PROCEEDINGS AND PAPERS

of the

Seventy-Sixth Annual Conference of the Mosquito and Vector Control Association of California

January 13-16, 2008

Held in Palm Springs, California

Editor: Dr. Richard Meyers

Reviewer:
Dr. Richard Meyers

Layout and Editorial Assistance: Arthur Wilfong and Ashley Paul, MVCAC

Mosquito and Vector Control Association of California
1215 K Street, Suite 2290
Sacramento, California 95814
Phone: (916) 440-0826 Fax: (916) 231-2141
E-mail: mvcac@mvcac.org

Published February 2009

2008
Mosquito and Vector Control Association of California

Catherine Smith, *Executive Director*
Dr. Richard Meyers, *Proceedings Editor*

BOARD OF DIRECTORS

President: David Brown
President Elect: John Rusmisel
Vice President: Jerry Davis
Past President: Steve West
Chair, Trustee Advisory Council: Lyndon Hawkins
Coastal Rep: Jim Wanderscheid
Sacramento Valley Rep: Mike Kimball
North San Joaquin Valley Rep: Lloyd Douglass
South San Joaquin Valley Rep: Michael Alburn
Southern California Rep: Mitchel R. Weinbaum

TRUSTEE ADVISORY COUNCIL

Chair: Lyndon Hawkins
Vice Chair: Chuck Balch
Secretary: Russ Bellici
Coastal Rep: Dennis Preger
Sacramento Valley Rep: Lyndon Hawkins
North San Joaquin Valley Rep: Chuck Balch
South San Joaquin Valley Rep: Richard Robinson
Southern California Rep: Henry Morgan

CORPORATE MEMBERS

Alameda County MAD
Alameda County VCSD
Contra Costa MVCD
Marin-Sonoma MVCD
Napa County MAD
No. Salinas Valley MAD
San Benito County Agri. Comm Office
San Francisco Pub. Health Env. Health
San Mateo County MAD
Santa Clara County VCD
Santa Cruz County MVC
Solano County MAD
Burney Basin MAD
Butte County MVCD
Colusa MAD
Durham MAD
El Dorado Co. Vector Control –CSA3
Glenn County MVCD
Lake County VCD
Nevada County Dept. of Agriculture
Oroville MAD
Pine Grove MAD
Placer MVCD
Sacramento-Yolo MVCD
Shasta MVCD
Sutter-Yuba MVCD
Tehama County MVCD
East Side MAD
Merced County MAD
Saddle Creek Community Svcs Dist.
San Joaquin County MVCD
Turlock MAD
Coalinga-Huron MAD
Consolidated MAD
Delano MAD
Delta VCD
Fresno MVCD
Fresno Westside MAD
Kern MVCD
Kings MAD
Madera County MVCD
San Luis Obispo County MAP
Tulare MAD
West Side MVCD
Antelope Valley MVCD
Coachella Valley MVCD
Compton Creek MAD
June Lake Public Utilities District
Long Beach – Vector Control Program
Los Angeles Co. W. VCD
Greater L.A. County VCD
Mammoth Lakes MAD
City of Moorpark Vector Control
Northwest MVCD
Orange County VCD
Owens Valley MAP
Riverside Co. Dept. Environ. Health
San Bernardino Co. VCP
San Gabriel Valley MVCD
MVMD of Santa Barbara County
West Valley MVCD

2008 MVCAC SUSTAINING MEMBERS

ADAPCO

Allpro Vector Group

Amvac

AMVAC Chemical Corporation

B&G Products of California, Inc.

B2E/Aquaprene

AMVAC Environmental Products

Clarke Mosquito Control Products, Inc.

Cognis Corporation

Curtis Dyna-Fog Ltd.

Dynamic Aviation

Harris & Associates

National Geographic Maps

Points West Argo, Inc.

Univar USA, Inc.

Valent BioSciences

Wellmark/Zoecon

Willdan Financial Services

Zoecon Professional Products

CONTENTS

Board of Directors, Trustee Advisory Council and Corporate Members.....ii
Sustaining Members.....iii

Conference Dedication

2008 MVCAC Conference Dedication, Dr. James "Jim" P. Webb, Jr.....1
Martine Jozan

Proceedings

2007 Year-in-Review: Integration of NASA's Meteorological Data into the California Response Plan.....7
Christopher M. Barker, Bborie K. Park, Forrest S. Melton, Bruce F. Eldridge, Vicki L. Kramer, and William K. Reisen

Aerial Adulticiding in Sacramento County, California, 2007 – How Was It Different from 2005 and 2006? 13
Paula A. Macedo, Marcia Reed, Kara Kelley, Gary W. Goodman, David A. Brown

Assessment of Barrier Applications of Demand® (Lambda-cyhalothrin) in Rural Landscapes in the Coachella Valley, California.....22
Hugh D. Lothrop, Branka B. Lothrop, William K. Reisen and Donald E. Gomsi

Building upon California's Surveillance Gateway.....27
Bborie K. Park, Bruce F. Eldridge, Christopher M. Barker, William K. Reisen

Comparing Mosquito Attractiveness to Bird-, Mammal-, and CO2-Baited Traps in Rural and Suburban Areas.....29
Marilou Thomas, Beatriz Perez, Paula A. Macedo, Stan Wright, David A. Brown

Evaluation of Arboviral Activity in Orange County, California, during 2007.....33
Robert Cummings, B. Fred Beams, Stephen G. Bennett, Karin De Collibus, Richard Evans, DVM, MS, Carrie Fogarty, Jim Francisco, Tunisia Hardy, Ralph Havickhorst, Catalina Herrera, Martine Jozan, MD, DrPH, Taylor Lura, Ivann Martinez, Toby McLaughlin, Tim Morgan, Kiet Nguyen, Tom Reynolds, Art Tilzer, Robert Velten, Josie Weir, and J.P. Webb, Jr.

Field Biology and Fieldwork – Challenges for a New Generation.....46
Richard M. Davis

Future Directions in Data Management.....50
Bruce F. Eldridge, Bborie K. Park, Christopher M. Barker, and William K. Reisen

Genetics and Pathogenesis of West Nile Virus.....54
Aaron C. Brault, Stanley A. Langevin, Payal D. Maharaj, Richard A. Bowen, Ying Fang and William K. Reisen

How Climate Affects Mosquito Biology and Arbovirus Transmission.....57
William K. Reisen

Human Cases of Flea-Borne Typhus in Orange County, California, during 2006 – 2008.....64
Robert Velten, Art Tilzer, Stephen G. Bennett, Robert Cummings, Carrie Fogarty, and Ralph Havickhorst

Human Health Risk Assessment of the Aerial Adulticiding Conducted in 2007 in Sacramento County.....71
Leslie M. Shama, Paula A. Macedo, Gary W Goodman, David A. Brown

Identifying the Reservoirs of the Lyme Disease Spirochete Borrelia burgdorferi (Sciurus Griseusi), in California: the Role of the Western Gray Squirrel.....73
Daniel J. Salkeld, Sarah Leonhard, Yvette A. Girard, Nina E. Hahn, Jeomhee Mun, Kerry A. Padgett, Robert S. Lane

Impact of West Nile Virus on California Birds.....77
Sarah S. Wheeler, C.M. Barker, B.D. Carroll, W.K. Reisen

Introduction of the Scorpion *Centruroides exilicauda* (Wood) into Southern California Communities: a Public Health Perspective.....80
Lawrence R. Bronson, Laura Krueger, and Kenn K. Fujioka

Introduction to symposium: Research on Arboviruses in California - Year 5.....87
William K. Reisen

Monitoring and Modeling Environmental Conditions Related to Mosquito Abundance and Virus Transmission Risk with the NASA Terrestrial Observation and Prediction System.....89
Forrest S. Melton, Rama R. Nemani, Andrew Michaelis, Christopher M. Barker, Bborie K. Park, William K. Reisen

Research on Arboviruses in California - Year 5: Some Concluding Thoughts.....94
William K. Reisen

Responding to West Nile Virus in Santa Clara County.....98
Noor S. Tietze, Tim Mulligan, Russ Parman and Nayer Zahiri

Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 2007.....108
Tina Feiszli, Stan Husted, Bborie Park, Bruce Eldridge, Ying Fang, William K. Reisen, Cynthia Jean, Cindi Cossen, Ryan Carney, Erin Parker, Claudia Erickson, Alana McQuarry, and Vicki Kramer

Symposium: Improving the Use of Climate Variation in Decision Support Systems – Introduction.....124
William K. Reisen

The California West Nile Virus Hotline as a Public Education Tool.....126
Long Her and Stan Husted

The Hunt for the New West Nile Virus in California.....127
Veronica Armijos, Andrew Chow, Payal D. Maharaj, Ying Fang, William K. Reisen and Aaron C. Brault

Use of the California Mosquito-Borne Virus Surveillance & Response Plan: Los Angeles - a Case Study.....129
Susanne Kluh

Using the Surveillance Gateway to Facilitate Recordkeeping.....137
Charles W. Smith, Jodi J. Holeman

West Nile Virus Activity in Kern County and the Factors Leading to the 2007 Outbreak.....138
Brian Carroll, Richard Takahashi and William Reisen

West Nile Virus State of Emergency: 2007.....146
Vicki Kramer, Tim Howard, Mark Novak, Renjie Hu, and Stan Husted

Where Have All the Western Equine Encephalomyelitis Cases Gone?.....155
Ying Fang, Aaron C. Brault and William K. Reisen

Who Found West Nile Virus Activity in Sacramento and Yolo Counties First...Mosquito, Chicken, or Pigeon?.....160
Kara Kelley, Paula A. Macedo, Marcia Reed, Gary W Goodman, David A. Brown

Guidelines for Contributors

Guidelines.....162

2008 MVCAC Conference Dedication
Dr. James "Jim" P. Webb, Jr.

Martine Jozan[1]
mjozan@ocvcd.org

[1]Orange County Vector Control District, Garden Grove, California, USA

The 76th Annual Conference of the Mosquito and Vector Control Association of California (MVCAC) is dedicated to James "Jim" P.Webb, who recently retired from the Orange County Vector Control District (OCVCD). As the Scientific Technical Director of the laboratory, Jim provided innovative leadership in the development of research projects designed for the surveillance and control of vector-borne diseases. By engaging in a cooperative dialogue with numerous academic institutions and other districts nationwide, he was able to foster new programs, expand the scope of smaller projects, and involve the participation of college students in long-term studies.

Jim was born in Long Beach, California, on November 30, 1941. He spent his toddler years in Moutainair, New Mexico, and lived, as a teenager, in Rib Lake, Wisconsin. His family eventually moved back to Harbor City, California, where he attended high school.

In 1970, he obtained a master's degree in biology from Long Beach State University, under the inspiring mentorship of Richard B. Loomis, who was then cataloguing the *Chiggers* of California. Jim became one of the 27 individuals who contributed directly to this extensive taxonomic study (Fig. 1). They formed a competitive team of students. The training was manifold, involving extensive field work and taxonomic expertise (Webb and Loomis 1969). Reports and papers had to be written and Jim acquired editorial skills, which he perfected later as the editor of the Bulletin of the Society for Vector Ecology (SOVE) and of the entire District.

Jim went to the Texas Tech University, Lubbock, for his Ph.D., granted in 1976. His interest had shifted from chiggers to ticks. His dissertation on the host-locating behavior of nymphal *Ornithodoros concanensis* was an elaborate ethological study (Webb 1977 and 1979). This newly acquired expertise of tick biology would prove invaluable in his later investigations of Lyme disease in California (Webb et al. 1990).

Two years of post-doctoral research followed at the University of California, Los Angeles, School of Public Health, where Telford Work was running an NIH-sponsored training program in field and laboratory techniques, especially tailored to the study of arthropod-borne viruses (arboviruses). This was still an emerging field, with little known about the transmission cycle of more than 400 characterized agents. Epidemiologic investigations, in search of western equine, St. Louis and California encephalitis virus foci, were carried out in Imperial County and Owens Valley and southern California. They required intensive field work, for one week or more each month, depending upon the season. Jim was in charge of mosquito collections and identification. At this time mosquito traps were still operated with 12 volt batteries. The adaptation to ordinary flashlight batteries by Don Rohe (State of California) brought a relief to field operations. Another way to assess virus transmission was to evaluate the circulation of antibodies in birds, natural hosts of local flaviviruses. Jim familiarized himself with the bleeding, banding, and releasing of a wide variety of avian species, as well as with various serological techniques. This experience laid the

foundation for a long-term arbovirus surveillance/control program, which was later set up at the District. Upon Telford Work's retirement, and thanks to Jim's foresight, equipment, supplies, and reagents were transferred to the newly constructed Orange County Vector Control District Laboratory.

From 1977 to 1981, Jim worked for Fluor, which at the time was planning to lay a natural gas pipeline along an environmentally sensitive corridor, from Prudhoe Bay, Alaska, to the Canadian Yukon border. He assumed leadership of a team responsible for the ecological and archeological mapping of the area. This helped him hone his managerial skills and analytical talents.

Upon the suggestion and recommendation of Jack Hazelrigg, Jim came to the Orange County Vector Control District in 1981. Recruited at first as a vector ecologist, he introduced new collecting methods to enhance the scope of virus isolations from mosquitoes and larvae as well (Beehler et al. 1993). Mosquito collections from oviposition traps and manholes (underground drains) became routine.

He became Technical Director in 1991, and from 2004 to 2007 served as the Scientific Technical Services Director, managing and overseeing a diverse array of projects. From the very beginning, and with the total confidence and support of Gilbert Challet, a gifted manager, Jim had the freedom to engage in imaginative projects, and thus expand the scope of services offered by the District. The occurrence of the 1983 SLE outbreak, in Long Beach, with 27 human cases, prompted him to put in place a long-term surveillance program, which incorporated the continuous year-round collection of large populations of passerines to canvass most of Orange County (Webb and Myers 1985). This program implemented by John Gruwell (Gruwell et al. 2000) is still the backbone of the District's surveillance 25 years later. These studies have provided a dynamic evaluation of virus transmission over time, and fostered the reassessment of control strategies as needed.

Early on, his unique contribution to the District has been his ability to engage a dialogue with numerous academic and governmental agencies, fostering cooperative research projects, and thus complementing the investigative abilities of the District with unique scientific expertise and technical resources (Fig. 2). These programs have also offered "students in residence" numerous training opportunities and new ideas for their masters degrees.

With his diverse interests, passions, and obstinate curiosity, it is not surprising that Jim became a pioneer in forensic entomology. In this emerging field, the developmental growth of insects is used as a diagnostic tool to illuminate the time and circumstances of death in homicide cases. One of his first investigations was notorious: In 1983 when a young woman was found murdered in a rural area of Ventura County, the boy friend, and prime suspect, denied having ever been near the crime scene. Yet, police investigators and suspect were covered with chigger bites. Chigger collections made on the site, and encountered nowhere else, contributed to a life conviction of the suspect (Webb et al., 1983). Jim was later called upon to assist with police investigations and testify in court as needed.

Although public complaints about insects are common ground for vector control districts, those coming from people affected with delusion of parasitosis (DOP) are unique. These individuals are convinced that mites, bugs, or worms are living on and/or inside their body. Their daily life is completely disrupted, impacting their working ability and social interaction. These irrational complaints could be overwhelming, but Jim was successful in developing a groundbreaking protocol for handling such cases, in cooperation with dermatologists and psychiatrists, to relieve the basic anxiety and obsession suffered by these patients (Webb 1991).

When West Nile virus made its unexpected incursion in New York in 1999, Jim was quick to recognize that the virus would eventually reach California. He immediately supported all efforts to develop, with the support

of the University of Queensland, Australia, a serological test, which could be used in active surveillance for early detection of specific WN antibodies. It took five years for the virus to leapfrog to the western states; and this new test, which had been evaluated in 2003, proved to be a useful and reliable diagnostic tool.

Jim pursued field and laboratory tasks with tremendous energy, aggravated stubbornness, and unrestrained imagination. He also taught at Saddleback and Chapman College, where he was both a lecturer and assistant director, overseeing the health science program.

For his friends, colleagues, and many students, Jim is a leader of innovative scientific programs, a mentor, a fierce and somewhat obstinate debater of ideas, and probably a restless thinker. This "man for all seasons" worked with endless energy, but he also knew how to play and enjoy a well-deserved beer at the end of a sweaty collecting day in the Mojave Desert.

Jim has just retreated to a new life of reflection on these many accomplishments, but we expect his intellectual contribution to continue.

He liked to gently admonish his students and possibly his staff, mentioning that "focusing on a single well-defined scientific question, a well-designed study with a thought out pathway, is better than trying to have your finger in too many pies, trying to answer several questions at the same time."

The dedication of this meeting to Jim is fitting, and I consider it an honor to have been chosen for this presentation.

REFERENCES CITED

Beehler, J. W., J. P. Webb and M .S. Mulla. 1993. Spatial and circadian oviposition patterns in an urban population of *Culex quinquefasciatus*. J. Am. Mosq. Control Assoc. 9(94): 385-388.

Gruwell, J. A., C. L. Fogarty, S. G. Bennett, G. L. Challet, K. S. Vanderpool, M. Jozan and J. P. Webb. 2000. Role of peridomestic birds in the transmission of St. Louis encephalitis virus in southern California. J. Wildl. Dis. 36(1): 13-34.

Webb, J. P. and R. B. Loomis. 1969. Another new species of *Speleocola lipovsky* (Acarina: Trombiculidae) off chiropterans from Sonora, Mexico. Bull. So. Calif. Acad. Sci. 68(2): 59-63.

Webb, J. P. 1977. Sound as a host-detection cue for the soft tick *Ornithodoros concanensis*. Nature 265(5593): 443-444.

Webb, J. P. 1979. Host-locating behavior of nymphal *Ornithodoros concanensis* (Acarina: Argasidae). J. Med. Entomol. 16(5): 437-447.

Webb, J. P., R. B. Loomis, M. B .Madon, S. G. Bennett and G. E. Greene. 1983. The chigger species *Eutrombicula belkini* Gould (Acari: Trombiculidae) as a forensic tool in a homicide investigation in Ventura County, California. Bull. Soc. Vector Ecol. 8(2): 141-146.

Webb, J. P. and C. H. Myers. 1985. Response of local agencies to the 1984 urban St. Louis encephalitis outbreak in Orange and Los Angeles Counties. Proc. & Papers Mosq. Vector Control Assoc. Calif. 53:22-29.

Webb, J. P., S. G. Bennett, J. I. Curry, C. L. Fogarty and M. B. Madon. 1990. *Ixodes pacificus* and Lyme disease in Orange County, California, 1989. Proc. & Papers Mosq. Vector Control Assoc. Calif. 58:53-54.

Webb, J. P. 1991a. Delusions of parasitosis: a symposium; coordination among entomologists, dermatologist, and psychiatrists. Bull. Soc. Vector Ecol. 18(1): 1-2.

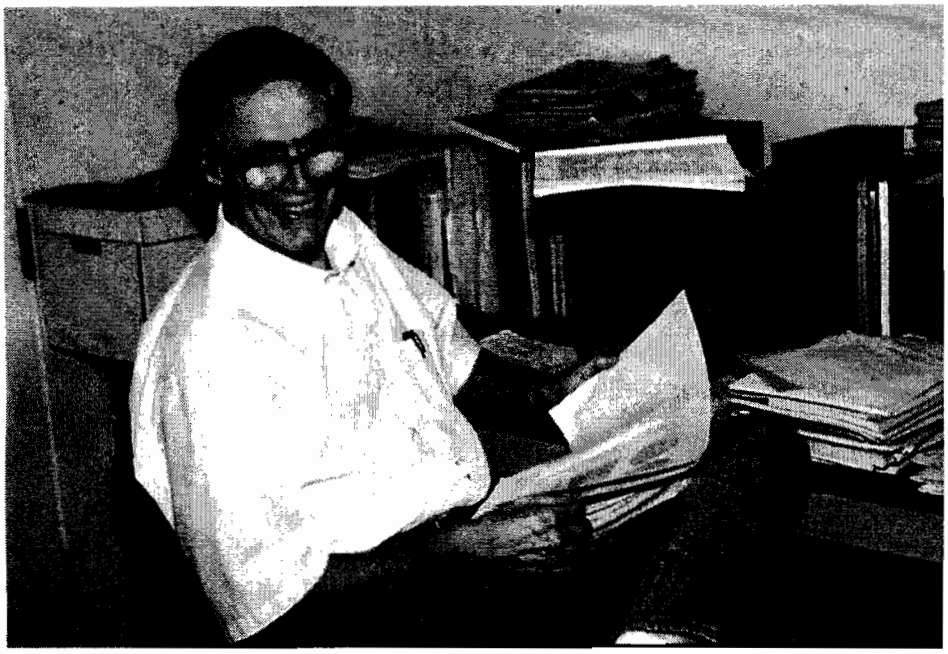
Webb, J. P. 1991b. Case histories of individuals with delusions of parasitosis in southern California and a proposed protocol for initiating effective medical assistance. Bull. Soc. Vector Ecol. 18(1): 16-25.

Directly or indirectly, 27 individuals developed the background, format, and information from these trips or the resulting data to write a masters degree thesis under Dick's critical and fair supervision. Their names and the year each finished his or her degree program are included here: Marilyn Bunnell (1957), Delmer E. Mangum (1958), Alvin H. DeYoung (1958), Kenneth Leith (1958), Charles M. Page (1961), William J. Wrenn (1965), W. Leon Hunter (1966), Ronald E. Somerby (1966), Julius C. Geest (1966), Lynell K. Tanigoshi (1968), Richard M. Davis (1968), Jerry L. Fowler (1968), Norman G. Puckett (1969), James P. Webb, Jr., (1970), James L. Lucas (1970), W. Calvin Welbourn (1972), M. Lee Goff (1974), Lawrence V. Pomeroy (1974), Sherburn R. Sanborn (1977), Stephen G. Bennett (1977), Hans Megens (1980), Charles S. Rau (1980), Steven D. Werman (1980), Kazuhiro Ando (1983), Lawrence L. C. Jones (1985), Noor S. Tietze (1986), and Gerald E. Greene (1986).

Figure 1. Student contribution to the taxonomy of California *Chiggers*.

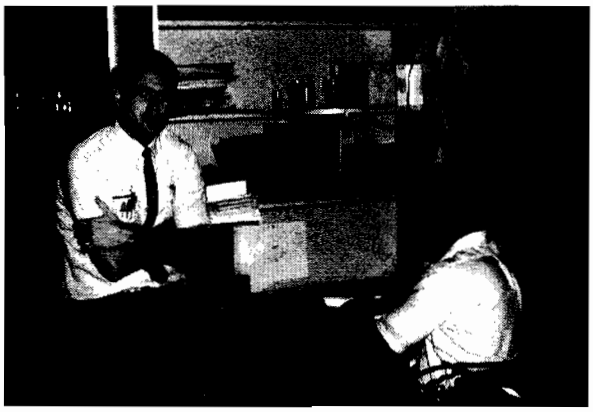
Tim Bradley, UCI: *Mosquito Ecology of Culex erythrothorax*
California Veterinary Laboratory, San Bernardino: *Salmonella in rats*
Jamie Childs, CDC: *Hepatitis E Virus*
Max Chu, CDC: *Tularemia*
Andy Comer, CDC: *Rickettsial pox*
Lance Durden, Georgia Southern University: *Lice I.D.'s*
Esteban Fernandez, Long Beach State: *Role of House Finch and WNV*
Durland Fish, Yale University School of Public Health: *Ticks and Lyme disease*
Charles Fulhorst, University of Texas Medical Branch, Galveston: *Arenaviruses*
Ken Gage, CDC: *Plague*
Roy Hall, University of Queensland, Australia: *West Nile virus*
Brian Hjelle, University of New Mexico: *Hantavirus*
Xi Yu Jia, UCI: *West Nile Virus Sequencing*
Anne Kjemtrup, University of California, Davis: *Babesia*
Michael Kosoy, CDC: *Bartonella*
Bob Lewis: *Fleas I.D's*
Lou Magravelli, Connecticut Agr. Experimental Station: *Alicia in ticks*
Chad McHugh, San Antonio: *Leishmania*
Bob McLean, National Wildlife Research Center, Ft. Collins, CO: *West Nile Virus Studies*
Darrell Paterson, Commonwealth University, Virginia: *Hepatitis B and C*
Robert Purcell, NIH: *Hepatitis E*
Telford Work, UCLA School of Public Health: *Flavivirus transmission*
Michael Yabsley, University of Georgia: *T. cruzi in rats*

Figure 2. Intercollegiate activities fostered by Jim Webb during his tenure at Orange County Vector Control District.



Jim at his desk, OCVD 1996, surrounded by his typical organized clutter.

Jim Webb at the microscope, 2000.



Jim Webb and Richard Davis, Long Beach State 1970.

2007 Year-in-Review: Integration of NASA's Meteorological Data into the California Response Plan

Christopher M. Barker[1,2], Bborie K. Park[2], Forrest S. Melton[3], Bruce F. Eldridge[1,2], Vicki L. Kramer[4], and William K. Reisen[2]

[1] *Department of Entomology, University of California, Davis, cmbarker@ucdavis.edu, (530) 752-0151*

[2] *Center for Vectorborne Diseases, University of California, Davis*

[4] *NASA Ames Research Center & California State University, Monterey Bay*

[3] *Vector-borne Disease Section, California Department of Public Health*

ABSTRACT: The California Mosquito-Borne Virus Surveillance and Response Plan recommends measured intervention responses based on assessed risk for arboviral epidemics. For West Nile virus (WNV), overall risk is based on temperatures and surveillance measures of vector and virus activity. In the current study, we assessed the value of temperature data acquired from NASA for predicting epidemic risk for WNV. We also considered whether the risk model would have provided adequate lead time for response and suggested ways in which the response plan's risk assessment model could be improved for prediction and assessment of current risk.

INTRODUCTION

The California Mosquito-Borne Virus Surveillance and Response Plan (CMVSRP) (California Department of Public Health et al. 2007) includes an epidemic risk assessment model for each of the three most important arboviruses in California and guidelines for local agency response during periods of heightened risk. The most recent incarnation of the CMVSRP was updated in preparation for the arrival of West Nile virus and has its roots in earlier documents with similar aims (California Department of Health Services 1983, Reeves 1969, Reisen 1995, Walsh 1987). Since the arrival of West Nile virus (Family Flaviviridae, genus *Flavivirus*, WNV), the Plan has been updated annually by the California Department of Public Health and collaborators.

The CMVSRP's risk assessment model includes environmental conditions favoring arboviral transmission. For West Nile virus, environmental risk is defined by temperature and is scored from 1—5. To determine risk for a given agency, one must obtain temperature data from a representative weather station and spatial resolution is limited by the locations of weather stations. Recently, NASA's Terrestrial Observation and Prediction System (TOPS) has been developed for interpolation of meteorological measurements based on slope, aspect, and other land attributes (Nemani et al. 2003, Thornton et al. 1997). The TOPS algorithm is currently being used to generate daily surfaces of meteorological variables at a 1-km resolution. In this study, we used TOPS and surveillance data from 2007 to determine (1) whether TOPS temperatures were predictive of epidemic risk for WNV, (2) whether the risk model would have provided adequate lead time for response, and (3) ways in which the risk model could be improved for prediction and assessment of current risk.

MATERIALS AND METHODS

Temperature data. Daily 1-km statewide minimum and maximum temperature

grids were acquired from NASA's TOPS (Nemani et al. 2003, Thornton et al. 1997) and stored in PostgreSQL v 8.2. Temperature minima and maxima were averaged to create daily 1-km grid of mean temperatures, which then were aggregated by half-month and agency using PostGIS. Temperature data were analyzed for all MVCAC member agencies.

Surveillance data. Vector and arbovirus surveillance data for 2007 from collaborating agencies, Kern MVCD and Sacramento-Yolo MVCD, were aggregated by half-month using the California Surveillance Gateway (http://gateway.calsurv.org). Other agencies with sufficient historical surveillance records (data not shown) also were analyzed, and results were e-mailed to each agency.

Risk calculations. Using the data sets described above, risk levels were calculated for each component of the WNV risk assessment model from the CMVSRP (Table 1). The plan was modified from the 2007 published version (California Department of Public Health et al. 2007) as follows: (1) temperature thresholds were modified to reflect epidemiologically relevant differences in extrinsic incubation periods (Reisen et al. 2006), (2) equine cases were removed from the risk model due to the low number of susceptible horses as a result of natural or vaccine-induced immunization, (3) chicken-related risk was simplified using only the numbers of flocks that seroconverted, and (4) instead of using the proximity of virus activity to the human population, risk was calculated separately for urban and rural areas based on abundance and infection rates for *Culens pipiens L./Cx. quinquefasciatus* Say and *Cx. tarsalis* Coquillett, respectively.

RESULTS

Temperature. Risk levels based on average daily temperature for each half-month and agency were mapped against regional onsets and peaks of WNV-attributed human case incidence (Fig. 1). During the half-month prior to the onset of human cases in each region, at least two agencies within the region reached a temperature risk level of 4 or greater. Prior to the peak in human cases, temperatures had reached the highest risk level of 5 in at least one agency in each region. Statewide, temperatures were indicative of spatial variation in WNV epidemic risk and provided 0.5 – 1 month lead time for response prior to the onset of human cases.

Vector and virus surveillance. Surveillance measures generally indicated concurrent WNV risk to humans (Fig. 2), but provided little lead time prior to the onset of cases within each agency. No single factor was sufficient for predicting human cases. Relative vector abundance provided the greatest lead time, but above-average abundance was not a necessary precursor to epidemics (Fig. 2). When adequate numbers of samples were tested, mosquito pools, chickens, and dead birds provided reasonable indications of concurrent risk for human cases and short lead times (0.5—1.5 months) for response prior to case onset.

DISCUSSION

Temperatures during 2007 were indicative of spatial variation in risk for WNV epidemics and high temperatures preceded occurrence of human cases in all MVCAC regions. However, lead time based on the current CMVSRP risk assessment model was limited to 0.5—1.5 months and more advance warning is needed. To improve lead time and allow comparisons of the current year's risk with that of other years, temperature anomalies and long-range forecasts during the early-season need to be integrated into the existing absolute temperature-based model.

Vector and virus surveillance measures were of variable value, depending partially on the number of samples tested for each agency. Human cases of WNV did not necessarily follow above-average vector abundance, and additional study is needed to relate vector abundance to risk for WNV transmission to humans. Based on the vectorial capacity equation, the force of transmission is expected to increase as abundance increases, and from earlier studies on

other arboviruses (Olson 1977, Olson et al. 1979, Reeves 1971), it seems likely that WNV transmission risk would have been higher if vector abundance had been higher. In many agencies, mosquito pools did not provide an early indication of virus activity prior to human-case onset because too few specimens were tested until the epidemic was realized. In other agencies where specimens were tested routinely, mosquito infection rate increases preceded or coincided with increases in human case incidence (e.g., Kern and Sacramento-Yolo MVCDs). Another factor during 2007 was that emergency funds were released by the State of California, allowing increased testing and better measurement of actual infection rates during the height of the epidemic.

The CMVSRP will continue to be revised based on these results and input from MVCAC member agencies. Improvements in forecasting of epidemic risk are needed, and a modular approach to risk assessment, separating forecasting components from measures of concurrent risk, may be a step in the right direction.

Acknowledgements

We thank Kern MVCD, Sacramento-Yolo MVCD, and other MVCAC member agencies for providing the surveillance data used in this study. Funding for this project was provided by the NOAA Office of Global Programs and the NASA Earth-Sun Science Applied Sciences Program.

REFERENCES CITED

California Department of Health Services. 1983. California's mosquitoborne encephalitis virus surveillance and control program Vector Biology and Control Branch, California Department of Health Services, Sacramento, CA.

California Department of Public Health, Mosquito and Vector Control Association of California, and University of California. 2007. California mosquito-borne virus surveillance and response plan. Web address: http://westnile.ca.gov/downloads.php?download_id=324&filename=2007%20CA%20Mosq%20Surv%20and%20Resp%20Plan%20JULY.pdf. Last accessed: 3/28/2008.

Nemani, R. R., M. A. White, L. V. Pierce, P. Votava, J. Coughlan and S. W. Running. 2003. Biospheric monitoring and ecological forecasting. Earth Obs. Mag. 12:6-8.

Olson, J. G. 1977. The impact of Culex tarsalis population density and physical environmental factors upon mosquito-borne encephalitis in humans and equines in California. Journal.

Olson, J. G., W. C. Reeves, R. W. Emmons and M. M. Milby. 1979. Correlation of *Culex tarsalis* indices with the incidence of St. Louis encephalitis and western equine encephalomyelitis in California. Am. J. Trop. Med. Hyg. 28:335-343.

Reeves, W. C. 1969. Evolving concepts of encephalitis prevention in California. Proc. Calif. Mosq. Control Assoc. 37:3-6.

Reeves, W. C. 1971. Mosquito vector and vertebrate interactions: the key to maintenance of certain arboviruses, pp. 223-230. *In* A. M. Fallis [ed.] The ecology and physiology of parasites. Univ. Toronto Press, Toronto.

Reisen, W. K. 1995. Report: Guidelines for surveillance and control of arboviral encephalitis in California. Web address. Last accessed.

Reisen, W. K., Y. Fang and V. M. Martinez. 2006. Effects of temperature on the transmission of West Nile virus by *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 43: 309-317.

Thornton, P. E., S. W. Running and M. A. White. 1997. Generating surfaces of daily meterological variables over large regions of complex terrain. J. Hydrol. 190:241-251.

Walsh, J. D. 1987. California's mosquito encephalitis virus surveillance and control program Vector-Borne Disease Section, California Department of Health Services, Sacramento, CA.

Table 1. Modified risk assessment model from the California Mosquito-borne Virus Surveillance and Response Plan as applied in this study.

Risk Level	Avg. Daily Temperature*	Adult mosquito abundance	Mosquito MIR/1,000	Chicken Seroconversions	Dead Bird Infections	Human Cases
1	<56°F	< 50% 5-yr. Avg.	0	0 in region	0 in region	0 in region
2	57-65°F	50-90% 5-yr. Avg.	0.1 – 1.0	≥ 1 in region, 0 in agency	≥ 1 in region, 0 in agency	
3	66-72°F	91-150% 5-yr. Avg.	1.1 – 2.0	1 flock in agency	1 in agency	≥ 1 in region, 0 in agency
4	73-79°F	151-300% 5-yr. Avg.	2.1 – 5.0	2 flocks in agency	2-5 in agency	1 in agency
5	>79°F	> 300% 5-yr. Avg.	> 5.0	>2 flocks in agency	>5 in agency	>1 in agency

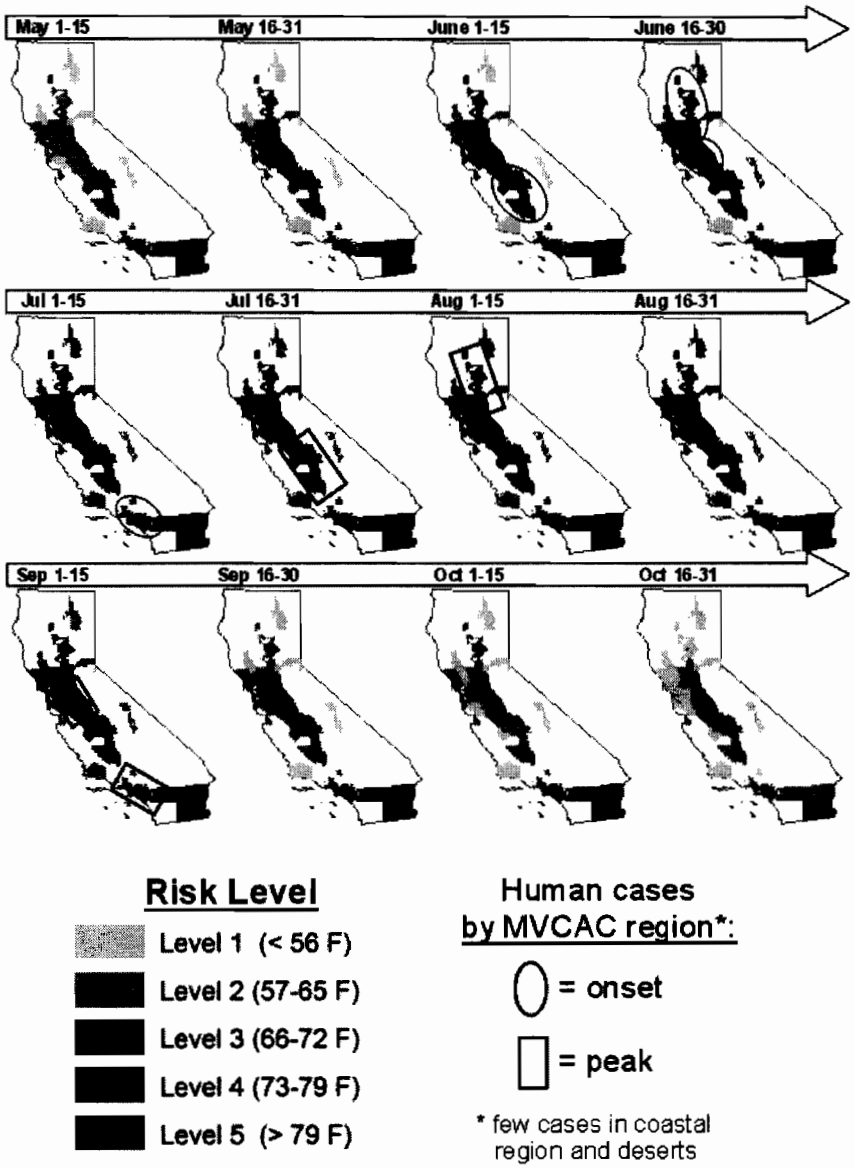


Fig. 1. Maps showing 2007 temperature-related risk by half-month and agency using revised thresholds according to Reisen et al. (2006). The onsets and peaks of human WNV-attributed case incidence within MVCAC regions are indicated by ovals and rectangles, respectively.

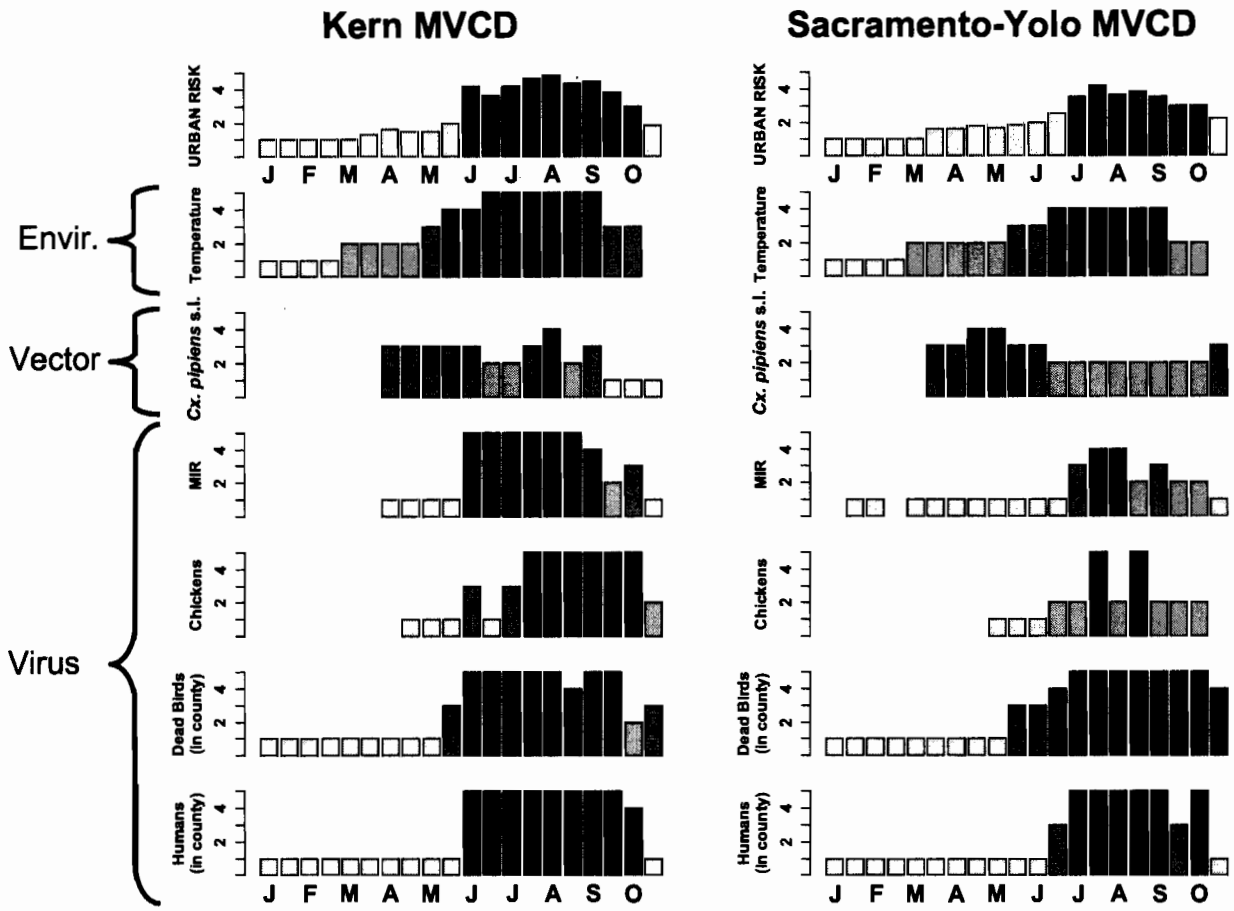


Fig. 2. 2007 WNV epidemic risk assessment by half-month for Kern and Sacramento-Yolo MVCDs. Overall risk for urban areas is indicated in the top row. Other rows contain risk levels for individual components of the assessment model. Rural risk (not shown) is based on abundance and MIRs for *Culex tarsalis* instead of *Cx. Pipiens/Cx. quinquefasciatus*.

Aerial Adulticiding in Sacramento County, California, 2007 – How Was It Different from 2005 and 2006?

Paula A. Macedo, Marcia Reed, Kara Kelley, Gary W Goodman, David A. Brown

Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Road, Elk Grove, CA 95624

ABSTRACT: With the increase in abundance and infection rates of *Culex tarsalis* and *Cx. pipiens* mosquitoes, the Sacramento-Yolo Mosquito and Vector Control District made the decision on July 26 to conduct aerial spraying over the North area of Sacramento County on the nights of July 30, 31, and August 1. At the same time, the District received notification of the first human case of West Nile virus (WNV) in the area. We conducted pre and post-trapping inside and outside the spray zone to evaluate mosquito abundance and infection rates after the spraying events. Results showed decreases in the abundance of *Cx. tarsalis* and *Cx. pipiens*, in the percent positive mosquito pools collected, and in the minimum infection rate for *Cx. tarsalis*. Differences among the aerial sprayings conducted in Sacramento County in 2005 and 2007, and Yolo County in 2006 are discussed.

The Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) conducts surveillance and control of mosquitoes in Sacramento and Yolo Counties in California. The District monitors weekly adult mosquito abundance with American light traps, gravid female traps, and Mosquito Magnet Traps® (SYMVCD 2006). We also use encephalitis virus surveillance (EVS) traps baited with CO_2 and gravid female traps to capture live mosquitoes to test for West Nile virus (Family Flaviviridae, genus *Flavivirus*, WVN), Saint Louis Encephalitis (SLE), and Western Equine Encephalitis (WEE). In 2004, WNV was associated with low level transmission to humans and horses (Armijos et al. 2005, Hom et al. 2005), but in 2005 there was a severe outbreak of WNV in Sacramento County, with 183 human cases and 40 equine cases (Elnaiem et al. 2006), and the District made the decision to conduct aerial spraying of a pyrethrin pesticide over the North and South areas of the county. In 2006, WNV reached epidemic levels in the cities of Woodland and Davis in Yolo County (Macedo et al. 2007). Analysis of data from both years showed that, although there was a significant decrease in numbers of human cases, abundance of mosquitoes, and infection rates, it could have been more effective if aerial sprayings had been conducted one or two weeks before, when abundance of mosquitoes and infection rates were higher, and transmission was possibly occurring to humans (Macedo et al. 2007). By the time the District receives notification of any human case, transmission is already well underway, with many people already infected, but not yet displaying symptoms, or presumptive cases not receiving definitive confirmation.

In 2007, the District identified the first positive mosquito pool on July 04, which was 5 days later than observed in 2005 and 2006, and the first positive sentinel chicken was tested on July 10 (Table 1). The District was also closely following weather patterns and changes in mosquito populations, and determined that *Cx. tarsalis* Coquillett and *Cx. pipiens* L. abundance peaks were 1 week to 10 days later than the previous year (Fig. 1). Mosquito abundance and infection rates were closely monitored. Despite all efforts from the SYMVCD's public information program and from it's intensive integrated pest management (IPM) program, which included environmental management, larviciding and biological control, infection rates for *Cx. tarsalis* and *Cx. pipiens* mosquitoes were high and reached 10.9 and 8.01 respectively, on

the week of July 24. Following the District's mosquito and mosquito-borne disease management plan (SYMVCD 2005), the District made the decision on July 26 to conduct aerial spraying of Evergreen® EC-60-6, over about 215 km^2 in the North area of Sacramento County on the nights of July 30, 31, and August 1. At that point, there were 21 trapping sites with one or multiple positive mosquito pools from the area delineated as the spray zone (Fig. 2A). On the same day that this decision was made, we received notification of the first human case in the area. In order to evaluate the success of the aerial spraying in decreasing mosquito abundance and infection rates, we conducted pre and post-trapping with CO_2-baited EVS traps and gravid traps inside and outside the spray zone.

Results show a decrease in the number of locations with positive pools inside the spray zone (Fig. 2B), as well as a decrease in abundance of *Cx. tarsalis* and *Cx. pipiens* (Fig. 3), a decrease in the percent positive mosquito pools collected (Fig. 4), and a decrease in the minimum infection rates for *Cx. tarsalis* (Table 2), which may have had an effect in decreasing WNV transmission.

As in 2005 and 2006, aerial applications in 2007 appeared to interrupt epidemic transmission. In 2007, the aerial spraying occurred at least one week before the ones in 2005 and 2006, and the peaks in the populations of *Cx. tarsalis* and *Cx. pipiens* were delayed approximately one week due to environmental conditions, which places the 2007 aerial spraying at least two weeks ahead of 2005 and 2006. We believe that this was one of the reasons why we did not observe as many human cases as in 2005 (Fig. 5). According to the Center for Disease Control and Prevention (CDC 2004), date of onset of symptoms after infection can vary from 2 to 14 days in humans. By the time we receive notification of the first human case, transmission for that particular case could have happened anywhere from 2 to 14 days before, with additional time for clinical diagnosis and notification. That means that we only learn about the case after transmission had already occurred with many others potentially infected. That was the case in 2005, 2006, and 2007. In 2007, the District learned about the first human case the same day it had made the decision to spray. After we conducted the aerial spraying of that area, we learned of five more human cases that may have had transmission occurring before the spraying events (Fig. 5). Our results underscore the importance of using all possible surveillance tools when making the decision about conducting aerial spraying of adulticides over a suburban or urban area. Some response plans may depend on the occurrence of human disease before a recommendation for aerial treatment is made. Our results suggest that it may be prudent to base the response on enzootic amplification, but we should use all available surveillance tools in order to make such informed decision.

REFERENCES CITED

Armijos, V., S. Wright, W. Reisen, K. Kelley, S. Yamamoto and D. Brown. 2005. West Nile Virus in Sacramento and Yolo Counties, 2004. Proc. & Papers Mosq. Vector Conrol Assoc. Calif. 73: 24-27.

Biggerstaff, B. J. 2004. Pooled infection rate (PooledInfRate 2.0), Center for Disease Control and Prevention, Ft Collins, CO. http://www.cdc.ncidod/dvbid/westnile/software.htm

Centers for Disease Control and Prevention (CDC). 2004. West Nile Virus (WNV). Information and Guidance for Clinicians. Available at: http://www.cdc.gov/ncidod/dvbid/westnile/clinicians/pdf/wnv-clinicaldescription.pdf

Elnaiem, D. A., K. Kelley, S. Wright, R. Laffey, G. Yoshimura, V. Armijos, M. Reed, M. Farley, G. Goodman, W.K. Reisen and D. Brown. 2006. Epidemic amplification of West Nile virus in Sacramento and Yolo Counties, June – September 2005. Proc. & Papers Mosq. Vector Conrol Assoc. Calif. 74: 8-20.

Hom, A., L. Marcus, V. L. Kramer, B. Cahoon, C. Glaser, C. Cossen, E. Baylis, C. Jean, E. Tu, B.F. Eldridge, R. Carney, K. Padgett, B. Sun, W. K. Reisen, L. Woods and S. Husted. 2005. Proc. & Papers Mosq. Vector Conrol Assoc. Calif. 73:66-77.

Macedo, P. A., C. F. Nielsen, M. Reed, K. Kelley, W. K. Reisen, G. W. Goodman and D. A. Brown. 2007. An evaluation of the aerial spraying conducted in response to West Nile virus activity in Yolo County. Proc. & Papers Mosq. Vector Conrol Assoc. Calif. 75:107-114.

Sacramento-Yolo Mosquito and Vector Control District (SYMVCD). 2005. Mosquito and Mosquito-Borne Virus Surveillance and Response Plan. Available at: http://www.fightthebite.net//download/Mosquito_Management_Plan.pdf

Sacramento-Yolo Mosquito and Vector Control District (SYMVCD). 2006. 2006 Annual Report. Available at: http://www.fightthebite.net//download/AnnualReport2006.pdf

Table 1. Order and dates for events prior to the aerial spraying events in 2005, 2006, and 2007.

WNV Detection	2005	2006	2007
1st pos. mosquito pool	Jun 29	Jun 29	Jul 04
1st pos. sentinel chicken	Jul 15	Jul 18	Jul 10
1st human case (notification)	Jul 21	Jul 26	Jul 26
Aerial spraying	Aug 8-10; 20-22	Aug 8-9	Jul 30-Aug 1

Table 2. Infection rates of WNV in *Culex. tarsalis* and *Cx. pipiens* mosquitoes collected the week before and the week after the aerial spraying, Sacramento County, 2007 (MLE, Bias-Corrected Maximum Likelihood estimate of infection rate/1000 mosquitoes, Biggerstaff 2004).

Treatment Status	*Cx. tarsalis*		*Cx. pipiens*	
	Before	After	Before	After
Spray zone	10.90	3.35	8.04	8.01
Control	3.58	5.66	6.29	3.15

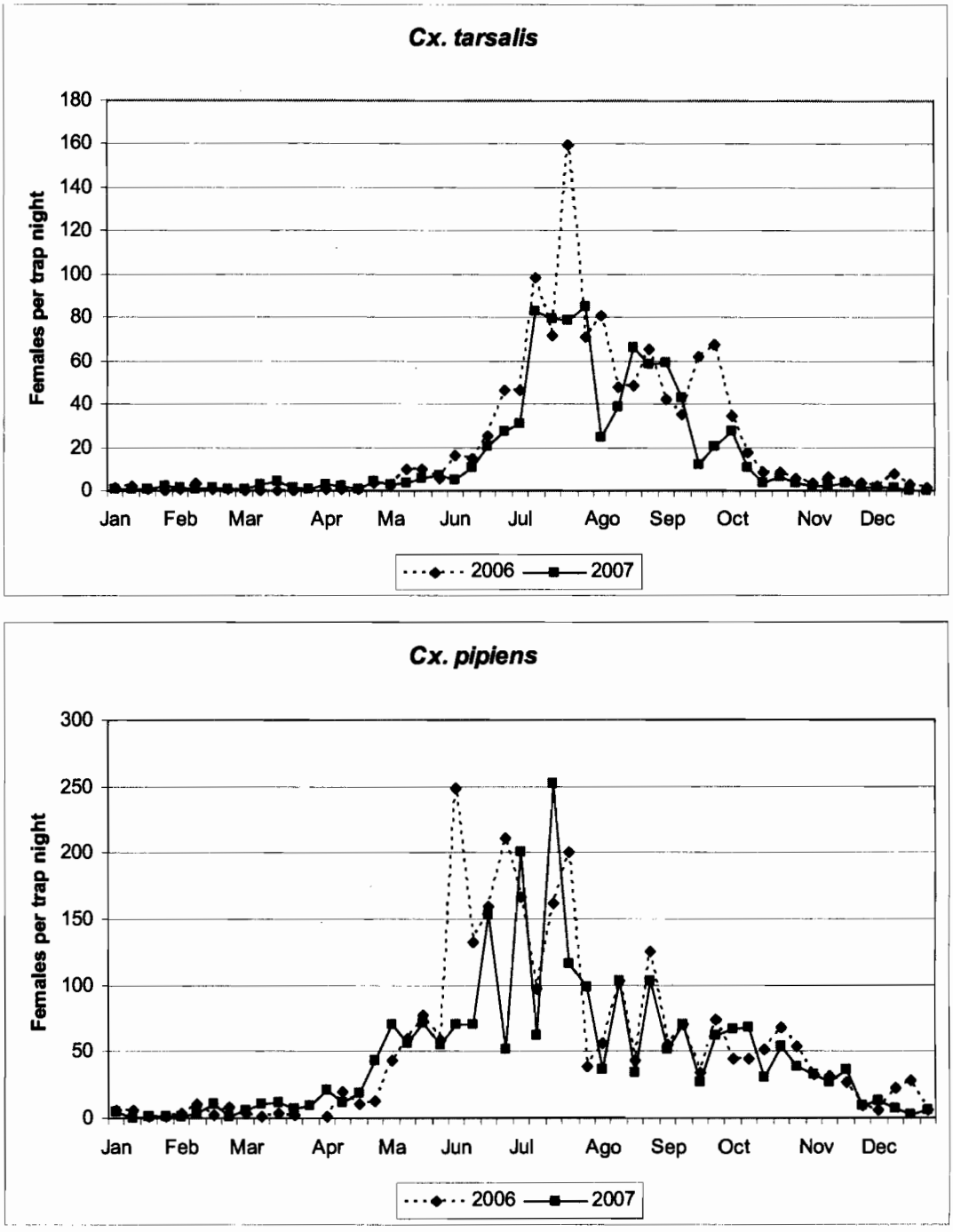
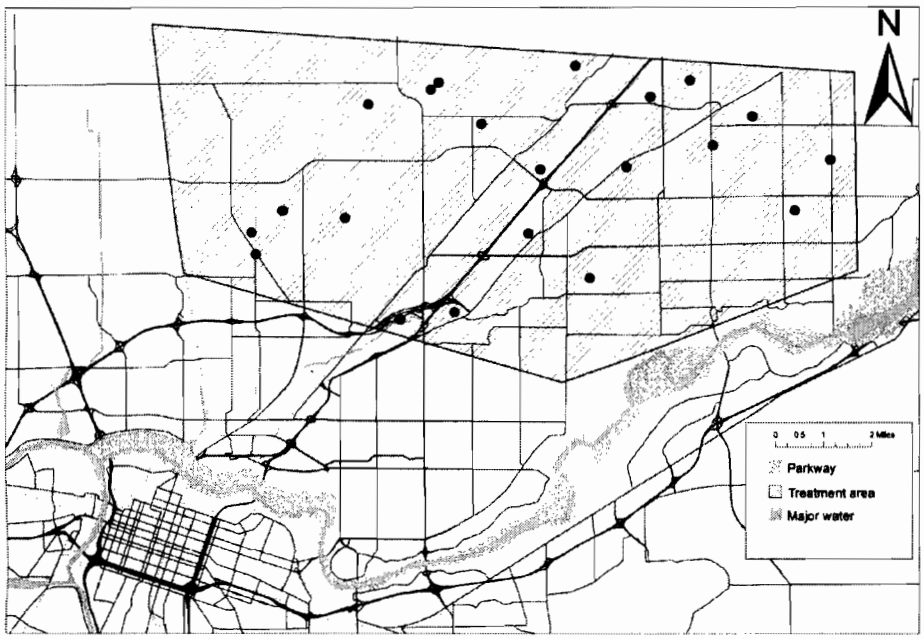


Figure 1. *Culex tarsalis* and *Cx. pipiens* abundance in 2006 and 2007 in Sacramento and Yolo Counties.

A



B

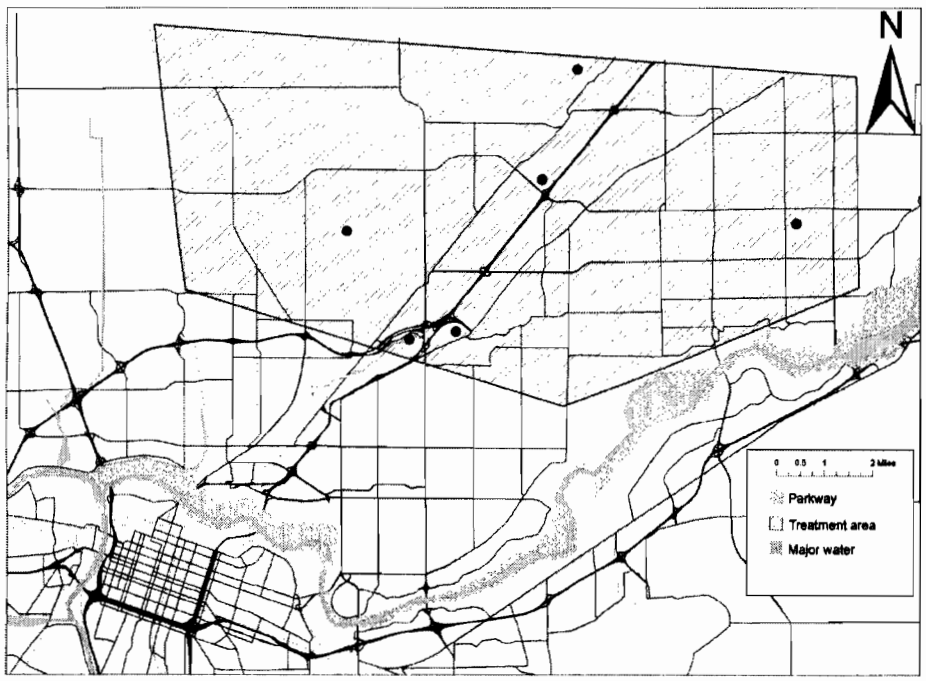


Figure 2. Locations (represented by dots) within the spray zone with positive mosquito pools in the week before (A) and the week after (B) the aerial spraying, Sacramento County, 2007.

