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PROCEEDINGS AND PAPERS

Volume 74 ..... January 29 - February 1, 2006

CONTENTS

Conference Dedication

Proceedings

Symposium: Invasion of California by West Nile Virus: Year 3

Symposium: Use of Rapid Antigen Detection Tests (RAMP/VecTest) for Surveillance of of West Nile Virus in Wild Birds and Mosquito Pools: Issues and Answers for 2005 and 2006

Surveillance

Biology/Ecology

Operational Strategies

Poster Presentations

The William C. Reeves New Investigator Award Competition

Submitted Papers

Guidelines for Contributors

Dedication of the Seventy-Fourth Annual MVCAC Conference to Patricia Gillies September 23, 1929 - May 11, 2004

Charles Myers

California Department of Health Services

The 2006 Annual Conference of the MVCAC is dedicated in memory of Patricia A. (Myers) Gillies in recognition of her many years of service to mosquito and vector control. Pat passed away in Fresno, California on May 11, 2004.

Pat was born in Berkeley, California on September 23, 1929. She grew up in Albany and graduated from El Cerrito High School. She also attended San Jose State College where she met and married Robert Gillies. They had two daughters, Catherine and Coila. Pat continued her education at Fresno State College, earning a secondary teaching credential and B.A. and M.A. degrees in Biology.

Following brief stints as a teacher, working at Fresno State and for the USDA, Pat embarked on a 33-year career as a Public Health Biologist with the California Department of Health Services, Bureau of Vector Control. During her long and varied career with DHS, Pat became best known for her work on pesticide resistance in medically important insects. She published numerous scientific papers in journals of a number of national and international professional societies of which she was a member.

Pat's commitment to mosquito control in California led to her appointment and service as Fresno County's trustee representative on the Consolidated Mosquito Abatement District's Board of Trustees in 1974. She served for six years until her DHS position moved her to Sacramento in 1980. Upon returning to Fresno in 1985 she was reappointed to the Board and served until her death. Pat was a dedicated trustee and was very supportive of the District employees.

The MVCAC recognized Pat for her exceptional, distinguished service in the interest of California mosquito and vector control by conferring Honorary Membership in 2002.

Pat Gillies should be remembered as a pioneer who blazed a trail in the masculine dominated field of mosquito and vector research and control that allowed easier access for women who followed.

In Memoriam
Glenn Yoshimura

[1]Stan Wright, [1]David Brown, [2]Minoo Madon, and [3]Lal Mian

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Glenn Yoshimura was born on September 8, 1947, in Hilo, Hawaii. He earned his BS degree in Entomology from the University of Hawaii, and MA in Entomology from Fresno State University. He served in the U.S. Army as a medic in both the States and in Vietnam. His professional employment included Research Associate at California State Public Health Laboratory, and positions as an Entomologist at both Kern MVCD and Sacramento-Yolo MVCD. In addition to his responsibilities at the Districts, he also served as Editor of the Proceedings and Papers of the Mosquito and Vector Control Association of California. He reviewed numerous research papers for the Journal of American Mosquito Control Association, and he co-authored Mosquitoes of Northern California.

Often a person's life is defined by such academic and professional accomplishments. However, Glenn's true worth can be far better defined in a more lasting manner by the ways he touched, guided and influenced others' lives. According to Glenn's mother he was a born naturalist. His interests existed very early and ranged through all of natural history but he especially loved insects. She recalls that Glenn expressed his fascination with the natural world very early in his childhood by raising tropical fish in an abandoned washing machine. Beyond insects and fish Glenn had interests in dog breeds and went to dog shows, he liked hiking and participated in nature tours and bird watching and enjoyed photographing nature. He also had geological interests in minerals and crystal formation, Native Americans and wildlife art. He of course collected insect specimens but also wildlife paintings, contemporary Native American art, geodes, fossils, rocks and minerals.

One of Glenn's most enduring attributes was that he gave most generously of himself. He was always on the lookout for an opportunity to share his skills and knowledge of science. He enjoyed mentoring, but his style of teaching required one to think. He didn't just step forward to solve a problem, even when he had an answer, but rather he guided you to come up with the correct answer yourself. This was often frustrating but ultimately would teach reasoning and problem solving.

Glenn had an impeccable memory. He could recall chemical names, citations including author and year of publication, or recite the diagnostic characters of his beloved insects or other invertebrates without even a hesitation for recall. He was an acknowledged expert at mosquito and flea identification along with most other common insects, and even other invertebrates such as fairy shrimps. Glenn was an encyclopedic resource and people would often go to him rather than consult the biological abstracts for supportive research.

His skills at editing manuscripts were astonishing - his spelling and grammar flawless, his knowledge of style and conventions always accurate and up-to-date and his analytical ability unrivaled.

Despite Glenn's many skills and abilities he was always very modest. He would readily share ideas, give direction and polish another's work without expectation of or often even the acceptance of acknowledgement. His analytical thinking has built the foundation of many projects, programs and publications in mosquito control and research. His contributions to mosquito control through his work on insecticide resistance and efficacy will stand the test of time. His field skills in mosquito sampling, recognition of mosquito habitat and his almost unnatural eye for mosquito-breeding sources will be painfully missed.

As a reviewer for the Proceedings & Papers of MVCAC, Glenn was extremely reliable and was always the first to respond with his impeccable editing style, grammar and checking references cited, paying attention to every minute detail. Glenn was an invaluable resource to the professional community.

Glenn passed away October 28th, 2005. Glenn was a dear friend to the many folks that knew him. Glenn will be missed in many ways as he touched numerous lives. He will be remembered for his gift of giving as much as his great learning and many academic accomplishments.

As a friend, colleague, editor and reviewer of the Proceedings & Papers of MVCAC, Glenn will be dearly missed!

Introduction

William K. Reisen

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West Nile virus invaded the southeastern deserts and then Los Angeles during 2003 (Reisen et al. 2004), successfully over wintered, and then amplified rapidly leading to epidemics in Riverside, San Bernardino and Los Angeles during 2004 (Hom et al. 2005). In 2004, virus traversed the Tehachapi Mountains, leading to epidemic transmission in the southern San Joaquin Valley centered in Bakersfield (Takahashi et al. 2005). By year's end, WNV was detected within every county of California. During 2005, WNV activity in southern California generally subsided as the epicenter moved north to the Sacramento area (Fig. 1). The largest numbers of human cases were recorded in Sacramento County, and this led to a large scale emergency adulticide application to interrupt transmission.

The Center for Vectorborne Diseases at the University of California, Davis, in collaboration with the Coachella Valley, Greater Los Angeles County, Kern, and Sacramento-Yolo Mosquito and Vector Control Districts and the California Department of Health Services have been studying the invasion of California by WNV collaboratively since 2003. At the 2004 and 2005 annual meetings of the Mosquito and Vector Control Association of California, we summarized our findings during the first and second years of this invasion and described selected aspects of the ecology of transmission. Our symposium during 2005 focused on the epidemic in Los Angeles and invasion of the Central Valley (Reisen 2005). The current symposium describes our research during the third year

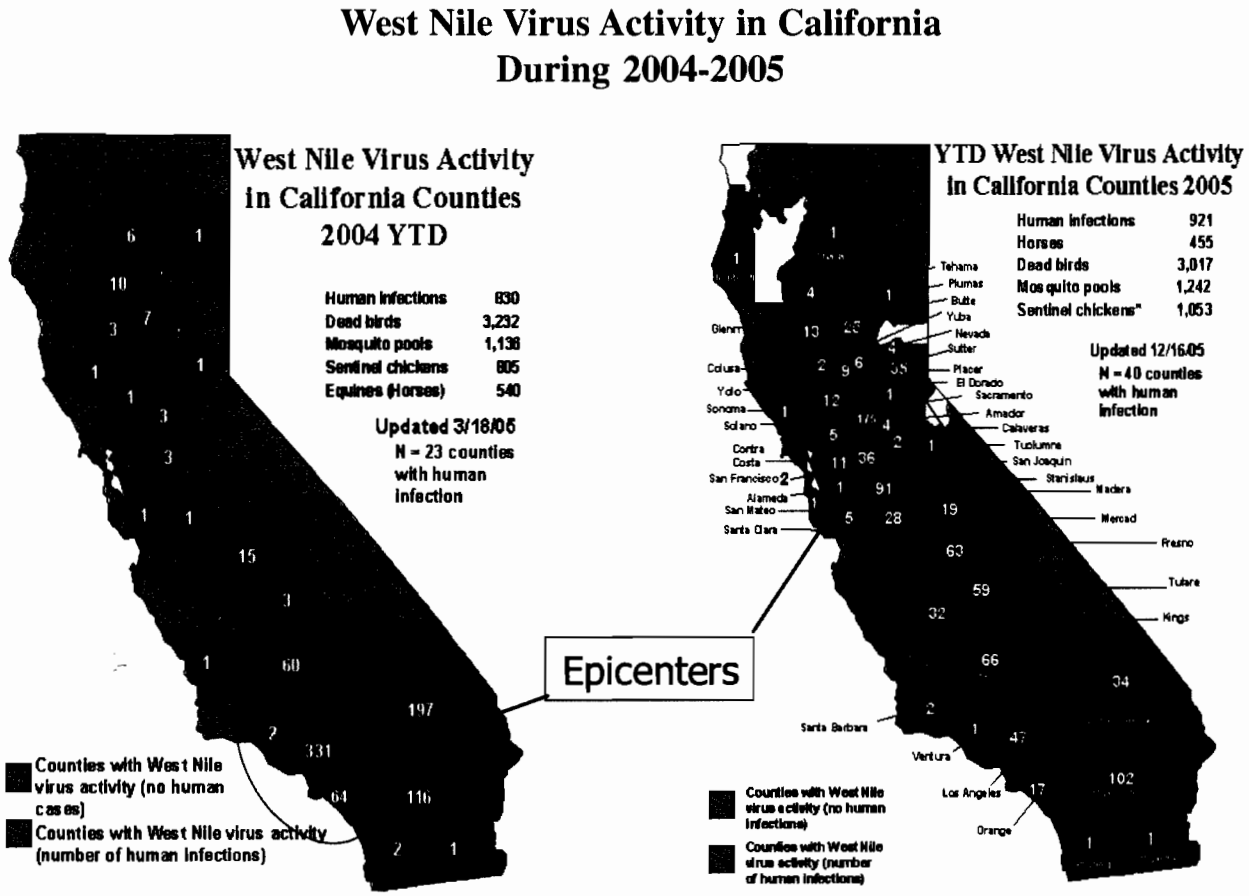

Figure 1. Summary of West Nile virus activity during 2004 and 2005 [maps from California Department of Health Services website http://westnile.ca.gov/].

of the invasion, focusing on factors enabling the success of this invading virus. The titles of the talks presented and the speakers are listed below.

- Southeastern California, Hugh Lothrop
- Los Angeles, J. Wilson
- Kern County, B.D. Carroll
- Rural transmission among free ranging birds in Sacramento County, S. Wright
- Epidemic amplification in Sacramento and Yolo Counties, Dia-Eldin Elnaiem
- Impact of temperature on West Nile virus transmission, W.K. Reisen
- Interactions with St. Louis encephalitis virus – partial cross protective immunity in House finches, Y. Fang
- Field evidence for vertical transmission, W.K. Reisen
- Role of Corvids, C.B. Barker
- Role of Ardeid birds, S.S. Wheeler
- Conclusions, W.K. Reisen

Acknowledgements

This research described in our symposium was funded by grants from the National Institutes of Allergy and Infectious Diseases, NIH, Centers for Disease Control and Prevention, Office of Global Programs, NOAA, California Department of Health Services, and the University-wide Mosquito Research Program. Additional funds, research space and logistical support were generously provided by the Coachella Valley MVCD, Greater Los Angeles Co VCD, Kern MVCD and the Sacramento/Yolo MVCD who hosted our field projects. Mosquito-pools were tested at the Center for Vectorborne Diseases (CVEC), University of California, Davis, by Marzieh Shafii, Nicole Kahl, Sira Ashtari and Kara Kelley (Sac/Yolo MVCD) under the direction of Barbara Cahoon-Young using multiplex technology developed by Aaron Brault at CVEC. Dead birds were necropsied by the California Animal Health and Food Safety laboratories in San Bernardino and Davis under the direction of Leslie Woods, with dead bird tissues tested at CVEC. Avian sera were tested by Kara Kelley (Sac/Yolo MVCD), Sandra Garcia and Ying Fang (CVEC). Sentinel chicken sera were tested by the CDHS Viral and Rickettsial Disease Laboratory under the direction of C. Cossen. Data on horse or human cases were provided by County Public Health Departments as well as by Cynthia Jean, CDHS.

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West Nile Virus Surveillance in the Imperial and Coachella Valleys, 2005

Hugh D. Lothrop[1], Marc Kensington[1], Arturo Gutierrez[2], Branka B. Lothrop[2] and William K. Reisen[1]

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GEOGRAPHY

Arbovirus surveillance in the Coachella and Imperial Valleys is a collaborative effort by the California Department of Health Services (CDHS), 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 positions of the two valleys relative to the Salton Sea can be seen in Figure 1. Darkened areas located in the northwest portion of the Coachella Valley, and scattered throughout the Imperial Valley, are created by dense road grids in residential and urban areas. Salt marshes and managed wetlands along the margin of the Salton Sea in both valleys historically have been productive sources for *Culex tarsalis* Coquillett. In the Coachella Valley these marshes and wetlands continue to be annual foci of arbovirus transmission (Reisen, et al. 1995); however, in the Imperial Valley these features have not been shown to be perennial foci for arbovirus activity (Lothrop, et al. 1994). West Nile virus was detected initially in Imperial County at or near these wetland sites during 2003 (Reisen et al. 2004).

SURVEILLANCE METHODS

Surveillance in the Coachella Valley consisted of 9 flocks of 10 chickens each with 2 corresponding CO_2-baited CDC style traps (EVS traps), a grid of 40 EVS traps around the northern shore of the Salton Sea, 16 gravid traps located in urban areas including Mecca and upland communities, and the DHS dead bird surveillance program. Surveillance in the Imperial Valley was divided between the Imperial County Health Department, with 4 flocks located at Seeley, El Centro, Brawley, and Holtville, and CVEC and CVMVCD, with 3 flocks located along the southern margin of the Salton Sea. Chickens maintained by CVEC and CVMVCD were replaced when they seroconverted. Selection of these sites was based upon historical arbovirus activity and juxtaposition with human populations. Flocks and EVS traps were sampled biweekly from March through November. Mosquitoes were identified, enumerated by species, pooled and sent to CVEC for virus testing.

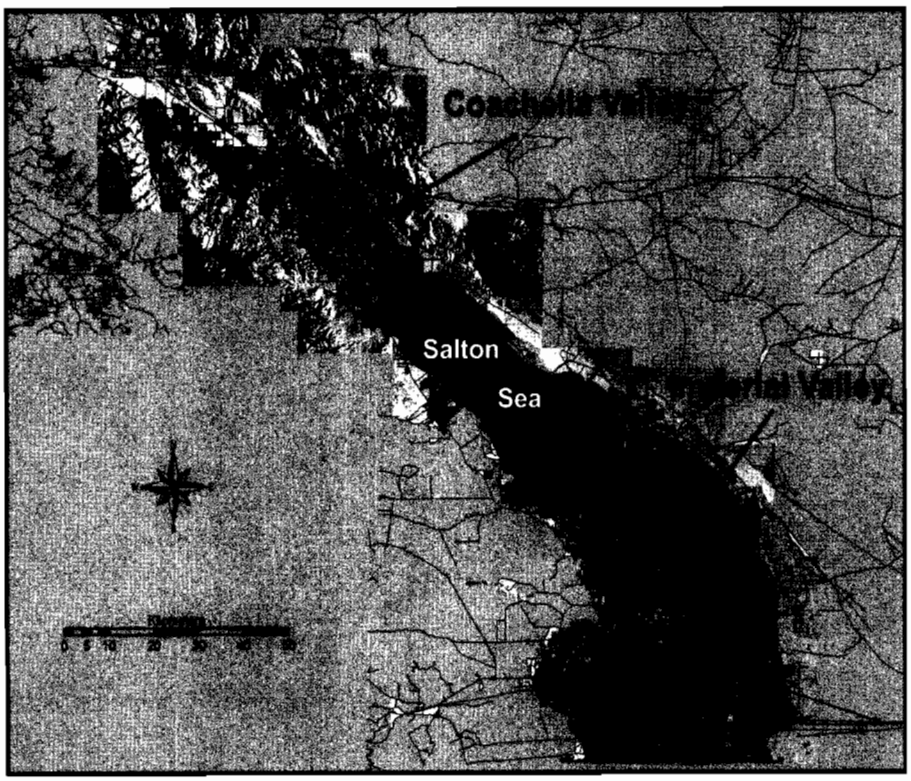

Figure 1. The Salton Sea basin showing the position of the Coachella and Imperial Valleys.

CHRONOLOGY

The first detection of WN virus in the Coachella Valley was found in 2 pools of *Cx. tarsalis* collected in EVS traps near North Shore on 29 May (Fig. 2). This site has been the historical early season focus of Saint Louis encephalitis virus and was the site of the first detection of WNV during 2004. In early June, one chicken near Indio seroconverted, followed in late June by five positive pools of *Culex quinquefasciatus* Say in Indio, Rancho Mirage, and Indian Wells, as well as two positive pools of *Cx.tarsalis* at Mecca and along the northern shore of the Salton Sea. During early to mid July, chickens continued to seroconvert in the lower valley (Indio, Thermal, and Mecca) and along the Salton Sea (4 flocks), with only one positive *Cx. tarsalis* pool collected near Mecca. During the same period 25 positive pools of *Cx. quinquefasciatus* were collected in Rancho Mirage, Indian Wells, and Palm Desert with chickens seroconverting in Palm Springs and Palm Desert. The first positive dead bird was a house finch submitted for testing on 25 July from Cathedral City, followed by a positive American crow from Palm Springs on 8 August. In early August, 2 positive pools of *Cx. tarsalis* were collected near North Shore and 4 positive pools of *Cx. quinquefasciatus* were collected in Cathedral City, Rancho Mirage, and Palm Desert, with a chicken seroconverting at Palm Springs. The July increase in enzootic transmission was associated with the appearance of human cases from Desert Hot Springs and from Rancho Mirage in early August. At mid August chickens had seroconverted at Palm Springs, Indio, Mecca, and along the Shore of the Salton Sea near Mecca. Another human case was reported from Indian Wells during this period. In late August one positive pool of *Cx. quinquefasciatus* was collected in Palm Desert and chickens had seroconverted at Palm Springs, Palm Desert, Indio, Mecca, near Mecca along the Salton Sea and the west shore. The third positive dead bird, a captive flamingo from Palm Desert, was submitted on September 1. During the first half of September, 2 positive pools of *Cx. quinquefasciatus* were collected from Palm Desert and Indian Wells while 13 positive pools of *Cx. tarsalis* were collected along the Salton Sea south of Mecca and the west shore. During this period a human case was reported from Palm Desert and chickens had seroconverted in Palm Springs, Palm Desert, Indio, and North Shore. In the second half of September one human case was reported from Palm Springs. During this period 1 positive pool of *Cx. quinquefasciatus* was collected west of Indio and 8 positive pools of *Cx. tarsalis* were collected from the area south and east of Mecca along the margin of the sea. At the same time chickens had seroconverted in Palm Springs and along the northern shore of the Salton Sea. In early October 1 positive pool of *Cx. quinquefasciatus* was collected in Indio and chickens had seroconverted in Mecca and south of Mecca along the west shore of the Salton Sea. In early November the last 2 positive pools of *Cx. tarsalis* were collected west of Mecca and along the northern margin of the Salton Sea. At this time, chickens had seroconverted along the northern margin and west shore of the Salton Sea. The last chicken had seroconverted at North Shore in late November.

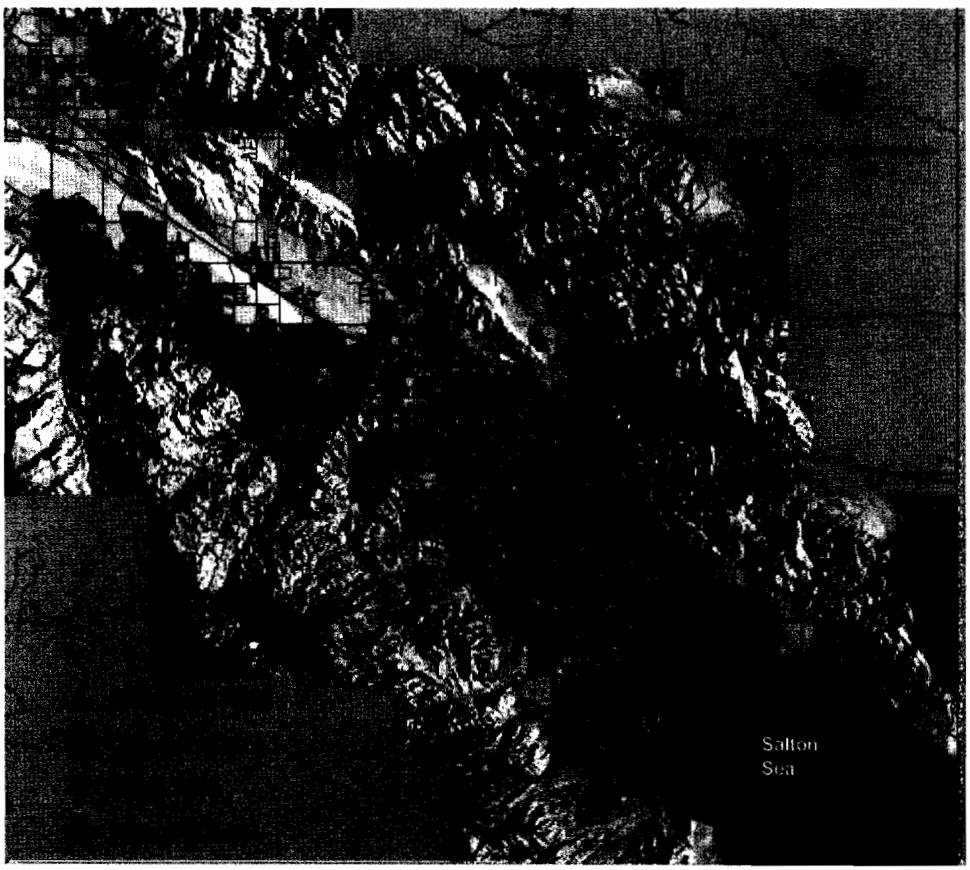

Figure 2. Coachella Valley surveillance. All symbols indicate WNV positive sites.

In the Imperial Valley, WNV was detected first at the Sonny Bono National Wildlife Refuge (SBNWR) on 25 May (Fig. 3) in 1 pool of *Cx. tarsalis*. Following this, WNV was not detected until early July when chickens seroconverted at Westmorland and Wister Wildlife Refuge (WWR), followed by chickens at Seeley in late July. In early August 1 positive pool of *Cx. tarsalis* was collected at SBNWR and 1 positive pool of *Cx. erythrothorax* Dyar was collected north of El Centro. During this period chickens seroconverted at SBNWR, Seeley, El Centro, Holtville, and Westmorland. In late August 3 positive pools of Cx. tarsalis were collected from WWR and 1 from Seeley. During this period chickens had seroconverted at WWR, Seeley, Holtville, Westmorland, and Brawley. In early September 1 positive pool was collected at WWR and chickens had seroconverted at SBNWR, Holtville, Brawley, and El Centro. In late September 2 positive pools were collected at Westmorland and SBNWR and chickens had seroconverted at El Centro and SBNWR. In early October chickens had seroconverted at the same sites. West Nile virus activity was last detected in late October in seroconverted chickens at Westmorland and SBNWR.

SUMMARY

In the Coachella Valley, WNV was first detected at the end of March 2005, more than a month later than in 2004, but again in pools of *Cx. tarsalis* at the same North Shore focus. Shortly thereafter, WNV appeared in *Cx. quinquefasciatus* in residential neighborhoods in the upper valley, contrasting with 2004 when positive pools of Cx. quinquefasciatus were not found in the upper valley until August (Lothrop et al. 2004). The level of activity throughout the valley remained low through June, and was detected in both *Cx. tarsalis* and *Cx. quinquefasciatus*. During this period only 1 chicken at Indio seroconverted. The highest infection rate (IR)(Biggerstaff 2003) in *Cx. quinquefasciatus* appeared in July (Fig. 4) at the a time of maximum temperatures but lowest relative abundance. Interestingly, scattered human cases began to appear in August while abundance remained low and *Cx. quinquefasciatus* IR declined. Two more human cases occurred in September as *Cx. quinquefasciatus* abundance rose slightly and IR declined. Throughout the season in the lower valley, the abundance of *Cx. tarsalis* was higher (Fig. 5) than *Cx. quinquefasciatus*, but the IR was lower and no human cases were reported in areas where *Cx. tarsalis* predominated. No positive pools of *Cx. quinquefasciatus* were collected from the lower valley, although this species was locally present in that region. In urban areas there was a disconnection between chicken seroconversions and IR in *Cx. quinquefasciatus*, with higher IR in June and July and higher infections in chickens in August and September. A similar pattern was seen for human infections. High IR in *Cx. quinquefasciatus*, between 5 and 20 per 1,000, preceded human infection by more than a month, although the highest IR, of 37, was detected within 3 weeks of the first case. The lack of human cases from the lower valley, with IR of 3 or less in *Cx. tarsalis,* might be associated to the predilection of the residents there to seek medical treatment in

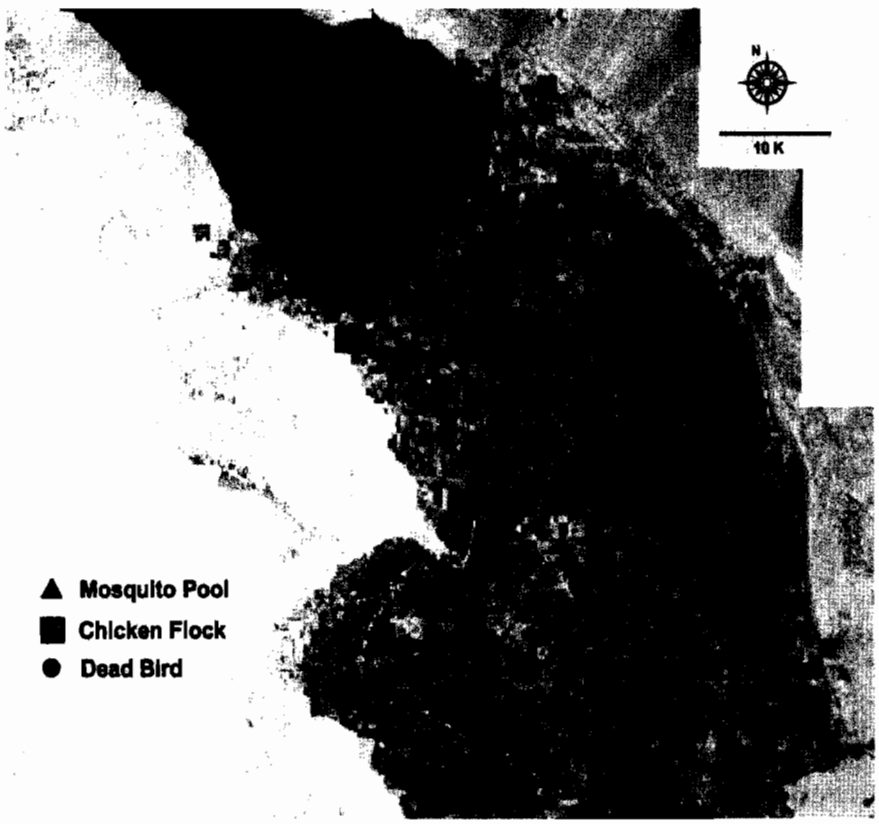

Figure 3. Imperial Valley surveillance. All symbols show WNV positive sites.

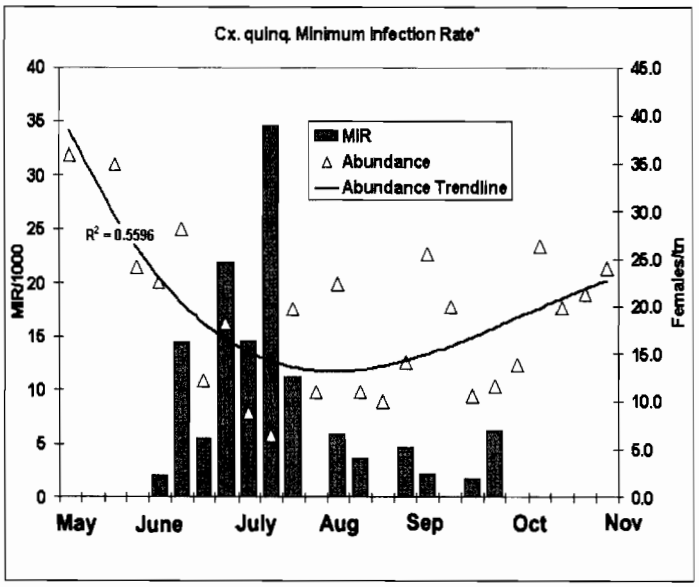

Figure 4. Minimum infection rate and females/trapnight (tn) of *Culex quinquefasciatus* in the Coachella Valley in 2005.

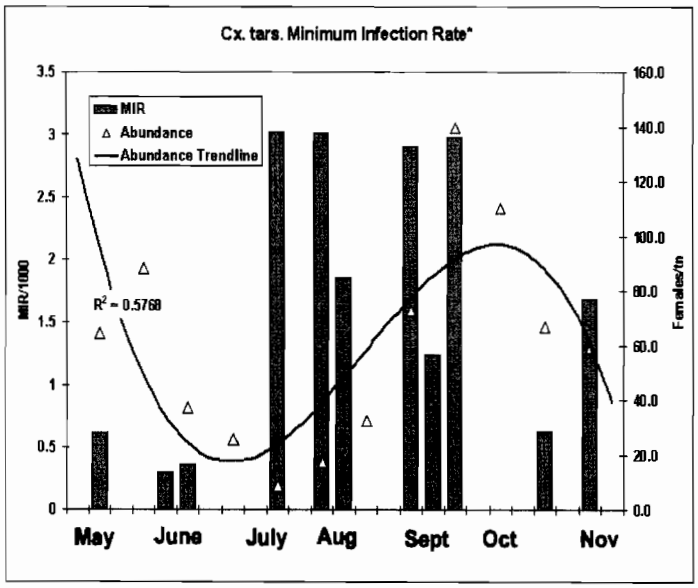

Figure 5. Minimum infection rate and females/trapnight (tn) of *Culex tarsalis* in the Coachella Valley in 2005.

Mexico. In this way local cases would go unreported. West Nile virus activity ended in November and was associated with *Cx. tarsalis* around the margin of the Salton Sea. At the end of the season, WNV had been detected at all surveillance sites throughout the valley.

In the Imperial Valley, WNV was first detected at SBNWR, the same site that it was detected last in 2004. As in the Coachella Valley, no WNV activity was detected in June. August and September were the months of highest WNV activity with seroconversions throughout the valley and the collection of 9 of 10 positive pools. Apart from the 3 sites managed by the CVMVCD along the margin of the Salton Sea, reliable mosquito pool data were lacking due to trapping failures in the rest of the valley. This leaves *Cx. quinquefasciatus* involvement in question since all but one positive pool consisted of *Cx. tarsalis*. Historically, SLEV activity has been linked with elevated *Cx. quinquefasciatus* along the New River (Work et al. 1977). West Nile virus activity was last detected along the margin of the Salton Sea in October, a month earlier than in the Coachella Valley, however the sensitivity of the surveillance was lower due to the accumulation of seroconverted chickens in the flocks not maintained by CVMVCD.

Western equine encephalomyelitis virus was detected in *Cx. tarsalis* pools from June and September in the Coachella Valley and May and June in the Imperial Valley. Chickens seroconverted in July, August and October in the Coachella Valley and June and July in the Imperial Valley. There were no positive mosquito pools or chicken seroconversions for Saint Louis encephalitis virus in either valley or the state of California during 2005. This was the third year that WNV amplification failed to attain epidemic levels, although widespread enzootic transmission was detected in both valleys.

Acknowledgements

This work was done in collaboration with the Coachella Valley Mosquito and Vector Control District, Donald Gomsi, Manager, who provided financial and logistical support. Additional funding came from the National Institutes of Health and Centers for Disease Control and Prevention. Testing of chicken sera was done by the California Department of Health Services. Mosquito pool and dead bird testing was done by the UC Davis Center for Vectorborne Diseases.

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Three Years of West Nile Virus in Greater Los Angeles County

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ABSTRACT: The 2005 season marked the third year of West Nile virus (WNV) invasion in the Greater Los Angeles area. This season showed the progression of viral transmission towards coastal regions, and the Santa Clarita valley, areas that had little viral activity in the two years prior. Conversely, areas with high levels of transmission during the 2004 season had lesser activity in 2005, which may be attributable to high antibody titers in free-ranging wild bird populations. Overall, there was a marked decrease in the numbers of WNV positive dead birds and mosquito pools reported throughout the Greater Los Angeles County Vector Control District's (GLACVCD) boundaries, which also corresponded in a human case reduction from ~200 in 2004 (7 fatal) to 29 cases (no fatalities) in 2005.

INTRODUCTION

West Nile virus (WNV) was first detected in the Greater Los Angeles area in September 2003. During this season of introduction there were a total of 25 positive dead birds and 6 WNV+ mosquito pools, all focused around the Whittier Narrows Nature Preserve crow roost and surrounding river basins. The 2004 season began in early March with the detection of antibodies in free-ranging peridomestic birds near the Whittier Narrows crow roost. This epizootic amplification continued to be detected in the surrounding communities, spreading southward towards the coast as evidenced by WNV+ dead birds and mosquito pools. By late May, WNV+ dead birds were detected in the San Fernando Valley, and infected mosquitoes were detected within a week. Both of these surveillance indicators preceeded human and horse cases in the 2004 season, whereas sentinel chicken seroconversions provided little or no early warning of disease transmission.

MATERIALS AND METHODS

Surveillance continued in the 2005 season with the monitoring of mosquito abundance using both CDC-style EVS traps baited with CO_2 and Gravid traps baited with alfalfa-yeast medium. These traps were set in transects along river basins and major highways as well as in sites established as "core" sites (Wilson et al. 2004, 2005) where sentinel chickens and wild bird sampling were also simultaneously continued. Mosquitoes were identified to species, separated by sex and submitted to the Center for Vector-borne Diseases (CVEC) at the University of California, Davis campus for screening of western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE) or WNV RNA by reverse transcriptase-polymerase chain reaction (RT-PCR). Maximum Likelihood Estimations (MLE) were calculated bi-weekly using PooledInfRate 2.0 software (Biggerstaff, 2004).

Wild Bird sampling continued bi-monthly throughout the 2005 season at seven sites using modified Australian crow traps baited with wild bird seed. Peridomestic birds were captured, banded, sampled by jugular venipuncture, withdrawal of 0.1cc blood, and released. The whole blood was diluted with 0.9cc saline (0.9% sodium chloride), centrifuged, and the sera were frozen at -70° C and shipped to CVEC for WNV, SLE, and WEE antibody screening by EIA.

Sentinel chicken samples were obtained by brachial venipuncture, samples were transferred onto filter paper and allowed to dry, then shipped to the California Department of Health Services (CDHS) Viral and Rickettsial Disease Laboratory (VRDL) for WNv, WEE, and SLE antibody screening by enzyme immuno assay (EIA) and indirect fluorescent assay (IFA). Dead birds were shipped for necropsy to the California Department of Animal Health and Food Safety (CAHFS), and subsequent tissue samples were sent to CVEC for WN viral RNA screening by RT-PCR.

Human cases were reported by the Los Angeles County Department of Health Services (LACo. DHS), Acute Communicable Disease Control (ACDC) and reported as epidemiologic information was made available. Equid cases were reported by the LACo. DHS, Veterinary Public Health.

RESULTS AND DISCUSSION

The foci of WNV activity in L.A. County during the 2005 season moved northward into the Santa Clarita area with the majority of WNV+ dead birds (73/146) and human cases (5/29). The Whittier Narrows crow roost, a previous hot spot of activity, meanwhile had no activity (Fig. 1).

The incidence of human cases sharply declined in the 2005 season, and when compared to the seroprevalence of antibodies in peridomestic birds over the 3 year period may be explained by the high numbers of non-susceptible reservoir hosts at the onset of the season. Later in the season, seroprevalence rates dropped and the human cases occurred (Fig. 2).

The abundance of *Culex quinquefasciatus* Say during the 2005 season peaked in May with 54.22 females/trap night (F/TN), MLEs peaked in August at 9.54/1,000 females. When compared to the 2004 season of widespread epidemic/ epizootic, abundance peaked later in June with 70.05 F/TN but MLEs peaked similarly in August at 26.14/1000 (Fig. 3).

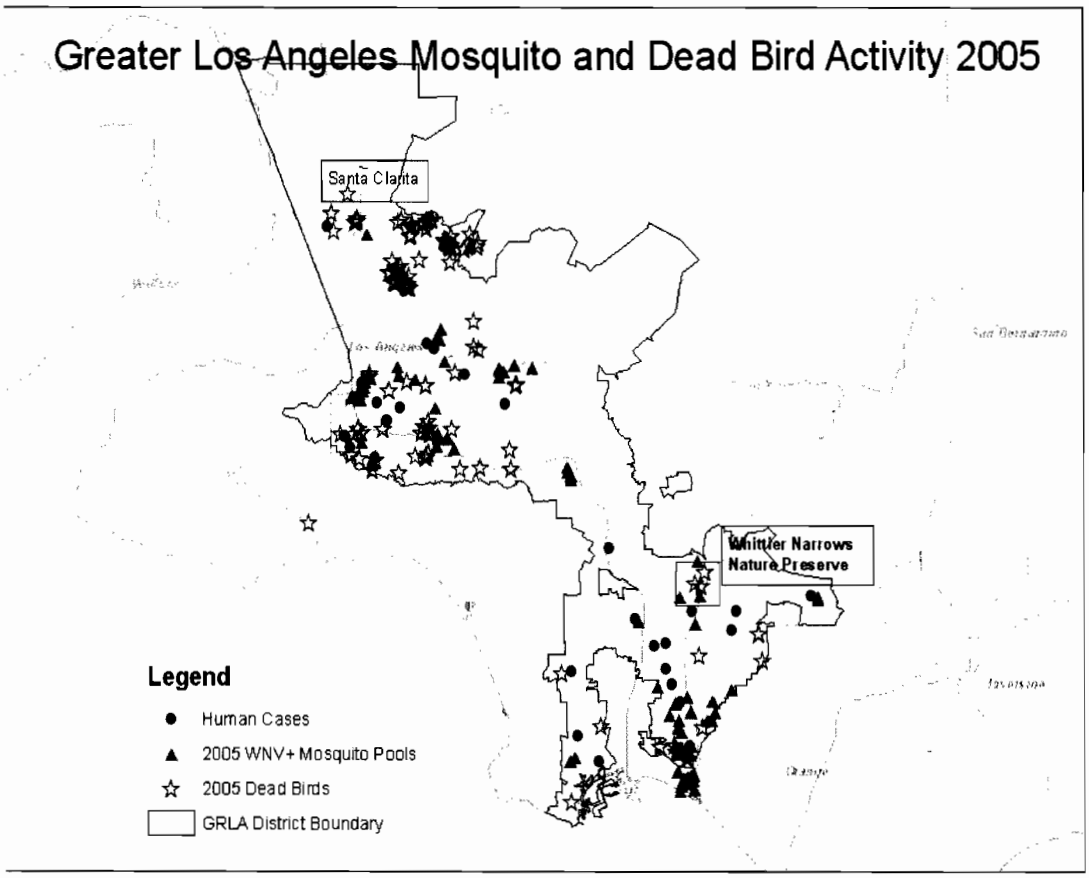

Figure 1. A map of the Greater Los Angeles County Vector Control District, with WNV activity mapped.

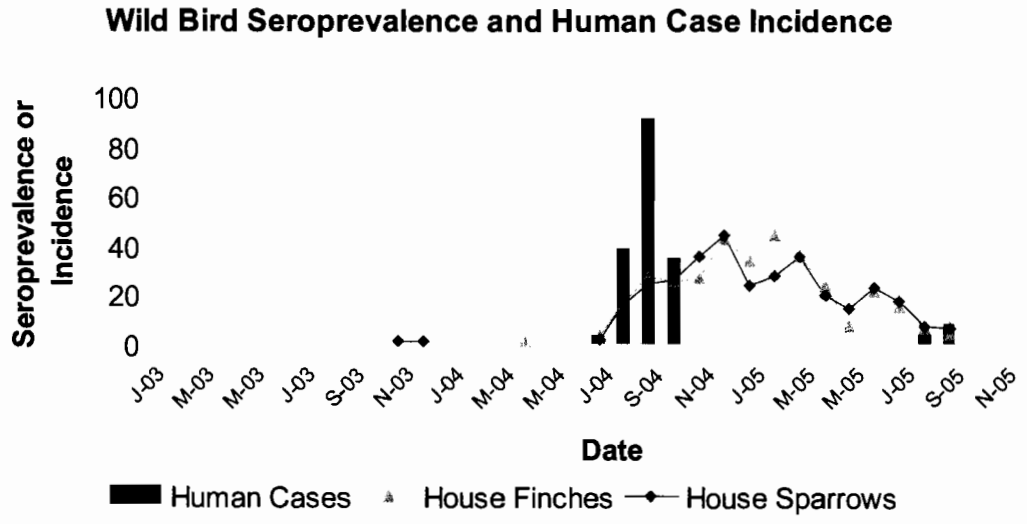

Figure 2. Data from 2003-2005 showing the incidence of human cases of WNV vs. the seroprevalence (#WNV+/ # bled) of antibodies in house sparrows and house finches.

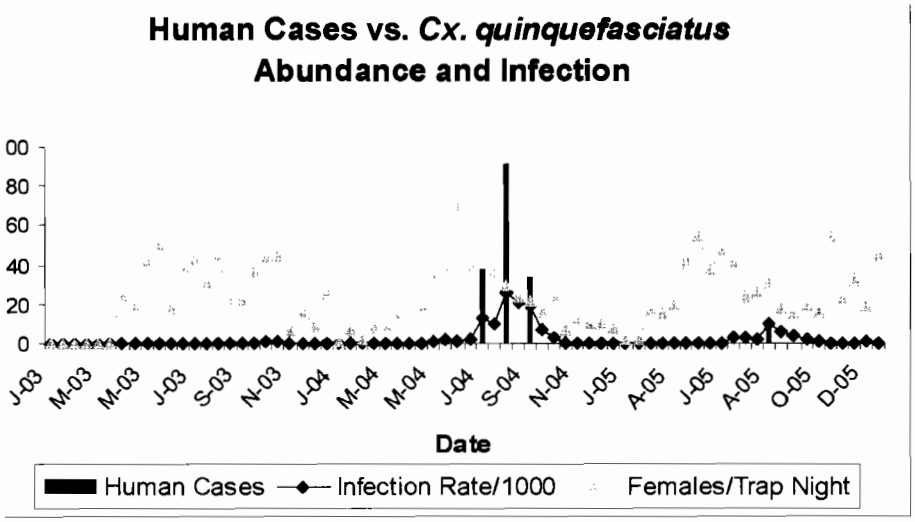

Figure 3. Data from 2003-2005 showing the incidence of human cases vs. the abundance and infection rate (MLE) of *Cx. quinquefasciatus*, the primary urban vector of WNV in Greater Los Angeles.

Of the total of 70 chickens (7 flocks) only 19 seroconverted during the 2005 season, which again were preceded by all other surveillance indicators.

CONCLUSION

After 3 years of West Nile virus activity in the Greater Los Angeles area, we have seen a pattern of introduction during the first season, amplification and transmission during the second season, and subsidence during the third season. The spread of introduction of the virus progressed from inland around the site of the Whittier Narrows crow roost, towards the coast, and then moved northwards.

When avian epizootic activity was occurring, as monitored by seroprevalence in house sparrows and house finches, as well as infection rates in *Cx. quinquefasciatus,* human cases were on the rise simultaneously. Once this transmission activity declined, we also saw a subsidence in human cases.

While 2005 was a period of epizootic/epidemic subsidence for most of the Greater Los Angeles area, human cases still occurred during period when herd immunity of peridomestic birds was low, and temperatures facilitated viral growth in the mosquito hosts.

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The Reappearance of West Nile Virus in Kern County During 2005

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ABSTRACT: West Nile Virus (WNV) reappeared in Kern County in mid-June 2005 and was detected by all surveillance methods. Human cases were detected within days of other transmission indicators. Activity during 2005 was similar to 2004, with infections detected in 66 humans, 26 equines, 121 sentinel chickens, 44 dead birds and 235 mosquito pools. In contrast to 2005, there were 412 EIA positive wild bird sera, almost three times the 157 positives detected in 2004. During this second year of virus activity WNV was found throughout the Central Valley portion of the county.

INTRODUCTION

During 2003 West Nile virus (WNV) activity was restricted to six counties south of the Tehachapi Mountains (Imperial, Los Angeles, Orange, Riverside, San Bernardino and San Diego)(Hom et al. 2004). By June 2004 there was evidence of WNV spread north of the Tehachapi mountain range and into the Bakersfield area (Takahashi et al. 2005). From there WNV quickly spread throughout the entire state and by the end of the year WNV had been detected in every county (Hom et al. 2005). The current paper discusses the reappearance of WNV in Kern County in 2005, its detection by various surveillance methods, how it spread through the county, and differences between the 2005 and 2004 surveillance years.

MATERIALS AND METHODS

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

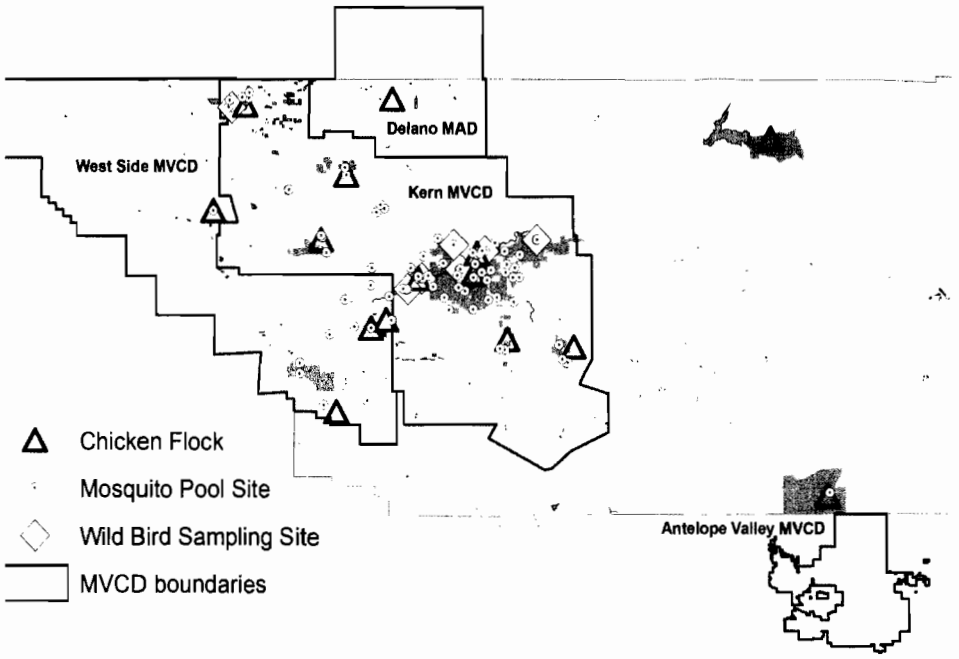

Figure 1. Surveillance sites in Kern County, 2005.

Dead Birds: Dead birds were reported by the public to the California Department of Health Services-Vectorborne Disease Surveillance hotline who forwarded pertinent information to the local vector control agency for bird pickup. Birds then were submitted to the California Animal Health and Food Safety (CAHFS) Central Laboratory at UCD for necropsy. Oral swabs and/or kidney tissue were sent to the UCD Center for Vectorborne Diseases (CVEC) laboratory for testing by singleplex reverse transcriptase-polymerase chain reaction (RT-PCR) and/or virus isolation on Vero cell culture.

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

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

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

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

RESULTS

WNV was detected initially in a dead house finch found in late February 2005 in Bakersfield. Two days later a barn owl found dead at Edwards Air Force Base tested positive. There was also a house sparrow found in late March. Based on the timing and location of these birds, there was no way to determine if these were new infections or residual RNA from chronic infections acquired the previous year.

During 2005, 235 of 1,596 mosquito pools from Kern County tested positive for WNV (Table 1). From late February through May, 2005, 1,835 *Culex erythrothorax* Dyar, 2,163 *Cx. quinquefasciatus* Say, 9,499 *Cx. tarsalis* Coquillett, and 2,032 *Aedes melanimon* Dyar were tested for virus infection in 390 pools, with negative findings. On 10 June seven pools of *Cx. quinquefasciatus* from a single gravid trap from the community of Greenfield on the southern edge of metropolitan Bakersfield tested positive for WNV. It took eleven more days for the virus to amplify and start spreading. On 21 June three more positive pools tested positive from three different sites. By 28 June 12 more pools from 5 new areas were added to the list. All of these were *Cx. tarsalis* and were collected by CDC traps. Of the six mosquito species submitted for testing, WNV was only detected in *Cx. quinquefasciatus* and *Cx. tarsalis*. Minimum infection rates per 1,000 (MIRs/1,000) for *Cx. quinquefasciatus* and *Cx. tarsalis* were ca. 2.0 during June, spiked to and remained around 11 through July and August, and then fell in September to 5 for *Cx. quinquefasciatus* and 1.1 for *Cx tarsalis*.

Table 1. Mosquito minimum infection rates in Kern County, 2005

Species	Pools	Total tested	WNV Positive	MIR/ 1000
Culex apicalis	1	5	0	0
Culex erythrothorax	50	2,079	0	0
*Culex quinquefasciatus**	417	15,129	104	6.8
Culex stigmatosoma	1	5	0	0
Culex tarsalis	703	31,329	131	4.2
Culex thriambus	1	8	0	0
Aedes melanimon	173	7,152	0	0
Total	1,346	55,707	235	

* Includes females collected by gravid traps.

Marked viral amplification was measured during last few weeks of June (Fig. 2) and by the end of the month there were 27 positive mosquito pools, 6 dead birds, 4 chicken seroconversions, 1 equine case and 32 free ranging bird seropositives, but 0 human cases. There was a sharp increase in virus activity in July associated with rising summer temperatures remained relatively constant through August. Virus activity then declined in September and came to an end in October and early November. Seroprevalence rates in free-ranging birds remained elevated with 41positives in October and 38 positives in November.

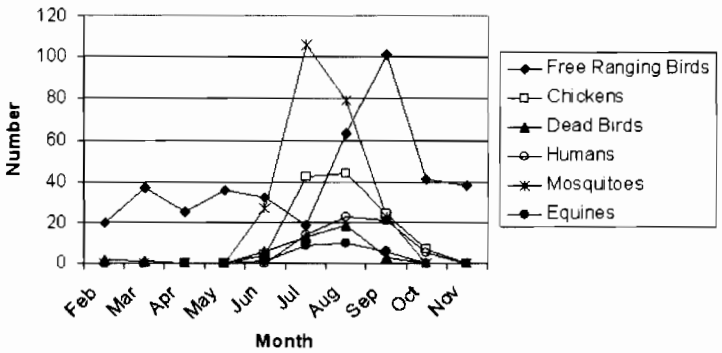

Figure 2. Positive surveillance measures plotted as a function of month during 2005.

A total of 110 chickens from 14 flocks seroconverted to WNV in Kern during the 2005 surveillance season (Table 2). The first chicken infections occurred before 27 June, with four positives from three flocks being confirmed. These flocks were in Arvin, southeast of Bakersfield, Bakersfield, and Pumpkin Center, just south of Bakersfield. By the end of July WNV had spread to almost every chicken flock in every area, with 42 new seroconversions. This trend continued into August with 47 new seroconversions. By September WNV infected chickens were detected at the mountain community of Weldon, some 45 miles northeast of Bakersfield at an elevation of 2653 ft. Flocks continued to seroconvert into late September and early October when surveillance was halted or there were no longer any replacement chickens available.

Table 2. Summary of positive surveillance results by cities within Kern County, 2005.

City	Mosquitoes	Chickens	Humans	Equines	Dead Bird	Free Ranging Birds
Arvin	16	23	2	0	0	6
Bakersfield	156	58	48	20	31	388
Buttonwillow	13	11	0	0	0	0
Delano	0	0	2	0	1	0
Edwards	0	0	0	0	6	0
Lamont	7	0	1	0	1	0
Lebec	0	0	0	2	1	0
Lost Hills	20	16	0	0	0	18
McFarland	0	0	4	0	0	0
Rosamond	0	0	0	2	1	0
Shafter	14	0	3	1	0	0
Taft	6	0	1	1	2	0
Wasco	3	12	1	0	1	0
Weldon	0	1	0	0	0	0
Total	235	121	66(4)*	26	44	412

* Includes 4 of unknown locations

The year ended with 66 laboratory confirmed human cases; there were no deaths in 2005. Forty eight of these cases were located in or around the metropolitan Bakersfield area. The rest were located in small agrarian communities on the valley floor (Table 2).

The year ended with 26 confirmed positive WNV equine cases, including 7 fatalities. Twenty of the 26 cases were located in metropolitan Bakersfield, two were in the mountains south of Bakersfield, just north of Los Angeles, and one was in the desert in eastern Kern County. The only other evidence of WNV activity in eastern Kern County was seven dead birds. There were no mosquito pools positive and no human cases.

Overall, 44 dead birds tested positive for WNV (Table 2). The most frequently reported dead bird species were western scrub-jays and American crows, each with 12 dead. Other species included house finches (5), barn owls (4), house sparrows (4), American kestrels (2), American robins (2), common raven (1), red-tailed hawk (1), and unidentified sparrow (1). Thirty-one of the 44 positive dead birds found in Kern County were found in or around metropolitan Bakersfield (Table 2) and five were found in the small cities of Delano, Wasco, Taft and Lamont. One was found in the mountain community of Lebec. There were seven found outside the San Joaquin Valley and East of the Tehachapi Mountain Range in Edwards AFB and Rosamond. Since the dead bird program relies on the public to find and report the dead birds, most of the dead birds were found in metropolitan Bakersfield. A sparse human population and large numbers of scavengers most likely reduced the effectiveness of the dead bird program in rural areas.

The free-ranging bird seroprevalence program detected 412 EIA positives during 2005 that represented 22 species (Table 3). Positivity rates ranged from 1% to 50%, depending upon species and residence status. As expected most of the transient migrants and winter residents had low seroprevalence rates compared to year-round resident species.

Table 3. Species of free ranging birds testing positive for *Flavivirus*, Kern County, 2005.

Species	Total tested	Total positive	% positive
Ash-throated flycatcher	9	2	22.2
Brewer's blackbird	25	4	16.0
Brown-headed cowbird	79	3	3.8
Bullock's oriole	40	4	10.0
California quail	257	72	28.0
California thrasher	10	1	10.0
Cooper's hawk	2	1	50.0
Golden-crowned sparrow	122	2	1.6
Greater road runner	4	1	25.0
House finch	326	58	17.8
House sparrow	147	12	8.2
Lazuli bunting	4	1	25.0
Loggerhead shrike	9	3	33.3
Marsh wren	26	1	3.8
Mourning dove	380	115	30.3
Nuttall's woodpecker	3	1	33.3
Red-winged blackbird	45	2	4.4
Rock pigeon	129	10	7.8
Song sparrow	311	7	2.3
Tricolored blackbird	44	1	2.3
Western Scrub-Jay	208	99	47.6
White-crowned sparrow	948	12	1.3
Total	3128	412	13.2

DISCUSSION

Examining the seasonality of positive surveillance indicators revealed several patterns. First, the dead birds that were positive in February, March and April most likely did not represent current transmission events and may have been infected during 2004, because there were no other indications of virus activity during late winter and early spring 2005. Previous studies have shown that birds surviving WNV infection frequently retain RNA within the kidney for as long as 8 wks (Reisen et al. 2006) and therefore these birds could have been chronically infected and died due to other causes. Second, as soon as mosquito pools became positive and increased in

number, there was a concurrent increase in the number of dead birds, chicken seroconversions and human cases. This trend was similar during viral subsidence in late summer; i.e., decreased mosquito infection rates were followed by decreases in the remaining surveillance indicators. Third, there was a decrease in seroprevalence rates among wild birds in June and July, perhaps due to natural mortality among older immune birds and increases in the numbers of hatching year birds diluting the numbers with antibody.

There were differences in WNV activity between 2005 and 2004 (Takahashi et al. 2005). In 2004 WNV activity started in the southeastern corner of the valley and moved into Bakersfield and then to the west side of the valley. In 2005 WNV activity first appeared in Bakersfield and then seemed to spread outward in all directions at a rapid pace, eventually affecting all monitoring sites all across the valley. It was not possible to determine if this apparent dispersal was the result of viral movement or delayed local amplification and detection. Virus activity slowed in September and finally subsided in October and November, when *Cx. tarsalis* enter diapause (Bellamy and Reeves 1963, Nelson 1964). By looking at the overall incidence and positivity rates, it appeared as if 2005 and 2004 were quite similar; however, the spatial and temporal patterns were quite different (Table 4). Although there were approximately the same number of human cases, sentinel chicken seroconversions, and mosquito pools positive, there was half the number of equine cases and dead birds. In addition, there was a large increase in seroprevalence among free ranging birds that may initiated the decline of WNV activity in September. With almost all of these being resident species, host-seeking infected mosquitoes likely had fewer antibody negative avian hosts to utilize in the transmission cycle. In summary, all surveillance sites were affected by WNV in 2005. It will be very interesting to see where it reemerges in 2006 and if the high level of seroprevalence within the avian community will dampen vernal amplification.

Table 4. Numbers of positives in Kern County during 2005 and 2004

		2005	2004
Human cases		66	60
Sentinel chickens		111	101
Mosquito pools	Positive	235	214
	Total Tested	1596	1367
Equine cases		26	46
Dead birds	Positive	44	87
	Total Tested	240	159
Wild birds	EIA Positive	412	157
	Total Tested	3476	3400

Acknowledgements

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Local Amplification of WNV in Wild Bird Populations in Sacramento and Yolo Counties, California

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ABSTRACT: West Nile Virus (WNV) first arrived in northern California in 2004 and amplified to epidemic levels in 2005. For several years prior and through the epidemic in Sacramento County, wild birds were sampled for evidence of WNV infection and amplification using mist nets, crow traps and ground traps. Each sampled bird was bled and banded according to protocols established by the U.S. Geological Survey, Bird Banding Laboratory in Laural, MD. Wild bird blood was tested for antibody to WNV by both an enzyme-linked immunosorbent assay (ELISA) and a plaque reduction neutralization test (PRNT). Sera that neutralized greater than 80% of the WNV plaque forming units at a dilution equal to or greater than 1:20 were considered serologically positive; end point titers ≥4X the competing virus were required for definitive identification of the infecting virus. During 2004 and 2005, 4,912 wild birds from 89 species were sampled for WNV antibody. Of these, antibody was detected in 12 of 28 families (Table 1) that included 24 species of wild birds (Table 2). The earliest detection of antibodies occurred in two species of Hirundinidae that are neo-tropical migrants, the purple martin and the cliff swallow. These birds provided an early warning of local virus activity weeks before the conventional detection of virus transmission in sentinel chickens and infection in mosquito pools. Within 6 weeks of the first detection in migrant birds antibody was detected in resident wild birds, where from 18 species, in 2004 74% and in 2005 89% of the antibody was detected. Some sites within our study area had a greater proportion of infected birds detected earlier in the year relative to other locations. Some infected species, such as ash-throated flycatchers appeared to decline in abundance, while other species showed no significant change in abundance relative to years prior to the WNV invasion. Peak months for the detection of WNV infections in wild birds were September in 2004 and in August of 2005. These months correspond with the greatest abundance of hatching year young from the resident bird populations. For many abundant resident bird species the juvenile part of the population had the highest proportion of antibody positive individuals. House finches appear to be significant contributors to local virus amplification as this species represented 46% of the antibody positive birds in 2005. Furthermore, 85% of the juvenile members of the house finch population were identified antibody positive for WNV. Considering that ca. 70% of experimentally infected individuals succumb to infection, our data indicate that most individuals became infected during 2005.

Table 1. Wild bird families sampled in Sacramento and Yolo Counties and the number and percent of antibody positive birds for WNV in 2004 and 2005.

Family of Wild Bird	#spp.	n	PRNT+	% pos.
Anatidae	4	107	2	1.9
Ardeidae	4	24	7	29.2
Columbidae	3	347	30	8.6
Accipitridae	4	12	5	41.7
Phasianidae	2	28	2	7.1
Tytonidae	1	8	0	0
Alcedinidae	1	5	0	0
Picidae	4	99	0	0
Tyrannidae	7	221	3	1.3
Corvidae	4	120	2	1.7
Hirundinidae	2	217	4	1.8
Paridae	1	71	0	0
Sturnidae	1	19	0	0
Sittidae	2	7	0	0

Family of Wild Bird	#spp.	n	PRNT+	% pos.
Troglodytidae	3	37	0	0
Turdinae	3	248	0	0
Timaliinae	1	206	0	0
Laniidae	1	2	0	0
Mimidae	1	10	0	0
Bombycillidae	1	1	0	0
Vireonidae	3	85	0	0
Parulidae	8	204	0	0
Thraupidae	1	21	0	0
Emberizae	13	1662	4	0.2
Cardinalidae	3	174	2	1.1
Icteridae	5	198	0	0
Fringillidae	4	917	29	3.2
Ploceidae	1	27	1	3.7

Table 2. Wild bird species that were antibody positive in 2004 and 2005.

