectares of citrus or dates. National and State wildlife refuges, covering roughly 1500 hectares, are located along the southeastern shore of the Salton Sea. Riparian corridors of the Alamo and New Rivers transect the valley from the Mexican border to the Salton Sea. None of these features have been shown to be perennial foci for arbovirus activity (Lothrop et al. 1994).

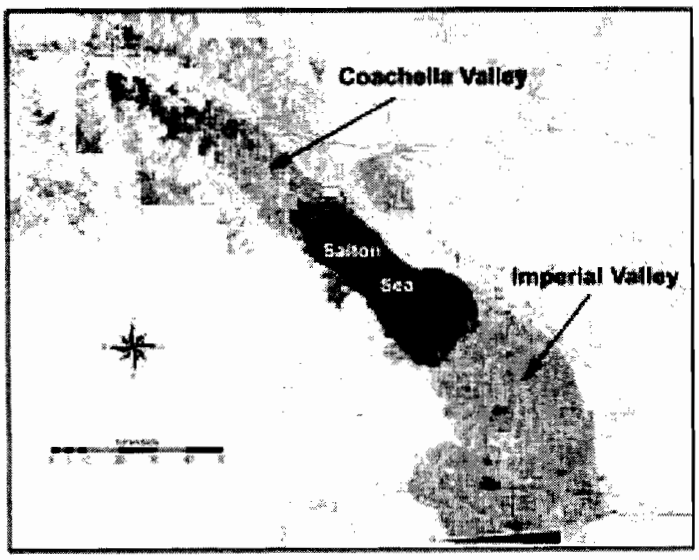

Figure 1. The Salton Sea basin showing the position of the Coachella and Imperial Valleys.

SURVEILLANCE METHODS

Surveillance in the Coachella Valley consisted of 10 flocks of chickens with 2 corresponding CO_2-baited CDC style traps (EVS traps), a grid of 40 EVS traps around the shore of the Salton Sea, 8 wild bird sampling sites, 8 gravid traps (located in urban areas), and the DHS dead bird surveillance program. Wild bird surveillance was discussed as a separate part of this symposium (Wheeler at al. 2004). Surveillance flocks and EVS traps were distributed at sites from Palm Springs to the margin of the Salton Sea (Fig. 2). To monitor the duration of arbovirus transmission, dead or seropositive chickens were replaced as needed throughout the season. Surveillance in the Imperial Valley was divided between 3 flocks located at Seeley, El Centro and Holtville, maintained by the Imperial County Health Department, and 3 flocks along the margin of the Salton Sea, maintained by UC Davis and the CVMVCD (Fig. 3). In early September, the Imperial County Health Dept. added one additional flock at the town of Brawley. The selection of these sites was based upon geographical and ecological parameters

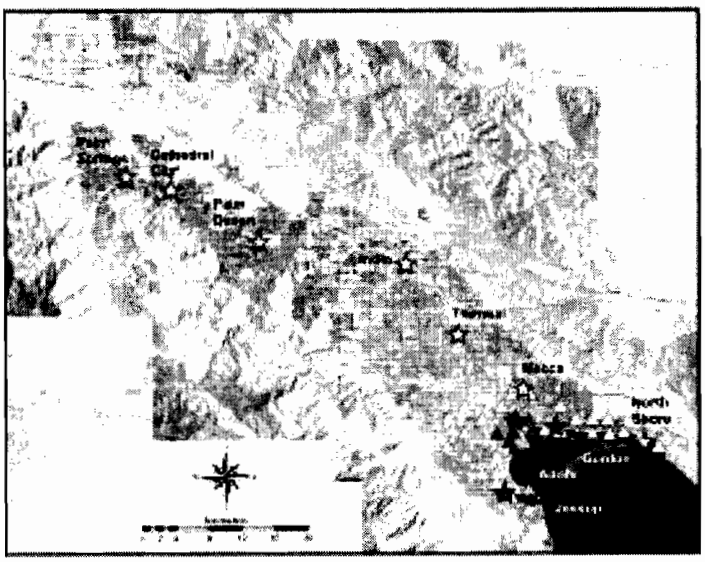

Figure 2. Coachella Valley surveillance showing flocks as stars and CO_2 traps as triangles. WN positive sites are shown with the symbol darkened. Note 2 darkened triangles at North Shore under the star.

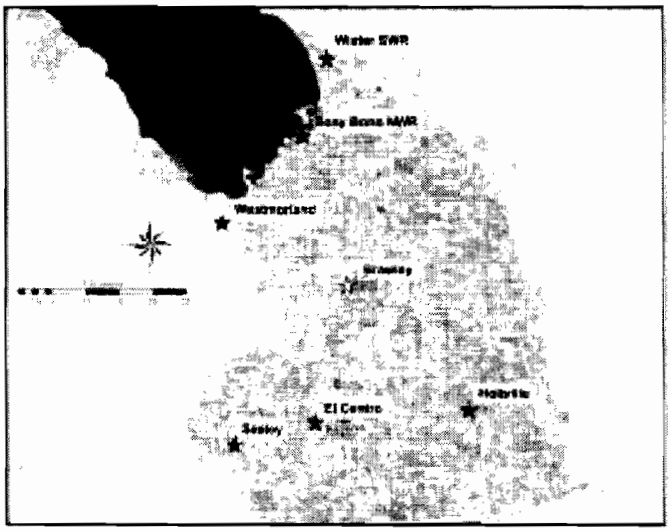

Figure 3. Imperial Valley surveillance showing flocks as stars. Note each flock had 2 CO_2 traps. WN positive sites are shown with the symbol darkened.

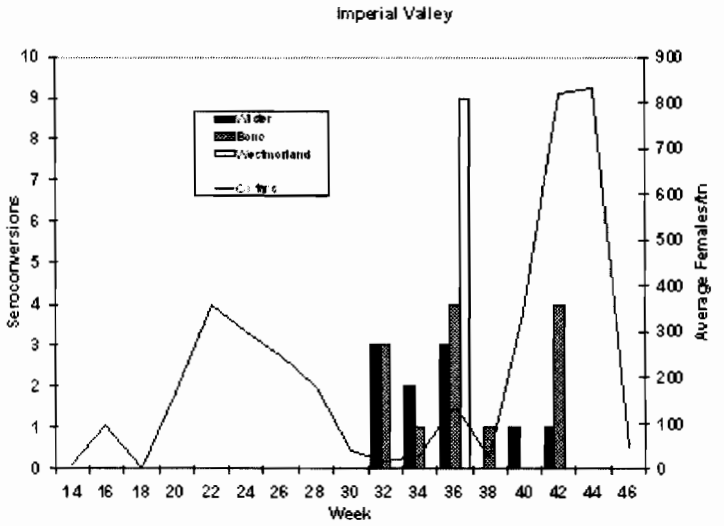

Figure 4. Seroconversions in Imperial Valley surveillance flocks maintained by CVEC/CVMVCD and average vector mosquitoes per trap night for the 3 sites.

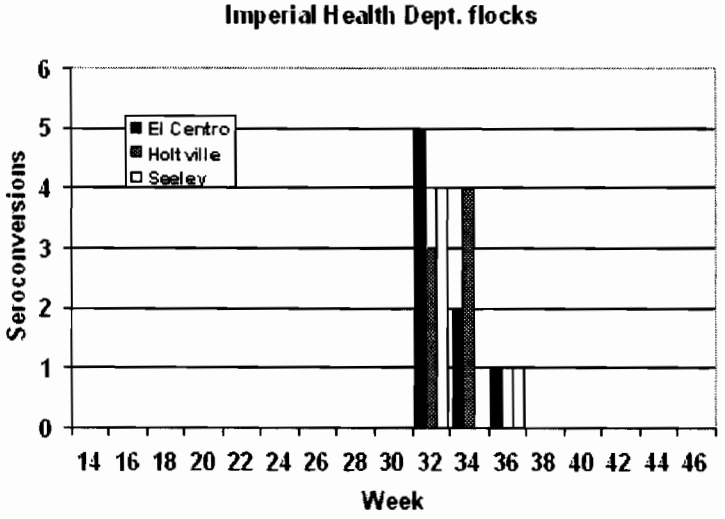

Figure 5. Seroconversions in Imperial Valley surveillance flocks maintained by Imperial Co. Health Dept.

supported by historical arbovirus activity in the region and juxtaposition to human populations. Flocks and EVS traps were sampled biweekly from March through November. Mosquitoes collected were identified, enumerated to species, pooled and sent to the UC Davis Center for Vectorborne Diseases for virus testing.

CHRONOLOGY

The first positive mosquito pool in Cochise County, southeastern Arizona (http://www.hs.state.az.us/phs/oids/vector/wnv_surv.htm), collected around July 1, was the first indication of the imminent entrance of WN virus into southern California. A positive mosquito pool, collected on August 4 in Wister State Wildlife Refuge near the town of Niland, was the first proof of WN presence in California, however, a pool collected on July 16 in El Centro was the actual first. Chickens seroconverted to WNV throughout Imperial Valley (El Centro, Holtville, Seeley, Wister State Wildlife Refuge and Sonny Bono National Wildlife Refuge) on August 4. West Nile virus transmission continued until October 13 at the refuges (Fig. 4), but appeared to span a shorter period at the Westmorland site and the 3 sites near the Mexican border (Fig. 5); WNV was not detected at the Brawley site established in September. The El Centro, Seeley, and Holtville sites received only one replacement of new chickens and may have lost late season sensitivity.

Transmission to sentinel flocks in the Coachella Valley lagged 2 weeks behind the Imperial Valley, with seroconversion of chickens at the Adohr flock on August 18 followed by the Gordon site on September 1 and Jessup on September 15. Virus transmission continued until October 13 at the Gordon site (Fig. 6). The first mosquito pool was collected August 26 at a trap site 2 kilometers west of the Adohr site followed one day later at a trap at North Shore at the extreme southeast of the

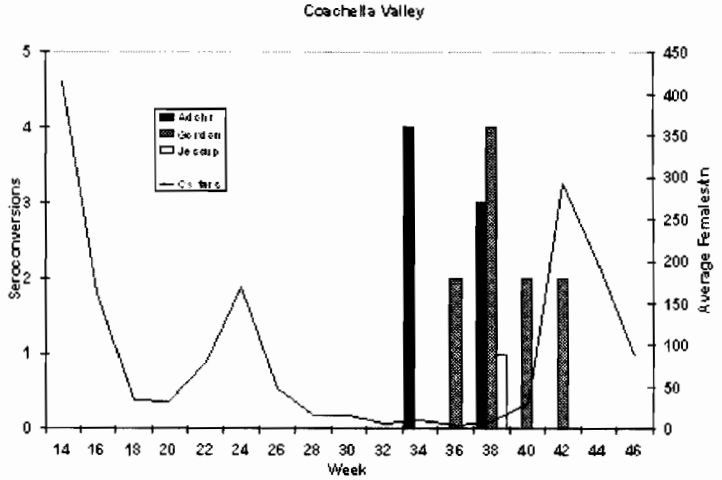

Figure 6. Seroconversions in Coachella Valley positive surveillance flocks and average vector mosquitoes per trap night for the 3 sites.

surveillance pattern. Transmission was most intense and prolonged at the Gordon site, but no positive mosquitoes were found in pools from this site.

SUMMARY

In the Imperial Valley, a total of 52 chickens at 6 of 6 flocks, set out at the start of the season, seroconverted to WN. One flock had 2 seroconversions to SLE, both dual WN/SLE infections. In the Coachella Valley, a total of 18 chickens at 3 of 10 flocks seroconverted to WN. One flock had 2 dual infections to WN and SLE. All seroconversions in the Coachella Valley were limited to sites near the Salton Sea. A summary of mosquitoes pooled is shown in Table 1 and was largely proportional to species abundance, although greater emphasis was given to species in low abundance. In the Imperial Valley there were 16 WN positive pools collected between July 16 and September 16 and 1 SLE positive pool collected on September 16. In the Coachella Valley there were 10 WN positive pools collected between August 26 and September 24 and 3 SLE positive pools collected between July 2 and October 2. All positive pools were collected near the Salton Sea.

In both valleys, the onset of virus transmission coincided with a period of low abundance of vector mosquitoes, agreeing with our previous studies on SLE and WEE viruses in this area. There was no increase in transmission related to the increase in abundance at the end of summer and early fall.

Based upon the collection of the earliest mosquito pool on July 16 and the extensive distribution of WN virus transmission, the Imperial Valley apparently was the site of introduction into California. The limited distribution of WN virus in the Coachella valley may have been due to introduction later in the season or enhanced mosquito control following introduction.

The activity level of SLE was below normal in the Imperial Valley, which usually shows transmission to every flock by season's end. In the Coachella Valley, SLE has not been detected by the surveillance system during some years, and therefore the low activity level was within the range of variability.

Acknowledgements

We wish to thank the Coachella Valley Mosquito and Vector Control District for logistical support. This research was funded by the Coachella Valley MVCD, National Institutes of Health, and the Centers for Disease Control and Prevention.

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Table 1. Summary of seasonal mosquito pooling for Imperial and Coachella Valleys. Italics indicate mosquitoes collected by Imperial Co. Health Dept., Vector Control.

Imperial		Coachella	
Species	# of Females	Species	# of Females
Aedes vexans	598	Aedes vexans	1128
Culiseta inornata	7	Culiseta inornata	149
Culex erraticus	258	Culex erythrothorax	6714
Culex erythrothorax	4145	Culex tarsalis	43340
Culex tarsalis	7440	Culex quinquefasciatus	9687
Subtotal	12448	Anopheles franciscanus	9
Aedes vexans	*151*	Ochlerotatus dorsalis	154
Culex Tarsalis	*3434*	Psorophora columbiae	141
Culex quinquefasciatus	*491*	Total	**61323**
Subtotal	*4076*		
Total	**16524**		

Invasion of Greater Los Angeles by West Nile Virus - 2003

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ABSTRACT: In response to the impending threat of West Nile virus (WNV) introduction into California, the Greater Los Angeles County Vector Control District (GLACVCD) in collaboration with the University of California, Davis (UCD), engaged in an extended arbovirus surveillance program in urban Los Angeles. The new program extended GLACVCD's surveillance of mosquitoes, wild birds and chickens, by implementing changes in mosquito trapping techniques, expanding surveillance to cover the entire district boundaries including transects along the 3 major rivers (Los Angeles, Rio Hondo, and San Gabriel River systems). From 16 September 2003 through the end of December, 6 *Culex quinquefasciatus* pools, 7 seropostive chickens from privately owned ranches, and 26 crow carcasses tested positive for WNV from the San Gabriel and Rio Hondo riparian corridors near the Whittier Dam, site of an enormous crow roost.

INTRODUCTION

The rapid spread of West Nile virus (WNV), since its introduction into the continental U.S.A. in 1999, necessitated the drastic restructuring of existing encephalitis virus surveillance programs to track a virus with both rural and urban transmission cycles. The Los Angeles basin provides an excellent area to study the introduction and establishment of a virus in an urban environment where surveillance methods have already been in place. It also provided an opportunity to evaluate current surveillance mechanisms for early WNV detection.

The mosquito-borne virus surveillance program at GLACVCD was developed in response to the St. Louis encephalitis virus (SLE) epidemic that occurred in the Los Angeles basin in 1984. It focused on both man-made and natural wetlands where the epizootic amplification would present first, and the risk for human infection was high. Because the SLE cycle focuses on *Culex tarsalis* Coquillett as the primary vector species, EVS/ CO_2 -baited traps were employed mostly at wetlands where amplification was anticipated.

After the 1999 WNV outbreak in New York, it was apparent that the occurrence of dead American crows may provide a particularly sensitive indication of WNV transmission (Eidson et al. 2001). It also was apparent that the primary urban vector could be *Culex quinquefasciatus* Say, the southern form of the *Culex pipiens* complex found to be of importance in the amplification and persistence of WNV in the northeast (Nasci et al. 2001).

Domestic chickens have been an integral component of arbovirus surveillance in California and due to frequent seroprevalence, may be useful as urban WNV sentinels (Komar et al. 2001) as shown during the 1999 WNV outbreak in New York. The same study indicated that house sparrows could be an important urban reservoir host.

In accordance with these findings, the 2003 surveillance program incorporated these surveillance methods at sites spread throughout GLACVCD's boundaries to evaluate their efficacy as early indicators of WNV activity. Urban mosquito trapping was augmented with Reiter traps to target gravid *Cx. quinquefasciatus* (Reiter 1983) and trap sites were organized to create transects along major riparian corridors and roadways.

MATERIALS AND METHODS

Five principal monitoring sites at Encino, Griffith Park, Machado Lake, Rowland Heights, and Whittier Narrows, were established to compare surveillance data from sentinel chickens, live wild bird seroprevalence, and mosquito populations sampled by EVS/ CO_2-baited and Reiter traps. Seven sentinel chicken flocks (10 chickens each) were sampled bi-weekly and sent to the California Department of Health Services Viral and Rickettsial Disease Laboratory for testing by enzyme immunoassay. Wild birds were collected over a 3-day period every 2 wks in 8 modified Australian crow traps baited with grain, bled by jugular puncture and sera tested by enzyme immunoassay. Mosquitoes were collected at biweekly or monthly intervals using 35 Reiter traps (Cummings 1992) baited with rabbit chow/ brewer's yeast infusion, and 100 EVS traps baited with ~1 lb. blocks dry ice (Newhouse et al. 1966). Transects of 8-10 Reiter traps were deployed along 6 north-south and 6 east-west transects as well as at the five principal monitoring sites. Mosquitoes were identified to species, pooled into lots of < 50 females, and then screened for WNV, SLE and western equine encephalomyelitis (WEE) infection by single-plex RT-PCR assays and *in situ* enzyme immunoassays. All dead birds were reported to the California Department of Health Services Dead Bird Program.

RESULTS AND DISCUSSION

The first indication that WNV had entered GLACVCD's boundaries was a *Cx. quinquefasciatus* pool collected on 16 Sep. 2003 in a Reiter trap placed along the Rio Hondo River (Table 1). This trap was part of an urban east-west transect along Whittier Boulevard in the city of Montebello.

Table 1: Summary of 2003 WNV isolations and avian serconversions at GLACVCD*.

Week	Human	Mosquito Pools	Chickens	Crows
15-Sep		1-*Cx. quinque*-Montebello		1 - Whittier
22-Sep				
29-Sep				2- Whittier, 2-Pico Rivera
6-Oct	1-Whittier	2-*Cx. quinque*-Montebello		1-Whittier, 2-Pico Rivera, 1-Van Nuys
13-Oct				
20-Oct		2-*Cx. quinque*-Pico Rivera, 1-*Cx. quinque*-Downey		1-Santa Fe Springs 1-Montebello
27-Oct				2-Whittier
3-Nov				3-Whittier, 1-Montebello, 2-Pico Rivera, 1-Cerritos, 1-Long Beach
10-Nov			4-Montebello**, 3-Pico Rivera**	1-Montebello, 1-Pico Rivera
17-Nov				1-Whittier
24-Nov				
1-Dec				1-Pico Rivera, 1-Montebello
8-Dec				1-Van Nuys

*Of the 1,519 Wild Bird Sera samples obtained, none was positive for WNV.
**Bled at private backyard ranches.

The second indication of WNV infection was a crow carcass collected in residential Whittier two days later. Five more infected crow carcasses were collected between 18 Sep. and 9 Oct., in Whittier and the bordering city of Pico Rivera.

On 9 Oct. 2003, two more pools of positive *Cx. quinquefasciatus* were collected in Montebello along the Rio Hondo River, using a Reiter trap placed in a residential neighborhood. The next detection of WNV was in Van Nuys where a crow was collected on 10 Oct. This crow was an isolated case, as it was 22 miles from the foci of activity in Montebello, and subsequent mosquito trapping in the local area yielded no other evidence of WNV.

There continued to be WNV activity in Pico Rivera, however, on 10 Oct., as 2 more crows collected at a residential park tested positive. An additional crow collected closer to the San Gabriel River corridor in Santa Fe Springs on 23 Oct. was WNV positive. That same night, the San Gabriel River corridor was trapped using EVS and Reiter traps and 15 pools of *Cx. quinquefasciatus* and 1 pool of *Cx. tarsalis* were submitted for viral testing. WNV was isolated from three pools of *Cx. quinquefasciatus;* one collected with an EVS trap in Pico Rivera, and two with Reiter traps in Pico Rivera and Downey.

Between 24 Oct. and 10 Nov., WNV was detected in 12 crow carcasses collected in the cities of Whittier (6), Montebello (3) and Pico Rivera (3). During this period, WNV was detected for the first time in crows from the cities of Cerritos (4 Nov.) and Long Beach (5 Nov.) along the San Gabriel River corridor.

Because positive mosquito pools and crow carcasses were concentrated in the Rio Hondo and San Gabriel River corridors, extended mosquito trapping was undertaken focally. The GLACVCD staff noted that this area had several chicken ranchers operating on properties adjacent to the riverbeds, and with the

cooperation of the owners, 78 private chickens, bantee roosters, and peacocks were bled on 12 Nov. In Montebello, along the Rio Hondo, 4 chickens tested positive for WNV antibody. In Pico Rivera, along the San Gabriel River, 3 additional chickens were positive for WNV antibody (Fig. 1).

Two more WNV infected crow carcasses were collected on 13 and 17 Nov. in Montebello and Whittier, respectively.

Virus activity shifted mid-November to include SLE in the San Fernando Valley. On 19 Nov. a Reiter trap collection of *Cx. quinquefasciatus* from Griffith Park provided an SLE positive pool in the city of Los Angeles.

In December, mosquito abundance declined as shown by EVS and Reiter trap collections (Figs. 2&3), but 3 additional crow carcasses were WNV positive from the previously positive areas of Pico Rivera, Montebello and Van Nuys.

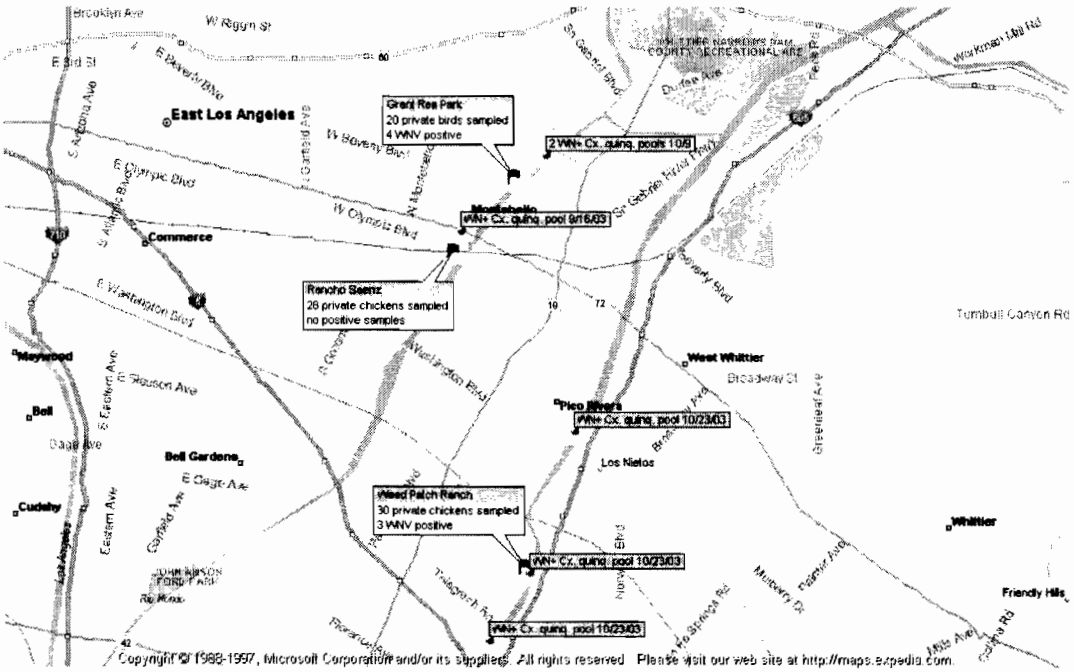

Figure 1: Distribution of positive mosquito pools relative to positive private chickens.

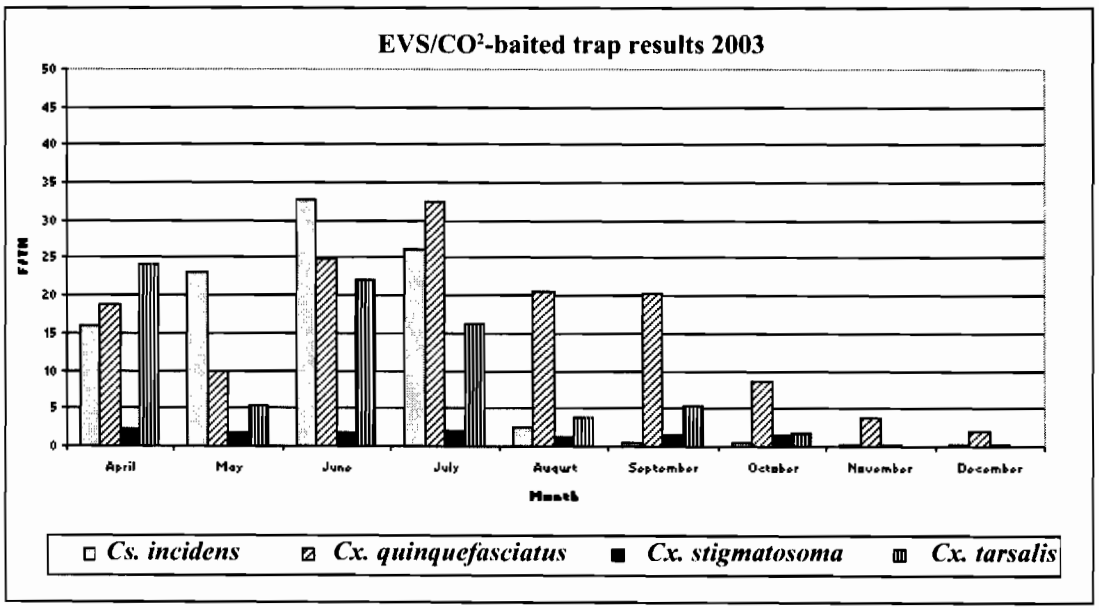

Figure 2: Mosquito abundance as females per trap night [F/TN] for each species collected in EVS/ CO_2-baited traps.

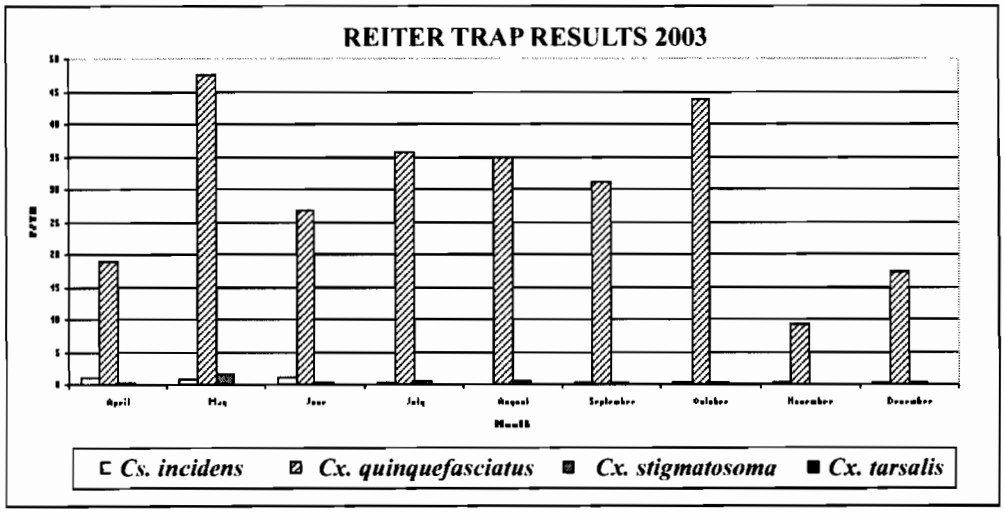

Figure 3: Mosquito abundance as females per trap night for each species, collected in Reiter gravid traps.

WNV activity was not detected at any of the 5 primary study areas and all sentinel chicken sera and mosquito pools at these sites tested negative. These data indicate the importance of widespread sampling in detecting WNV activity, especially when *Cx. quinquefasciatus* may be the primary urban vector. Ten species of wild birds (primarily House Finches, English Sparrows, Mourning Doves, and White-crowned Sparrows) were collected in the cities of Bellflower, Encino, Harbor City, Montebello, Pacoima, Santa Fe Springs, South El Monte, and Rowland Heights (Fig. 4). A total of 1,519 sera samples was screened for antibodies to WNV, SLE, and WEE by EIA. High mortality rates among these passeriform species after infection with WNV (Komar et al. 2003) may have made them a poor sentinel system, and therefore sampling during the 2004 season will be expanded to include doves and pigeons, species that produce a low-titer viremia, survive infection and produce elevated antibody titers (Komar et al. 2003).

Mosquito abundance during the 2003 season is shown for the primary vector species collected (Figs. 2&3). EVS trap results showed a distinct decrease in host-seeking female abundance from October through the end of the year when other indicators (i.e., crow carcasses, chickens, gravid mosquito collections, etc.) began showing signs of WNV invasion into this region. The WNV positive

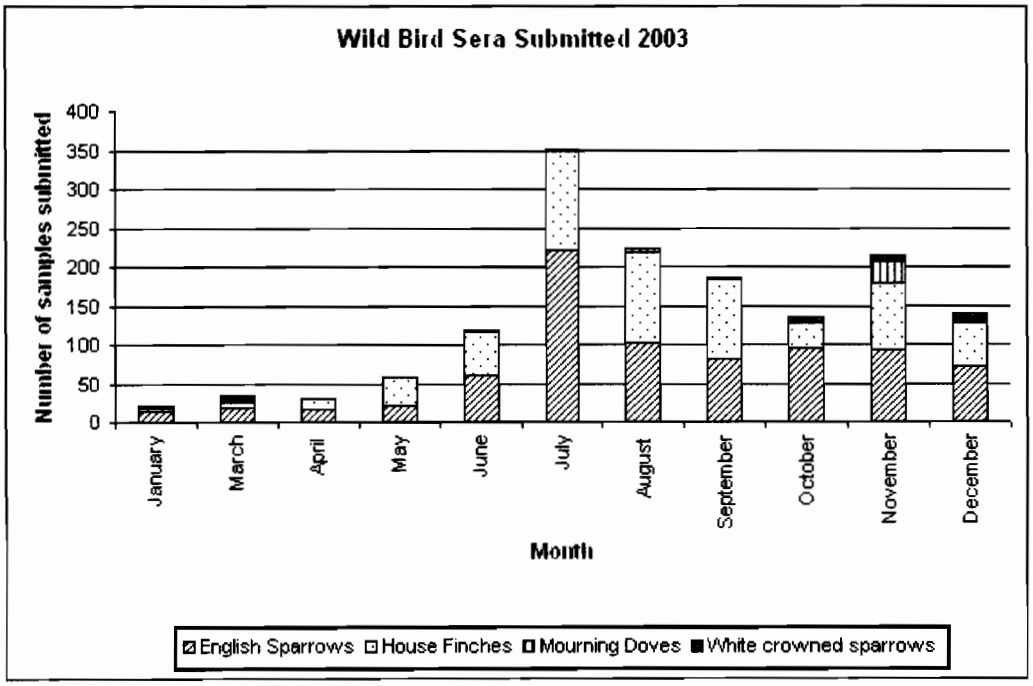

Figure 4: Wild bird sera collections by species.

mosquito collections coincided with positive bird carcasses collected from the same cities (Fig. 5) near the Whittier Narrows Nature Reserve. An area in the adjoining Montebello oil fields was identified as the nocturnal roosting site for large numbers of crows. On-going winter surveillance has focused on the importance of this crow roost as a site of WNV persistence and early season amplification.

SUMMARY

Changes in GLACVCD's surveillance program were advantageous for the detection of WNV in Los Angeles County. The increased use of Reiter traps in urban areas facilitated collections of gravid *Cx. quinquefasciatus* and greatly increased the number of mosquito pools sent for testing. Transect trapping spatially increased the areas surveyed and led to the early detection of WNV in the Whittier area of urban Los Angeles.

Cx. quinquefasciatus was the only positive mosquito species detected in Los Angeles County (Table 2), verifying the need for implementing a modified surveillance and population management program to focus on this species. Traditionally surveyed mosquito species such as *Cx. tarsalis* and *Cx. stigmatosoma* are localized at residual wetlands, whereas *Cx. quinquefasciatus* is pervasive throughout an urban environment. The exploitation of peridomestic habitats by this vector species and its avian and mammalian host selection pattern (Reisen et al. 1992) may provide a difficult

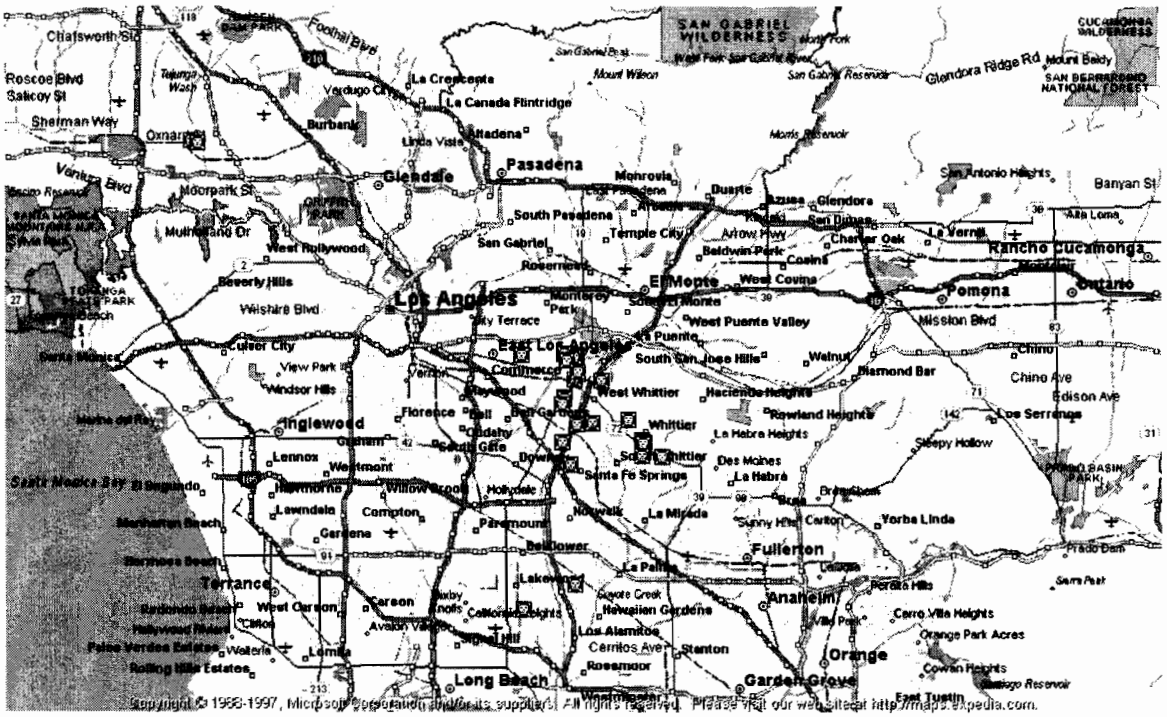

Figure 5: Dead crow distribution relative to the Whittier Narrows crow roost.

Table 2: Pools submitted to the Center for Vectorborne Diseases at the University of California, Davis.