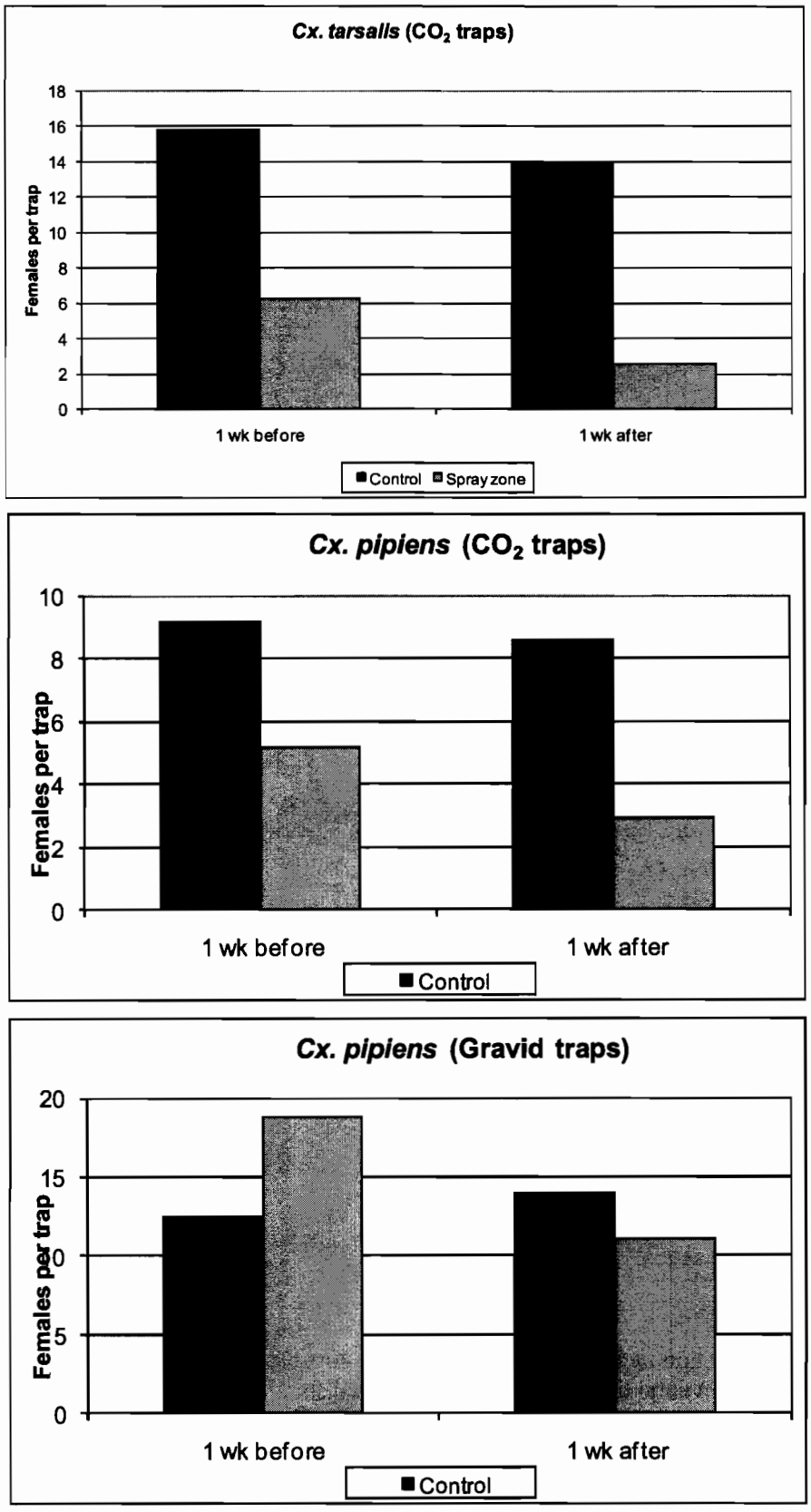


Figure 3. *Culex tarsalis* and *Cx. pipiens* females per trap night collected during the week before and the week after the aerial spraying in Sacramento County, 2007.

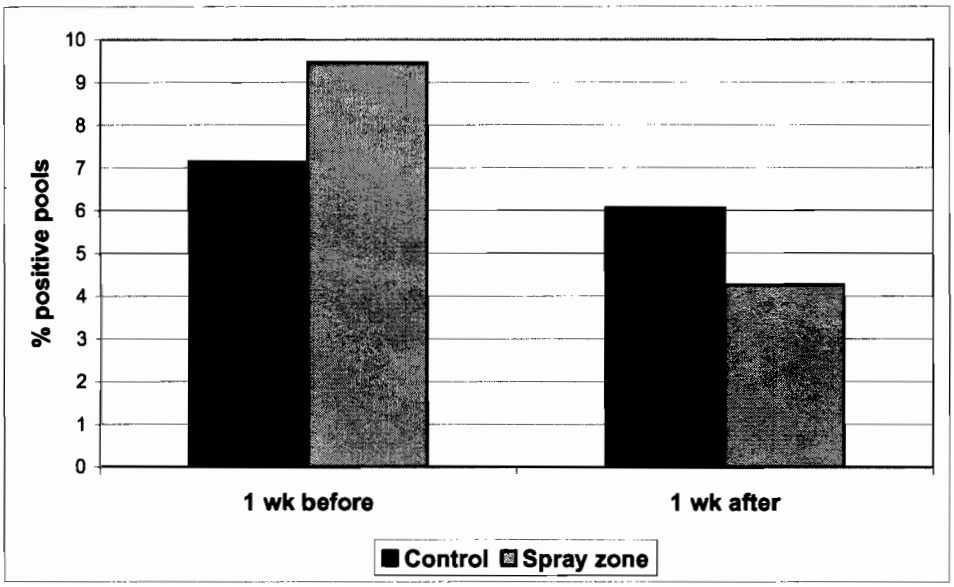
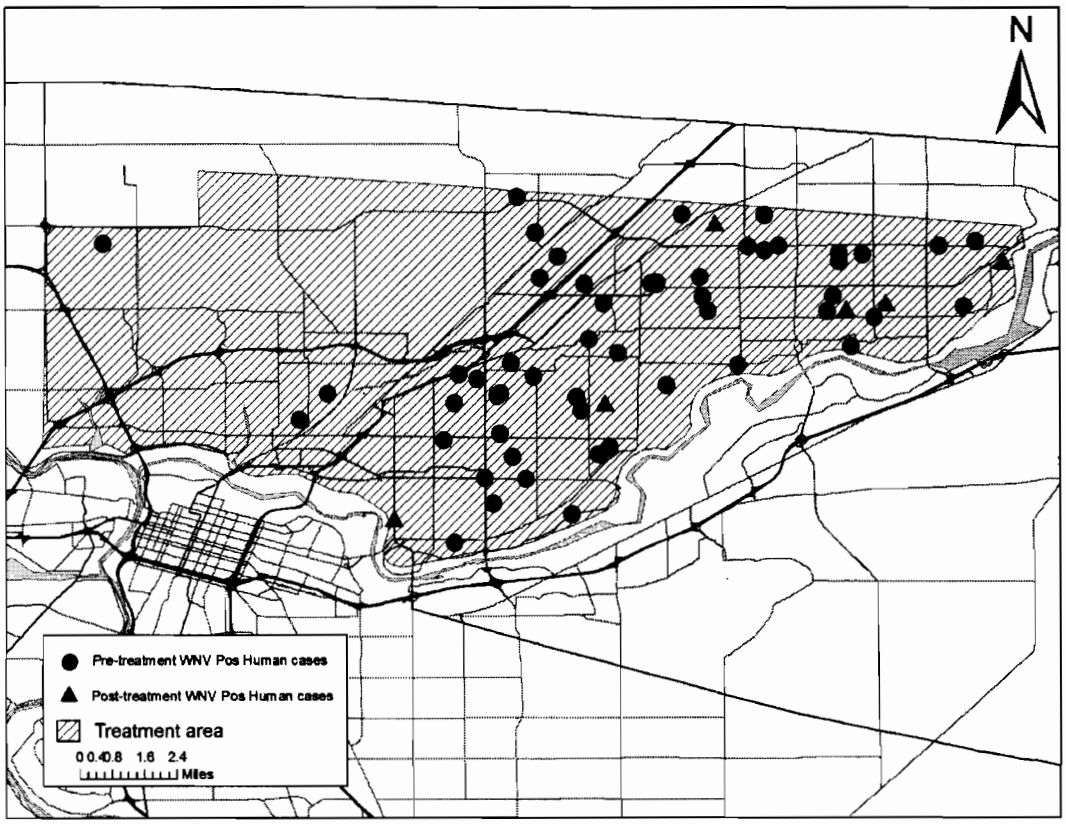


Figure 4. Percent positive mosquito pools before and after spraying, Sacramento County, 2007.



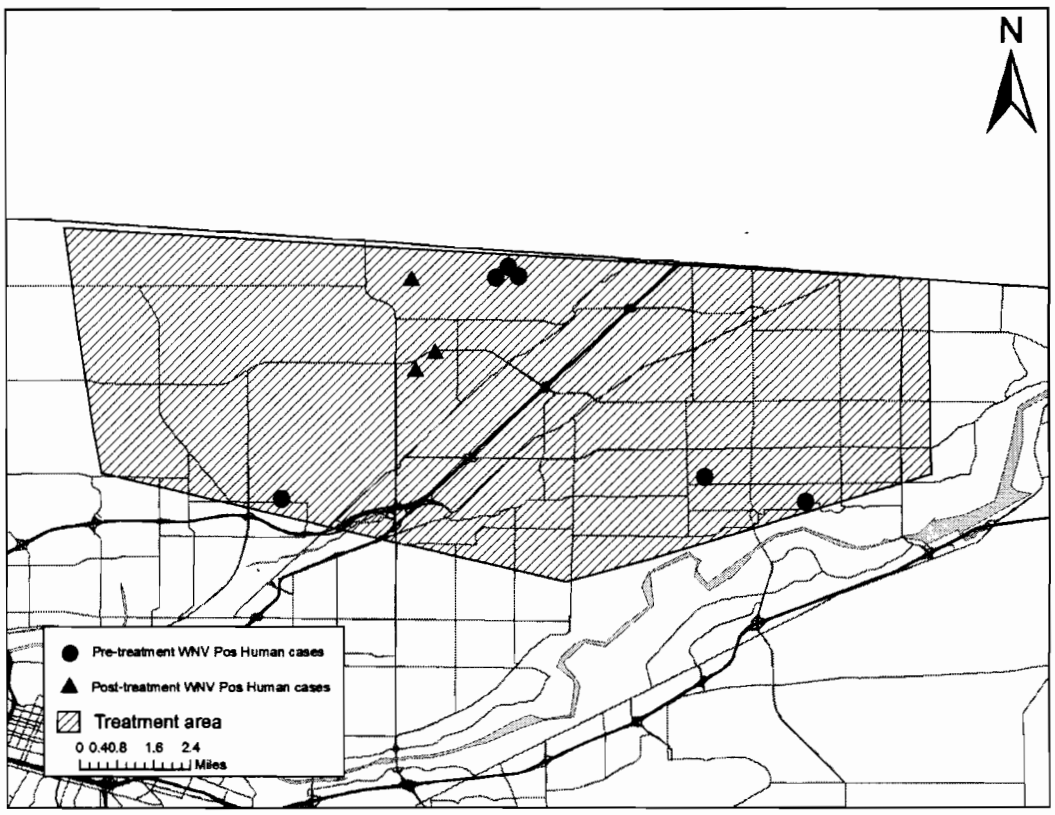


Figure 5. Human case within the spray zone i 2005 (A) and 2007 (B) Dots represent cases with transmission occurring before the spraying events, and triangles represent human cases occurring after the spraying events.

Assessment of Barrier Applications of Demand® (Lambda-cyhalothrin) in Rural Landscapes in the Coachella Valley, California.

Hugh D. Lothrop[1], Branka B. Lothrop[2], William K. Reisen[1] and Donald E. Gomsi[2].

[1]*Arbovirus Research Unit, Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, Davis, CA 95616, hdlothrop@ucdavis.edu.*

[2]*Coachella Valley Mosquito and Vector Control District, 43420 Trader Place, Indio, CA 92201.*

ABSTRACT: Applications of Demand® (Lambda-cyhalothrin) as a barrier to mosquito dispersal were assessed in a rural area of the Coachella Valley. Ecotonal vegetation was treated in two replicates in an area 1 mile wide east to west and ½ mile deep. Approximately 80% of mosquito breeding sites were located south of the barrier. No significant reduction in mosquito abundance was detected north of the barrier, although there was epidemiological evidence that northward dispersal of West Nile virus had been interrupted, as indicated by reduced mosquito infection rates and seroconversions of sentinel chickens. To assess permeability of a single layer barrier, the dense vegetation on the perimeter of a pond was treated. Wild caught *Culex tarsalis* were marked and released outside the barrier and unmarked females abundance was measured to assess penetration. Abundance was decreased 73% and 86% during the 2 nights post-treatment and dispersal of marked mosquitoes through the barrier was reduced from a ratio of 2:1 to 1:2 (inside: outside) pre-treatment to post-treatment, respectively.

INTRODUCTION

Research on microhabitat distribution patterns of mosquitoes in the Coachella Valley have shown that the majority of flying mosquitoes are found at vegetative ecotones (Lothrop and Reisen 1998). It is likely that mosquitoes spend some time in these areas resting on the vegetation and treating these ecotones with contact pesticide should result in mortality. How much mortality and whether it is sufficient to act as a barrier to dispersing mosquitoes, and more importantly the dissemination of mosquito-borne viruses, is the focus of this study. The objectives of our research were to assess the effect of operational scale treatments on mosquito abundance and virus dissemination and to determine the permeability of a single layer continuous barrier to dispersing mosquitoes.

MATERIALS AND METHODS

Two replicate treatments, Trials 1 and 2, of 1 mile in length and ½ mile in depth were done in the northern duck club region in the Coachella Valley on 19 Sept and 10 Oct. 2006 after flooding (Fig. 1). Demand® (Lambda-cyhalothrin, Syngenta Crop Protection, Inc. Greensboro, North Carolina) was applied at 0.1 oz AI/min at a speed of 5 mph using a Micronair AU8115M, Micron Sprayers Ltd, Bromyard Industrial Estate, Bromyard, Herefordshire, UK. The Micronair nozzle was oriented at 70 degrees to the vehicle path with rotational speed set to produce droplets of 200 μ, according to the manufacturers chart. CO_2-baited CDC style traps (CO2T) were used to monitor changes in abundance. Mosquito pools were tested for western equine encephalomyelitis (WEEV), Saint Louis encephalitis (SLEV), and West Nile (WNV) viruses by RT-PCR. Sentinel chickens

were sampled serologically by filter paper blot and tested by EIA followed by PRNT to differentiate SLEV from WNV antibodies. To compare treatment effect, traps were grouped into regions north, south and east. Regions north and south were separated by the treated barrier zone. Each region was represented by 3 selected traps. Assuming unbiased dispersal from south to east and north, we used east as a control.

A perimeter treatment, Trial 3, was done in 2 passes with the nozzle adjusted to cover 0 to 5 feet and 5 to 10 feet, respectively (Fig. 2). Demand was applied at 0.027 oz AI/min at 5 mph. Effectiveness was assessed using mark-release-recapture and abundance sampling. Nine CO2Ts inside and 8 outside the perimeter were used to recapture marked mosquitoes released 300 feet outside to the east. Two releases were done, numbering 15,700 *Cx. tarsalis* Coquillett pre-treatment and 18,600 post-treatment. Abundance sampling and recapture was done on 2 nights post release.

Potential mortality from contact with treated surfaces was assessed using cages made from 1.25 X 2 inch polyvinyl chloride pipe lined with #2 Whatman filter paper treated with Demand® at 10 oz/acre of surface area. Although coverage in field applications was not completely uniform the 10 oz/acre rate is equivalent to those used in the barrier treatments. Twenty colony *Cx. quinquefasciatus* Say were exposed for 5 minutes and mortality noted at 1 hour.

RESULTS AND DISCUSSION

In Trial 1, there was no reduction in dispersal from south to north, although abundance doubled south of the barrier following treatment (Fig. 3). In Trial 2, treatment resulted in 75% control using Mulla's formula (Mulla et al. 1971) on the night following application. Abundance data for the following week were confounded by 3 nights of aerial adulticide applied to the south region. Epidemiological data during Trial 2 included 8 WNV positive pools out of 56 collected south and southwest of the barrier and the seroconversion of 4 of 10 sentinel chickens, located 200 yards south of the barrier. No positives were found in 103 pools collected inside or north of the barrier and in a flock of 10 chickens 1.5 miles north of the barrier. These data provided a stronger indication of the effectiveness of the barrier than changes in abundance, because mosquito breeding sources north of the barrier could have masked decreases in the dispersal of females from sources in the south.

Landscape features in the previous experiment prevented treating a continuous barrier and thereby containment/exclusion of dispersing mosquitoes. The treatment of the dense vegetation around the perimeter of a pond in Trial 3, gave us confidence in using abundance as a measure of effectiveness. Using Mulla's formula, abundance was decreased 73% and 86% during the 2 nights post treatment and dispersal of marked mosquitoes through the barrier was reduced from an inside to outside ratio of 2:1 to a ratio of 1:2, pre-treatment to post-treatment respectively.

Mortality following 5-minute exposure in the cylinder cages was 43.5% at a 10 oz/acre rate, estimated to approximate the field treatment rate. This indicates that short term exposure of mosquitoes resting on treated vegetation in the field may result in mortality.

The results of these trials indicate this method can impact mosquito dispersal and thereby dispersal of mosquito-borne pathogens. Barrier treatments constrain pesticide applications to limited features of the landscape, primarily ecotones of vegetation. While this method impacts smaller areas than ultra-low volume applications, there may be higher non-target mortality due to the higher toxicity and rates of the pesticides used. This aspect needs to be examined before barrier treatment becomes widely used.

Acknowledgments

This was a collaborative effort between the Coachella Valley Mosquito and Vector Control District and the University of California,

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

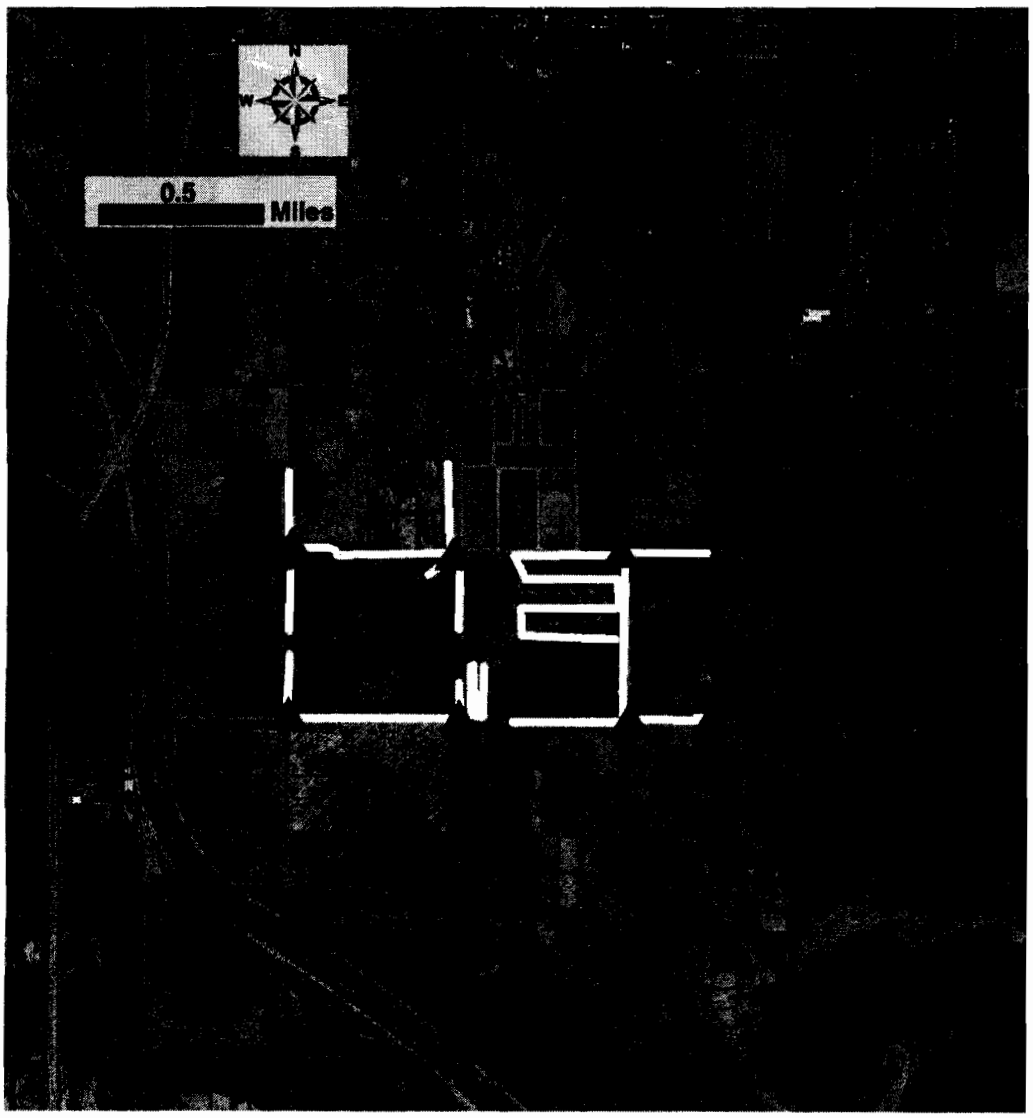


Figure 1. Barrier in depth treatments. Lines represent treated barriers and triangles represent CO2-baited style trap sites.

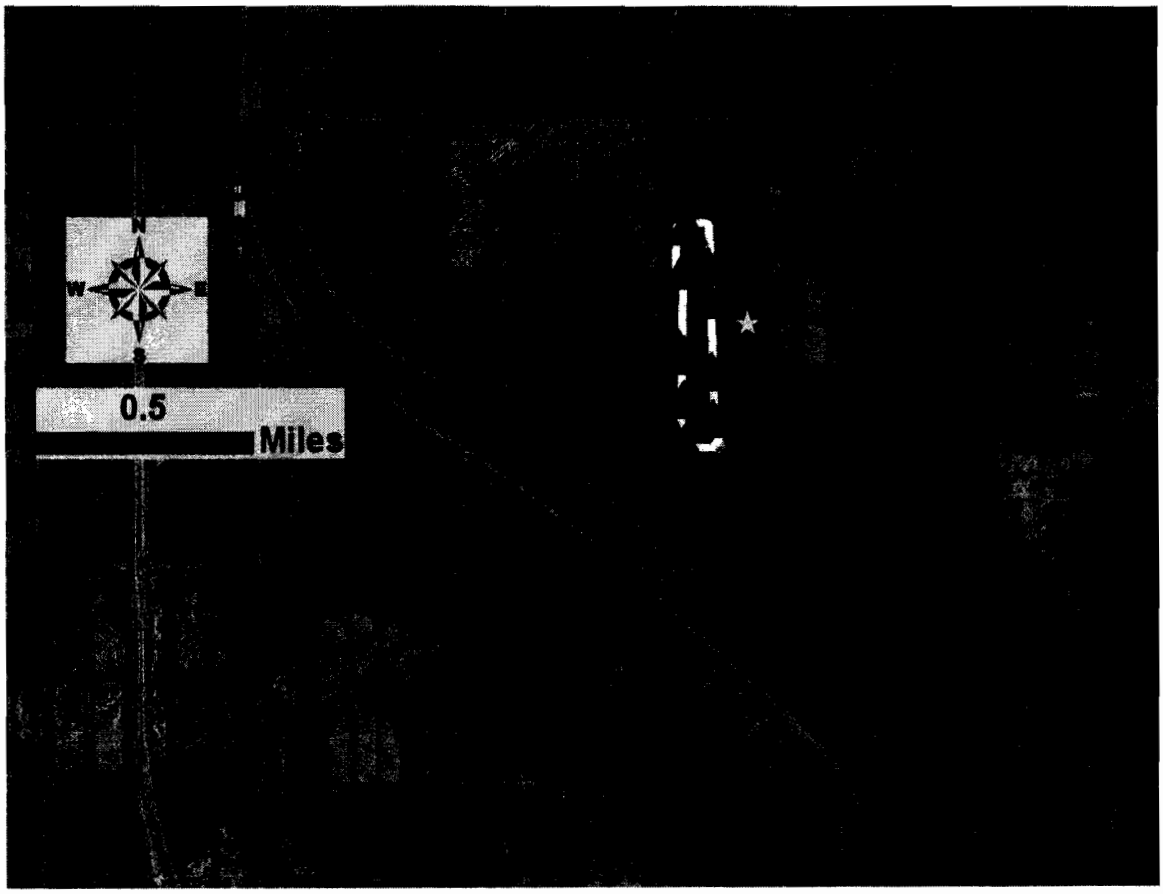


Figure 2. Perimeter treatment. Triangles represent CO_2-baited CDC style trap sites. White line under dense trap pattern represents the barrier treatment.

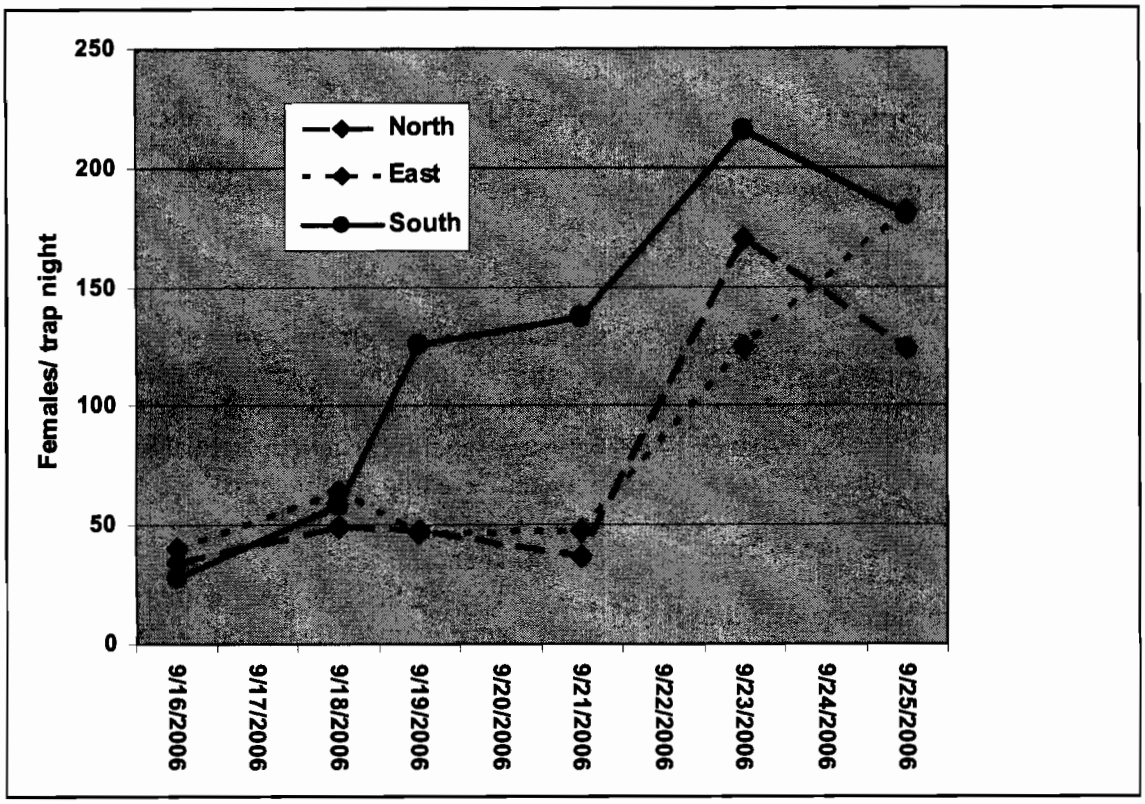


Figure 3. *Culex tarsalis* females per trap night in zones north, east, and south.

REFERENCES CITED

Lothrop, H. D. and W. K. Reisen. 1998. Host-seeking abundance of *Culex tarsalis* in microhabitats of the Coachella Valley, California. Proc. Mosq. Vector Control Assoc. Calif. 66: 31-32.

Mulla, M. S., R. L. Norland, D. M. Fanara, H. A. Darwezeh and D. W. McKean. 1971. Control of chironomid midges in recreational lakes. J. Econ. Entomol. 64: 300-307.

Building upon California's Surveillance Gateway

Bborie K. Park, Bruce F. Eldridge, Christopher M. Barker, William K. Reisen

Environmental Assessment and Information Technology Program Center for Vectorborne Diseases, UC Davis

Historically, vector control agencies in California used a diverse set of tools to manage and store their surveillance data. These tools varied from pen-and-paper to Microsoft Excel and Access to commercial products. The focus of these tools was to store data, not to apply the data in assisting the agency's surveillance operations.

The methods used to store the data did not allow rapid communication and data exchange between the local agencies and state bodies. The flow of data typically was slow, of small quantity and required intensive human involvement. This resulted in long turnaround times for testing of surveillance samples, inaccuracy of datasets provided to state and federal bodies, and hindered improvements to California's surveillance activities.

Starting in 2006, several "centralized" web applications were introduced to supplement and improve the operations of local agencies. Using the Internet provides several enhancements:

1. Data is stored in one place, in a standardized structure and provides the opportunity to build longitudinal or historical datasets.
2. The flow of data between agencies is accelerated because all users know where the data is and how to access it.
3. Users can access the data from any location that has a connection to the Internet.

The California Vectorborne Disease Surveillance Gateway (herein called CSG) was one of the applications introduced in 2006. The CSG was designed to supplement an agency's in-house surveillance data management systems by providing additional capabilities and overcoming existing limitations. By focusing on functionality, easing the ability to share data between agencies and establishing links between research and practice, the CSG has been welcomed by California's local vector control agencies.

The CSG is a comprehensive surveillance management tool. It achieves this by providing four major components: (1) surveillance sites description, (2) mosquito abundance, (3) mosquito pool submission and test results, (4) sentinel chicken band management, (5) blood sample submission and test results, and (6) dead bird reporting and testing. Each of these data covers one of the core enzootic surveillance measures. Several utilities are provided to facilitate data transfers to and from the CSG, calculate key parameters such as abundance anomalies based on 5 year means and infection rates, output reference information, and perform common spatial tasks.

Data coming into the CSG are stored and made immediately available for a variety of analysis tools and products. The primary tools are the mosquito abundance and the mosquito infection rate calculators. These two calculators provide users the ability to analyze their agency's mosquito datasets both spatially and temporally. One of the products currently available is the real-time mapping of surveillance activities – overall and for positive samples – on the Internet and the desktop.

As of the end of 2007, the CSG was

fully adopted by 50% of California's vector control agencies. Test results from 70% of submitted mosquito pools are available within 48 hours of delivery. Weekly snapshots of the data are made available for uploading to the CDC's ArboNET.

With the success and acceptance of the California Vectorborne Disease Surveillance Gateway, efforts are underway to provide decision support capabilities using all of the available surveillance indicators. This will be done using the Risk Assessment Model found in the California's Mosquito-borne Virus Surveillance and Response Plan. Climate data, the one component of the Risk Assessment Model not found in the CSG, will be acquired from NASA's TOPS dataset. To help facilitate the addition of decision support to the CSG, spatial capabilities are also being added throughout the application. This will permit end-users to visually 'mine' data on a map rather than a textual interface.

In 2009, the CSG will be overhauled to take advantage of new technologies to improve the user experience. Several new data components will also be added, including pesticide use and resistance. With these additions and enhancements, the California Vectorborne Disease Surveillance Gateway will be able to serve, adapt and enhance surveillance data to meet the needs of local agencies.

Acknowledgments

We thank the individual mosquito control agencies throughout California who adopted, contributed to, and continue to help improve the CSG. We would also like to the California Department of Public Health for their continued support in encouraging agencies to take advantage of the CSG. Major funding for the CSG was provided by the NOAA Office of Global Programs and NASA Earth-Sun Science Applied Sciences Program.

Comparing Mosquito Attractiveness to Bird-, Mammal-, and CO_2-Baited Traps in Rural and Suburban Areas

Marilou Thomas, Beatriz Perez, Paula A. Macedo, Stan Wright, David A. Brown

Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Road, Elk Grove, CA 95624

In 2006, the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) initiated a study comparing mosquito attractiveness to the standard CO_2-baited traps and the bird-baited traps (BBTs) (Perez et al. 2007). Although a greater number of *Culex* mosquitoes were attracted to BBTs than to CO_2 traps in both rural and suburban sites, diversity was greater in the CO_2-baited traps. For the 2006 study, only four trap sites were utilized (two rural and two suburban) which precluded a more complete analysis of the data. In 2007, we expanded the previous study by incorporating more trap sites and mammal-baited traps (MBTs) in order to determine the host selectivity of mosquitoes in rural and suburban areas of Sacramento County and to compare the attractiveness of all three trap types to different mosquito species.

The study was conducted weekly from June through September. Once a week, one trap of each type (BBT, MBT, and CO_2) was set up in 12 sites (6 rural and 6 suburban) in the afternoon and collected the following morning. We evaluated mosquito abundance, diversity, and tested mosquito pools for the presence of West Nile virus (family Faviviridae, genus *Flavivirus*, WNV). Pigeons (*Columba livia*) captured at the SYMVCD property were use as attractants in the BBTs. Guinea Pigs (*Cavia porcellus*) were used as attractants in the MBTs. The animals used in this study were housed at SYMVCD and provided with food and water as needed. All trap types had the same basic configuration and followed design described by Perez et al. (2007).

Overall, our results agree with the previous study, where a greater number of *Culex* mosquitoes were captured with the BBTs than with the CO_2-baited traps, while the CO_2-baited traps captured a greater diversity of species. As shown in Table 1, *Culex pipiens L.* were the most abundant species collected in CO_2 traps in rural sites, but they were more abundant in the BBTs than in the CO_2 traps in suburban sites. One hypothesis for that is that pigeons are an established and common urbanized bird, and they may be more attractive due to the mosquito species feeding habits in such areas. Interestingly, *Cx. tarsalis* Coquillett were more abundant in BBTs at rural sites, but in CO_2 traps at suburban sites. Most *Aedes vexans* (Meigen) (98%) and *Ae. melanimon* (Dyar) (98.1%) were captured by CO_2 traps in rural sites, while most (82%) *Culiseta incidens* (Thomson) were captured by CO_2 traps in suburban sites. *Cx. pipiens* and *Cx. tarsalis* were the only two species common to all three trap types in both rural and suburban sites.

As *Cx. pipiens* and *Cx. tarsalis* were the common factor to all of the traps, we compared their abundance over time in the three trap types (Fig. 1). Overall, *Cx. pipiens* were more attracted to BBTs, and *Cx.*

tarsalis were attracted to both CO_2-baited traps and BBTs depending on the month during the season (Fig. 1). As expected, both species seem to be less attracted to the MBTs.

Statistical analysis (SAS 2005) of total abundance data showed no significant differences between CO_2-baited traps and BBTs in rural sites, and MBTs collected significantly fewer mosquitoes than the two other trap types (Table 2). There was no significant difference among the three trap types in suburban sites. When we compared the diversity of species captured by the three trap types, CO_2-baited traps captured significantly more species than BBTs, which captured more than MBTs in both rural and suburban sites (Table 3).

We tested over 950 mosquito pools for this study, but only seven were positive for WNV. Of the seven, four were mosquitoes from BBTs, two from CO_2-baited traps and one was from a MBT. We were unable to perform a complete evaluation of this component of the study due to the limited number of positive mosquito pools.

Implementing a greater variety of surveillance tools may increase the chances of early WNV detection. Since *Cx. pipiens* and *Cx. tarsalis* are the main vectors of WNV in California, BBTs may be a useful tool in capturing these mosquitoes for virus testing, especially in suburban areas. In the future, we plan to expand this study to compare trap placement at different canopy elevations in suburban sites.

Acknowledgements

The authors wish to thank the laboratory staff at SYMVCD for helping set up traps, identify mosquitoes, and test them for WNv. Thanks are also extended to Beatrix Treiterer and the staff at Stone Lakes National Wildlife Refuge.

REFERENCES CITED

Perez, B. L., S. A. Wright, D. E. A. Elnaiem, P. A. Macedo and D. A. Brown. 2007. A preliminary evaluation of mosquito attractiveness for bird-baited traps. Proc. & Papers Mosq. Vector Control Assoc. Calif. 75:65-67.

SAS Institute Inc. 2005. SAS® version 9.1.3. Cary, NC: SAS Institute Inc.

Table 1. Mosquito species diversity and abundance captured in CO_2 baited traps (CO2), bird-baited traps (BBT), and mammal-baited traps (MMT) in rural and suburban areas of Sacramento County 2007.

Species	Rural			Suburban		
	CO2	BBT	MBT	CO2	BBT	MBT
Culex pipiens	3471	2768	580	583	867	105
Cx. tarsalis	2036	4566	734	760	556	124
Cx. stigmatosoma	12	28	1	0	2	0
Cx. erythrothorax	1021	373	158	4	1	0
Anopheles freeborni	113	0	0	32	0	0
An. franciscanus	3	0	0	0	0	0
An. punctipennis	7	0	0	0	0	0
Aedes nigromaculis	2	0	0	0	0	0
Ae. melanimon	53	0	1	0	0	0
Ae. sierrensis	3	0	5	1	0	0
Ae. vexans	743	9	5	1	0	0
Culiseta incidens	17	1	0	91	2	0
Cs. inornata	4	0	0	0	0	0
Orthopodomyia signifera	1	0	0	0	0	0

Table 2. Mean abundance ± SE of all the total number of mosquitoes captured by the three trap types in Sacramento County, 2007.

Trap Type	Rural	Suburban
CO2	83.49 ± 6.34 a	17.26 ± 6.49 a
BBT	89.32 ± 6.41 a	17.01 ± 6.53 a
MBT	21.57 ±7.10 b	4.38 ± 7.65 a

Means followed by the same letter in the same column are not significantly different.

Table 3. Species diversity mean ± SE of mosquitoes captured by the three trap types in Sacramento County, 2007.

Trap Type	Rural	Suburban
CO2	3.4 ±0.1 a	2.04 ± 0.1 a
BBT	2.4 ± 0.1 b	1.66 ± 0.1 b
MBT	1.96 ± 0.1 c	0.88 ± 0.1 c

Means followed by the same letter in the same column are not significantly different.

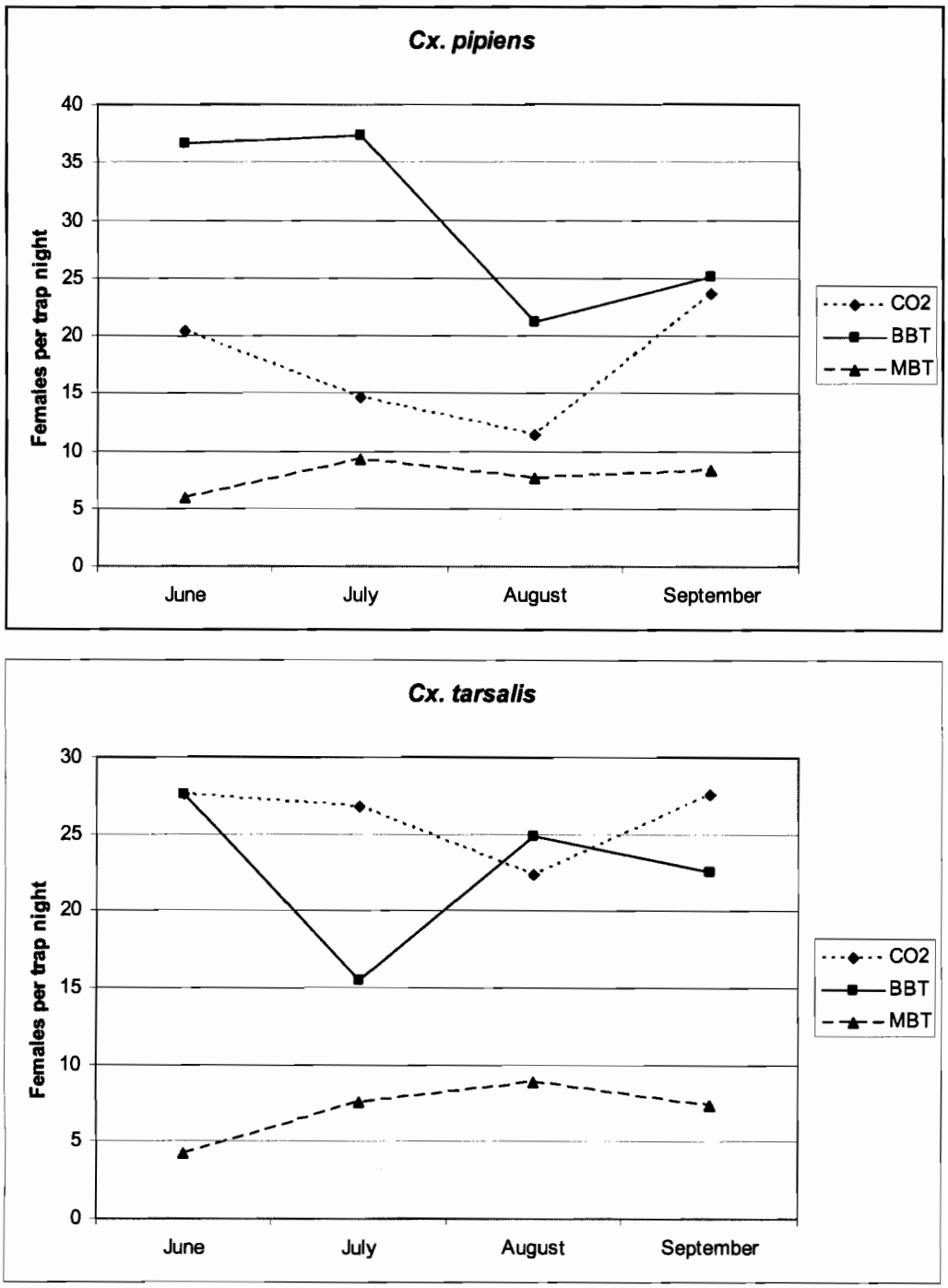


Figure 1. Average abundance of *Culex pipiens* and *Cx. tarsalis* captured in CO_2 traps(CO2), bird-baited traps (BBT), and mammal-baited traps (MBT) from June to September in Sacramento County, 2007.

Evaluation of Arboviral Activity in Orange County, California, during 2007

Robert Cummings, B. Fred Beams, Stephen G. Bennett, Karin De Collibus, Richard Evans, DVM, MS, Carrie Fogarty, Jim Francisco, Tunisia Hardy, Ralph Havickhorst, Catalina Herrera, Martine Jozan, MD, DrPH, Taylor Lura, Ivann Martinez, Toby McLaughlin, Tim Morgan, Kiet Nguyen, Tom Reynolds, Art Tilzer, Robert Velten, Josie Weir, and J.P. Webb, Jr.

Orange County Vector Control District, 13001 Garden Grove Blvd., Garden Grove, CA 92843
rcummings@ocvcd.org (714) 971-2421, ext. 138

ABSTRACT: The Orange County Vector Control District continued its arbovirus surveillance program in 2007 by testing mosquitoes, avian blood samples from free-ranging wild birds and sentinel chickens, and dead birds collected from various animal control agencies and the public. Evidence of West Nile virus (WNV) infection was detected in mosquito pools (27 of 997), wild birds (58 of 3,556) and dead birds (34 of 263). No sentinel chickens in a flock of ten birds tested positive for WNV antibodies. Nine non-fatal human cases of WNV infection were reported in the county during 2007. *Culex quinquefasciatus* was the most frequently trapped mosquito, accounting for more than half of the submitted pools (554 of 997) and positive pools (24 of 27). House Finches (*Carpodacus mexicanus*) and House Sparrows (*Passer domesticus*) accounted for 50.6% (173 of 342) of *Cx. quinquefasciatus* blood meals, while American Crows (*Corvus brachyrhynchos*) represented only 3.1% (10 of 342). Correspondingly, house finches and house sparrows comprised the majority of the WNV-seropositive free-ranging wild birds (54 of 58). American Crows made up the majority (79.1%) of positive dead birds (34 of 43). The seasonal (May - October), maximum likelihood estimation (MLE) in *Cx. quinquefasciatus* was comparatively greater in 2007 than in 2006 (3.1/1,000 vs. 0.7/1,000, respectively), as was the number of reported human cases (9 vs. 6). In contrast, the WNV-seropositive rate in the sampled wild bird population and the percent of WNV-positive dead birds decreased in comparison to 2006 (0.8% vs. 1.5%, and 13.8% vs. 19.4%, respectively). Critical threshold (Ct) values of RT-PCR-tested, WNV-positive mosquito pools and dead bird tissues, when compared to *in situ* ELISA results from the same specimens, indicated that recoverable virus could be obtained for PCR critical threshold values up to 31. Similarly, when WNV-positive immunohistochemistry (IHC) test results were compared to RT-PCR WNV-positive dead bird tissue determinations, 90% (18 of 20) of the IHC results agreed with the PCR findings.

INTRODUCTION

The Orange County Vector Control District (District) encompasses approximately 789 square miles (all of Orange County), and approximately 3.1 million residents reside within the borders of the county (US Census Bureau 2007). Most of the District is comprised 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 the county are several natural mosquito-producing fresh and salt-water wetlands. Four important vectors of West Nile virus (Family Flaviviridae, genus *Flavivirus*, WNV), *Culex tarsalis* Coquillett, *Culex quinquefasciatus* Say, *Culex stigmatosoma* Dyar, and *Culex erythrothorax* Theobald (Goddard et al. 2002,

Reisen et al. 2005) are routinely collected in the county (Gruwell et al. 1988). The District employed an integrated arboviral disease surveillance system throughout the year, comprised of avian serosurveillance (sentinel chickens and wild birds), testing dead birds and mosquitoes, and monitoring veterinarian and physician reports for WNV infections in animals and humans.

MATERIAL AND METHODS

Mosquito Trapping: Mosquitoes were collected weekly from a total of 75-80 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). Mosquitoes from these sites were identified and pooled for testing by TaqMan Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) (Applied Biosystems 7300) using West Nile virus (WNV)-specific primers (Lanciotti et al. 2000). Maximum Likelihood Estimations (MLEs) were calculated weekly using PooledInfRate 2.0 software (Biggerstaff 2004).

Collection of Blood-fed mosquitoes: As part of a collaborative, multi-agency study, blood-fed mosquitoes were collected in CO_2-baited traps, gravid traps, and aspirated at known mosquito resting sites by personnel from Northwest (Riverside County), West Valley (San Bernardino County), and Orange (Orange County) Mosquito and Vector Control Districts. Specimens were quickly identified to species and placed into sequentially numbered vials for each site and date, held in a low-temperature freezer at -80°C, and sent on dry-ice to the Connecticut Agricultural Experimental Station (CAES) for PCR analysis of products of the *cytochrome b* gene in blood meals (Molaei et al. 2006).

Wild Bird Serosurveillance: Free-ranging wild birds were trapped in ten modified Australian crow traps (McClure 1984) at sites used to sample the adult mosquito population. Six of the ten trap sites were located in riparian corridors or wetland areas surrounded by suburban development. Birds were sampled at each site on alternate weeks (5 sites/week). Newly captured birds were banded, aged, sexed (if possible), bled and released. Blood samples (0.2ml) were taken from the jugular vein with a 1.0-ml syringe and a 28-gauge needle, dispensed immediately in 1.8 ml phosphate buffered saline (PBS) diluent with 0.75% bovine serum albumin and stored on ice until processed at the District's laboratory for detection of antibodies.

Sentinel Chicken Serosurveillance: The District maintained one flock of ten sentinel 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 St. Louis encephalitis (SLE), western equine encephalomyelitis (WEE) and WNV antibodies by the California Department of Health Services' Viral and Rickettsial Disease laboratory (CDHS/VRDL) by enzymatic immunoassay (EIA) (Chiles and Reisen 1998).

Serological Testing at the District: Serology was performed using a blocking ELISA, according to the protocol established at the University of Queensland, Australia (Hall et al. 1995) and evaluated by Jozan et al. (2003), on blood taken from free-ranging wild birds and sentinel chickens. Laboratory staff used a baculovirus-Kunjin epitope NS1 recombinant antigen, and the specific West Nile anti-NS1 monoclonal antibody 31112G. Testing for antibodies to SLE and WEE viruses were by EIA (Chiles and Reisen 1998).

Dead Bird Surveillance: Dead birds were collected in response to reports from the public via dead bird phone calls and through cooperation with various animal control agencies. Tissue samples (kidney, liver and spleen) were tested by immuno-histochemistry (Steele et al. 2000) and RT-PCR.

Tissue Culture Isolation: Cells: Vero cells (source: R. Poston at Louisiana State University) in MEM, 8% fetal calf serum and 1% 1M Hepes, PSEK (R. Hall, University of Queensland) in 199 plus 1% Hepes and 8 % FCS. Two-to-three-days-old monolayers were grown in either 16 x 100 borosilicate glass tubes

with hermetic screw cap, or 24 multiwell Falcon plates, in medium supplemented with 2.5% 1M Hepes and 0.2% 1M Tris. Cultures were inoculated with 0.1ml of undiluted specimen. Following an adsorption of 45 minutes at 37° C, fresh medium was added, and cells were incubated at 37° C. Cytopathic effect (CPE) was recorded daily, and cells and medium were harvested when cell destruction was multifocal.

***In Situ* ELISA:** One-day-old cell monolayers in 96-well plates were inoculated with specimen, undiluted and at 1:10. Cells exhibiting multifocal CPE were fixed by the addition of 70% PBS-bovalbumin-acetone buffer at room temperature for 1 hour, from which the fixative was later aspirated, and plates were incubated overnight at 37° C. After incubation, each fixed monolayer was washed twice in PBS-Tween and blocked with TENTC for an hour at room temperature. Exactly 0.05 ml of specific West Nile anti-NS1 monoclonal antibody 31112G was then added to the prescribed wells, plates were incubated at 37° C for an hour, washed four times, and an anti-mouse peroxidase conjugate was added and incubated for an hour at 37° C. Finally, the plate was washed six times, and ABTS substrate was added for 15-30 minutes. Plate readings were made with a spectrophotometer (Broom et al. 1998, Hunt et al. 2002).

RESULTS AND DISCUSSION

Mosquitoes: Pools of *Cx. quinquefasciatus* made up the largest component of the specimens collected (554 of 997) (Table 1). Of the 997 pools, 250 were submitted to the Center for Vector-borne Diseases (CEVC) at the University of California, Davis for arbovirus testing by multiplex real time RT-PCR; none tested positive for any arboviruses. The remaining 747 mosquito pools were tested at the District's laboratory by singleplex RT-PCR for WNV; 27 were WNV-positive (critical thresholds < 30). *Culex quinquefasciatus* comprised the majority of the positive pools (24 of 27). WNV-positive pools appeared in late July and continued until early November. MLEs peaked in early September at 20.1 (Figure 1).

Mosquito Blood Meal Analyses: PCR analyses of blood meals from *Cx. quinquefasciatus* females collected in CO_2-baited EVS and gravid traps, and from resting sites in Orange, Riverside, and San Bernardino counties during 2006 - 2007, indicated that collectively, House Finches (*Carpodacus mexicanus* Say) and House Sparrows (*Passer domesticus* L.) comprised approximately half (50.6%) of all blood meals (Figure 2). House Finches were fed upon nearly equally across urban/suburban (40.5%), riparian (35.7%), and wetland (36.4%) habitats, while house sparrows accounted for 29% of the blood meals from urban/suburban neighborhoods of Orange and Riverside counties (Figures 3 – 5). One blood fed *Cx. quinquefasciatus* (collected in Fullerton on July 26, 2006) that had fed on a house finch also tested positive for WNV.

Since PCR-based methods of blood meal identification allow for direct estimates of vector contact with different avian species (Molaei et al. 2006), information obtained in this study can be useful in evaluating the potential amplification hosts of WNV. These blood fed mosquito data clearly demonstrate the important role House Finches and House Sparrows play as primary WNV reservoirs, based on the ability of the virus to amplify in these avian hosts (Komar et al. 2003, Reisen et al. 2005). West Nile virus-infected House Finches and House Sparrows probably have a greater influence on WNV enzootic/epizootic maintenance, amplification and dispersal than infections in American Crows (*Corvus brachyrhynchos* Brehm) and Western Scrub Jays (*Aphelocoma californica* Vigors) in Orange, and portions of Riverside and San Bernardino counties, since corvid-derived blood meals for *Cx. quinquefasciatus* made up only 3.2% (11 of 342) of the total (Table 2). Furthermore, House Finches and House Sparrows are widely abundant across a variety of Southern California habitats and comprise a large proportion of the avian community (Great Backyard Bird Count 2005 – 2007), while crows

are highly concentrated in focal nighttime roosts when most mosquitoes feed.

The District is conducting a study of mosquito feeding preferences with the University of California, Riverside, and focusing on making blood fed mosquito collections at crow roost sites in Orange County. Observations of bird communities at crow roosts in the county indicate that there are a variety of other avian WNV reservoirs present at these roosts, and their role needs to be evaluated. These investigations will help determine if the relatively high proportion of *Cx. quinquefasciatus* feeding on House Finches, Mourning Doves, and House Sparrows (74%, or 253 of 342 blood meals, Table 2) is due to host abundance, preference, or a combination of the two factors. A host preference-WNV enzootic transmission cycle may exist between *Cx. quinquefasciatus* and House Finches in southern California similar to one between *Cx. pipiens* and American Robins (*Turdus migratorius* L.) in Maryland, where Kilpatrick et al. (2006) found that preferential host-seeking by *Cx. pipiens* on the relatively uncommon American Robin was responsible for the majority of WNV-infected mosquitoes.

Results on the host-seeking preferences of *Cx. tarsalis* are incomplete. Only 21 blood fed samples have been identified (Table 2) at the time of this writing; results are pending on additional *Cx. tarsalis* specimens.

Wild Bird Surveillance: Of 3,556 wild bird samples, 58 showed evidence of WNV antibodies (1.6%): 46 House Finches, 8 House Sparrows, 3 Rock Doves, and 1 Rufous-crowned Sparrow (Table 3). No wild birds tested positive for either SLE or WEE antibodies.

Sentinel Chickens: None of the sentinel chickens tested positive for any arbovirus.

Dead Bird Surveillance: Of the 458 birds collected, only 263 were suitable for testing, and 43 of these were found positive for WNV antigen by immunohistochemistry and/or RT-PCR (Table 4). Rates of WNV-positive dead birds declined from 19.6% (49/250) during 2006 to 16.3% (43/263). The proportion of WNV-positive non-corvids to total WNV-positive dead birds declined from 30.6% (15/49) in 2006 to 20.9 % (9/43). Figure 6 shows the location of WNV-positive dead birds, free-ranging wild birds, mosquito pools, and cities with human cases; Figure 7 depicts a timeline of WNV activity during 2007.

***In Situ* ELISA:** The *in situ* ELISA was performed with original tissue suspensions from RT-PCR-determined, WNV-positive dead birds (25) and mosquito pools (27). In all tests, the highest RT-PCR critical threshold (Ct) reading, which resulted in recovery of live virus, was 30.4; the sample came from kidney tissue necropsied from a dead crow. The *in situ* ELISA offered a back up system of confirmation for RT-PCR readings and warrants more examination on a larger sample size to pinpoint upper critical thresholds for borderline PCR results.

Acknowledgements

We gratefully acknowledge the assistance of Sue Koenig for data processing, and Lawrence Shaw, Assistant Manager/Director of Operations, and Gerard Goedhart, District Manager, for operational and financial support.

REFERENCES CITED

Biggerstaff, B. 2004. PooledInfRate 2.0. Web address: http//www.cdc.gov/ncidod/dvbid/westnile/software.htm

Broom, A. K., R. A. Hall, A. Johansen, N. Oliveira, M. A. Howard, M. D. Lindsay, B. H. Kay and J. S. Mackenzie. 1998. Identification of Australian arboviruses in inoculated cell cultures using monoclonal antibodies in ELISA. Pathology 30: 286-288.

Chiles, R. E. and W. K. Reisen. 1998. A new enzyme immunoassay to detect antibodies to arboviruses in the blood of wild birds. J. Vector Ecol. 23:123-125.

Cummings, R. F. 1992. The design and use of a modified Reiter gravid mosquito trap for

mosquito-borne encephalitis surveillance in Los Angeles County, California, 1987. Proc. Calif. Mosq. Vector Control Assoc. 56:58-68.

Goddard, L., A. Roth, W. K. Reisen and T. W. Scott. 2002. Vector competence of California mosquitoes for West Nile virus. Emerg. Infect. Dis. 8:1385-1391.

Great Backyard Bird Count (GBBC). Web address: http://www.birdsource.org/gbbc

Gruwell, J. A., B. L. Brown and J. P. Webb Jr. 1988. Passeriform birds as a surveillance method for arbovirus activity in rural and suburban sites in Orange County, California, 1987. Proc. Calif. Mosq. Vector Control Assoc. 60:170-176.

Hall, R. 1995. Immunodominant epitopes on the NS1 protein of Murray Valley encephalitis and Kunjin viruses serve as targets for a blocking ELISA to detect virus specific antibodies in sentinel animal serum. J. Virol. Methods. 51:201-210.