Wild Bird Species	n	PRNT +	%
Black-crowned night heron, *Nycticorax nycticorax*	9	4	44.0
Snowy egret, *Egretta thula*	6	3	50.0
Snow goose, *Chen caerulescens*	9	1	11.0
Mallard, *Anas platyrhynchos*	87	1	1.1
Cooper's hawk, *Accipiter cooperi*	4	1	25.0
Red-shouldered hawk, *Buteo lineatus*	4	3	75.0
Red-tailed hawk, *Buteo jamaicensis*	1	1	100.0
Califonria quail, *Callipepla californica*	20	1	5.0
Ring-necked pheasant, *Phasianus colchicus*	8	1	12.5
Rock pigeon, *Columba livia*	325	27	8.3
Mourning dove, *Zenaida macroura*	21	3	14.0
Cliff swallow, *Hirundo pyrrhonota*	145	3	2.1
Purple martin, *Progne subis*	22	1	4.5
Western scrub-jay, *Aphelocoma coerulescens*	88	2	2.3
Ash-throated flycatcher, *Myiarchus cinerascens*	21	2	9.5
Black phoebe, *Sayornis saya*	141	1	0.7
Black-headed Grosbeak, *Pheucticus melanocephalus*	143	2	1.4
Spotted towhee, *Pipilo maculatus*	288	1	0.3
Song sparrow, *Melospiza melodia*	448	1	0.2
Fox sparrow, *Passerella iliaca*	201	1	0.5
Golden-crowned sparrow, *Zonotrichia atricapilla*	501	1	0.2
American goldfinch, *Carduelis tristis*	40	1	2.5
House finch, *Carpodacus mexicanus*	785	28	3.6
House parrow, *Passer domesticus*	27	1	3.7

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Epidemic Amplification of West Nile Virus in Sacramento and Yolo Counties, June- September 2005

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West Nile Virus (WNV) is a mosquito-borne Flavivirus (Family *Flaviviridae*, genus: *Flavivirus*) that may cause a number of disease syndromes in humans and may lead to death in some cases (Hayes 2005). In nature, WNV is maintained and amplified within a mosquito—wild bird transmission cycle (Hayes 2001). Humans contract the infection when bitten by an infected female mosquito. In California, WNV activity was first detected in July 2003, in a pool of *Culex tarsalis* Coquillett collected near El Centro, Imperial County (Reisen et al. 2004). During 2004, WNV amplified to epidemic proportions in Southern California and spread northward to all of the 58 counties in CA (Hom *et al.* 2005). The initial invasion and amplification of WNV into Sacramento and Yolo Counties occurred in 2004 (Armijos et al. 2005). The first evidence of WNV activity in these counties was obtained on the 24th of June 2004, when sera from 1 of 10 purple martins and 2 of 20 Cliff Swallows tested positive for WNV antibody. First detection of the virus was in a dead western scrub-jay found in the town of Wilton on July 8th, 2004. Active transmission was confirmed by detection of the virus in 2 pools of *Culex tarsalis* and one pool of *Cx. pipiens* L. collected on July 23. West Nile Virus continued to amplify during August and September 2004, with 18 more mosquito pools and 130 dead birds testing positive. First detection of WNV antibodies in sentinel chickens was obtained in September when 19 chickens from 4 flocks tested positive for the virus. The number of positive pools and dead birds was greatly reduced in October. No evidence for transmission was obtained after October. However, WNV antibodies were detected in December from winter resident golden-crowned and fox sparrows. Overall during 2004, WNV was detected in 21 of 1,231 mosquito pools (2%), 165 out of 446 dead birds (37%), and 26 out of 100 sentinel chickens (26%). Here, we describe the epidemic amplification of WNV in Sacramento and Yolo Counties during 2005.

WNV activity in Sacramento and Yolo Counties was monitored as described previously (Armijos *et al.* 2005). Our surveillance methods consisted of dead bird reports supported by limited laboratory testing, 10 flocks of 11 sentinel chickens (total of 110 chickens), mosquito trapping using gravid traps (Cummings 1992) and CO_2 - baited traps (Rohe and Fall 1979). Sentinel chicken sera were screened for Flavivirus antibody using an enzyme immunoassay and confirmed using a plaque reduction neutralization test (Reisen et al. 1994). Mosquito pools were tested for western equine encephalomyelitis, St. Louis encephalitis and WNV RNA by a multiplex RT-PCR (Brault et al. unpublished).

Sacramento was the epicenter of WNV activity in California in 2005. Between July and October 2005, 175 cases were reported from Sacramento and 13 from Yolo County (approx. incidence rate = 1.1 per 10,000). Of these one patient died of WNV complications, 51 cases had neuroinvasive disease and 15 were asymptomatic blood donors. Additionally, a total of 40 equine cases and 19,429 dead birds were reported from the area.

The first evidence of WNV activity in Sacramento County came early in February 2005, when the virus was detected in a dead yellow-billed magpie, collected in Sacramento. This was followed on March 25th by a report of WNV positive dead western scrub jay in Elk Grove (Sacramento County). Both corvid species succumb rapidly after infection providing evidence of recent transmission. No additional positive dead birds were reported from Sacramento County until June 6th and June 7th, when two positive dead American crows were found in Elk Grove. In Yolo County, the first WN positive dead bird was a Brewer's blackbird found on February 2 in Davis. This was followed by 3 positive American crows on July 17th.

The amplification of WNV in Sacramento and Yolo Counties was accompanied by unprecedented mortality in wild birds. Figure 1 shows the number and distribution of dead birds reported by the public from January to September 2005. Because of the high number of reports received and limitations in personnel available for picking up dead birds, only small samples of the carcasses were submitted for laboratory testing. In these samples, the overall confirmed WNV infection was 47% (79 out of 168 birds). The infection rate in tested dead birds increased from 8.1% (4 out of 37) in January-April to 14% (4/29) in May – June and reached a peak of 84% (70 out of 83) in July-August. Active transmission of WNV subsided in Sacramento and Yolo Counties towards the end of September. In Nov-Dec, no WNV infection was found in seven dead birds that were submitted for testing.

Infection of WNV in mosquitoes of Sacramento and Yolo Counties was first detected on 29 Jun and 1 Jul, in 7 pools of *Cx pipiens* and three pools of *Cx tarsalis* mosquitoes collected from Arden Park, Brook Tree Park, Beach Lake Stable, Grand Rio and Rusch Park areas of Sacramento County and Knights Landing/HWY 45 area of Yolo County. Infection in mosquitoes reached their highest levels in July and August and then subsided sharply. Last sample of WNV positive mosquitoes was detected on the 27th of September.

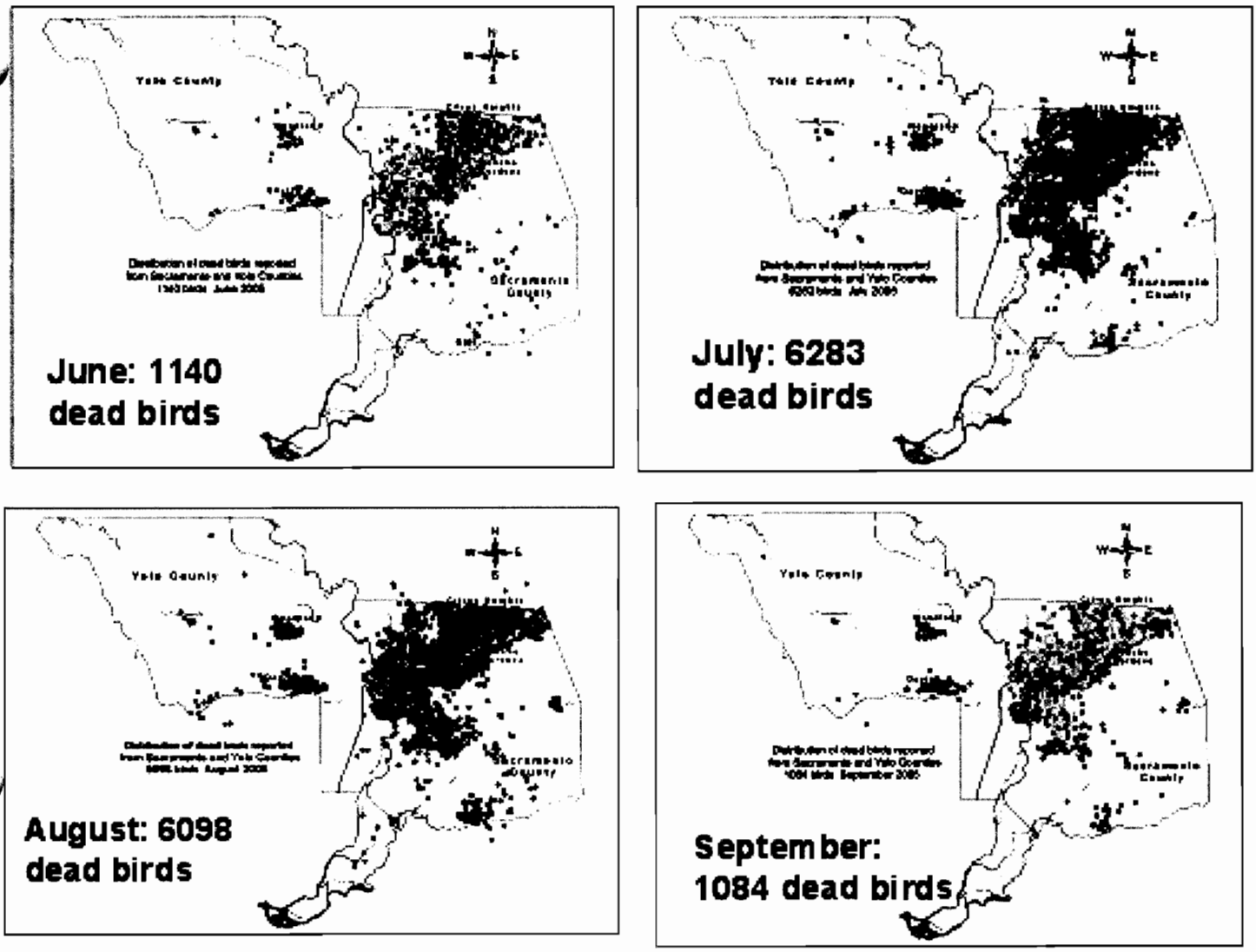

Figure 1. Dead bird reports in Sacramento and Yolo Counties, CA, June-September 2005.

Table 1 shows infection rates of WNV in the mosquito pools collected from Sacramento and Yolo Counties. According to MIR estimates, the highest infection rate was observed in *Cx. stigmatosoma* Dyar followed by *Cx. pipiens* and *Cx tarsalis*. The high MIR detected in *Cx. stigmatosoma* may be due to the fact most pools of this species were collected by GT traps (data not

Table.1: Infection rates of WNV in mosquitoes collected from Sacramento and Yolo Counties, California, June-October 2005

Mosquito species	No. of pools tested tested	Total number of females tested	No. of positive pools	% positive pools	Minimum infection rate / 1000 females
Aedes vexans	13	203	0	0	0
Culex erythrothorax	72	1780	1	1.4	0.56
Cx. pipiens	904	12539	95	10.5	7.6
Cx. stigmatosoma	57	144	2	3.5	13.9
Cx. tarsalis	782	18167	40	5.1	2.2
Cx. thriambus	2	11	1	-	-
Ochlerotatus melanimon	66	1542	0	0	0
Total	1896	34386	139	7.3	4.0

shown), which attracts larger proportions of female mosquitoes that have already obtained a blood meal and are more likely to be infected than the host seeking females attracted to CO2-baited traps. This factor may also be responsible for the high infection rate detected in *Cx. pipiens*. However, for *Cx. pipiens*, infection rates in CO2-baited traps were also higher than any other species, including *Cx. tarsalis*. This result indicates that *Culx pipiens* was a main amplifying vector of WNV in the area.

Interestingly, out of two pools containing a total of 11 females of *Cx. thriambus* Dyar, we found one pool positive for WNV. The small numbers collected of *Cx thriambus* may be due to a small population size of this species or deficiency in the trapping method. More work is needed to determine the role played by *Cx. thriambus* in amplifying WNV.

Although infections of WNV in wild birds and mosquitoes were detected by the end of June, human cases and seroconversions in sentinel chickens were not observed until the last week of July. These results support the previous findings that surveillance of WNV infection in wild birds and mosquito pools are better methods in providing early warning of virus activity, than sentinel chickens which take a long time to seroconvert (Armijos et al. 2005).

Our surveillance in Sacramento and Yolo Counties described the onset and amplification of WNV that led to a severe epidemic in 2005. The magnitude of transmission was measured by the high number of human cases and the high rate of seroconversions in sentinel chickens (53%) compared to 2004. Although WNV transmission was much more intense and commenced one month earlier, subsidence of the epidemic began in September 2005, one month earlier than observed in 2004. Rapid subsidence have been due to focal depopulation of corvids, acquired herd immunity among surviving peridomestic passeriform birds, and intensive control efforts exerted by the Sac Yolo MVCD during July-September 2005.

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Impact of Temperature on the Transmission of West Nile Virus

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Arboviruses must be able to grow under a wide range of temperatures because infected birds have body temperatures that exceed 40°C, whereas mosquitoes have body temperatures that approximate ambient conditions. These latter mosquito temperature regimens can be as low as freezing in winter hibernacula and exceed 40°C during summer (Meyer et al. 1990). Different arboviruses vary markedly in their ability to grow under this range of temperature conditions, and these temperature requirements may delineate distribution in time and space. Previously, we developed degree-day models to describe the extrinsic incubation of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) in *Culex tarsalis* Coquillettt and then used these models to show how the responses of these viruses to temperature delineated their seasonality (Reisen et al. 1993), based on estimates of mosquito body temperature (Meyer et al. 1990). West Nile virus (WNV) transmission has been recorded from the plains of Canada to the tropics of Central and South America, suggesting that invading genotypes must be able to grow effectively under a wide range of temperature conditions. Our present research describes temperature studies to develop a degree-model for the extrinsic incubation period of WNV in *Cx. tarsalis* (Reisen et al. 2006).

Culex tarsalis females were infected by feeding on passerine birds viremic with the NY99 strain of WNV and then were incubated under constant temperatures of 10 – 30°C. At selected time intervals, transmission was attempted using an *in vitro* capillary tube assay (Aitken 1977). The time from imbibing an infectious blood meal until 50% of infected females first transmitted was converted to a rate and regressed as a function of incubation temperature from 14-30°C (Fig. 1). The extrinsic incubation period (EIP) was estimated to require 109 degree days and the point of zero virus development (X-intercept) was estimated to be 14.3°C. This degree day model for WNV closely approximated our previous estimates for SLE, indicating that the NY99 WNV strain must require warm ambient temperatures for efficient transmission.

The time for completion of the EIP was estimated monthly from temperatures recorded at Coachella Valley, Los Angeles and Kern County, California, during the 2004 epidemic year and was related to the duration of the *Cx. tarsalis* gonotrophic cycle and measures of WNV activity. Enzootic WNV activity commenced only after temperatures increased, the duration of the EIP decreased, and virus potentially was transmitted in less than gonotrophic cycles. Examination of temperature anomalies throughout the United States during the epidemic summers of 2002 – 2004 indicated that WNV dispersal and resulting epicenters were linked closely to above average summer temperatures. Possibly, long-lead forecasts of temperature such as shown in Fig. 2 could provide an early warning for WNV epidemic transmission at northern latitudes.

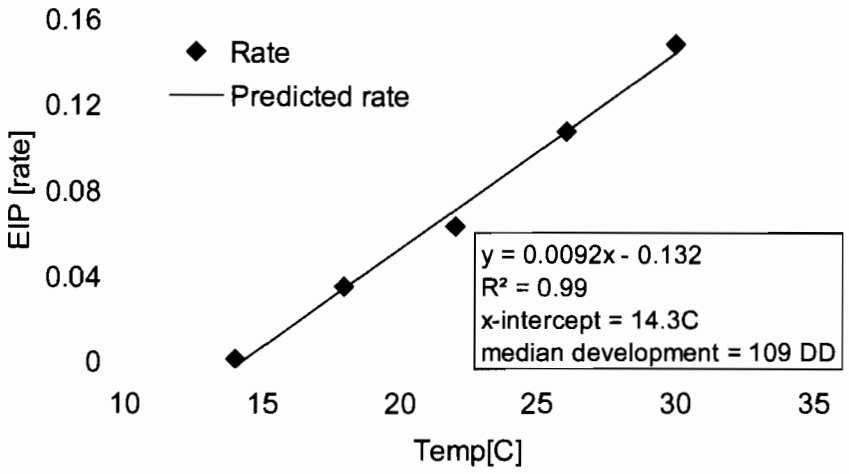

Figure 1. Regression fit of the inverse of the median extrinsic incubation period in days (EIP rate) as a function of incubation temperature. X-intercept is the virus 0 growth point and the heat required until median transmission is estimated in degree-days (DD).

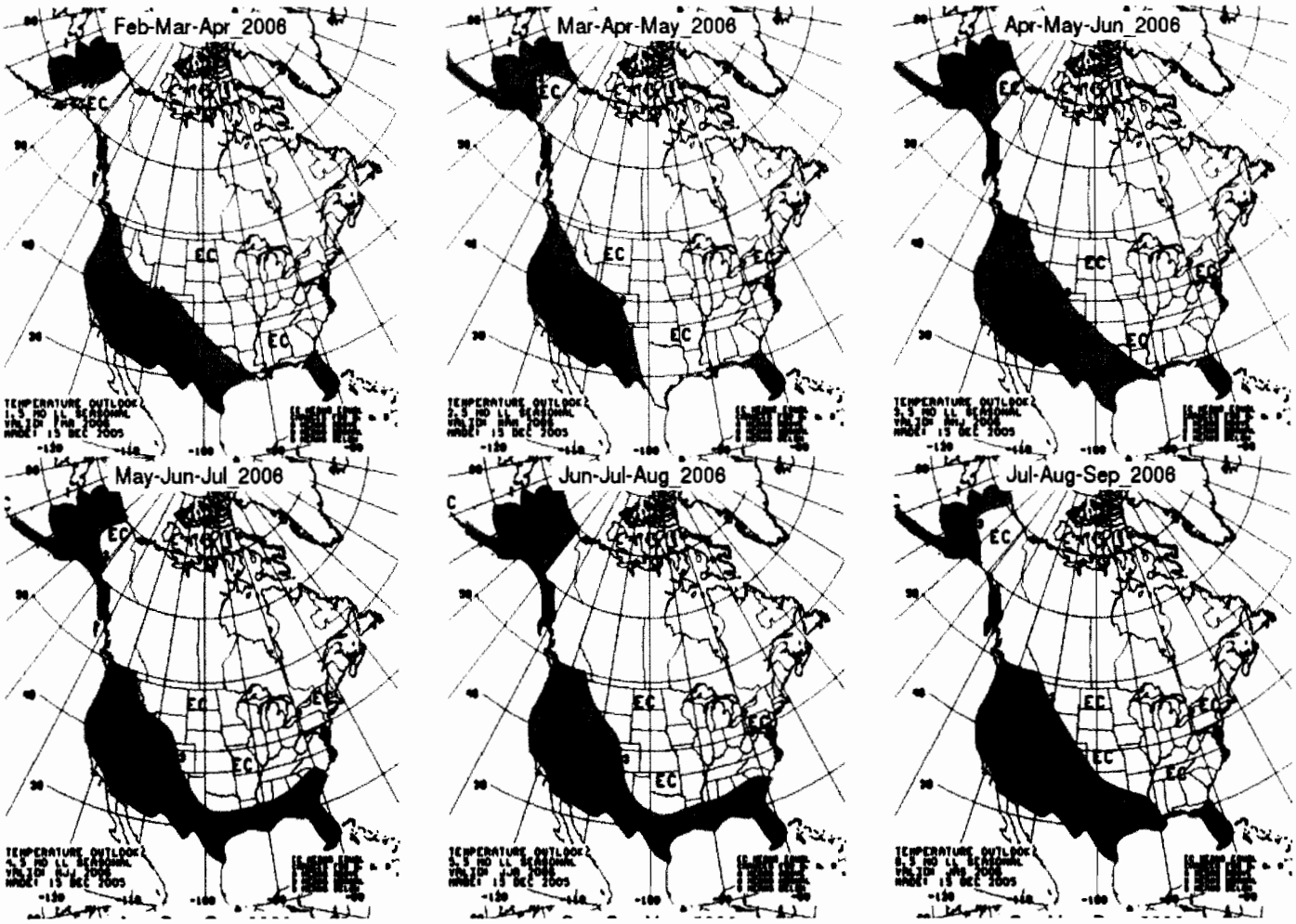

Figure 2. Long-lead forecasts of temperature anomalies for the United States made 15 December 2005 cropped for the spring and summer months of 2006. From Climate Prediction Center, NCEP, NOAA [http://www.cpc.ncep.noaa.gov/products/predictions/multi_season/13_seasonal_outlooks/color/churchill.html].

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WNV Interactions with SLE: Partial Cross Protective Immunity in House Finches[1]

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SUMMARY

After West Nile virus [WNV] invaded California during the summer of 2003, St. Louis encephalitis virus [SLE] disappeared and has not been detected in >30,000 mosquito pools or 70,000 sentinel chicken sera tested during 2004 and 2005. WNV and SLE are closely related, classified with the Japanese encephalitis virus serocomplex, and cross react in serological assays. Recently, viruses within this complex were found to provide cross protection using a Hamster model (Tesh et al. 2002); however, similar studies have not been done with birds, even though these are the natural hosts for both viruses.

We selected house finches as a representative avian host for our experiments, because they are competent hosts for both WNV and SLE, frequently become infected during outbreaks, and are easy to maintain in captivity. House finches were infected initially with either WNV or SLE and then challenged 6 weeks post infection with either homologous or heterologous viruses. WNV virulence in house finches exceeded SLE as indicated by elevated mortality [overall WNV = 65%, SLE = 0%, n = 20] and viremia titers. Previous infection with either SLE or WNV protected house finches from mortality during subsequent challenge. Protective immunity during homologous and heterologous challenge with SLE prevented detection of viremia; i.e., viremia remained <1.7 $\log_{10}$ PFU/mL, our threshold of detection using Vero cell plaque assay. In contrast, heterologous challenge with WNV in birds previously infected with SLE resulted in peak viremias that ranged from 2.7 – 6.4 $\log_{10}$ PFU/mL. Viremias >5 $\log_{10}$ PFU/mL were considered sufficiently elevated to infect susceptible populations of California *Culex* mosquitoes (Goddard et al. 2002; Reisen et al. 2005). Interestingly, infection with SLE following recovery from WNV infection elicited a consistent and significant rise in WNV PRNT but not SLE PRNT titers, perhaps because protective immunity prevented the immunological response associated with a second viremia episode. In contrast, infection with WNV following recovery from SLE produced very high antibody titers and a non-specific response that was highly variable among individual birds within this treatment group. Differences here were attributed to differential virulence associated with primary and secondary SLE and WNV infection.

Epidemic transmission of WNV in periurban areas has been associated with elevated infection rates in *Culex* vectors and a variety of bird species, including house finches and house sparrows. As pointed out in papers in our symposium, herd immunity among free-ranging birds in Los Angeles during 2004 exceeded 40% and in Bakersfield during 2005 exceeded 35%, and these levels were associated with the termination of epidemic transmission by late summer. Our current cross immunity study also indicated that herd immunity to WNV at these elevated rates may be sufficient to prevent the reintroduction of SLE into California. In recent years, SLE has been detected in Coachella Valley in southeastern California at consistent but varying levels of enzootic activity (Reisen et al. 2002). Although genotypes have become extinct over time, recolonization events occasionally have led to elevated focal enzootic transmission at sites near the Salton Sea and to outbreaks as far north as Bakersfield. We continue to monitor arbovirus activity at the south and north shores of the Salton Sea to detect the next incursion of SLE into California.

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[1] The collection and infection of House finches was done under Protocol 11184 approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis, California Resident Scientific Collection Permit No. 801049-02 from the State of California Department of Fish and Game, and Federal Fish and Wildlife Permit No. MB082812-0 from the Department of the Interior. Use of arboviruses was approved under Biological Use Authorization #0554 by Environmental Health and Safety of the University of California, Davis, and USDA Permit #47901.

Field Evidence for Vertical Transmission of West Nile Virus

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INTRODUCTION

Among the potential mechanisms for the persistence of West Nile virus (WNV) in California (Reeves 1987, Rosen 1987, Reeves 1990), three seem most plausible and have been supported by limited field data: 1) vertical transmission to females destined for reproductive diapause that may carry WNV through winter and then transmit virus to avian hosts during spring, 2) continued transmission and 3) chronic infection of WNV in avian hosts that relapse during spring (Reisen et al. 2006). The current report focuses on the role of vertical transmission in virus persistence during winter as well as amplification during summer. WNV has been recovered from diapausing *Culex pipiens pipiens* in New York (Nasci et al. 2001), New Jersey (Farajollahi et al. 2005) and Pennsylvania (Bugbee and Forte 2004). Because females destined for diapause do not blood feed (Mitchell 1981, Mitchell and Briegel 1989, Spielman 2001), infection presumably occurs by vertical transmission (Rosen 1987). However, attempts to recover WNV from overwintering *Culex tarsalis* following the Colorado epidemic were unsuccessful (Moore et al. 2005), questioning whether WNV persists in diapausing females of other species and/or strains of *Culex*. Laboratory experiments where female *Culex* were infected by intrathoracic inoculation and their progeny reared *en masse* demonstrated vertical passage of the NY99 strain of WNV by several North American *Culex* species (Dohm et al. 2002, Goddard et al. 2003). Our recent studies showed that 24% of Yolo County *Cx. tarsalis* females tested individually transmitted virus vertically after being experimentally infected *per os* (Reisen et al. 2006). In addition, WNV was isolated from field-collected *Cx. univitattus* males from Kenya (Miller et al. 2000) and from *Cx. quinquefasciatus* collected during summer in California (Reisen et al. 2006), indicating vertical transmission can occur under field conditions. The present paper describes our attempts to detect vertical infection in nature during the summer transmission and during the fall – winter diapause seasons in California.

MATERIALS AND METHODS

We attempted to find evidence for natural vertical transmission of WNV at our four study areas in Coachella Valley, Los Angeles, Kern and Sacramento counties using four methods:

1. Field-collected immatures. Larvae and pupae were collected opportunistically on multiple occasions and from different sites and reared either outdoors (Los Angeles) or in an insectary at 22-24°C (Coachella Valley, Kern County, Sacramento County). Adults of both sexes were allowed to emerge *en mass* and then were maintained on 10% sucrose for >5 days until frozen at -80°C for later testing.

2. F-1 progeny. Adult host-seeking females were blood fed on an uninfected chick, held for 5 days on 10% sucrose and then allowed to lay eggs. Additional egg rafts were collected from gravid traps. Three – four egg rafts were reared in each standard tray to pupation and then allowed to emerge under insectary conditions. Many of these females were used in vector competence experiments (not shown here), and the males were frozen for later virus testing.

3. Adult males. Collections were made from gravid traps (Los Angeles, Kern) or from suction traps placed near nectar sources (Coachella Valley). Males were speciated into pools of d"50 and then frozen for virus testing.

4. Overwintering adults. Unfed females and males were collected by aspiration in Kern County from a variety of hibernacula (bridges, park restrooms, farm buildings) during the fall and early winter months of 2004 and 2005. Specimens were sorted to species and pooled by species, sex and reproductive status for later virus testing.

Mosquito pools were tested for virus infection with a single or multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) using robotic RNA extraction and a real-time TaqMan diagnostic platform. Primers for WNV RT-PCR have been published previously (Lanciotti et al. 2000).

RESULTS

During 2004 and 2005, we tested a total of 39,900 adult males and females of four *Culex* species in 1,198 pools (Table 1). Evidence for natural vertical transmission was detected in 3 pools of *Cx. quinquefasciatus* collected during the summer of 2004 (Reisen et al. 2006). One of these pools consisted of adult males collected from a gravid trap in Los Angeles, one was adults reared from an egg raft collected from a gravid trap in Los Angeles, and one was from field-collected larvae collected in Kern County. During the summer of 2005, six pools of adult *Cx. tarsalis* males reared from eggs laid by host-seeking females collected in Coachella Valley (one pool) and Kern County (five pools) tested positive. To date, none of the pools from *Cx. pipiens* or *Cx. stigmatosoma* have tested positive and none of the pools consisting of *Culex* adults collected during fall and early winter have tested positive.

DISCUSSION

Natural occurrence of WNV vertical transmission was detected for the first time in *Cx. quinquefasciatus* and *Cx. tarsalis*. These results have important implications for virus amplification and persistence. Vertically infected females have disseminated infections

Table 1. Summary of field-collected mosquitoes tested to detect vertical passage of WNV.

Location	Date	Culex species	Method	Sex	No. Pools	Total
2004						
Coachella	Jun-Aug	tarsalis	Suction trap	M	49	1,865
			Reared	M	31	1,198
			Reared	F	34	1,366
Los Angeles	Jun-Aug	quinquefasciatus	Gravid traps	M	15	428*
			Reared	F	26	1,065*
			Reared	M	39	1,806
Kern	Jul-Sep	quinquefasciatus	Gravid trap	M	8	102
			Reared	M	30	1,405*
			Reared	F	30	1,395
		stigmatosoma	Reared	M	10	390
			Reared	F	12	431
		tarsalis	Reared	M	11	388
			Reared	F	9	360
	Oct-Dec	quinquefasciatus	Resting	M	32	440
			Resting	F	73	858
		stigmatosoma	Resting	M	5	26
			Resting	F	22	209
		tarsalis	Resting	M	12	119
			Resting	F	32	369
		Subtotal		Total	480	11,322
2005						
Coachella	Aug	tarsalis	Reared	M	11	512*
			Reared	F	4	186
	Jun	quinquefasciatus	Reared	M	20	1,000
Los Angeles	May - Jun	quinquefasciatus	Reared	M	30	1,476
		stigmatosoma	Reared	M	13	614
			Reared	F	1	13
		tarsalis	Reared	M	2	81
			Reared	F	1	23
		thriambus	Reared	M	13	650
			Reared	F	3	148
Kern	Aug - Sep	quinquefasciatus	Reared	M	19	820
		tarsalis	Reared	M	22	1,073**
			Reared	F	6	276
	Oct-Nov	quinquefasciatus	Resting	M	13	410
			Resting	F	25	389
		stigmatosoma	Resting	M	3	18
			Resting	F	9	42
		tarsalis	Resting	M	16	481
			Resting	F	18	277
Sacramento-Yolo	Aug-Oct	pipiens	Reared	M	31	1,322
			Reared	F	40	1,768
	Mar-Sep	tarsalis	Reared	M	219	9,640
			Reared	F	199	8,944
		subtotal			718	28,578
		Total			1,198	39,900

*Single pools positive
**Five pools positive

at emergence and theoretically can transmit virus by bite during their initial blood meal. Therefore, these females potentially transmit by bite during the first and each subsequent blood meal increasing the chances of horizontal transmission. Because as many as 25% of the infected females in some populations such as Yolo County *Cx. tarsalis* may transmit WNV vertically, we feel that this mechanism could be responsible, in part, for the rapid amplification of WNV.

As indicated previously, vertical infection of females destined for diapause due to temperature – photoperiod conditions could provide one mechanism for virus overwintering. Presumably vertically transmitted infections were the source of WNV infection in *Cx. pipiens* collected during winter in the east. Interestingly, we were not able to find evidence for natural vertical transmission in *Cx. pipiens* during the epidemic season of 2005 in Sacramento or during our previous laboratory attempt (Goddard et al. 2003). Although we were able to repeatedly detect vertical transmission during the summer months when epidemic transmission occurred in Kern County (Hom et al. 2004, 2005), we were not able to detect virus in over wintering adults of *Cx. tarsalis* or *Cx. quinquefasciatus*. These adult males and females were tested immediately upon return from the field, because we felt that our multiplex RT-PCR should be sufficiently sensitive to detect low level RNA. However, previous studies have shown that SLE detection in over wintering females was enhanced if these females first were held at summer conditions to terminate diapause and then allowed to blood feed (Bailey et al. 1978). In agreement, viral infection rates in experimentally infected females held under winter conditions and tested by RT-PCR were lower than rates determined after terminating diapause and holding females at warm temperatures (Reisen et al. 2002). Therefore, our future attempts to find WNV in overwintering *Culex* will be done after terminating diapause in the laboratory.

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Invasion of California by West Nile Virus: Role of Corvids

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INTRODUCTION

As West Nile virus (WNV) has advanced westward across North America, birds in the family Corvidae have been frequently implicated as competent amplifying hosts, and high mortality resulting from WNV infection has made them useful indicators of local virus presence (Eidson et al. 2001; Hom et al. 2004, 2005). In California, corvids include species with different degrees of aggregation and their distribution is not uniform among or within ecological zones (United States Geological Survey 2006). The most abundant corvid species in southern California are American crows (*Corvus brachyrhynchos*) and western scrub-jays (*Aphelocoma californica*). American Crows are densest in urban areas close to the southern coast and in nearby inland valleys, and western scrub-jays are broadly distributed at moderate densities in the same areas inhabited by crows as well as the southern end of the Central Valley. Within a region, crows aggregate in large communal roosts, particularly during the cooler seasons of the year, whereas jays are territorial and disperse more evenly across the habitat. These differences in distributions and behaviors allowed us to conduct a natural experiment to test the hypothesis that corvids played an important role in WNV transmission during the invasion of southern California by WNV during 2004. The data presented here are based on Reisen et al. (2006).

MATERIALS AND METHODS

Study areas. A natural experiment was designed to compare three study areas: 1) the Kern Mosquito and Vector Control District (KMVCD) with few American crows and moderate populations of western scrub-jays, 2) the Greater Los Angeles Vector Control District (GLACVCD) with many American crows and moderate populations of western scrub-jays, and 3) the Coachella Valley Mosquito and Vector Control District (CVMVCD) with few corvids, except occasional common ravens.

Surveillance. In each of the three study areas, mosquitoes were monitored throughout the transmission season for WNV infection. Bird mortality was also monitored passively through public reports to the California Department of Health Services Dead Bird Hotline. Suitable specimens reported to the hotline were tested for the WNV infection.

Spatial analysis. Zip code-level records for dead birds and human cases were obtained from the California Department of Health Services' Vector-Borne Disease Section and Viral and Rickettsial Diseases Laboratory, respectively. We analyzed these records with the spatial scan statistic in SaTScan software (Kulldorff and Nagarwalla 1995; Kulldorff 1997) using an underlying Bernoulli probability distribution to identify areas with clusters of dead birds. All identified clusters were characterized by central zip code and radius, and clusters of dead birds were compared with those of human cases to determine whether there was overlap. Also, human WNV-attributed case incidence and infection rates for *Culex quinquefasciatus* were compared within and outside the dead bird clusters in the Greater Los Angeles Vector Control District.

RESULTS AND DISCUSSION

Human incidence of WNV-attributed illness was highest in Kern County, where there is a combination of highly competent amplifying hosts (western scrub-jays) and susceptible mosquitoes (*Culex tarsalis*). Coachella Valley, with few corvids but many *Cx. tarsalis*, had the lowest human case incidence. Los Angeles had a human case incidence between that of Coachella Valley and Kern County, despite having relatively few *Cx. tarsalis*. This is probably because the abundant corvid amplifying hosts in Los Angeles developed sufficient viremias to infect moderately susceptible mosquitoes, such as *Cx. quinquefasciatus*.

Clusters of WNV-infected dead corvids identified by the spatial scan procedure in the Los Angeles urban areas were located near large American crow roosts and their radii were consistent with dispersal distances from communal roosts. Clusters of human cases were closely aligned with these dead bird clusters, and no dead bird or human case clusters were identified in Kern County or the Coachella Valley. Apparently, the more uniform dispersal pattern of the territorial scrub jays in Kern County resulted in intense but less focal virus transmission than that observed in the Los Angeles area, where clusters were focused around the large American Crow roosts. Comparisons between areas inside and outside dead corvid clusters showed that human WNV-attributed case incidence in the study areas and *Cx. quinquefasciatus* infection rates in Los Angeles were higher inside the dead bird clusters than outside them.

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Role of Ardeid Birds in the Spread of WNV

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Two colonies of nesting Ardeid birds were sampled to determine their involvement in the amplification of West Nile Virus (WNV). One colony, located at the Finney-Ramer Wildlife Refuge in Imperial County, California, did not appear to be a focus of WNV amplification during the summer of 2004 (Reisen et al. 2005). Blood samples taken during June and July from 155 nestlings of four species of Ardeid birds (cattle egrets, black-crowned night herons, great egrets, and snowy egrets) and five nestling double-crested cormorants yielded a single WNV isolation from a 3-week-old cattle egret. Antibody was detected by enzyme immunoassay from 20 nestlings (13%), 14 (70%) of which were confirmed as positive by plaque reduction neutralization test (PRNT). However, titration end points against WNV and St. Louis encephalitis virus (SLE) were similar precluding viral identification. The grouping of positives within few nests, highest PRNT titers in youngest birds (<1 weeks of age), the decline of titer with nestling age, and the lack of antibody specificity indicated that antibody may have been acquired maternally and did not represent new infections. Infection rates in *Culex tarsalis* mosquitoes collected near the Ardeid colony at Ramer Lake (3.1 per 1,000) were statistically similar to rates estimated at the nearby Wister Unit wetlands (5.3 per 1,000) that lacked an Ardeid nesting colony. Black-crowned night heron nestlings experimentally infected with the NY99 strain of WNV produced elevated peak viremias that ranged from 5.3 to 7.6 $\log_{10}$ plaque forming units (PFU)/mL and were considered to be moderately competent hosts, whereas cattle egret nestlings had peak viremias that ranged from 2.4 to 5.3 $\log_{10}$ PFU/mL and were considered to be incompetent hosts.

Initial data from a second Ardeid nesting colony located on the University of California campus in Davis, California provide contrasting results from that of the Finney-Ramer colony. Beginning in July and ending in August 2005, 23 Ardeid birds where sampled from the arboretum, including 11 black-crowned night herons, 7 cattle egrets, and 5 snowy egrets. All of the cattle egrets were antibody negative, however, 4 black-crowned night herons and 1 snowy egret were found antibody positive for WNV. One snowy egret and 2 black-crowned night herons submitted to the California Dead Bird Program were found WNV positive. Mosquito collections were attempted at ground level using dry ice-baited traps and oviposition traps, and at nest level in the canopy using dry ice-baited traps. The canopy traps were run 3 traps at a time for 3 trap nights once a week beginning 21 July 2005, the canopy traps were nonproductive, catching no mosquitoes. Three dry ice-baited traps run at ground level and a single oviposition trap were run once a week beginning 1 August 2005. This effort produced 11 WNV negative pools of *Culex* mosquitos at a combined average of 17 female *Culex* mosquitoes per trap night. Although initial results show some WNV activity at the UC Davis Ardeid colony, more data will be required in 2006 to determine the role of this Ardeid nesting colony in focal amplification of WNV.

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Symposium title: Invasion of California by West Nile virus: Year 3 Summary Thoughts

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West Nile virus [WNV] was detected throughout California during 2005, although the intensity and patterns of transmission varied markedly among our study areas in the Coachella Valley, Los Angeles, Kern and Sacramento Counties, depending upon previous levels of transmission activity. In the irrigated desert of Coachella Valley in southeastern California, enzootic WNV transmission continued at modest levels in rural habitats near the Salton Sea. Transmission in residential habitats in the northwestern portion of the valley was associated with unexpectedly elevated infection rates in *Cule quinquefasciatus* Say and scattered human cases. High infection rates within *Cx. quinquefasciatus* populations in the absence of large corvid populations differed from results from previous years (Reisen et al. 2006a) and may have been associated with an increase in *Cx. quinquefasciatus* susceptibility to infection in comparison with pre-WNV baseline data from 2003 (Reisen et al. 2005a).

In Los Angeles, there was a general subsidence of enzootic and epidemic transmission at upland sites affected by the Whittier crow roost when compared to 2004 (Wilson et al. 2005). Relatively few dead crows were reported by the public or were observed during our surveillance in this area. Free-ranging peridomestic passerines including house finches and house sparrows developed elevated seroprevalence rates at the end of the 2004 transmission season, and these elevated rates persisted though winter into the early spring of 2005 when the attrition of after hatching year birds and dilution by the addition of hatching year birds decreased overall 'herd immunity' to less than 10%. Human infection in Los Angeles during 2005 occurred in Long Beach, where enzootic activity was minimal during 2004, and at scattered upland sites after 'herd immunity' declined by midsummer 2005.

Activity in Kern County during 2005 was remarkably similar to 2004 (Takahashi et al. 2005), with an elevated case incidence rate of 8.8 per 100,000. Resurgence was related to a wet spring which rapidly increased vernal *Cx. tarsalis* Coquillett populations and to relatively low 'herd immunity' in free ranging birds which remained <10% at the end of 2004. In marked contrast, the overall seroprevalence rate in wild birds in Kern County during 2005 exceeded 30% in all species and approached 50% in surviving Western scrub-jays, the most important corvid species in and around Bakersfield. We are continuing to monitor seroprevalence in the avian population to determine if elevated immunity will be associated with a reduced incidence of human infection during 2006 as we observed in Los Angeles during 2005.

Sacramento County was the 2005 WNV epicenter. Early avian infection was noted in rural areas, but most infections where documented in year round resident bird species. These early indications of enzootic transmission were followed by a large epiornitic in American crows over much of northern urban Sacramento. Infection rates in *Cx. pipiens* L. in periurban areas were greater than in *Cx. tarsalis,* which were similar to *Cx. stigmatosoma* Dyar. Human infection followed closely behind extensive crow mortality and triggered emergency adulticiding which appeared to arrest transmission. Long term studies on several resident bird species at Stone Lakes National Wildlife Refuge documented shifts in population age structure, with proportionately greater numbers of hatching year birds collected since the introduction of WNV. These data indicated that high mortality rates among older birds may have changed the age structure as well as possibly enhanced brooding success due to reduced competition.

Research concurrent with our field ecology studies focused on mechanisms that may have enabled the introduction and maintenance of WNV in California. Laboratory studies described the impact of temperature on virus growth in *Cx. tarsalis* and le to the construction of a degree-day model describing the extrinsic incubation period (or the time from imbibing an infectious blood meal until 50% of the infected females were able to transmit virus by bite). The model for WNV (Reisen et al. 2006c) was strikingly similar to that developed previously for St. Louis encephalitis [SLE] (Reisen et al. 1993) and indicated that transmission was most effective under elevated summer temperatures. At northern latitudes or cooler maritime climates in coastal California transmission may be limited to periods with above normal temperatures that may be predictable from long lead climate forecasts.

SLE was absent from California for the 2nd consecutive year and was not detected in pools of mosquitoes, sera of sentinel chickens or humans, despite the enhanced surveillance effort associated with tracking WNV. This disappearance perhaps was related, in part, to cross protective immunity in birds. In one experiment, house finches surviving previous infection with WNV were completely protected against infection with SLE; i.e., viremia could not be detected after experimental challenge. In contrast, although previous SLE infection prevented mortality, WNV infection still resulted in a viremia that was sufficiently elevated to infect mosquitoes. This incomplete cross protection may provide an advantage to WNV and may have contributed to the current competitive displacement of SLE in California.

Field studies in southern California provided the first documentation of natural vertical transmission of WNV by both *Cx. quinquefasciatus* and *Cx. tarsalis*. These findings ha important implications for amplification and over wintering (Reisen et al. 2006b). If newly emerged and vertically infected females

can transmit WNV during their initial and subsequent blood meals, viral amplification may be dramatically enhanced during summer by multiple infectious vector host contacts and the elimination of delays and survival losses related to extrinsic incubation. If females that emerge during fall destined for diapause are infected vertically, these females may retain their infection through winter and renew the transmission cycle in late winter of the following year when they terminate diapause and take their initial blood meal.

Historically, ardeid birds have been implicated as important vertebrate hosts of Japanese encephalitis virus (Buescher et al. 1959) and Murray Valley encephalitis (Boyle et al. 1983). However, communally nesting ardeid birds (cattle egrets, black-crowned night herons, snowy egrets, great egrets) in Imperial (Reisen et al. 2005b) and Yolo Counties did not form a major focus for WNV amplification, even though WNV infections were detected in mosquitoes and birds collected at these nesting sites. After experimental WNV infection, nestling black-crowned night herons produced elevated viremias, whereas cattle egrets were moderately competent hosts; both species exhibited elevated mortality rates after experimental infection.

WNV infection in corvids and their resulting elevated viremias were critical for the efficient infection of peridomestic *Cx. quinquefasciatus* and for epidemic WNV transmission in periurban settings. Examination of bird distributions and mosquito infection rates showed that during 2004 *Cx. quinquefasciatus* infection rates were significantly higher in Los Angeles and Kern Counties with elevated American crow and western scrub-jay populations than in Coachella Valley where corvid populations were less abundant or absent (Reisen et al. 2006a). Dead bird test results were used to delineate the radius of effective foraging areas used by American crows from 6 roosts in southern California. *Culex quinquefasciatus* infection rates and the incidence of human infection were significantly higher within these clusters of dead American crows than without, indicating the epidemiological importance of dying American crows in residential landscapes.

Collectively, our research is starting to direct our understanding of WNV transmission in California (Figure 1). The basic rural enzootic maintenance cycle seems to involve *Cx. tarsalis* and perhaps other susceptible *Culex* species and passerine birds such as house finches and house sparrows. Elevated herd immunity within these populations seems to dampen transmission and may have effectively arrested the 2004 WNV epidemic and 2005 amplification within upland Los Angeles and perhaps the 2005 outbreak in Bakersfield. The majority of human cases have occurred within urban or periurban residential landscapes in association with elevated *Cx. pipiens* or *Cx. quinquefasciatus* infection rates. This urban amplification undoubtedly is facilitated by WNV infection in corvids that results in elevated viremias, sickness and death and effective *Cx. pipiens* complex vector infection clustered around communal American crow roosts. Mosquito-mosquito vertical transmission as well as bird-bird transmission at these heavily populated roosts also may contribute to rapid amplification to epidemic levels. Although rabbit and squirrel infections have been documented, there does not, as yet, seem to be a mammal-*Aedes* cycle similar to that recognized for western equine encephalomyelitis virus (Hardy 1987) and therefore horse and human infections most likely have originated from the bite of *Culex* mosquitoes.

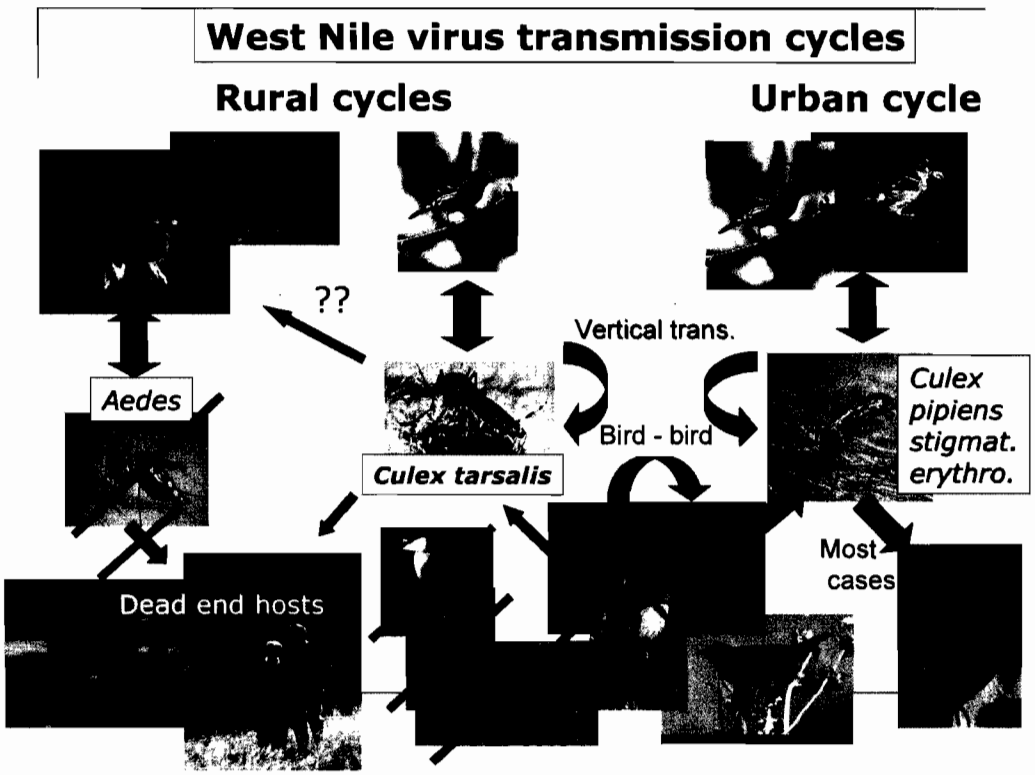

Figure 1. Complexity of West Nile virus transmission cycles in California. Diagonal lines indicate portions of the proposed cycle and tentative host groups that remain undocumented or unlikely based on current results.

Based on our research and the collective experience of California agencies with WNV over the past 3 years, we feel that during 2006:

- The WNV epidemic should begin to subside, with fewer human and horse cases throughout the state.
- Intense enzootic transmission and human cases may not be concentrated at large urban epicenters, but rather be dispersed as WNV tracks populations of non-immune birds.
- Focal outbreaks may continue throughout California during periods of hot weather as WNV tracks pockets of largely non-immune avian populations. Incidence of human disease within these outbreaks may be elevated, especially in rural communities.
- Periurban corvid epiornitics may subside due to depopulation and associated passerine herd immunity, decreasing the value of dead bird reports and increasing the need for bird necropsy and testing.

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Antigen Detection Tests: "The Good, The Bad and The Ugly"

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To facilitate the rapid screening of field-collected pools of mosquitoes for St. Louis encephalitis virus, the Centers for Disease Control and Prevention (CDC) at Ft. Collins developed an antigen capture enzyme immunoassay (EIA) (Tsai et al. 1987). This EIA was useful for large scale field programs where full laboratory support was difficult (Tsai et al. 1988), but was decidedly less sensitive than viral isolation using suckling mouse brain inoculation, the gold standard for virus isolation at that time. EIA technology concurrently was modified to improve sensitivity by first growing virus on Vero cell culture (Graham et al. 1986) and then for high through put using a 96-well format (Chiles et al. 2004b). However, this latter assay required Biosafety Level 3 (BSL3) laboratory conditions restricting use for most Flaviviruses and the detector antibody did not clearly distinguish between West Nile virus (WNV) and St. Louis encephalitis (SLE) (Chiles et al. 2004a). In response to the WNV epidemic and the growing need for diagnostics in areas of the country without sufficient centralized laboratory support, the CDC sponsored development of a wicking antigen assay (VecTest) by Medical Analysis Systems, Inc. Although specific, this assay was less sensitive than plaque or reverse transcriptase-polymerase chain reaction (RT-PCR) assays in laboratory (Ryan et al. 2003) and field (Nasci et al. 2002) evaluations. Because of the large amounts of WNV found in dead American crows, the VecTest also was found to be useful for testing oral swabs from these birds (Komar et al. 2002, Stone et al. 2004). However, in our hands this method was less sensitive than RT-PCR and did not work well on other avian species (Padgett et al. 2006). To improve upon visualization and to quantify results, this wicking technology was modified into the RAMP test. Although somewhat improved and specific, this assay still was less sensitive than RT-PCR assays and can only detect a single virus per test. The development of RT-PCR assays for use at the Center for Vectorborne Diseases (CVEC) has been described elsewhere (Chiles et al. 2004b). The current paper summarizes differences in sensitivity among antigen capture assays and the RT-PCR multiplex used by CVEC and describes the impact of using less sensitive assays on the time from infection to detection and estimates of field infection rates.

SENSITIVITY

Serial 10-fold dilutions of WNV grown in Vero cell culture were tested concurrently by the VecTest, RAMP, in situ EIA and RT-PCR assays to determine comparative sensitivity (Figure 1). RT-PCR following RNA extraction using the RNEasy (Qiagen) kit with a Qiagen manifold system was most sensitive, followed in descending order by RT-PCR following ABI robotic RNA extraction, in situ EIA, RAMP and VecTest. The range in sensitivity was decreased from ca. 6 plaque forming units (PFU) of WNV per mL for the RT-PCR to ca. 200,000 PFU for the VecTest.

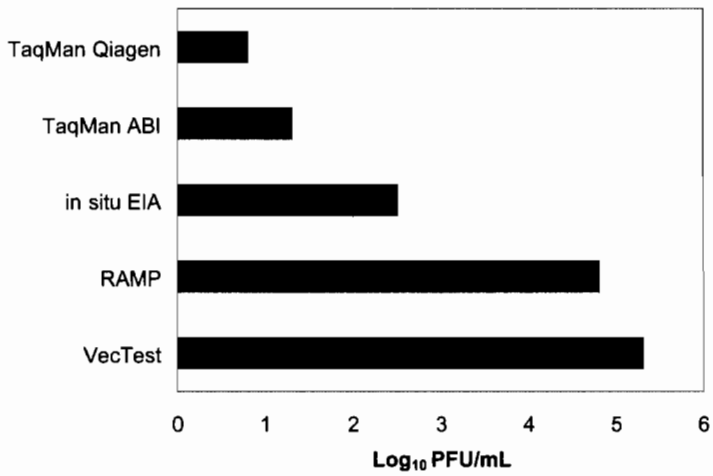

Figure 1. Sensitivity in $\log_{10}$ plaque forming units (PFU) of West Nile virus determined by plaque assays detected by TaqMan RT-PCR after RNA extraction using the Qiagen manifold or the ABI 6700 robotic systems, the *in situ* enzyme immunoassay (EIA) or the RAMP or VecTest antigen capture assays.

The time from detection of WNV after mosquito infection varied as a function of assay sensitivity and temperature. Following infection mosquitoes exhibited a wide range of virus titers that increased as a function of time in days post infection (dpi) (Fig. 2). The rate of increase in body virus titer was faster under warm temperatures similar to those found during summer than those found under cool temperatures similar to those experienced during spring. Based on the sensitivity of the different assays estimated in Fig. 1, RT-PCR would be able to detect infection in all female mosquitoes when they were collected on 3 – 4 dpi, whereas the RAMP and VecTest would require viral titers to increase to more than 4.8 or 5.3 $\log_{10}$ PFU, respectively. Under warm conditions (e.g., 30°C), viral growth exceeded these thresholds in as little of as 5 dpi; however, at cool temperatures (e.g., 22°C), viral growth to exceed these detection thresholds required more than 21 dpi.

Transmission of WNV by *Culex tarsalis* Coquillett occurs only after body titers exceed a minimum threshold of around 4.6 $\log_{10}$ PFU (Fig. 2). Therefore, RAMP and VecTest detect positive pools that contain 1 or more females that have body titers sufficiently elevated to transmit virus. From a surveillance standpoint, an early warning is compromised because detection is limited to females that have begun transmitting virus.

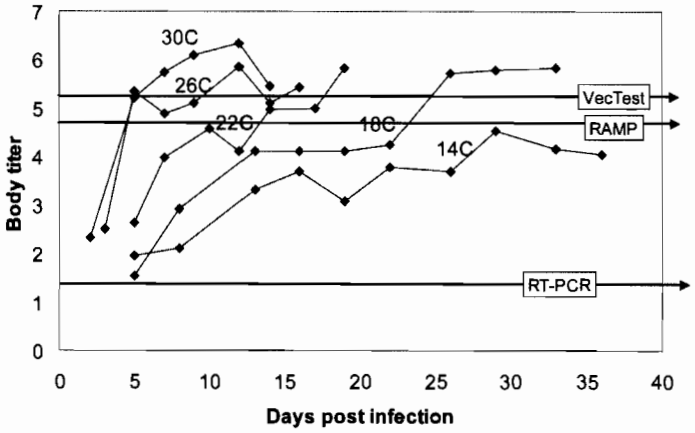

Figure 2. Increase in WNV titer in *Culex tarsalis* plotted as a function of time in days after infection for females maintained at each of 5 temperatures (redrawn from Reisen et al. 2006). Horizontal lines show the thresholds for the detection of virus using RT-PCR, RAMP and VecTest based on sensitivity estimates from Fig. 1.

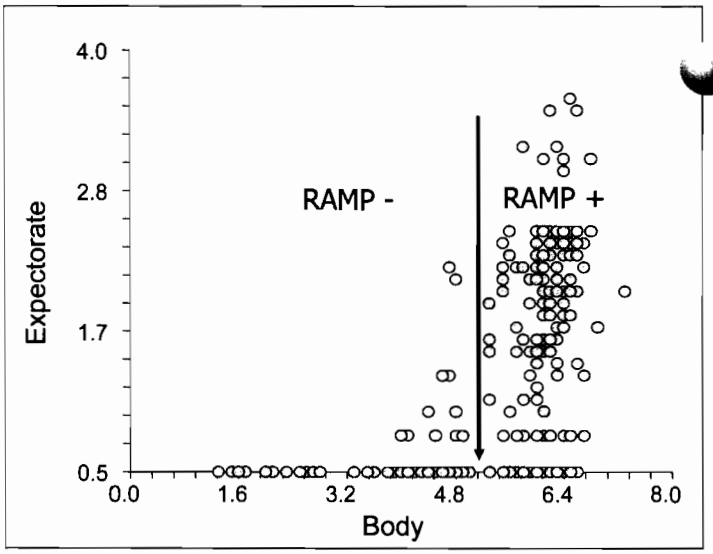

Figure 3. Quantity of WNV in the expectorate or bodies of infected *Culex tarsalis* expressed as $\log_{10}$ plaque forming units per sample (redrawn from Reisen et al. 2006). The vertical line shows the expected demarcation of RAMP positive (right) and negative (left) specimens based on the sensitivity estimates from Fig. 1.

SAMPLING AND RISK ASSESSMENT

Temperature markedly affects not only WNV growth within the mosquito host (Fig. 3), but also daily mosquito survival (Reeves et al. 1994), the duration of the gonotrophic cycle and therefore the frequency of blood feeding (Reisen 1995), and vectorial capacity, or the case dissemination rate (Smith 1987). Assay sensitivity markedly alters the detection of virus occurrence, because mosquitoes must survive long enough to amplify virus to detectable levels. In Fig. 4, for example, an imaginary cohort of 1,000 *Culex* females all become infected feeding on a viremic bird on day 0 during early summer when temperature averages 72°F (22°C). If this cohort had a constant daily survivorship of 80% and a 6 day gonotrophic cycle, then 262 and 69 females would survive to complete gonotrophic cycles 1 and 2 and were available for trap collection when attempting to refeed when 1 and 2 parous, respectively. Therefore, based on assay sensitivity (Fig. 1) and viral growth at 22°C (Fig. 2), 262 1-pars and 69 2-pars would be available for testing; however, testing by antigen capture technology only would detect infection within the 2-pars. To retain the same level of surveillance sensitivity, it would be necessary to collect and test almost 4 times the number of mosquitoes to compensate for the decrease in assay sensitivity.

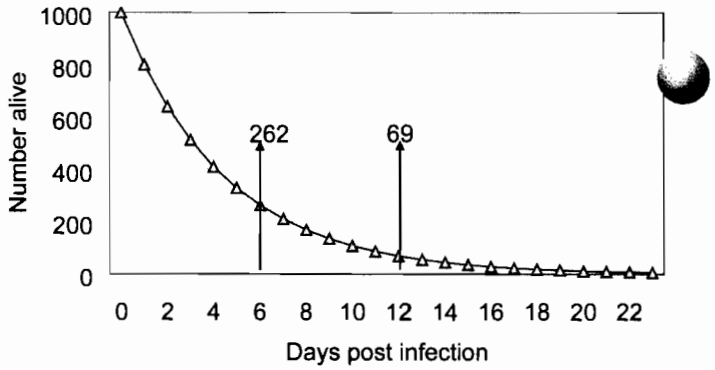

Fig. 4. Theoretical number of host-seeking females alive out of 1,000 infected on day 0 that were available for collection by dry ice-baited traps after 1 and 2 gonotrophic cycles [GC] at 72F. Survival curve based on 80% daily survivorship and a 6 day GC calculated from previous degree-day models for *Cx. tarsalis* (Reisen 1995).

Decreased assay sensitivity and commensurate decreased detection of positive mosquito pools most likely would lead to an underestimate of the minimum infection rate (MIR) used to measure risk in the state guidelines (Kramer 2001). In these guidelines, MIRs for *Culex* range from 0 to >5 per 1,000, indicating escalating risk from normal season to epidemic conditions. This range seems appropriate for *Cx. tarsalis* and *Cx. pipiens* complex based on our observations in California, where epidemics have been associated with elevated MIR >5 per 1,000 (Wilson et al. 2005, Takahashi et al. 2005). During spring and early summer when temperatures are cool (72°F), RAMP and VecTest would miss ca. 3 of 4 positive pools and therefore MIRs would be underestimated proportionately. Therefore, a sample of 4 positives in 20 pools of 50 females each would provide a minimum infection rate estimate of 4 per 1,000 and be at the emergency planning stage, whereas if only 1 of these 4 pools tested positive, risk level would be at normal season.

TURN-AROUND TIME VS SENSITIVITY

Currently mosquito pools submitted to CVEC by Wednesday are tested and reported by Friday. This high through put and rapid turn-around-time were improved by frequent reporting and maintained throughout the summer of 2005 (Kahl et al. 2005). Therefore, if mosquito traps were operated on Monday, picked-up and processed on Tuesday, samples sent to CVEC by overnight FedEx on Tuesday, they would be tested on Wednesday or Thursday and reported by Friday. During spring districts potentially would know if virus was present 2 weeks before the virus grew to levels detectable by RAMP or VecTest (based on our estimates in Fig. 2).

SUMMARY: the Good, the Bad and the Ugly

Antigen capture tests were developed with "good" intentions to fill a laboratory support void in some areas, because they can be done by personnel with minimal training and equipment and results are available within minutes providing the appearances of rapid data acquisition. If the supplied commercial buffers are used, these contain detergent that reputedly inactivates virus, making testing relatively safe. The tests work well when done properly and provide specific results. However, these tests are "bad" in that they are less sensitive and therefore underestimate true infection rates in mosquito populations, especially during spring and early summer or in relatively cool environments when arboviruses within mosquito hosts grow slowly and are present at low levels. Low assay sensitivity would require expanded sampling to detect virus present at low infection rates and at low titers. From a surveillance and abatement standpoint, these tests become "ugly" when they do not detect virus infections until the mosquitoes are capable of transmission. In addition, minimum infection rate estimates and therefore disease risk may be underestimated, delaying timely intervention to prevent human infection. By this time proactive abatement may be too late and only an emergency response possible.

Acknowledgements

Emily Green of CVEC estimated sensitivity levels of the various assays. Laboratory studies referenced in this report were funded by the National Institutes of Allergy and Infectious Diseases, NIH, and the CDC.

Application of Rapid Antigen Detection Tests in 2005

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ABSTRACT: After six years of anticipation, West Nile Virus (WNV) finally arrived in San Joaquin County in 2004. The extent of virus in our county was minimal but it allowed us to see how overwhelmed the State surveillance system was during its first epidemic year. With all predictors indicating that northern California was going to be the epicenter of virus in 2005, San Joaquin County Mosquito and Vector Control District opted to change the surveillance practices to increase turn-around time and to reduce the load going to the California Department of Health Services (CDHS) . All dead birds, mosquito pools, and chicken sera would be tested in-house during 2005 in order to expedite our control efforts in the areas where virus was discovered. Rapid Analyte Measuring Platform (RAMP®, Response Biomedical) was used to test for virus in dead birds as well as in mosquito pools. Approximately 50 encephalitis virus surveillance (EVS) traps were routinely placed around the county. Mosquito pools were tested for virus within 24-48 hours from the point of collection. District personnel were notified of positive pools immediately and adulticiding efforts in that area were planned for that evening. Although sensitivity, as it relates to RT-PCR, was lost during this year, the District feels that the rapid response that we were able to achieve doing in-house testing played an important factor in reducing the number of human cases in our county.

INTRODUCTION

West Nile Virus (WNV) is a mosquito-borne virus that spread rapidly across the United States eventually making its way to the state of California in 2003. California was expected to be the epicenter for the virus in 2004. Although southern California initially experienced the brunt of the virus, every county in the state eventually reported virus.