Species	Number of Mosquitoes Tested	Number of Pools Submitted	Number of Positive Pools & Virus
An. hermsi	1,922	48	0
Cs. incidens	4,915	110	0
Cs. inornata	1,470	32	0
Cs. particeps	297	10	0
Cx. erythrothorax	12,311	259	0
Cx. quinquefasciatus	42,243	1,051	6 WNV/ 1 SLEV
Cx. stigmatosoma	788	28	0
Cx. tarsalis	4,897	121	0
Cx. thriambus	590	18	0
Oc. sierrensis	12	1	0

challenge in suppressing a WNV epidemic, as contact with vector mosquitoes may be difficult to limit by adulticiding and adjusting hours of recreation. The widespread urban habitats of *Cx. quinquefasciatus* also include underground storm drain systems which may provide an overwintering opportunity for WNV (Nasci et al. 2001) throughout the Los Angeles basin. Mosquito surveillance and treatment of these systems will be intensified during winter and early spring to suppress mosquito populations and avoid WNV persistence in early summer.

Acknowledgements

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West Nile Infections in Free Ranging Wild Birds in the Coachella Valley, Riverside Co., California

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The wild bird arbovirus surveillance program in the Coachella Valley of California was successful in detecting West Nile virus (WNV) antibodies in 9 species of free ranging birds during 2003. Antibodies against western equine encephalomyelitis (WEE) and Saint Louis encephalitis (SLE) were also detected but less frequently than WNV.

In 2003, mist nets or grain-baited wire traps were used for wild bird sampling[2] at 9 sites throughout the Coachella Valley (Fig. 1). Mist nets caught the widest variety of birds, whereas the grain-baited traps generally only caught species that were attracted to the bait seed inside, including sparrows, finches, doves, pigeons, blackbirds and quail. Each bird was identified to species and banded with the proper United States Geological Survey (USGS) band (aside from the Gambel's quail and rock pigeons which were banded with non-USGS bands). In addition age, sex, weight and wing chord measurements were recorded. A 0.1 ml sample of blood was obtained by jugular or brachial puncture and then combined with 0.9 ml of physiological saline. Blood samples were centrifuged and the sera sent to the Arbovirus Research Laboratory where they were screened for antibodies to WEE, SLE and WNV using an enzyme immunoassay (EIA) (Chiles and Reisen 1998). EIA positive samples were confirmed with a plaque reduction neutralization test (PRNT). The PRNT allowed for the separation of antibodies attributable to SLE and WNV, closely related members of the Japanese encephalitis complex in the Flaviviridae, which are not clearly distinguishable by EIA. Samples that tested EIA positive for either SLE or WNV, but not confirmed by PRNT, were considered *Flavivirus* positive. Overall, there were 2 confirmed dual WNV/SLE infections, 28 confirmed WNV infections, 4 confirmed SLE infections, 46 *Flavivirus* positives, and 3 WEE positives.

In 2003, 3,455 samples were collected from 63 different species and 25 different families of birds, with 95% of the species tested 20 or more times. Few corvids, raptors, waterfowl, and shorebirds were sampled (aside from 1 common raven, *Corvus corax*, 1 sharp-shinned hawk, *Accipiter striatus*, 1 Cooper's hawk, *Accipiter cooperii*, 10 least bitterns, *Ixobrychus exilis*, 4 green herons, *Butorides virescens*, 3 least sandpipers, *Calidris minutilla*, and 1 American coot, *Fulica americana*). Although we sampled birds from 25 different families, 97% of the birds captured in 2003 belonged to eight families (Fig. 2).

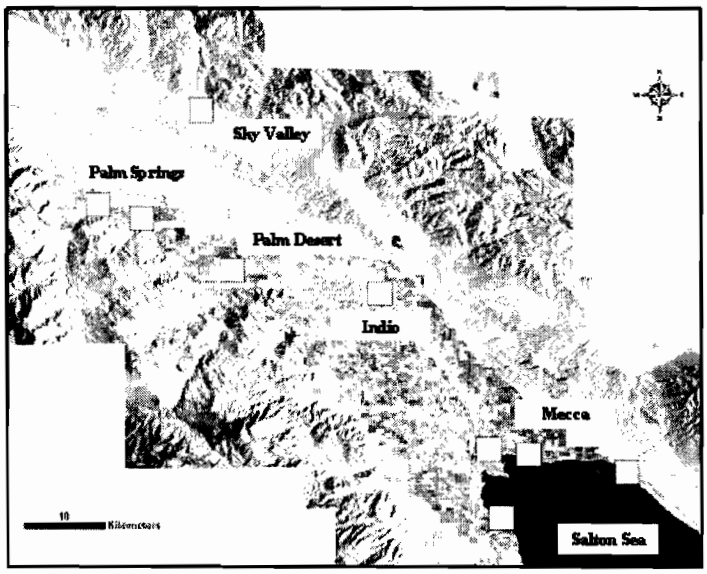

Figure 1. Map of Coachella Valley study sites

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[2] The collection, banding and bleeding of wild birds were conducted under Protocol 8141 approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis, California Resident Scientific Collection Permit 801049-02 by the State of California Department of Fish and Game, and Master Station Federal Bird Marking and Salvage Permit No. 22067 from the U.S. Geological Survey Bird Banding Laboratory

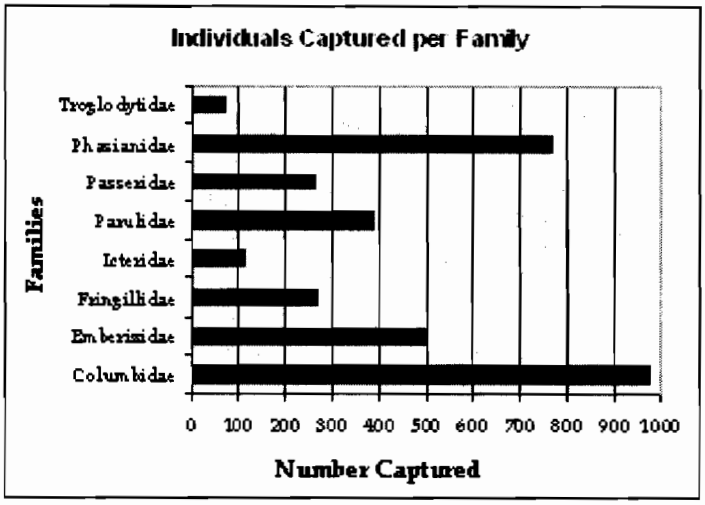

Figure 2. Number of individuals captured per family

To investigate the role of migratory birds in the spread of WNV, the residency status of the birds captured were broken into three categories (Fig. 3): migratory (the bird does not stay in the Coachella Valley to breed or over winter, but just passes through), seasonal resident (the bird overwinters or breeds in the Valley, but not both), and year-round resident (the bird spends its entire life in the Valley). All antibody positive species were year-round residents, except for the white-winged dove (*Zenaida asiatica)* which is a spring and summer seasonal resident (Table 1). Results during 2003 were similar to previous years (Reisen et al. 2000, Reisen et al. 2002, Wheeler et al. 2003), except for the emergence of WNV and the decreased number of *Flavivirus* positive Passeriforms. The seroconversion patterns of sentinel chickens agreed well with wild bird seroprevalence in that there was a similarity in frequency, location and seasonality of positives. As in previous years, seropositive wild birds were not found north of the town of Mecca, except for a *Flavivirus* positive mourning dove *(Zenaida macroura)*

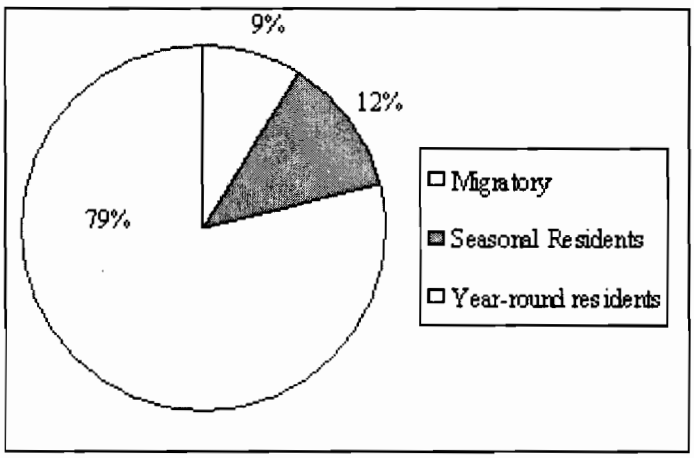

Figure 3. Percentage of birds out of 3,455 caught in 2003 that belong in each residency group, based on time spent in the Coachella Valley.

Table 1. The number of *Flavivirus* positive birds by species.

***Flavivirus* Positive Species**	
Abert's Towhee *Pipilo aberti*	1
Common Ground-dove *Columbina passerina*	13
Common Yellowthroat *Geothlypis trichas*	2
Gambel's Quail *Callipepla gambelii*	40
House Finch *Carpodacus mexicanus*	1
Least Bittern *Ixobrychus exilis*	1
Mourning Dove *Zenaida macroura*	11
Rock Pigeon *Columbia livia*	10
White-winged Dove *Zenaida asiatica*	1
Grand Total	80

collected in Sky Valley in February 2003. This bird was most likely infected in 2002 and at the time sampled was still antibody positive, however where this bird was infected is still a question.

Only 4 *Flavivirus* positive passeriforms or songbirds were detected in 2003, only one of which, the Abert's towhee (*Pipilo aberti)*, was confirmed by PRNT. Columbiforms, the dove family, made up 35% of *Flavivirus* positives, and Gambel's quail made up nearly half of the positive species. It is important to note that, because we were testing for antibody, only birds that survived infection were detected in our study. The California WNV dead bird surveillance program provides information about birds dying from infection.

No migratory birds tested positive for *Flavivirus* antibody in 2003, possibly because the majority of migrants were caught in May before the first confirmed positives in August. Alternatively, many migratory species may not survive the WNV infection. The large number of positive columbiforms and quail was expected, because we catch large numbers of these birds and they survive WNV infection. Sampling will continue in 2004 at the current locations in the Coachella Valley with efforts focused on colony roosting birds, resident species and migrants.

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Surveillance for Arboviruses in California Mosquito Pools: Current and Future Protocols

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INTRODUCTION

Although fourteen mosquito-borne viruses were known to occur in California prior to 2003, only St. Louis encephalitis (SLE) and western equine encephalomyelitis (WEE) viruses were known to cause significant human and/or equine disease (Reeves 1990) and were the focus of diagnostics at the Arbovirus Research Unit at the University of California, Davis (UC Davis). West Nile virus (WNV) was first isolated in the southern portion of the state in July 2003 and is expected to increase its geographic distribution within the state during the 2004 transmission season. The westward progression of WNV across the North American continent has resulted in the increased submission of mosquito pools by mosquito control districts to diagnostic laboratories, necessitating increased diagnostic effort and efficiency (Nasci et al. 2003). Anticipating the incursion of WNV into California and the associated increased numbers of mosquito pools submitted for viral detection, the Center for Vectorborne Diseases (CVEC) at UC Davis expanded throughput and employed a combination of standard and new molecular methods to evaluate more than 10,000 mosquito pools submitted during the 2003 surveillance season. This represented >100% increase in comparison to submissions during the past 5 years of surveillance (Table 1). The introduction of new rapid, high throughput testing paradigms for arboviruses in mosquito pools that maintain the same levels of sensitivity and specificity as contemporary assays have been evaluated.

Mosquito pools submitted to CVEC by California Mosquito and Vector Control Districts (MVCD) were assayed for the presence of WNV, WEE, SLE and California encephalitis (CE) viruses by the *in situ*-enzyme immunoassay (EIA) in 2003 (Chiles 2004, Graham 1986). This assay detects viral antigen produced following infection of tissue cultures with mosquito homogenates. Because some arboviruses require several days to induce cytopathogenicity and to produce sufficient viral antigen for detection, an incubation of several days is required before the test can be read. Additionally, this test requires the use of antibodies to react with the viral antigen. Serological cross-reactivity of flaviviruses (SLE and WNV) necessitates further testing to differentiate the viral antigen detected (Baba et al. 1998). Given the time required for processing of samples and the lack of specificity, nucleotide detection assays were evaluated. Reverse transcription polymerase chain reaction (RT-PCR) detects viral RNA by a rapid method of amplification that can yield virus-specific results within hours and previously has demonstrated higher sensitivity for arboviruses than standard viral isolation techniques (Lanciotti et al. 2000). Therefore, mosquito pools from four selected regions (Coachella Valley, Kern, Greater Los Angeles, and Sacramento/Yolo Mosquito and Vector Control Districts) were assayed for the presence of WNV, SLE and WEE RNA by TaqMan RT-PCR assays.

Table 1. Numbers of mosquito pools tested for virus by the Arbovirus Unit at the Center for Vectorborne Diseases at UC Davis.

Year	Number of pools sampled
1998	4,266
1999	3,746
2000	4,325
2001	3,686
2002	4,900
2003	10,111

METHODS

Mosquitoes were field sorted by species and sex and enumerated into pools of up to 50 individuals and placed in a mixer-mill tube containing two BBs. Mosquitoes were frozen and shipped on dry ice and then stored at -85°C until tested. Frozen mosquito pools were homogenized in a Spex Certiprep 8000D mixer-mill for three minutes. Diluent containing 20% fetal bovine serum and a full complement of the antibiotics penicillin, streptomycin and mycostatin was added. After homogenization an aliquot of the mosquito slurry was removed for RNA extraction (see below).

in-situ EIA

Ninety-six well plates were seeded with Vero (African Green Monkey) cells and allowed to grow for 24 hours. These cultures then were inoculated with 100 µl of the centrifuged mosquito pool homogenate and allowed to incubate at 38°C for a fixed time period, depending upon the virus. WEE was allowed to incubate 3-5 days, SLE 5-7 days, CE 3-7 days and WNV 3-5 days. The plates then were fixed in cold methanol and processed for viral identification utilizing a series of primary antibodies, hyper-immune polyclonal mouse ascites fluids, or monoclonal antibodies to the viruses being tested. A blocking buffer was used to eliminate non-specific absorbance to the plate. The blocker was followed by the addition of a secondary specific anti-mouse conjugated antibody, followed by the appropriate rinsing steps. The substrate was then applied for color development to render the virus-antibody reaction visible.

Plates were read microscopically for evidence of viral antibody reactions as demonstrated by a dark brownish focal staining against a clear background.

Initially, testing of all mosquito pools was performed by both *in-situ* and the RT-PCR methods in 2003. Because of the close relationship among flaviviruses, serological differentiation of WNV and SLE was anticipated to be problematic, denoting a limitation of the *in-situ* EIA detection method (Chiles 2004). By August, it was evident that throughput was inadequate, and subsequently only material from Coachella Valley, Kern, Greater Los Angeles and Sacramento/Yolo MVCDs continued to be tested by both methods; remaining pools were tested only by the *in situ*-EIA method. All WNV or SLE isolations were from these four districts and therefore were confirmed by RT-PCR. The anticipated continued spread of WNV from southern California to the Central Valley and the problems associated with cross reactivity between WNV and SLE necessitated the transition from traditional virus isolation and the use of the *in situ*-EIA to more rapid and sensitive RNA molecular diagnostics (below).

RNA extraction/ TaqMan RT-PCR

Extracted RNA was used as template for a one-step TaqMan RT-PCR reaction using an Applied Biosystems 7900 system. cDNA was generated by reverse transcription of RNA and amplification was performed by the binding of viral specific primers to the cDNA template (Lanciotti et al. 2000). Extension of amplification products from the primers produced a virus-specific amplification product that was detected by binding of fluorescently labeled viral sequence-specific probes. Extensive analyses were performed to identify the optimal primer sequences that would maintain the highest levels of sensitivity and specificity for viral strains presently and historically circulating in California. Specificity and sensitivity comparisons were made with alternative sets of primers. Primer sets that were determined to have the highest level of sensitivity were designated as screening primers, whereas additional primer sets targeting an alternative portion of the viral genome were utilized as confirmatory primers.

RESULTS

In 2003, an unprecedented number of agencies submitted mosquito pools to CVEC (Table 1). Overall, 39 different agencies submitted pools of 25 different species of mosquitoes. A total of 44 mosquito pools was determined to be positive by *in situ*-EIA for at least one arbovirus: thirty-two WNV, six SLE, one WEE and five California group viruses. One mosquito pool was identified by *in situ*-EIA to have contained both WNV and SLE (Table 2).

West Nile viruses have demonstrated very low genetic variability since introduction into North America in 1999 (Lanciotti et al. 2002). A primer/probe set previously designed against the envelope gene of a WNV isolate from 1999 demonstrated a sensitivity level of 0.1 plaque forming units (PFU) per mosquito pool and was designated as the screening primer for WNV. A primer/probe combination from the NS1 gene region was demonstrated to have a sensitivity of 1.0 PFU and was used for confirmation of positives by the envelope set (Lanciotti et al. 2000). Unlike WNV, multiple genotypes of SLE have been identified to circulate in California (Kramer et al. 1997, Reisen et al. 2002). The SLE TaqMan assay system had a detection level of less than a single PFU for all of the circulating SLE viral genotypes; however, reduced sensitivity was identified for viral genotypes that differed from the prototype strain from which the primers were designed. New primer and probes were designed for WEE, because the previously published reagents were unable to identify all the strains known to have circulated in California. Alignments of fifty-five partial sequences from the E2 envelope glycoprotein of Californian WEE isolates were performed and two primer/probe sets were identified that detected WEE at a sensitivity level equal to or greater than 0.01 PFU.

Twenty-eight of the thirty-one (90%) mosquito pools identified by *in situ*-EIA as positive for WNV antigen were confirmed by TaqMan RT-PCR (Table 2). Three additional mosquito pools were positive for WNV RNA by RT-PCR but were *in situ*-EIA negative. Similarly, two additional mosquito pools were positive by RT-PCR for SLE, but were negative by *in situ*-EIA. TaqMan RT-PCR was

Table 2. Comparison of surveillance results for the *in situ*-EIA and RT-PCR methods by the Center for Vectorborne Diseases in 2003.

	Arbovirus				
Method	WNV	SLE	WNV/SLE	WEE	CE
in situ-EIA	31*	5	1	1	5
RT-PCR	28	5	NT	NT	NT

* 3 *in situ*-EIA positive pools failed to be confirmed by RT-PCR (COAV 1121, IMPR 116, COAV 1183). NT; Not Tested. COAV; Coachella Valley. IMPR; Imperial Valley.

determined to have a higher degree of sensitivity than *in situ*-EIA in control assays, demonstrating a potential explanation for the TaqMan positive, *in situ*-EIA negative data. Interestingly, RT-PCR failed to confirm three *in situ*-EIA positive WNV mosquito pools. Genetic variation across the primer/probe binding areas of viruses from *in situ*-EIA positives could be a potential explanation for the failure to identify some of the *in situ*-EIA positive pools by TaqMan RT-PCR. Genetic sequencing of potential viral isolates made from the *in situ*-EIA cultures materials will be performed to assess this hypothesis. The single WEE-positive pool identified by *in situ*-EIA was not tested by TaqMan RT-PCR, because the pool was made from a geographic area outside of the four districts used for assay validation and was not tested by both *in situ*-EIA and RT-PCR screening methods. All CE positives were assayed strictly by the *in situ*-EIA assay because CE has not been added to the TaqMan screening panel. Efforts are currently in progress to develop primers and probes for the incorporation of California encephalitis group viruses into our molecular assays.

As a further measure to deal with the increased specimen testing load, we have developed a TaqMan RT-PCR based multiplex assay for the concurrent identification of viral RNA for three encephalitis viruses (WNV, WEE, SLE) from individual mosquito pools. This will be performed through the use of differentially labeled probes that will distinguish viral-specific amplification products. Preliminary data have indicated that multiplex assays will be capable of detecting viral RNA of multiple arboviruses within the same reaction. Currently, we have been successful in detecting and differentiating either SLE or WNV in individual reactions of culture samples that contain either single or mixed agents.

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Avian and Mosquito Host Competence for West Nile Virus

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INTRODUCTION

Information on host competence is critical to understanding arbovirus epidemiology. This is especially true for the North American encephalitides, including West Nile virus (WN), where a wide variety of avian and mosquito hosts participate in virus transmission. During 2003 WN virus invaded southern California and was isolated from *Culex tarsalis* Coquillett collected from rural Imperial and Coachella Valleys and from *Culex quinquefasciatus* Say collected from the Whittier Narrows area of urban Los Angeles (Reisen et al. 2004). Previous vector competence studies indicated that *Cx. tarsalis* was a competent vector when fed the elevated infectious dose of 7 $\log_{10}$ plaque forming units (PFU) of WN virus/ml, but less competent when fed the lower infectious dose of 4.9 $\log_{10}$ PFU/ml (Goddard et al. 2002). In contrast, *Cx. quinquefasciatus* from Coachella Valley seemed incompetent over the range of doses tested, although both *Culex* species appeared to be more susceptible to infection than *Cx. pipiens* L. from the midwest and east coast of the United States (Turell et al. 2000, Turell et al. 2001, Dohm et al. 2002). In these studies, female mosquitoes were exposed to fixed doses of virus, and therefore minimum and median thresholds for infection were not determined. Based on the eastern US studies a threshold of 5 $\log_{10}$ PFU/ml was used to determine avian host competence and a regression function calculated to estimate mosquito infection rates in response to different avian viremias (Komar et al. 2003). The current study exposed females of 3 species of California *Culex* mosquitoes to dilution series of WN virus and St Louis encephalitis virus (SLE) to determine the minimum and median infectious doses during a period of active WN virus transmission in southern California.

Birds also vary widely in their response to infection with WN virus (Komar et al. 2003) and SLE virus (Reisen et al. 2003), and therefore in their importance as a source of virus to infect mosquitoes. The amount of WN virus expectorated by *Culex* mosquitoes is unknown, but was anticipated to vary considerably based on studies with SLE virus (Reisen et al. 2000). A second objective of the current study was to ascertain the impact of varying infectious doses on the infection response of avian hosts and to measure their viremia response to estimate host competence. Our study focused on house finches, house sparrows, mourning doves, common ground doves, and quail, because these species were found seropositive to SLE virus in previous antibody surveys (Reisen et al. 2003) and to WN virus during 2003 (Wheeler et al. 2003).

MATERIALS AND METHODS

Virus. The NY WN virus strain and the Kern217 SLE virus strain were used throughout. The NY strain of WN virus was isolated from a Flamingo that died at the Bronx Zoo during the 1999 outbreak. The Kern217 strain of SLE virus was isolated from *Cx. tarsalis* collected during the 1989 outbreak in Kern County.

Mosquitoes. *Culex* mosquitoes were collected from localities in Coachella Valley, Los Angeles and Kern County and transported to the Arbovirus Field Station where all experimental infections were conducted. Mosquitoes (either the F1 progeny of field-collected females or adults emerging from field-collected immatures) were reared to adults under standard insectary conditions (22 –25°C, 14:10 L:D photoperiod), held for 3 – 5 d on 10% sucrose, starved for 24 h, and then infected by feeding on cotton pledgets soaked with 10 fold dilutions of sweetened (2.5% sucrose) defibrinated blood - virus mixtures. Engorged females were sorted, enumerated and then maintained for 2 wks at 26°C, after which transmission rates were measured for females fed the highest concentration of virus using the capillary tube method (Aitken 1977). Bodies and expectorate samples from transmission attempts and all remaining mosquitoes were frozen individually at –80°C and shipped to the Arbovirus Laboratory at the University of California Davis where they were tested for virus using a plaque assay on Vero cells (Kramer et al. 2002). Specimens tested for WN virus were incubated for 4 d whereas those tested for SLE virus were incubated for 7-8 d, after which the plates were fixed and plaque forming units (PFU) counted. For comparison, *Cx. tarsalis* from Kern, Coachella and Los Angeles were infected concurrently by feeding on viremic house finches and transmission assessed.

Birds. Representative avian species were collected in Kern County using grain-baited ground traps and then transported to the Arbovirus Field Station where they were bled to determine previous infection. Common ground doves were from our breeding colony originating from Coachella Valley during 2000. Birds were infected by subcutaneous inoculation with ca. 1,000 PFU of WN virus in the cervical region, and then bled daily for 5 – 7 d to monitor viremia response. House finches, a representative passeriform, and mourning doves, a representative columbiform, were infected with a 10 fold dilution series of WN virus to determine the minimal dose required for infection.

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RESULTS

Mosquitoes. *Culex* populations from southern California varied markedly in their susceptibility to oral infection with WN virus (Table 1) but generally appeared to be somewhat less susceptible than determined previously (Goddard et al. 2002). Virus doses in Table 1 were estimated by interpolation from titers of virus per ml offered to females on the cotton pledgets. *Cx. stigmatosoma* was most susceptible (i.e., required the least amount of virus to infect 5 and 50% of the population sampled), followed by *Cx. tarsalis* and then *Cx. quinquefasciatus*. *Cx. stigmatosoma* from San Fernando, Los Angeles, also were highly susceptible to SLE virus with <2.2 and 4.1 $\log_{10}$ PFU/ml required to infect 5 and 50% of the population, respectively; concurrently collected *Cx. tarsalis* required 3.8 and >4.9 $\log_{10}$ PFU/ml, respectively. *Cx. tarsalis* females that fed on viremic house finches with 5.4 - 5.9 $\log_{10}$ PFU/ml viremias were infected and transmitted virus more readily than females infected by feeding on comparable or higher concentrations of WN virus presented as artificial meals on pledgets (Fig. 1). Similar results were shown previously for WN virus strains from South Africa (Cornel and Jupp 1989).

Table 1. Susceptibility of Culex from southern California to infection with WN virus.

Culex species	County	Site	Infectious dose* ($\log^{10}$ PFU/ml) 5%	50%
quinquefasciatus	Los Angeles	San Fernando	6.0	>6.3
		Los Angeles	4.9	6.3
		Machado Lake	6.1	>6.3
	Kern	Bakersfield	7.3	>7.3
stigmatosoma	Los Angeles	San Fernando	4.6	5.1
tarsalis	Kern	Kern NWR	<4.3	5.4
	Coachella	North Shore	4.6	>6.3
	Los Angeles	San Fernando	4.2	>5.8

*Amount of virus required to infect 5 and 50% of the population estimated by interpolation.

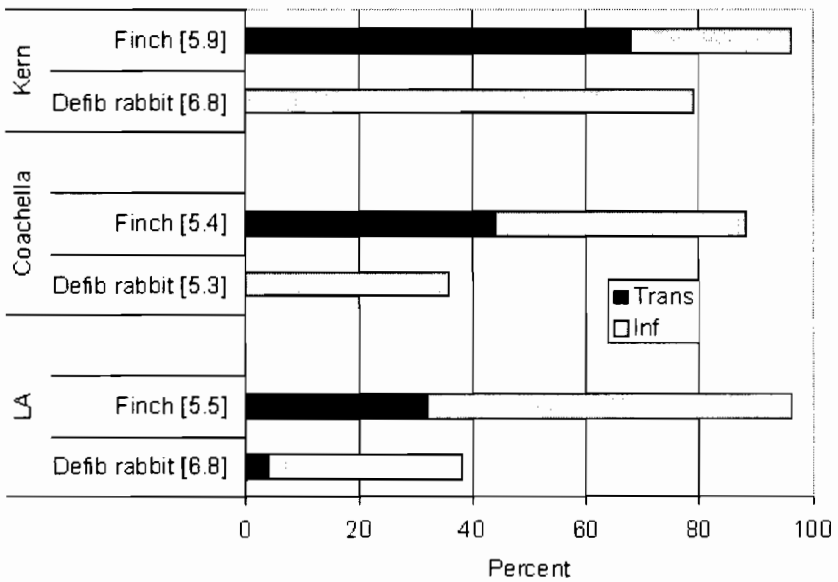

Figure 1. Percentage of *Cx. tarsalis* females infected and transmitting West Nile virus after imbibing natural blood meals from a viremia house finch or an artificial blood meal (sweetened defibrinated rabbit blood mixed with virus). Numbers in brackets are the concentration of virus as $\log_{10}$ plaque forming units per ml.

Birds. House finches became infected with WN virus and developed viremia titers peaking at 6 – 8 $\log_{10}$ PFU/ml on days 2 - 3 post infection, regardless of the titer of virus in the subcutaneous inoculum. Birds remained viremic until 7 d post infection when they either began to die or cleared their infection. Overall, 16 of 20 birds died by day 10 PI, but unexpectedly mortality was not dose related, being lowest at 2.3 $\log_{10}$ PFU/0.1 ml infectious dose (1/4 dead) and greatest at 4 and <0.3 $\log_{10}$ PFU/0.1 ml infectious dose (4/4 dead). In contrast, all 20 mourning doves inoculated with the same decreasing dilution series of virus survived infection and produced viremias peaking at 4 - 6 $\log_{10}$ PFU/ml on days 1 - 3. A summary of the remaining birds tested was shown in Table 2. Similar to crows, scrub jays were highly susceptible, succumbing to infection at 5 d post inoculation. Most house sparrows, common ground doves and California quail survived infection, but house sparrows produced very high viremias peaking at 9 $\log_{10}$ PFU/ml.

Table 2. Host competence of experimentally infected California birds for WN virus.

Bird Species	Infecting Dose	Mortality Rate Infected	Mortality Rate Dead	Viremia Response Days PI	Viremia Response Titer
House finch	<0.3-4.0	20	15	2-4	6-8
House sparrow	3.5	6	1	1-3	6-9
Western scrub jay	3.3	5	5	2-5	7-8
Mourning dove	<0.3-4.2	20	0	1-3	4-6
Common ground dove		6	0	1-4	4-6

DISCUSSION

Collectively our data indicated that California *Culex* mosquitoes generally were more susceptible to infection with WN virus than *Culex* tested from the midwestern and eastern US (Turell et al. 2001, Turell et al. 2002). However, the quantity of virus required for infection was generally greater than required to produce comparable infections with endemic SLE virus. Interestingly, birds developed much higher viremia levels following experimental infection with WN virus than SLE virus (Reisen et al. 2003), perhaps indicating that evolution may lead to a more susceptible vector and a less susceptible vertebrate host.

Acknowledgements

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Application of a Risk Model and Response

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INTRODUCTION

The California Mosquito-borne Virus Response Plan (CMBVRP) was initiated in 2001 to assist vector control agencies in structuring their response to mosquito-borne disease based upon a list of characterized factors. The Plan is used to define risk factors and high risk population groups or areas, identify levels of surveillance, prevention and control, demonstrate the need for public health intervention and allocate resources.

The scope of the Plan includes surveillance factors, mosquito control methods, response levels, characterization of conditions and responses and appendices with applied guidelines and procedures.

The Coachella Valley Mosquito and Vector Control District (CVMVCD) implemented the Response Plan in 2001 and supplemented it with the Action Plan in 2003. In September 2003, CDHS published a supplemental document to the Plan to coordinate action between the CDHS and partner agencies in responding to a mosquito-borne disease emergency, using the Standardized Emergency Management System – SEMS – organizational chart for response (CDHS 2003). An evaluation of the CMBVRP was reported by Barker et al. (2002, 2003).

The primary goal of this paper is to assess how well the State Plan can be adapted to conditions at the local level.

METHOD

Six to eight environmental and epidemiological factors presented in the benchmarks of the Plan are used to determine the risk of human infection. Certain factors are modified to fit conditions in each region. For the Coachella Valley Risk Assessment Plan, environmental factors were modified, average values of mosquito abundance were calculated for 5 years, and details of surveillance methods were specified.

Surveillance factors in the Coachella Valley include:

1. Environmental conditions – Salton Sea level, duck club flooding, average air temperature for the region.
2. Adult mosquito vector abundance — collection from 8 CDC-CO_2 traps placed strategically at saline and freshwater wetlands along the north and west shore of the Salton Sea, with over 5 years of history at the same location.
3. Virus isolation – MIR (minimum infection rate)/1000 – mosquito pools collected all year in 2003; previous years March-mid November.
4. Sentinel chickens – 10 flocks with 10 chickens each, from April to mid November, 6 flocks with 10 chickens for the rest of 2003 and beginning of 2004.

The rest of the surveillance factors conform to the State Plan. Each factor is scored from 1 (least severe) to 5 (most severe). The mean score of the factors relates to a response level of, Normal season 1.0-2.5, Emergency planning 2.6- 4.0, Epidemic 4.1- 5.0.

Each risk level has a defined response activity that describes the necessary response actions. Appropriate and timely response to surveillance data is the key to prevention of human infections. The response must be immediate and include effective mosquito control and intensified public outreach.

RESULTS

Case Study

To demonstrate the operation of the risk assessment process, biweekly tables are presented showing values used during the 2003 season before and during the period of introduction of WN virus. Risk factors evaluated included environmental factors - Salton Sea level, duck club flooding, and temperature as well as adult vector mosquito abundance and virus isolation rate from mosquito pools as minimum infection rate (MIR). Other risk factors, including equine cases, human cases, and proximity to urban/suburban regions were calculated in the same manner as in the State Risk Assessment Plan.

The mosquito abundance, temperature, and action taken, from April to October, are presented in Figure 1. The data in the figure correspond to the response level that was calculated biweekly and presented in Tables 1-6.

Beginning on week 33, in August, (Table 1) the response level of 1.0 indicated Normal season. However, at that time there was already an elevated risk because mosquitoes currently being collected were tested positive two weeks later. The response level for week 35 was elevated to 1.6, but remained in the range of Normal season (Table 2). Again, lag in test results hid additional risk factors. Week 37 rose to 2.6, Emergency planning (Table 3). Week 39 dropped back to 2.4, and indicated Normal season (Table 4). Risk factors in week 41 rose elevating the response to 2.6, Emergency planning (Table 5). There were no additional positive mosquito pools following this and week 43 dropped to the risk level dropped 2.5, Normal season for week 43 where it remained to the conclusion of the season (Table 6).

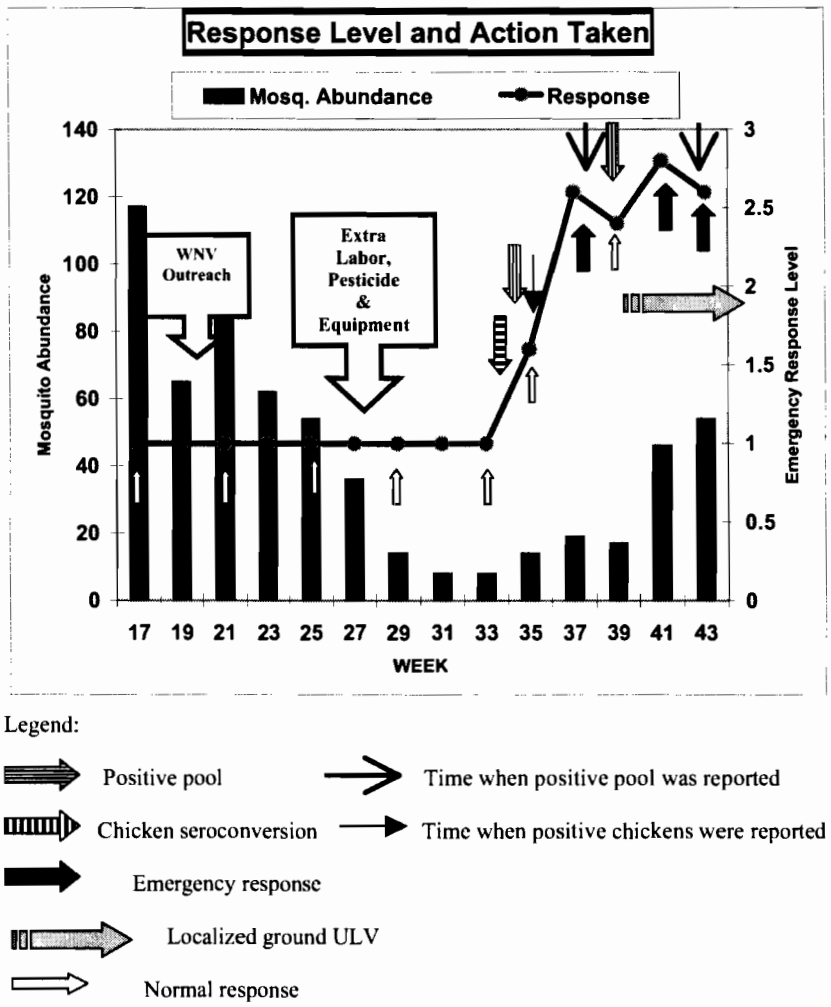

Figure 1. West Nile detection and response during April-October 2003 season in the Coachella Valley.