Hunt, A. R., R. A. Hall, A. J. Kerst, R. S. Nasci, H. S. Savage, N. A. Panella, K. L Gottfired, K. L. Burkhalter and J. T. Roehrig. 2002. Detection of West Nile virus antigen in mosquitoes and avian tissues by a monoclonal antibody-based capture enzyme immunoassay. J. Clinical Microbiol. 40:2023-2030.

Jozan, M., R. Evans, R. McLean, R. Hall, B. Tangredi, L. Reed and J. Scott. 2003. Detection of West Nile virus infection in birds in the United States by Blocking ELISA and Imunohistochemisty. Vector-Borne and Zoon. Dis. 3:99-110.

Kilpatrick, A. M., P. Daszak, M. J. Jones, P. Marra and L.D. Kramer. 2006. Host heterogeneity dominates West Nile virus transmission. Proc. Royal Soc. B: Biol. Sci. 273: 2327-2333.

Komar, N. S., S. Langevin, N. Hinten, E. Nemeth, D. Edwards, B. Hettler, R. Davis, M. Bowen and M. Bunning. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg. Infec. Dis. 9:311-322.

Lanciotti, R. S., A. J. Kerst, R. S. Nasci, C. J. Godsey, C. J. Mitchell, H. S. Savage, N. Komar, N. A. Panella, B. C. Allen, K. E. Volpe, B. S. Davis and J. T. Roehrig. 2000. Rapid detection of West Nile virus from human clinical specimens, field collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J. Clin. Microbiol. 38:4066-4071.

McClure, E. Bird Banding. 1984. Boxwood Press, Pacific Grove, CA, pp. 493-496.

Molaei, G., J. Oliver, T. G. Andreadis, P. M. Armstrong and J. J. Howard. 2006. Molecular identification of blood-meal sources in *Culiseta melanura* and *Culiseta morsitans* from an endemic focus of eastern equine encephalitis virus in New York. Am. J. Trop. Med. Hyg. 75:1140-1147.

Reisen, W. K., Y. Fang and M. Martinez. 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. J. Med. Entomol. 42:367-375.

Rohe, D. L. and R. P. Fall. 1979. A miniature battery-powered CO_2-baited trap for mosquito-borne encephalitis surveillance. Bull. Soc. Vector Ecol. 4:24-27.

Steele, K. E., M. J. Linn, R. J. Schoepp, N. Komar, T. W. Geisbert, R. M. Manduca, P. P. Calle, B. L. Raphael, T. L. Lippinger and T. McNamara. 2000. Pathology of fatal West Nile Virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. Vet. Pathol. 37:208-224.

U.S. Census Bureau. 2007. California QuickFacts; Orange County, California. URL: http://quickfacts.census.gov/qfd/states/06/06059.html

Table 1. Comparison of mosquito collection numbers and maximum likelihood estimations (MLE) by species for peak months of West Nile virus activity, 2007.

Species	Total Mosquitoes	WNV Positive Pools	MLE (May-Oct)
Cx. quinquefasciatus	11,690	24	3.1
Cx. erythrothorax	9,846	1	0.1
Cx. tarsalis	2203	1	0.5
Cx. stigmatosoma	481	1	2.1
Others	180	0	0
Annual Totals	24,400	27	n/a

Table 2. Blood meal sources for *Cx. quinquefasciatus, Cx. tarsalis, and Cx. erythrothorax* collected from Orange, Riverside, and San Bernardino Counties, 2005 –2007. N = 342 host sources from 333 individual *Cx. quinquefasciatus* females (9 with multiple sources).

Host Blood Meal Source	*Cx. quinque*	Percent	*Cx. tarsalis*	Percent	*Cx. erythro*	Percent
House Finch	113	33.9	8	38.0	9	19.5
Mourning Dove	77	23.1	6	28.4		
House Sparrow	57	17.1	1	4.8		
American Robin	17	5.1	1	4.8	1	2.2
American Crow	10	3.1	1	4.8		
Virginia Opossum	8	2.4			3	6.5
Domestic Cat	6	1.8				
Human	6	1.8	1	4.8	1	2.2
Song Sparrow	4	1.2				
California Thrasher	4	1.2				
Domestic Dog	4	1.2				
Western Tanager	3	0.9				
Western Bluebird	3	0.9				
Mourning Dove / Cat	3/3	0.9				
Green Heron	2	0.6			2	4.4
Cedar Waxwing	1	0.3				
Rock Dove	1	0.3				
Scrub Jay	1	0.3				
Anna's Hummingbird	1	0.3				
Wild Turkey	1	0.3				
Lincoln's Sparrow	1	0.3				
Ruby-crowned Kinglet	1	0.3				
House Wren	1	0.3				
Mule Deer	1	0.3				
Cottontail Rabbit	1	0.3			3	6.5
American Robin / Cat	1/1	0.3				
House Finch / Human	1/1	0.3				
House Finch / Rabbit	1/1	0.3				
House Finch / Cow	1/1	0.3				
Cliff Swallow / Myotis bat	1/1	0.3				
American Robin / Dog	1/1	0.3				
Cooper's Hawk			1	4.8		
Red-winged Blackbird					1	2.2
Wood Duck					1	2.2
Cinnamon Teal					1	2.2
Mallard			1	4.8	4	8.7
Marsh Wren					1	2.2
Yellow-breasted Chat			1	4.8		
Black-crowned Night Heron					3	6.5
Savannah Sparrow					1	2.2
Coyote					1	2.2
Dusky-footed Wood Rat					6	13.0
Roof Rat					8	17.3
Grand Total	333 (342)	100.00	21	100.00	46	100.00

Bird Species	Total Blood Samples	WNV Positive	Percent Positive
House Finch	2,350	46	2.0%
House Sparrow	926	8	0.5%
Rock Dove	162	3	1.9%
Others	118	1	0.8%
Totals	3,556	58	1.8%

Table 3. Results for free-ranging wild bird seroprevalence for 2007.

Table 4: Numbers of dead birds received, tested, and WNV-positive per month during 2007.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	Percent
Received	15	19	29	30	54	46	44	69	54	57	29	12	458	N/A
Tested	10	13	17	22	37	21	17	25	31	36	23	11	263	57.4% of rec'd
Positive	0	2	1	3	2	1	0	6	16	10	2	0	43	16.3% of tested

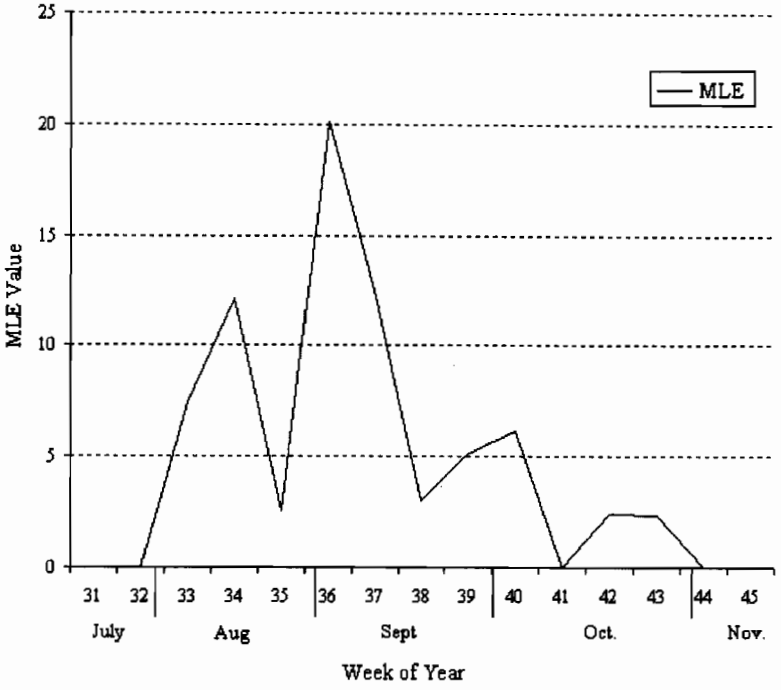


Figure 1: Weekly maximum likelihood estimations (MLEs) for WNV-positive *Cx. quinquefasciatus* in Orange County during July – November during 2007.

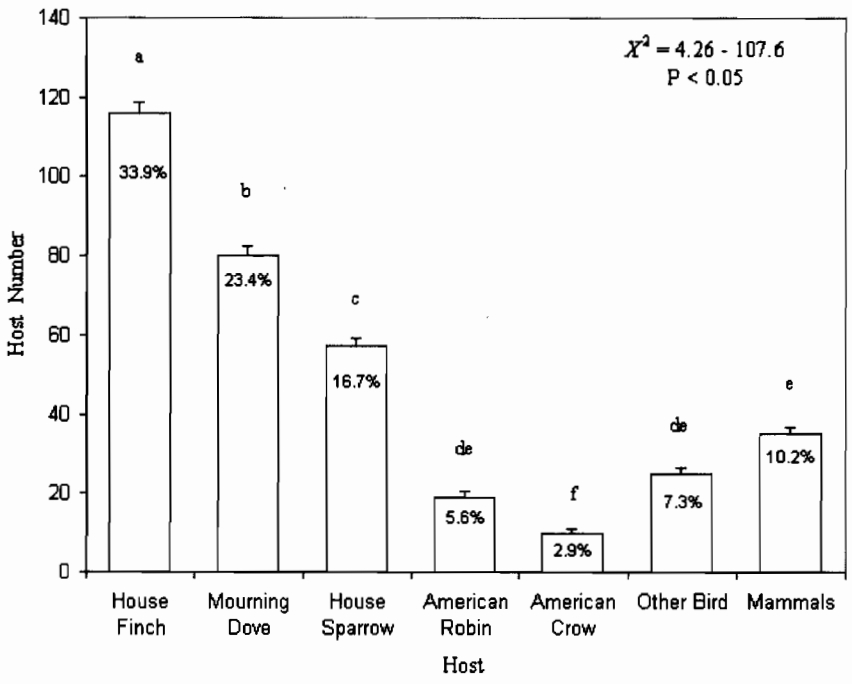


Figure 2: Host sources of *Cx. quinquefasciatus* in Orange, Riverside, and San Bernardino counties, 2006 – 2007. N = 342 blood meals from 333 mosquitoes (9 with multiple sources).

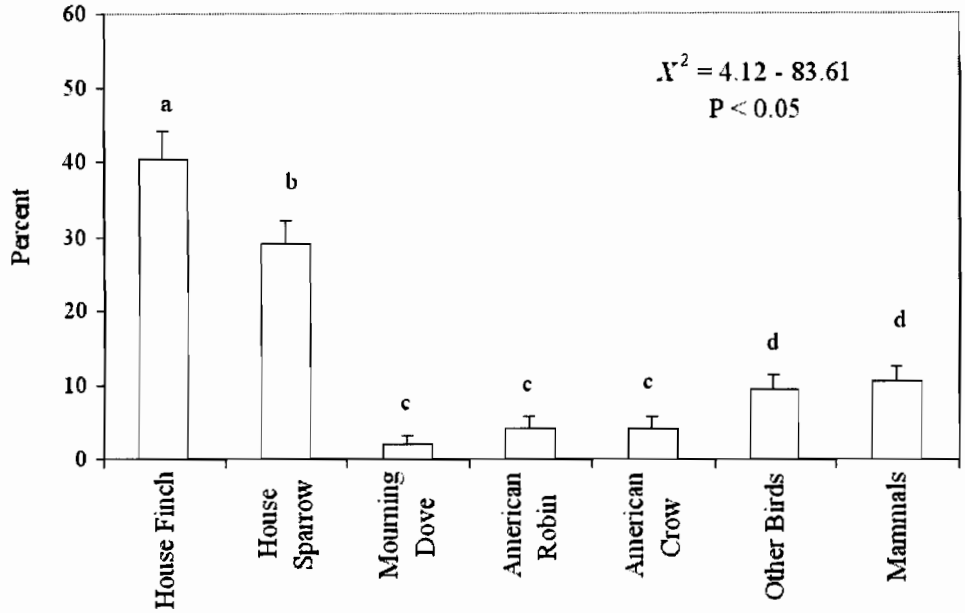


Figure 3: Host sources of *Cx. quinquefasciatus* in urban/suburban habitats of Orange and Northwest Mosquito and Vector Control Districts (West Valley Mosquito and Vector Control District data not included) during 2006 – 2007. N = 298 blood meals.

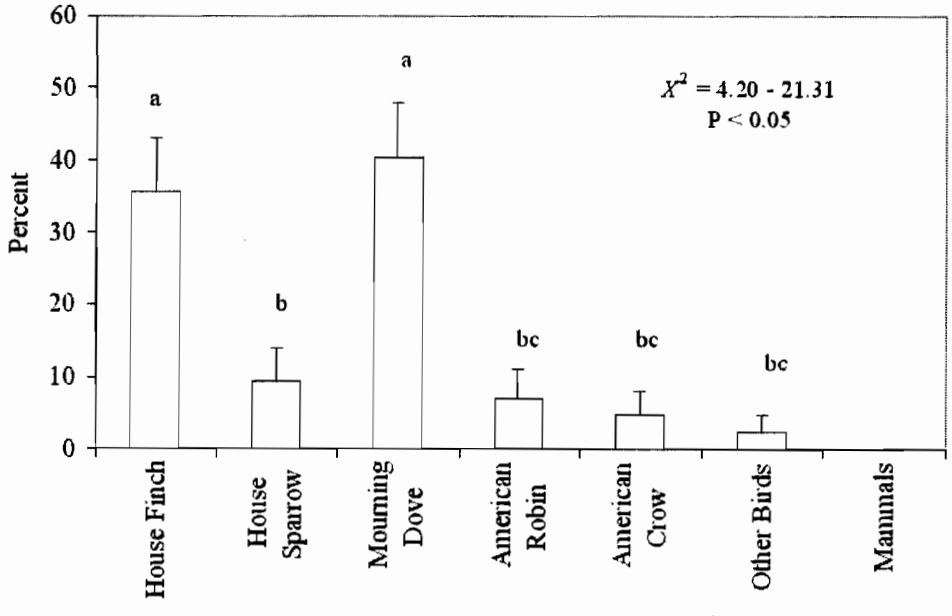


Figure 4: Host sources of *Cx. quinquefasciatus* in riparian habitats of Orange and Northwest Mosquito and Vector Control Districts (West Valley Mosquito and Vector Control District data not included) during 2006 – 2007. N = 298 blood meals.

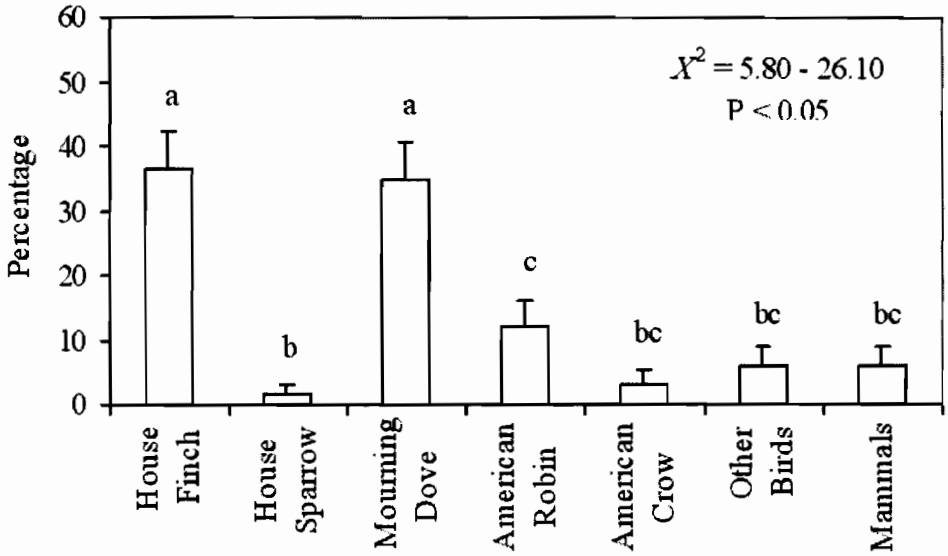


Figure 5: Host sources of *Cx. quinquefasciatus* in wetland habitats of Orange and Northwest Mosquito and Vector Control Districts (West Valley Mosquito and Vector Control District data not included) during 2006 – 2007. N = 298 blood meals.

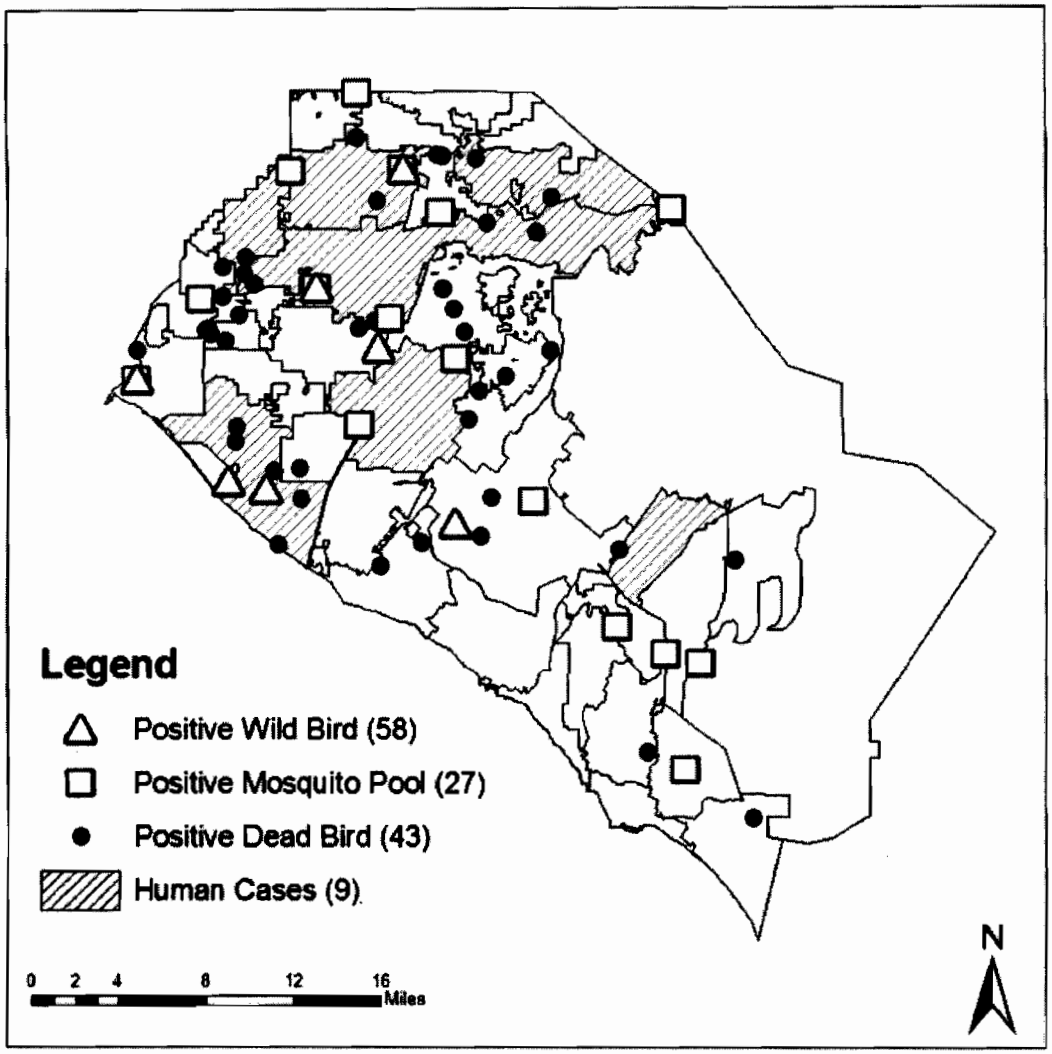


Figure 6: Distribution of WNV-positive wild birds, mosquito pools, dead birds, and human cases in Orange County, 2007. Human cases per city: Anaheim (1), Buena Park (1), Fullerton (2), Huntington Beach (1), Lake Forest (1), Santa Ana (2), and Yorba Linda (1).

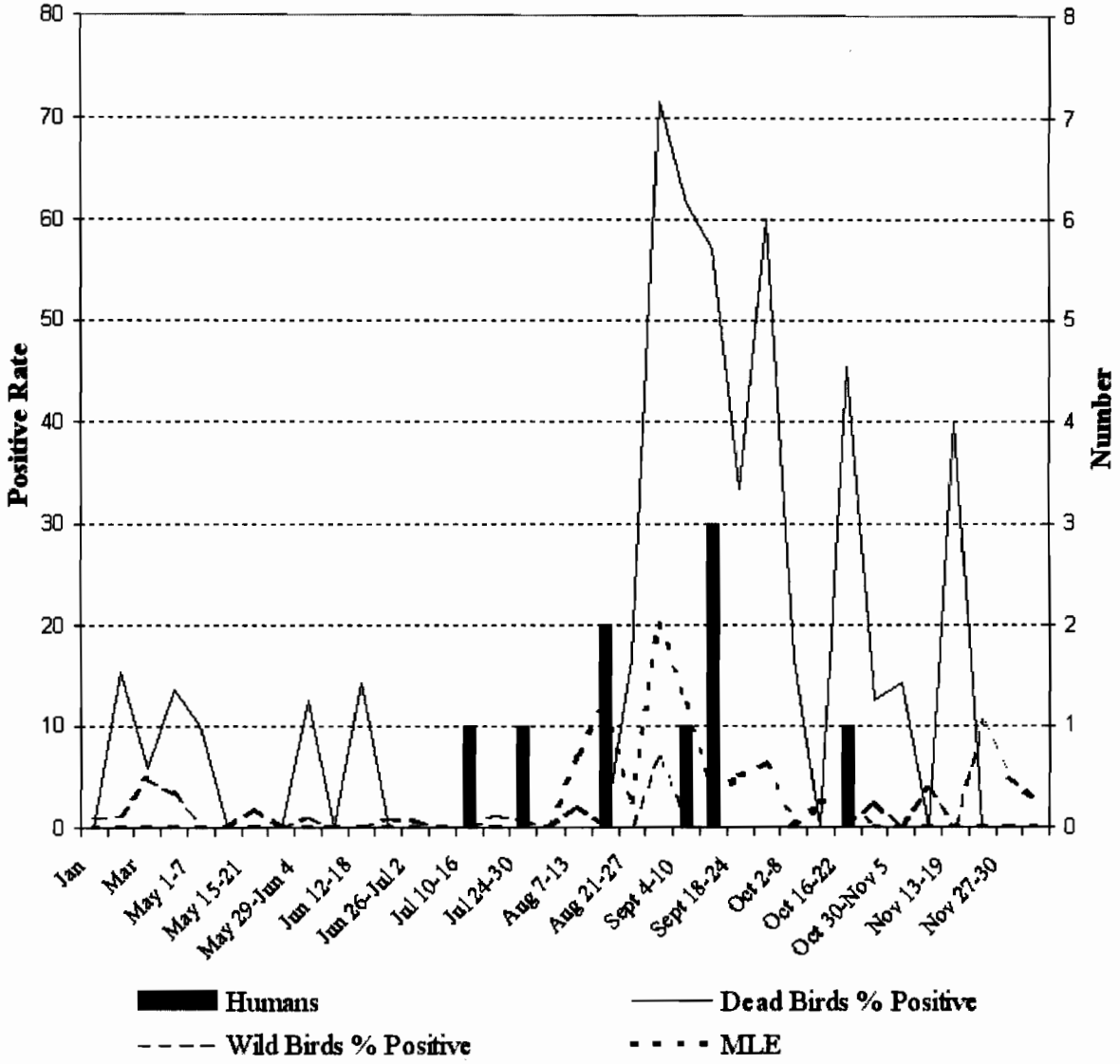


Figure 7. Timeline of WNV activity in dead birds, wild birds, mosquitoes, and humans in Orange County during 2007.

Field Biology and Fieldwork – Challenges for a New Generation

Richard M. Davis

Vector-Borne Disease Section, California Department of Public Health, 342 Tyrus Court, Nipomo, CA 93444; Richard.Davis@cdph.ca.gov

ABSTRACT: Increased social, institutional, and bureaucratic changes over the past decade have led to a decline in field-based studies of biology and may eventually threaten the ability to conduct fieldwork and train new field technicians. These changes include increased number and complexity of bureaucratic regulations, increased acceptance of animal rights ideologies, increased fear of pesticides, increased fear of nature, and a poor understanding of risk in a society seeking to avoid all risks. It can no longer be assumed that young biologists and new or prospective employees possess a set of basic outdoor skills that will safeguard them in the field. On the contrary, many aspiring biologists and new employees are inexperienced in nature and must be trained on fieldwork from the ground up, and need to be approached honestly, directly, and with a new understanding.

INTRODUCTION

Most field biologists never forget the excitement and wonder of their first experiences in the "wild," whether it was catching lightning bugs or butterflies, collecting worms for fishing, or catching, holding, and wondering in amazement at a frog, toad, or lizard. Many biologists are born out of these experiences and it is often assumed that similar field experiences early in the lives of bright and receptive youngsters stimulate the next generation to pursue professional careers in the biological sciences, or at least provide them with a much greater perception of nature. However, as academic institutions continue to lose or replace their natural history programs, many biologists today are concerned that the opportunity for students to experience the outdoors will be lost (Hafner 2007, Schmidly 2005). To complicate matters, new bureaucratic regulations, changing ideologies, and irrational fears based in poor understanding have further contributed to the decline in field-based studies of biology, ecology, and conservation, and may eventually threaten the ability to conduct fieldwork and train new field technicians. The ability to understand, protect, or even control the fauna and flora being studied is rapidly becoming lost because young biologists are ignorant of the diversity and complexity of nature (Mares 2002).

DISCUSSION

The following discussion will attempt to provide a better basis for understanding the many fundamental threats to field biology in an effort to strive for better training of field biologists, ecologists, and field technicians.

Increased number and complexity of regulations. Bureaucratic regulations represent a relatively small, but increasingly complex obstacle to fieldwork. Although the need for most regulations is well-justified, their complexity often makes it difficult for field biologists to know if they are in full compliance. Faced with these uncertainties, some types of fieldwork may be abandoned. In addition, colleges and universities rely heavily on a relatively new addition to the regulatory maze, the Institutional Animal Care and Use Committee (IACUC). All student projects or projects conducted in conjunction with a

university that involve animals, must be approved by the IACUC. The beneficial role of the IACUC is beyond question, but the ability of these committees to distinguish between good and bad field practices varies considerably (Laber et al. 2007).

Increased visibility of animal rights groups. Animal rights activists have become increasingly organized and vocal. These activists advocate the humane treatment of all animals, especially those used in research studies. These activists do not always pose a serious impediment to field-based studies, but they represent an obstacle with messages and ideologies that may dissuade younger generations from any form of animal research, laboratory or field-based.

Increased fear of pesticides. A large portion of the general population view all pesticides as bad, and is unable to distinguish differences between them, or any beneficial role (Dunlap and Beus 1992). Many of these people become even more intractable with attempts to clarify the differences or needs, and are unwilling to listen to any reason. In addition, the media is ineffective in allaying any fears of pesticide spraying, and in effect may even exacerbate those fears (Roche 2002).

Increased fear of disease. Hypochondrias and medical phobias have increased significantly in recent decades. Witness the increased use of hand sanitizers and the widespread use of antibiotics (Gray and Ropeik 2002) and "cure-alls" to treat anything and everything. The use of the internet has become a leading source of "diagnostic and treatment" information of all possible ailments, and the media often sensationalizes stories by focusing on worst-case scenarios. Things that would not have bothered us a generation ago certainly bother us now (Winik 2006).

Increased fear of nature. The fear of outdoors is keeping many families indoors, especially young families with children. Those who venture into our national, state, and local parks and forests face a frightening barrage of signs and pamphlets warning about bears, mountain lions, bison, snakes, poison oak, bubonic plague, and a host of other disclaimers required in an increasingly risk-adverse and litigious society. The media also sensationalizes reports of attacks by all kinds of animals, large and small. Parents are even keeping their children inside more because of fears of kidnapping and crime (Luov 2005). Indoor electronic games are rapidly replacing outdoor games. Luov (2005) envisions an entire generation of children raised indoors under virtual house arrest. The "nature-deficit disorder" in children today establishes the psychological and emotional foundations for a "fear of nature" later in life.

Decline in natural history studies. The increased commercialization and for-profit activities in our universities have significantly devalued research and study in natural history (Schmidly 2005). As a result, biology departments have willingly participated in a major shift from outdoor to indoor studies. Field biologists are suddenly in the minority, and their field-based studies and scientific worth have been significantly devalued in favor of computer modeling and lab-based research.

Failure to understand risk. Although a vast literature exists on the subject of "risk," few people understand risk and fewer yet realize that our minds make countless risk assessments every day (Adams 1995). Human perception of risk is predominantly emotional (Gray and Ropeik 2002), and people tend to fear sudden dangers more than slowly unfolding dangers. People also tend to fear new and exotic threats, such as avian flu, more than familiar diseases, such as the common flu, which kills around 20,000 in the United States every year. The common theme relevant to fear is that there exists a tendency to react rapidly and aggressively to new and unpleasant stimuli, but eventually habituate when exposed to the same stimulus repeatedly (Slovic et al. 2004). Habituation to a real threat can be dangerous, but it can be beneficial if the initial reaction is excessive but rational (Slovic et al. 2004). Recent popular books, such as State of Fear by Michael Crichton, and the present political

environment, continue to press our "fear button."

Risk management (communication about risk, risk mitigation, and decision making) is as much a political process as it is an analytical process. The purpose of risk analysis is risk reduction, but the ultimate objective of many regulatory agencies appears to be the absolute removal of all risk (Adams 1995). Although well-intentioned, this ignores the fact that many people willingly take risks of some degree every day. It also fails to distinguish between those who engage in risk by choice, and those who are exposed involuntarily. Disputes about risk and risk reduction usually stem from differences in premise, cultural background, personal risk tolerance, life experiences, or goals of risk reduction.

Risk and fieldwork. Fieldwork and field biology exposes researchers, biologists, and technicians to a long list of potential dangers they would not normally encounter at home, in the office, or in the laboratory. Some of these dangers include falling rocks and trees; Africanized bees, wasps, ticks, and spiders; attacks by mountain lions, bears, and dogs; sunburn and heat stroke; poison oak; rattlesnakes, bubonic plague, West Nile virus, hantavirus, tularemia, and rabies; rodent bites; accidental needle sticks from syringes; and working on wet, slippery, or unsteady ground. Add to this list the risk of a vehicle accident on the way to or from the field, and one might begin to wonder why fieldwork is done at all. Obviously, the benefits of fieldwork generally far outweigh the potential risks, especially since the dangers are quite low for the safety-conscious person.

In general, those who work in the field make a conscious decision to accept risk, or some degree of "calculated" risk. However, two new kinds of prospective employees are now often encountered: those who are nature-deprived and do not understand the risks of fieldwork, or those who are afraid of nature and refuse to participate.

CONCLUSIONS

Recent social and bureaucratic changes have made it increasingly difficult to conduct fieldwork. Fieldworkers must run a gauntlet of an increasingly complex set of regulations. In addition, there exist an escalating number of prospective employees who are afraid or ignorant of the natural world. Efforts to conduct fieldwork are also thwarted by an ever increasing risk-adverse society. It is imperative to realize that a large and growing proportion of the younger generation today have an understanding of nature that may be fundamentally different from that of the past. It must not be assumed that many prospective employees have a set of basic outdoor skills that will safeguard them in the field. These younger people need to be taught about fieldwork from the ground up. They must be approached and trained honestly, directly, and with care and understanding. Attempts to embellish exploits in the field or the use of frightening words and statements should be avoided for fear of unintentionally contributing to the amplification of risk perception. New employees must be trained to recognize and understand risks, and how to resolve or manage the risk potential. Effective communication empowers people to make wiser choices in their own lives (Gray and Ropeik 2002).

Last, but not least, ensure that policies, procedures, and protocols have not made any major assumptions relative to these new basic understandings. Policy statements such as the following make some of these major assumptions: "must accept risks associated with the natural world"; "hazards associated with the natural world are relatively easy to identify and manage"; and "often instinct and experience can help avoid problems". With a new increased awareness of the potential shortcomings in newer or prospective employees, there exists a need to ensure that such policy statements are clearer and less ambiguous.

Acknowledgements

Thanks to Professor Mark Hafner, Lousiana State University, for the opportunity to further discuss the subject, and to Paloma Nieto and Marco Metzger for their critical reviews.

REFERENCES CITED

Adams, J. 1995. Risk. Routledge, London, United Kingdom.

Dunlap, R. E. and C. E. Beus. 1992. Understanding public concerns about pesticides: an empirical examination. J. Cons. Affairs 26:418-438.

Gray, G. M. and D. P. Ropeik. 2002. Dealing with the dangers of fear. The role of risk communication. Health Affairs 21:106-116.

Hafner, M. S. 2007. Field research in mammalogy: an enterprise in peril. J. Mammal. 88:1119-1128.

Laber, K., B. W. Kennedy and L. Young. 2007. Field studies and the IACUC: protocol review, oversight, and occupational health and safety considerations. Lab Animal 36:27-33.

Luov, R. 2005. Last child in the woods: saving our children from nature-deficit disorder. Algonquin Books, Chapel Hill, North Carolina.

Mares, M. A. 2002. A desert calling: life in a forbidding landscape. Harvard Univ. Press, Cambridge, Massachusetts.

Roche, J. P. 2002. Print media coverage of risk-risk tradeoffs associated with West Nile encephalitis and pesticide spraying. J. Urban Health 79:482-490.

Schmidly, D. J. 2005. What it means to be a naturalist and the future of natural history at American universities. J. Mammal. 86:449-456.

Slovic, P., M. L. Finucane, E. Peters and D. G. MacGregor. 2004. Risk as analysis and risk as feelings: some thoughts about affect, reason, risk, and rationality. Risk Anal. 24: 311-322.

Winik, L. W. 2006. A nation of hypochondriacs? Parade Magazine May 21:21.

Future Directions in Data Management

Bruce F. Eldridge[1,2], Bborie K. Park[1], Christopher M. Barker[1,2], and William K. Reisen[1]

[1]*Environmental Assessment and Information Technology Program, Center for Vectorborne Diseases, and*

[2]*Department of Entomology, University of California, Davis, CA 95616*

It was inevitable that a significant public health situation involving a vectorborne disease would occur at a time when economics, environmental awareness, public suspicion of science and scientists, and survival mechanisms of vectors such as pesticide resistance would make vector control a challenge in California to an extent not seen for many years. However, one consequence of this situation is the vital need for mosquito and vector control agencies to have access to current, accurate, and persuasive data pertaining to all aspects of vector surveillance and control. The need for well designed data management plans in vectorborne disease prevention and control operations seems imperative given today's climate of concern by people representing a variety of viewpoints, vocations, and agency affiliations. Data on pesticide usage are required by government agencies. Surveillance data to justify operations are expected by the public and required by mosquito and vector control agencies to direct and evaluate the effectiveness of control operations and to develop novel approaches to mosquito and vector control.

Many changes have occurred in the methods and capabilities for over the past 20 years. Table 1 contrasts the most common methods used in data management in 1988 with the most common methods used in 2008.

These changes have not occurred in all agencies at the same rate, nor to the same extent. If you can visualize a gradual shift from the use of typewritten paper media, to single-organization electronic data management, to large inter-agency electronic networks, you will recognize that today some California agencies will be in each of the data management modes, or some transitional combination of them. This is due to various economic factors, including agency size and the population it serves.

Regardless of where individual agencies are currently, all will eventually move toward electronic data management eventually. Management systems will be guided by the following principles:

- Systems must be tailored to the requirements of individual agencies
- There must be increasing emphasis on data integrity, cost effectiveness, and integration into preventive and abatement operations
- There should be improvement of research programs through better and more accessible data
- There will be better hardware and software
- There will be financial support and job security for information technologists

These are the characteristics of modern and efficient relational database servers that we believe will bring about progress during the coming years:

- Must be spatially enabled to provide mapping services
- Must be accessible by a variety of methods
- Must not be vendor-specific
- Must be secure and persistent

Figure 1 represents a theoretical system that we are developing as an example of what future data management systems may look like. We have implemented our model system using currently-available open source software. Although the system works well with the software we have used, improved products may

become available in the future and we will continue to evaluate new products. MapServer and GeoServer are open source programs that anyone can download at no cost. GeoServer is a product that can take data from a spatially-enabled database such as PostgreSQL and provide maps over the Internet. For users having relatively modest computer resources, a web browser will suffice to view maps created by GeoServer or MapServer. This would be a case of a 'thick server' and a 'thin client', where most of the computer processing workload takes place on the server. However, GeoServer can also serve as a Web Feature Service (WFS). A WFS provides only binary data that are interpreted on client computers to produce maps using programs such as ArcGIS or UDig. This would be an example of using a thick client. In this case, most of the processing workload is done on the client computer. Where high-speed Internet connections are used, this method can produce very fast and efficient results. The primary advantage of this kind of system is that a single database can be used to accept, store, and serve mapping and other data, thus fulfilling the characteristics for data tools that we mentioned earlier. The end result for this kind of system is economy of scale, and great savings in personnel costs. It satisfies one of the principle mantras in our efforts of IT development: No data must be entered and verified more than once.

Another area that has seen great change just over the past 5 years is in the creation and management of websites. This is an area that is closely related to data management but has some significant differences. The days of static sites with a single or a few webmasters to manage content appear to have passed. Most new websites will use a content management system (CMS) in which the management of specific content is spread among many specialists. The CalSurv surveillance website and the Center for Vectorborne Diseases website use Drupal, a CMS.

CalSurv is a vectorborne disease surveillance system operated jointly by California mosquito and vector control agencies, the California Department of Public Health, and the University of California, Davis. Content management is handled by about 10 different vector biologists. For mapping, the CalSurv website uses a thick server and thin client approach, with Google maps as the server and a browser of user choice as the client. Google maps permits viewing maps either as simple graphics or as satellite images. Full zoom capability is available in either case.

An example of this mapping capability can be seen by going to the CalSurv website (http://www.calsurv.org) and going to the malaria section (Vectorborne diseases → Mosquitoborne diseases →Malaria). There is a story on malaria outbreaks in California since 1945. The story includes a map there containing several blue "balloons". Clicking on the "balloons" brings up a record pertaining to the particular mapped item from a database.

Other possibilities for these kinds of data management systems are limited only by our collective imaginations. We are currently working on several projects for future incorporation into CalSurv and the California Vectorborne Surveillance Gateway, including pesticide usage reporting, pesticide resistance mapping for resistance management, vectorborne disease predictive modeling, and mosquito and vector control decision support systems. The websites and underlying databases will continue to improve with the support and input of collaborating agencies.

Acknowledgments

Funding was provided by the National Oceanic and Atmospheric Administration (NOAA) Office of Global Programs and the National Aeronautics and Space Administration (NASA) Earth-Sun Science Applied Sciences Program. We gratefully acknowledge the collaboration of the Scripps Institution of Oceanography, UC San Diego, the many California mosquito and vector control agencies, and the California Department of Public Health.

Table 1. Data management methods 1988 vs. 2008

1988	2008
Nearly all data stored in paper records	Majority of records stored electronically
Analyses of large data sets are complex, laborious, and expensive	Analyses of large data sets are straight-forward, easy, and inexpensive
Nearly all reporting is done in paper journals or books; color renditions are expensive, therefore used sparingly	Electronic journals growing, use of color is now routine
Maps, tables, and graphs are prepared mostly by hand	Maps, tables, and graphs are prepared automatically and on demand
Data sets are usually prepared for single purposes (a report, a graph, a paper, etc.)	Data sets are designed for multiple purposes and integrated using an informatics-based approach

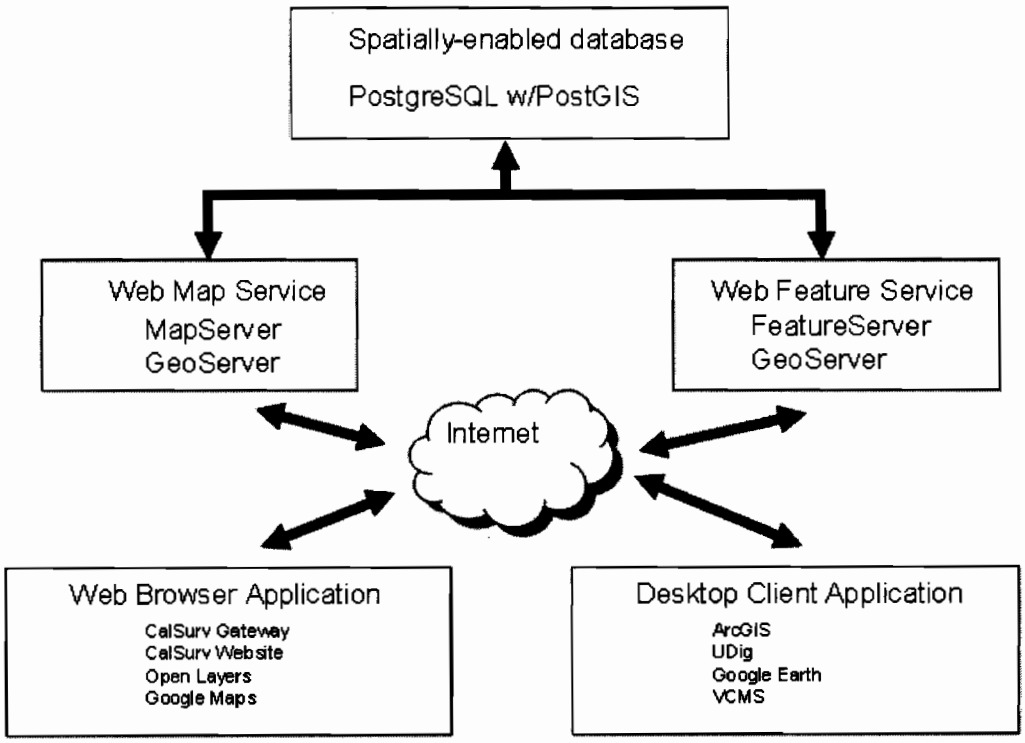


Figure 1. A theoretical web-based system for vectorborne disease data exchange.

Genetics and Pathogenesis of West Nile Virus

Aaron C. Brault, Stanley A. Langevin, Payal D. Maharaj, Richard A. Bowen, Ying Fang and William K. Reisen

Center for Vectorborne Diseases and Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis 95616, acbrault@ucdavis.edu, (530) 754-8359

West Nile virus (Family Flaviviridae, genus *Flavivirus,* WNV) transmission in North America (NA) has been characterized by high replication in passerine birds, with corvids [American Crows (*Corvus brachyrhynchos*)(AMCRs), ravens and jays] replicating the virus to the highest titers and subsequently being the most susceptible to infection (Figure 1)(Kramer et al. 2007). In order to determine if the WNV genotype introduced into NA has an increased virulence phenotype in avian hosts or whether NA bird species are merely more sensitive to infection with the virus, experimental AMCR infections have been performed with strains of WNV from Africa and Australia. Results of these experimental infection studies indicated that the strain introduced into NA in 1999 was significantly more virulent to AMCRs than other WNV strains (Brault et al. 2004). Selection modeling using 21 complete WNV genomes identified a single WNV genetic locus (NS3-249) to be under the effect of positive selection. Furthermore, experimental infection of AMCRs with recombinant WNVs from strains with variable avian virulence phenotypes implicated the same amino acid residue within the helicase domain [a threonine amino acid to a proline (NS3-P249T)] to be instrumental in the viremia and mortality response in this species (Brault et al. 2007). The NS3-P249T virus was nearly completely attenuated in AMCRs, with 12.5% mortality and peak viremia of 5 $\log_{10}$ PFU/mL, whereas the proline mutation at NS3-249 in the virus inflicted 100% mortality with a peak viremia of 9.3 $\log_{10}$ PFU/mL. These results confirmed the vital role of this locus for avian virulence potential and indicate the selective advantage of the NS3-249P for increased AMCR replication within the NY99 WNV genetic backbone (Brault et al. 2007). The importance of this genetic mutation is also signified by the fact that this substitution has arisen on three independent occasions, all of which were associated with outbreaks of human disease [Egypt in the 1950's (Hurlbut et al. 1956), Romania (Savage et al. 1999) and Russia in 1996 and 1999 (Platonov et al. 2001), respectively and the Israeli genotype in 1997 (Lanciotti et al. 1999, 2002) that was subsequently introduced into NA]. Furthermore, these results indicated that a single amino acid substitution could have a rather dramatic effect on viral replication phenotype and contribute to altered transmissibility in the field.

In contrast to explosive epidemic/epiornitic WNV transmission in the United States and Canada, circulation of the virus in Mexico and Central America has been characterized by little disease in humans, equines or avian hosts (Deardorff et al. 2006). A WNV isolate (TM171-03) made from a raven in Tabasco state, Mexico in 2003 (Estrada-Franco et al. 2003) exhibited reduced replication and lower mortality as compared to other North American isolates following experimental infection of AMCRs, House Sparrows (HOSP) and House Finches (*Carpodacus mexicanus* Say) (HOFI) (Brault et al. unpublished data). To identify the genetic determinants responsible for the 5 $\log_{10}$ PFU/mL reduction in viremia observed for the Mexican strain, an infectious

cDNA clone of the TM171-03 virus was engineered from a NY99 progenitor clone (Kinney et al. 2006) and used to generate recombinant WNVs for *in vivo* phenotypic comparisons with clone-derived, virulent NY99 virus using our AMCR virulence model. Four amino acid substitutions were identified in the premembrane protein, envelope protein and nonstructural proteins 4B and 5 (prM-I141T, E-S156P, NS4B-I245V and NS5-T898I)(Beasley et al 2004). The envelope mutation (E-S156P; N-Y-P) encoding an ablation of the N-linked glycosylation site (N-Y-S)] and three 5'NCR and four 3'NCR mutations were identified between the avian virulent NY99 and the lesser virulent TM171-03 strain (Beasley et al 2004). Only chimeric viruses with altered prM and/or E mutations modulated viremia and virulence potential in AMCRs. The E-S156P mutant exhibited reduced virulence (75% with average survival times extended by approximately 2 days; about 800-fold reduction in mean peak viremia titer) as compared to the NY99 parental strain in which 100% of AMCRs died. The prM-I141T mutant resulted in 80% mortality and a 40-fold reduction in mean peak viremia titer. The prM-I141T + E-S156P combination mutant resulted in a virus that elicited a 38,000-fold drop in peak viremia titer and a 50% survivorship, not significantly different from the parental Mexican strain. Similar results were identified with these constructs in HOSP and HOFI, indicating that these genetic residues encoded an attenuative phenotype that was consistent across numerous avian taxa. These data indicate that the combined effects of the prM-I141T and E-S156P mutations encode the reduced avian replication phenotype of the TM171-03 virus, and perhaps of related strains, in Latin America, and that these genetic determinants might contribute to diminished WNV transmission south of the United States-Mexican border.

Acknowledgements

This work was supported by the National Institutes of Health (AI061822), the Centers for Disease Control and Prevention (CI000235) and the Pacific Southwest Regional Center for Excellence (PSWRCE U54 AI65359).

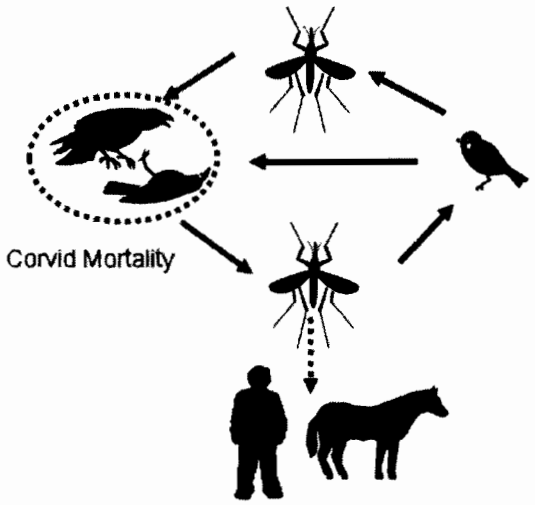


Figure 1. Transmission cycle of West Nile virus (WNV) illustrating its impact on producing corvid mortality.

REFERENCES CITED:

Beasley, D. W., C. T. Davis, J. Estrada-Franco, R. Navarro-Lopez, A. Campomanes-Cortes, R. B. Tesh, S. C. Weaver, and A. D. Barrett. 2004. Genome sequence and attenuating mutations in West Nile virus isolate from Mexico. Emerg. Infect. Dis. 10: 2221-2224.

Brault, A. C., C. Y. Huang, S. A. Langevin, R. M. Kinney, R. A. Bowen, W. N. Ramey, N. A. Panella, E. C. Holmes, A. M. Powers and B. R. Miller. 2007. A single positively selected West Nile viral mutation confers increased virogenesis in American crows. Nat. Genet. 39: 1162-1166.

Brault, A. C., S. A. Langevin, R. A. Bowen, N. A. Panella, B. J. Biggerstaff, B. R. Miller and N. Komar. 2004. Differential virulence of West Nile strains for American crows. Emerg. Infect. Dis. 10: 2161-2168.

Deardorff, E., J. Estrada-Franco, A. C. Brault, R. Navarro-Lopez, A. Campomanes-Cortes, P. Paz-Ramirez, M. Solis-Hernandez, W. N. Ramey, C. T. Davis, D. W. Beasley, R. B. Tesh, A. D. Barrett and S. C. Weaver. 2006. Introductions of West Nile virus strains to Mexico. Emerg. Infect. Dis. 12: 314-318.

Estrada-Franco, J. G., R. Navarro-Lopez, D. W. Beasley, L. Coffey, A. S. Carrara, A. Travassos da Rosa, T. Clements, E. Wang, G. V. Ludwig, A. C. Cortes, P. P. Ramirez, R. B. Tesh, A. D. Barrett and S. C. Weaver. 2003. West Nile virus in Mexico: evidence of widespread circulation since July 2002. Emerg. Infect. Dis. 9: 1604-1607.

Hurlbut, H. S., F. Rizk, R. M. Taylor and T. H. Work. 1956. A study of the ecology of West Nile virus in Egypt. Am. J. Trop. Med. Hyg. 5: 579-620.

Kinney, R. M., C. Y. Huang, M. C. Whiteman, R. A. Bowen, S. A. Langevin, B. R. Miller and A. C. Brault. 2006. Avian virulence and thermostable replication of the North American strain of West Nile virus. J. Gen. Virol. 87: 3611-3622.

Kramer, L. D., J. Li and P. Y. Shi. 2007. West Nile virus. Lancet Neurol. 6: 171-181.

Lanciotti, R. S., G. D. Ebel, V. Deubel, A. J. Kerst, S. Murri, R. Meyer, M. Bowen, N. McKinney, W. E. Morrill, M. B. Crabtree, L. D. Kramer and J. T. Roehrig. 2002. Complete genome sequences and phylogenetic analysis of West Nile virus strains isolated from the United States, Europe, and the Middle East. Virol. 298: 96-105.

Lanciotti, R. S., J. T. Roehrig, V. Deubel, J. Smith, M. Parker, K. Steele, B. Crise, K. E. Volpe, M. B. Crabtree, J. H. Scherret, R. A. Hall, J. S. MacKenzie, C. B. Cropp, B. Panigrahy, E. Ostlund, B. Schmitt, M. Malkinson, C. Banet, J. Weissman, N. Komar, H. M. Savage, W. Stone, T. McNamara and D. J. Gubler. 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 286: 2333-2337.

Platonov, A. E., G. A. Shipulin, O. Y. Shipulina, E. N. Tyutyunnik, T. I. Frolochkina, R. S. Lanciotti, S. Yazyshina, O. V. Platonova, I. L. Obukhov, A. N. Zhukov, Y. Y. Vengerov and V. I. Pokrovskii. 2001. Outbreak of West Nile virus infection, Volgograd Region, Russia, 1999. Emerg. Infect. Dis .7: 128-132.

Savage, H. M., C. Ceianu, G. Nicolescu, N. Karabatsos, R. Lanciotti, A. Vladimirescu, L. Laiv, A. Ungureanu, C. Romanca and T. F. Tsai. 1999. Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. Am. J. Trop. Med. Hyg. 61: 600-611.

How Climate Affects Mosquito Biology and Arbovirus Transmission

William K. Reisen

Center for Vectorborne Diseases and Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, (530) 752-0124, arbo123@pacbell.net

ABSTRACT: This brief overview outlines how climate change at different temporal scales affects the dynamics of mosquito populations and arbovirus transmission in California. In general, above normal precipitation decreases mosquito abundance in wet areas such as the Sacramento Valley and increases abundance in dry areas such as the San Joaquin Valley, whereas above normal temperature enhances the risk of virus amplification.

Climate describes average weather conditions in a defined region over relatively long periods of time and includes measures of precipitation and temperature. For mosquitoes, precipitation determines the amount of surface water habitat available for female oviposition and larval development and therefore correlates well with population size. In contrast, temperature governs the rate at which life history events occur, because mosquito temperature more or less approximates that of their microenvironment during resting and activity periods (Meyer et al. 1990). Both population size and the rate at which events such as the duration of the gonotrophic cycle and the extrinsic incubation period of pathogens affect key elements of vectorial capacity (Reisen 1989) and therefore arbovirus amplification and the force of transmission.

The impact of temperature on the duration of biological events can best be studied under controlled laboratory conditions and these data may be used to construct degree-day (DD) models (Fig. 1). In these models, the median duration of the biological events were calculated by probit or other analyses, the times in days inverted to a rate, and then plotted as a function of temperature. A linear regression model was fit, and the extrapolated intercept of the x-axis used to approximate the temperature at which no development would occur and the inverse of the slope used to estimate the number of degree days which is considered to be constant. These models have been completed for *Culex tarsalis* Coquillett (Fig. 1) and describe the effect of temperature on the rate of immature development from eclosion to emergence (Reisen et al. 1984), the duration of the gonotrophic cycle from blood feeding to oviposition (Reisen 1995), and the duration of the extrinsic incubation period (EIP) of encephalitis viruses from ingestion of the infectious blood meal until transmission (Reisen et al. 2006). In combination, these models can estimate the duration of generation time, the frequency of blood feeding, and the time from infection to transmission under varying field temperatures.

Climate variation can be examined at several temporal scales: weeks or months within seasons, years within decades, or among decades. Short term changes are close to what we think of as 'weather'. Interannual patterns show changes in global circulation patterns such as the El Niño/southern oscillation, whereas interdecadal change may depict long term climate trends or global warming. Some arboviruses (Family Flaviviridae) such as West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) and some mosquito species such as *Cx. pipiens* L. *and Cx. quinquefasciatus* L. seem to do best under hot and dry conditions and

are linked to interannual cycles of extraordinarily warm temperatures. In Fig. 2, for example, cooling of the Pacific was associated with warm and dry La Niña conditions in southern California and increases in the occurrence of SLEV infection in pools of *Cx. tarsalis* mosquitoes. Although California has not had a long history of WNV occurrence, we anticipate a similar cycling of WNV activity, because both WNV and SLEV have similar EIP thermodynamics in their mosquito vectors (Reisen et al. 2006), utilize the same avian hosts, and are closely related genetically, being classified within the same Japanese encephalitis virus serocomplex.

Significant warming trends were documented over the past 50 years at representative weather stations in Los Angeles and Coachella Valley (Indio), but not in Kern and Sacramento (Fig. 3). The >1°C increase documented in Los Angeles was attributed climate change related to urbanization and the increased surface area covered by concrete (Kalnay and Cai 2003). The potential impact of these temperature changes in the emergence of the F1 progeny from overwintering *Cx. tarsalis* was estimated for data from Los Angeles presuming that diapause was terminated in December (Reisen et al. 1995) and eggs were oviposited on 1 Jan 1951 or 2006. Curves compared the relative rates of development based on temperature data from those years. After F1 emergence, we presumed that the first blood meal was taken from a viremic bird and then plotted the time until first transmission was possible based on the EIP model. As anticipated, mosquitoes emerged and transmitted virus earlier in 2006 than 1951. However, these data could be biased by unseasonably hot or cool individual years, respectively, so we then compared mean temperatures for the 1951-1960 and 1996-2005 decades and got essentially similar results (Fig. 4). Here, spring temperatures were noticeably warmer leading to shorter larval developmental periods and earlier potential virus transmission events earlier in the season. Interestingly, temperatures and event timings were similar during midsummer. Therefore, it seemed that the main effects of temperature change were the elongation of the transmission season, the potential for earlier mosquito population emergence and virus amplification, and therefore a greater risk of tangential transmission to humans during summer because virus would have a longer and warmer period to amplify within the enzootic bird – mosquito cycle.

In summary, climate change has and will continue to have a major impact on the dynamics of mosquito populations (Reisen et al. 2008) and the epidemiology of the pathogens they transmit at varying time scales. In general warming temperatures will alter:

1. Duration of diapause, moving the time of mosquito emergence to earlier in spring.
2. Duration of larval development, increasing the rate of mosquito population growth and shortening generation times.
3. Duration of the gonotrophic cycle, increasing the frequency of blood feeding and therefore the frequency of host contact and the chances of acquiring and distributing viruses, and thereby increasing arbovirus amplification rates.
4. Duration of the extrinsic incubation period, increasing the transmission rate.
5. Duration of the transmission season, elongating the season enabling transmission earlier in the spring and later in the fall.
6. Distribution of virus activity, expanding virus transmission into marginal habitat in northern latitudes and higher elevations.

Precipitation effects are less direct and affect different ecosystems and mosquito species in different ways; both too much and too little precipitation can have negative effects. Precipitation can effect mosquito populations by altering the amount of larval habitat at different times of the year.