In 2004, the San Joaquin County Mosquito and Vector Control District (SJCMVCD) along with many other northern California districts had their first experience with West Nile Virus (WNV). These northern California districts also had an opportunity to step back and reflect on the extent of virus in southern California as well as their response to the disease. Many northern California districts met to reevaluate their 2005 game plans. It was during this reevaluation that many complaints began surfacing regarding the turn-around time with respect to the dead birds and the mosquito pools. San Joaquin County was just one of many districts that had positive samples reported 2 weeks or more after the initial collection date. Amplification of WNV could have been worse in our district considering that we were unable to stay ahead of the virus.

JUSTIFICATION FOR IN-HOUSE TESTING

SJCMVCD was able to reevaluate every aspect of our surveillance and control efforts during our first year with virus. One issue was that we could not afford to wait five or more days to get WNV positive information to our field staff. With northern California expecting to be the epicenter of WNV in 2005, we knew that time was of the essence. In addition, we couldn't predict how much WNV would remain in southern California and whether these districts would continue to add additional workloads to the two key labs [UC Davis Center for Vector-borne Disease (CVEC) and the CDHS Viral and Rickettsial Disease Laboratory (VRDL)]. We needed to find a way to expedite all of our surveillance and testing so that management staff could effectively plan for our control. In-house testing seemed like the best solution to expedite the turn-around time of our samples.

SJCMVCD had already begun intensifying its surveillance program by incorporating the use of RAMP® as a tool to detect WNV. During 2005, the district employed RAMP® to test all dead birds and mosquito pools collected as well as testing our own chicken sera for flavivirus and Western Equine Encephalitis using the enzyme linked immunosorbent assay (ELISA).

MOSQUITO POOL TESTING

SJCMVCD employed the Rapid Analyte Measuring Platform (RAMP) to test 1699 mosquito pools during the 2005 mosquito season. Adult mosquitoes were pooled and tested within 24 hours of collection. Thirty-eight pools tested positive for WNV. The first two pools were submitted to CVEC and confirmed positive by multiplex RT-PCR. The remaining 36 pools were held at the district office in a -70 C freezer until the end of the season. Marin-Sonoma Mosquito and Vector Control District confirmed 34 of the remaining pools using RT-PCR. It's unclear whether the two pools that tested negative were true negatives or whether the RNA within the samples may have degraded in the RAMP buffer. Table 1 shows the RAMP values of all the pools and their correlating CT-values. Both pools testing negative by RT-PCR had RAMP values >640 units and pools collected later from both of these locations were confirmed positive, which leads us to believe that the RNA was probably degraded in the samples. A significant finding during this study was two pools that were confirmed positive had RAMP values in the 30-40 unit range (30 units = positive pool). All samples were tested using the same protocol and same biologist.

Table 1. RAMP positive mosquito pools confirmed by RT-PCR by Marin-Sonoma MVCD.

Pool#	Spp.	Date Collected	Site Code	# in Pool	RAMP Results	RAMP units	RT-PCR Results	Ct Values	StDevCt
821	Cx. tarsalis	7/20/2005	8080	39	positive	>640	negative	undet./38.01	n/a
825	Cx. pipiens	7/25/2005	8081	20	positive	>640	negative	38.44/38.79	0.244
831	Cx. pipiens	7/27/2005	8083	50	positive	>640	positive	23.38/23.36	0.017
842	Cx. tars/Cx. pip	7/26/2005	8070	6/7	positive	163.6	positive	26.01/25.48	0.376
843	Cx. tarsalis	7/26/2005	8056	11	positive	68.8	positive	24.65/24.47	0.129
890	Cx. pipiens	8/3/2005	8083	50	positive	150.4	positive	20.29/20.26	0.024
898	Cx. tarsalis	8/3/2005	8027	43	positive	>640	positive	17.81/17.60	0.15
905	Cx. pipiens	8/8/2005	8083	50	positive	31.9	positive	22.99/22.94	0.037
920	Cx. tarsalis	8/9/2005	8075	50	positive	>640	positive	20.58/20.62	0.031
932	Cx. pipiens	8/10/2005	8029	17	positive	286.6	positive	22.68/22.59	0.068
938	Cx. pipiens	8/10/2005	8079	20	positive	158.8	positive	22.88/22.33	0.385
944	Cx. tarsalis	8/9/2005	8082	22	positive	>640	positive	19.73/19.71	0.016
946	Cx. tarsalis	8/9/2005	8021	16	positive	522.1	positive	31.43/31.76	0.232
954	Cx. tarsalis	8/10/2005	8085	50	positive	39.6	positive	31.88/31.59	0.206
960	Cx. tarsalis	8/11/2005	8018	50	positive	>640	positive	18.75/18.81	0.04
962	Cx. tarsalis	8/11/2005	8018	50	positive	461.8	positive	18.60/18.57	0.025
976	Cx. tarsalis	8/11/2005	8005	29	positive	>640	positive	25.96/26.41	0.319
978	Cx. pipiens	8/11/2005	8005	7	positive	396.5	positive	21.63/21.66	0.017
987	Cx. pipiens	8/15/2005	8083	43	positive	>640	positive	19.67/19.65	0.009
1007	Cx. tarsalis	8/15/2005	8075	30	positive	207.6	positive	23.03/23.05	0.015
1053	Cx. pipiens	8/15/2005	8085	50	positive	>640	positive	19.66/19.69	0.017
1056	Cx. tarsalis	8/15/2005	8085	50	positive	601.9	positive	21.21/21.24	0.023
1077	Cx. tarsalis	8/15/2005	8004	50	positive	248.5	positive	25.90/25.06	0.592
1085	Cx. tarsalis	8/17/2005	8022	26	positive	510.5	positive	20.53/20.73	0.138
1092	Cx. pipiens	8/22/2005	8083	12	positive	120.9	positive	20.65/20.68	0.024
1110	Cx. pipiens	8/23/2005	8084	17	positive	>640	positive	19.36/19.36	0.006
1113	Cx. tarsalis	8/23/2005	8026	18	positive	105.8	positive	18.31/18.31	0.004
1118	Cx. pipiens	8/23/2005	8020	14	positive	>640	positive	19.77/19.76	0.011
1126	Cx. pipiens	8/23/2005	8070	3	positive	>640	positive	22.62/22.54	0.058
1127	Cx. pipiens	8/23/2005	8056	50	positive	>640	positive	19.17/19.51	0.241
1128	Cx. pipiens	8/23/2005	8056	48	positive	132.8	positive	22.14/22.29	0.102
1144	Cx. Tar/Cx. Pip	8/25/2005	8027	27/6	positive	160.2	positive	32.33/32.10	0.157
1172	Cx. tarsalis	8/28/2005	8018	39	positive	>640	positive	19.49/19.51	0.013
1261	Cx. pipiens	9/7/2005	8029	47	positive	>640	positive	18.19/18.23	0.032
1345	Cx. tarsalis	9/15/2005	8045	8	positive	287.1	positive	22.53/22.72	0.133
1524	Cx. pipiens	10/3/2005	8080	12	positive	40.1	positive	25.87/25.95	0.055

RESPONSE

The San Joaquin County WNV Task Force, which consists of the Mosquito and Vector Control District, the Office of Emergency Services (OES), the Local Health Department, the Ag Commissioner's Office, and the County Environmental Health, began implementing the California Mosquito-borne Virus Surveillance and Response Plan and evaluating the WNV risk assessment on a weekly basis once virus moved into northern California. Table 2 shows a sample of the District's weekly assessment. During the week of July 22 the district was functioning in a normal season mode with all employees maintaining surveillance and control of their assigned zones. Within four weeks the District's risk level was elevated to epidemic conditions and

Table 2. WNV risk assessment during July and August 2005.

DATE	AVG. RATING	RESPONSE
July 22	2.15	Normal Season
July 29	3.375	Emergency Planning
August 1	3.625	Emergency Planning
August 8	4.0*	Emergency Planning
August 15	4.375	Epidemic Conditions

aerial contractors had to be employed.

Figure 1 shows the number of positive mosquito pools for WNV and the week that corresponds with the collection and testing. Between weeks 32 and 33, eleven mosquito pools tested positive for WNV pushing San Joaquin County into epidemic conditions. A riparian brush rabbit, raised at a propagation facility in San Joaquin County, tested positive for WNV antibodies during this same time period. The rabbit was later relocated to another area of our county. One mosquito pool and three chickens located near the propagation facility tested positive for WNV within two weeks following this discovery.

Within 24-48 hours of detecting virus, management was notified of the positive locations. Field technicians were sent to the positive trap locations to perform thorough inspections; if during the inspection, an abundance of adult mosquitoes was found, ground and/or aerial spraying operations were planned. In addition, every member of our Task Force as well as the local news media was notified. Spray maps were generated by the management team and given to our local OES to be posted on our website as well as given to our field technicians. During our emergency planning stages, an additional aerial applicator was hired to assist with large scale spraying operations. Vector Disease Control Incorporated (VDCI) was the applicator chosen to assist our current aerial contractors (Precissi Flying Service Inc) to treat areas measuring >5000 acres. Routine inspections and treatments of 5000 sources occurred simultaneously with routine ULV adulticiding of small areas (<5000 acres) by ground crews. Figure 2 shows an overlay of our adulticiding operations in response to the number of positive

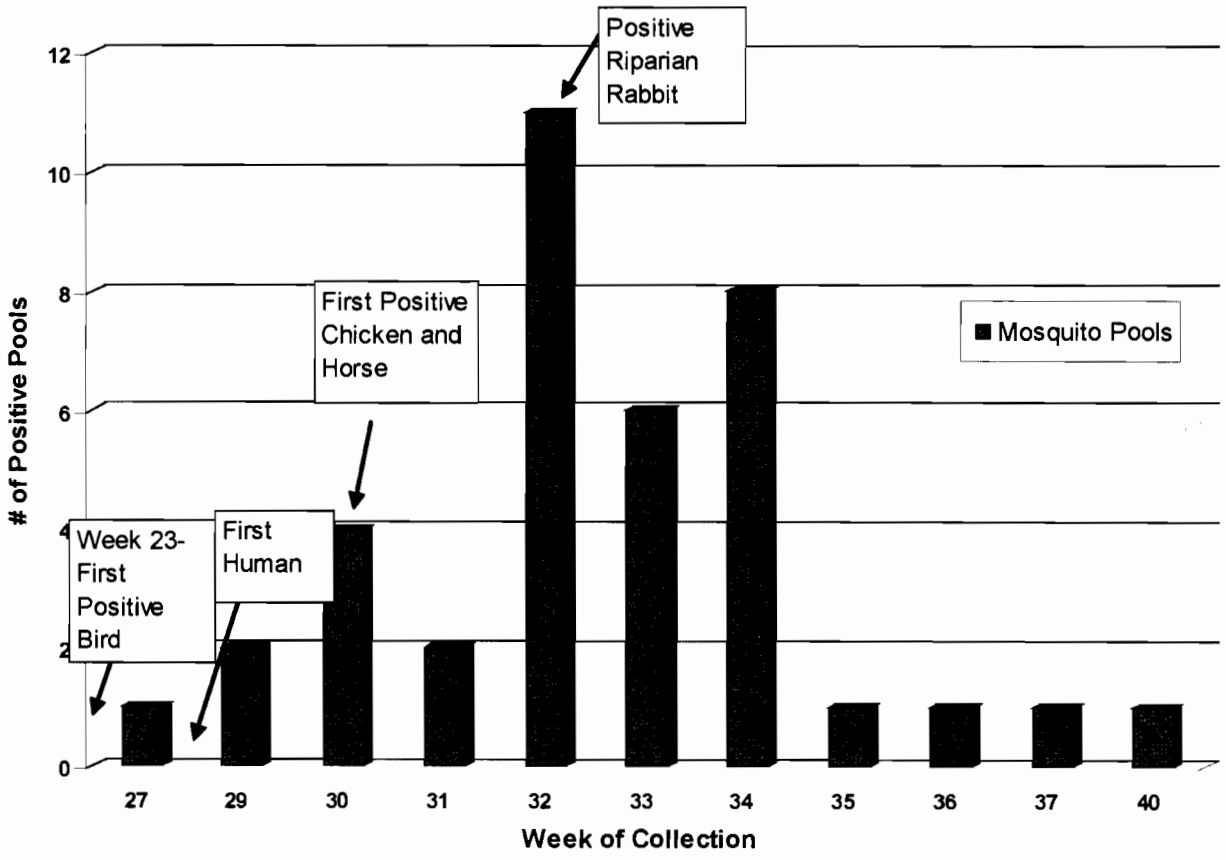

Figure 1. Temporal distribution of positive mosquito pools in San Joaquin County and a comparison of WNV activity in all samples.

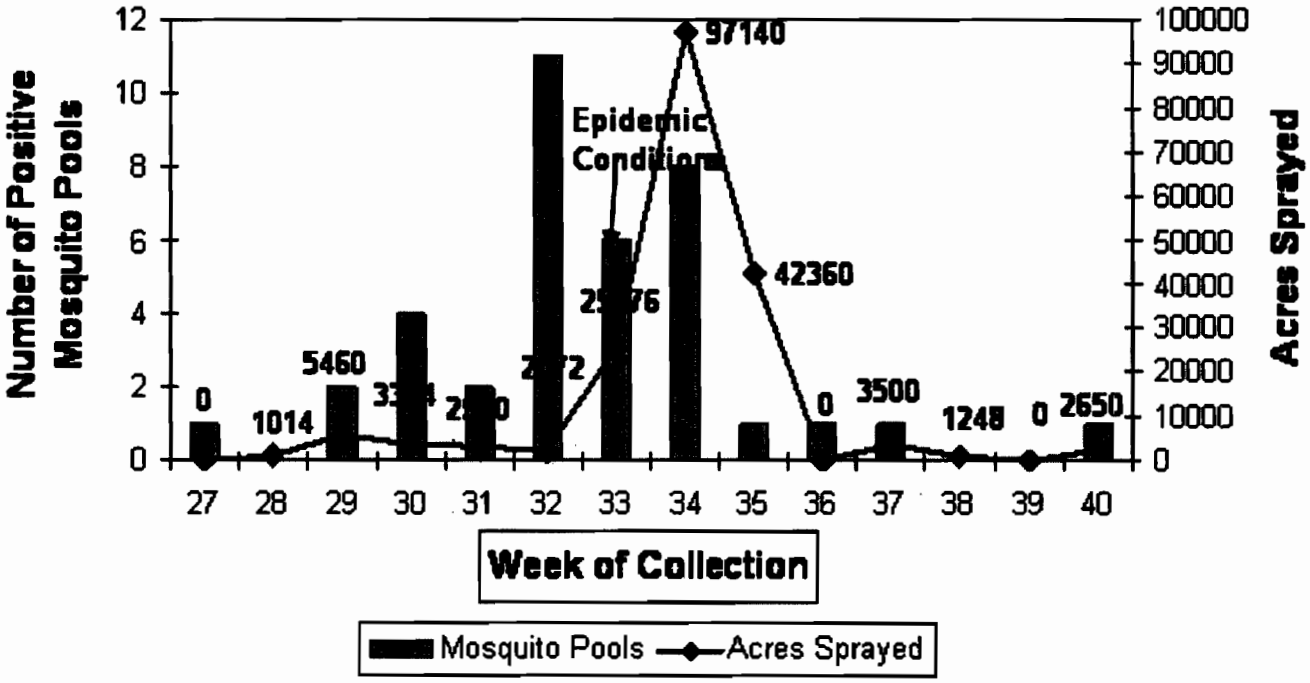

Figure 2. Number of acres treated in response to positive mosquito pools.

mosquito pools. SJCMVCD was functioning in a normal season mode when the first positive mosquito pool was collected on Brack Tract. A reevaluation of our weekly risk assessment placed SJCMVCD in emergency planning after our first positive mosquito pool was announced. Within 5 weeks of our first positive mosquito pool, 19 more pools tested positive for WNV, advancing the district into epidemic conditions.

CONCLUSION

San Joaquin County MVCD began implementing in-house testing during the 2005 season to increase the turn-around time as well as the response time by the technicians. All members of our county WNV Task Force were kept abreast of all changes in virus activity. The cooperation by all members of the District and the Task Force helped reduce the additional stresses that could have occurred with the media coverage and workloads. Response time by the employees was usually within 48 hours. Field technicians continued to inspect and larvicide their assigned work zones in addition to ULV adulticiding in the evenings. Between ground and aerial work, a total of 187,074 acres were treated in response to positive mosquito pools. The intensive control efforts after week 33 resulted in a significant reduction of positive mosquito pools.

The result, we feel, was significant compared to our neighboring counties; only 36 human cases, with one fatality, and only 19 equine cases were reported for San Joaquin County.

Rapid Detection of West Nile Virus in Fox Squirrels (*Sciurus niger*) Using RAMP® Test

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ABSTRACT: A trial was conducted to determine whether the RAMP® rapid antigen test could detect West Nile virus in oral swabs from dead fox squirrels. Although only two squirrels out of 15 tested were RAMP® positive using the standard cutoff value of R = 50, six out of seven squirrels with R values greater than zero were confirmed to be PCR positive while all squirrels with R = 0 were PCR negative. Test results on corvids were species-specific; results were more accurate for American crows than for western scrub-jays. Adjusting the test conditions or lowering the cutoff value for positive results might improve the utility of RAMP® for squirrel and scrub-jay testing.

INTRODUCTION

During 2005, the Contra Costa Mosquito and Vector Control District utilized the RAMP® rapid antigen test for early detection of West Nile virus (WNV) in corvid oral swab samples. While we considered our use of these tests to be supplemental to PCR testing by the University of California Davis Center for Vectorborne Diseases (CVEC), the California Department of Health Services (CDHS) accepted RAMP® test results on dead crows as accurate enough to be considered 'official' for reporting purposes.

While corvids and certain other bird species are known to be susceptible to WNV, data collected by CDHS in 2004 suggested that tree squirrels may also be susceptible. The fox squirrel *Sciurus niger*, an introduced species, is very common in densely populated residential areas of the San Francisco Bay area (Byrne 1979), has a limited home range (Flyger and Gates 1982), and therefore could prove to be a useful sentinel for local virus transmission in urban areas. The California Department of Health Services, in collaboration with the Lindsay Wildlife Museum, a large wildlife rehabilitation center located in central Contra Costa County, had already initiated a study of WNV in squirrels submitted to the wildlife hospital (Padgett et al. 2005). Suspect squirrels reported to the CDHS statewide WNV hotline by the Museum were picked up by District personnel for shipment to the California Animal Health and Food Safety Laboratory (CAHFS) for necropsy and CVEC for PCR testing, a process which took up to 14 days. Since rapid testing is capable of providing same-day results and a much quicker operational response, we decided to investigate whether RAMP® could detect WNV in squirrel oral swabs.

MATERIALS AND METHODS

We used the standard RAMP® procedures recommended by the manufacturer, Response Biomedical, for testing oral swabs from corvids and squirrels and the standard cutoff value of R ≥50 (determined automatically by the RAMP® reader from the ratio of a test band to a control) to decide whether a test result was positive (Response Biomedical Corp., 2005). All squirrels tested by RAMP® were subsequently submitted to CVEC for PCR testing. Western scrub-jays (*Aphelocoma californica*) testing positive (R ≥ 50) were reported to DHS but not submitted for further testing while those testing negative were submitted for PCR testing. Since RAMP® results on crows (*Corvus brachyrhynchos*) were considered definitive by CDHS, we did not submit the majority of crows we tested for PCR confirmation.

RESULTS

A total of 15 fox squirrels were RAMP® tested. Of these, only two had R values above the standard cutoff value of 50 recommended for corvids. These were both confirmed by CVEC to be PCR positive, as were five additional squirrels with R values less than 50 (Table 1). Therefore, using the standard cutoff value, 87% of our squirrel test results were false negatives. However, further examination of the data revealed that all seven of the PCR positive squirrels had RAMP® R values greater than zero, and all but one of eight PCR negative squirrels had R = 0. R values for PCR positive squirrels ranged from 8.6 to > 640 (mean = 134.6, standard error = 101.6) (Table 2).

Table 1. Fox squirrel RAMP test results. Samples with R >= 50 were considered WNV positive.

Tested	RAMP +	RAMP -	False +	False -
15	2 (13%)	13 (87%)	0 (0%)	13 (87%)

Table 2. RAMP test results for individual fox squirrels with R > 0.

Date	DHS ID Number	City	RAMP	R	PCR
9/26	Q5-196	Concord	negative	8.6	positive
9/26	Q5-195	Walnut Creek	negative	33.4	positive
9/28	Q5-201	Pleasant Hill	positive	81.3	positive
10/4	Q5-213	Martinez	negative	19.0	positive
10/11	Q5-225	Martinez	positive	> 640	positive
10/18	Q5-232	Concord	negative	19.9	negative
10/21	Q5-233	Concord	negative	24.7	positive

Examination of RAMP® test results on corvids showed that sensitivity of the test varied with species (Table 3). Of 31 crows tested, nine were RAMP® positive and 22 RAMP® negative. Thirteen of the RAMP® negative crows were submitted for PCR testing, with a single false negative (7.6%). Of 67 western scrub-jays tested, 31 were RAMP® positive and 36 RAMP® negative. All of the negative jays were submitted for PCR testing, which revealed 16 false negatives (24%). However, all but five of the false negative jays had R values greater than zero.

Table 3. Corvid test results. *out of 13 crows submitted for PCR confirmation

Species	Tested	RAMP +	RAMP -	False -
Western scrub-jay	67	31 (46%)	36 (54%)	16 (24%)
American crow	31	9 (29%)	22 (71%)	1 (7.6%)*

DISCUSSION

Despite the low sensitivity of RAMP® relative to PCR testing (Cahoon-Young et at. 2005), the ability to rapidly screen samples makes it a useful tool for vector control agencies desiring a quick operational response to evidence of local virus transmission. Using the standard cutoff value of 50, RAMP® failed to detect the majority of WNV positive fox squirrels. However, the observation that all of the squirrels with $R < 0$ were PCR negative and all but one with $R > 0$ were PCR positive suggests that despite the lower apparent viremias in squirrels RAMP® may be still be useful for rapid WNV screening, perhaps with adjustments in the cutoff value and/or the test procedure to improve sensitivity. The accuracy of RAMP® also varied with corvid species but it still detected the majority of positive crows and scrub-jays and enabled us to respond operationally while waiting for PCR confirmation of negative results. As with squirrels. adjustment of cutoff values might improve the accuracy of RAMP® for testing scrub jays. Additional testing is planned in 2006.

Acknowledgment

We thank Kerry Padgett of DHS and the staff of the Lindsay Wildlife Museum for arranging for submission and PCR testing of squirrels, the staffs at CAHFS and CVEC for conducting necropsies and PCR tests, and Gary Bogue of the Contra Costa Times for helping get the word to the public that dead squirrels as well as birds should be reported.

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West Nile Virus Rapid Diagnostic Test: False Negative, False Positive

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ABSTRACT: During 2005, over 1,750 RAMP test cartridges were used for detection of West Nile virus (WNV) in mosquito samples and dead birds at the West Valley MVCD. Some mosquito and bird specimens also were tested by RT-PCR. Concurrent testing with VecTest was done on all bird specimens. The RAMP and VecTest tests are viral antigen based tests, and RT-PCR is a viral RNA test.

While the RAMP test greatly shortened the turn-around time for mosquito test results, occasional conflicting test results between the RAMP and RT-PCR were seen. We evaluated the potential sources of discrepancies. We also investigated the possible causes of WNV false positive and false positive negative results with the RAMP tests, such as reagent lots, the manufacturer suggested cutoff value (15 vs. 30 RAMP units) for positive RAMP results, the homogeneity and viscosity of mosquito sample homogenates, and the sample volume applied to the test cartridges.

Surveillance for Mosquito-borne Encephalitis Virus Activity and Human Disease, Including West Nile Virus in California, 2005

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

In 2005, the surveillance program elements included the following:

(1) Diagnostic testing of specimens from human patients exhibiting symptoms of encephalitis, aseptic meningitis, acute flaccid paralysis, or with unexplained febrile illness of more than seven days;

(2) Enrollment of patients diagnosed with encephalitis into the CDHS California Encephalitis Project, which evaluates 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 western equine encephalomyelitis (WEE), West Nile virus (WNV), and other arboviruses as appropriate;

(4) Monitoring and testing of mosquitoes for the presence of St. Louis encephalitis (SLE), WEE, and WNV. Tests were also done for dengue and other arboviruses, as appropriate;

(5) Serological monitoring of sentinel chickens for SLE, WEE, and WNV antibodies;

(6) Surveillance and diagnostic testing of dead birds, especially crows and other species in the family Corvidae, for infection with WNV;

(7) Weekly reporting in the CDHS Arbovirus Surveillance Bulletin of arbovirus testing results in California and arbovirus activity throughout the United States;

(8) Reporting of WNV data bi-weekly on the California WNV website: www.westnile.ca.gov, and the California Vectorborne Disease Surveillance System: http://vector.ucdavis.edu/arbo.html, including test results, reports, maps, and public education materials;

(9) A pilot study to predict areas of human risk of acquiring WNV using dead bird reports analyzed with the Dynamic Continuous-Area Space-Time (DYCAST) model.

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

Arbovirus diagnostic procedures used in 2005 in California are summarized in Table 2.

HUMAN DISEASE SURVEILLANCE

In 2002, the VRDL initiated a regional public health laboratory network with 33 local county public health laboratories to enhance human WNV testing and surveillance efforts in California. The local laboratories tested for WNV using the IgM or IgG immunofluorescent assay (IFA) and/or the IgM or IgG enzyme immunoassay (EIA). Laboratories forwarded positive specimens to the VRDL for verification or confirmatory testing. Additional suspect cases of WNV were identified through the California Encephalitis Project, the Kaiser Permanente regional laboratories, four major commercial reference laboratories, and blood donation centers.

Over 2,800 specimens from 2,222 individuals were tested for WNV at the VRDL in 2005. The first case of 2005 was a 47-year-old male from Tulare County who had onset of West Nile fever in early June. In total, 935 human WNV infections were identified among the residents of 40 counties in California (Fig. 1), an increase of 105 infections from 23 counties identified in 2004. One hundred one of the 935 WNV infections were detected in blood donors, 42 of whom later developed clinical symptoms consistent with West Nile fever. Four asymptomatic WNV-infected individuals were identified through testing at local health departments.

Of the 880 cases (symptomatic infections), 534 were classified as West Nile fever, 305 were identified as neuroinvasive disease cases (i.e. encephalitis, meningitis, or acute flaccid paralysis), and 41 were of unknown clinical presentation. Males represented 483 (55%) of 880 cases. The median age for all cases for whom data were available was 51 years (range: 2-95 years). The median age for West Nile fever cases was 49 (range: 2-93), and for neuroinvasive disease cases, 55 years (range: 4-95 years). The median age of the 19 WNV-associated fatalities was 78 years (range: 56-92 years).

Table 1. Summary of WNV Surveillance in California, 2005

County	Humans	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens
Alameda	1	2	48	8	
Alpine					
Amador	4	7	24		
Butte	25	7	79	4	15
Calaveras	2	6	10		1
Colusa	2	2	14		3
Contra Costa	11	10	93	1	18
Del Norte					
El Dorado	1	5	67		
Fresno	68	33	97	71	47
Glenn	13	2	30	5	9
Humboldt	1		4		
Imperial	1			10	54
Inyo		2	12	2	
Kern	68	26	44	235	113
Kings	32	5	73	3	19
Lake		10	32	32	4
Lassen		1	3		
Los Angeles	47	10	173	218	60
Madera	19	20	22	5	7
Marin			14		
Mariposa		2	6		
Mendocino		1	34		
Merced	28	25	171	34	14
Modoc			1		
Mono		1	1		
Monterey		1	2		
Napa			44		2
Nevada	4	5	27		
Orange	17		302	83	2
Placer	35	23	84	20	2
Plumas	1	1			
Riverside	104	46	111	111	154
Sacramento	177	33	70	122	16
San Benito		2	1		
San Bernardino	35	3	74	40	79
San Diego	1		160		
San Francisco	2		2		
San Joaquin	36	19	24	1	21
San Luis Obispo		13	41	2	4
San Mateo	1		10		
Santa Barbara	2	6	73	21	8
Santa Clara	5	1	144	3	
Santa Cruz			2		
Shasta	1	2	28		9
Sierra					
Siskiyou			6		
Solano	5	16	44	4	23
Sonoma	1	1	78		
Stanislaus	92	46	235	114	31
Sutter	9	1	9	43	6
Tehama	4	3	47		1
Trinity					
Tulare	60	22	226	16	44
Tuolumne	1	3	59		
Ventura	1	8	62	2	2
Yolo	12	14	17	28	12
Yuba	6	10	12	4	10
State Totals	**935**	**456**	**3046**	**1242**	**790**

EQUINE SURVEILLANCE

Serum or brain tissue specimens from 1,295 horses displaying neurological signs were submitted to the California Animal Health & Safety Laboratory (CAHFS) for arboviral testing at CAHFS, VRDL, and the United States Department of Agriculture, National Veterinary Services Laboratory. WNV infection was detected in 456 horses from 43 counties (Table 1, Fig. 2). Of the 456 infected horses, 200 (44%) died or were euthanized. Twenty horses were properly vaccinated with the WNV vaccine, 78 were improperly vaccinated, 342 were unvaccinated, and the vaccination history was unknown for 16.

ADULT MOSQUITO SURVEILLANCE AND TESTING

In April, surveillance data began to be disseminated using the Adult Mosquito Occurrence Report (AMOR). Forty-three weekly Arbovirus Surveillance bulletins and 44 AMORs, including a five-year average AMOR summary, were disseminated statewide to all program participants. Forty-one local agencies from 32 counties collected mosquitoes using New Jersey light traps in 2005. Additionally, some districts used encephalitis virus surveillance traps (EVS) and gravid traps. The mosquito counts from all three traps, where applicable, were forwarded to VBDS and summarized weekly into the AMOR from April 9 to November 19.

Fifty local mosquito control agencies submitted a total of 752,401 mosquitoes (20,270 mosquito pools) to CVEC for virus isolations (Tables 3,4,5). WNV was detected in 1,242 of 20,270 mosquito pools from 29 counties (Table 3, Fig. 3). In 2005, West Nile virus was first detected from a pool of *Culex quinquefasciatus* Say collected on January 25 in Orange County. The last detection of WNV in mosquitoes in 2005 was from a pool of *Cx. quinquefasciatus* collected on November 30 in Los Angeles County. WNV was identified from six *Culex* species: *Cx. erythrothorax*Dyar, *Cx. pipiens* L., *Cx. quinquefasciatus*, *Cx. stigmatosoma* Dyar, *Cx. tarsalis* Coquillett, and *Cx. thriambus* Dyar. For the first time, WNV was identified from *Anopheles freeborni* Atken in 2005.

In 2005, eight local agencies (Contra Costa, Marin/Sonoma, Sacramento/Yolo, San Gabriel Valley, Shasta, San Joaquin, Santa Clara, and West Valley) tested a total of 129,778 mosquitoes (4,887 mosquito pools) using RAMP and Vec Test. Results are not included in the summary results because the tests were not confirmed by CVEC.

Western equine encephalomyelitis (WEE) was detected in *Cx. tarsalis* pools from three counties: Imperial (7), Kern (25), and Riverside (19), respectively (Table 3). Saint Louis encephalitis (SLE) virus was not detected in mosquito pools in 2005.

CHICKEN SEROSURVEILLANCE

The 2005 surveillance season began in March with the deployment of sentinel chicken flocks in southern California counties. Fifty-five local mosquito and vector control agencies in 40 counties maintained 271 sentinel chicken flocks (Table 3, Fig. 4). Blood samples were collected from chickens every other week. A slight change to the sentinel chicken testing protocol was made in 2005 to hasten reporting. A chicken found to be positive to flavivirus was

Table 2. Arbovirus diagnostic procedures for California.

	Screening	Primary Test	Confirmatory Test	Virus Tested		
				SLE	WNV	WEE
Human sera	Screened by local public health labs and VRDL	EIA IgM and IgG for WNV (VRDL)	PRNT (VRDL)	–	x	–
Human cerebrospinal fluid	Screened by VRDL	EIA IgM for WNV (VRDL)	If CSF is IgM-positive by EIA, a serum sample is requested	–	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	Rapid Assay either VecTest or RAMP (local agency) or RT-PCR using a primary set of primers on kidney tissue and cell culture on organ pools (CVEC)	RT-PCR using a set of secondary primers (CVEC)	–	x	–
Other animals sera	Screened by VPHS	PRNT for sera (CVEC), virus isolation (CVEC)		–	x	–
Mosquito pools	Collections by local agencies	RAMP (by agency)	*in-situ* EIA using vero cell cultures (CVEC)	x	x	x
Chicken sera	Local agency sentinel flocks	EIA (VRDL)	IFA (PRNT as needed - 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

Viruses:
SLE, St. Louis encephalitis
WEE, western equine encephalomyelitis
WNV, West Nile virus

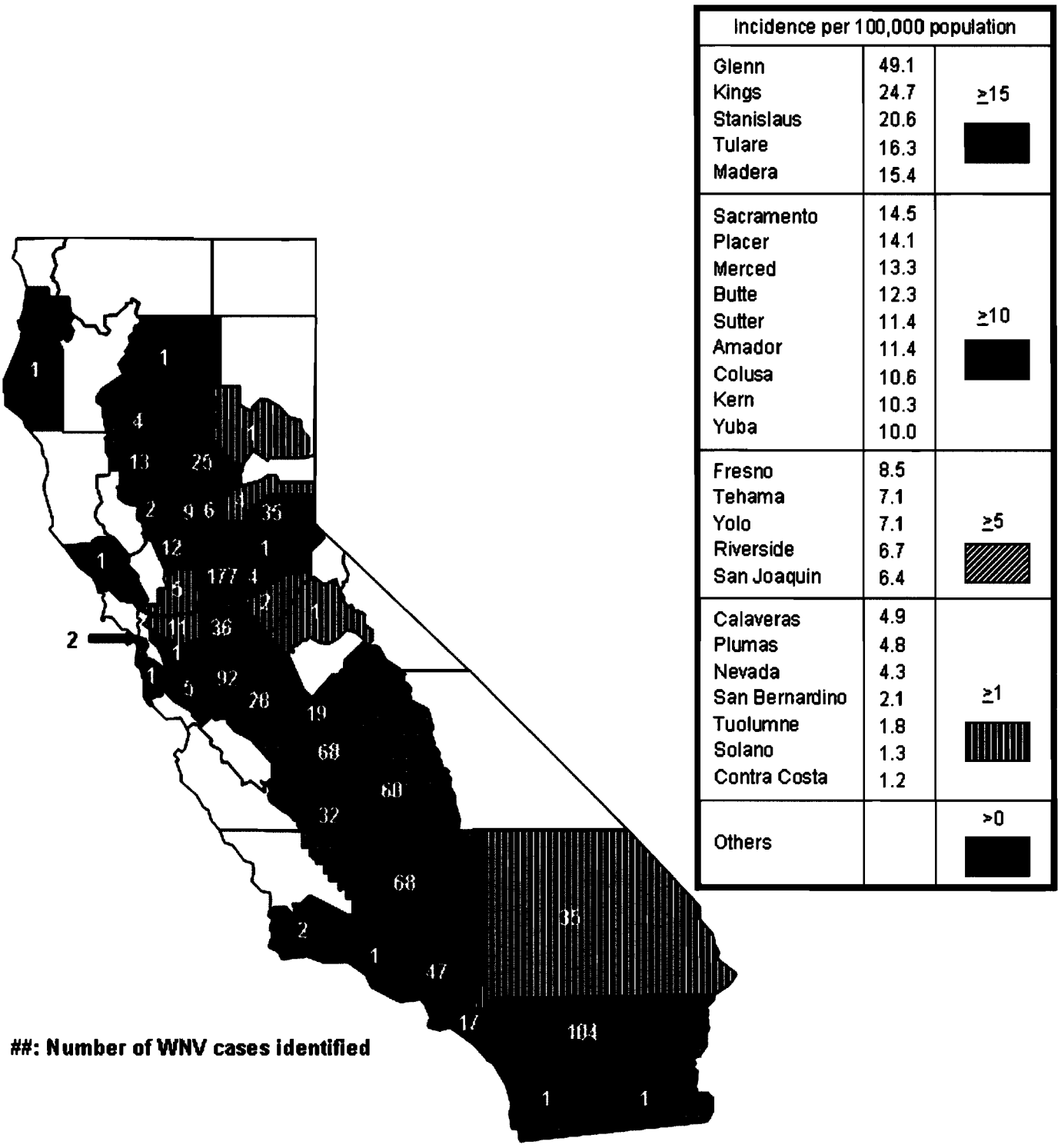

Figure 1. Human Cases of WNV and Incidence per 100,000 by California County, 2005

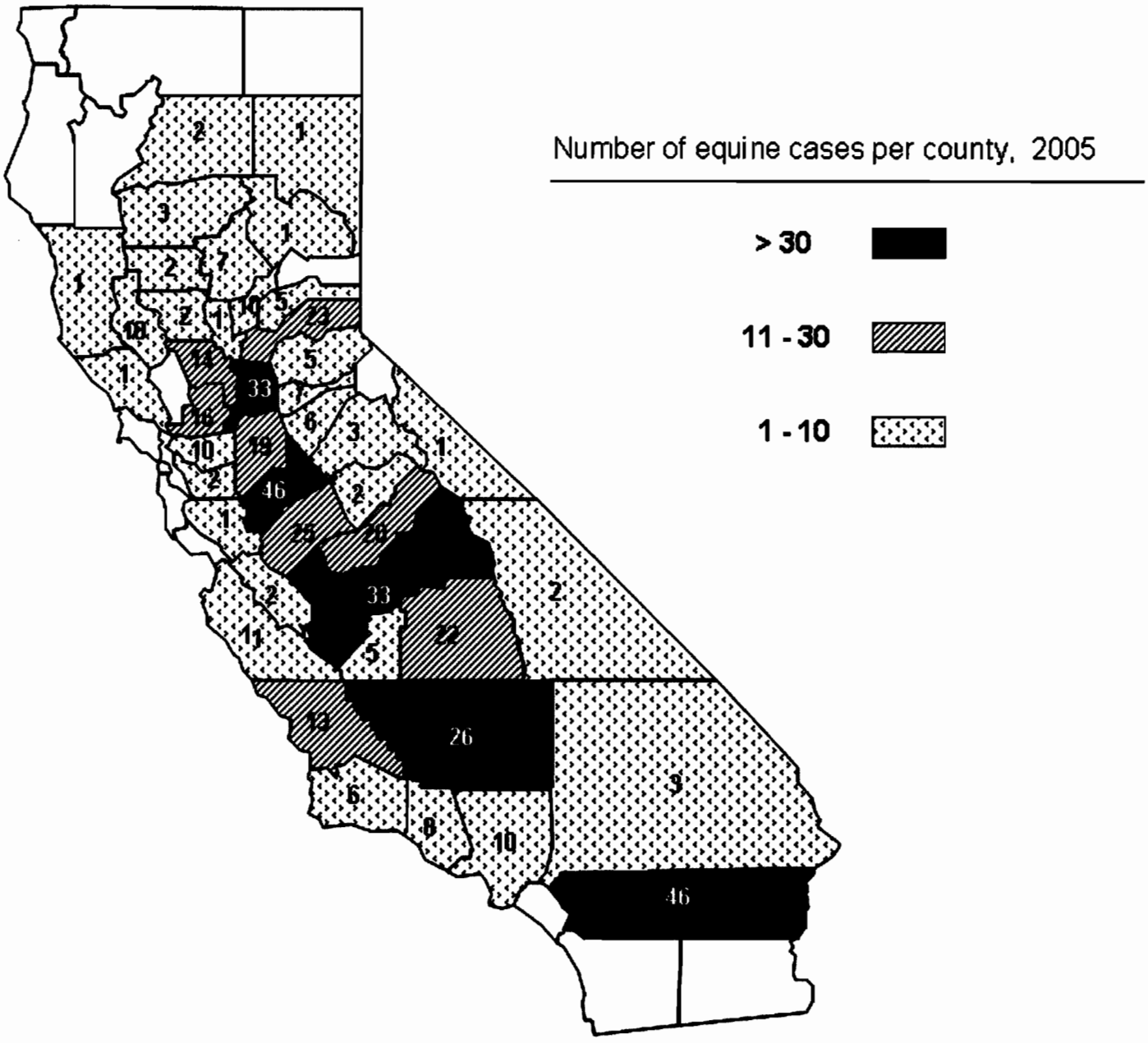

Figure 2. Number of Equine Cases of West Nile Virus by California County, 2005

Table 3. Mosquito pools and sentinel chicken flocks tested for West Nile virus, 2005.

County	Agency	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	WEE + pools	No. flocks	No. chickens	No. sera tested	WNV + sera	WEE + sera
Alameda	Alameda Co. MAD	16173	398	8		3	21	310	0	
Alameda	Alameda Co. Vector Control	839	33							
Alpine										
Amador										
Butte	Butte Co. MVCD	2542	63	4		7	77	1,169	15	
Calaveras						1	10	127	1	
Colusa	Colusa MAD			0		1	10	129	3	
Contra Costa	Contra Costa MVCD	19586	423	1		5	60	758	18	
Del Norte										
El Dorado		73	3							
Fresno	Consolidated MAD	10587	323	57		6	65	849	47	
Fresno	Fresno MVCD	1566	38	4		2	22	267	0	
Fresno	Fresno Westside MAD	2862	71	10		2	20	320	0	
Glenn	Glenn Co. MVCD	2350	47	5		1	13	184	9	
Humboldt										
Imperial	Coachella Valley MVCD	7868	171	0	4	12	120	530	29	26
Imperial	Imperial Co.EH	5868	163	10	3	4	40	550	25	26
Inyo	Owens Valley MAP	6091	127	2		0	0	0	0	
Kern	Antelope Valley MVCD	10	1					54		
Kern	Delano MAD		0	0		2	20	263	18	1
Kern	Kern MVCD	65704	1,585	223	25	9	90	1,269	84	35
Kern	South Fork MAD		0	0		1	10	129	1	
Kern	Westside MVCD	12902	278	12		3	30	402	10	
Kings	Consolidated MAD	127	4							
Kings	Kings MAD	1853	62	3		4	40	430	19	
Lake	Lake Co. VCD	14399	359	32		2	20	295	4	
Lassen										
Los Angeles	Antelope Valley MVCD	1613	56	1		8	48	638	15	4
Los Angeles	Greater Los Angeles Co. VCD	101796	2,879	181		7	70	1,249	18	
Los Angeles	Long Beach EH	12707	449	33		4	40	420	7	
Los Angeles	Los Angeles Co. West VCD	13793	457	1		20	120	18	15	
Los Angeles	San Gabriel Valley MVCD	55	2	2		11	44	49	5	
Madera	Consolidated MAD	24	1							
Madera	Fresno Westside MAD	27	1							
Madera	Madera Co. MVCD	1100	22	5		2	20	197	7	
Marin	Marin-Sonoma MVCD	939	56			3	33	409		
Mariposa										
Mendocino										
Merced	Merced Co. MAD	21907	619	34		8	48	485	14	
Merced	Turlock MAD	7986	242							
Modoc										
Mono										
Monterey	North Salinas MAD		0	0		3	33	438	0	
Napa	Napa MAD		0	0		5	55	695	2	
Nevada		27	2	0		2	20	295	0	
Orange	Orange Co. VCD	90924	2,841	83		1	10	137	2	
Placer	Placer Co. VCD	4130	268	20		7	42	2	2	
Plumas										
Riverside	Coachella Valley MVCD	109646	2,794	86	19	12	120	1,623	66	19
Riverside	Northwest MVCD	17261	486	6		6	60	832	33	
Riverside	Riverside Co. EH	18496	439	19		6	123	1,140	55	17
Sacramento	Sacramento-Yolo MVCD	19898	762	122		5	50	10	16	
San Benito										
San Bernadino	San Bernardino Co. VCP	20427	567	20		11	110	1,877	59	11
San Bernardino	West Valley MVCD	2437	57	20		8	32	528	20	
San Diego	San Diego Co. Dept of Health	6997	148	0		3	30	470	0	
San Francisco	EMDP		1							
San Francisco	Dept. Environment		1							
San Francisco	San Mateo Co. MAD	1436	29							
San Francisco	PRTR	423	12							
San Joaquin	San Joaquin Co. MVCD	4511	98	1		6	60	211	21	
San Luis Obispo	San Luis Obispo Co.	1970	48	2		3	30	391	4	
San Mateo	San Mateo Co. MAD	4982	112	0		4	40	518	0	
Santa Barbara	Santa Barbara Coastal VCD	16016	399	21		6	58	1,005	8	

continued>

Table 3. (continued)

County	Agency	No. mosquitoes tested	No. mosquito pools tested	WNV + pools	WEE + pools	No. flocks	No. chickens	No. sera tested	WNV + sera	WEE + sera
Santa Clara	Santa Clara Co. VCD	68	3	3		4	40	1,067	0	
Santa Cruz	Santa Cruz Co. MVCD	925	18	0		1	10	121	0	
Shasta	Burney Basin MAD		0	0		2	20	202	2	
Shasta	Shasta MVCD	1631	52	0		5	55	695	7	
Sierra										
Siskiyou										
Solano	Solano Co. MAD		0			3	36	471	23	
Solano	Sacramento-Yolo MVCD	6383	238	4						
Sonoma	Marin-Sonoma MVCD	343	9			4	44	628		
Stanislaus	East Side MAD					2	20	224		
Stanislaus	Turlock MAD	33623	1,107	114		7	84	1,134	31	
Sutter	Sutter-Yuba MVCD	18235	398	43		5	50	692	6	
Tehama	Tehama Co. MVCD	50	3	0		2	20	232	1	
Trinity										
Tulare	Delta VCD	3533	100	16		6	60	572	36	
Tulare	Tulare MAD		0	0		2	20	302	8	
Tuolumne										
Ventura	City of Moorpark		0	0		1	6	107	0	
Ventura	Orange Co. VCD	202	8							
Ventura	Ventura Co. EH	1786	37	2		4	40	637	2	
Yolo	Sacramento-Yolo MVCD	8357	300	28		5	50	14	12	
Yuba	Sutter-Yuba MVCD	913		4		2	20	281	10	
Total		**728,977**	**20,270**	**1,242**	**51**	**271**	**2,539**	**29,051**	**790**	**139**

Table 4. Mosquito pools (*Culex* spp.) tested for WNV, 2005.

	Cx erythrothorax		*Cx pipiens*		*Cx quinquefasciatus*		*Cx stigmatosoma*		*Cx tarsalis*		Other *Culex* spp.	
County	pools	WNV +	pools	WNV +	pools	WNV +	pools	WNV +	pools	WNV +	pools	WNV +
Alameda	197	6	125	1	1				67	1		
Butte	1		12				1		24	4		
Contra Costa	115		25						230	1		
Eldorado									1			
Fresno	15				198	33	4	3	117	35		
Glenn									41	5		
Imperial	28	1			12		1		268	9	3	
Inyo	10								117	2		
Kern	54				568	104	2		991	131	2	
Kings					43	3			22			
Lake	65	1					27	7	214	23		
Los Angeles	207				2,729	177	150	8	378	28	95	5
Madera			14	2	1	1			9	2		
Marin	19											
Merced	54		388	23			4	1	330	10	2	
Orange	418	1			1,441	67	191	7	295	8	27	
Placer	5		33	6			2		47	14		
Riverside	399	9			767	56	61	2	2,339	43	8	1
Sacramento	88	2	290	84			5		218	36		
San Bernardino	20				246	20	38		252	20		
San Diego	45		5		14		6		67		1	
San Francisco	33		3						1			
San Joaquin			4						25	1		
San Luis Obispo	17		1				7	1	6	1		
San Mateo	16		81						6			
Santa Barbara	130	4			43		24		97	17	2	
Santa Clara			1	1					2	2		
Santa Cruz	16		5									
Shasta	1		28				10		10			
Solano			178	2					53	2		
Sonoma			6				1		1			
Stanislaus	80	1	583	103			2		359	10		
Sutter			35	3					311	40		
Tehama									2			
Tulare	8				64	16	1		27			
Ventura	12	1	7		1				23	1		
Yolo			80	14			2	1	183	12		1
Yuba			5						18	4		
Total	**2053**	**26**	**1909**	**239**	**6128**	**477**	**539**	**30**	**7151**	**462**	**140**	**7**

Table 5. Mosquito pools (*Aedes* spp., *Anopheles* spp., *Coquillettidia perterbans*, *Culiseta* spp., *Psorophora columbiae*) tested for WNV, 2005.

Mosquito Species	*Ae dorsalis*	*Ae melanimon*	*Ae squamiger*	*Ae taeniorhynchus*	*Ae vexans*	*Ae washinoi*	Other *Aedes*	*Aedes* spp. Subtotal	*An franciscanus*	*An freeborni*	*An hermsi*	*An punctipennis*	*Anopheles* spp. Subtotal	*Coquillettida perterbans*	*Cs incidens*	*Cs inornata*	*Cs particeps*	*Ps columbiae*	Other spp. Subtotal
County																			
Alameda	1							1		3			3						
Butte		18			2			20							4	1			5
Contra Costa		38						38											
El Dorado							3	3											
Fresno		9			1			10							5				5
Glenn		1						1		5			5						
Imperial	5				1			6	1				1					7	7
Kern		235						235											
Kings								0		1			1						
Lake		3					10	13	1	23[a]		1	25	2	1	11			14
Los Angeles							1	1			4		4		110	1			111
Merced		65						65								3			3
Orange			9			19		28	4		58		62		182	74	6		262
Placer		5					1	6		15			15		1				1
Riverside	2				50			52	1		8		9		4	34	5		43
Sacramento		28			35		4	67		1			1		41				41
San Bernardino					1			1	3		4		7		43	12			55
San Diego			4				3	7			2		2		1				
San Francisco								0							4				4
San Joaquin		12			52			64							1	4			5
San Luis Obispo	14							14											
San Mateo						4		4							3				3
Santa Barbara			2	8		25	3	38	20		21		41		9	2	12		23
Shasta		1						1											
Solano							3	3							4				4
Sonoma								0								1			1
Stanislaus		54			5			59							6	17			23
Sutter		43			7		2	52								1			
Ventura					4			4	2				2		6				6
Yolo		7					5	12		12			12		7				7
Total	22	519	15	8	158	48	35	805	32	37	97	1	190	2	432	161	23	7	623

[a] One pool positive for WNV

reported immediately to the submitting agency before the confirmatory test was performed. In areas where SLE has never been documented, when at least one bird in a flock was confirmed positive to WNV, subsequent flavivirus positive chickens from the same flock were assumed to be infected with WNV, and no further confirmatory testing was necessary. In SLE areas, all flavivirus positive samples were further characterized as WNV or SLE. Chicken samples that were only flavivirus positive were not included in Table 3.

The VRDL tested 29,051 chicken sera for antibodies to SLE, WEE, and WNV. The Sacramento-Yolo Mosquito and Vector Control District (1,916 samples), Los Angeles City West Vector Control District (2,534 samples), San Joaquin County Mosquito and Vector Control District (665 samples), and the San Gabriel Valley Mosquito and Vector Control District (553 samples) tested their own sentinel chicken flocks.

A total of 790 seroconversions to WNV were detected among 136 flocks from 31 counties (Table 3, Fig. 4). The first WNV seroconversions in sentinel chicken flocks were detected on February 25 from San Bernardino County.

A total of 139 WEE seroconversions were detected among 20 flocks from five counties: Imperial (52), Kern (36), Los Angeles (4), Riverside (36), and San Bernardino (11). No SLE seroconversions were detected in 2005.

DEAD BIRD SURVEILLANCE FOR WEST NILE VIRUS

The WNV dead bird surveillance program, a collaborative program between CDHS and over 130 local agencies and supported by a CDC grant, was established in 2000. In 2005, CDHS fielded 124,876 calls to the West Nile virus hotline with 109,361 reports of dead birds from 58 counties yielding 11,480 carcasses submitted for testing. Of 9,227 carcasses deemed suitable for testing, WNV was detected in 3,046 carcasses from 52 counties (Table 6, Fig. 5). Retrieval and testing of dead birds was continued at the discretion of the local agency following the initial detection of WNV in its jurisdiction.

In 2004, local agencies began to screen birds for WNV using two commercially available rapid assays— RAMP® (Rapid Analyte Measurement Platform), Response Biomedical Corp., and VecTest™,

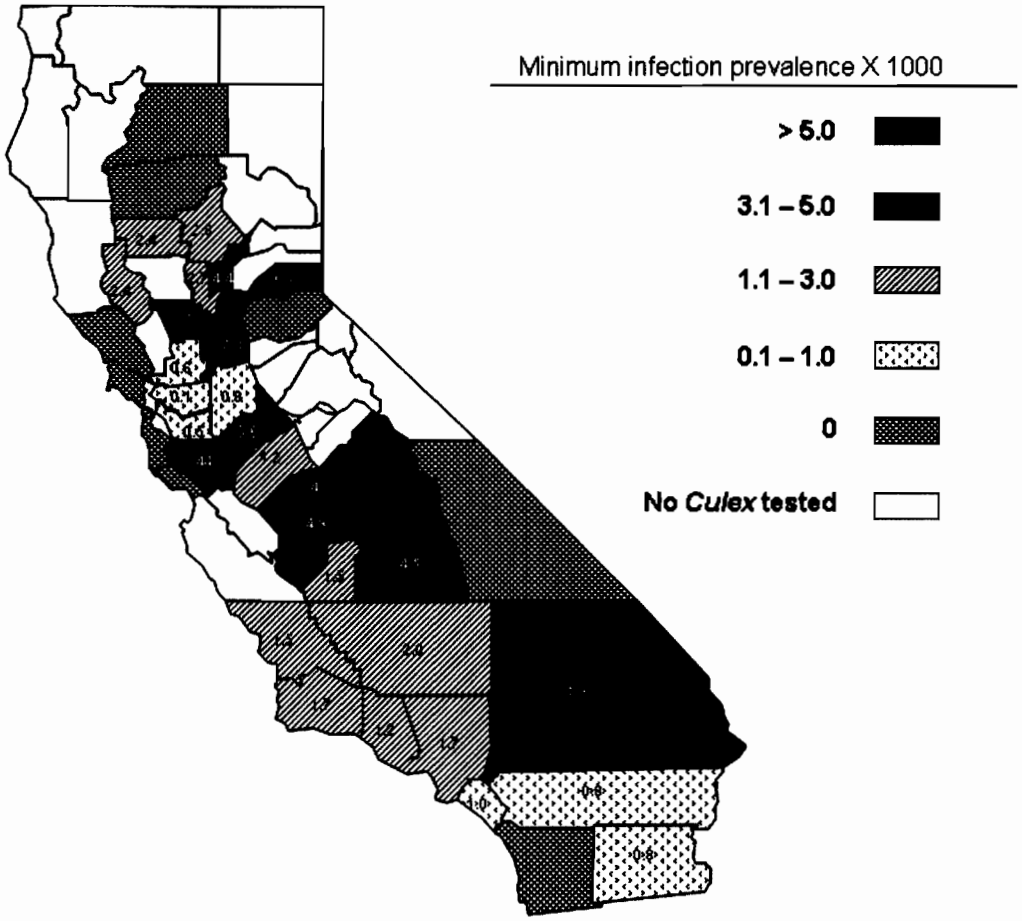

Figure 3. Minimum Infection Prevalence [No. pools positive X (No. mosquitoes tested)$^{-1}$ X 1000] of WNV in *Culex* spp., 2005.

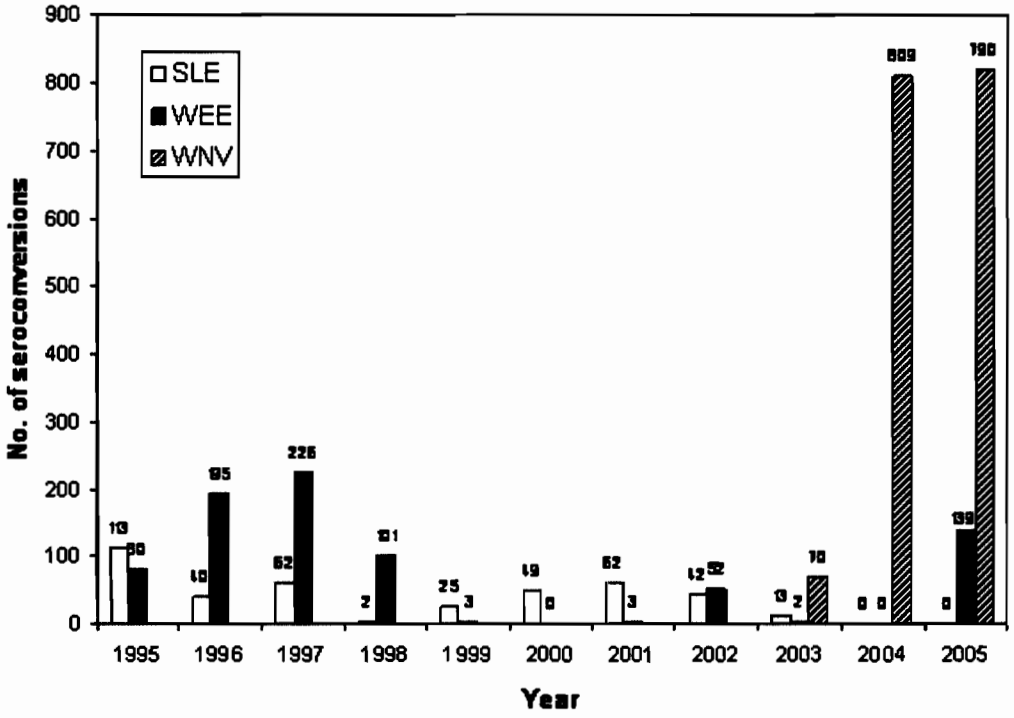

Figure 4. SLE, WEE, and WN Virus Sentinel Chicken Seroconversions 1995-2005

Table 6. Dead birds tested and reported for West Nile virus, 2005.

	Corvid[a]			Non-Corvids			All Birds		
County	Reported	Tested	Positive	Reported	Tested	Positive	Reported	Tested	Positive
Alameda	579	130	34	2015	231	14	2594	361	48
Alpine	2	0	0	0	0	0	2	0	0
Amador	185	16	12	307	50	12	492	66	24
Butte	1069	93	69	664	59	10	1733	152	79
Calaveras	211	9	6	405	26	4	616	35	10
Colusa	96	15	12	40	4	2	136	19	14
Contra Costa	1935	132	60	4271	385	33	6206	517	93
Del Norte	7	2	0	25	7	0	32	9	0
El Dorado	792	71	47	1138	122	20	1930	193	67
Fresno	3049	165	80	4079	212	17	7128	377	97
Glenn	108	42	28	58	10	2	166	52	30
Humboldt	46	11	0	170	38	4	216	49	4
Imperial	4	0	0	28	1	0	32	1	0
Inyo	73	13	11	122	17	1	195	30	12
Kern	472	76	25	1689	164	19	2161	240	44
Kings	840	76	58	716	53	15	1556	129	73
Lake	338	45	32	306	31	0	644	76	32
Lassen	7	3	3	54	8	0	61	11	3
Los Angeles	1879	349	150	3116	345	23	4995	694	173
Madera	387	31	20	341	17	2	728	48	22
Marin	491	34	8	770	88	6	1261	122	14
Mariposa	63	9	6	102	18	0	165	27	6
Mendocino	411	47	33	231	27	1	642	74	34
Merced	2400	198	161	823	61	10	3223	259	171
Modoc	2	0	0	26	8	1	28	8	1
Mono	14	4	1	56	10	0	70	14	1
Monterey	139	11	1	426	59	1	565	70	2
Napa	225	58	40	273	76	4	498	134	44
Nevada	289	21	16	571	74	11	860	95	27
Orange	1058	415	270	702	245	32	1760	660	302
Placer	2124	86	64	2262	85	20	4386	171	84
Plumas	26	6	0	117	22	0	143	28	0
Riverside	3054	124	100	2006	135	11	5060	259	111
Sacramento	10260	79	58	6380	48	12	16640	127	70
San Benito	35	5	1	140	14	0	175	19	1
San Bernardino	1222	116	66	2110	176	8	3332	292	74
San Diego	661	413	150	510	122	10	1171	535	160
San Francisco	48	8	0	357	46	2	405	54	2
San Joaquin	3638	53	23	1662	36	1	5300	89	24
San Luis Obispo	600	57	37	654	84	4	1254	141	41
San Mateo	231	33	1	1115	147	9	1346	180	10
Santa Barbara	631	102	68	465	50	5	1096	152	73
Santa Clara	1094	275	135	2179	221	9	3273	496	144
Santa Cruz	94	6	0	700	33	2	794	39	2
Shasta	203	43	19	439	69	9	642	112	28
Sierra	1	0	0	19	5	0	20	5	0
Siskiyou	16	7	2	53	30	4	69	37	6
Solano	1175	60	38	1218	68	6	2393	128	44
Sonoma	1244	173	42	1690	284	36	2934	457	78
Stanislaus	5344	320	224	2125	88	11	7469	408	235
Sutter	740	19	7	306	6	2	1046	25	9
Tehama	118	52	38	137	53	9	255	105	47
Trinity	1	1	0	15	2	0	16	3	0
Tulare	1987	245	191	1521	157	35	3508	402	226
Tuolumne	103	69	44	108	70	15	211	139	59
Unknown	243	1	0	367	5	0	610	6	0
Ventura	1003	117	56	861	104	6	1864	221	62
Yolo	2002	39	14	789	11	3	2791	50	17
Yuba	236	14	11	227	11	1	463	25	12
Totals	55305	4599	2572	54056	4628	474	109361	9227	3046

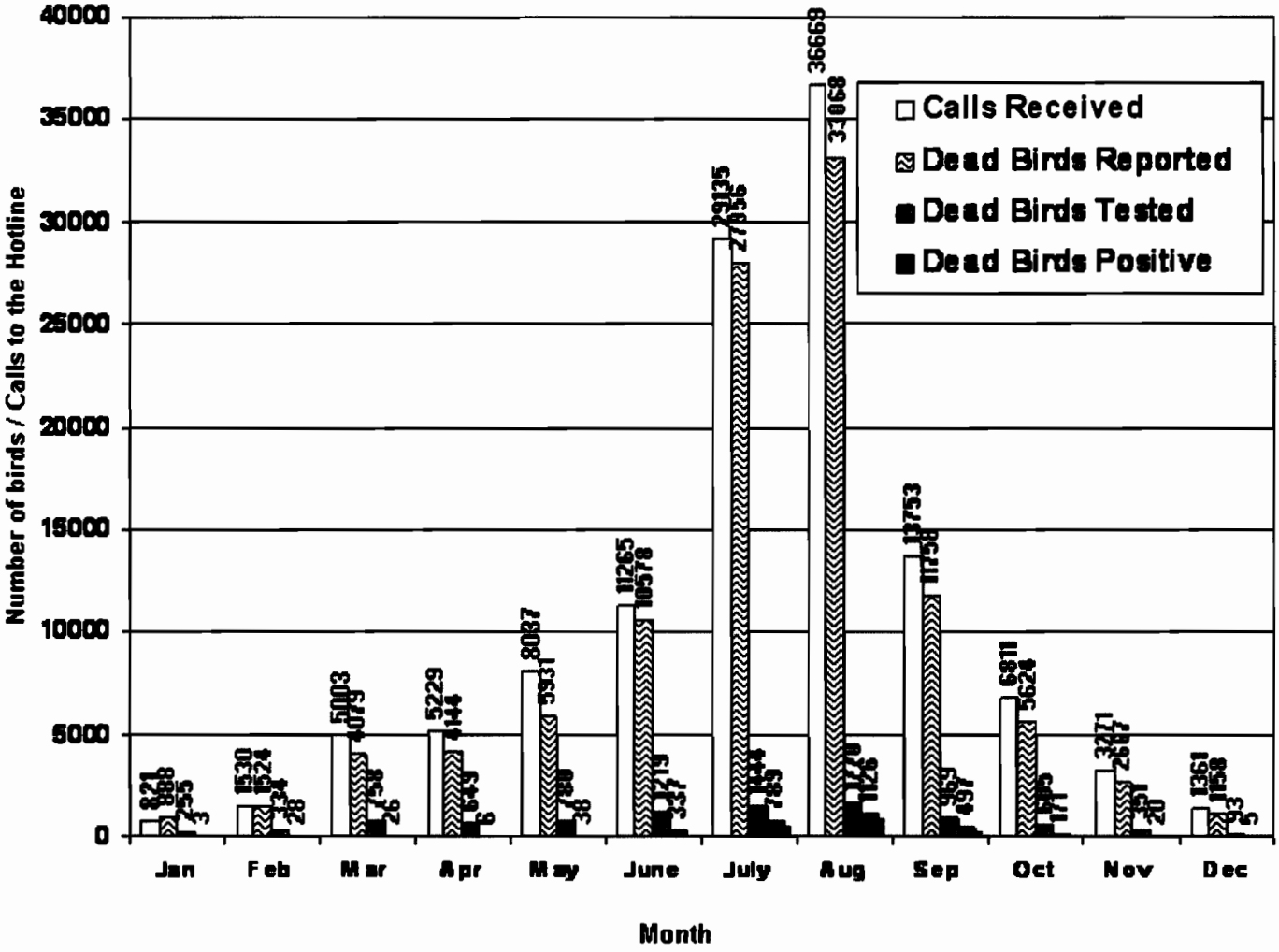

Figure 5. Calls and Reports to the CDHS West Nile virus "Dead Bird Hotline," 2005.