Table 1. Risk model scores for week 33-34.

Environmental factors	1	Dead bird	1
Adult abundance	1	Equine cases	1
Virus isolation Rate (MIR)	1	Human cases	1
Sentinel chicken seroconversion rate	1	Proximity to urban/suburban regions	1
RESPONSE LEVEL		**1.0 - NORMAL SEASON**	

Table 2. Risk model scores for week 35-36.

Environmental factors	3	Dead bird	1
Adult abundance	2	Equine cases	1
Virus isolation Rate (MIR)	1	Human cases	1
Sentinel chicken seroconversion rate	2	Proximity to urban/suburban regions	2
RESPONSE LEVEL		**1.6 - NORMAL SEASON**	

Table 3. Risk model scores for week 37-38.

Environmental factors	4	Dead bird	3
Adult abundance	3	Equine cases	1
Virus isolation Rate (MIR)	5	Human cases	1
Sentinel chicken seroconversion rate	2	Proximity to urban/suburban regions	2
RESPONSE LEVEL		**2.6 - EMERGENCY PLANNING**	

Table 4. Risk model scores for week 39-40.

Environmental factors	3	Dead bird	3
Adult abundance	3	Equine cases	1
Virus isolation Rate (MIR)	3	Human cases	1
Sentinel chicken seroconversion rate	3	Proximity to urban/suburban regions	2
RESPONSE LEVEL		**2.4 - NORMAL SEASON**	

Table 5. Risk model scores for week 41-42.

Environmental factors	3	Dead bird	3
Adult abundance	3	Equine cases	3
Virus isolation Rate (MIR)	3	Human cases	3
Sentinel chicken seroconversion rate	1	Proximity to urban/suburban regions	2
RESPONSE LEVEL		**2.6 – EMERGENCY PLANNING**	

Table 6. Risk model scores for week 43-44.

Environmental factors	3	Dead bird	3
Adult abundance	3	Equine cases	3
Virus isolation Rate (MIR)	3	Human cases	3
Sentinel chicken seroconversion rate	1	Proximity to urban/suburban regions	2
RESPONSE LEVEL		**2.5 - NORMAL SEASON**	

SUMMARY

For our District it was important to secure additional funding, control products, equipment, labor, and adjust mosquito control before the risk assessment indicated an emergency planning level for the Coachella Valley. However, the use of the Risk Assessment plan during the period of WN virus detection in the Coachella Valley confirmed that:

· Environmental factors and relative adult mosquito abundance may be classified as an early warning system, particularly if historical data are available. Both these factors helped launch the public outreach program early in the season to inform the public about the possible impact of West Nile virus and necessary control measures that the District needed to take to reduce the risk of arboviral infection in the residents of the Coachella Valley.

· The epidemiological factors, infections of mosquitoes, infections in other animals and humans, seroconversions of chickens, free ranging birds, and dead bird surveillance – lagged as triggers for increased risk level and adequate response in the Coachella Valley. At the time when these factors elevated the risk level to emergency planning, the District had all responses that corresponded to that risk level in place for two months.

It was necessary for the District to act at the level of emergency planning before the risk assessment indicated that condition, because:

· Increased surveillance and control of vector species in early season may reduce the potential of virus amplification.

· The process necessary to get approval for additional funding, purchases of mosquito control products and additional equipment, contracts with commercial applicators, information/or permits for adulticiding is lengthy.

Consistent activity of WEE and/or SLE in the Coachella Valley in the past, coupled with the mentioned risk factors, were the most important influences on the District's response. As a part of early response to environmental factors and the historical data of two other viruses, the District initiated the formation of the West Nile Task Force for the Riverside County, including other districts in the region, the local health department and emergency offices. The Response Plan was used in each region of the county and assessment values were used to establish a response level for Riverside County as a whole. In the future, historical WN virus data for the region will be a major guideline for planning surveillance, outreach, prevention, and control programs.

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Invasion of Southern California by West Nile Virus: Conclusions

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From the excellent papers presented in this symposium, we advance the following conclusions and synthesis:

- West Nile Virus (WNV) most likely was introduced into SE California from the Colorado/Nebraska epicenter, although the mechanism of dispersal and introduction was unclear. Arizona surveillance detected WNV concurrent with SE California, but Baja, Mexico did not report virus until November. Migratory birds were ruled out as the mechanism of introduction into California, because there was no WNV activity detected along the Pacific flyway in the NW USA or Canada or along the Pacific Coast of Mexico prior to detection in California. In addition, all avian migrants tested to date have been antibody negative, whereas resident birds developed WNV-antibody during late summer. However, dispersal by post nesting vagrants or summer residents cannot be ruled out. Alternatively, introduction by infected mosquitoes may have been facilitated by summer monsoonal storm tracks moving clockwise around high pressures over Nevada.
- Introduction of WNV has complicated laboratory diagnostics, especially serology. Separating WNV from SLE antibody positives now requires additional testing by plaque reduction neutralization tests, delaying virus identification and reporting of sentinel chicken enzyme immunoassay results.
- WNV was tracked effectively by different surveillance methods in different areas. In rural southeastern California deserts, WNV was tracked best by testing pools of *Cx. tarsalis* collected in dry-ice baited traps and by testing sera from sentinel chickens. However, in urban Los Angeles virus was tracked best by testing dead birds, especially corvids, and by testing pools of *Cx. quinquefasciatus* collected in gravid female traps. Virus activity seemed to be associated with rural wetlands and urban crow roosts.
- Species of free-ranging birds most frequently antibody positive were residents that survived infection, such as mourning and common ground doves, pigeons, and quail. House finches and house sparrows were not found seropositive to WNV in Coachella Valley and in Los Angeles.
- Thresholds of *Cx. tarsalis* abundance necessary for WNV transmission in SE California were very low. During midsummer when WNV invaded California, temperatures were exceptionally hot and *Cx. tarsalis* abundance was very low (ca. <25 females per CDC trap-night) compared to the spring or fall maxima (>500 - 700 females/trap-night). This timing was similar to that observed for SLE activity in this area. These data imply that targets for suppression may be difficult to attain and that low density actually may facilitate transmission efficiency.
- Based on the pattern of WNV invasion and amplification in other areas of North America, California may expect extensive amplification in southern California and the introduction of virus into the Central Valley during 2004. The challenge for vector control districts will be to control amplification at levels where human infections are minimal.

Getting the Most Out of Interactive Mapping

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The California Vectorborne Disease Surveillance Program is a joint effort between the Mosquito and Vector Control Association of California and its member mosquito and vector abatement agencies, the California Department of Health Services, and the University of California. One of the elements of this program is the operation of a public website displaying the most current information on several arbovirus surveillance indicators, including virus antibody detection in sentinel chickens and virus isolation from mosquito pools. With the recent presence of West Nile virus in the state, dead bird testing for this virus has been added. Since its inception in the early 1990s, the public website has undergone gradual improvement. Last year, interactive mapping was tried on a trial basis, and because of its wide acceptance by users of the website, it will replace completely the static maps previously used. The purpose of this paper is to provide information on the use of controls for the maps, and to demonstrate ways in which users can change various elements of the map display from within their browsers. The ability to make changes by users is the basis for interactivity of the maps. As this paper is read, users should set their browsers to **http://vector.ucdavis.edu**, and try each control as it is described.

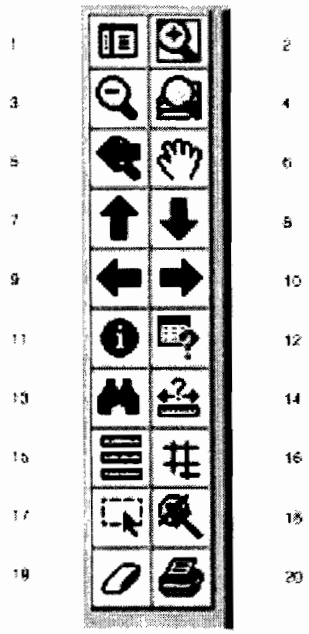

Figure 1.

THE TOOL BAR

The interactive maps use a program called ArcIMS (Environmental Systems Research Institute, Redlands, CA). Along the left side of every map displayed is a toolbar containing 20 buttons. These are the means by which users can control the appearance and coverage of the maps. The function of each button will be described below. The descriptions are numbered to coincide with the numbers of the buttons shown in Fig. 1.

1. THE LAYER-LEGEND TOGGLE BUTTON. This button toggles the panel to the right of the map between the layer display and the legend display (Fig. 2). The use of both of these views is essential to the function of the map. Most computerized maps are made up of layers. In the case of the surveillance maps, each arbovirus shown is contained on a separate layer, and there are also layers for states, counties, and bodies of water. The layer panel permits users to turn each of these layers on or off (to be visible or not after refreshing the map), and to make one of the layers "active." This last feature is necessary for use of some of the other buttons on the toolbar. Usually, when one of these buttons doesn't work the way one thinks it should, it is because the appropriate layer has not been made active on the layer panel.

The legend display is a listing of the symbols used on the map. For example, the symbol for the most recent sign of viral activity is

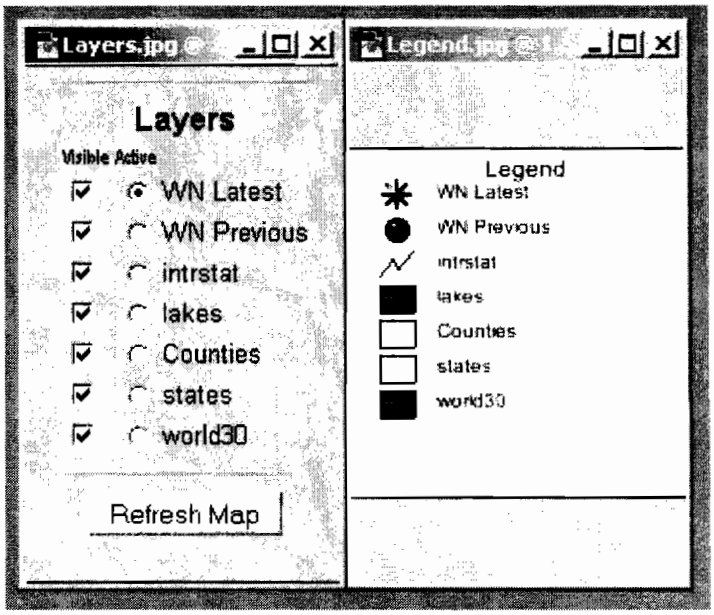

Figure 2.

a starburst; previous signs of activity are plain circles. Each virus has its own color.

2. ZOOM IN BUTTON. This button does just what its name suggests: it permits users to increase the size and decrease the extent of the map displayed. Also, as one zooms in, labels appear that were not visible at the maximum extent.

3. ZOOM OUT BUTTON. This is another button whose purpose is self-evident.

4. ZOOM TO FULL EXTENT BUTTON. Each map on the surveillance website has had its maximum extent property set by the map designer. In the case of the California surveillance maps, the extent includes western North America, and the user can not zoom beyond that extent. This button provides a way for the user to go directly to the maximum extent without having to use the zoom out button.

5. ZOOM TO PREVIOUS EXTENT BUTTON. This button allows users to go back to the most recent extent used.

6. PAN (DRAG) BUTTON. The pan button will permit dragging the entire map in any direction.

7. PAN N BUTTON. This button has the same purpose as the pan button, except that the direction of panning is always north.

8. PAN S BUTTON. The direction of panning is south.

9. PAN W BUTTON. The direction of panning is west.

10. PAN E BUTTON. The direction of panning is east.

11. INFORMATION BUTTON. This button provides specific information on a feature on the map. To use it, first toggle to the layer display, then make the layer active that contains the features of interest. Next, click on the information button, then on the feature (usually a dot). In the panel below the map, specific details concerning that feature will appear.

12. QUERY BUTTON. This button will select one or more features depending on the query written in the panel that appears. This is done by using various drop-down lists rather than having to write the query from scratch. When one presses the "execute" button below the query screen, the query will run, the selected features will change color, and a list of features matching the search will appear below the map. This is an extremely useful tool.

13. FIND (LOOKUP) BUTTON. The function of this button is similar to the query button, except that lookup strings are much easier to write. To use this button, make the layer active that contains the information of interest, then enter a data string in the box that appears below the map. The mapping software will look for all features on the layer matching the string you entered (e.g., a zip code).

14. MEASURE BUTTON. This button is for measuring the distance between 2 points selected, or the combined distance of multiple features selected.

15. UNITS BUTTON. This button is usually used in conjunction with the measure button, and permits selection of feet, miles, meters, or kilometers.

16. BUFFER BUTTON. The buffer button is a complex feature, and directions for its use is difficult to explain. The short explanation is that this button permits the drawing of an area around some point (such as a human case of West Nile fever). Depending on how large you set the buffer all features (on the active layer) within the buffer will appear below the map. A possible surveillance use for this button would be to determine the number of schools within 3 miles of a WN-positive sentinel chicken.

17. SELECT BY RECTANGLE BUTTON. This useful button permits selecting features on a layer by dragging a rectangle around an area with a mouse. A table showing information on all features that fall within the rectangle will appear in a pane below the map.

18. SELECT BY POLYGON BUTTON. This does the same thing as the select by rectangle, except users can construct irregularly-shaped polygons.

19. CLEAR SELECTION BUTTON. Once features on a layer are selected by one of the methods described above, they can be unselected with this button.

20. PRINT BUTTON. Clicking on this button causes printing of the map (at the extent selected) to be printed on the default printer.

An Improved System for Objective Statewide Trap Stratification Based on Human Population Density

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ABSTRACT: An objective surveillance site stratification system was developed for California that utilized GIS and 2000 United States Census Bureau human population density data to stratify sites into urban, suburban, and rural categories. Block groups were selected as the geographic units for analysis because they were small enough to delineate populated areas accurately, and they were the smallest units for which the data were manageable using commonly available computer software. Urban, suburban, and rural areas were defined as block groups having ≤800, 801—4,000, or > 4,000 persons per sq mi, respectively. Based on these thresholds, all surveillance sites registered in the California Surveillance Site Registration Database were assigned an urban, suburban, or rural designation.

INTRODUCTION

Historically, California mosquito control agencies have designated trap locations as urban, suburban, or suburban/rural in an effort to make trap counts comparable among agencies statewide. These designations have applied primarily to New Jersey light trap (NJLT) locations from which mosquitoes have been collected and reported in the weekly Adult Mosquito Occurrence Report. These determinations have been made by personnel at each local agency using the following criteria: urban areas > 1 mile inside a densely populated area; suburban areas ¼ to 1 mile inside a densely populated area; or suburban/rural areas outside of or < ¼ mile inside a densely populated area. However, the determination of what constitutes a densely populated area is subjective and undoubtedly varies among agencies.

The trapping methods employed by California's mosquito control agencies have diversified considerably during recent years, and there is a demand for an objective trap stratification system that will meet a variety of surveillance needs. Among these are needs for continued comparability of trap counts among agencies, stratification based on the level of competition from external light sources, quantification of the epidemic risk level once virus transmission has been detected, and targeted surveillance in "rural" areas, such as embedded wetlands or vector dispersal corridors, within otherwise urban areas.

GIS-based methods now permit objective trap stratification that can be applied to the entire state at once. With the required human population census data, GIS layers, and exact trap location information, traps can be stratified rapidly and objectively at any time as new traps are added or as new human population information becomes available. The objectives of this paper were 1) to define an appropriate spatial scale for use in trap stratification, 2) to determine whether suburban/rural areas should be defined by population density or as a buffer around urban areas, 3) to define population density thresholds for urban, suburban, and suburban/rural areas, and 4) to stratify current surveillance sites into the 3 population density categories.

PROCEDURES

Data Sources. 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 tract, block group, and block were provided by personnel at the USCB. Using ArcView 8.3 (Environmental Systems Research Institute (ESRI), Redlands, CA), these census and land area data were combined with USCB TIGER/Line files in the form of ESRI shapefiles for census tracts, block groups, and blocks so that human population density in persons/mi2 could be mapped throughout California. Census TIGER/Line shapefiles for county boundaries, streets, water bodies, and landmarks also were mapped as reference layers (http://www.geographynetwork.com). Surveillance site location information came from the California Surveillance Site Registration Database maintained at the University of California, Davis.

Spatial Scale. The 2000 census data were examined to determine the appropriate spatial scale for trap stratification. We examined the data at the block, block group, and tract levels to identify the level at which the human population density values adequately fit the actual distribution of human residences, as represented by the presence of streets. Another practical consideration was that we needed to identify a spatial scale at which

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the data volume would be manageable in common computer software, such as Microsoft Office products (Microsoft Corporation, Redmond, WA).

Census blocks are the smallest unit into which the USCB divides human population data. Blocks are grouped into block groups, which are in turn grouped into tracts. In 2000, California was divided into 533,159 blocks, 22,133 block groups, and 7,049 tracts with an average of 64, 1,530, and 4,805 persons per block, block group, or tract, respectively. In Los Angeles County alone, there were 86,614 blocks, a number which is not manageable in software such as Microsoft Excel that can accommodate a maximum of 65,536 rows.

We chose block groups for use in trap stratification because they represented the smallest units for which the data were manageable, and they covered a small enough area to delineate populated areas accurately. The land area covered by block groups ranged from an average of 0.2 mi^2 in densely populated areas to approximately 50 mi^2 in sparsely populated areas.

Definition of Suburban Areas. Until now, urban and rural areas have been defined based on a subjective population density threshold, and suburban areas have been defined as those areas within a specified distance of the urban areas. To better meet current surveillance needs in California, including the need for quantification of the epidemic risk level once virus transmission has been detected (California Department of Health Services et al. 2003) and the need for identification of "rural" areas within otherwise urban areas, an alternative method for definition of suburban areas was considered. This method defined urban, suburban, and rural areas strictly by population density so that the actual human population density was represented in all areas, and 2 thresholds were defined: one separating urban and suburban areas and another separating suburban and rural areas.

Use of this objective system will improve the comparability of trap counts among agencies and will allow assessment of virus transmission risk to the human population once virus activity has been detected, while still providing an indication of the intensity of competing light sources for NJLT collections. Also, this system will help to identify rural and suburban areas, particularly those that provide increased opportunity for vector-amplifying host contact, within urban areas, so that these areas may be targeted for placement of surveillance sites.

Choice of Human Population Density Thresholds. The USCB defines urban areas, in part, as a central place(s) and adjacent territory with a general population density of at least 1,000 persons/ mi^2. Using the USCB criteria, all areas outside of urban areas are designated as rural, and no suburban category exists.

By plotting a histogram of block groups categorized by population density and examining the level at which each density range was represented, we were able to determine a logical population density range for suburban areas, with urban and rural areas defined as block groups with population densities above and below the suburban range, respectively. Urban areas contain large numbers of block groups because the area covered by each block group becomes smaller as population density increases, and rural areas contain large numbers of block groups because most of California is sparsely populated, even though the area covered by each individual block group in rural areas is relatively large (Fig. 1). Suburban areas are represented by smaller numbers of block groups because they represent areas that cover a smaller amount of total land area than rural areas and a larger amount of land area per block group than urban areas.

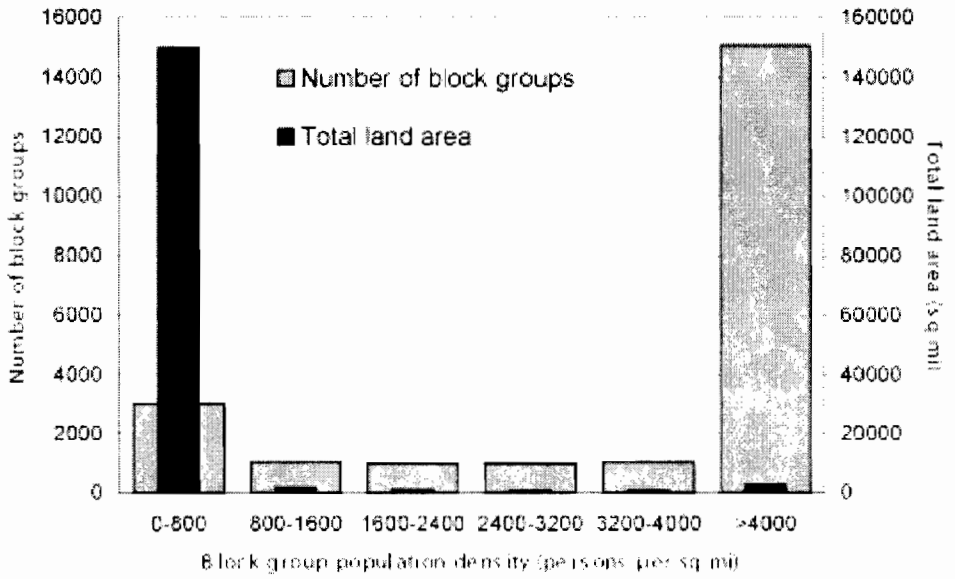

Figure 1. Numbers of block groups and total land area by human population density strata showing the large amount of land area covered by rural (0-800 persons per sq mi) block groups and the large number of urban (>4,000 persons pers sq mi) block groups covering a small proportion of the total land area.

The histogram of block groups by population density (Fig. 2) shows that there are two peaks separated by a range of population densities for which there are lower numbers of block groups (approximately 800—4,000 persons/mi^2). The population density range between the peaks was selected to represent suburban areas (inset, Fig. 2) for the reasons outlined in the previous paragraph. Rural block groups were defined as having no more than 800 persons/mi^2, and urban block groups were those with > 4,000 persons/mi^2. After choosing thresholds based on the histogram, the stratified block groups were mapped with an overlay showing streets to determine how well the strata matched the actual human population distribution, as indicated by the density of streets in a given area. In nearly all areas, the areas defined as urban and suburban were approximately the same as those areas in which streets were present (e.g., Fig. 3).

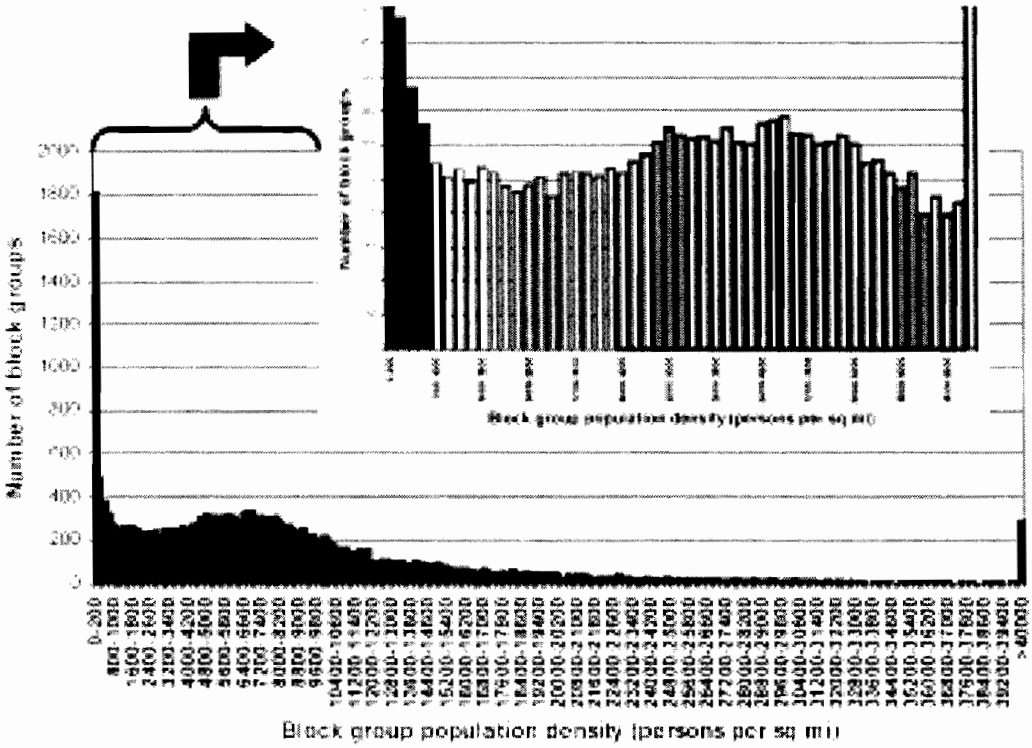

Figure 2. Histogram showing numbers of block groups by population density for California, 2000. The inset is an enlargement for population densities from 0—10,000 persons per sq mi and shows block groups designated as rural (black columns), suburban (gray columns), and urban (white columns).

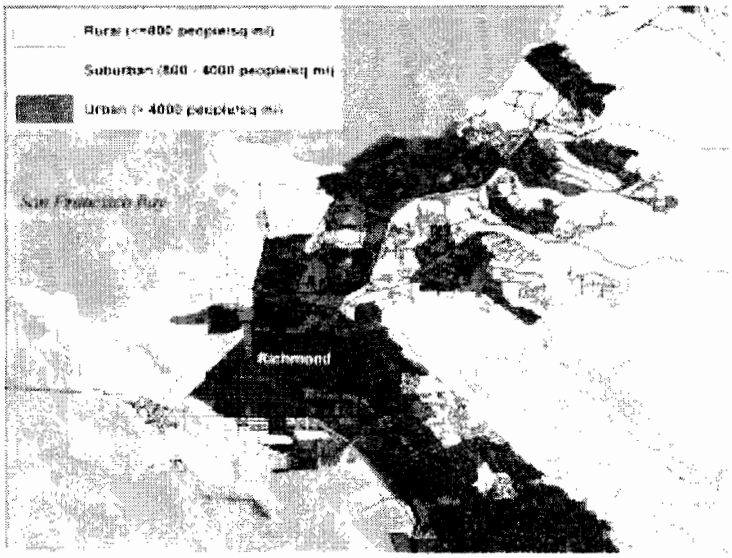

Figure 3. Map of Richmond, California (Contra Costa County) showing areas designated as urban, suburban, and rural. Streets are also shown as an indication of the distribution of the human population.

Stratification of Registered Surveillance Sites. After decisions had been made on appropriate spatial scale and population density thresholds defining urban, suburban, and rural areas, three polygon shapefiles were generated from the TIGER/Line block group polygons that were linked to block-group level population density and land area data. We used a query to select all block groups within the respective block group population density ranges for urban, suburban, and rural areas and exported each selection as a new shapefile for each of the 3 density strata.

Using the new shapefiles generated for urban, suburban, and rural areas and the California Surveillance Site Registration Database, we selected all surveillance sites within the boundary of each shapefile and assigned the urban, suburban, or rural designation to all sites selected for each respective population density stratum (e.g., Fig. 4). The resulting percentages of surveillance sites in each stratum for all agencies are presented in Table 1.

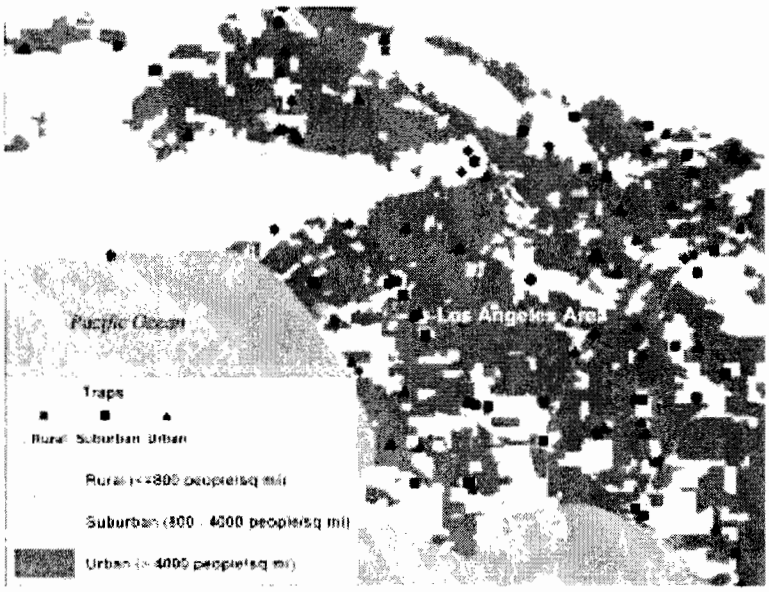

Figure 4. Map of Los Angeles, California and surrounding areas showing areas designated as urban, suburban, and rural, along with designations assigned to registered surveillance sites.

Table 1. Percentages of sites classified as rural, suburban, and urban by agency for all sites registered in the California Surveillance Site Registration Database as of November 2003.

Agency Code	Urban	Suburban	Rural	Agency Code	Urban	Suburban	Rural
ACVC	88.9%	11.1%	0.0%	MOOR	0.0%	0.0%	100.0%
AFSB	2.7%	13.5%	83.8%	NAPA	20.0%	0.0%	80.0%
ALCO	12.5%	25.0%	62.5%	NSAL	0.0%	50.0%	50.0%
ANTV	40.0%	33.3%	26.7%	NWST	18.2%	36.4%	45.5%
BUCO	0.0%	11.1%	88.9%	ORCO	22.7%	50.0%	27.3%
BURN	0.0%	0.0%	100.0%	PASA	20.0%	80.0%	0.0%
CLSA	0.0%	0.0%	100.0%	PLCR	3.1%	18.8%	78.1%
CNSL	3.4%	0.0%	96.6%	RIVR	0.0%	13.3%	86.7%
CNTR	16.1%	29.0%	54.8%	SANB	0.0%	80.0%	20.0%
COAV	3.4%	15.9%	80.7%	SAND	7.1%	71.4%	21.4%
DLNO	0.0%	33.3%	66.7%	SANM	33.3%	33.3%	33.3%
DLTA	16.7%	16.7%	66.7%	SAYO	11.6%	17.2%	71.2%
DNOR	0.0%	0.0%	100.0%	SBCO	25.0%	25.0%	50.0%
EAST	0.0%	0.0%	100.0%	SCRZ	23.5%	29.4%	47.1%
FRNO	9.1%	9.1%	81.8%	SFMO	0.0%	0.0%	100.0%
FRWS	0.0%	25.0%	75.0%	SGVA	56.0%	28.0%	16.0%
GLEN	0.0%	12.5%	87.5%	SHAS	0.0%	13.5%	86.5%
GLVY	10.0%	40.0%	50.0%	SJCM	11.1%	8.1%	80.7%
GRLA	33.3%	30.2%	36.5%	SOLA	0.0%	0.0%	100.0%
IMPR	0.0%	14.3%	85.7%	STCL	24.0%	24.0%	52.0%
INYO	0.0%	0.0%	100.0%	SUYA	7.1%	2.9%	90.0%
KERN	11.2%	14.0%	74.8%	TEHA	0.0%	0.0%	100.0%
KNGS	0.0%	25.0%	75.0%	TLRE	8.3%	8.3%	83.3%
LACW	51.5%	21.2%	27.3%	TRLK	1.8%	8.1%	90.1%
LAKE	0.0%	12.5%	87.5%	VENT	23.5%	26.5%	50.0%
MADR	10.0%	40.0%	50.0%	WEST	0.0%	0.0%	100.0%
MARN	0.0%	8.3%	91.7%	WVAL	0.0%	42.9%	57.1%
MERC	11.1%	0.0%	88.9%				

SUMMARY

There are many advantages to an objective statewide trap stratification system, as compared with the current subjective system. Although the value of having a person present at a surveillance site to survey the surroundings cannot be discounted and the decadal frequency of the U.S. Census is a limitation, the system proposed here is much more consistent among agencies and allows nearly instantaneous stratification of all traps throughout the state, once the requisite datasets have been assembled. This objective system meets a broader range of surveillance needs than the previous system, and sites can easily be restratified at any time if population density thresholds defining strata are modified, new traps are added, or new census data become available.

Acknowledgements

We especially thank Marie Pees at the Population Division, United States Census Bureau, for providing 2000 census population and land area values for California. Funding was provided by a grant from the Office of Global Programs, National Oceanic and Atmospheric Administration.

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Surveillance for Mosquito-borne Encephalitis Virus Activity and Human Disease, Including West Nile Virus in California, 2003

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The California Arbovirus Surveillance program is a cooperative effort of the California Department of Health Services (CDHS), the University of California at Davis, Center for Vectorborne Diseases (CVEC), the Mosquito and Vector Control Association of California, local mosquito and vector control agencies, county and local public health departments, physicians, and veterinarians throughout California. Local agencies that participated in the statewide mosquito-borne encephalitis surveillance program are listed in Table 1. Additional collaborating agencies in the West Nile virus (WN) surveillance program included the California Department of Food and Agriculture, California Animal Health and Food Safety Laboratory (CAHFS), California Department of Fish and Game, the U.S. Fish and Wildlife Service, and the Centers for Disease Control and Prevention (CDC).

In anticipation of the arrival of WN in California, the surveillance program was expanded significantly in 2003. The local mosquito and vector control agencies submitted more than twice the number of chicken sera and mosquito pools for testing than in any previous year. The use of sentinel chickens, mosquito collections, and dead bird reporting validated the effectiveness of the surveillance program in the detection of WN activities in CA, provided early warning of virus activities in various regions of the state, and helped to focus on mosquito control efforts in the most critical areas.

Surveillance program elements include: 1) diagnostic testing of specimens from human patients exhibiting symptoms of viral meningitis, encephalitis, acute flaccid paralysis/atypical Guillain-Barré, and febrile illness; 2) enrollment of patients diagnosed with encephalitis into the California Encephalitis Project, which evaluates clinical course, demographics, exposure to arthropods, and laboratory evidence to determine etiology; 3) diagnostic testing of specimens from equids that exhibit clinical signs of viral neurologic disease compatible with arboviral infection, including western equine encephalomyelitis (WEE), WN, and other arbovirus as appropriate; 4) monitoring and testing of mosquitoes for the presence of St. Louis encephalitis (SLE), WEE, and WN. Tests were also performed for California encephalitis (CE), dengue, and other arboviruses as appropriate; 5) serological monitoring of sentinel chickens for SLE, WEE, and WN antibodies; 6) surveillance and diagnostic testing of dead birds, especially crows and ravens, for infection with WN; 7) weekly reporting in the CDHS Arbovirus Surveillance Bulletin and on the website (**www.westnile.ca.gov**) of arbovirus testing results in California and arbovirus activity throughout the United States. Diagnostic procedures used in 2003 in California are summarized in Table 2.

The 2003 surveillance season began in April with the deployment of sentinel chicken flocks and the beginning of mosquito collection data for the Adult Mosquito Occurrence Report (AMOR). Thirty-five weekly Arbovirus Surveillance bulletins and thirty-one adult mosquito occurrence reports were distributed to all program participants to provide detailed surveillance summaries. Positive findings including chicken serology, mosquitoes, and dead birds were communicated immediately to submitting agencies, local health departments, and the appropriate mosquito and vector control districts.

WN was first detected in a mosquito pool collected on July 16, 2003 in Imperial County (Tables 3 and 4). Sentinel chicken seroconversions were first detected in chickens bled on August 4, 2003, in Imperial County; however, there was no sera collected in July so there might have been seroconversions earlier than August 4 (Tables 4 and 5). Riverside County had its first positive WN test results in sentinel chickens bled on August 25 and in mosquito pools August 26, 2003. Los Angeles County was the first county with a positive WN dead bird reported on September 3, 2003 (Tables 4 and 6). Positive human WN results were limited to Imperial, Riverside, and Los Angeles counties between September 28 and October 8, 2003 (Tables 4 and 7). At least one of the surveillance elements (chicken, mosquito pools, and dead birds) indicated WN activity at least one month prior to the human case in each county (Table 4). Table 8 is a summary of WN positives by county and surveillance element for 2003.