Winter rains create surface water oviposition habitat for overwintering females terminating diapause. Spring population abundance generally is positively correlated with increased winter or spring rain. High run-off in spring increases surface water. High snow pack can affect agriculture by altering planting dates and irrigation costs. There are major differences in the way precipitation affects rural and urban mosquitoes. For rural *Cx. tarsalis* populations, increased precipitation during winter is positively correlated in dry California south of Sacramento, but negatively correlated in wet California north of Sacramento and in some coastal areas. In dry areas, above normal winter rain creates suitable larval habitats in intermittent riparian systems and is used to flood retention ponds for underground aquifer recharge. Moderate flooding also creates new habitat in adjacent inundated areas. In contrast very high rainfall can 'flush' riparian systems and wash out larvae in wet areas, thereby negatively impacting early season mosquito populations. In contrast, for urban *Cx. pipiens* populations, winter rains fill artificial containers such as abandoned swimming pools creating new habitats, while eliminating underground breeding sources which are 'flushed out' by street run-off. In general, elevated rainfall enhances rural *Cx. tarsalis* populations and decreases urban *Cx. pipiens* and *Cx. quinquefsciatus* complex populations.

In summary, climate changes can:

1. Alter mosquito and virus distributions at continental and local scales.
2. Change mosquito phenology and population dynamics.
3. Impact ecosystem dynamics and thereby alter the size and age structure of vertebrate host populations.
4. Impact viral amplification dynamics by altering the intensity of vector-host contact, vector population size and the duration of the extrinsic incubation period.

A thorough understanding of these dynamics can be utilized for forecasting mosquito abundance and virus transmission risk in decision support systems for mosquito control which is the focus of our on-going research.

REFRENCES CITED

Kalnay, E. and M. Cai. 2003. Impact of urbanization and land-use change on climate. *Nature* 423:528-531.

Meyer, R. P., J. L. Hardy and W. K. Reisen. 1990. Diel changes in adult mosquito microhabitat temperatures and their relationship to the extrinsic incubation of arboviruses in mosquitoes in Kern County, California, U.S.A. *J. Med. Entomol.* 27:607-614.

Reisen, W. K. 1989. Estimation of vectorial capacity: relationship to disease transmission by malaria and arbovirus vectors. *Bull. Soc. Vector Ecol.* 14:39-40.

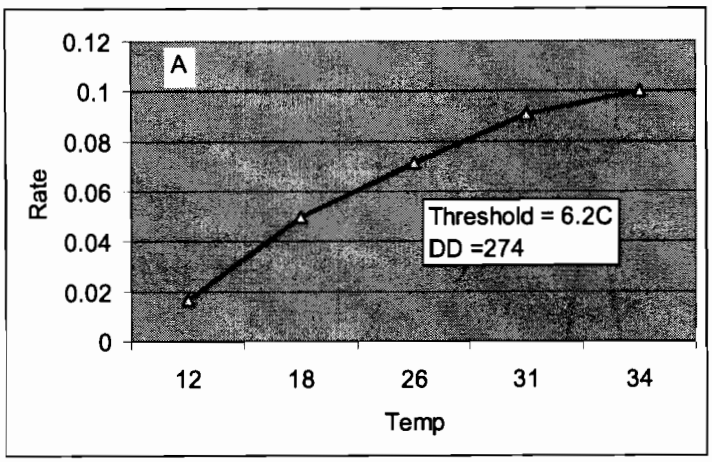
Reisen, W. K. 1995. Effect of temperature on *Culex tarsalis* (Diptera: Culicidae) from the Coachella and San Joaquin Valleys of California. *J. Med. Entomol.* 32:636-645.

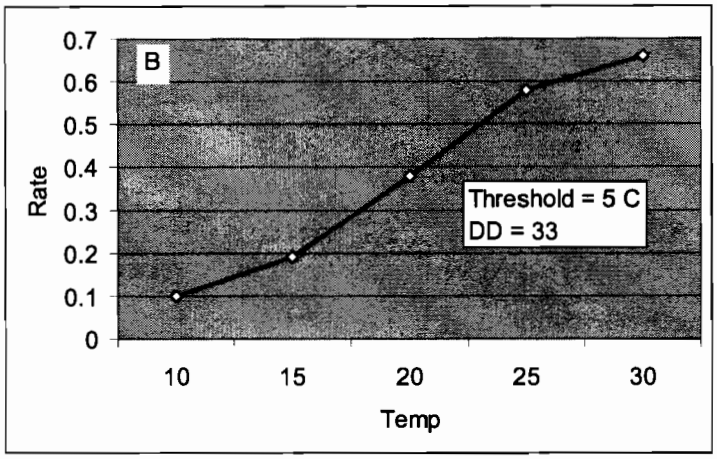
Reisen, W. K., D. Cayan, M. Tyree, C. M. Barker, B. F. Eldridge and M. Dettinger. 2008. Impact of climate variation on mosquito abundance in California. *J. Soc. Vector Ecol.* in press.

Reisen, W. K., Y. Fang and V. M. Martinez. 2006. Effects of temperature on the transmission of West Nile virus by *Culex tarsalis* (Diptera: Culicidae). *J Med. Entomol.* 43:309-317.

Reisen, W. K., M. M. Milby and M. E. Bock. 1984. The effects of immature stress on selected events in the life history of *Culex tarsalis*. *Mosq. News* 44:385-395.

Reisen, W. K., P. T. Smith and H. D. Lothrop. 1995. Short term reproductive diapause by *Culex tarsalis* (Diptera: Culicidae) in the Coachella Valley of California. *J. Med. Entomol.* 32:654-662.





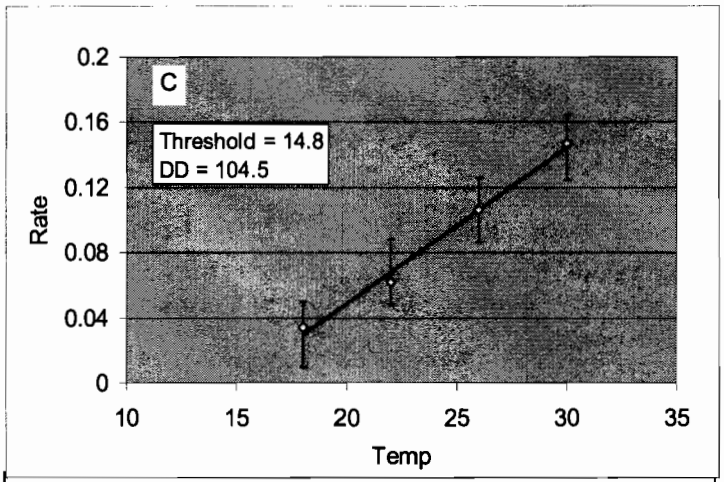


Fig. 1. Degree-day functions showing the rate of A) development from eclosion to emergence, B) duration of the gonotrophic cycle and C) duration of extrinsic incubation of WNV in *Cx. tarsalis* plotted as a function of incubation temperature [ºC].

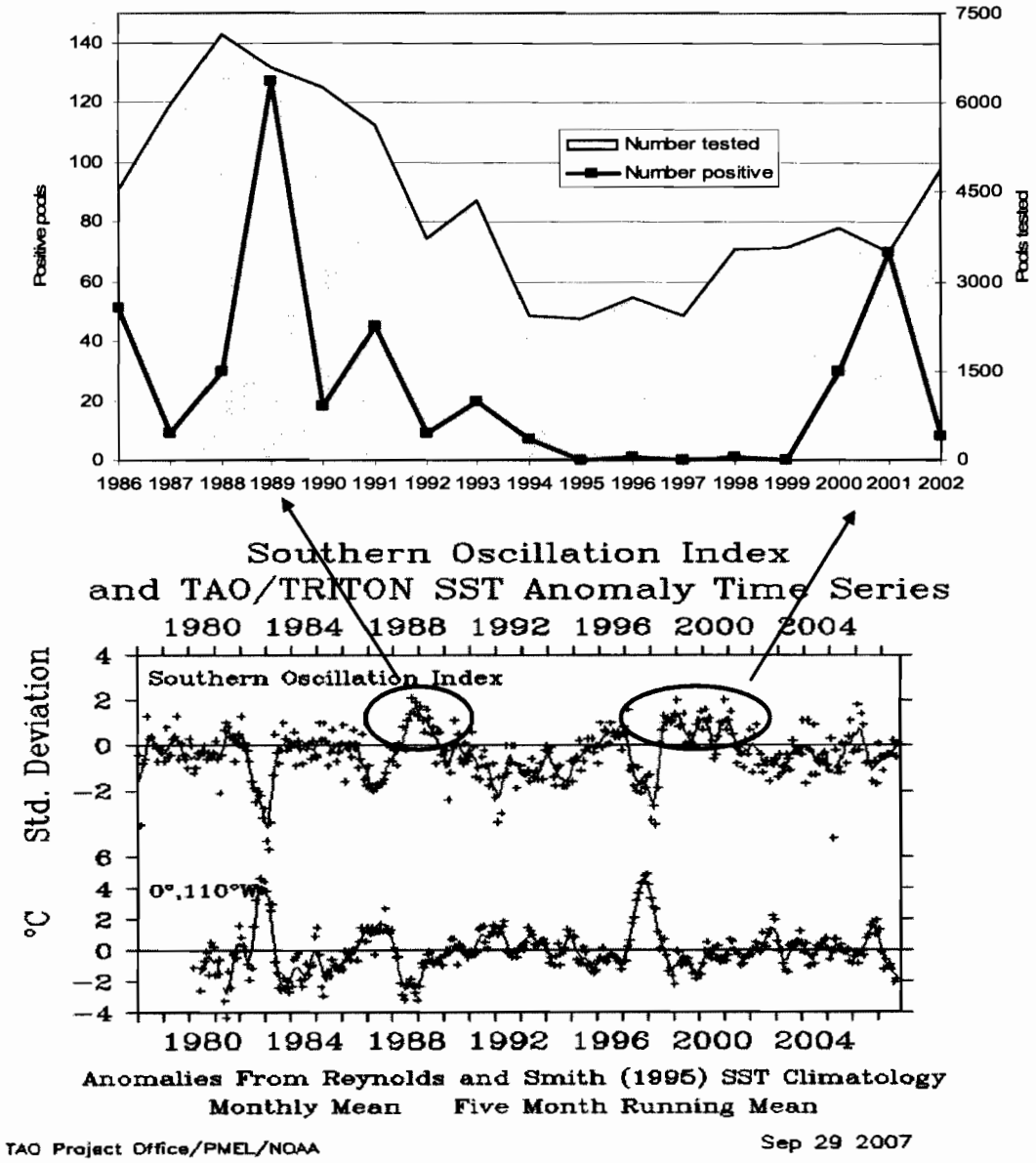


Fig. 2. Number of mosquito pools tested and positive for SLEV virus in California and the Southern Oscillation Index and Pacific Ocean temperature anomalies from 1978 – 2006. Arrows show periods with increased Southern Oscillation Index associated with increased SLEV activity.

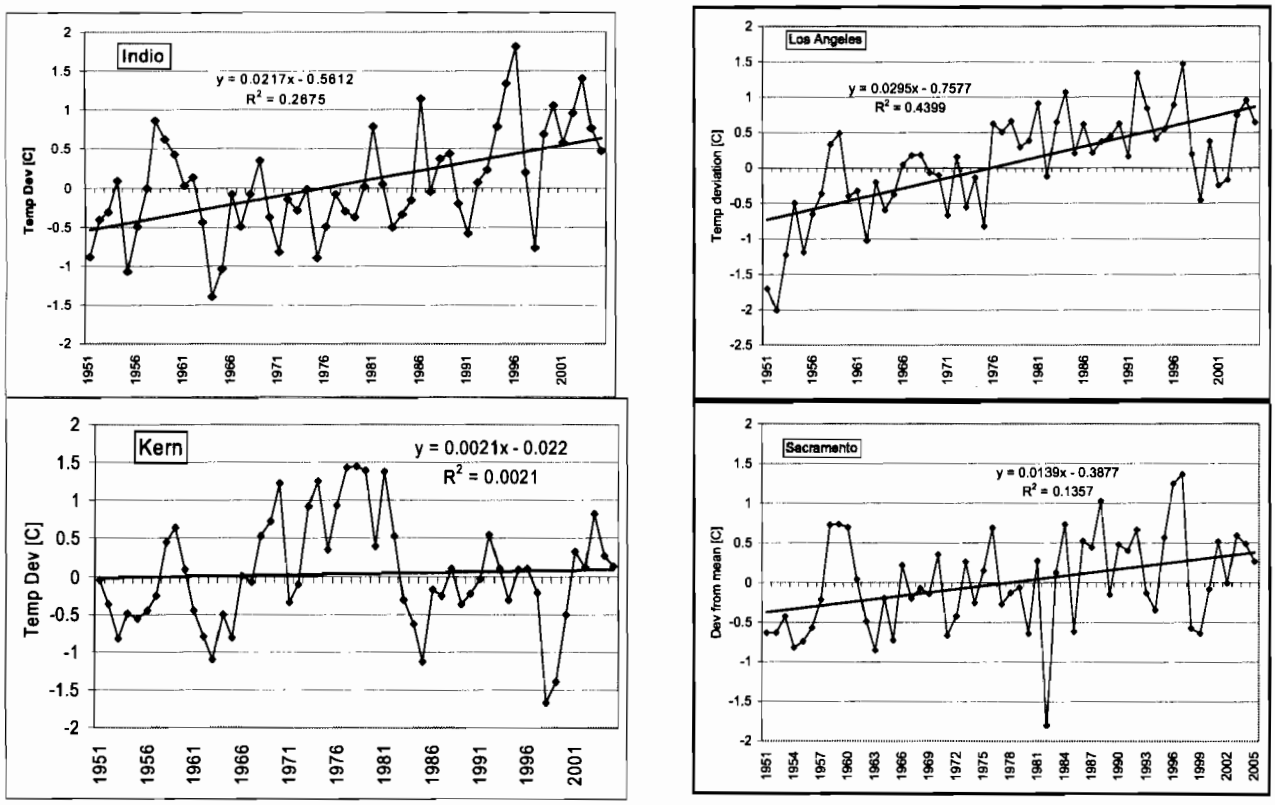


Fig. 3. Deviations from mean 50 year temperature plotted as a function of time in years. Regressions of deviations in Indio and Los Angeles were significant, indicating climate change.

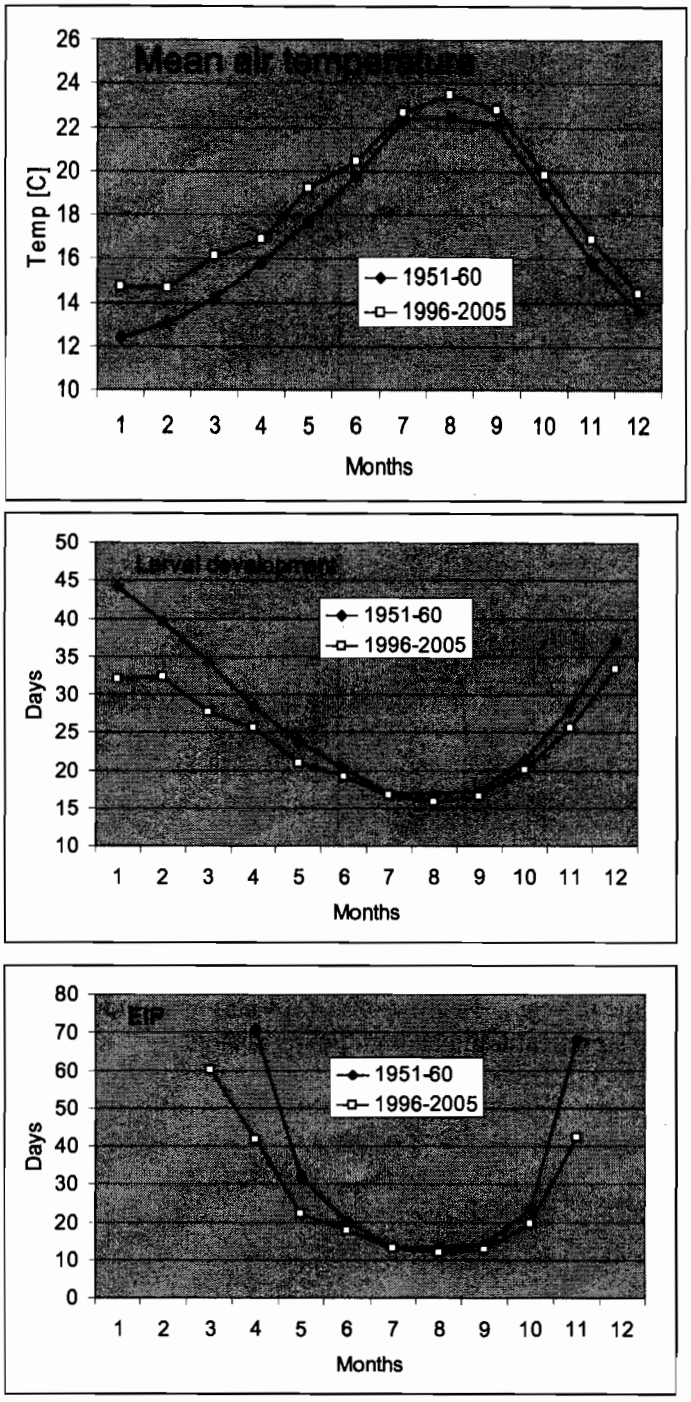


Fig. 4. Change in mean air temperature in Los Angeles and its impact on the duration of larval development and the extrinsic incubation period [EIP].

Human Cases of Flea-Borne Typhus in Orange County, California, during 2006 – 2008

Robert Velten, Art Tilzer, Stephen G. Bennett, Robert Cummings, Carrie Fogarty, and Ralph Havickhorst

Orange County Vector Control District, 13001 Garden Grove Blvd., Garden Grove, CA 92843
rvelten@ocvcd.org (714) 971-2421, ext. 138

ABSTRACT: Orange County, California, experienced 14 confirmed and two probable cases of flea-bone typhus from December 2006 to January 2008. The majority of cases had some association with patient exposure to feral cats and/or opossums infested with cat fleas. Surveillance efforts around case homes yielded 29 Virginia Opossums (*Didelphis virginiana*) and 13 Roof Rats (*Rattus rattus*). Blood and flea samples were taken from 13 House Cats (*Felis domesticus*) associated with several of the cases. Of 12 opossums with complete test results on organ tissue (adrenal, lung, liver, spleen, and kidney), flea pools, and blood samples taken from each animal, four opossums had at least one organ sample test PCR-positive for *Rickettsia felis*, all had at least one flea pool test PCR-positive for *R. felis*, and all blood samples tested negative via IFA and PCR for evidence of infection with any rickettsial agents. All 13 rats and a single flea collected from one rat were negative for the presence of either *R. felis* or *R. typhi* DNA. Results are pending on the remaining specimens (flea pools, organ tissue and blood samples from 17 opossums and 13 cats).

INTRODUCTION

In December 2006, the first case of flea-borne typhus in Orange County since 1993 was diagnosed in a 35-year-old female residing in Huntington Beach. By January 2007, a total of three cases had been diagnosed (an additional probable case associated with a confirmed case was treated but not tested via immunoassay) and the Orange County Vector Control District (District) was called by the Orange County Health Care Agency to investigate the probable enzootic sources of the outbreak. Trapping of mammals likely to host infected fleas was performed for several weeks in the vicinity of the human victims. By January 2008, an additional 11 confirmed and one more probable case had occurred, prompting further trapping and sample collections. This paper is an overview of the District's surveillance efforts and the results of cooperative investigations with California Department of Public Health (CDPH), Vector Borne Disease Section personnel, and testing by the Centers for Disease Control and Prevention Rickettsial Zoonoses Branch (CDC RZB).

MATERIALS AND METHODS

Human cases were diagnosed by health care providers based upon symptomology and confirmation using commercial *Rickettsia*-panel, immunofluorescent antibody (IFA) tests. Suspect patients were reported to Orange County Health Department personnel, who subsequently informed the Orange County Vector Control District (OCVCD). OCVCD staff conducted patient interviews and surveys of each victim's home and surrounding neighborhood to determine possible modes of exposure to flea-borne typhus. Baited live-animal traps were located as close to patient residences as was feasible, giving consideration to trap security and habitat suitability. OCVCD personnel took blood samples from captured Virginia Opossums (*Didelphis virginiana* Grey) and Roof Rats (*Rattus rattus* Waldheim) via

cardiac puncture of euthanized animals; fleas were removed by brushing, identified to species, counted, pooled, and stored at -80° C. Veterinarians from the Orange County Animal Care Services took samples of organ tissue (lung, spleen, kidney, and liver) and assessed the overall state of health of each animal during necropsy. When applicable, blood and flea samples from House Cats (*Felis domesticus* L.) associated with human typhus cases were taken by private practice veterinarians chosen by the patients. Animal and flea testing was carried out by the CDC RZB for discrimination between *Rickettsia felis* (endemic typhus) and *R. typhi* (murine typhus) via IFA and polymerase chain reaction (PCR) of blood, organ tissue, and flea samples.

RESULTS

Of 16 suspected typhus cases, 14 were confirmed with elevated antibody titers, and symptoms resolved with appropriate antibiotic (doxycycline) therapy. Two cases were unconfirmed, but were treated presumptively with antibiotics, since each patient was associated with at least one other confirmed case in the family and exhibited symptoms similar to those of other infected members.

Overall, the incidence of flea-borne typhus cases in Orange County in 2007 was less than one case per 100,000 residents (incidence = 0.52). Seven females and nine males, ranging in ages from 3 to 81, were afflicted during the months of December (7), January (4), June (1) and July (4) from December 2006 to January 2008. Symptoms were somewhat variable, but all were characterized by sustained high fever, headache and fatigue. Rash, nausea/vomiting, muscle aches and overall malaise manifested in some, but not all cases. Hospitalization averaged 5 days, and delay in proper diagnoses and treatment was responsible for extended stays of up to 9 days in some patients. Symptoms of most patients resolved within one day of corrective antibiotic treatment.

Interviews of human cases regarding potential exposure to infected fleas indicated probative vector/host encounters for 15 of 16 victims. A total of 1,427 fleas were collected from 29 opossums, 13 roof rats, and 13 pet cats. Ectoparasite brushings from 29 *D. virginiana* yielded 1,402 fleas (2 – 216 per animal), comprising 1,295 *Ctenocephalides felis,* 2 *Echidnophaga gallinacea,* 2 *Oropsylla* (*Diamanus*) *montanus,* and 105 *Pulex irritans*. Collections from 13 *R. rattus* yielded only 1 flea specimen, *Leptopsylla segnis.* Only 24 fleas (all *C. felis*) were collected from 13 *F. domesticus*, or found in their bedding. Flea samples were submitted to the CDC RZB for PCR detection and characterization of *R. felis* and *R. typhi*.

As of February 2008, blood samples from 12 *R. rattus* and 16 *D. virginiana* were found negative for *Rickettsia* via IFA and PCR of buffy coat cells. All tested *D. virginiana* flea collections had at least one pool of *C. felis* test positive for *R. felis* via PCR; the Minimum Infection Rate (MIR) for *C. felis* varied from 6% to 45%. For *P. irritans*, 2 out of 3 pools were found with *R. felis* DNA, and had a comparatively low MIR of 3%. A single *D.* virginiana carried 4 flea species: *C. felis*, *P. irritans*, *O. montana* and *E. gallinacea*. Of these fleas, two *O. montanus* were PCR-positive for *R. felis*, while two *E. gallinacea* were both negative. Additional specimens (~60% of fleas collected, including those from *F. domesticus* associated with human cases) are pending with the CDC RZB.

Organ samples from four *D. virginiana* tested PCR-positive for *R. felis*: 2 adrenal glands (2 animals), kidney (1 animal), liver (1 animal), and lung (1 animal). Positive fleas were recovered from two of these tissue-positive *D. virginiana*, while results are pending on fleas taken from the other two. The type of organ sampled varied by animal, depending on the veterinarian performing the necropsy.

DISCUSSION

This relatively large number of flea-borne typhus cases within a limited time frame (13 months) was a major departure from past

outbreaks in Orange County. In the 60 years between 1933 and 1993, the average annual number of cases was approximately one. Marked increases were noted in four outbreaks (1944, 1948, 1949 and 1974), during which 6 – 9 cases occurred per year. This pattern held until the early 1990s when no typhus cases were diagnosed for 12 years. The prevalence and improved efficacy of flea control measures on pet animals may explain this decrease, and the subsequent laxity in flea control due to the drastic reduction in flea populations may have contributed to the current resurgence of human infection.

Victims in the current outbreak varied from 3 - 81 years of age, with 7 females and 9 males involved. Out of 16 cases, 10 had one or more House Cats (*F. domesticus*), none of which had flea control measures instituted, and five of the ten had an association with adoption of a stray cat. Five of the 16 owned a pet dog(s) (*Canis familiaris*). In one incident, a patient's pet dog brought a dead *D. virginiana* into the victim's house, possibly exposing the owner to infected fleas that had fallen off the dead animal. One other case was associated with a dead D. *virginiana* in the attic. Only one of the 16 typhus patients had no known animal association. None of the victims recalled being bitten by fleas or noticed the presence of fleas in common living areas of their homes.

An interesting cluster of four typhus cases occurred in a single family in the city of Westminster during July 2007. The family adopted a flea-infested, pregnant "stray" *F. catus* (cat), which subsequently gave birth to a litter of kittens in the garage. Each victim participated in the rearing of the litter and had ample exposure to fleas living on the cats and the animal bedding. Symptoms occurred in four family members within a span of three weeks after adopting the stray cat. All patients recovered, but the first two victims were initially misdiagnosed as having a severe case of the flu. Once proper treatment began, symptoms resolved within a day. When an association of cases was made, treatment was instigated early in the course of the infection, and no hospitalization was required for the fourth victim. As has been observed elsewhere with flea-borne typhus cases, most victims experience significant delay in diagnoses and subsequent appropriate antibiotic therapy due to the complexity in arriving at a diagnosis (Civen and Ngo 2008).

The change in distribution of typhus cases from the first half of the 20th century compared to this recent outbreak (Figures 1 and 2), in association with cases in Long Beach (Prelesnik 2007), is curious. Historically, Los Angeles County has had annual outbreaks of what is now recognized as endemic typhus (Adams et. al. 1970, Azad et al. 1992, Williams et. al. 1992), as seen elsewhere, particularly in Texas (Boostrom et al. 2002). Increased urbanization and channelization of hydrological drainage systems may have had a detrimental effect upon flea hosts, especially opossums, until neighborhoods matured and resources became more readily available. Previously, north-central Orange County was characterized by agricultural land use, with small, developed areas, until suburban sprawl took over in the latter half of the 20th century. Assignment of these early cases to murine [*R. typhi*, in the Oriental Rat Flea (*Xenopsylla cheopsis)*] or endemic (*R. felis* in *C. felis*) typhus etiologic agents is not definitively possible in retrospect. However, given the character of human distribution and known vector/zoonotic host associations, most of the recognized past cases could reasonably be assumed to be due to the latter pathogen.

The bimodal, temporal incidence of infection in the recent cases (Figure 3) may be related to flea/host interactions, with host reproductive and dispersal phases. Additionally, infection appears to be highly variable and focal within individual opossums. Surveillance for fleas on *D. virginiana* and *F. catus* throughout the year would help elucidate flea population trends and rickettsial infection rates in relation to human case occurrence. The information derived could provide impetus for preventive flea control in cooperation with public health agencies and veterinarians. Unfortunately, no local or state agency tests for flea-borne typhus

at this time, and CDC RZB will only test samples associated with human cases. Given this agency's staffing and funding difficulties, the District has experienced a delay of six months or more between submission of samples and receipt of test results. At this time, OCVCD has undertaken cooperative relationships with researchers at local universities to develop in-house testing protocols for the detection and differentiation of rickettsial pathogens.

Lack of evidence of infection in one opossum, with 3 of 4 flea species testing positive for *R. felis,* raises the possibility of lateral transmission to larval fleas in nesting/den sites via infected adult flea feces. New, whole-genome sequence data for several *Rickettsia* species, including *R. felis* and *R. typhi,* could provide the means to assess differences in vector/pathogen lineages, and give evidence of line fidelity via vertical transmission in comparison to lateral acquisition/dissemination. As more molecular surveillance research is conducted, *R. felis* is increasingly being found in new arthropod species, including native flea species (Stevenson et al. 2005), Trombiculid mites (Choi et al. 2007), and some ticks (Ishikura et. al. 2003, Reeves et al. 2006). Co-infection with other *Rickettsia* species, combined with evidence of potential genetic exchange within *Rickettsia* (Ogata et al. 2005), raises the risk of shifts in infectivity and virulence in what were thought to be isolated lineages of these obligate intra-cellular parasites.

Acknowledgements

We gratefully acknowledge the assistance of Renji Hu and Laura Krueger with the California Department of Public Health for coordinating testing with the CDC, and Marina Eremeeva of the CDC RZB.

REFERENCES CITED

Adams, W. H., R. W. Emmons and J. E. Brooks. 1970. The changing ecology of murine (endemic) typhus in Southern California. Am. J. Trop. Med. Hyg. 19:311-318.

Azad, A. F., J. B. Sacci Jr., W. M. Nelson, G. A. Dasch, E. T. Schmidtmann and M. Carl. 1992. Genetic characterization and transovarial transmission of a typhus-like rickettsia found in cat fleas. Proc. Natl. Acad. Sci. USA. 89:43-46.

Boostrom, A. B., M. S. Beier, J. A. Macaluso, K. R. Macaluso, D. Sprenger, J. Hayes, S. Radulovic and A. Azad. 2002. Geographic association of *Rickettsia felis*-infected opossums with human murine typhus, Texas. Emerg. Inf. Dis. 8:549-553.

Choi, Y. J., E. M. Lee, J. M. Park, K. M. Lee, S. H. Han, J. K. Kim, S. H. Lee, H. J. Song, M. S. Choi, I. S. Kim, K. H. Park and W. J. Jang. 2007. Molecular detection of various Rickettsiae in mites (Acari: Trombiculidae) in Southern Jeolla Province, Korea. Microbiol. Immunol. 51(3):307-312.

Civen, R. and V. Ngo. 2008. Murine Typhus: An unrecognized suburban vectorborne disease. Clin. Infec. Dis. 45 (15 March): 913-918.

Ishikura, M. S., Y. Ando, K. Shinagawa, S. Matsuura, T. Hasegawa, H. Nakayama, H. Fujita and M. Watanabe. 2003. Phylogenetic analysis of spotted fever group Rickettsiae based on gltA, 17-kDa, and rOmpA genes amplified by nested PCR from ticks in Japan. Microbiol. Immunol. 47(11):823-832.

Ogata, H., P. Renesto, S. Audic, C. Robert, G. Blanc, P. E. Fournier, H. Parinello, J. M. Claverie and D. Raoult. 2005. The genome structure of *Rickettsia felis* identifies the first putative conjugative plasmid in an obligate intracellular parasite. Plos. Biol. 3(8):1-12.

Prelesnik, L. K. 2007. Murine typhus in Southern California: Epidemiologic investigation update. Proc. & Papers Mosq. Vector Control Assoc. Calif. 75:92-95.

Reeves, W. K., A. D. Loftis, F. Sanders, M. D. Spinks, W. Wills, A. M. Denison and G. A. Dasch. 2006. *Borrelia, Coxiella,* and *Rickettsia* in *Carios capensis* (Acari: Argasidae) form a brown pelican

(*Pelecanus occidentalis*) rookery in South Carolina, USA. Exp. Appl. Acarol. 39:321-329.

Stevenson, H. L., M. B. Labruna, J. A. Montenieri, M. Y. Kosoy, K. L. Gage and D. H. Walker. 2005. Detection of *Rickettsia felis* in a New World flea species, *Anomiopsyllus nudata* (Siphonaptera: Ctenophthalmidae). J. Med. Entomol. 42(2):163-167.

Williams, S. G., J. B. Sacci Jr., M. E. Schriefer, E. M. Andersen, K. K. Fujioka, F. J. Sorvillo, A. R. Barr and A. F. Azad. 1992. Typhus and typhus-like Rickettsiae associated with opossums and their fleas in Los Angeles County, California. J. Clin. Microb. 30 (7):1758-1762.

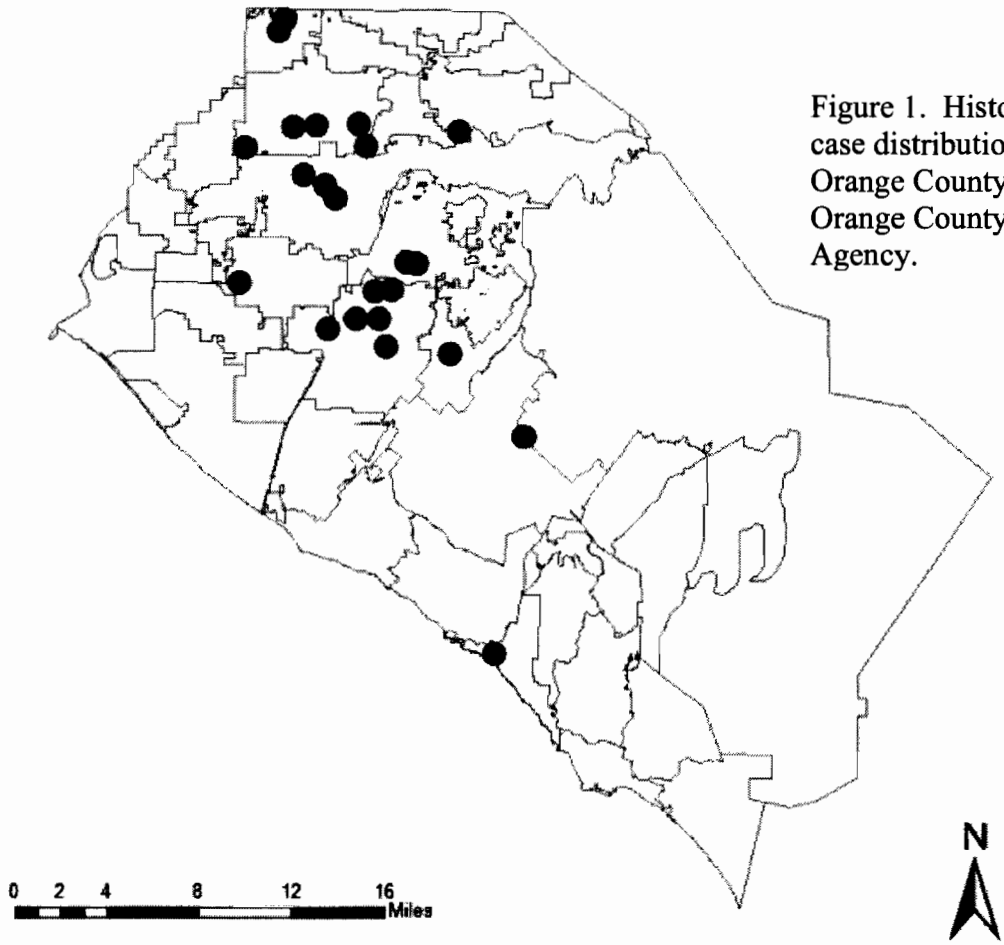


Figure 1. Historical human typhus case distribution (1933-1949), in Orange County, CA. Source: Orange County Health Care Agency.

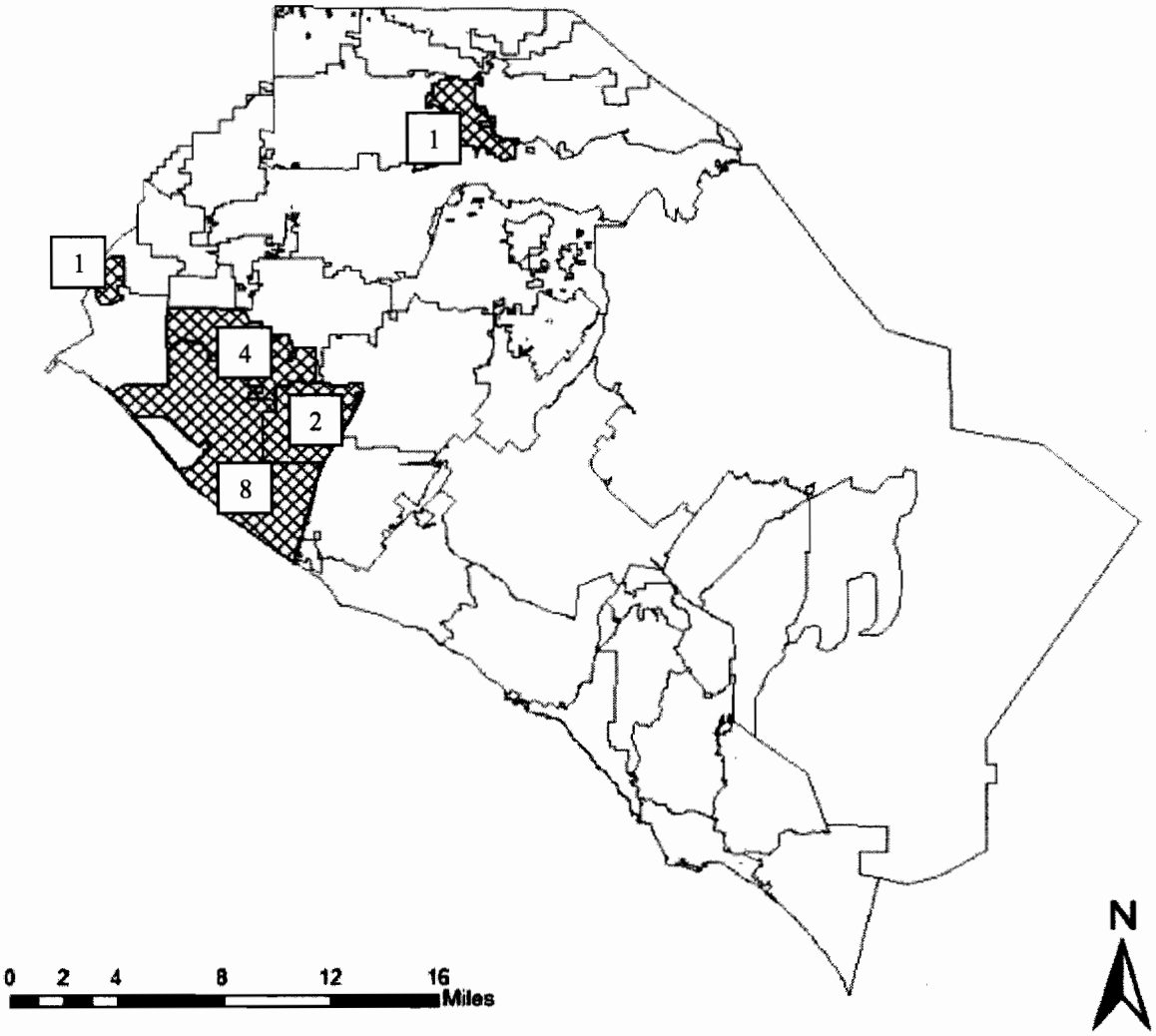


Figure 2: Human typhus case distribution in Orange County, California, 2006 – 2008. Cities with cases, including two presumptive: Huntington Beach (8), Westminster (4), Fountain Valley (2), Rossmoor (1), and Placentia (1).

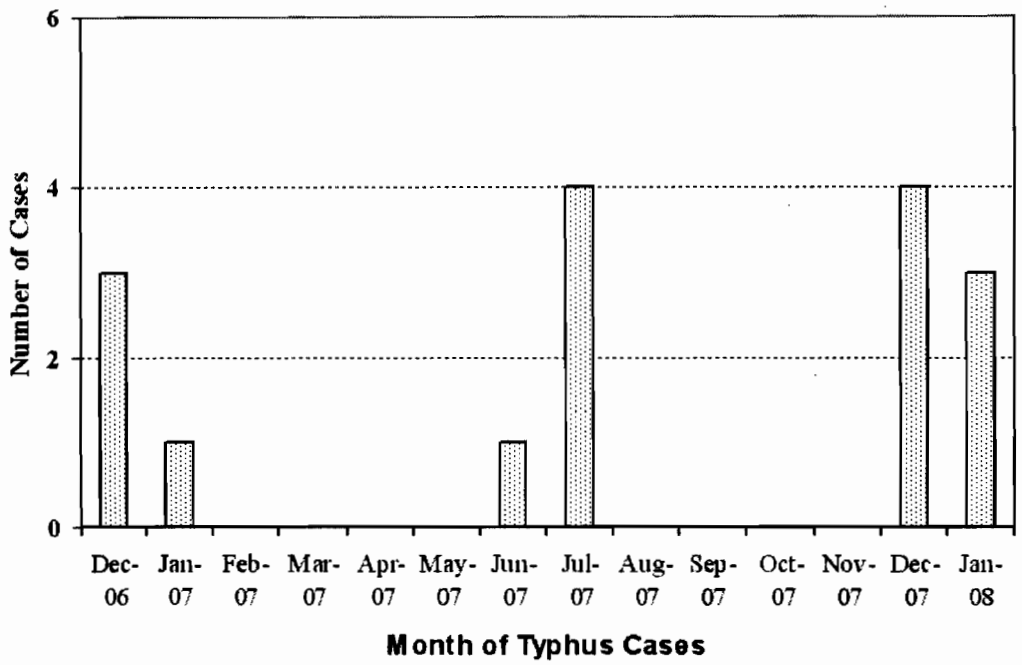


Figure 3: Timeline of human typhus cases in Orange County, California, by month during 2006 – 2008. Total = 16 cases, 14 confirmed and two probable.

Human Health Risk Assessment of the Aerial Adulticiding Conducted in 2007 in Sacramento County

Leslie M. Shama, Paula A. Macedo, Gary W Goodman, David A. Brown

Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Road, Elk Grove, CA 95624

Since the first outbreak in the Western Hemisphere in New York City in 1999, West Nile virus (family Flaviviridae, genus *Flavivirus*, WNV) has become a major concern in the United States. It has spread throughout the country since then, reaching California in the summer of 2003 (Reisen et al. 2004). WNV was first detected in Sacramento County in the summer of 2004, when it was associated with low level transmission to humans and horses (Armijos et al. 2005). In 2005, there was a severe outbreak of WNV in Sacramento County (Elnaiem et al. 2006), which prompted management of mosquitoes through aerial spraying of pyrethrins over the northern part of the county. The use of insecticides in areas where they have traditionally not been used or have been used less frequently has raised concerns by the public about health risks from insecticide use (Peterson et al. 2006). Likewise, the aerial spraying events in Sacramento County seemed to have generated concerns about the safety of the product used by Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) to manage adult mosquito populations in the 2005 outbreak.

In 2007, after considering the increase in numbers and infection rates of *Culex tarsalis* Coquillett and *Cx. pipiens* L. mosquitoes in the north area of Sacramento County, the District conducted aerial spraying of Evergreen® EC-60-6 over about 215 km^2 on the nights of July 30, 31, and August 01 using a fixed wing Piper Aztec aircraft. Human health risk assessments had been previously modeled for truck-mounted applications of pyrethrins and piperonyl butoxide (PBO) at higher rates than the ones used by SYMVCD, and different spraying schedule (Peterson et al. 2006). We conducted a human health risk assessment for six different subgroups after exposure from aerially applied pyrethrins and PBO at 0.0025 and 0.025 lbs/acre respectively, over 3 days, in Sacramento County, using more recent deposition data. In this study we compare these results to the ones reported by the human health risk assessment conducted by Peterson and colleagues (2006) (Table 1).

The values from the previous study represented a conservative approach where risks were most likely overestimated (Peterson et al. 2006) and the authors concluded that human health risk from residential exposure to insecticides used to control adult mosquitoes were low and not likely to exceed levels of concern. Our assessment was based on the aerial application conducted in Sacramento County in 2007, and our risk quotients were 2.4 to 5.4 times lower than the ones reported by Peterson and colleagues for pyrethrins, and 1.5 to 3.3 times lower for PBO. We conclude that human exposure would most likely result in negligible risk. Our study and the current weight of scientific evidence do not support the perceptions that human health risks from exposure to the pyrethrins insecticide applied by SYMVCD in Sacramento County in 2007 may be greater than the risk from WNV.

Table 1. Acute risk quotients (RQs)[1] for pyrethrins and piperonyl butoxide (PBO) for each subgroup.

	Peterson et al. 2006		Sacramento Co. 2007	
Subgroups	**Pyrethrins**	**PBO**	**Pyrethrins**	**PBO**
Adult males	0.0081	0.0004	0.0015	0.0002
Adult females	0.0085	0.0004	0.0018	0.0002
Children (10-12 yrs)	0.0113	0.0006	0.0021	0.0004
Children (5-6 yrs)	0.0190	0.0009	0.0040	0.0006
Toddlers (2-3 yrs)	0.0245	0.0012	0.0064	0.0009
Infants (0.5-1.5 yrs)	0.0218	0.0010	0.0091	0.0003

[1]RQs = total acute potential exposure ÷ reference dose (RfD), representing exposure ÷ toxic endpoint.

REFERENCES CITED

Armijos, V., S. Wright, W. Reisen, K. Kelley, S. Yamamoto and D. Brown. 2005. West Nile Virus in Sacramento and Yolo Counties, 2004. Proc. & Papers Mosq. Vectr Control Assoc. Calif. 73: 24-27.

Elnaiem, D.A., K. Kelley, S. Wright, R. Laffey, G. Yoshimura, V. Armijos, M. Reed, M. Farley, G. Goodman, W.K. Reisen and D. Brown. 2006. Epidemic amplification of West Nile virus in Sacramento and Yolo Counties, June – September 2005. Proc. & Papers Mosq. Vectr Control Assoc. Calif. 74: 18-20.

Peterson, R.K.D., P.A. Macedo, and R.S. Davis. 2006. A human-health risk assessment for West Nile virus and insecticides used in mosquito management. Environ. Health Perspect. 114 (3): 366-372.

Reisen, W.K., H.D. Lothrop, R.E. Chiles, M.B. Madon, C. Cossen, L. Woods, S. Husted, V.L. Kramer and J.D. Edman. 2004. Invasion of California by West Nile virus. Emerg. Infect. Dis. 10: 1369-1378.

Identifying the Reservoirs of the Lyme Disease Spirochete *Borrelia burgdorferi* (Sciurus Griseusi), in California: the Role of the Western Gray Squirrel

Daniel J. Salkeld[1,2], Sarah Leonhard[1], Yvette A. Girard[1], Nina E. Hahn[3], Jeomhee Mun[1], Kerry A. Padgett[4], Robert S. Lane[1]

[1]Department of Environmental Science, Policy & Management, University of California, Berkeley, CA 94720, USA

[2]IUCN – The World Conservation Union, Washington DC 20009, USA

[3]Office of Laboratory Animal Care, University of California, Berkeley, CA 94720, USA

[4]Vector-Borne Disease Section, Division of Communicable Disease Control, California Department of Public Health, Richmond, CA 94804, USA

INTRODUCTION

The spirochete *Borrelia burgdorferi* sensu stricto (s.s.) is the causative agent of Lyme disease in North America. A study of Western Gray Squirrels (*Sciurus griseus*) in oak woodlands in Mendocino County, California, revealed that squirrels are commonly infected with *B. burgdorferi* s.s. (80%), and that larval *Ixodes pacificus* ticks are capable of acquiring the infection (47% of larvae were infected) (Lane et al. 2005). Because western gray squirrels may be an important regional reservoir of the Lyme disease spirochete, we surveyed populations of squirrels throughout northern California, and performed xenodiagnostic and transmission experiments using *I. pacificus* to determine whether Western Gray Squirrels are indeed competent wildlife reservoirs.

Squirrel Infection Prevalence

Western gray squirrels were either live-trapped or collected as road-kills, or by shooting. Ear-punch biopsies (EPB) were tested for presence of *B. burgdorferi* using PCR techniques (Lane et al. 2005).

We tested a total of 227 individual western gray squirrels from California, of which, approximately 30% of individuals were positive for *B. burgdorferi* (Table 1). Prevalence of infection varied with geographic location and was highest in the north-western counties of the state, predominantly Humboldt, Mendocino and Trinity Counties (Table 1, Fig. 1).

We sequenced *B. burgdorferi*-positive 5S-23S intergenic spacer region amplicons from squirrel EPBs sampled in Humboldt (n = 3), Lake (n = 1), Mendocino (n = 4), Placer (n = 1), Sonoma (n = 3) and Trinity (n = 4) Counties. All amplicons were identified as *B. burgdorferi* s.s., using the neighbor-joining method with uncorrected (*p*) distances. No co-infections with other *Borrelia* genospecies were detected.

Although we tested EPBs from other *Sciurus* species in California, few animals were positive for *B. burgdorferi*. One of 14 Eastern Gray squirrels (*S. carolinensis*) was infected from Santa Cruz County and one fox squirrel (*S. niger*) was positive (1.5%) from Alameda County. None of two Northern Flying squirrels (*Glaucomys sabrinus*) from Humboldt County was infected. However, infection prevalence among Douglas squirrels (*Tamiasciurus douglasii*) was much higher with 6 of 11 animals infected in Humboldt County and 2 of 3 animals infected (66%) from Mendocino County. Also, we found evidence of *B. burgdorferi* infection in 1 of 6 Virginia opossums (*Didelphis virginiana*). The one positive opossum was collected in Sonoma County. There was no evidence of infection (0/5) in skunks (*Mephitis mephitis*). The one positive opossum was collected in Sonoma County.

Transmission experiments

To determine whether *I. pacificus* ticks can become infected with *B. burgdorferi* by squirrels, wild squirrels captured at the Hopland Research & Extension Center, Mendocino

county, and determined to be PCR-positive for *B. burgdorferi,* were exposed to approximately 200 laboratory-reared, non-infected *I. pacificus* larvae. The transmission rate of *B. burgdorferi* to ticks was measured by examining molted nymphs using PCR. Overall, a mean (± S.D.) of 59.4 (64.0) larvae successfully fed on individual squirrels, and a maximum of 241 larvae dropped off one squirrel. Most larvae fed for four or five days before falling off the host. Infection status, as measured by PCR testing of EPBs, varied over time. Squirrels infected 10 to 50% of *I. pacificus* larvae that had fed on them.

To confirm transmission of B. burgdorferi from squirrel to tick to squirrel, we exposed an uninfected wild-caught squirrel to infected nymphs. At one, two and four months after exposure to one confirmed infected nymph, PCR tests of EPBs were subsequently positive, negative and positive, respectively. Sequencing of the B. burgdorferi amplicon from the first month's positive EPB revealed that it was identical to the amplicon-sequence harvested from the positive nymph that had fed on the squirrel.

CONCLUSION

Recent investigations into the ecology of the Lyme disease spirochete *B. burgdorferi* s.s. suggested that the western gray squirrel (*S. griseus*) is an important wildlife reservoir in California (Lane et al. 2005, Brown et al. 2006). Here, we have demonstrated that *B. burgdorferi* s.s. is widespread in western gray squirrel populations in northern California. We showed that laboratory-infected larvae could maintain the spirochete trans-stadially, and were able to transmit the infection to an uninfected squirrel as nymphs, thereby illustrating the viability of the squirrel-tick-squirrel transmission cycle. Consequently, we are confident that western gray squirrels have a definitive role in the ecology of Lyme disease in the western USA in oak-dominated dense woodlands, and that an awareness of squirrel-*B. burgdorferi* ecology will be important for controlling and understanding this zoonotic disease (Lane et al. 2005, Eisen et al. 2006).

Acknowledgements

We thank Esther Olsen, Joan Wallace, Walter Brown and Bob Murray at the University of California - Berkeley. We thank Nate Nieto, Janet Foley, Rick Brown, Desiree Early, Robert and Lise Jones for additional squirrel ear samples. We thank all the extremely helpful staff at the various collecting sites including China Camp, Annadel and Clear Lake State Parks, especially Bob Birkland; Hopland Research and Extension Center, especially Bob Timm; the Audubon Canyon Ranch - Bolinas Lagoon Preserve, especially Gwen Heistand; the Donald and Sylvia McLaughlin Natural Reserve, especially Cathy Koehler; Quail Ridge Reserve; Bidwell Park; and Les Dodge of Dodge Pheasant Farms; and we do not blame any of the aforementioned for our calamitous lack of success in catching squirrels at most of these locations. This research was funded by grant AI22501 from the National Institutes of Health.

REFERENCES CITED

Brown, R. N., M. A. Peot and R. S. Lane. 2006. Sylvatic maintenance of *Borrelia burgdorferi* (Spirochaetales) in northern California: untangling the web of transmission. J. Med. Entomol. 43:743-751.

Eisen, R. J., R. S. Lane, C. L. Fritz and L. Eisen. 2006. Spatial patterns of Lyme disease risk in California based on disease incidence data and modeling of vector-tick exposure. Am. J. Trop. Med. Hyg. 75:669-676.

Lane, R. S., J. Mun, R. J. Eisen and L. Eisen. 2005. Western Gray Squirrel (Rodentia: Sciuridae): a primary reservoir host of *Borrelia burgdorferi* in California oak woodlands. J. Med. Entomol. 42:388-396.

Impact of West Nile Virus on California Birds

Sarah S. Wheeler, C.M. Barker, B.D. Carroll, W.K. Reisen

Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, Old Davis Rd, Davis, CA 95616, sswheeler@ucdavis.edu

The presence of dead American Crows has been the calling card of the West Nile virus (Family Flaviviridae, genus *Flavivirus*, WNV) invasion into North America. The especially high virulence of the invading and now circulating strains of WVN has been attributed to a mutation in the viral helicase gene (Brault et al. 2007). However, not all bird species responded equally to WNV, because of differences in susceptibility to infection, habitat selection, and mosquito host selection patterns. To better quantify the effects of WNV on the avifauna of California (CA) during the post-WNV invasion period (2004-2007), and in an attempt to identify species at greater risk of population decline due to WNV, four datasets were evaluated with respect to WNV-associated risk and combined to create a risk assessment for 23 CA bird species. The four datasets evaluated were: 1) the presence of antibodies against WNV in free-ranging birds, 2) percentage of dead birds tested and found WNV positive by the California dead bird program, 3) WNV-associated mortality determined from experimental WNV infections, and 4) population declines detected by Bayesian regression analyzed data from 1980-2003 (pre-WNV) to extrapolate population trends for the 2004-2007 post-WNV period (LaDeau et al. 2007). Declines in the BBS data were considered significant if they dropped below 95% confidence intervals (CI) generated by the model. Since the model was based on pre-WNV population trends, significant declines from the expected 95% CI, in areas of epizootic WNV transmission, were attributed to the negative impact of WNV. Risk was assessed and scored for each of the four data sets and then averaged into an overall risk score (Table 1). This risk score allowed for species to be compared based on WNV-associated risk. Scores ranged from 1.00 for the Pigeon (*Columba livia*) through 3.40 for Yellow-billed Magpies *(Pica nuttalli)* and 3.60 for American Crows *(Corvus brachyrhynchos)*. Other species potentially at high risk for WNV included the House Finch *(Caprodacus mexicanus)*, Black-crowned Night Heron *(Nycticorax nycticorax)*, Yellow Warbler *(Dendroica petechia)* and Western Scrub-Jay *(Aphelocoma coerulescens)*. Significant population declines in competent host species may alter avian community structure, allow for the increase in highly efficient competing species such as House Sparrows (*Passer domesticus)*, and alter the dynamics of WNV transmission by changing host availability for host-seeking mosquitos.

Acknowledgements

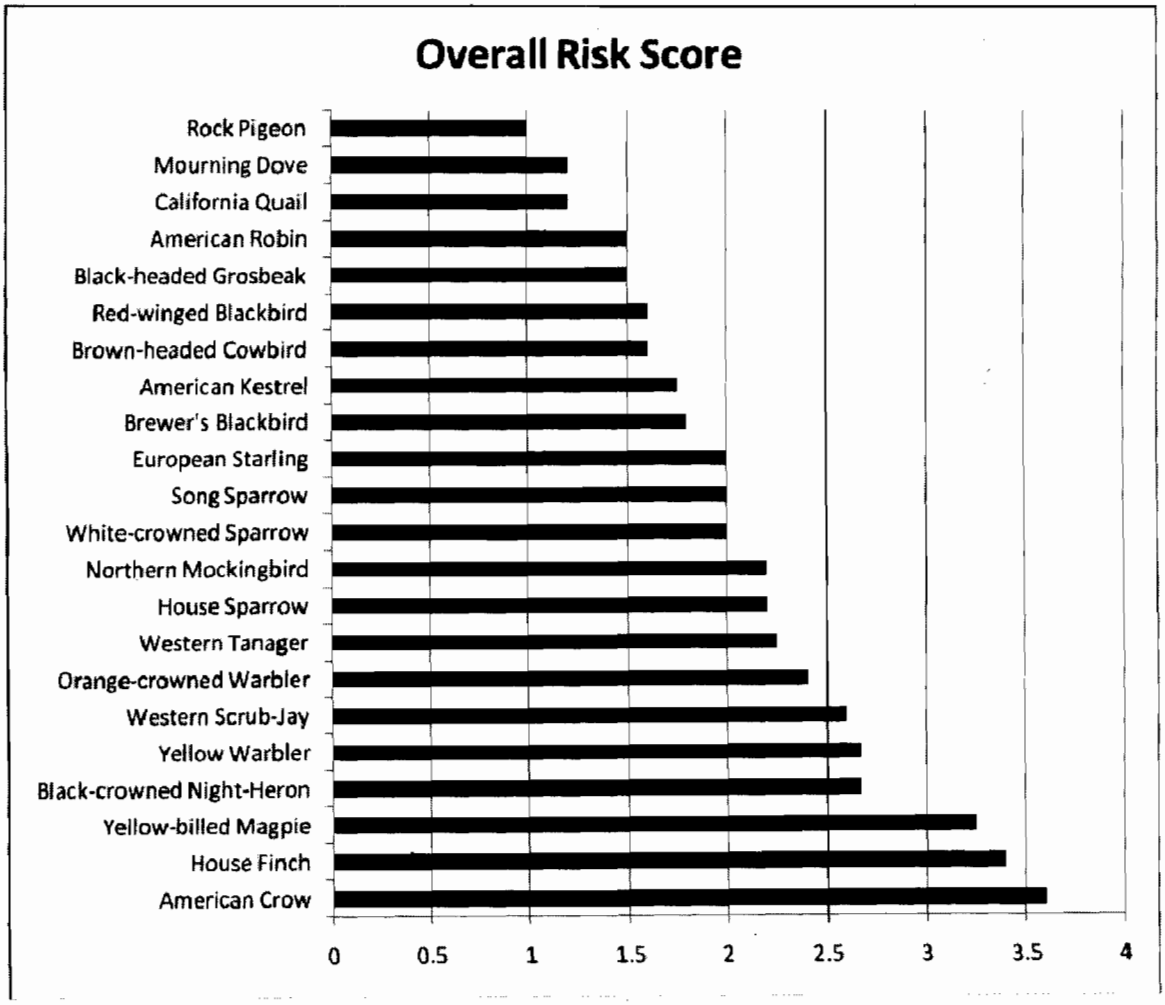
We especially thank Y. Fang and S. Garcia, M. (CVEC Arbovirus Laboratory); and K. McCaughey, R. Carney and Dead Bird Hotline staff (California Department of Public Health, Vectorborne Disease section, VL Kramer Chief); and L Woods and staff (UC Davis, California Animal Health and Food Service) for dead bird necropsies. This research was funded, in part, by ELC Funding from the Centers for Disease Control and Protection, CDC, Research Grant AI55607 from the National Institute of Allergy and Infectious Diseases, NIH, the Climate Variability and Human Health Program, Office of Global Programs, NOAA, the Coachella Valley, Kern County, and Yolo County.