Medical Analysis Systems. Of the 2,566 American crows that were positive for WNV in 2005, 413 were tested via VecTest™ and 105 were tested by RAMP®. Positive corvids tested by rapid assays were not confirmed by further testing. All negative rapid assay results were confirmed to prevent reporting of false negatives.

In 2005, CDHS used a model called the California Dynamic Continuous-Area Space-Time (DYCAST) program to forecast areas of increased WNV risk to humans based on incidence and geographical occurrence of dead bird reports. This model was developed in cooperation with the Center for Advanced Research of Spatial Information at Hunter College, City University of New York. Risk maps were made available to mosquito and vector control agencies via a password-protected website, to provide information useful for focusing mosquito control operations, public education campaigns, and surveillance activities. Of those human cases with a known onset date and that could be geo-coded (332), 274 (83%) occurred within a quarter square mile of areas that were predicted by the DYCAST system to be high WNV infection risk to humans. One hundred sixty-six (50%) of these cases occurring in areas identified as high risk approximately one month prior to onset, indicating that DYCAST may be an effective early warning system for WNV risk to humans.

REPORTS, PUBLIC EDUCATION, AGENCY OUTREACH

During 2005, CDHS distributed weekly arbovirus surveillance data and national WNV activity updates. Surveillance bulletins were distributed to local, state, and federal public health agencies and universities in California. Bulletins and other reports were posted on the California West Nile virus website, www.westnile.ca.gov and the California Vector borne Disease Surveillance System, http://vector.ucdavis.edu/arbo.html. The California West Nile virus website received over 3 million "hits" in 2005 and was extremely valuable in communicating up-to-date information to the media, local agencies, and the general public.

Fight the Bite educational material starter bundles were distributed by CDHS to California public health and mosquito abatement agencies during early spring 2005. The agencies

customized the English and Spanish materials and distributed nearly 252,000 wallet cards, 8,400 posters, 42,000 bookmarks, and 126,000 *Fight the Bite* brochures. "*Fight the Bite*" brochures were also available in six other languages: Tagalog, Vietnamese, Hmong, Lao, Cambodian, and Russian. A second distribution of approximately 70,000 pieces of *Fight the Bite* materials took place during June. In addition, over 10,000 WNV Surveillance brochures in English and Spanish were distributed to local agencies during 2005.

The CDHS Office of Public Affairs (OPA) issued eleven WNV press releases during 2005. These provided information to the public on human WNV-related fatalities and the spread of WNV throughout the state. Two of the press releases were translated into Spanish. To help ensure a consistent message, WNV Press Kits were assembled by OPA and distributed to the media during various events statewide. Four roundtable discussions with leaders of the mosquito abatement and public health communities were coordinated by the Governor's Office, OPA and CDHS during the 2005 WNV season.

West Nile virus television and radio PSAs were made available statewide to broadcasters for downloading from mid-April to mid-May 2005. Wal-Mart® stores aired a WNV prevention message in their stores throughout California for several months in 2005.

The CDHS hosted meetings with representatives from local mosquito and vector control districts, universities, and other local agencies to share and review current WNV information and develop surveillance and prevention strategies. During the summer of 2005, Statewide WNV teleconferences were conducted by the CDHS every three weeks to discuss issues, solutions, and management strategies. In December, the CDHS hosted the WNV Steering Committee meeting in West Sacramento to review WNV prevention efforts during the year and plan strategies for the next season.

During 2005, VBDS staff gave more than 40 formal presentations and provided innumerable consultations by phone and in person to local agencies, health care providers, and the public on many WNV related topics.

WEST NILE VIRUS IN THE UNITED STATES

By the end of 2005, WNv activity was reported form 48 states and the District of Columbia. The 2005 WNV epidemic resulted in reports of 2,999 human cases of WNV disease. Of the 2,999 cases, 1,291 were defined as Neuroinvasive diseases (WNND) and 1,606 as WN fever cases (WNF). There were 119 reported WNV-related deaths.

In addition, 108,766 dead birds were reported and 5,392 were WNV-positive from 45 states. Fourteen bird species were newly reported in 2005. WNV infections were 1,181 horses; 1,214 sentinel chickens in 16 states, and 11,815 mosquito pools from 39 species in 42 states and DC. Forty-nine of the 55 WNV-positive squirrels were from California which also had one black-tailed jackrabbit, two alpaca, three sheep, and one llama test positive.

Acknowledgements

The authors gratefully acknowledge the cooperation and assistance of the local mosquito and vector control agencies in the collection and submission of samples for testing and their financial support to the testing laboratories; the local public health laboratories which tested samples; the many physicians and veterinarians who submitted specimens from clinical cases, and the valuable contributions of the staffs of MVCAC, CVEC (especially Nicole Kahl), CAHFS, VRDL, CDFA Animal Health Branch, the Infectious Disease Branch, and VBDS of CDHS (especially Alicia Scribner, Lindsie Goss, and Curtis Fritz). Surveillance funding was augmented by generous support from the Centers for Disease Control and Prevention.

Use of Sentinel Chickens in California for Arbovirus Surveillance, 1962-2005: Data Aggregation and Analysis

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ABSTRACT: An electronic dataset has been created comprising all known records of sentinel chicken samples from California that were tested for arboviral antibodies during the years 1962 to 2005. The dataset also contains some records of samples submitted for testing by states surrounding California. A total of 411,571 samples were tested, mostly by the Viral and Rickettsial Disease Laboratory, California Department of Health Services. Arboviral antibodies were detected in 3.9% of these samples. West Nile viral antibodies were the most frequently detected (5,861, 1.4%), followed by western equine encephalomyelitis (5,178, 1.3%) and St. Louis encephalitis (3,940, 1.0%). There were 899 samples (0.2%) reported as "Flavivirus" that most likely represented tests later confirmed as West Nile viral antibodies. The role of sentinel chickens in arbovirus disease surveillance programs is discussed briefly, as is the potential use of the dataset for assessing the effectiveness of various surveillance components.

ARBOVIRUS SURVEILLANCE

Arbovirus surveillance has been a principal component of prevention and control of mosquitoborne viral diseases in California for more than 50 years. In California, it is a collaborative activity among approximately 60 local mosquito abatement agencies, the California Department of Health Services, and the University of California. Surveillance is a complex activity, and its effective use depends upon periodic review and analysis of components and procedures. The California system has steadily evolved over the years as improvements have been made in sampling and testing procedures, and in electronic data aggregation, analysis, and reporting.

COMPONENTS OF SURVEILLANCE

The components of arbovirus surveillance and the rationale for their use were described by Eldridge (1987). Factors relating to weather include temperature and precipitation, snow depth, and flood forecasts. These affect adult mosquito abundance, which is estimated using various types of traps. Viral infections in mosquitoes are estimated by testing of pools of mosquitoes, and viral transmission by mosquitoes is estimated by testing of sera from sentinel chickens. In some cases, sera from wild birds are tested instead of or in addition to sentinel chickens. Since the invasion of West Nile virus in California, dead birds have been tested for evidence of infection by that virus. Guidelines for the compilation of these surveillance measures into a risk level estimate have been developed (CDHS et al. 2006).

SENTINEL CHICKENS

Sentinel chickens are an important component of arbovirus surveillance programs, and have characteristics that are not matched by other components. Because chickens are confined to coops, the place of infection can be established with greater accuracy than is possible for tests of birds found dead, or birds trapped alive. Although not exact, time of infection can be estimated with good accuracy, depending primarily on the frequency of sampling. Seropositive sentinel chickens are direct evidence of the presence of infective mosquitoes, whereas positive mosquito pools are evidence only of infected mosquitoes. Scott et al. (2001) showed that the use of sentinel chickens is also cost-effective in comparison with some other surveillance methods.

THE HISTORICAL SENTINEL CHICKEN DATABASE

Historical chicken data were accumulated from several sources. Before 1979, sentinel chickens were not used statewide, and data were available only from research programs conducted by the University of California Berkeley, School of Public Health. These data originally were stored electronically on reels of 2-inch tape, but were converted to standard text files several years ago. Some mosquito abatement agencies used sentinel chickens for surveillance before 1979, and had paper records for these years. After 1979, chickens were used as a statewide surveillance tool, and data were made available in weekly surveillance bulletins published by the California Department of Health Services. Positive results have been summarized (Hui et al. 1999, Steinlein et al. 2003). Since 1999, data for individual tests have been stored electronically. Data from these sources, covering the years 1962 to 2005, were aggregated into a single database table in Microsoft SQL Server 2000® and hosted by a server maintained by the Environmental Assessment and Information Technology Program (EAIT) of the Center for Vectorborne Diseases, UC Davis. No data were available for the years 1964, 1974, 1975, and 1976.

The data represent 411,571 individual samples. Approximately 4% of these samples were positive for either St. Louis encephalitis virus (SLE), West Nile virus (WN), or western equine encephalomyelitis virus (WEE). These cannot be interpreted as

numbers of seroconversions, because in many instances, the positive samples represent retesting of sera from chickens that had seroconverted earlier. The historical chicken dataset contains data on tests conducted from California, Nevada, Utah, Oregon, Washington, Utah, and Arizona.

Fig. 1 shows the total number of tests conducted on sentinel chicken sera collected in California from 1962 to 2005. Fig. 2 shows the number of sites where chicken flocks were maintained in California for this period, plus the number of mosquito abatement agencies maintaining sentinel chicken flocks. The number of agencies maintaining flocks increased steadily after 1979, and leveled out after 1994.

The number of flocks maintained in California by each agency varied from one MVCAC region to another. During the 1980s districts typically operated only 1-2 flocks consisting of 25 hens but beginning in 1990, agencies increased their coverage by operating 3 or more flocks each, and in some cases, as many as 10. These increases were related to decreasing flock size from 20 to 10 hens and to changes in sampling that made blood collection easier. Sacramento Valley region districts tended to operate the greatest number of flocks per district.

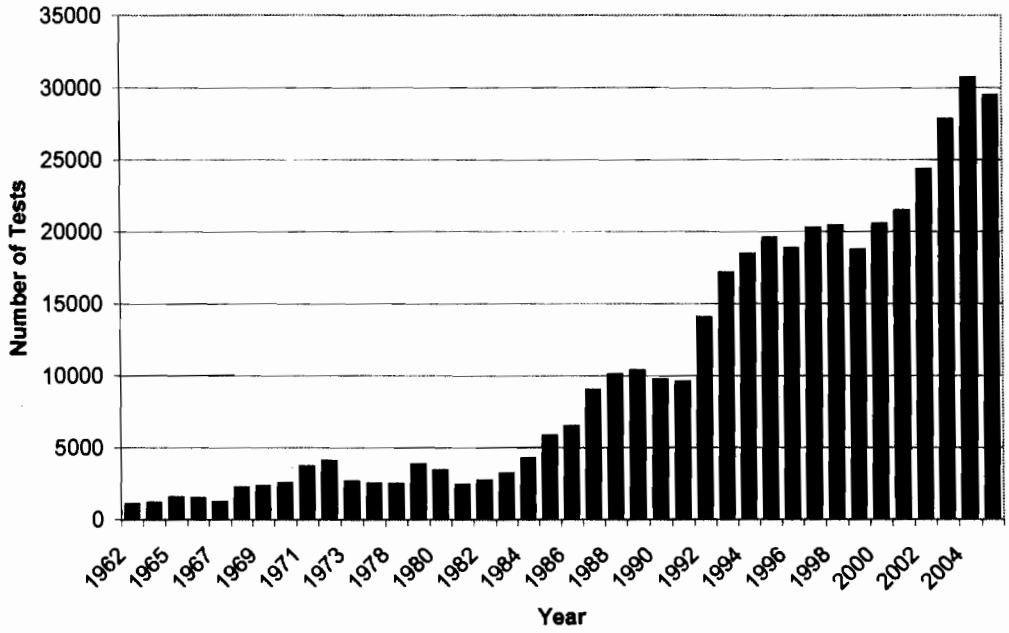

Figure 1. Total arbovirus antibody tests conducted on serum samples from sentinel chickens in California, 1962-2005.

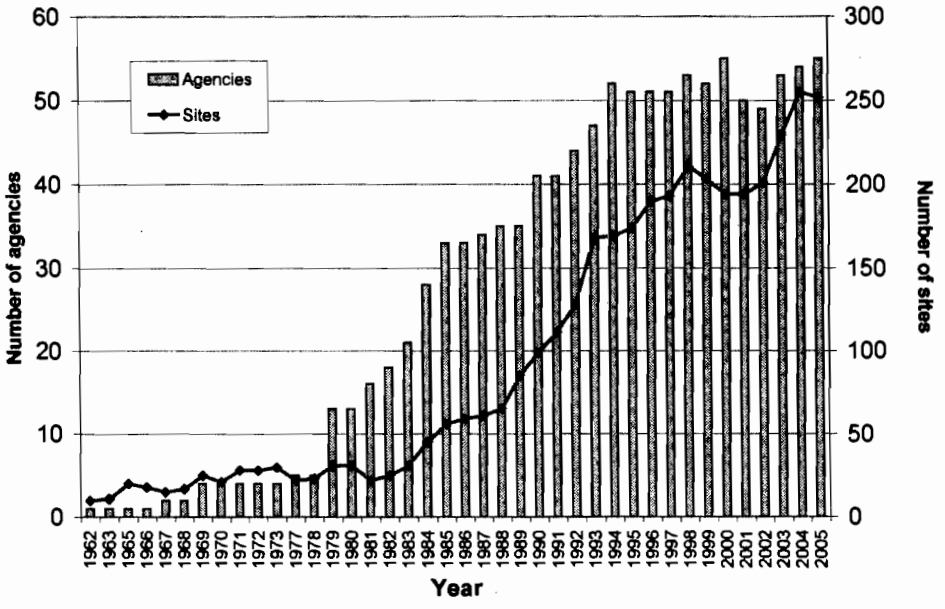

Figure 2. Sentinel chicken flocks maintained by mosquito abatement agencies in California, 1962-2005.

Table 1 shows the breakdown of the entire dataset based on the test outcome. Approximately 96% of the tests were negative. Among the positive tests for viral antibodies, the most common was WN, comprising 1.4% of the tests, followed by WEE, 1.3%, and SLE, 1.0%. In addition to the WN-positive tests, 899 (0.2%) were recorded as "Flavivirus". If some of these were later confirmed to be WN-positive samples, the percentage of WN-positive tests would be 1.4-1.6%. All of the WN-positive tests were for the years 2003-2005.

Table 1. Results of all tests of serum samples from sentinel chickens in California and surrounding states, 1962-2005.

Results	Number Tests	Percent
SLE	3,940	1.0
WEE	5,178	1.3
Dual infections (WEE/SLE)	258	<0.1
WN	5,861	1.4
Flavivirus*	899	0.2
Unknown	11	<0.1
Negative	395,424	96.1
Total Tests	411,571	100.0*

Includes samples later confirmed as WN

There were also 258 samples that tested positive for both SLE and WEE. These were from Kern and Imperial Counties. The Kern County samples were from the 1960s, collected in conjunction with research activities of the UC Berkeley School of Public Health programs. These are regarded as dual infections in chickens contracted over the course of their exposure.

The breakdown in tests and test results by state is shown in Table 2. The bulk of tests are from samples submitted from California agencies. The nearly complete lack of WN-positive samples from outside California reflects the fact that after WN invaded the western USA, states that had submitted samples to California for testing before 2003 used WN-related federal funds to establish their own testing capabilities.

Table 2. Results of tests of serum samples from sentinel chickens in California and surrounding states, 1962-2005. Dual infections are WEE and SLE.

State	SLE	WEE	Dual infections	WN*	Total tests
Arizona	30	6	0	0	346
California	3,906	5,161	258	6,737	398,238
Nevada	3	10	0	0	7,869
Oregon	0	1	0	0	3,199
Utah	1	0	0	23	1,456
Washington	0	0	0	0	463
Total Positives	3,940	5,178	258	6,760	411,571

*includes 899 samples originally reported as "Flavivirus"

Numbers of tests from 5 geographic regions of California (equivalent to the regions of the Mosquito and Vector Control Association of California) are shown in Table 3. Most of these tests are from agencies that are members of MVCAC. The large number of both positive and negative tests from the Southern Region reflects the relatively longer period of warm weather favoring mosquito breeding and viral activity in this region; the large numbers of WN-positive tests from this region is inflated by the fact that California WN activity started there first.

Table 3. Results of tests of serum samples from sentinel chickens in California by geographic region (based on regions of Mosquito and Vector Control Association of California, but includes tests from non-member agencies).

Geographic Region	SLE	WEE	WN*	Total tests
Coastal	183	334	272	44,020
N San Joaquin	0	52	242	19,347
S San Joaquin	862	1,492	1,863	105,577
Sacramento V	909	1,386	1,178	84,323
Southern	1,952	1,897	3,182	144,971
Total Positives	3,906	5,161	6,737	398,238

*includes 899 samples originally reported as "Flavivirus"

DISCUSSION

There are many uses for historical datasets of vectorborne disease surveillance components, including data associated with sentinel chickens. If datasets include data gathered over long time periods and represent broad geographical coverage, they can be used to assess the effectiveness of a given component in comparison with other components. In the case of sentinel chickens, they can be evaluated for sensitivity to viral activity, and the temporal distribution of seroconversions can be compared to other surveillance components such as climate variation to determine their relative value as an early warning system for viral activity.

There are uses of sentinel chicken datasets that extend beyond comparisons with other surveillance components. The relationship between flock density in a surveillance region (or mosquito abatement district) and sensitivity to detect viral activity is extremely important from the standpoint of both effectiveness as a surveillance indicator, and also cost effectiveness. Any agency conducting surveillance must continually balance considerations of both value and cost. From an epidemiological viewpoint, such datasets can form the basis of studies that examine the relationship between factors such as land use and viral activity, and relative effectiveness of chicken flocks in a variety of ecological and sociological settings.

Acknowledgements

The data comprising the historical sentinel chicken dataset were produced by the combined activities of a number of state and local agencies. Chickens were maintained and periodically bled by California mosquito abatement agencies and similar agencies with surveillance programs. Some of these data were from local agencies outside California. The sentinel chicken program is administered by the California Mosquito and Vector Control Association. Chicken sera were tested by the Viral and Rickettsial Disease Laboratory Branch, California Department of Health Services, and results of tests were originally reported by the Vector-borne Disease Section, Infectious Disease Branch, CDHS. The dataset was assembled by the Environmental Assessment and Information Technology Program, Center for Vectorborne Diseases, University of California, Davis in collaboration with all agencies listed above.

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Why Was West Nile Virus Where it Was in Contra Costa County?

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ABSTRACT: We examined the association of dead bird reports with human population density, microclimate and the distribution of human and equine West Nile virus cases in Contra Costa County during 2005. The incidence of dead bird reports (reports per 1000 residents) was strongly correlated with average high summer temperatures and was roughly predictive of the actual distribution of human and equine cases. Currently available risk assessment models such as DYCAST do not take population density or microclimate variation into account.

INTRODUCTION

During 2005, the Contra Costa Mosquito and Vector Control District (CCMVCD) relied heavily on mapping dead bird reports received by the California Department of Health Services (CDHS) Statewide West Nile virus (WNV) hotline to identify areas of our county at higher risk for WNV transmission. It became apparent early in the season that higher numbers of calls were being received from areas of the County with the highest human population, and therefore that human population density might be confounding our efforts to estimate actual differences in the risk of human cases. In addition to fairly large variations in population density, the San Francisco Bay area, where our District is located, has large variations in microclimate as one moves from the Bay towards inland. During midsummer, there may be as much as a 20° F difference in average high temperatures from the west side to the east side of Contra Costa County. Since ambient temperature is known to have a strong influence on the extrinsic incubation rate of West Nile virus (Reisen et al. 2006), it might have a significant effect on virus transmission rates across the County. We therefore decided to examine the distribution of dead bird reports during 2005, with regard to variations in human population density, temperature and evidence of actual virus transmission as indicated by human and equine WNV cases and sentinel chicken seroconversions.

MATERIALS AND METHODS

Information on the location of dead bird reports was compiled and reported to us weekly by the staff of the CDHS WNV hotline. Human population data for incorporated municipalities was obtained from 2000 United States Census data (U.S. Census Bureau 2000). The incidence of dead bird reports for each of eleven cities was calculated by dividing the number of dead bird reports for the season by the total human population. Average summer high temperatures for various cities were obtained from The Weather Channel Interactive (2005). The relationship between temperature and dead bird report incidence was examined by regression of log (incidence) vs. published average high temperature. Monthly climate maps from the Western Regional Climate Center California Climate Data Archive (2005) were used to identify specific microclimate zones within the county which were then overlaid on maps of WNV positive human, equine and sentinel chicken locations. Information on the location of human cases was provided by the Contra Costa County Department of Health Services (CCCDHS), and equine case locations by the California Department of Food and Agriculture (CDFA). Sentinel chicken serum samples were collected biweekly from five flocks of 10 chickens each and tested at the CDHS Viral and Rickettsial Disease Laboratory (VRDL) in Richmond, CA.

RESULTS AND DISCUSSION

A comparison of the number of dead bird reports received from each of 10 cities with the incidence of reports (number of reports per 1000 residents) revealed some interesting differences (Fig. 1). Although the highest numbers of reports were received from Concord and Walnut Creek, which are located in the central part of the county, the incidence of reports from those communities was relatively low compared with the incidence in Brentwood and Oakley, located in the east. Communities on the west side including Richmond, Pinole and San Pablo showed a very low incidence of reports suggesting that incidence increased as one moved inland from the Bay and hence from areas of cooler to warmer microclimate. Linear regression analysis indicated that the log of dead bird report incidence was strongly correlated ($r^2 = 0.72$, $p<0.05$) with average summer high temperatures for each of the 10 cities (Fig. 2).

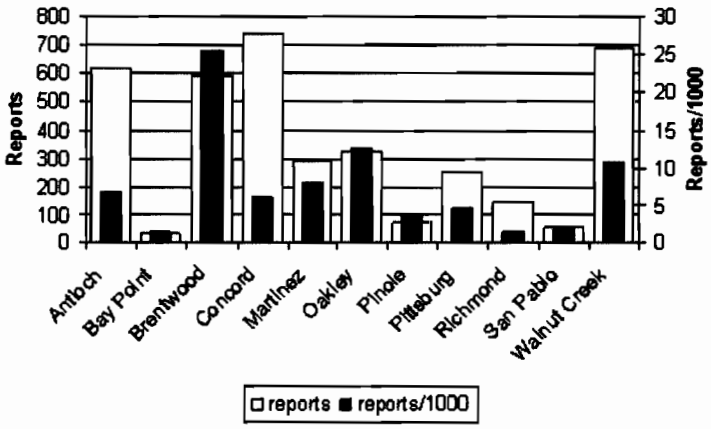

Figure 1. Number of dead bird reports per city during 2005 (left axis) vs. reports per 1,000 residents (right axis) for 11 cities on Contra Costa County

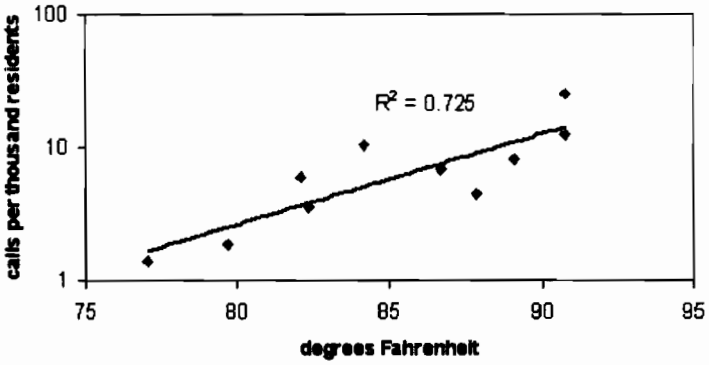

Figure 2. Log of dead bird calls per thousand residents vs. average summer high temperature for ten cities in Contra Costa County.

During 2005, there were 11 human and 10 equine cases of WNV in our county. A map of these cases, superimposed on a map showing average daily high temperatures in August 2005, revealed that virtually all cases occurred in areas of the county where the average daily high temperature was 85° F or above (Fig. 3). There was a single human case in Richmond, the westernmost city in the county, but according to information received from the Contra Costa County Department of Health Services this patient had a history of recent travel to another part of the state where virus incidence was much higher, and was presumed to have been infected outside the area. Seroconversions in sentinel chickens followed a similar pattern, with the majority of chickens (seven out of 10) seroconverting in flocks located in Knightsen and Oakley, three out of 10 in the Walnut Creek, a single chicken in Martinez and none in Hercules (Fig. 4). The concentration of human and equine cases and seropositive chickens in the eastern part of the county suggests that dead bird report incidence, rather than the raw distribution of reports, was giving us a more accurate indication of the risk of virus transmission.

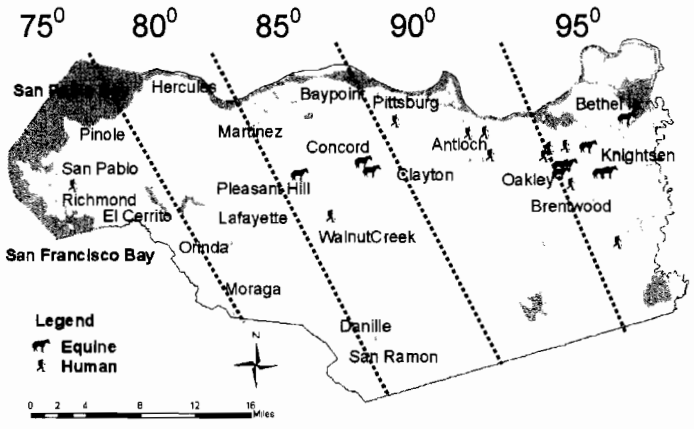

Figure 3. Locations of human and equine WNV cases in Contra Costa County, 2005 in relation to average daily high temperatures (⁰F) in August.

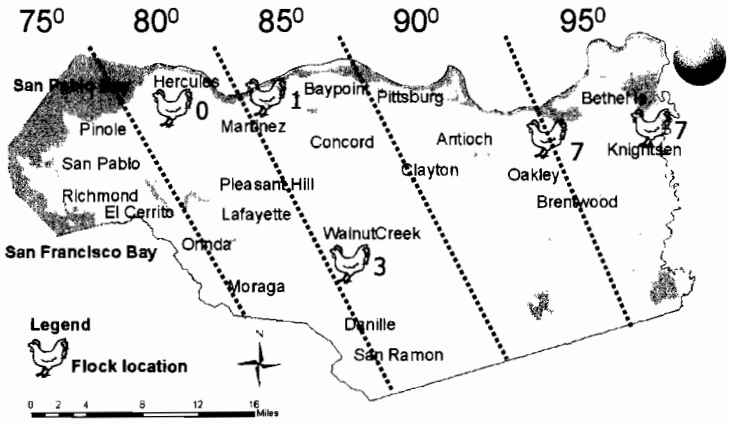

Figure 4. Number of seroconversions at five sentinel chicken flock locations in Contra Costa County, 2005 in relation to average daily temperatures (⁰F) in August. There were ten chickens per flock.

Existing WNV risk assessment models like DYCAST (Theophilides et al. 2002) analyze the spatial and temporal variation in the distribution of dead bird reports, but do not take human population density into account. Our data suggest that this may result in an overestimate of risk in more densely populated communities and an underestimate in less populated areas, resulting in less than optimal allocation of often limited field resources. In addition, in areas with large variations in microclimate difference in ambient temperature may have a significant influence on the local risk of virus transmission. These factors may be important in the development and refinement of future risk assessment tools.

Acknowledgements

We wish to acknowledge the staff of the WNV hotline for quickly and efficiently compiling and transmitting dead bird report information, the staffs of CDFA and CCCDHS for providing information on equine and human cases, and the DHS Viral and Rickettsial Disease laboratory for testing sentinel chicken serum samples.

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Abundance of West Nile Virus and Surveillance Programs in the Consolidated Mosquito Abatement District

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ABSTRACT: The Consolidated Mosquito Abatement District (District) conducted surveillance to detect West Nile Virus (WNV) in 2004, the first year of disease occurrence within the District, and in 2005. Detection of WNV was accomplished through the collection and testing of dead birds, mosquito pools, and sentinel chicken blood. Additional data of WNV occurrence was obtained from equine and human cases that were confirmed by the California Department of Food and Agriculture and the Fresno County Department of Environmental Health, respectively. Dead birds collected by District personnel were submitted to the local California Animal Health and Food Safety Laboratory for testing. Pools of adult mosquitoes collected in gravid and CO_2-baited traps and submitted to the University of California, Davis Center for Vectorborne Diseases for testing. Blood samples obtained from sentinel chickens were submitted to the Viral Rickettsial Disease Laboratory of the California Department of Health Services for testing. The data indicated varying degree of sensitivity in the detection of WNV among the surveillance programs. WNV was first detected from dead birds, followed by mosquito pools, equine and human cases, and sentinel chickens.

INTRODUCTION

The Consolidated Mosquito Abatement District (District) covers about 1,058 square miles—approximately one-sixth of the total area of Fresno County and a small portion of Kings County. The combined area serviced by the District shall be referred to as "the County" (CMAD 2006). About 450,000 people live in the District among the nearly 900,000 residents of the County. The District comprises a variety of ecotypes, including urban, suburban, rural residential, irrigated pastures and alfalfa, orchards, vineyards, row crops, dairies, riparian watercourses, and foothill woodlands (CDFDRU 2005).

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

WNV was first discovered in the County in wild birds during the summer of 2004. Viral infections were subsequently confirmed in humans, horses, mosquito pools, and sentinel chickens in that order. In 2005, the virus was detected in dead birds before the onset of spring and in mosquitoes during mid-spring, followed by infections in humans, horses, and chickens during mid-summer. The extent of WNV activity in 2004 and 2005 is illustrated in Figure 1.

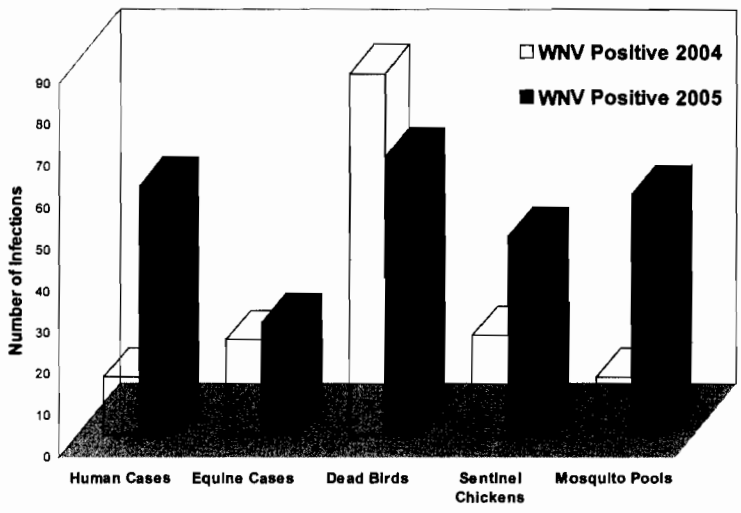

Figure 1. Evidence of WNV in the District during 2004 and 2005.

DISTRICT SURVEILLANCE PROGRAM

Dead Birds: Dead birds suitable for testing are collected and delivered to the local CAHFS facility. During 2004, the District utilized two commercial antigen-based immunochromatic assays—VecTest® and RAMP®—to test crows and jays (Family *Corvidae*) prior to submitting the carcasses to CAHFS. By arrangement, CAHFS used the District's RAMP® equipment to test corvids during 2005 in order to provide the District with immediate results. All birds were eventually sent to the CAHFS laboratory in Davis for final testing. Figure 2 compares the dead birds that tested positive for WNV in 2004 and 2005.

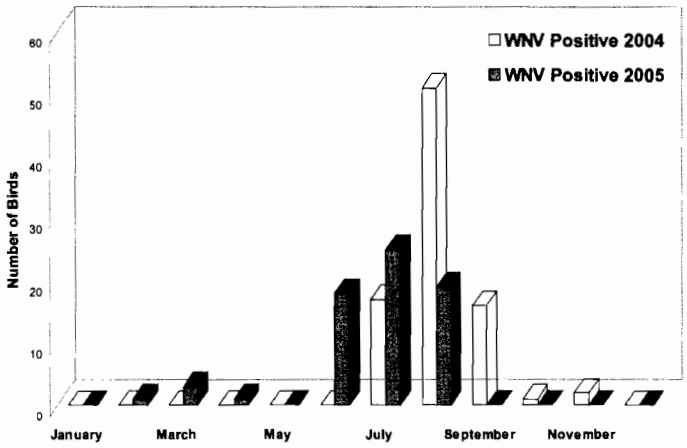

Figure 2. Comparison of dead birds testing positive for WNV in the District during 2004 and 2005.

The District substantially reduced the collection of dead birds by Mid-August of 2005 when it was evident that 80% of submitted specimens were infected. This accounts for the greater number of infected birds during the corresponding time period in 2004 when the District continued to collect birds. It should also be noted that the District collected birds in the Sierra Foothill communities (Foothills) adjacent to the eastern boundary of the jurisdiction. While only one dead bird from this area tested positive for WNV in 2004, several birds tested positive in 2005.

Sentinel Chickens: The District maintains six flocks, each containing ten chickens, placed throughout its jurisdiction as part of the California statewide encephalitis virus surveillance program. A total of 24 chickens in four of the flocks became infected with WNV during August through November, 2004 (Fig. 3). During the same period in 2005, 48 chickens in five of the flocks became infected with the virus. During each year, WNV was confirmed in August after the virus had already infected wild birds, mosquitoes, humans, and horses.

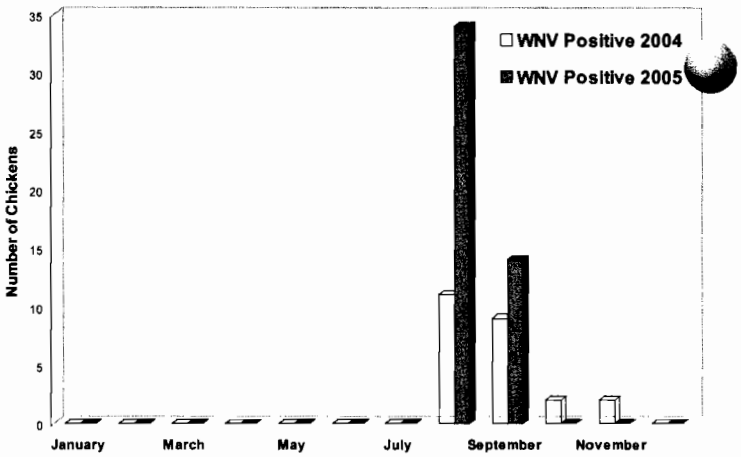

Figure 3. Sentinel chickens sero positive for WNV in the District during 2004 and 2005.

Mosquitoes: The District employs carbon dioxide-baited (CO_2) and gravid traps to collect mosquitoes to be tested for WNV. Of the 123 mosquito pools submitted in 2004, 14 tested positive for WNV. In 2005, 58 out of 323 pools were found positive, including one collected from the Foothills. Positive pools were much more widely distributed in 2005 than in 2004 (Fig. 4).

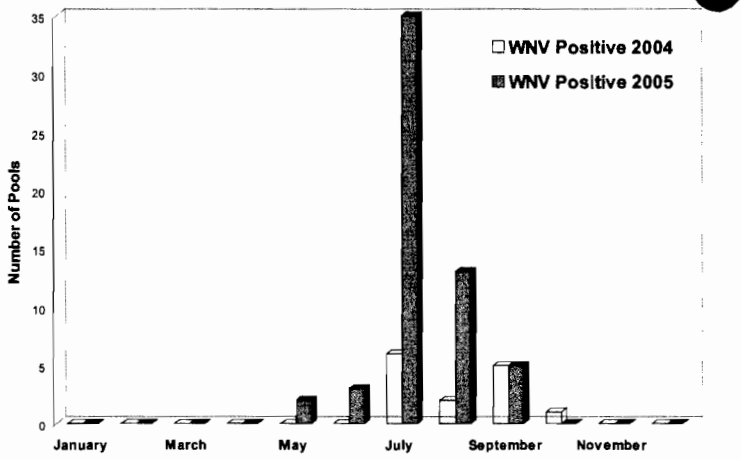

Figure 4. Mosquito pools that tested positive for WNV in the District during 2004 and 2005.

Data on virus isolation from different mosquito species are presented in Table 1. WNV was isolated only from *Culex quinquefasciatus* in 2004. In 2005, the virus was detected in *Culex quinquefasciatus* (18.9%), *Cx. tarsalis* (75.0%), and *Cx. stigmatosoma* (17.8%).

Table 1. Data on WNV occurrence in mosquito pools of different species in the District during 2004 and 2005.

	Pools in 2004			Pools in 2005		
Mosquito Species	Submitted	WNV+	%+	Submitted	WNV+	%+
Ae. vexans				1	0	
An. freeborni				1	0	
Cx. erythrothorax	7	0	0	16	0	0
Cx. quinquefasciatus	75	14	18.7	180	34	18.9
Cx. stigmatosoma	0	0	0	4	3	75
Cx. tarsalis	40	0	0	118	21	17.8
Cs. incidens	1	0	0	3	0	0

SUPPLEMENTARY SURVEILLANCE INFORMATION

Human Cases: The District was informed of human infections of WNV by the County Environmental Health Department and from the California Department of Health Services. Fourteen human infections were confirmed within the District in 2004, with no fatalities, as compared with 45 cases in 2005, which included two fatalities (Fig. 5). It should be noted that two of the cases in 2005 occurred outside the District in the Foothills.

Equine Cases: The District was informed of equine infections of WNV by the California Department of Food and Agriculture. During the period of July through November, 2004 the District had 23 horses infected with WNV, and six of them either died or were euthanized. In 2005, there were 27 horses infected with the virus, with eleven fatalities. Approximately one-third of the horse cases in 2005 occurred in the Foothills. In each year, the highest numbers of cases were reported during August (Fig. 6).

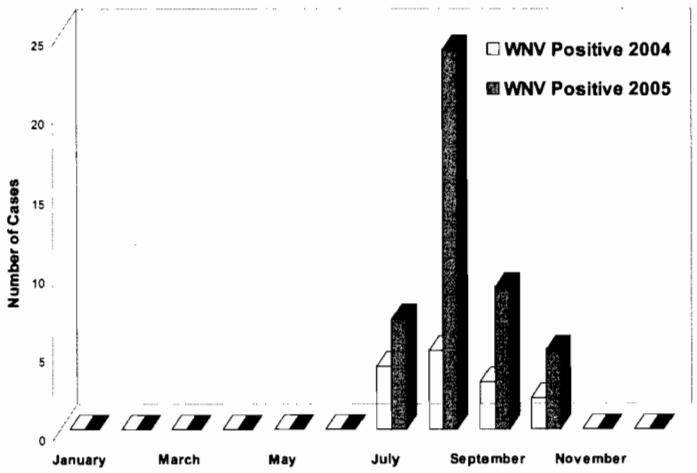

Figure 5. Human cases of WNV in the District during 2004 and 2005.

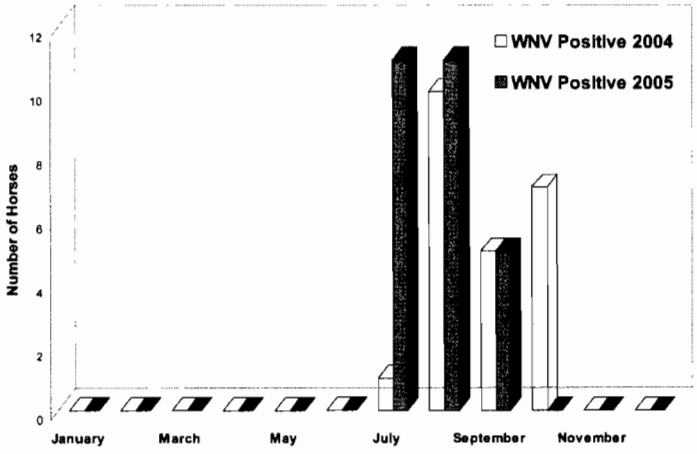

Figure 6. Equine cases of WNV in the District during 2004 and 2005.

DISCUSSION AND CONCLUSIONS

WNV activity in the District and the County not only increased substantially in 2005 from its introduction in 2004, but the virus also appeared in the District earlier in 2005 than in 2004. In each year, the first evidence of WNV was discovered in dead wild birds, followed by mosquitoes, humans, and horses by July. The first detection of WNV in mosquitoes in 2005 occurred in May. Sentinel chicken infections were not discovered until August in each year.

By the middle of August in each year, 80% of dead birds tested were infected with WNV. Western scrub jays represented about half of the infected birds while American crows represented about one-third of the total. Many of the corvids that were RAMP®-tested by the Fresno CAHFS facility tested positive for WNV.

Frequency of WNV infection in mosquito pools appeared highest in July of each year. Surveillance information compiled by the District combined with data analysis from the Department of Health Services suggests that both the minimum infection rate of WNV in mosquitoes and the human case incidence within the County increased substantially in 2005 from 2004. Not only were more mosquitoes that tested positive for WNV captured in 2005, but also the infected specimens were more widely distributed between both gravid and CO_2-baited traps. Almost all the positive pools were processed from gravid traps in 2004 in which WNV was isolated only from *Culex quinquefasciatus*. In 2005, the virus was isolated from pools of *Cx. stigmatosoma*, and *Cx. tarsalis*, in addition to *Cx. quinquefasciatus*.

Although the sentinel chickens became infected with WNV after evidence of the virus was discovered in other hosts, the number of infected chickens in 2005 was double the number in 2004, suggesting that the virus had greatly amplified.

WNV infections in horses appeared to be slightly higher in 2005 over 2004, with nearly twice as many fatalities in 2005. The substantially higher human caseload in 2005 included two fatalities. The higher number of human, horse, and dead bird infection discovered in the Foothills during 2005 combined with widespread isolations in mosquito pools throughout the District provide reasonable evidence that WNV has spread dramatically and affected more hosts during 2005 than was the case in 2004.

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Jodi Holeman, Emma Lee, Victor Maggi, Ken Klemme, Tokuo Fukuda, Henry Doll, and Steve Mulligan of Consolidated Mosquito Abatement District are greatly appreciated for their assistance in data collection, equipment maintenance, and guidance. Others who contributed data include David Farley, Fresno Mosquito and Vector Control District, Ryan Carney, California Department of Health Services, and Chris Barker , University of California, Davis.

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Evaluation of Mosquito and Arboviral Activity in Orange County, California, During 2005

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ABSTRACT: The Orange County Vector Control District (OCVCD) continued its arbovirus surveillance in 2005 by collecting and pooling mosquitoes, testing avian blood samples drawn from wild birds and sentinel chickens, as well as testing dead birds collected from various animal control agencies and the public. Evidence of West Nile virus (WNV) infection was detected in wild birds (189 of 3,708), dead birds (302 of 666), mosquito pools (113 of 3,017), and 2 of the 10 sentinel chickens in the County. By year's end, 17 nonfatal human cases were reported due to WNV infection. *Culex quinquefasciatus* Say was the most abundantly trapped mosquito, accounting for the majority of submitted pools (1,680 of 3,017) and positive pools (94 of 113). House finches (*Carpodacus mexicanus* Say) and house sparrows (*Passer domesticus* L.) were the most frequently sampled wild birds (3,360 of 3,708) and together, comprised most of the WNV-seropositive samples (185 of 189). Comparatively, fewer human cases of WNV occurred in 2005 than 2004 (17 vs. 64, respectively) and the minimum infection rate (MIR) in *Cx. quinquefasciatus* was also lower in 2005 than 2004 (1.6 vs. 3.7, respectively). No St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) virus activity was detected by any of our surveillance methods.

INTRODUCTION

The Orange County Vector Control District (District) encompasses approximately 789 square miles (all of Orange County), and slightly over 3 million residents reside within its borders (US Census Bureau 2005). Most of the District consists of urban/suburban habitats with a variety of residential mosquito-breeding sources: improperly maintained swimming pools and ponds, debris-choked drainage channels, and other man-made habitats. Interspersed within this development are several natural, mosquito-producing fresh and salt-water wetlands. Three important encephalitis vectors are collected in the county: *Culex tarsalis* Coquillett, *Culex quinquefasciatus* Say, C*ulex stigmatosoma* Dyar and, for West Nile virus (WNV), *Culex erythrothorax* Theobald. The District employed an integrated arboviral disease surveillance system throughout the year, comprised of avian serosurveillance (sentinel chickens and wild birds), testing dead birds and mosquitoes, and monitoring veterinarian and physician reports for WNV infections in animals and humans.

MOSQUITO SURVEILLANCE

Mosquitoes were collected weekly from a total of 88 traps throughout the District, combining CDC/CO_2 - style, host-seeking EVS traps (Rohe and Fall 1979) and Reiter/Cummings gravid female, ovipositional traps (Cummings 1992). Due to the high level of West Nile activity in 2004 (Schwedes et al. 2005), 38 new mosquito sites were added (Figure 1) to improve the spatial distribution of the trap grid and increase collections from previously under-sampled areas of south Orange County. Blood-fed mosquitoes were also aspirated at known resting sites, and at locations of service requests.

Culex quinquefasciatus comprised the majority of the specimens collected (60,120 of 103,162) (Table 1). Of 3,017 mosquito pools submitted for arbovirus testing by mutiplex reverse transcriptase-polymerase chain reaction (RT-PCR), 2,917 pools were tested by the Center for Vector-borne Diseases (CEVC) at the University of California, Davis, resulting in 84 WNV-positive pools; 100 pools were tested by the Marin-Sonoma Mosquito and Vector Control District, of which 29 were found to be WNV-positive. Altogether, 113 WNV-positive pools were found in 2005; *Cx. quinquefasciatus* comprised the majority of these (94 of 113) and had the highest minimum infection rate (MIR) (Table 1).

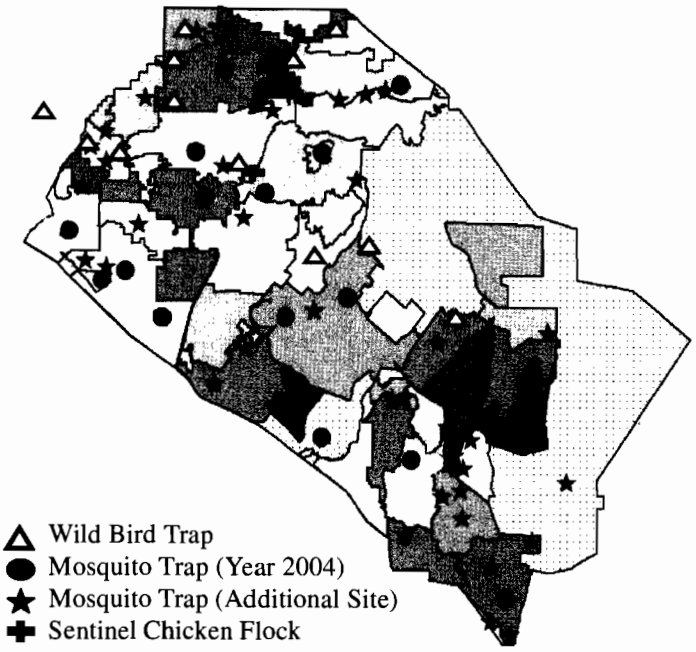

Figure 1. Map of Orange County with bird and mosquito trap locations.

Table 1: Mosquito collection data and minimum infection rates (MIR) for 2004 and 2005 by species.

Species	Total Mosquitoes (2004)	Total Mosquitoes 2005	WNV Positive Pools (2004)	WNV Positive Pools 2005	MIR (2004)	MIR 2005
Cx. quinquefasciatus	*(41,858)*	60,120	*(153)*	94	*(3.7)*	1.6
Cx. erythrothorax	*(10,320)*	18,369	*(1)*	1	*(0.1)*	0.1
Cx. tarsalis	*(5,315)*	10,157	*(4)*	7	*(0.8)*	0.7
Cx. stigmatosoma	*(927)*	5,647	*(6)*	11	*(6.5)*	1.9
Others	*(5,529)*	8,869	*(0)*	0	*(0.0)*	0.0
Total	***(63,949)***	**103,162**	***(164)***	**113**	***(2.5)***	**1.1**

The West Nile virus MIR for *Cx. quinquefasciatus* increased to its highest level in August in 2005, but overall, rates were lower in most months when compared to 2004 levels (Figure 2). The highest MIR for *Cx. quinquefasciatus* peaked at 13.2 at one site in the city of Anaheim in 2005.

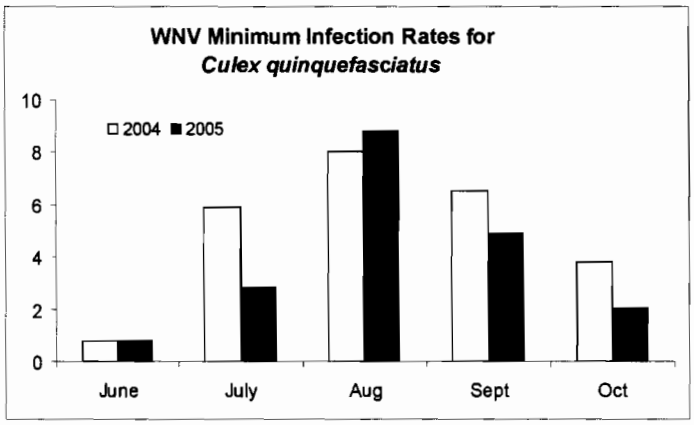

Figure 2: Comparison of minimum infection rates for *Culex quinquefasciatus* in peak months, 2004 and 2005.

One WNV-positive pool of *Cx. quinquefasciatus* was collected in the city of Santa Ana during the month of January (1/25/05). Additionally, two WNV-positive pools of *Cx. stigmatosoma* adults (50 males, 41 females) were reared from larvae collected in July (7/22/05) from an ornamental pond in the city of Cypress, indicating transovarial transmission of WNV in this species. No mosquito pools tested positive for either St. Louis encephalitis (SLE) or western equine encephalomyelitis (WEE) viruses.

SENTINEL CHICKENS

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

WILD BIRD SEROSURVEILLANCE

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

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

Of the 2,412 house finches sampled in 2005, 139 birds (5.7%) tested positive for WNV antibodies; forty-six (4.8%) of the 948 house sparrow samples and four birds of other species (1.1%) were WNV-positive during the year (Table 2). Antibody-positive birds were detected in every month of 2005 (Figure 3). However, unlike 2004, WNV-seropositive rates held constant around 4.2% during the summer/fall months of 2005. Increasing percentages of antibody-positive wild birds were seen only in immunologically-naïve immature house finches and house sparrows during the months of May – October 2005 (Figure 4). Approximately 12% of these hatching-year birds were seropositive by October, similar to the pattern seen in 2004 (Figure 3).

Table 2: Wild bird data and seropositive rates by species, 2004 and 2005.

Species	Blood samples (2004)	Blood samples 2005	WNV positive (2004)	WNV positive 2005	% positive (2004)	% positive 2005
House finch	(2,296)	2,412	119	139	(5.2%)	5.7%
House sparrow	(612)	948	40	46	(6.5%)	4.8%
Others**	(976)	348	21	4	(2.2%)	1.1%
Total	**(3,884)**	**3,708**	**180**	**189**	**(4.6%)**	**5.1%**

** Positive species: nutmeg mannikin, white-crowned sparrow, green heron, brown-headed cowbird

Non-positive species: black-headed grosbeak, scrub jay, song sparrow, doves, hawks, warblers

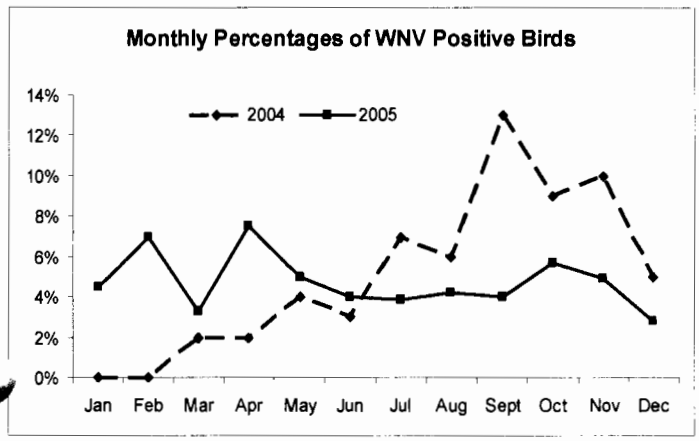

Figure 3: Percentages of positive wild birds by month, 2004 and 2005.

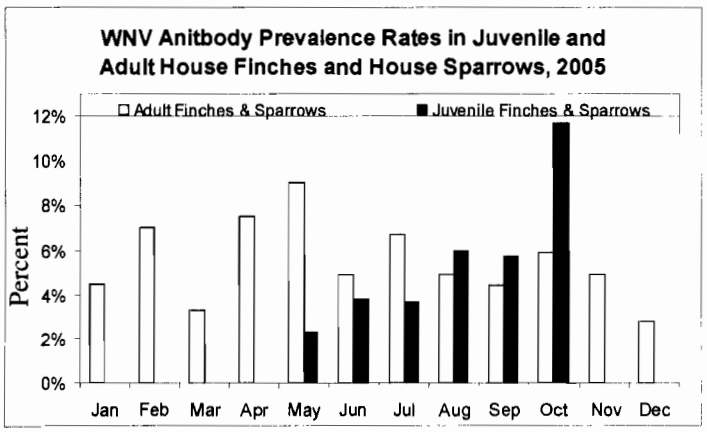

Figure 4: Comparison of seropositive rates between adult and immature house sparrow and house finch, 2004 and 2005.

DEAD BIRD SURVEILLANCE

Dead birds were collected from the public via dead bird call-ins and through cooperation with various animal control agencies. Of the 1,124 birds collected, only 666 were suitable for testing, and 302 of these were found positive for WNV antigen by immunohistochemistry (Steele et al. 2000) (Figure 5).

Staining of tissue allows a precise analysis of the cell and tissue types being infected by the virus (Figure 6). The lower sensitivity of immunohistochemistry may screen out specimens that have or do not have a patent viral infection, but perhaps died of other causes. Immunohistochemistry also preserves a stable specimen that can be re-examined if there is any doubt as to the test results, allowing for ease of confirmation.

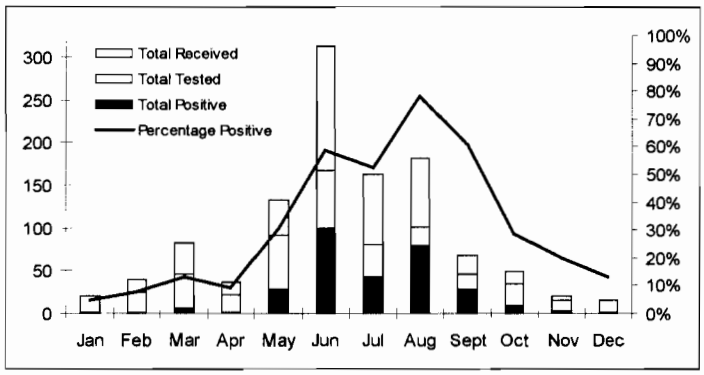

Figure 5. Numbers of dead birds collected, tested, and positive by month. Positives were found from January through December 2005.

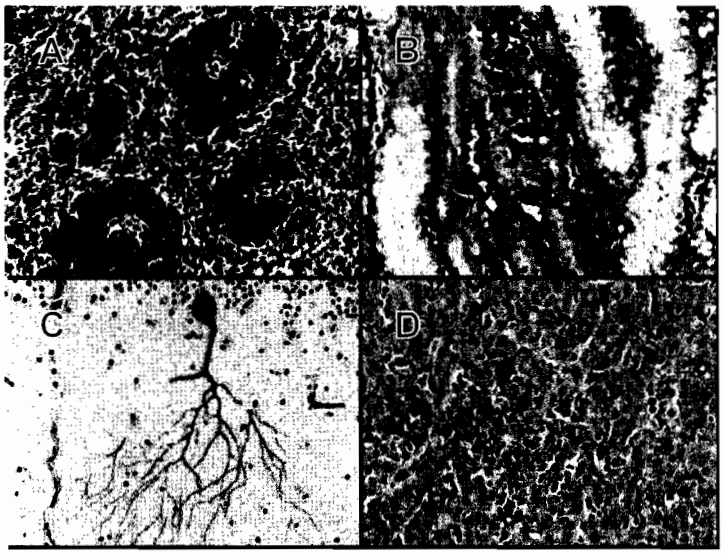

Figure 6. WNV positive crow tissue sections: A) Spleen showing WNV positive macrophages surrounding arterioles. B) Kidney macrophages positively stained for WNV antigen. C) Purkinje cell in the cerebellum. D) Liver macrophages

CLIMATOLOGICAL DATA

Temperature and precipitation data were obtained through the National Oceanic and Atmospheric Administration (NOAA) to determine potential differences in weather patterns between 2004 and 2005. There were no apparent differences between average monthly high and low temperatures for 2004 and 2005 (Figure 7). Rainfall, however, was significantly higher in 2005 (Figure 8).

RESULTS AND DISCUSSION

The District's arboviral disease surveillance program demonstrated the usefulness of a comprehensive WNV monitoring system involving serological testing of WNV infection in free-ranging wild birds, PCR identification of WNV in mosquitoes, and the detection, distribution and quantification of WNV antigen in dead birds by WNV-specific immunohistochemistry. Data from all three surveillance methods for January – June of 2005 suggested that the public was at a potentially greater risk for WNVinfection than in 2004. Near-record rainfall in 2005 resulted in the production of

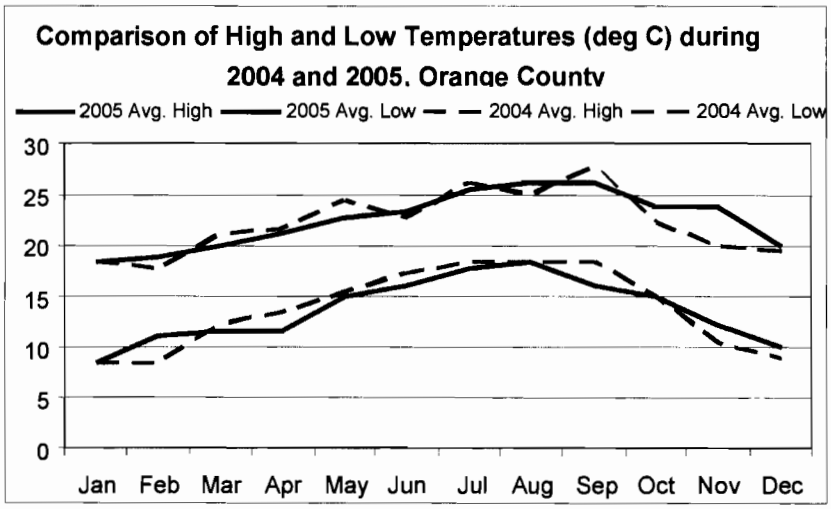

Figure 7: Temperature comparisons for 2004 and 2005, Santa Ana, California.

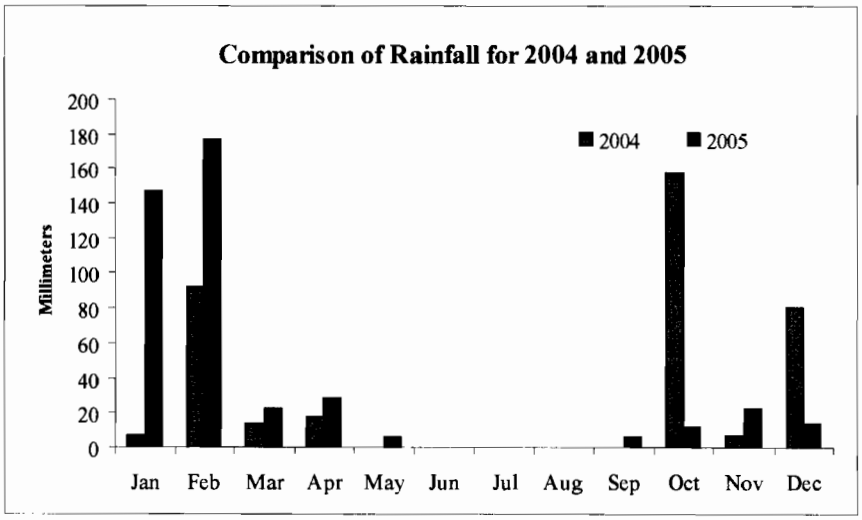

Figure 8: Precipitation comparisons for 2004 and 2005.

large numbers of *Cx. tarsalis* from coastal wetlands and ample numbers of *Cx. quinquefasciatus* from a variety of rain-filled, peridomestic mosquito breeding sources in Orange County. Unlike 2004, most WNV-infected dead birds were found in late spring/ early summer clustered along the District's coastal wetlands. Additionally, seven pools of WNV-positive *Cx. tarsalis* mosquitoes were collected in June from these wetlands. Dead bird infections early in the season and dissemination of West Nile virus to other areas of the County (Figure 9) could have been attributed to above-normal levels of *Cx. tarsalis* females from these habitats.