HUMAN SURVEILLANCE

The DHS Viral and Rickettsial Disease Laboratory (VRDL) tested sera and/or cerebrospinal fluid specimens from 1,112 patients for antibodies to WEE, WN, and SLE. Cases represented 312 cases of encephalitis, 490 cases of aseptic meningitis, 11 cases of acute flaccid paralysis/atypical Guillain-Barré syndrome, and 299 cases of febrile illness. Of these, sera from 352 patients were first screened, for immunoglobulin M (IgM) antibodies against WN by

Table 1. Participation by local agencies in the statewide mosquito-borne encephalitis surveillance program, 2003.

County	Agency	Agency code	New Jersey Light Traps	Mosquito Pools	No. flocks	No. chickens	No. serum samples	Birds reported	Birds tested
Alameda	Alameda Co. MAD	ALCO	13	75	3	26	254	227	31
Amador								26	4
Butte	Butte Co. MVCD	BUCO	26	10	7	70	985	239	30
Calaveras								15	2
Colusa	Colusa MAD	CLSA	3		1	10	130	11	2
Contra Costa	Contra Costa MVCD	CNTR	22	365	4	43	721	456	54
Del Norte								3	1
El Dorado								144	13
Fresno	Consolidated MAD	CNSL	12	74	5	51	679	180	21
Fresno	Fresno MVCD	FRNO	9	27	2	25	150		
Fresno	Fresno Westside MAD	FRWS	10	38	2	28	208		
Glenn	Glenn Co. MVCD	GLEN	5	22	2	26	358	9	4
Humboldt								21	5
Imperial	Coachella Valley MVCD	IMPR			3	30	676	28	8
Imperial	Imperial Co. Environmental Health	IMPR		532	2	38	232		
Inyo	Owens Valley MAP	INYO		175	3	31	403	72	10
Kern	Arbovirus Field Station	AFSB		122					
Kern	Delano MAD	DLNO	8		2	20	220	70	13
Kern	Kern MVCD	KERN	20	558	9	92	1,259		
Kern	South Fork MAD	SFMO		1					
Kern	Westside MVCD	WEST	17		3	30	438		
Kings	Kings MAD	KNGS	9		3	30	304	20	2
Lake	Lake Co. VCD	LAKE		202	2	20	259	19	3
Los Angeles	Antelope Valley MVCD	ANTV	13		5	35	463	1,619	343
Los Angeles	Greater Los Angeles Co. VCD	GRLA	17	1,659	5	50	870		
Los Angeles	Long Beach Environmental Health	LONG		328	4	37	626		
Los Angeles	Los Angeles Co. West VCD	LACW		179	20	120	1,827		
Los Angeles	San Gabriel Valley MVCD	SGVA		37	11	64	1,476		
Madera	Madera Co. MVCD	MADR	5	21	2	22	199	31	1
Marin	Marin-Sonoma MVCD	MARN	29		5	55	680	99	15
Mariposa								9	1
Mendocino								33	5
Merced	Merced Co. MAD	MERC	18	5	6	36	509	159	21
Merced	Turlock MAD	TRLK		224					
Mono								11	2
Monterey	North Salinas MAD	NSAL	17		1	10	161	54	12
Napa	Napa MAD	NAPA			2	11	141	30	6
Nevada								76	3
Orange	Orange Co. VCD	ORCO		571	1	10	180	414	119
Placer	Placer Co. VCD	PLCR	15	16	5	55	768	150	19
Riverside	Coachella Valley MVCD	COAV	10	1,461	10	100	1,563	606	184
Riverside	Northwest MVCD	NWST	12	541	6	70	1,008		
Riverside	Riverside Co. Environmental Health	RIVR	13	104	6	66	1,190		
Sacramento	Sacramento-Yolo MVCD	SAYO	40	657	5	50	770	757	135
San Benito								13	2
San Bernadino	San Bernardino Co. VCP	SANB	22	123	7	70	1,211	611	121
San Bernardino	West Valley MVCD	WVAL		120	5	30	478		
San Diego	San Diego Co. Dept of Health	SAND		98	3	30	540	498	255
San Francisco								50	5
San Joaquin	San Joaquin Co. MVCD	SJCM	50	330	5	60	723	153	21
San Luis Obispo	San Luis Obispo Co.	SLOC		137	2	21	221	169	16
San Mateo	San Mateo Co. MAD	SANM			3	30	447	149	25
Santa Barbara	Santa Barbara Coastal VCD	SBCO		220	5	50	756	80	15
Santa Clara	Santa Clara Co. VCD	STCL	36	115	4	60	589	223	41
Santa Cruz	Santa Cruz Co. MVCD	SCRZ	7	50	1	9	126	54	2
Shasta	Burney Basin MAD	BURN	6		2	19	201	61	9
Shasta	Shasta MVCD	SHAS	26	94	5	61	800		
Solano	Solano Co. MAD	SOLA	25		2	22	233	91	5
Solano	Sacramento-Yolo MVCD	SAYO		297					
Sonoma	Marin-Sonoma MVCD	MARN	*		2	22	308	160	18
Stanislaus	East Side MAD	EAST			2	22	310	164	29
Stanislaus	Turlock MAD	TRLK	21	78	4	48	643		
Sutter	Sutter-Yuba MVCD	SUYA	40	274	5	50	695	69	19
Tehama	Tehama Co. MVCD	TEHA			2	22	201	17	1
Tulare	Delta VCD	DLTA	12	33	6	60	783	53	9
Tulare	Tulare MAD	TLRE	10		2	20	273		
Tuolumne								11	1
Ventura	City of Moorpark	MOOR	4		1	10	180	137	32
Ventura	Ventura Co. Environmental Health	VENT	20	39	4	40	759		
Yolo	Sacramento-Yolo MVCD	SAYO	*	253	5	50	798	239	65

Table 2. Arbovirus diagnostic procedures for California.

	Criteria	Primary Test	Confirmatory Test	Virus Tested			
				SLE	WN	WEE	CE
Mosquito pools	Collections by local agencies		*in-situ* EIA using vero cell cultures (CVEC)	x	x	x	x
Chicken sera	Local agency sentinel flocks	EIA (VRDL)	IFA (PRNT as needed - VRDL)	x	x	x	–
Equine sera	Per request of the veterinarian	EIA (CVEC)	PRNT (CVEC)	–	x	x	–
Equine tissue	Screened by VPHS and CDFA	Virus isolation in VERO cells (CVEC)		–	x	x	–
Bird carcasses	Screened by VBDS; necropsy and tissue removal by CAHFS	RT-PCR using a primary set of primers on kidney tissue and cell culture on organ pools (CVEC)	RT-PCR using a secondary primers (CVEC)	–	x	–	–
Other animals	Screened by VPHS	PRNT for sera (CVEC), virus isolation (CVEC)		–	x	–	–
Human sera	Screened by local public health labs and VRDL	EIA (for SLE and WEE), IgM-EIA (for WN) (VRDL)	PRNT (CVEC/VRDL)	x	x	x	–
Human cerebrospinal fluid	Screened by VRDL	EIA (for SLE and WEE), IgM-EIA (for WN) (VRDL)	PRNT (CVEC/VRDL)	x	x	x	–

Abbreviations:

Agencies: CAHFS, California Animal Health and Food Safety Laboratory
CVEC, University of California, Davis, Center for Vector-Borne Disease
VBDS, Vector-Borne Disease Section
VPHS, Veterinary Public Health Section
VRDL, Viral and Rickettsial Disease Laboratory

Assays: EIA, enzyme immunoassay
PRNT, plaque reduction neutralization test
IFA, immunofluorescent antibody
IgM-EIA, immunoglobulin M enzyme immunoassay
RT-PCR, reverse transcription polymerase chain reaction

Table 3. CE, SLE, WEE, and WN isolated from mosquito pools during 2003.

Mosquito Species	Date Collected	County	Agency	Virus Isolated							
				CE		SLE		WEE		WN	
				pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Culex quinquefasciatus	16-Sep	Los Angeles	GRLA							1	50
	9-Oct	Los Angeles	GRLA							2	95
	23-Oct	Los Angeles	GRLA							3	129
	19-Nov	Los Angeles	GRLA			1	25				
Culex tarsalis	2-Jul	Riverside	COAV			1	50				
	16-Jul	Imperial	IMPR							1	50
	4-Aug	Imperial	IMPR							1	27
	7-Aug	San Diego	SAND					1	50		
	11-Aug	Riverside	COAV			1	25				
	19-Aug	Imperial	IMPR							4	160
	26-Aug	Riverside	COAV							1	45
	27-Aug	Riverside	COAV							1	23
	2-Sep	Imperial	IMPR							6	300
	4-Sep	Riverside	COAV							1	27
	9-Sep	Riverside	COAV							2	71
	11-Sep	Riverside	COAV							3	93
	16-Sep	Imperial	IMPR			1	11			4	161
	23-Sep	Riverside	COAV							1	50
	24-Sep	Riverside	COAV							1	50
	2-Oct	Riverside	COAV			1	50				
Ochlerotatus melanimon	12-Jun	Kern	KERN	3	150						
	19-Jun	Kern	KERN	1	50						
	13-Aug	Inyo	INYO	1	33						
Totals				**5**	**233**	**5**	**161**	**1**	**50**	**32**	**1,331**

Table 4. Biweekly timetable of WN infections week beginning July 6, 2003

Positive Species	Jul-6	Jul-20	Aug-3	Aug-17	Aug-31	Sep-14	Sep-28	Oct-12	Oct-26	Nov-9	Nov-23	Dec-7
Human							RIV, IMP, LA					
Mosquito Pools	IMP		IMP	RIV, IMP	RIV, IMP	RIV, IMP, LA	LA	LA	IMP			
Chicken			IMP	IMP, RIV	IMP, RIV		IMP	IMP, RIV				
Dead Birds					LA	LA	LA, RIV, SB, SD	LA, OR, RIV, SD	LA, OR, RIV, SB, SD	LA, RIV, SB	LA, SB	LA, SB
Horses								SD				

Legend: IMP=Imperial LA=Los Angeles OR=Orange RIV=Riverside SB=San Bernardino SD=San Diego

Table 5. Chicken seroconversions to SLE, WEE, and WN by location and week (Monday of week shown below) bled, 2003

SLE

County	Agency	Site	City	Location	9/1	9/15	9/22	9/29	10/13	Total
Imperial	IMPR	IMPR0012	Westmorlan	West Mo	2					2
Los Angeles	SGVA	SGVA0002	Monterey P	City Yard				2		2
Riverside	COAV	COAV0122	Mecca	Gordon		2				2
Riverside	RIVR	RIVR0006	Blythe	4th Avenue		2		3	1	6
San Bernard	SANB	SANB0002	Redlands	Treatment Plant			1			1
				Total	2	4	1	5	1	**13**

WEE

County	Agency	Site	City	Location	11/4	Total
San Diego	SAND	SAND0016	Carlsbad	Bov Lagoor	2	2
				Total	2	**2**

WN

County	Agency	Site	City	Location	8/4	8/18	8/25	9/1	9/15	9/29	10/13	10/27	Total
Imperial	IMPR	IMPR0002	Seeley	Campbell		4		2					6
Imperial	IMPR	IMPR0003	El Centro	Nichols		7				1			8
Imperial	IMPR	IMPR0010	Niland	Wister	3	2		2	1	1	1	1	11
Imperial	IMPR	IMPR0011	Niland	Bono Wildl	3	1		3	2	4*			13
Imperial	IMPR	IMPR0012	Westmorlan	West Mo				3	5	1			9
Imperial	IMPR	IMPR0016	Holtville	Zenos		7							7
Riverside	COAV	COAV0035	Mecca	Adohr			4		3				7
Riverside	COAV	COAV0122	Mecca	Gordon				2	4		2		8
Riverside	COAV	COAV0131	Oasis	Jessup					1				1
				Total	6	21	4	12	16	3	7	1	**70**

*In some flocks when a chicken has seroconverted, it is replaced by a non-infeeted chicken

Table 6. WN positive dead birds (Monday of week shown below)

Species	Week	Los Angeles	Orange	Riverside	San Bernardino	San Diego	Total
American crow	9/1	1					1
	9/8	2					2
	9/15	6					6
	9/22	5					5
	9/29	6		2	1		9
	10/6	7		1	1		9
	10/13	4		1		1	6
	10/20	2	2	1		1	6
	10/27	9		3			12
	11/3	10			4		14
	11/10	4		1			5
	11/24				1		1
	12/1	4					4
	12/8	2			1		3
	12/15				1		1
Blackbird	9/29			1			1
Common raven	10/27	1					1
House finch	10/13					1	1
	10/20			1			1
Mockingbird	10/6					1	1
Northern flicker	11/3		1				1
Sparrow	9/1			1			1
	10/6				1		1
	10/20	1					1
	10/27					1	1
Western scrub-jay	10/6	1		1			2
Totals		**65**	**3**	**13**	**10**	**5**	**96**

immunofluorescence assay (IFA) at one of 30 county public health laboratories.

Of the 1,112 patients tested, 294 were enrolled in the California Encephalitis Project. For each patient enrolled, a battery of tests was conducted, including polymerase chain reaction (PCR), serology, and viral isolation for 15 agents, including WN. Testing for additional agents was pursued as clinical symptoms and exposure history warranted; extensive testing for arboviruses was conducted for cases with known mosquito exposure and those with a travel history to an area of WN activity.

Three human cases of WN with likely exposure in California were identified in 2003. A 31-year-old male from Riverside County was diagnosed with aseptic meningitis on September 28, 2003. A 46-year-old male male resident of Imperial County was diagnosed with aseptic meningitis on October 18, 2003. A 61-year-old male resident of Los Angeles County was diagnosed with a febrile illness on October 18, 2003 (Table 7). Specimens from two of the cases (Imperial and Riverside Counties) were screened for total antibody at the local public health laboratory and forwarded to VRDL. VRDL detected antibody titers to WN that were higher than those for SLE, WEE, and dengue in sera from all three patients. Plaque reduction neutralization tests (PRNT's) confirmed acute WN infection for all three cases. All three patients survived.

The California Encephalitis Project detected 20 WN infections acquired outside of California in 2003. Eighteen cases were California residents who were exposed and had traveled to a WN

Table 7. Human cases of infection with West Nile virus, 2003.

	Age	Gender	Onset	Place of Residence	Imported	Diagnosis
Locally Acquired	31	M	9/28/2003	Riverside	--	Confirmed WN Meningitis
	46	M	10/5/2003	Imperial	--	Confirmed WN Meningitis
	61	M	10/8/2003	Los Angeles	--	Confirmed WN Fever
Imported	64	M	7/21/2003	Los Angeles	Louisiana	Confirmed WN Meningoencephalitis
	60	F	7/28/2003	San Diego	Mexico	*Secondary Flavivirus Infection
	55	F	8/1/2003	Kern	Colorado	Confirmed WN Fever
	47	F	8/7/2003	Alameda	Colorado	Confirmed WN Acute Flacid Paralysis
	30	F	8/12/2003	Shasta	Colorado	Confirmed WN Fever
	68	F	8/15/2003	Riverside	Colorado, Indiana	Confirmed WN Encephalitis
	67	M	8/19/2003	Sacramento	Pennsylvania	Confirmed WN Meningitis
	57	F	8/19/2003	Alameda	Pennsylvania	Confirmed WN Encephalitis
	24	M	8/19/2003	Ventura	Colorado	Confirmed WN Fever
	62	M	8/21/2003	San Mateo	South Dakota, Utah, Colorado	Confirmed WN Meningoencephalitis
	79	F	8/22/2003	San Diego	South Dakota	Confirmed WN Meningitis
	48	M	8/24/2003	Los Angeles	Colorado	**Confirmed WN Fever
	41	M	8/24/2003	Los Angeles	Massachusetts	Confirmed WN Encephalitis
	19	F	8/24/2003	Sonoma	Wyoming	Confirmed WN Meningitis
	41	M	8/29/2003	Los Angeles	Colorado	Confirmed WN Meningoencephalitis
	19	M	8/29/2003	Los Angeles	Saskatchewan, Canada	Confirmed WN Fever
	70	M	9/8/2003	Kern	Colorado	Confirmed WN Encephalitis
	75	F	9/23/2003	Los Angeles	Arizona and New Jersey	Confirmed WN Meningitis
	9	M	9/28/2003	Colorado	Riverside, CA	Confirmed WN Fever
	62	M	10/5/2003	Florida	San Diego, CA	Confirmed WN Meningoencephalitis

* PRNT did not distinguish flaviviurses

**Blood Donor

Table 8. Summary of West Nile virus, Positives, 2003.

WN Results							
	Imperial	Los Angeles	Orange	Riverside	San Bernardino	San Diego	**State Total**
Humans	1	1	0	1	0	0	**3**
Horses	0	0	0	0	0	1	**1**
Birds	0	65	3	13	10	5	**96**
Sentinel Chickens	54	0	0	16	0	0	**70**
Mosquito Pools	16	6	0	10	0	0	**32**

endemic area and two were residents of WN endemic states who became ill in California. Extensive testing and case follow-up were initiated on each of these cases to confirm WN infection (Table 7).

Blood banks tested all donations for WN in California and reported positives to CDHS. One blood donor, from Los Angeles County, became clinically ill after donation, although most likely acquired the infection in Colorado.

No cases of WEE and SLE were identified in California in 2003.

EQUINE SURVEILLANCE

Serum and brain tissue specimens from 208 horses displaying neurological signs were submitted to CAHFS and CVEC for arboviral testing.

The first confirmed locally acquired equine WN case in California was reported from San Diego County in a 20 year-old Missouri Fox trotter gelding. The horse was not vaccinated for WN and had not traveled outside California. The horse developed clinical signs on October 17; WN antibody was detected by IgM capture ELISA and PRNT on serum samples. The horse recovered (Table 4).

Two imported equine WN cases were reported in 2003. The first case was a 3 year-old American Quarter horse stallion imported from Toyah, Texas on July 15 that developed neurological signs, including ataxia and facial paralysis, consistent with WN on July 17. Serum antibodies to WN were detected by IgM capture EIA and PRNT, collected from the horse stabled in Alameda County.

The second imported case was a 10 year-old American Quarter horse gelding in Riverside County. The horse resided in Arizona and traveled to California for one day before developing illness on October 15. The horse had two WN vaccinations in 2002 and had received a booster in August 2003. Serum IgM antibody to WN was detected by both capture ELISA and PRNT. The horse was euthanized due to neurologic impairment.

ADULT MOSQUITO SURVEILLANCE

Thirty-five local agencies from 29 counties began to collect mosquitoes using a total of 622 New Jersey Light traps in April 2003 (Table 1). Data from these sources were forwarded to VBDS and collated into the adult mosquito occurrence report (AMOR), distributed weekly from April 3 to November 5.

MOSQUITO TESTING

Forty-two local mosquito control agencies submitted a total of 422,388 mosquitoes (10,297 mosquito pools) to CVEC for virus isolations (Figure 1 and Tables 9-13). This submission rate represents an increase of twice the number of pools submitted from any previous year. Mosquito pools were tested for arboviruses at CVEC by *in situ* enzyme immunoassay using Vero cell culture.

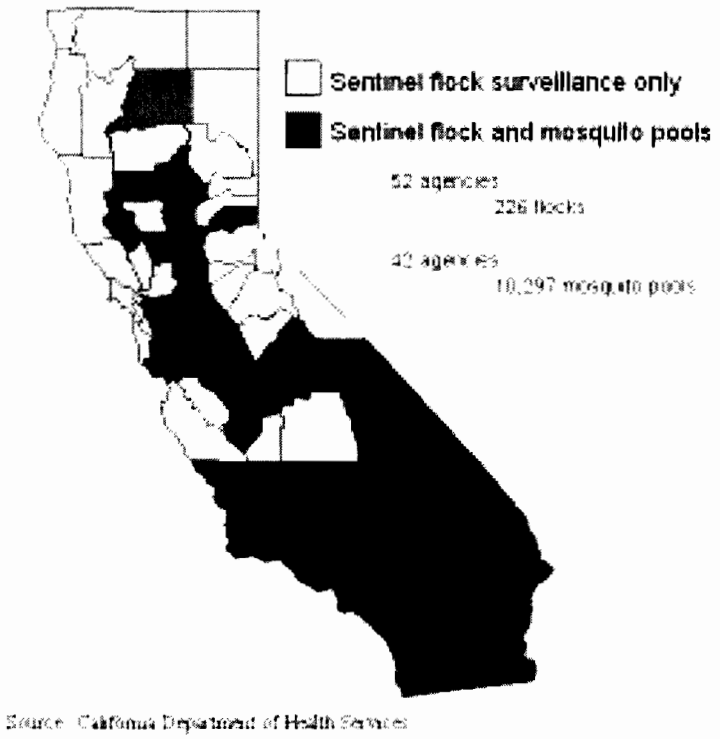


Figure 1. Counties which submitted chicken sera and/or mosquito pools for SLE, WEE, WN, and CE testing, California, 2003.

Table 9. Mosquitoes (*Culex* spp.) tested for WN, WEE, and SLE by submitting county and agency, 2003.

		Cx erythrothorax		*Cx pipiens*		*Cx quinquefasciatus*		*Cx stigmatosoma*		*Cx tarsalis*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Alameda	ALCO	55	2,703	16	696					3	34	74	3,433
Butte	BUCO									4	172	4	172
Contra Costa	CNTR	43	2,150			9	380	1	26	309	15,352	362	17,908
Fresno	CNSL	4	137			20	905			50	2,181	74	3,223
Fresno	FRNO									27	1,139	27	1,139
Fresno	FRWS									38	1,871	38	1,871
Glenn	GLEN									21	1,050	21	1,050
Imperial	IMPR	106	4,973			55	1,521			325	12,315	486	18,809
Inyo	INYO	28	1,066							66	2,632	94	3,698
Kern	AFSB					27	534	2	36	49	1,859	78	2,429
Kern	KERN	8	363			215	6,961			273	10,180	496	17,504
Kern	SFMO	1	22									1	22
Lake	LAKE	8	400					18	775	160	7,736	186	8,911
Los Angeles	GRLA	260	12,431	2	100	998	40,386	26	708	146	5,918	1,432	59,543
Los Angeles	LACW	17	807			50	2,307			11	494	78	3,608
Los Angeles	LONG	2	66			238	10,636	1	15	87	3,365	328	14,082
Los Angeles	SGVA					25	873			12	372	37	1,245
Madera	MADR	3	150	3	137					15	730	21	1,017
Merced	MERC									5	250	5	250
Merced	TRLK	56	2,536	14	572					131	5,682	201	8,790
Orange	ORCO	100	4,384			296	9,236	6	111	134	4,197	536	17,928
Placer	PLCR			3	150					13	427	16	577
Riverside	COAV	128	5,675			276	8,976			1,007	45,042	1,411	59,693
Riverside	NWST	150	7,096	1	50	185	7,357	56	1,720	146	5,470	538	21,693
Riverside	RIVR	29	1,313			10	183	4	60	61	2,270	104	3,826
Sacramento	SAYO	28	1,091	161	5,490			4	96	305	13,272	498	19,949
San Bernardino	SANB	18	701			32	1,017	14	123	46	1,486	110	3,327
San Bernardino	WVAL					85	3,961	1	17	34	1,574	120	5,552
San Diego	SAND	47	2,344							50	2,333	97	4,677
San Joaquin	SJCM			116	3,745	1	12			176	6,863	293	10,620
San Luis Obispo	SLOC	105	5,141	2	100					17	830	124	6,071
Santa Barbara	SBCO	47	1,945			36	1,497	6	136	62	2,738	151	6,316
Santa Clara	STCL			68	3,293					25	1,064	93	4,357
Santa Cruz	SCRZ	31	1,390	17	478					2	33	50	1,901
Shasta	SHAS	5	248	42	2,037					44	1,899	91	4,184
Solano	SAYO			238	9,201			4	64	43	1,239	285	10,504
Stanislaus	TRLK	7	283	19	658			1	41	34	1,191	61	2,173
Sutter	SUYA			11	311					262	13,007	273	13,318
Tulare	DLTA	6	208			6	241			21	739	33	1,188
Ventura	VENT	5	193							34	1,508	39	1,701
Yolo	SAYO			12	263					196	9,306	208	9,569
Yuba	SUYA			14	463					18	678	32	1,141
Total		**1,297**	**59,816**	**739**	**27,744**	**2,564**	**96,983**	**144**	**3,928**	**4,462**	**190,498**	**9,206**	**375,969**

Table 10. Mosquitoes (*Culex spp.*) tested for WN, WEE, and SLE by submitting county and agency, 2003.

		Cx erraticus		*Cx restuans*		*Cx thriambus*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Imperial	IMPR	7	320					7	320
Los Angeles	GRLA			4	149	16	567	20	716
Total		**7**	**320**	**4**	**149**	**16**	**567**	**27**	**1,036**

Table 11. Mosquitoes (*Aedes vexans, Coquillettidia perturbans, Culiseta* spp., *Orthopodomyia signifera*, and *Psorophora columbiae* (tested for WN, WEE, and SLE by submitting county and agency, 2003.

		Ae vexans		*Cq perturbans*		*Cs incidens*		*Cs inornata*		*Cs particeps*		*Or signifera*		*Ps columbiae*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Imperial	IMPR	31	1,010					8	111							39	1,121
Inyo	INYO							6	50	1	5					7	55
Kern	AFSB											1	10			1	10
Kern	KERN									1	29					1	29
Los Angeles	GRLA					108	4,916	32	1,433	10	288					150	6,637
Los Angeles	LACW					101	4,679									101	4,679
Merced	TRLK	1	50													1	50
Orange	ORCO					7	115	2	24	3	37					12	176
Riverside	COAV	30	1,013					11	221					2	91	43	1,325
Riverside	NWST									2	38					2	38
Sacramento	SAYO	41	1,656			20	616	2	41							63	2,313
San Bernardino	SANB	2	50					4	50					3	9	9	109
San Joaquin	SJCM	21	938					1	36	2	31					24	1,005
Santa Barbara	SBCO					2	68	4	109	2	61					8	238
Santa Clara	STCL					1	49	1	13	1	49					3	111
Shasta	SHAS			3	141											3	141
Solano	SAYO					6	81									6	81
Stanislaus	TRLK	16	687													16	687
Yolo	SAYO	4	114			1	7									5	121
Total		**146**	**5,518**	**3**	**141**	**246**	**10,531**	**71**	**2,088**	**22**	**538**	**1**	**10**	**5**	**100**	**494**	**18,926**

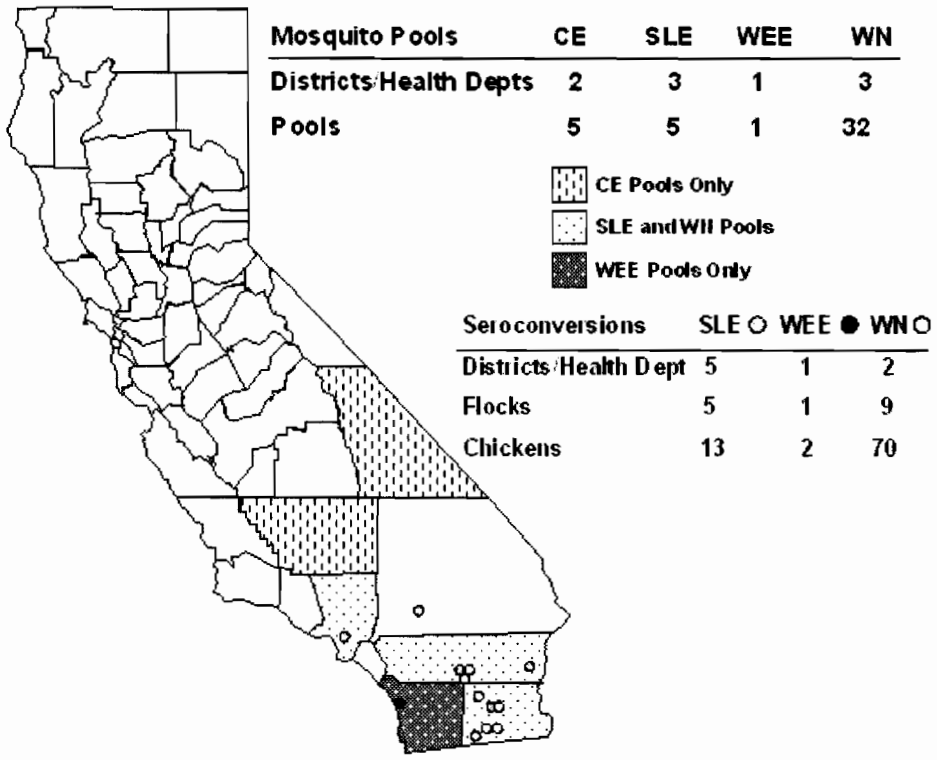
Table 12. Mosquitoes (*Ochlerotatus* spp.) tested for WN, WEE, and SLE by submitting county and agency, 2003.

		Oc dorsalis		*Oc melanimon*		*Oc nigromaculis*		*Oc sierrensis*		*Oc taeniorhynchus*		*Oc washinoi*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Alameda	ALCO	1	50											1	50
Butte	BUCO			6	300									6	300
Contra Costa	CNTR	3	131											3	131
Glenn	GLEN			1	50									1	50
Inyo	INYO			71	3,262									71	3,262
Kern	AFSB			43	2,075									43	2,075
Kern	KERN			59	2,519									59	2,519
Lake	LAKE			16	793									16	793
Los Angeles	GRLA							1	12			7	317	8	329
Merced	TRLK			22	962									22	962
Riverside	COAV	6	154											6	154
Sacramento	SAYO			75	3,121	1	50	3	77					79	3,248
San Joaquin	SJCM			13	395									13	395
San Luis Obispo	SLOC	13	650											13	650
Santa Barbara	SBCO									4	143	43	2,046	47	2,189
Santa Clara	STCL	17	731					1	12			1	50	19	793
Solano	SAYO			1	13									1	13
Sutter	SUYA			1	43									1	43
Yolo	SAYO			15	567			1	13					16	580
Total		**40**	**1,716**	**323**	**14,100**	**1**	**50**	**6**	**114**	**4**	**143**	**51**	**2,413**	**425**	**18,536**

Table 13. Mosquitoes (*Anopheles spp.*) tested for WN, WEE, and SLE by submitting county and agency, 2003.

		An franciscanus		*An freeborni*		*An hermsi*		*An occidentalis*		*An punctipennis*		Total	
County	Agency	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.	pools	mosqs.
Inyo	INYO			3	112							3	112
Kern	KERN					2	95					2	95
Los Angeles	GRLA					49	2,034					49	2,034
Orange	ORCO					23	574					23	574
Riverside	COAV	1	9									1	9
Riverside	NWST					1	22					1	22
Sacramento	SAYO			15	533					2	99	17	632
San Bernardino	SANB	3	19			1	10					4	29
San Diego	SAND					1	7					1	7
Santa Barbara	SBCO	1	10			13	496					14	506
Solano	SAYO			5	51							5	51
Stanislaus	TRLK							1	24			1	24
Yolo	SAYO			24	826							24	826
Total		**5**	**38**	**47**	**1,522**	**90**	**3,238**	**1**	**24**	**2**	**99**	**145**	**4,921**

West Nile virus was first detected in California in a mosquito pool of *Culex tarsalis* collected on July 16 from El Centro, Imperial County. In total, WN was detected in 32 mosquito pools—26 of 4,462 pools of *Culex tarsalis* and 6 of 2,564 pools of *Culex quinquefasciatus* from sites in Los Angeles County (Table 3 and Figures 2 and 3). St. Louis encephalitis was detected in four pools of *Culex tarsalis* from Imperial and Riverside counties and a single pool of *Culex quinquefasciatus* from Los Angeles County in 2003. Five pools of *Ochlerotatus melanimon* collected from Kern (4) and Inyo (1) counties tested positive for the California encephalitis group virus (CE). A single pool of *Culex tarsalis* collected in San Diego was positive for WEE.



Source: California Department of Health Services

Figure 2. Collection site of mosquito pools positive for SLE, WEE, WN, or CE, and location of sentinel chicken flocks with at least one or more seroconversions to SLE, WEE, or WN, California, 2003.

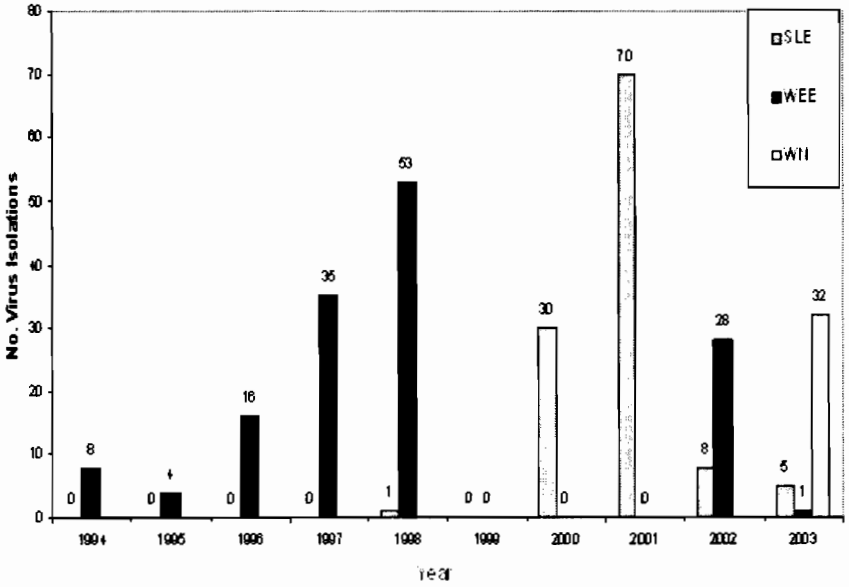


Figure 3. Isolations of WN, SLE, and WEE from pooled mosquitoes in California, 1994-2003.

SENTINEL CHICKEN SURVEILLANCE

Fifty-two local mosquito and vector control agencies in 36 counties maintained 226 sentinel chicken flocks (Figure 1 and Table 5). Blood samples were collected from chickens every other week between April 16 and October 23, 2003, with some local agencies submitting sera samples through December. The VRDL tested 30,798 chicken sera for antibodies to SLE, WN, and WEE by EIA. The Sacramento-Yolo Mosquito and Vector Control District (1,568 samples) and the San Gabriel Valley Mosquito and Vector Control District (1,464 samples) tested their own sentinel chicken flocks for antibodies, representing an additional 3,032 serum samples, all of which tested negative. VRDL also tested eight flocks maintained by New Mexico (6) and Utah (2).

A total of 70 seroconversions to WN were detected among nine flocks from Imperial (54) and Riverside (16) counties (Figures 2 and 4 and Table 5). The first WN seroconversions were provisionally detected by EIA of filter paper strips obtained from six chickens in two Imperial County flocks on August 4. WN was confirmed by PRNT on whole blood collected on August 20.

Seroconversions to SLE were identified in flocks from Imperial (2), Los Angeles (2), Riverside (8) and San Bernardino (1) Counties. The first SLE seroconversion was detected in specimens obtained on September 2 from a flock in Imperial County. The last seroconversions for 2003 were detected in specimens obtained on October 16 from a flock in Riverside County (Figures 2 and 4 and Table 5).