REFERENCES CITED

Brault, A. C., C. Y. H. Huang, S. A. Langevin, R. M. Kinney, R. A. Bowen, W. N. Ramey, N. A. Panella, E. C. Holmes, A. M. Powers and B. R. Miller. 2007. A single positively selected West Nile viral mutation confers increased virogenesis in American crows. Nat. Genet. 39: 1162-1166.

LaDeau, S. L., A. M. Kilpatrick and P. P. Marra. 2007. West Nile virus emergence and large-scale declines of North American bird populations. Nature 447:710-713.

Table 1: Overall WNV-associated risk score for 23 species of California birds. Risk was assessed based on four indicators: wild bird serology from Kern, Coachella and Yolo Counties, the percent of each species that tested positive from the CA dead bird program, the outcome of experimental infection studies, and bird population declines that fell below 95% confidence intervals generated by our BBS regression model. The overall risk score is an average of each of the four indicators; the BBS model was given a double weight because it reflected actual change in bird populations.



Introduction of the Scorpion *Centruroides exilicauda* (Wood) into Southern California Communities: a Public Health Perspective

Lawrence R. Bronson [1], Laura Krueger [2], and Kenn K. Fujioka [3]
[1]California Department of Public Health, Vector-Borne Disease Section,
2135 Civic Center Drive, Redding, CA 96001
[2]Orange County Vector Control District,
13001 Garden Grove Blvd., Garden Grove, CA 92843
[3]San Gabriel Valley Mosquito and Vector Control District,
1145 Azusa Canyon Road, West Covina, CA 91790

ABSTRACT: *Centruroides exilicauda* (Wood) is endemic to Arizona, southwestern New Mexico, and southeastern California along the Colorado River corridor. This scorpion is considered to be the only medically important species found in the United States due to the toxicity of its venom. Past documented introductions of this species into non-endemic Southern California communities have apparently been isolated events. We report on the discovery of an established population of *C. exilicauda* that has adapted to life in a suburban Los Angeles County neighborhood. The initial results from our investigation are presented and the public health implications of established localized populations of this species are discussed.

INTRODUCTION

Centruroides exilicauda (Wood) is endemic to the arid and semi-arid regions of Arizona, southwestern New Mexico, and in southeastern California along the Colorado River corridor. This scorpion is considered the only medically important scorpion found in the United States due to the toxicity of its venom (Keegan, 1980). Introductions of this scorpion into Southern California communities have been documented as isolated events associated with human transport. Here we report our initial discovery of an established population of *C. exilicauda* in suburban West Covina, Los Angeles County, California and discuss the potential public health hazard associated with this introduction.

The genus *Centruroides* is a New World taxon that is distinguished from other genera by its overall slender form, a triangular shaped sternum, and a subaculeor tooth or tubercle on the telson at the base of the stinger. *Centruroides exilicauda* (Fig. 1) is a non-burrowing species that is an adept climber and is often seen perched above ground. Commonly known as the "Arizona bark scorpion", it takes refuge in cracks and crevices, under bark on standing or fallen trees, and in structures affording access. Though a denizen of arid environments, the bark scorpion is attracted to moisture and is often associated with riparian corridors, drainages, or man-made sources of water. This scorpion appears to thrive equally in natural habitats and lush suburban developments. In Arizona, *C. exilicauda* is the most commonly encountered scorpion inside houses (Scorpions, University of Arizona Cooperative Extension, 2001).

Envenomation by *C. exilicauda* follows a classic clinical course unlike envenomation by other scorpion genera. Intense local pain is followed by numbness, increased salivation, agitation, respiratory difficulties, tachycardia, hypertension, and muscle spasms. Respiratory paralysis and death are possible. The last verified death in Arizona by *C. exilicauda* envenomation occurred in 1968 (Stahnke, 1972). Because antivenin is now available and patient support and treatment has improved, the risk of fatalities from stings has decreased. In Arizona

where *C. exilicauda* is commonly encountered in urbanized areas, Arizona Poison Control Centers receive 6000-7000 calls a year concerning scorpions and actively track bark scorpion envenomations (Banner Poison Control Center, Phoenix, AZ).

Investigation

In July 2007 a resident of West Covina, Los Angeles County California submitted three dead scorpions (2 adult and 1 immature), to the San Gabriel Valley Mosquito and Vector Control District (SGVMVCD) for identification and assistance in managing an ongoing scorpion infestation at their property (Fig. 2). The resident reported finding at least 9 scorpions indoors in 2007 and one unremarkable stinging incident. Scorpions were also found in the yard on exterior walls, the ceiling of a covered patio, and in the garage over the last 4-5 years. The resident recalled that relatives from Arizona occasionally visited and parked a recreational vehicle at their home. No scorpions were encountered prior to these visits. The resident also reported that neighbors on either side recently noticed scorpions on their properties, with one encountering scorpions indoors. Staff of the SGVMVCD identified the scorpions as *Centruroides exilicauda* and contacted the California Department of Public Health's Vector-Borne Disease Section (VBDS) with concerns regarding the potential public health implications. Staff from the SGVMVCD and the VBDS contacted the resident to inspect the property and provide guidelines (Scorpions, University of California Statewide IPM Program, 2003) to manage the infestation.

In early November, the site was inspected on two evenings with handheld portable black lights (BioQuip® Products, Rancho Dominguez, CA). Scorpions fluoresce in ultraviolet light and are readily detected while foraging at night (Williams, 1968). Despite the cooler fall temperatures, active *C. exilicauda* were observed on both occasions only at the property where the scorpions were probably first introduced. On 1 November four *C. exilicauda* were detected. One immature was collected from the trunk of an ornamental shrub planted against the front of the house. Three adult scorpions were observed on the wood shingled roofs covering the house and detached garage. The air temperature at 2000 hours was 59° F(15°C). Daytime high temperatures reached 76°F(25°C). On 15 November, 11 *C. exilicauda* were observed and five were collected. Eight were observed on the wood shingled roofs of the house and garage, two in the garage, and one was collected on the front yard lawn. The air temperature at 2000 hours was 66° F(19°C). The daytime high temperature reached 92°F(34°C). The scorpions appeared to prefer the wood shingled roof areas as habitat (Fig. 3). Besides providing crevices for refuge, the west and south facing slopes collect and retain heat during the cooler months allowing activity that may not be possible at ground level during cooler temperatures.

Residences were evaluated for points that would allow scorpions to enter. Gaps under doors, missing or poorly fitted window screens, unsealed openings around pipes, conduits, window or wall mounted air conditioning units, and missing or damaged screening on attic or foundation vents were noted. Ornamental landscaping positioned against walls or overhanging roofs were also noted because they provide climbing scorpions with cover proximal to the structure (Fig. 4). These findings along with simple, inexpensive remedies were discussed with each resident.

The resident at the focus of the *C. exilicauda* infestation had previously hired a pest control company to treat in and outdoors for scorpions but was discouraged by the lack of results. Guidelines for controlling the infestation emphasized modifying the area around the home as the first step in control. In addition, control included general scorpion awareness, exclusion from interior spaces, and physical removal instead of relying on pesticides.

On January 16, 2008, one active scorpion was found on the roof of the primary residence. The air temperature at 2000 hours was 58°F(15°C) with windy conditions. Daytime

high temperatures reached 68° F(20°C). Detecting one active scorpion on a cool January night confirmed our suspicions that favorable climatic conditions could support a population of *C. exilicauda* throughout the year in the Los Angeles Basin. No other scorpions were detected and residents reported no recent activity. Glue boards (Tomcat Scorpion Glue Board, Motomco, Clearwater, FL) were deployed indoors in the kitchen, utility room, and living room areas where scorpion activity had been previously noted. Two days before our inspection SGVMVCD staff produced and distributed a fact sheet and survey to 19 homes in the neighborhood. Three properties requested inspections, though none reported seeing scorpions.

Discussion

Adult scorpions are notoriously difficult to control solely with pesticides. Though many products are registered for use against scorpions, their secretive behavior and nearly impervious exoskeleton render barrier treatments largely ineffective. Scorpion management guidelines universally recommend proactive integrated strategies including awareness of potential encounters in the home environment, exclusion, physical removal, and if pesticides are warranted, contracting with a qualified structural pest control company to apply indoor and outdoor barrier treatments. The pesticide label must be followed if the homeowner chooses to apply products themselves. Modifying the structural environment by removing debris and pruning vegetation around the foundation and roof significantly reduces cover for scorpions. Also, using yellow outdoor lighting may reduce scorpion foraging near potential home entry points.

Envenomation by the bark scorpion can potentially be life threatening although the vast majority of stinging incidents do not require medical assistance. Children under five years of age are at greatest risk for severe reactions requiring medical assistance. A review of *C. exilicauda* envenomation in Arizona found that all patients under the age of one were either admitted to hospital care or seen in an emergency facility. A similar high rate of emergency room or inpatient hospital care was evident in children 1-5 years old (Likes et al., 1984). *Centruroides exilicauda* readily enters homes which increases the risk of encounters. This species displays a negative geotaxis and may be found clinging to the underside of objects as well as on walls or ceilings in the home. They are attracted to moisture in and around the home and are often found trapped in sinks, bathtubs, or around pool areas. Their ability to climb almost any surface may lead to unexpected encounters on furniture, tables, lamps, bookshelves, clothing, or bedding.

Introductions of *Centruroides exilicauda*, formerly known as *C. sculpturatus* (Williams, 1980), into Southern California historically were identified from stinging incidents or specimens submitted by residents. Russell and Madon (1984) reported *C. exilicauda* introductions from Los Angeles, Riverside, San Bernardino, and Orange counties. They also described four cases of *Centruroides* envenomation seen at the University of Southern California Medical Center during the 1970s. Geck (1980) reported on five specimens of *C. exilicauda* that were submitted to the Orange County Vector Control District (OCVCD) by residents during the mid 1970s. The common thread in these accounts was people and equipment traveling between the Colorado River corridor and their neighborhoods. Both reports raised concerns about the public health hazard if *C. exilicauda* became established in Southern California communities. Neither article intimated that *C. exilicauda* was established at the time.

The Orange County Vector Control District documented limited infestations by *C. exilicauda* in Fountain Valley, Garden Grove, Huntington Beach, and Irvine in the 1970s and 1980s. In both the Fountain Valley and Irvine infestations the OCVCD treated properties in the neighborhoods with a pesticide. The Irvine infestation was originally identified and treated in 1984-85; *C. exilicauda* reappeared in the

neighborhood during 1991-93. Forty suburban properties were infested; two attics of homes harbored scorpion populations. The Huntington Beach infestation involved a single property where *C. exilicauda* inhabited a wood shingled roof much like the current infestation in West Covina. Control efforts by the homeowner were unsuccessful until the wood shingles were removed. Several stinging incidents were reported by workers involved in the roof removal (OCVCD pers. comm., 2008).

Concerns raised by Russell (1984) and Geck (1980) regarding the potential for *C. exilicauda* to become established in Southern California communities appear justified. Suburban residents are unlikely to encounter other scorpion species in their home so an introduction of *C. exilicauda* creates a need for public education. In response to the current investigation, the SGVMVCD and the Los Angeles County Agricultural Commissioner's office produced *Centruroides exilicauda* fact sheets that are available on their websites. In Arizona where *C. exilicauda* is largely endemic, educational material is well developed and plentiful.

Since Southern California residents use the border area along the Colorado River year round it is likely that bark scorpions will continue to be imported from this region. Likewise, travelers to and from other endemic regions in Arizona such as Phoenix and Tucson may inadvertently transport this scorpion. Though the probability is low that "hitchhiking" scorpions will establish a viable population, thirty years of periodic infestations validates this possibility. As participants in California's regional surveillance system for vectors, local agencies should respond to public inquires about scorpions especially when they are found indoors where none had been previously encountered. Recognizing early infestations of *C. exilicauda* may help prevent localized populations from becoming established and reduce the risk of stinging incidents.

Introductions of this species may be far more common than previously suspected. Information obtained from the California Poison Control System documented 663 confirmed scorpion envenomation contacts from Los Angeles County residents between 1997 and 2007. Though the vast majority of envenomations were determined to present little or no effect and were treated at home, 84 (13%) contacts were referred to health care facilities (HCF) for treatment/evaluation. Three patients were admitted to critical care facilities with what were termed major effects. One patient was admitted to a non-critical care unit with moderate effects. Though we could not determine the offending scorpion species from these records the number of calls to Poison Control each year in Los Angeles County concerning scorpion envenomation is surprisingly large.

Overall, it appears that few properties in West Covina are infested with Bark Scorpions even several years after it was introduced. The population is localized and composed of multiple age cohorts indicating continued breeding. Scorpions do not colonize new physical surroundings rapidly because of their biology and behavior. However, the high population of *C. exilicauda* at one residence currently poses a potential risk to its occupants. Our initial investigations were conducted when scorpion activity was limited by cooler temperatures. The CDPH and SGVMVCD plan to reinspect the neighborhood in summer to confirm the initial assessment and finalize a course of action.

Acknowledgments

The authors would like to thank Steve Bennett, Hazel Wallace, Minoo Madon, and Larry Shaw for providing historical data of infestations in Orange and Los Angeles County. Thanks to Gail VanGordon for identifying the initial specimens and referring the infestation to local and state personnel. Gevork Arakelian developed a very useful *Centruroides* pest information sheet for Los Angeles County available on the Los Angeles County Agricultural Commissioner's pest web. Much appreciation is due to Kelly Middleton for

developing a public information flyer distributed to the affected neighborhood. Joe Burns, Marco Metzger and Justin Harbison aided in the collection of specimens. Last but not least, the authors acknowledge the homeowners of the index house for allowing us full access to their property.

REFERENCES CITED

Geck, R. 1980. Introduction of scorpions to Orange County. Proc. Calif. Mosq. Vector Contr. Assoc. 48: 136.

Keegan, H.L. 1980. Scorpions of medical importance. University Press of Mississippi.

Likes, K., W. Banner Jr. and M. Chaves. 1984. Centruroides exilicauda envenomation in Arizona. West. J. Med. 141: 634-637.

Russell, F. E. and M. B. Madon. 1984. Introduction of the scorpion Centruroides exilicauda into California and its public health significance. Toxicon. 22:658-664.

Stahnke, H. L. 1972. Arizona's lethal scorpion. Ariz. Med. 29:490-493.

University of Arizona, College of Agriculture and Life Sciences. 2001. Scorpions. Web address: http://ag.arizona.edu/pubs/insects/az1223.pdf.

University of California Statewide IPM Program. 2003. Scorpions. Web address: http://imp.ucdavis.edu/pmg/pestnotes/pn74110.html.

Williams, S. C. 1968. Methods of sampling scorpion populations. Proc. Calif. Acad. Sci. 36: 221-230.

Williams, S. C. 1980. Scorpions of Baja California, Mexico, and adjacent islands. 135: 1-127.

Figure 1: The Arizona bark scorpion, *Centruroides exilicauda.*

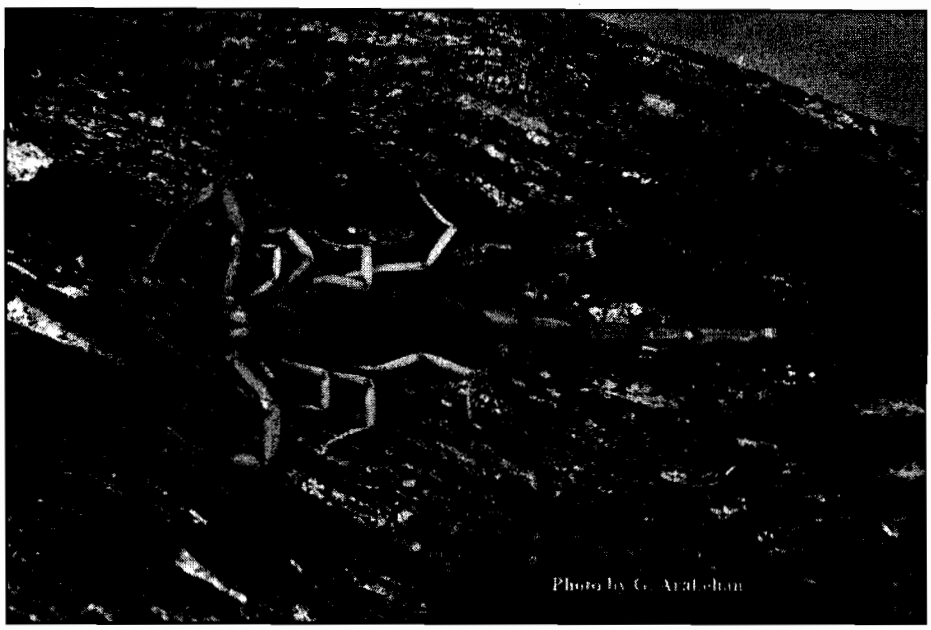


Figure 2: Suburban neighborhood introduction site.

Figure 3: Wood shingled roof: a novel *C. exilicauda* niche.

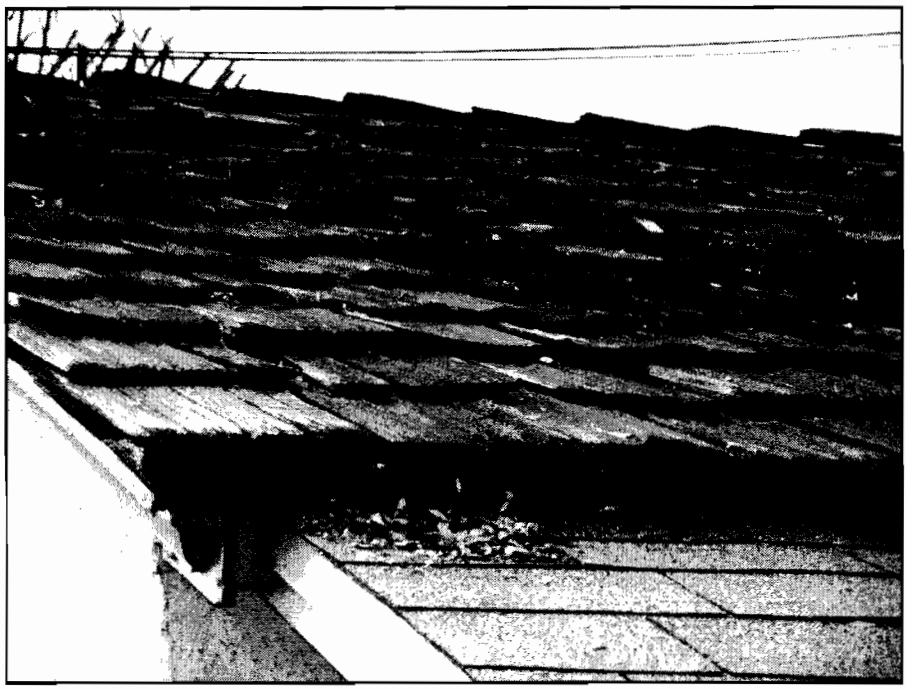


Figure 4: Foundation plantings provide *C. exilicauda* foraging cover and potential contact points for indoor access.

Introduction to symposium: Research on Arboviruses in California - Year 5

William K. Reisen

Center for Vectorborne Diseases and Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, (530) 752-0124, arbo123@pacbell.net

West Nile virus (Family Flaviviridae, genus *Flavivirus*, WNV) continued to be active throughout California during 2007 (Table 1). Although the number of human cases increased by 36% over 2006, most of the enzootic measures of virus activity remained relatively constant. The increase in human cases were largely attributed to an urban outbreak with 138 confirmed human cases centered in Bakersfield, whereas most of the activity during 2006 occurred in rural areas or small towns such as Davis (Nielsen et al. 2008). As WNV transitions from an invading 'virgin soil' epidemic to an endemic virus, different questions addressed by our research group included:

1. What conditions enable WNV amplification to outbreak levels? We addressed this question by investigating factors leading up to the Bakersfield outbreak in 2007, studying temporal and spatial changes in the vector competence of *Culex*, evaluating the impact of herd immunity in urban and rural areas, and determining the impact of WNV on bird populations throughout California with the idea that depopulation of key species could limit amplification.
2. How is WNV adapting genetically to California? We were especially interested in possible change in virus genetics that may lead to decreased or perhaps even increased avian virulence or transmissibility.
3. Hunt for newly emerging viruses. West Nile virus will not be the last emerging problem in California. In addition to the three viruses tested by our multiplex RT-PCR [western equine encephalomyelitis (WEEV), St Louis encephalitis (SLEV), West Nile (WNV)], there are 10 other viruses known to occur in California, including some that have been linked to human illness. We tested >1,000 pools of *Aedes* and *Culiseta* by Vero cell plaque assay during 2005-06 and made 45 isolations that are being characterized. In addition, during 2007 we have tested 4,000 pools of *Culex* from portals of entry into California and have made 2 isolations of an unidentified virus. In addition, a Kamati River-like virus was found to frequently infect field populations of *Cx. tarsalis*.
4. What happened to other viruses such as western equine encephalomyelitis virus (WEEV)? Historically, WEEV caused repeated outbreaks of human cases in the Central Valley of California and other areas of the West, but recently these cases and WEEV have all but disappeared. We compared avian virulence and vector competence of isolates made each decade from 1953 through 2005.

Table 1. West Nile virus activity in California [from http://westnile.ca.gov/]

Element	2003	2004	2005	2006	2007	Total
Human cases	3	779	880	278	378	2,318
Horses	12	540	456	58	28	1,094
Dead birds	96	3,232	3,046	1,446	1,395	9,215
Mosquito pools	32	1,136	1,242	832	1,007	4,249
Chickens	70	809	1,053	640	510	3,082
Squirrels	nd	49	48	32	26	155

Human and horse cases reported by practicioners
Dead birds reported by the public, necropsied and kidney tested by RT-PCR or oral swap teste
Mosquito pools tested by RT-PCR
Chickens, seroconversions with sera screened by EIA
Squirrels, necropsied and tissues tested by RT-PCR

Acknowledgements

Research reported in our symposium was funded, in part, by the National Institutes of Allergy and Infectious Diseases, NIH, the Centers for Disease Control and Prevention, CDC, and the Coachella Valley, Greater Los Angeles County, Kern and Sacramento/Yolo Mosquito and Vector Control Districts.

Participants from the Center for Vectorborne Diseases at UC Davis include:
Arbovirus Lab, Davis: AC Brault, Y Fang, S Garcia, M Dannen, K Domer, A Chow, J Jones
Arbovirus Field Station, Bakersfield: VM Martinez, BD Carroll, S Hallam
Coachella Valley: HD Lothrop, M Kennsington, P Miller
Los Angeles: J Wilson*
Sacramento-Yolo: C Nielsen*, S Wheeler*, V Armijos*, T Thiemann*
Environmental Assessment & Information Technology: BF Eldridge, B Park, CM Barker*

Collaborators included personnel from the following Mosquito and Vector Control Districts:
Kern: R Takahashi, G Abbott, R Quiring
Coachella Valley: B Lothrop, A Guitierrez, D Gomsi
Greater Los Angeles Co: M Maddon, S Kluh, P O'Connor, H Morales, T Posey
Sac/Yolo: S Wright, K Kelly, M Reed, P Macedo, D Brown
California Department of Public Health: Vectorborne Disease Section, V Kramer, Head, and Viral and Rickettsial Diseases Laboratory, C Glaser, Head.
University of California, San Diego, Scripps Institution of Oceanography, Climate Division: D Cayan, M Dettinger, M Tyree
NASA Ames Research Division: F Melton, B Lobitz, R. Nemani

REFERENCES CITED

Nielsen, C.F., V. Armijos, S.S. Wheeler, T. E. Carpenter, K. Kelley, W. M. Boyce, D. A. Brown and W. K. Reisen. 2008. Risk factors associated with the 2006 West Nile virus outbreak in Davis, a residential community in Northern California. Am. J. Trop. Med. Hyg. 78:53-62.

Monitoring and Modeling Environmental Conditions Related to Mosquito Abundance and Virus Transmission Risk with the NASA Terrestrial Observation and Prediction System

Forrest S. Melton[1,2], Rama R. Nemani[2], Andrew Michaelis[1,2], Christopher M. Barker[3,4], Bborie K. Park[4], William K. Reisen[4]

[1] *California State University, Monterey Bay,* CSUMB / NASA Coop, Mail Stop 242-4, Moffett Field, CA 94035, (650)604-2787, forrest.s.melton@nasa.gov

[2] *NASA Ames Research Center*

[3] *Department of Entomology, University of California, Davis*

[4] *Center for Vectorborne Diseases, University of California, Davis*

ABSTRACT: The NASA Terrestrial Observation and Prediction System (TOPS) is a modeling framework that integrates satellite observations, meteorological observations, and ancillary data to support monitoring and modeling of ecosystem and land surface conditions in near real-time. TOPS provides spatially continuous gridded estimates of a suite of measurements describing environmental conditions, and these data products are currently being applied to support the development of new models capable of forecasting estimates of mosquito abundance and transmission risk for mosquito-borne diseases such as West Nile virus (WNV).

INTRODUCTION

One significant barrier to progress in modeling mosquito abundance and risk of transmission of West Nile virus (Family Flaviviridae, genus *Flavivirus,* WNV) and other encephalitis viruses has been the lack of consistent, spatially continuous observations of climate and landscape condition at suitable spatial and temporal scales. The record of mosquito trap counts collected by mosquito and vector control districts in California and recently compiled and digitized by researchers at UC Davis is a unique and valuable dataset. However, many traps in California are located a significant distance from the nearest meteorological station, and measurements of soil moisture and vegetation condition have been limited to a few regional or plot level studies, or labor intensive analyses based on Landsat TM and other satellite sensors (e.g., Wood et al. 1991). The few existing statewide datasets, such as the National Land Cover Database 2001 (Homer et al. 2004), are not updated at a sufficient frequency to support dynamic modeling and forecasting at temporal resolutions finer than years to decades. The datasets provided by the Terrestrial Observation and Prediction System (TOPS) make a substantial contribution to filling this data gap, and can assist in the development of models capable of forecasting mosquito abundance and virus transmission risk for the state of California.

THE TOPS FRAMEWORK

TOPS is a modeling framework that provides capabilities for automated ingestion and processing of heterogeneous data sources for use in modeling ecosystem processes and estimating land surface conditions in near real-time (Nemani et al. 2003a, 2008). TOPS currently includes capabilities for automated acquisition and processing of data from orbiting satellites, networks of meteorological stations, hydrologic gauging stations, and numerous ancillary data sources (Figure 1). Currently, a modified version of the BIOME-BGC model (White and Nemani 2004, Running et al. 1997) is used to estimate various water (evaporation, transpiration, stream flows, and soil water), carbon (net photosynthesis, plant growth) and nutrient flux (uptake and mineralization) processes. TOPS forecasts parameters at a variety of spatial scales, from global net primary productivity (NPP) anomalies at 0.5 x 0.5-degree resolution (Nemani et al. 2003b) to local estimates of ecosystem parameters at resolutions as fine as 250m. At each spatial resolution, TOPS uses different sources of satellite data [Moderate Resolution Imaging Spectroradiometer [MODIS] to Ikonos] and meteorological data (single weather station to global atmospheric model outputs).

Using TOPS, scientists at NASA Ames Research Center currently produce a comprehensive suite of over thirty variables describing land surface conditions. These products are generated daily for California at a spatial resolution of 1 km in both nowcast and forecast modes to facilitate near real-time monitoring of ecosystem conditions (Figure 2). Products include satellite (land cover, snow cover, surface temperature, vegetation density, vegetation productivity), surface weather (max/min temperatures, humidity, solar radiation and rainfall), and modeled fluxes (soil moisture, vegetation stress). TOPS also maintains an extensive historical record of observations for California and the western US, including climate data that extend from 1950 onwards, and remote sensing data that extend from the beginning of the NOAA Advanced Very High Resolution Radiometer (AVHRR) satellite data in 1982 to the present. This data record allows TOPS to calculate historical averages for all products to identify and track anomalies in climate and ecosystem parameters.

APPLICATIONS OF TOPS FOR MODELING MOSQUITO ABUNDANCE AND VIRUS TRANSMISSION RISK

Many of the data products generated by TOPS for California are potentially important inputs to models of mosquito abundance and virus transmission risk. For example, temperature directly affects larval development, rates of adult survival, and the rate of virus amplification for West Nile virus (Reisen et al. 2006). Surface water is required for mosquito reproduction, and the timing and extent of surface water availability directly influences rates of mosquito reproduction. Surface water availability and soil moisture levels also affect ecosystem dynamics, primary productivity, and bird population dynamics, and thus may also indirectly affect rates of arboviral infection in avian hosts. Measures of vegetation condition may assist in identifying potential habitat for some mosquito species and in discriminating between high and low risk areas for virus transmission. Barker and colleagues (in these proceedings) describe the success of initial modeling efforts which incorporate TOPS data on maximum and minimum temperature, and they have applied the initial model to support mapping of estimated virus transmission risk by county at a semi-monthly timestep. Our current research effort is focused on extending these initial results to incorporate estimates of vegetation condition, soil moisture, and the timing of snow melt in California watersheds.

Acknowledgements

Funding for this project was provided by a Decision Support through Earth Science Research Results grant from the NASA Applied Sciences Program.

REFERENCES CITED

Homer, C., C. Huang, L. Yang, B. Wylie and M. Coan. 2004. Development of a 2001 National Landcover Database for the United States. Photo. Eng. and Remote Sens. 70(7): 829-840.

Nemani, R. R., M. A. White, L. V. Pierce, P. Votava, J. Coughlan and S. W. Running. 2003a. Biospheric monitoring and ecological forecasting. Earth Obs. Mag. 12:6-8.

Nemani, R. R., C. D. Keeling, H. H. Hashimoto, W. J. Jolly, S. C. Piper, C. J. Tucker, R. B. Myneni and S.W. Running. 2003b. Climate-Driven Increases in Global Terrestrial Net Primary Production from 1982 to 1999. Science 300(5625):1560.

Nemani, R. R., P. Votava, A. Michaelis, M. White, F. Melton, J. Coughlan, K. Golden, H. Hashimoto, K. Ichii, L. Johnson, M. Jolly, C. Milesi, R. Myneni, L. Pierce, S. Running, C. Tague and W. Pang. 2008. Terrestrial Observation and Prediction System (TOPS): Developing ecological nowcasts and forecasts by integrating surface, satellite and climate data with simulation models, in Research and Economic Applications of Remote Sensing Data Products. Aswathanarayana, U. and R. Balaii (eds.). American Geophysical Union.

Reisen, W. K., Y. Fang and V. M. Martinez. 2006. Effects of temperature on the transmission of West Nile virus by Culex tarsalis (Diptera: Culicidae). J. Med. Entomol. 43:309-317.

Running, S. W., P. E. Thornton and M. A. White. 1997. Generating surfaces of daily meteorological variables over large regions of complex terrain. J. Hydrol. 190:241-251.

White M. A. and Nemani R. R. 2004. Soil water forecasting in the continental United States: relative forcing by meterology versus leaf area index and the effects of meteorological forecast errors. Can. J. Remote Sens. 30:717-730.

Wood, B., L. Beck, R. Washino, S. Palchick and P. Sebesta. 1991. Spectral and spatial characterization of rice field mosquito habitat. Int. J. Remote Sens. 12(3):621-626.

Figure 1. An overview of the Terrestrial Observation and Prediction System (TOPS) framework. TOPS provides capabilities for automated retrieval and processing of observations from satellites, aircraft, ground-based networks, and ancillary data such as soil texture maps and digital elevation maps. Using the Java Distributed Application Framework, these data sources are ingested by a suite of ecosystem models, which in turn can be driven by weather and climate forecasts to estimate land surface conditions related to public health and vectorborne disease, agricultural water demand, ecosystem productivity, and other applications.

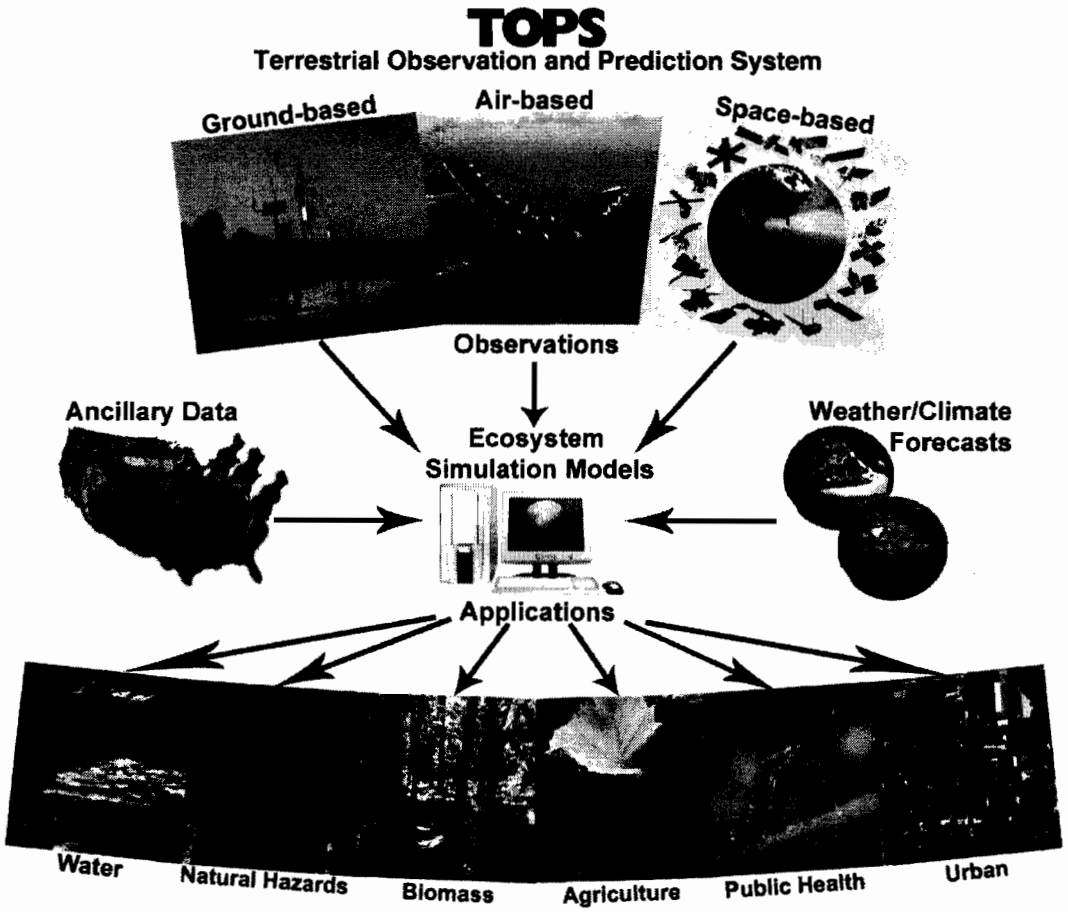
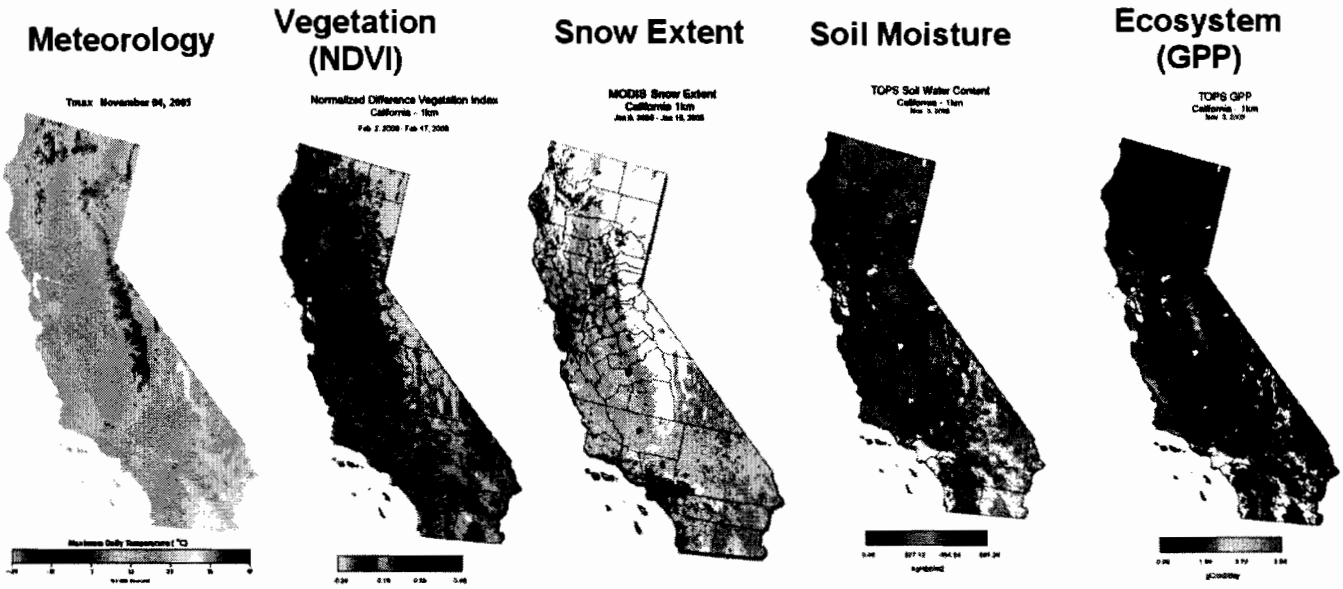


Figure 2. Examples of the TOPS California data products, which include measures of meteorological conditions, vegetation conditions, snow extent, soil moisture, and gross primary productivity. These products are being produced by TOPS on a daily to weekly basis for California at a spatial resolution of 1km, and currently being used in research on the development of new models for forecasting mosquito abundance and virus transmission risk.



Research on Arboviruses in California - Year 5: Some Concluding Thoughts

William K. Reisen

Center for Vectorborne Diseases and Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, (530) 752-0124, arbo123@pacbell.net

West Nile virus (Family Flaviviridae, genus *Flavivirus*, WNV) has now transitioned from an invading to an endemic arbovirus and most likely will remain a public, veterinary and wildlife health problem in California for years to come. In accordance, our research has been redirected towards understanding conditions that trigger and enable enzootic vernal amplification and subsequent summer outbreaks, especially climate variation and passerine seroprevalence or 'herd immunity'. However, other, often unanticipated, factors also may come into play, such as those observed during the 2007 Bakersfield, Kern County, outbreak. Here, high herd immunity in critical avian hosts at the end of the previous season and a very dry winter led us to believe that 2007 would be a year with minimal WNV activity; however, several unforeseen factors combined to produce the largest encephalitis outbreak documented in Bakersfield since the 1952 WEEV epidemic (Reeves and Hammon 1962): 1) peridomestic water sources in the form of abandoned swimming pools were created at homes in foreclosure during to the collapse of the housing market leading to extensive new domestic/urban mosquito (*Culex pipiens* L. and *Cx. quinquefasciatus* Say) production sites, 2) above normal spring temperatures led to early amplification of WNV in urban mosquito populations, 3) a decline in the House Finch population led to a marked increase in House Sparrow populations, and 4) an overall reduction in bird abundance may have led to an increased number of blood meals being taken from alternate hosts, including humans, thereby enhancing tangential transmission. These factors coalesced by early June into an unexpected outbreak.

Other epidemiological factors were investigated. Vector susceptibility to infection varied markedly over time and space and among different species of *Culex*, but seemed, at best, to play a minor role in infection rates and outbreaks in California. We also were not able to ascribe changes in vector competence to overriding ecological factors such a climate, perhaps indicating that our measures of vector competence were not precise enough to detect the impact of extrinsic factors. Herd immunity among peridomestic passerines, especially House Finches and House Sparrows, seemed to be a critical factor affecting the slope and height of the vernal amplification curve and the rate of subsidence in late summer – early fall. What is not understood is the level of herd immunity within critical avian host populations that is necessary to arrest transmission. Our data seemed to indicate that in diversified natural ecosystems such as the Stone Lakes Refuge this critical level of herd immunity may be considerably lower (ca. 10%) than in simplified urban environments such as Los Angeles (ca. 25 – 30%) where most blood meals probably come from competent hosts. Seroprevalence as high as 20% in House Finches in Bakersfield did not limit amplification during 2007. Similarly it has been difficult to understand the impact of depopulation of important amplification hosts such as American Crows or Western Scrub-jays

on virus dynamics. Detailed analyses indicate that both species were at high risk of decline, but California populations seem less affected and rebounded more rapidly than observed elsewhere (Caffrey et al. 2003, Caffrey et al. 2005, Ladeau et al. 2007).

WNV is now endemic in California and much of the New World and genetic changes are anticipated as WNV adapts to different climate patterns and vector-host transmission cycles. Already the invading NY99 has been replaced by a new North American strain (Davis et al. 2005) that apparently is capable of more rapid dissemination and transmission by *Cx. pipiens* (Kramer et al. 2008) while retaining its virulence for birds (Brault et al. 2007). To attenuate this virulence and maintain fitness, there will have to be an increased susceptibility in the primary *Culex* vectors, a parameter that has not changed noticeably in California since the invasion of WNV in 2003. Outbreaks most likely will continue to be urban or peri-urban and associated with above normal temperature, lowered avian herd immunity and elevated infection in *Cx. pipiens* and Cx. quinquefasciatus. In order to plan for effective intervention, the California State Mosquito-borne Virus Surveillance and Response Plan (Kramer 2007) will need to be altered to place shared emphasis on forecasts and as well as the current nowcasts. Because of the speed of data collection, specimen testing and reporting, nowcasts based on surveillance parameters are always unavoidably offset in time.

Unfortunately, WNV is not the only mosquitoborne virus of public health importance in California, although the comparative health importance of the St Louis encephalitis (SLEV) and western equine encephalomyelitis (WEEV) viruses seem minimal compared to WNV. SLEV has not been detected since the arrival of WNV in 2003 and seems to remain displaced by cross-protective immunity to WNV in birds (Fang and Reisen 2006). What is not understood is what level of WNV transmission and herd immunity in birds are necessary to permit this virus to become re-established. Historically, California strains of SLEV maintained by highly susceptible *Cx. tarsalis* Coquillett have been somewhat attenuated (Bowen et al. 1980) compared with those from the Eastern US transmitted by *Cx. pipiens/quinquefasciatus*, but that may not be the case in the future. WEEV remains active, being transmitted enzootically within the *Cx. tarsalis*-bird cycle without spreading to the *Aedes*-rabbit cycle (Hardy 1987) or to humans. WEEV has remained phenotypically similar in its ability to infect *Cx. tarsalis* and passerine birds, so it may be that other epidemiological factors or mosquito control have constrained amplification.

With several large shipping and airports, extensive ground traffic and commerce with Mexico, extensive tourism and travel, a mild climate, and varied landscapes supporting a diverse mosquito and animal fauna, California would seem highly susceptible for invasion by new mosquitoes and the pathogens they transmit. *Aedes albopictus* (Skuse) has been introduced on several occasions (Linthicum et al. 2003, Madon et al. 2002), but has been eradicated by vigilant mosquito control districts. *Aedes aegypti L.* has become endemic in neighboring Arizona and is a threat to invade California. Both species have been responsible for transmission during the on-going and widespread Chikungunya virus epidemic (Powers and Logue 2007) and viremic travelers have been detected in the US (Lanciotti et al. 2007). Japanese encephalitis virus (JEV) has been expanding its distribution in Asia (Mackenzie et al. 2001, 2002) and easily could reach California where local mosquitoes and birds have been found to be competent hosts (Hammon et al. 1951, Reeves and Hammon 1946). Other viruses that have been considered include Rift Valley fever from Africa, Venezuelan equine encephalomyelitis from the Neotropics, and Ross River and Barmah Forrest from Australia. Current surveillance programs focus on the sensitive, high throughput detection of the endemic viruses and need to be expanded to detect newly emerging or invading viruses. Recent attempts at providing this coverage have incorporated Vero cell culture, but research into new technology such as Luminex would seem

warranted. In addition, the risk for establishment should be investigated by evaluating the vector competence of California's mosquitoes for these potentially invading viruses. The rapid and extensive dispersal of WNV throughout the US and California has demonstrated the ease with which an invading virus can spread as well as shown the devastating impact (Holloway 2000) on mosquito control and health resources as well as human, veterinary and wildlife health.

REFERENCES CITED

Bowen, G. S., T. P. Monath, G. E. Kemp, J. H. Kerschner and L. J. Kirk. 1980. Geographic variation among St. Louis encephalitis virus strains in the viremic responses of avian hosts. Am. J. Trop. Med. Hyg. 29:1411-1419.

Brault, A. C., C. Y. Huang, S. A. Langevin, R. M. Kinney, R. A. Bowen, W. N. Ramey, N. A. Panella, E. C. Holmes, A. M. Powers and B. R. Miller. 2007. A single positively selected West Nile viral mutation confers increased virogenesis in American crows. Nat. Genet. 39:1162-1166.

Caffrey, C. L., T. J. Weston and S. C. R. Smith. 2003. High mortality among marked crows subsequent to the arrival of West Nile virus. Wildl. Soc. Bull. 31:870-872.

Caffrey, C. L., S. C. R. Smith and T. J. Weston. 2005. West Nile virus devastates an American crow population. The Condor 107:128-132.

Davis, C. T., G. D. Ebel, R. S. Lanciotti, A. C. Brault, H. Guzman, M. Siirin, A. Lambert, R. E. Parsons, D. W. Beasley, R. J. Novak, D. Elizondo-Quiroga, E. N. Green, D. S. Young, L. M. Stark, M. A. Drebot, H. Artsob, R. B. Tesh, L. D. Kramer and A. D. Barrett. 2005. Phylogenetic analysis of North American West Nile virus isolates, 2001-2004: Evidence for the emergence of a dominant genotype. Virology 342:252-265.

Fang, Y. and Reisen, W. K. 2006. Previous infection with West Nile or St. Louis encephalitis viruses provides cross protection during reinfection in house finches. Am. J. Trop. Med. Hyg. 75:480-485.

Hammon, W. M., W. C. Reeves and G. E. Sather. 1951. Japanese B encephalitis virus in the blood of experimentally inoculated birds. Am. J. Hyg. 53:249-261.

Hardy, J. L. 1987. The ecology of western equine encephalomyelitis virus in the Central Valley of California, 1945-1985. Am. J. Trop. Med. Hyg. 37:18s-32s.

Holloway, M. 2000. Outbreak not contained. West Nile virus triggers a reevaluation of public health surveillance. Sci. Am. 282:20-22.

Kramer, V. L. 2007. California State mosquito-borne virus surveillance and response plan. California Dept Hlth Svc

Kramer, L. D., L. M. Styer and G. D. Ebel. 2008. A Global Perspective on the Epidemiology of West Nile Virus. Annu. Rev. Entomol. 53:61-81.

Ladeau, S. L., A. M. Kilpatrick and P. P. Marra. 2007. West Nile virus emergence and large-scale declines of North American bird populations. Nature 447:710-713.

Lanciotti, R. S., O. L. Kosoy, J. J. Laven, A. J. Panella, J. O. Velez, A. J. Lambert and G. L. 2007. Chikungunya virus in US travelers returning from India, 2006. Emerg. Infect. Dis. 13:764-767.

Linthicum, K. J., V. L. Kramer, M. B. Madon, K. Fujioka. 2003. Introduction and potential establishment of Aedes albopictus in California in 2001. J. Am. Mosq. Control Assoc. 19:301-308.

Mackenzie, J. S., K. B. Chua, P. W. Daniels, B. T. Eaton, H. E. Field, R. A. Hall, K. Halpin, C. A. Johansen, P. D. Kirkland, S. K. Lam, P. McMinn, D. J. Nisbet, R. Paru, A. T. Pyke, S. A. Ritchie, P. Siba, D. W. Smith, G. A. Smith, A. F. van Den Hurk and L. F. Wang. 2001. Emerging viral diseases of Southeast Asia and the Western Pacific. Emerg. Infect. Dis. 7:497-504.

Mackenzie, J. S., C. A. Johansen, S. A. Ritchie, A. F. van Den Hurk and R. A. Hall. 2002. Japanese encephalitis as an emerging virus:

the emergence and spread of Japanese encephalitis virus in Australasia. Curr. Top. Microbiol. Immunol. 267:49-73.

Madon, M. B., M. S. Mulla, M. W. Shaw, S. Kluh and J. E. Hazelrigg. 2002. Introduction of Aedes albopictus (Skuse) in southern California and potential for its establishment. J. Vector Ecol. 27:149-154.

Powers, A. M. and C. H. Logue. 2007. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J. Gen. Virol. 88:2363-2377.

Reeves, W. C. and W. M. Hammon. 1946. Laboratory transmission of Japanese B encephalitis virus by seven species (three genera) of North American mosquitoes. J. Exp. Med. 83:185-194.

Reeves, W. C. and W. M. Hammon. 1962. Epidemiology of the arthropod-borne viral encephalitides in Kern County, California, 1943-1952. Univ. Calif. Berkeley Publ. Hlth. 4:1-257.

Responding to West Nile Virus in Santa Clara County

Noor S. Tietze, Tim Mulligan, Russ Parman and Nayer Zahiri

Santa Clara County Vector Control District

1555 Berger Drive, San Jose, CA 95112

ABSTRACT: The Santa Clara County Vector Control District developed a program to detect and operationally respond to West Nile virus foci during the past 3-4 years. The WNV surveillance program was configured to provide an efficient monitoring system and trigger for community-based alerts, meetings and small scale, truck-based mosquito adulticide applications. During the last three years, 1,347 samples consisting of birds, mosquitoes, and squirrels were recovered from 16 municipalities and the unincorporated regions of Santa Clara County and resulted in 538 positive detections for WNV. Mosquito trapping was modified to biased trap placement around positive bird and squirrel detection sites. During the years 2005, 2006 and 2007, a GIS based process of identifying WNV foci was developed and foci were designated on two, three and six occasions each year, respectively. The focus areas averaged 2.75 mi^2 and totaled 24.7 mi^2 during the three-year interval.

INTRODUCTION

In 1999 West Nile virus (Family Flaviviridae, genus *Flavivirus*, WNV) was first detected in the United States in New York City and only five years later it was found in Santa Clara County in American Crows (*Corvus brachyrhynchos*), Western Scrub Jays (*Aphelocoma coerulescens*) and various local hawk species. In California, WNV was first detected in 2003 in southern California (Reisen et al. 2004) and during the next four years, spread throughout the state. There have been 14 human cases of WNV in Santa Clara County during the last three years.

Santa Clara County is located south of the San Francisco Bay and is represented by a variety of biomes including grassland to the east, wetlands of the San Francisco Bay, and the oak woodlands of the Diablo Range and redwoods of the Santa Cruz Mountains. The county is home to 1.68 million residents living in 1,304 mi^2, primarily in suburban neighborhoods located in Campbell, Cupertino, Gilroy, Los Altos, Los Altos Hills, Los Gatos, Milpitas, Monte Sereno, Morgan Hill, Palo Alto and San Jose, Santa Clara, Saratoga and Sunnyvale.

The Santa Clara County Vector Control District (SCCVCD) was created to provide mosquito control to county residents and protect them from vector-borne diseases. The District's WNV Response Plan encompassed disease surveillance, public outreach and education, and of course, larval and adult mosquito surveillance and suppression programs. An important element of the plan was accurate and timely designation of WNV foci to help guide district efforts during these epidemics. Thresholds were needed to identify when and where intensified larval mosquito monitoring and control should be implemented as well as targeting public outreach as WNV foci were delineated.

This report summarizes the SCCVCD WNV surveillance activities during the years 2005 through 2007 and explains the decision-making process adopted for designating WNV foci. Setting clear thresholds are believed to improve the District's response time in providing disease suppression and mosquito control services, particularly when extensive public outreach is prerequisite to fogging.

MATERIALS AND METHODS

The SCCVCD adopted a procedure to respond to the presence of WNV based on positive detections in birds, squirrels and mosquitoes. During 2007, weekly GIS-based checks were conducted for locations meeting or exceeding a 2.0 "positive detections" per square mile threshold; our threshold for designating a WNV "high risk zone". Unlike the Dead Crow Density Model presented by Eidson et al. (2005) that recommended a threshold value of 0.1 positive dead crows per square mile of county in New York, the SCCVCD assessed dead bird density on a smaller geographic scale. This system also differed from that of the California Dynamic Continuous-Area Space-Time (i.e., DYCAST) model developed by Center for Advanced Research of Spatial Information at Hunter College, City University of New York, which based results on all bird reports (i.e., positive, negative or untested dead birds) as opposed to only positive detections. The high risk zones were targeted for intensified larval surveillance and control and adult mosquito surveillance and WNV testing. Subsequent detection of WNV positive mosquitoes were used as a trigger to designate WNV foci, prepare for community meetings and mosquito adulticiding events.

In-house laboratory assays for WNV, using the VecTest WNV antigen assay (Microgenics Corp., Fremont, CA) provided early detection in crows and jays while the RAMP® WNV test (Response Biomedical Corp.,Burnaby, BC) was utilized for rapid assessment in adult mosquitoes. While these techniques offered poorer sensitivity than that of PCR, the high viremia in crows resulting from acute infection (Komar et al. 2003 and Reisen et al. 2005) was easily detected using VecTest™ and the RAMP test was found to be proficient at detecting WNV in mosquitoes at a minimal concentration of 4 log10 PFU/mL (William Reisen, pers. comm.).

Dead Bird/Squirrel Program

Dead bird reports were received directly from the public or sometimes via California Department of Public Health Vector-Borne Disease Services (VBDS) Dead Bird Hotline. This consisted of dispatching these reports to field technicians to recover and test in-house or package and ship out for necropsy at California Animal Health and Food Safety Laboratory (CAHFS) and subsequent WNV testing by VBDS. During 2005 all bird species were considered for recovery and testing, whereas in 2006 and 2007 the program was streamlined to the Corvidae and raptors. VBDS implemented squirrel testing in 2006. In-house testing of corvids was conducted in a biosafety cabinet (NuAir Inc.Plymouth, MN), where the birds were orally swabbed using Dacron-tipped disposable swabs and processed according to VecTest™ instructions. Western Scrub Jays (*A. coerulescens*) were tested using the VecTest™ and when found negative were also submitted for confirmation to CAHFS for necropsy and subsequent testing at VBDS. Infected and uninfected dead crows were stored in a large freezer (4 °F) and eventually incinerated (Koefran Inc., Sacramento, CA). Test data including results were recorded on file cards and later entered into a computer database.

Mosquito Surveillance

From the 2005 through 2007 the SCCVCD implemented intensified mosquito surveys at "sentinel" WNV sites (i.e., positive bird/squirrel detection sites) as recommended by Gu and Novak (2004). The latter study found that, given the spatial and temporal variability of infected mosquitoes in the field, agencies should target "sentinel" sites with intensified sampling to effectively double the probability of detection. This biased sampling system facilitated the recovery of positive mosquitoes detected using the RAMP® WNV test which subsequently formed the decisive trigger for implementing adult mosquito control operations. During 2005 through 2006, at least two positive mosquito

detections were deemed necessary to trigger adult mosquito control, whereas in 2007 that threshold was reduced to one positive mosquito detection.

Mosquito surveys were conducted using fabricated carbon dioxide-baited traps designed by Orange County VCD (Bob Cummings, pers. com.) as well as commercially-available EVS traps (BioQuip Products, Ranch Domingeuz, CA). Carbon dioxide bait was supplied as about five pounds (2 kg) of block or pelletized dry ice (Air Products, San Carlos, CA) placed in metal or plastic one gallon paint buckets hung above the trap. Mosquito traps were placed proximal to recent WNV positive detections in birds and squirrels.

Traps were placed during the afternoon and recovered on the following morning. Adult mosquitoes were euthanized using triethyl amine, sorted under a dissecting microscope, and prepared for RAMP® WNV testing. Mosquito samples were homogenized using a vortex for one minute and centrifuged for five minutes. The RAMP® WNV results were assessed about 90 minutes after preparation of the samples. Sample data were entered into an Approach database system capturing fields: geographic coordinates, date, species and number and test results.

Data Processing and Geographic Information System

Upon receiving test results for WNV (in-house or outsourced), mosquito, bird and squirrel data were entered into a database system including address or geographic coordinates, animal tested, date of collection and results. The data were exported to geographic information system (GIS) ArcGIS 9.2 (ESRI, Redlands, CA) for assessment of WNV risk level, printing of public outreach maps (website), staff work assignments, and ultimately designation of adulticide application zones.