West Nile virus-seropositive rates in wild birds were also comparatively higher in the first six months of 2005 than 2004 (Figure 5). The detection of WNV antibody-positive wild birds usually foreshadowed the detection of WNV in mosquitoes and dead birds, ultimately indicating the emergence of multiple WNV transmission foci throughout Orange County. Infections in wild birds, however, may have indicated antibody persistence in adult passerine birds as the result of infection to WNV from the previous year (Schell et al. 2006). Antibody persistence and "herd immunity" in wild bird populations may have contributed to a dampening of WNV transmission for the second half of 2005. From June onwards, WNV antibody-positive rates declined and leveled off in house finches and house sparrows (Figure 5). Correspondingly, mosquito infection rates were lower overall in 2005 than 2004 (Table 1).

From June through September, 17 human cases (no deaths) were reported in 2005, a dramatic decline from the 64 cases (4 deaths) reported in 2004. There was a huge gap between the first evidence of West Nile activity and the onset of human disease (Figure 10). The reduction in human cases may have been the result of a combination of wild bird immunity, lower mosquito infection rates, better mosquito control and public awareness of disease prevention.

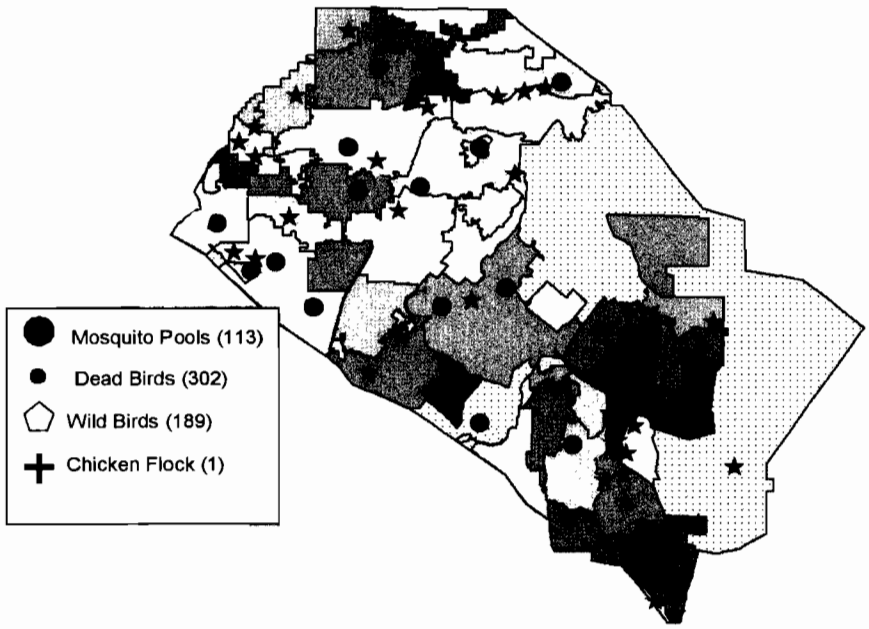

Figure 9. Map of Orange County, with distribution of WNV positive samples collected in 2005

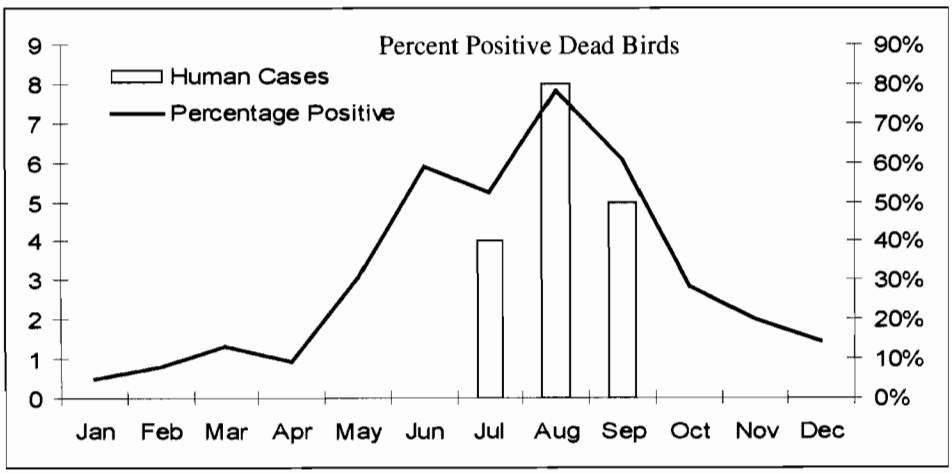

Figure 10. Timeline of arbovirus events by month, including human cases and positive dead birds.

In conclusion, WNV is entrenched in the suburban *Cx. quinquefasciatus*-peridomestic small bird cycle of Orange County and remains a threat to the public. Its abundance is likely to undergo yearly oscillations, deceasing and increasing with changes in avian immunity and mosquito counts. Since large numbers of immunologically-naïve passerine wild birds are produced in urban habitats each year, sufficient quantities of susceptible avian hosts are likely to be available for continued arboviral persistence in the future. The role of transovarial transmission in mosquitoes, the importance of viral persistence in chronically infected wild birds, and the attenuation of viral amplification via herd immunity in avian hosts for the maintenance of WNV in nature remain to be determined.

Acknowledgements

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West Nile Antibodies in Naturally Infected House Finches and House Sparrows Orange County Vector Control District, 2005

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ABSTRACT: In 2005, 3,708 sera were collected from wild birds at 12 different locations, as part of the continuous arbovirus surveillance program of the Orange County Vector Control District (OCVCD). Sera were screened by the blocking ELISA for specific diagnosis of West Nile/Kunjin NS1 antibodies. Twenty two passerines which had tested positive for the first time were held in a mosquito-proof cage, and were tested every 2 weeks, to assess the duration of immunity. The duration of antibodies ranged from 3 to 15 months. Some birds maintained a titer over 1:40 after 9 months; others showed a gradual decrease of immunity and remained negative for 5 months. All sequential sera tested between April and July were negative by PCR, showing that there was no circulating virus at the time. A puzzling pattern of alternative reactions from positive to negative and back to positive was observed in 12 individuals held between 13 and 15 months. The possible explanations for such events are still conjecturable. Controls remained negative all through the study precluding transmission from bird to bird or by exposure to feces and aerosols. A 2002-2003 study of great-horned owls, *Bubo virginianus*, offered some contrast in the duration of their immune response. The patterns of immunity observed in these studies once more emphasized the need to have a serological test capable of distinguishing between recent and past infections, particularly for avian sera.

INTRODUCTION

During the initial epidemiological studies of West Nile Virus (WNV) transmission in Egypt, antibodies were detected in various avian species, predominantly hooded crows, *Corvus corone*, and house sparrows, *Passer domesticus*. Subsequently, Virus transmission by experimentally infected mosquitoes showed that both avian species develop a viremia of 3 to 8 logs within 3 days, with a mortality of 69% and 100%, respectively. In contrast, the viremia in kestrels, *Falco tinnunculus*, buff-backed herons, *Bulbucus ibis*, and palm doves, *Streptopelia senegalensis*, similarly inoculated, was never higher than 4 logs and all the birds survived (Work et al. 1955). The number and duration of infectious mosquito bites had no correlation with the rate of viremia. The detection of antibodies in crows, collected in the field, was instrumental in characterizing areas of transmission as endemic, transitional, and non-endemic. Collections made in late spring showed low incidence of immunity, reflecting the introduction of non-immune juveniles (Taylor et al. 1956). In North America, the introduction of WNV in 1999 in New York and its 5 years dispersal nationwide has mostly demonstrated that crows, suffering from a high mortality rate, are not very suitable for long term antibody studies. House finches and house sparrows which have been used since the seventies for the surveillance of arboviruses, namely St. Louis encephalitis (SLE) and Western equine encephalitis (WEE) (Gruwell et al. 2000; Holden et al. 1973), may provide early indication of WNV transmission (Nevarez et al. 2005). The duration of SLE antibodies in experimentally infected birds is well documented (Mclean, 2005), but more remains to be done with WNV. A recent study of rock doves naturally infected with WNV and held in a mosquito-free environment for 60 weeks has provided some insight about the duration of antibodies in this species, which would average 15 months at least. Of particular interest is the observation of persistent maternal antibodies in squabs for an average of 28 days (Gibbs et al. 2005).

MATERIALS AND METHODS

Trapping and blood collection

Wild birds were captured in modified crow traps (Gruwell et al. 2000) located at 12 different sites in Orange County, California. A total of 3,708 birds was collected. Serum collection was performed from the jugular vein. A sample of 0.2ml of blood was diluted immediately 1:10 in 1.8ml of 0.75% Phosphate Buffer Saline with pH 7.2, and transported to the laboratory on ice, where it was centrifuged at +8°C for 15 minutes at 2800rpm.

Selection of birds for captivity (house finches and house sparrows)

There was a total of 594 recaptured birds, with 61 birds exhibiting WN antibodies. Seventeen birds tested positive between November 2004 and April 2005, 5 between May and June 2005, and all were transferred to a mosquito-proof cage (10x8 ft), for long term observation of immunity patterns. Five negative birds were included in that group for control. Serum collections were obtained every 2 weeks from each bird.

Great-horned owl study

Blood spots on filter paper were collected between August 2002 and April 2003 from 49 birds admitted to a rehabilitation center in Illinois in 2002, with a diagnosis of possible WNV infection. Samples were eluted at 1:10 in bovalbumin-PBS diluent, and similarly centrifuged.

Testing

Testing was done by Blocking ELISA using as antigen a baculovirus- Kunjin recombinant, epitope NS1, and the whole cell lyzate WN antigen, and as a competitive antibody the broad flavivirus monoclonal 3H6 and the WN-KUN specific anti-NS1 3112G monoclonal. The test was performed according to the protocol

established at the University of Queensland Australia (Hall et al. 1995) which provided us with all the reagents. The technique was extensively evaluated at the District for its use with avian sera (Jozan et al. 2003). Sera were screened at a dilution of 1:20, and all positive samples were titrated up to 1:1280. A percent inhibition greater than 45% was the threshold set for a positive result. A 30-45% inhibition was deemed inconclusive and repeated. Any test showing an inhibition equal or below 30% was considered negative.

RESULTS AND DISCUSSION

Passerine study

Of the 22 positive birds confined in a mosquito-proof cage, seven birds died within 3 to 9 months during the course of the study. 13 birds are still under observation. Persistence of antibodies was found in 5 birds at 12, 13, 14 (2 birds) and 15 months (Table 1). Titers as high as 1:640 were observed, and 1:1280 in one instance. A gradual decrease of immunity was observed in 7 birds and 1 bird which have remained negative for 5 and 11 months, respectively. The exact duration of immunity was unknown for all but 2 of these birds, because of the lack of samples prior to January. A seroconversion in sequential sera collected within 8 to 15 days of each other was observed in 3 birds collected in February, May and July 2005. Six birds were positive in 2004 between November and December, but samples were too far apart to determine the time of seroconversion. Similarly, there was no past history on 13 positive birds which entered the study in January, and it was not known how long they might have had this antibody. The duration of antibodies ranged from 3 to 15 months. Antibodies were consistently observed in 2 birds still under observation, and held respectively for 13 and 15 months. Four birds held respectively for 3 (2 birds), 6 and 8 months showed persistent antibodies also. Given the short lifespan of finches and sparrows (Milby and Wright, 1976) the persistence of antibodies for 12 to 15 months would indicate that some of them develop a lifetime immunity. The number of sparrows (7) in the study was too small to contrast the antibody results with that of the house finches (15).

An antibody pattern alternating from positive to negative to positive was observed in 12 birds held between 10 and 15 months (Table 1). A similar observation has been made with naturally infected chickens (Vuong, 2006 pers. Commun.) Explanations for this event vary and all are conjectural: the possible failure of the test is the first to come to mind, but these samples were repeated with the same results. The normal fluctuation of antibody from day to day is another possible consideration, yet unsatisfactory. A chronic steady infection, latent infection or integrated infection could trigger the pulsation of antibodies from time to time. To test such hypothesis, it would be necessary to search for the presence of virus in various tissues. An experimental study on rock doves infected with Sindbis virus (alphavirus) and 2 flaviviruses (Tick-borne and West Nile) have shown similar fluctuations occurring with West Nile Virus (Chunikhin et al. 1972). The authors interpreted it as a chronic infection triggering viremia from time to time. Serum from all confined birds were tested by PCR from April to July, with a total of 8 sequential samples per bird. Virus was not found in either positive or negative birds. Therefore, there was not detectable circulating virus. Other factors such as stress from overcrowding, competition for food, or changes in temperature could modulate the antibody response, but these conditions are minimized in the large confined area of the study. The puzzle remains to be solved. During the duration of this study none of the five negative controls which were in constant contact with the positive birds seroconverted. Viral transmission from bird to bird, from aerosols or feces was thus nonexistent under these particular circumstances.

An attempt was made to analyze all the birds recaptured in the field, but the average duration of antibody could not be determined because of too few recapture samples per bird (data not shown). Only one bird was consistently trapped and showed antibody for 6 months.

Great-horned Owl study

This 2002-2003 study (Table 2) offers some contrast with the above results. Samples were sequentially obtained from 49 birds which were admitted to the rehabilitation center with a variety of neurological syndromes, ranging from lethargy, to blindness and ataxia. All but 2 birds, which died in 2002, were released within 9 months of the study. Eight birds were negative upon admission, and remained so at the end of the observation period. A total of 39 birds was positive. For 13 individuals only one specimen was available for testing. 2 to 6 sequential samples were obtained from the remaining 26 birds. Seven birds tested negative upon entry but, seroconverted within a month. Due to the lack of prior samples, such a seroconversion might reflect the rise of antibodies during the incubation period of the disease. It could also be the result of an infection acquired from the other sick birds or their fomites. The average duration of antibodies for sequential specimens (3 to 6 collections) was 6 months. Four birds were still positive at the time of release in April 2003, with an average titer of 1:40. Antibody fluctuation from positive to negative to positive was not observed but cannot be excluded since blood samples were collected 2 months apart on the average. A rise in titer was observed with all specimens and the progression of immunity was marked by the appearance of SLE antibodies in 12 instances. A booster reaction to a previous infection with SLE known to have been circulating in Illinois could explain such reaction. The life span of raptors would accommodate this double exposure. It might also reflect the increase of cross reactions which often characterize the progression of a flavivirus immune response. This positive broad flavivirus reaction was not observed in the passerine study.

CONCLUSION

The near lifetime duration of antibodies in passerines affects our interpretation of positive serological results for WNV during the winter months. WN antibodies detected in a single serum collection might only reflect an immunity acquired in the preceding fall. The neutralization test often fails to separate WNV from SLE, a problem which will be compounded if both viruses circulate at the same time. Furthermore, the fluctuations from positive to negative and back to positive, observed during this fifteen months study would indicate that negative specimens might just be in the fluctuation phase and thus the predictive value of the system for virus transmission would appear jeopardized. In the absence of a seroconversion

Table 1. Duration of West Nile antibody in house finches and house sparrows confined to a mosquito-proof cage from January 2004 to February 2005.

Month →			2004	January 05	February	March	April	May	June	July
Band #	Species	Pos / # Tested	Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)
01-624	HF	3/27	NS	0/2	**1/2 (40)**	0/2	**0/2***	**0/2***	**0/2***	**1/2* (20)**
03-0510	HF	21/31	NS	**2/2 (160,40)**	**2/3 (160,160)**	**3/3 (160,160,40)**	**1/2* (40)**	**1/2* (80)**	**1/2* (80)**	**2/2* (40,20)**
03-0577	HF	19/30	NS	**2/2 (160,40)**	**1/2 (80)**	**3/3 (160,20,40)**	**1/1* (20)**	**1/2* (80)**	**2/2* (40,40)**	**2/2* (40,20)**
03-0702	HF	6/20	NS	NS	**1/1 (20)**	NS	NS	NS	**1/2* (20)**	**2/2* (20,40)**
03-0932	HF	5/19	NS	NS	NS	NS	NS	NS	**1/2* (20)**	**1/2* (40)**
04-1287	HF	12/29	NS	**1/2 (160)**	**2/2 (80,160)**	**3/3 (80,160,40)**	**1/2* (80)**	**0/2***	**0/2***	**1/2* (20)**
04-1478	HF	6/10	**3/3 (160,640,640)**	**1/1 (640)**	0/1	**2/3 (80,80)**	Died			
06-0384 Control	HF	0/30	NS	**0/2**	0/2	0/3	0/2	0/2	0/2	0/2
04-1491	HF	15/25	NS	**2/2 (320,320)**	**2/2 (80,40)**	**2/3 (80,40)**	**1/2* (40)**	**0/2***	**1/2* (40)**	**2/2* (40,40)**
05-0443	HF	15/18	NS	NS	**1/1 (160)**	**4/4 (320,320, 160, 160)**	**1/2* (20)**	**1/2* (20)**	**2/2* (160,160)**	**2/2* (160,160)**
21-0011	HF	4/5	**3/4 (160,320,640)**	**1/1 (640)**	Died					
05-0444 Control	HF	0/11	NS	NS	NS	NS	NS	NS	0/2	0/2
05-0606	HF	2/27	NS	NS	**1/1 (40)**	**1/3 (20)**	0/2	0/2	0/2	0/2
05-0616	HF	24/28	NS	NS	**2/2 (160,160)**	**3/3 (320,320,320)**	**2/2* (320,40)**	**1/2* (20)**	**2/2* (80,80)**	**3/3* (40,80,160)**
05-0698	HF	4/8	NS	NS	NS	NS	NS	NS	**1/3**	**1/2* (20)**
06-0684 Control	HF	0/29	NS	**0/2**	0/2	0/3	0/2	0/2	0/2	0/2*
07-882	HF	2/25	NS	NS	0/1	0/1	0/2	**1/2 (320)**	**0/3***	0/2*
08-2316	HF	6/8	NS	NS	NS	NS	NS	0/1	0/1	**2/2* (40,40)**
01-0555 Control	HS	0/10	NS	NS	NS	NS	NS	NS	NS	NS
03-0699	HS	28/32	**1/2 (80)**	**2/2 (640,40)**	**2/2 (20,40)**	**3/3 (40,80,40)**	**2/2* (80,40)**	**1/2* (20)**	**2/2* (20,320)**	**2/2* (40,40)**
03-812	HS	8/30	NS	**1/2 (640)**	**1/2 (40)**	0/3	**1/2* (20)**	0/2*	**1/2* (20)**	**1/2* (20)**
01-707 Control	HS	0/7	NS	NS	NS	NS	NS	NS	NS	NS
03-858	HS	11/12	NS	NS	**1/1 (160)**	**3/3 (320,320,320)**	**2/4* (160,160)**	**1/2* (40)**	**2/2* (80,160)**	**2/2* (160,80)**
03-0891	HS	12/19	NS	NS	NS	NS	NS	**2/2* (640,640)**	NS	**2/2* (80,80)**
05-0424	HS	4/30	**1/3 (640)**	**0/2**	0/2	**1/2* (20)**	**1/2* (80)**	0/2*	0/2*	0/2*
05-0509	HS	5/12	**4/4 (80,80,20,20)**	**0/2**	0/2	**1/2* (20)**	0/2	Died		
05-0546	HS	11/18	**2/4 (80,320)**	**2/2 (640,320)**	**2/2 (80,80)**	**2/3 (40,40)**	**1/2* (40)**	0/2*	**2/2* (40,20)**	0/2*

*: Serum tested by PCR for West Nile Virus

Known Sero-conversion within 8-15 days

HF: house finch

Duration of captivity

HS: house sparrow

>> Table 1. *(continued)*

August	September	October	November	December	January	February
Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)	Pos / # Tested (Titer)
0/3	**1/1 (40)**	0/2	0/2	0/1	0/2	0/2
2/3 (40,320)	**2/2 (40)**	**1/2 (20)**	0/1	0/1	**2/2 (40,20)**	**1/2 (40)**
2/3 (20,40)	**2/2 (20,20)**	0/2	**2/2 (20,40)**	0/2	0/2	**1/2 (40)**
1/3 (40)	**1/2 (20)**	0/2	0/2	0/1	0/2	0/2
2/3 (80,40)	**1/2 (40)**	0/2	0/2	0/1	0/2	0/2
2/3 (20,320)	**2/2 (40,20)**	0/2	0/2	0/1	0/2	0/2
0/3	0/2	0/2	0/2	0/2	0/2	0/2
2/2 (20,20)	**2/3 (40,40)**	0/2	**1/2 (20)**	0/1		
2/3 (80/40)	**2/2 (40,80)**	Died				
0/2	0/2	0/2	Died			
0/3	0/2	0/2	0/2	0/2	0/2	0/2
1/2 (160)	**1/2 (40,20)**	**2/2 (40,20)**	**2/2 (40,80)**	**1/2 (160)**	**2/2 (20,20)**	**1/2 (20)**
2/3 (20,80)	Died					
0/2	0/2	0/2	0/2	0/2	0/2	0/2
0/3	**1/2 (80)**	0/2	0/2	0/2	0/2	0/2
2/2 (40,40)	**2/2 (40,40)**	Died				
NS	NS	0/2	0/2	0/2	0/2	0/2
2/3 (40,160)	**2/2 (20,20)**	**2/2 (80,20)**	**2/2 (20,40)**	**1/2 (20)**	**2/2 (40,20)**	**2/2 (40,40)**
1/ 2 (80)	**2/2 (20,20)**	0/2	0/2	0/2	0/2	0/2
NS	0/1	0/2	0/2	0/2		
Died						
2/3 (80,80)	NS	0/2	**2/2 (20,80)**	0/2	**2/2 (20,20)**	**1/2 (40)**
1/3 (80)	**1/2 (160)**	0/2	0/2	0/2	0/2	0/2
Died						

NS: No Sample

Positive reading are indicated by bold face type

Table 2. Duration of WN antibodies in captive great-horned owls from Aug. 2002 - Apr. 2003.

Specimen #	Duration of Captivity	Duration of Immunity	Positive /Total Samples	Percent Positive
A-258-02	6 mo.	6 mo.	4/4	100%
A-279-02	9 mo.	7 mo.	5/6	83%
A-288-02	8 mo.	7 mo.	4/5	80%
A-294-02	7 mo.	5 mo.	3/4*	75%
A-295-02	7 mo.	5 mo.	3/4	75%
A-303-02	7 mo.	7 mo.	5/5	100%
A-305-02	7 mo.	5 mo.	4/5*	80%
A-307-02	7 mo.	7 mo.	5/5	100%
A-308-02	6 mo.	5 mo.	3/4	75%
A-309-02	3 mo.	2 mo.	2/3*	67%
A-314-02	7 mo.	7 mo.	5/5	100%
A-338-02	7 mo.	5 mo.	4/5*	80%
A-355-02	7 mo.	5 mo.	3/4	75%
A-357-02	7 mo.	4 mo.	4/6	67%
A-360-02	7 mo.	5 mo.	5/5	100%
A-362-02	7 mo.	7 mo.	5/5	100%

* known sero conversion within 30 days

observed in sequential sera no more than 10 to 15 days apart, there is a need for a reliable method to determine if the antibody is recent or not. The IgM capture test in birds does not answer this need. The predominant immunoglobulin of birds is IgY, an ancestral immunoglobulin, characterized by the absence of a hinge seen in mammals, henceforth a limited flexibility, an inability to activate the complement, and a very low avidity/affinity (Warr et al. 1995). As the immune response progresses, so does the increase in avidity, which in mammalian sera can be readily measured and has been studied with West Nile and Dengue (Fox et al. 2004; de Souza et al. 2004). At this time similar studies have not been conducted with avian sera. Designing a test which ultimately would identify a recent antibody from an old one would solve the riddle of virus transmission in a timely and predictive fashion, paramount to any efficient surveillance.

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Seasonal Evaluation of West Nile Virus in Lake County, California in 2005

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ABSTRACT: West Nile Virus (WNV) was first documented in Lake County in August of 2004. Both the duration of activity and prevalence of WNV among avian, equine, sentinel chicken, and mosquito indicators increased during 2005, the second year of its presence in the county. There were no human cases of WNV in 2005, a decrease from one in 2004. Overall, a measurable amplification of WNV in Lake County occurred during the 2005 mosquito season. However, the increased level of activity was lower than that observed in some other regions of California.

INTRODUCTION

Lake County is a mountainous region between the Pacific Coast and the Central Valley of California. Most of the county's 64,000 residents reside in the Clear Lake Basin, around the shoreline of Clear Lake. This polymictic, eutrophic lake occupies more than 43,000 acres and provides habitat for immature mosquitoes in aquatic weeds, and marshes along the shoreline. Agricultural and public lands dominate the landscape with rural residences punctuated by some suburban development. The two most prolific breeding areas for mosquitoes in Lake County are the wild rice fields, near Upper Lake at the north end of the basin, and Anderson Marsh near Lower Lake to the south. West Nile Virus (WNV) was first detected in Lake County in 2004, the same year that most of northern California was invaded. This paper investigates progression of the virus in Lake County between the 2004 and 2005 seasons.

MATERIALS AND METHODS

Mosquitoes

Mosquito collection was performed with CDC suction traps (Sudia and Chamberlain, 1962) modified by removal of the light and by the addition of CO_2 bait and by use of Large Red Boxes (LRB). CO_2 traps were hung 1.5m above ground and baited with 3.2kg of dry ice. The traps were operated from mid afternoon until mid morning of the following day on each sample date. LRBs were 1.8 by 1.2 by 1.2 m red plywood boxes enclosed on four sides (bottom and the front are open). These were positioned to assure morning shade and resting mosquitoes were captured during early morning hours via vacuum. Live females were immobilized on a chill table and sorted to species with a dissecting microscope. Pools of 12 to 50 mosquitoes were shipped to the University of California-Davis Center for Vectorborne Diseases (CVEC) for WNV testing via multiplex RT-PCR (Chiles et al. 2004). Minimum Infection Rate (MIR), the minimum number of infected mosquitoes per one thousand tested, was calculated for each species of mosquito tested. MIR was determined by: [(total # of WNV positive pools) / (total # of mosquitoes tested)] x 1000.

Mosquito surveillance was also conducted at each site where WNV positive birds and horses were located. One CO_2 trap and a Small Red Box (SRB) (0.6m^3 cardboard box) were placed overnight at each location. Adult mosquitoes were collected from SRBs by the same vacuum method used for LRBs.

Birds

Dead birds were tested for WNV as part of the California WNV Dead Bird Surveillance Program sponsored by the California Department of Health Services, Vector-Borne Disease Section (CDHS-VBDS). Birds reported by the public and deemed acceptable for testing were collected by the Lake County Vector Control District (LCVCD). All non-corvids were necropsied at the California Animal Health and Food Safety (CAHFS) laboratory at the University of California, Davis (UCD). Tests for WNV were conducted on extracted tissues via reverse transcriptase-polymerase chain reaction (RT-PCR) at the UCD Center for Vectorborne Diseases laboratory. For American Crows (*Corvus brachyrhynchos*), Western Scrub-Jays (*Aphelocoma coerulescens*) and Steller's Jays (*Cyanocitta stelleri*) oral swab samples were first tested via VecTest at the LCVCD laboratory. Negative and indeterminate buffer samples were forwarded to CVEC for further evaluation via RT-PCR.

The LCVCD accepted birds for WNV testing throughout the entire mosquito season. No areas were excluded so bird data were comparable throughout each year.

Sentinel Chickens

Ten chickens were kept in each of two coops near the wild rice fields in Upper Lake and on a hill above Anderson Marsh, near Lower Lake. Blood samples were collected biweekly from each chicken from April 20th to November 16th of each year. Samples were submitted to the California Department of Health Services (CDHS), Richmond Campus for arbovirus testing. Collection and submission of blood samples was conducted as per CDHS guidelines in "Instructions for Sentinel Blood Samples."

Equines and Humans

Equine WNV cases were preliminarily reported to LCVCD by the case veterinarian and confirmed by CDHS, Veterinary Public Health Section, Division of Communicable Disease Control (DCDC). Human WNV cases were reported by Lake County Public Health Department and the CDHS.

RESULTS

WNV was first detected in Lake County in a Western Scrub-Jay, collected on August 2nd, 2004. The next week a pool of *Culex tarsalis* Coquillett mosquitoes tested positive for the virus. WNV

activity peaked at the end of August, 2004 (Fig. 1). This included positive birds, mosquito pools, the first equine case for the season and, to date, Lake County's sole human case of WNV. Sentinel chicken seroconversion lagged behind other 2004 indicators for the presence of WNV. A single chicken tested positive on September 29th. There were no positive indicators for WNV after October 8th during the 2004 season. Positive dead birds were located throughout populated regions of the county.

In 2005 WNV was detected in dead birds, mosquito pools, horses, and sentinel chickens throughout the Clear Lake basin (Fig. 2). The initial detection of WNV occurred two weeks earlier than in 2004 in a crow found in Lucerne on July 20th. Peak WNV activity occurred in mid August, approximately one week earlier than during the 2004 season. A positive mosquito pool marked the end of the season in October 13, 2005, a week later than the last indicator in 2004 (Fig.1). The seasonal peak in weekly mean maximum air

Figure 1

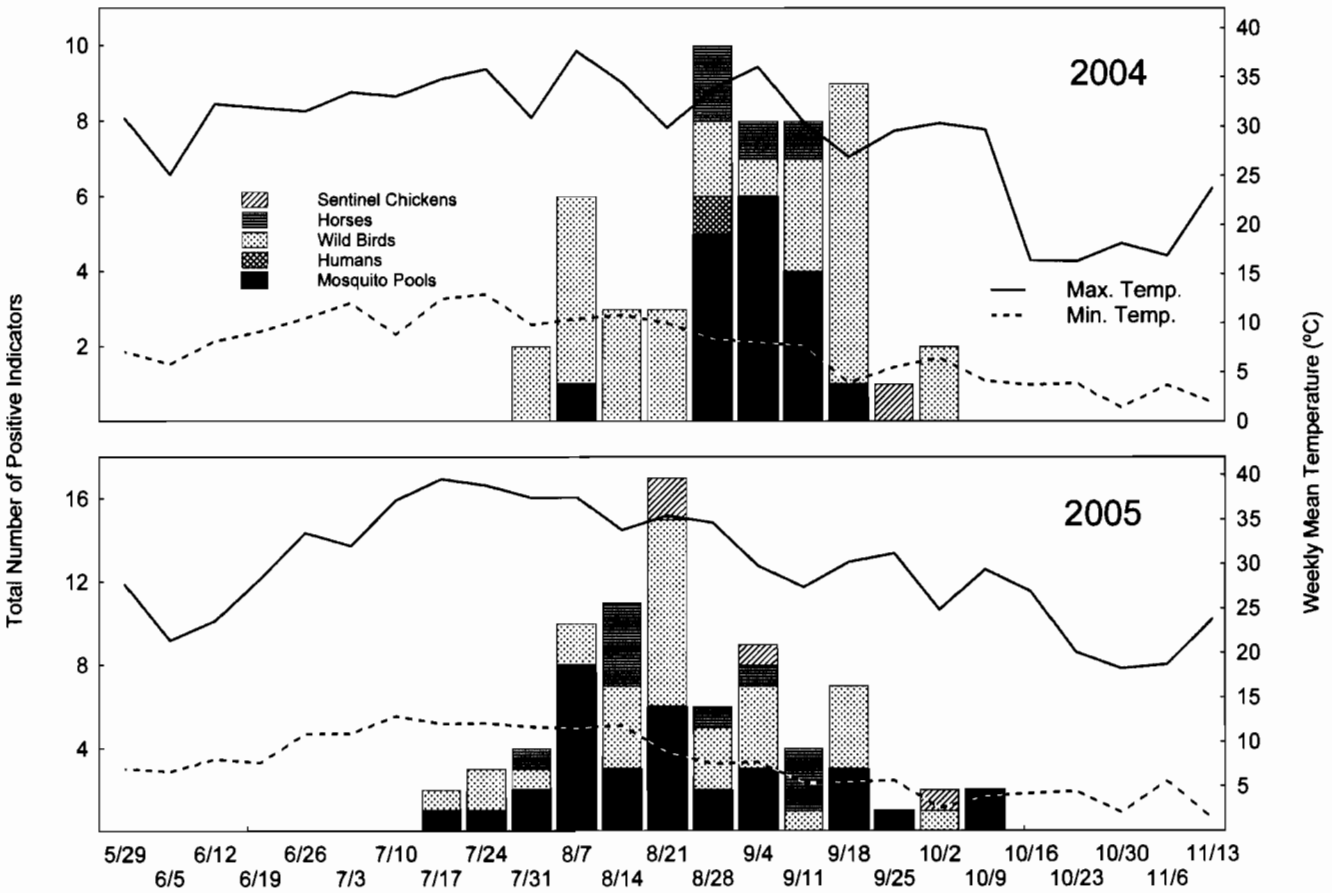
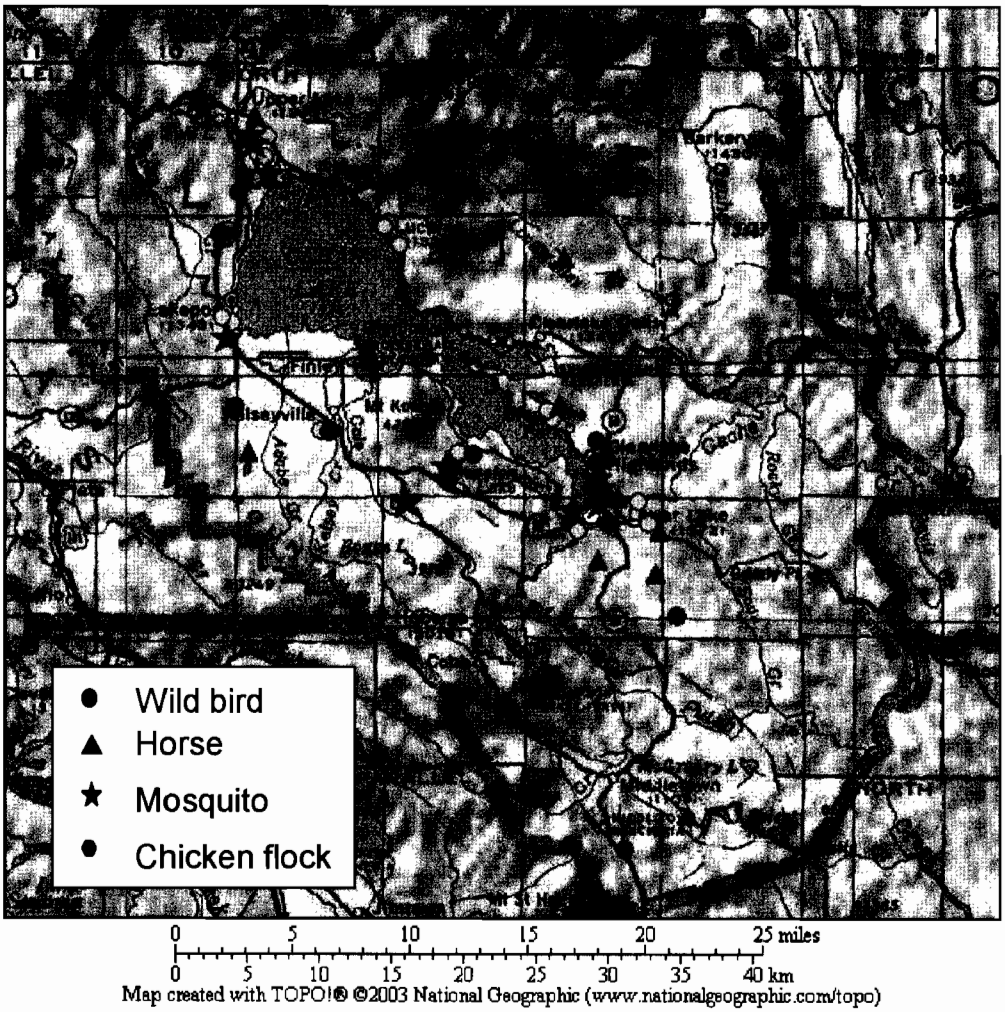


Figure 1. Weekly comparison of cumulative WNV positive indicators in Lake County, California during the 2004 and 2005 mosquito seasons. Weekly mean maximum and minimum air temperatures are shown for each year. Temperature data were recorded in Kelseyville.



temperatures also occurred earlier in 2005 (Fig.1). Both seasons experienced mean temperatures during the months of June through October that were lower than the fifty year average. In addition to a longer season, there was greater amplitude of positive indicators for WNV in 2005. Positive mosquito pools increased from 17 in 2004 to 32 in 2005. The number of horse cases increased from four to ten and WNV positive sentinel chickens increased from one to four in the Upper Lake flock. Dead bird positives remained similar in both species composition and magnitude (29 positive in 2004 and 32 in 2005). Crows, scrub jays, and California Quail (*Callipepla californica*) tested positive each season. Additionally, in 2005 one Steller's Jay was positive.

The total number of crows tested and the percentage that were WNV positive were similar for each season; 13 of 16 crows were positive in 2004 and 15 of 19 were positive in 2005. All 15 scrub jays tested between July and September of each year were positive for WNV. Although high percentages of crovids tested were positive for WNV, mosquito abundance at the sites where these birds were collected was often low. At positive scrub jay sites an average of 22.5 *Culex tarsalis* mosquitoes per CO_2 trap night were collected. *Culex tarsalis* mosquitoes were collected at 13 of the 15 trap sites.

Table 1. Minimum Infection Rates of the mosquito species tested for WNV from Lake County, California in 2005

Mosquito species	Number Tested	Positive Pools	MIR*
Anopheles franciscanus	23	0	0.00
Anopheles freeborni	692	1	1.45
Anopheles punctipennis	50	0	0.00
Coquillettidia perturbans	29	0	0.00
Culex erythrothorax	3009	1	0.33
Culex stigmatosoma	537	7	13.04
Culex tarsalis	9131	23	2.52
Culiseta incidens	16	0	0.00
Culiseta inornata	428	0	0.00
Ochlerotatus melanimon	46	0	0.00
Ochlerotatus sierrensis	374	0	0.00

*MIR = minimum number of infected mosquitoes per 1000 tested

Positive crow sites had a higher mean *Culex tarsalis* count (44.4) than scrub jay sites, but *Culex tarsalis* mosquitoes were found at only 7 of 14 sites. Less than one adult female *Cx. stigmatosoma* Dyar mosquito was retrieved from SRBs at positive scrub jay sites and zero were collected from positive crow sites. A total of 24 bird species were tested in 2005, 96% of positive birds were corvids. Only a few crow roosting sites of forty or fewer birds were found in Lake County, but searches of these areas did not locate sick or dead birds.

Eleven mosquito species were submitted from Lake County for WNV testing in 2005. Consistent with past mosquito seasons, no *Culex pipiens* L. mosquitoes were identified in Lake County in 2005. *Culex stigmatosoma,* collected strictly from LRBs, exhibited the highest seasonal MIR of 13.04 (Table 1). In greatest abundance were *Culex tarsalis* mosquitoes collected from both CO_2 traps and LRBs. Twenty three positive pools from 9131 tested individuals of *Culex tarsalis* yielded a seasonal MIR of 2.52. One pool of *Culex erythrothorax* Dyar (MIR 0.33) and one of *Anopheles freeborni* (MIR 1.45) mosquitoes were also positive. After the first positive mosquito pool in mid July of 2005, an upward trend in the number of positive mosquito pools occurred through the week of August 7th (Fig. 1). The seasonal weekly high MIR was 6.29 for *Culex tarsalis* and 90.91 for *Culex stigmatosoma.* These weekly high MIRs occurred during the last week of September and the second week of October, respectively.

In 2005, five equine cases of WNV were concentrated within a 7 km radius of Anderson Marsh. The remaining five equine cases occured throughout the county. Four horses contracted WNV the week of August 14th and three others, the week of September 11th (Fig. 1). Horses with similar dates of onset of symptoms were dispersed throughout the county rather than grouped geographically. Although *Culex sp.* abundance ranged from 0-25 females per CO_2 trap night, at positive equine sites, *Culex* mosquitoes were captured at only six of the ten sites. Female *Culicoides sonorensis* ($\bar{x}$ = 23.4 per CO_2 trap) were collected from eight horse sites and *Culicoides occidentalis* (1159 female gnats) were collected at one other site.

The first seroconversion among sentinel chickens postdated every other WNV indicator in 2005 by three weeks. A total of four chickens seroconverted between August 24th and October 5th, from the Upper Lake flock (Fig. 1).

Lake County had no human cases of WNV in 2005, despite positive birds and mosquito pools in and around the more densely populated regions of the county. Four residential areas of concern were Lakeport, Clearlake, Upper Lake and Clearlake Rivera. The western rim of Clear Lake, near Lakeport, typically provides abundant *Culex sp.* larval habitat in Creeping Water Primrose (*Ludwigia peploides*). Even so, in 2005 mosquito abundance near Lakeport was low in comparison to other sampling locations (Fig. 3). The communities of Upper Lake and Nice, on the northern margin of the lake, border 800 acres of wild rice. The Clearlake Rivera is located on a ridge above irrigated pasture land and was the site of the 2004 human case. The city of Clearlake borders the Anderson Marsh wetlands. Each mosquito larval habitat is within 5 km of the corresponding residential area. Mosquito abundance varied by location, but all had *Culex tarsalis* MIRs of 4.05 or higher during the peak WNV activity period of July 17 through October 12 in 2005 (Table 1). Mosquitoes trapped in the pasture below the Clearlake Riviera had the highest MIR (9.90) of any site tested.

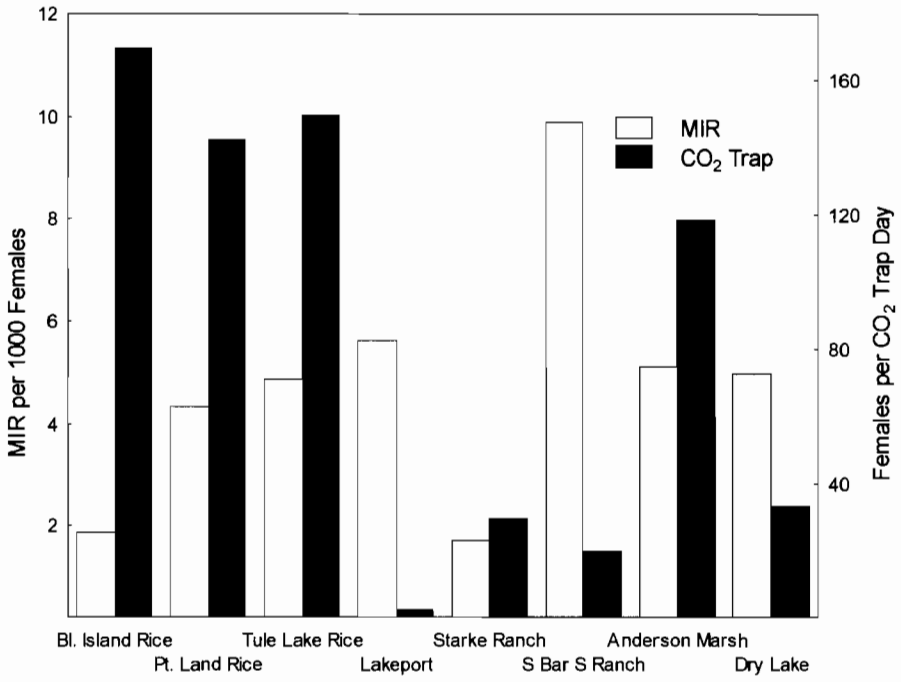


Figure 3. Abundance of *Culex tarsalis* mosquitoes collected in CO_2 traps and the associated MIRs for six locations in Lake County during 2005.

DISCUSSION

The number of positive indicators for WNV increased both in duration and amplitude from the 2004 to the 2005 season. Among dead bird surveillance, mosquito pools, equine cases and sentinel chickens, the chickens were the least useful tool for detecting the presence of WNV in human occupied areas of Lake County. Seroconversions in the Upper Lake flock lagged behind other indicators by four weeks in 2004 and three weeks in 2005. No seoconversions occurred in the Lower Lake flock in either year despite WNV positive mosquitoes and dead birds in the immediate vicinity of the flock.

Mosquitoes were most abundant in Lake County in proximity to wild rice fields and marshlands. However, monitoring near residential areas and positive wild bird and equine sites showed there was not a strong relationship between mosquito abundance and WNV prevalence.

Culex sp. mosquitoes are the most competent vectors of WNV in California (Reisen et al. 2005). In Lake County, *Culex tarsalis* was the most abundantly collected WNV positive species in CO_2 traps. However, *Culex stigmatosoma* females, collected only from LRBs, had the highest MIR in Lake County. An increased LRB surveillance system might further elucidate the importance of this mosquito relative to WNV dissemination and maintenance in Lake County. A single WNV positive *Anopheles freeborni* pool was collected in Lake County. This was the only positive *Anopheles sp.* pool collected in California in 2005. In 2004, a WNV positive *Anopheles hermsi* pool was collected from Los Angeles, California (O'Connor, 2005). Transmission rates for these mosquitoes are undocumented.

The biting midge, *Culicoides sonorensis,* has been implicated in the transmission cycle of WNV in Sage Grouse (*Centrocercus urophasianus*) (Naugle, 2004). In Lake County, equine cases of WNV were more often associated with the presence of *Culicoides* than any *Culex sp.* However, none of the *Culicoides* populations in Lake County have been tested for competence as WNV vectors. Further research into the possible involvement of these flies in WNV transmission is needed.

West Nile Virus is particularly destructive to crow populations; possibly incurring 65% mortality in a wild population (Caffrey et al. 2005) and nearly 100 % mortality in a laboratory study (McLean et al. 2001). Crows are widespread and commonly live amongst people. As a result, they have become sentinels for the presence and abundance of WNV (Eidson et al. 2001) and have been associated with clusters of human cases (Reisen et al., 2006A). The paucity of large roosting sites and moderate crow densities in Lake County (Sauer et al. 2005) may have slowed WNV amplification in 2005. While Lake County did experience an increase in total positive WNV indicators and a longer season in 2005, there was no demonstrable change in crow mortality. The incidence of human cases also showed no measurable increase during the second WNV season.

A recent study found that warmer than average temperatures have been associated with intense WNV activity as the virus traveled across North America (Reisen et al. 2006B). Cooler than average seasonal temperatures in Lake County during the 2005 WNV season combined with the low density of an important avian host, the crow, may help explain the more limited amplification of WNV in Lake County than was observed in some other regions of California.

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West Nile Virus Surveillance and Control in the West Valley Mosquito and Vector Control District in 2005

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ABSTRACT: West Nile virus (WNV) enzootic transmission remained highly active in the jurisdiction of West valley MVCD and its vicinity in 2005. in total, 68 positive mosquito samples were detected among 1759 samples tested. Twenty-five dead birds were positive among 85 tested. Seven out of the eight sentinel chicken coops sero-converted with a total of 19 positive chickens. Two equine cases and 6 human cases were confirmed in 2005. In response to this WNV epidemic, intensive breeding source inspection, reduction and treatment for larval control, and ground ULV for adult control in selected communities were conducted during active transmission season. Media campaign and community fair participation were intensified to maintain effective public relations and public education.

An Overview of Arboviral Activity in Orange County in 2005

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ABSTRACT: The Orange County Vector Control District continued its arbovirus surveillance program throughout 2005 by collecting blood samples from wild birds and sentinel chickens, and submitting mosquito pools and dead birds for WNV testing. Over one hundred wild birds tested positive for WNV antibodies by blocking ELISA, over 80 mosquito pools tested positive, and two sentinel chickens from one flock tested WNV-positive. No chickens, wild birds or mosquito pools were found positive for any other arbovirus in 2005.

Modified Lard-Can Traps and Arbovirus Surveillance: An Alternative to Chicken Flocks

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ABSTRACT: For decades, chicken flocks have been the "gold standard" for sentinel arbovirus surveillance. Use of modified lard-can traps in New York City from 2001-2003, showed how known enzootic foci can be adequately monitored using an alternative method and sentinel species for the presence of West Nile virus. Elevating the traps into tree canopies may improve detection sensitivity.

A Temporal Analysis of Corvids and the Transmission Cycle

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ABSTRACT: Analysis of data collected via the California Dead Bird Surveillance Program (DBSP) and serological data from live capture of wild birds revealed a temporal difference in avian species impacted by West Nile virus (WNV) in 2004 and 2005. In 2004, the dominant species affected by WNV were corvids, representing 84% (2706/3232) of dead birds testing positive for WNV. As evidence of WNV transmission in vectors and humans decreased over the winter months, dead birds that tested positive for WNV were predominantly non-corvid. Between January and April of 2005, non-corvids represented 73% (46/63) of dead birds that tested positive for WNV. Serological tests of wild birds indicated that they survived previous infection with WNV until captured. Interestingly, the balance shifted back towards the corvids in May as other evidence of increased transmission, such as WNV positive mosquito pools and human infections, increased. Throughout the winter months the majority of WNV positive dead birds were non-corvids and it is likely that the DBSP detected chronic infections; mortality may have been due to another primary illness, as other evidence of active WNV transmission was lacking.

Preliminary Findings of a Fort-Morgan-like Alphavirus From Cliff Swallow Bugs [*Oeciacus vicarius* (Hemiptera: Cimididae)] in Sacramento County

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ABSTRACT: During the summer of 2005, arbovirus studies were conducted in proximity to a cliff swallow (*Petrochelidon pyrrhonota*) colony on the Stone Lakes National Wildlife Refuge within Sacramento County. Swallow bugs (*Oeciacus vicarius*) were removed from mist-netted cliff swallows, and cliff swallow nests, and segregated into pools of fifteen to twenty individuals that were ground in tissue culture media and inoculated onto Vero and C6/36 cells. Cytopathic effects (CPE) were evident in three Vero cell cultures. Screening of the culture supernatants by TaqMan RT-PCR for Western Equine encephalitis (WEE) virus, West Nile virus or St. Louis encephalitis viruses yielded negative results. Growth characteristics and plaque sizes were consistent with that of an alphavirus. Previous isolation of Fort Morgan virus (FMV), Buggy Creek virus (BCV) and Bijou Bridge virus (BBV) alphaviruses from cliff swallow bugs further incriminated an alphavirus as the potential unknown agent. Therefore, broadly reactive, alphavirus-specific primers were used for the amplification of a 1.2 kilobase fragment of the E1 through 3'untranslated region (3'UTR) using a degenerate forward primer in the E1 gene region as well as a reverse primer complementary to the 3' poly-A tail present within all alphaviruses. Amplicons were successfully generated from Vero cultures with variable results from C6/36 cultures. Direct sequence analyses of the amplicon products demonstrated a 4.4 % and 5.6% sequence diversity from FMV and BCV, respectively. Phylogenetic analyses clearly demonstrated that these viruses were closely related to the distinct group of viruses within the WEE serocomplex that have all been isolated from cliff swallow bugs. Sequence analyses further demonstrated the virus to be most closely associated with FMV; however, serological analyses are pending that will determine if this is a new subtype virus or an isolate of FMV. This represents the first finding of an alphavirus other than WEE naturally occurring in California and represents the first isolation of a FMV west of the continental divide.

The Center for Medical, Agricultural, and Veterinary Entomology: Developing New Mosquito Surveillance and Control Products

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ABSTRACT: The Center for Medical, Agricultural and Veterinary Entomology (CMAVE), U.S. Department of Agriculture – Agricultural Research Service conducts specific research directed at reducing or eliminating the harm caused by insects to humans, animals, and crops. CMAVE is an internationally renowned research institution that is located adjacent to the campus of the University of Florida in Gainesville, Florida. Research is directed not only at the insect pests such as mosquitoes themselves but also at pathogens they may transmit and at identifying inherent protective mechanisms in nature. CMAVE work emphasizes both control and prevention. The Center has 4 research units, and each unit has specific goals, although there are several commonalities. The Mosquito and Fly Research Unit conducts research on insects of medical and veterinary importance with the goal of achieving control of pest and vector species through the development of environmentally acceptable approaches. New mosquito surveillance and control products are developed and tested.

INTRODUCTION

Mosquitoes are a serious threat to human health and our agricultural economy because they serve as significant vectors of arthropod-borne pathogens. Mosquitoes transmit diseases such as West Nile virus, dengue, malaria, leishmaniasis and filarial worms to humans and animals. There has been a significant increase in arbovirus transmission by mosquitoes in the U.S. and the world exemplified by the introduction and rapid spread of West Nile virus in the U.S (CDC 2004). A constant threat from emerging arthropod-borne disease such as Rift Valley fever, either accidentally or intentionally introduced, cannot be ignored (Anyamba et al. 2005). High mosquito populations also inhibit human activities, diminish the productivity of livestock and reduce the effectiveness of US military personnel (Chretien et al. 2005).

Effective general and specific surveillance traps, and new trap technologies are needed to provide early warning of mosquito-borne diseases that can trigger specific and appropriately timed control responses to protect animals and humans from arthropod attack and the transmission of disease agents (Brownstein et al. 2004). To meet this need, the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) at the Center for Medical Agricultural and Veterinary Entomology (CMAVE) directs scientists in the Mosquito and Fly Research Unit to develop new surveillance and control tools that can fit into an integrated pest abatement system to safely and effectively manage mosquitoes.

Personal protection is the first line of defense against disease transmitting mosquitoes and can be significantly improved by the development of new classes of topical and area repellents to protect the general public and military personnel (Bernier 2006). Vector control can be an effective means of preventing outbreaks of arthropod-borne disease but the availability of public health pesticides has been greatly reduced due to resistance development, and environmental concerns and regulations (Rose 2001). New public health vector control strategies require evaluation of new chemistries, development of innovative control methods, improved application techniques and targeted pesticide use. Advancements in personal protection and vector control research will enhance the effectiveness of Integrated Pest Management (IPM) programs and improve the capacity to prevent disease transmission by mosquitoes.

CENTER FOR MEDICAL, AGRICULTURAL AND VETERINARY ENTOMOLOGY

CMAVE conducts specific research directed at reducing or eliminating the harm caused by insects to humans, animals, and crops. The Center is an internationally renowned research institution that is located adjacent to the campus of the University of Florida in Gainesville, Florida. Within the Center there are more than 200 personnel, including nearly 50 doctoral level scientists working in 150,000 sq ft^2 of state-of-the-art laboratories and field sites throughout the United States and internationally. Research is directed not only at insect pests such as mosquitoes but also at the pathogens that they may transmit and at identifying inherent protective mechanisms in nature. The Center has four research units, and each unit has specific goals; however, there are several commonalities among the units. CMAVE work emphasizes both control and prevention. Effective prevention depends on developing rapid, sensitive methods for detection and surveillance. Second, all our research includes a basic component, because historically the best methods of protection come from understanding how things work at the molecular level. Third, CMAVE work emphasizes biological and IPM techniques that put less pressure on the environment and may be self-sustaining. As the only ARS laboratory that has a research unit dedicated to studying mosquitoes, CMAVE conducts research directed at identification of insect pheromones and attractants that can be used in traps, the production of genetically altered insects for population eradication, the isolation of biological control agents,

the development of innovative methods of detecting mosquitoes in traps, and the discovery and development of new classes of insecticides that are environmentally safe and targeted at mosquito species that are either pests or vectors of disease. All CMAVE scientists strive to develop biologically-based alternative controls, personal protection tools and new materials and methods for surveillance that fit into integrated pest abatement systems to control and prevent mosquito vectors. A summary of CMAVE research activities focused on new mosquito repellent and control products are described below.

MOSQUITO AND FLY RESEARCH UNIT (MFRU)

The MFRU conducts research on insects of medical and veterinary importance with the goal of achieving control of pest and vector species through the development of environmentally acceptable approaches. Protection of humans and animals from diseases caused by arthropods of medical importance is a major priority.

The design of disease intervention strategies by the MFRU involves developing novel arthropod control including the following:

- New Repellent compounds
- Novel Inhibitors/masking agents
- New mosquito larvicides and adulticides
- Enhanced insecticide ULV/barrier treatment strategies
- New highly efficient attractant traps to reduce population
- Novel strategies for sustained control

Sophisticated chemical instruments such as the Gas Chromatograph - Mass Spectrometer shown in Figure 1 are used to identify and quantify volatile organic compounds which are potential kairomonal and allomonal compounds, e.g. attractants in host odors and repellents (Bernier et al. 1999, 2000, 2002). Likely candidate repellent compounds are evaluated in standardized and specialized bioassays, some of which involve the use of a triple-cage dual-port olfactometer (Figure 2) used to assess efficacy of candidate spatial repellent compounds (Bernier et al. 2005).

Figure 1. Gas Chromatograph - Mass Spectrometer used to identify and quantify volatile organic compounds which are potential attractants in host odors and repellents.

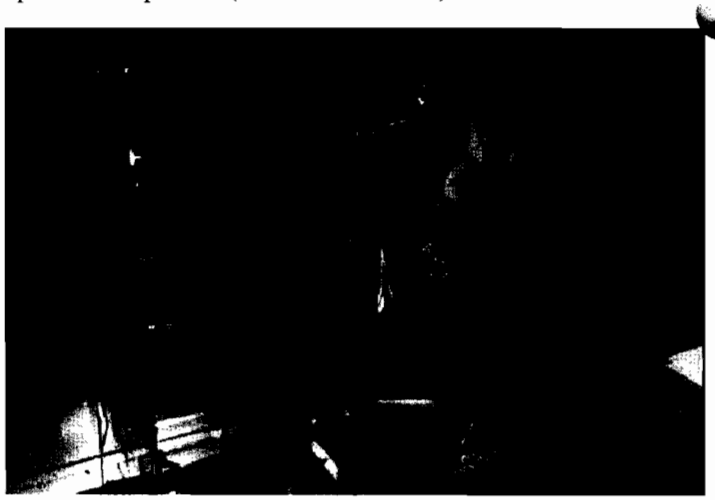

Figure 2. A triple-cage dual-port olfactometer used to assess efficacy of candidate spatial repellent compounds by observing the behavior of host-seeking female mosquitoes.

Effective attractant blends for mosquitoes have been discovered based upon components emanated from human skin (Bernier et al. 2001, 2003). These blends are being developed so that they can be used in commercial traps to improve mosquito surveillance and enhance killing trap collection of a targeted mosquito species. Human-produced attraction-inhibitors were discovered in human skin emanations, and are being further elucidated and characterized. These compounds function by masking the chemical cues (kairomones) used by mosquitoes for host location. The inhibitors can be used in a push-pull type system to enhance trap catches and minimize attraction of mosquitoes to humans and livestock in a local area.

As part of an effort to assess the efficacy of "Attract and Kill" systems to control mosquitoes. Modified Mosquito Magnet - Pro traps (Figure 3) were systematically arrayed on an isolated island

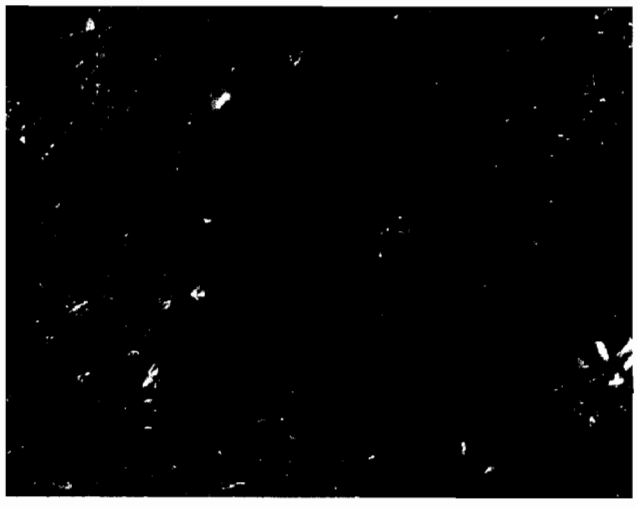

Figure 3. Modified Mosquito Magnet Pro Trap used to conduct research on novel strategies to attract and kill mosquitoes for control purposes on an isolated island along the Gulf Coast of Florida.

along the Gulf Coast of Florida. A preliminary analysis indicates excellent sustained control of *Aedes taeniornhychus*.

Standardized lures for gravid female traps for *Culex* species are being developed to replace highly variable and unstable hay infusion as attractants. Volatile chemicals from hay infusions have been collected and characterized (Allan and Kline 2004). Methods are being designed to release volatile compounds that attract *Culex* mosquitoes to the trap. Mixtures of chemical that mimic the hay infusion can then be used with traps to assure consistent and effective collection of gravid *Culex*. After evaluation in the laboratory preliminary field studies are conducted in large outdoor screened cages that are subject to natural ambient weather conditions and natural vegetation (Figure 4). Five similar outdoor screened cages are located on the grounds of CMAVE.

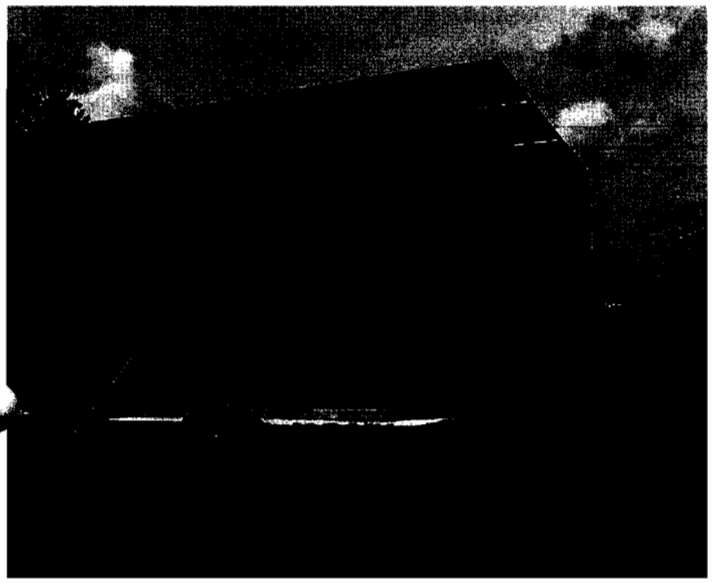

Figure 4. A large screened cage where preliminary field experiments can be conducted under ambient weather conditions and natural vegetation with controlled numbers of mosquitoes.

In collaboration with the U.S. Department of Transportation "Air Curtains" have been designed to prevent mosquitoes and flies from entering aircraft. Air curtain units mounted above and on both sides of simulated (Figure 5) and actual jet ways and doorways of aircraft prevented the passage of essentially 100% of the test insects through the doorway (Carlson et al. 2006). This accomplishment was important because many airline companies are looking for non-pesticidal methods for destroying insects on commercial aircraft without the use of residual or aerosol pesticides. Additionally, since infected and exotic mosquitoes potentially pose a security and health risk to U.S. citizens, this new technology may be of significance to state and local Public Health and Vector Control Officials.