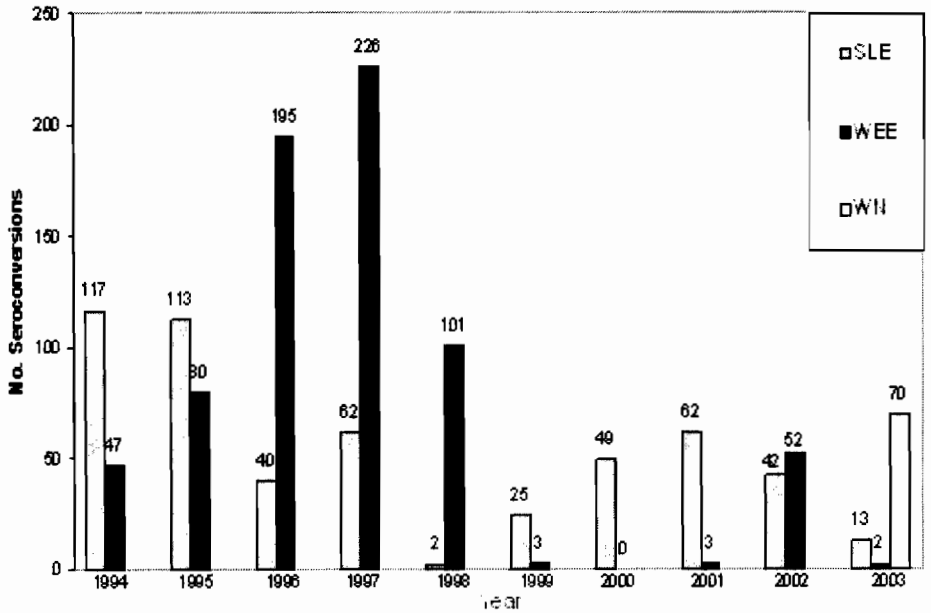


Figure 4. Seroconversions to WN, SLE, and WEE in sentinel chicken flocks in California, 1994-2003.

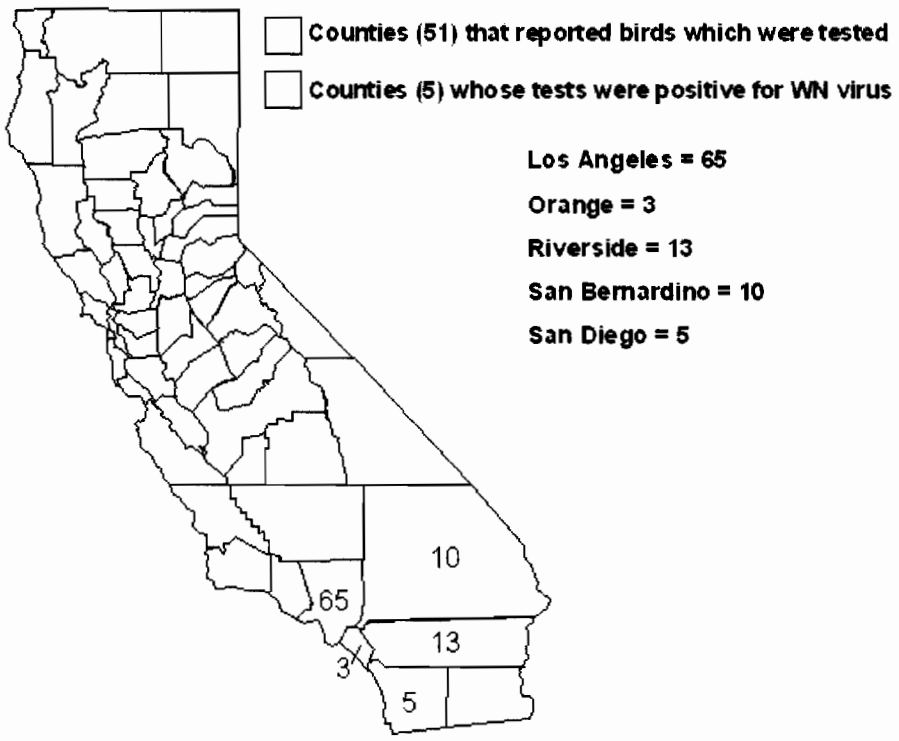
The first WEE seroconversions were detected in specimens obtained on November 4 from two chickens in a sentinel flock in San Diego County (Figure 2 and Table 5).

DEAD BIRD SURVEILLANCE

The CDHS West Nile Virus dead bird surveillance program, a collaborative program with over 130 local agencies and supported by a CDC grant, was established in 2000 and expanded in 2003. The hotline received 3,666 reports of dead birds from 56 counties during 2002; 653 birds from 45 counties were tested for WN. In 2003, 8,650 calls were received from 57 counties reporting dead birds (McCaughey et al. 2003). Of these, 1,765 birds from 51 counties were tested for West Nile virus (Figure 5 and Table 14).

A toll-free hotline (1-877-WNV-BIRD) was created for dead bird reporting by the public in April 2002 (McCaughey et al. 2003). In 2003, a call router was created for the hotline that allowed CDHS to give information in Spanish to callers, record more complete information from callers during non-business hours through the use of a voice form, enable direct calls, and optimize the time of hotline staff. Additionally, presentations by CDHS biologists were given to local agencies and the general public to educate and encourage participation in the dead bird surveillance program. CDHS also published a brochure on West Nile virus for distribution to the general public containing information on the virus, mosquito control, and reporting dead birds. The website complemented the hotline for the purpose of information retrieval and dispersion on dead bird surveillance.

The criteria for WN testing were that the bird must have been dead for less than 24 - 48 hours at the time of the report and it was one of the target species. During the first seven months of 2003, the species selected for WN testing were limited to raptors and corvids: American crow, western Scrub jay, Steller's jay, yellow-billed magpie, and common raven.



Source: California Department of Health Services

Figure 5. State map of dead birds reported and tested for WN by county, 2003

Table 14. Dead birds tested and reported for West Nile virus, 2003.

County	American crow		Common raven		Other species		Total	
	Reported	Tested	Reported	Tested	Reported	Tested	Reported	Tested
Alameda	48	14	6	2	173	15	227	31
Alpine	0	0	0	0	4	0	4	0
Amador	3	0	0	0	23	4	26	4
Butte	33	7	3	1	203	22	239	30
Calaveras	0	0	0	0	15	2	15	2
Colusa	2	1	0	0	9	1	11	2
Contra Costa	60	4	6	3	390	47	456	54
Del Norte	0	0	1	1	2	0	3	1
El Dorado	3	0	0	0	141	13	144	13
Fresno	42	7	5	3	133	11	180	21
Glenn	3	3	0	0	6	1	9	4
Humboldt	6	2	6	3	9	0	21	5
Imperial	2	0	0	0	26	8	28	8
Inyo	14	3	10	2	48	5	72	10
Kern	9	2	11	2	50	9	70	13
Kings	8	2	0	0	12	0	20	2
Lake	7	2	1	0	12	1	20	3
Lassen	1	0	0	0	1	0	2	0
Los Angeles	778	196	43	14	795	135	1616	345
Madera	15	0	0	0	16	1	31	1
Marin	35	9	4	2	60	4	99	15
Mariposa	3	1	0	0	6	0	9	1
Mendocino	9	0	3	2	21	3	33	5
Merced	37	6	1	1	120	14	158	21
Mono	1	0	1	0	9	2	11	2
Monterey	27	9	1	0	26	3	54	12
Napa	8	4	0	0	22	2	30	6
Nevada	4	1	0	0	72	2	76	3
Orange*	223	65	9	4	178	50	410	119
Placer	16	3	1	0	133	16	150	19
Plumas	0	0	0	0	10	0	10	0
Riverside	203	77	6	3	397	106	606	186
Sacramento	163	35	6	1	588	89	757	125
San Benito	2	2	0	0	11	0	13	2
San Bernardino	209	58	18	5	383	58	610	121
San Diego	128	62	10	8	359	187	497	257
San Francisco	7	1	0	0	43	4	50	5
San Joaquin	50	8	3	0	100	13	153	21
San Luis Obispo	24	5	2	1	143	10	169	16
San Mateo	17	6	7	3	125	17	149	26
Santa Barbara	28	8	3	3	49	4	80	15
Santa Clara	57	17	1	1	165	23	223	41
Santa Cruz	3	0	0	0	51	2	54	2
Shasta	10	4	0	0	51	5	61	9
Sierra	0	0	0	0	2	0	2	0
Siskiyou	0	0	1	0	7	0	8	0
Solano	9	0	0	0	82	5	91	5
Sonoma	48	6	3	2	109	10	160	18
Stanislaus	35	8	0	0	129	21	164	29
Sutter	17	5	1	0	51	14	69	19
Tehema	5	0	0	0	12	1	17	1
Trinity	0	0	0	0	7	0	7	0
Tulare	14	2	1	0	38	7	53	9
Tuolumne	0	0	1	0	10	1	11	1
Ventura	42	8	5	3	90	21	137	32
Yolo	90	30	1	0	146	34	237	64
Yuba	5	0	1	1	32	8	38	9
TOTAL	2563	683	182	71	5905	1011	**8650**	**1765**

* Note, Orange County tested with VecTest.

In August of 2003, the target species list was expanded to include finches, sparrows, and blackbirds. The accepted species list was expanded again in September of 2003 for the counties of Imperial, San Diego, San Bernardino, Los Angeles, San Diego, Riverside and Santa Barbara to include all wild birds except pigeons and doves (Figure 6).

Beginning in November, only crows and ravens were accepted for WN testing except in San Diego, San Bernardino, and Riverside counties where they continued to collect all species.

Necropsies of submitted carcasses were performed by CAHFS Central in Davis and San Bernardino. CAHFS Fresno and Turlock still accepted deliveries of dead birds for WN testing and shipped the carcasses to CAHFS Central. Kidney tissues were forwarded to CVEC for testing via RT-PCR. PCR has a sensitivity that can detect virus in birds that have been dead for up to four days. However, in the field the duration of sensitivity was reduced due to the hot temperatures advancing decomposition along with maggot infestations destroying internal organs. Viral isolation was performed by CVEC on tissues that tested positive for WN by PCR.

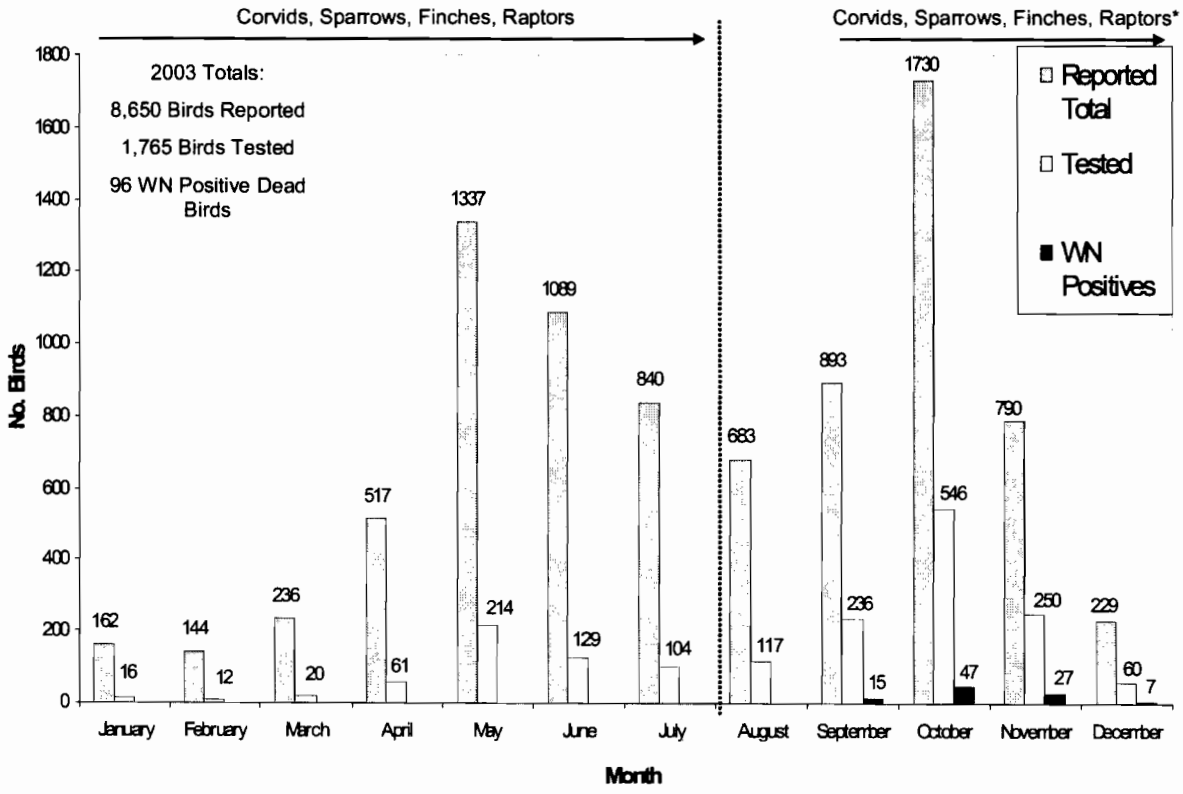
In total, WN was detected in 96 bird carcasses from Los Angeles, Orange, Riverside, San Bernardino, and San Diego Counties (Table 6).

Weekly Arbovirus Surveillance Bulletin and Website

Between April 18 and December 30, CDHS published weekly bulletins that reported results of arbovirus tests from human, equids, mosquitoes, sentinel chickens, and dead birds, as well as updates on national WN activity. These bulletins were distributed to local, state, and federal public health agencies, universities, and other state health departments; the bulletins were also posted on the California WN website (www.westnile.ca.gov). The website also provided WN facts, press releases, maps of WN activity, an on-line dead bird reporting form, and links to related websites. Pictures of birds were added to the website to assist the public to better identify bird species when reporting bird carcasses.

Response Activities to West Nile virus

The *California Mosquito-Borne Virus Surveillance and Response Plan* (CDHS et al. 2003) was revised in 2003 and distributed to all local mosquito control agencies. To provide a semi-quantitative estimate of the virus transmission risk that could be used by local agencies to plan and conduct control activities, independent models were developed to account for the different ecological dynamics of WEE, SLE, and WN transmission in California.



* Southern California counties began testing all birds, except pigeons and doves, after the first WN positives were detected.

Figure 6. Dead birds reported, tested, or positive by month to the CDHS WN Hotline, California, 2003.

An *Operational Plan for Emergency Response to Mosquito-Borne Disease Outbreaks* (CDHS et al. 2003a), written as a supplement to the *Response Plan* by CDHS in collaboration with the Governor's Office of Emergency Services (OES), was completed in 2003 and distributed to all local mosquito control agencies. This document identifies the coordination between DHS and partner agencies in responding to a mosquito-borne disease emergency. It is consistent with the CDHS Emergency Plan, Departmental Administrative Order, and the State Emergency Plan. This document expands on the roles of the agencies mentioned in the *Response Plan* and provides the policy basis for mosquito-borne disease outbreak planning, response, recovery, and mitigation actions. The document includes the following information: (1) Description of how CDHS and federal, state, and local agencies function together in a coordinated escalating emergency response, (2) The progression from normal to emergency operations; and (3) The emergency management structure (Standardized Emergency Management System [SEMS] organization chart for CDHS response), notification system, responsibilities for the various agencies involved in the response, and anticipated agency roles at each jurisdictional (federal, state, local) level.

In response to the transmission of WN in 6 counties in southern California, CDHS participated in the formation of WN Task Forces, composed of representatives from such agencies as the County Health and Environmental Health Departments, County OES, County Agricultural Commissioners, county law enforcement, and vector control agencies. These Task Forces, using the *Response Plan* as a guide, developed WN plans and coordinated surveillance, response, education, and communication.

West Nile virus in the United States

By the end of 2003, WN activity had been identified in 45 states and the District of Columbia (http://www.cdc.gov/ncidod/dvbid/westnile/). The WN epidemic and epizootic resulted in 9,862 reported human cases of WN disease and 264 deaths. Significant human disease activity was recorded from these following states: Colorado (2,947), Nebraska (1,942), South Dakota (1,039), and Texas (720). The 2003 WN epidemic was the largest recognized arboviral meningoencephalitis epidemic in the Western Hemisphere and the largest WN meningoencephalitis epidemic ever recorded (CDC, 2003)[2].

Moreover, 11,115 WN positive dead birds had been reported from 42 states, the District of Columbia, and New York City. Horse infections numbered 4,084 from 41 states. Finally, WN seroconversions in 1,377 sentinel chicken flocks from 15 states, and a total of 7,602 WN-positive mosquito pools were reported from 38 states, the District of Columbia, and New York City.

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Special thanks to the Mosquito and Vector Control Association of California and other participating agencies for financial support of laboratory testing and submissions of samples.

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Mosquito and Arbovirus Surveillance in Northwest Mosquito and Vector Control District in 2003

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ABSTRACT: The mosquito and arbovirus surveillance program at Northwest Mosquito and Vector Control District (WMVCD) includes the standard weekly mosquito trapping, collection of dead birds and biweekly testing of sentinel chicken flocks for St. Louis (SLE), western equine (WEE), California (CE) and West Nile (WN) encephalitis viruses. We also trap and test wild birds for the SLE, WEE and WN viruses. In 2003, we collected 62,703 mosquitoes and tested 520 mosquito pools. We trapped and tested 398 wild birds and collected 60 dead crows, sparrows, finches and other bird species. None of the mosquito pools or blood samples from sentinel chickens tested positive for any of the arboviruses. However, one live house finch tested positive for the antibody to the WN virus (WNV) and 4 dead birds (three crows and one house finch) were positive for the WNV.

INTRODUCTION

A multifaceted mosquito and encephalitis virus surveillance (EVS) program has been conducted by the Northwest Mosquito and Vector Control District (NWMVCD) since its inception in 1959. In 2003, the program included mosquito collections with New Jersey-style light traps (NJLT), carbon dioxide-baited EVS traps and gravid mosquito traps and incorporated testing of mosquito pools, sentinel chickens, wild birds and bird carcasses collected throughout the District to test for SLE, WEE, CE and WN viruses.

The NWMVCD encompasses approximately 240 square miles and services close to 400,000 residents. The District's service area includes the cities of Norco, Corona, Lake Elsinore, parts of the city of Riverside and several adjoining unincorporated communities. Disease and vector surveillance programs are part of the District's coordinated effort to best serve the community by detecting and controlling vector-borne diseases in our area.

MATERIALS AND METHODS

New Jersey-Style Light Traps

The population dynamics of adult mosquitoes were monitored with NJLT (Mulhern 1942) 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). The traps were equipped with 25-watt incandescent light bulbs (235 lumens) and placed approximately 2.4 m above ground level. The mosquitoes trapped were counted and sorted according to sex and species with a report submitted to the California Department of Health Services to be included in the state-wide adult mosquito occurrence report.

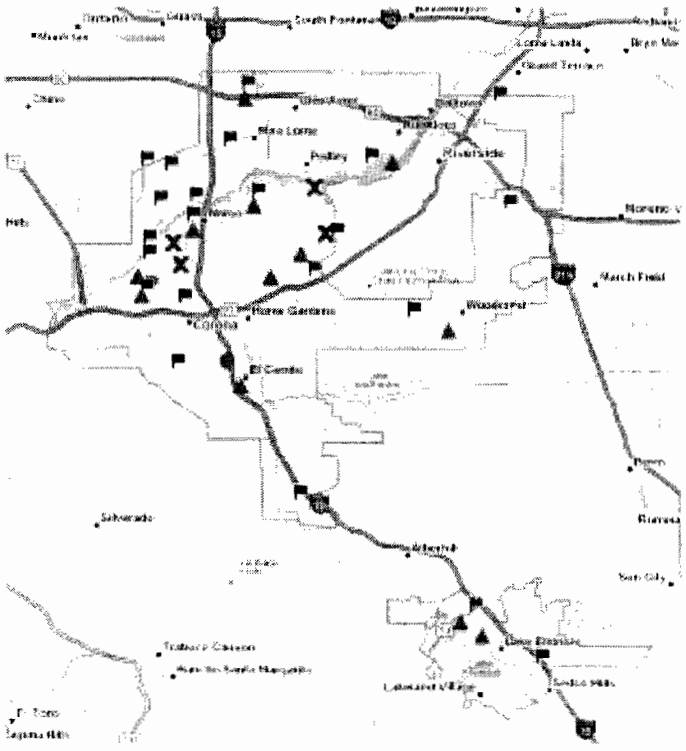

Figure 1. Distribution of NJLTs (triangles), EVS traps (flags) and gravid traps (crosses) within the boundaries of the NWMVCD (dark gray polygons) in 2003.

Encephalitis Virus Surveillance Traps

Host-seeking female mosquitoes were monitored using carbon dioxide-baited EVS traps 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 18 cm above trap entry.

A total of 25 fixed trap locations were selected to best monitor mosquito-infested areas within the five service zones at the District (Fig. 1). The traps were operated weekly from dusk to dawn. The mosquitoes collected were anesthetized with triethylamine (TEA) and sorted by species and sex. Pools of 12 to 50 mosquitoes were then shipped on dry ice overnight to the University of California Davis Arbovirus Research Unit (DARU) for testing. Female *Culex erythrothorax* Dyar, *Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar and *Culex tarsalis* Coquillett were included.

Gravid Traps

In 2003, gravid female mosquitoes were collected at four locations (Fig. 1), two above ground and two in underground storm drains. Traps designed by Reiter (1987) and modified by Cummings (1992) were baited with alfalfa infusion (Reiter 1983) and operated weekly overnight. The above-ground traps were used from August through December and the underground ones from September through December. The gravid mosquitoes collected were anasthetized with TEA and sorted by species. Pools of *Cx. quinquefasciatus* and *Cx. stigmatosoma* were submitted to DARU for arbovirus testing (as described above).

Sentinel Chicken Flocks

Six sentinel chicken flocks of ten white leghorn birds each were maintained from April through December at different locations throughout the District (Fig. 2). Blood samples were collected biweekly from the wing vein. The samples were placed on filter-paper strips, air dried and submitted to DARU for testing.

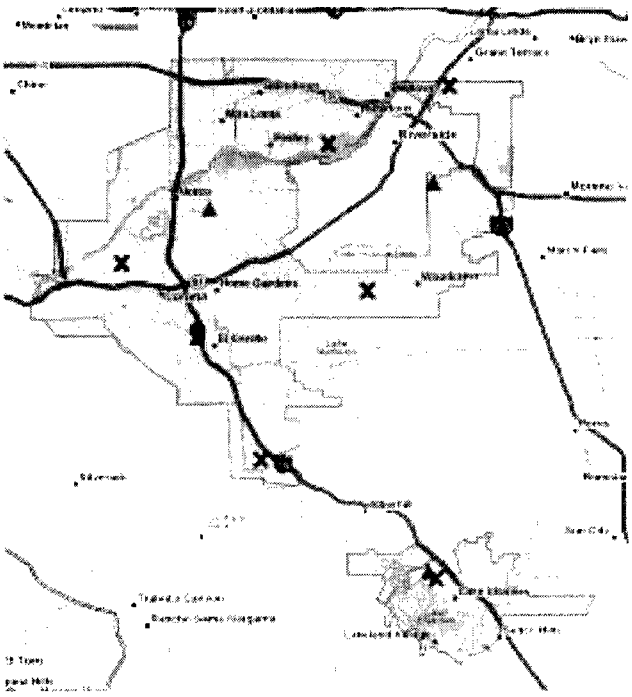

Figure 2. Distribution of sentinel chicken flocks (triangles) and wild bird traps (crosses) within the boundaries of NWMVCD (dark gray polygons) in 2003.

Wild Birds

Beginning in April 2004, four modified Australian crow traps (McClure 1984) were built and set up in Corona, Norco, Canyon Crest and Lake Elsinore (Fig. 2). The traps were baited with wild bird seed (Golden State Commodities, Oakdale, CA) and water to attract house finches (*Carpodacus mexicanus*) and house sparrows (*Passer domesticus*). They were checked twice a week for birds. The birds 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*) obtained from modified Australian crow traps operated by the Least Bells Vireo Conservation Project of the Santa Ana Watershed Association. 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 inhibiton as described by Gruwell at al. (2000). The samples were also tested for antibodies specific to the West Nile virus by a blocking ELISA developed by Jozan et al. (2003).

Dead Birds

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.

Data Analysis

Mosquito abundance data were blocked by month and analyzed using repeated measures ANOVA with the collection month as the main effect. Abundance measurements were repeated within each trap location. Student-Newman-Keuls method was utilized for multiple comparisons of means. Species composition data for each trap type were analyzed using Kruskal-Wallis one way ANOVA with Dunn's pairwise multiple comparisons procedure.

RESULTS

Mosquito Surveillance

In 2003, a total of 4,766 female mosquitoes were colleced in NJLTs. *Culex quinquefasciatus* dominated the trap catch ($p < 0.05$), followed by *Cx. tarsalis, Cx. stigmatosoma* and *Cx. erythrothorax* (Fig. 3). Other species collected included *Culiseta inornata* Williston, *Culiseta particeps* (Adams), *Culiseta incidens* (Thomson), *Anopheles hermsi* Barr & Guptavanji, *Anopheles franciscanus* McCracken and *Ochlerotatus washinoi* Lanzaro & Eldridge. Rural habitats produced most mosquitoes ($p < 0.05$) (Fig.

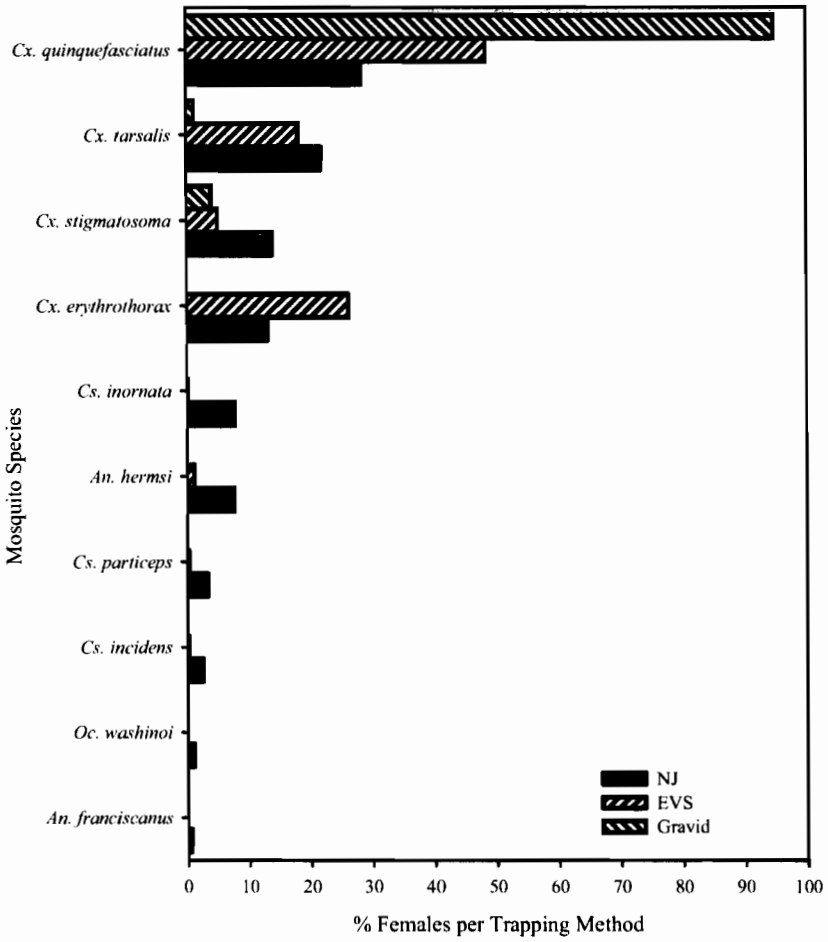

Figure 3. A comparison of species composition of female mosquitoes collected in NJLTs (black bars), EVS traps (light gray bars) and gravid traps (dark gray bars) in 2003.

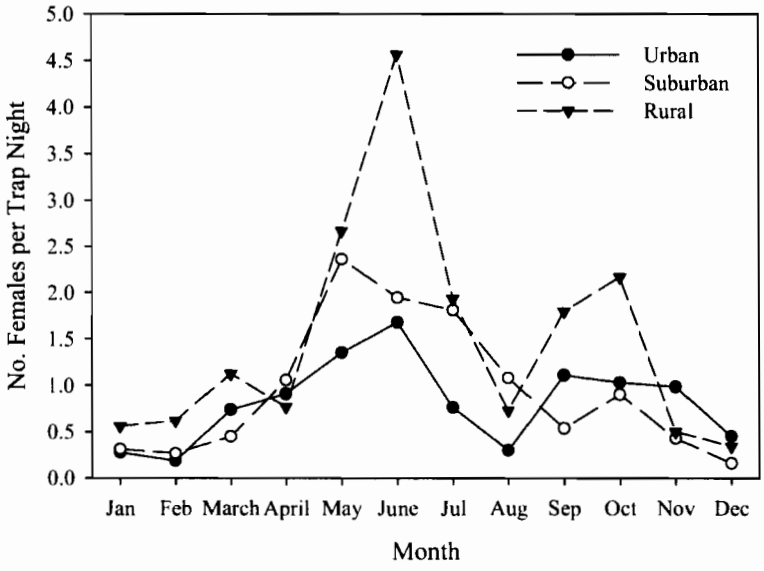

Figure 4. Relative numbers of female mosquitoes collected in NJLTs in urban (dark circles), suburban (clear circles) and rural (dark triangles) habitats.

4). Mosquito abundance in suburban and urban areas was not significantly different. Numerically, mosquito numbers captured in NJLTs in 2003 were consistently lower than the 5, 10 and 15-year averages (Fig. 5). In NJLTs, all four *Culex* species peaked in April through August and there was a second peak in September through October for *Cx. tarsalis* and *Cx. quinquefasciatus* (Fig. 6A).

The CO_2-baited traps yielded 57,050 mosquitoes. As in the NJLTs, *Cx. quinquefasciatus* was the most abundant species ($p < 0.05$) followed by *Cx. erythrothorax, Cx. tarsalis* and *Cx. stigmatosoma* (Fig. 3). *Culex erythrothorax* were most abundant in May while the numbers of *Cx. quinquefasciatus* peaked in July (Fig. 6B). The highest numbers of *Cx. tarsalis* were collected in September.

The total number of mosquitoes collected in the gravid traps was 887 with the majority (841) being *Cx. quinquefasciatus* ($p < 0.05$) followed by *Cx. stigmatosoma* and *Cx. tarsalis* (Fig. 3). The overall catch for all gravid traps combined decreased from August through November (Fig. 6C). When considered separately, the above-ground traps produced more mosquitoes (mean number of females per trap night = 32) in August and September while an average of 14 mosquitoes per trap night were consistently collected from underground sources from September through December.

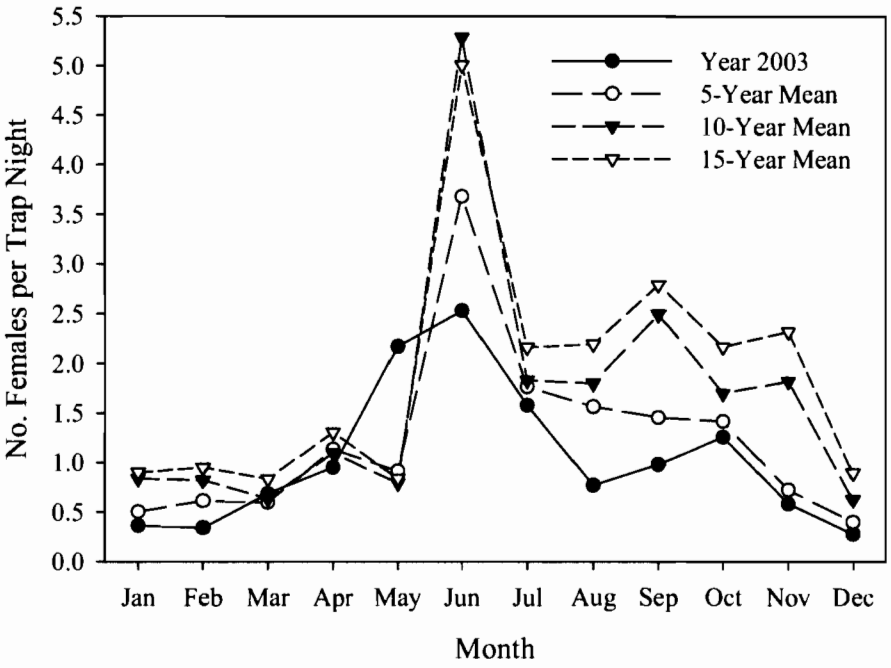

Figure 5. A comparison of the mean numbers of female mosquitoes collected in NJLTs in 2003 to the 5 (clear circles), 10 (dark triangles) and 15 (clear triangles) year averages.

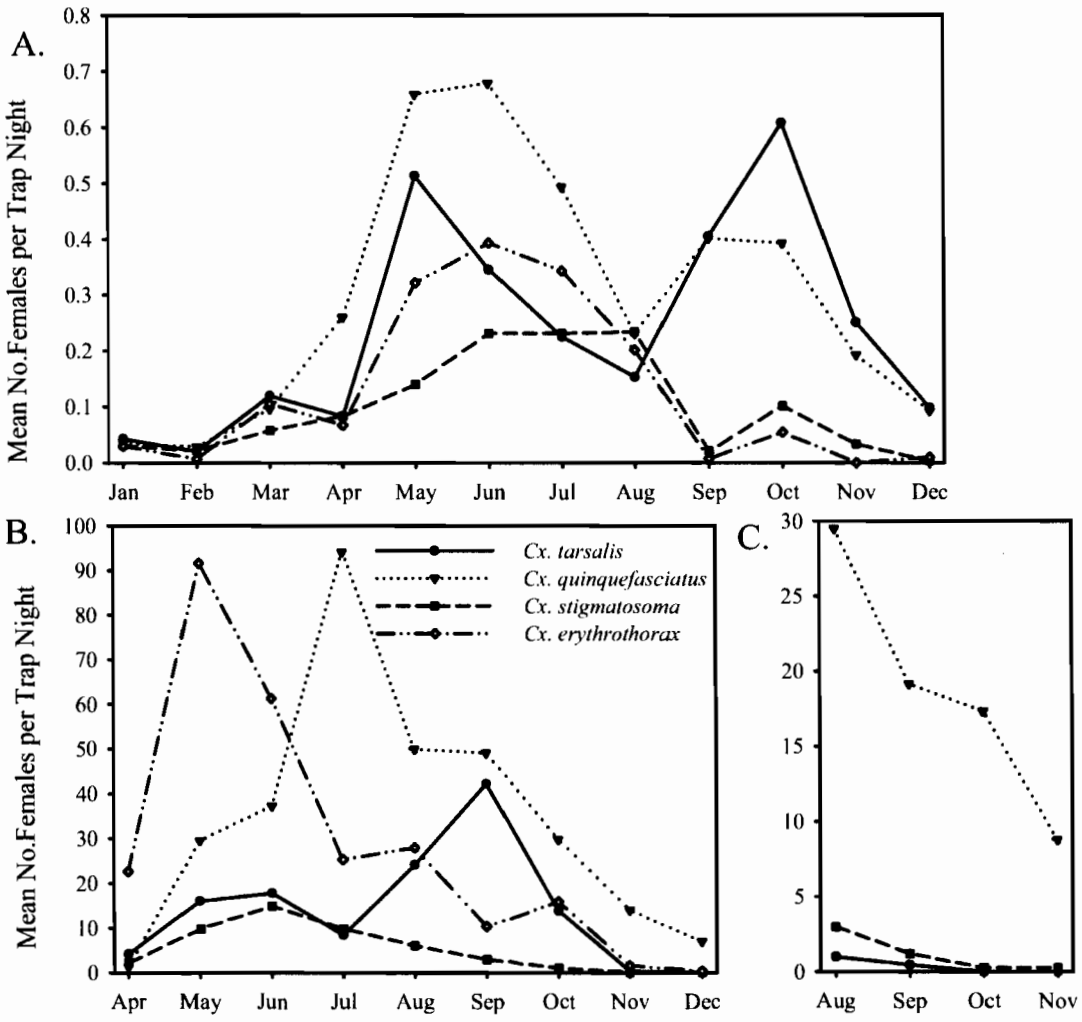

Figure 6. Relative abundance of the four *Culex* species collected throughout 2003 in NJLTs (A), EVS traps (B) and gravid traps (C).