Each new positive bird detection was address-matched using the ArcGIS 9.2, Streetmap USA (ESRI, Redlands, CA) address locator. By labeling week of collection, positive detections were assessed to determine if there were more than two positives per square mile during the last 3 weeks. The ESRI module, Spatial Analyst was used to calculate positive detection density (Population Field=None; Density Type=Kernel; Search Radius 0.016 miles; Output Cell Size=0.00187).

Positive dead bird density was also calculated retrospectively within each designated focus by selecting positive detections and charting cumulative density over week of the year. Focus site area was calculated using an ESRI-compatible acreage tool (Bennett and Peters, Inc.).

WNV Focus Delineation

Foci were delineated based on a central point or points consisting of WNV positive mosquito detections. A circle formed from a 0.75 mile radius was plotted using Buffer feature (ESRI). A shapefile was created manually based on a polygon layout viewed along with a street shapefile. The edges of the spray zone were modified to encompass all residential parcels within the 0.75 mile radius while taking into account major roads and logistical considerations for the spray route. Once the focus polygon was completed, parcels within the latter polygon were "selected by location" and the selected parcel table was exported to generate a site address mailing label list as well as a "look up table" saved as a "dbf" file for reference during the upcoming community meeting. Adulticide application statistics were calculated based on a 300 foot swath buffer by using ArcGis buffer feature by specifying 150 ft buffer size field for all street segments within the focus area. Using the street buffer area value, pesticide volume, time needed to spray, and number of spray trucks could be calculated.

Community Meetings

As part of the procedure, community meetings were held to assist residents with questions regarding (1) public health concerns of WNV, (2) questions about pesticides, or (3)

other aspects of our operation. During 2005 through 2006 community meetings were held at community centers or school gymnasiums within or near the delineated zone, where short presentations were given on the above topics followed by questions directed from the public. This approach was later modified to an "open house" meeting format where residents could select among several "booths" where questions/discussions were held on the above topics.

RESULTS AND DISCUSSION

This SCCVCD surveillance program recovered 168, 649 and 577 specimens for WNV testing during 2005, 2006 and 2007, respectively (Table 1). Although a total of 42 species were sampled, American crow and the two mosquito species, *Culex pipiens* L. and *Culex tarsalis* Coquillett comprised the bulk of the samples (Table 1). During that time there were 28 WNV positive mosquitoes, 496 positive birds, eight positive squirrels, two positive horses and 14 human cases.

During 2005 to 2007 in Santa Clara County there were 11 WNV foci/adulticiding zones identified by the District's Disease Surveillance Program (Table 2). The foci encompassed portions of San Jose, Saratoga, Campbell, Los Gatos and Cupertino. Foci were identified between July 7 and September 1st and shifted from south San Jose in 2005 northwest to Campbell-west San Jose-Saratoga in 2006 and Saratoga-Cupertino-Los Gatos in 2007. Excluding the July 13, 2007 adulticiding zone that resulted in equipment failure, the foci varied from 1.6 to 4.5 mi^2 with an average of 2.51mi^2. An average of 5067 parcels existed per focus/adulticiding zone. Maintaining an operationally optimal area was important since our mission was to complete adult mosquito control within each area during a single night.

Response time for adult mosquito suppression in designated WNV foci improved during the three year period from an average of 28 days in 2005 to 6.6 in 2007. A major factor in determining response time was the scheduling and conduct of community meetings. In one case in 2006 the need for a community meeting was waived and adult mosquito treatments were conducted one day after detecting the positive mosquitoes. Changing the operational threshold from two positive mosquito detections to one as a trigger for designating WNV foci allowed for earlier focus delineation, but subsequent positive mosquito finds caused shifting and/or overlapping focus delineations. As a result certain areas were adulticided up to three times during the course of the season. The operational consequences to lowering this trigger thus needs to be weighed against response time, a critical factor in mosquito-borne disease prevention.

As reported by the Santa Clara County Public Health, human onset dates for WNV cases residing in the county were on weeks 34, 37, 39 (2x) and 40 in 2005; 28 (2x), 30 and 31 in 2006; and 27, 28, 35 and 38 in 2007. Thus onset dates between 2005 and 2006 were significantly ($P<0.05$) different, whereas there were no significant differences between week of onset when comparing 2005 and 2007 or 2006 and 2007. It is assumed that certain number of the human cases acquired WNV outside the County, potentially those early onset dates on weeks 27 and 28, when the virus has much greater activity in the warmer Central Valley region of California. The overall average human onset date for Santa Clara County based on the past three years was week 32.8 or the second week of August.

The retrospective analysis of cumulative dead bird/squirrel density within foci yielded positive results supporting the adopted "high risk zone" identification procedure. In 2005 the 2.0 density threshold was exceeded at both foci on week 30 (Figure 1); in 2006 it was exceeded on weeks 26 and 31 (Figure 2); and during 2007 it was week 28, 30 and 33 (Figure 3). These dates indicate the inception points for "high risk zone" designations and validated operational targeting these areas prior to the establishment of WNV foci. Human onset dates occurred four to eight weeks after high risk zone thresholds were met in 2005, but only three to six weeks after the threshold was first met in 2006 and zero

to 11 weeks later in 2007. In spite of these variations in human onset dates, the 2.0 positive detections per square mile threshold is believed to be useful tool in our decision matrix when it comes to gearing up for mosquito control at specific locations in the county. An optimal response would be to conduct adult mosquito suppression at least one week prior to any potential human onset, which occurred about 83% of time in the last three years.

Acknowledgements

The authors would like to thank the staff of the Santa Clara County Vector Control District, namely Bill Shipway, Michael Stephenson, Mark Marden, Caroline Dunkelberger, Menou Thaupreseuth and Andy Hardwick for their involvement in this program.

REFERENCES CITED

Eidson, E., K. Schmit, Y. Hagiwara, M. Anand, P. Backenson, I. Gotham and L. Kramer. 2005. Dead crow density and West Nile Virus Monitoring, New York. Emerg. Infect. Dis. 11:1370-1375.

Gu, W. and R. J. Novak. 2004. Short Report: Detection probability of arbovirus infection in mosquito populations. Am. J. Trop. Med. Hyg. 7(5):636-638.

Komar, N., S. Langevin, S. Hinten, N. Nemeth, E. Edwards, D. Hettler, B. Davis, R. Bowen and M. Bunning. 2003. Experimental infection of North American birds with the New York 1999 strain on West Nile Virus. Emerg. Infect. Dis. 9:311-322.

Reisen, W. K., H. Lothrop, R. Chiles, M. Madon, C. Cossen, L. Woods, S. Husted, V. Kramer and J. Edman. 2004. West Nile Virus in California. Emerg. Infect. Dis. 8:1369-1378.

Reisen, W. K., Y. Fang and V. M. Martinez. 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. J. Med. Entomol. 42:367-375.

Table 1. List of Sampled organisms processed by Santa Clara County Vector Control District during 2005 through 2007.

Species Sampled	2005	2006	2007
Aedes Squamiger	0	1	0
American crow	118	355	206
American kestrel	0	1	0
American robin	0	1	1
Anna's hummingbird	0	0	1
Barn owl	1	2	2
Brewer's blackbird	1	1	0
Brown-headed Cowbird	0	0	1
California towhee	0	1	0
Cedar waxwing	0	1	0
Common raven	0	6	5
Cooper's hawk	1	17	13
Culex pipiens	4	100	166
Culex tarsalis	4	65	99
Eastern-gray squirrel	0	1	23
Horse	1	0	0
House finch	0	1	0
House sparrow	1	0	0
Lesser goldfinch	1	0	0
Mockingbird	0	0	1
Northern flicker	0	0	1
Northern harrier	0	0	1
Nuttail's woodpecker	0	1	0
Oak titmouse	0	0	1
Owl	0	2	0
Quail	1	0	0
Red-fox squirrel	0	10	6
Red-shouldered hawk	0	3	0
Red-tailed hawk	0	1	4
Redwing blackbird	0	1	0
Screech owl	1	0	0
Sharp-shinned hawk	0	1	2
Sparrow	0	1	0
Squirrel	0	7	13
Steller's Jay	0	6	2
Swainson's thrush	0	0	1
Varied thrush	0	0	1
Western gray squirrel	0	0	2
Western scrub jay	17	61	25
Yellow-rumped warbler	0	1	0
Yellow-billed magpie	1	0	0
Zebra finch	1	0	0
Total	158	649	577

Table 2. West Nile Virus Foci/mosquito adulticide treatment zones in Santa Clara County 2005 through 2007. Applications were truck-based synergized pyrethrin (Pyrenone 25-5) applied at a rate of 0.0025 lbs/acre.

Year	Municipality	Positive Mosquito Trigger date[1]	Date Adulticided	Focus Size (mi^2)	Response Time(days)	Number of Parcels	Community Meeting Held
2005	San Jose (Mia Circle)	8/3/05	9/1/05	1.6	29	2916	yes
2005	San Jose (La Colina Park)	8/5/05	9/1/05	1.7	27	3883	yes
2006	West San Jose- Saratoga	6/27/06	7/7/06	3.0	10	6411	yes
2006	Campbell-San Jose	8/4/06	8/14/06	3.1	10	6798	yes
2006	Saratoga	8/22/06	8/23/06	4.5	1	5637	no
2007	San Jose[2]	7/13/07	7/27/07	0.5	14	1178	yes
2007	Campbell- San Jose-Los Gatos	7/19/07	8/1/07	2.5	13	6045	yes
2007	San Jose-Los Gatos	7/25/07	8/1/07	1.3	7	2741	yes
2007	San Jose-Los Gatos	8/3/07	8/13/07	2.1	10	4415	yes
2007	San Jose-Campbell-Saratoga-Cupertino	8/15/07	8/22/07	3.2	7	5642	yes
2007	Campbell-San Jose-Saratoga-Cupertino	8/24/07	8/27/07	2.1	3	6178	yes

[1]During 2005-2006 two positive mosquito samples triggered fogging events; in 2007 this was changed to one positive sample.
[2]Spray equipment failure

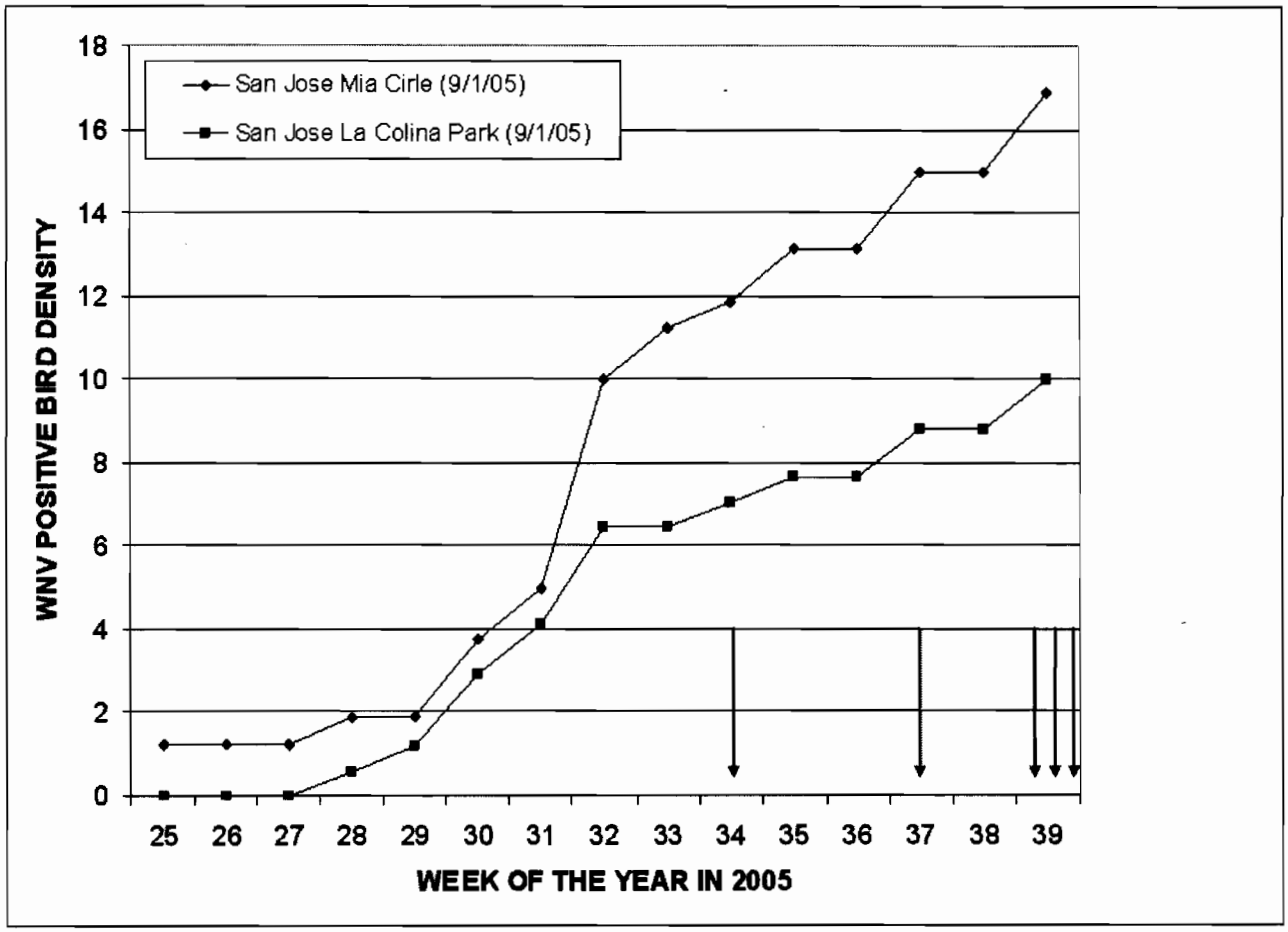


Figure 1. Cumulative density of WNV positive dead birds by designated focus in 2005. Arrows indicate human case onset dates.

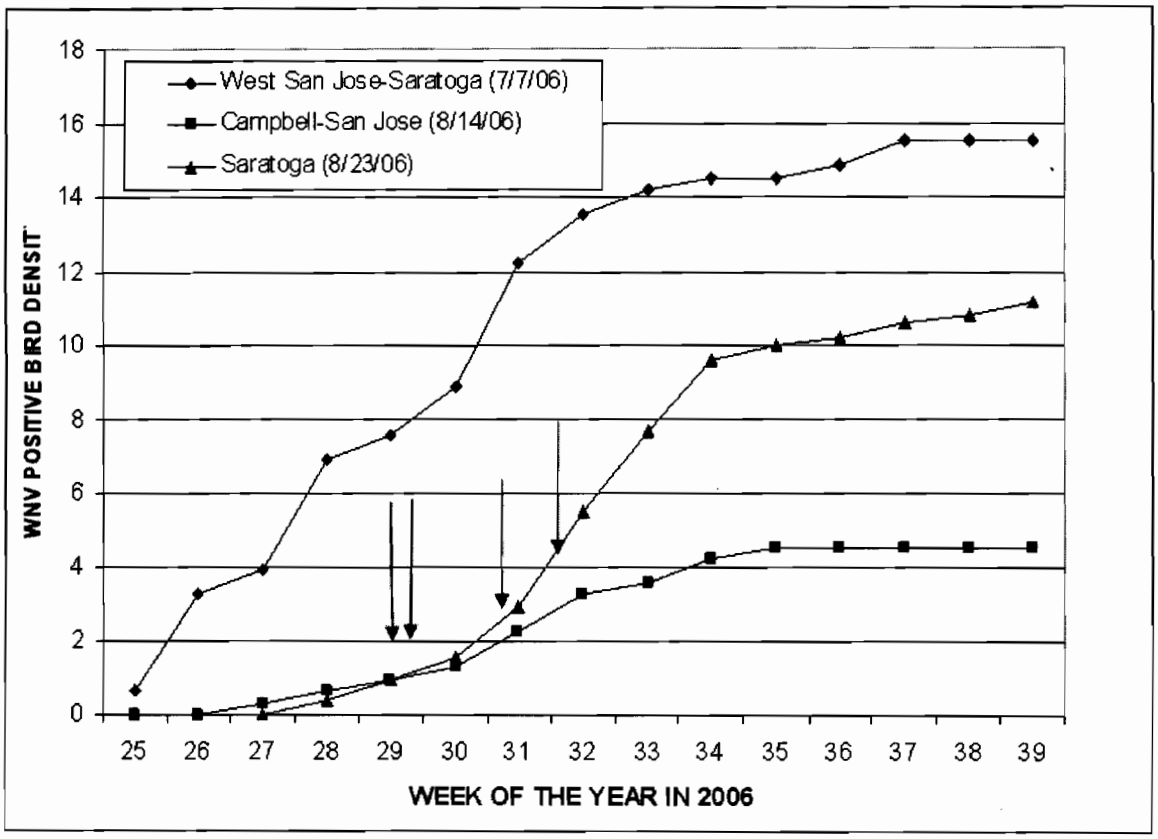


Figure 2. Cumulative density of WNV positive detections by designated focus in 2006. Arrows indicate Human case onset dates.

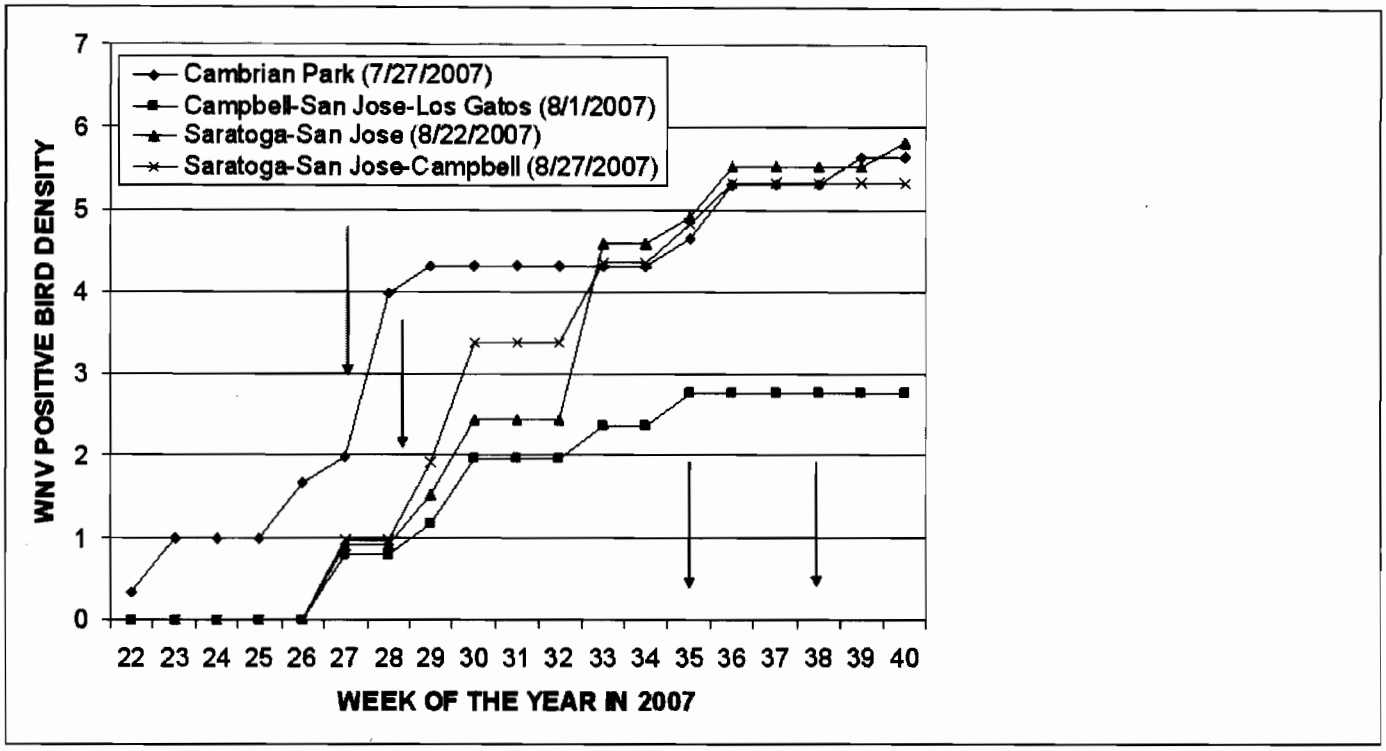


Figure 3. Cumulative density of WNV positive detections by designated focus in 2007. Arrows indicate human case onset dates.

Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 2007

Tina Feiszli[1], Stan Husted[1], Bborie Park[2], Bruce Eldridge[2], Ying Fang[2], William K. Reisen[2], Cynthia Jean[1], Cindi Cossen[1], Ryan Carney[1], Erin Parker[1], Claudia Erickson[1], Alana McQuarry[3], and Vicki Kramer[1].

[1] *California Department Public Health, 1616 Capital Ave. Sacramento, CA 95899*
[2]*U.C. Davis Center for Vectorborne Diseases, University of California, Davis, CA, 95616*
[3]*California Department of Food and Agriculture, Sacramento, CA 95814*

The California Arbovirus Surveillance program is a cooperative effort of the California Department of Public Health (CDPH), the University of California at Davis Center for Vectorborne Diseases (CVEC), the Mosquito and Vector Control Association of California (MVCAC), local mosquito abatement and vector control agencies, county and local public health departments, and physicians and veterinarians throughout California. Additional local, state, and federal agencies collaborated upon, and contributed to, the West Nile virus (Family *Flaviviridae*, genus *Flavivirus*,WNV) component of the arbovirus surveillance program.

In 2007, 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) Diagnostic testing of specimens from horses exhibiting clinical signs of viral neurologic disease compatible with western equine encephalomyelitis virus (Family *Togoviridae*, genus *Alphavirus*, WEEV), WNV, and other arboviruses as appropriate;
(3) Monitoring and testing of mosquitoes for the presence of St. Louis encephalitis virus (Family *Flaviviridae*, genus *Flavivirus*, SLEV), WEEV, and WNV; testing for other arboviruses, as appropriate;
(4) Serological monitoring of sentinel chickens for SLE, WEE, and WNV antibodies;
(5) Surveillance and diagnostic testing of tree squirrels and dead birds, especially crows and other birds in the family Corvidae, for infection with WNV;
(6) Weekly reporting in the CDPH Arbovirus Surveillance Bulletin of arbovirus test results in California and arbovirus activity throughout the United States;
(7) Bi-weekly posting of WNV information, including test results, reports, maps, and public education materials on the California WNV website: www.westnile.ca.gov;
(8) Identifying reported dead bird clusters using the WNV Dynamic Continuous-Area Space-Time (DYCAST) model to identify areas of peak WNV activity;
(9) Data management and reporting through the California Surveillance Gateway, a web application used by local agencies, CDPH, CVEC, and VRDL.

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

HUMAN DISEASE SURVEILLANCE

A regional public health laboratory network was implemented in 2002 to enhance human WNV testing and surveillance efforts in California. The laboratory network consists of the CDPH Viral and Rickettsial Disease Laboratory (VRDL) and 29 local county public health laboratories that are also able to perform WNV testing. Local laboratories test for WNV using an IgM or IgG immunofluorescent assay (IFA) and/or an IgM enzyme immunoassay (EIA). Specimens with inconclusive results are

forwarded to VRDL for further testing or confirmation with a plaque reduction neutralization test (PRNT). Additional WNV infections are identified through testing performed at reference laboratories or blood donation centers.

In 2007, specimens from 1,333 individuals were tested for WNV infection at VRDL. Additionally, over 1,000 specimens were tested at local public health laboratories. The earliest WNV symptom onset date reported in 2007 was for a 27-year-old female from Kern County who developed symptoms compatible with West Nile fever (WNF) on April 15. In total, 380 human WNV infections were identified among the residents of 30 counties in California (Table 1 & Figure 1), a 37% increase from the 278 cases reported in 2006. Of the 380 WNV cases, 220 (58%) were classified as West Nile fever, 156 (41%) were neuroinvasive disease (i.e. encephalitis, meningitis, or acute flaccid paralysis), and four (1%) were of unknown clinical presentation. The median age for all cases from whom data were available was 55 years (range: 2-96 years) and 211 (56%) were male. The median age for West Nile fever and neuroinvasive cases was 49 (range: 2-86) and 61 years (range: 8-96 years), respectively. The median age of the 20 WNV-associated fatalities was 75 years (range: 50-96 years).

EQUINE SURVEILLANCE

Serum or brain tissue specimens from 352 horses displaying neurological signs were submitted to the California Animal Health & Safety Laboratory (CAHFS) and CVEC for arboviral testing. WNV infection was detected in 28 horses from 14 counties (Table 1). Prior to onset, two horses were currently vaccinated with the WNV vaccine, three had not completed the recommended vaccine dosage schedule, and 20 were unvaccinated; vaccination history was unknown for three horses. Fourteen (50%) of the horses died or were euthanatized as a result of their infection.

ADULT MOSQUITO SURVEILLANCE

From April to November, statewide adult mosquito abundance was monitored weekly by 44 local agencies from 33 counties which contributed trap collection data to the CDPH weekly adult mosquito occurrence reports (AMOR). Local agencies submitted mosquito data from New Jersey light trap collections (35 agencies), carbon-dioxide baited trap collections (33 agencies), and gravid trap collections (18 agencies). The weekly AMOR reports and the accompanying 5-year AMOR summaries were used by agencies to compare mosquito abundance with neighboring districts, measure the effectiveness of their larval control programs, help identify unknown breeding sources, and establish thresholds as part of the state response plan.

Fifty-one agencies in 41 counties collected a total of 704,348 mosquitoes (23,870 pools) which were tested by a real-time polymerase chain reaction test (RT-PCR) for SLEV, WEEV, and WNV viral RNA (Table 2) at CVEC and the Sacramento-Yolo Mosquito and Vector Control District. An additional 100,965 mosquitoes (4,214 pools) were tested for only WNV by eight local agencies using either RT-PCR or a commercial rapid assay-RAMP® (Rapid Analyte Measurement Platform, Response Biomedical Corp).

West Nile virus was detected in 1,003 of 28,084 mosquito pools from 30 counties (Table 1 & Figure 2); 821 were positive by RT-PCR and 182 were positive by RAMP only. WNV was identified from five *Culex* species (*Cx. erythrothorax*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. Stigmatosoma* and *Cx. tarsalis)*, and three other species (*Aedes melanimon, Culiseta incidens* and *Culiseta particeps*) (Table 3). The first detection of WNV in mosquitoes in 2007 was from a pool of *Culex tarsalis* collected on January 10 in Los Angeles County. The last detection of WNV in mosquitoes in 2007 was from a pool of *Cx. quinquefasciatus* collected on December 6 in Los Angeles County.

WEE virus was detected in 16 mosquito pools of *Cx. tarsalis* collected from Kern (15 pools) and Fresno (one pool) counties. The first and last WEE positive pools were collected in Kern County on June 19 and September 12, respectively. SLE virus was not detected in mosquito pools in 2007. WEEV and other arbovirus activity for the past 10 years is shown in Fig. 3.

CHICKEN SEROSURVEILLANCE

Fifty-two local mosquito and vector control agencies in 39 counties maintained 247 sentinel chicken flocks (Table 2). From April through November, blood samples were collected from chickens every other week and tested for antibodies to SLEV, WNV, and WEEV using an EIA and IFA. Detection of *Flavivirus* or WEEV antibody was confirmed with western-blot or a plaque reduction neutralization test. In areas where SLEV has never been documented, flavivirus positive chickens from the same flock where at least two WNV confirmed positive chickens had been identified were assumed to be infected with WNV and confirmatory testing was not performed.

VRDL and four local mosquito and vector control agencies tested 30,664 chicken sera samples for antibodies to SLEV, WEEV, and WNV (Table 2). A total of 510 seroconversions to WNV were detected among 117 flocks from 27 counties (Table 2 & Figure 4). In 2007, the first WNV seroconversion was detected in Imperial County on January 2. The last WNV seroconversions were detected on November 5 from chickens located in Merced and Riverside Counties.

Thirteen WEE seroconversions were detected among nine flocks from two counties: Kern (10) and Los Angeles (3). The first and last WEE seroconversions were detected in Kern County on July 24 and October 15, respectively. No SLE seroconversions were detected in 2007. WEEV and other arbovirus activity for the past 10 years is shown in Fig. 5.

DEAD BIRD AND TREE SQUIRREL SURVEILLANCE FOR WEST NILE VIRUS

Established in 2000, the WNV dead bird surveillance program is a collaborative program between CDPH and over 130 local agencies. In 2007, the WNV Hotline (877-WNV-BIRD) operated seven days a week from 8am to 5pm. Staff fielded 36,164 calls in English and Spanish and obtained 32,203 reports—26,228 through the hotline and 5,975 through the website. Carcasses deemed suitable for testing were tested at CVEC by RT-PCR, at CAHFS by immunohistochemistry (IHC), or at one of 25 local agencies by IHC, RAMP, or VecTest (Medical Analysis Systems Inc., Camarillo, CA). In 2007, out of 6,002 tested carcasses, WNV was detected in 1,395 (23.2%) carcasses from 50 counties: 1,045 by RT-PCR, 278 by VecTest, 47 by RAMP, and 25 by IHC (Table 4 & Figure 6).

Based upon public dead bird reports, CDPH was also able to detect WNV activity using the Dynamic Continuous-Area Space-Time system (DYCAST). This early warning system, developed in cooperation with the Center for Advanced Research of Spatial Information (CARSI) at Hunter College, City University of New York, generates daily maps of high WNV activity by analyzing the incidence in space and time of dead bird reports. Local agencies used the maps to help focus surveillance and public education activities, and to help establish priority areas for mosquito control. Maps were made available on the California Surveillance Gateway website, and a real-time alert system was instituted to provide counties with custom reports about WNV transmission.

Tree squirrels (Sciurus spp.) have been included as a WNV surveillance element since 2004, based upon evidence they were susceptible to WNV mortality and could provide information on localized WNV transmission. In 2007, 736 dead tree squirrels were reported through the WNV Hotline and suitable carcasses were tested at CVEC. Out of 227 tested

carcasses, antibodies to WNV were detected in 26 (11.5%) carcasses from 10 counties (Table 1). These included 11 fox squirrels (*Sciurus niger*), 7 eastern gray squirrels (*S. carolinensis*), five western gray squirrels (*S. griseus*), and three squirrels of undetermined species.

PUBLIC EDUCATION AND REPORTS

The "Fight the Bite" WNV prevention campaign was adopted by CDPH in 2004 from the Colorado Department of Health and Environment and continues to be the main theme for prevention activities. In 2007, CDPH continued distribution of "Fight the Bite" educational materials in multiple languages to public health and vector control agencies in all 58 California counties. Press releases, media advisories, and events were used extensively to inform the public on the spread of WNV throughout the state and to promote personal protection measures. In 2007, CDPH also developed and distributed the following new WNV educational materials: a 3-minute WNV survivor interview DVD; an 18 month Fight the Bite calendar; fliers to encourage residents to report dead birds and to inform residents about the potential danger of mosquito breeding in neglected swimming pools; and an information packet for real estate managers on the management of green pools for properties in foreclosure.

Throughout the year, CDPH published weekly bulletins reporting statewide arbovirus surveillance data and national WNV activity. Surveillance bulletins were distributed to local, state, and federal public health agencies and universities in California, posted on the California West Nile virus website (www.westnile.ca.gov) and on the California Vector-Borne Disease Surveillance System (http://calsurv.org). The WNV website provided information to the public on WNV prevention and contained an online submission form for reporting dead birds directly to the WNV hotline. The site posted up-to-date county specific information on WNV activity and provided comparison surveillance data from 2006, both which were used extensively by the media. Reports, educational materials, and presentations were also made available for local agencies.

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, CARSI, CVEC (especially Maureen Dannen, Keira Simmons, and Andrew Chow), CAHFS (especially Jacquelyn Parker), and CDFA Animal Health Branch. From CDPH, we thank the VRDL (especially Carol Glaser), the Veterinary Public Health Section (especially Ben Sun), and VBDS (especially Renjie Hu, Mark Novak, Kerry Padgett, Ervic Aquino, Long Her, Jamie Riggs-Nagy, Rachel Owens, and the WNV Hotline staff).

Surveillance funding was augmented by generous support from the Centers for Disease Control and Prevention.

Table 1. Infections with West Nile virus in California, 2007

County	Humans	Horses	Dead Birds	Mosquito Pools	Sentinel Chickens	Dead Squirrels
Alameda	0	0	19	1	0	1
Alpine	0	0	0	0	0	0
Amador	0	0	1	1	0	0
Butte	16	0	27	5	32	0
Calaveras	0	0	3	0	0	0
Colusa	2	0	4	1	2	0
Contra Costa	3	0	28	28	5	5
Del Norte	0	0	0	0	0	0
El Dorado	0	0	7	0	0	2
Fresno	17	1	114	63	46	0
Glenn	7	0	36	1	8	4
Humboldt	0	0	2	0	0	0
Imperial	3	0	0	4	17	0
Inyo	0	0	1	0	0	0
Kern	140	4	124	206	82	0
Kings	7	2	9	30	22	0
Lake	0	0	3	8	2	0
Lassen	0	0	2	0	0	0
Los Angeles	36	0	164	94	27	3
Madera	2	0	6	8	4	0
Marin	0	0	4	0	0	0
Mariposa	0	0	0	0	0	0
Mendocino	2	0	3	0	0	1
Merced	4	0	34	2	11	0
Modoc	0	0	1	0	0	0
Mono	0	0	0	0	0	0
Monterey	0	0	0	0	0	0
Napa	1	0	2	0	0	0
Nevada	0	0	5	0	0	0
Orange	9	0	43	26	0	0
Placer	4	1	16	25	13	0
Plumas	0	0	1	0	0	0
Riverside	17	0	6	30	44	0
Sacramento	25	2	142	135	19	2
San Benito	0	0	0	0	0	0
San Bernardino	4	0	26	6	35	0
San Diego	15	4	108	6	1	0
San Francisco	0	0	1	0	0	0
San Joaquin	10	1	46	154	24	1
San Luis Obispo	0	1	9	0	0	0
San Mateo	0	0	2	0	0	1
Santa Barbara	0	0	1	0	0	0
Santa Clara	4	0	83	10	0	6
Santa Cruz	0	0	6	0	0	0
Shasta	9	3	48	17	7	0
Sierra	0	0	0	0	0	0
Siskiyou	0	2	4	0	0	0
Solano	1	1	3	0	7	0
Sonoma	1	2	19	1	0	0
Stanislaus	21	0	130	81	35	0
Sutter	3	0	1	23	17	0
Tehama	4	2	20	2	8	0
Trinity	0	0	1	0	0	0
Tulare	10	2	38	23	27	0
Tuolumne	0	0	1	0	0	0
Ventura	1	0	15	0	1	0
Yolo	2	0	25	10	4	0
Yuba	0	0	1	2	10	0
State Totals	**380**	**28**	**1,395**	**1,093**	**510**	**26**

Table 2. Mosquitoes and sentinel chickens tested for St. Louis encephalitis (SLE) a, western equine encephalomyelitis (WEE), and West Nile (WNV) viruses, California 2007.

County	Agency	No. mosquitoes tested[a]	No. mosquito pools tested	WNV + pools	WEE + pools	No. flocks	No. chickens	No. sera tested[a]	WNV + sera	WEE + sera
Alameda	Alameda Co. MAD	11,525	331	1	0	3	21	315	0	0
Alameda	Alameda Co. Vector Control	1,032	21	0	0	0	0	0	0	0
Alpine		0	0			0	0	0	0	0
Amador	Amador Co. Dept. Agriculture	145	13	1	0	0	0	0	0	0
Butte	Butte Co. MVCD	1,774	43	5	0	7	77	1,047	32	0
Calaveras	Saddle Creek Comm. Serv	82	8	0	0	1	10	146	0	0
Colusa	Colusa MAD	9,516	200	1	0	1	10	130	2	0
Contra Costa	Contra Costa MVCD	18,728	439	0	0	5	60	639	5	0
Del Norte		0				0	0	0	0	0
El Dorado	El Dorado Co. Vector Control	0				0	0	0	0	0
Fresno	Consolidated MAD	14,028	458	56	0	6	64	714	34	0
Fresno	Fresno MVCD	2,155	49	5	0	2	20	300	12	0
Fresno	Fresno Westside MAD	3,311	76	0	1	2	21	204	0	0
Glenn	Glenn Co. MVCD	1,244	26	1	0	1	13	120	8	0
Humboldt		0				0	0	0	0	0
Imperial	Coachella Valley MVCD	11,340	240	4	0	3	30	281	1	0
Imperial	Imperial Valley VCD	0				4	20	200	16	0
Inyo		0				0				
Kern	Delano MAD	0				1	10	123	4	0
Kern	Kern MVCD	21,210	549	152	15	9	109	1,278	77	10
Kern	South Fork MAD	0				1	10	150	0	0
Kern	UCD Field Station	20,674	572	54	0	0	0	0	0	0
Kern	Westside MVCD	106	4	0	0	3	30	395	1	0
Kings	Consolidated MAD	216	9	1	0	0	0	0	0	0
Kings	Kings MAD	9,303	223	29	0	4	24	322	22	0
Lake	Lake Co. VCD	10,961	280	8	0	2	20	281	2	0
Lassen	Lassen Co. Dept. of Agric.	267	8	0	0	0	0	0	0	0
Los Angeles	Antelope Valley MVCD	2,919	65	0	0	8	48	704	6	3
Los Angeles	Greater Los Angeles Co. VCD	73,883	2,254	89	0	7	60	1,339	13	0
Los Angeles	Long Beach EH	5,836	189	0	0	3	29	385	0	0
Los Angeles	Los Angeles Co. West VCD	16,277	402	2	0	21	130	2,186	6	0
Los Angeles	San Gabriel Valley MVCD	797	27	3	0	11	45	41	2	0
Madera	Fresno Westside MAD	48	2	0	0	0	0	0	0	0
Madera	Madera Co. MVCD	2,335	48	8	0	2	21	220	4	0
Marin	Marin-Sonoma MVCD	0	0	0	0	2	20	279	0	0
Mariposa		0				0	0	0	0	0
Mendocino		0				0	0	0	0	0
Merced	Merced Co. MAD	2,422	83	2	0	8	48	608	11	0
Merced	Turlock MAD	11,122	310	0	0	0	0	0	0	0
Modoc	California Dept Public Health	69	4	0	0	0	0	0	0	0
Mono		0				0	0	0	0	0
Monterey	North Salinas MAD	0				2	22	284	0	0
Napa	Napa Co. MAD	4,359	109	0	0	3	33	390	0	0
Nevada		0				2	20	180	0	0
Orange	Orange Co. VCD	7,612	253	0	0	1	10	175	0	0
Placer	Placer Co. MVCD	18,724	619	25	0	7	42	506	13	0
Plumas		0				0	0	0	0	0

Table 2. (Continued)

Riverside	Coachella Valley MVCD	62,832	1,763	26	0	9	90	1,732	27	0
Riverside	Northwest MVCD	10,772	317	3	0	5	87	890	17	0
Riverside	Riverside Co. EH	32,623	753	1	0	6	60	953	0	0
Sacramento	Sacramento-Yolo MVCD	90,865	5,427	135	0	8	48	1,332	19	0
San Benito		0				0	0	0	0	0
San Bernadino	San Bernardino Co. VCP	9,706	425	3	0	10	106	1,936	33	0
San Bernardino	West Valley MVCD	15,287	622	3	0	8	25	434	2	0
San Diego	San Diego Co. Dept of Health	4,750	154	6	0	4	40	625	1	0
San Francisco	Presidio Trust	149	8	0	0	0	0	0	0	0
San Joaquin	San Joaquin Co. MVCD	24,024	694	15	0	4	40	481	24	0
San Luis Obispo	San Luis Odispo Co. EH	446	10	0	0	0	0	0	0	0
San Mateo	San Mateo Co. MAD	3,294	93	0	0	1	10	119	0	0
Santa Barbara	Santa Barbara Coastal VCD	9,329	208	0	0	4	40	666	0	0
Santa Clara	Santa Clara Co. VCD	25	2	0	0	2	20	128	0	0
Santa Cruz	Santa Cruz Co. MVCD	2,398	64	0	0	2	20	267	0	0
Shasta	Burney Basin MAD	0				2	20	154	0	0
Shasta	Shasta MVCD	19,886	434	17	0	5	55	626	7	0
Sierra		0				0	0	0	0	0
Siskiyou		0				0	0	0	0	0
Solano	Solano Co. MAD	2,032	52	0	0	3	35	366	7	0
Sonoma	Marin-Sonoma MVCD	56	2	1	0	4	40	557	0	0
Stanislaus	East Side MAD	356	10	8	0	2	16	207	10	0
Stanislaus	Turlock MAD	55,027	1,898	69	0	7	85	994	25	0
Sutter	Sutter-Yuba MVCD	10,683	254	23	0	5	50	584	17	0
Tehama	Tehama Co. MVCD	790	23	2	0	3	30	387	8	0
Trinity		0				0	0	0	0	0
Tulare	Delano MAD	0				1	10	110	3	0
Tulare	Delta VCD	15,767	375	22	0	3	30	439	7	0
Tulare	Tulare MAD	0				2	20	301	17	0
Tulare	Kings MAD	364	8	1	0	0	0	0	0	0
Tuolumne		0				0	0	0	0	0
Ventura	City of Moorpark	0				1	9	153	0	0
Ventura	Ventura Co. EH	1,465	34	0	0	4	40	662	1	0
Yolo	Sacramento-Yolo MVCD	46,630	2,200	10	0	7	42	1,381	4	0
Yuba	Sutter-Yuba MVCD	722	25	2	0	2	20	238	10	0
Total		**704,348**	**23,870**	**795**	**16**	**247**	**2,186**	**30,664**	**510**	**13**

Table 3. Mosquitoes tested for West Nile virus (WNV), California, 2007.

***Culex* species**	**Pools**	**No. mosquitoes**	**WNV +**	**Prevalence[a]**
Cx boharti	3	13	0	0.00
Cx erraticus	2	21	0	0.00
Cx erythrothorax	1,651	64,003	4	0.06
Cx pipiens	7,285	179,638	279	1.55
Cx quinquefasciatus	5,992	180,101	342	1.90
Cx restuans	4	78	0	0.00
Cx stigmatosoma	595	4,535	5	1.10
Cx tarsalis	10,747	342,209	368	1.08
Cx thriambus	32	933	0	0.00
Cx unknown	3	65	1	15.38
All *Culex*	**26,314**	**771,596**	**999**	**1.29**

***Anopheles* species**	**Pools**	**No. mosquitoes**	**WNV +**	**Prevalence**
An franciscanus	13	323	0	0.00
An freeborni	120	4,625	0	0.00
An hermsi	55	1,470	0	0.00
An punctipennis	5	34	0	0.00
All Anopheles	**193**	**6,452**	**0**	**0.00**

***Aedes* species**	**Pools**	**No. mosquitoes**	**WNV +**	**Prevalence**
Ae dorsalis	18	661	0	0.00
Ae melanimon	739	16,968	2	0.12
Ae nigromaculis	16	155	0	0.00
Ae sierrensis	3	8	0	0.00
Ae squamiger	1	14	0	0.00
Ae taeniorhynchus	5	164	0	0.00
Ae vexans	105	1,866	0	0.00
Ae washinoi	59	1,887	0	0.00
All *Aedes*	**946**	**21,723**	**2**	**0.09**

Other species	**Pools**	**No. mosquitoes**	**WNV +**	**Prevalence**
Culiseta incidens	568	4,387	1	0.23
Culiseta inornata	26	126	0	0.00
Culiseta particeps	6	114	1	8.77
Coquilletidia peturbans	8	253	0	0.00
Unknown species	23	662	0	0.00
All other	**631**	**5,542**	**2**	**0.41**

[a] Prevalence = (No. pools positive/No. mosquitoes tested) X 1000

Table 4. Dead birds reported, testeda, and positive for West Nile virus, California 2007.

	Corvid[b]				Non-Corvids			
County	Reported	Tested	Positive	Percent Positive	Reported	Tested	Positive	Percent Positive
Alameda	190	56	8	14.29	820	189	11	5.82
Alpine	1	1	0	0.00	5	1	0	0.00
Amador	10	2	0	0.00	102	29	1	3.45
Butte	405	60	24	40.00	606	64	3	4.69
Calaveras	10	0		0.00	183	39	3	7.69
Colusa	14	5	4	80.00	36	7	0	0.00
Contra Costa	362	96	22	22.92	1680	218	6	2.75
Del Norte	5	3			20	7	0	0.00
El Dorado	76	18	2	11.11	365	89	5	5.62
Fresno	656	133	99	74.44	1284	161	15	9.32
Glenn	72	33	28	84.85	76	32	8	25.00
Humboldt	38	22	1	4.55	89	24	1	4.17
Imperial	0				11	2	0	0.00
Inyo	11	4	0	0.00	21	1	1	100.00
Kern	301	67	49	73.13	1497	266	75	28.20
Kings	63	16	8	50.00	127	19	1	5.26
Lake	14	8	2	25.00	58	14	1	7.14
Lassen	20	4	0	0.00	64	21	2	9.52
Los Angeles	858	264	124	46.97	1554	411	40	9.73
Madera	54	12	6	50.00	89	23	0	0.00
Marin	155	19	0	0.00	276	53	4	7.55
Mariposa	3	1	0	0.00	46	8	0	0.00
Mendocino	31	8	2	25.00	84	19	1	5.26
Merced	203	46	30	65.22	281	51	4	7.84
Modoc	1	1	0	0.00	18	6	1	16.67
Mono	4	2	0	0.00	36	2	0	0.00
Monterey	43	9	0	0.00	189	48	0	0.00
Napa	43	4	2	50.00	86	4	0	0.00
Nevada	55	14	2	14.29	261	72	3	4.17
Orange	251	90	32	35.56	584	150	11	7.33
Placer	140	20	10	50.00	752	78	6	7.69
Plumas	11	6	0	0.00	50	19	1	5.26
Riverside	125	11	3	27.27	423	28	3	10.71
Sacramento	1053	172	119	69.19	2232	209	23	11.00
San Benito	12	6	0	0.00	44	18	0	0.00
San Bernardino	175	43	13	30.23	560	118	13	11.02
San Diego	430	249	107	42.97	421	91	1	1.10
San Francisco	18	6	0	0.00	133	34	1	2.94
San Joaquin	821	54	41	75.93	759	41	5	12.20
San Luis Obispo	39	11	1	9.09	283	73	8	10.96
San Mateo	124	43	0	0.00	451	107	2	1.87
Santa Barbara	38	9	0	0.00	99	20	1	5.00
Santa Clara	644	236	82	34.75	1309	39	1	2.56
Santa Cruz	55	11	1	9.09	249	74	5	6.76
Shasta	348	75	44	58.67	460	60	4	6.67

Table 4. (Continued)

	Corvid[b]				Non-Corvids			
County	Reported	Tested	Positive	Percent Positive	Reported	Tested	Positive	Percent Positive
Sierra	7	2	0	0.00	6	2	0	0.00
Siskiyou	12	7	4	57.14	22	2	0	0.00
Solano	134	16	3	18.75	405	0		0.00
Sonoma	227	57	12	21.05	575	102	7	6.86
Stanislaus	665	153	111	72.55	780	109	19	17.43
Sutter	103	2	1	50.00	120	4	0	0.00
Tehama	64	30	16	53.33	135	23	4	17.39
Trinity	20	2	0	0.00	40	12	1	8.33
Tulare	150	49	27	55.10	372	87	11	12.64
Tuolumne	12	1	0	0.00	124	35	1	2.86
Ventura	228	65	4	6.15	390	106	11	10.38
Yolo	280	46	17	36.96	367	93	8	8.60
Yuba	29	2	1	50.00	91	6	0	0.00
Totals	**9,913**	**2,382**	**1,062**	**44.58**	**22,200**	**3,620**	**333**	**9.20**

[a]Tested by University of California at Davis Center for Vectorborne Diseases or local mosquito/vector control agency

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

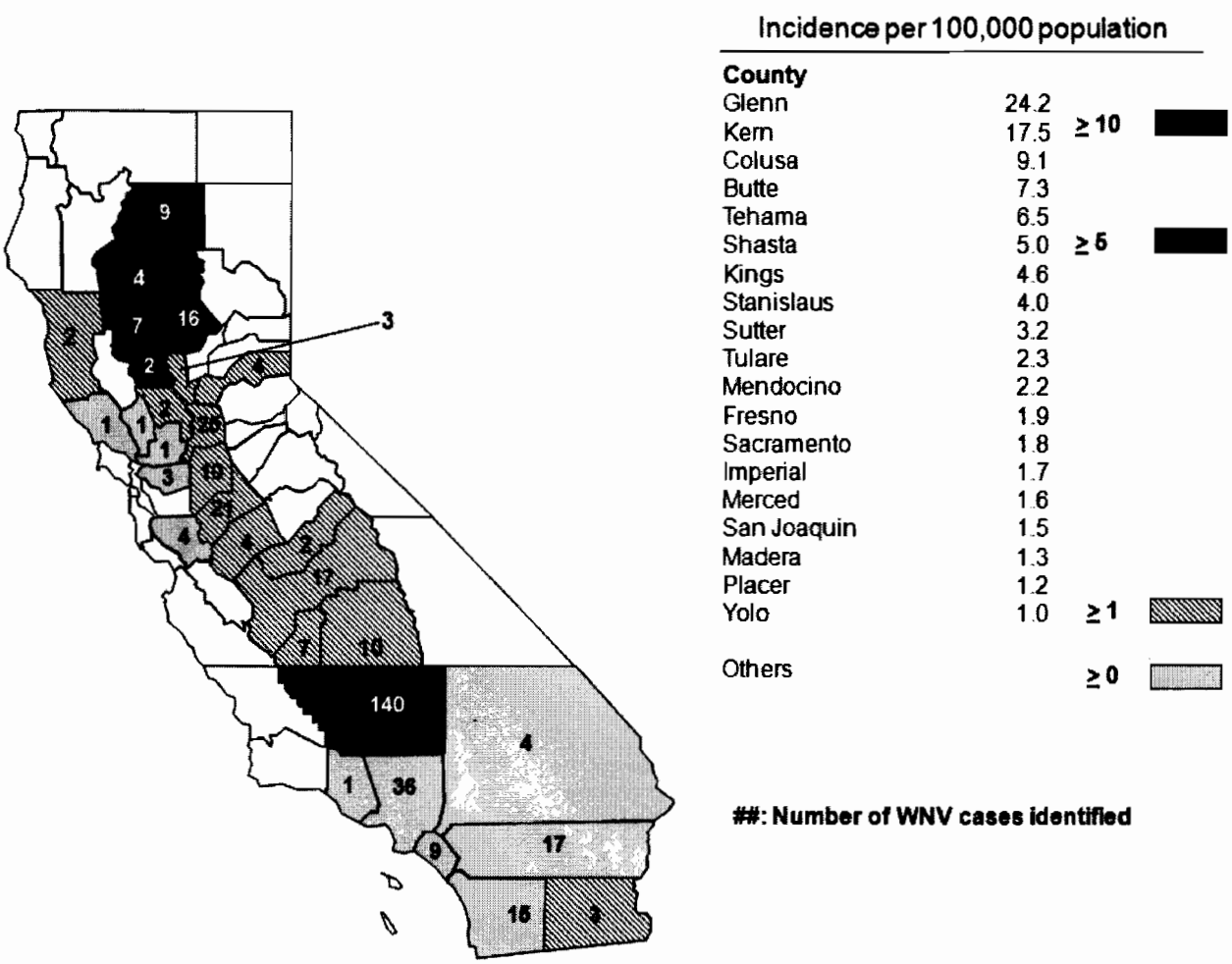


Figure 1: Human cases of West Nile virus infection, California 2007

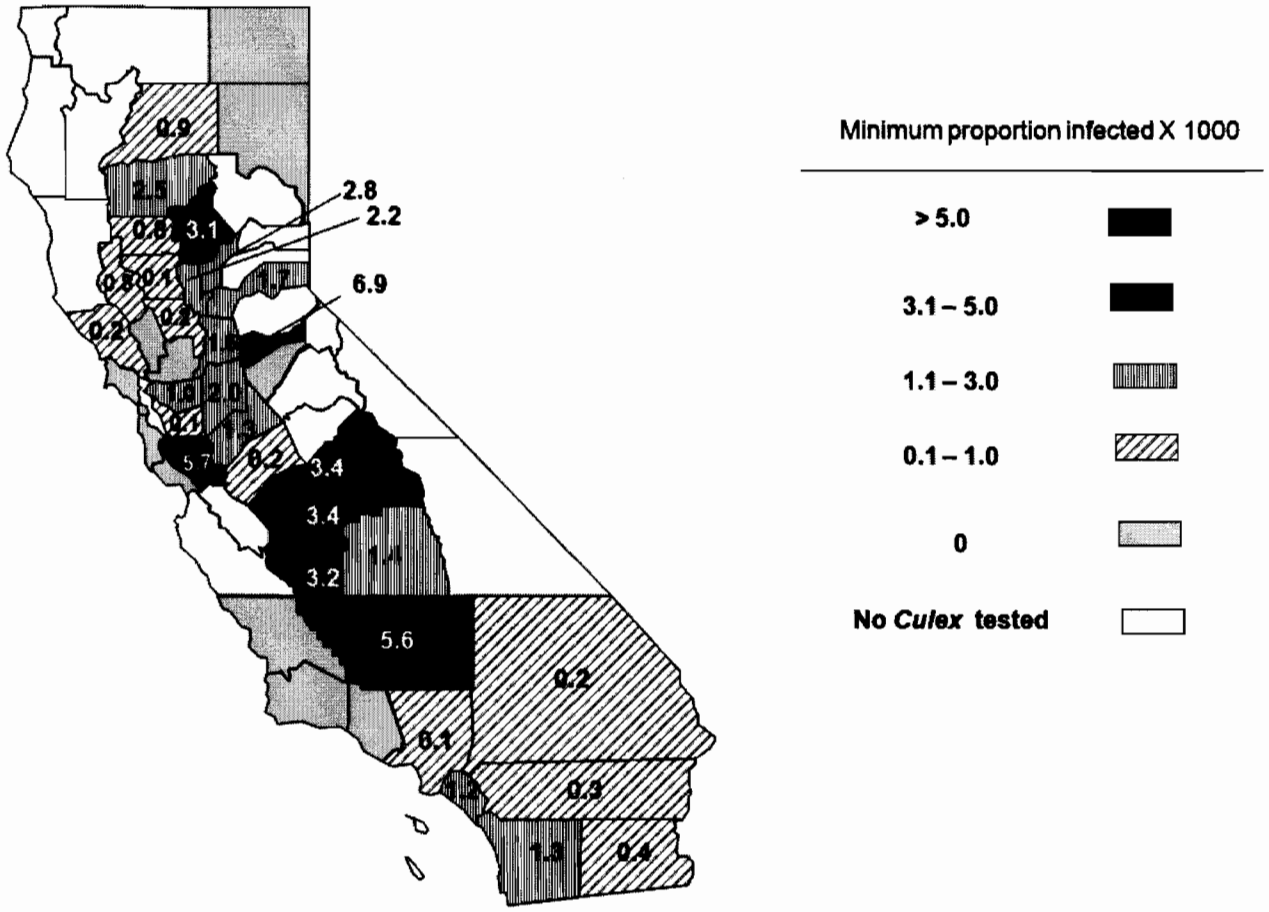


Figure 2: West Nile virus in *Culex* spp., California, 2007. Minimum proportion infected = number of positive pools/number of mosquitoes tested X 1,000

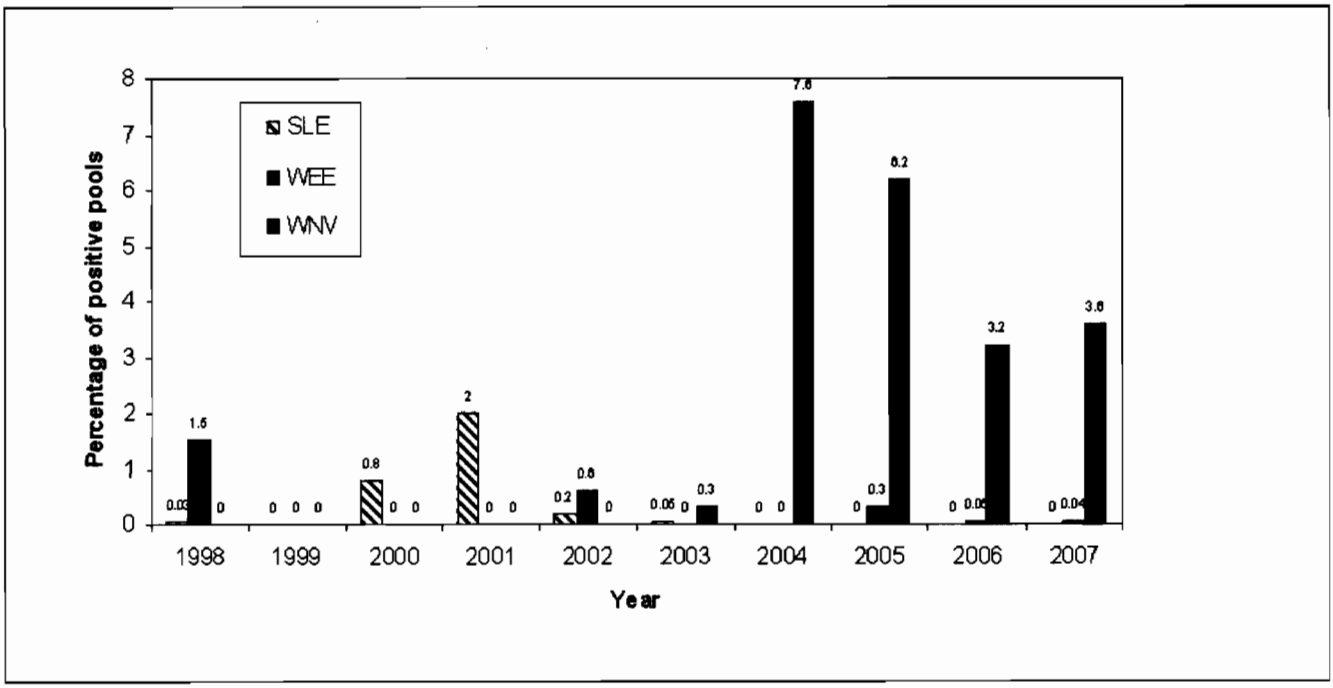


Figure 3: Percentage of mosquito pools testing positive to St. Louis encephalitis virus (SLE), western equine encephalomyelitis virus (WEE), and West Nile virus (WNV), 1998-2007