New viruses that are pathogenic to mosquitoes are being evaluated as potential mosquito control agents. Two new cytoplasmic polyhedrosis viruses (CPV), known as cypoviruses, have been isolated. One of the cypoviruses isolated from the mosquito *Uranotaenia sapphirina* has been characterized both morphologically

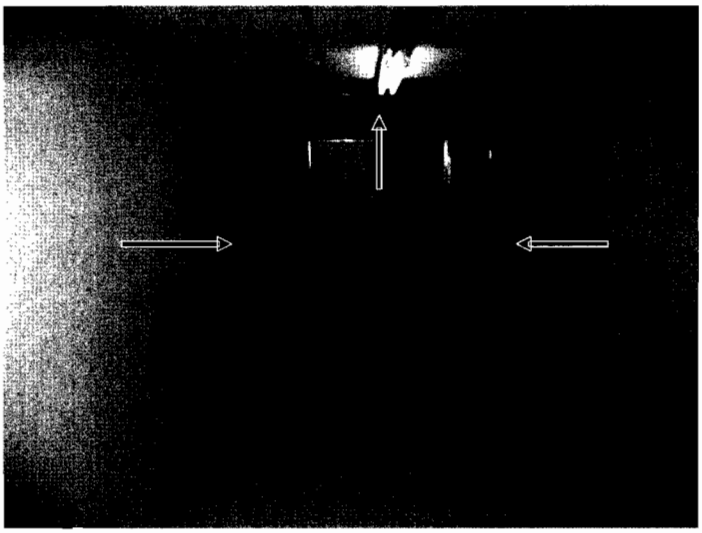

Figure 5. Simulated aircraft jet way where 3 "'Air Curtains" (shown at arrows) positioned near doorway prevent mosquitoes and flies from entering aircraft as passengers enter aircraft.

and molecularly (Shapiro et al. 2005). Another new CPV from the mosquito *Culex restuans* has been isolated and the biological and morphological characteristics determined. This virus infects a number of important mosquitoes such as *Culex quinquefasciatus*, a vector of West Nile virus. The discovery of these and other viral pathogens of mosquitoes expands the genetic diversity base required to investigate and better understand the basic mechanisms involved in infectivity and host range that will enhance capabilities to use these viruses for mosquito control.

As a major component of the Department of Defense Deployed War Fighter Protection Research Program (DWFP) established by the Armed Forces Pest Management Board in 2004 the MFRU continues a tradition started in the 1940's by working with U.S. military to make some of the world's most important advances in controlling disease carrying insects that pose a risk to deployed military troops. The primary components of this research are as follows (AFPMB 2006):

1. Develop new public health pesticides and/or develop new formulations for EPA-registered active ingredients to protect U.S. military deployed abroad form disease-carrying insects.
2. Develop better products for personal protection.
3. Develop new, or improve pesticide application technologies for increasing efficacy of pesticide dispersal and/or reducing the amount of active ingredient needed for effective control.
4. Support the registration/re-registration of military-unique uses of EPA-registered public health pesticides.

In support of the DWFP insecticides are being developed for military use that are fast acting against adult mosquitoes, sand flies and flies, moderately persistent, easy to store and apply, not toxic to vertebrates, and slow to cause resistance. Quantitative structure activity relationships are being modeled to predict chemical structures, from more than 8,000 previously screened compounds, that will provide better repellents, inhibitors, and insecticides. The

longevity of military uniforms and tents treated with insect repellents are being evaluated. New and improved ultra-low-volume and barrier spray pesticide applications are being developed in collaboration with the U.S. Navy, universities, and private contractors.

SUMMARY

The scope of mosquito surveillance and control research at CMAVE is entirely within the scope of USDA-ARS National Program 104, Veterinary, Medical, and Urban Entomology Research, and addresses the goals of program including: efforts to provide new and improved surveillance tools; knowledge of mosquito behavior, host-pathogen interactions, and neural and sensory pathways; repellents; and biological and chemical control strategies. Customers and collaborators in these research efforts include State and local Departments of Health and Agriculture, Mosquito Abatement and Control Districts, the Armed Forces Pest Management Board of the Department of Defense, the U.S. Agency for International Development, the World Health Organization, International Atomic Energy Agency, Livestock Producers, Equine Industry, Pesticide Control Industry, and the General Public.

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Re-emergence of *Ochlerotatus dorsalis* in San Mateo County, California

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ABSTRACT: *Ochlerotatus dorsalis*, the summer salt marsh mosquito, was once abundant in San Mateo County and was a major source of citizen complaints. This mosquito was a focus of the mosquito control operations by the San Mateo County Mosquito Abatement District (SMCMAD) and, as a result of its importance, was featured on the District's seal. During the 1990's this mosquito virtually disappeared from San Mateo County. Light and carbon dioxide-baited traps did not collect a single *Oc. dorsalis* adult and immatures were completely absent from dip samples in larval sources. In 2001, *Oc. dorsalis* adults appeared in carbon dioxide-baited traps at two locations. Its occurrence in traps increased and by 2004, they had colonized a newly-restored tidal marsh in Redwood City. A fly-off of *Oc. dorsalis* adults in 2004 and 2005 spurred emergency applications of adulticides. This paper describes the possible causes of the decline and re-emergence of *Oc. dorsalis* in San Mateo County, and discusses the efficacy of the adulticide applications in 2004 and 2005.

INTRODUCTION

Ochlerotatus dorsalis is one of two species in the genus that commonly develop in salt marshes fringing San Francisco Bay. Larval development occurs from March through October and is often tied to monthly tide cycles. Eggs are laid in moist sediment in depressions in the salt marsh. Larvae hatch when these areas are flooded by monthly high tides or occasional rainfall. This species is multivoltine, producing several generations during warmer months. Development to the adult stage can occur in 7-10 days at 75-80°F (Rees and Nielsen 1947). Females are voracious biters, can survive up to 90 days after emergence and will fly up to 30 miles inland in search of a blood meal (Rees and Nielsen 1947). They can have a significant impact on neighboring communities because female *Oc. dorsalis* feed preferentially on mammals, attacking indoors and outdoors from dawn to dusk, and actively pursue hosts even under moderately windy conditions.

Records from SMCMAD light trap collections from 1955 through 1994 documented the presence of *Oc. dorsalis* in moderate numbers throughout the District (Fig. 1). However, from 1995 through 2003, this species was absent from light trap collections. The District began using carbon dioxide-baited traps for adult

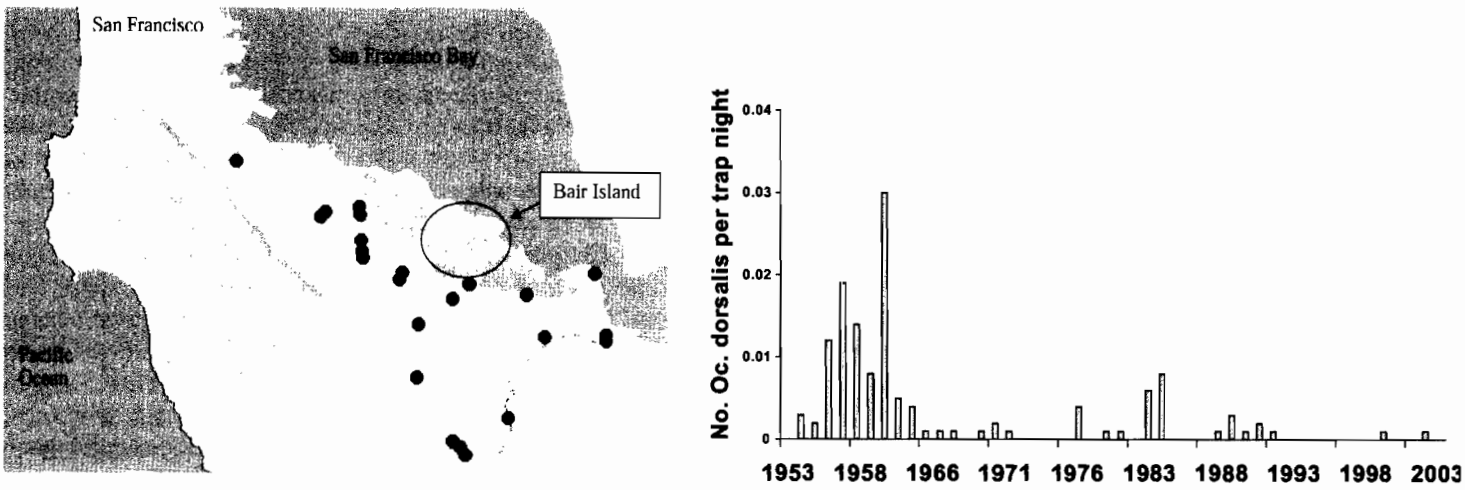

Figure 1. Distribution of *Ochlerotatus dorsalis* in light traps in San Mateo County between 1953 and 1990.

mosquito surveillance in 2000, and by 2003 light traps had been mostly discontinued. *Ochlerotatus dorsalis* adults were detected for the first time in carbon dioxide-baited traps in the cities of East Palo Alto and Redwood Shores in 2001. Over the next 5 years, the number of cities in which this species was detected increased (Fig. 2). In 2004 and 2005, adults were seen in traps throughout the cities of Redwood Shores and Foster City.

Oc. dorsalis, females are voracious biters and feed preferentially on mammals. They will bite from dawn to dusk, both indoors and outdoors. This species develops in rainwater impoundments that form in the autumn and evaporate in late spring. One of the largest sources of *Oc. squamiger* has been Bair Island, a 3,000-acre parcel of diked, reclaimed salt marsh in Redwood City (Fig. 1). This parcel was developed into salt evaporation ponds in the 1940's. In 1965, salt production ceased and the ponds were pumped dry. Large quantities of rainwater now collect in the ponds during winter months and remains until May or June. The interior of the ponds is

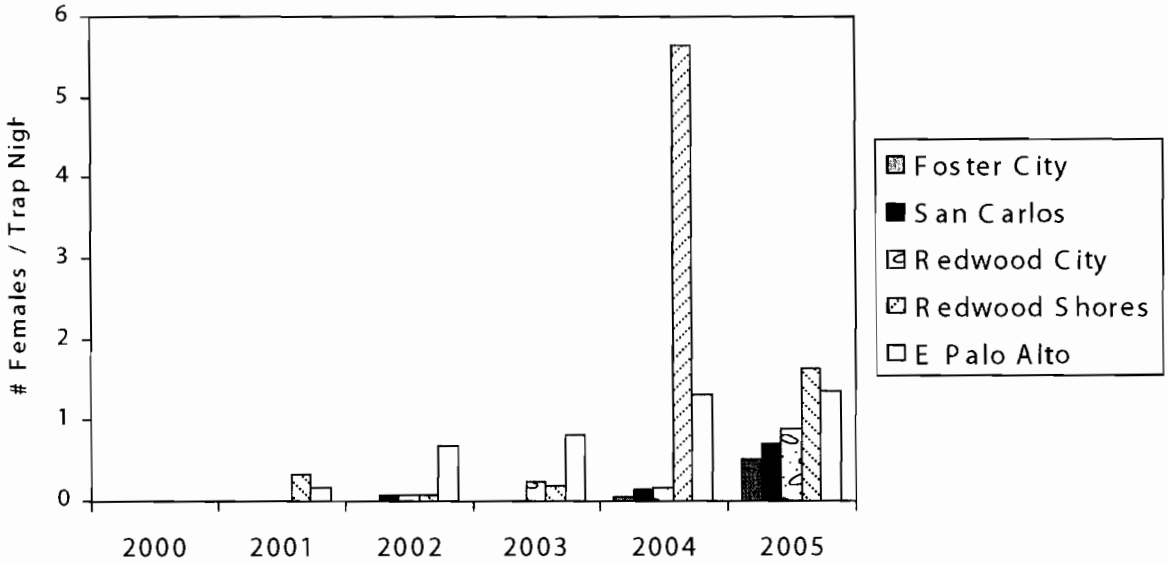

Figure 2 . Collections of *Ochlerotatus dorsalis* in carbon dioxide-baited traps between 2000 and 2005.

The decline of *Oc. dorsalis* in San Mateo County is probably related to changes in larval habitat. During the 1970's and 1980's, *Oc. dorsalis* larvae were frequently collected in tidewater impoundments in East Palo Alto, East Menlo Park, San Carlos, Redwood City, Foster City, and San Mateo. Urban development during the last 20 years drastically changed the developmental sites. Many of the sources in which larvae occurred have been eliminated. Others have either been completely cut off from tidal influence or altered to receive strong tidal flow.

While *Oc. dorsalis* declined in recent years, the winter salt marsh mosquito, *Oc. squamiger*, increased in abundance. From 1970 through 2006, *Oc. squamiger* was the most commonly encountered species in San Francisco Bay marshes in San Mateo County. This species is univoltine, with larvae developing during winter months (Carpenter and LaCasse 1955). Eggs are laid in spring and early summer on moist ground in the salt marsh and hatch when winter rains flood these areas (usually November-December). Larvae develop slowly during winter and may take several months before reaching the adult stage. Adults generally emerge within a 2-week period in late March or early April. Females disperse along creeks to obtain blood meals and may travel up to 30 miles inland. Like isolated from tidal influence by levees. The ponds usually dry out completely by the end of June, and remain dry throughout the summer. Therefore, they do not provide developmental sites for *Oc. dorsalis* larvae.

In 2003, a restoration project was completed in one of the former salt ponds at the eastern end of Bair Island (Fig.1). The project consisted of a single, shallow opening in the dike and construction of a shallow channel extending into the center of the pond. This breach allowed water from San Francisco Bay to enter the pond at high tide, but did not allow water to flow back out readily. For the first time in 30 years, impounded water was present in large shallow depressions in the salt marshes of Bair Island during summer months.

The year following completion of this project, a flood of service requests was received in August from the residential communities of Redwood Shores and Foster City, located on the northeast boundary of Bair Island. A total of 688 calls were received during the month of August that year, primarily from these two cities. This was a marked increase from the typical 200 calls per month received throughout the district during the same period in previous years (Fig. 3). Carbon dioxide-baited traps in the area yielded *Oc. dorsalis* adults in significant numbers and an inspection of the newly-restored

portion of Bair Island revealed *Oc. dorsalis* larvae. Ultra low volume (ULV) application of pyrethrum (Pyrenone 25-5) was conducted on August 17, 20, and 25, 2004. The material was applied at 2.25 ounces per acre using two truck-mounted foggers. Applications were made from 2:00 AM to 6:00 AM.

The effectiveness of the application on mosquito populations in the spray area was reflected in the results of carbon dioxide-baited trapping conducted before and after ULV application. Twenty carbon dioxide-baited traps were set at sites throughout the communities of Redwood Shores and Foster City at approximately weekly intervals, as part of the District's regular surveillance program. Traps were set in the afternoon and picked up the following day. Two species of mosquitoes (*Oc. dorsalis* and *Culex pipiens*) were collected in these traps (Fig. 4). The density of *Oc. dorsalis* decreased significantly after the second spray event on August 20. Trapping on September 7 and 20 collected 0.33 and 1 *Oc. dorsalis* female per trap night, respectively.

In contrast, the density of *Cx. pipiens* in traps decreased very little after the application of adulticides and even increased slightly by September 7 (Fig. 4). There are several possible explanations for this difference. *Culex pipiens* females tend to feed at night, they commonly enter homes and often rest in underground sources such as storm drains. This is the species most frequently submitted by county residents who collect biting mosquitoes from inside their homes. *Ochlerotatus dorsalis* females do not enter homes as readily and are frequently encountered outdoors during daylight hours. This was the primary species collected during informal landing/biting counts conducted by District technicians immediately prior to the spraying. Additionally, *Cx. pipiens* develop in storm drain systems and can emerge as adults in 7 to 10 days in this area during summer months. Adults survive for only 1-2 weeks. The catch basins in Redwood Shores and Foster City have the potential of continuously producing *Cx. pipiens* adults. The SMCMAD has an ongoing

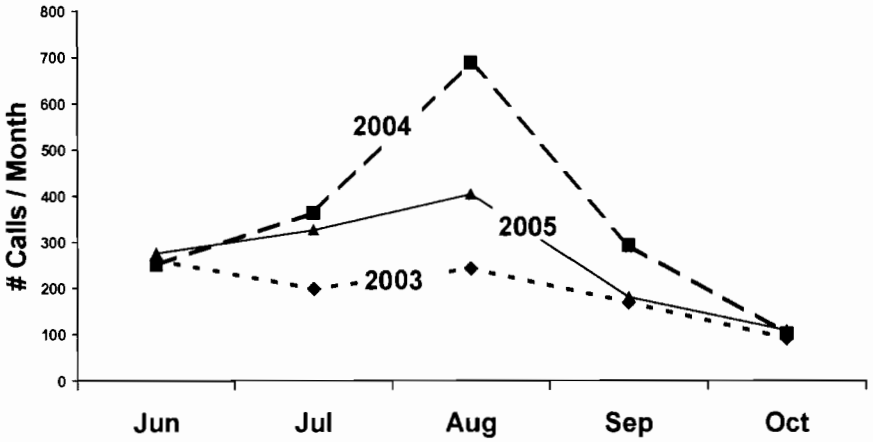

Figure 3. Number of service requests related to mosquitoes during the period of adult activity for *Ochlerotatus dorsalis* in San Mateo County in 2003, 2004 and 2005.

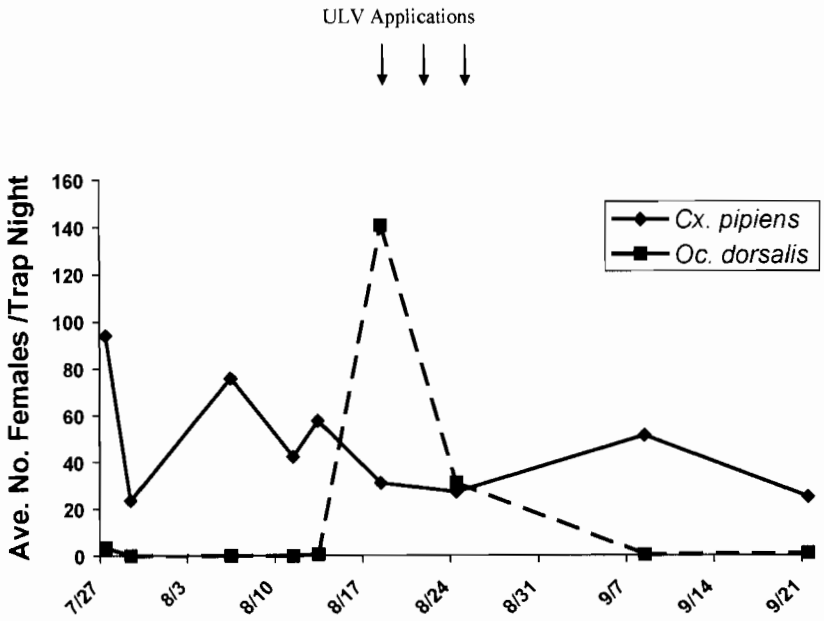

Figure 4. Effects of Ultra Low Volume (ULV) application of Pyrenone 25-5 on collections of *Ochlerotatus dorsalis* and *Culex pipiens* in 2004.

program to apply larvicides to catch basins in these areas. Following observation of the number of *Cx. pipiens* collected in carbon dioxide-baited traps, the catch basin program was reviewed and applications increased in number and frequency.

In 2005, there was another emergence of *Oc. dorsalis* adults from the newly-restored area of Bair Island. Late-stage larvae were discovered on August 1 and ULV applications were conducted on August 5 and 11. The emergence was detected and treated earlier this time and abundance of *Oc. dorsalis* in carbon dioxide-baited traps (7.2 per trap night during the week of August 1) did not reach the level seen in 2004 (140.7 per trap night during the week of August 13). Service requests reached a peak of 404 calls per month in August 2005. This was about 60% of the number of calls received in 2004, but approximately twice the number received during the same time period in previous. Following the ULV application, the density of *Oc. dorsalis* was reduced to less than 1 per trap night. However, *Cx. pipiens* were reduced by only 32 %. A carbon dioxide-baited trap was left in place at one of the regular monitoring sites on the afternoon before spraying commenced. The following morning this trap was examined and found to contain 105 dead and 1 live *Cx. pipiens* females. Based on this limited information, it does not appear that pesticide resistance was the cause for the low rate of reduction in *Cx. pipiens* populations.

DISPERSAL PATTERNS

The dispersal pattern seen for adults of *Oc. dorsalis* in 2004 and 2005 differed from that of *Oc. squamiger* (Fig. 5, 6). Most of the *Oc. dorsalis* females were collected in traps placed in the residential neighborhoods directly adjacent to Bair Island. This was also the location of the majority of service requests during this time period. In contrast, collections of *Oc. squamiger* in these and other years were highest along the course of creeks that empty into San Francisco Bay near the larval source. Most of the service requests in which *Oc. squamiger* adults have been identified have come from homes along the same creeks. Prior to 1990, *Oc. dorsalis* were collected regularly from light traps located at sites throughout the County (Fig. 1). Therefore, *Oc. dorsalis* adults have migrated to the hills west of their larval source in this county in the past.

Figure 5. Occurrence of *Ochlerotatus dorsalis* in carbon dioxide traps in 2004 and 2005.

Figure 6. Distribution of *Ochlerotatus squamiger* in carbon dioxide-baited traps in 2004 and 2005.

CONCLUSIONS

Ochlerotatus dorsalis is now firmly established on Outer Bair Island and the SMCMAD conducts inspections of this site throughout summer. It is hoped that this will allow the area to be treated with larvicides in the future and that further applications of ULV materials to communities bordering Bair Island will not be necessary. In the long term, the SMCMAD is working with USFW and California Department of Fish and Game to increase the tidal flow at this site. The re-emergence of *Oc. dorsalis* at this site serves as a reminder of the enormous impact that can result from small changes in the ecology of a single site. This site demonstrates that tidal restoration projects will only reduce mosquito problems if there is a significant amount of water flow and the site flushes out completely on a daily basis. The number of restoration projects being carried out in the San Francisco Bay is increasing in recent years. The most ambitious of these is the South Bay Salt Pond Project, in which 15,000 acres of restoration are planned. Such a project has the potential of producing vast numbers of salt marsh mosquitoes if the degree of tidal flow is too weak.

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Fairview Project – the Case for Urban Constructed Wetlands

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ABSTRACT: The "Fairview Project" includes the creation of a constructed wetlands within the coastal city of Costa Mesa in Orange County, California. Design and maintenance features of the "wetlands" will include aspects of basin design, vegetation management, and water quality to be presented in a manner than minimizes mosquito production. Management of the "wetlands" will require routine inspection by local vector control to assure quality control of basin, vegetation, and water quality elements. The use of biorational larvicides also are included in the wetlands management guidelines.

Control of Invasive Cordgrass, *Spartina alterniflora*, in Tidal Saltmarsh of San Francisco Bay

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ABSTRACT: Invasive cordgrass, *Spartina alterniflora*, was introduced into California wetlands in the early 1970s. It grows higher and lower in the tidal zone than the native cordgrass *Spartina foliosa*, and has begun colonizing mudflats and pickleweed marshes throughout San Francisco Bay. The potential for spread of this invasive plant has prevented the restoration of tidal flow to diked, reclaimed marshes in some areas and hence impacts mosquito distribution. The San Mateo County Mosquito Abatement District is working with the Coastal Conservancy and U.S. Fish and Wildlife Service to eliminate invasive cordgrass in the south bay. The materials, methods and equipment used will be described. Problems involved in this work include negotiating tidal marshes with all-terrain vehicles, and dealing with water quality issues and endangered species.

INTRODUCTION

Cordgrasses (*Spartina* spp.) are perennial grasses that grow in the lower intertidal zone of saltmarshes worldwide. They have hollow stems and tolerate high levels of salinity (10 to 60 ppt) (Landin 1991). These traits allow the plants to survive when submerged for several hours every day in tidal areas. *Spartina foliosa* is native to the Pacific coast of North America and is an important part of the ecosystem of San Francisco Bay. It occupies a band in the intertidal zone of salt marshes throughout the estuary. Stands of cordgrass are habitat for the endangered California Clapper Rail, *Rallus longirostrus obsoletus*.

In 1976, *Spartina alterniflora* Loisel, a species native to the Atlantic coast of North America was introduced into San Francisco Bay by the Army Corps of Engineers. Plants were imported from Maryland as part of a salt marsh restoration project in the city of Alameda. They were used to stabilize the shoreline (Faber 2000). Atlantic cordgrass grows up to six feet in height, twice the height of native Pacific cordgrass, and has roots that extend much deeper (up to three feet). The additional height and root depth allow *S. alterniflora* to grow much lower in the intertidal zone than the native species. The plant can persist as long as some part of the leaves is exposed during low tide. Invasive cordgrass is able to colonize portions of the Bay's mudflats that would otherwise remain unvegetated. These mudflats are essential foraging grounds for the vast numbers of shorebirds that reside in or migrate through the San Francisco Bay Estuary every year.

The Atlantic species of cordgrass also colonizes higher in the tidal zone than the native, invading areas that are usually occupied primarily by pickleweed, *Salicornia virginica*. This habitat contains another endangered species, the salt marsh harvest mouse, *Rheithrodontomys flaviventris*. Conversion of the pickleweed marsh to cordgrass reduces the amount of habitat available to salt marsh harvest mice and further threatens their existence.

The invasive nature of Atlantic cordgrass is due in part to its ability to spread rapidly. Cordgrasses propagate by both seeds and vegetative growth. Plants are wind pollinated. Seeds drop in autumn and germinate in the spring after soaking for six weeks. Seeds can be widely dispersed by the tides, spreading to distant mudflats where they take root and rapidly colonize. The plants die back in mid-September to early October, but rhizomes continue to grow throughout the winter.

Cordgrass tends to grow as "clones," circular patches of the same plant expanding laterally through rhizomes that grow up to three feet per year. These roots trap sediment, eventually causing gradually sloping mud flats to become elevated marsh. Although small stands may initially provide shelter for birds such as the Clapper Rail, in the long term their growth results in a loss of shallow water habitat.

Whereas native Pacific cordgrass grows in small clumps, smooth cordgrass forms large clumps that merge to form dense uniform meadows, choking out all other plant species including the native cordgrass. Additionally, the Atlantic species hybridizes readily with native Pacific cordgrass. Hybrids vary across a wide spectrum of growth habits and characteristics, but most are more invasive than the native species. As the total acreage occupied by invasive cordgrass increases, it tends to flood neighboring plants with its pollen, increasing the proportion of seed produced carrying non-native characteristics.

A diverse array of techniques has been applied to the control of invasive cordgrasses on the Pacific Coast (Major III et al. 2003). Many of these were developed or tested in Willapa Bay, Washington, which currently has the largest expanse of this plant and the biggest control program on the Pacific coast. Control techniques include physical measures, such as mowing, hand-pulling, covering stands with thick cloth or plastic, or burning and chemical methods. Hand-pulling and covering are labor-intensive procedures and are only applicable to small isolated stands. Mowing requires the use of large mechanized equipment in the intertidal zone and is not feasible under the conditions present in San Francisco Bay. Application of herbicides from ground-based equipment or helicopters is the most practical method for large-scale control work in the San Francisco Bay and this is the method used most commonly on the San Francisco Peninsula.

In 2003, the San Mateo County Mosquito Abatement District became involved in the control of invasive cordgrass, *Spartina alterniflora*, in tidal salt marshes of San Francisco Bay. The presence of invasive cordgrass in these areas is a barrier to the implementation

of restoration projects that would return tidal flow to diked marshes along the Bay. The District offered to assist in the control of invasive cordgrass in order to facilitate the restoration of tidal flow and reduce the area needing to be treated for mosquito larvae. This paper describes the district's involvement in the program, the materials and methods that have been used, and some of the challenges presented by the project.

DISTRICT CONTROL PROGRAM

The San Mateo County Mosquito Abatement District (SMCMAD), with equipment and personnel trained in the application of control materials in salt marshes, as well as a general familiarity with the marshes, began working on cordgrass in 1999. The District offered to assist in the control of invasive cordgrass in order to facilitate the restoration of tidal flow to diked, reclaimed marshes. Diked salt marshes lining the southern portion of San Francisco Bay are major sites for the development of salt marsh mosquitoes. The largest of these sites in San Mateo County is Bair Island, a 3,000 acre parcel of former salt ponds on the western shore of San Francisco Bay between the San Mateo Bridge and the Dumbarton Bridge. It is part of the Don Edwards Wildlife Refuge, managed by the U.S. Fish and Wildlife Service. Bair Island has been a major source of mosquito development since the 1970's. The District spends up to $80,000 annually for aerial application of larvicides to Bair Island. A plan has been developed for restoration of tidal flow to most of Bair Island and this would greatly curtail the area needing treatment. However, the threat of colonization by invasive cordgrass from surrounding marshes is a barrier to implementation of this project. The dikes surrounding Bair Island will not be breached until the threat of invasive cordgrass has been removed. This paper highlights the efforts applied to control *S. alterniflora* in the tidal marshes of the San Francisco Bay.

MATERIALS

Early applications included an aquatic formulation of the herbicide glyphosate (Rodeo™). This was the only product registered for use in aquatic estuaries in the United States in 1999, when the project began. Rodeo was combined with a non-ionic surfactant (R-11®, Wilbur-Ellis Co) to increase the adherence to the plants and facilitate its absorption. A marker dye (Blazon Spray Pattern Indicator "Blue," Milliken Chemical) was also used to better visualize the areas treated.

Glyphosate, applied to the foliage, is absorbed and translocated to the root. In the root, glyphosate inhibits protein synthesis, halting future growth. The material is most effective when applied to the plant at a receding tide so that it remains on the plant long enough to be absorbed before being washed off. The label calls for 6 hours of drying time before tidal waters submerge the plant.

Glyphosate can work quickly at high application rates (5 - 8%). However, in our experience, it has not been very effective at controlling invasive Spartina, with cordgrass resprouting the following year in treated locations. In 2005, a promising new product imazapyr (Habitat®), became available for treatment of cordgrass in California. Imazapyr inhibits an enzyme, acetolactate synthase, found only in plants. This enzyme is used by plants to synthesiz specific amino acids required for protein synthesis and cell growt Because these enzymes occur only in plants, imazapyr is not toxic to birds, mammals, fish or insects. Imazapyr is quickly absorbed, but slow acting. Effects are not apparent until the following spring. Imazapyr was combined with a modified crop oil (Competitor®, Wilbur-Ellis), which increased adherence and absorption. A marker dye was also added to the tank mixture.

APPLICATION VEHICLES

A variety of equipment was used to apply the material, depending on location and accessibility. In areas where the plants were accessible by land, applications were made from Argo all-terrain vehicles. The District owns 4 Argos: two older, smaller Argo Conquests and two newer, larger Avengers. All of the Argos are equipped with 8 wheels with tracks. They apply only one pound pressure per square foot, which minimizes damage to the marsh vegetation.

The Argo Avenger can operate in deeper water and has better traction in soft mud. However, the smaller size of the Conquest makes it more maneuverable in tight spaces.

A 14-foot Klamath boat was used to make applications in sloughs and on parts of the marsh cut off from the mainland by channels. This boat has a modified front end with a railing and hose reel, allowing the applicator to stand on the transom and apply material with a power sprayer over the bow. A second person operates t outboard motor. Due to its small size, this boat can navigate ver narrow channels to reach patches of cordgrass deep in the center of the marsh. The boat is equipped with an adjustable nozzle gun on a 150-foot hose so that the applicator can walk out to patches that would otherwise be inaccessible. Boat applications were made during high tides, when the small sloughs are navigable. Applications from Argos were made at low tide, when more of the low marsh is exposed. Both types of applications are often made on the same day at different times in the tidal cycle.

The District also owns an American RX7 hovercraft, which will be used in 2006 to reach stands of cordgrass that are separated from the shore by mudflats. In these areas the water is too shallow for navigation by boat even at high tide. At low tide the mud is too soft and deep for travel on foot or by Argos.

Large areas where invasive cordgrass has become the dominant plant species are treated by helicopter. The District contracts with an agricultural applicator that uses a Jet Ranger helicopter with a boom sprayer.

The helicopter flies 8-10 feet above ground and applies the material to a 40-50 foot swath. This provides maximum coverage, but at high speeds, some material is lost to drift. Therefore, the helicopter applies at the highest label rate of material per acre (6 pts/A).

The additional material needed, combined with the cost of contracting the equipment ($1,100 per hour in 2005) make thi method more expensive than ground application. However, t helicopter can treat up to 300 acres in a single day.

In comparison, four Argos working for 8 hours can treat only about 40 acres under optimal conditions. Helicopter application is also the least invasive means of treating cordgrass and has minimal impact on wildlife due to the short time the equipment is in the marsh.

In 2004, 150 acres in San Mateo County were treated by helicopter; in 2005 this was increased to 266 acres.

SPRAY EQUIPMENT

The boat and hovercraft use an interchangeable 25-gallon tank with a 70-psi gas powered motor. The Argos are also equipped with 25-gallon tanks and have 60-psi Sureflo pumps.

The material in each 25-gallon tank treats 2 acres. The District uses a nurse trailer equipped with three 50-gallon tanks to carry additional water to the site, mixing material as needed. The truck towing this trailer carries additional water in a 50-gallon tank.

TIMING OF TREATMENT

The window of time during which cordgrass control can be conducted each year is very short, restricted by the growing season and the presence of endangered species. Glyphosate and imazapyr must be applied to actively growing plants to be effective. The growing season for cordgrass generally extends from May through September, depending on weather. The plants flower in late summer, set seed, and then die back until the following spring.

The nesting season for the endangered California Clapper Rail covers most of the plant's growing season, extending from February 1 to August 30. Wherever Clapper rails are present, ground applications for cordgrass control cannot begin until the end of the nesting season. Therefore, ground applications to cordgrass must be conducted between September 1 and the end of the growing season. This gives a window of only a few weeks to a month for control. Control is further hindered by the fact that the plants have often begun to set seed before the control season has begun. Plant dispersal may continue despite the successful applications that kill existing plants.

CONCLUSION

The District has been treating cordgrass for six years with mixed success. Early work (1999-2000) was conducted entirely on the Don Edwards National Wildlife Refuge. At that time, invasive Spartina existed almost entirely in small, restricted clumps, less than 40 ft in diameter. The District was able to make glyphosate applications to all of these patches (100 acres total) within 2 weeks. In some parts of the high marsh, up to 80% control was achieved. Control was much less effective in parts of the low marsh. In some areas only 20% of the plants were removed. Poor control in the low marsh may be due to the short time that the material was on the plants before tidewater returned and washed it off. Also, in areas treated by boat at high tide, only a small portion on the upper portions of the leaves received material because the rest of the plant was underwater at the time. To be effective, glyphosate must be applied to most of the plant's surface. Therefore, the area covered when applications are made at high tide may not be sufficient for glyphosate to kill the plant.

No control work was conducted during 2001 to 2003 due to new requirements for permits for applications of herbicides to water. In 2003, the Coastal Conservancy completed an environmental impact report and obtained a National Pollution Discharge Elimination System (NPDES) permit. Control work began again in 2004. However, during the intervening three years the area covered by invasive cordgrass had grown to over 600 acres. The outlook brightened in 2005, when imazapyr became available. This product shows great promise. Tests in Willapa Bay (Patten 2003) show that it is much more active, giving good control when applied to only the upper 10 inches of foliage. The results of applications of imazapyr in 2005 will not become evident until June of 2006.

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Deposition of Pyrenone® 25-5 During Characterization of Aerial Ultra-Low Volume Tests for Mosquito Control in the Coachella Valley, CA

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Ultra-low volume (ULV) applications of adulticides remain the primary method to interrupt mosquito-borne encephalitis epidemic transmission (Gubler et al. 2000). Because of their short persistence in the environment (Angioni et al. 2005; Antonious et al. 2001), high efficacy against vector *Culex* mosquitoes and lack of residue limits, pyrethrins are frequently the compound of choice for use in agricultural and residential environments. A major concern associated with these applications for epidemic management in and around residences arises from perceived health effects related to aerosols at application and residues following treatments. Because applications for public health utilize extremely low doses, residues typically have been difficult to detect and document in soil and water (Antonious et al. 1997).

Our ongoing research in the Coachella Valley, Riverside County, CA evaluates the efficacy of ULV adulticide applications over desert, citrus and date orchard, vineyard, and managed duck marsh habitats, north east of the Salton Sea. These habitats typically support elevated populations of *Culex* mosquitoes and encephalitis virus transmission that are managed by the Coachella Valley Mosquito and Vector District [CVMVCD]. In the event of high adult mosquito abundance or the detection of West Nile virus, the CVMVCD applies ULV adulticides by air or ground to pre-empt or interrupt virus transmission.

During initial aerial applications in these desert environments, desiccating conditions were found to preclude aqueous droplet descent and reduce sentinel mosquito mortality. To facilitate droplet integrity and improve efficacy, we conducted a series of aerial applications designed to optimize application efficacy. To reduce evaporation during droplet descent, Pyrenone 25-5® was mixed with BVA mineral oil. To estimate deposition following ULV aerial applications of pyrethrins, a new method was developed for separating the six esters from natural pyrethrum and commercial Pyrenone®. The esters of chrysanthemic acid [cinerin and jasmolin I] are known as the pyrethrin I group, whereas the esters of pyrethric acid [cinerin II and jasmolin II] form the pyrethrin II group. Among the 6 esters, pyrethrin I and II are major insecticidal components. Pyrenone® 25-5 is a commercial formulation containing the above-mentioned 6 esters (5%) and the synergist piperonyl butoxide (PBO 25%).

Applications of Pyrenone 25-5® and BVA Spray 13® oil were done with a single-engine, fixed wing aircraft equipped with two Micronair® AU5000 atomizers. Meteorological conditions were recorded by a portable weather station, with temperature probes at 1 and 6 m. Temperature was also recorded from the aircraft at several elevations to characterize the air column at the level of the application. Teflon® coated glass slides (76 x 25 mm) on slide rotators were positioned along transects to collect and measure droplets, and mortality was monitored using Indio colony *Culex quinquefasciatus* mosquitoes exposed in sentinel cages (Townzen and Natvig 1973). Adulticide residue was collected using 24 cm diameter filter papers positioned along transects, with 3 positive controls held outside of the treated zone. The trace amounts of two major insecticidal components (pyrethrin I and II) and the synergist, PBO, were detected from samples near the center of the spray zone by High Performance Liquid Chromatography (HPLC).

Wind conditions for the 3 trials were similar, with wind velocity below 1.6 km/h, but humidity and temperature varied (Table 1). Deposition of Pyrethrins was highest for the 1:3 ratio of insecticide to BVA oil, with a maximum of 156 $\mu g/m^2$ and an average of 45.1 $\mu g/m^2$ and was not detectable beyond 60 m from the center of the

Table 1. Temperature and relative humidity data at 1-, 6-, and 30-m levels during the oil:Pyrenone applications.

Ratio Oil:Pyrenone	Relative Humidity	Temp-1m	Temp-6m	Temp-30m
1:1	30%	32 °C	33.6 °C	38.3 °C
1:2	51%	25 °C	28.3 °C	35 °C
1:3	54%	32 °C	35 °C	N/A

swath (Table 2). PBO deposition for this trial was a maximum of 5,213 µg/m² and an average of 2,530 µg/m², and was not detectable beyond 150 m from swath center. The 1:2 mixture, which was determined to be the best for operations, had a maximum deposition of 30 µg/m² and an average of 21.1 µg/m², and was not detectable beyond 60 m from swath center. PBO deposition for this trial was a maximum of 2,350 µg/m² and an average of 1,875 µg/m², and was not detectable beyond 120 m from swath center. The ratio of 1:1 resulted in sentinel mortality of less than 2% average for the swath. In general, detection of residues agreed with the dispersion pattern of droplets measured by spinning Teflon® slides and mortality among sentinel mosquitoes, indicating HPLC may be useful in detecting post spray residues. The curve of deposition was what would be expected for fallout of larger droplets, whereas mortality in caged sentinels conformed to what would be expected for drift of suspended smaller droplets.

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Table 2. Deposition of spray droplets of oil: Pyrenone mixture (micrograms/ m²) with maximum and average deposition for three different swaths.

	Pyrethrin I		Pyrethrin II		PBO		
Ratio Oil:Pyrenone	Max	Average	Max	Average	Max	Average	Swath
1:1	0.0	0.0	11.4	3.7	795	378	390m
1:2	3.0	1.7	27.0	19.4	2350	1875	210m
1:3	64.8	13.6	91.3	31.5	5213	2530	340m

Assessment of Aerial Ultra-Low Volume Adulticide Efficacy in the Coachella Valley, California

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ABSTRACT: Aerial ultra-low volume (ULV) application of adulticide is widely used to interrupt transmission of mosquito-borne viruses and suppress nuisance mosquito populations. The diversity of weather conditions and landscapes treated with this method affect the ability of pesticide particles to reach the target. In the Coachella Valley, high temperatures and low humidity have required mixing oil with Pyrenone® 25-5 to reduce evaporation and permit ULV particles to reach ground level. Pyrenone® 25-5 was mixed with BVA oil, in a 1 to 2 ratio (Pyrenone to oil), and delivered with 2 Micronair® AU5000 mounted on a Cessna Agtruck. Coverage and drift were measured by bioassay using caged sentinel mosquitoes and rotating 1 inch slide impingers. Suppression of wild populations of vector mosquitoes was measured using CO_2-baited CDC style traps and included mark-release-recapture to indicate dispersal into and out of the core of the treated area. Sentinel flocks of chickens and pools of mosquitoes were used to indicate virus transmission activity. Results were highly variable and, overall, had no significant impact on virus transmission or suppression of vector abundance. Mortality of sentinel caged mosquitoes set in the lee of vegetation indicated a shadow effect and cages set in the open showed variable mortality, indicating inconsistencies in the ULV fog. Droplet densities on slide impingers were low, but generally correlated with mortality in caged sentinel mosquitoes.

INTRODUCTION

In many regions ultra-low volume (ULV) aerial applications are the only practical methods to apply adulticides due to the large size of the treatment area and/or lack of road access. However, few recent studies have assessed the effectiveness of aerial ULV applications in suppressing vector abundance and interrupting virus transmission. Previous evaluations (Mount et al. 1996) focused on use of organophosphate compounds that are no longer used in California due to environmental and health considerations. Documentation of method effectiveness is important so that the expenditure of resources for applications can be justified and that the concerns expressed by the public about pesticide exposure versus the public health benefit, can be addressed. Variations in efficacy related to weather and landscape also require that assessment be done under these differing conditions. The human population in the desert southwest of the United States has grown dramatically and aged within recent years (Rogerson and Kim 2005). Expanded residential irrigation and water management have been accompanied by increased mosquito populations and the arboviruses they transmit, making evaluation of adult control necessary. In the southwest deserts of the United States, atmospheric conditions can be problematic for aerial application and required trials to optimize application methods before beginning our block assessment trials.

MATERIALS AND METHODS

Pyrenone® 25-5 (25:5 mixture of pyrethrins: piperonyl butoxide) mixed with BVA oil (1:2) was applied with a Cessna Agtruck flying at 160 kph and 30 meters. Two Micronair® AU5000 nozzles with standard propeller blades, set to 35° pitch were mounted on a boom located below and aft of the wing. Distribution of the pesticide fog was assessed by bioassay using caged sentinel *Culex* mosquitoes. Sentinel hoop cages were constructed of 16 cm diameter white PVC pipe cut to 4 cm lengths and covered with fiberglass window screen. Hock® slide rotators were set near 5 bioassay cages to measure droplet impingement on rotating Teflon® slides. Abundance of vector mosquitoes was monitored using CO_2-baited CDC style light traps (EVS traps) without lights. EVS traps were placed in a crosshair pattern spanning the treated area, with inner controls set on the ends of the crosshairs, 0.8 km beyond the edge of the treatment zone (Fig. 1). Outer controls were set 2 km outside of the treated area. The effect of adulticide applications on target

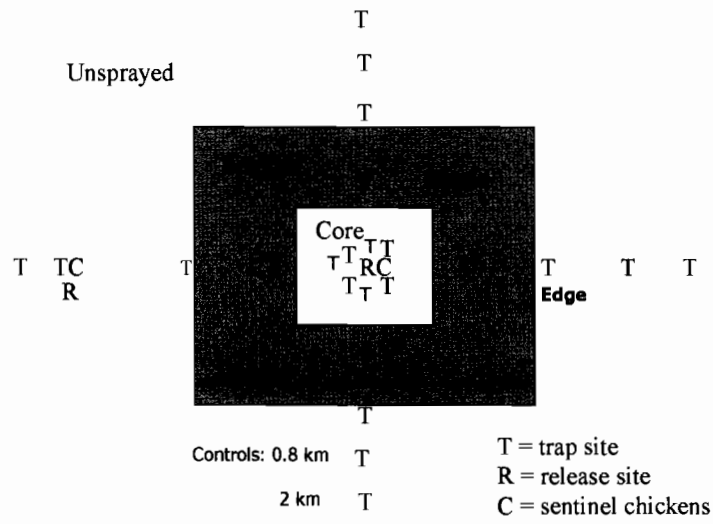

Figure 1. EVS trap placement for block treatment design. Control distance is from the edge of the treatment zone.

population abundance was estimated by calculating percent control comparing the ratio of abundance at treated to untreated negative control traps pre and post application (Mulla et al. 1971), where % reduction= 100-[C_1/T_1 x T_2/C_2]100 and C_1=pretreatment control, C_2= posttreatment control, T_1=pretreatment treated, and T_2=posttreatment treated. Treatments began at sunset on 3 alternate nights and abundance sampling was done before, during, and after the treatment period, on alternate nights to treatments. Marked wild-caught mosquitoes were released on the first or second night of treatment in an attempt to measure interruption of dispersal into and out of the treatment area. Three treatment areas were used. Two were 260 ha sections in agricultural and duck club habitats, and a third was an elongated strip of marsh and associated housing along the margin of the Salton Sea at the community of North Shore. Agricultural habitat was treated in March, duck clubs in June and September, and the shoreline strip in June. Trials at the duck club habitat compared the effects of weather conditions during different seasons. Weather conditions during each trial are presented in Table 1.

Table 1. Weather conditions during aerial adulticiding applications in various habitats.[1]

Habitat	Temp. C°	RH %	Wind (direction/speed km/h)	Inversion
Agriculture	20	50	NNW / < 1.6	+
	21	60	Variable / < 1.6	+
	24	45	NNW / < 1.6	+
Duck club June	33	30	N / < 1.6	+
	38	12	NNW / 9	+/-
	34	26	N W / < 1.6	+/-
Duck club September	26	47	N / < 3	+
	26	47	No detectable wind	-
	25	56	NNW / < 1.6	+/-
Shoreline June	32	25	NNW / 3 to 6	+/-
	32	16	NNW / 5	+/-
	36	15	No detectable wind	+

[1] +/- represents presence/absence of inversion.

RESULTS

Documenting the interruption of enzootic WNV transmission was problematic due to the non-uniform and sporadic pattern of virus activity measured by sentinel chickens and positive mosquito pools. Recapture of marked mosquitoes was 0.36% for the March trial, which limited the value of this tool in estimating the interruption of dispersal. Recapture of 3.5 % for the June duck club trial was closer to previous mark-release-recapture results for untreated dispersal studies, but recapture at the control and core traps ceased after the first trapping. Therefore assessment focused on sentinel mosquitoes to bioassay droplet dispersal and distribution and on changes in abundance to measure the impact of treatment on the target population. Droplet density estimated by rotating slides generally was correlated with sentinel mosquito mortality.

The block treatment in agricultural habitat yielded mixed results in both control and bioassay (Tables 2 & 3) despite suitable atmospheric conditions for all treatments (Table 1). In all 3 treatments abundance at inner control traps fluctuated concurrently with abundance at treatment traps, perhaps indicating that the ULV spray affected abundance beyond the target area. The first treatment resulted in negative control of abundance, meaning abundance in the treated zone increased disproportionately to abundance in the outer control traps. The second treatment resulted in good control of abundance, while the third had mixed results, with higher abundance at the core of the treated area relative to the other zones. Bioassay indicated minimal penetration of the landscape by the ULV droplets because mortality in cages set at 1 m height in vineyard and citrus was much lower than in adjacent unprotected sites.

Table 2. Percent reduction in mosquito abundance collected in CO_2-baited traps in mixed agricultural habitat for three treatments.

Post-treatment	Core	Mid	Edge	Inn-Cont
1	-60%	40%	-5%	-86%
2	71%	87%	72%	42%
3	-80%	85%	35%	55%

Table 3. Mean[1] mortality of sentinel caged mosquitoes in vineyard and citrus habitats for cages set inside vegetation and in adjacent open areas.

Habitat	In	Out
Vineyard	23%	49%
Citrus	13%	40%

[1]mean of three treatments.

The June treatment in duck club habitat also resulted in mixed results, with general reduction of abundance on treatments 1 and 3, but control failure on treatment 2. Although the wind speed for treatment 2 was within label specifications, it may have been sufficiently elevated to suppress mosquito flight (Reisen et al. 2003) and the treatment did not impact the wild population (Table 4).

Table 4. Percent reduction in mosquito abundance collected in CO_2-baited traps in duck club habitat in June and September.

	Post-treatment	Core	Mid	Edge	Inn-Cont
JUNE	1	28%	35%	18%	-10%
	2	-67%	-48%	-28%	15%
	3	-7%	66%	48%	57%
SEPTEMBER	1	2%	28%	5%	41%
	2	-73%	-124%	-140%	-129%
	3	-120%	-151%	-231%	-300%

Mortality of sentinel mosquitoes was highly variable at open sites (Table 5). The September evaluation at the same site resulted in a poorer reduction in abundance and lower bioassay mortality.

Treatment of the margin of the Salton Sea at North Shore required only 2 passes for planned coverage and care had to be taken to offset the flight paths so that the adulticide would drift into the community of Desert Shores without impinging upon the surface of the Salton Sea. Although there was no inversion on the second treatment, sentinel mortality indicated more uniform penetration into the residential landscape than achieved by treatment 1 or 3 (Table 6). Overall, change in abundance did not indicate that control was achieved after 3 consecutive treatments and sentinel mortality was sporadic within and among treatments.

DISCUSSION

Light wind seemed critical for dispersing spray droplets throughout un-vegetated portions of the target area as indicated by sentinel mosquito mortality. Winds also produced a cumulative dosing effect by pushing multiple spray swaths through the same target area. Conversely, wind seemed to compromise our measure of control within the target areas, perhaps by limiting flight activity, enhancing wind shadows and expanding drift into inner control areas.

To interrupt virus transmission, most infected mosquitoes must be eliminated over a 6-day period, the duration of viremia in infected avian hosts. To provide suppression over this period, we treated on three alternate nights. However, our results indicated that nightly treatment would be prudent, because weather conditions would provide better control on some nights than others and more frequent repetition would achieve some cumulative mortality over time. Our study was conducted in a hot arid climate. We strongly recommend that similar assessments of ULV methods be made in other regions under different climatic and landscape conditions.

Acknowledgements

This work was done in collaboration with the Coachella Valley Mosquito and Vector Control District and Bayer Environmental Sciences. Funding came from the National Institutes of Health and Centers for Disease Control. Thanks are due to Mark Palmer, Sarah Wheeler, Marc Kensington (Center for Vectorborne Diseases, UC Davis), and Arturo Gutierrez (Coachella Valley MVCD) for technical assistance.

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Table 5. Variable mortality of caged sentinel mosquitoes set at 1 m in open terrain in duck clubs in June and September.

	Treat #	Open Sites								
JUNE	1	3%	5%	88%	14%	26%	26%	68%	80%	44%
	2	37%	77%	75%	77%	52%	-20%	77%	77%	74%
	3	3%	-1%	61%	27%	1%	-4%	21%	10%	-4%
SEPTEMBER	1	2%	11%	63%	-4%	28%	31%	48%	65%	33%
	2	42%	18%	0%	0%	0%	4%	0%	0%	0%
	3	50%	24%	34%	7%	7%	16%	31%	63%	48%

Table 6. Mortality of cated sentinel mosquitoes set as 1 m within the community of Desert Shores in North Shore.[1]

	Cage site											
Treat #	A	B	C	D	E	F	G	H	I	J	K	L
1	-3%	-3%	-3%	-3%	1%	-3%	21%	-3%	97%	1%	97%	43%
2	34%	18%	41%	14%	36%	26%	15%	-1%	3%	-5%	15%	-5%
3	0%	5%	0%	0%	10%	0%	NP	NP	5%	4%	0%	0%

[1] NP – no cages placed.

Comparison of Dilute and Neat Formulations of ANVIL 10+10®

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[2]*University of Arkansas, Agri 319, University of Arkansas, Fayetteville, 72701*

ABSTRACT: The study compared ANVIL 10+10® applied neat (undiluted) as a ground ULV aerosol to ANVIL 10+10 diluted in mineral oil (1:3.46) against caged *Anopheles quadrimaculatus* in a series of evening outdoor trials. In order to be able to detect differences, the application rate was below the level expected to provide 100% control with this species. Both formulations were applied at the same rate (0.00173 lb actual insecticide per acre).

The undiluted application yielded 86% - 90% mortality, with no recovery after the initial knockdown. The diluted formulation yielded 67% - 77% initial knockdown, but after 24 h the average mortality fell to 62%. Based on these trials, dilution of ANVIL 10+10 can be expected to reduce product effectiveness.

The Impact of ULV Applications of Resmethrin on West Nile Virus Transmission Cycle in the McFarland Oil Field Area of Long Beach, California

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[1] *California Department of Health Services (CDHS/VBDS, CDC West Nile Grant)*
[2] *Center for Vector borne Diseases, University of California, Davis*
[3] *Greater Los Angeles County Vector Control District*

ABSTRACT: The effect of adulticiding applications on mosquito abundance and its impact on the transmission of West Nile virus (WNV) was evaluated at the McFarland Oil Field area of Long Beach, California. Using two ULV ground foggers, resmethrin (18%) diluted 1:3 with mineral oil was applied at a rate of 6.5 oz/acre. Extensive pre- and post-treatment trapping was conducted to monitor and evaluate mosquito abundance and infection rates in the treated zone as well as a neighboring untreated area of Long Beach. Comparisons of the treated and untreated areas showed that infection rates in mosquitoes were significantly higher in the untreated area and WNV activity persisted longer than in the treated area. The first indication of WN V activity at the oil field area were WNV+ *Culex tarsalis* pools, but pre-treatment trapping showed that the *Culex quinquefasciatus* population was already infected. Treatment achieved a significant reduction of the mosquito population in the treated area as well as a decrease in the mosquito infection rate.

INTRODUCTION

During June 2005, five pools of *Culex tarsalis* Coquillett with a Maximum Likelihood Estimation (MLE) value of 4.61 from McFarland Oil Field in the city of Long Beach, California tested positive for WNV, indicating zoonotic transmission. Conditions seemed ideal to determine if an adulticiding application could prevent the crossover of the virus into the *Cx. quinquefasciatus* population to minimize the risk of human infection. This paper presents data on the evaluation of resmethrin applications on adult mosquito populations and its impact of WNV reansmission by mosquitoes in the test area.

MATERIALS AND METHODS

Extensive mosquito surveillance was conducted to determine mosquito-borne virus activity and mosquito abundance in McFarland Oil Field and an untreated neighborhood in Long Beach. At the McFarland Oil Field, a series of pre-treatment and post-treatment trappings were conducted monitoring adult mosquito abundance using EVS/CO2 and Gravid traps (Cummings 1992). The McFarland Oil Field area was divided into 3 zones: core, treated, and untreated (Fig. 1). The overall size of the treatment area was 1 square mile. A total of 16 trap sites were selected, with four in the core area, eight in the treated area, and four sites in the untreated area. Each site contained both, an EVS/CO_2 and a gravid trap. Pooled Mosquitoes were submitted to the Center for Vectorborne Diseases, University of California, Davi (CVEC) for virus testing.

Adult mosquito trapping was conducted on days -4, -2, -1, 0, 1, 2, 3, 7, 9, and 15 (negative numbers representing pre-treatment trapping, 0 being the first day of treatment and days 1 through 15 are post-treatment days). Treatment was conducted on June 24, 26, and 28, 2005

Two different ULV foggers were used for the application of 1:3 resmethrin (Scourge®,18% Resmethrin) and mineral oil at a rate of 6.5 oz per acre: a U.S. Navy multi-headed fogger, Big Bertha and a Pro-Mist. Big Bertha was operating at an output rate of 12 oz/min and a speed of 10 miles/hour, while the output rate of the Pro-Mist was 3 oz/min at a speed of 3 miles/hour. Big bertha was used to treat the majority of the oil field area, and the Pro-Mist was used to treat the neighboring parking lots, housing and the shopping center.

A total of 12 sentinel mosquito cages were placed in the treatment path of Big Bertha and 17 sentinel mosquito cages in the path of the Pro-Mist. Adult *Culex quinquefasciatus* Say were laboratory-raised for use as sentinel mosquitoes. The sentinel cages were made of ~3 in. wide sections of PVC pipe, covered with bridal veil netting secured with rubber bands. Each cage contained ~20 mosquitoes and were mounted at a height of 3 ft with the exception of five cages, which were placed 10 feet off the ground.

Sentinel mosquito cages for Big Bertha were placed in a line perpendicular to the path of the fogging truck. Downwind sentinel cages were set up at 100 ft, 200 ft, 250 ft, 300 ft, 350 ft, and 400 ft from the driveline of the fogger. At the 200 ft mark, a 10 ft tall sentinel cage was set next to the 3 ft cage. Upwind, cages were set at 100 ft, 200 ft, 250 ft, and 300 ft from the driveline of the fogger, with an additional 10 ft tall cage was again placed at the 200 ft mark.

Sentinel mosquito cages for the Pro-Mist were placed in a open parking lot, as well as, in a breezeway, and an adjacent courtyard to determine whether buildings were hampering the movement of the adulticidal material. Upwind cages were set at 50 ft and 100 ft while the cages downwind were located at 50 ft, 100 ft, 150 ft, 200 ft, 250 ft, 300 ft and 350 ft. The cages at the 50 ft and 150 ft mark also had a 10 ft tall cage in addition to the 3 ft tall sentinel mosquito cage. The cages in the breezeway were set at a distance of 50 ft, 100 ft, and 150 ft from the fogger. A 10 ft tall sentinel cage was also placed at the 100 ft mark. Two cages were set behind the building at the 150 ft mark to help understand the effect that structures might have on the flow of the adulticiding material.

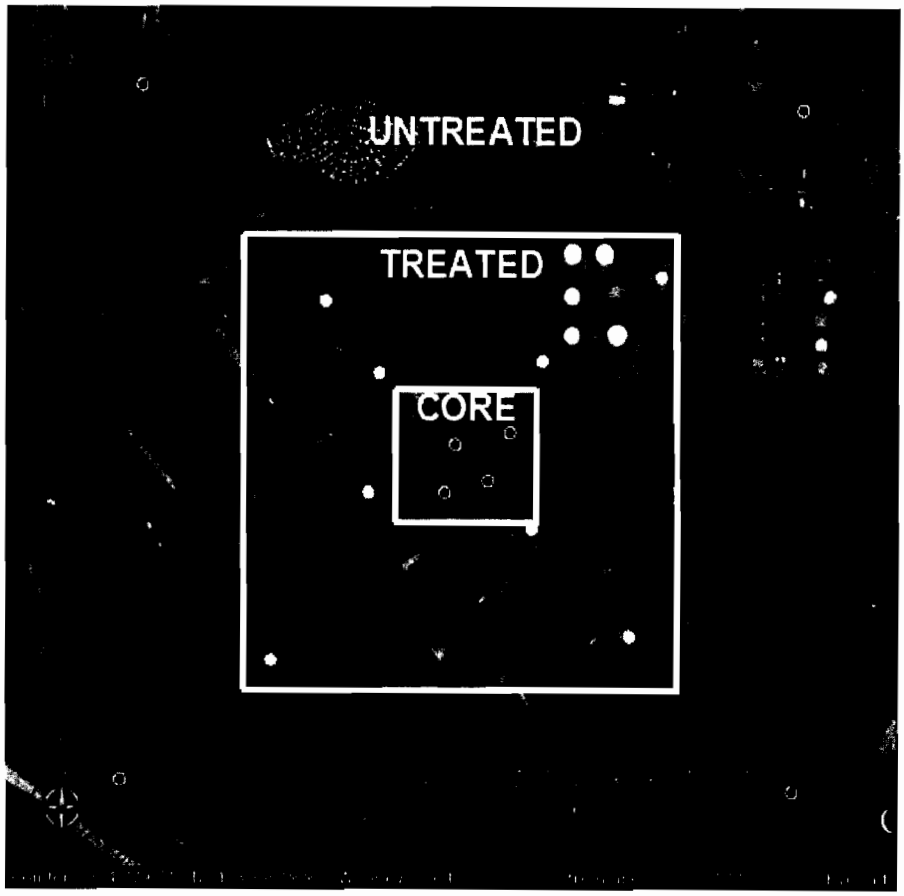

Figure 1: McFarland Oil Field treatment area with various trap sites indicated by small dark circles four each in the core and untreated area, and 8 small white circles in the treated area.

McFarland Oil Field as well as the untreated Long Beach neighborhood were monitored for a period of 5 months (April through September 2005). For both sites, mosquito abundance for gravid and EVS traps were calculated along with MLE of the mosquito infection rate from pooled samples. MLEs were calculated using Biggerstaff (2004) software.

Calculations for McFarland were done for each of the 3 zones: core, treated, and untreated in which females/trap-night (F/TN) were calculated. The data was analyzed by calculating a percent reduction of mosquitoes during pre- and post-treatment.

RESULTS AND DISCUSSION

The evaluation of the sentinel mosquito cages showed that Big Bertha achieved much better adulticide distribution and reached significantly further than the Pro-Mist. Sentinel mosquito cages set in the pathway of Big Bertha resulted in an average mortality of 92%. In the downwind direction, the fog reached as far as 400 ft with a mortality of 88%. Traps facing upwind had a 93% kill at a distance of 300 ft from the driveline (probably due to exposure during additional passes further upwind). Cages set in the Pro-Mist treated shopping center parking lot area achieved an average mortality rate of only 15%. The highest mortality of 48% was obtained at a distance of 50 ft in a cage that was 3 ft off the ground. Traps set at 300 ft and 350 ft had 0% mortality. In the breezeway, the average mortality rate was 27%; this was reduced significantly because the 2 cages were placed directly behind the building and had an average of 11% mortality.

Mosquito abundance data from both the EVS/CO_2 as well as the gravid traps demonstrate that post-treatment population levels were significantly reduced (Fig. 1&2). The dominant species collected in the gravid traps was *Cx. quinquefaciatus*. The treatment achieved a significant reduction of 86% gravid females in the core and a 5% reduction in the treated zone. In both zones, mosquito numbers remained significantly lower through the end of July. The untreated zone, however, showed a 37% increase in gravid mosquito abundance (Fig. 2).