Arbovirus Surveillance

From the EVS traps, a total of 520 mosquito pools were submitted to DARU for testing. This included 135 pools of *Cx. tarsalis,* 186 pools of *Cx. quinquefasciatus,* 134 pools of *Cx. erythrothorax* and 65 pools of *Cx. stigmatosoma.* From the gravid traps, 18 pools of *Cx. quinquefasciatus* were processed for testing. None were positive for any of the arboviruses.

None of the blood samples from the sentinel chickens tested positive for any of the arboviruses. Out of a total of 355 live wild birds tested (Table 1), one adult male house finch was positive for the WN virus antibody. Upon its first capture in the Canyon Crest trap in the City of Riverside on Sep. 22nd the blocking ELISA test showed 60% inhibition of color development (the bird was 60% positive for the WNV antibody). On Oct. 3rd the house finch was recaptured and subsequent blocking ELISA test was 54% positive.

Table 1. Birds collected in wild bird traps from April through December 2003 (for single recaptures, the same birds trapped multiple times were counted only once; they were counted each time in the multiple recaptures.)

	Number of Birds Bled										
Bird Species	**April**	**May**	**June**	**July**	**August**	**Sept.**	**Oct.**	**Dec.**	**Total**	**No. Recap. (single)**	**No. Recap. (multiple)**
House finch	0	47	38	30	68	10	12	3	**208**	**28**	**75**
House sparrow	0	31	22	10	2	0	0	14	**79**	**5**	**10**
Brown-headed cowbird	12	12	3	10	0	0	23	0	**60**	**N/A**	**N/A**
California towhee	1	0	4	0	0	0	0	0	**5**	**0**	**0**
Spotted towhee	0	0	0	1	0	0	0	0	**1**	**0**	**0**
Red-winged blackbird	0	2	0	0	0	0	0	0	**2**	**0**	**0**
Total	**13**	**92**	**67**	**51**	**70**	**10**	**35**	**17**	**355**	**9**	**24**

During 2003, 53 dead birds were submitted to the CAHFS laboratory. Of these, three American crows (one collected Oct. 20th and two Nov 3rd) and a house finch (collected Oct. 22nd) tested positive for WNV. All four birds were found in the portion of the City of Riverside served by the District.

DISCUSSION

Mosquito Surveillance

While *Cx. quinquefasciatus* dominated trap catch for all three mosquito trap types, for the remaining mosquito species different trapping methods produced different species composition patterns and drastically different abundances (Figs 3 and 6). The number of *Cx. quinquefasciatus* collected in the EVS traps was highest in July while it was highest in May and June in the NJLTs. *Culex. erythrothorax* abundance peaked in May in the EVS traps while it did so in May through July in the NJLTs. *Culex tarsalis* were most abundant later in the season as indicated by both NJLT and EVS trap catch, while the numbers of *Cx. stigmatosoma* remained low throughout the year even though they did rise slightly during the summer.

As expected, EVS and gravid traps proved more effective in trapping *Culex* mosquitoes than NJLTs (Fig. 6, A-C). This drastic difference may be attributed to trap placement, with the EVS and gravid traps being positioned in more mosquito-infested areas since they are more easily deployed due to their light weight and independence from AC power sources. The highest catch in the EVS and gravid traps may also be due to *Cx. quinquefasciatus* being the dominant mosquito species collected. Populations of *Culex quinquefasciatus* are typically underrepresented in light trap catch (Barr et al. 1960). An additional factor contributing to the low NJLT count may be increased background illumination from other light sources especially in the urban and suburban trapping areas (Milby and Reeves 1989). The highest mosquito numbers collected in the rural areas for NJLTs (Fig. 4) and the lowest number of mosquitoes collected in NJLTs in 2003 as compared to the 5, 10 and 15-year averages (Fig. 5) supports this idea. As the urban and suburban trapping sites within the District become more urbanized over time, background illumination increases resulting in an increased number of competing light sources and lower numbers of mosquitoes being trapped in NJLTs.

The mean number of mosquitoes captured per trap night in the EVS traps was also higher than in the gravid traps. However, gravid traps captured mostly *Cx. quinquefasciatus* as would be expected due to the fermented alfalfa infusion being used as an attractant (Reisen and Meyer 1990) and *Cx. quinquefasciatus* being the dominant mosquito species in our District (Fig. 2) and the most prevalent species collected in underground storm drains in southern California (Su at al. 2003). The gravid traps were only deployed from August through December. During those months, populations of adult *Cx. quinquefasciatus* (as indicated by the EVS and NJLT catch) were already declining (Fig. 6 C).

Arbovirus Surveillance

Not surprisingly, collection and testing of dead birds was the quickest and most effective way of detecting WNV in the area. Trapping and testing live wild birds produced one WNVpositive house finch out of 355 birds trapped, indicating that this method might hold promise for WN surveillance. Even though none of the mosquito pools or blood samples from sentinel chickens tested positive for WNV testing sentinel chickens and mosquito pools for arboviruses still remain the methods of choice for SLE, WEE and

CE surveillance as well as for further detection of WNV activity in the District service area.

Acknowledgements

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Surveillance for Rodent-borne Pathogens in Northwestern Riverside County in 2003

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ABSTRACT: As part of the disease surveillance, rodent trapping was carried out at 17 sites in northwestern Riverside County in 2003. Of 392 rodents trapped, 139 (35%) were *Peromyscus maniculatus*. In overnight traps at 12 sites, seven had hantavirus-positive rodents and one had two arenavirus-positive *P. maniculatus*. Hantavirus positive species included *P. maniculatus* (23), *Peromyscus eremicus*, *Peromyscus californicus*, *Microtus californicus* and *Neotoma lepida*. *Peromyscus eremicus* showed the highest rate of infection (20.8%). In rodent plague surveys, sera from 57 *Spermophilus beecheyi* tested negative for the plague antibody. The plague antibody was also not detected in any of the rats, mice or voles collected.

INTRODUCTION

The Northwest Mosquito and Vector Control District (NWMVCD) provides vector control services to 400,000 residents within an area of approximately 240 square miles that includes the cities of Norco, Corona, Lake Elsinore, parts of the city of Riverside and several adjoining unincorporated communities (Fig. 1). The disease and vector surveillance program is part of the District's coordinated effort to best service the community by detecting and controlling vector-borne diseases in the area. Surveillance for hantaviruses and plague has been carried out at the NWMVCD for over a decade whereby rodents have been trapped throughout the District service area and their blood samples tested. In 2003, serological testing for arenaviruses was added to the program.

Hantaviruses are responsible for two types of human diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). These viruses are most commonly transmitted to humans through aerosolization of rodent excreta, but secondary aerosols, mucous membrane contact, and skin breaches are also a consideration. Nearly 366 cases of HPS, a severe disease with 37% mortality, have been reported in North America while HFRS has proved to be very uncommon. A great number of New World rodent species, including deermice, *P. maniculatus*, wood rats, *Neotoma spp.*, voles, *Microtus spp.* and *Clethrionomys spp.*, and rats, *Rattus rattus* and *Rattus norvegicus*, have been found to have antibodies to hantaviruses. Sin Nombre virus, mostly carried by *P. maniculatus*, is responsible for the human HPS cases in the western United States. Other hantaviruses may also be vectored by other rodent species. These include the El Moro Canyon virus carried by the western harvest mouse, *Reithrodontomys megalotis* and the Isla Vista virus vectored by the California vole, *Microtus californicus* (Bennett et al. 1999).

Arenaviruses are associated with human disease worldwide. As with hantaviruses, arenavirus infections in humans may result from inhalation of aerosols of rodent excreta or from direct contact of rodent excreta with open skin and mucous membranes. Person

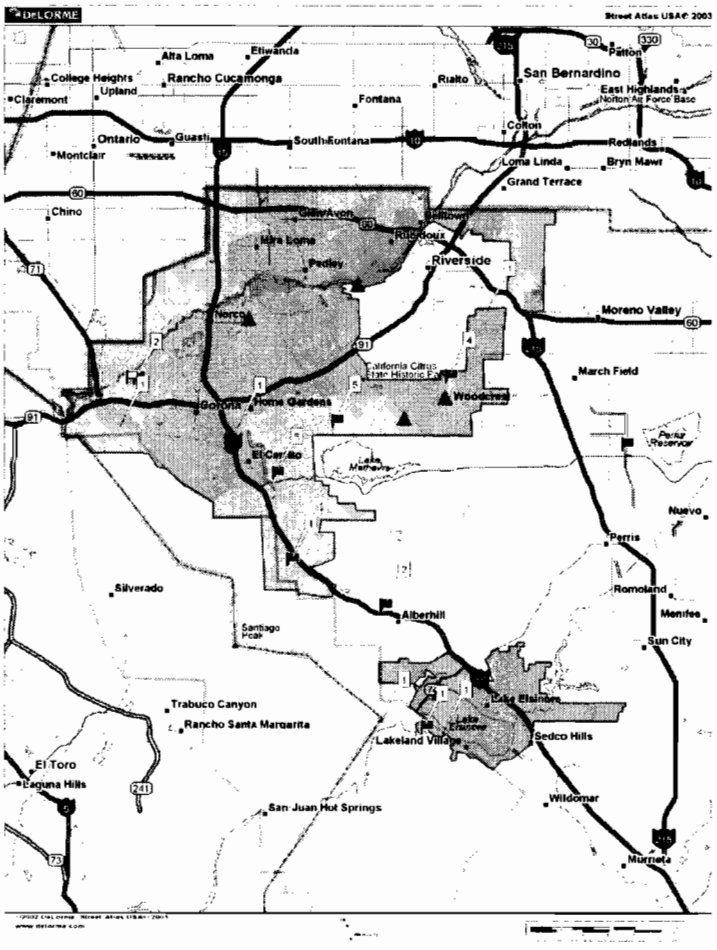

Figure 1. Map of rodent trap sites showing white tags for rats and mice and dark triangles for ground squirrels within the NWMVCD service areas (dark grey polygons). Numbers within the tags indicate trap nights for each site sampled in 2003. Dark flags indicate hantavirus-positive sites and the light grey flag marks the arenavirus-positive site.

to person transmission may occur upon direct contact with infective fluids and contaminated materials, such as medical equipment. Ingestion of contaminated foods with rodent excreta may also result in an infection. Arenaviruses known to occur in North America include Lymphocytic Choriomeningitis (LCM), White Water Arroyo (WWA) and Tamiami (TAM) viruses (Childs and Peters 1993). The LCMV, vectored principally by the house mouse, *Mus musculus*, causes meningitis, encephalitis, or both. The disease is usually not fatal but there are no specific treatments. Wood rats in the southwestern United States are the principal hosts of WWA virus which is an agent of hemorrhagic fever in humans. The TAM virus transmitted by the hispid cotton rat, *Sigmodon hispidus*, in Florida causes Tamiami virus encephalitis.

In southern California, antibodies against Pichinde (PIC) and TAM viruses have been found in dusky-footed wood rats, *N. fuscipes* and desert wood rats (Kosoy et al. 1996). Bennett et al. (2000) found antibodies to the Amapari (AMA) and/or WWA viruses in desert wood rats, dusky-footed wood rats, brush mice (*Peromyscus boylii*), California mice (*Peromyscus californicus)*, deermice, cactus mice and harvest mice collected in the Los Angeles, Orange and Northwestern San Diego Counties. Later, Fulhorst et al. (2002) isolated a new arenavirus, Bear Canyon virus that belongs to the Tacaribe serocomplex. It was found in *P. californicus* collected in the Cleveland National Forest close to the Orange County and Riverside County line.

Plague was introduced to North America in 1900, when Norway rats carrying plague-infected fleas escaped from a ship from Hong Kong docked in San Francisco. Since then, eighteen rodent species in California have been implicated in the epidemiological cycle of plague. The causative agent of plague, the bacterium *Yersinia pestis*, is maintained in wild rodents and other small mammals and transmitted within and among species by their fleas. The host species of plague include relatively resistant enzootic (maintenance) hosts and the susceptible epizootic (amplification) hosts. The enzootic hosts include *Peromyscus spp.* and voles. *Peromyscus maniculatus* and *M. californicus* are most significant in this respect (Davis et al. 2002). The epizootic host species include California ground squirrels, *Spermophilus beecheyi*, wood rats, *Neotoma* spp., and chipmunks, *Tamias* spp.. The ground squirrels and their fleas are most often associated with human plague cases in California (Nelson 1980).

Based on routine disease surveillance activities, this paper presents data on rodent-borne pathogens at various sites in the northwestern Riverside County during 2003.

MATERIALS AND METHODS

Small rodents including rats, mice and voles were trapped at 12 locations throughout the Northwestern Riverside County (Fig. 1). Based on previous rodent surveillance studies at NWMVCD (unpublished data), locations with the highest trap success for *Peromyscus spp.* are open fields containing some human refuse and scattered vegetation. For the present study, sites were selected based on these criteria. Locations were selected within each of the five service zones of the NWMVCD. Additionally, California ground squirrels were trapped for plague surveillance at 5 locations throughout the District (Fig. 1). Ground squirrel trapping locations were selected based on field observations of high squirrel activity by field technicians within each of the five District service zones. On each sampling occasion 40 traps were set in stations.

In overnight surveys, Sherman traps (7.6 x 8.9 x 22.9 cm) were used at different locations throughout the year (Fig. 1). Each trap was baited with 3 g of rolled oats. Squirrels were trapped in Tomahawk live traps (12.5x12.5x40 cm, Tomahawk, WI) baited with peanut butter and rolled oats mixed together to form 27 g balls (dia. 3.5 cm). The Tomahawk traps were set throughout the year in the midmorning and collected on the same day in the early afternoon.

All rodents were euthanized with carbon dioxide within hours after trap collection. The cardiac puncture technique was used to collect blood samples. For hantavirus antibody testing, whole blood samples collected from rats, mice and voles were shipped overnight to the California Department of Health Services - Vector-Borne Disease Section (CDHS-VBDS) Laboratory. To test for arenaviruses, blood serum was separated through centrifugation at 4500 rpm for 20 min and stored at -70°C until enough samples were accumulated for shipment. The samples were shipped on dry ice to the University of Texas Medical Branch (UTMB), Department of Pathology for analysis. Whole blood samples adsorbed onto Nobuto filter strips were shipped to CDHS-VBDS for plague antibody detection.

RESULTS AND DISCUSSION

A total of 335 rodents were collected over 920 trap nights at the12 surveillance sites throughout the year (Table 1). Seven out of the 12 sites had hantavirus positive rodents (Fig. 1). Most of the positive sites were resampled at least once (Fig. 1). A total of 57 ground squirrels were collected at 9 different sites.

Of the 335 rodents collected, 39 (11.6%) tested positive for hantavirus and two for arenavirus. Most rodents collected were *P. maniculatus* followed by *N. lepida, Chaetodipus californicus, P. californicus, P. eremicus, N. fuscipes, M. californicus and M. musculus* (Table 1). All except *C. californicus*, *M. musculus* and *N. fuscipes* had some individuals that were positive for the hantavirus antibody. Two *P. maniculatus* were positive for the arenavirus antibody. Surprisingly, *P. eremicus* had the highest rate of hantavirus infection (20.8 %) and not *P. maniculatus* (16.5%) but the small sample size for *P. eremicus* may have influenced this result. Additionally, two *M. californicus* found positive for hantavirus antibody were most likely infected with the Isla Vista virus, characteristic to this species.

All 57 *S. beecheyi* as well as noctural rodents (rats, mice and voles) tested negative for the plague antibody.

The above data show the presence of rodent-borne pathogens and the associated potential risk of exposure. Although we did not find plague activity in the rodent samples tested in our area, plague enzootics in ground squirrels have been reported in the adjoining areas of Riverside County from time to time (Dr. J.C. Hitchcock, personal communication). Evidently, we need to expand our rodent

Table 1. Data on the surveillance for rodent-borne viruses in northwestern Riverside County in 2003.

Rodent species	# collected	#/trap-night	#(%) AV*	#(%) HV**
Chaetodipus californicus	48	0.05	0	0
Microtus californicus	4	0.00	0	2(50)
Mus musculus	2	0.00	N/A	0
Neotoma fuscipes	22	0.02	0	0
Neotoma lepida	58	0.06	0	6(10.3)
Peromyscus californicus	38	0.04	0	3(7.9)
Peromyscus eremicus	24	0.03	0	5(20.8)
Peromyscus maniculatus	139	0.15	2(1.4)	23(16.5)
Total	**335**	**0.35**	**2(<0.1)**	**39(11.6)**

*AV—Arenavirus
**HV—Hantavirus

surveys to new areas beyond our existing sampling sites. We also plan to continue our collaboration with Dr. Charles Fulhorst at UTMB to further investigate the arenaviruses found in local rodent populations within the District's territory.

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Preventive Measures for Reducing the Risk of Tick-Borne Relapsing Fever in Mono and Inyo Counties, California

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ABSTRACT: There were 5 reported and 12 suspect tick-borne relapsing fever (TBRF) cases reported in Mono and Inyo Counties during the period 2000-2002. These cases are thought to be associated with 6 putative exposure sites within these 2 counties. Individuals responsible for each of the sites of case exposure were contacted in July and August 2003, and we documented the preventive measures that were implemented after the occurrence of illnesses. At 4 of the 6 sites, concerted efforts were taken to reduce the risk of TBRF. One site strictly concentrated on renovation and rodent proofing. At the other 3 sites, actions included one or more of the following: prohibiting use of structures, destroying or rodent proofing structures, rodent control, and insecticidal/acaricidal applications. Rodent proofing structures is an intrinsic part of TBRF prevention, and the preventive measures taken at each of the 4 sites should effectively lower the risk of TBRF at the sites.

INTRODUCTION

Tick-borne relapsing fever (TBRF) is a reportable disease in California caused by the spirochetal bacterium, *Borrelia hermsii* (Davis). In the western United States at elevations above 1500 m, *B. hermsii* is transmitted by the argasid or soft tick, *Ornithodoros hermsi* Wheeler, Herms and Meyer (Herms and Wheeler 1936). Historically in California, the majority of TBRF cases have consistently occurred in the vicinity of Big Bear Lake (San Bernardino County) and Lake Tahoe (Placer and the surrounding counties), although scattered cases have been reported from 20 of the 58 counties in the state. Relative to these two apparent disease foci, the risk of contracting TBRF in Mono and Inyo Counties appears to be relatively low. A historical review of unpublished reports showed that only 4 of 140 reported cases of TBRF between 1921-1935 and 1951-1965 were from Mono County, and none from Inyo County. From 1991-2000, 88 cases were reported of which 10 were from Mono and Inyo Counties (State of California Department of Public Health 1936, California Department of Health Services [CDHS] 2001, CDHS unpublished data).

There were 5 reported and 12 suspect tick-borne relapsing fever (TBRF) cases reported from Mono and Inyo Counties between 2000-2002 (CDHS unpublished data). Each of these cases was thought to be associated with one of 6 putative exposure sites within these 2 counties, and Walker et al. (2003) provided a detailed description of these sites. Individuals responsible for each of the putative exposure sites were contacted during July and August of 2003. Queries included the following questions: (1) Has anyone stayed at the sites after the initial TBRF illness or TBRF-like illness occurred?; (2) Has anyone contracted a febrile illness after staying at the site after the initial illness?; (3) Have actions been taken to prevent rodent access to the structure?; (4) Have efforts been taken to reduce potential or existing rodent habitats proximal to the structure?; and (5) Have tick or rodent control been conducted. Based on responses, concerted efforts were taken to reduce the risk of TBRF at 4 of the 6 sites. Summaries of site activities are given below.

MONO COUNTY

Site #1: Crestview Fire Station.

This site, located on the grounds of an Inyo National Forest Fire Station, United States Forest Service (USFS), includes 2 putative exposure structures: (1) a barracks building (Bldg. 1343) and (2) a cabin (Bldg. 1120). Prevention measures against TBRF were initiated in August 2001 and included: (1) prohibiting individuals from staying overnight in Bldg. 1343 and Bldg. 1120; (2) contracting a commercial pest control company to administer rodenticides to the crawl spaces under the buildings; (3) control of rodent ectoparasites with bait stations containing 2% diazinon dust (Gold Crest Diazinon 2D Insecticidal Dust, Roussel Bio Corporation, Englewood, NJ); (4) renovation and rodent proofing of buildings on the facility; (5) trapping and removal of 85 rodents (73 chipmunks [*Tamius* spp.] and 12 golden-mantled ground squirrels [*Spermophilus lateralis* (Taylor)]) during TBRF surveillance procedures. Building 1343 was deemed unrepairable due to its age and large size, thus was purposely destroyed by burning by USFS staff in May 2003 in an attempt to eliminate a potential site of TBRF infections. Rodent surveys indicated that the chipmunk population was high (average trap success = 1 animal/trap) at the time of the initial site investigations during the summer of 2001.

Sites #2 and #3: Crowley Lake Cabins.

These sites were located near Crowley Lake and had a history of TBRF cases prior to those that occurred in 2002 (USFS Staff, personal communication). At site #2, the cabin was occupied several times after the cases were reported in the spring of 2002; no febrile illnesses were reported after these occupancies. Prevention measures against TBRF included renovating the cabin during the summer of 2002, and efforts were made to exclude rodents from entering the structure. During renovation, large amounts of rodent droppings were observed and rodent nests were removed from the wall opposite the front entrance. Extensive renovation of the cabin did not include specific rodent exclusion efforts. At site #3, the cabin was maintained in a very clean and organized manner. Prevention measures against TBRF included rodent proofing the structure and use of rodenticides to control rodents. The cabin was occupied as a summer residence, and prior to and after summer occupancy, the cabin was treated, in accordance to the label instructions, with an over-the-counter indoor insecticide/acaricide fogger.

Sites #4 and #5: Mammoth Lakes and Lake George.

The cabins at both sites were seasonally occupied during the warm months of the year. Each of the cabins was maintained in a clean and clutter-free manner, and measures were regularly taken by the owners to control the occasional rodent. Based on communication with individuals responsible for the exposure sites, it appeared that these rodent control or TBRF prevention measures were not any different than before the illnesses occurred. No previous history of TBRF cases at the sites was mentioned. At site #4, a probable chipmunk or wood rat nest was discovered in a storage area under the eve of the second story, but there was no report of complaints or any observations suggestive of severe rodent problems in the structure. This nest was upstairs and on the opposite side of the cabin from where the case individuals slept in 2002. Observations indicated that populations of golden-mantled ground squirrels and chipmunks appeared high in the vicinity of the cabin; however, no active surveillance activities were conducted at this site. At site #5, no wood rat or chipmunk nests were found in the cabin, and mice were reported to not be a problem, but occasionally a mouse was trapped. No additional TBRF preventive measures were conducted at either site.

INYO COUNTY

Site #6: Inyo County Cabin.

The cabin, located west of Bishop, was occupied several times after the initial 2 cases occurred in 2002, and no febrile illnesses were reported after any of the stays. The initial cases were the first and only cases ever reported from the cabin. Tick-borne relapsing fever preventive measures implemented at the cabin, which were taken shortly after the illnesses occurred, included: (1) reduction of potential rodent harborage or clutter on the exterior and interior of the structure; (2) conduct of rodent proofing; (3) application of rodenticides and snap traps for rodent control; and (4) treatment of the cabin with an over-the-counter indoor insecticide/acaricide fogger according to the label instructions. Rodenticides were also used prior to the illnesses. No pre- or post-treatment surveillance activities were conducted at this site.

DISCUSSION

Quite often TBRF cases re-occur at sites that have a history of human infection. By implementing several basic TBRF preventive measures such as reducing rodent harborage in and around structures, rodent proofing structures and conducting routine rodent and tick control, the risk of TBRF infection may be reduced. Rodent proofing, including removal of accessible rodent nesting material from the cabins, and insecticidal/acaricidal treatments of cabins were found to be effective in preventing further TBRF cases for up to 17 years at the North Rim of Grand Canyon National Park (Boyer et al. 1977, Paul et al. 2002). Recently, an over-the-counter insecticidal/acaricidal indoor fogger was reported to be effective in controlling *O. hermsi* (Schwan et al. 2003). We implemented these control methods in combination at those sites to reduce the exposure risk of TBRF. Follow-up investigations, like those described for site #1, are warranted to evaluate the efficacy of the TBRF preventive measures taken at each of the other sites discussed in this report. We believe that rodent proofing structures is an intrinsic part of TBRF prevention, and that the preventive measures taken at 4 of the 6 sites should effectively lower the risk of TBRF at the sites.

Acknowledgements

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Temporal Distribution of Adult *Ixodes pacificus* at Griffith Park, Los Angeles County

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ABSTRACT: Tick-borne Lyme disease (LD) is currently the most commonly reported vector-borne disease in the United States. In California, *Ixodes pacificus* is the principal vector responsible for transmission of the LD-causing spirochete, *Borrelia burgdorferi* from wild animals to humans. In the southern portion of the state, *I. pacificus* has been often encountered in natural habitats in mountains and along the foothills; however, well-designed long-term studies on the seasonality of the tick and the transmission risk of LD are lacking. In late 2001, we initiated such studies to fill knowledge gaps. Here we report the temporal distribution of adult *I. pacificus* observed at Griffith Park, Los Angeles County from December 2001 through October 2003.

INTRODUCTION

Tick-borne Lyme disease (LD) was first recognized as a new disease from Lyme, Connecticut in 1975 (Steere et al. 1977). Since then, it has become the most frequently reported vector-borne disease in the United States (CDC 2002). In California from 1989 to 2003, a total of 2,309 cases was reported from 54 out of 58 counties throughout the state. Humans acquire the LD-causing spirochete, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt, and Brenner (Johnson et al. 1984), primarily through the bite of an infected tick. In the western United States including California and Oregon, the western black-legged tick, *Ixodes pacificus* Cooley and Kohls, is the principal vector responsible for the transmission of *B. burgdorferi* from wild animals to humans (Burgdorfer and Keirans 1983; Burgdorfer et al. 1985; Lane et al. 1991; Clover and Lane 1995). This tick is a 3-host species and reportedly feeds on about 80 species of lizards, birds, and mammals (Arthur and Snow 1968; Furman and Loomis 1984) and has been collected from 55 out of 58 counties in the state.

In southern California, human LD cases have been reported from every county and *B. burgdorferi* has also been detected in *I. pacificus* in the region (Webb et al. 1992). There were 8 cases reported in Los Angeles County in 2002, all of which were contracted from ticks outside the county. But ticks carrying *B. burgdorferi* have been reported in the county in the past (pers. comm. with D. Heft). Of the 48 cases of Lyme disease reported in Los Angeles County since 1989, 16 are believed to be caused by ticks in the county. Our tick surveillance data have indicated that *I. pacificus* is frequently found in natural habitats in mountains and along the foothills throughout the region. Information about the seasonal activity of *I. pacificus* is, however, lacking. In late 2001, we initiated a longitudinal study to determine the seasonal activity of *I. pacificus* and the transmission risk of LD in southern California. Here we report the field observations of adult *I. pacificus* activity at Griffith Park, Los Angeles County.

METHODS AND MATERIALS

Griffith Park is the largest urban municipal park in the U.S. and is located at the far eastern end of the Santa Monica Mountains with elevations ranged between 100 to 500 meters. It covers an area of 4,107 acres with abundant species of wildlife. During the summer of 2001, we conducted site evaluations and determined that this site was ideal for a long-term study based on historical records of the presence of *I. pacificus*, and its potential public health importance as demonstrated by the park's access to and extensive use by humans (Hu et al. 2003).

Tick sampling was conducted at the Griffith Park twice/month starting in December 2001 through October 2003. Collections were made through collaborative efforts of the California Department of Health Services, Vector-Borne Disease Section and the Los Angeles County Department of Health Services, Vector Management Program. Ticks were collected by using the standard flagging technique, i.e., a square meter flannel material dragged over low vegetation (brushy and grassy area) or leaf litter. One area at the site was designated for a tick seasonal activity study and a "non-removal tick sampling method" was applied (Hu et al. 2003). Ticks were sampled along roads and trails for a minimum period of 1 person-hour of active flagging (i.e. 1 person for 60 min., 2 people for 30 min. each, etc.). The flag was examined periodically (~5-min. intervals). To minimize the direct impact of tick sampling procedure on the abundance assessment, ticks on the flag were identified to species, their developmental stages and sexes, counted, and released at the site of collection. Tick abundance was expressed as the total number of ticks collected /person hr. of active flagging. This was used as quantitative data to determine the seasonal activity of ticks for the site.

RESULTS AND DISCUSSION

From December 2001 to October 2003, a total of 46 tick collections was made at the study site to determine the seasonal

activity of *I. pacificus*. The temporal distribution of adult *I. pacificus* at Griffith Park during the sampling time period is presented in Fig. 1. In California, *I. pacificus* adults are most active between November and March. Because the first tick collections started in December 2001, our data did not include the specific time of first appearance of *I. pacificus* adults in the fall of 2001. Adults were active through the winter months until the end of May 2002. In the fall of 2002, adults occurred first on November 13 when 20 individuals were collected. Adults were continuously collected in the field until June 12, 2003, although only a single adult *I. pacificus* was collected on May 21 and June 12.

It is worthy to note that 483 adult *I. pacificus* were collected during 2001-2002 and only 230 were collected during 2002-2003, representing less than half the number collected in the previous adult season. Our data also showed that adult *I. pacificus* could be very abundant at the site, for instance, a total of 136 ticks was collected on December 13, 2001. This number is comparable to those obtained from other areas in the state with known high *I. pacificus* infestations (pers. comm. with R.S. Lane). Environmental parameters such as temperature, humidity, vegetation cover, and habitat type are important for the survival of *I. pacificus*. We are currently analyzing the meteorological data to establish its correlation to the seasonal abundance of the ticks. The data presented here demonstrate that *I. pacificus* adults are active during the winter months into late spring at Griffith Park. We strongly recommend that people who visit Griffith Park during that time period take personal protection measures, including the wearing of long-sleeve shirts and long pants, and the use of repellents containing DEET to avoid the ticks.

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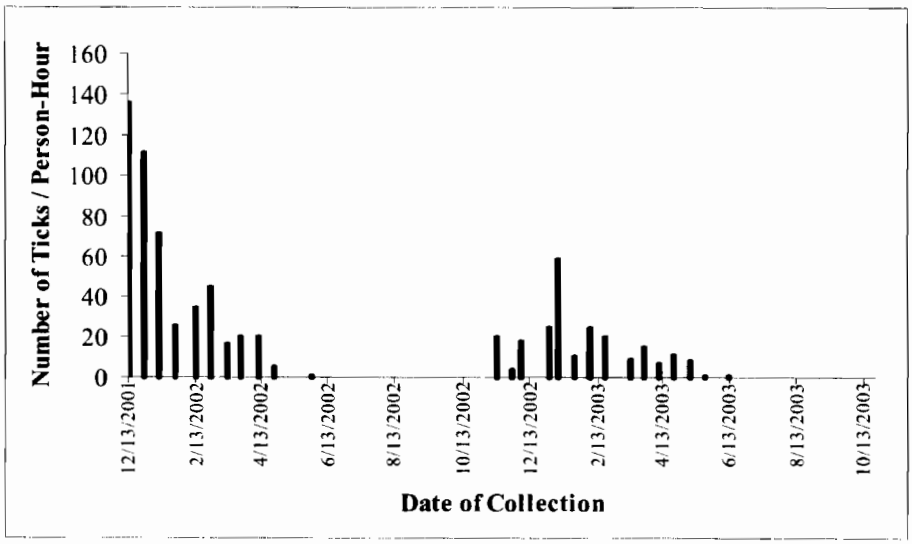

Figure 1. Temporal Distribution of Adult *Ixodes pacificus* Observed at Griffith Park, Los Angeles County from December 2001 to October 2003.

Creeping Water Primrose (*Ludwigia hexapetala*) and *Culex*: An Invasion of the Laguna de Santa Rosa Wetlands

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INTRODUCTION

The Laguna de Santa Rosa (the Laguna) is an extensive wetland ecosystem located in Sonoma County that consists of a main channel fourteen-miles in length and a 7,000-acre floodplain. The Laguna has a mosaic of seasonal wetlands and vernal pools, is the largest freshwater marsh in Northern California, and drains a watershed of 160,000 acres including five cities. The Laguna ecosystem has been invaded by an alien aquatic plant *Ludwigia hexapetala* (Hooker & Arnott) Zardini, Gu & Raven, commonly known as creeping water primrose. *L. hexapetala* has completely covered an approximate three-mile section of the Laguna main channel and a large portion of the Laguna floodplain totaling approximately 1,452 acres.

In summer 2002, the Marin/Sonoma Mosquito & Vector Control District (MSMVCD) received multiple service requests for abundant mosquito problems from private property owners living in the Laguna floodplain. Adult mosquito surveillance conducted at the edge of the Laguna riparian corridor using Faye traps, indicated *Culex erythrothorax* Dyar and *Culiseta particeps* (Adams) were present in large numbers. Operations staff cut their way through dense riparian corridor to reach the Laguna main channel to sample larval populations. In the main channel it was observed that *Ludwigia* was at 100% cover and standing 5.5 feet off of the water surface. It was also observed that when the *Ludwigia* was disturbed, *C. erythrothorax* mosquitoes emerged in large numbers. The suspicion was *Ludwigia* was providing habitat for *Cx. erythrothorax* larvae and adults.

ACCESS

Access into the Laguna through the dense stand of *Ludwigia* was especially difficult and problematic (Figure 1). MSMVCD operations staff tested several different types of equipment in the Laguna to gain access. Argo Conquest® and Argo Centaur® amphibious vehicles were useful in portions of the Laguna with moderate *Ludwigia* density, shallow water, and minimal sediment. When sediment was deep or the Argos floated, the vehicles were very difficult to maneuver and would get stuck.

Kayaks were useful for accessing areas in the Laguna with moderate to dense *Ludwigia*. In areas with the densest *Ludwigia* canopy cover and root masses, the kayaks were physically exhausting to propel and maneuver.

In late summer of 2003 MSMVCD purchased an airboat (Diamondback Airboats, Cocoa, FL.). Throughout the fall and winter 2003/2004 the airboat enabled MSMVCD staff to access previously unreachable areas of the Laguna (Figure 2). The airboat has potential to be useful as a surveillance platform, as well as a vehicle for larvaciding in the Laguna and several other wetlands in Marin and Sonoma County.

Figure 1. Marin/Sonoma Mosquito & Vector Control Operations staff wading through *Ludwigia* to access the Laguna de Santa Rosa.

SURVEILLANCE

Larval mosquito surveillance within the *Ludwigia* was difficult, frustrating, and at times dangerous. To wade through *Ludwigia* canopy cover, intertwined root masses, and thick sediment was physically challenging and exhausting. Moving through *Ludwigia* was dangerous in deep water and when negotiating numerous submerged obstacles.

Obtaining larval dip samples through *Ludwigia* root masses was problematic and time consuming. Disturbed *Ludwigia* roots sent shock waves across the water surface 10 to 20 feet in every direction and forcing a dipper through the root mass was difficult to impossible. Bio-Quip® mosquito larval traps, placed throughout the Laguna, were unsuccessful in attracting and capturing mosquito larvae in *Ludwigia*. Given the difficulty of sampling larvae from the Laguna, dry ice baited-Faye traps were the most efficient and effective means of sampling mosquito populations. Pyramid style

Figure 2. Marin/Sonoma Mosquito & Vector Control District's airboat traveling in the main channel of the Laguna de Santa Rosa over *Ludwigia*.

emergence traps (Walton et. al. 1999) placed in dense *Ludwigia* were unsuccessful in capturing emerging adult mosquitoes.

Larval and adult mosquito surveillance results showed an abundance of *Cx. erythrothorax*, *Culex pipiens* Linnaeus, *Culex tarsalis* Coquillett, and *Cs. particeps* were being produced in the *Ludwigia* habitat within the Laguna. The presence of *C. pipiens* was surprising to MSMVCD staff and suggested poor water quality in the Laguna.