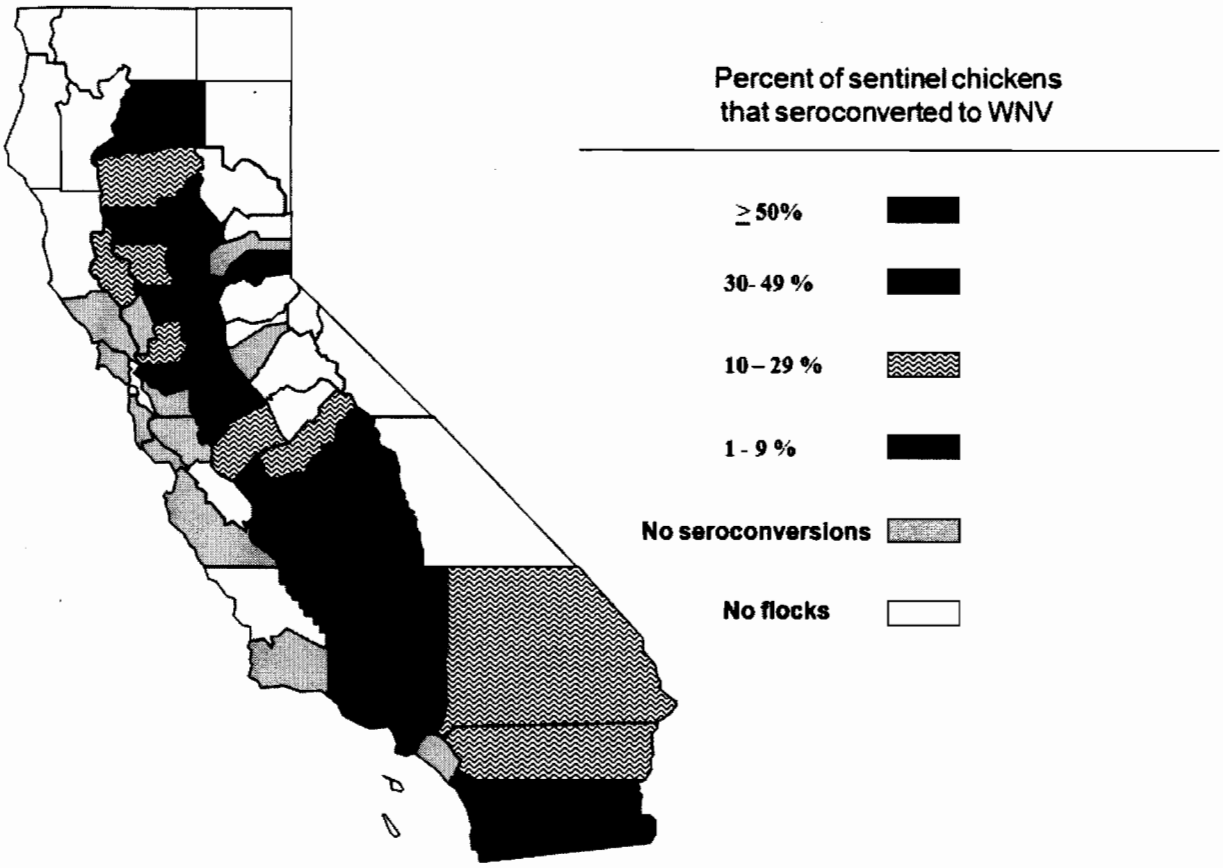


Figure 4: West Nile virus detection by sentinel chickens, California, 2007

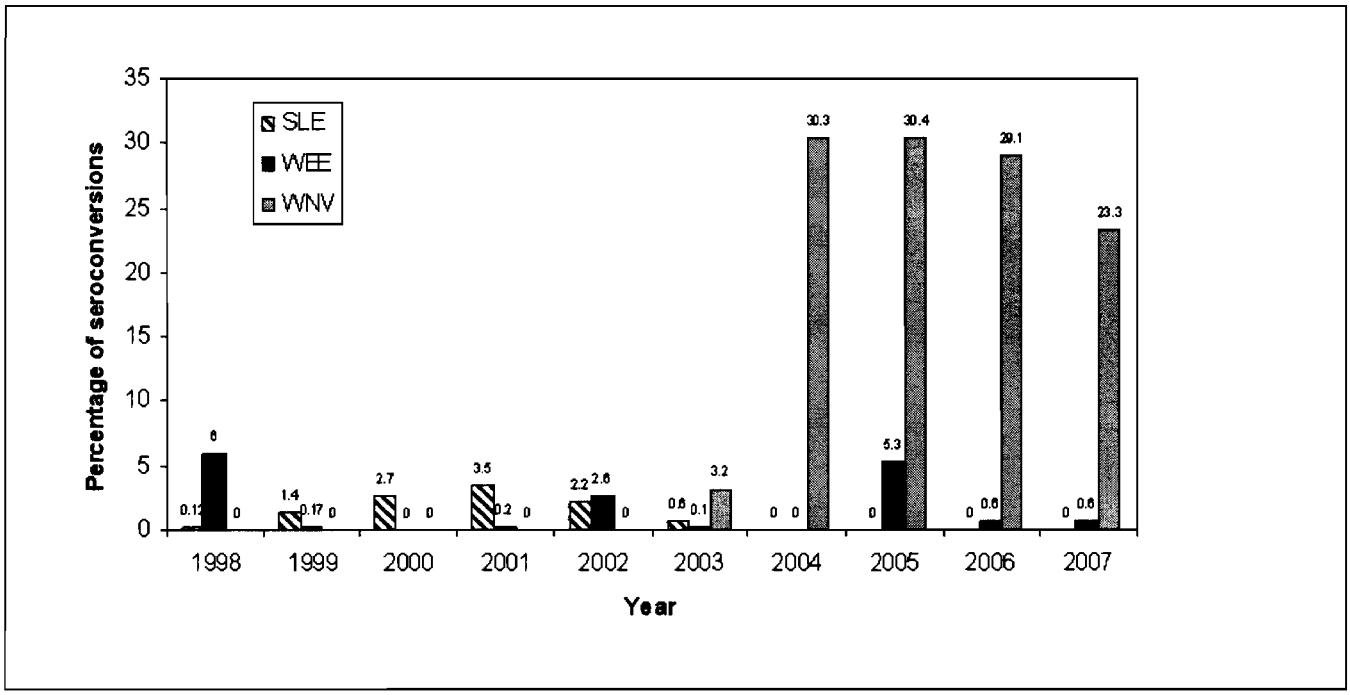


Figure 5: Percentage of sentinel chicken seroconversions to St. Louis encephalitis virus (SLE), western equine encephalomyelitis virus (WEE), and West Nile virus (WNV), 1998-2007

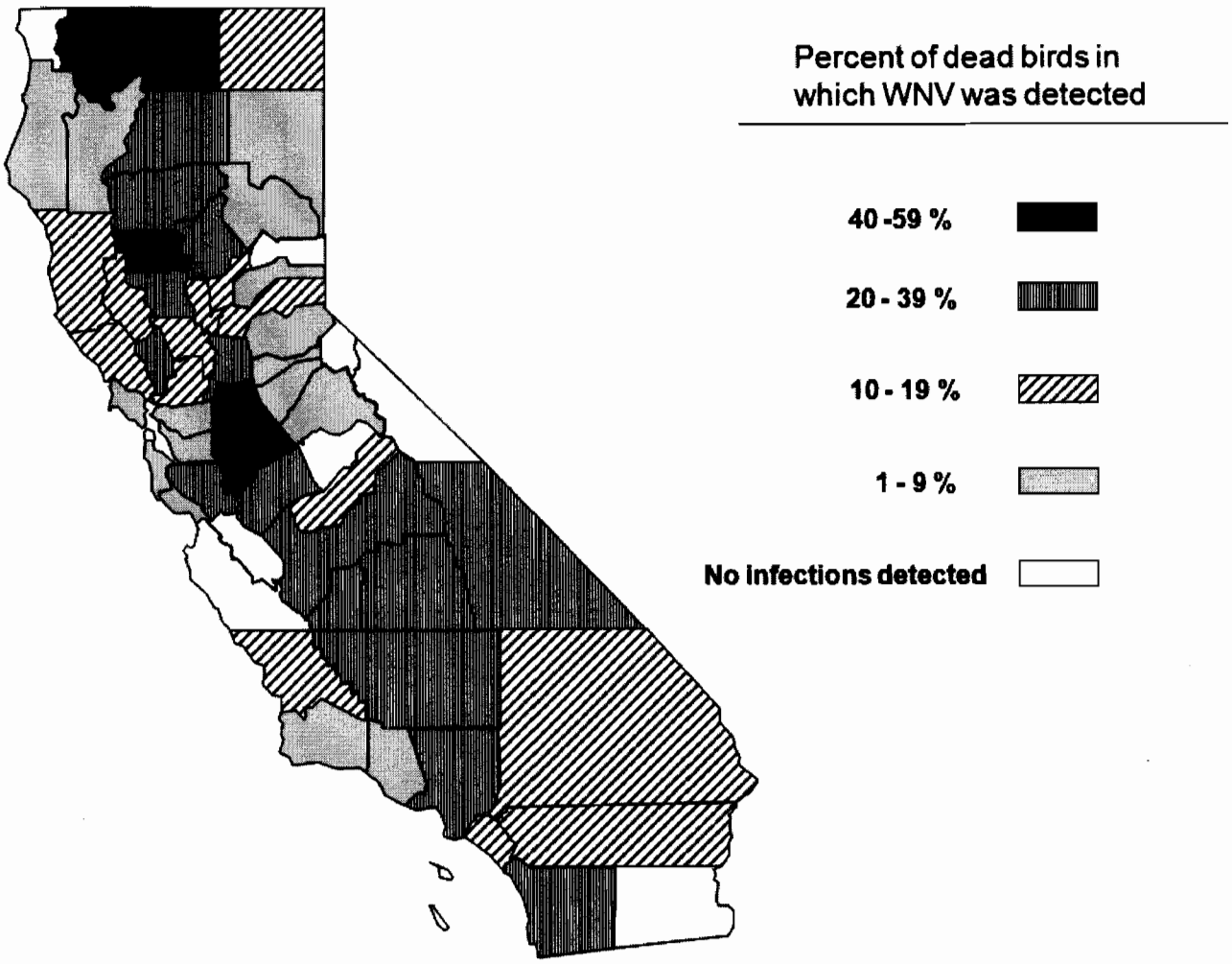


Figure 6: Prevalence of West Nile virus infection in dead birds, California, 2007

Symposium: Improving the Use of Climate Variation in Decision Support Systems – Introduction

William K. Reisen

Center for Vectorborne Diseases and Department of Pathology, Microbiology and Immunology
School of Veterinary Medicine, University of California, Davis, CA 95616, (530) 752-0124, arbo123@pacbell.net

Over the past 7 years our research sponsored by the National Oceanic and Atmospheric Administration [NOAA] and National AeroSpace Agency [NASA] has focused on forging linkages among climate variation and measures of encephalitis virus risk, especially mosquito vector abundance. The purpose of our recent research was to exploit these linkages to provide improved decision support systems for mosquito and encephalitis virus intervention programs in California and the West. Applications would be used for forecasting during the winter – spring period and for now casts during the summer transmission season.

The current symposium summarizes research among our climate collaborators at the Scripps Institution and NASA, the Mosquito and Vector Control Districts and the California Department of Public Health who collect surveillance data, and the Environmental Assessment and Information Technology program within the Center for Vectorborne Diseases at the University of California who develop new applications. Topics addressed in our symposium include:

- Overview of research on climate variation and patterns focusing on recent changes related to global warming.
- Use of climate variation in forecasting and understanding mosquito population dynamics.
- Incorporation climate variation, models and forecasting into the California Mosquitoborne Virus Surveillance and Response Plan.
- Current and planned data management and decision support tools.

Acknowledgements

Research and the symposium was funded, in part, by NASA Applied Sciences Program: REASoN Award, Decision Support through Earth Science Research Results; NOAA Office of Global Programs: Climate and Health Program; NIAID, National Institutes of Health; CDC West Nile support funds; Kern, Coachella Valley, Greater Los Angeles and Sacramento/Yolo MVCDs.

Participants in this research include:

UC Davis: Environmental Assessment and Information Technology Program

Center for Vectorborne Diseases: Bruce Eldridge, Bborie Park, Christopher Barker Arbovirus Laboratory

Center for Vectorborne Diseases: Aaron C. Brault, Ying Fang, Keira Simmons and staff

Mosquito and Vector Control Association of California, Steve West current and David Brown incoming presidents, Steve Mulligan and Min-Lee Chang former and current Chairs of the Research Committee

California Department of Public Health: Vector-borne Disease Section, Head: Vicki

Kramer; Viral and Rickettsial Diseases Laboratory, Head: Carol Glaser

UC San Diego: Scripps Institution of Oceanography, Climate Division: Daniel Cayan, Director, Michael Dettinger, Mary Tyree

NASA Ames Research Division: R. Nemani, Head, Forrest Melton, Brad Lobitz

The California West Nile Virus Hotline as a Public Education Tool

Long Her and Stan Husted

California Department of Public Health, Vector Borne-Disease Section, 1616 Capitol Ave. MS 7307,

P.O. Box 997377, Sacramento, CA 95899

The West Nile virus (Family Flaviviridae, genus *Flavivirus*, WNV) and Dead Bird hotline at the California Department of Public Health in Richmond, CA, received over 36,000 calls in 2007. Of the 51 WNV positive counties detected in 2007 in California, 42 (82%) first detected WNV by testing dead birds. Although the primary purpose of the hotline is to take dead bird reports for surveillance, the hotline operators have observed that these calls also present an excellent opportunity to educate the public. Due to the passive nature of dead bird surveillance, public participation is vital to the overall success of the program. Once callers understand WNV risk and the importance of dead bird reports, they may be more likely to call back when they find another dead bird.

The hotline is a unique public education tool because it provides a medium through which the public has direct contact with both state and local agencies. In reporting a dead bird, it is not uncommon for callers to have multiple questions about WNV or other services provided by state and local agencies. Hotline calls are answered seven days a week by staff, many of whom are science or public health college graduates and undergraduates. The hotline is staffed from 8 am until 5 pm; however, voicemail and internet reporting is also available 24 hours a day. All reports receive a call back within 24 hours of the initial report.

In addition to the information available from the hotline staff, public education can also be obtained through voicemail prompts and the California WNV website (www.westnile.ca.gov). Information on WNV topics, such as prevention, symptoms, infections in pets, repellents, and local agency contact information, is readily available via the voicemail prompt.

Although WNV has received extensive media coverage, callers reporting dead birds still have many misconceptions and questions about WNV. In general, callers want to know what they can do to protect their families from infected birds. Common questions include:

- I touched a dead bird. Do I have WNV?
- How do I properly dispose of a dead bird?
- Do I have to worry about the feathers in the yard?
- My dog had a dead bird in its mouth. Will it die?
- I saw 50 dead birds in the park. Did WNV do that?
- I found a sick bird in my yard. What do I do?
- My neighbor has a green pool. Who do I contact?
- What is MAD? What is MVCD?
- Does this bird have avian influenza?

The three primary goals for 2008 are to (1) increase local advertisement of the hotline number, (2) increase prevention through knowledge, and (3) improve and enhance public outreach. The number one complaint from callers is: "Your number is too hard to find". The hotline continually advocates and educates the public about their local agency and available resources and has the number registered with the 411 operators statewide. The more the hotline is advertised, the more it can assist callers and local agencies. Once the public is informed that WNV is in their community, they are more likely to practice personal prevention and reduce their risk of infection. The contributions and dedication of the hotline staff will continue to increase public awareness of WNV.

The Hunt for the New West Nile Virus in California

Veronica Armijos, Andrew Chow, Payal D. Maharaj, Ying Fang, William K. Reisen and Aaron C. Brault

Center for Vectorborne Disease Research and Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616

ABSTRACT: West Nile virus (WNV) is one of the many vector-borne diseases that has emerged or resurged in the Western Hemisphere in the past century. It was first isolated in California in 2003 from a *Culex tarsalis* mosquito pool collected in Imperial County, and subsequently spread throughout the remainder of the state. The invasion of WNV, periodic introduction of exotic mosquito vectors, and the presence of continually reintroduced vector-borne agents like St. Louis encephalitis virus (SLEV) and western equine encephalomyelitis virus (WEEV) are indicative of the potential that vector-borne agents could be introduced or reemerge in California. As part of the continuing surveillance efforts and in an effort to identify novel vector-borne disease agents for which current assays are not designed, *Culex, Culiseta, Aedes* and *Anopheles* species mosquitoes from 2005, 2006 and 2007 were screened for unknown viruses by plaque assay. Special attention was paid to areas that could be potential ports of entrance for new arboviruses into California such as Los Angeles, Coachella Valley and the San Francisco Bay area (Alameda, San Mateo and Contra Counties).

Mosquito pools tested for the years 2005 and 2006, included *Culiseta spp.* (n=192) and *Aedes spp.* (n=418). For 2007, *Culex spp.* mosquito pools (n=4,041), *Culiseta spp.* (n=4), *Aedes spp.* (n=1), and *Anopheles spp* (n=1) were tested. Mosquito pools were initially screened for WNV, SLEV and WEEV RNA using multiplex real time RT-PCR. Multiplex-negative pools were then screened by plaque assay to identify cytopathic effects in Vero cells. RNA was extracted from the plaque formation positive mosquito pools, using a QIAamp viral RNA extraction kit (Qiagen, Valencia, CA). RT-PCR was performed using California encephalitis virus (CEV) and Bunyawera serogroup-specific primers for the 2005 and 2006 mosquito pools and consensus Flavivirus, Bunyanwera and California serogroup primers were used for the 2007 pools. Amplicons were visualized by electrophoresis on agarose gels. Positive DNA fragments were extracted using the QUIAquick PCR purification kit (Qiagen) and sequenced using an ABI 3730 DNA sequencer (Applied Biosystems). Nucleic acid sequences were screened against the GenBank database using the BLAST program and analyzed using the software Sequencher™ version 4.8 (Gene Codes Corporation, Ann Arbor, MI).

A total of 45 *Aedes spp.* mosquito pools were positive by plaque assay for 2005 and 2006, and 35 *Ae. melanimon* mosquito pools generated amplicons. Of the 35 pools that generated amplicons, 33 were sequenced and amplicons were confirmed by GenBank BLAST searches as CEV. For 2007, a total of 15 *Culex spp.* mosquito pools were positive by plaque assay, and 14 of these pools were confirmed as WNV. Sequencing of these positives failed to identify mutations within the primer or probe binding regions. One *Culex quinquefasciatus* mosquito pool did not generate amplicons and current studies are underway to identify and characterize this isolate.

California, with a large human population, is the point of entry for travelers and commerce from around the world, making it vulnerable to the introduction of resurgent arboviruses like dengue virus (DENV), Murray Valley encephalitis virus, Ross River virus and Chikungunya viruses (CHIKV) that are spreading geographically. The invasion of WNV in California, illustrates how new vector-borne agents can enter the state despite the

extensive mosquito control and public health system. Furthermore, the rapid worldwide spread and previous introductions into California of *Ae. albopictus* mosquitoes, an efficient vector for DENV and CHIKV, highlights the potential of introduction of novel vector-borne disease agents and the necessity for expansion of viral testing paradigms for new disease agents.

Use of the California Mosquito-Borne Virus Surveillance & Response Plan: Los Angeles - a Case Study

Susanne Kluh
Greater Los Angeles County Vector Control District
12545 Florence Ave, Santa Fe Springs, CA

ABSTRACT: In the ongoing attempt to provide local vector control agencies with better tools to predict impending arboviral disease outbreaks, the California Department of Public Health (CDPH) published the California Mosquito-Borne Virus Surveillance & Response Plan, a tool to interpret surveillance as well as environmental factors, predict disease risk and provide control guidelines. This paper discusses an assessment of the effectiveness of the West Nile virus (WNV) Risk Assessment Model for the Greater Los Angeles Vector Control District (GLACVCD) service area in 2006 and 2007. It was found that the current CDPH WNV Risk Assessment Model is a simple and useful data interpretation tool in an attempt to predict disease risk using environmental and virus surveillance indicators. However, some modifications should be considered to achieve a more accurate prediction.

INTRODUCTION

In the early 1930's, three new mosquito-borne encephalitis viruses were discovered: western equine encephalomyelitis (WEEV) in California (Meyer et al. 1931, Karabatsos 1985), eastern equine encephalomyelitis (EEEV) on the East Coast (TenBroeck and Merrill 1933) and Saint Louis encephalitis (SLEV) in Missouri (Muckenfuss et al. 1934). Two of these viruses, SLEV and WEEV, would eventually become endemic in California, recurring over many decades in minor epizootics or major epidemics. Early it was recognized that in case of these mosquito borne diseases, surveillance and reporting of clinical human cases alone would not assist in prediction of probable case occurrence in the future, since human infection is not an essential component of the disease transmission cycle (Reeves 1990).

A statewide comprehensive surveillance program was first established in 1969 (Reeves 1990) and surveillance and interagency response guidelines were published by the California Department of Public Health (CDPH) (Walsh 1987) as well as the Mosquito and Vector Control Association of California (Reisen 1995).

Since the detection of West Nile virus (Family Flaviviridae, genus *Flavivirus*, WNV) in New York in 1999, California health and vector control agencies have been preparing for the arrival of this "new" mosquito-borne disease. The California Mosquito-Borne Virus Surveillance & Response Plan represents a continued effort to develop better models to effectively predict and lessen or prevent mosquito borne disease outbreaks in California. The following is an assessment of the effectiveness of the WNV Risk Assessment Model for the Greater Los Angeles Vector Control District (GLACVCD) service area in 2006 and 2007.

MATERIALS AND METHODS

The WNV Risk Assessment Table used in this evaluation was provided by the CDPH in the 2007 edition of the California Mosquito-Borne Virus Surveillance & Response Plan. In this table, eight surveillance and environmental factors are assigned values between one and five according to their potential role in WNV amplification. These values are added up and divided by eight. This average is used as risk assessment figure to establish the level of disease risk as well as the level of response warranted by vector control agencies (Response

Level/ Average Rating: Normal Season 1.0-2.5, Emergency Planning 2.6-4.0, Epidemic 4.1-5).

Surveillance and environmental factors were accumulated on a monthly basis throughout the surveillance year. Temperature data was acquired from 3 public weather stations available online at Weather Underground (http://www.wunderground.com/) to represent different parts of the GLACVCD service area (Whittier, Glendale and Van Nuys). Adult mosquito abundance was evaluated through EVS/CO_2 and Reiter gravid trapping conducted by GLACVCD scientific-technical staff and compared to the five year average in the same surveillance area. Minimum Infection Rates (MIRs) were calculated using the Microsoft Excel add-in for Pooled Infection Rate (http://www.cdc.gov/ncidod/dvbid/westnile/software.htm), as specified in the Risk Assessment Table. Mosquito samples were collected by GLACVCD scientific-technical staff and tested at the Center for Vectorborne Diseases, University of California, Davis. Sentinel chicken blood samples were collected from seven flocks in the Greater Los Angeles area and analyzed at the CDPH laboratory in Richmond, CA. Information on WNV positive dead birds was provided through the CDPH Dead Bird Hotline. Equine cases are reported to GLACVCD by the Los Angeles County Department of Public Health Veterinary Services and the numbers of human cases by the Los Angeles County Department of Public Health, Acute Communicable Disease Control. Please note that all areas within Los Angeles County were considered operationally urban.

RESULTS AND DISCUSSION

In 2006, most of the WNV activity within GLACVCD boundary was observed in the San Fernando Valley area and only a few WNV+ dead birds and mosquito pools were detected in other parts of the District (Fig.1). Surveillance data was aggregated by month and risk levels were calculated District-wide. The model predicted elevated levels of WNV risk for June, after the detection of two positive mosquito pools in May, suggesting that control operations should enter the emergency planning phase. The District's first human case for that season was reported in July and more cases followed every month through October, resulting in a final count of seven human cases of WNV within GLACVCD boundary in 2006 (Fig.2). Values in the Risk Assessment Model peaked at 3.75 in September, indicating that disease risk never reached epidemic levels, and it could well be argued that seven human cases of an endemic disease in an area as densely populated as Los Angeles County should be expected and not be considered an epidemic.

In 2007, the San Fernando Valley again experienced higher numbers of WNV+ dead birds, mosquito pools and consequently human cases than remaining District areas (Fig.3). District wide risk levels predicted by the CDPH model remained below the epidemic threshold, even though 29 human cases were recorded within GLACVCD boundary between July and September 2007, certainly more cases than had been expected after previous years' experience. In hopes to see the model better reflect local risk levels, it was considered to separate different portions of the District and calculate risk levels locally. WNV activity was heavily focused in the San Fernando Valley which could potentially be caused by differences in environmental factors, such as temperature. Surprisingly, an evaluation of temperature did not show a big difference between the San Fernando Valley and the warmer areas of the Los Angeles basin (Fig.4).

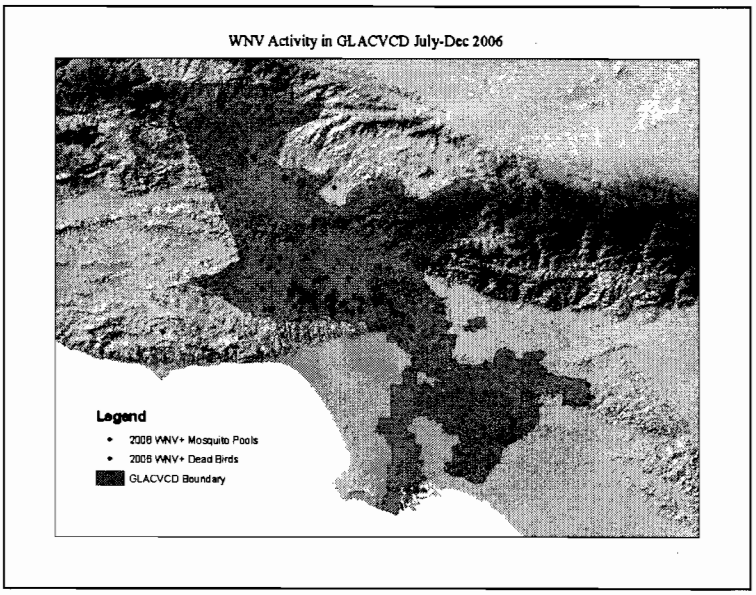


Figure 1. Map of WNV+ dead birds and mosquito pools within GLACVCD boundary, 2006.

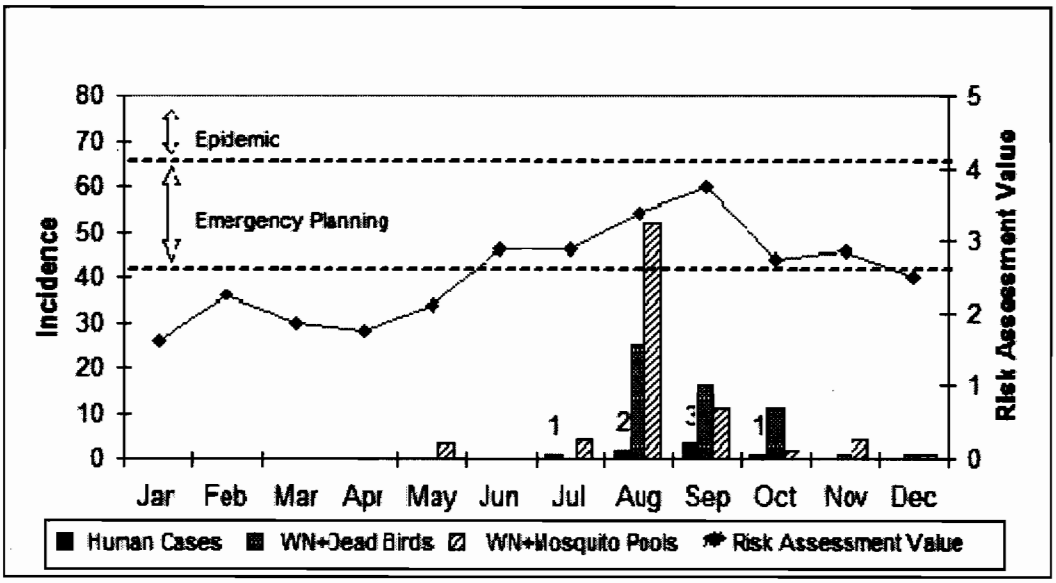


Figure 2. GLACVCD WNV incidence in humans, dead birds and mosquito pools in relationship to risk assessment value, 2006.

Glendale temperatures were used in the District-wide risk evaluation. Due to its central location and distance from the ocean (Fig.5), temperatures were believed to best reflect overall District conditions. It was somewhat unexpected to see that Glendale temperatures were cooler than temperatures in both the San Fernando Valley or the warmer areas of the Los Angeles basin. However, overall differences between all three sites never exceeded a ten degree range and levels of estimated risk ascend in steps of eight degrees, so temperature differences did not account for the model's failure to predict the epidemic. Calculating the entire risk level separately for the San Fernando Valley and the Los Angeles basin did not result in higher risk levels for the San Fernando Valley, but demonstrated that a District wide assessment predicted the highest levels of risk overall. This result was expected since both subsections of GLACVCD are ecologically similar, and thus a larger evaluation area would improve predictions due to the increased potential for data points (Fig.6).

One of the surveillance factors in the model is the number of equine cases reported. However, due to the effectiveness and widespread use of vaccinations for horses, this surveillance indicator is no longer available. No WNV+ horses have been recorded in 2006 or 2007 in the Los Angeles County area. The overall assessment of risk as it is calculated in this model is an average of the risk ratings of all surveillance factors, thus including horse cases unjustifiably lowers this average. By removing horse cases from the list of surveillance factors in 2006 and 2007 risk assessment calculation for the entire GLACVCD area, the model predicts epidemic conditions in September 2006 and in August and September 2007 (Fig.7).

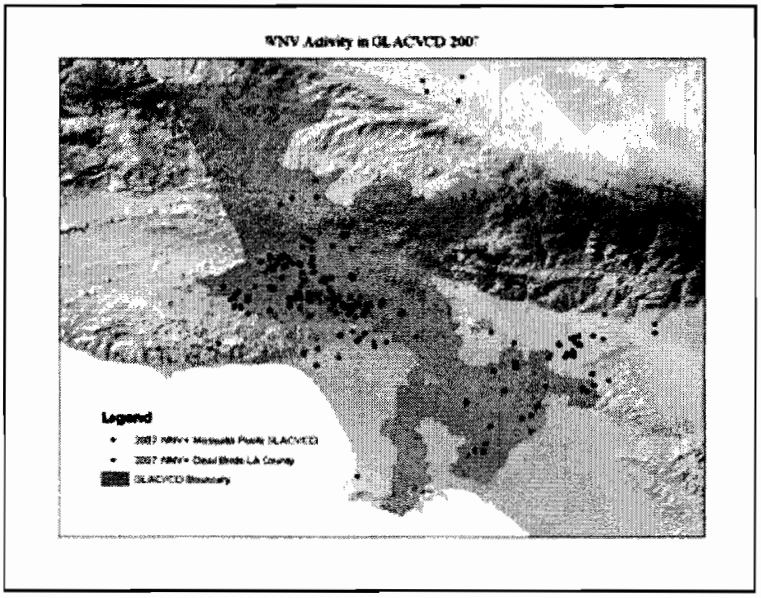


Figure 3. Map of WNV+ dead birds and mosquito pools within GLACVCD boundary, 2007.

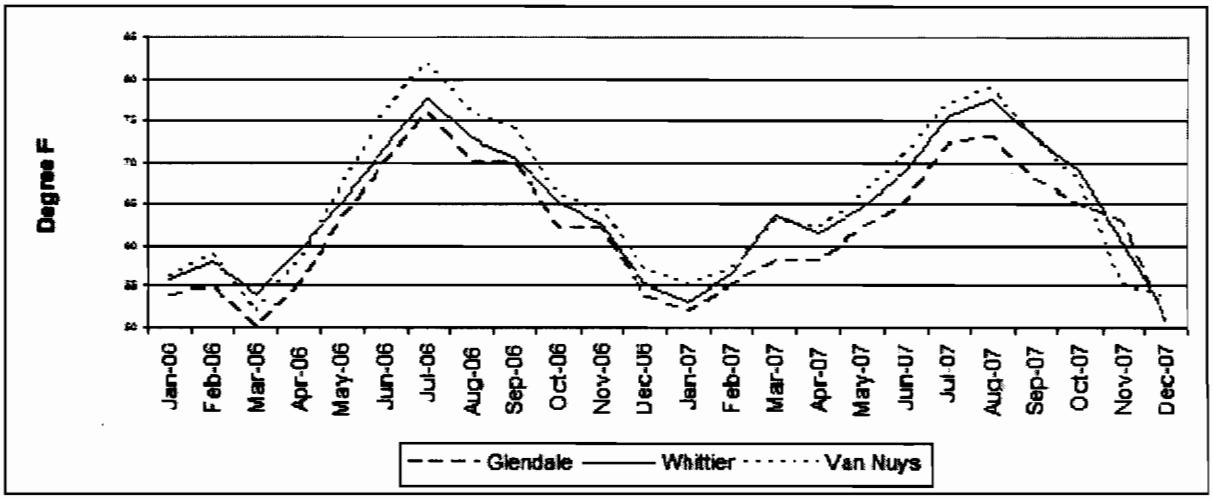


Figure 4. Average monthly temperature in Glendale, Whittier and Van Nuys, 2006 and 2007.

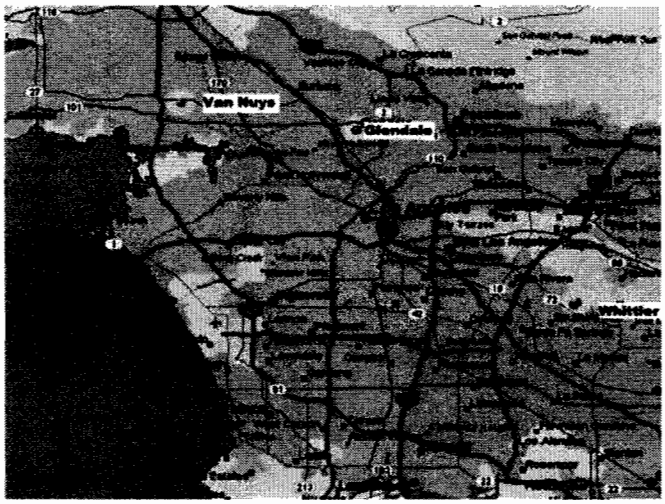


Figure 5. Los Angeles area map with locations of weather stations in Glendale, Whittier and Van Nuys.

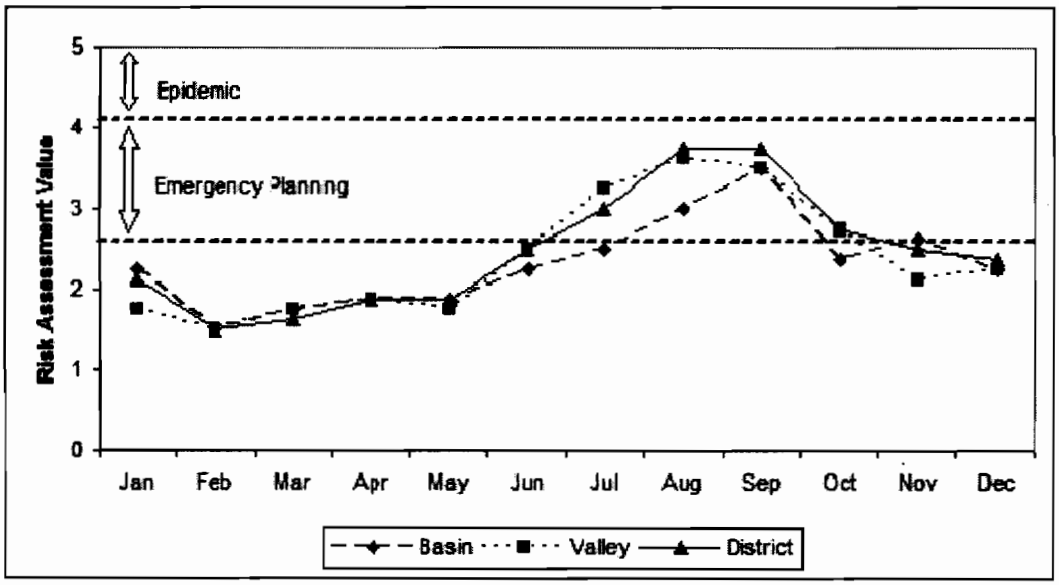


Figure 6. Risk assessment calculations for Los Angeles basin, San Fernando Valley and GLACVCD

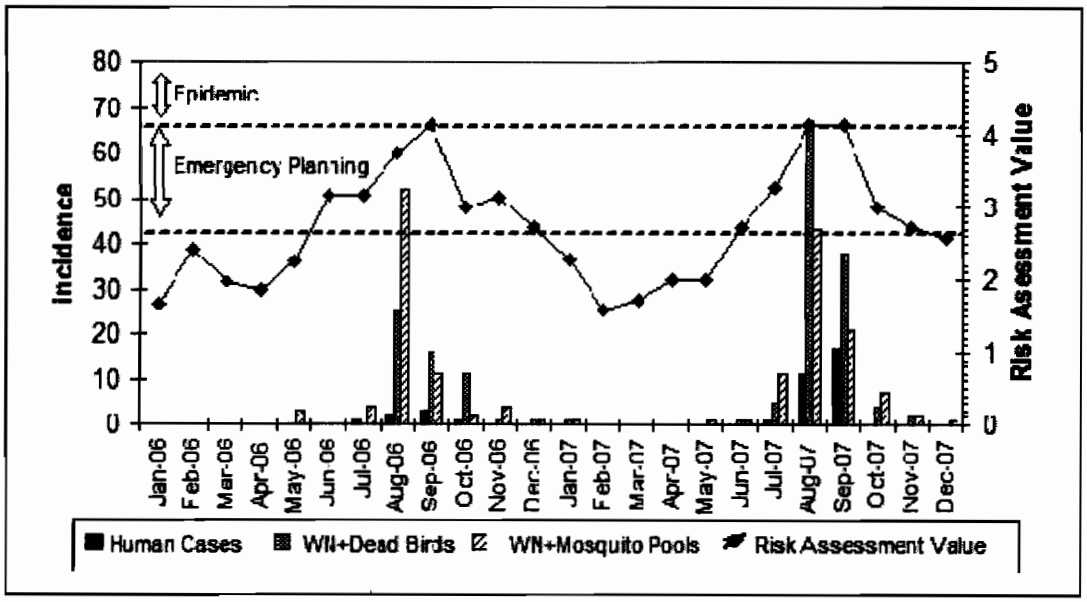


Figure 7. GLACVCD WNV incidence in humans, dead birds and mosquito pools in relationship to risk assessment value, 2006 and 2007.

In 2006 and 2007, the model predicts risk levels in the "Emergency Planning" range for one month prior to the occurrence of the first human case, allowing some time for increased control measures. There is however, a discrepancy in rating the importance of WNV+ mosquito pools in spring versus fall. Minimum Infection Rate (MIR) is used to gage the risk due to infected mosquito presence assuming higher risk levels for higher infection rates. Since the MIR is calculated as minimum infection rate in mosquito pools of 50 and per 1000 females, 2 positive pools in May when mosquito numbers are high, will result in a lower MIR than 2 positive pools in November when mosquito abundance is low. This directly causes the model to assess lower risk levels as a result of positive mosquito pools in May than in November (Fig.7), when indeed the threat due to potential virus amplification is much greater in early summer than in late fall. Mosquito infection rate is no doubt a very important indicator of virus transmission and the detection of virus positive pools should influence predicted risk levels independent of mosquito abundance.

CONCLUSION

The current CDPH WNV Risk Assessment Model is a simple and useful data interpretation tool in an attempt to predict disease risk using environmental and virus surveillance indicators. However, some modifications should be considered to achieve a more accurate prediction. In regions where the rate of vaccinated horses is high, for example, reports of horse cases should no longer be included as a surveillance indicator. Once horse cases were removed from the calculation, the model predicted risk levels to warrant emergency planning in June, one month before the occurrence of the first human cases in both 2006 and 2007. But positive mosquito samples in May of both years actually triggered emergency planning for GLACVCD operations. It would be useful if the model could mirror this perception of risk. It seems this lag time is due to the use of MIR to assess the importance of positive mosquito samples, since high abundance numbers in spring "dilute" infection rates. Any revision of this Risk Assessment Model should attempt to also evaluate risk in relationship to the time of the year those infected mosquitoes were detected.

Acknowledgements

My special thanks to the GLACVCD scientific-technical department staff, Jacquie and Paul O'Connor, Harold Morales, Tanya Posey and Minoo Madon, for helping to collect all the data used in the risk assessment, to Jennifer Wilson, UC Davis, for all her help in data collection and processing and to William Reisen, UC Davis, and the NIH for funding. Thank also to Jennifer and Minoo for reviewing the manuscript.

REFERENCES CITED

Karabatsos, N. 1985. *International Catalogue of Arboviruses*, 3rd ed. Am. Soc. Trop. Med. Hyg. San Antonio, TX.

Meyer, F. K., C. M. Haring and B. Howitt. 1931. The etiology of epizootic encephalomyelitis in horses in the San Joaquin Valley, 1930. Science 74:227-228.

Muckenfuss, R. S., C. Armstrong and L.T. Webster. 1934. Etiology of the 1933 epidemic of encephalitis. J. Am. Med. Assoc. 103:731-733.

Reeves, W. C. 1990. Epidemiology and control of mosquito-borne arboviruses in California, 1943-1987. Calif. Mosq. Vector Control Assoc., Sacramento, CA.

Reisen, W. K. 1995. Guidelines for surveillance and control of arbovirus encephalitis in California. pp. 1-34 in: Interagency guidelines for the surveillance and control of selected vector-borne pathogens in California. Calif. Mosq. Vector Control Assoc., Inc., Sacramento, CA.

TenBroeck, C. and M. Merrill. 1933. A serological difference between eastern and western equine encephalomyelitis virus. Proc. Soc. Exp. Biol. Med. 31:217-220.

Walsh, J. D., Ed. 1987. *California's Mosquitoborne Encephalitis Virus Surveillance and Control Program.* Calif. Dept. of Health Serv., Vector Surv. and Control Branch, Sacramento, CA.

Using the Surveillance Gateway to Facilitate Recordkeeping

Charles W. Smith, Jodi J. Holeman

Consolidated Mosquito Abatement District, 2425 Floral Avenue, Selma, CA 93662
Mailing Address: P.O. Box 278, Selma, CA 93662
Phone: (559) 896-1085
Email: cwsmad@pacbell.net

ABSTRACT: The Consolidated Mosquito Abatement District (District) conducts surveillance and collects information on sources of mosquito breeding, mosquito abundance, and mosquito-borne disease. Mosquito abundance is determined by surveillance of sources and identification of both larval and adult collections. Disease surveillance is conducted by collection and testing of dead birds, analysis of sentinel chicken blood samples, and testing of mosquito pools. Beginning in 2006, the District has used the California Vector-Borne Disease Surveillance Gateway (Gateway) to facilitate reporting and organizing disease and abundance data. Trap collections of adult mosquitoes, submission of mosquito pools and sentinel chicken blood samples, calculation of minimum infection rates in mosquitoes, extraction of data on recorded dead birds, and geo-coding of surveillance sites are all processed using the Gateway. The Gateway facilitates the registration and management of District surveillance sites. These sites can be integrated into the District's aerial mapping program, providing spatial relationships between sites, source locations, and arboviral infections. Worksheets, reports, and spreadsheets containing surveillance data can be easily generated from the Gateway. Use of the Gateway by adjacent mosquito abatement districts in Fresno County facilitates comprehensive data analysis and sharing of information with other local agencies.

Acknowledgements

We wish to acknowledge Bborie Park and Bruce Eldridge of the University of California, Davis for development and assistance in the use of the Surveillance Gateway, Kriss Lynn-Patterson and James Languille of the University of California Kearney Agricultural Center for development and assistance of the District's aerial mapping program, and Niki Frye and Victor Maggi for assistance in the collection and processing of all surveillance data.

West Nile Virus Activity in Kern County and the Factors Leading to the 2007 Outbreak

Brian Carroll[1], Richard Takahashi[2] and William Reisen[3]

[1]*Arbovirus Field Station, Center for Vectorborne Diseases, School of Veterinary Medicine, 4705 Allen Rd, Bakersfield, CA 93314*
[2]*Kern Mosquito and Vector Control District, 4705 Allen Rd, Bakersfield, CA 93314*
[3]*Center for Vectorborne Diseases, School of Veterinary Medicine, University of California, Davis, CA 95616*

ABSTRACT: West Nile virus (Family Flaviviridae, genus *Flavivirus*, WNV) reappeared in Kern County in late-May 2007, amplified rapidly and was detected concurrently by all surveillance methods. Enzootic activity during 2007 had some similarities to that of the previous three years, with 77 seropositive sentinel chickens in 9 flocks, 207 positive mosquito pools, 124 dead birds that tested positive, and 168 seropositive wild birds. WNV disease in equines remained infrequent, with only 4 cases reported. In contrast, Kern County had a significant increase in human disease, with 138 laboratory confirmed fever and neuroinvasive cases, combined incidence = 17.8 per 100,000 population. The standard surveillance indicators, sentinel chickens and mosquito pools, indicated that WNV enzootic activity was on the decline, yet there were epidemic numbers of human cases. During this fourth year of virus activity, WNV was found throughout Kern County on the floor of the Central Valley.

INTRODUCTION

West Nile virus (WNV) activity in California began in 2003 and was limited to six counties south of the Tehachapi Mountains (Hom et al. 2004). By June 2004 WNV activity had spread north of the Tehachapi Mountain range and into the Bakersfield area of Kern County in the southern San Joaquin Valley (Takahashi et al. 2005). WNV quickly spread from there and by the end of the year was detected in every county of the state (Hom et al. 2005). During 2005 WNV activity was focused primarily within the city of Bakersfield (Carroll et al. 2006), while activity in 2006 was widespread throughout Kern County on the valley floor (Carroll et al. 2007). The current paper discusses the reappearance of WNV in Kern County in 2007 and describes detection by various surveillance methods, its spread through the county, differences between 2007 and the previous three years, and factors that possibly led to the human epidemic.

MATERIALS AND METHODS

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

Dead Birds: Dead birds were reported by the public to the CDHS-VBDS hotline who forwarded pertinent information to the KMVCD

for bird pickup. Birds were submitted to the California Animal Health and Food Safety (CAHFS) Central Laboratory at UCD for necropsy. Oral swabs and/or kidney tissue were sent to the UCD Center for Vector-borne Diseases (CVEC) laboratory for testing by reverse transcriptase-polymerase chain reaction (RT-PCR).

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

Chickens: Sera were collected biweekly from 10 hens within each of 9 flocks within the KMVCD. Individual blood samples were collected on strips of filter paper and then sent to California Department of Public Health (CDPH) Viral and Rickettsial Disease Laboratory (VRDL) for testing for IgG antibody by an indirect enzyme immunoassay (EIA) (Reisen et al. 1994). Positives were confirmed by indirect fluorescent antibody (IFA) and end-point plaque reduction neutralization tests (PRNT).

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

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

RESULTS

WNV was initially detected in a dead American Crow (*Corvus Brachyrhynchos*) collected on May 25th. Within the next two weeks virus activity also was detected by positive mosquito pools, seroconverted sentinel chickens and additional dead birds. This activity was localized around the city of Bakersfield. By the end of June virus activity had begun to move out of the city and by the end of the season was active in all of the surrounding communities.

From March through early November 2007, 6,111 *Aedes melanimon* Dyar, 21,240 *Culex quinquefasciatus* Say, and 15,298 *Culex tarsalis* Coquillett mosquitoes from Kern MVCD were tested for virus infection in 1,264 pools, of which 207 were positive for WNV. Only one *Ae. melanimon* pool tested positive and we felt that this species did not play a significant role in virus maintenance or amplification. *Cx. quinquefasciatus* and *Cx. tarsalis* were the major vectors for WNV transmission activity in 2007 (Table 1). Infection rates per 1,000 (MIRs/1000) for *Cx. quinquefasciatus* and *Cx. tarsalis* exceeded the epidemic threshold of 5.0 during June in Bakersfield, and stayed above this threshold until August. Epidemic levels were not attained until July in the southeast and August in the northwest parts of Kern County. Epidemic levels dropped below the epidemic threshold in September in Bakersfield and the Southeast and October in the northwest.

In 2007, 124 out of 332 dead birds tested positive for WNV (Table 2). The most frequently reported bird was for the first time not the American Crow *(C. brachyrynchos),* but the House sparrow (*Passer domesticus*). Ninety-three of the 124 positives were represented by four species of birds, House Sparrows (*P. domesticus*) (28), American Crows (*C. brachyrynchos*) (26), Western Scrub-Jays (*Aphelocoma coerulescens*) (23), and House Finches (*Carpodacus mexicanus*) (16). 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.

A total of 77 chickens from 9 flocks seroconverted to WNV during the 2007 surveillance season (Table 2). The first chicken infections occurred before 11 June, with 2 chickens from one flock within Bakersfield confirmed. By the end of the season WNV had spread throughout all 9 flocks generating 75 additional seroconversions. July and August had the most seroconversions with 28 and 21, while September and October had significantly fewer with 15 and 11, respectively. This reduction in seroconversions was attributed to the lack of availability of replacement chickens.

The free-ranging bird seroprevalence program detected 164 EIA positives during 2007 that were represented by 5 species of birds (Table 3). There were 4 additional positives among the other 33 species tested. Positivity rates of the five main species ranged from 4% to 57%. As expected the five species that were infected most frequently were year round residents.

Only four confirmed positive WNV equine cases were detected, with two fatalities. All four of these cases were in the metropolitan Bakersfield area. This decrease in positive cases most likely was due to increases in planned as well as natural immunization of the equine population in Kern County.

Overall, 139 laboratory confirmed human cases were reported, with four fatalities. One hundred thirteen 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).

DISCUSSION

Surveillance indicators detected virus activity at approximately the same time throughout the Bakersfield area in late May and early June. All indicators not only increased throughout the summer, but spread to the outlying areas of the floor of the central valley of Kern County. Indicators continued to detect virus activity throughout the summer, finally subsiding in late September. There were a few chickens and one mosquito pool that tested positive in early October, but these most likely were infected in late September.

There were some distinct differences in WNV activity in 2007 compared to 2004 - 2006. In 2004, WNV activity started in the southeastern corner of the valley, moved into Bakersfield and then to the west side of the valley. In 2005, WNV activity appeared first within the city of Bakersfield and then spread outward, finally affecting every surveillance site across the valley floor. In 2006, there was one early positive and then a six week period of negative activity, before activity began increasing. During 2007, virus transmission was intense and amplification rapid. There were no gaps as in 2006 and activity spread very quickly, unlike 2004 and 2005. Within a 4 week period in 2007, WNV was detected across the entire valley portion of Kern County. While the virus was active in all areas of Kern County, transmission was most intense within the greater Bakersfield area.

Overall, mosquito infection and sentinel chicken seroconversion rates were similar to previous virus years (Table 4); however, carefully examining the timing and distribution of these data during 2007 indicated important differences. Warm spring temperatures led to elevated mosquito infection rates within Bakersfield during June that was followed closely by human cases that rose to epidemic levels by July. The number of free ranging bird positives may have declined, but a closer look reveals that 13.5 % of the overall birds tested in 2007 had seroconverted, compared to 20% in 2006. This can be partly attributed to the 70% decrease in birds tested. We feel that this decrease in birds tested is related to not only the increased virus activity and the natural increased mortality that comes with it, but to the natural fluctuations in the bird populations. Significant decreases in bird populations may have increased the risk of tangential transmission to humans. In agreement, the number of human cases in Kern County increased 175% compared

to 2006, with 138 cases with 4 fatalities, placing the county in at an epidemic status with an overall incidence of 17.8 per 100,000 population. Virus activity slowed in late September and finally subsided in October, when *Cx. tarsalis* entered diapause (Bellamy and Reeves 1963, Nelson 1964).

We feel that there were two significant factors that helped drive increases in *Cx. tarsalis* and *Cx. quinquefasciatus* population abundance and virus activity. The first of these was the natural fluctuation in the environmental conditions of precipitation, temperature, and river flow. In 2007 Kern County had 2.5 inches of rain, about 40% of the average, and was in drought conditions. There were two periods of moderately heavy precipitation, one in late February and early March, and again in late April and early May, which left large amounts of surface water as breeding sources for *Cx. tarsalis* just prior to the normal transmission season. Kern County also had a considerable number of days with temperatures that were well above average from February through July. This early period of above average temperatures may have expedited the development of the F1 generation of the *Cx. tarsalis* as well as the overwintering *Cx. quinquefasciatus* larvae. This expedited development combined with the abundance of untreated breeding sources led to a higher mosquito abundance at the beginning of transmission season. The main source of water in Bakersfield other than rainfall is the Kern River, whose source is in the Sierra Nevadas. With the lack of a plentiful snow pack the previous winter, the river through Bakersfield and to the SW portion of the valley remained completely dry throughout the entire year. As the earlier mentioned untreated breeding sources dried up, it combined with the lack of river water to minimize the amount of sources for the production of *Cx. tarsalis*.

A second significant factor was the drastic rise in foreclosed homes in the Bakersfield area. From April to September of 2007 there were 2,080 homes foreclosed in the Bakersfield area, compared to 91 for the same time period in 2006. This increase of over 2,000% left many abandoned swimming pools turning "green" during the transmission season. We feel that the lack of natural breeding sources and the abundance of "green" pools created ideal conditions for the expansion of *Cx. quinquefasciatus* within the urban area. These increases prompted Kern MVCD to perform aerial surveys to identify "green" pools in August. They also accepted the publics help in reporting "green" pools. The KMVCD treated 809 pools in 2007 compared to 398 in 2006, indicating that with the aerial survey and the public complaints were able to identify many more "green" pools in 2007 that needed treatment.

In summary, enzootic activity was detected by all surveillance methods in all areas within Kern County during 2007. There were some unique conditions, both natural and man made that led to an increase of mosquito abundance and virus transmission. It will be interesting to see where WNV will reemerge during 2008 and whether or not the predictions of another long hot summer and the continued rise in foreclosure rates continue to have a significant impact on the virus cycle and create another epidemic year.

Acknowledgements

We thank Vincent Martinez and Scott Hallam, Arbovirus Field Station, for help with collecting and processing the mosquitoes, and Ying Fang, Sandra Garcia, Maureen Dannen, Keira Simmons and Andrew Chow, CVEC, for help with selected laboratory assays. This research was funded, in part, by Research Grant AI55607 from the National Institute of Allergy and Infectious Diseases, NIH, the Climate Variability and Human Health program, Office of Global Programs, NOAA, and funds from the Center for Disease Control Prevention.

REFERENCES CITED

Bellamy, R. E. and W. C. Reeves. 1963. The winter biology of Culex tarsalis

(Diptera:Culicidae) in Kern County, California. Ann. Entomol. Soc. Am. 56:314-323.

Carroll, B., R. Takahashi, C. Barker, and W. K. Reisen. 2006. The Reappearance of West Nile Virus in Kern County During 2005. Proc. & Papers Mosq. Vector Control Assoc. Calif. 74: 12-15.

Carroll, B., R. Takahashi and W. K. Reisen. 2007. West Nile Virus Activity in Kern County during 2006. Proc. & Papers Mosq. Vector Control Assoc. Calif. 75:15-20.

Chiles, R. E., E. N. Green, Y. Fang, W. K. Reisen, J. D. Edman and A. C. Brault. 2004. Surveillance for arboviruses in California mosquito pools: Current and future protocols. Proc. & Papers Mosq. Vector Control Assoc. Calif. 72:15-17.

Chiles, R. E. and W. K. Reisen. 1998. A new enzyme immunoassay to detect antibodies to arboviruses in the blood of wild birds. J. Vector Ecol. 23:123-135.

Cummings, R. F. 1992. Design and use of a modified Reiter gravid mosquito trap for mosquito-borne encephalitis surveillance in Los Angeles County, California. Proc.& Papers Mosq. Vector Control Assoc. Calif. 60: 170-176.

Hom, A., A. Houchin, K. McCaughey, V. L. Kramer, R. E. Chiles, W. K. Reisen, E. Tu, C. Glaser, C. Cossen, E. Baylis, B. F. Eldridge, B. Sun, K. Padgett, L. Woods, L. Marcus, L. T. Hui, M. Castro and S. Husted. 2004. Surveillance for mosquito-borne encephalitis activity and human disease, including West Nile virus in California, 2003. Proc.& Papers Mosq. Vector Control Assoc. Calif. 72: 48-54.

Hom, A., L. Marcus, V. L. Kramer, B. Cahoon, C. Glaser, C. Cossen, E. Baylis, C. Jean, E. Tu, B. F. Eldridge, R. Carney, K. Padgett, B. Sun, W. K. Reisen, L. Woods and S. Husted. 2005. Surveillance for mosquito-borne encephalitis virus activity and human disease, including West Nile virus, in California, 2004. Proc.& Papers Mosq. Vector Control Assoc. Calif. 73:66-77.

Nelson, R. L. 1964. Parity in winter populations of *Culex tarsalis* Coquillett in Kern County, California. Am. J. Hyg. 80: 242-253.