In the EVS/CO_2 traps, *Cx. quinquefaciatus* was the most prevalent species collected, with smaller numbers of *Cx. tarsalis Coquillett* and *Cx. stigmatosoma Dyar*. Mosquito abundance in the core area was reduced by 12% for *Cx. quinquefaciatus* and 84% for *Cx. tarsalis*. In the treated zone, there was a reduction of 83% for *Cx. quinquefaciatus* and 96% for *Cx. tarsalis*. In the EVS/CO_2, traps there was 72% reduction of *Cx. quinquefaciatus* and 50% reduction of *Cx. tarsalis* even in the untreated zone. Immediately following the treatment, mosquito abundance for the entire area monitored remained low, subsequently increasing towards the end of the surveillance. Mosquito numbers first increased in the untreated

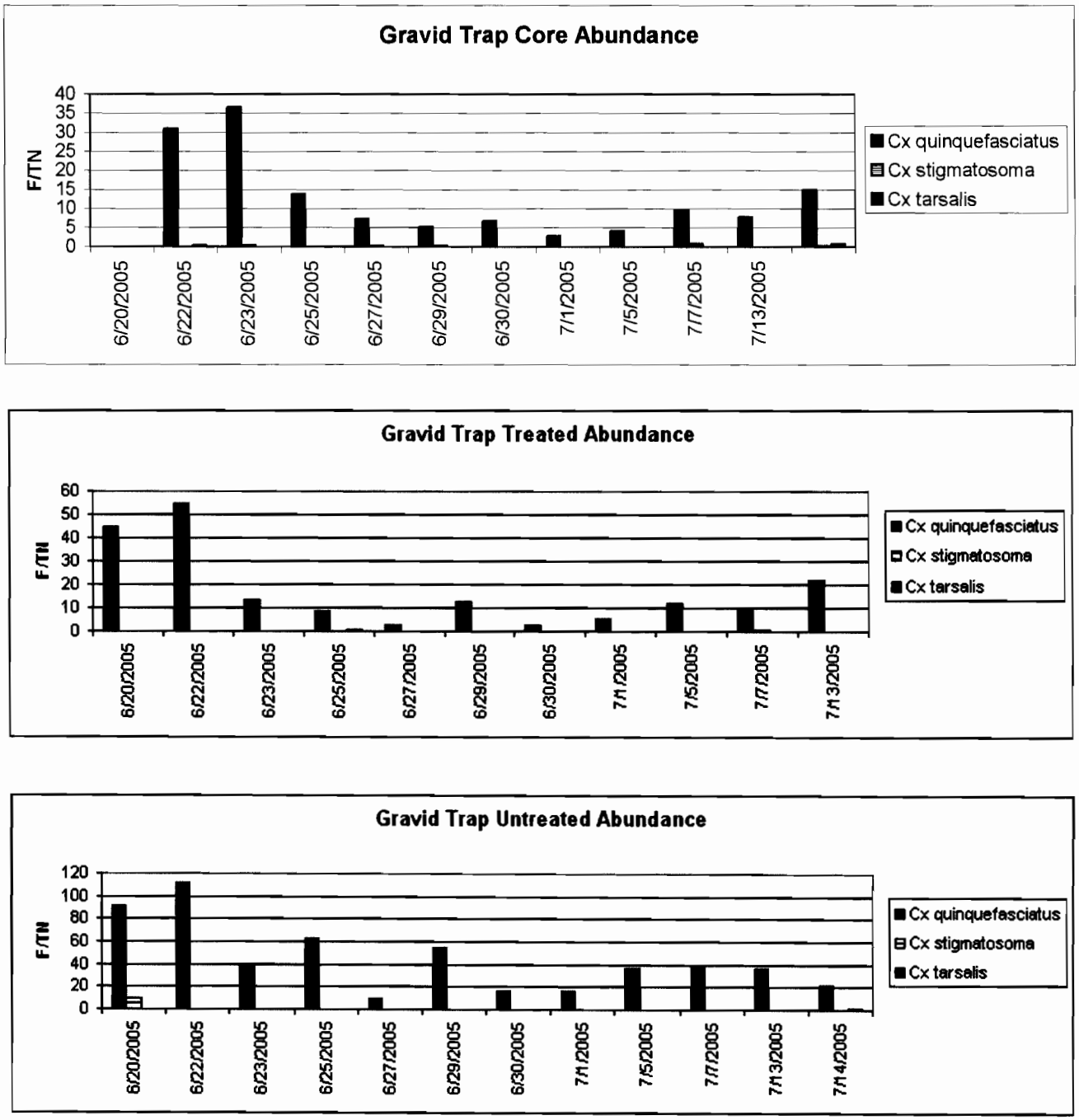

Figure 2: Gravid female mosquitoes trapped at various locations in the test area.

area, where decreases following the treatment remained unexplained; next mosquito populations recovered in the treated area, probably due to new hatches and migration from neighboring areas. Lastly, mosquito numbers remained low for the longest period in the central core area, where there was no migration pressure from the treated area towards the center (Fig. 3).

MLE values for McFarland were calculated separately for the core, treated, and untreated zone for both pre-treatment and post-treatment time periods. The core MLE pre-treatment was 0 and post-treatment was 3.4. The treated zone had a pre-treatment MLE value of 7.9 and a post-treatment of 6.6 representing a 16% reduction in the infection rate. For the untreated zone, there was a

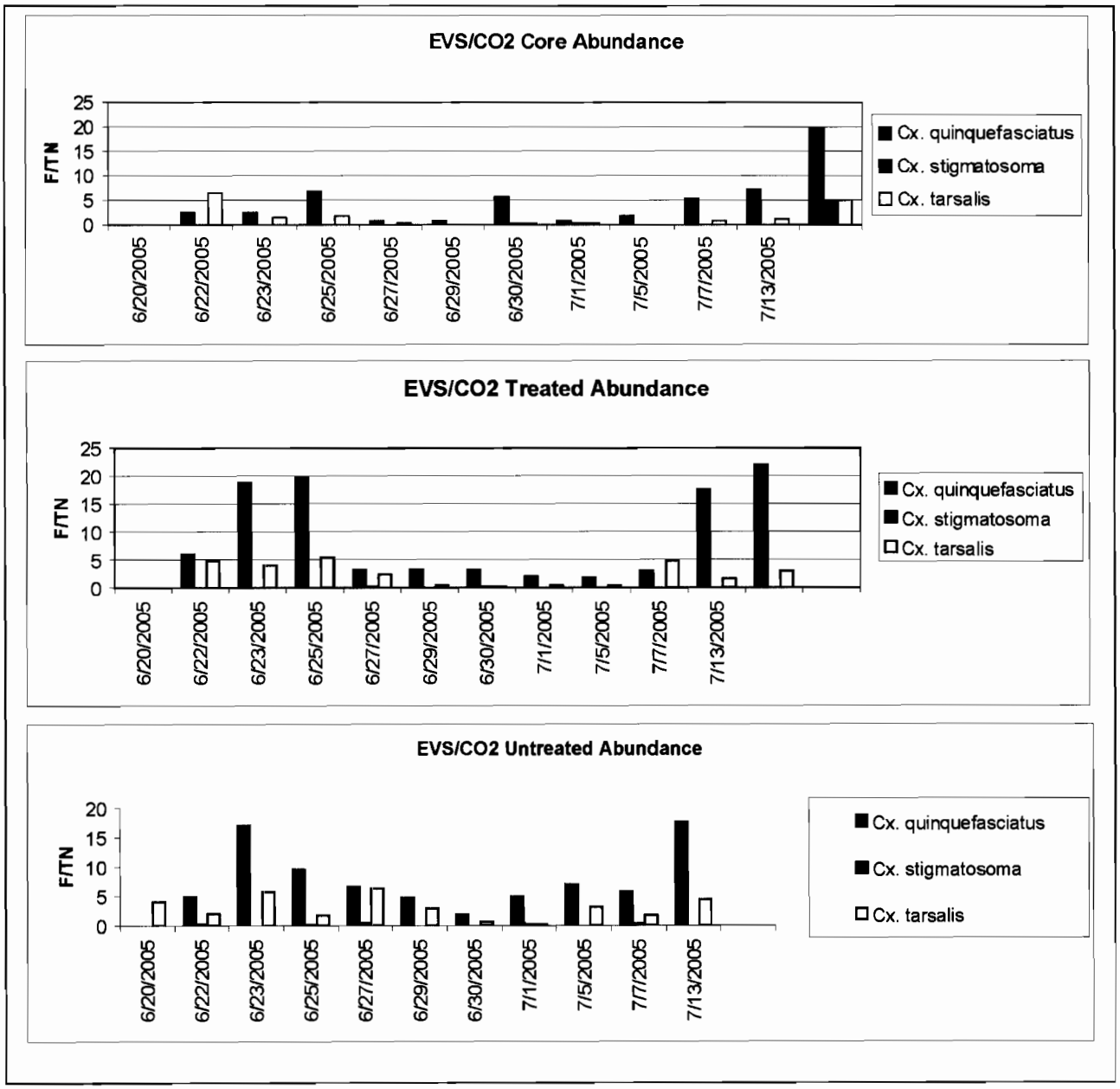

Figure 3. Data on adult mosquitoes collected in EVS/CO2 traps at various sites in the test area.

pre-treatment MLE value of 5.6 and a post-treatment of 14.1, showing an increase in MLE by 151% (Fig. 4). These numbers clearly show that the adulticiding application significantly curbed mosquito infection rates in the treated area compared to those in the untreated trial zone. This result is further strengthened by a comparison of infection rates in the treated oil field area and those of a neighboring untreated Long Beach neighborhood. MLEs for the untreated Long Beach neighborhood were consistently higher with an average MLE of 10.17 and a high of 19.83 (Fig. 5). McFarland Oil Field's MLEs, on the other hand, remained significantly lower with an average MLE of 6.54 and never even reached a MLE of 8.00. In addition transmission persisted for a longer period of time in the Long Beach neighborhood as opposed to McFarland (Fig.4&5)

In conclusion, the application of resmethrin achieved a significant reduction of the mosquito population in the treated area as well as a decrease in the mosquito infection rate. Big Bertha's output of material was more efficient than that of the Pro-Mist. Sentinel mosquito cages behind structures were not affected by adulticiding application. MLE values were significantly lowered in the core and treated areas of McFarland Oil Field while they increased in untreated areas. Overall, MLEs values were significantly lower in the adulticided area of McFarland Oli Field as opposed to the untreated neighborhood of Long Beach.

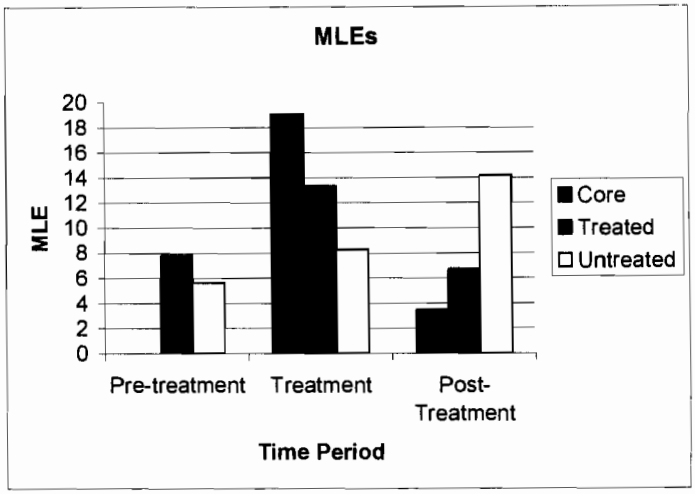

Figure 4. Maximum Likelihood Estimation values at theMcFarland Oil Field

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We thank Roger Nasci, CDC Fort Collins, CO for funding this project, Vicki Kramer and Renjie Hu, California Department of Health Services, Jack Hazelrigg, Greater Los Angels County Vector Control District; and William Reisen,Center for Vectorborne Diseases, UC Davis, for their support and encouragement received.

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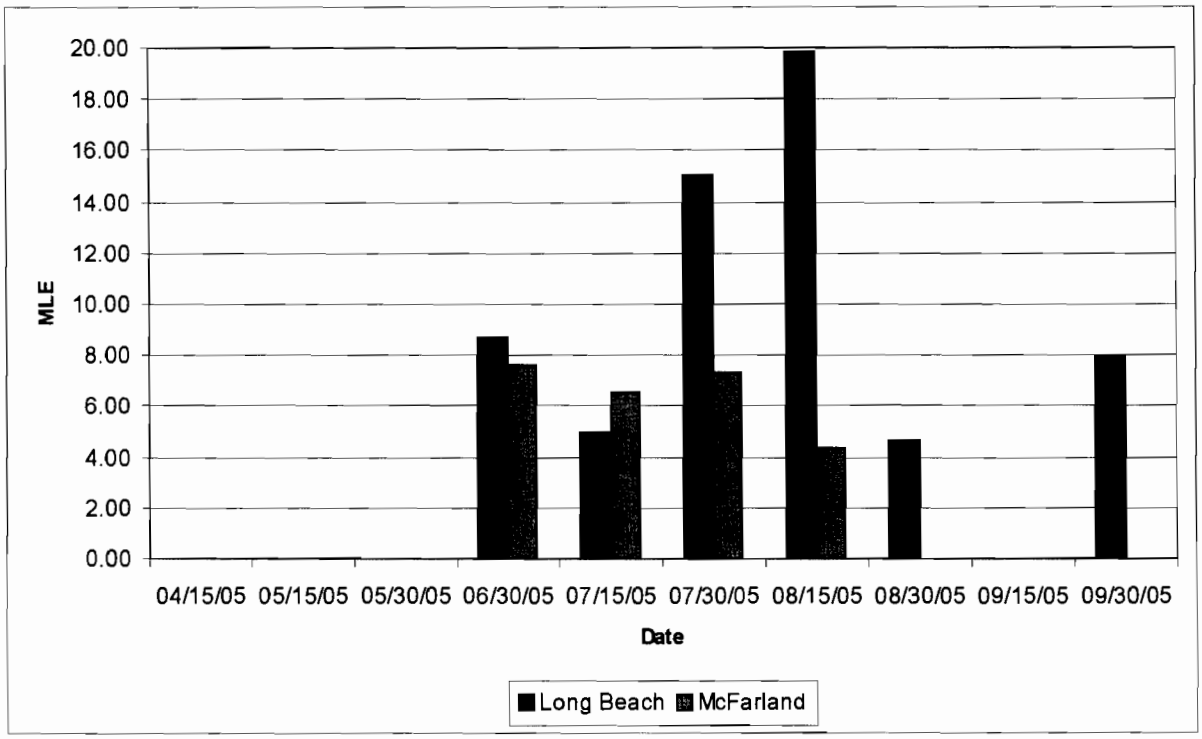

Figure 5. Infection rates of mosquitoes collected in the Long Beach neighborhood and McFarland Oil Field.

Control of *Culex erythrothorax* in Cattail Marshes: an Emerging Problem in Urbanized Areas in San Mateo County, California

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ABSTRACT: *Culex erythrothorax*, once a rare mosquito of little significance, is an increasingly important problem in San Mateo County. The San Mateo County Mosquito Abatement District conducted helicopter applications on 3 marshes located adjacent to heavily populated areas. This marsh habitat presents unique problems in that it is covered by dense emergent vegetation and is often occupied by endangered species. This paper discusses the evolution of the problem and the methods that have been used to combat it.

INTRODUCTION

Culex erythrothorax, the tule mosquito, develops primarily in standing water colonized by tall, emergent vegetation such as cattails (*Typha* spp.) or tules (*Scirpus* spp.) (Chapman, 1962; Bohart and Washino, 1978). Once a rare mosquito of little public health importance, it has become a significant control issue in urbanized areas on the San Francisco Peninsula. This paper reviews the challenges presented by this mosquito in San Mateo County and describes the methods used to control it by the San Mateo County Mosquito Abatement District (SMCMAD).

San Mateo County is located on the San Francisco Peninsula, just south of the city of San Francisco. The county has a population of approximately 700,000. Most of development in the county has occurred east of the Santa Cruz Mountains, along the shore of San Francisco Bay. Like other mosquito control districts in California, the SMCMAD focuses control primarily on the larval stages of mosquitoes. The species with the greatest impact on residents of the county have traditionally included the northern house mosquito (*Culex pipiens*), salt marsh mosquitoes (*Ochlerotatus squamiger* and *Oc. dorsalis*), and *Culiseta incidens* and *Cs. inornata*. Until 2003, *Culex erythrothorax* was extremely rare and not a focus of control operations. Adults of this species were rarely collected in surveillance traps (Figure 1) and larval development was restricted to a few isolated ponds, remote from human activity. Adults of this species emerge from mid-June through October in San Mateo County. They do not generally travel far from the larval source (Walton et al. 1999) and therefore, cattail marshes that are not located near human

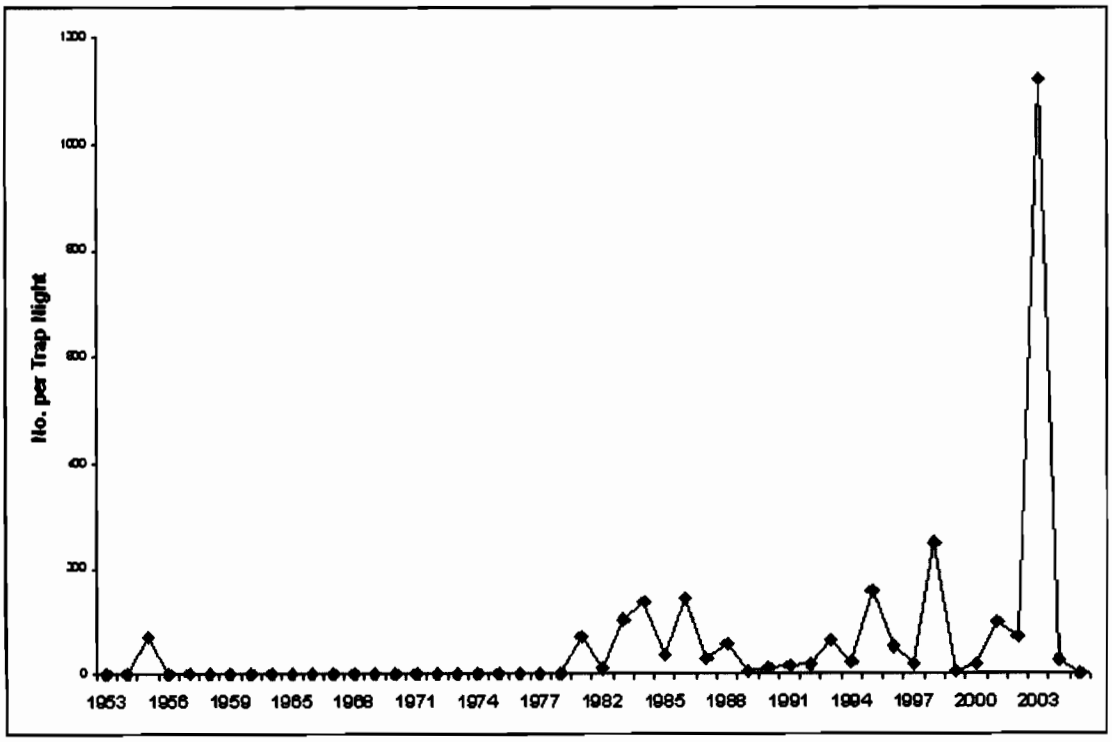

Figure 1. Occurrence of *Culex erythrothorax* adults in New Jersey light traps in San Mateo County from 1953 to 2005.

residences are not considered a significant public health issue.

In 2003, *Cx. erythrothorax* adults began to appear in traps at sites in residential neighborhoods in the county. The problem increased and by 2004 the District was applying larvicides by helicopter for *Cx. erythrothorax* at three sites (Figure 2). This mosquito is also increasingly found in roadside ditches and flood control channels in the urbanized portions of the county. Many public works agencies have stopped clearing vegetation from urban waterways. The dense stands of cattails and tules, which now occupy these sites, are becoming significant sources of *Cx. erythrothorax* development.

PROBLEMS PRESENTED FOR CONTROL BY *CULEX ERYTHROTHORAX*

The biology of *Cx. erythrothorax* is closely tied to the presence of tall, emergent vegetation (Chapman 1962, Bohart and Washino 1978), making surveillance and control challenging. Larvae of *Cx. erythrothorax* are extremely difficult to sample, due to the density of the vegetation in their sites of development. Monitoring for this species is often only possible through the use of traps that capture the adult mosquitoes after they emerge. *Culex erythrothorax* adults do not generally disperse more than a few hundred feet from the larval source. This is particularly true when open fields, devoid of trees, surround larval sources. However, riparian areas adjacent to tule ponds can provide a cool, moist environment that can serve as a corridor, allowing adult mosquitoes to travel greater distances and move into nearby residential neighborhoods. Because they are potential vectors of West Nile virus (Goddard et al. 2002), these adults can present a significant threat to public health. Adults often emerge in very high numbers because larvae are protected from natural predators by the dense vegetation in which they develop. A 2-acre cattail marsh can produce thousands of adult mosquitoes per trap night.

The tall, dense vegetation in *Cx. erythrothorax* sources also hampers the application of larvicides by ground-based equipment. Most of the material remains on the vegetation and therefore never reaches the water. In one case, a 10 foot-wide ditch was sprayed repeatedly with a variety of different materials [Bacillus thuringiensis israelensis (B.t.i.) liquid suspensions and granules, Golden Bear Oil (G.B. 1111)] using truck-mounted power sprayers with very little reduction seen in subsequent collections of carbon dioxide-baited traps. Only after the ditch was cleared of cattails did the application of larvicides have a significant impact. However, in the San Francisco

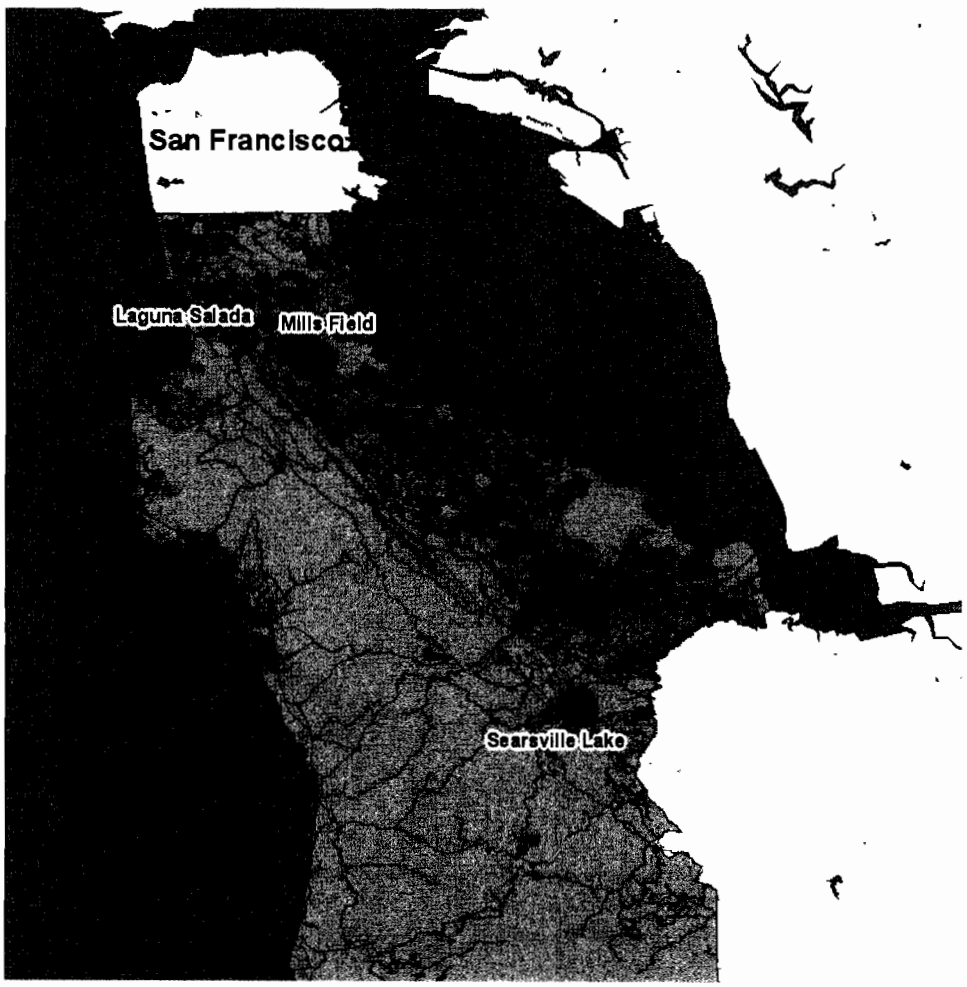

Figure 2. Sites in San Mateo County treated by helicopter for immature stages of *Culex erythrothorax*.

Bay area, most wetlands are occupied by endangered species and physical control of emergent vegetation is not possible. Development on the San Francisco Peninsula has resulted in a drastic loss of wetland habitat. This loss has driven some of the species that rely on wetlands to become threatened or endangered. Most of the remaining wetlands are occupied by at least one endangered species.

EMERGENCE OF PROBLEM SITES

Problems related to tule mosquitoes first emerged in 2003 at Searsville Lake in the southern end of the county. The lake is situated in the Jasper Ridge Biological Preserve, a field research facility owned and operated by Leland Stanford University. The SMCMAD has maintained a light trap at this location since 1977. The lake is fed by 2 creeks, which enter on the western side. It was once a major source of *Anopheles* mosquitoes, due to dense mats of introduced Parrot's Feather (*Myriophyllum aquaticum*) that covered much of the surface of the west side of the lake. During the 1990's a series of heavy storms resulted in the deposition of large quantities of sediment in this area. The parrot's feather that once dominated this portion of the lake has largely been replaced by cattails. The change in vegetation has been followed by a shift in the species of mosquitoes developing there. Between 1977 and 1990, *Anopheles* species, particularly *An. punctipennis*, dominated light trap collections along with *Culiseta particeps*. Collections of *Cx. erythrothorax* averaged from 0-8 per trap night, with a maximum of 20 to 40 per trap night in August of some years (Figure 1). In August of 2003, collections of *Cx. erythrothorax* in the light trap suddenly rose to 273 per trap night. The District conducted follow-up surveillance with carbon dioxide-baited traps. Adults of *Cx. erythrothorax* were collected throughout the woodlands on the western side of the lake, reaching nearby residences surrounding the preserve. The SMCMAD began conducting helicopter applications of larvicides to the lake in September of 2003.

Culex erythrothorax adults also appeared in high numbers in 2003 in a carbon dioxide-baited trap located just across the highway from the San Francisco International Airport. This trap is located on Mills Field, a property owned by the City and County of San Francisco. The south end of the property lies in the city of Millbrae. In the north, it extends into the city of San Bruno. Although residential housing surrounds it, this site cannot be developed, due to its proximity to airport runways and the presence of the largest remaining population of the San Francisco garter snake (*Thamnophis sirtalis tetrataenia*). This snake is endemic to the San Francisco Peninsula (Fox 1951). Loss of wetlands on the San Francisco peninsula has led them to the brink of extinction and they are listed as endangered under both state and federal regulations (US Fish and Wildlife Service 1985, Wharton 1989).

Laguna Salada, in the center of the Sharp Park Municipal Golf Course, is another important habitat for San Francisco garter snakes and another significant source of *Cx. erythrothorax* (McGinnis 1984). Laguna Salada is a freshwater marsh located at the mouth of San Pedro Creek next to the Pacific Ocean. Sharp Park is owned by the City and County of San Francisco. It falls under the jurisdiction of the Department of Parks and Recreation. Situated in the city of Pacifica, the site consists of a lagoon located at the mouth of San Pedro Creek. A sea wall separates the golf course from the beach. The property along the south boundary of the golf course is part of the Golden Gate National Recreation Area and is being actively restored for both San Francisco Garter snake and the equally endangered red-legged frog (*Rana aurora draytonii).*

Laguna Salada empties into to a small reservoir with a pump station that was once used to regulate water levels and prevent flooding. However, because of the presence of both San Francisco garter snakes and red-legged frogs, the City has stopped doing any maintenance on the lagoon or reservoir. Both sites are rapidly becoming inundated by dense stands of cattails and tules. The property is bordered on the east by high-density residential housing. Downtown Pacifica lies on its northern boundary. In July of 2004, eleven carbon dioxide-baited traps were set around the perimeter of the lagoon and reservoir. Several of the traps collected high numbers of *Cx. erythrothorax* adults (up to 583 individuals per trap night, average 150 per trap night). Helicopter treatment at this site commenced in August of 2004.

TREATMENT OF CATTAIL MARSHES

The three sites described here are currently treated by helicopter from mid-June through October every year. Applications are made every 3 weeks using Vectolex CG (20 lbs per acre) or Altosid XRG (20 lbs per acre). Altosid XRG is applied at every third application, in order to prevent the development of resistance. The materials are applied from a hopper suspended under the helicopter. After the material has been applied, the hopper is disconnected and the helicopter flies over the treated area at very low elevation (approximately 20 feet) so that the downdraft from the propellers will blow the granular material off the vegetation and into the water column. Helicopter treatment of these sites has been very effective at reducing adult mosquito density (Figure 3 and 4). In particular, the additional step of using the propeller to move material off the cattails, reduced trap counts to less than 10 mosquitoes per trap night in 2004 and 2005 at all three sites. However, helicopter treatment is very expensive and should not be a long-term solution to the problem. The SMCMAD does not own a helicopter, but rather contracts with an agricultural applicator from the Central Valley. Helicopter time currently costs $1,100 per hour, with a 2-hour minimum charge. In addition, there is a charge for travel to the district (approximately $400 for each application). Larvicides represent another expense, currently approximately $100-150 per acre, depending on the material applied. Helicopter applications are conducted at each site every three weeks for 5 months. The property owners at each site currently pay for the material and helicopter service.

NECESSITY OF PHYSICAL CONTROL

Ultimately, physical alteration of these sites, with removal of some vegetation, is the only measure that will reliably solve the mosquito problems. Unfortunately, the presence of endangered species at two of the sites makes such alteration of the property extremely complicated. The third site, Searsville Lake, also supports endangered red-legged frogs and is part of a biological research station on a university campus. Physical changes to that site are almost out of the question.

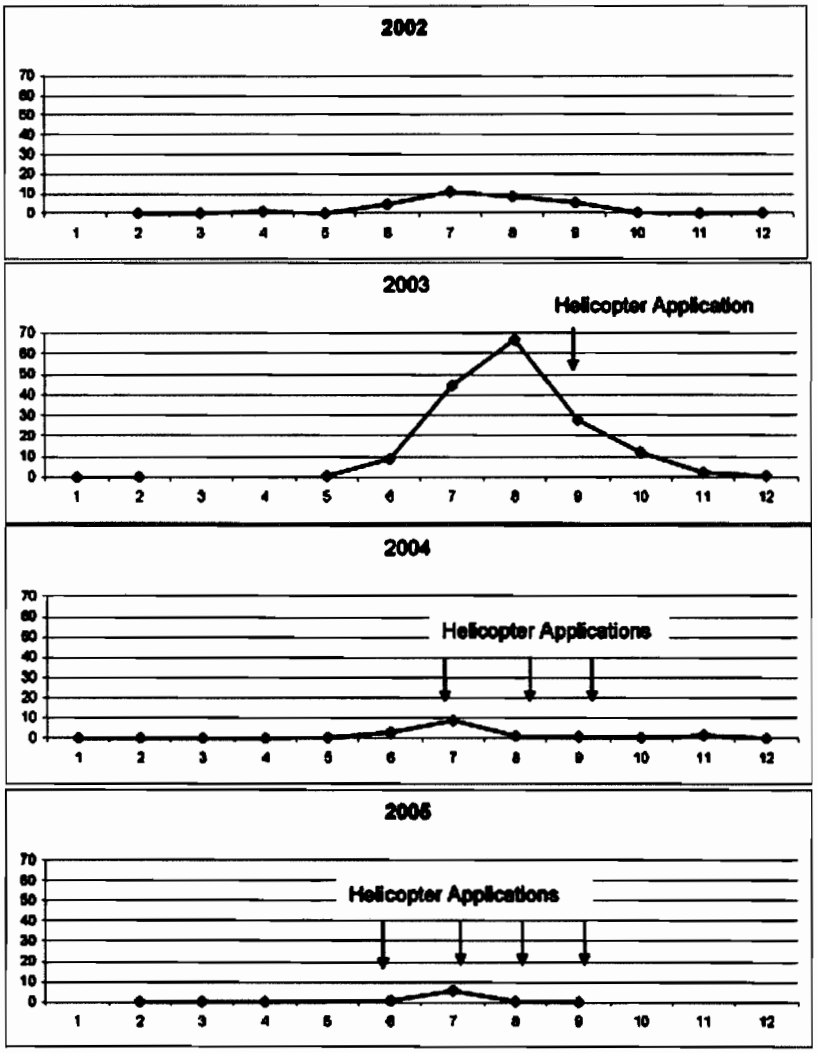

Figure 3. Impact of helicopter applications of larvicides on density of *Culex erythrothorax* females in carbon dioxide-baited traps at Searsville Lake, Woodside, California

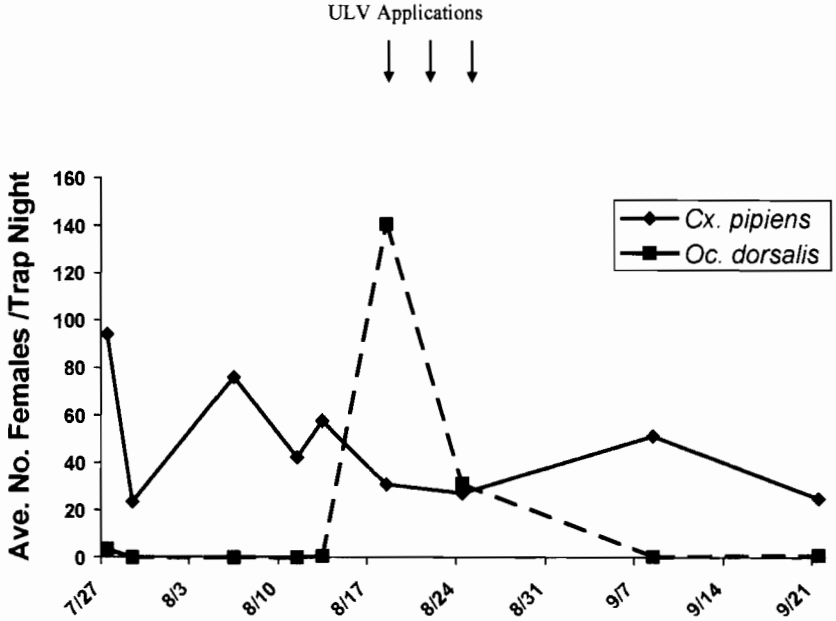

Figure 4. Impact of helicopter applications of larvicides on *Culex erythrothorax* at Mills Field, San Bruno California.

Cattail growth and the accumulation of silt in wetlands are part of the natural process of succession in which ponds are eventually converted to meadows. Before the onset of widespread development, new wetlands were continuously produced to replace ones that were lost to this process. On ranches, farms and other developed sites, cattails were regularly removed from ponds such as these, so that open water could be maintained. Extensive cattail growth actually reduces the amount of aquatic habitat available for both snakes and frogs in the long term, and maintaining the remaining habitat will require some kind of vegetation management. However, once cattail growth has become extensive, the methods that would be useful in reducing them (burning, mechanized equipment) will also result in some take of endangered species. Most landowners do not have the resources to develop a habitat conservation plan or produce the environmental impact analysis that is required by regulatory agencies such as the U.S. Fish and Wildlife Service or California Department of Fish and Game. Therefore, no work is done on the problem at all, to the detriment of all sides. The problem is now extending to roadside drainage ditches throughout the San Francisco Bay area. Many of the local cities lack the resources required to comply with regulations on waterways, such as obtaining NPDES permits for application of herbicides to water or completing an endangered species consultations and stream alteration permits for the use of mechanized equipment in waterways. Therefore, many cities have dropped their maintenance operations and allowed drainages to fill with vegetation. In some cases, this includes invasive weeds such as *Arundo donax*. Once heavy vegetation develops in the ditches, they are considered wetlands and potential habitat for endangered species. Once that has occurred, maintenance becomes even more difficult.

At present, the District is trying to bring property owners together with biologists from the Endangered Species Recovery Office of the U.S. Fish and Wildlife Service. The hope is that all parties will work together toward developing a recovery plan for endangered species on each property. Such plans could enhance and maintain habitat for endangered species while alleviating other problems caused by the dense vegetation. This work is in the early stages and will require cooperation from all sides. Whether it will be successful in the long run remains to be seen, but it is the only chance for arriving at a permanent solution to the problem.

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Utilizing Retail Nurseries as Mosquitofish Distribution Centers in Marin and Sonoma County

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ABSTRACT: This paper outlines the implementation and maintenance of retail nurseries as mosquitofish distribution centers in Marin and Sonoma County. Impacts on District resources, mosquitofish availability, etc. are discussed, as well as the advantages, disadvantages, and overall public response to the program.

INTRODUCTION

Marin/Sonoma Mosquito and Vector Control District (MSMVCD) has historically used the Mosquitofish Program to distribute fish to the public in two ways: the first method involves residents coming to the District office to pick up fish; the second a District field technician driving to the resident's home to deliver the mosquitofish. Beginning in 2002, the District introduced a new way to provide fish to the public by supplying fish to certain nurseries located in Marin and Sonoma County. This supplemented the District's efforts of distributing fish throughout these counties.

The District considered the Nursery Program as another means to educate the public about mosquito control, especially in anticipation of the arrival and spread of West Nile virus. The District also viewed this program as a way to decrease fish-related service requests while increasing surveillance and control, particularly during the busy season. It can take from seven to ten days for a technician to respond to a fish request and for many residents this isn't expedient enough from a public relations standpoint. Furthermore, for some residents, it's not convenient for District staff to be on their property and they are unable to drive to our District office during regular hours to pick up fish.

PROGRAM IMPLEMENTATION AND MAINTENANCE

The District identified the distribution sites situated within an easy access to Marin and Sonoma County residents and District staff. Thus, in 2003 and 2004, the District supplied fish to 10 participating nurseries, three in Sonoma County and seven in Marin County. By 2005, 13 nurseries participated, seven in Sonoma County and six in Marin County. Sloat Garden Centers, a chain located throughout Marin County, have played a key role due to their expertise and notoriety.

Early in the year, these specific nurseries were contacted to confirm that they had a suitable fish tank when the season started (Figure 1). In April the District stocked each nursery with a substantial amount of fish, depending on the size of the fish tank. The District also provided these nurseries brochures on mosquitofish and West Nile virus, along with a "Fight The Bite" poster for distribution to the public. Furthermore, each nursery was given containers so residents could transport their fish. All the containers were affixed with a label outlining the appropriate sources in which to put mosquitofish, and stating it is unlawful to put mosquitofish in waterways without a permit, pursuant to Title 14 CCR, Fish and Game Code, Section 1.63, Section 6400, and Section238.5. In order to keep track of these new mosquitofish sources, the District supplied a log book in which residents signed their names, addresses, and where they were placing the fish.

Figure 1. A holding tank for fish at a Sloat Garden Center in Mill Valley, CA.

Once the District had all of the nurseries set up for the season, maintaining the program was quite simple. Each nursery was contacted once a week to see if they needed more fish, brochures, or containers. Most often, the nurseries needed to be restocked every other week, at which time the District also collected any finished log book pages, making sure the nursery still had blank pages for residents to fill out.

PROGRAM IMPACT ON DISTRICT RESOURCES

This program mainly affected District staff time. In 2003, the District gave out about 60 lb (27.3 kg) of fish, at approximately 700 fish per pound. Of the 60 lb total, 24% were residential pick ups from the office, 19% were delivered by field technicians, and 57% were distributed through the nurseries. In 2004, the District supplied more than twice the amount of fish from the previous year, totaling 135 lb (61.2 kg) of fish. Fifteen percent were given to residents who came to the office. Nurseries gave away 80%, which was a substantial increase from 2003, lowering the amount of fish related service requests for the District field technicians. Consequently, only 5% were delivered by field technicians in this particular year. In 2005, the District didn't see much change in the quantity of fish supplied to residents compared to 2004. The District used 127 lb (57.6 kg) of fish and again, most of the fish were stocked at nurseries (55% of the total), and 13% were supplied from the District office.

Conversely, the District saw an increase in the amount of fish the technicians stocked, up to 32% of the 2005 total as opposed to 5% from the previous year. Unfortunately, all the factors that affect this difference are difficult to measure, but one could consider that the fish were made available to the technicians earlier in the year than the nurseries. In addition, at the end of 2004 residents voted to expand service to the entire population of both counties. The area covered grew from 960 to 2,300 square miles. As a result, the District wanted to thoroughly inspect its newly annexed areas for mosquito breeding sources, so they increased service requests for fish in these areas instead of referring these residents to a participating nursery. When technicians responded to these particular fish requests, it enabled them to look for other mosquito habitats in the surrounding area. In addition to the nurseries receiving less referrals from our District office, we hired five new technicians, so there were more technicians in the field doing surveillance and delivering fish.

Aside from the impacts of the Nursery Program on field technicians, the District's laboratory staff organized the implementation and maintenance of the program. In turn, time was needed to create databases for the fish and the nursery log books in order to record the effects of this program and to track fish distribution. The Mosquitofish Database breaks down how many fish are harvested, how they are distributed and approximately how many fish the District has at a given moment. The Log Book Database is formatted so the field technicians can back-check all of the new fish sources from the nursery log books and from the fish pick-ups at the office.

ADVANTAGES AND DISADVANTAGES

The most apparent advantage, in regards to District resources, was the cost-effectiveness of the program. First, it's free for the District to collect mosquitofish, while the purchase of pesticides can be quite expensive. With more residents using fish, there is also the potential to limit pesticide resistance of mosquitoes. Instead of technicians using travel time to respond to each fish request that may be in a far reaching area, residents drove to their local participating nursery to receive their fish. This allowed the technicians more time for surveillance and control; while residents liked it because it was convenient and free.

In addition, the Nursery Program met one of the District's main goals: educating the public about mosquito control. In turn, the District built a good rapport with the community, and more residents became aware of the District services that are offered. Also, the nurseries saw mosquito control as an important concern amidst the spread of West Nile virus and were happy to facilitate this proactive approach, especially since more and more residents have set up backyard ponds in recent years.

Although there are many advantages to this Nursery Program, fish availability, unknown sources, and responsible fish application posed challenges. The District starts receiving fish requests as early as February and March, but at this time the fish aren't feeding as readily as mid-summer and the District doesn't have an abundant supply to give away. In addition, the technicians weren't able to check the fish sources from the nursery log books in a timely manner, so they were unaware of many new fish sources. From 2004 and 2005 alone, there were about 4,500 new fish sources that originated from these log books.

Another disadvantage was the concern that responsible fish application rested with the public. All our fish containers were labeled, as mentioned before, but someone could easily say they were putting their fish in a water trough, when actually they intended to put them in a creek instead. As many people know, mosquitofish are a non-native species. They are extremely effective if applied in an appropriate source; however, the District realizes the environmental impact of placing non-native fish in natural waterways, where they have the potential to out-compete other native aquatic wildlife. In addition, since the District's annexation, certain agencies and residents have objected to the distribution of mosquitofish through retail nurseries. They fear mosquitofish dispersal is not controlled enough and the District's Nursery Program raises the risk of these fish entering natural waterways especially in the National Parks system. This is a valid concern, although many don't realize mosquitofish are already present in some waterways. They were first introduced into California in 1922 and have since spread throughout the state (Swanson et al. 1996). Unfortunately, this same gamble is encountered when residents get fish from the District office, or even when a technician leaves a person's property. Nevertheless, MSMVCD prides itself on being environmentally sensitive, so for 2006, we have elected to modify our Mosquitofish Program.

2006 MOSQUITOFISH PROGRAM

The District decided to no longer distribute mosquitofish through retail nurseries. Instead, the District field technicians will respond to all fish-related service requests and the public will still be able to come to the main office and receive fish. In addition, when residents come to the office to pick up fish they will be required to sign an Acknowledgement Form, which will include the person's information, such as address and where they are putting the fish. This form summarizes and cites California Fish and Game's regulations on appropriate fish application; furthermore, it states a field technician will back-check the resident's source. They also receive some information about fish care and pond design. Then,

once a week, service requests will be generated from the completed forms, so the District database will stay current and technicians will be aware of new sources. Technicians will also visit residents based on the information entered in our Log Book Database from previous years.

Furthermore, the District wants to continue to utilize the nurseries as an educational tool. Thus, MSMVCD will continue to have a "Fight The Bite" display with a poster and brochures. Also, most nurseries, depending on their set-up, will have a weather-proof sign to post outside. The sign gives residents tips on different things they can do with their pond to reduce mosquito breeding habitats, such as aeration, bio-control (Bti products and mosquitofish), and recommends consultation with nursery staff on plant selection. Nursery staff will also have a significant supply of our District business cards to give to residents so they can contact us if they have questions or want fish. In turn, if a service request is made, a District technician will visit the property and assess any mosquito sources and the best way to treat them.

The last component of the modifications to the Mosquitofish Program is the education of the District staff. Since the annexation, as mentioned before, the District increased the number of employees. As a result, the District thought it would be a good idea to have an educational workshop for the new employees about mosquitofish. It also refreshed the knowledge of the technicians that have been here longer. The workshop was titled "*Gambusia affinis* 101." It focused on mosquitofish biology, fish protocols at the District office, and appropriate sources for mosquitofish.

CONCLUSION

Utilizing retail nurseries as mosquitofish distribution centers in Marin and Sonoma counties was a success for the most part. The overall public response was satisfactory. It was convenient for residents, the nurseries enjoyed their ability to be proactive, and the program raised public awareness of the District. It was also convenient and cost-effective for District staff, especially during a time when workloads increased dramatically due to the District expanding its boundaries and the arrival of West Nile virus. In addition, the program forced the District to re-evaluate certain aspects of its Mosquitofish Program in order to address valid concerns in regards to responsible fish distribution.

Acknowledgements

Marin/Sonoma Mosquito and Vector Control District would like to thank the participating nurseries throughout Marin and Sonoma County for their continued support. The author thanks Piper Kimball and the staff at Marin/Sonoma Mosquito and Vector Control District for their creativity, suggestions, and guidance during this program.

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An Alternative Larval Control Method for Cemeteries: One Year Update

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ABSTRACT: The recent addition of a new floral policy at Butte County cemeteries has significantly reduced adult mosquito populations, district man-hours, and control costs. Effective larval control in cemetery vases was met by the cemeteries applying Agrosoke Watering Crystals. Lab experiments, field tests and trap counts demonstrated the effectiveness and length of control of Argosoke Watering Crystals.

A Rapid Method of Locating Un-maintained Swimming Pools

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ABSTRACT: Butte County Mosquito and Vector Control District was able to quickly detect and accurately locate un-maintained swimming pools that are often a source of Culex mosquitoes by using photographs taken from a sheriff's helicopter and laser equipped GPS.

Mosquito Management in a New Generation of Stormwater Structures

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ABSTRACT: Federal, state, and local clean water regulations are forcing tremendous changes in the treatment and management of urban and stormwater runoff. Implementation of structural treatment devices known as best management practices (BMPs) is required under existing laws in an effort to improve the quality of water runoff before it enters receiving waters. An unintended consequence of BMP implementation is the creation of mosquito habitats when not properly designed and/or maintained. Since 1998, the California Department of Health Services, Vector-Borne Disease Section (CDHS-VBDS) has led a series of collaborative studies to investigate mosquito breeding in stormwater BMPs built by the California Department of Transportation (Caltrans). The results of these efforts, fueled in part by the arrival and rapid spread of West Nile virus, have contributed greatly to how subsequent BMPs have been designed, implemented, and maintained. In 2004, a new generation of Caltrans BMPs, representing the latest treatment technologies, was completed along the 21 mile San Joaquin Hills Transportation Corridor in Orange County, State Route 73. CDHS-VBDS and Orange County Vector Control District staff monitored 19 structures biweekly for presence of mosquitoes and/or mosquito habitat beginning in October of 2004. During the first year of monitoring, appropriate BMP alterations and design modifications were recommended to reduce or eliminate standing water, however, many critical problems remain unresolved. This paper will provide an overview of our findings during the first year on the SR-73 project and discuss strategies implemented by Caltrans to minimize the breeding potential of their newest BMPs.

No Two Years Are Alike: Operational Challenges of Controlling Adult Mosquitoes

Fran Krenick, Jim McNelly and Ben Goudie

Clarke Mosquito Control, 159 North Garden Avenue, Roselle, IL 60172

ABSTRACT: Successful control of adult mosquitoes remains one of the most challenging aspects of our industry. This paper demonstrates how critical it is to reassess the dynamics influencing a program not only each year, but location to location. This presentation will address critical components of adult mosquito control as well as the logistical challenges posed by emergency operations.

Clarke Mosquito Control's Partnership with Mosquito Control Districts in the 2005 Natural Disaster Area: Expectations and Guidelines for the Future

[1]Clark Wood, [1]Jim McNelly, [1]Frank Krenick, [2]Aaron Lorson, and [2]Michael Rosolina

[1]Clarke Mosquito Control, Roselle, Illinois
[2]Dynamic Aviation, Bridgewater, Virginia

ABSTRACT: This paper will focus on the important interaction that develops between local mosquito control districts and private industry during a mosquito control emergency. This complex process is dynamic in scope and integrates many layers of responsibilities, expertise and commitment for successful operations. As well as discussing the natural disaster of 2005, this presentation will address expectations and guidelines for future emergency operations.

Update on Recent Local Vector Control Efforts...and What about the 3 Million People in California without Mosquito Control?

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ABSTRACT: Recently successful local ballot measures in California for mosquito and vector control services will be reviewed with emphasis on changing community priorities. In addition, over 3 million people in California currently do not receive regular mosquito control services. This presentation will also examine which areas of the State do not provide mosquito control services and what options and solutions could be implemented to better protect the public health all residents in the State.

The Infamous Lincoln Log Decks – An Update on Mosquito Control in a Challenging Habitat

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WHAT'S THE PROBLEM?

Sierra Pacific Industries (SPI) operates the largest sawmill in California in Lincoln, CA. Over the last 10 years, the City of Lincoln has grown rapidly, and homes, two parks, and an elementary school now abut the SPI property. Markham Ravine runs east-west through the SPI property and provides a mosquito flyway into the residential neighborhood to the west, and a drainage channel provides another flyway into the public park and homes to the south (Fig. 1).

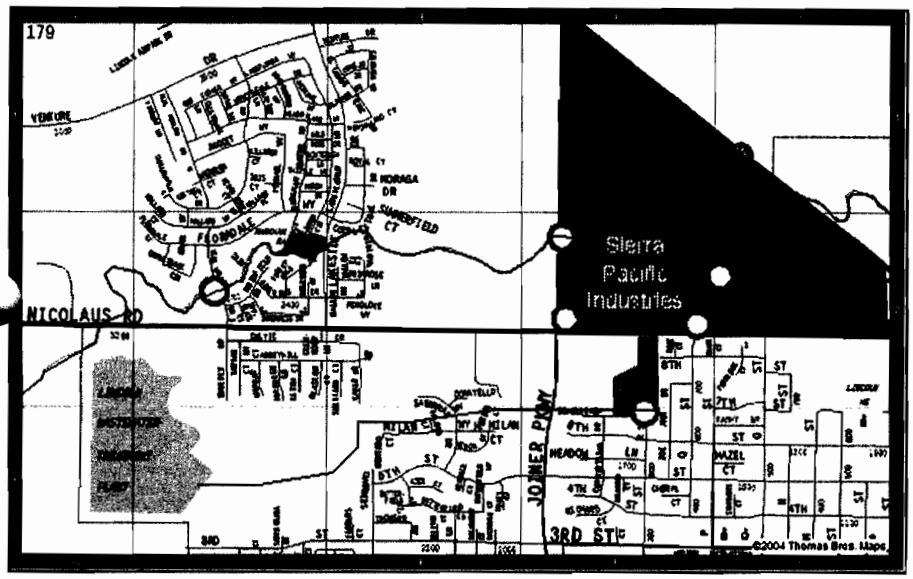

Figure 1. The SPI Log Decks are bordered by homes, an elementary school, and two parks to the west and south sides. The six dots indicate weekly Fay-Prince trap locations.

Figure 2. A typical log desk reaches 60 feet high (18.3 m) and covers an area of 0.50 to 0.75 acres (0.20 to 0.30 ha). This unfinished log deck is about 60' at its tallest point.

Each day – weather permitting – 100 to 300 trucks deliver logs harvested from the Sierras to SPI's site in western Lincoln, where the logs are stacked in "decks" that measure up to 60 feet high and cover 0.50 to 0.75 acres each (Fig. 2). The Lincoln site can accommodate up to 36 log decks covering an area of approximately 20 acres.

To prevent splitting and maintain the quality of the wood, the logs are kept saturated with water through a system of irrigation pipes with sprinkler heads that run across the top of each deck (Fig. 3). The irrigation system runs 24 hours a day, 7 days a week. Runoff water is collected through a series of ditches and ponds, and reused. None of the log deck water is allowed to drain into natural waterways or leave the SPI property.

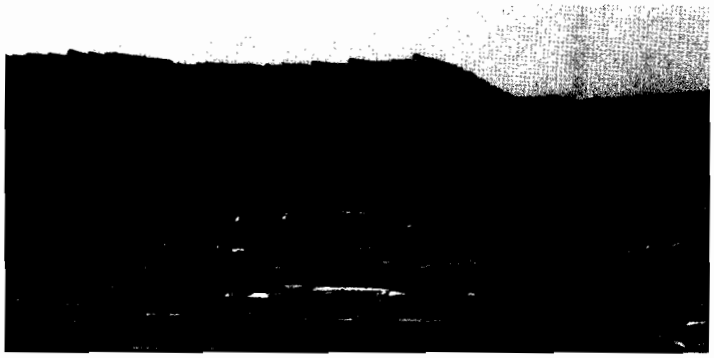

Figure 3. Log decks are irrigated with recycled water to prevent the logs from drying out and splitting, which reduces their economic value. The constant irrigation also produce tremendous larval mosquito habitat within the log decks where water is trapped between the logs. In 2003 and early 2004, the log decks were treated with VectoBac 12 AS and VectoLex WDG applied through this irrigation system, but this did not provide adequate larval control.

MOSQUITO PRODUCTION IN THE LOG DECKS

The SPI log decks are the single largest source of *Culex* mosquitoes in Placer County. The majority of mosquito production occurs in the decks themselves, where water is trapped between the stacked logs (Fig. 4), with smaller numbers of mosquitoes produced from the edges of log ponds, ditches, and in water trapped in the log debris. *Culex pipiens* is the most common mosquito collected from the log deck traps, with smaller numbers of *Cx. tarsalis* and *Cx. stigmatosoma* (Fig. 5). Low, but increasing, numbers of *Cx. erythrothorax* are also collected regularly, but these originate from the cattails in the associated ponds, not from the decks themselves.

Figure 4. Irrigation water trapped between the logs produces tremendous larval mosquito habitat.

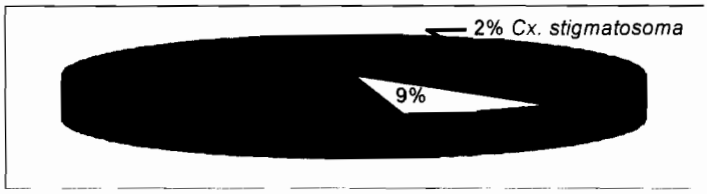

Figure 5. Mosquito species composition at the Log Deck , Lincoln.

WEST NILE VIRUS ACTIVITY ASSOCIATED WITH THE LOG DECKS

West Nile virus (WNV) was first detected in Placer County in 2004, mainly in dead birds and sentinel chickens, with a few WNV+ pools of mosquitoes, 26 equine cases, and a single human case. Significantly higher WNV activity occurred in Placer County in 2005 when, countywide, there were 35 human cases of WNV infection, 23 equine cases, 84 WNV+ dead birds, 20 WNV-positive mosquito pools, and 5 of 7 sentinel chicken flocks that seroconverted.

Four human cases of WNV infection occurred within 0.5 mile of the SPI log decks, and three pools of mosquitoes (2 pools of *Cx. pipiens* and 1 pool of *Cx. tarsalis*) from the log deck traps were WNV+. Additionally, in August, we saw an increase in dead bird reports from Lincoln, and the state's DYCAST risk analysis identified western Lincoln as one of the high risk areas in Placer County for human infection with WNV.

MOSQUITO SURVEILLANCE AND CONTROL CHALLENGES AT THE SPI LOG DECKS

Working around the log decks is inherently dangerous because of the sheer size of the logs and log decks, as well as the high volume of large equipment that operates continuously from dawn to sunset, 6 days/ week. Larval sampling is difficult and dangerous at the log decks. Most of the larval production occurs in pockets of water trapped between the logs within the decks, making sampling difficult at best (Fig. 4). When combined with the inherent danger of potentially unstable logs and heavy, fast-moving machinery, the risks to employees outweigh the benefits of collecting larval samples. Instead, we assess adult mosquito populations using six CO_2-baited Fay-Prince traps (John W. Hock Co., Gainesville, FL). Four traps are placed around the log decks (Fig. 1) so that changes in the wind direction will have a minimal impact on the total collection; additionally two traps are placed along flyways that lead into the adjacent residential neighborhoods. The trap collections are identified to species and counted, and subsamples are pooled for virus testing. The per trap-night averages are shown (Fig. 6).

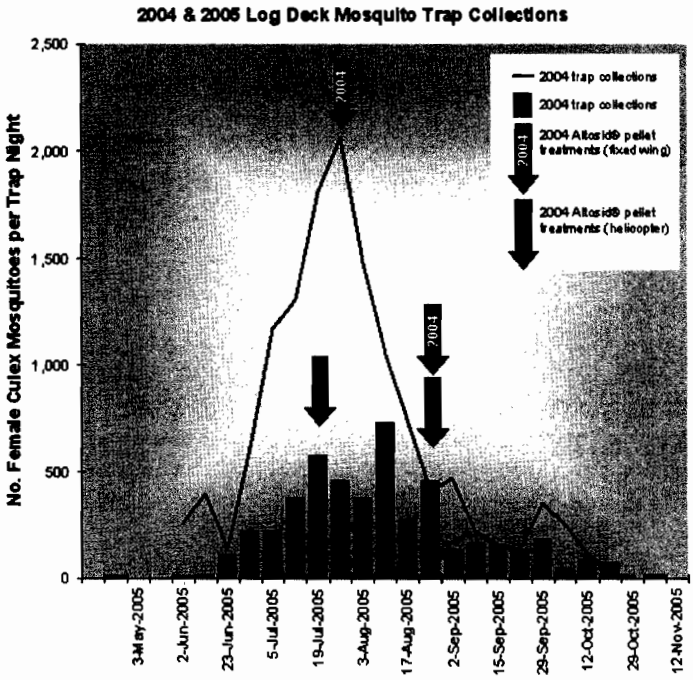

Figure 6. Mean number of adult mosquitoes collected per trap night at the Log Deck during 2004 and 2005.

WHAT MOSQUITO CONTROL MEASURES HAVE BEEN TAKEN AT THE LOG DECKS?

Sierra Pacific Industries bears all costs for mosquito abatement on their property. The cost of aerial applications was a concern for both SPI and the District. Because the mosquito production at the SPI log decks is a long-term problem, the Placer Mosquito Abatement District (PMAD) wanted to establish and maintain a good working relationship with SPI. Thus our goal was to find the most cost-effective way to keep mosquito populations below the threshold where disease transmission is likely to occur. Initially, we sought a mosquito control method for the log decks that was more cost-effective than aerial applications.

2002

In 2002, Placer MAD's first year of operations, mosquito control efforts in the decks were limited to ground-based applications of the larvicidal oil (GB-1111) and adulticide resmethrin plus piperonyl butoxide (Scourge^{R}). It quickly became clear that the size of the decks made the traditional ground application of mosquito larvicides unfeasible. We also discovered that ground-applied adulticides did not effectively penetrate the log decks because of the air currents produced by the constant evaporation of water from the log decks.

2003

By 2003, it was evident that the log decks were simply too large for traditional ground-based product applications, and the cost of aerial applications was still a concern. As an alternative, we sought a more cost-effective control method that had been used successfully at other – albeit smaller – log deck arrays: a chemigation program using *Bacillus thuringiensis israelensis* aqueous suspension (VectoBac 12AS) and *Bacillus sphaericus* (VectoLex WDG) injected directly into the irrigation system was initiated (Fig. 3). In the early summer of 2003, injections were made once weekly, and adult mosquito collections continued to increase. In August 2003, chemigation was increased to twice weekly, and a modest decline in mosquito trap counts was noted, but because of the lateness in the season and the absence of long-term historical mosquito population data, we could not determine if the drop in mosquito numbers was due to our treatment or to the natural decline in *Culex* populations that occurs in the late summer/ early fall.

2004

In April 2004, we began biweekly chemigation of the decks with VectoBac 12-AS, hoping to see a reduction in mosquito in mosquito populations similar to that seen at the end of the previous summer. By the end of June 2004, it was apparent that the VectoBac 12-AS chemigation was not adequately controlling the mosquitoes being produced in the log decks, so in July we contracted a local aerial applicator (Dibble Aviation, Marysville, CA) to apply methoprene (Altosid® pellets) over the decks and associated ponds and debris. In 2004, two applications of methoprene were made at the maximum label rate (10 lbs/ acre) using a fixed wing aircraft, and trap collections declined following each application (Fig. 6).

2005

Based on the apparent success of the previous year's aerial methoprene applications, we planned to continue aerial applications in 2005. However because of the encroaching development, we used a helicopter with a bucket spreader instead of a fixed-wing aircraft to apply the pellets. The number of *Culex* mosquitoes collected dropped from 12,363 to 8,900 mosquitoes – a 28% reduction at 1-wk and – and to 6,322 (50% at 2-wk post-treatment.reduction) (Fig. 6). At this time of the year, we were confident that the reduction in mosquito numbers was attributable to the application and not to a natural seasonal decline in the population that occurs later in the season. We made a second aerial application of methoprene in August to maintain low numbers of mosquitoes during the peak of West Nile virus activity.

DISCUSSION

The number of mosquitoes collected in 2005 was low, relative to the same time period in 2004. The wet spring and late snowmelt reduced the number of logs that could be harvested and transported in the spring, and consequently, there were < 6 log decks at any one time through the end of June 2005. This represented a substantial reduction in the number of log decks from the 2004 season, and thus the available mosquito habitat over previous years. Adult mosquito collections remained low until about-mid July, and when the mosquito numbers started to increase, we made our first methoprene application on July 24, 2005. Three weeks after that application, trap counts began to increase again (the very low mosquito trap count on August 17 was due to the high winds that evening), and a second application was made on August 21, 2005. Mosquito trap collections dropped off rapidly following the second application, and numbers remained low for the rest of the season.

The estimated 30-day residual effect of the methoprene pellets appears to be reduced to about 21 days, presumably due to the constant irrigation of the decks. In 2006, PMAD plans to monitor mosquito populations with the same weekly trap set, and will begin aerial applications of methoprene pellets when *Culex* spp. numbers reach 400 mosquitoes per trap night, or possibly at a lower threshold, depending on West Nile virus activity.