LARVACIDING

Applying larvacide to the Laguna was an issue that underwent lengthy discussion and consideration amongst MSMVCD staff. Difficult access to the Laguna, limited success with equipment, safety concern, potential penetration and effectiveness of larvacides in the *Ludwigia* habitat, and cost effectiveness of larvacide application were all issues that were deliberated. It was decided that on August 12, 2003 MSMVCD would larvacide the Laguna by helicopter.

Methoprene:

Methoprene (Altosid XRG®) was the larvacide used for the first helicopter treatment of the Laguna. The XRG formulation was selected for its granular properties to penetrate dense stands of *Ludwigia*. It was also selected for its potential to provide long-term twenty-one day control.

MSMVCD treated 102 acres in the Laguna with XRG at the label rate of 20 lb./acre. A total of 2,040 lb. of XRG was applied to the Laguna, at a cost of $170/ acre with a total cost of $17,340, excluding helicopter time.

Turkey sized (12.0 in. x 16.5 in.) roasting tins were placed below the *Ludwigia* canopy in several locations within the treatment area to evaluate XRG penetration of *Ludwigia*. Results showed all roasting tins contained large numbers of XRG granules. Post treatment observations indicated XRG granules did not adhere to *Ludwigia* canopy or roots.

A small number (approximately 150) of pupae were sampled post treatment and brought back to the laboratory for observation. There were pupae in the lab that died or hatched as abnormal adults, however, there were also pupae that hatched as normal adults. Healthy pupae and a continuous abundance of adult mosquitoes were also observed in the field after the XRG treatment. Three weeks post treatment Faye trap results indicated an increase in adult mosquito populations within the treated area.

Bacillus sphaericus (Vectolex CG):

The XRG treatment of the Laguna did not result in the desired level of mosquito control. MSMVCD management realized that the mosquito breeding cycle in the Laguna needed to be broken quickly. Pressure was also being placed by the media and the public to break the mosquito breeding cycle in the Laguna in fear of the potential arrival of West Nile virus. MSMVCD management decided on a second larvacide application to the Laguna by helicopter using Vectolex CG® (CG). CG, like XRG, was selected because of its granular properties to penetrate dense *Ludwigia* canopy and root structures. CG with *Bacillus sphaericus* as the active ingredient also provided potential for rapid control and for the bacteria to recycle in the mosquito populations in the Laguna, thus, providing long-term control. MSMVCD treated 112 acres in the Laguna at the label rate of 20 lb./acre. A total of 2,240 lb. of CG was applied at a cost $85/ acre with a total cost of $9,520.

Glue boards (5.0 in. x 10.5 in.) were placed above and below the *Ludwigia* canopy to evaluate CG penetration. Post-treatment results showed a thirty percent difference in the amount of CG granules in glue boards at the top of the canopy compared to glue boards at the bottom of the canopy. Post-treatment it was observed that CG granules had adhered to *Ludwigia* leaves and stems (Figure 3). The helicopter pilot flew over the treatment area for a second

Figure 3. Vectolex CG® adhering to a *Ludwigia* leaf.

time, as low as possible, and used rotor wash to shake CG granules from the *Ludwigia* to the water surface. Field observations indicated that this method worked quite well.

Two plots (10.0 ft. x 10.0 ft.) were established prior to the CG application to evaluate pre- and post-treatment larval abundance. Plot one showed an average of sixteen larvae per dip pre-treatment and zero larvae per dip post-treatment for twenty-five dips. Plot two showed an average of three larvae per dip pre-treatment and zero larvae per dip post-treatment for twenty-five dips. A set of Argo tracks existed in the treatment area with *Ludwigia* pushed below the surface of the water. Prior to the CG treatment, larvae could be readily dipped in the Argo tracks and observed by the thousands. After the CG treatment larvae could not be dipped nor observed in the Argo tracks. The CG application provided mosquito control in the Laguna for a three-week period and to the end of the mosquito-breeding season.

The situation in the Laguna de Santa Rosa is troubling from a mosquito control as well as, an ecological standpoint. The invasion of the Laguna by *Ludwigia* is a symptom of a much larger problem. The MSMVCD has sponsored an internship with Sonoma State University to study the growth dynamics, abundance, and potential control measures for *Ludwigia*. The Sonoma State intern is also studying water quality in the Laguna. The MSMVCD is also a member of the *Ludwigia* Task Force that is charged with developing a management and restoration plan for the Laguna system.

Acknowledgements

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Diel Periodicity of Host-seeking by *Ochlerotatus sierrensis*, the Western Treehole Mosquito

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ABSTRACT: Time-segregated CO_2-trap sampling detected activity by both adult sexes of *Ochlerotatus sierrensis* throughout the diel cycle in two oak woodlands that had moderate tree canopy closure in Lake County, CA. Low levels of adult activity were detected during the most brightly sunlit midday periods in both woodlands. On days with maximum temperatures <30°C at Seigler Springs, host-seeking females were most active during 2-hr periods before sunset and after sunrise, and activity during those periods increased with increasing temperatures. A seasonal study at Lakeport showed that diel patterns of activity by females changed as temperatures increased from May until August. On hot summer days (maximum temperatures >30°C), most females were caught during cooler parts of the day that occurred after sundown, during the night and during early morning hours. Male adults were most active during the 2-hr period ending at sunset in both woodlands, except on hot days when peak activity continued into the 2-hr period after sunset. Very few males were caught during the night regardless of temperature or location.

INTRODUCTION

Ochlerotatus sierrensis (Ludlow), the western treehole mosquito, is widely distributed in forested areas of California (Bohart and Washino 1978), but the most abundant populations occur in lower elevation woodlands of the Coast Range and the Sierra Nevada. One unusual trait of the species is that both adult sexes are attracted to mammals where mates are located and females obtain bloodmeals (Washburn et al. 1992). Females can be severe biting pests for humans (Woodward et al. 2003) and they are important vectors of two filarial nematodes, *Dirofilaria immitis* (Leidy), the canine heartworm (Sacks et al. 2003) and *Setaria yehi* (Rudolphi), the deer bodyworm (Lee 1971). In laboratory experiments, females have transmitted viruses that cause encephalitis in humans including West Nile virus (Goddard et al. 2002), western equine encephalomyelitis virus (Reeves and Hammon 1962) and California encephalitis virus (Berge 1975). Accurate determination of the daily periodicity of host-seeking would indicate periods when transmission of pathogens is most likely to occur (Reisen et al. 1997). In addition, since adults are known to use protected resting sites such as treeholes (Lee 1971) and rodent burrows (Bennett 1978), the time of host-seeking may delineate periods when adults are more exposed and therefore more vulnerable to control (Reisen et al. 1997).

Despite the biting and vector potentials of *Oc. sierrensis*, the daily periodicity of host-seeking has never been completely described. Lee (1971) studied the time of host-seeking by making human sentinel collections at various times of day, but he did not examine entire daily cycles nor did he attempt many collections after darkness, possibly because the method was labor-intensive and dependent upon human vision to capture adults. The primary purpose of the present study was to determine the daily periodicity of adults using CO_2-baited suction traps in conjunction with collection bottle rotators that segregated daily catches among predetermined periods of time. A major advantage of this method over the use of human sentinel collections was that the mechanical traps could be operated continually during entire daily cycles. Since both adult sexes were attracted to the CO_2-baited traps (Garcia et al. 1989, Washburn et al. 1992, Woodward et. al. 2003) the method was used to examine the activity periods of females and males.

MATERIALS AND METHODS

The study was conducted at two sites in Lake County, California including an oak woodland (ca. 60% tree canopy closure) dominated by California black oak (*Quercus kelloggii* Newberry) and interior live oak (*Q. wislizenii* Candolle) near Seigler Springs (N38°52', W122°41', elevation 844 m). The site was studied in 2001 during eight diel cycles that began on May 7, 8, 9, 10, 14, 15, 16 and 17. A John W. Hock Company model 1512 Collection Bottle Rotator® (CBR), capable of segregating insect catches among eight collection bottles during programmed time intervals, was mounted on a support pipe 0.5 m above ground at each of two locations (55 m apart) in the woodland. Each CBR was fitted with eight numbered polyethylene collection bottles (500 ml) holding 100 ml of 50% alcohol as a killing agent and a CDC-style (Sudia and Chamberlain 1962) suction trap that was modified by removal of both the light source and screen, and by painting the exterior black on the top half and white below. Compressed gas cylinders, two-stage regulators and 3 mm inside diameter polyethylene tubes were used to release carbon dioxide at a constant rate of 1000 ml per minute at a point 5.0 cm above the top of each suction trap during all sample periods. Each CBR was programmed to operate the CO_2-trap continually and to rotate a new collection bottle into position under the trap at the following times on each day: 1) 2 hr before sunrise, 2) sunrise, 3) 2 hr after sunrise, 4) 5 hr after sunrise, 5) 4 hr

before sunset, 6) 2 hr before sunset, 7) sunset and 8) 2 hr after sunset. The last period ended 2 hr before sunrise to complete each 24 hr cycle. When sampling was conducted on consecutive days, the traps were turned off for servicing and programmed to resume operation 10 minutes after workers left the vicinity to minimize sample bias. The time that the trap was not operated was subtracted from the length of the appropriate sample period. At the end of each daily cycle the collection bottles were returned to the laboratory where captured mosquitoes were identified (Bohart and Washino 1978) and counted under a dissecting microscope (6-30X magnification). Temperature and relative humidity at the study site were recorded at 30 minute intervals with an Onset Hobo® Pro Series datalogger. Light intensity was monitored at the same intervals with an Onset Hobo® LI datalogger.

A second study was conducted during 1998 in a blue oak (*Q. douglasii* Hooker and Arnott) woodland (ca. 57% tree canopy closure) near Lakeport (N39°01', W122°55', elevation 433 m). One CBR was used to examine the periodicity of host-seeking during 25 diel cycles. The data were grouped and analyzed among cool days (maximum air temperature 14-22°C) on May 18, 19, 20, 21, 22; June 8, 10, and 11, warm days (maximum air temperature 23-30°C) on June 5, 17, 18, 22, 25, 29; July 1, 3, and 10; and hot days (maximum air temperature 31-41°C) on July 7, 13, 15, 16, 18, 23, 27 and August 4. The methods used were the same as those described for the Seigler Springs site except that the CBR was programmed to rotate a new bottle into position under the CO_2-trap at: 1) 2 hr before sunrise, 2) sunrise, 3) 2 hr after sunrise, 4) 4 hr after sunrise, 5) 6 hr before sunset, 6) 2 hr before sunset, 7) sunset and 8) 2 hr after sunset. The last period ended at 2 hr before sunrise to complete each 24 hr cycle. In addition, carbon dioxide was released at a point 5.0 cm above the suction trap from a 1.9 cm diameter hole in the bottom of a white ice chest (4 liter) holding 4.5 kg of dry ice. Between 1.1 and 1.8 kg of dry ice remained in the ice chests at the end of each diel cycle. Air temperature was continually recorded during study periods with a Cole-Parmer Instrument Company thermograph.

Statistical Analysis

Data were analyzed according to methods described by Zar (1980). Mean numbers of adult *Oc. sierrensis* collected per hour during diel cycles were transformed by $\log_{10}[x+1]$ to normalize variances that were heteroscedastic and then compared by 1-way ANOVA followed by Duncan's multiple range tests. Linear regression analysis was used to calculate coefficients of determination (R^2) between capture rates of adults (log transformed) and temperature, relative humidity, sunlight and moonlight data.

RESULTS

Time-segregated CO_2-trap collections of *Oc. sierrensis* adults at Seigler Springs totaled 1130 females and 922 males. Daily maximum air temperatures ranged from 17-29°C and there was no precipitation during the study. Under those conditions, both sexes of adults were caught throughout the diel cycle, but there were significant differences in the numbers of adults caught per hour at different times of day (Table 1).

Table 1. Statistical comparison of the mean numbers of *Oc. sierrensis* adults caught per hour with time-segregated CO_2-traps at Seigler Springs, Lake County, CA. Two traps were operated on each of eight diel cycles between May 8 and May 17, 2001.

Daily Interval	Mean Adults per Hour	
	Females	Males
SR-2h to SR[1]	2.55 c	2.99 b
SR to SR+2h	7.62 b	3.78 b
SR+2h to SR+5h	3.48 c	2.72 b
SR+5h to SS-4h	0.26 d	0.26 c
SS-4h to SS-2h	1.89 c	2.14 b
SS-2h to SS	14.37 a	13.05 a
SS to SS+2h	2.49 c	1.80 b
SS+2h to SR-2h	0.24 d	0.15 c

The data were transformed by $\log_{10}[x+1]$ and compared with a 1-way ANOVA. Means within columns followed by the same letter were not significantly different (P>0.05) by a Duncan's multiple range test.

[1] SR=sunrise, SS=sunset, h=hours

Females exhibited two daily peaks in activity, the largest in the 2-hr period ending at sunset and a smaller peak in the 2-hr period beginning at sunrise (Fig. 1). Captures of females during those peak periods of activity accounted for 40% and 21% of the total number collected, respectively. Males exhibited a single daily peak in activity during the 2-hr period ending at sunset that accounted for 45% of all of the males that were caught. Adults exhibited low levels of activity both at midday, the hottest and most brightly sunlit period, and during the cool, dark hours of the night. Females caught during the dark represent the first reported evidence of nocturnal host-seeking activity by *Oc. sierrensis,* but the possible effects of temperature and moonlight on nocturnal activity could not be separated during the eight nights of the study at Seigler Springs. More than 90% of the females were collected on the four warmest nights (mean air temperatures > 18°C during the dark period), but those same nights also had the longest periods with moonlight (> 4 hours per night).

Overall, the daily periodicity of *Oc. sierrensis* adults that were attracted to carbon dioxide did not show a significant linear correlation to diel changes in air temperature (females, R^2=0.00, P=0.95; males, R^2=0.03, P=0.69), relative humidity (females, R^2=0.02, P=0.71; males, R^2=0.00, P=0.97), or sunlight (females,

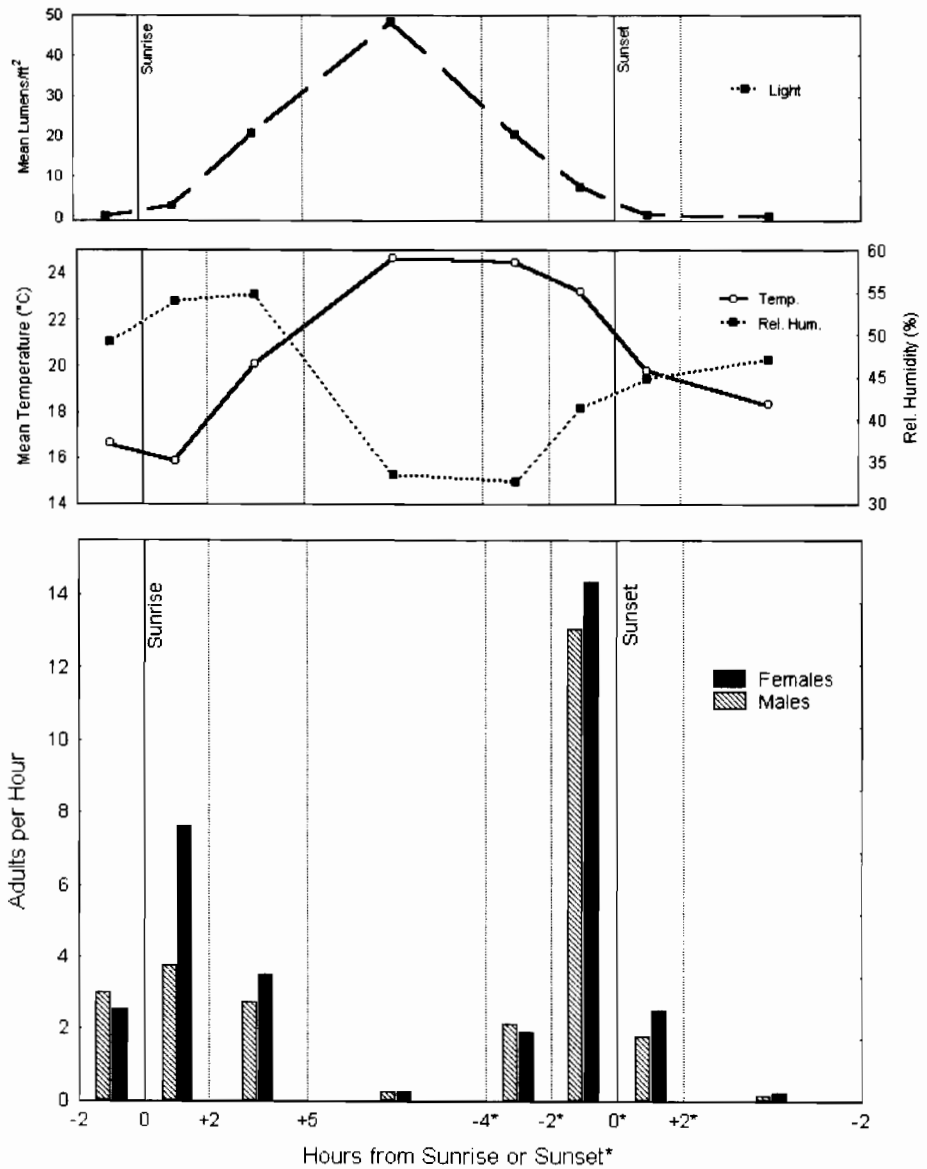

Figure 1. The diel pattern of activity by adults of *Oc. sierrensis* collected with two time-segregated CO_2-traps on eight dates between May 8 and May 17, 2001 at Seigler Springs, Lake County, CA is shown in the bottom panel. Diel changes in mean air temperature, mean relative humidity (middle panel) and illumination from sunlight (top panel) are also shown.

R^2=0.08, P=0.50; males, R^2=0.05, P=0.50) (Fig. 1). Although the diel pattern of activity by females did not track diel changes in temperature, mean females caught per hour during the peak periods of activity (the 2-hr period beginning at sunrise and the 2-hr period ending at sunset) did show a highly significant linear correlation (R^2=0.64, P<0.001), to temperature during the eight days of the study (Fig. 2).

The effect of seasonal changes in air temperature on the daily patterns of activity by *Oc. sierrensis* adults was examined with the study at Lakeport. The time-segregated CO_2-trap operated during 25 diel cycles collected a total of 899 females and 1477 males. Those data were grouped among days with cool, warm and hot temperatures (Fig. 3), and the statistical analysis showed there were

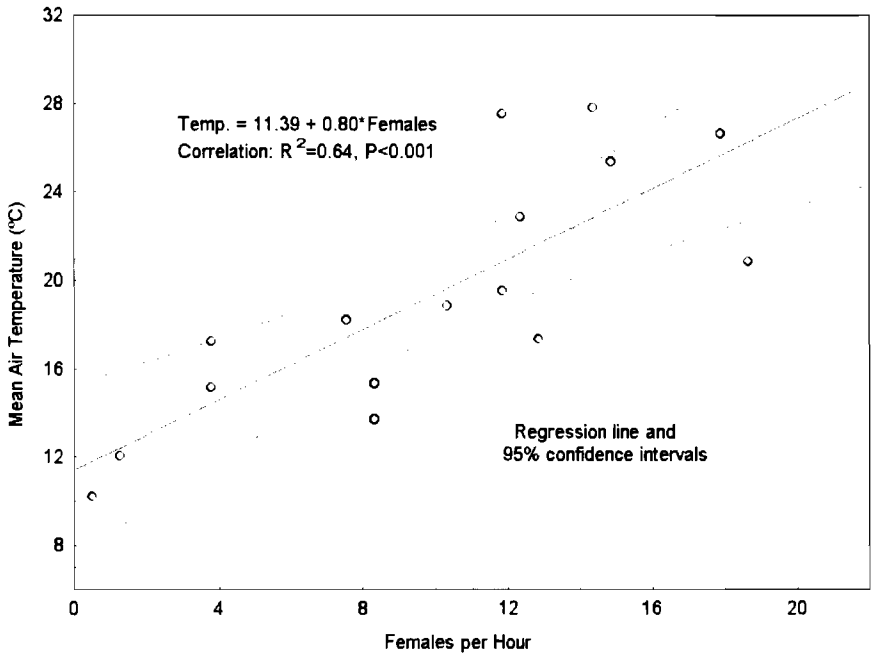

Figure 2. Linear regression analysis that compared CO_2-trap catches of *Oc. sierrensis* females with air temperature during peak periods of activity on eight days between May 8 and May 17, 2001 at Seigler Springs, Lake County, CA. Data collected during morning (2 hr period beginning at sunrise) and evening (2 hr period ending at sunset) crepuscular periods were combined for the analysis.

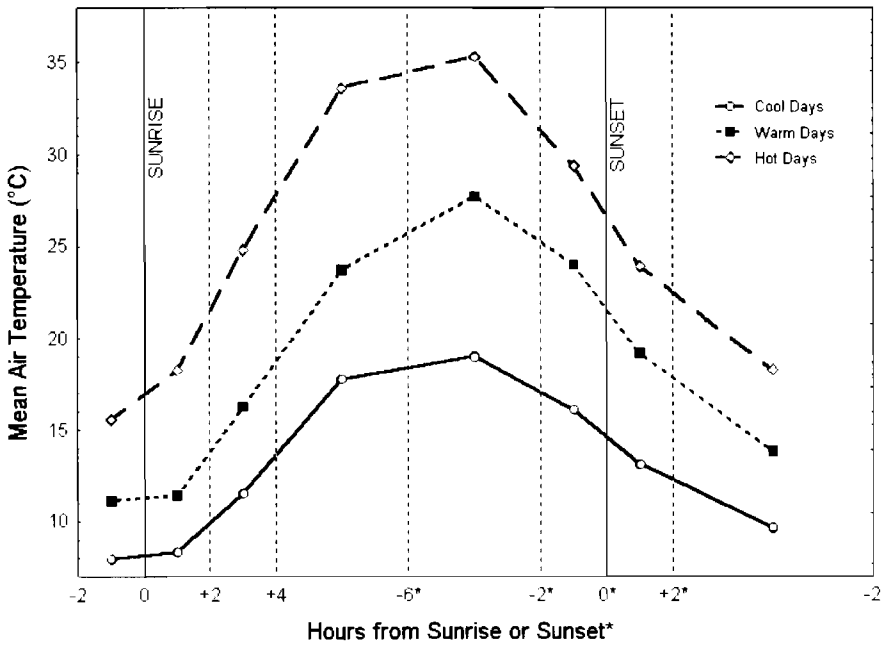

Figure 3. Diel variability in air temperature on cool (n=8), warm (n=9) and hot (n=8) days on 25 study dates between May 18 and August 4, 1998 at Lakeport, Lake County, CA.

significant differences in the daily timing of peak periods of activity among the three groups (Table 2). Differences in the mean numbers of adults caught per hour between the cool days group and the hot days group reflect seasonal declines in population size between May and August.

On cool days, host-seeking females were active throughout the day, but there was a peak in activity during the 2-hr period ending at sunset that included 43% of the total catch of females (Fig. 4). The evening peak in host-seeking activity on warm days included the periods from 2 hr before until 2 hr after sundown. Host-seeking activity also increased during the morning hours relative to midday hours on warm days. On hot days, just 7% of females were caught during the 2-hr period ending at sunset. Most females were active during cooler parts of the day including the 2-hr period after sunset and 2-hr periods before and after sunrise. Overall, the percentage of females caught during midday periods decreased with increasing temperatures while the percentage caught after sundown and during early morning hours increased as daily temperatures increased. The percentage of females caught at night (2 hr after sunset until 2 hr before sunrise) increased from 5% on cool days to 26% on hot days. Females were caught on nights with and without moonlight, but there was not a significant correlation between the numbers of females caught at night and either hours of moonlight (R^2=0.03, P=0.42) or the percentage of the moon illuminated (R^2=0.02, P=0.58).

Table 2. Statistical comparison of the mean numbers of *Oc. sierrensis* adults caught per hour with a time-segregated CO_2 -trap at Lakeport, Lake County, CA. Maximum air temperatures were 14-22°C on cool days (n=8), 23-30°C on warm days (n=9), and 31-41°C on hot days (n=8) between May 18 and August 4, 1998.

Daily Interval	Females per Hour			Males per Hour		
	Cool	Warm	Hot	Cool	Warm	Hot
SR-2h to SR[1]	0.69 d	0.22 cd	0.76 ab	0.25 c	0.00 c	0.13 b
SR to SR+2h	1.63 bcd	0.78 cd	1.45 a	1.82 bc	0.39 c	0.19 b
SR+2h to SR+4h	1.80 bcd	1.77 bc	0.83 b	1.78 bc	1.31 bc	0.16 b
SR+4h to SS-6h	0.97 cd	0.16 d	0.00 c	1.25 bc	0.14 c	0.00 b
SS-6h to SS-2h	2.57 bc	0.67 cd	0.00 c	3.58 b	1.11 bc	0.00 b
SS-2h to SS	13.86 a	5.99 a	0.50 b	43.30 a	18.26 a	0.57 a
SS to SS+2h	3.47 b	4.48 ab	1.45 a	5.10 b	2.35 b	0.63 a
SS+2h to SR-2h	0.47 d	0.74 cd	0.65 b	0.16 c	0.02 c	0.00 b

The data were transformed by $\log_{10}[x+1]$ and compared with a 1-way ANOVA. Means within columns followed by the same letter were not significantly different (P>0.05) by a Duncan's multiple range test.

[1] SR=sunrise, SS=sunset, h=hours

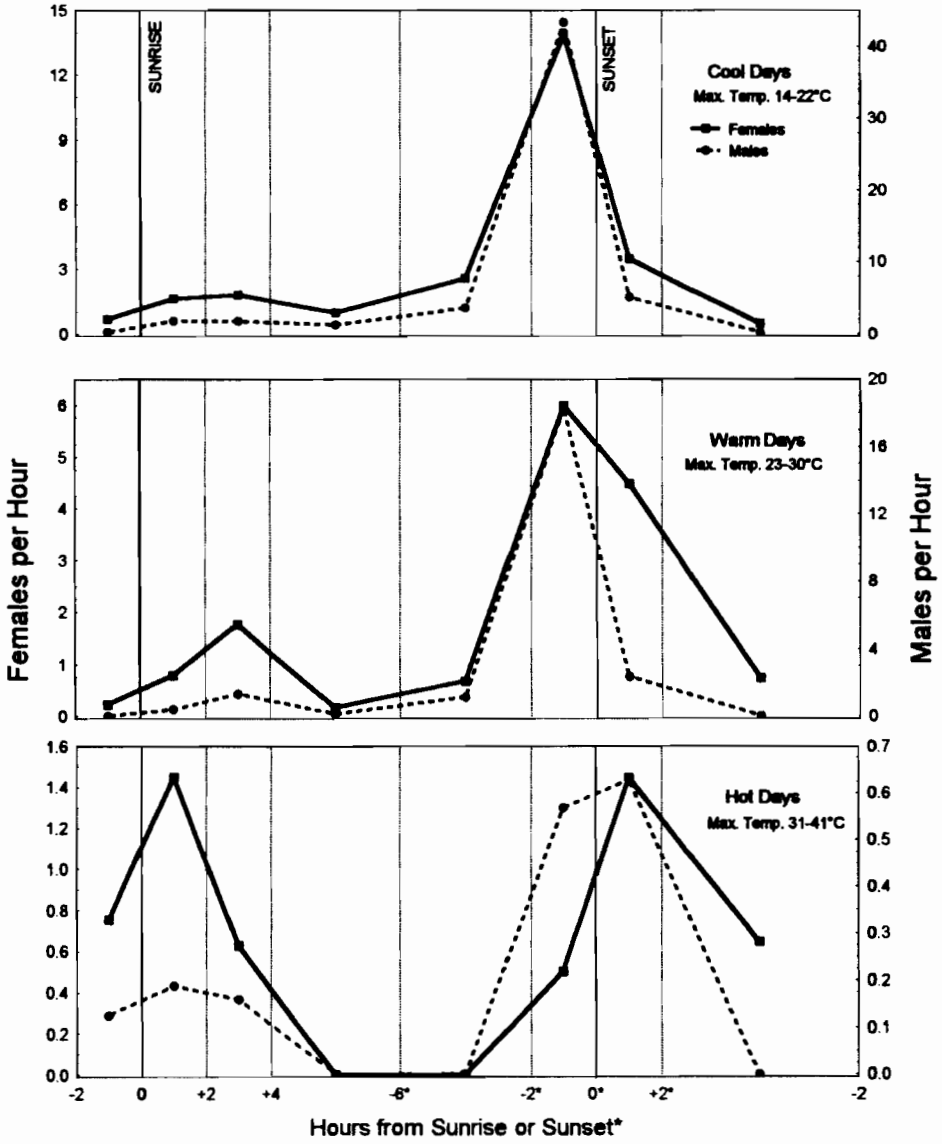

Figure 4. The diel patterns of activity by adults of *Oc. sierrensis* collected with a time-segregated CO_2-trap on cool (n=8), warm (n=9) and hot (n=8) days between May 18 and August 4, 1998 in Lakeport, CA.

In comparison to females, variability in daily temperatures did not cause males of *Oc. sierrensis* to exhibit such marked changes in daily periodicity (Fig. 4). Males were most active during the 2-hr period ending at sunset on both cool and warm days. On hot days the evening peak in activity included the 2-hr periods before and after sunset. Males were inactive or minimally active at night regardless of temperature.

DISCUSSION

Females of *Oc. sierrensis* exhibited host-seeking behavior throughout the day and during the night both at Seigler Springs and at Lakeport. Peak activity at Seigler Springs during May occurred prior to sunset and after sunrise, periods characterized by low light intensity from the sun (Fig. 1) and air temperatures between 10 and 28°C (Figs. 1 and 2). Within that temperature range, host-seeking activity during the crepuscular periods increased with increasing temperatures (Fig. 2), a finding that largely explains why females were more active prior to sunset than after sunrise. At Lakeport, the daily periodicity of host-seeking females showed seasonal changes as temperatures increased from mid May until early August (Figs. 3 and 4). Considering entire diel cycles, the percentage of females that sought hosts at night and during the morning hours increased when temperatures became too hot during evening crepuscular periods. The peak in activity prior to sunset that occurred on days with maximum temperatures <30°C was not

evident on hot days when maximum temperatures were >30°C. The very minimal nocturnal activity by males, even on hot days (Fig. 4), indicates there is probably a visual component to their searches for mates that cannot be accomplished during the dark. The low level of nocturnal activity by males also indicates that females that were attracted to carbon dioxide during the night were searching for hosts, not mates.

The results of this study agree with those of Lee (1971) in most respects. He conducted the only previous study of the daily periodicity of host-seeking activity by a wild population of *Oc. sierrensis* and also found that females will bite throughout the day, but that shifts to morning and late afternoon periods occur on hot days. He collected a larger percentage of females during midday periods than during the present study, but the dense canopy cover at his Mendocino County study site may have lowered the intensity of sunlight all day long. Lee (1971) did not attempt to collect host-seeking females more than an hour after sunset, but nocturnal host-seeking activity has been previously reported for *Oc. triseriatus*, the eastern treehole mosquito (Aziz and Hayes 1987) and many other North American species of *Ochlerotatus* (e. g., Nelson and Spadoni 1972, Mitchell 1982).

The results of the present study indicate humans may be exposed to pathogens vectored by *Oc. sierrensis* females at any time of day or night during spring and summer months in Lake County. The nocturnal host-seeking activities of females, particularly on hot days that occurred in July and August, have implications for dog owners concerned with canine heartworm transmission. Females of *Oc. sierrensis* are the primary vectors of heartworm in many areas of northern California (Sacks et al. 2003, Sacks et al. 2004). Although females are active from April to October (Woodward et al. 2003), a long-term study (Sacks et al. 2003) showed 95% of transmission to coyotes occurred between July 1 and September 14, periods when temperatures were hot enough for heartworm larvae to develop to the infective stage in the mosquito vectors. The results of the present study indicate those same hot summer months are periods when *Oc. sierrensis* females are most likely to engage in late evening and nocturnal host-seeking activity.

Acknowledgements

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Seasonal Abundance of Adult *Ixodes pacificus* in the San Jacinto Mountains, Riverside County

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ABSTRACT: Lyme disease (LD) is a tick-transmitted human illness that is now the most commonly reported vector-borne disease in the United States. In California, *Ixodes pacificus* is known as the principal vector transmitting the LD pathogen, *Borrelia burgdorferi* from wild animals to humans. In the southern portion of the state, *I. pacificus* has been often encountered in natural habitats in mountains and along the foothills; however, well-planned longitudinal studies on the seasonality of the tick and the transmission risk of LD are lacking. In the summer of 2001, we determined that three sites (Santa Rosa Mountain, the Spitler Peak Trail, and Thomas Mountain) in the San Jacinto Mountains in Riverside County were ideal for such studies to fill our knowledge gaps. Our data on the seasonal abundance of adult *I. pacificus* between November 2001-May 2002 and November 2002-May 2003 showed tick activity during the winter months through late spring at all 3 sites.

INTRODUCTION

Lyme disease (LD), a tick-transmitted human illness, was first recognized as a new disease from Lyme, Connecticut in 1975 (Steere et al. 1977). Since then, it has become the most commonly reported vector-borne disease in the United States (CDC 2002). From 1989 to 2003, a total of 2,309 LD cases was reported from 54 out of 58 counties in California. Humans acquire the LD-causing spirochete, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt, and Brenner (Johnson et al. 1984), primarily through the bite of an infected tick. In the western United States, the western black-legged tick (*Ixodes pacificus* Cooley and Kohls) is known as the principal vector transmitting *B. burgdorferi* from wild animals to humans (Burgdorfer et al. 1985, Lane et al. 1991, Clover and Lane 1995). This is a 3-host tick species and reportedly feeds on about 80 species of lizards, birds, and mammals (Arthur and Snow 1968; Furman and Loomis 1984). Specimens of *I. pacificus* have been collected from 55 out of 58 counties in the state.

In southern California, human cases of LD have been documented from every county and *B. burgdorferi* has also been detected in *I. pacificus* in the region (Webb et al. 1992). Our tick surveillance data have indicated that *I. pacificus* is frequently collected in natural habitats in mountains and along the foothills throughout the southern portion of the state. Information about the seasonal activity of *I. pacificus* is, however, lacking. In Riverside County, tick and tick-borne disease surveillance have been conducted in the central portion of the county by the Riverside County Department of Environmental Health, Vector Control Program since 1992. Specimens of *I. pacificus* collected have been sent to the collaborative agencies including the California Department of Health Services, Vector-Borne Disease Section, for the detection of *B. burgdorferi* as well as *Ehrilichia* spp. From 1992 to the present, there have been only 2 pools of *I. pacificus*, both collected at Santa Rosa Mountain in March 2001, which have tested positive for *Borrelia* species, more closely related to the relapsing fever group than *B. burgdorferi*.

It is evident that *I. pacificus* is present in Riverside County and sometime may be potentially infected with pathogens causing human diseases. However, well-planned longitudinal studies determining the seasonality of the tick and the transmission risk of LD in the county are lacking. In the summer of 2001, we concluded that 3 sites in San Jacinto Mountains were ideal for such studies. Here we report results on the seasonal abundance of adult *I. pacificus* in Santa Rosa Mountain, the Spitler Peak Trail, and Thomas Mountain.

MATERIALS AND METHODS

Since 1992, tick and tick-borne disease surveillance have been conducted in 5 areas that were strategically selected throughout the unincorporated areas of Riverside County. Each area is referred to as a group, and each group has 3 specific sampling sites. After detailed site evaluations during the summer of 2001, we determined that group 2, located within the San Jacinto Mountains, was ideal for a long-term study based on historical records of *I. pacificus*, the detection of *Borrelia* infection in ticks, and its distinctive ecological habitats (Hu et al. 2003). This group consists of sites at Santa Rosa Mountain, the Spitler Peak Trail, and Thomas Mountain.