Reisen, W. K., S. B. Presser, J. Lin, B. Enge, J. L. Hardy and R. W. Emmons. 1994. Viremia and serological responses in adult chickens infected with western equine encephalomyelitis and St. Louis encephalitis viruses. J. Am. Mosq. Control Assoc. 10: 549-555.

Sudia, W. D. and R. W. Chamberlain. 1962. Battery-operated light trap, an improved model. Mosq. News 22: 126-129.

Takahashi, R. M., W. K. Reisen and C. M. Barker. 2005. Invasion of Kern County by West Nile virus. Proc.& Papers Mosq. Vector Control Assoc. Calif. 73: 20-23.

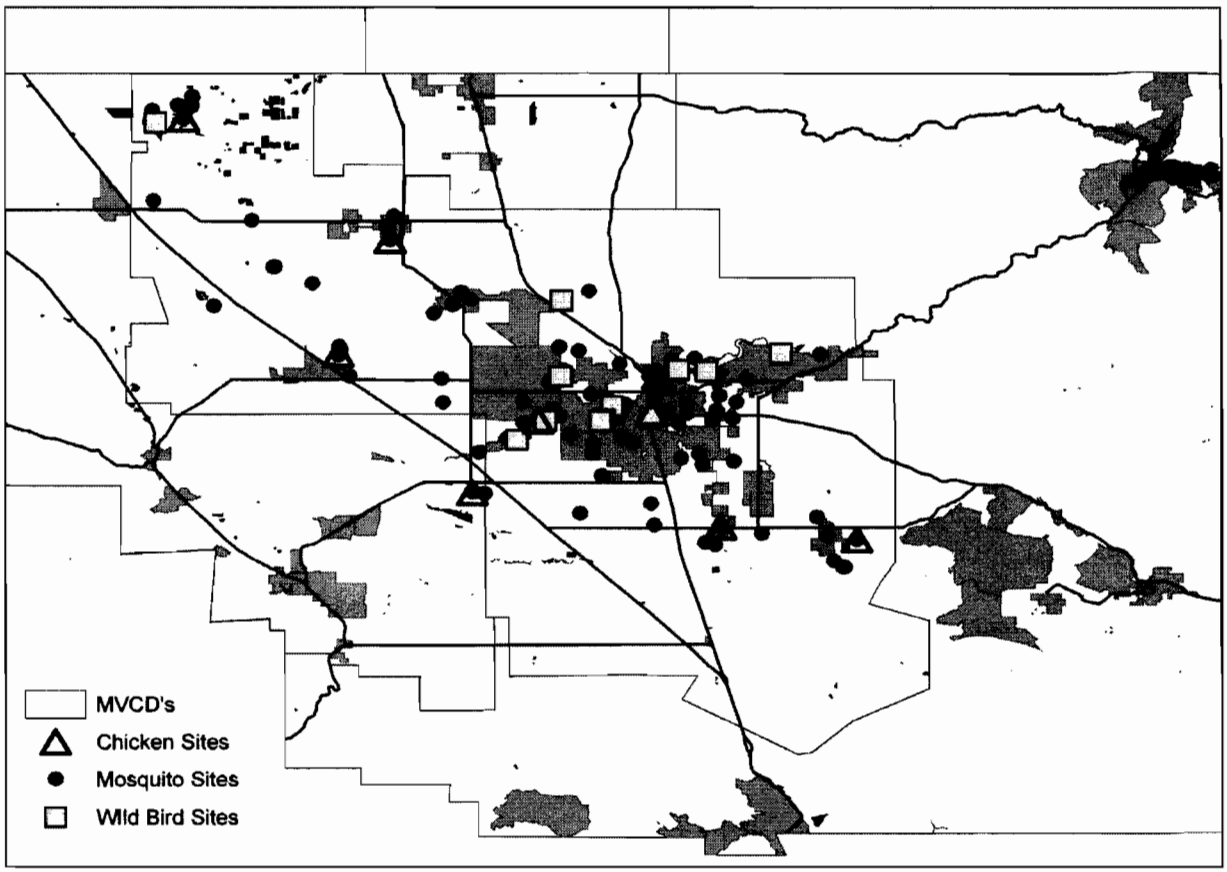


Figure 1. Surveillance sites in Kern County 2007.

Table 1. Mosquito infection rates (MIR) in Kern County, 2007.

Species	Pools	Total Tested	WNV Positive	MIR/1000
Aedes melanimon	137	6,111	1	.2
Culex quinquefasciatus	693	21,240	140	6.6
Culex tarsalis	409	15,298	65	4.2
Total	1,264	43,649	206	4.7

Table 2. Summary of positive surveillance results by cities within Kern County, 2007.

City	Mosquitoes (Pos pools)	Chickens (Seroconversions)	Dead Birds pos. for WNV	Equine WNV cases	Human WNV cases	Free Ranging Birds (number seropositive)
Arvin	30	20	0	0	6	0
Bakersfield	158	33	115	4	113	167
Buttonwillow	5	6	1	0	2	0
Delano	0	0	2	0	0	0
Fellows	0	0	1	0	0	0
Frazier Park	0	0	0	0	1	0
Lamont	5	0	0	0	8	0
Lake Isabella	0	0	1	0	0	0
Lost Hills	4	9	0	0	0	1
McFarland	0	0	0	0	1	0
Shafter	3	0	0	0	3	0
Taft	0	0	1	0	0	0
Tehachapi	0	0	1	0	0	0
Wasco	2	9	0	0	4	0
Weldon	0	0	1	0	0	0
Wofford Heights	0	0	1	0	0	0
Totals	206	77	124	4	138	168

Table 3. Species of free ranging birds testing positive for Flavivirus, Kern County, 2007.

Species	# Tested	# Positive	Percent Positive
California Quail (Resident)	57	23	43.5
House Finch (Resident)	182	40	20.9
House Sparrow (Resident)	311	20	4.1
Mourning Dove (Semi-Resident)	204	69	39.3
Western Scrub Jay (Resident)	27	12	57.3
Others (33 Species)	385	4	2.0
Totals	864	168	21.7

Table 4. Number of Surveillance methods positive for WNV in Kern County, 2004-2008.

Surveillance Methods		2004	2005	2006	2007
Positive Human Cases		60	68	50	138
Positive Equine Cases		47	26	4	4
Positive Sentinel Chicken Seroconversions		101	121	89	77
Dead Birds	Tested	159	240	118	332
	Positive	87	44	24	124
Mosquito Pools	Tested	1367	1596	1868	1264
	Positive	214	235	217	207
Free Ranging Birds	Tested	3400	3476	4036	1242
	EIA positive	157	412	811	168

West Nile Virus State of Emergency: 2007

Vicki Kramer, Tim Howard, Mark Novak, Renjie Hu, and Stan Husted

Vector-Borne Disease Section, California Department of Public Health, 1616 Capitol Ave., MS 7307, P.O. Box 997377, Sacramento, CA 95899-7377

On August 2, 2007, Governor Arnold Schwarzenegger declared a State of Emergency due to increasing risk of West Nile virus (Family Flaviviridae, genus *Flavivirus*, WNV) transmission in California. Through the end of July, 56 human cases of WNV had been reported to the California Department of Public Health (CDPH), a number significantly greater than the 16 cases reported at the same time in 2006, and comparable to 2004 (54 cases) and 2005 (56 cases) when ultimately 779 and 880 cases were reported, respectively, and a total of 48 people died. It appeared that California was on a trajectory similar to 2004 and 2005 when morbidity and mortality from WNV were extensive in many regions of the state. The intent of the Emergency Proclamation was to provide additional resources to local agencies to assist with WNV prevention, surveillance, and control, and ultimately minimize the risk of California residents and visitors from becoming infected with WNV.

The August 2nd Proclamation was followed by three Executive Orders. The sequence of events included:

- August 2, 2007: State of Emergency Proclamation
  Instructed CDPH to carry-out eleven specific orders, as detailed below, to reduce WNV transmission. Orders 1 and 2 allocated up to $1.35 million for mosquito control and WNV surveillance.
- August 13, 2007: Governor's Executive Order (S-10-07)
  Allocated up to $10 million in additional funding for mosquito control and WNV surveillance.
- August 20, 2007: Governor's Executive Order (S-11-07)
  Allocated up to $500,000 to the California Department of Fish and Game for vegetation and water management on state-owned wetland wildlife areas.
- September 10, 2007: Governor's Executive Order (S-12-07)
  Allowed funds previously allocated through the Emergency Proclamation and Executive Order (S-10-07) to be awarded to local agencies to enhance education of the general public on WNV prevention, expand outreach to the medical community, and intensify human case surveillance.

Partial text from the Emergency Proclamation and Executive Orders is provided below, along with a description of response activities carried out under the leadership of the Vector-Borne Disease Section, CDPH. Full text of the Proclamation and Executive Orders can be found at http://westnile.ca.gov (under Resources).

Emergency Order 1

IT IS ORDERED that the Department of Public Health shall allocate up to $1 million dollars as needed, to local vector control agencies to identify potential mosquito habitat and to treat those areas to prevent the spread of West Nile Virus in the three above-listed counties (e.g. Kern, Colusa, and San Joaquin) and other counties identified by the Department of Public Health.

Emergency Order 2

IT IS FURTHER ORDERED that the Department of Public Health shall allocate up to $350,000 to local vector control agencies for surveillance purposes to provide an early warning of the incidence of West Nile Virus so that proper control measures can be taken by the local vector control agencies to prevent the spread of West Nile Virus in the three above-listed counties and other counties identified by the Department of Public Health.

Executive Order S-10-07 (8/13/07)

IT IS ORDERED that the Department of Public Health shall allocate up to an additional $10 million, as needed, to local vector control agencies to identify potential mosquito habitat and to treat those areas to prevent the spread of West Nile Virus and/or for surveillance purposes to provide an early warning of the incidence of West Nile Virus so that proper control measures can be taken by the local vector control agencies to prevent the spread of West Nile Virus in counties identified by the Department of Public Health.

Executive Order S-12-07 (9/10/07)

IT IS ORDERED that the Department of Public Health shall allocate funds previously provided through the Governor's August 2, 2007 Emergency Proclamation and Executive Order S-10-07, as needed, to local agencies involved with West Nile Virus response to (1) enhance and expand public education on West Nile Virus prevention, (2) enhance outreach to the medical community, and/or (3) conduct active surveillance or epidemiological investigations of human West Nile Virus cases in counties identified by the Department of Public Health to be at elevated risk of West Nile Virus transmission.

Orders 1 and 2 and Executive Order S-10-07 appropriated up to $11.35 million to assist local agencies with WNV prevention, surveillance, and control. Funds totaling $6,214,219 were allocated over 10 funding phases to 67 agencies in 36 counties.

Award process:

Counties identified in the Governor's Emergency Proclamation at highest risk of WNV transmission were notified immediately and by Tuesday, August 7, letters of intent to allocate base funding (total $400,000) for mosquito control were provided to Kern, San Joaquin, and Colusa counties; $53,000 was also immediately allocated to Glenn County. Also on August 7, emergency funding applications were distributed to all local vector control and public health agencies and a conference call was held to explain the application process. Emergency award funding was based primarily on the risk of WNV transmission and on immediate resource needs of the applicant agency. Other evaluation criteria included the ability of an agency to use additional resources in a timely manner to impact current WNV activity and the population size afforded protection by additional resources. Funds were distributed via local assistance awards following the evaluation and approval of an award application submitted by the local agency.

Per the August 2nd Proclamation and Executive Order S-10-07, funds could only be used for emergency mosquito control or WNV surveillance. Allowable expenditures included: 1) salary for temporary (seasonal) personnel engaged in surveillance or mosquito control activities or for overtime not previously budgeted for existing staff, 2) mosquito control products, 3) mosquito control or surveillance equipment, and 4) contracts for aerial application of mosquito control products or aerial surveillance for neglected swimming pools or other mosquito producing habitat. Subsequent to Executive Order S-12-07 (September 10), agencies could also apply for funding for WNV public education, medical community outreach, and human case surveillance. Allowable expenditures included: 1) temporary personnel or overtime not previously budgeted, 2) costs associated with

public education and outreach (e.g. advertising, printing), and 3) costs associated with human case surveillance (e.g. lab supplies).

Applicants were required to submit a justification for requested funds, including a description of their current WNV risk and how the funds would reduce the risk of virus transmission. Specifically, applicants detailed why their current resources were insufficient; how additional staff would be used; which problem habitats required enhanced control or surveillance; what sources of mosquitoes were currently not controlled; why additional equipment or contractual services were needed; and how WNV public education, outreach to the medical community and/or human case surveillance would be enhanced. Current WNV risk was estimated using the risk assessment table in the California Mosquito-Borne Virus Surveillance and Response Plan (see http://westnile.ca.gov). This assessment table provided a semi-quantitative measure of WNV transmission risk based on eight surveillance factors (environmental conditions, adult mosquito vector abundance, virus infection rate in mosquitoes, sentinel chicken seroconversions, fatal infections in birds, infections in horses, infections in humans, and proximity of detected virus activity to urban or suburban regions).

The initial deadline for receipt of applications subsequent to the Emergency Proclamation was Wednesday, August 8. Subsequent to Executive Order S-10-07, there was no application deadline; applications were processed as received by the Vector-Borne Disease Section (VBDS) of CDPH to allow maximum responsiveness to changing WNV risks throughout the state. CDPH issued a Letter of Award to recipient agencies indicating the level of funding being granted. Upon signature and return of this letter to CDPH, agencies were eligible to spend against the award. Funds were distributed to agencies via lump sum payments and had to be expended by December 31, 2007. Award recipient agencies were required to submit a final report indicating budgeted expenditures and provide a narrative; award expenditures were subject to audit.

Award funding:

Initial requests for award funding far exceeded the emergency proclamation allocation of $1.35 million. The Governor rapidly responded via Executive Order S-10-07 which provided additional funding. This funding was a ceiling amount; funds could only be awarded based on the strict criteria mentioned above and had to address the current emergency. The need for funds to support public education and human case surveillance was addressed subsequent to the immediate needs of mosquito control and WNV surveillance via Executive Order S-12-07.

Of the approximately $6.2 million allocated to 67 local agencies in 36 counties from August 7 to October 15, 2007 (Table 1), approximately $4.3 million was directed toward enhancing mosquito control, $725,000 for WNV surveillance, $1.2 million for WNV public education, and $40,000 for human case surveillance (Table 2). The amount allocated to an individual county ranged from $3,000 to $813,244.

The bulk of the mosquito control and WNV surveillance funding (Figure 1) was used for mosquito adulticides (34%) and larvicides (25%); followed by equipment (19%), contract applications of pesticides (10%), labor (7%), and aerial surveillance for neglected swimming pools (5%). Of the $1.9 million allocated for public education, outreach to the medical community, and human case surveillance (Figure 2), over half of the funding was used for radio and television advertising (55%). Funds were also used for printed materials (23%), promotional items (10%), displays (9%), and labor (3%).

Based on the final reports received from recipient agencies, the award funding succeeded in providing for the timely acquisition of critical resources that reduced the local risk of WNV transmission. Of the $6,214,219 allocated, only $111,480 was unspent as planned and returned to CDPH for deposit in the State general fund.

The funds allocated in 2007 augmented the $15 million provided by the state during the

previous two fiscal years. The prior funds were dedicated to enhancing and expanding mosquito control in California and were not provided through an emergency declaration. The $21.2 million provided over three years to local agencies effectively strengthened the mosquito control infrastructural in the state and was instrumental in establishing services to regions of California previously without vector control.

Emergency Order 3

IT IS FURTHER ORDERED that the Department of Public Health shall coordinate with the State and Consumer Services Agency, the Resources Agency and the Department of Food and Agriculture to develop a plan using best management practices for implementation by the appropriate state agencies for the early detection of West Nile Virus on state-owned properties and appropriate mitigation and abatement measures. Funds in the amount up to $150,000 shall be allocated for the purpose of developing this plan.

The Emergency Proclamation ordered CDPH to develop a best management practices (BMP) plan for mosquito control on state-owned properties. In response, CDPH immediately contacted partnering state agencies and the Mosquito and Vector Control Association of California to form a steering committee and initiate plan development. On September 12, a contract was executed allowing CDPH to hire staff to develop the BMP plan in concert with partnering agencies and under the leadership of VBDS.

A stakeholder meeting was held on October 18, 2007; thirteen agencies were represented. Stakeholders provided input on development of the BMP plan and established a timetable for plan completion, dissemination, and evaluation. A draft plan was subsequently reviewed by members of the steering committee and the document finalized in June, 2008. The printed document "Best Management Practices for Mosquito Control on California State Properties" was distributed to appropriate state and local agencies in July and follow-up contact made with state agencies to help ensure plan implementation. The document can be found at http://westnile.ca.gov (under Resources).

Emergency Order 4

IT IS FURTHER ORDERED that the Department of Public Health and the Department of Food and Agriculture shall work with the Mosquito Research Program at the University of California, Davis, to determine what resources are needed to further advance the research on the ecology and the epidemiology of West Nile Virus.

In consultation with the UC Davis Center for Vectorborne Diseases and the University of California Mosquito Research Program, CDPH developed a funding proposal to 1) promote research on the ecology and epidemiology of WNV through a comprehensive, sustainable surveillance system and 2) promote a broad spectrum of research on WNV and mosquito biology and control at all UC campuses.

The proposal was submitted by VBDS for review and evaluation by key staff at CDPH, Health and Human Services Agency, and the Governor's Office. The proposal was well received but ultimately, due to the state budget deficit projected for fiscal year 2008-09, funds were not available for proposal implementation.

Emergency Order 5

IT IS FURTHER ORDERED that the Department of Public Health shall work with (1) local vector control districts to utilize their existing power pursuant to Health and Safety code section 2053 to inspect and abate vector or public nuisances, with special emphasis on the removal of standing water in untended pools and containers on vacant property; and (2) the Business, Transportation and Housing Agency and local public health departments to notify lenders, realtors, mortgage brokers and others whose responsibilities include managing vacant homes to ensure that pools and other containers

that can hold water are drained and maintained empty to prevent the spread of West Nile Virus.

In 2007, the rate of home foreclosures increased dramatically leaving many backyard pools untended. These pools provided excellent habitat for mosquito breeding and increased the risk of WNV transmission in urban areas. In recognition of this problem, the Emergency Proclamation instructed CDPH to address mosquito breeding in neglected pools in collaboration with local vector control agencies and the Business, Transportation and Housing Agency, which includes the Department of Real Estate.

Within a week of the Emergency Proclamation, CDPH developed and distributed a "neglected pool" information flyer to agencies and the general public. CDPH also developed and distributed a Question and Answer sheet for public agencies on mosquito management strategies for untended pools and vacant properties. All documents were posted on the CDPH website.

CDPH contacted the Business, Housing and Transportation Agency who subsequently 1) sent an electronic alert to all Department of Financial Institutions alerting them of the emergency declaration and encouraging those with property management responsibilities to drain and maintain pools and other containers that may breed mosquitoes, 2) prepared a list of contacts and stakeholder groups who would benefit from the information, and 3) sent a letter to all real estate brokers in affected counties with information about the declaration and actions to take. The Department of Real Estate also posted the general notification on their website.

Order 6 instructed CDPH to provide technical assistance to local agencies as needed to minimize the risk of WNV transmission. **Orders 7, 8, and 9** pertained to deployment of state resources, administration of contracts, and exemptions from certain government codes to allow rapid facilitation of the Governor's emergency orders. **Order 10** required CDPH to consult with county agricultural commissioners prior to certain pesticide applications, but such consultation was not necessary as CDPH did not apply pesticides for mosquito control. **Order 11** was not relevant to WNV; it required CDPH to provide consultation to local agencies on Valley Fever (coccidioidomycosis).

Governor's Executive Order, August 20, 2007 (S-11-07)

IT IS ORDERED that the Department of Public Health shall allocate up to $500,000 in additional funds to the Department of Fish and Game. The Department of Fish and Game shall use these funds for vegetation management on the following wetland wildlife areas managed by the Department of Fish and Game and located in counties determined by the Department of Public Health to be at high risk of West Nile Virus transmission: Mendota Wildlife Area (Fresno County); Los Banos and North Grasslands wildlife areas (Merced County); Upper Butte Basin Wildlife Area (Butte and Glenn counties); and Gray Lodge Wildlife Area (Butte and Sutter counties). In addition to these wetland wildlife areas, the Department of Fish and Game shall use these funds for vegetation management on other wetland wildlife areas identified in consultation with the Department of Public Health as being located within counties that are moderate to high risk of West Nile Virus transmission. The Department of Fish and Game shall consult with the Department of Public Health regarding best practices for vegetation management to prevent West Nile Virus, including best water management practices, vegetation control, wetland infrastructure maintenance, wetland enhancement features, and biological controls.

On August 20, 2007, CDPH was ordered to allocate funds to the California Department of Fish and Game (DFG) for vegetation and water management in wetland wildlife areas to reduce mosquito production and WNV transmission risk. CDPH consulted with DFG and local vector control agencies and provided an initial allocation of $100,000 to DFG on August 22 to conduct immediate vegetation management on the five wetland

wildlife areas indicated in the Executive Order. An additional allocation of $400,000 to DFG was provided on August 30 to conduct vegetation and water management and wetland infrastructure maintenance on eight wetland wildlife areas in eight counties.

Ultimately in 2007, 380 human cases (20 fatal) of West Nile virus were reported in California, far fewer than projected in late July. Resources provided through the Emergency Proclamation and subsequent Executive Orders likely contributed to minimizing illness and death from WNV in 2007. The emergency funding, coupled with state funding provided to enhance mosquito control infrastructure during the prior two fiscal years, has vastly improved California's ability to respond to the statewide WNV invasion. Continued collaboration between state and local public health and vector control agencies, as exemplified during the emergency proclamation response, is essential as we collectively strive to protect California residents and visitors from the ongoing threat of WNV infection.

Acknowledgments

The authors acknowledge all VBDS staff, particularly administrative staff that facilitated the rapid and often complex allocation of award funds: Linda Parsons, Janey Butner, and Jesse Laxton. We thank Anne Kjemtrup and Claudia Erickson for their contributions in developing public education materials addressing mosquito breeding in neglected swimming pools, and Carrie Nielsen for taking the lead in developing a best management practices plan for mosquito control on state-owned properties. Cynthia Jean and Carol Glaser provided assistance in reviewing applications that dealt with human case surveillance. From UC Davis, Bill Reisen and Greg Lanzaro contributed to development of the funding proposal to enhance WNV research. The support of Gil Chavez, Interim Chief, CDPH Division of Communicable Disease Control, was instrumental in ensuring prompt execution of the emergency orders.

Table 1. West Nile virus (WNV) emergency funding over ten phases: August 7 to October 15, 2007. Total amount allocated: $6,214,219.

Funding Phase	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6	Phase 7	Phase 8	Phase 9	Phase 10
Date	8-7-07	8-10-07	8-13-07	8-22-07	8-30-07	9-6-07	9-20-07	9-27-07	10-9-07	10-15-07
Amount	$453,000	$897,000	$562,036	$1,397,771	$382,452	$741,526	$1,009,075	$269,373	$411,935	$90,051
No. of Agencies	4	28	20	22	6	12	13	9	13	11
No. of Counties	4	21	17	15	6	11	13	7	11	9

Table 2: West Nile virus (WNV) emergency funding by county and allocation category.

COUNTY	TOTAL	MOSQUITO CONTROL	SURVEILLANCE	PUBLIC EDUCATION	HUMAN CASE SURV.
ALAMEDA	$35,360	$0	$35,360	$0	$0
AMADOR	$34,876	$26,376	$8,500	$0	$0
BUTTE	$428,608	$373,620	$30,538	$24,450	$0
CALAVERAS	$49,161	$30,664	$16,637	$1,860	$0
COLUSA	$442,860	$355,830	$38,904	$48,126	$0
CONTRA COSTA	$10,000	$0	$0	$700	$9,300
EL DORADO	$11,430	$11,430	$0	$0	$0
FRESNO	$277,510	$169,542	$53,268	$44,500	$10,200
GLENN	$278,103	$233,184	$25,618	$19,300	$0
IMPERIAL	$61,162	$37,933	$5,030	$18,199	$0
INYO	$20,041	$15,041	$5,000	$0	$0
KERN	$813,244	$381,884	$62,126	$369,234	$0
LAKE	$64,963	$57,963	$7,000	$0	$0
LOS ANGELES	$301,032	$118,728	$27,365	$154,939	$0
MARIN-SONOMA	$14,115	$0	$14,115	$0	$0
MERCED	$251,097	$235,852	$9,695	$5,550	$0
MODOC	$21,050	$15,208	$0	$5,842	$0
MONO	$15,000	$15,000	$0	$0	$0
NAPA	$44,427	$32,591	$11,836	$0	$0
NEVADA	$3,000	$2,450	$550	$0	$0
PLACER	$146,804	$91,990	$20,470	$34,344	$0
RIVERSIDE	$228,080	$136,334	$46,632	$41,514	$3,600
SACRAMENTO-YOLO	$552,010	$211,760	$45,250	$295,000	$0
SAN BENITO	$17,085	$10,200	$910	$5,975	$0
SAN BERNARDINO	$241,802	$107,929	$119,598	$14,275	$0
SAN JOAQUIN	$691,259	$667,929	$23,330	$0	$0
SAN LUIS OBISPO	$68,798	$35,800	$15,998	$17,000	$0
SANTA CLARA	$69,700	$47,461	$19,983	$0	$2,256
SANTA CRUZ	$26,605	$9,580	$9,875	$7,150	$0
SHASTA	$331,549	$245,053	$31,031	$55,465	$0
SOLANO	$52,429	$52,429	$0	$0	$0
STANISLAUS	$93,775	$71,113	$11,925	$4,300	$6,437
SUTTER-YUBA	$174,041	$174,041	$0	$0	$0
TEHAMA	$183,573	$166,110	$17,463	$0	$0
TULARE	$155,747	$109,559	$10,646	$26,620	$8,922
VENTURA	$3,923	$3,638	$285	$0	$0
TOTAL	$6,214,219	$4,254,222	$724,938	$1,194,343	$40,715

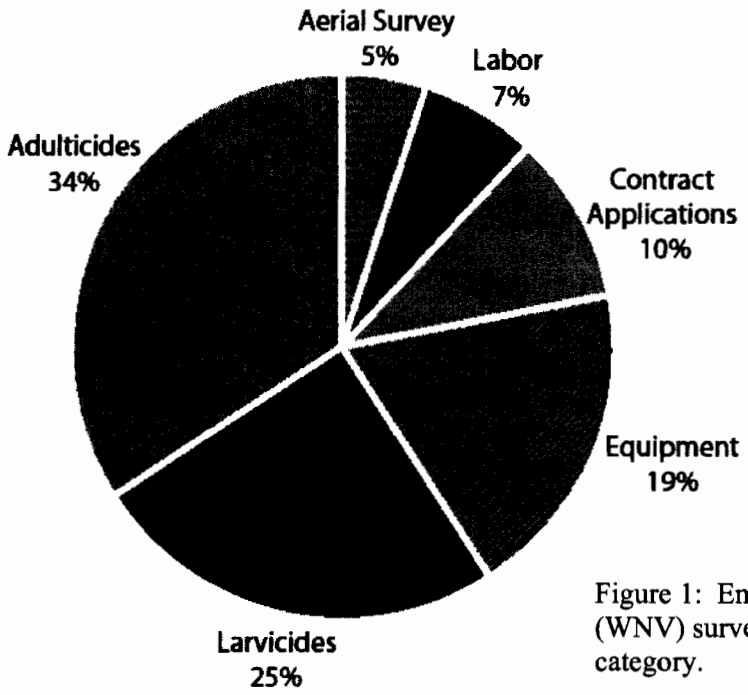


Figure 1: Emergency mosquito control and West Nile virus (WNV) surveillance funding; percent of total funding by category.

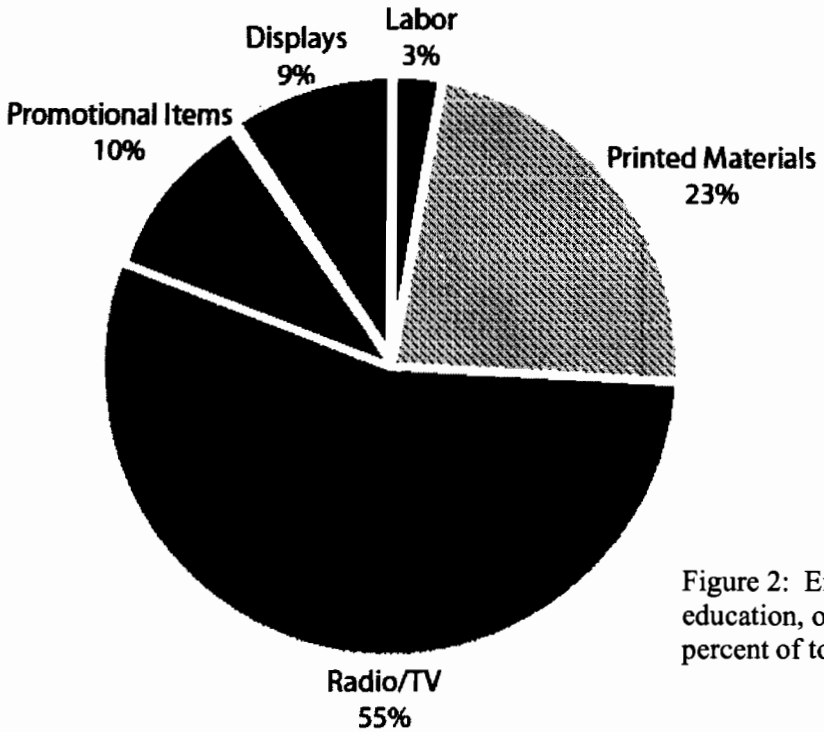


Figure 2: Emergency West Nile virus (WNV) public education, outreach, and human case surveillance funding; percent of total funding by category.

Where Have All the Western Equine Encephalomyelitis Cases Gone?[1]

Ying Fang, Aaron C. Brault and William K. Reisen

Arbovirus Research Unit, Center for Vectorborne Diseases, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, arbo123@pacbell.net

ABSTRACT: Historically, western equine encephalomyelitis virus (WEEV) has caused large equine and human epidemics from Canada to Argentina. Despite recent enhanced surveillance for West Nile virus, there have been no reports of equine or human cases and little documented WEEV enzootic activity. During the past three years, WEEV has been active again enzootically in California, with 85 positive pools and 135 sentinel seroconversions, but without human or equine cases. In the current study, we compared host and vector competence of representative WEEV isolates made during each decade over the past 60 years, using White-crowned sparrows (*Zonotrichia leucophrys*), House Sparrows (*Passer domesticus*) and *Culex tarsalis* as representative hosts. Results indicated limited time-related change in virulence among WEEV strains in birds and vector competence in *Cx. tarsalis*. Although temporal and spatial genetic changes have been documented, these seem to present limited phenotypic change in host competence and cannot explain the absence of equine and human cases.

INTRODUCTION

Historically, Western equine encephalomyelitis virus (Family Togaviridae, genus *Alphavirus*, WEEV) has caused devastating epidemics of neurological disease among young children and equines in the Central Valley of California (Reeves et al. 1990). Improved flood control projects along the western slope of the Sierra Nevadas, better housing with air conditioning, changes in human lifestyle and improved mosquito control undoubtedly have combined to reduce the numbers of WEEV encephalitis cases, whereas equine cases have been prevented by extensive immunization. However, the same factors have not prevented West Nile virus (WNV, Family Flaviviridae, genus *Flavivirus*) infection in rural human populations, most likely transmitted, in part, by the same vector mosquito, *Culex tarsalis* Coquillett. Since 2005, there has been a resurgence of enzootic transmission of WEEV in California, with 85 positive pools of *Cx. tarsalis* and 135 positive sentinel chicken sera detected in Coachella Valley and Kern County. Interestingly, there have been no human cases identified. The detection of enzootic transmission in *Culex* without spread to *Aedes* or tangential transmission to humans potentially could be caused by genetic changes in recent strains of the virus that limit amplification. To test this notion, we conducted experiments to:

1. Determine WEEV avian virulence. We compared a well-characterized isolate made in Imperial Valley in 2005 to isolates made historically between 1953 and 1992 using House sparrows and White-crowned sparrows as model bird hosts.
2. Determine if WEEV infectiousness for *Cx. tarsalis* has changed by comparing the 2005 and 1953 isolates.
3. Determine if previous WNV infection in some way 'blocks' or alters WEEV infection.

[1] This research has been accepted for publication in the American Journal of Tropical Medicine and Hygiene

MATERIALS AND METHODS

Viruses. We used strains of WEEV isolated from pools of *Cx. tarsalis* collected during each decade from 1953 through 2005. Prior to 1990 isolations were made by suckling mouse intracerebral inoculation, whereas after 1990 all isolates made in Vero cell culture. All strains were at suckling mouse or Vero cell passage 2 at the time of experimentation.

Mosquitoes. *Culex tarsalis* colonies recently were established from the Kern National Wildlife Refuge (KNWR) in 2003 and the Yolo by-pass of the Sacramento River in Yolo County in 2005. A total of 60-80 females that were within 3 - 8 d post-emergence were sorted into 0.6L infection cartons and starved for 24 h prior to infection attempts.

Birds. White-crowned sparrows (WCSP) (*Zonotrichia leucophrys*) and House Sparrows (HOSP) (*Passer domesticus*) were selected as experimental hosts in which to compare WEEV strains because they were highly and moderately susceptible, respectively (Reisen et al. 2003), relatively easy to cage adapt, and abundant near our laboratory in Kern County. WNV-antibody positive Western Scrub-jays (WESJ) (*Aphelocoma coerulescens*) and House finches (HOFI) (*Carpodacus mexicanus*) collected at the Kern River near Bakersfield were used to determine the effects of previous WNV infection on the viremia response.

Experiments. Viruses were compared during the following experiments:

Exp. 1. Vector competence. *Cx. tarsalis* from the Yolo and KNWR colonies were offered a 10-fold dilution series of BFS1703 1953 or IMP181 2005 viruses in heparinized chicken blood containing 2.5% sucrose. Females were allowed to feed for up to 1 hr, after which engorged females were transferred to clean 0.6L cartons and maintained for 14 d in an incubator at 26°C. Following the 14 d extrinsic incubation period, females exposed to the highest titer of virus were tested for their ability to transmit virus using an *in vitro* method (Aitken 1977). Expectorate samples and bodies of these females as well as those exposed to lower concentrations of WEEV were placed in individual cryovials and stored at -80°C until tested.

Exp. 2. Host competence. Groups of 4 to 8 WCSP or HOSP were inoculated subcutaneously in the cervical region with 2 to 3 $\log_{10}$ PFU/0.1 mL of each WEEV strain. To monitor viremia, birds were bled daily by jugular puncture and samples frozen immediately at -80°C until assessed for viral titer by titration by plaque assay on Vero cells.

Exp. 3. Effect of WNV antibody on WEEV infection. Four field-collected HOFI and 6 WESJ that tested positive for WNV by EIA and an equal number of birds that were antibody negative, each were inoculated subcutaneously in the cervical region with a 2.7 $\log_{10}$ PFU/0.1mL inoculum of the KERN5547 strain of WEEV. Birds were bled daily for 5 d to assess viremia.

RESULTS

Exp. 1. Vector competence. The median infection-dose estimated for the KNWR colony of *Cx. tarsalis* ingesting a 10-fold dilution series of either the BFS1703 1953 or the IMP181 2005 strains of WEEV were almost identical (3.35±0.18 and 3.16±0.15 $\log_{10}$ plaque forming units (PFU) per mL, respectively). When assessed using the *in vitro* capillary tube method (Aitken 1977), there were no significant differences in the ability of either the KNWR or YOLO strains of *Cx. tarsalis* to transmit the 1953 or 2005 strains of WEEV (Fig. 1). Collectively, these data indicated that there were minimal differences in the vector competence of *Cx. tarsalis* for the 1953 and 2005 WEEV strains.

Exp 2. Host competence. All birds tested negative by EIA at capture for antibodies against WEEV and SLEV. Overall, 2 of 8 WCSP and 1 of 8 HOSP infected with the 1953 strain died during acute infection, significantly more (Chi square = 11.1, $P<0.001$) than the remaining 57 birds that survived infection with the other strains. When tested using a repeated

measures ANOVA (Hintze 1998), there were significant differences in mean viremias on 1 and 2 days post inoculation [dpi] among WEEV strains within bird species (WCSP: $F = 6.23$, $df = 5, 40$, $P < 0.001$; HOSP: $F = 12.79$, $df = 3, 23$, $P < 0.001$) (Fig. 2), but there was no time-related trend.

Exp 3. WNV immunity. All birds tested negative by EIA at capture for antibodies against WEEV. Four House Finches (*Carpodacus mexicanus*) and 6 Western Scrub Jays (*Aphelocoma coerulescens*) showed evidence of previous WNV and were paired with an equal number of birds without detectable antibody, and then infected with WEEV. As expected, previous infection with WNV had little effect on the course of WEEV infection in WESJ and HOFI naturally immune to WNV. WEEV and WNV are in different viral families, do not cross react serologically, and do not provide cross-protective immunity.

DISCUSSION

There did not appear to be temporal patterns in vector or host competence that would account for decreased WEEV enzootic amplification and the reduction of equine and human cases. There were essentially no differences in *Cx. tarsalis* vector competence for the 1953 and the 2005 strains of WEEV. There were significant differences among mean viremias following infection with different WEEV isolates, but these strains did not assort by time. The BFS1703 strain isolated in 1953 from Kern County exhibited among the highest viremias and was the only strain that produced mortality in birds, agreeing with previous studies (Hardy and Reeves 1990, Reisen et al. 2003). The COA592 strain isolated from Coachella Valley in 1992 produced statistically similar viremia titers, but without mortality. The greatest viremia decrease was seen in the response of HOSP to the 2005 strain (mean titer = 3.6 $\log_{10}$ PFU/mL). Further research will be necessary to elucidate factors leading to variation in virulence among avian but not mosquito hosts.

In the same mosquito and avian hosts, WEEV had greater vector susceptibility (i.e., lower ID_{50}) and lower avian viremia compared to the invading WNV. In addition, WEEV replicates far better in *Cx. tarsalis* under cool temperatures than WNV and therefore potentially has a longer transmission season than WNV (Reisen et al. 2006). With a lower mosquito ID_{50} and lower thermal threshold for replication, *Cx. tarsalis* would be expected to be more readily infected and transmit more efficiently at the avian viremia titers produced by WEEV than at the markedly higher titers for WNV. However, this has not been the case and, although WNV has continued to be transmitted at epidemic levels in California, WEEV has remained at low enzootic levels without reported human cases during the past three years (Feiszli et al. 2007, Hom et al. 2006). Apparently, the decline in human cases must be related to epidemiological factors such as improved housing and the use of air conditioning, whereas the decline in equine cases most likely is related to extensive vaccination.

Acknowledgements

Sandra Garcia, Arbovirus Research Laboratory, and Vincent Martinez, Brian Carroll and Scott Hallam, Arbovirus Field Station, Center for Vectorborne Diseases, provided excellent technical assistance in the laboratory and field, respectively.

The collection and infection of wild birds with encephalitis viruses was done under Protocol 11184 approved by the Institutional Animal Care and Use Committee of the University of California, Davis, California Resident Scientific Collection Permit 801049-02 by the State of California Department of Fish and Game, and Federal Fish and Wildlife Permit No. MB082812-0. 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.

REFERENCES CITED

Aitken, T. H. G. 1977. An in vitro feeding technique for artificially demonstrating virus transmission by mosquitoes. Mosq. News 37:130-133.

Feiszli, T., B. Park, V. L. Kramer, A. Kjemtrup, B. F. Eldridge, Y. Fang, W. K. Reisen, E. Baylis, C. Jean, J. Glover, R. Carney, K. Padgett, C. Erickson and S. Husted. 2007. Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 2006. Proc. & Papers Mosq. Vector Control Assoc. Calif. 75:48-59.

Hardy, J. L. and W. C. Reeves. 1990. Experimental studies on infection in vertebrate hosts In: W. C. Reeves, editor. *Epidemiology and control of mosquito-borne arboviruses in California, 1943-1987.* Sacramento, Calif.: Calif. Mosq. Vector Control Assoc. pp 66-127.

Hintze, J. 1998. NCSS Statistical Software (computer program). 2000. Kaysville, UT: NCSS.

Hom, A., D. Bonilla, A. Kjemtrup, V. L. Kramer, B. Cahoon-Young, C. M. Barker, L. Marcus, C. Glaser, C. Cossen, E. Baylis, C. Jean, B. F. Eldridge, R. Carney, K. Padgett, B. Sun, W. K. Reisen, L. Woods, J. Glover, C. Erickson and C. Barclay. 2006. Surveillance for mosquito-borne encephalitis virus activity and human disease, including West Nile virus, in California, 2005. Proc. & Papers Mosq. Vector Control Assoc. Calif.74:43-55.

Reeves, W. C., S. M. Asman, J. L. Hardy, M. M. Milby and W. K. Reisen. 1990. *Epidemiology and control of mosquito-borne arboviruses in California, 1943-1987.* Sacramento, Calif: Calif.Mosq.Vector Control Assoc.

Reisen, W. K., R. E. Chiles, V. M. Martinez, Y. Fang, and E. N. Green. 2003. Experimental infection of California birds with western equine encephalomyelitis and St. Louis encephalitis viruses. J. Med. Entomol. 40:968-982.

Reisen, W. K., Y. Fang, and V. M. Martinez. 2006. Effects of temperature on the transmission of West Nile virus by *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 43:309-317.

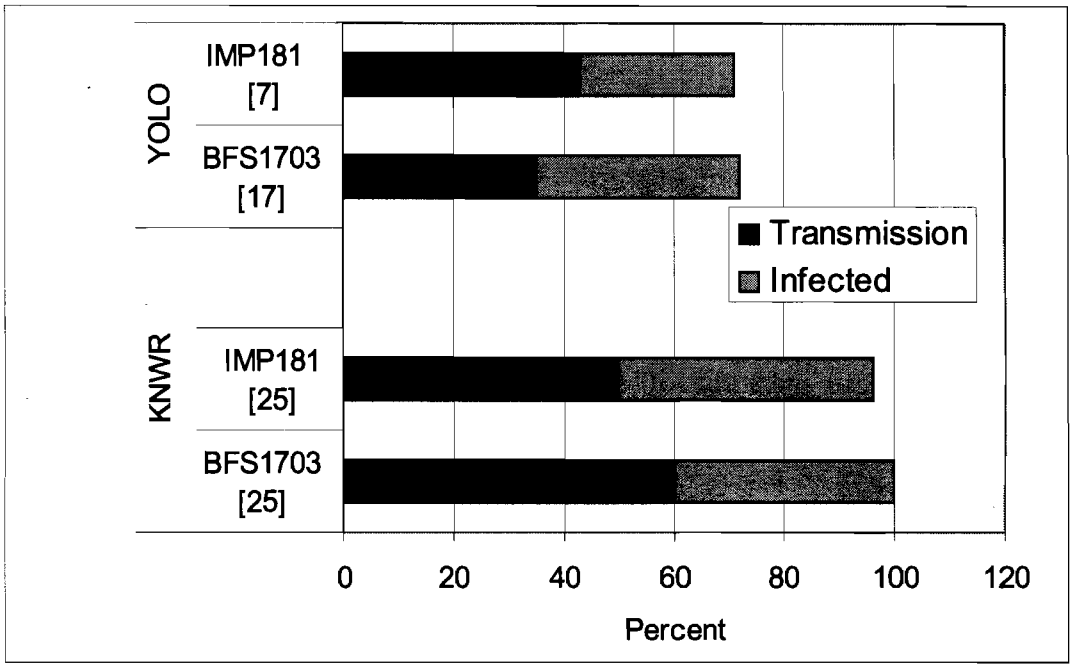


Fig. 1. Vector competence of the Yolo and KNWR colonies of *Culex tarsalis* for the 1953 BFS1703 and 2005 IMP181 strains of WEEV. Data show the percentage of females infected and transmitting virus using the capillary tube method.

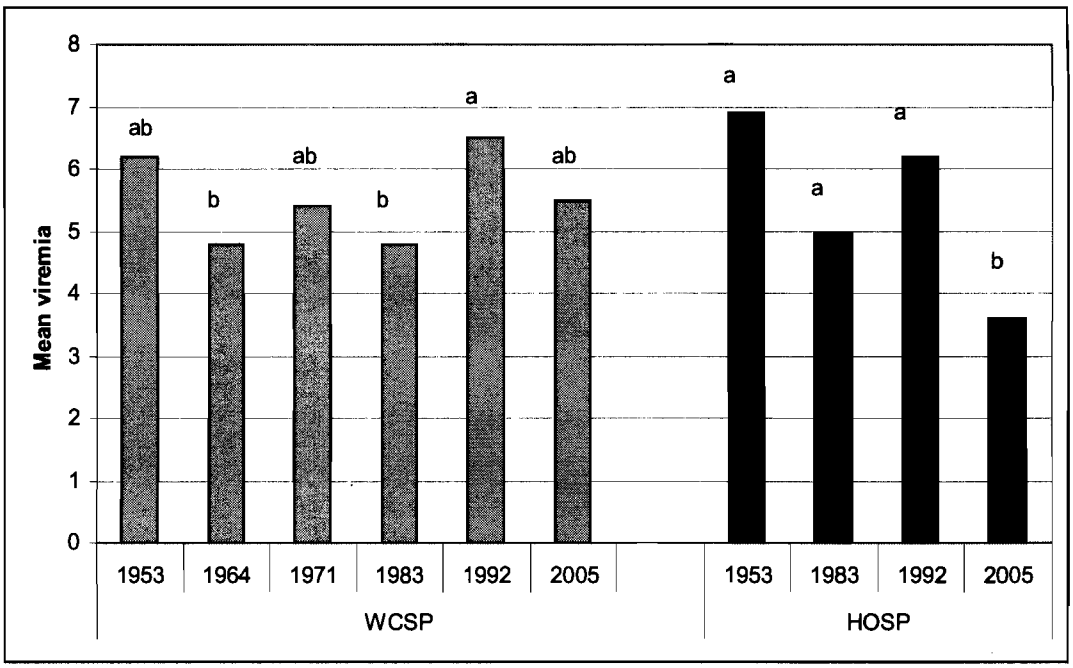


Fig. 2. Mean viremia in $\log_{10}$ PFU/mL at 1 and 2 days post infection for White-crowned Sparrows (WCSP) (*Zonotrichia leucophrys*) and House Sparrows (HOSP) (*Passer domesticus*) infected with WEEV strains from 1953 – 2005. Bars with similar letters above them were not significantly different when tested by a Fisher's least significant difference multiple comparison test ($P>0.05$).

Who Found West Nile Virus Activity in Sacramento and Yolo Counties First...Mosquito, Chicken, or Pigeon?

Kara Kelley, Paula A. Macedo, Marcia Reed, Gary W Goodman, David A. Brown

Sacramento-Yolo Mosquito and Vector Control District, 8631 Bond Road, Elk Grove, CA 95624

Sentinel chicken flocks have been used to detect arbovirus activity throughout the country, but have not been an effective early indicator of West Nile virus (Family Flaviviridae, genus Flavivirus, WNV) activity in Sacramento and Yolo Counties. In 2007 the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) made changes to the surveillance program in order to evaluate the efficacy of the sentinel chicken program. Until 2006 the sentinel chicken surveillance program consisted of 10 flocks of 10 chickens each, distributed mostly throughout the rural areas in both counties. The District increased the number of flocks from 10 to 15 in order to incorporate suburban sites. Six chickens were distributed to each of the 15 flocks. We also incorporated pigeons (*Columba livia*) in our sentinel program at 6 of the 15 flock sites. One urban site and five rural sites were selected for the sentinel pigeons due to easy access and space to accommodate both the chickens and pigeons cages in the same property. The third change in the program was the placement of 3 encephalitis virus surveillance (EVS) traps and 1 gravid female trap in each of the 15 chicken flock sites, to determine if antibodies would be found in the sentinel birds before virus detection in the mosquitoes. Blood samples from the chickens and pigeons were collected weekly from March through October. EVS and gravid female traps at each location were placed and collected following the same schedule, with subsequent testing of the mosquitoes for the presence of WNV.

When comparing mosquito pools to the sentinel chickens in urbanized areas, the study showed that the mosquito pools indicated WNV activity approximately four weeks prior to the first sentinel chicken conversion (Fig. 1). In the single urbanized site, the sentinel pigeons showed antibodies to WNV five weeks before any of the sentinel chickens demonstrated antibody conversion. All mosquito pools collected and tested from that site were negative for WNV.

In contrast, the rural sites revealed that sentinel chickens showed antibodies to WNV three weeks prior to the first WNV positive mosquito pool, and at some of the sites, sentinel chickens were the only indicators of WNV activity (Fig. 1). In addition, sentinel chickens indicated WNV activity between one and three weeks before the first sentinel pigeon showed antibodies.

From our results, we conclude that mosquito pools seem to be a more effective indicator of early WNV activity in suburban environments, but not in rural environments, where chickens seem to be a better early indicator. Unfortunately we only had one urban site with pigeons in our study; therefore further studies are necessary to determine if the sentinel pigeons would be a more effective indicator of WNV activity than the sentinel chickens in such environments. One hypothesis is that, with the pigeon being an established and common urbanized bird, it may be a more attractive sentinel due to the mosquito species feeding habits in such areas.

Overall, implementing a greater number and variety of sentinel tools increases the chances of early detection of WNV activity at the sentinel sites. With more timely information, the District's control efforts were more effective and, in turn, lowered potential risk to the public at large. Our mission is to continue enhancement of all of the District's virus surveillance programs. With the expansion of the surveillance programs, there is a greater

probability of early detection of virus activity, which therefore translates to increased protection from arbovirus transmission.

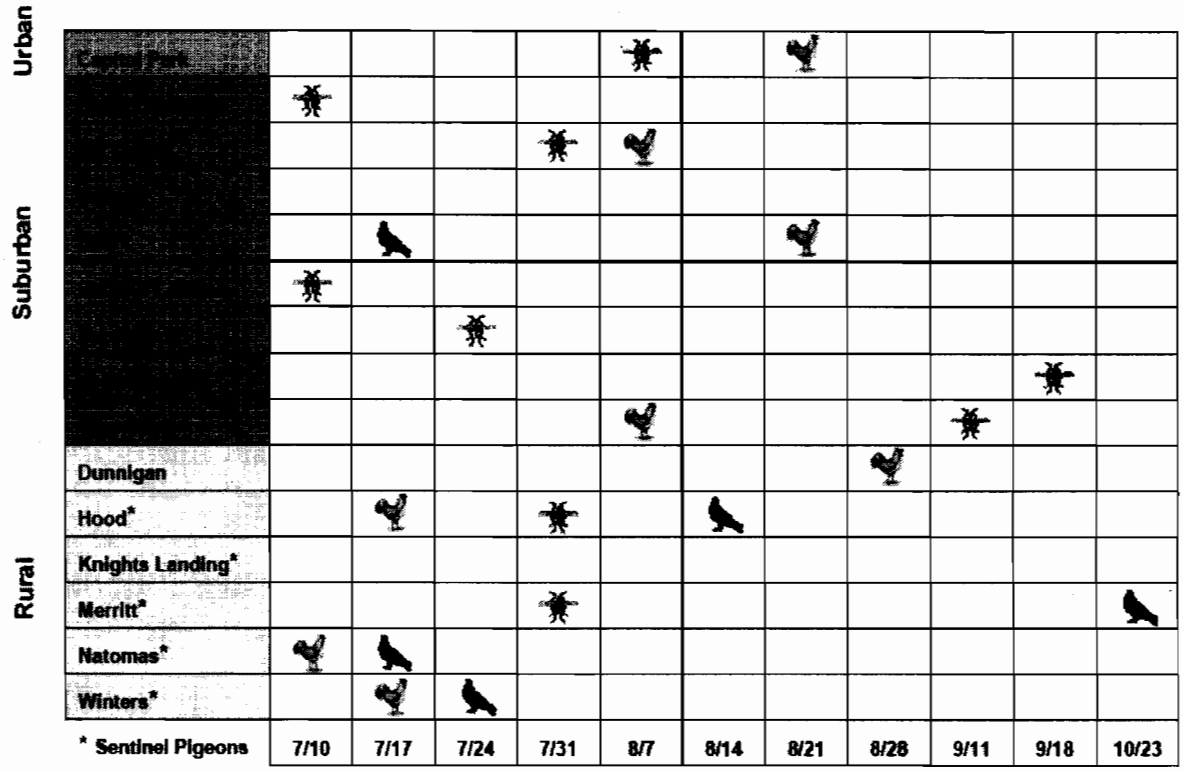


Figure 1. Initial indication of West Nile virus activity in Sacramento and Yolo Counties, 2007.

Guidelines for Contributors

Proceedings and Papers of the Mosquito and Vector Control Association of California

Proceedings and Papers, also referred to as *Proceedings*, is the official, professional publication of the Mosquito and Vector Control Association of California. It is printed one volume each year and included papers based on presentations, including young investigators award competition, given at the Association's annual conference. Publication of submitted papers is also encouraged. It publishes articles on the biology, ecology and control of mosquito and other vectors of disease.

CONTRIBUTIONS: A manuscript for publication in the *Proceedings* is encouraged from every speaker. Articles should be original contributions in the field of mosquito and vector ecology and control and provide information to benefit the diverse interests in scientific and technical development, operations and programs, and management documentation. Please do not submit papers that have been previously published or are being considered for publication elsewhere. An excessive number of pages on one subject or by any one author are generally discouraged. Although preference is given to papers accepted on the program agenda, acceptability for publication rests on merit determined on review by the editor. A non-member author wishing to publish in the *Proceedings* is required to pay the registration fee for the conference.

MANUSCRIPT FORMAT: Manuscripts must be typed double-spaced only on one side of the page with one-inch margins on all sides. A 3-1/2" floppy diskette or compact disk should also be submitted which includes, besides manuscript, images of all tables, figures or photographs. Common IBM compatible word processing programs such as Microsoft Word or WordPerfect, or Excel for charts is preferred. One hard copy plus two copies of the tables, figures and/or photographs should accompany the diskette. Electronic submission of manuscripts is encouraged. These should be submitted to the incoming editor within 60 days following the end of the conference. Articles received after that time may be returned for resubmission for the next issue of the *Proceedings*. Authors should refer to recent issues of the *Proceedings and Papers of the Mosquito and Vector Control Association of California* for style and format and the *Journal of the American Mosquito Control Association* for guidance on scientific names.

The *Proceedings* subscribed to the scientific abbreviations of mosquito generic named used by the American Mosquito Control Association. The usage and a list of these scientific names are discussed in the Journal of the American Mosquito Control Association, 5:485 (1989). Bi-letter generic abbreviations are used for Culicdae. Common abbreviations (et al., i.e., etc.) are not italicized. Use of the metric system (with English measurements in parenthesis) is encourages. Avoid footnotes in text.

The papers in the *Proceedings* will appear, for the most part, as submitted. Editorial liberties will be exercised in those instanced where improved clarity is needed and where style is incorrect. Articles requiring extensive editing and not conforming o style and instructions will be returned to the author for correction.

SUBMITTED PAPERS: Manuscripts (other than presentations at the conference) submitted for publication in the *Proceedings* will be treated as "Refereed or Peer Reviewed Articles." These will be sent for review to at least two or more scientists proficient in the subject. Following their comments and advice, the Editor will determine whether these should be published as Peer Reviewed articles.

TITLE: The title, author's name(s), organization, mailing address, e-mail address,

and telephone number should appear at the top of the first page.

ABSTRACT: An Abstract is required, and should provide a brief summary of the paper. The Editor may refuse to publish Abstracts or Summaries alone.

PAGE NUMBERING: Number pages consecutively, including tables and figures. Insert the tables and figures as separate pages following the first place they are referenced in the text.

TABLES: Tables should be typed on separate sheets placed in correct sequence in the text and should be limited to those strictly necessary. Titles should appear at the top of the tables. Tables should be prepared with regard to the ultimate printed size of one (3") or two columns (6-1/4"). Each table should be referenced at some point within the text. Avoid long and complex tables.

ILLUSTRATIONS: Figures, graphs, line drawings and photographs must be mailed flat. Figures should be numbered consecutively. Titles, legends, or other headings should be typed double-spaced on a separate sheet of paper. Titles when provided with illustrative materials should be at the bottom of the figure, graph or picture. As with tables, illustrative materials must be planned to fit reasonably within a one or two column format. Figure numbers, in addition to the author's name, should be written in blue pencil on the back of each illustration. Figures generated on dot matrix printers, or photocopies reproduced poorly will not be acceptable for publication. Since most figured may be reduced to one column width, the original lines and printing must be legible when reduction becomes necessary. Patterns in black/gray instead of colors should be used in maps, charts and other illustrative materials.

REFERENCES CITED: Alphabetize references by the author's surnames. Within the alphabetical order, sequence should be by year beginning with the earliest publication date. Include only publications that are cited in the text, and the style of the citations should conform to the format in the latest issue of the *Proceedings*.

PROOF AND REPRINTS: Authors will receive a galley proof, as well as order forms for reprints. Major revisions at this stage will not be acceptable. Proofs with corrections, if any, and reprint order forms should be returned within 7 days to the MVCAC office (Electronic submission of both proof corrections and reprint orders is highly desirable):

Mosquito and Vector Control
Association of California (MVCAC)
Attention: Ashley Paul
1215 K Street, Suite 2290
Sacramento, California 95814
Phone: (916) 440-0826 Fax: (916) 231-2141
E-mail: apaul@mvcac.org

Incoming Editor: Jan Washburn, Ph.D.
c/o
Alameda County Mosquito Abatement District
23187 Connecticut St.
Hayward, CA 94545-1605
Phone: (510) 783-7744
E-mail: j.washburn@mosquitoes.org