Additionally, Placer MAD continues to recommend the following practices at the SPI Lincoln facility to reduce mosquito habitat as part of an integrated mosquito management (IMM) program:

- Regular removal of the tree bark and debris that accumulate around the base of the log decks, drainage channels, and ponds to increase the rate of drainage of water from the log decks and reduce the standing water
- Removal of emergent vegetation (cattails, tules, and similar plants) from ponds
- Maintain the irrigation ponds with steep sides
- Maintain drainage ditches so that they are open, free of vegetation, and fast-flowing.

Placer MAD continues to work with Sierra Pacific Industries to look for economical alternatives to controlling the mosquitoes their log decks property in Lincoln.

Acknowledgements

The authors thank the PMAD Board of Trustees for their support, Grant Mitchell, Sierra Pacific Industries, Lincoln, CA for his cooperation, and Gary Dibble, Dibble, Inc. Aviation Services for his assistance with the aerial applications.

Gone Fishin': A Survey of Mosquito Fish Use & Production Capacity in California

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ABSTRACT: In 2004, the Integrated Pest Management (IPM) Committee was charged with determining whether there were enough mosquito fish in California to stock all the rice fields in the state. To determine the mosquito fish capacity and how mosquito fish are presently used throughout the state, a 1-page survey was sent to all to all MVCAC member agencies in the spring of 2005. Fifty-one member agencies responded, and the results are reported here.

MOSQUITO FISH APPLICATIONS

Fifty of 51 responding Mosquito and Vector Control Districts (MVCDs) reported that they use mosquito fish. The one agency that did not use fish is located within the Lake Tahoe watershed.

California MVCDs stock mosquito fish in a wide variety of sources. The most common sources are ornamental (lined) ponds (86% of reporting MVCDs), manmade (unlined) ponds (86%), stock tanks (74%), rice fields (74% of reporting MVCDs with rice fields), unmaintained swimming pools, spas, etc. (74%), and water gardens (64%) (Fig. 1).

Other sources where MVCDs report using fish include:

- any discrete source without an inlet or outlet, that is NOT within the 10-20 year floodplain
- any place with standing water where the fish can survive
- any water that remains for 6 months or longer
- borrow pits
- by resident's request
- creeks
- dead end catch basins
- decomp rice
- ditches
- drains
- dredge ponds
- duck clubs
- wherever circumstances justify utilization, except vernal pools where fairy shrimp are present
- flood channels
- groundwater recharge ponds
- incidental agricultural and domestic sources
- intermittent sources that hold water for 1-2 months
- irrigation ditches
- log deck ponds
- natural wetlands
- organic rice
- oxidation ponds
- perennial ponds
- ponding basins
- retention & detention basins
- RR bar ditches
- sloughs
- State & Federal wildlife areas
- storm drain sumps
- various other flooded areas
- wetland-like wildlife areas
- wildlife areas

CALIFORNIA MOSQUITO FISH PRODUCTION

Presently, 29 MVCDs produce or harvest from existing sources a combined total of 14,432 to 14,850 pounds of mosquito fish per year. These MVCDs estimated that they could produce/ harvest a maximum 21,569 pounds of *Gambusia* annually with their existing facilities, and several districts reported that they were either building new fish-rearing facilities, or planning to improve or build fish-rearing facilities to increase their mosquito fish production. The greatest quantities of *Gambusia* are produced by MVCDs that have large acreages of rice fields within their borders.

Eighteen districts reported buying a combined total of 1,215-1,296 pounds of fish per year from other districts, and one district reported that this year they purchased 2,000 pounds of mosquito fish from a commercial catfish company.

Over one-half of responding districts reported that they harvest mosquito fish from existing sources; many of these districts neither rear nor purchase their *Gambusia*, but instead rely solely on mosquito fish harvested from existing sources within their districts.

Only two agencies (Contra Costa and Sac-Yolo MVCDs) reported using fish other than *Gambusia* for mosquito control.

STOCKING LIMITATIONS AND PUBLIC PERCEPTIONS

Except for Inyo, Mono, San Bernardino, Riverside and Imperial counties, where *Gambusia* may not be planted without the written concurrence of the California Department of Fish & Game (CAC, Title 14, Section 238.5(f)), the majority of MVCDs (45 of 50 districts) allow residents to pick up fish from the District office to stock sources on their privately owned property. Most agencies provide supplemental information about mosquito fish, including where mosquito fish can legally be planted.

Most MVCDs (41/50) reported that they perceive that there is an increased demand from the public for mosquito fish this year over previous years, and several districts reported that they had seen up to a four-fold increase in fish requests from their residents. The public's awareness of West Nile virus in California appears to be driving the increased demand for mosquito fish.

MVCAC Mosquito Fish Survey- Summary of Results

Please complete this survey and return it to Jamie Scott at jjscott@placermosquito.org
or FAX it to 916-435-8171 or post it to PO Box 216, Lincoln, CA 95648

What is the name of your district/ agency? **51 MVCAC Member Agencies returned competed surveys.**

98% Yes Does your district use mosquito fish? If no, why not? **The single district that does not use *Gambusia* is located within the Lake Tahoe watershed.**
If yes, what are your major application(s) for mosquito fish?

- ☐ Water gardens **(64%)**
- ☐ Stock tanks **(74%)**
- ☐ Rice fields **(74% of MVCDS with rice)**
- ☐ Unmaintained swimming pools, spas, etc. **(74%)**
- ☐ Ornamental ponds (lined) **(86%)**
- ☐ Manmade ponds (natural bottom) **(86%)**
- ☐ Other: **See attached report (50%)**
- ☐ Other: _____
- ☐ Other: _____

4% Yes Does your district use fish – other mosquito fish – for mosquito control? If yes, what other fish do you use, and where do you use them? **Sacramento Perch (in experimental ponds); Guppies (in koi ponds & acidic sources); Sticklebacks (in swimming pools in the fall for mosquito control; in landscape lakes in the spring for midge control)**

36% Yes Does your district purchase mosquito fish from another district? If yes, approximately how many pounds of mosquito fish do you purchase per year? **3,203-3,283 pounds/ year (includes 2,000 pounds purchased from Superior Catfish)**

36% Yes Does your district rear its own mosquito fish? If yes, approximately how many pounds of mosquito fish do you produce per year? **14,410 to 14,826 lbs per year**
What would you estimate is the maximum quantity of mosquito fish that your district could produce? **21,545 lbs/ year maximum (with existing facilities)**

73% Yes ***(Of 19 MVCDs with rice fields)*** Does your district use mosquito fish in rice fields? If yes, approximately how many acres of rice do you stock with mosquito fish? **31,852 to 41,453 acres per year**
Approximately how many pounds of fish does your district stock per acre of rice? **0.2 to 3.0 pounds/ acre**

79% Yes ***Of 19 MVCDs with rice fields)*** Would your district stock fish in all of its rice fields if enough fish were available? Why or why not? **See attached report**

90% Yes Do you permit residents to pick up mosquito fish from your district so that they can stock their own sources?

82% Yes Do you perceive that there is more demand from your residents for mosquito fish this year (2005) than in previous years?

86%Yes Do you anticipate using more mosquito fish in your district this year (2005) than in previous years?

If you need more space, please feel free to add comments on the back of this sheet or add more sheets as needed. Thank you for taking the time to answer these questions!

Figure 1. The mosquito survey that was sent to all MVCAC member districts and the summary of results are shown here.

MOSQUITO FISH IN RICE FIELDS

Fourteen of the 19 MVCDs with rice fields in their jurisdictions reported using mosquito fish in their rice fields, and 15 of them replied that they would stock all of their rice fields with mosquito fish if enough fish were available. A few MVCDs reported that they already stock mosquito fish in all of their rice fields, or in all of their organic rice fields (each of these districts is responsible for mosquito abatement of a few hundred acres up to 5,000 acres of rice). Four MVCDs replied that they would not necessarily plant mosquito fish in all of their rice fields:

- "We use mosquito fish only in organic rice."
- "We only stock (rice) fields that are producing mosquitoes above our threshold levels."
- "Our past data indicates that mosquito fish save 1-2 larviciding treatments per season. Once *Culex tarsalis* and *Anopheles freeborni* reach treatment thresholds mosquito fish cannot keep pace so we rely on larvicides. I would not be averse to trying fish at different (higher) stocking rates."

Stocking rates of reporting districts varied from 0.2 to 3.0 pounds of fish/ acre.

In 2003, approximately 507,000 acres of rice were harvested in California (California Field Crop Review, Vol. 25 No. 1, January 21, 2004, California Agricultural Statistics Service). At the lowest reported stocking rate of 0.2 pounds/acre, 101,400 pounds of mosquito fish would be required to stock all rice fields in California. This is approximately 4.7 times the estimated maximum mosquito fish production capacity of all Districts in California, and nearly 7 times the actual quantity of *Gambusia* reared and harvested per year by MVCDs. If mosquito fish were planted in all rice fields in California at the highest reported stocking rate – 3.0 lbs/ acre – over 1.5 million pounds of mosquito fish would be required, or 70 times the maximum estimated annual production capacity in California.

CONCLUSIONS

The majority of MVCDs reported that they consider *Gambusia* an important component of their Integrated Mosquito Management (IMM) program – including the control of mosquitoes originating from rice fields. There were a few agencies that reported that they did not consider mosquito fish to play a major role in their IMM programs, however they did not specify why.

Clearly, even if it were economically feasible, there are not enough *Gambusia* reared by MVCDs in California to stock all rice fields, even at the lowest rate of 0.2 lb/ acre. However many Districts reported that the use of mosquito fish within their rice fields reduced the need for larviciding in these fields, particularly at stocking rates of 1.0 lb/acre or greater. Although stocking all rice fields in California with mosquito fish is not presently possible, the selective stocking of some fields – particularly organic rice fields, fields with physical barriers (such as power lines) that interfere with aerial applications of larvicides, or those fields that have historically produced more mosquitoes than adjacent fields – may be an important part of IMM in rice.

West Nile Virus Education in "The Second Year"

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ABSTRACT: The California Department of Health Services (CDHS) and many local agencies including health departments, mosquito and vector control districts, and environmental health agencies developed and implemented West Nile virus (WNV) prevention education campaigns in 2004 in response to the spread of WNV throughout the state. These campaigns continued into 2005 while the focus of WNV activity shifted from southern California in 2004 to north-central California in 2005. Sacramento County reported the highest number of cases per county in the state (175). The purpose of this study was to evaluate the efficacy of local WNV prevention education programs and assess whether agencies faced issues different in the northern part of the state as opposed the southern part of the state. In a case-study of a mosquito and vector control agency's experience with WNV prevention education in the Sacramento Valley, an online survey was sent to 100 local agencies. Questions focused on materials, major prevention messages used, methods of distribution, and cost. Additionally, agencies were asked to assess the perception of the public to their messages, and to expand on future plans for their WNV prevention education programs.Over 30% of queried agencies responded as of December 1, 2005. Most agencies (>90%) used the "Fight the Bite" materials offered by CDHS and half of them developed their own materials to target their specific populations. Materials were distributed in at least nine languages. Print material was the most common way to distribute information, although unique approaches such as visiting migrant workers' job sites and medical care facilities were also implemented. Northern California agencies faced similar problems in 2005 in educating their public about WNV as did agencies last year in southern California. Reaching California's diverse communities continues to be a challenge for effective WNV prevention education.

Public perception surrounding aerial adulticing became important in 2005, particularly in northern California. Sacramento-Yolo County Mosquito Vector Control increased efforts to inform their residents not only about WNV prevention but also about urban aerial adulticiding. Urban aerial adulticiding was a major educational obstacle in 2005 and will likely be an important issue for WNV educators in 2006. Specific approaches in educating the pubic will be presented.

Distribution of Resistance Genes in Mosquitoes: A Case Study of *Anopheles gambiae* on Bioko Island

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Pyrethroids are the fastest growing class of insecticides and are used worldwide for both agriculture and vector control. According to the World Health Organization, vector resistance to insecticides is the most outstanding technical problem impeding the development of vector control programs. This problem is further exacerbated if the targeted vector has a complex population structure as seen in *Culex pipiens* (Barr 1982), *Cx. tarsalis* (Gimnig et al. 1999), *Anopheles gambiae* (Davidson 1964) and *An. freeborni* (Porter and Collins 1996). With these species not only is it important for us to monitor resistance and develop novel strategies for efficient vector control, but we also must understand the complicated factors that influence the spread of resistance through populations.

The insect sodium channel is the primary target site for pyrethroid insecticides and DDT. The insecticide binds to the sodium channel and this perturbation of nerve action results in exhaustion and rapid "knockdown." Knockdown resistance (*kdr*) is conferred by a single point mutation in the sodium channel gene that prevents insecticide binding (Chandre et al. 1999). Similar mechanisms of genetic resistance have evolved in a number of mosquito species including *Cx. pipens, Ae. aegypti, An. gambiae* as well as house flies and agricultural pests.

In order to understand the distribution of insecticide resistance in *An. gambiae*, we must first consider its complex genetic structure. There are two subpopulations, designated M and S molecular forms. Like many mosquito subpopulations, they are morphologically identical and can only be distinguished by PCR. Although they occur in sympatry, the forms exhibit strong positive assortative mating and are considered by many to be incipient species (Lanzaro and Tripet 2003). In West Africa, *kdr* has been strongly associated with the S molecular form even at sites where S and M forms are sympatric. Resistance in the S form has been reported from the Ivory Coast (Weill et al. 2000, della Torre et al. 2001), Benin (della Torre et al. 2001), Nigeria (Awolola et al. 2003), Mali (Fanello et al. 2003) and Burkina Faso (Diabate et al. 2003). More recently, *kdr* has been found in the M form in Benin (Weill et al. 2000, della Torre et al. 2001), Burkina Faso (Diabate et al. 2004) and Ghana (Yawson et al. 2004). In these cases, although mating is limited, it has been suggested that the *kdr* mutation reached the M form through mating with resistant S form individuals (Weill et al. 2000). Evidence to support introgression was found by similarities in the intron near the *kdr* gene.

Bioko Island is a small island 75 km off the coast of Cameroon. In 2001 no *kdr*-associated resistance was detected anywhere on the island (Berzosa et al. 2002) or in Cameroon in either form (Etang et al. 2003, Gentile et al. 2004, Weill et al. 2000). This finding led to the launch of two malaria control campaigns on the island: one using pyrethroid treated bednets, the other to spray all homes in the capitol (25,000) with deltamethrin (MCDI Newsletter, March 2004). Mosquito abatement personnel began noticing resistance and were reporting control failures. Since this population was susceptible prior to the campaigns, we wanted to characterize the amount of resistance and determine its origin. For improved control measures it is important to distinguish whether it arose independently on the island after pyrethroid exposure, or if it arose through mating with a nearby resistant population. For comparison with the mainland population, we also chose to study Tiko, the nearest mainland site in Cameroon.

Through analysis of microsatellite molecular markers we determined that the island population is reproductively isolated from the mainland and that on the island the M and S forms are reproductively isolated. We then performed a PCR diagnostic to detect the *kdr* mutation. Although we expected to see some level of resistance based on the reports from abatement personnel, our results were very surprising (Reimer et al. 2005). Eighty percent of M form mosquitoes (n=36) on Bioko Island carried at least one copy of the resistant allele, while no resistance was detected in the S form (n=25). No resistance was found in either form in Tiko (n=68). Based on these results it appears that resistance arose independently in the M form. This is a very different pattern than what was seen in mainland West Africa where resistance arose in the S form and over time, through limited mating entered the M form. We then sequenced this gene and the polymorphic intron that precedes it in order to confirm our findings and to see if there were any patterns that might suggest that resistance could have entered from an existing resistant population from the mainland. The sequence data suggests that *kdr* indeed arose independently in the M form.

On Bioko Island the M and S subpopulations were exposed to the same amount of pyrethroids and were collected from the same locations. However, knockdown resistance was only seen in the M form, and most likely arose independently. Furthermore, resistance in the M form arose within two years, probably in response to intensive and extensive pyrethroid application through insecticide treated bednets and indoor residual spraying.

The application of chemical insecticides remains an important component for vector control. However, many mosquito species that occur around the world have complicated population structure, including *An. freeborni, An. quadrimaculatus* and *Cx. pipiens*. These species have morphologically identical subpopulations that are reproductively isolated and genes, including those that confer resistance, will not be distributed uniformly. As we saw on Bioko Island, resistance in a population can occur independently and very

quickly in the presence of insecticides. This case study emphasizes the integral role of population genetics in not only monitoring and predicting, but also in managing the spread of insecticide resistance for more effective mosquito control.

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Efficacy and Residual Activity of the Adulticide, Deltamethrin, against Mosquitoes in Underground Storm Drain Systems

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ABSTRACT: The purpose of these trials was to assess the efficacy of adulticide applications for mosquito control in Underground Storm Drain Systems (USDS). Deltamethrin was applied to the upper three feet of manhole walls of 3 treated systems within the city of Pico Rivera, Los Angeles County. A fourth untreated system served as control. Due to toxicity to fresh water as well as estuarine fish and invertebrates, contamination of water in the laterals had to be prevented. To achieve this, a unique technique was employed. The treated area of each manhole chamber was sealed tightly with an inflated beach ball to prevent material introduction into the laterals. Treatment efficacy and residual treatment effects were assessed with EVS/CO_2 light traps and exposure of adult mosquitoes to treated and untreated wall surfaces respectively. Adult mosquitoes exposed to treated wall surfaces showed high mortality rates for up to 3 months.

INTRODUCTION

The USDS in urban Los Angeles is a complex network of drains, catch basins and manhole chambers. Within the City of Los Angeles alone, there are 1500 miles of storm drains, 6700 miles of street drains and 34,000 catch basins (G. Lee Moore, pers. comm.). The USDS is historically known to be capable of producing large numbers of mosquitoes including *Culex quinquefasciatus* Say, the predominant species in USDS in California (Schaeffer and Mulligan, 1980, 1981, Dhillon and Mulla, 1982, 1983, 1984, Dhillon, Mulla and Chaney 1985) and determined to be the primary vector of SLE in the Houston epidemic of 1964 (Pigford 1964). Due to restricted accessibility of breeding sources in these systems, controlling mosquito populations in USDS continues to be a challenging task for local mosquito and vector control districts.

Larviciding trials using the L.A. Vector/USDS Larvicide Applicator (developed at Greater Los Angeles County Vector Control District) demonstrated that while using this new method, breeding within a system could be effectively controlled. EVS/CO_2 trapping still documented high numbers of female mosquitoes using the USDS as sheltered resting sites (Kluh et al. 2001). Earlier studies demonstrated that most adult resting activity took place on the vertical walls of the upper manhole chambers (Dhillon and Mulla, 1982, 1983, 1984). Application of a residual adulticide to the upper three feet of wall in all manholes could, therefore, potentially significantly reduce the number of resting females.

MATERIALS AND METHODS

The three selected trial sites as well as the untreated control site were selected from sites used in previous trials in order to allow limited comparability of results. These four USDS are known to historically produce mosquitoes and are located within a 5 sq mi area in the L.A. Basin (Fig. 1). Two sets of trials were performed during the summer and fall of 2002. While the first trial consisted of an adulticide application only, the second combined adulticiding and larviciding.

On June 4, 2002, deltamethrin (Suspend SC®) was applied to the upper three feet of all manhole chamber walls at three sites. Applications were made at the lowest label recommended rate of 0.75 fl oz/gal applied at a rate of 1 gal/1000sq ft using a BFG pressure sprayer with a fan nozzle. Due to toxicity of deltamethrin to fresh water and estuarine fish and invertebrates, contamination of the storm drain runoff during application was prevented by employing a unique method by sealing the lower part of the manhole chamber with an inflated beach ball, padded with absorbent cloth (Fig. 2). Treatment efficacy and residual adulticide activity was monitored with EVS/CO_2 traps and by exposure of caged adult female mosquitoes to treated and untreated wall surfaces (Fig. 3).

On September 26, 2002, the three trial sites were first treated with a combination of *Bacillus sphaericus* (VectoLex WDG®) and *Bacillus thuringiensis israelensis* (VectoBac 12 AS®) (1:1) at a dilution rate of 1 lb/1.33 gal of water and applied at a rate of 2 gal/acre using the L.A. Vector/USDS Larvicide Applicator, followed by an application of deltamethrin at the highest recommended rate of 1.5 fl oz/gal applied at a rate of 1 gal/1000 sq ft. The efficacy and residual activity of deltamethrin was monitored as described earlier.

RESULTS AND DISCUSSION

Figure 5 illustrates the results of the adult monitoring in the untreated control system at Lambert Ave throughout the duration of the first trial. Low numbers of adults were initially present due to prior treatment of this system. During the following four weeks, however, adult mosquito numbers increased rapidly to 400 females/trap-night, necessitating flushing of the system with water in order to limit the impact of the trial on mosquito abundance in the

Figure 1. Location of trial sites 1. Pico Vista Rd (north), treated 3. Bequette Ave, treated (L.A. County)2. Pico Vista Rd (south), treated 4. Lambert Ave, "control"

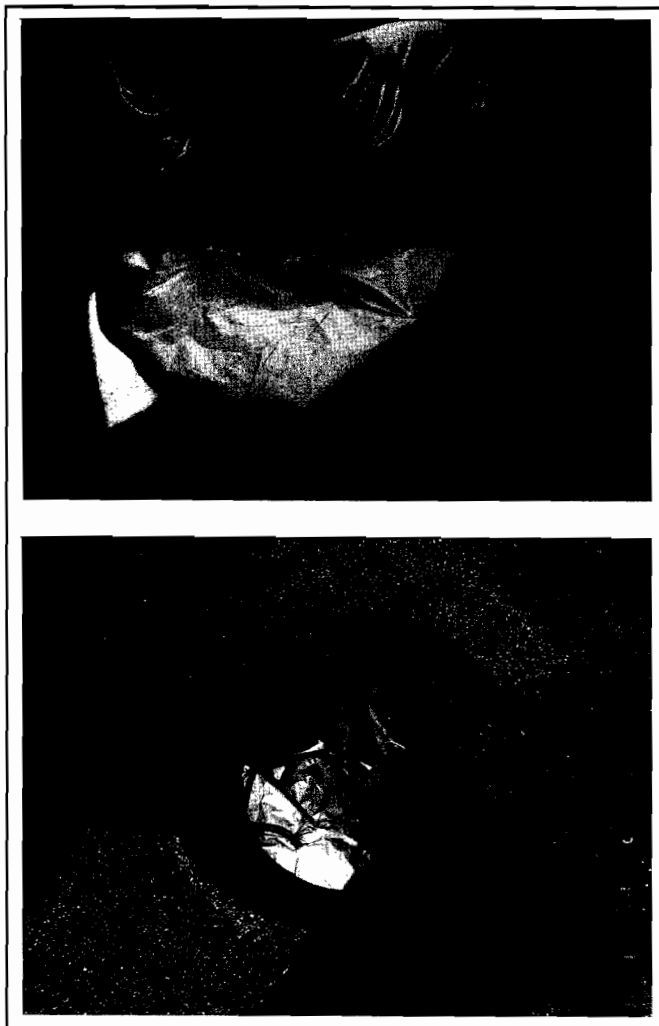

Figure 2. Manhole with inflated beach ball and absorbent padding.

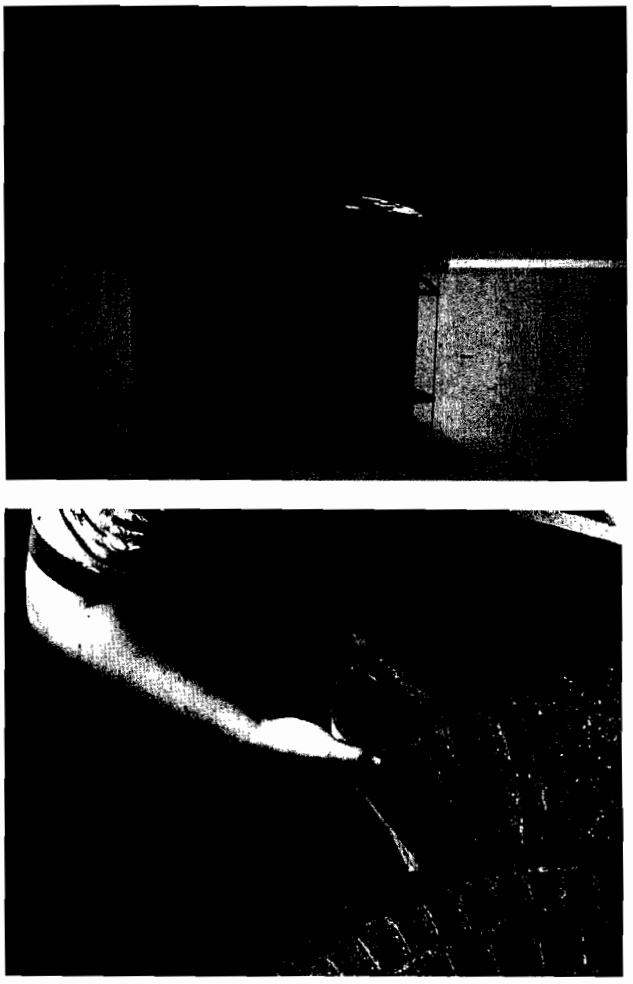

Figure 3. Cages for controlled exposure of adult female mosquitoes to treated and untreated wall surfaces

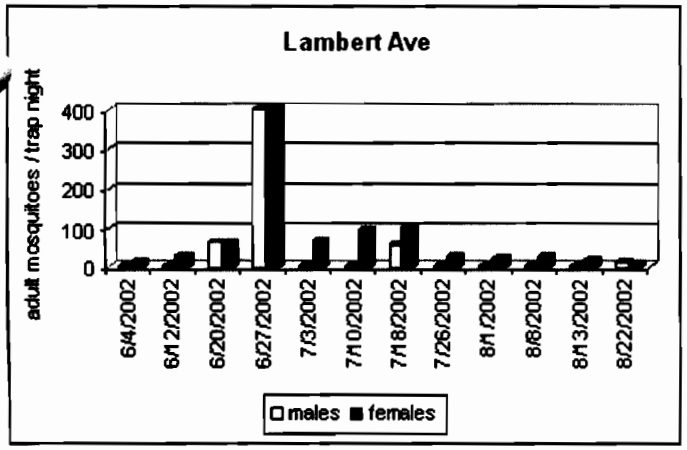

Figure 5. EVS/CO_2 trap results at the untreated control site.

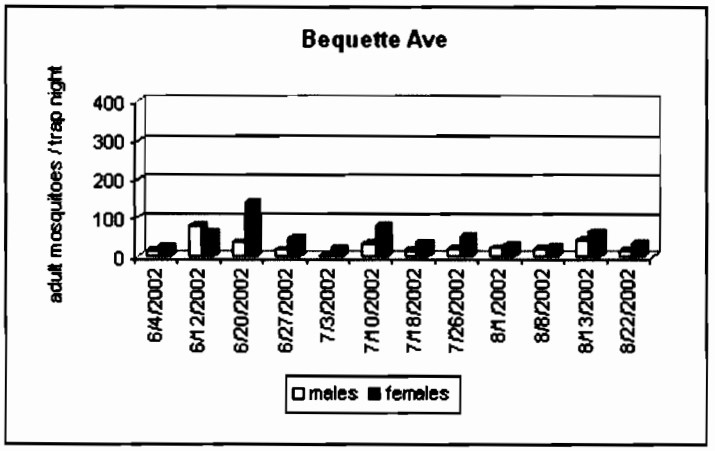

Figure 6. EVS/CO_2 trap data on adult mosquitoes at the Bequette Ave treatment site.

surrounding neighborhood without influencing the system's mosquito productivity for an extended period. The flushing resulted in a reduction of adults, but adult male and female numbers increased once again during the following weeks. Between July 18 and 26, 2002, we observed the presence of used motor oil dumped into the USDS by a resident, which may have effectively prevented mosquito breeding.

Figures 6 through 8 present the results of EVS/CO2 trapping within the treated systems. While adult numbers at Bequette Ave (Fig. 6) and N. Pico Vista Rd (Fig. 7) mostly remained <100 females/trap-night; adult counts at S. Pico Vista Rd (Fig. 8) during the observation period, exceeded 200 females/trap-night. Adult counts at these treated sites never reached the high levels of the untreated control, but the level of control achieved by residual adulticide application at resting sites was unsatisfactory. Mortality rates in caged sentinel mosquitoes (Fig. 9) exposed to the treated manhole walls show, however, that the lack of control success was not due to the ineffectiveness of the adulticide. On June 27, 2002 mortality rates of the exposed adults were 100% at Bequette Ave and S. Pico Vista Rd and 96% at N. Pico Vista Rd; 33% of the untreated control site mosquitoes at Lambert Ave also died, due to hot and dry weather conditions. Survival rate of unexposed mosquitoes improved during the following weeks, as transport and holding conditions of the samples from the field trials were enhanced. Mortality at Bequette Ave and S. Pico Vista Rd remained high through the end of August, while the residual activity at N. Pico Vista Rd decreased and eventually completely subsided. These results demonstrate that mortality was due to mosquitoes resting on the treated walls of the systems, but the population decrease thus achieved was not satisfactory. As expected, the EVS/CO2 trapping results demonstrate a high presence of male adults in all traps throughout the trial period. Since males are not attracted to the EVS/CO2 traps, their presence indicates the proximity of the source and confirms continuity of breeding in the USDS. Thus it became obvious that application of a residual adulticide would have to be combined with larvicidal treatments to achieve acceptable levels of control.

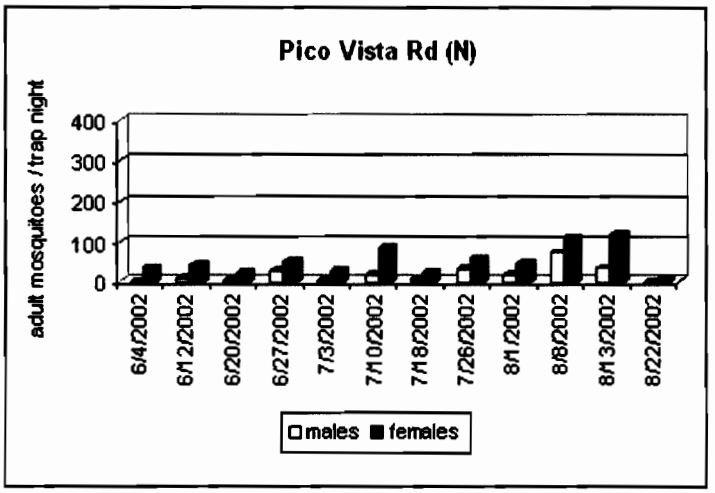

Figure 7. EVS/CO_2 trap data on adult mosquitoes at the Pico Vista Rd north treatment site.

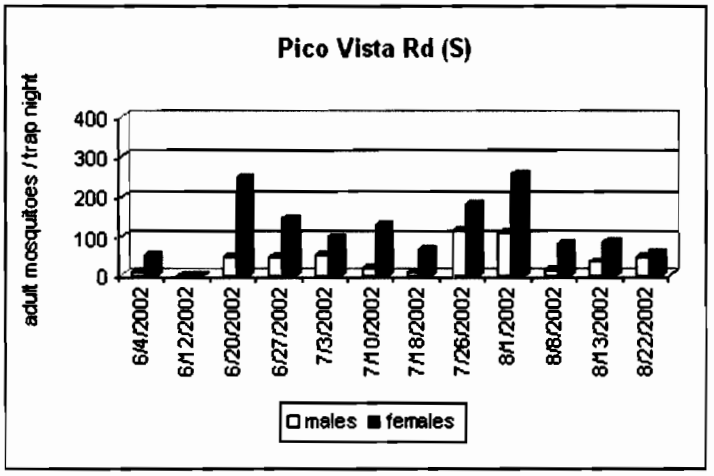

Figure 8. EVS/CO_2 trap data on mosquitoes at the Pico Vista Rd south treatment site.

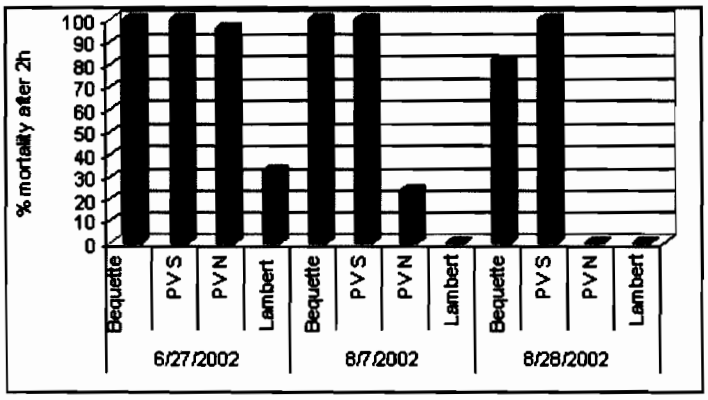

Figure 9. Mortality rate of adult mosquitoes 2 hours after exposure to treated and untreated wall surfaces

The second trial combined larvicidal and adulticidal efforts. Figure 10 illustrates EVS/CO2 trapping results at N. Pico Vista Rd during a larvicidal trial in fall 2000. Low adult male mosquito occurrence demonstrated the success of the larvicidal applications in controlling the immediate breeding source. Female counts, however, remained high due to attraction to the CO2 source from surrounding systems or perhaps from neighboring above-ground areas. Figure 11 shows the results of the combined treatments in 2002. Male as well as female mosquito numbers/trap-night was <100. Although the reduction in the number of males due to effective larviciding had previously been achieved, this level of reduction of fly-in females, however, due to the adulticide treatment of resting surfaces, had not been achieved before.

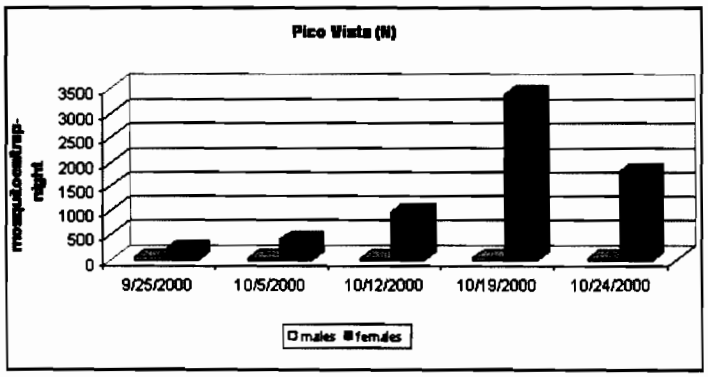

Figure 10. EVS/CO_2 trap data on mosquitoes at the Pico Vista Rd north treatment site.

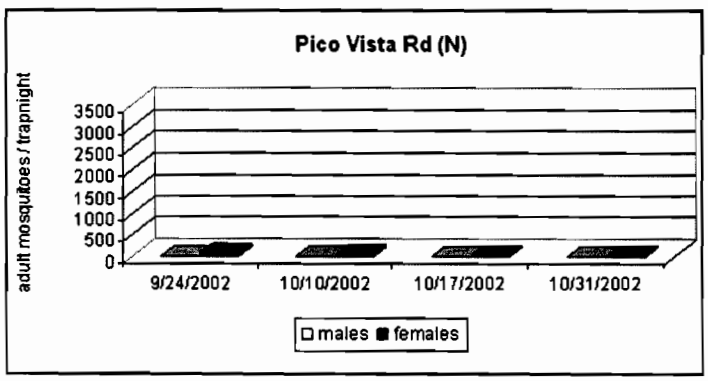

Figure 11. EVS/CO_2 trap data on mosquitoes at the Pico Vista Rd north treatment site.

CONCLUSIONS

Our results demonstrate that residual adulticidal application alone to resting areas within USDS may reduce mosquito populations, but not to satisfactory levels. Larvicidal applications within the USDS effectively prevents mosquito breeding in the treated systems, but warm and moist conditions below-ground still provide attractive resting sites for adults that may have originated from elsewhere. A combination of larviciding and adulticiding appears to achieve satisfactory levels of control. Occasional flushing of the USDS with water significantly enhances control efforts by removal of accumulating debris contributing to stagnant pockets of water. Since USDS are known to be important overwintering shelters, an adulticide application to the resting surfaces in late fall may significantly reduce overwintering mosquitoes, this would result in lower numbers of mosquitoes the following spring. Successful control efforts could potentially reduce not only populations, but also have a positive affect on the number of overwintering females, eventually leading to protection from exposure to arboviral diseases.

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The History of Plague in California: 1900 – 1949

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ABSTRACT: Due to our preoccupation with current emerging infections such as West Nile Virus, Hantavirus, and Lyme disease, we need to be vigilant about pre-existing zoonotic diseases such as plague. The paper presents a general overview of the history of plague and provides an in-depth account of the early history of plague in California. The paper also lists all of the 411 cases of human plague in the state by year, month, locality, sex and outcome for the first 50 years: 1900-1949. It also summarizes the movements and dynamics of both human and animal plague chronologically within the state for those years. Much of the information presented is not covered in recent reviews on this subject.

INTRODUCTION

Plague caused by the bacterium *Yersinia pestis* (Yersin) is a zoonotic disease involving numerous mammalian hosts, primarily rodents, and their fleas. Plague presents in three forms: bubonic; septicemic; and pneumonic. Plague is probably the most feared of all zoonotic diseases known.

There have been several major plague pandemics recorded over the last 1500 years: the "Justinian" plague of 500 to 600 AD; the "Black Death" from the mid 1300s to the mid 1700s, sometimes divided into the "Black Death" from the mid 1300s to late 1500s followed by a reappearance in the mid 1600s to the mid 1700s; and our current plague pandemic, which began in the Yunan Province of China showing up in Hong Kong by 1894 and Calcutta, India by 1895. This recent pandemic spread rapidly around the world, greatly facilitated by the increasing transport of cargo by steamships (Dickie 1926, Link 1955, Twigg 1978). The history of plague will cover the first 50 years (1900-1949) including a list of all human plague cases, in 19 counties and 42 localities within the state. The first indigenous case officially reported as human plague for California (as well as North America) was in San Francisco, a fatal case in a male aged 41, with an onset 7 Feb and death on 6 Mar 1900. This case was followed by three additional fatal cases in Mar, and signaled the onset of the first epidemic in the United States.

Most of the early work on plague in California was done by officers of the United States Public Health and Marine Hospital Services (USPHMHS), and the U.S. Public Health Services Plague Laboratory (USPHSPL) in San Francisco, CA. Link (1955) lists 310 publications, just on plague, by Public Health Service officers from 1897 to 1951, with well over one half, 179 (58%) published by 1916 and 217 (70%) by 1929.

Prior to "recent" publications, a number of taxonomic synonyms exist in literature reviews for an historical background. A few examples related to our search include: the plague bacillus – *Yersinia pestis* (Yersin), *(Bacillus pestis* and *Pasteurella pestis);* the California ground squirrel – *Spermophilus beecheyi* (Richardson), *(Otospermophilus beecheyi* and *Citellus beecheyi);* and the ground squirrel flea – *Oropsylla montana* (Baker), *(Ceratophyllus acutus* and *Diamanus montanus);* even the Norway rat – *Rattus norvegicus* (Berkenhout) in early papers was *Mus norvegicus,* and the black rat—*Rattus rattus* (Linnaeus) was *Mus rattus.*

EARLY HISTORY

There are three excellent reviews on the early history of plague in California: Wherry (1908); Dickie (1926); and Link (1955). The first plague reported for the United States was from the Japanese ship, S.S. Nippon Maru, that arrived in San Francisco, 27 Jun 1899, from Hong Kong via Honolulu. Interestingly, almost 11 years later, 18 Jun 1910, the same ship provided the first isolation of plague from a rat onboard a ship arriving in the United States – also San Francisco (Link 1955).

William B. Wherry (1908) with the USPHMS (Oakland) provides a must-read and excellent, comprehensive and detailed review of plague work and workers in California: "Plague Among Ground Squirrels of California." It goes well beyond the title. Plague was first detected in San Francisco's Chinatown in 1900 with 22 fatalities; 25 in 1901; 41 in 1902; and 17 fatalities in 1903, all cases from San Francisco. One of the early cases in Aug 1903, was a blacksmith from Pacheco, Contra Costa County (CCC) who had been squirrel hunting 3-4 days before onset. When he became sick, he went to German Hospital, San Francisco and died of plague. Rupert Blue, in charge of plague control in San Francisco while investigating one of the cases said: "I was impressed with the possibility of ground squirrel infection in Contra Costa County..." suggesting that these two cases were possibly related to ground squirrel transmission. It had been rumored in 1903 that an epizootic was devastating the California ground squirrels (CGS) in CCC the previous year. D. H. Curry of the plague laboratory, in 1904, was first to show experimentally that CGSs were susceptible to plague (Wherry 1908). Among early cases of plague reviewed, was a boy that died of plague, 15 JUL 1908, on a ranch in CCC. This case resulted in the first confirmation of naturally infected CGSs by McCoy (1908) in a squirrel from the same ranch on 5 Aug 1908, and by Wherry (In: McCoy 1908) on 24 Aug 1908. In the same Public Health Reports (i.e. 11 Sep 1908), Annon. (1908) provides the first plague case from Southern California (Los Angeles) a boy bitten on the finger by a CGS on 5 or 6 Aug, sickened on 11 Aug and seen by

a physician on 12 Aug. This case was followed up by McCoy (Wherry 1908) who established the boy's age as 10 and that the bite occurred in Elysian Park (not Buena Vista Park), near the Southern Pacific Railroad yard. The sick CGS was apparently killed by a dog or cat, however, on 21 Aug, a plague positive dead CGS was found within 50 yards of the bite location. According to Dickie (1926) the infected CGSs were probably carried from the San Francisco area south by train to the railroad yard near Elysian Park. In respect to the first probable squirrel-bite transmission, Currie (Wherry 1908) showed that a considerable percentage of plague inoculated CGSs develop plague pneumonia, and that the mucus in the posterior nares was proven infectious, suggesting that the bite of a plague-sick CGS could result in transmission. A worker named Klein, in 1906 (Wherry 1908) suggested that the initial human case of a pneumonic plague outbreak may arise from a non-human pneumonic host. As well as reviewing the early cases, Wherry (1908) covers the epidemiologic, pathologic and bacteriologic factors; the natural and experimental aspects; and the interrelationships of the CGSs, rats, humans, and their fleas. Besides the CGS and rat fleas, it also includes the human flea, *Pulex irritans* Linnaeus. He mentions that a plague infected squirrel hunter could return to an urban area and infect rats via squirrel fleas, or by the human himself being the source of infection for human fleas. At the time, *P. irritans* was relatively common and could sometimes be found in great numbers on Norway rats. In a footnote he states that they found an infected rat in the basement of a vacant house in Oakland:

"It [the basement] was riddled with rat runways...it was so heavily infested with fleas that the dust on the floor could be seen to pulsate with their movements. Four sheets of flypaper were placed on the floor of the basement for one minute and then removed. ...one with 100 fleas...another...115 fleas...[the third] about 95...the fourth about 75 fleas...at least one of these [P. irritans] was plague infected."

This observation is of interest because the human flea could have played a role in the plague outbreaks of the San Francisco Bay area. Ell (1980) in an interesting (but seldom cited) study, developed a relatively strong case that the major inter-human transmission of medieval plague was by *P. irritans,* not a rat flea or pneumonically, even though both were involved in inter-human transmission (see also Gottfried 1985). It was also known that the ground squirrel flea, *O. montana* and *Hoplopsyllus anomalus* (Baker) had been identified from Norway rats by R. W. Doane in Aug 1908 (Wherry 1908) and that it showed a possible connection between rats and CGSs.

An outstanding worker at the time was G. W. McCoy, USPHMHS (San Francisco) who published 37 papers on plague (as either sole or senior author); 35 were published between 1908 and 1914. His work covered all aspects of plague including seminal papers on wild rodent plague, for example: the first demonstration of a naturally plague positive CGS (1908); followed up by showing that ground squirrel fleas, *O. montana* and *H. anomalus* would bite humans (Wherry 1908); virulence studies on various plague cultures (1909a); rodent susceptibility to plague in gophers, field mice (voles) and CGSs (1909b); immunity of San Francisco rats to plague (1909c); the first case of squirrel plague in man which came to autopsy in America, with Wherry (1909); pathology and bacteriology of plague in naturally and experimentally infected CGS (1909d); and among other studies he proved conclusively that *O. montana* transmits plague from one CGS to another (1910), also a first. He also followed up the first case of plague in southern California (Los Angeles).

William M. Dickie (1926) from the California State Board of Health (Sacramento) covers the period 1900 – 1925. About 2/3 is devoted to the Los Angeles pneumonic plague outbreak of Oct 1924, where 30 of 32 cases were fatal. The first pneumonic outbreak was in Oakland during Sep 1919, when 13 of 14 cases were fatal, Los Angeles was the second. By 1926, 10 counties in California had reported human plague associated with the CGS. It was suggested that the second plague outbreak in the San Francisco Bay area, commencing in 1907, was due to transmission from infected CGSs to rats. Of interest, it was noted that over the years, tons of CGSs were shipped into San Francisco and Oakland for food, at $0.75 to $1.25 per dozen (big money for the late 1800s and early 1900s). Higher prices were offered by fancy restaurants, since they could obtain four gourmet "frog legs" from a single CGS. This practice could have contributed to plague in the San Francisco Bay area.

Vernon B. Link (1955), PHSPL, San Francisco, authored Public Health Monograph No. 26; a very detailed history of plague in the United States. It has great vintage photographs, and much of it is particularly relevant to plague in California.

We have selected these earlier days of plague in California at greater detail to show both the breadth and depth of detective work and solid research undertaken, primarily during the first two decades of the Twentieth Century. We also wanted to showcase several of the many early plague workers and some of their interesting thoughts and deductions. Now-a-days, in this computer age, there is a tendency to think that research done 10-20 years ago is of little or no value. Our review shows that most of the basic groundwork of plague in California was completed 90-100 years ago.

Human plague cases were reported from 19 California counties from 1900-49 and are listed by fatal/total cases at a decreasing case rate: San Francisco 196/289, Los Angeles 36/42, Alameda 28/41, Contra Costa 13/15, San Benito 3/4, Siskiyou 3/4, Monterey 1/2, Santa Cruz 0/2, Stanislaus 0/2, Fresno 1/1, Modoc 1/1, Santa Barbara 1/1, Tulare 1/1, Placer 0/1, San Bernardino 0/1, San Joaquin 0/1, San Luis Obispo 0/1, Santa Clara 0/1, and Sonoma 0/1.

The human plague cases in California from the first 50 years, 1900-49 are shown in Table 1. The 411 cases from California are presented as an annual summary with accumulated totals by five year intervals from 1900-09, 10 year intervals from 1910-29, and the 20 years 1930-49 as the last interval. The data for the list have been extracted and modified from an appendix in Link (1955), which listed all of the individual human plague cases for the entire United States from 1900-51.

Summary of the first five years 1900-4. The index case for the San Francisco epidemic was a 41 year old male with an onset 6 Feb and death on 6 Mar. '00. This first epidemic in San Francisco was primarily restricted to Chinatown and lasted until Feb '04. There were 125 cases reported during this period with a mortality rate of 97.6%. All cases were from San Francisco until 1903 (3 ½ years after the index case) when 3 cases, all male, all fatal, occurred, Aug

Table 1. Human plague cases in California 1900-49.

YEAR (S)	MALE			FEMALE			? M/F			TOTAL		
	F	R	T	F	R	T	F	R	T	F	R	T
1900	17	0	17	5	0	5				22	0	22
1901	20	0	20	7	1	8	3	1	4	30	2	32
1902	37	0	37	5	0	5				42	0	42
1903	13	0	13	6	0	6	1	0	1	20	0	20
1904	4	0	4	4	1	5				8	1	9
1900-1904	91	0	91	27	2	29	4	1	5	122	3	125
% FATAL		100.0			93.1			80.0			97.6	
% SEX/125		72.8			23.2			4.0				
% SEX/120 Adj for ?		75.8			24.2			0				
1905	N/A											
1906	0	1	1	0	0	0				0	1	1
1907	61	66	127	28	29	57				89	95	184
1908	3	4	7	2	0	2				5	4	9
1909	2	2	4	0	0	0				2	2	4
1905-1909	66	73	139	30	29	59	0	0	0	96	102	198
% FATAL		47.5			50.8			N/A			48.5	
% SEX/198		70.2			29.8			N/A				
1910	1	0	1	0	1	1				1	1	2
1911	1	4	5	0	0	0				1	4	5
1912	N/A											
1913	1	0	1	1	0	1				2	0	2
1914	0	1	1	0	0	0				0	1	1
1915	1	0	1	0	0	0				1	0	1
1916	N/A											
1917	N/A											
1918	N/A											
1919	10	0	10	3	1	4				13	1	14
1910-1919	14	5	19	4	2	6	0	0	0	18	7	25
% FATAL		73.7			66.7			N/A			72.0	
% SEX/25		76.0			24.0			N/A				
1920	0	0	0	1	0	1				1	0	1
1921	1	1	2	0	0	0				1	1	2
1922	1	1	2	0	0	0				1	1	2
1923	0	0	0	0	1	1				0	1	1
1924	22	2	24	12	2	14				34	4	38
1925	1	0	1	0	1	1				1	1	2
1926	N/A											
1927	1	0	1	0	0	0				1	0	1
1928	1	1	2	1	0	1				2	1	3
1929	N/A											
1920-1929	27	5	32	14	4	18	0	0	0	41	9	50
% FATAL		84.4			77.8			N/A			82.0	
% SEX/50		64.0			36.0			N/A				
1930-1932	N/A											
1933	1	0	1	0	0	0				1	0	1
1934	1	0	1	0	0	0				1	0	1
1935	N/A											
1936	0	3	3	0	1	1				0	4	4
1937	0	0	0	1	0	1				1	0	1
1938-1940	N/A											
1941	2	0	2	0	0	0				2	0	2
1942	0	0	0	1	0	1				1	0	1
1943	0	1	1	0	0	0				0	1	1
1944	0	1	1	0	0	0				0	1	1
1945-1946	N/A											
1947	1	0	1	0	0	0				1	0	1
1948-1949	N/A											
1930-1949	5	5	10	2	1	3	0	0	0	7	6	13
% FATAL		50.0			66.7			N/A			53.8	
% SEX/13		76.9			23.1			N/A				
1900-1949	203	88	291	77	38	115	4	1	5	284	127	411
% FATAL		69.8			67.0			80.0			69.1	
% SEX/411		70.8			28.0			1.2				
% SEX/406 Adj for ?		71.7			28.3			N/A				

Pacheco, Sep Pinole and San Ramon, followed by a fourth case in Feb '04, a fatal Concord female. Thus only 3.2% of the cases from Feb '00 to Feb '04 were located outside of San Francisco. The first non-fatal case did not show up until a female with onset 9 Jul '01. A second non-fatal case, also female, whose onset was 7 Feb '04 and represented the last human case of the first epidemic. There was, however, an additional non-fatal case among the five "sex not known" individuals, onset 12 Dec '01. Therefore only three of 125 cases (2.4%) recovered from plague from Feb '00 to Feb '04.

The next five years, 1905-09, included a second epidemic in San Francisco following the earthquake in 1906. There was also a small epidemic in Oakland in '07. No cases were recorded in '05 and only one in '06 at Oakland, a male 14 onset in Apr and represents the very first male to survive in California from plague, and it was also the first case for Oakland. This second San Francisco outbreak 1907-08, was widespread, compared to the first, the index case a male 56, onset 24 May, death 26 May. The last case was a male 19 non-fatal, onset 7 Mar '08. There was a total of 167 cases and unlike the first epidemic, there were more non-fatal (89) than fatal cases (78) i.e. 53.3% and 46.7%, respectively. By sex the mortality rates were similar: male 47.1% (56/119) and female 45.8% (22/48). Recall that the mortality rate for the first epidemic was 97.6% and only 3 of 125 or 2.4% recovered. The epidemic in Oakland was primarily in 1907 and the total cases during the period '05-09 for Oakland were 20 of which 10 were fatal, 50.0%, male 41.7% (5/12) and female 62.5% (5/8). During the five year period, there were 198 cases, 96 fatal (48.5%); the male rate was 47.5% (66/139) and female 50.8% (30/59). There were 11 cases in 9 localities outside of San Francisco and Oakland of which 72.7% (8/11) were fatal. Eight of the 9 localities were in the Bay area, the ninth locality was '08 At Los Angeles (the boy bitten by the CGS), representing the first locali outside of the Bay area and the first case from southern California. This case is most likely associated with the Bay area plague outbreaks since the squirrels involved probably hitched a ride on a train bound south to the train yard near Elysian Park.

The ten years 1910-19 provided 8 new localities. There were no cases reported in '12, '16, '17 and '18. With the exception of the first southern California case in '08, the first California cases reported from localities outside of the San Francisco Bay area were in 1910, Jun Hollister and Aug Coyote. The first cases reported from the Central Valley were 1911, Apr and Jul Modesto, and Sep Ripon. The first pneumonic plague outbreak in North America was in Oakland from 15 Aug to 25 Sep 1919, 92.9% (13/14) fatal. It was a classical well-documented outbreak. The index case had just returned from squirrel hunting, a 32 year old male onset 15 Aug, died 19 Aug. A male 32 that lived in the same house had an onset 25 Aug, died 2 Sep and presumably infected 5 others, 3 of which infected 7 others. The chain of 14 cases with association, date of onset and outcome are summarized in Table 2 (Dickie 1926, Link 1955).

The ten years, 1920-29, also provides 8 new localities. No cases were recorded for '12, and '15-19. The second pneumonic plague epidemic in California occurred at Los Angeles in 1924 Oct-Nov. With the exception of the Los Angeles outbreak, all of the 10 localities involved a single case 7 male, 3 female, 6/10 were fatal. All localities were from the East Bay south to Santa Ynez. The Los Angeles outbreak was covered in detail in both Dickie (1926) and Link (1955 Of the 40 cases from 10 Oct to 15 Jan 1925, 32 were pneumonic, of which 2 recovered, 93.8% fatal, the remainder were bubonic. A 55

Table 2. Summary of first pneumonic plague outbreak in Oakland in 1919.

Case No.	Gender*	Date of onset	Association
1	M	15 – 19 Aug	Squirrel hunter
2	M	25 Aug – 2 Sep	Same house as 1
3	F	29 Aug (non-fatal	Wife of 2
4	M	29 Aug – 2 Sep	Landlord of 2
5	M	30 Aug – 2 Sep	Visited 2
6	M	31 – 25 Sep	Visited 2
7	F	1 – 4 Sep	Nurse of 2
8	M	5 – 9 Sep	Doctor of 4
9	M	5 – 8 Sep	Cousin of 5, rode to hospital with 5
10	M	5 – 8 Sep	Visited 5 in hospital
11	M	5 – 8 Sep	Same house as 6
12	M	5 – 8 Sep	Doctor of 6
13	F	6 – 10 Sep	Same house as 6
14	F	6 – 11 Sep	Nurse of 6

*M—male, F—female

year old male was responsible for the beginning of the outbreak with onset 1 Oct, non-fatal. His 14 year old daughter onset 1 Oct, died 5 Oct was the origin of the pneumonic cases when her bubonic plague, became secondary pneumonic plague. Like the Oakland chain of events, most of cases were: relations, friends, boarders, nurses, a priest, even an ambulance driver. Several had "no contact known" including the last three cases following 11 Nov. This was a port-associated outbreak, primarily involving rats.

The next 10 years, 1930-39, added 8 new localities even though there were 6 years with no human plague cases, '30-32, '35 and '38-39. The new cases included: Santa Rosa APR '36, representing the first case north of San Francisco Bay and west of the Sacramento River—occurring 37 years after the outbreak just to the south; San Bernardino Jul "36 represents the first case in the Inland Empire; and Lake Tahoe Jul '36 the first case on the east side of the Sierra Nevada. The following year there was an additional case at Lake Tahoe May '37 but on the Nevada side, resulting in the first human case in Nevada and undoubtedly a result of the eastward movement of plague out of California.

The ten years 1940-49 had 5 years without plague cases, '40, '45-46 and '48-49. The 6 cases recorded included 5 new localities with a noticeable move north. San Francisco had its first case in 32 years, a 36 year old male, onset 30 Mar '44, non-fatal. Mt. Shasta City Aug '41 and Yreka NOV '42 exemplify the northern shift. The Alturas case Jun '47 was not only the farthest north in the first 50 years, it was also the last case of human plague in the first 50 years.

There were 411 human cases in 19 different counties and 42 different localities in California: from Alturas near the Oregon border to San Diego bordering Mexico; the Pacific to the east side of the Sierra Nevada and the Great Basin; and a few feet above sea level to well over one mile in elevation.

The overall mortality rates for male (M), female (F) and overall (411 cases) were: 69.8%, 67.0% and 69.1% respectively. The rates 1900-29 between the M and F by accumulated years, shows a slightly higher level among the Ms at 7 percentage points, with the exception of the 1905-09 cases when the female rate was 3.3% above the Ms. The highest mortality rates related to: 1) the first San Francisco epidemic at 97.6%, M 91 all fatal, and F 27/29 at 93.1%; and 2) the second related to the Los Angeles pneumonic outbreak at 82.0%, M 27/32 at 84.4% and F 14/18 at 77.8%. The high recovery rates during the second San Francisco epidemic and Oakland resulted in less than 50% mortality: 96 of 198 at 48%; M 69/139 at 47.5%; and F 30/59 at 50.8%. The reciprocal yields the recovery rate 51.5%, compared to 2.4% 1900-05, 28.0% for 1910-19, 18.0% for 1920-29 and 46.2% for 1930-49.

The overall percentage of male (M) to female (F) was 71.7% to 28.3% a ratio of 2.5:1. All of the accumulated percentages were Ms in the 70%s, ranging from 70.2% 1905-09 to 76.0% 1910-19, with the exception of the years 1920-29 which included the Los Angeles outbreak where the Ms dropped to 64.0%. The ratios of M to F for each of the accumulated years were: 1900-04 3.1:1; '05-09 2.4:1; '10-19 3.2:1; '20-29 1.8:1; and '30-49 3.3:1. The lower ratio of '05-09 and '20-29 relate to the second San Francisco and Oakland outbreaks, and the Los Angeles outbreak, both of which had ratios less than 3:1, showing relatively higher F cases compared to M cases in those outbreaks.

Isolations of plague from animals were reported from 35 counties in California during the first 50 years of plague (Table 3). Isolations were made between 1902-48, however first isolations only occurred during 17 of these years. The first in 1902 and the last in 1946.

The first isolations for San Francisco '02 and Alameda '07, were from rats. The following year, '08 provided the first isolations from wild rodents – CGS in AUG: 1) from a ranch in Contra Costa 5 Aug; and 2) a couple weeks later in Los Angeles, related to the squirrel bite case. These 2 isolations demonstrated the first real evidence of wild animal plague in the United States, and as a result, antiplague work shifted from rats to infected wild animals, especially CGS. Organized plague surveys were instituted and produced 3 new counties in '09 and 5 more in '10, all south of San Francisco. The

Table 3. Chronology of first isolation of plague organism by county in California.

Year	County
1902	San Francisco
1907	Alameda
1908	Contra Costa, Los Angeles
1909	San Benito, Santa Clara, Santa Cruz
1910	Merced, Monterey, San Joaquin, San Luis Obispo, Stanislaus
1911	Fresno
1916	San Mateo
1928	Ventura
1929	Santa Barbara
1934	Kern, Modoc, Tulare
1935	Lassen
1936	Eldorado, Placer, San Bernardino
1938	Plumas
1941	Shasta, Siskiyou
1942	Alpine, Marin, Mono, Riverside, San Diego
1943	Inyo, Kings, Nevada
1946	Orange

Central Valley had its first counties added in 1910. San Mateo, the county bordering San Francisco to the south finally was added in 1916, 14 years after the first San Francisco isolation. Ventura '28 and Santa Barbara '29 filled in the coastal counties from San Francisco to Los Angeles. A big jump occurred when Modoc '34, in the northeast corner of the State, was declared plague positive by wild animal isolation. This coincided with the first human case from Oregon at Lake View just across the border. Lassen '35 joined Modoc the following year. Two Sierra Nevada counties were added in '36, another in '38. The far north added two in '41. The next year '42, produced 5 counties scattered all over the State: Alpine, a Sierra Nevada location; Marin just across the Golden Gate Bridge from San Francisco, 40 years after San Francisco; Mono became the first entirely on the east side of the Sierras, followed the next year by Inyo '43; also in '42 Riverside in the southeast and San Diego in the southwest corner of the State, bordering Mexico. The last county to show up on the list was Orange '46, finally filling in the only gap in the coastal counties from San Francisco to San Diego. No counties were added during the next 3 years, '47-49 thus rounding out the first 50 years of animal plague in California.

To better understand the history of plague in California, we should recognize the timing and the extent of the human plague cases and outbreaks that occurred, out of state, from 1900-49.The chronological sequence of the other 10 states is: MI; WA; LA; FL; TX; OR; UT: NV: ID: and NM. A summary of the states that imported human plague cases follows: 1) MI '01 Apr Ann Arbor recovered (R)—this case resulted from plague specimens sent from San Francisco to a laboratory in Ann Arbor; 2) WA '07 Oct Seattle 7 fatal F, '13 Dec 1 F; 3) LA '14 Jun-Sep, '15 Aug, '19 Oct-Dec New Orleans 18 F 33 R; 4) FL '20 Jun-Jul Pensacola 7 F 6 R; 5) TX '20 Jun-Oct Beaumont, Galveston, Houston, Port Arthur 17 F 14 R; 6) OR '34 May Lake View 1 F; 7) UT '36 Jun Beaver 1 R; 8) NV '39 May Lake Tahoe 1 R; 9) ID '40 Sep Emmitt 1 F; and 10) NM '49 Jul, Nov Cerro, Placitas, Patrico 1 F 2 R (also '50 Jan-Jul Maljamar, Gloricia, Canada, and 1951 Jan Hobbs 1 F 3 R). The WA, LA, FL and TX cases were port related primarily from murine (rat) plague, while OR, UT, NV, and NM were from wild animal plague.

The Hawaiian Islands (like Alaska) did not attain statehood until 1959, consequently was not included in the human plague cases in the United States. Plague in the Territory of Hawaii closely paralleled our human plague in California. The first human plague cases in what is now the United States were in the Territory of Hawaii during Dec 1899, with 11 of 12 F. The index case died on 11 Dec, 3 months before the first fatality in California 6 Mar '01 (onset 11 Feb '01). Interestingly the same ship, the SS Nippon Maru, that may have brought the plague to California, first docked in Honolulu enroute to docking in San Francisco, on 27 Jun 1899. Outbreaks and cases of plague were recorded from 1899 to 1949, resulting in 410 cases, 375 fatal F (91.5%) on the islands of Oahu, Hawaii, Maui and Kauai. A summary of the 410 human cases by island is: Oahu 1899-1910, 204/278 F (89.5%); Hawaii-Hilo '00-18, 34/43 F (79.1%) and Hamakau '10-49, 111/112 F (99.1%); Maui '00 and '30-38, 15/16 F (93.8%); and Kauai '01-02, 11 all F. The Oahu, Hilo, Maui '00, and Kauai outbreaks were port related while the Hamakau district on the big island and Maui '30-38 were primarily due to non-commensal rodents.

The Territory of Puerto Rico also recorded human plague cases 62 of 81 (76.5%) in two epidemics: 1912, 36 of 55 fatal (65.5%) and 1921 with 26 cases all fatal.

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