Santa Rosa Mountain, at an elev. of ~1600 m, has a flora consisting primarily of oak, pinon juniper, manzanita, and desert transition flora. Mammals occurring in the area include mainly mule deer, deer mice, wood rats, ground squirrels, rabbits, big horn sheep, and mountain lions. The average rainfall is typically 15-17.5 cm.

The Spitler Peak Trail, at an elev. of ~1600 m, has a fora consisting primarily of coniferous forest, scrub oak, sage, manzanita, and annual grasses. Mammals occurring in the area included mainly mule deer, deer mice, wood rats, ground squirrels, tree squirrels, and mountain lions. The average rainfall is 27.5-30 cm.

Thomas Mountain, at an elev. of ~1400 m, has a flora consisting primarily of coniferous forest, oak, sage, manzanita, and annual grasses. Mammals occurring in the area include mainly mule deer, deer mice, wood rats, ground squirrels, tree squirrels, and mountain lions. The average rainfall is 17.5-20 cm.

Starting in November 2001, tick sampling was conducted twice/month at the 3 sites described above. Tick sampling was only conducted from November 2001 to May 2002 and November 2002 to May 2003. Collections were made through collaborative efforts of the California Department of Health Services, Vector-Borne Disease Section and the Riverside County Department of Environmental Health, Vector Control Program. Ticks were collected by using the standard flagging technique, i.e., a square meter flannel material dragged over low vegetation (brushy and grassy area) or leaf litter. One area at each site was designated for a tick seasonal activity study and a "non-removal tick sampling method" was applied (Hu et al. 2003). Ticks were sampled for a minimum period of 1 person hr. of active flagging (i.e., 1 person for 60 min, 2 people for 30 min each, etc.). To minimize the direct impact of tick sampling procedure on abundance determination, ticks on the flag were identified to species, developmental stage, sex; and number before they were released at the site of collection. Tick abundance was expressed as the total number of ticks collected/ person hr. of active flagging. This was used as a quantitative measure to determine the seasonal activity of ticks at each site.

RESULTS AND DISCUSSION

To determine the seasonality of *I. pacificus,* a total of 28 tick collections was made at each study site between November 2001-May 2002 and November 2002-May 2003. The seasonal abundance of adult *I. pacificus* at Santa Rosa Mountain, the Spitler Peak Trail, and Thomas Mountain established during the sampling time period are presented in Fig. 1, 2, and 3, respectively. In California, *I. pacificus* adults are most common during November and March. Although our tick collections were conducted during this period, we were unable to record the specific time for the first and last appearance of adult ticks. If we extend our time period for tick sampling in the future, we should be able to obtain more details in regard to the seasonal activity of *I. pacificus* adults.

From November 2001 to May 2002, a total of 83, 83, and 98 *I. pacificus* adults were collected from Santa Rosa Mountain, the Spitler Peak Trail, and Thomas Mountain, respectively. However, only 43, 35, and 58 were collected from these respective sites from November 2002 to May 2003, representing approximately half the number collected in the previous season. These findings are similar to what we found at Griffith Park, Los Angeles County (Hu et al. 2004). It is well known that environmental parameters such as temperature, humidity, vegetation cover, and habitat type are important for the survival of ixodid ticks (Eisen et al. 2003, Hubalek et al. 2003). We are currently analyzing the meteorological data to establish their correlation to the seasonal abundance of the ticks for these sites. The data presented here have demonstrated that *I. pacificus* adults remain active during the winter months through late spring at the 3 sites in the San Jacinto Mountains. We recommend that people conducting outdoor activities in these areas

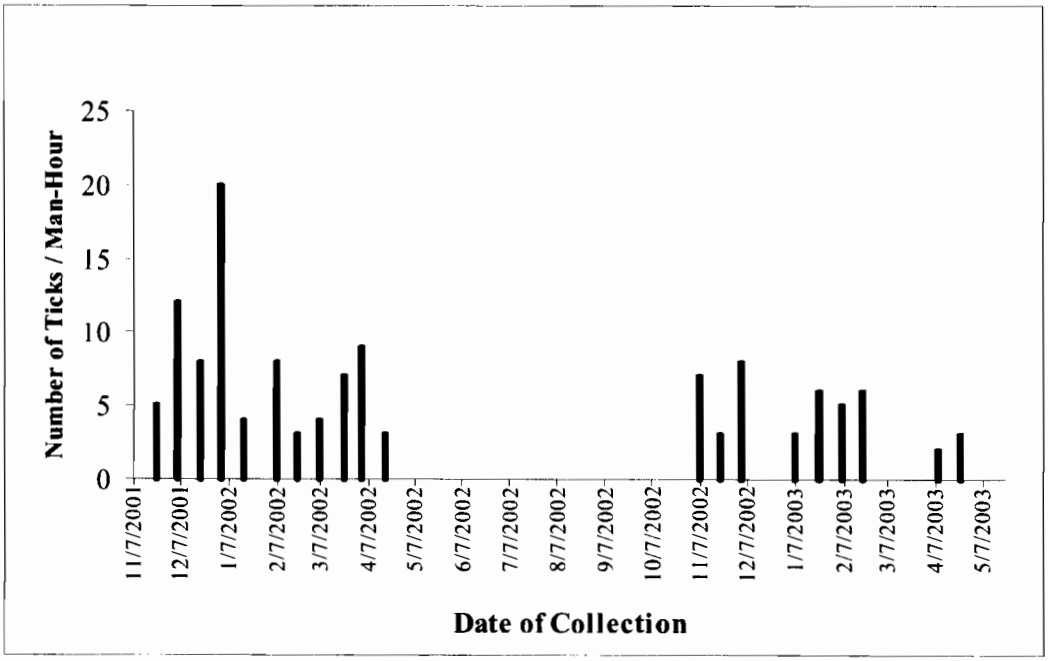

Figure 1. The seasonal abundance of adult *I. pacificus* at Santa Rosa Mountain, Riverside County, between November 2001-May 2002 and November 2002-May 2003 (No tick sampling was conducted between June-October 2002).

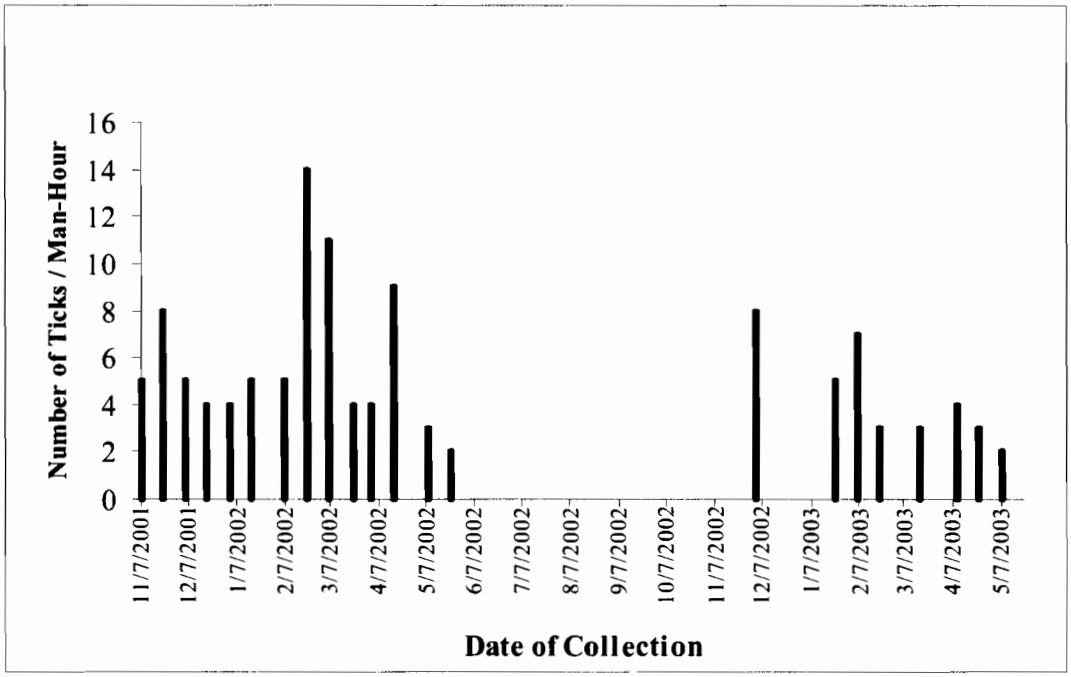

Figure 2. The seasonal abundance of adult *I. pacificus* at the Spitler Peak Trail, Riverside County, between November 2001-May 2002 and November 2002-May 2003 (No tick sampling was conducted between June-October 2002).

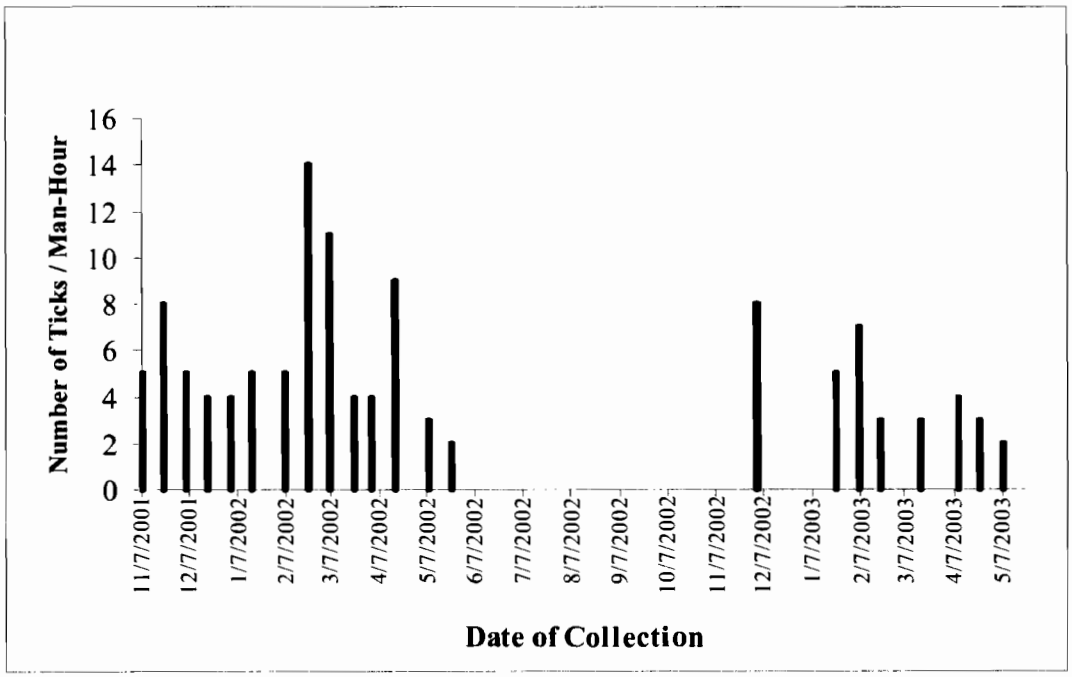

Figure 3. The seasonal abundance of adult *I. pacificus* at Thomas Mountain, Riverside County, between November 2001-May 2002 and November 2002-May 2003 (No tick sampling was conducted between June-October 2002).

during that time period take personal protection measures including the wearing of long-sleeve shirts and long pants as well as the use of repellents containing DEET to avoid the ticks.

Acknowledgements

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Summary of *Ixodes pacificus* Surveillance and Testing for *Borrelia burgdorferi* in California

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ABSTRACT: The California Department of Health Services (CDHS) has conducted tick surveillance for *Ixodes pacificus* since the early 1900s. The purpose of this document is to summarize the surveillance information gathered through 2003 from the Vector-Borne Disease Section (VBDS) and many collaborating agencies. The data include: a distribution map of *Ixodes pacificus* found and tested positive for *Borrelia burgdorferi*, a summary of the adult and immature *I. pacificus* collected from 70 different sources, a table of selected vector-borne diseases acquired or reported in California from 1980 to 2003, and a table of positive *B. burgdorferi* identified from *I. pacificus* by using different laboratory techniques from CDHS and collaborating agencies from 1985 to 2003.

INTRODUCTION

Lyme disease (LD) has been a rapidly emerging vector-borne infectious disease in the United States since 1975 (Steere et al. 1977). The Centers for Disease Control and Prevention (CDC) initiated nationwide surveillance reporting in 1982. The Council of State and Territorial Epidemiologists designated LD a nationally notifiable disease in 1991 (CDC 1991). In California during the 1970s, tick-borne diseases constituted 75% of the reported vector-borne diseases. Mosquitoes and fleas were identified as the source of vector-borne diseases in 14% and 10% of the cases, respectively (Lane and Murray 1980). As such, ticks were considered the most important group of zoonotic disease carrying arthropods. Tick-borne diseases were a low public health concern for Californians until the first autochthonous LD case was recognized in a hiker from Sonoma County in 1978 (Naversen and Gardner 1978). In March of 1989, LD was made a reportable disease in California (as outlined in Title 17 of the California Code of Regulations). From 1989 to 2003, a total of 2,252 LD cases were reported from 53 counties (VBDS 2003).

From a public health standpoint, ticks currently represent the most important group of arthropods in California. The human cases of selected vector-borne diseases acquired or reported in California by year since 1989 are summarized in Table 1. Before LD or Hantavirus Pulmonary Syndrome (HPS) were declared reportable diseases, the mosquito-borne diseases, [western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE)], were the most prevalent vector-borne diseases in California with a total of 1,068 cases (640 cases of WEE and 428 cases of SLE) reported from 1950 to 1968 (Emmons et al. 1972 , Emmons and Grodhaus 1976). In contrast, in the 15-year period since 1989, out of 2,523 human cases of vector-borne disease reported, 2,252 cases (89%) were LD while only 10 cases of WEE and 107 cases of SLE were reported from 1969 to 1997 (Hui et al. 1999); and no cases of WEE and SLE were reported from 1998 to 2002 (Steinlein et al. 2003).

Tick Surveillance

Of the 49 tick species recorded in California (Furman and Loomis 1984), only four species in the Ixodidae family (comprised of the hard ticks, *Dermacentor andersoni* Stiles, *D. occidentalis* Marx, *D. variabilis* Say, and *Ixodes pacificus* Cooley and Kohl), and two species in the Argasidae family (comprised of the soft ticks, *Ornithodoros coriaceus* Koch and *O. hermsi* Wheeler, Herms, and Meyer), are known to transmit pathogenic agents to humans. Before the western black-legged tick, *I. pacificus*, was identified as the vector of *Borrelia burgdorferi* in 1986 (Lane and Burgdorfer 1987), it was not known to be an important vector for transmitting human pathogens in California. Hence, there was no active survey to collect *I. pacificus* in the early 1900s. Most of the early records of *I. pacificus* documented in the VBDS database were from the following sources: 1) published literature (Ryckman et al. 1955), 2) special research projects associated with Q fever, Colorado tick fever, and plague surveillance, 3) records from the Ticks of California (Furman and Loomis 1984), and 4) ticks removed from animals, rodents, birds or humans and documented by the VBDS. Tick collection and surveillance by flagging and dragging was not initiated until the mid 1980s. The VBDS database consists of 2,538 records representing the following ten species: *Dermacentor albipictus*, *D. andersoni*, *D. occidentalis*, *D. variabilis*, *Haemaphysalis leporispalustris*, *Ixodes angustus*, *I. pacificus*, *I. spinipalpis*, *Ornithodoros hermsi*, and *Otobius megnini*.

Ixodes pacificus was the most commonly encountered *Ixodes* species in California (Furman and Loomis 1984). Out of the 2,538 records, 2,225 are *I. pacificus*. A total of 48,719 adults, 1,687 nymphs and 1,086 larvae were identified from 70 different sources (Table 2) in 56 counties. In California, only Modoc and Alpine Counties have not reported *I. pacificus* (Figure 1). Multiple distribution locations were recorded in all counties except Mono County. The first and only record of *I. pacificus* in Mono County was two nymphs removed from the wall voids next to the nests of a woodpecker and chipmunk during follow-up investigation of a human relapsing fever case in 2003.

Table 1. Human cases of selected vector-borne diseases in California, 1989-2003.

	Tick-borne							Mosquito-borne			Flea-borne	Rodent-borne	
Year	Lyme[1]	Babesiosis[2]	HGE[2]	HME[2]	Relapsing fever[1]	RMSF[1]	Tularemia[1]	SLE[2]	WEE[2]	WNV[2]	Plague[2]	HPS[2]	Total
1989	270				N/R	2	2	29	0		0		303
1990	347				10	1	0	2	0		0		360
1991	265	1			6	0	2	1	0		0		275
1992	228	1			6	3	2	2	0		1	2	245
1993	134	1			5	0	4	3	0		0	1	148
1994	68	1		1	3	5	2	1	0		2	3	86
1995	80	0	2	0	5	0	2	0	0		2	5	96
1996	65	1	0	0	19	1	1	0	0		0	1	88
1997	154	0	1	1	7	2	4	1	0		2	2	174
1998	135	0	2	1	7	1	3	0	0		1	3	153
1999	139	0	1	0	8	1	3	0	0		0	6	158
2000	95	1	1	0	9	1	1	0	0		0	8	116
2001	93	0	1	0	7	0	1	0	0		0	0	102
2002	97	0	1	0	18	2	1	0	0	1	0	2	122
2003	82	0	0	0	5	1	1	0	0	3	0	5	97
Total	**2252**	**6**	**9**	**3**	**115**	**20**	**29**	**39**	**0**	**4**	**8**	**38**	**2523**

1= Reported cases, locale of exposure not necessarily known. Source: California Department of Health Services, Surveillance and Statistics Section

2= Reported cases where exposure is known. Source: California Department of Health Services, Vector-Borne Disease Section

HGE= human granulocytic ehrlichioses, HME= human monocytic ehrlichiosis, HPS= hantavirus pulmonary syndrome, RMSF= Rocky Mountain spotted fever, SLE= St. Louis encephalitis, WEE= western equine encephalomyelitis, WNV= West Nile virus

Table 2. Sources of *Ixodes pacificus* recovered in California.

Source[1]				
	Adults	**Nymphs**	**Larvae**	**Total records**
Badger (*taxidea taxus*)	4			1
Bear (*Ursus americanus*)	229			39
Bobcat (*Lynx rufus*)	123			21
Brush	290			3
Brush mouse (*Peromyscus boylii*)		1	1	2
CA ground squirrel (*Spermophilus beechyi*)	7	12		9
Cat	12		5	7
Chipmunk (*Tamias quadrimaculatus*)		1		1
Chipmunk Nest				1
CO2	1			1
Cottontail rabbit	1			1
Cow	31			11
Coyote (*Canis latrans*)	60			14
Deer	1623	31	46	88
Deer mouse (*Peromyscus maniculatus*)			18	8
Dog (*Canis familiaris*)	401			112
Drag	1995	1		63
Elk	1			1
Feral Pig	4			2
Flag	37318	1091	605	1223
Flag-Drag	95			2
Flag-Vegetation	3347	68		72

Table 2 is continued on following page»

Table 2, continued. Sources of *Ixodes pacificus* recovered in California.

Source[1]	Adults	Nymphs	Larvae	Total records
Flicker		1		1
Fox	27			5
Gray fox (*Urocyon cinereoargenteus*)	23			5
Hand pick	21			14
Hare	1	1	7	2
Horse	124			41
House	2			2
Human (*Homo sapiens*)	208	17		166
Island fox	7			1
Jackrabbit (*Lepus californicus*)	14	1	7	2
Jay		7		1
Junco (*Junco hyemalis*)	14	2		3
Kangaroo rat	5	1		3
Lab reared ticks	28			1
Lizard		141	198	42
Lizard (*Sceloporus graciosus*)		153	16	13
Lizard (*Sceloporus occidentalis*)		84	74	15
Meadow Vole (*Microtus californicus*)	1	3	2	4
Mole			2	1
Mouse			31	16
Mt. Lion (*Felis concolor*)	35			7
Mule	1			1
Opossum (*Didelphis virginiana*)			1	1
Pinon Mouse (*Peromyscus truei*)		6	1	2
Pocket Mouse		1		1
Quail (*Callipepla californica*)		1		1
Rabbit	1			1
Raccoon (*Procyon lotor*)		1		1
Shrew			1	1
Sparrow		15	8	11
Sparrow (*Zonotrichia atricapilla*)		2		1
Squirrel	3	6	2	5
Squirrel (*Sciurus griseus*)			1	1
Tent	1			1
Thrush (*Catharus ustulatus*)		3		3
Unknown	203	27	16	81
Vegetation	2446		8	61
Vole	1			1
Wall voids		2		1
Warbler		1		1
Weasel (*Mustela frenata*)	1			1
White-footed mouse (*Peromyscus* spp.)	1		10	4
Wild goat	2			1
Wood rat	1	1	24	8
Wood rat (*Neotoma fuscipes*)	5		2	4
Wood rat Nest		3		1
Wren		2		2
Wren (*Thryomanes bewickii*)	1			1

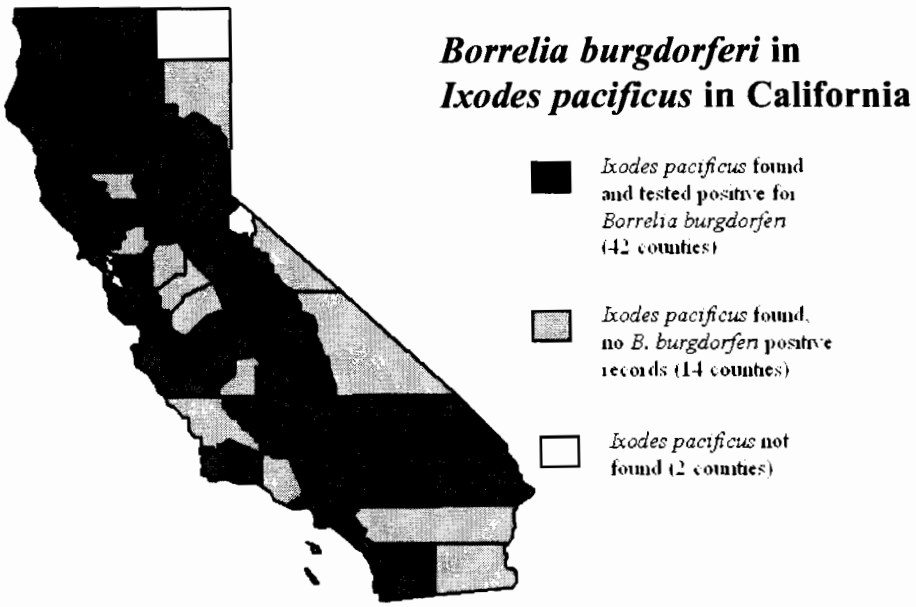

Figure 1.

The number of specimens in each record varied from one to 448 adult ticks. The most common collecting method was flagging (1,223 records), the second was removed from humans (166 records) and the third removed from dogs (112 records). Flagging is a commonly used surveillance method to collect large numbers of ticks. The VBDS database contains 37,318 adults, 1,091 nymphs and 605 larvae collected by flagging. Ticks removed from humans usually resulted in one or two specimens, however, there were three unusual records of ticks recovered from humans: nine males and twelve females were collected from Steelhead in Humboldt County in 1943, nine males and five females from Pilot Hill in El Dorado County in 1951, and five males and four females from San Rafael in Marin County in 1952. The earliest records of *I. pacificus* in the VBDS database are from 1894: two males collected from a mountain lion in San Benito County on December 13, 1894 and six males and seven females from another mountain lion in Santa Clara County on December 14, 1894. J. M. Loomis identified the ticks. Neither record identified the collector.

Tick Testing

After the discovery of LD in Lyme, Connecticut in 1977, CDHS made a concerted effort from 1989 to 1991 to study the disease in California. Three-year special funding was granted to VBDS and the Microbial Diseases Laboratory (MDL) of CDHS to establish the distribution of *I. pacificus* in each county and identify the *Borrelia burgdorferi*, the causative agent of LD in the tick population. The funding provided for two public health biologist positions in VBDS for statewide tick surveillance and for the costs of laboratory support in MDL for testing the agents. The overall purpose was to enhance the knowledge of *I. pacificus* distribution and to obtain data on the incidence of *B. burgdorferi* in the vector in order to assess the risks associated with locality, habitat, and tick populations.

Ticks collected for distribution data were accessed, cataloged, and curated. All distribution records and testing data were initially recorded in a SMARTWare® Informix Software database, converted to Ashton-Tate® dBase IV at a later date, and converted from dBase IV conversion to Excel and into a Microsoft® Access application in 1996. An annual summary of distribution data with maps was provided to the VBDS staff and the Centers for Disease Control and Prevention (CDC).

Ticks submitted to MDL for lab testing were alive and pooled (10 ticks/pool). MDL tested for *B. burgdorferi* using an indirect fluorescent antibody (IFA) and confirmed results by culturing spirochetes from the pooled ticks in Babour Stoenner Kelly (BSK) medium. The collection protocol and testing criteria for LD surveillance were developed by the LD Surveillance Coordinator during 1989-1991. Three hundred ticks sampled per site and ten pools per designated county were recommended in order to obtain estimates of percent infectivity. A total of 2,474 pools from 38 counties were tested by MDL in the three-year study period; evidence of *B. burgdorferi* by culture was identified in 54 pools from 31 counties.

After the special funding ended in 1991, VBDS has turned to outside collaborating laboratories to test ticks for *Borrelia* spirochetes. These laboratories use different testing techniques (culture, direct fluorescent antibody [DFA], IFA, and PCR), that vary in specificity or sensitivity for *B. burgdorferi*. The collaborations have resulted in identification of infected ticks from additional counties. From 1987 to 2001, the Rocky Mountain Laboratory of the National Institutes of Health tested 377 pools of *I. pacificus*; evidence of *B. burgdorferi* was identified from 13 pools by IFA and seven pools by culture in Butte, Glenn, Los Angeles,

Mariposa, Plumas, Tehama, Tulare, and Yolo Counties. From 1991 to 2003, the U.S. Army Center for Health Promotion & Preventive Medicine-West tested 535 pools of *I. pacificus;* evidence of *B. burgdorferi* was identified in 14 pools by IFA or polymerase chain reaction (PCR) from Monterey, San Diego, Santa Barbara, Sonoma and Tulare Counties. From 1995 to 1998, Butte County Mosquito and Vector District tested 550 pools; evidence of *B. burgdorferi* was identified in 12 pools by IFA from Mariposa, Shasta, Sierra, Tehama, and Yuba Counties. From 2001 to 2003, Washoe County Environmental Health of Nevada tested 1,162 pools of *I. pacificus*; evidence of *B. burgdorferi* was identified by IFA in 38 pools from Butte, Del Norte and Shasta Counties. One record in 1991 is an adult tick that was IFA-positive when tested at Orange County Vector Control District and confirmed culture-positive for *B. burgdorferi* by MDL.

Statewide Database

In November 2001, VBDS requested local agencies to participate in creating a statewide database for tick surveillance for *B. burgdorferi.* The information collected from Alameda County Vector Control Services District, Contra Costa County Mosquito and Vector District, Los Angeles County West Vector Control District, Placer County Public Health Laboratory, Sacramento/Yolo Mosquito and Vector Control District, and San Diego County Environmental Health was incorporated into the VBDS database. These laboratories also use a variety of techniques to test for *B. burgdorferi* in ticks. Individual laboratories commonly "pool" or group ticks together from one to ten ticks per pool. A complete listing of all the *I. pacificus* ticks tested for *B. burgdorferi* by county and by laboratory is shown in Table 3. Specimens were collected and tested for *B. burgdorferi* from 46 counties; evidence of *B. burgdorferi* was identified in 40 counties. A total of 27,417 *I. pacificus* in 8,268 pools were tested. Adult ticks comprised 98% of the testing records with 26,786 adults collected from 46 counties in 7,962 pools. Ticks positive for *B. burgdorferi* were identified from 40 counties in 242 pools; the minimum infection prevalence was 0.9%. Only 631 nymphs in 306 pools from Humboldt, Mendocino, Monterey, Nevada, Placer, Santa Barbara, Santa Cruz, Shasta, Sonoma, and Yolo Counties were tested for *B. burgdorferi.* Evidence of *B. burgdorferi* infection was identified in 38 pools from Mendocino and Yolo Counties only; the minimum infection prevalence statewide in nymphs was 6%, much higher than in adults. The overall minimum infection prevalence for both adults and nymphs was 1.02%.

VBDS has no records of specimens submitted for testing from Marin and Sutter Counties, however, positive *B. burgdorferi* ticks have been identified from *I. pacificus* from Marin (Burgdorfer et al. 1985, Lane 1992) and Sutter (Wright et al. 2003) counties. These two additions make a total of 42 counties in California that have evidence of *B. burgdorferi* infection in ticks (Figure 1). The testing results for *I. pacificus* contained in the tick database may be found on the CDHS website under the Detection of the Lyme_Disease Agent in California Ticks: http://dhs.ca.gov/ps/dcdc/disb/pdf/tick%20map2.pdf.

Other Aspects of the Surveillance Program

In 1992, VBDS entered into a Cost-Share Agreement with the Pacific Southwest Region of the United States Department of Agriculture Forest Service to maintain cooperative surveillance and control of vector-borne diseases within the National Forests. In accordance with the agreement, VBDS has conducted tick surveillance for LD in Angeles, Cleveland, Inyo, Klamath, Lassen, Los Padres, Mendocino, Plumas, San Bernardino, Sequoia, Shasta, Sierra, Six Rivers, Tahoe, and Trinity National Forests. Evidence of *B. burgdorferi* was identified in Klamath, Lassen, Plumas, Shasta, Six Rivers, Sierra, and Tahoe National Forests. All surveillance activities are published in the VBDS United State National Forest Annual Report and theVBDS Annual Report.

Knowledge of tick distribution and infection with *B. burgdorferi* has prompted VBDS to expand their tick-borne disease information program. The purpose of the program is to provide information on tick-borne diseases to the public, physicians, and government agencies in California. Recent activities included updating the "Lyme Disease in California" brochure, releasing press announcements about tick awareness two times a year to coincide with increased adult and nymphal tick activity, writing and distributing radio public service announcements, posting the tick testing data on the CDHS website, and giving presentations to the public and physician groups as well as state agencies. Specific activities for physician education included publication of two epidemiology updates in the California Medical Board's *Action Alert* newsletter, organizing a public health grand rounds for public health agencies, and surveying physician awareness of tick-borne diseases via a questionnaire in the *Action Alert.* VBDS receives input and advice on the tick- borne disease education from a nine-member Lyme Disease Advisory Committee (LDAC), created in 2000. The mission of the committee is to "make recommendations to the California Department of Health Services on strategies to enhance the awareness of the public and the medical community about Lyme disease in California, and thereby reduce exposure to, and suffering from, this and other tick-borne diseases."

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Table 3. Ixodes Pacificus ticks tested for Borrelia burgdorferi, California, 1985 - 2003.

County	#Ticks	#Pools	Positive					Testing Laboratories	Minimum Infection Prevalence
			Culture	DFA	IFA	PCR	Total		
Alameda	839	266	3		6		9	APHL,CDHS, SPHL, UCB	1.07%
Amador	47	5	1				1	CDHS	2.12%
Butte	766	504	1		27		28	CDHS, RML, WCEH	3.65%
Calaveras	44	4	1				1	CDHS	2.27%
Contra Costa	1504	1152			24		24	CCMVCD, CDHS	1.60%
Del Norte	125	35	2		1		3	CDHS, WCEH	2.40%
El Dorado	112	11	1				1	CDHS	0.89%
Fresno	84	49	1				1	CDHS, RML, USARMY	1.19%
Glenn	84	12	2				2	RML	2.38%
*Humboldt**	671	454	6				6	CDHS	0.89%
Inyo	22	17					0	RML, UCB	0
Kern	206	26	2				2	CDHS, RML, USARMY	0.97%
Lake	278	99	11		9		11	CDHS, Sac/Yolo MVCD	3.96%
Los Angeles	5769	862	5	14			19	PPHL, RML, OrangeVCD, USARMY	0.33%
Madera	252	27	1				1	CDHS, RML, USARMY	0.04%
Mariposa	518	287	4	5			9	BMVCD, CDHS, RML	1.73%
*Mendocino**	1104	474	45				45	CDHS, UCB	4.08%
*Monterey**	387	70			1		1	CDHS, USARMY	0.26%
Napa	209	108	2				2	CDHS	0.96%
*Nevada**	570	62	3		3		6	CDHS, PPHL	1.05%
Orange	364	73	1				1	CDHS, Orange VCD	0.27%
*Placer**	740	131	4				4	CDHS, Sac/Yolo MVCD, USARMY	0.54%
Plumas	154	118			1		1	BMVCD, RML	0.65%
Riverside	1691	475					0	CDHS, RML, USARMY	0
Sacramento	1392	206	6	2	2		10	CDHS, Sac/Yolo MVCD	0.72%
San Benito	258	113	1				1	CDHS	0.39%
San Bernardino	342	71	2				2	CDHS, USARMY	0.58%
San Diego	594	185	5		19		24	SDEH, USARMY	4.04%
San Joaquin	54	18					0	Sac/Yolo MVCD	0
San Luis Obispo	944	188					0	CDHS, SLO HD, USARMY	0
San Mateo	160	40	3				3	CDHS	1.88%
*Santa Barbara**	1079	318				2	2	CDHS, RML, USARMY, SbLab	0.19%
Santa Clara	54	5	1				1	CDHS	1.85%
*Santa Cruz**	234	68	1				1	CDHS, USARMY	0.43%
*Shasta**	1166	831	1		16		17	BMVCD, CDHS, RML, WCEH	1.46%
Sierra	93	23			4		4	BMVCD	4.30%
Siskiyou	114	11	1				1	CDHS	0.88%
Solano	121	75					0	CDHS, NY Med College	0
*Sonoma**	985	224	9			2	11	CDHS, USARMY	1.12%
Tehama	243	24	1		1		2	CDHS, BMVCD, RML	0.82%
Trinity	900	177	2				2	CDHS, USARMY	0.22%
Tulare	462	126	2			1	3	CDHS, RML, USARMY, WCEH	0.65%
Tuolumne	130	31	2				2	CDHS	1.54%
Ventura	355	55					0	CDHS	0
*Yolo**	1121	140	5	4	8	2	14	CDHS, RML, Sac/Yolo MVCD	1.25%
Yuba	76	18			2		2	BMVCD, CDHS	2.63%
Total	**27417**	**8268**	**138**	**25**	**124**	**7**	**280**		**1.02%**

APHL=Alameda Co. Public Health Laboratory
BMVCD=Butte Co. Mosquito & Vector District
CDHS=California Department of Health Services
CCMVCD=Contra Costa Co. Mosquito & Vector District
ConnAg=Connecticut Agriculture Experiment Station
NY Med College=New York Medical College
Orange VCD=Orange Co. Vector Control District
PPHL=Placer Co. Public Health Laboratory
RML=Rocky Mountain Lab, National Institute of Health
Sac/Yolo MVCD=Sac/Yolo Mosquito & Vector Control
SbLab=Santa Barbara Co. Laboratory
SDEH=San Diego Co. Environmental Health
SLO HD=San Luis Obispo Co. Health Department
SPHL=Sonoma Co. Public Health Laboratory
UCB=University of Berkeley
WCEH=Washoe Co. Environmental Health, Nevada
USARMY=US Army Center for Health Promotion & Preventive Medicine-West
DFA=direct fluorescent antibody IFA=indirect fluorescent antibody PCR=polymerase chain reaction
* nymphal ticks were tested

Lake, Sacramento and Yolo counties: multiple tests were performed on the tick pools, the same tick pool may be positive by one, two or three methods, the total numbers of positive were adjusted (underlined) to reflect the true infection prevalance

Vector Control Program; San Diego County Environmental Health; Santa Barbara Coastal Vector Control District; Santa Cruz County Environmental Health; Sonoma County Public Health Laboratory; University of California, Berkeley; U. S. Army Center for Health Promotion and Preventive Medicine-West; and the Washoe County Environmental Health of Nevada.

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